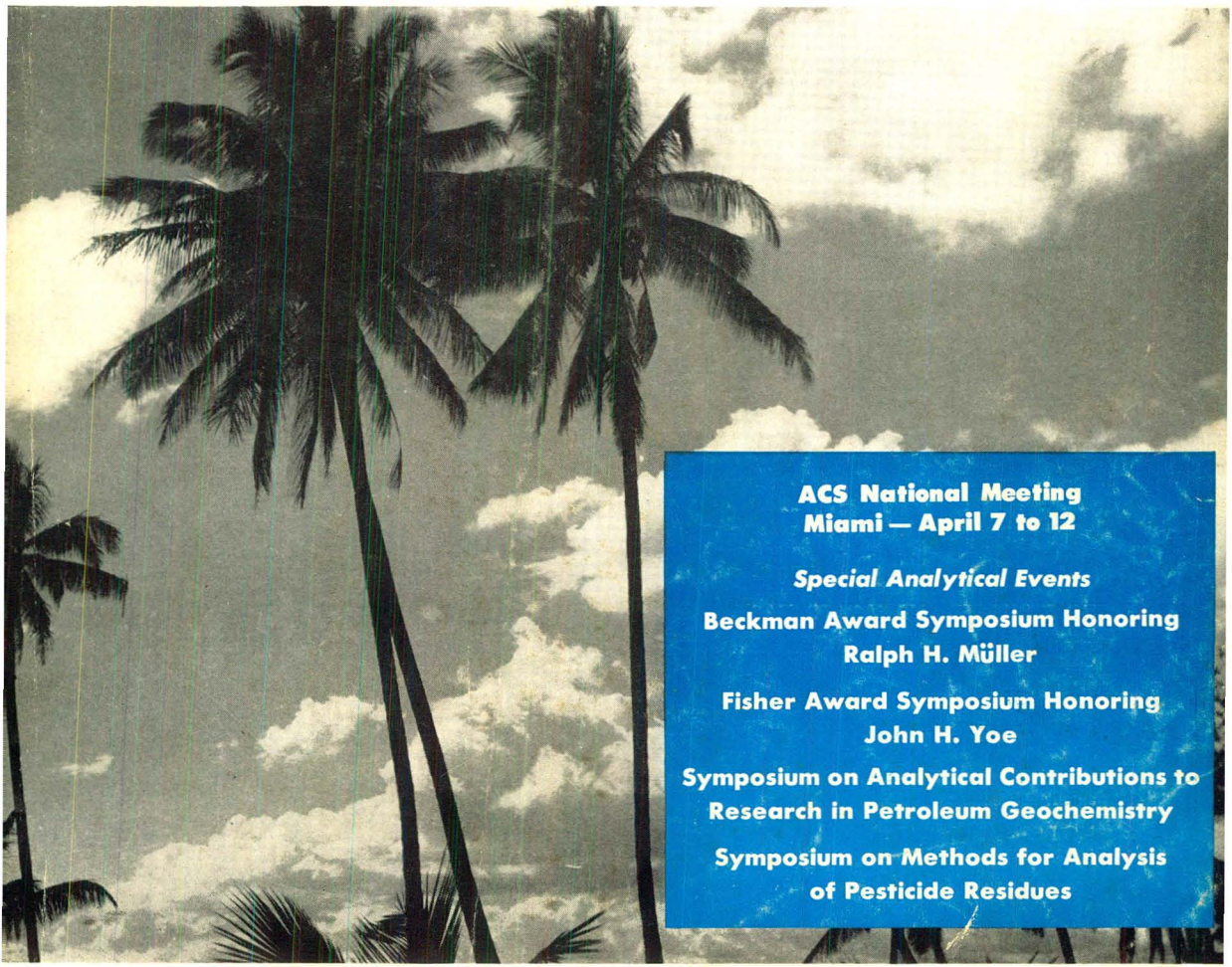
March 1957

ANALYTICAL CHEMISTRY



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FULL PROGRAM OF ANALYTICAL DIVISION APPEARS ON PAGE 29 A

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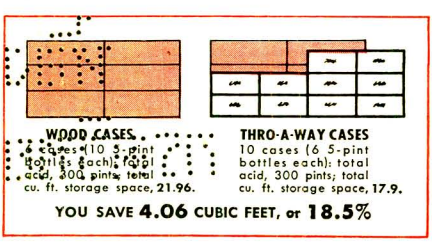

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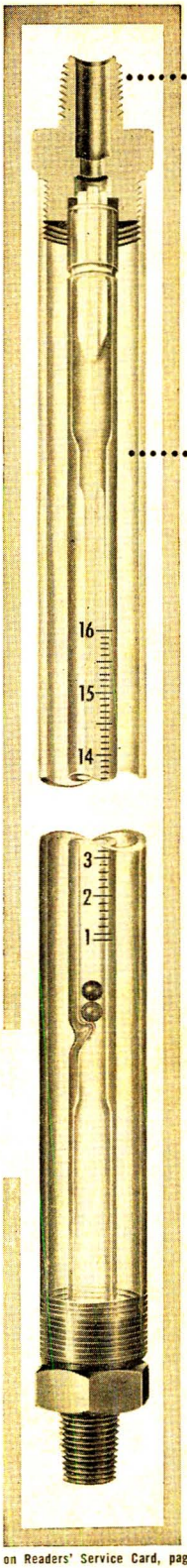

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ANALYTICAL CHEMISTRY

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THIS MONTH'S COVER

A diversified program with a large number of papers on topics of current interest is scheduled for the Division of Analytical Chemistry at the ACS National Meeting at Miami, April 7 to 12. In addition to symposia on analytical contributions in petroleum geochemistry and analysis of pesticide residues, the division will present symposia honoring Beckman Award winner Ralph H. Müller and Fisher Award winner John H. Yoe. The complete program appears on page 29 A.

Volume 29, No. 3

Issued February 20, 1957

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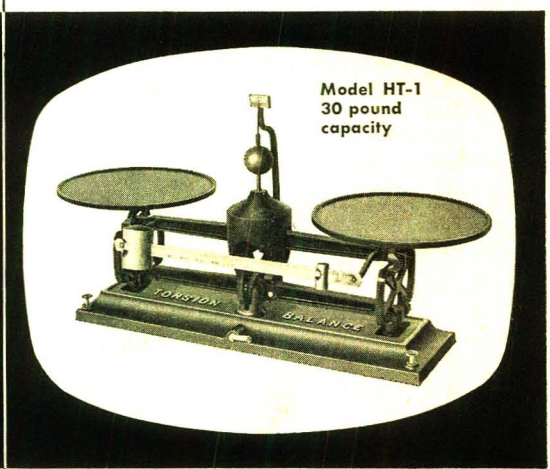

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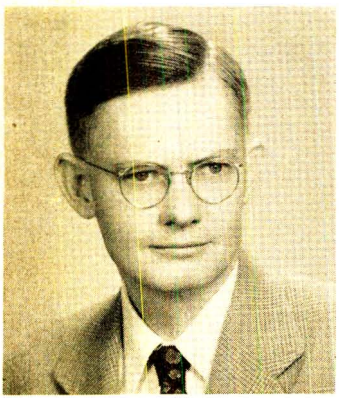
REPORT FOR ANALYSTS

Classification of Analytical Methods

Between absorption spectrometry and zymometry there are a host of other analytical techniques, the number of which is increasing steadily. An alphabetical listing of these methods and techniques, while impressive in size, is disheartening to those who are expected to master analytical techniques. Over the years, various classification systems have been proposed to systematize these techniques and methods. The author and some of his associates proposed one such system seven years ago. Subsequent experience has established its usefulness, the authors feel, in the organization of courses and the indexing of papers. Progress in the field of analysis, however, has made modification desirable. The modified system and the reasons for it are the subject of this month's "Report for Analysts."



AFTER COMPLETING an elementary course in quantitative analysis, a student may feel that the whole field is included under the heading gravimetric and titrimetric methods. He gets a rude awakening when he is confronted in advanced courses or in work after graduation by a whole new array of techniques and methods.

If he should attempt to list known methods, a partial list might read:

Absorption spectrography
Absorption spectrometry
Amperometric titrimetry
Biological assay
Capillary analysis
Colorimetry
Combustion analysis
Conductometric titrimetry
Coulometric analysis
Critical solution temperature method
Densitometry
Electrical conductivity method
Electrodeposition
Electrographic analysis
Emission flame spectrophotometry

Frederick C. Strong III, 39-year-old associate professor of chemistry at Stevens Institute of Technology is a native of Denver. He received his B.A. degree with high honors from Swarthmore College in 1939 and his M.S. in analytical chemistry at Lehigh University in 1941. He did some graduate work at Bryn Mawr in 1947. Except for a few short periods of industrial experience as a research chemist, he has taught chemistry. He was an assistant at Wesleyan University in 1943, an instructor at Cedar Crest College, Allentown, Pa., assistant professor at Villanova in 1947 and has been at Stevens Institute of Technology since 1951. His teaching at present includes courses in sophomore analytical chemistry, physical chemistry and a graduate course in instrumental methods of analysis.

In addition to an article on "Trends in Quantitative Analysis" (ANAL. CHEM., 19, 968–71, 1947) and "The Theoretical Basis of the Bouguer-Beer Absorption Law" (ANAL. CHEM. 24, 338–42, 1952), he has prepared a textbook "Qualitative Analysis" to be published shortly by McGraw-Hill Book Co. He is also editor of "Applied Spectroscopy," publication of the Society for Applied Spectroscopy.

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Polarography
Potentiometric titrimetry
Raman spectrography
Refractometry
Sonic methods
Spectrophotometry
Thermal conductivity method
Titrimetry
Turbidimetry
Viscometry
Volume of liquid distilled
Volume of precipitate
X-ray diffraction analysis
Zymometry[a]

[a] It seemed fitting to conclude the list with a Z so the author's associates invented Zymometry, the "determination of enzymes."

After he has made up such a list, he is not much better off. The correlations, if any, between the methods are not evident. What is needed is a classification method to show how each method fits into the whole scheme of analysis.

This problem is not new. It has been recognized for many years. Many competent analysts have made efforts to devise a classification system. In an earlier article (*3*), the author described the virtues and faults of several of these.

Requisites of an Ideal System

Requirements of an ideal classification system are:

1. A division must be exhaustive.

2. The constituent species of the genus must exclude one another.

3. A division must proceed at every stage upon one principle, the *fundamentum divisionis*.

4. It must be based upon generally accepted divisions of chemistry and physics.

5. It must use a generally accepted vocabulary.

6. It must be of definite utility.

7. It must not involve theoretical knowledge on a level above that of an undergraduate in chemistry.

8. The criteria for differentiation must be simply and clearly defined.

9. It must be capable of expansion and intensification without the necessity for radical revision.

A practical system, even though it does not meet all these criteria, can still be a very useful guide.

One problem in most classification systems arises from the careless use of "physicochemical."

Requisites for Practical System

A classification system, to be most useful, the author feels, should be based on the operations involved—that is, the fundamental nature of the operations which are carried out upon the material to be analyzed. It can be physical, chemical, or biological in nature. The objective of using an operational (functional) basis is to clarify the fundamental nature of the operation itself, as this is a source of confusion in the organization of the science of analytical chemistry.

The next step is to differentiate between operations involved in preparation and operations involved in measurements.

Definition. "Method of analysis" is defined as a procedure or sequence of operations which makes possible a quantitative estimation of the chemical composition of any material. "Determination" denotes an operation which involves a measurement of amount. "Preparation" denotes any operation to which the material must be subjected before a measurement can be applied.

Classification of Quantitative Analytical Methods

The classification system proposed for quantitative analytical methods is based on (1) methods of preparation of sample, and (2) methods of determination of the desired constituent.

Preparational operation can generally be differentiated on the basis of being either essentially physical or essentially chemical. Such a distinction, while difficult to make on a fundamental scientific basis, is relatively simple when based on traditional considerations. The distinction between a chemical and physical process is made by considering whether or not the chemical composition of the system changes as a result of the process. To be useful, a classification of the methods available for measurements must achieve a high degree of differentiation.

In this connection, biological methods of measurement constitute a large subdivision. Whether or not a method is biological depends on (1) whether the

determination is based primarily on response of a living organism and, if not, (2) whether the chemical composition of system changes as a necessary result of the determination.

Similar types of questions help break the classification system into biological, chemical, or physical.

Revised System

The system developed by the author, which is a revision of his 1950 system, appears below. One major change has been to make the classification more specific, particularly with regard to qualitative analysis. Previously, qualitative analysis was considered as a necessary preliminary and came under the heading of sample preparation. The author now feels that this is unrealistic suppression of an important part of analysis, particularly in such fields as emission and absorption spectrometry.

The previous concept of analysis is retained. This involves a three-step procedure: sampling, qualitative analysis, and quantitative analysis. Such a system is consistent with the truism that an analysis that is purely qualitative is of no value; there must be at least a semiquantitative estimate of the substance identified. This concept is at variance with some definitions of analysis.

In working with his original system, this author concluded that it was too general in allowing space for all conceivable methods of quantitative analysis, without naming or amplifying important methods now in use.

The revised version lists only methods of current importance or those promising to be important in the near future. There is some elaboration on their variations.

One significant change is the shift of gravimetric methods from a chemical to a physical operation of measurement. The previous inclusion of the separational steps as inherent in the connotation of the term *gravimetric* (thus making it chemical in nature) has justification, but an expansion of the section on *preliminary operations* and a grouping of methods of *separation* make the previous arrangement inconsistent. The new position for gravimetric methods is the one advocated by Patterson and Mellon (*2*). However, the author cannot accept their assertion that all methods of measurement are essentially physical. For example, it appears to the author that the measuring step in a titration is the process of titration itself, the standard solution being the yardstick, not the buret readings. Concurring with this point of view, the Nomen-

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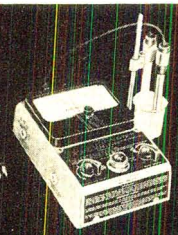

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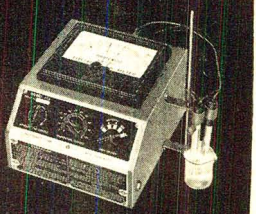

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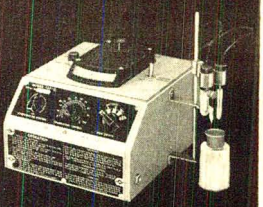

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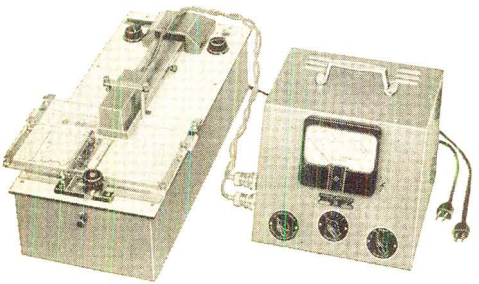

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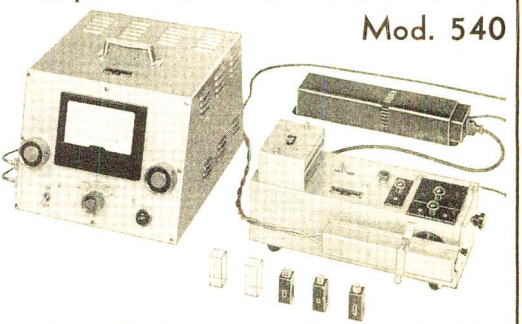

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clature Committee of the Division of Analytical Chemistry of the AMERICAN CHEMICAL SOCIETY says in its latest report (*1*):

Titration is a determination of the reactive capacity, usually of a solution. Titrimetric signifies measurement by titration.

This point of view sanctions the picturesque term, *coulometric titration.* Other definitely chemical methods are those measured by reaction kinetics and by oxidation potential. On the other hand, observations of color and odor are dependent upon physiological reactions of the observer and are therefore biological operations.

In classifying so-called electrochemical methods of analysis, distinction is made between those that merely involve electrical indication of titration end points and complete analytical methods such as polarography and electrodeposition.

Undoubtedly, any classification will have aspects that invite revision by the reader, either for special purposes or on a fundamental basis. The one presented herewith is the product of many revisions by the author and will probably soon be changed again.

Summary

A classification system for analytical methods gives boundaries to the field and brings order to the list of methods. A system is useful to teachers, students, and librarians. It also points out areas where the opportunity exists for devising new methods.

A classification system makes possible cross referencing and developing an indexing system such as the decimal method.

Such a classification system permits a rapid and accurate comparison of analytical methods on the basis of general theory; characteristics such as rapidity, accuracy, sensitivity, complexity (mathematical or operational), cost (initial and operational); and applicability.

Literature Cited

(1) Hughes, H. K., *et al.*, ANAL. CHEM. 24, 1349–54 (1952).
(2) Patterson, G. D., Jr., Mellon, M. G., *J. Chem. Educ.* 26, 468–71 (1949).
(3) Serfass, E. J., Steinhardt, R. G., Jr., Strong, F. C., III, ANAL. CHEM. 22, 966–9 (1950).

See classification system, page 27 A ▶

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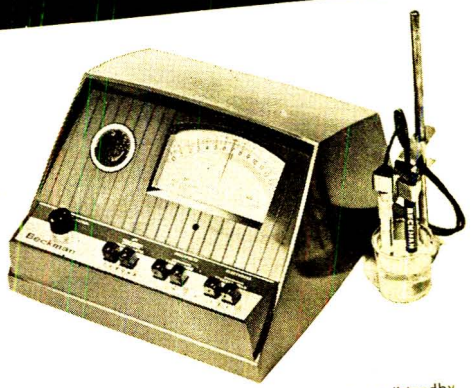

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Model 16

Features of the newly redesigned *Thelco* Ovens and Incubators include: black heat elements . . . dependable hydraulic thermostat . . . centralized controls . . . adjustable air exhaust . . . and many more.

Thelco Ovens have a temperature range to 200°C. Thermostat sensitivity is ±0.25°C. In Models 16 and 17, with gravity convection heat transfer, heat-up time to maximum temperature without load is 1 hour 30 minutes. Recovery time (open door 1 minute) is 40 minutes. Overall uniformity is ±1.4 to ±1.7°C. throughout temperature range. In Model 18, with mechanical convection, heat-up time is reduced to 40 minutes and recovery time to 25 minutes. Temperature uniformity is ±1.1°C. throughout chamber.

Cat. No.	Model	Working Chamber	Volts	Watts	Price
0-8265	16	13"w x 14"d x 13"h	115/230	650	$149.00
0-8270	17	19"w x 14"d x 19"h	115/230	1100	195.00
0-8275A	18	18"w x 14"d x 19"h	115	1160	250.00
0-8275B	18	18"w x 14"d x 19"h	230	1160	250.00

WRITE FOR COMPLETE INFORMATION ON THESE AND OTHER MODELS.

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For further information, circle numbers 25 A-1, 25 A-2 on Readers' Service Card, page 75 A

CLASSIFICATION OF ANALYTICAL METHODS

Sample preparation

Sampling
- Drilling, filing, chipping, etc.

Reduction of particle size
- Crushing
- Grinding
- Sieving

Mixing

Reduction of sample size

Qualitative analysis

Preliminary operations
- **Physical operations**
  - Dissolution
  - Fusion
  - Separation

Filtration
Adsorption (physical adsorption)
Adsorption chromatography
Extraction
Simple extraction
Continuous extraction
Partition chromatography
Leaching
Distillation
Sublimation
Dialysis
Electrophoresis
Flotation
Magnetic separation
- **Chemical operations**
  - Dissolution
  - Fusion
  - Acidification
  - Alkalization
  - Neutralization
  - Oxidation

Wet oxidation
Combustion
- Reduction
- Complexation
- Degradation by electron bombardment (in mass spectrometry)
- Separation

Precipitation
Chemical reagent
Electrodeposition
Adsorption (Chemisorption)
Gas fractionation
Chromatography
Adsorption chromatography
Ion exchange chromatography
Gas evolution
Gas absorption
Operations of identification
- **Physical operations of measurement or observation**
  - Mechanical

Density, specific gravity
- Electrical

Impedance
Photoelectron spectrometry
- Thermal

Melting point, freezing point
Boiling point
- Optical

Refractive index (refractometry)
Emission spectrum (emission spectrometry)
Arc and spark spectrometry
Flame spectrometry
Fluorescence spectrometry
Absorption spectrum (absorption spectrometry)
Diffraction measurements
X-ray diffraction
Electron diffraction
- **Chemical operations of measurement or observation**
  - Simple chemical observations

Formation of characteristic precipitate
Formation of characteristic solution
Formation of characteristic gas
- Electrochemical measurements

Gaseous ion discharge (mass spectrometry)
Half-wave potential (qualitative polarography)
- **Biological operations of observation**
  - Odor
  - Color

Quantitative analysis

Preliminary operations
- (as in qualitative analysis)

Operations of measurement
- **Physical measurements** (Do not require chemical alteration of the treated sample as an integral part of the measurement)
  - Mechanical measurements

Mass (gravimetric methods)
Volume (volumetric methods)
Gas analysis
Evolution methods
Entrainment methods
Density, specific gravity (densitometry)
Pressure (manometric methods)
Viscosity (viscometry)
- Electrical measurements

Photoelectron spectrometry
- Thermal measurements

Temperature of phase change
Boiling point (of mixture)
Melting point, freezing point (of mixture)
Critical solution temperature
Thermal conductance
- Optical measurements

Photometric methods
Transmitted radiation measured
- Absorptiometry
  - Visual matching
  - Instrumental measurement
    - X-ray absorption
    - Ultraviolet absorption spectrophotometry
    - Light absorption (visible range)
    - Colorimetry
    - Spectrophotometry
    - Infrared absorption
    - Microwave absorption
- Turbidimetry

Scattered radiation measured
- Nephelometry
- Raman spectrometry

Emitted radiation measured
- Arc and spark spectrophotometry
- Flame spectrophotometry
- Fluorescence spectrophotometry

Diffracted radiation measured
- X-ray diffraction
- Electron diffraction

Measurement based on radiation velocity
Refractometry
Interferometry
Rotation of polarized radiation measured (polarimetry)
- **Chemical measurements** (require at least a small amount of chemical reaction as an integral part of the measurement)
  - Oxidation potential measured—e.g., pH measurement
  - Electrochemical current measured

Gaseous ions discharged (mass spectrometry)
Ions in solution discharged
Unpolarized current (conductance methods)
Polarized current (quantitative polarography)
- **Reaction rate measured (kinetic methods)**
- **Reaction capacity measured (titrimetry)**

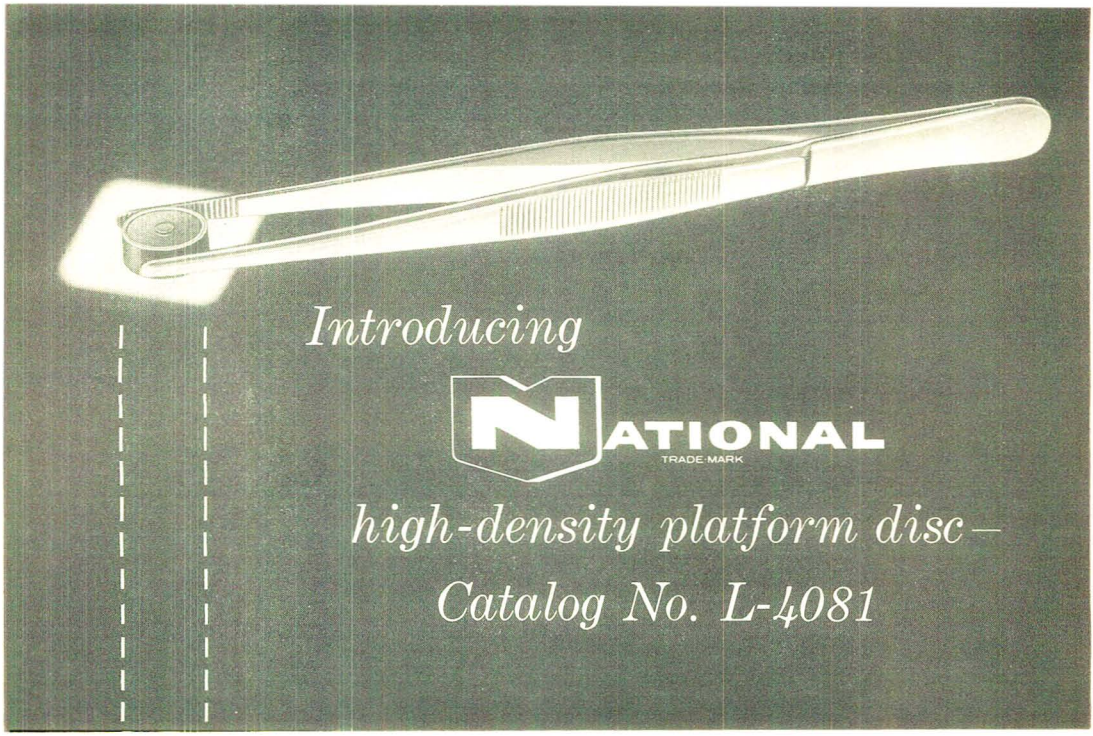
Volume of reagent measured (volumetric titrimetry)
Stoichiometric point determined visually
- Self-indicator
- Added indicator
- External indicator

Stoichiometric point determined photometrically
Stoichiometric point determined electrochemically
- Conductometric
- Potentiometric
- Amperometric
- High frequency

Mass of reagent measured (gravimetric titrimetry)
Subdivisions as above
Charge of reagent measured (coulometric titrimetry)
Heat of reaction measured (colorimetric methods)
- **Biological methods**
  - Growth measurements

Microbiological
Population methods
Growth product determinations
Macrobiological
- Killing power

Microbiological (bactericidal methods,
Macrobiological



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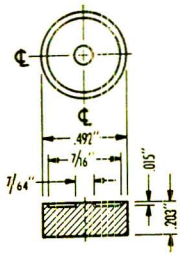

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ANALYTICAL CHEMISTRY NEWS
March 1957

Miami Meeting Features Strong Analytical Program

ACS analytical program includes symposia on analysis in petroleum geochemistry and pesticide residues and honors to Müller and Yoe

A STRONG technical program at a world-renowned resort, Miami, is a major attraction for analysts at the 131st national meeting of the AMERICAN CHEMICAL SOCIETY to be held April 7 to 12.

The program of 125 papers will include such highlights as the Beckman Award Symposium honoring Ralph H. Müller, the Fisher Award Symposium honoring John H. Yoe, a symposium on Analytical Contributions to Research in Petroleum Geochemistry, held jointly with the Division of Petroleum Chemistry, and a symposium on Methods for Analysis of Pesticide Residues, held jointly with the Division of Agricultural and Food Chemistry. Another highlight will be the divisional dinner which will feature Paul M. Gross, vice president, Duke University, as speaker. The final program of the Division of Analytical Chemistry appears below. The complete program for the 131st national ACS meeting appeared in the February 18 issue of *Chemical and Engineering News*.

PROGRAM

R. P. CHAPMAN, *Chairman;* WARREN W. BRANDT, *Secretary*

Monday Morning and Afternoon, April 8

BECKMAN AWARD SYMPOSIUM HONORING RALPH H. MÜLLER

R. L. GARMAN, *Presiding*

9:00— 1. R. L. GARMAN. Introductory Remarks.
9:10— 2. D. J. FISHER AND M. T. KELLEY. Instrumental Methods of Derivative Polarography.
9:30— 3. HAROLD H. STRAIN. Simple Apparatus for Continuous Electrochromatography.
9:50— 4. VAN ZANDT WILLIAMS. Infrared Spectrophotometer for the Organic Chemist.
10:10— 5. CLEMENT J. RODDEN. Automatic Determination of Uranium in Process Streams.
10:25— 6. GILBERT F. KINNEY AND R. A. REINHARDT. Operational Analogs for Kinetic Studies.
10:40— 7. SEYMOUR T. ZENCHELSKY. Derivative Thermometric Titrations.
10:55— Recess.
11:00— 8. R. L. GARMAN. Introduction of Beckman Award Medalist.
11:10— 9. RALPH H. MÜLLER. (Beckman Award in Chemical Instrumentation address). Adventures in Instrumentation.
11:50— Discussion
2:00— 10. CLEMENT CAMPBELL AND SAUL GORDON. Differential Thermoanalytical Techniques. Instrumentation and Applications.
2:20— 11. BENJAMIN CARROLL, HAROLD B. TILLEM, AND ELI S. FREEMAN. Rouy Method for Photoelectric Polarimetry.
2:40— 12. ELI S. FREEMAN AND BENJAMIN CARROLL. Magnetic Transmission-Type Continuously Recording Testut Thermobalance.

Monday Afternoon

GENERAL

R. P. CHAPMAN, *Presiding*

3:00— 13. R. P. CHAPMAN. Introductory Remarks.
3:05— 14. P. W. COLLYER, P. B. MOSELEY, AND R. H. OSBORN. New Differential Refractometer and Its Application to Chromatography.
3:25— 15. PHILIP J. ELVING AND EDWARD C. OLSON. Electrochemical Behavior of Aromatic *N*-Nitrosohydroxylamines.
3:45— 16. FRANCIS E. CRANE. Potentiometric Determination of Tetraphenylborate with Silver Nitrate.
4:05— 17. JOSEPH GLICKSTEIN AND RALPH H. MÜLLER. Automatic Integrator for Coulometric Analysis.
4:20— 18. C. A. GENGE. Mass Spectrometric Analysis of the Methyl Esters of Tall Oil Fatty Acids.
4:40— 19. EDWARD L. SIMONS. Automatic Cryoscopic Determination of Molecular Weights.

Tuesday Morning and Afternoon

FISHER AWARD SYMPOSIUM HONORING JOHN H. YOE

R. T. HALL, *Presiding*

9:00— 20. R. T. Hall. Introductory Remarks.
9:10— 21. A. D. MAYNES AND W. A. E. MCBRYDE. Determination of Traces of Lead in Igneous Minerals.
9:35— 22. JAMES S. PARSONS. Permselective Membrane Electrodes. Theory and Analytical Applications.
10:00— 23. MAXWELL L. CLUETT AND JOHN H. YOE. Spectrophotometric Method for Determination of Submicrogram Amounts of Nickel in Human Blood.
10:25— 24. RICHARD M. RUSH, FREDERICK NELSON, AND KURT A. KRAUS. Anion Exchange Studies of a Number of Elements of Groups III, IV, and V in HCl and HCl-HF Solutions.
10:45— 25. EVERETT C. COGBILL AND JOHN H. YOE. Spectrophotometric Determination of Boron in Plant Tissue with Derivatives of Anthranilin and Chrysazin.
11:05— Recess.
11:10— 26. THOMAS B. CRUMPLER. John Howe Yoe, Teacher and Investigator.
11:25— 27. JOHN H. YOE. (Fisher Award in Analytical Chemistry Address). Colorimetric Analysis with Organic Reagents.
2:00— 28. D. M. ROSIE AND R. L. GROB. Thermal Conductivity Behavior and Its Importance in Quantitative Gas Chromatography.
2:20— 29. EDGAR L. STEELE AND JOHN H. YOE. New Class of Organic Reagents for Spectrophotometric Determination of Trace Amounts of Osmium.

Tuesday Afternoon

GENERAL

GORDON O. GUERRANT, *Presiding*

2:35— 30. JAMES C. STERNBERG. Simplification of Calculations in Spectrophotometric Analysis of Multicomponent Systems.
2:55— 31. M. F. KRANC, D. KADAVY, AND H. GARRIGAN. Differential Spectrophotometric Analysis of Phthalocyanine Blue Base.
3:20— 32. BURTON F. PEASE AND MAX B. WILLIAMS. Spectrophotometric Investigation of the Analytical Reagent 1-(2-Pyridylazo)-2-naphthol, PAN, and Its Copper Chelate.
3:35— 33. TAFT Y. TOEIBARA, RUTH S. HELLARD, AND PRISCILLA A. DEWEY. Determination of Beryllium in Biological Material.
3:50— 34. R. B. PENLAND, E. P. BERTIN, S. MIZUSHIMA, BROTHER COLUMBA CURRAN, AND J. V. QUAGLIANO. Absorption Spectra of Cobalt(III) Amine Complexes Containing Some Coordinated Anions.
4:10— 35. HENRY J. HOENES, JR., AND K. G. STONE. Bis-(α-benzoinoximo)-dioxomolybdenum(VI) as a Weighing Form for Determination of Molybdenum.

4:30— 36. Glen A. Thommes and Elmer Leininger. Fluorometric Determination of *o*- and *m*-Hydroxybenzoic Acids in Mixtures.
4:45— 37. Louis Silverman and Rachel L. Seitz. Determination of Microgram Amounts of Cobalt in Sodium by 2-Nitroso-1-naphthol.

Wednesday Morning

SECTION A

SYMPOSIUM ON ANALYTICAL CONTRIBUTIONS TO RESEARCH IN PETROLEUM GEOCHEMISTRY

(Joint with Division of Petroleum Chemistry)

NELSON P. STEVENS, *Presiding*

9:00— 38. Nelson P. Stevens. Introductory Remarks.
9:10— 39. Wilson L. Orr and John R. Grady. Quantitative Determination of Chlorophyll Derivatives in Marine Sediments.
9:30— 40. H. N. Dunning and J. W. Moore. Analytical Methods and Geochemical Correlations of Porphyrin Research.
9:55— 41. Gordon W. Hodgson and Bruce L. Baker. Vanadium, Nickel, and Porphyrins in the Thermal Geochemistry of Petroleum.
10:15— 42. R. J. Grabowski. Spectrochemical Analysis of Petroleum.
10:35— 43. E. D. Evans, G. S. Kenny, W. G. Meinschein, and E. E. Bray. Separations of Saturated Hydrocarbons Extracted from Recent Marine Sediments.
10:55— 44. M. C. Brenneman. Studies of the Carbon Number Distribution of *n*-Paraffins from Quaternary Sediments.
11:15— 45. J. P. Forsman and J. M. Hunt. Analytical Techniques in the Separation of Organic Matter from Rocks.
11:35— 46. G. O. Guerrant. Ultramicromethod for Molecular Weight Determination.

Wednesday Morning

SECTION B

GENERAL

JOHN H. YOE, *Presiding*

9:00— 47. G. G. Long and A. H. Gropp. Anodic Polarography.
9:15— 48. James F. Miller. Polarographic Determination of Lead in Sodium Acetate–Acetic Acid System.
9:30— 49. I. Rosenthal, G. J. Frisone and R. J. LaCoste. Polarographic Behavior of 1,1-Di-*p*-chlorophenyl-1,2,2,2-tetrachloroethane.
9:50— 50. W. S. Lyon. Method for Determination of Neptunium-239 Counting Efficiency.
10:05— 51. Arno H. A. Heyn and Harmon L. Finston. Separation of Magnesium from Sodium and Potassium. A Tracer Study.
10:30— 52. Duane N. Sunderman and W. Wayne Meinke. Radiochemical Separations of Silver.
10:45— 53. E. Richard Nightingale, Jr. Poised Oxidation Reduction Systems. Quantitative Evaluation of Redox Poising Capacity and Its Relation to the Feasibility of Redox Titrations.
11:20— 54. Alan F. Clifford. Prediction of Solubility Product Constants.
11:40— 55. Glenn L. Booman, Maxine C. Elliott, Robert B. Kimball, Fred O. Cartan, and James E. Rein. Determination of Free Acid in the Presence of Hydrolyzable Ions.

Wednesday Afternoon

SYMPOSIUM ON ANALYTICAL CONTRIBUTIONS TO RESEARCH IN PETROLEUM GEOCHEMISTRY

(Joint with Division of Petroleum Chemistry)

NELSON P. STEVENS, *Presiding*

2:00— 56. Max Blumer. Removal of Elemental Sulfur from Hydrocarbon Fractions.
2:15— 57. John S. Ball and H. M. Smith. Bureau of Mines Crude Oil Analysis as a Tool for Geological Correlation.

GENERAL

CHARLES N. REILLEY, *Presiding*

2:30— 58. R. W. Schmid and Charles N. Reilley. New Complexon for Titration of Calcium in the Presence of Magnesium.
2:50— 59. D. M. West and D. A. Skoog. Oxidation of Some Organic Compounds with Standard Solutions of Quinquevalent Vanadium.
3:05— 60. James S. Fritz, William J. Lane, and Ann Sutton Bystroff. Complexometric Titrations Using Azoxine Indicators.
3:25— 61. Leo Levi. Location of Unsaturation in Terpenes and Terpenoids by Infrared and Chemical Analysis.
4:00— 62. Frank E. Critchfield and James B. Johnson. Aliphatic Primary Amino Nitrogen Compounds. Determination by Reaction With 2,4-Pentanedione.
4:15— 63. C. Eugene Bennett and F. J. Debbrecht. A Rapid Method for Organic Halogen Analysis.
4:35— 64. A. J. Martin. Potentiometric Titration of Halide Mixtures.
4:50— Divisional Business Meeting for Members.
6:30— Divisional Dinner. Speaker. Paul M. Gross, Vice President, Duke University.

Thursday Morning and Afternoon

SYMPOSIUM ON METHODS FOR ANALYSIS OF PESTICIDE RESIDUES

(Joint with Division of Agricultural and Food Chemistry)

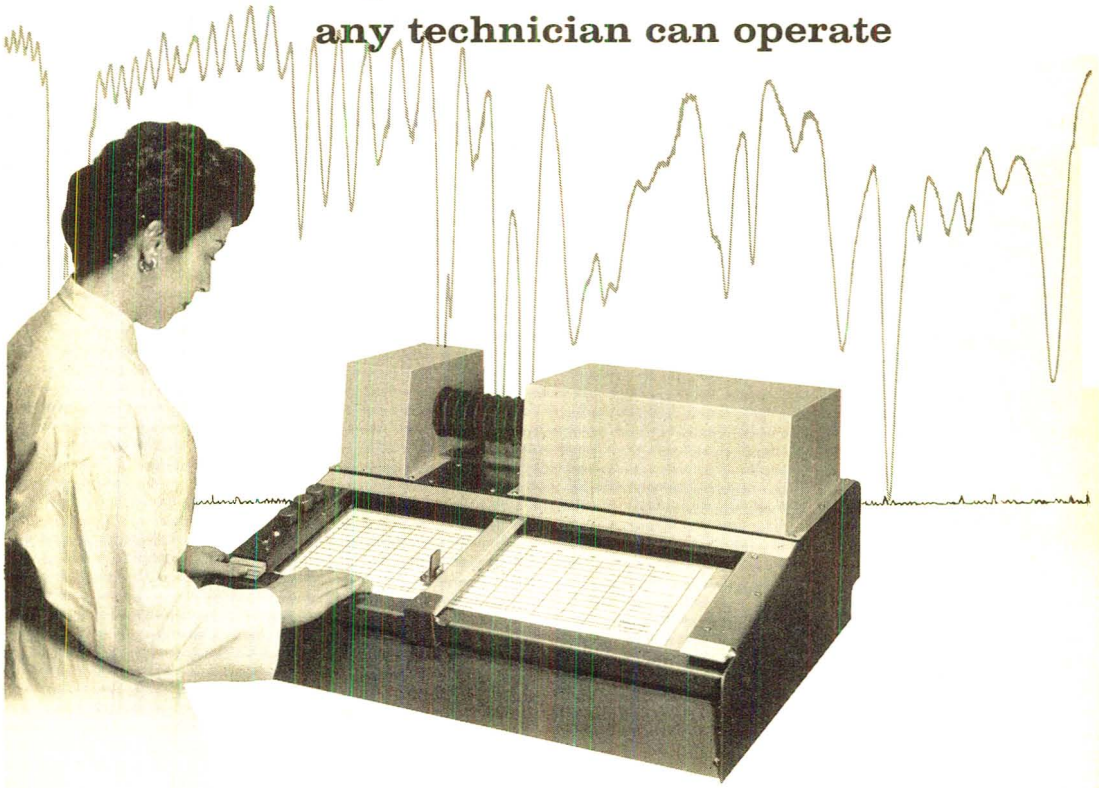
LOUIS LYKKEN, *Presiding*

9:00— 65. Louis Lykken. Introductory Remarks.
9:05— 66. F. A. Gunther. Development and Status of Modern Analytical Methods for Pesticide Residues in Crops and in Foods.
9:25— 67. T. H. Harris. Analytical Method and Residue Data Requirements for Federal Registration of Pesticide Formulations.
9:45— 68. J. A. Noone. Residue Determinations, a Limiting Factor in Pesticide Usage.
10:05— 69. C. H. Van Middelem. Some Basic Principles Involved in Obtaining Valid, Useful Pesticide Residue Data.
10:25— 70. Louis Lykken, L. E. Mitchell, and S. M. Woogerd. Important Considerations in Collecting and Preparing Samples for Residue Analysis.
10:45— 71. J. M. Bann. Extraction and Cleanup Techniques in Residue Analysis.
11:05— 72. R. H. Carter. Determination of Organic Chlorine Residues Resulting from Insecticide Applications.
11:25— 73. M. S. Schechter. Colorimetric Methods for the Determination of Pesticide Residues.
11:45— Discussion

C. H. VAN MIDDELEM, *Presiding*

2:00— 74. C. H. Van Middelem. Introductory Remarks.
2:05— 75. P. A. Giang. Enzymatic Methods for Analysis of Organophosphorus Insecticides.
2:25— 76. J. E. Dewey. Utility of Bioassay in Determination of Pesticide Residues.
2:45— 77. G. E. Pollard. Application of Infrared Spectrophotometry to Determination of Pesticide Residues.
3:05— 78. H. P. Burchfield and P. H. Schuldt. Applications of Zincke Reaction to Analysis of Pesticides Containing Active Halogen Atoms.
3:25— Discussion.
3:35— 79. J. R. Lane, D. K. Gullstrom, and J. E. Newell. Adaptation of Residue Methods to Include New Vegetables or to Extend the Sensitivity Range. Extension of Residue Methods for Maleic Hydrazide and Alanap.
3:50— 80. J. M. Bann, S. C. Lau, J. C. Potter, H. W. Johnson, Jr., A. E. O'Donnell, and F. T. Weiss. Determination of Endrin in Agricultural Products and Animal Tissues.
4:05— 81. T. G. Bowery and F. E. Guthrie. TDE and Endrin Residues on Tobacco. Isolation and

An Infrared Spectrophotometer any lab can afford – any technician can operate



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For further information, circle number 31 A on Readers' Service Card, page 75 A

3 NEW TOOLS FOR THE ANALYTICAL CHEMIST

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The new Beckman GC-2 Gas Chromatograph – with its expanded temperature range – is easily the most advanced, most practical way to utilize the promising technique of gas chromatography.

Temperature control settings on the GC-2 range from 40°C. to 225°C., allowing analysis of gases and liquids with boiling points up to 300°C.

Conduction heating keyed to *proportional control* gives rapid response to temperature setting changes – thus the analyst can perform more determinations on more different types of compounds per day because there's minimum delay while temperature stabilizes. This rapid-response temperature control makes "downtime" for column interchange and regain of operating temperature, a matter of minutes.

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For further information, circle number 32 A on Readers' Service Card, page 75 A

Identification of TDE Residues in Cigarette Smoke.

4:20— 82. H. C. Austin, Jr., and F. L. Bonner. Determination of Trace Quantities of Lindane in Poultry Tissue.

4:35— 83. E. L. Stanley, I. Rosenthal, and C. F. Gordon. Microdetermination of Rhothane (TDE, DDD) in Spray Residues.

4:50— Discussion.

Thursday Afternoon

SECTION B

GENERAL

WARREN W. BRANDT, *Presiding*

2:00— 84. Allen A. Duswalt and Warren W. Brandt. Determination of Fluoride Ion by Turbidimetric Titration.

2:15— 85. Leon W. Gamble, Walter E. Price, and William H. Jones. Rapid Determination of Fluoride in Silica-Alumina Catalyst by Steam Hydrolysis.

2:35— 86. K. A. Kubitz. Determination of Traces of Isocyanate in Urethane Based Polymers.

2:50— 87. George A. Huff and Fred H. Tingey. Statistically Designed Training and Testing Program for an Analytical Control Laboratory.

3:05— 88. J. H. Karchmer and Marjorie T. Walker. Modification of the Acetic Acid–Zinc Reflux Method for Determining Disulfides in Petroleum Naphtha.

3:20— 89. R. D. Schwartz and D. J. Brasseaux. Determination of Normal Paraffins in Olefin-Free Petroleum Distillates by Molecular Sieve Sorption and Refractometry.

3:40— 90. C. Eugene Bennett, S. Dal Nogare, L. W. Safranski, and O. D. Lewis. Trace Analysis by Gas Chromatography.

4:00— 91. George Kyryacos and Cecil E. Boord. Gas Absorption Chromatography in the Analysis of Cool-Flame Combustion Products.

4:20— 92. John L. Dolphin and Thomas W. Stanley. Vapor Phase Chromatography in Air Pollution Studies Column Evaluation.

4:40— 93. Nathaniel Brenner. Modification of a Gas Chromatography Instrument for Special Laboratory Problems.

Friday Morning

SECTION A

SYMPOSIUM ON METHODS FOR ANALYSIS OF PESTICIDE RESIDUES

(Joint with Division of Agricultural and Food Chemistry)

LOUIS LYKKEN, *Presiding*

9:00— 94. Louis Lykken. Introductory Remarks.

9:05— 95. G. R. Boyd. Determination of Residues of *O*-2,4-Dichlorophenyl *O,O*-Diethyl Phosphorothioate (V-C 13 Nemacide) by Cholinesterase Inhibition.

9:20— 96. L. E. Palmer and E. F. Williams. Analysis of Thimet and Its Metabolites.

9:35— 97. C. H. Van Middelem and R. E. Waites. Pesticide Residues. Enzymatic Determination of Systox in Collards, Lettuce, and Mustard by Use of Cholinesterase Inhibition Technique.

9:50— 98. C. L. Dunn. Analytical Method for Determination of 2,3-*p*-Dioxanedithiol *S,S-Bis*(*O,O*-Diethyl Phosphoro-dithioate) (Hercules 528).

10:05— 99. P. A. Giang and M. S. Schechter. Colorimetric Method for Estimation of Guthion Present in Cottonseed Residues.

10:20—100. R. P. Gigger. Determination of Diazinon Residues.

10:35—101. W. E. Whitehurst and J. B. Johnson. Determination of CRAG DCU Residues in Crops and Soil.

10:50—102. J. R. Lane. Colorimetric Microdetermination of Spergon (2,3,5,6-Tetrachlorobenzoquinone) Residues on Food Crops.

For further information, circle number 35 A on Readers' Service Card, page 75 A →

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SPECIFICATIONS AS MAXIMUM

Reagent Sulfuric Acid

NO_3	0.00005%
Cl	0.00002%
As	0.000000%
NH_4	0.0001%
Fe	0.00002%
Residue on Ignition	0.0004%
Heavy Metals (as Pb)	0.00008%
Substances Reducing $KMnO_4$ (as SO_2)	0.0001%

Reagent Ammonium Hydroxide

Cl	0.00005%
Pyridine	Free
PO_4	0.0000%
Heavy Metals (as Pb)	0.0001%
CO_2	0.002%
Residue on Ignition	0.0003%
Sulfates (SO_4)	0.0003%
Fe	0.00003%
Substances Reducing $KMnO_4$ (as SO_2)	0.002%

Reagent Glacial Acetic Acid

Residue on Ignition	0.0008%
Cl	0.0000%
Sulfurous Acid (as SO_2)	0.000%
Sulfates (SO_4)	0.0000%
Fe	0.00002%
Heavy Metals (as Pb)	0.00005%
Substances Reducing $KMnO_4$	Passes A.C.S. Test
Dilution Test	Passes A.C.S. Test

Reagent Hydrochloric Acid

Free Cl	0.0000%
Sulfites (SO_2)	0.00008%
Sulfates (SO_4)	0.00008%
Heavy Metals (as Pb)	0.0001%
Residue on Ignition	0.0004%
Fe	0.00001%
As	0.000001%
NH_4	0.0003%

Reagent Nitric Acid*

Cl	0.00005%
Residue on Ignition	0.0004%
Heavy Metals (as Pb)	0.00002%
Sulfates (SO_4)	0.00008%
Fe	0.00002%
As	0.000000%

*Nitric Acid is currently available only in returnable containers.

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DU PONT REAGENT	SINGLE-TRIP			RETURNABLE											
	6— 5-pt. Bottles			24— 1-lb. Bottles			10— 5-pt. Bottles			6½-gal. Carboy			13-gal. Carboy		
	Gross lbs.	Tare lbs.	Net lbs.	Gross lbs.	Tare lbs.	Net lbs.	Gross lbs.	Tare lbs.	Net lbs.	Gross lbs.	Tare lbs.	Net lbs.	Gross lbs.	Tare lbs.	Net lbs.
Sulfuric Acid	74	20	54	50	26	24	140	50	90	138	40	98	262	67	195
Ammonium Hydroxide	44	20	24	59	35	24	90	50	40	88	40	48	163	67	96
Glacial Acetic Acid	50	20	30	59	35	24	100	50	50	97	40	57	180	67	113
Hydrochloric Acid	56	20	36	56	32	24	110	50	60	104	40	64	194	67	127
Nitric Acid	—	—	—	56	32	24	126	56	70	115	40	75	219	69	150

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IN CANADA—DU PONT COMPANY OF CANADA (1956) LIMITED, Box 660, Montreal, P. Q., Canada.

11:05—103. R. J. Lacoste and G. T. Myers. Colorimetric Determination of Dithiocarbamate Residues.
11:20—104. J. E. Newell, R. J. Mazaika, and W. J. Cook. Microdetermination of Phygon in Water.
11:35—105. J. R. Lane. Colorimetric Microdetermination of Phygon (2,3-Dichloro-1,4-naphthaquinone) Residues on Food Crops.
11:50 Discussion

SECTION B

GENERAL

JOHN MITCHELL, JR., *Presiding*

9:00—106. W. B. Chess and D. N. Bernhart. Determination of Small Amounts of Pyrophosphate in Disodium Phosphate.
9:15—107. Eugene Sawicki and Robert Miller. Detection of Pyrene and Benzo(*a*)-pyrene in the Atmosphere.
9:30—108. W. H. Power, W. C. McCluggage, G. D. Nelson, and J. H. Payne, Jr. Separation of Radium and Barium by Ion Exchange Elution.
9:45—109. R. B. Hahn and P. T. Joseph. Ultraviolet Spectrophotometric Determination of Zirconium Using Bromanilic Acid.
10:00—110. J. E. Fagel, Jr., P. D. Zemany and H. A. Liebhafsky. Tungsten or Molybdenum by X-Ray Emission Spectrography.
10:20—111. Morton Schmall and E. G. Wollish. Determination of Panthenol and Pantothenates in Multivitamin Preparations.
10:40—112. I. B. Eisdorfer and W. C. Ellenbogen. Separation and Determination of Triiodothyronine, Thyroxine, and Some Related Amino Acids Using Circular Paper Chromatography.
11:00—113. Dean J. Veal, Frank C. Haas, and M. D. Grimes. New Colorimetric Method for Determination of Traces of Methanol in Ethylene.
11:20—114. L. S. Harrow, J. T. Butler, F. E. Resnik, Anne Estes, Margaret Bill, and R. B. Seligman. Spectrophotometric Determination of Total Carbonyl Content Utilizing Integrated Absorbance Measurements.
11:40—115. R. C. Blinn and F. A. Gunther. Simple Determination of Ammonia with Cupric Carbonate.

Friday Afternoon

SYMPOSIUM ON METHODS FOR ANALYSIS OF PESTICIDE RESIDUES

(*Joint with Division of Agricultural and Food Chemistry*)

C. H. VAN MIDDELEM, *Presiding*

2:00—116. C. H. Van Middelem. Introductory Remarks.
2:05—117. J. H. Brumbaugh and D. E. Stallard. Colorimetric Method for Perchloroethylene. Determination of Residual Perchloroethylene in Fumigated Wheat.
2:20—118. D. E. Stallard and J. H. Brumbaugh. Study of Residual Benzene in Wheat Following Fumigation.
2:35—119. R. H. Carter. Method for Determination of Ethylene Dibromide Residues in Fumigated Materials.
2:50—120. Calvin Menzie. Determination of *m*-Dinitrophenol.
3:05—121. B. D. Hilton and M. H. J. Weiden. Modification of the Analysis for Ovex on Apples.
3:20—122. J. R. Lane, D. K. Gullstrom, and J. E. Newell. Determination of Duraset (*N*-Metatolyl Phthalamic Acid) Residues in Fruit Crops.
3:35—123. R. B. Bruce, J. W. Howard, and J. B. Zink. Determination of Diphenylamine Residues on Apples.
3:50—124. F. A. Gunther, R. C. Blinn, M. J. Kolbezen, C. W. Wilson, and R. A. Conkin. Ammonia. Spectrophotometric Techniques and Equipment for Evaluating Concentrations of Spectrally Absorbing Vapors in Dynamic Systems.
4:05—125. H. C. Austin, Jr., F. L. Bonner, and E. A. Epps, Jr. Spectrophotometric Determination of Arsenicals on Plant Material.
4:20 Discussion.

Wolfgang Kirsten of Sweden outlines some highlights in his paper to Philip W. West, LSU symposium chairman

Outstanding foreign analysts on the LSU program included Hermann Flaschka, Egypt; Clement Duval, France; Wolfgang Schöniger, Switzerland

LSU Symposium Draws Record Attendance

H. W. Patton, Tennessee Eastman, foresees excellent future for gas phase chromatography

An attendance of more than 425 and a strong technical program made the 10th Annual Symposium on Modern Methods of Analytical Chemistry one of the most successful ever held. The meeting, sponsored by Louisiana State University, January 28 to January 31, featured lectures by world-famous analysts from the United States and many foreign nations.

The statement that the gas phase chromatograph is the poor man's mass spectrometer and the chemist's dream may not be too far from the truth, according to Hugh Patton of Tennessee Eastman. He pointed to the fact that within a year of the appearance of the first commercial unit, eight different instrument companies were offering them for sale. Although gas adsorption chromatography is much more versatile, powerful, and reliable, because of the large variety of partitioning liquids available and the symmetrical band shapes obtained, he emphasized that virtually any specific vaporizable mixture can be separated by GLPC if enough time and effort can be applied to the problem. Looking to the future, Patton foresaw two more extensive uses for gas chromatography: large-scale purification of valuable materials and process control.

Complexones. Speaking of complexones, Hermann Flaschka, National Research Center of Egypt, stated that there are at present available complexometric titrations for virtually the entire periodic table, for many organic materials, and even for gases. The big problem now, he said, lies in obtaining the requisite selectivity. Manipulation of pH and the use of masking agents have been useful approaches in obtaining this selectivity, but much more work remains to be done. Flaschka presented a mathematical treatment of the titration reaction, which showed that calculation of the stability constant of a given complex should predict its applicability in a given titration. The predicted applicabilities are in close agreement with experiment. The use of complex chemical replacement reactions is not limited to titrimetric methods. Flaschka illustrated this point with the example of the polarographic determination of thorium, using Pb-EDTA as the replacement complex.

Solvent Extraction. The impor-

tant field of solvent extraction was discussed by Harry Freiser, University of Pittsburgh, and George H. Morrison, Sylvania Electric. Freiser noted the tremendous resurgence of separation methods in the past 15 years—homogeneous precipitation, ion exchange, chromatographic separations, and solvent extraction. Solvent extraction, being extremely "clean," fast, and convenient, and applicable over a very wide concentration range, is an especially powerful technique. Morrison, in listing the techniques available in solvent extraction, called special attention to the power of the "salting-out" approach, which has been used with great success in the extraction of uranium. This approach, whose potential has been largely unrealized until recently, should be applicable to all the actinides and to many other elements. Morrison and Freiser described extractable species as falling into two general types: ion-association complexes and chelates. Morrison, speaking of the first type, classified them in 15 groups depending on the media involved in complex formation (F^-, Cl^-, $COOH^-$, etc.). He stressed the great importance of the proper choice of acid concentration, solvent and oxidation conditions for any system. Freiser, discussing the chelate type, stated that the wider use of mixed solvents and masking agents should greatly extend the present scope of analytical extractions. He also pointed out that a great virtue of the chelates was their high optical absorbancy, which often allows a direct photometric determination (especially ultraviolet) of the extracted material.

Automation. Howard V. Malmstadt, University of Illinois, described how some or all of the manipulations inherent in the titration procedure can be performed automatically. Automatic methods can provide a great saving in time and often an increase in precision and accuracy. The general characteristics of existing automatic titration systems and the specific features of a derivative control unit were presented. It was shown that most titrations can be automatically performed with a minimum of pre-titration considerations by using a second or third derivative control unit in conjunction with potentiometric and spectrophotometric end point detection systems; other end point detection devices can also be used in specific cases. Malmstadt said that "virtually all of the known 'visual indicator' titrations can be put on an automatic basis with a simple attachment to the second derivative control unit, consisting of a tungsten light source, a series of filters, and a barrier layer detector."

Nonaqueous Polarography. Oklahoma A&M's Paul Arthur described the present status of nonaqueous polarography. The circuit serves two functions as shown by the equation: E applied = E effective + IR. In conventional polarography, the IR portion is usually negligible; in nonaqueous work, however, it is usually larger than E effective, since solution resistances are of the order of megohms. This condition causes great distortion of the polarographic wave with conventional equipment, rendering it useless for analytical purposes. He described two approaches to this situation. In the first, the IR drop is compensated with an equivalent voltage. In the second approach (which Arthur developed), E effective is measured separately by using a second reference electrode. The output of this circuit is fed to the chart circuit of a function plotter, while the output of the conventional part of his apparatus is fed to the pen circuit. Arthur described the application of nonaqueous polarography to qualitative and quantitative analysis of organic compounds.

Acid-Base Titrations. Speaking of acid-base titrations in nonaqueous solvents, John Riddick, Commercial Solvents, stated that they are accurate, fast, inexpensive, and applicable to a tremendous variety of materials. Drawing from experiences in his own laboratory, he showed their great utility in following reactions and in process control. Most physicochemical and thermodynamic expressions of acid strengths appearing in the literature are too complicated and too restricted for practical use, he said. However, he has been able to develop certain rather simple expressions which allow him to relate acid strengths from one solvent to another. For practical analytical purposes, Riddick favors the Lewis concept of acids, incorporating Bronsted's theories. He listed six acid-base phenomena which he has found most helpful in his interpretation of reactions in nonaqueous solvents: hydrogen and atomic bonding, and resonance, electrostatic, steric, and solvent effects.

Thermogravimetry. Clement Duval presented a general survey of researches made during the past few years with the thermobalance. He outlined the chief methods of gravi-

ANALYST'S CALENDAR

March 4 - 8	**8th Annual Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy and Exposition of Modern Laboratory Equipment,** Penn-Sheraton Hotel, Pittsburgh, Pa. ANAL. REF.: Feb. 1957, pages 39A and 51A.
March 5	**Society for Applied Spectroscopy,** dinner meeting, Hotel New Yorker, N. Y. Henry Hemmendinger, Davidson and Hemmendinger, "Spectrophotometric Calibration of the Dependence of Color on Colorant Compositions."
March 5 - 8	**1957 Conference on High Speed Computers,** Louisiana State University, Baton Rouge, La.
March 11 - 15	**1957 Nuclear Congress and Atomic Exposition,** Convention Hall, Philadelphia, Pa.
March 19	**Society for Applied Spectroscopy,** dinner meeting, Philadelphia College of Pharmacy and Science, Philadelphia, Pa. Britton Chance, University of Pennsylvania, "Spectrophotometric Studies of Enzymes in Living Cells."
Apr. 2.	**Society for Applied Spectroscopy,** dinner meeting, Hotel New Yorker, N. Y. B. H. Carroll, Eastman Kodak, "Photographic Process in Emission Spectroscopy."
Apr. 7 - 12	**131st National Meeting,** ACS, Miami, Fla. ANAL. REF.: Feb. 1957, page 29A.
Apr. 16	**Society for Applied Spectroscopy,** dinner meeting, Philadelphia College of Pharmacy and Science, Philadelphia, Pa. Cyrus Feldman, Oak Ridge National Laboratories, "1000 Ways to Use a Spectrograph—Applications in Atomic Energy."
Apr. 16 - 18	**Symposium on Nondestructive Tests Developed in the Field of Nuclear Energy,** American Institute of Chemical Engineers, American Nuclear Society, American Society for Testing Materials, Society for Nondestructive Testing. Morrison Hotel, Chicago, Ill. Declassified information on testing methods and techniques for reactor components. *Contact:* J. L. Swanson, Aircraft Nuclear Propulsion Dept., General Electric Co., Cincinnati 15, Ohio.
Apr. 26 - 27	**Twelfth Annual Microchemical Symposium and Exhibition.** Metropolitan Microchemical Society, American Museum of Natural History, New York, N. Y. General papers and round table session on microdetermination of halogens. *Contact:* Erik R. Hoffmann, Ethicon, Inc., U.S. 22, Somerville, N. J.
Apr. 27- May 2	**Scientific Apparatus Makers Association,** 39th Annual Meeting, Greenbrier Hotel, White Sulphur Springs, W. Va.
Apr. 29- May 1	**Eighth Annual Spectroscopy Symposium and Exhibit,** American Association of Spectrographers, Hotel LaSalle, Chicago, Ill. *Contact:* Theodore H. Zink, H. Cohn & Sons, 4528 West Division St., Chicago 51, Ill.
	48th Annual Meeting, American Oil Chemists' Society, Roosevelt Hotel, New Orleans, La. *Contact:* Lucy R. Hawkins, Executive Secretary, American Oil Chemists' Society, 35 E. Wacker Drive, Chicago 1, Ill.

Coming Events

May 13 to 15—12th Purdue Industrial Waste Conference, Purdue University, Lafayette, Ind.

May 20 to 24—5th Meeting on Mass Spectrometry, ASTM Committee E-14, Commodore Hotel, New York, N. Y.

June 10 to 14—Symposium on Molecular Structure and Spectroscopy, The Ohio State University, Columbus, Ohio.

June 13 to 15—10th Summer Symposium, ACS Analytical Chemistry Division and ANALYTICAL CHEMISTRY, Purdue University, Lafayette, Ind.

June 24 to 28—Congress on Modern Analytical Chemistry in Industry, St. Andrew's University, Scotland.

July 10 to 17—4th General Assembly and International Congress, International Union of Crystallography, McGill University, Montreal, Canada.

July 16 to 25—XIXth Conference and XVI Congress, International Union of Pure and Applied Chemistry, Paris, France.

Sept. 8 to 13—132nd National Meeting, ACS, New York, N. Y.

Sept. 11 to 13—Fourth Ottawa Symposium on Applied Spectroscopy, Canadian Association for Applied Spectroscopy, Victoria Museum, Ottawa.

Oct. 14 to 16—Association of Official Agricultural Chemists, Annual Meeting, Shoreham Hotel, Washington, D. C.

NEWS

metric analysis, new compounds discovered with thermolysis curves, reactions in the solid state, new procedures for gravimetry, construction of families of isotherms, explanations of some errors, and the use of thermogravimetry in organic chemistry. He also described for the first time an infrared procedure for inorganic identifications for use on a single drop of solution.

A special cell made of thallium bromoiodide is used and a special range of 6 to 10 microns employed. Perkin Elmer 12C and double-beam spectrometers equipped with rock salt prisms were used in the studies reported. He listed the chief anions encountered in inorganic chemistry and gave the wave lengths of the selected bands to be used for their qualitative identification—bisulfate in the presence of sulfate, persulfate, and sulfate, etc. Then, he explained how an experiment of catalysis may be followed and how the existence of uranyl ions may no longer be explained with the formula UO_2^{++}.

Organic Microanalysis. A review of the development of the organic microanalysis—i.e., methods which use 3 to 10 mg. of substance—was presented by Wolfgang Schöniger of Sandoz, Ltd., Switzerland. He pointed out that nearly all final determinations are now done by volumetric methods, and that only for the carbon and hydrogen determinations is gravimetry still in use.

A method for the simultaneous determination of carbon, hydrogen, and nitrogen, which also allows working without a weighed amount of substance has been developed in his laboratory. The substance is burned in an evacuated system and the milligrams of carbon, hydrogen, and nitrogen are determined manometrically. Out of these numbers the atomic ratios of carbon, hydrogen, and nitrogen can be calculated in the usual way.

He also described a new method for the determination of hydrogen and sulfur in organic compounds. The substance is burned in a conical flask in an oxygen atmosphere. The combustion products are absorbed and the final determination of halogens and sulfur can be done in the same flask. Highly halogenated compounds also will give good results, and only liquids with a low boiling point cannot be analyzed.

Organic Elementary Analysis. Wolfgang Kirsten of Sweden discussed the determination of carbon, hydrogen, nitrogen, sulfur, and halogens in small amounts of organic material. Combustion methods are used in all cases for the decomposition of the samples, and the advantages and

disadvantages of wet combustions, combustions in tubes in a gas flow, sealed tube combustions, and metal bomb combustions were discussed with regard to the species problem. The usefulness of the sodium phenate-hypochlorite colorimetric reactions for the Kjeldahl nitrogen determination was stressed. Sulfur is determined by combustion and following hydrogenation. Fractions of micrograms of sulfur can thus be determined spectrophotometrically in small or large samples of organic as well as inorganic material in a very short time. Several spectrophotometric methods for the determination of halides were discussed.

For the determination of carbon, hydrogen, and nitrogen simultaneously in one sample, a sealed-tube-combustion and manometric–gas volumetric measuring method was described and procedures for the analyses of 0.1- to 0.2-mg. samples were presented.

Summer Computer Program

The special summer computer program, being offered for the fifth time by Wayne University, will consist of three courses.

The first course, June 3 to 8, will be an introduction to computers and their applications; the second, June 10 to 15, will cover data processing in business and industry; and the third, Sept. 9 to 14, will be concerned with industrial and management computer applications.

The third course will cover engineering, physical, and social sciences as well as management problems.

Details are available from A. W. Jacobson, Director, Computation Laboratory, Wayne State University, Detroit 2, Mich.

Spectrography Course at Boston College

A special two-week intensive course in modern industrial spectrography will be given at Boston College, July 15 to 26. The course is designed for industrial chemists and physicists interested in the techniques of emission spectroscopy as an analytical tool.

Details are available from James J. Devlin, S.J., Physics Department, Boston College, Chestnut Hill 67, Mass.

New Spectroscopy Journal

Publication of a new "Journal of Molecular Spectroscopy" will be initiated in May, Academic Press reports. Editor is Harald H. Nielsen, Ohio State University.

The journal will include original research papers on such topics as: molecular spectra in emission and absorption; molecular spectra in the ultraviolet, the visible, the near and far infrared, and in the microwave region; and Raman spectroscopy and radiofrequency spectroscopy (including nuclear magnetic resonance spectroscopy); intensity measurements and line width measurements as well as interpretation of spectra, molecular dynamics, and the electronic energies of molecules; and experimental and theoretical aspects of molecular spectroscopy.

Volume I, to consist of 4 issues, will be published in 1957. Subscriptions are $10, payable to the publishers, Academic Press, Inc., 111 Fifth Ave., New York 3, N. Y.

Microchemistry Symposium at ACS Fall Meeting

A Symposium on Microchemistry will be part of the Division of Analytical Chemistry program at the National ACS Meeting to be held in New York, Sept. 8 to 13. Included will be papers on micromanipulation and organic quantitative analysis, as well as general papers. Those wishing to present papers are requested to submit titles, author's name and address, and preliminary abstract by May 1. They may be sent to K. B. Streeter, Merck-Sharp and Dohme Research Laboratories, West Point, Pa.

Ottawa Spectroscopy Symposium

The Fourth Ottawa Symposium on Applied Spectroscopy will be held Sept. 11 to 13 at the Victoria Museum, Ottawa. Sponsor is the Canadian Association for Applied Spectroscopy.

Papers are invited on applied spectroscopy and related fields of instrumental analysis. Titles and brief abstracts should be submitted to J. H. D. Howarth, Canada Metal Co., Ltd., 721 Eastern Ave., Toronto, Ont., Canada, by June 15.

NEW BOOKS

Quantitative Chemical Analysis. 11th edition revised. *Robert Alexander Chalmers*, editor. xvi + 540 pages. Oliver and Boyd, Ltd., 39A Welbeck St., W. I. London, England. 1956. 30s. net.

Released 8 years after the publication of the 10th edition of this book, the new volume incorporates considerable revised and added material. Major alterations include the rewriting of the section on colorimetry, the writing of a brief account of the theory of precipitation and contamination of precipitates, and the inclusion of a short and elementary account of some of the physicochemical methods now used more and more often.

A new feature is the inclusion of short lists of suggested reading matter following each of the eight parts. The entire text has been revised and brought up to date, providing a reference work for the student of quantitative chemistry.

pH Measurements: Their Theory and Practice. *Victor Gold.* 125 pages. Methuen & Co., Ltd., 36 Essex St., London, W. C. 2, England, 1956. 9s. 6d. net.

Starting at an elementary level, this book describes the basic theory of pH and leading experimental techniques, both electrometric and optical, for its measurement. It explains the correct significance of pH measurements in relation to ionization equilibria and reaction velocities in aqueous solution, and points out the limitations of the concept of pH.

The theory of proton transfer equilibria and the theory of galvanic cells are also explained in an effort to make this a useful handbook for students and research workers not basically trained in chemistry.

Tables of numerical data for pH determinations are included as appendixes.

Elements of X-Ray Diffraction. *B. D. Cullity.* xiv + 514 pages. Addison-Wesley Publishing Co., Inc., Reading, Mass., 1956. $10.

The theory, experimental methods, and principal applications of x-ray diffraction, primarily for those who are not familiar with the technique, are outlined in this volume. Although the author, a metallurgist, has confined most applications to metals and alloys, the principles are applicable to nonmetallic materials. Chapters are included on chemical analysis by diffraction and fluorescence and the diffractometer.

Quality Control and Applied Statistics Abstracts Service. Vol. I, Issue 1. *Robert S. Titchen, Arnold J. Rosenthal, Bruce Bollerman,* and *Frank Nistico,* editors. 37 abstracts, 79 pages. Interscience Publishers, Inc., 250 Fifth Ave., New York 1, N. Y., 1956. Subscription: $60 per year. 12 issues per year, approximately 1000 pages.

This new abstract service is intended to bring together information on quality control which currently appears in a wide variety of journals. It reviews new contributions appearing in approximately 400 journals in 1- to 2-page abstracts which are printed in loose leaf forms. Each is classified to facilitate subject filing.

ASTM Standards on Rubber Products (with related information). Methods of Testing Specifications. xiv + 746 pages. American Society for Testing Materials, 1916 Race St., Philadelphia 3, Pa., 1956. $5.75.

This paper-bound volume gives every ASTM standard pertaining to rubber and related products compiled in latest form. Methods of tests, specifications, recommended procedures, and definitions are included in this convenient reference book.

Spectroscopy at Radio and Microwave Frequencies. *D. J. E. Ingram.* xii + 332 pages. Philosophical Library, Inc., 15 East 40th St., New York 16, N. Y., 1956. $15.

In view of the rapid development of microwave and radiofrequency spectroscopy and its increasing applications to physics and chemistry, the author undertakes in this book to give a general review of this specialized branch of spectroscopy. Although a thorough knowledge of physics and electronics is required for complete understanding of spectroscopy at these frequencies, the book is aimed at presenting a broad outline for those not specializing in the field, but interested in it as background.

Theory is explained, research carried out thus far is summarized, and experimental apparatus is described in detail. Production and detection of microwaves are discussed, as well as wave guide techniques. Gaseous microwave spectroscopy, electron paramagnetic resonance, and nuclear radiofrequency resonance are all treated at some length.

The final chapter comprises various applications of radiofrequency and microwave spectroscopy to both those of fundamental research and of a practical nature.

Symposium on Impact Testing. ASTM Special Technical Publication No. 176. 170 pages. American Society for Testing Materials, 1916 Race St., Philadelphia 3, Pa., 1956. $3.50

Technical papers on impact and shock tests for parts, components, and complete structures present latest theoretical and practical developments in this expanding field. Symposium report also contains several additional papers considered timely and appropriate to the subject. Graphs, charts, photographs, and several bibliographies are included.

Clinical Chemistry: Principles and Procedures. *Joseph S. Annino.* xxi + 280 pages. Medical Book Department, Little, Brown and Co., Boston 5, Mass. 1956. $7.50.

Directed toward those concerned with setting up and controlling methods in the hospital chemistry laboratory, this book provides fundamental information on laboratory techniques and methods. In Part I, pertinent apparatus and standard solutions are described, and individual sections are devoted to colorimetry and quantitative analysis. The second part of the book includes detailed methods of analysis of carbon dioxide, chloride, sodium and potassium, nitrogen, calcium, and phosphorus, as well as various biological substances.

Instruments for the Study of Atmospheric Pollution. 2nd edition, 8 pages. Committee on Air Pollution Controls, American Society of Mechanical Engineers, New York 18, N. Y. Available from: ASME Order Department, 29 West 39th St., New York 18, N. Y., 1956. Paper $2.00.

Instruments, ranging from simple filter papers to electrostatic precipitators are among the hundreds of devices useful in studying air pollution listed in this second edition. Names and addresses of manufacturers are given. Items covered include those for measuring smoke density, collecting air samples, analyzing foreign substances, detecting irritating or dangerous gases, and measuring and predicting weather conditions.

Crystallographic Data for the Calcium Silicates. Sponsored by the Department of Scientific and Industrial Research, Building Research Station. *L. Heller* and *H. F. W. Taylor.* vi + 79 pages. Her Majesty's Stationery Office. Can be purchased from York House, Kingsway, London, W. C. 2, England. 1956. 10s. 6d. ($1.89).

In view of the considerable amount of recent research on the calcium silicates, the authors have compiled crystallographic data for these compounds in reference form for use in identification and other purposes. This booklet comprises the optical and x-ray data including observations not previously published on most of the known calcium silicates, both artificial and naturally-occurring. The compounds are discussed singly, with uniform headings under each describing composition, occurrence, synthesis, appearance, optical properties, density, and unit-cell.

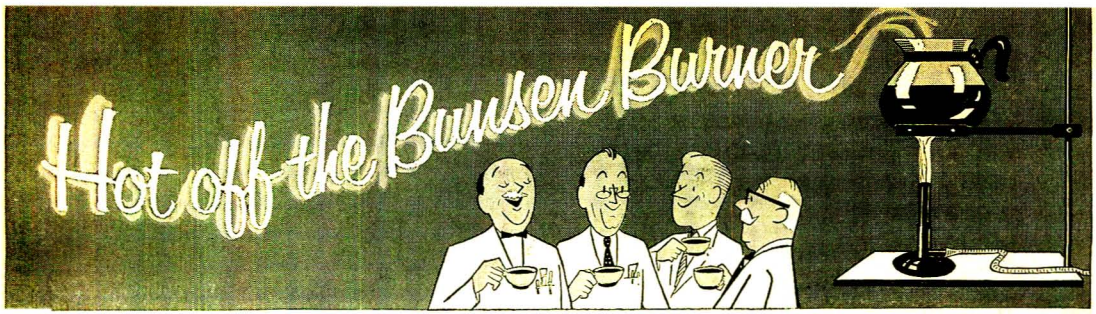
Kwalitatieve Chemische Analyse. *C. J. Van Nieuwenburg* and *J. W. L. Van Ligten.* 326 pages. D. B. Centen's Uitgeversmaatschappij N.V., 1 e Weteringplantsoen 9, Amsterdam-C., The Netherlands. 1956. Hfl 14.50.

This book is actually a laboratory manual for the student of qualitative chemical analysis. Following an introductory chapter which covers a general background, definitions, apparatus, and theory, the book goes into the reactions of specific cations and anions. Systematic analyses of these ions are described, as well as other analytical procedures.

Bibliography of Polarographic Literature 1922–1955. Edited by *Clark L. Schmitz* and *Edward F. Ewen.* 192 pages. E. H. Sargent & Co., 4647 West Foster Ave., Chicago 30, Ill., 1956. $5.00.

Here is an up-to-date bibliography of written material in the field of polarography which should prove to be of value to analysts. The first 144 pages comprise an alphabetical listing of authors, together with complete titles of each author's work. Where abstracts of papers are available, references to them are cited. Each of the 6800 entries is numbered, and this number is used in the cross-indexing.

A 48-page subject index with 14,000 references follows the author listing, referring again to the number designating each entry. Within this subject index, helpful breakdowns on apparatus, instrumentation, and specific types of polarography are included.



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the ANALYST'S column

L.T. Hallett, Editor

ALAN T. THOMAS, Brown-Forman Distillers Corp., Louisville, Ky., has developed a formula for approximating the cost of operating a laboratory. While Thomas' concept is probably not the whole or final answer, it is food for thought for those concerned with economics of analysis.

In this condensation, Thomas outlines his general cost approximation formula; applications will be discussed in a subsequent column.

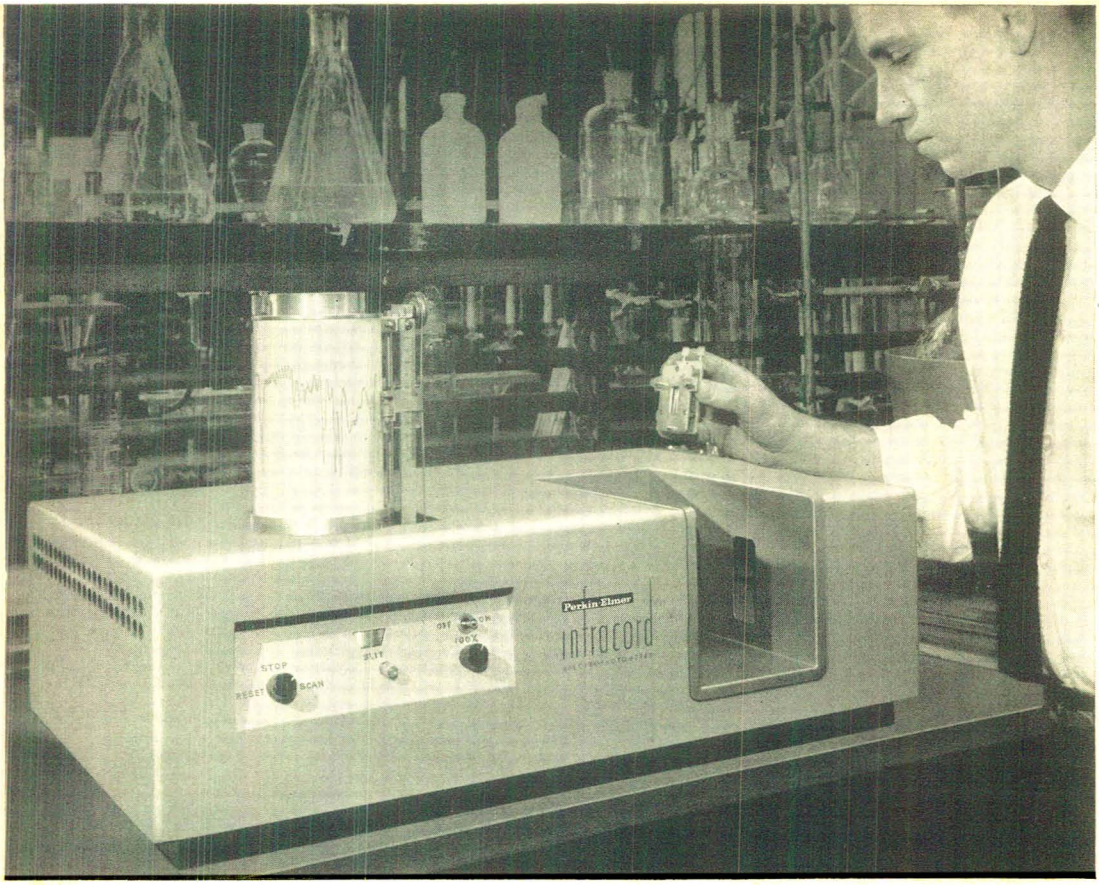
Literature on variables affecting efficiency of laboratory operations is sparse. Industrial Engineers and Cost Controllers have contributed information that might be useful in such an analysis, e.g., the Gilbreths' (*2*) concept of "therbligs" or fundamental motions and Tuttle's (*4*) emphasis upon what he calls "dynamic variable cost control"; but the most powerful tool to apply to such a problem is the operational approach as outlined by Ackoff (*1*).

This paper develops an operating cost approximation formula of a general nature to aid laboratory management. It is presupposed that laboratory operations have been studied and standard test methods developed so that time requirements of each test are known.

A generalized cost approximation formula must possess certain characteristics to be of value: 1. the equation must be dimensionally consistent; 2. have as broad a range of application as possible; 3. be simple to use; and 4. obtainable data should be expressible in the terms of the equation.

Although a number of relationships might fulfill these criteria to varying degrees, the one that best satisfies all four should involve time per unit of effort, cost per unit of time and the number units of effort. These can be combined in the formula: $C = tnc$ where C is the cost of the test, t is the time requirement per determination, n is the number of determinations, and c is the cost per unit time. It is frequently unnecessary to break the product tn into its components. In actual comparison of two or more costs, ratios can often be formed allowing cancellation of common factors.

Each independent variable in this equation is a function of other variables. These parameters may vary from laboratory to laboratory or under different situations, but the basic formula is still applicable. Estimates of parameters are necessary to permit adequate evaluation of test cost. Confidence limits can be established on the cost estimate if the possible errors in the parameter estimates are known.



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"Major variables affecting t are test method u and the particular analyst a. The analyst's time requirement for correctly performing a given test is determined by his skill and effort. Methods of rating these factors are given in time and motion study textbooks (*3*).

The c is a function of technician salary, instrument depreciation, insurance costs, maintenance costs, and miscellaneous fixed and variable costs such as administrative costs and prorated utilities costs. The composition and importance of the unit cost factor depend largely upon the accounting system.

The number of test determinations is a function of the production schedule, the type of test, and sometimes the history of past results. Multiple and sequential sampling plans are examples of a test's dependence upon previous samples. In these cases n contains a statistically determined component dependent upon a measurable characteristic or variable of the item tested.

Tests can be broken down into two categories: (1) Type A, those that fluctuate with capacity or production rate and (2) Type B, those that are a function of operating time or producing units.

Items Tested In Laboratory

Items Tested	Type of Test	Function of
Incoming grain	A	Cars received
Grinds of grain	B	Shifts worked
Beers	B	No. fermentors set
Alcohols	B	No. fermentors set
By-products	A&B	Shifts worked & shipments made
New whisky	B	Days produced
Warehoused whisky	B	Days produced
Regage	A	Dumps made
Bottling house supplies	A	Shipments received

Increases in production will cause a proportionate increase in the work time spent on Type A tests, other things remaining constant. The work load with Type B tests will form discrete plateaus with increasing or decreasing production. An extra shift, new machines or equipment, or an additional plant would cause a shift in plateau.

Fortunately, in a given application, the multitude of possible variables can be partitioned into significant and nonsignificant and the analysis can be completed. One indirect advantage of an operations research type approach of fitting information into a generalized model is that the investigator is forced to formulate his problem clearly, define its scope and be sure that he has the pertinent facts relating to its solution.

(1) Ackoff, R. L., *Operations Research* **4**, 265 (1956).
(2) Gilbreth, F. B., Gilbreth, L. M., *Management and Administration* **8**, 151 (1924).
(3) Lowry, S. M., Maynard, H. B., Stegemerten, G. J., "Time and Motion Study," McGraw-Hill, New York, (1940).
(4) Tuttle, F. G., *The Controller* **XXIV**, 62 (1956).

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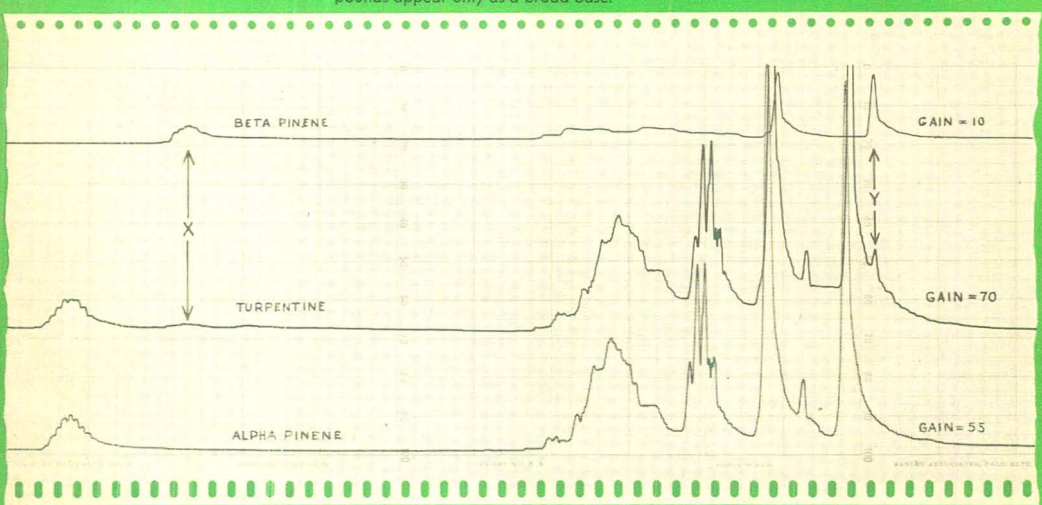
ANALYSIS OF TURPENTINE

As an example of the procedure to be followed in the analysis of a mixture of hydrogen-containing compounds, N-M-R spectra of three samples are compared below. Clearly, α-pinene has left its telltale "fingerprint" and represents the major component in turpentine. Peaks X and Y can be identified with β-pinene which is present in approximately 4.9 percent concentration. From the ratio of the gain settings required to produce comparable amplitudes for corresponding peaks in the turpentine and α-pinene spectra, it is apparent that this turpentine contains approximately 69% of α-pinene. The remaining material, which consists of other terpenes, is too diverse to give well-defined spectra and the resonances from these compounds appear only as a broad base.



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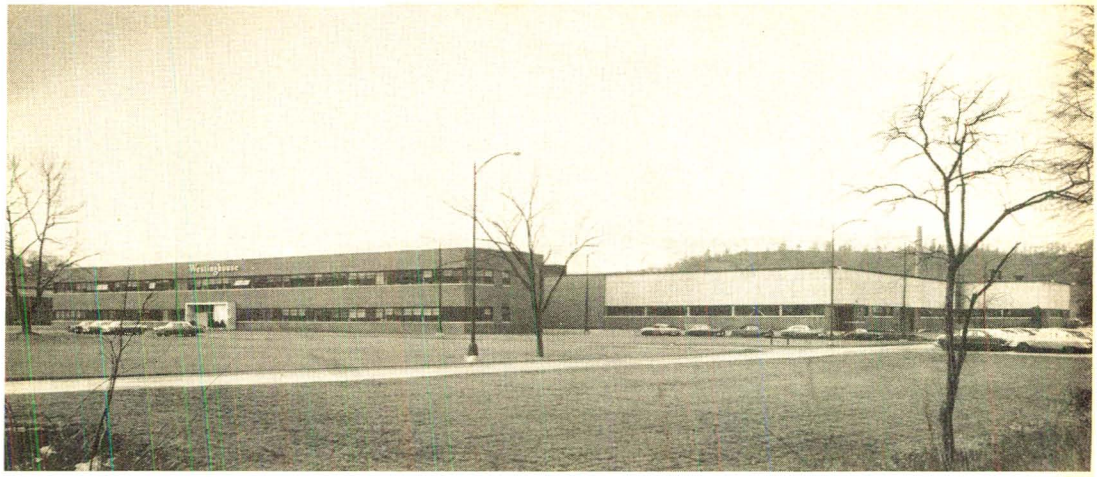


Extensive analytical facilities as well as all the equipment necessary for the development and manufacture of semiconductor devices are housed in Westinghouse's new Semiconductor Department

Harnessing Semiconductors Requires Close Analytical Control

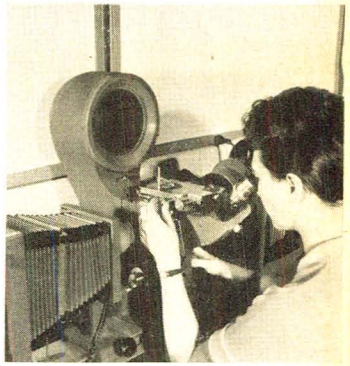
THE CHEMICAL and metallurgical aspects of semiconductor manufacture pose many analytical problems. Since the emphasis is on purity, it is necessary to employ very refined analytical techniques, or to develop new ones when none are available, in order to detect and determine trace elements at the parts per million (or less) level.

The new Semiconductor Department of Westinghouse Electric Corp., recognizing that quality control is essential in manufacturing a good product, has created the Analytical Control Laboratories for the purpose of safeguarding quality from the chemical and metallurgical standpoints. These laboratories are part of the Statistical Control Department, which also includes an Experiment Design and Statistical Analysis Section, a Computing and Tabulating Laboratory, Quality Control Engineering, and a Product Test and Inspection Section.

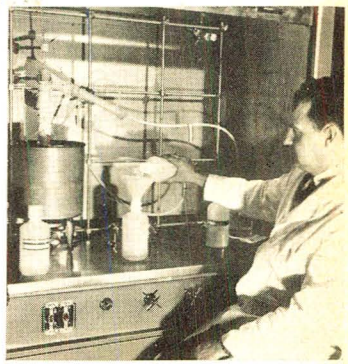
The plant, completely air conditioned and humidity controlled, is located in Youngwood, Pa., about 30 miles east of Pittsburgh. Housed in its 165,000 square feet are extensive semiconductor development and applications engineering laboratories as well as up-to-date manufacturing facilities. The administrative and engineering offices occupy a two-story plant area.

The Analytical Control Laboratories are divided into sections comprising: analytical (wet) chemistry, emission spectroscopy, x-ray diffraction, metallurgy, and photography. The floor area is 3,200 square feet.

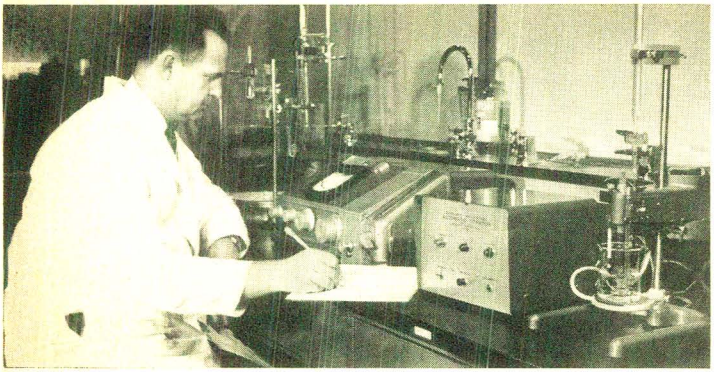
Each laboratory features such items as chemical hoods (where necessary), shadowless lighting and service lines of hot, cold, and deionized-distilled water, vacuum, gas, and compressed air. The various laboratories are divided by the use of removable partitions for easy rearrangement.



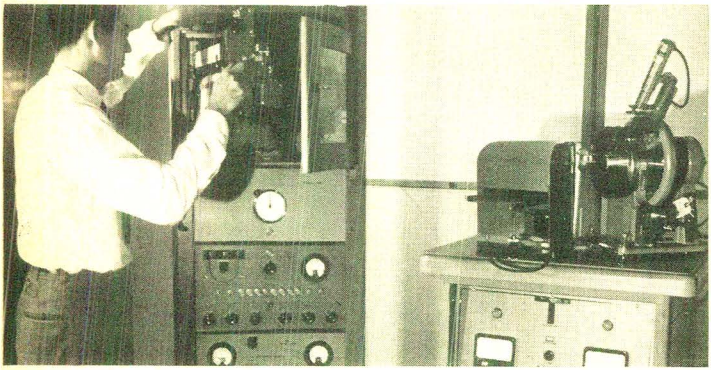
The Balphot metallograph is used in many metallurgical studies of semiconductor materials. Here, the p — n junction of a large silicon diode is being examined. This laboratory also includes equipment for sample preparation, microphotography, and melting point determination of alloys



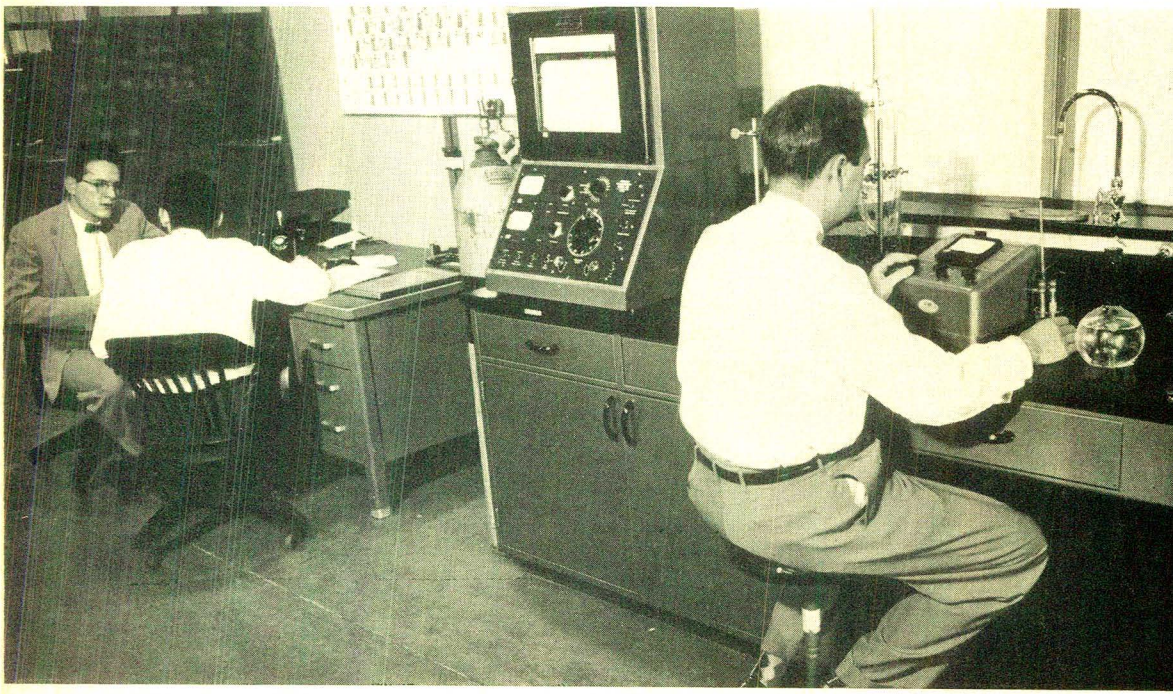
For analysis of trace elements in silicon and other materials at the parts per billion level, it is necessary to remove all contaminants from the reagents used. Here, purification of one of the reagents is carried out in a laboratory-made polyethylene still. Quartz ware or polyethylene has to be used throughout the analysis, since high blanks result from glassware



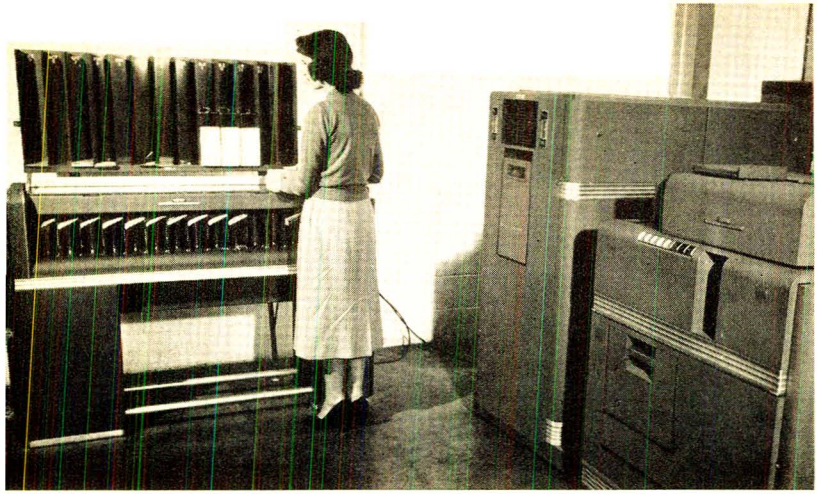
Spectrophotometry and titrimetry are employed for analysis of those elements that do not lend themselves readily to emission spectroscopy. Here, a chemist measures the absorption of the colored complex of phosphorus for its determination in silicon



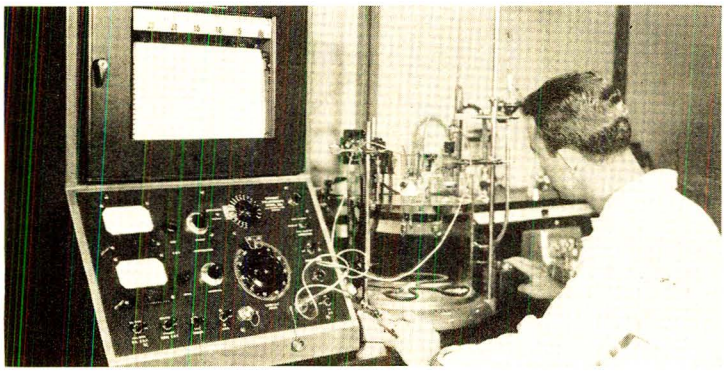
In order to manufacture transistors with the desired electrical characteristics, nearly perfect single crystals of germanium and silicon have to be grown from a melt. X-ray diffraction is used in the laboratory as a powerful tool in the study of imperfections in single crystals as well as to assist in their orientation on a proper crystallographic plane prior to slicing



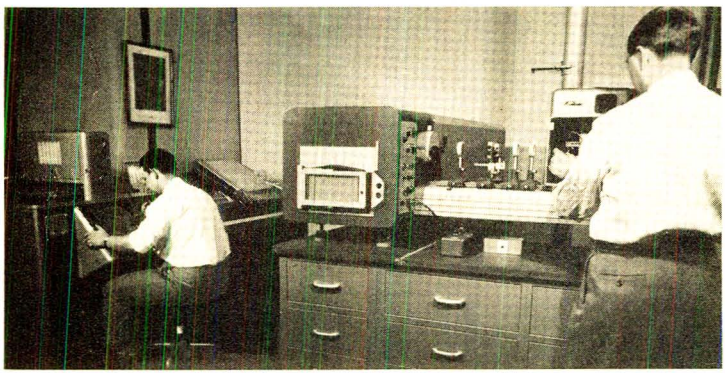
Many engineering and production problems of chemical and metallurgical nature occur in the manufacture of semiconductors. Some of the instrumentation employed by the Analytical Control Laboratories to solve these problems is shown



The laboratories employ IBM equipment for their record keeping as well as for analyzing and tabulating certain experimental results. The equipment is also used for many other control purposes



Very complete facilities are available in the emission spectrographic laboratory. A dual grating spectrograph is employed for trace analysis in many metals and alloys. A recording microphotometer is used (left) for the measurement of the spectral line intensities



The polarograph has proved to be very useful for the analysis of some alloys and solutions. It is also employed in studying the electrochemistry of the rarer elements like germanium, gallium, and indium

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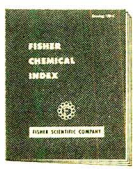

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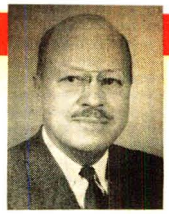

by Ralph H. Müller

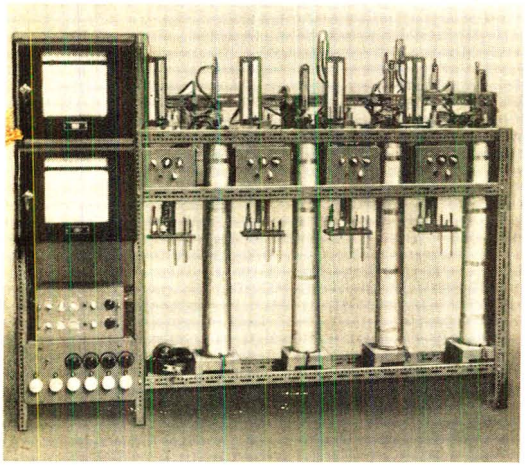

Figure 1. Shandon flame chromatograph performs automatic analyses

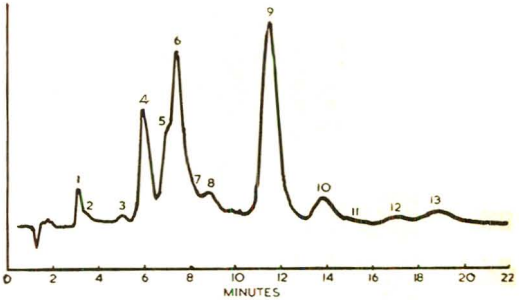

Figure 2. Chromatogram of National Benzole mixture motor spirit: column charge 1.5 mg.; column temperature 101 °C.

Vapor phase chromatography used to perform automatic analyses

SOME months ago in reviewing the monograph on Gas Chromatography by Courtenay Phillips we had occasion to refer to Scott's hydrogen flame detector [*Nature* **176,** 793 (1955)]. In this class of differential detector used in gas chromatography, hydrogen is used as the mobile phase. As described by Phillips:

The exit gas from the column is burnt at a small vertical jet, and a thermocouple junction is placed so as to be slightly above the normal hydrogen flame. When an organic vapour is present in the gas, the flame lengthens and engulfs the thermojunction. The output from the thermocouple is fed through a suitable potentiometric network to a recorder. The thermocouple may be made of 32 S.W.G. iron and constantan wires, brazed together with silver-bronze Brazotectic (m.p. 875° C.) leaving a globule of alloy at the junction about 1.5 mm. in diameter. The metal globule increases the thermal inertia of the couple and results in greater zero stability. The jet is made of Pyrex glass capillary and has a bore of about

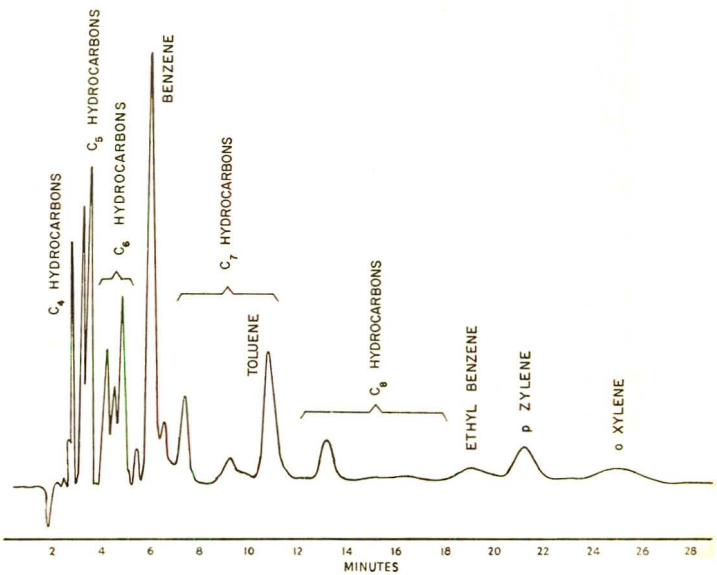

Figure 3. Lavender Oil: column charge 1.4 mg.; column temperature 176° C. Constituents unidentified

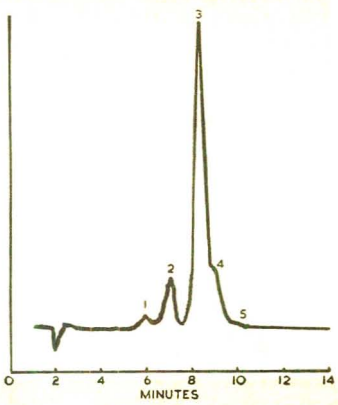

Figure 4. Limonene: column charge 0.4 mg.; column temperature 176° C. Constituents unidentified

0.2 mm. The detector incorporates a number of baffle plates which are essential to reduce the effects of draughts. With high temperature columns it has been found necessary to replace the iron constantan thermocouple with one of platinum/14 per cent rhodium platinum. The theory of the detector is not yet clear, but it depends upon the heat of combustion and rate of burning of the vapour. A linear relationship has been found between peak area and weight of a hydrocarbon vapour producing the peak. The detector has a small volume, is easily constructed, and is particularly suitable for use with high temperature columns. In its present form it will easily detect down to about 4 micrograms (μg) of a vapour.

We are happy to be informed that this device, which one might be inclined to regard as tricky or temperamental, has been developed and improved to the point where it forms the basis of an important and highly reliable industrial instrument. Through the courtesy of H. Freedman, Shandon Scientific Co., Ltd., 6 Cromwell Place, London, S.W. 7,

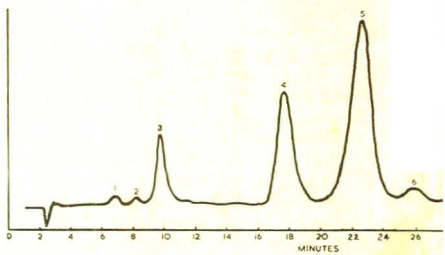

Figure 5. Peppermint Oil (American); column charge 1.4 mg.; column temperature 176° C. Constituents: (1) (2) (3) terpenes; (4) menthone; (5) menthol; (6) menthyl acetate

we are able to describe some features of the Shandon flame chromatograph, an instrument for performing automatic analyses by vapor phase chromatography.

One example of the instrument, which is in use at the laboratories of the National Benzole Co., is shown in Figure 1. The heated chromatographic columns are shown on the rack together with flowmeters and control units. Honeywell-Brown recorders of 1 mv. span and pen-response time of 1 second are used to record changes in the hydrogen flame detector. A chart speed of about 0.5 inch per minute is used. A unique sampling system is employed. The sample is contained in a small glass capillary which is held in a thin metal dart. This dart is placed in the sample chamber, one end of which can be sealed, the other end leading directly to the column via a wide-bore stopcock. After the sample is placed in the sample chamber, the stopcock is then opened and the metal dart is plunged by magnetic control into the column packing, whence the contents of the capillary are instantaneously discharged. Instantaneous discharge and reproducible samples are the major features of the sampling system.

A typical motor fuel analysis is illustrated in Figure 2, an elegant analysis performed in less than a half hour. Similar results are obtained for mixtures of alcohols and paraffins and for chlorinated hydrocarbons.

Figures 3, 4, and 5 show some typical results with essential oils. It is said that these chromatograms have caused quite a stir in the essential oil field and it may be that at last the skill and experience of the "taste and smell" chemist can be correlated with results obtained by an analytical, chromatographic technique.

Analysts in this country will view this fine achievement with great interest. Although thermal conductance detection is preferred by the majority of American chromatographers, it is by no means certain that it is the best method. Almost every physical phenomenon has been tried in the problem of detection; thermal conductance is probably one of the most convenient and simple. Modern chromatography is practically a British science; the example which we have described briefly represents an outstanding development and to continue the tradition, the distinguished A. J. P. Martin has greatly extended and improved the gas-density balance principle. This mode of detection has also been incorporated in a commercially available instrument. Martin delivered a lecture on these general principles at the Lisbon Congress, and publication will be awaited by all analysts.

ANALYTICAL CHEMISTRY

March 1957, Vol. 29, No. 3
Issued February 20, 1957

Radiation — A Potential Hazard

The dangers of radiation resulting from fall-out from atomic weapons are generally recognized as a potential health hazard, particularly to germinal tissues and thus to future generations. There are, however, other more common sources of radiation, such as use of x-rays and radioisotopes for medical purposes, whose possible harmful effects are becoming a matter of some concern.

Before the atomic age, the amount of radiation to which the individual was exposed was limited generally to medical fields and then in relatively small amounts.

The Scientific Committee on the Effects of Atomic Radiation of the United Nations is releasing a report on the matter of medical use of x-rays. Preliminary information indicates that this report shows that there are certain undesirable effects, particularly genetic, that the most important artificial source of radiation is use of radiological methods of diagnosis, and that more data are needed concerning the extent of exposure from this source.

This leads us to the observation that the steady increase in use of research and testing equipment which incorporates x-ray and radioisotope sources, presents potential hazards to analysts.

The extent of the increased analytical interest in nuclear fields, including use of isotopes, instrumentation, power, and education, will be covered in considerable detail at the summer symposium, sponsored by the Division of Analytical Chemistry and ANALYTICAL CHEMISTRY at Purdue University, Lafayette, Ind., June 13 to 15, 1957. The field is considered so important that the entire symposium will be directed to the topic "Nucleonics and Analytical Chemistry Ten Years After."

We know from personal experience that analysts, while fully cognizant of these hazards, sometimes grow careless. Other workers in areas where such equipment is used, also tend to be a little careless. Because effects of radiation appear to be cumulative, there is no room in the laboratory for laxity in using such equipment.

It behooves those who are exposed to radiation in their work to advise their doctors when medical and diagnostic use of x-rays are indicated.

Radiation, whether from x-rays or radioisotope sources, is a powerful tool. In the hands of those familiar not only with its uses but also with its hazards, it can accomplish results not obtainable by other means. In the hands of the careless or indifferent worker, it is a lethal tool.

Application of Gas-Liquid Chromatography to Analysis of Liquid Petroleum Fractions

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▶ Gas-liquid chromatography has many potential applications in the separation and analysis of volatile fractions from petroleum. In the work described, the gas density balance apparatus has been used to determine the retention volumes of a large number of hydrocarbons and some sulfur compounds boiling in the range from 30° to 150° C., using stationary phase liquids of differing structural type. Identification and estimation of minor impurities in reference fuel iso-octane and in a less pure iso-octane are described. Tentative identifications from retention volume data are confirmed by mass spectrometric examination of concentrates of these impurities, obtained by condensing the vapor contained in selected portions of the chromatograms. The analysis appears to be the most complete reference fuel analysis carried out to date, yielding information not obtainable by distillation, infrared, or mass spectrometric techniques.

THE CHROMATOGRAPHY of gases and vapors, in which a moving gas phase passes through a stationary column of sorbent, has great promise for separation and analysis of volatile mixtures. Although adsorption methods have been employed (*1, 11, 22, 27–29*), the partition technique, originally proposed by Martin and Synge as early as 1941 (*26*) and subsequently developed by James and Martin (*13, 16, 17, 21*) is much more versatile and has received the greater attention (*2, 5, 11, 14, 15, 18–20, 22–24, 26–30, 32*). It has been applied to problems in chemical kinetics (*4*) and to the examination of the thermal decomposition products of polymers (*7*). The extension of the method to higher boiling compounds, with operating temperatures of 200° C. and above, has recently been described (*6, 8*), and details of new detectors have been given (*12, 14, 18, 25, 31*).

In the analysis of volatile petroleum mixtures the technique is especially valuable because of its speed, accuracy, high separation efficiency with small samples, and the comparative simplicity of the apparatus required. The present investigation is concerned with the determination of retention volume data for a number of hydrocarbons and sulfur compounds boiling in the range from 30° to 150° C., using two stationary phases. One of the phases, a high boiling normal paraffin, separates substantially according to volatility only, while with the other—a high molecular weight aromatic—molecular type as well as boiling point influence the separation. The apparatus developed by James and Martin, employing a gas density balance as detector (*14, 18*), has been used. Similar retention volume data have recently been reported by these authors (*20*).

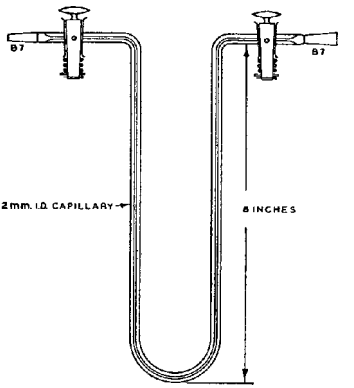


Figure 1. Capillary condensation trap

The technique and data described in the first part of this work have been used to analyze the minor impurities present in two samples of reference fuel iso-octane (2,2,4 - trimethylpentane). These two samples originated in a program set up by the Section on Reference Fuel Analysis of Committee D 2, ASTM, to determine the nature of the hydrocarbon impurities commonly present in the fuel. One was the final certified product and the other an "impure" sample taken at an intermediate point in the manufacture of the first, which was analyzed to increase the likelihood of detection of typical impurities. The samples were provided by the Phillips Petroleum Co., Bartlesville, Okla. who stated that impurities normally associated with their iso-octane in its manufacture were 2,3-dimethylpentane, 2- and 3-methylhexane, 2,4- and 2,5-dimethylhexane, and 2,2,3-, 2,3,3- and 2,3,4-trimethylpentane. An examination of the samples carried out in the laboratories of the Shell Development Co., Emeryville, Calif., had shown the impure sample to be 99.77 mole % pure by a cryoscopic calorimetric technique. A combination of analytical distillation and infrared spectroscopic techniques revealed only an estimated 0.1 mole % of 2,3-dimethylpentane. The certified sample was 99.93 mole % pure and no impurities in it could be detected by these techniques.

RETENTION VOLUMES OF HYDROCARBONS AND SULFUR COMPOUNDS

Materials Employed. The volatile hydrocarbons and sulfur compounds employed in this work were available as the result of syntheses carried out over a number of years. The purity of nearly all was above 99 mole %. A complete list of physical properties is not given here, as only the boiling points are significant in the determination and correlation of retention volume data described.

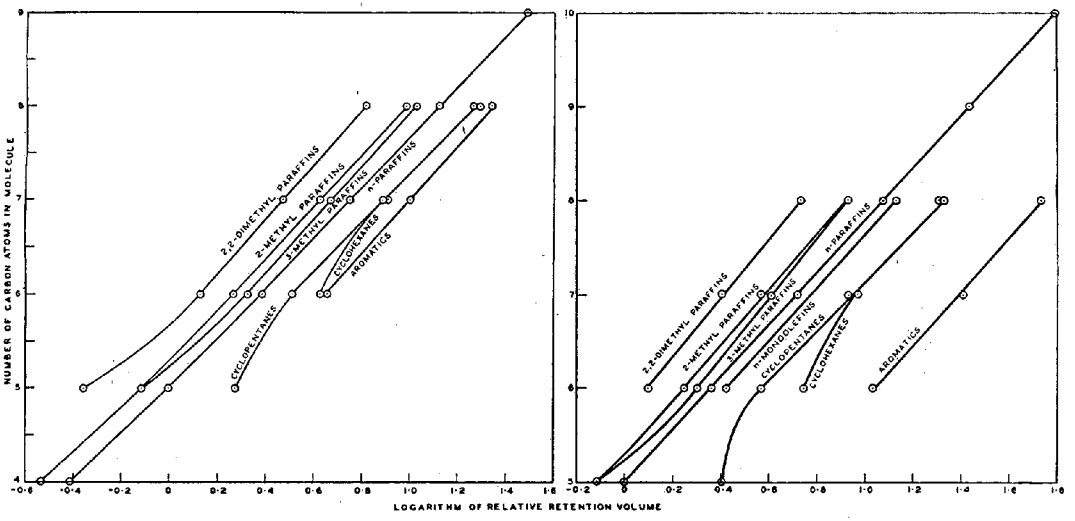
The two high molecular weight hydrocarbons used as stationary phase liquids were synthesized by conventional methods; the *n*-hexatriacontane by the Würtz reaction fron *n*-octadecyl bromide and the benzyldiphenyl by the reaction of benzyl chloride with diphenyl (*10*). It was necessary to use a mixture of the ortho and para isomers of benzyldiphenyl because the melting point of the pure para isomer, 85° C., is above the operating temperature employed.

Kieselguhr, a compact diatomaceous silica (Celite 545, Johns Manville Corp.), was prepared for use as the stationary phase support according to the procedure described by James and Martin (*17*). The eluent gas was an oxygen-free grade of nitrogen containing less than 10 p.p.m. of oxygen.

Equipment and Procedure. The apparatus used was essentially that described recently by James and Martin (*18*). Some slight modifications were

made in the construction of the density balance but these have no significant effect on the performance of the detector. When it is necessary to carry out a further examination of the vapor contained in a particular peak of the chromatogram, a capillary condensation trap (Figure 1), immersed in liquid nitrogen, is connected to the outlet of the density balance. The stopcocks are lubricated with the minimum quantity of silicone grease and the sample is kept frozen in the lower portion of the trap except during transfer onto another column or into the mass spectrometer.

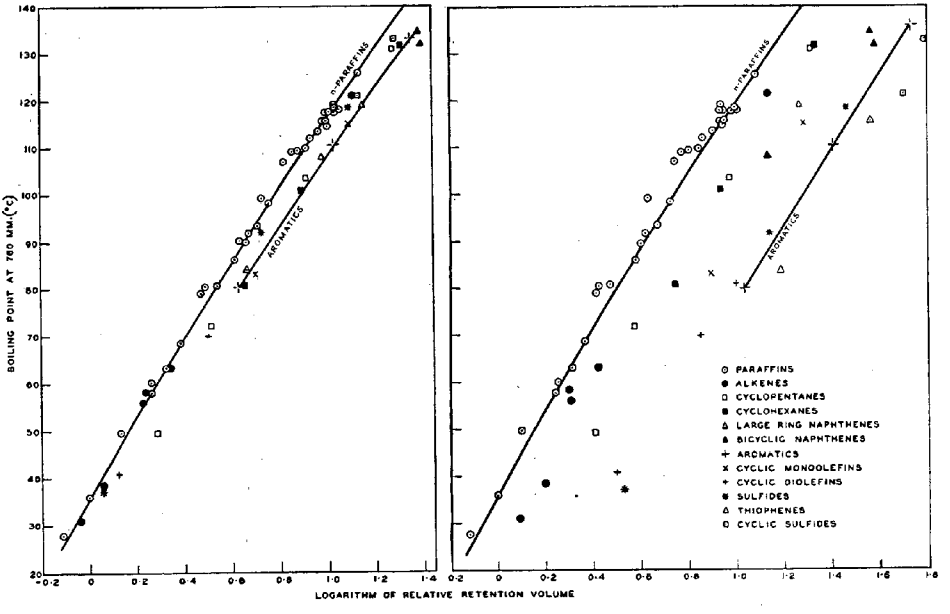
Experimental. In carrying out this investigation with compounds boiling



Relation between relative retention volumes of hydrocarbon structures and number of carbon atoms in molecule

Figure 2. *n*-Hexatriacontane stationary phase
Operating temperature, 78.5° C.

Figure 3. Benzyldiphenyl stationary phase
Operating temperature, 78.5° C.



Relation between relative retention volume and boiling point

Figure 4. *n*-Hexatriacontane stationary phase
Operating temperature, 78.5° C.

Figure 5. Benzyldiphenyl stationary phase
Operating temperature, 78.5° C.

in the range from 30° to 150° C., it was found convenient to operate with the apparatus at a temperature of 78.5° C. by refluxing ethyl alcohol in the vapor jacket. This temperature allowed the higher boiling compounds to pass through in a reasonable time (about 1.5 hours) without reducing the retention volume of the most volatile ones too much.

The relative retention volumes of 81 hydrocarbons and seven sulfur compounds have been determined on both hexatriacontane and benzyldiphenyl, using *n*-pentane as the reference substance throughout. The results are given in Table I. These values have been found to be repeatable to better than 1% on any particular column at a fixed flow rate; for a range of flow rates from 10 to 50 ml. per minute the variation is not greater than 2%. The operating times represented by the relative retention volumes given all fall between 1 and 90 minutes.

The efficiencies of the two columns used in this work have been calculated by the method given by James and Martin (*17*). The hexatriacontane column gives about 1200 plates (flow rate, 50 ml. per minute) while the benzyldiphenyl column gives about 320 plates (flow rate, 40 ml. per minute).

DISCUSSION

The logarithms of the relative retention volumes given in Table I have been plotted in Figures 2 and 3 *vs.* the number of carbon atoms in the molecule. Each homologous series produces a straight line approximately parallel to the others, showing that the energy of interaction with the stationary phase increases by a constant amount for each CH_2 added. Where the addition creates a methyl group, as in the case of the first step of the naphthene series, the line for this first step diverges from that for the remainder of the series. Benzene appears to be anomalous in this respect. At the lower molecular weight end of the paraffin series some divergences also appear—e.g., neopentane in the 2,2-dimethyl series—presumably because of some symmetry factors.

Figures 4 and 5 are plots of the logarithms of the relative retention volume *vs.* the boiling points. With the *n*-hexatriacontane column the separation is substantially dependent only on volatility, although a small type separation, equivalent to about 8° to 9° C. in boiling point, separates the aromatics and naphthenes from the paraffins. With the benzyldiphenyl column the separation is much more pronounced, the aromatics being separated from the paraffins by an amount equivalent to nearly 40° C.

The three chromatograms shown in Figure 6 illustrate these separations. Chromatogram 1 is that of a 14-component synthetic mixture of C_5 to C_8 hydrocarbons, including paraffins, naphthenes, and aromatics, on *n*-hexatriacontane as stationary phase. Three pairs of peaks have similar retention volumes:

3-methylhexane and 3-ethylpentane;

2,3 - dimethylbutane and cyclopentane;

2,2,3-trimethylpentane and toluene.

To improve the resolution of the first pair it would be necessary to increase the length of the column, as the separation of the paraffins is similar on both stationary phases. The second pair, a paraffin and naphthene, overlap completely, the type separation exactly compensating for the difference in volatility. Chromatogram 2 shows that these two hydrocarbons can be almost

Table I. Relative Retention Volumes of Hydrocarbons and Sulfur Compounds

(Operating temperature, 78.5° C.; *n*-pentane = 1)

No.	Boiling Pt., ° C. (760 mm.)	Compound	Stationary Phase: *n*-Hexatriacontane R.r.v.	*n*-Hexatriacontane Log_{10} r.r.v.	Benzyldiphenyl R.r.v.	Benzyldiphenyl Log_{10} r.r.v.
		PARAFFINS				
1	− 11.73	Isobutane	0.29	−0.538	...	...
2	− 0.50	*n*-Butane	0.39	−0.409	...	...
3	+ 9.50	Neopentane	0.44	−0.356	...	...
4	27.85	Isopentane	0.77	−0.113	0.76	−0.119
5	36.07	*n*-Pentane	1.00	0	1.00	0
6	49.74	2,2-Dimethylbutane	1.34	+0.127	1.26	+0.100
7	57.99	2,3-Dimethylbutane	1.83	0.263	1.74	0.241
8	60.27	2-Methylpentane	1.83	0.263	1.79	0.253
9	63.28	3-Methylpentane	2.12	0.326	2.05	0.312
10	68.74	*n*-Hexane	2.42	0.384	2.31	0.364
11	79.20	2,2-Dimethylpentane	3.00	0.477	2.57	0.410
12	80.50	2,4-Dimethylpentane	3.08	0.489	2.63	0.420
13	80.88	2,2,3-Trimethylbutane	3.49	0.543	2.94	0.468
14	86.06	3,3-Dimethylpentane	4.13	0.616	3.84	0.584
15	89.78	2,3-Dimethylpentane	4.55	0.658	4.03	0.605
16	90.05	2-Methylhexane	4.27	0.630	3.74	0.573
17	91.85	3-Methylhexane	4.68	0.670	4.14	0.617
18	93.48	3-Ethylpentane	5.18	0.714	4.63	0.666
19	98.43	*n*-Heptane	5.73	0.758	5.26	0.721
20	99.24	2,2,4-Trimethylpentane	5.28	0.723	4.26	0.629
21	106.84	2,2-Dimethylhexane	6.57	0.818	5.49	0.740
22	109.10	2,5-Dimethylhexane	7.13	0.853	5.94	0.774
23	109.43	2,4-Dimethylhexane	7.45	0.872	6.23	0.795
24	109.84	2,2,3-Trimethylpentane	8.10	0.909	6.89	0.838
25	111.97	3,3-Dimethylhexane	8.49	0.929	7.17	0.856
26	113.47	2,3,4-Trimethylpentane	9.21	0.964	8.03	0.905
27	114.76	2,3,3-Trimethylpentane	10.00	1.000	8.71	0.940
28	115.61	2,3-Dimethylhexane	9.63	0.984	8.51	0.930
29	115.65	2-Methyl-3-ethylpentane	9.84	0.993	8.83	0.946
30	117.65	2-Methylheptane	9.83	0.993	8.54	0.932
31	117.71	4-Methylheptane	10.12	1.005	8.83	0.946
32	117.73	3,4-Dimethylhexane	10.73	1.030	9.67	0.985
33	118.26	3-Methyl-3-ethylpentane	11.33	1.054	10.09	1.004
34	118.53	3-Ethylhexane	10.69	1.029	9.66	0.985
35	118.93	3-Methylheptane	10.58	1.025	8.54	0.932
36	125.67	*n*-Octane	13.42	1.128	12.00	1.079
37	150.80	*n*-Nonane	30.96	1.491	27.14	1.434
38	174.12	*n*-Decane	...	...	61.20	1.787
		AROMATICS				
39	80.10	Benzene	4.19	0.622	10.77	1.032
40	110.63	Toluene	10.50	1.021	25.46	1.406
41	136.19	Ethylbenzene	21.97	1.342	53.97	1.732
42	138.35	*p*-Xylene	26.02	1.416	57.78	1.762
43	139.10	*m*-Xylene	25.72	1.410	55.63	1.745
44	144.41	*o*-Xylene	30.72	1.487	73.19	1.864
		CYCLOPENTANES				
45	49.26	Cyclopentane	1.90	0.279	2.54	0.405
46	71.81	Methylcyclopentane	3.25	0.512	3.74	0.573
47	87.85	1,1-Dimethylcyclopentane	4.77	0.679	5.00	0.699
48	90.77	*cis*-1,3-dimethylcyclopentane	5.18	0.714	5.66	0.753
49	91.87	*trans*-1,2-dimethylcyclopentane	5.49	0.740	5.71	0.757
50	99.53	*cis*-1,2-dimethylcyclopentane	7.25	0.860	8.03	0.905
51	103.47	Ethylcyclopentane	8.18	0.913	9.31	0.969
52	105.05	1,1,3-Trimethylcyclopentane	10.39	1.017	10.43	1.018
53	109.40	1,2,4-Trimethylcyclopentane (low boiling isomer)	8.53	0.931	8.03	0.903

Table I. Relative Retention Volumes of Hydrocarbons and Sulfur Compounds (*Continued*)

(Operating temperature, 78.5° C.; *n*-pentane = 1)

No.	Boiling Pt., ° C. (760 mm.)	Compound	Stationary Phase: *n*-Hexatriacone R.r.v.	*n*-Hexatriacone Log_{10} r.r.v.	Benzyldiphenyl R.r.v.	Benzyldiphenyl Log_{10} r.r.v.
		CYCLOPENTANES, (*Continued*)				
54	116.95	1,2,4-Trimethylcyclopentane (high boiling isomer)	11.37	1.056	11.32	1.054
55	121.50	1-Methyl-1-ethylcyclopentane	13.61	1.134	14.93	1.174
56	126.95	Isopropylcyclopentane	16.20	1.210	17.14	1.234
57	130.95	*n*-Propylcyclopentane	18.43	1.266	20.49	1.312
		CYCLOHEXANES				
58	80.74	Cyclohexane	4.50	0.653	5.54	0.744
59	100.94	Methylcyclohexane	7.77	0.890	8.46	0.927
60	119.54	1,1-Dimethylcyclohexane	13.05	1.115	13.46	1.129
61	120.10	*cis*-1,3-dimethylcyclohexane	12.95	1.112	12.80	1.107
62	131.78	Ethylcyclohexane	19.61	1.293	21.46	1.331
		OTHER SATURATED CYCLICS				
63	118.9	Cycloheptane	14.00	1.146	18.09	1.257
64	151	Cyclo-octane	38.72	1.588	51.43	1.711
65	108	Norbornylane	9.45	0.975	13.55	1.132
66	132	*trans*-(3,3,0)-bicyclo-octane	24.53	1.389	38.13	1.581
67	135	*cis*-(3,3,0)-bicyclo-octane	24.39	1.387	36.41	1.561
		OLEFINS				
		Alkenes				
68	31.16	2-Methyl-1-butene	0.91	−0.041	1.25	0.097
69	38.57	2-Methyl-2-butene	1.16	+0.065	1.55	0.190
70	56.30	*cis*-4-methyl-2-pentene	1.68	0.225	2.06	0.314
71	58.55	*trans*-4-methyl-2-pentene	1.73	0.238	1.97	0.295
72	63.49	1-Hexene	2.13	0.328	2.73	0.426
73	72.5	4,4-Dimethyl-1-pentene	2.59	0.413	2.61	0.417
74	101.44	2,4,4-Trimethyl-1-pentene	6.09	0.785	6.23	0.795
75	104.91	2,4,4-Trimethyl-2-pentene	6.34	0.802	7.33	0.865
76	121.28	1-Octene	11.70	1.068	13.40	1.127
		Cyclic Olefins				
77	82.98	Cyclohexene	5.03	0.702	7.83	0.894
78	115	Cycloheptene	12.41	1.094	19.12	1.281
		Cyclic Diolefins				
79	41	Cyclopentadiene	1.32	0.121	3.17	0.501
80	70	Methylcyclopentadiene	3.20	0.505	7.09	0.851
81	81	Cyclohexadiene	4.27	0.630	10.02	1.009
		SULFUR COMPOUNDS				
		Alkane Sulfides				
82	37.28	2-Thiapropane	1.14	0.057	3.38	0.529
83	92.06	3-Thiapentane	5.22	0.718	13.81	1.140
84	118.50	3-Thiahexane	11.67	1.087	29.13	1.464
		Cyclic Sulfides				
85	121.45	Thiacyclopentane	13.33	1.125	50.59	1.704
86	133.23	2-Methylthiacyclopentane	18.85	1.275	61.19	1.787
		Thiophenes				
87	84.10	Thiophene	4.58	0.661	15.06	1.178
88	115.44	3-Methylthiophene	10.04	1.001	35.28	1.548

completely separated on benzyldiphenyl. Chromatogram 3 shows that the last pair, a paraffin and aromatic, have a much larger separation on benzyldiphenyl than on *n*-hexatriacontane.

By plotting the relative retention volumes for the two stationary phases against each other, lines radiating from the origin are obtained (one for each homologous series). The slopes of these lines are dependent on the relative interactions of each series with the two stationary phases. Radial areas on this diagram may then be allocated to the various types of hydrocarbon structure.

A disadvantage of this plot is that points are spaced along the lines in a distribution logarithmic to molecular weight, and thus tend to be crowded into the bottom left hand corner. By plotting the logarithms of the relative retention volumes against each other, a corresponding series of approximately parallel lines is obtained, with points spaced linearly with molecular weight. This is shown in Figure 7 for all the data given in Table I. By plotting the retention volume data for an unknown hydrocarbon on the two stationary phases an approximate value for its molecular weight can be obtained and its structure defined broadly as paraffinic, naphthenic, aromatic, or the like. In some favorable cases it is possible to identify an unknown unambiguously as a single hydrocarbon.

There is a difficulty in the practical application of this dual stationary phase technique to mixtures containing more than a few well-resolved components, in that it is difficult to pair corresponding peaks on the two chromatograms. This difficulty has often been overcome by condensing the vapor in particular peaks of the chromatogram using a *n*-hexatriacontane column and running these condensed peaks individually on the benzyldiphenyl column. By a similar technique it has been possible to to examine the hydrocarbons in individual peaks in the mass spectrometer and thus confirm a tentative identification based on the retention volume data.

The method described by James and Martin (*17*) for calculating the separation efficiency of a gas-liquid partition column gives very high figures for the number of theoretical plates but it seems doubtful whether these figures have any real significance as compared with the theoretical plate efficiency obtained for fractionating columns. It is difficult to make a direct comparison, because even with *n*-hexatriacontane there is a small type separation. The 4-foot *n*-hexatriacontane column with a nominal efficiency of about 1200 theoretical plates gives only a poor resolution between 2,4- and 2,5-dimethylhexane (boiling 0.33° C. apart), while a comparable partial separation is accomplished with a 200-plate fractionating column. The method, however, does enable various partition columns to be compared. The benzyldiphenyl column has about one fourth the efficiency of the *n*-hexatriacontane column, presumably because of the higher viscosity of the former hydrocarbon.

ANALYSIS OF ISO-OCTANE FUEL SAMPLES

From a preliminary examination of the two iso-octane fuels it was obvious that it would be necessary to use a large sample with considerable overloading of the columns before the chromatogram would show the small amounts of impurities. In spite of the overloading, however, it was soon apparent that the impure sample contained a group of impurities boiling

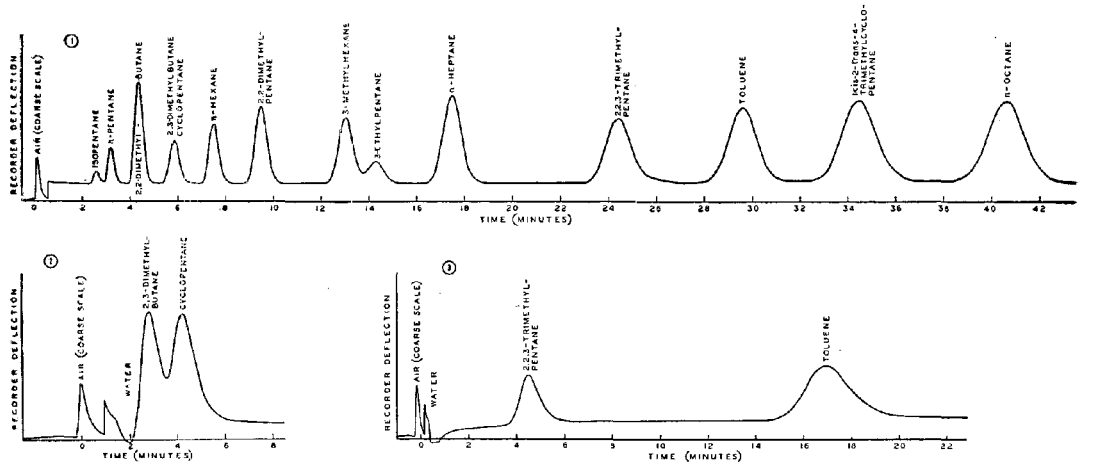


Figure 6. Chromatograms of hydrocarbon mixtures

1. C_6 to C_8 hydrocarbon mixture on *n*-hexatriacontane column
   Pressure, 610 mm. Hg; flow, 45 ml./min.; jacket temperature, 78.5° C.
2. 2,3-Dimethylbutane and cyclopentane on benzyldiphenyl column
   Pressure, 320 mm. Hg; flow, 12.5 ml./min.; jacket temperature, 78.5° C.
3. 2,2,3-Trimethylpentane and toluene on benzyldiphenyl column
   Pressure, 740 mm. Hg; flow, 40 ml./min.; jacket temperature, 78.5° C.

Table II. Relative Retention Volumes of Components of Reference Fuel Samples

Component	Stationary Phase[a] *n*-Hexatriacontane	Benzyldiphenyl
Lower boiling impurities	4.6, 4.3 (4)	4.0 to 4.5 (6)
Main component iso-octane	5.4 (4), 5.3 (5)	4.3 (6)
Higher boiling impurity	7.7 (4), 7.7 (5)	8.5 (6)

[a] Numbers in brackets indicate chromatogram (Figures 8, 9) from which each result was obtained.

lower than iso-octane which were absent from the certified sample, and that both samples contained a small quantity of a higher boiling impurity.

In order that the chromatograms of the two samples should be directly comparable, an accurately measured sample of 0.100 ml. was delivered by a micrometer syringe onto the column.

The results obtained for the impure and certified samples using a *n*-hexatricontane column are shown in chromatograms 1 and 2 (Figure 8); the overloading is obvious. The very large iso-octane peak, which has been recorded only after reducing the sensitivity of the amplifier by a factor of 100, is about 50 chart widths high on the normal sensitivity. It has a steep front and long tail, presumably caused by curvature of the isotherm at high concentrations; the top flattens as the saturation vapor pressure is approached. In spite of the large, wide peak due to the main component the impurities still may be seen easily. The absence of the lower boiling impurities in the certified sample contrasts strikingly with the similar amounts of the higher boiling impurity in both the impure and certified samples.

As the retention volumes measured with such heavy overloading are unreliable, it was necessary to obtain a concentrate of the impurities from several chromatograms by condensing the vapors issuing from the density balance for the periods corresponding to the impurities. This is illustrated in chromatogram 3 (Figure 8) where vapor has been condensed between *AA* and *BB*. The condensed material may be re-examined by connecting the trap to the top of the column, allowing it to warm up, and ejecting the condensed material with the stream of cluent gas. Chromatogram 4 shows a rerun of the impurity concentrate from four preliminary runs using a 0.100-ml. sample as shown in chromatogram 3. It is interesting that the two air "pellets" at the ends of the trap, introduced together with the hydrocarbon vapors into the eluent gas stream, move individually through the column and produce two separate peaks. The procedure has been carried one step further in chromatogram 5, in which the second peak only has been separated during chromatogram 4 and rerun, reducing the iso-octane concentration by a further substantial factor.

To obtain retention volume data for the impurities using a second stationary phase, an impurity concentrate similar to that obtained for chromatogram 4 was rerun on a benzyldiphenyl column to produce chromatogram 6 (Figure 9).

The relative retention volumes of the impurities from the various chromatograms were measured using *n*-pentane as standard. The values obtained (Table II) for the lower boiling impurities were consistent with the three lower boiling impurities suggested by the makers—i.e., 2- and 3-methylhexane and 2,3-dimethylpentane. The latter two isomers would not be resolved, and a small partially resolved peak in the leading edge of the first peak of chromatogram 4 gives a relative retention volume which agrees well with that of 2-methylhexane. On benzyldiphenyl none of these hexanes would be resolved from large quantities of iso-octanes, as has been observed in chromatogram 6 (Figure 9).

The higher boiling impurity proved more difficult to identify. The logarithms of the relative retention volumes measured on the two stationary phases, when plotted on a large version

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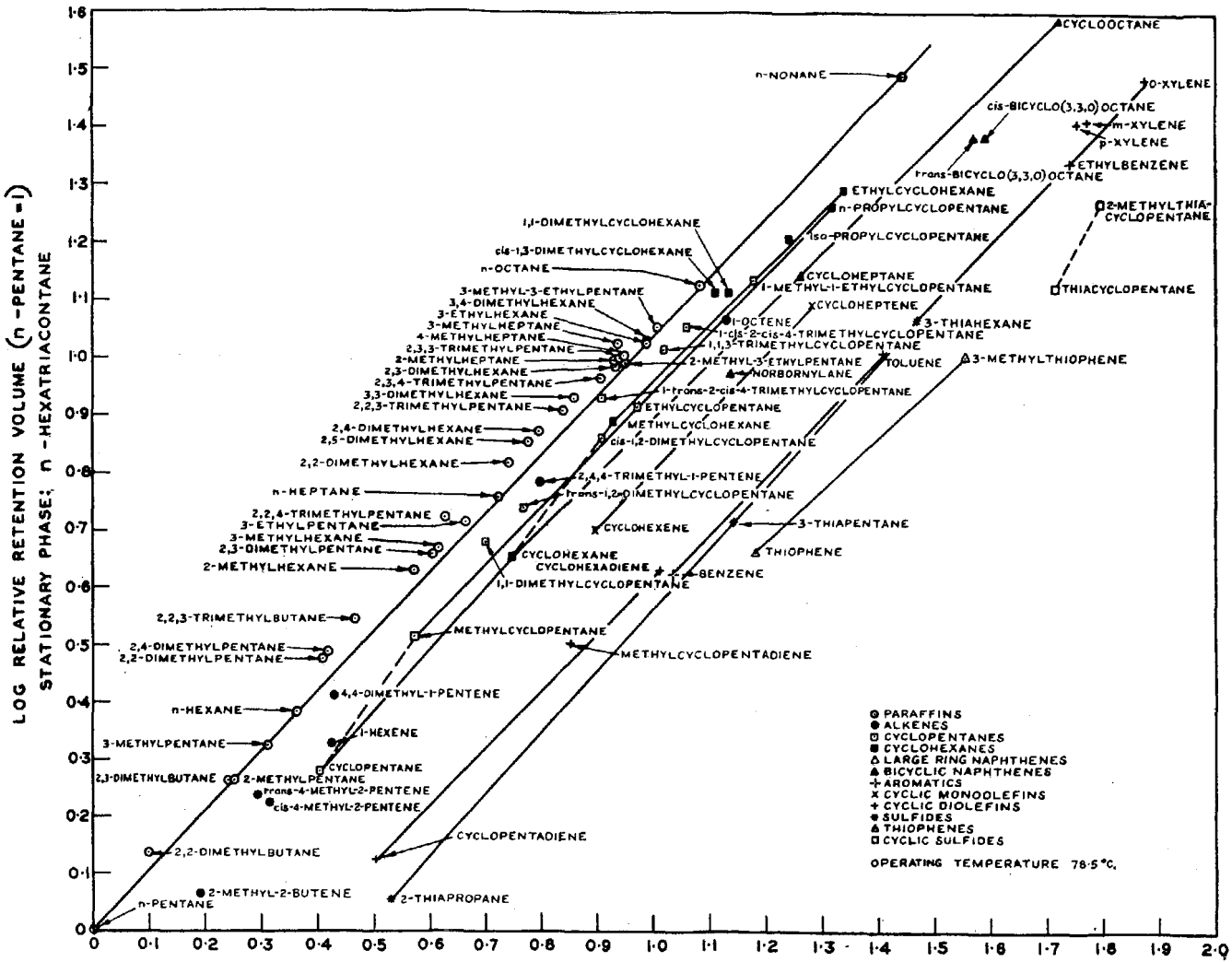
Figure 7. Plot of logarithm of relative retention volume on *n*-hexatriacontane vs. logarithm of relative retention volume on benzyldiphenyl


LOG RELATIVE RETENTION VOLUME (n-PENTANE = 1)
STATIONARY PHASE; n-HEXATRIACONTANE
LOG RELATIVE RETENTION VOLUME (n-PENTANE = 1); STATIONARY PHASE: BENZYLDIPHENYL
n-PENTANE
2-METHYL-2-BUTENE
2,2-DIMETHYLBUTANE
CYCLOPENTADIENE
2-THIAPROPANE
2,3-DIMETHYLBUTANE
2-METHYLPENTANE
trans-4-METHYL-2-PENTENE
cis-4-METHYL-2-PENTENE
3-METHYLPENTANE
n-HEXANE
CYCLOPENTANE
1-HEXENE
4,4-DIMETHYL-1-PENTENE
2,4-DIMETHYLPENTANE
2,2-DIMETHYLPENTANE
2,2,3-TRIMETHYLBUTANE
METHYLCYCLOPENTANE
METHYLCYCLOPENTADIENE
2-METHYLHEXANE
2,3-DIMETHYLPENTANE
3-METHYLHEXANE
3-ETHYLPENTANE
2,2,4-TRIMETHYLPENTANE
n-HEPTANE
2,2-DIMETHYLHEXANE
2,5-DIMETHYLHEXANE
2,4-DIMETHYLHEXANE
2,2,3-TRIMETHYLPENTANE
3,3-DIMETHYLHEXANE
2,3,4-TRIMETHYLPENTANE
2,3-DIMETHYLHEXANE
2-METHYLHEPTANE
2,3,3-TRIMETHYLPENTANE
4-METHYLHEPTANE
3-METHYLHEPTANE
3-ETHYLHEXANE
3,4-DIMETHYLHEXANE
3-METHYL-3-ETHYLPENTANE
n-OCTANE
cis-1,3-DIMETHYLCYCLOHEXANE
1,1-DIMETHYLCYCLOHEXANE
CYCLOHEXANE
CYCLOHEXADIENE
1,1-DIMETHYLCYCLOPENTANE
CYCLOHEXENE
BENZENE
THIOPHENE
3-THIAPENTANE
METHYLCYCLOHEXANE
ETHYLCYCLOPENTANE
cis-1,2-DIMETHYLCYCLOPENTANE
2,4,4-TRIMETHYL-1-PENTENE
trans-1,2-DIMETHYLCYCLOPENTANE
1-trans-2-cis-4-TRIMETHYLCYCLOPENTANE
2-METHYL-3-ETHYLPENTANE
NORBORNYLANE
TOLUENE
1-cis-2-cis-4-TRIMETHYLCYCLOPENTANE
1,1,3-TRIMETHYLCYCLOPENTANE
1-OCTENE
CYCLOHEPTENE
3-THIAHEXANE
3-METHYLTHIOPHENE
1-METHYL-1-ETHYLCYCLOPENTANE
CYCLOHEPTANE
THIACYCLOPENTANE
2-METHYLTHIACYCLOPENTANE
n-PROPYLCYCLOPENTANE
iso-PROPYLCYCLOPENTANE
ETHYLCYCLOHEXANE
trans-BICYCLO(3,3,0)OCTANE
cis-BICYCLO(3,3,0)OCTANE
ETHYLBENZENE
p-XYLENE
m-XYLENE
o-XYLENE
n-NONANE
CYCLOOCTANE
PARAFFINS
ALKENES
CYCLOPENTANES
CYCLOHEXANES
LARGE RING NAPHTHENES
BICYCLIC NAPHTHENES
AROMATICS
CYCLIC MONOOLEFINS
CYCLIC DIOLEFINS
SULFIDES
THIOPHENES
CYCLIC SULFIDES
OPERATING TEMPERATURE 78·5°C.

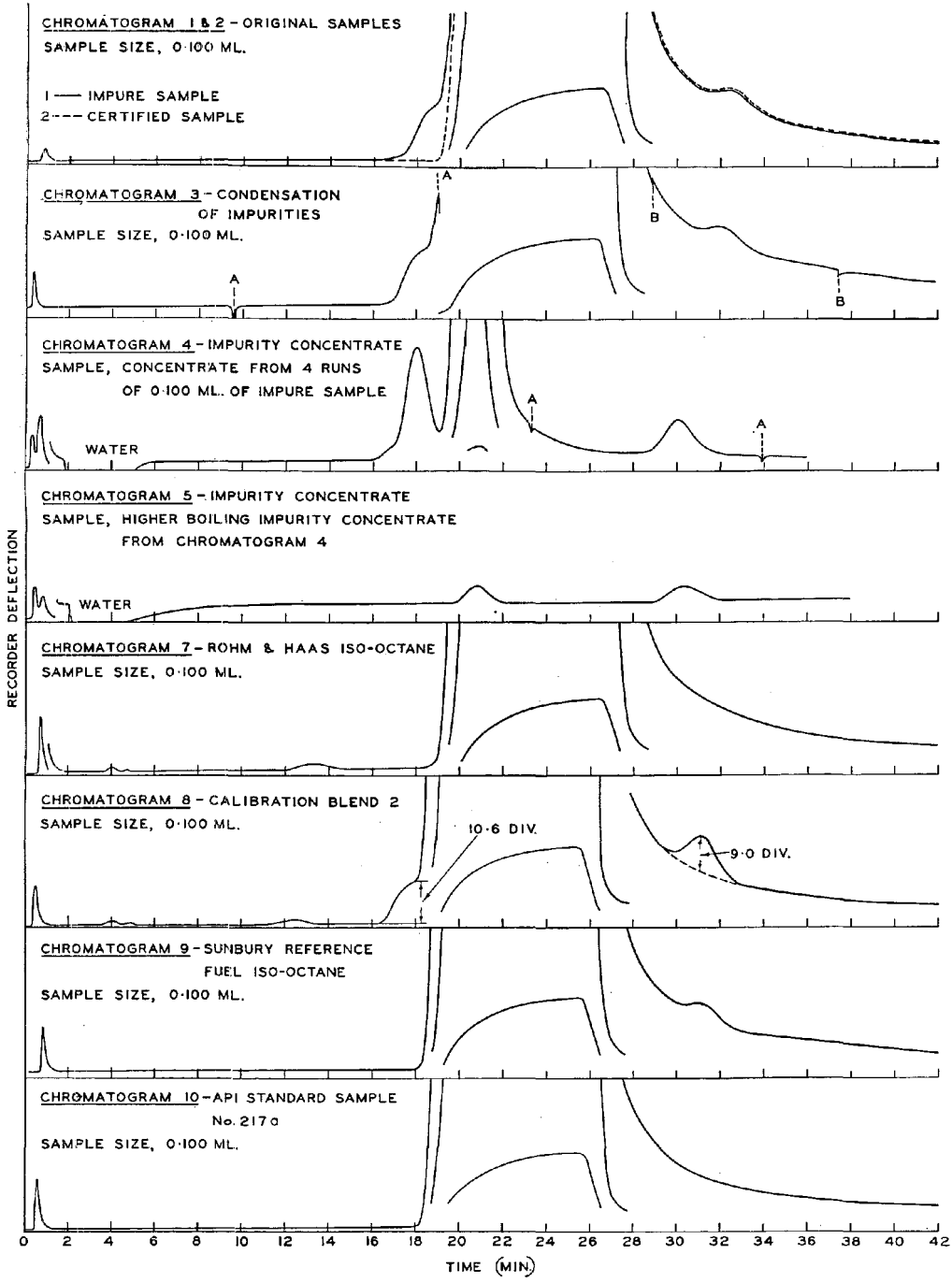


Figure 8. Chromatograms

Stationary phase, *n*-hexatriacontane; temperature 78.5° C.; flow, 45 ml./min.

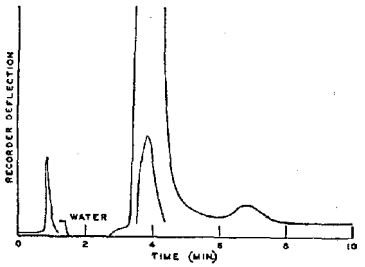


Figure 9. Chromatogram 6 of impurity concentrate sample of both higher and lower boiling impurities

Stationary phase, benzyldiphenyl; temperature, 78.5° C.; flow, 40 ml./min.

of the plot shown in Figure 7, showed that it was probably methylcyclohexane. The relative retention volumes of none of the paraffins suggested by the makers matched the data for the impurity, nor did those of the only other likely impurities, 2,4,4-trimethyl-1- and -2-pentene.

In order to check the values used, most of the relative retention volumes were redetermined on the actual columns used for this work. The data obtained are shown in Table III together with the corresponding values from Table I. The agreement is better than 2% in spite of the fact that the latter values were obtained on different columns some months earlier.

The presence of methylcyclohexane in reference fuel iso-octane seemed so improbable that it was decided to confirm the tentative identifications made from retention volume data by examination of separate concentrates of the low and high boiling impurities in the mass spectrometer. The concentrates were obtained by condensation in capillary traps and these were attached directly to the mass spectrometer inlet. It proved necessary, because of the very small amount of material available (a few milligrams), to construct a new uncontaminated inlet system having a smaller volume than is usually employed. The components identified are shown in Table IV; the agreement with the identifications made chromatographically is good. It proved possible to carry out a quantitative analysis of both concentrates from the mass spectrometric data. These are also given in Table IV, together with values calculated on an iso-octane–free basis.

As the amounts of the high boiling impurity in the certified and impure samples were identical (see chromatograms 1 and 2, Figure 8), it was necessary to determine only the concentrations of the impurities in the impure sample to complete the analysis.

Table III. Relative Retention Volume Data for Suspected Hydrocarbons

Hydrocarbon	Stationary Phase: *n*-Hexatriacontane, New values	*n*-Hexatriacontane, Old values	Benzyldiphenyl, New values	Benzyldiphenyl, Old values
2,4,4-Trimethyl-2-pentene	6.34	6.34	7.30	7.33
Methylcyclohexane	7.63	7.77	8.60	8.46
2,4-Dimethylhexane	7.33	7.45	..	..
2,5-Dimethylhexane	7.03	7.13	..	..
2,2,3-Trimethylpentane	7.95	8.10	..	..
2,3-Dimethylpentane	4.54	4.55	..	..
3-Methylhexane	4.60	4.68	..	..

A sample of iso-octane free of these impurities was needed in order to prepare a number of synthetic blends for calibration purposes. It was not possible to use the direct area measurement method because the resolution was inadequate with the column so heavily overloaded. Also, with the high concentration of vapor in the peak for the main component, the response of the density balance was probably no longer proportional to the density difference between the eluent gas and the vapor. A sample of iso-octane prepared some years ago by fractionation of a commercial material manufactured by the Rohm & Haas Co. was examined and found to be substantially free of these particular impurities, although significant amounts of others at different positions in the chromatogram were detected (chromatogram 7, Figure 8).

Calibration blends were made up gravimetrically using 2,3-dimethylpentane and methylcyclohexane with this iso-octane. It did not seem necessary to use 2- and 3-methylhexanes as these were poorly resolved from the dimethylpentane under the conditions used. In order to limit the amounts of the pure hydrocarbons used and to reduce inaccuracies due to evaporative losses during weighing and manipulation, a method of successive dilutions was employed.

The chromatogram of each blend, using an 0.100-ml. sample, was obtained with a *n*-hexatriacontane column under the same operating conditions as used for the samples. The curve produced by Blend 2 is shown in chromatogram 8 (Figure 8). For the light impurity the height from the zero line to the point of inflection was taken as a measure of the peak height, while the peak height of the methylcyclohexane was measured from the line the curve would have followed in the absence of this component. The results obtained are given in Table V and have been plotted in Figures 10 and 11. The accuracy of molar proportions read from these plots has been given as the maximum divergence at the particular level. The results obtained from three successive runs on both samples are given in Table VI, together with the corresponding molar proportions of the impurities. The complete results of the analysis of the impure and certified samples have been summarized in Table VII.

Table IV. Mass Spectrometric Analysis of Concentrates of Lower and Higher Boiling Impurities

Component	Concn., Mole % Detd.	Concn., Mole % Corr.
Lower Boiling Impurities		
2,2,4-Trimethylpentane	34	
2-Methylhexane	6	9
3-Methylhexane	7	11
2,3-Dimethylpentane	53	80
Higher Boiling Impurities		
2,2,4-Trimethylpentane	50	
C_7 Naphthene (almost certainly methylcyclohexane)	45	90
Unidentified paraffins	5	10

Table V. Calibration Blends of Iso-octane with 2,3-Dimethylpentane and Methylcyclohexane

Blend No.	2,3-Dimethylpentane, Peak height (chart div.)	2,3-Dimethylpentane, Mole %	Methylcyclohexane, Peak height (chart div.)	Methylcyclohexane, Mole %
1	...	...	5.7	0.028
2	10.6	0.140	9.0	0.057
3	13.0	0.166	19.3	0.091
4	107.0	1.075	..	...
5	12.5	0.148	..	...
6	8.0	0.087	..	...
7	4.5	0.051	..	...

The presence of methylcyclohexane is surprising. This hydrocarbon was not detected during the complete analyses of a large range of alkylates by API Research Project 6 (*9*) or in an earlier investigation of a crude synthetic iso-octane derived from hydrogenated diisobutene (*3*). Chromatogram 9 (Figure 8) shows that the curve obtained with a reference fuel sample from a batch being currently used at Sunbury (supplied by Enjay Co., Elizabeth, N. J.) is similar to that of the certified sample. The Rohm & Haas sample was manufactured over 15 years ago and was probably obtained from a hydrogenated diisobutene feedstock. No impurities can be detected in API sample 217A (chromatogram 10, Figure 8), probably because of the rigorous purification by both regular and azeotropic fractionation.

The total impurity measured by gas partition chromatography for both samples tends to be lower by about 0.05 mole % than the corresponding figures obtained by the freezing point method. There are two possible sources of error in the chromatographic procedure which may contribute to the difference.

Impurities have not been detected because they are not resolved from the main iso-octane peak. Only 3-ethylpentane, *n*-heptane, and possibly 2,2-dimethylhexane among the paraffins would fail to be resolved under the conditions used.

A number of impurities, such as other branched-chain octanes, which would be resolved are present in such small amounts that their individual concentrations in the chromatogram are below the sensitivity of the detector. The small proportion of paraffin detected by the mass spectrometer in the higher boiling impurity is interesting in this connection.

In spite of this small discrepancy, however, the gas-liquid chromatographic method, in conjunction with the mass spectrometer, has provided the most complete analysis to date.

CONCLUSIONS

The potentialities of gas-liquid chromatography in the detection, identification, and estimation of minor impurities in nearly pure materials are, in some respects, more striking than its application to the analysis of more conventional volatile mixtures.

The measurement of total impurity by cryoscopic methods has been developed in recent years to give reliable and usually extremely accurate results, but often their value is reduced by the fact that no information of the nature of the impurities may be obtained. Gas-liquid chromatography, however, enables the impurities to be concentrated and in some cases entirely separated

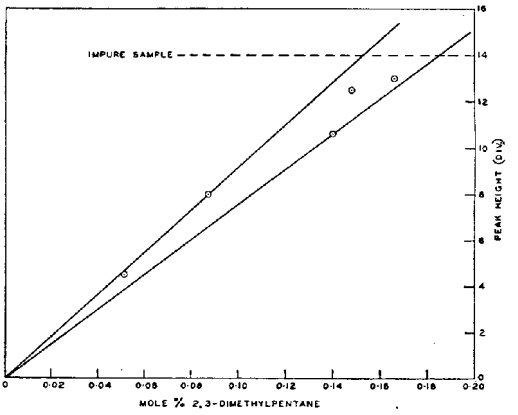


Figure 10. Peak height *vs.* mole per cent 2,3-dimethylpentane in 2,2,4-trimethylpentane

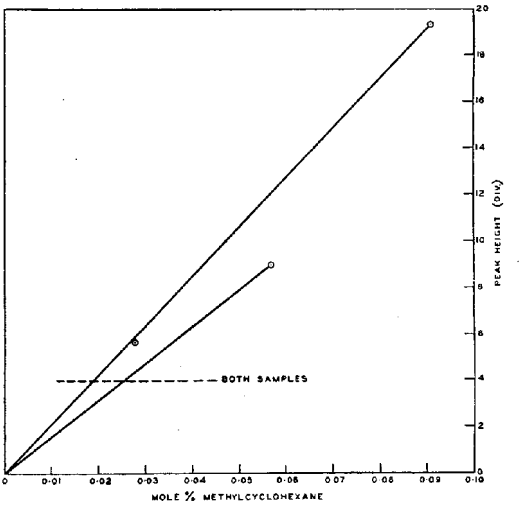


Figure 11. Peak height *vs.* mole per cent methylcyclohexane in 2,2,4-trimethylpentane

Table VI. Determination of Impurity Concentration in Impure and Certified Samples

Sample	Lower Boiling Impurity		Higher Boiling Impurity	
	Peak height (div.)	Mole %	Peak height (div.)	Mole %
Impure	13.9	0.165	3.9	0.022
	13.8	±0.02	4.0	±0.01
	14.4		3.9	
	Av. 14.0		3.9	
			3.9	0.022
Certified	Nil	Nil	4.0	±0.01
			4.0	
	Av.		4.0	

before identification and estimation. Where a complete analysis of the impurity concentrates is not possible by chromatography, as with the analysis of the iso-octane samples described, gas-liquid chromatography enables the scope of existing analytical methods to be extended to lower concentration levels.

ACKNOWLEDGMENT

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Table VII. Complete Analysis of Impure and Certified Samples

	Impure Sample			Certified Sample		
Impurities	Mole %	Composition, molar ratio		Mole %	Composition, molar ratio	
Lower boiling	0.165 ±0.02	2,3-Dimethylpentane	80	Nil	Nil	
		3-Methylhexane	11			
		2-Methylhexane	9			
Higher boiling	0.022 ±0.01	C_7 Naphthene (methylcyclohexane)	90	0.022 ±0.01	C_7 Naphthene (methylcyclohexane)	90
		Paraffins	10		Paraffins	10
Total	0.187 ±0.03			0.022 ±0.01		

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Characterization of Long-Chain Fatty Acids by Infrared Spectroscopy

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▷ Fatty acids may be readily identified by infrared spectrophotometry of the solid compounds. Examination of the spectra of soaps as well as of the free acids by the potassium bromide disk technique has definite advantages, chief among which is the revelation of the "rule of two" relationship between the so-called band progressions and chain lengths. Infrared spectra of synthetic solid fatty acids of exceptionally high purity from C_{10} to C_{36} are presented, as well as the band progressions for soaps from C_3 to C_{36}, to demonstrate this relationship and provide a catalog of spectra for direct identification.

The effective use of infrared spectroscopy for positive identification of the fatty acids has been subject to several difficulties. The liquid state offers few unique bands. In the solid state the spectra contain many bands, but slight differences in preparatory techniques result in spectral discrepancies (*6*). Gore and Waight (*4*) have distinguished the shorter acids by measurement of the ratio of intensities of C—H stretching bands due to CH_2 and CH_3 groups, but this technique becomes impractical for C_{18} and longer chains because of the smaller difference between homologs.

In 1952 Jones, McKay, and Sinclair

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(*5*, *9*) showed that the fatty acids had unique infrared spectra as Nujol mulls, and could be qualitatively distinguished from each other. They also called attention to a remarkable spectral feature in the 8-micron region: a series of uniformly spaced bands whose number increased with chain length. The term "band progression" was applied to such a series of bands. Brown, Sheppard, and Simpson (*1*, *2*) showed that these bands are attributable to CH_2 wagging.

Findings in this laboratory indicate that comparison of the spectra of crystalline films may possess certain drawbacks. Because of the tendency of fatty acids to occur in several polymorphic forms and the frequent coexistence of two or more of these forms in a single film, different film preparations of the same acid may show spectral discrepancies if the solidified capillary film technique is used. A second difficulty is caused by orientation of the solid film and partial polarization of the radiation within a spectrophotometer. In this case significant intensity changes in several bands can be observed merely by repositioning a single film preparation within a cell holder. Such spectral differences as may occur raise doubt about the validity of an identification. The present authors have, therefore, sought to overcome these difficulties by adopting a reproducible sample-handling technique, and basing identification not merely on the ability to obtain an identical spectrum, but on the correlation of a prominent spectral feature with the chain length.

Correlation of chain length with the number of bands in the band progression series of solid mulls of fatty acids was first made by Jones and his coworkers (*5*, *9*). They recognized three bands in lauric acid (C_{12}), nine bands in heneicosanoic acid (C_{21}), and generalized for the series C_{16} to C_{21} that the number of apparent progression bands increases by one for each additional methylene group. Primas and Günthard (*7*) have shown that the number of bands due to CH_2 wagging in compounds of the type $R'\text{-}CO(CH_2CH_2)_nCOR''$ bears an approximate relationship of one band for each four consecutive methylene groups. In this laboratory, it has been observed for fatty acid chains longer than twelve carbons that one band is, in fact, present for every two carbons in the chain. The band progression region of the soaps may be defined as 7.43 to 8.47 microns (Figure 4), and the bands crowd together when they become more numerous.

Childers and Struthers (*3*) have prepared the sodium soaps of some short-chain fatty acids. The spectra had sharp, unique peaks, and the preparation of the soaps opened the way to reproducible spectra. The samples were prepared as quantitative mulls, and

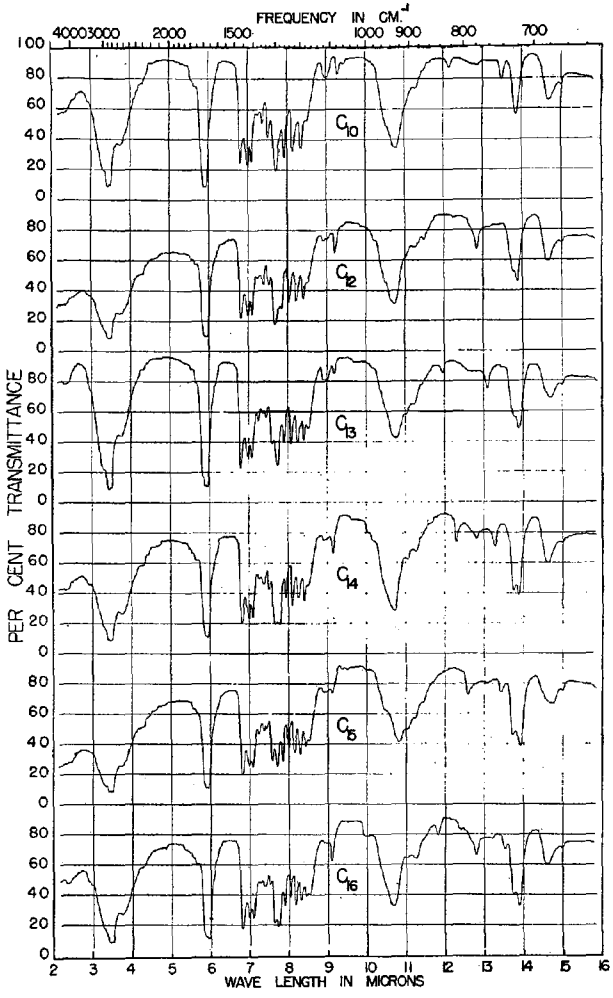

Figure 1. Spectra of normal aliphatic acids in potassium bromide matrix

0.01 mmole of fatty acid in 12-mm. diameter circular disk

both qualitative and quantitative determinations were shown to be practicable.

The sodium, barium, and silver soaps have been prepared in this laboratory, and excellent spectra have been obtained for their potassium bromide disks. More of the progression bands are apparent for a soap than for the corresponding acid, and this is due to the removal of an interfering peak at 7.65 microns attributable to the C—O—H grouping. The progression bands of different soaps of the same acid show no alteration in position.

The work reported here proposes that the spectrum of an unknown free acid be obtained by the potassium bromide pellet technique, and then compared with a reference spectrum for identification. If doubt as to the identity persists, the soap should be prepared, mixed into a potassium bromide disk, and the progression bands in the spectrum counted. An acid with an odd number of carbons may be distinguished from the next longer even-numbered acid which has the same number of progression bands by the shift in wave length of the entire set. Comparison of the progression bands enables identification of a straight-chain fatty acid even if a reference spectrum is not available. Branched acids give similar bands which are nonuniformly spaced, so that the straight and branched types may be readily distinguished. A group of spec-

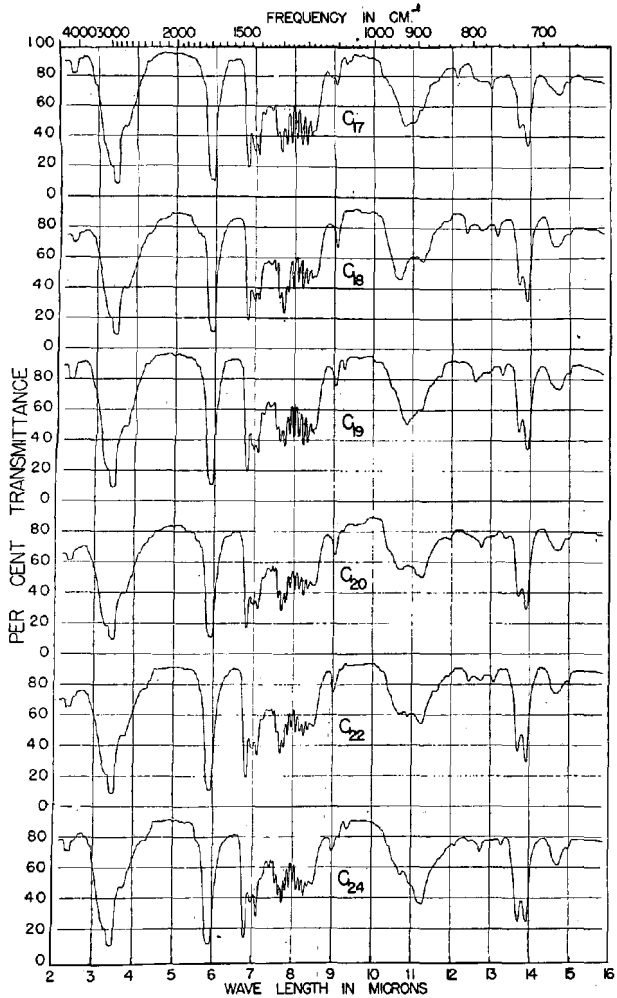

Figure 2. Spectra of normal aliphatic acids in potassium bromide matrix

0.01 mmole of fatty acid in 12-mm. diameter circular disk

tra of normal aliphatic acids from C_{10} to C_{36} is presented for reference in Figures 1, 2, and 3.

MATERIALS

The C_{10} to C_{36} normal aliphatic acids were prepared synthetically by Schuette and coworkers (*8*) at the University of Wisconsin. The methods and intermediates used in the syntheses were selected so as to leave no doubt about the purity and structure. Estimated purity was 99.9% or better, as established by melting point data and cooling curve observations. The shorter acids, C_3, C_4, and C_8, used only in the form of soaps to extend the family of progression bands, were generally of lower purity. Barium chloride dihydrate, silver nitrate, and anhydrous methanol used in the preparation of the soaps were all of reagent grade.

PROCEDURES

Preparation of Potassium Bromide Pellets. Freeze-dried potassium bromide (200 mg.) was mixed with 0.01 mmole of acid or soap. A small amount of carbon tetrachloride was added to the powders in an agate mortar and triturated to form a smooth paste. After evaporation of the carbon tetrachloride in 1 minute, trituration was resumed for 1 minute longer. The powder was pressed in a hardened tool steel die under a force of 10 tons for 2 minutes in vacuo to form a flat disk about 1 mm. thick and 12 mm. in diameter.

Preparation of Soaps. BARIUM. Several milligrams of the free acid was dissolved in about 3 ml. of methanol. One milliliter of a 1% solution of barium chloride dihydrate in methanol was added. Concentrated aqueous ammonia (28%) then was added dropwise to the hot methanolic solution until no additional precipitate was formed. The precipitate was centrifuged, washed twice with 3-ml. portions of hot methanol, and dried at 110° C. The barium soaps of the C_8 to C_{36} acids were made.

SODIUM. Aqueous sodium hydroxide, 1*N*, was used to titrate an ethanolic solution of the acid to a phenolphthalein end point. The solution was then evaporated to dryness at 110° C. The sodium soaps of only the C_3, C_4, and C_8 acids were prepared.

SILVER. Silver nitrate (1.7 grams) was dissolved in 5 ml. of distilled water, and 80 ml. of absolute ethyl alcohol was added. Concentrated aqueous ammonia was added dropwise until the brown turbidity which first formed just disappeared, and the solution was made up to 100 ml. with ethyl alcohol. The silver solution was added dropwise to the hot methanolic solution of the acid until no additional precipitate formed. The white precipitate was centrifuged, washed several times with hot methanol, and dried in a vacuum oven at 60° C. The silver soaps of the C_8 to C_{20} acids were made.

APPARATUS

The spectra shown were obtained by use of the double-beam Baird Associates Model B infrared spectrophotometer. The reference used in all cases was a polished rock salt plate. All spectra reproduced herein are the originals. Figure 4 is a montage of actual curves obtained by use of a calcium fluoride prism, but all other work was accomplished with a sodium chloride prism.

RESULTS AND DISCUSSION

The spectra presented in Figures 1, 2, and 3, obtained from potassium bromide disks, show greater detail than spectra usually afforded by solid films. An important reason for this difference is the minimization of polymorphism in the former. In addition, instrumental polarization of the infrared radiation can cause nonreproducible spectral variations when the specimen exhibits overall crystallographic orientation, as may frequently happen in solid films. The more nearly random orientation of sample in the potassium bromide disk minimizes the orientation-polarization effect. This technique, therefore, has the advantages of yielding sharper and more reproducible spectra and is recommended over the solid film technique for those fatty acids obtained as crystals.

The 7.4- to 8.5-micron region deserves special attention. The number of bands

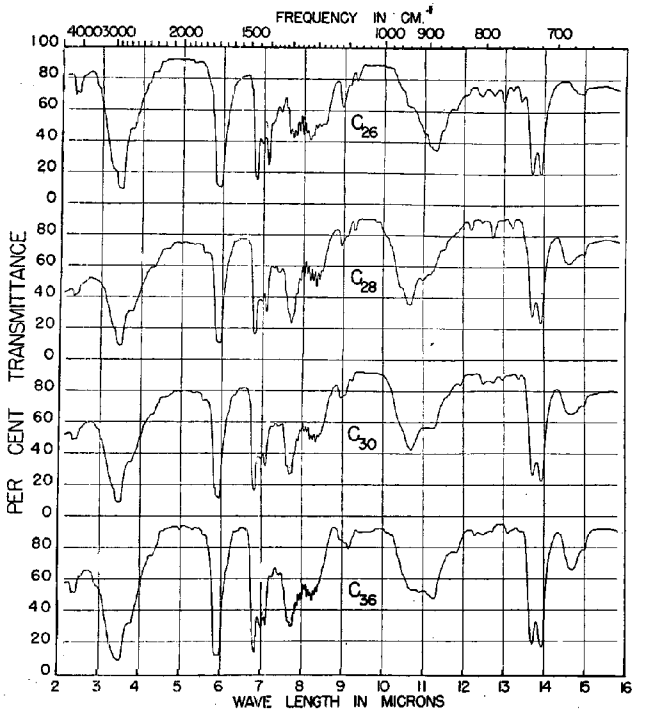

Figure 3. Spectra of normal aliphatic acids in potassium bromide matrix

0.01 mmole of fatty acid in 12-mm. diameter circular disk

in the progression series is approximately equal to half the number of carbons in the chains. By empirically defining the region as 7.43 to 8.47 microns, the following relationship exists for the straight-chain fatty acids with an even number of carbons,

$$\text{Number of bands in progression series} = \frac{\text{Number of carbons in chain}}{2}$$

For the acids with an odd number of carbons,

$$\text{Number of bands in progression series} = \frac{\text{Number of carbons in chain} + 1}{2}$$

This rule of two relationship is obvious from the spectra of the solid acids from C_{10} through C_{26}, but in the longer acids only the bands at the long wave length end of this region remain distinct. That the shoulders on the broad band at 7.65 microns are really members of the band progressions can be brought out more clearly by preparation of soaps of the acids. In this way, the band at 7.65 microns is eliminated, and the rest of the progression bands are unmasked.

The patterns exhibited by the progression bands are shown in Figures 4 and 5. Their continuity in the spectra of the soaps from C_3 to C_{36} is most striking. The family of curves obtained from the free acids is not identical with, although it is similar to, the family of curves obtained from the soaps, revealing an influence on all of the bands by the end group. Close examination of the band progressions of the soaps as given in Figure 5 reveals the following anomalies.

In the soaps below C_{16}, weaker intermediate bands can be seen which are not regularly spaced. Their intensities gradually become relatively negligible in chains longer than C_{12}. To use the rule of two, these intermediate bands must be disregarded. Their existence in the shorter acids indicates that one band is probably produced for each methylene group, but that limitations on resolution allow detection of only half of them in chains longer than C_{16}.

The band at the shortest wave length is always closer to the second band than the spacing of the remainder of the progression bands in each set. This effect may be related to the extraordinary

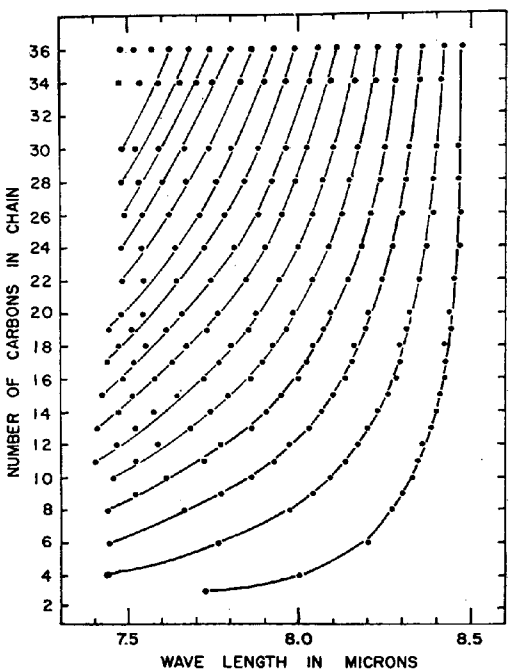

Figure 4. Family of curves relating progression bands of soaps

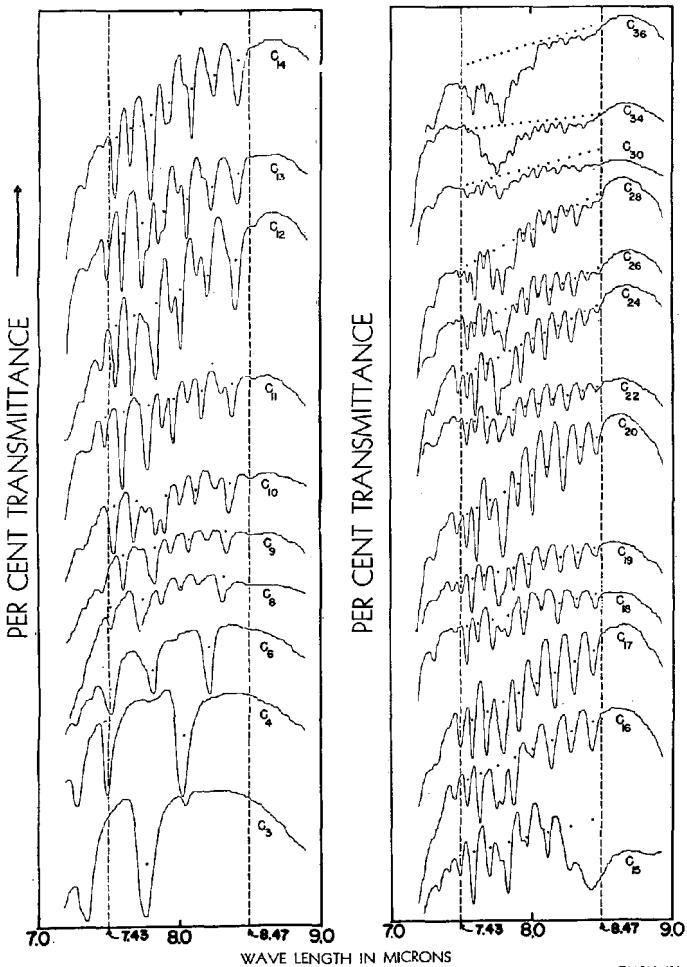

Figure 5. Band progressions of soaps of fatty acids

All from barium soaps except for C_3, C_4, C_6, for which sodium soaps were used

chemical reactivity of the α-methylene group, which would be concomitant with a weaker C—H bond and/or a greater C—H distance, causing a methylene wagging slower than otherwise would be expected.

The rule has utility even if used solely on an empirical basis, provided the above-mentioned disparities are taken into account.

It may be difficult to decide, solely on the basis of the spectrum of a mixture, which component acids are present unless other information is available. More than one combination of mixed fatty acids might give nearly indistinguishable composite spectra. In such cases, the mixture of acids may be converted to a mixture of soaps, and the spectrum obtained. The irregular series of progression bands resulting then may be visually unscrambled into sets of uniformly spaced bands. The separated sets can be used to help identify the components. Verification is made by comparison of the remainder of the spectrum with reference spectra. Figure 6 shows two specific examples of this application.

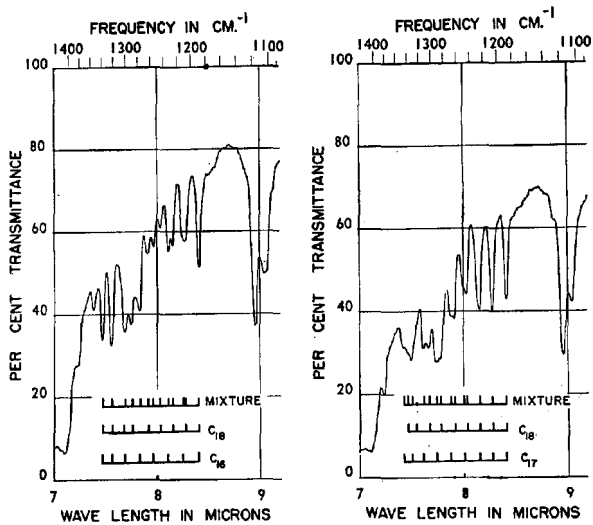

Figure 6. Identification of half-and-half mixture of soaps by separation into uniformly spaced progression bands

CONCLUSIONS

The use of the potassium bromide disk technique, in which melting of the crystallized fatty acid is avoided, results in sharper, more reproducible spectra. If an unknown fatty acid is not crystalline or is somewhat impure, preparation of the soap will still permit rapid evaluation of the chain length if progression bands are counted and the rule of two is applied. Even in half-and-half mixtures, the components can be identified by a visual unscrambling of the two sets of uniformly spaced bands.

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Infrared Absorption of Aldehydic C=H Group

Ortho-Substituted Benzaldehydes

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▷ Sixteen benzaldehydes, ten of which were substituted in the ortho position, were measured in the aldehydic C—H region in order to test the assumption that the increase in the C—H frequencies of benzaldehydes containing a proton acceptor in the ortho position is due to hydrogen bonding. All these aldehydes obey the observed regularities and fall into two groups. The first, containing unbonded benzaldehydes, absorbs at 2720 to 2745 and 2812 to 2832 cm.$^{-1}$. The second, containing hydrogen-bonded aldehydic C—H groups, absorbs at 2747 to 2765 and 2860 to 2900 cm.$^{-1}$. The origin of the second band is discussed. The results verify the assumption that the cause of the rise in the C—H frequencies is a hydrogen bond affecting the aldehydic C—H group. Thus a clear-cut differentiation between an ortho-substituted and a nonsubstituted benzaldehyde is possible in many cases.

ALTHOUGH the C—H stretching frequency of the formyl group in various substituted benzaldehydes is usually in the narrow region of 2720 to 2740 cm.$^{-1}$, in the case of a number of ortho-substituted benzaldehydes it rises to about 2760 cm.$^{-1}$ (*21*). The other characteristic band for the formyl group at about 2820 cm.$^{-1}$ also rises in these benzaldehydes to the region of 2860 to 2890 cm.$^{-1}$. As this increase in frequency could be correlated with neither the electronic effects of the ortho substituents nor their steric effects and because this increase was observed only with benzaldehydes that were substituted by a proton acceptor but not with *o*-tolualdehyde, it was tentatively assumed to be due to some kind of hydrogen bonding between the polar hydrogen atom of the formyl group and the substituting proton acceptor. As such a hydrogen bond involving a C—H group seemed somewhat uncommon and the number of the "anomalous" benzaldehydes already studied then was small (only five) it seemed worth while to study the infrared spectra in this region of more benzaldehydes, especially ortho-substituted ones, in order to find whether this assumption is consistent with more experimental data. It was also interesting to see if a frequency of about 2760 cm.$^{-1}$ for the formyl C—H stretching can really be taken as a reliable proof of the presence of a proton-accepting atom or group in an ortho position, in an unknown substituted benzaldehyde. An additional number of benzaldehydes were therefore measured in the infrared region.

EXPERIMENTAL

Most of the measurements were made with a Perkin-Elmer infrared spectrometer, Model 12 C. Some of them were made with a direct current Perkin-Elmer spectrometer, Model 12 B. Unless otherwise stated, measurements were carried out with a sodium chloride prism. The materials studied were commercial of the highest available purity, used without further purification, were obtained as a gift from various organic chemists, or were synthesized according to the literature.

2-Chloro-3- and 2-chloro-5-hydroxybenzaldehyde were synthesized by (quick) chlorination of *m*-hydroxybenzaldehyde in acetic acid (which contained some water). Under these conditions, contrary to Lock and Hosaeus (*17*), both isomers are formed, the latter in larger quantities. They were separated by dissolving the mixture in a sodium carbonate solution and precipitating with hydrochloric acid. 2-Chloro-3-hydroxybenzaldehyde came out of the solution first and melted after crystallization from dilute acetic acid, at 136° C. According to Hodgson and Beard (*11*) it melts at 139°. 2-Chloro-5-hydroxybenzaldehyde (6-chloro-*m*-hydroxybenzaldehyde) was then precipitated from the filtrate on addition of more acid and standing. After crystallization from dilute acetic acid it melted at 113.5–114.5°. Hodgson and Beard (*11*) give 111° as its melting point.

2-Nitro-5-fluorobenzaldehyde was synthesized by José Schwarcz (*24*).

RESULTS

The results of the measurements of the infrared absorption in the formylic C—H region in the case of 16 benzaldehydes (ten of which can be described as ortho-substituted) are summarized in Table I.

Apart from the measurements in this region, some materials were also studied in the whole 2.8- to 12-micron region, in order to detect possible irregularities in the spectra of the anomalous benzaldehydes. The main bands observed are presented in Table II. Experimental conditions are the same as those of Table I.

The results for salicylaldehyde are in good agreement with the values read from the curve given by Barnes and associates (*5*) for the 1050 to 1800-cm.$^{-1}$ region and the value of 1666 cm.$^{-1}$ found for its C=O frequency in carbon tetrachloride solution agrees well with the value of 1661 cm.$^{-1}$ given by Bellamy (*6*) for this frequency in chloroform solution. The value of 1698 cm.$^{-1}$ for this frequency in 2-naphthaldehyde also is in accord with the value of 1702 cm.$^{-1}$ given by Hunsberger (*12*). Both 3-chlorobenzaldehyde and its 4-isomer have been measured by Lecomte (*16*) in the region up to 1200 cm.$^{-1}$; the results are comparable.

DISCUSSION

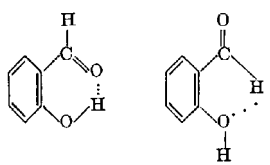
2730-Cm.$^{-1}$ Frequency. As can be seen from Table I, all the benzaldehydes in which no hydrogen bond that

Table I. Aldehydic C—H Frequencies in Various Benzaldehydes

Material	Concentration	Cell Thickness, Mm.	Frequencies[a], Cm.$^{-1}$	
o-Bromobenzaldehyde[b]	0.007 g./1 cc. CCl_4	8	2747 (s)	2860 (vs)
Salicylaldehyde	0.040 g. + 1 cc. CCl_4	0.2	2745 (9)	2830 (22)
Salicylaldehyde[c]	0.002 g./1 cc. CCl_4	8	2744 (s)	2832 (s) [2562 (s), 2610, 2683 (vw), 2778 (vw)]
Phthalaldehyde	0.11 g. + 1 cc. $CHCl_3$	0.1	2740 (23)	2830 (24)
2-Chloro-3-hydroxybenzaldehyde[c]	Satd. soln. in CCl_4[d]	8	2750	2860 (s) [2628, 2716 (w), 2823]
2-Chloro-3-hydroxybenzaldehyde	0.011 g. + 1 cc. $CHCl_3$	1.4	2760	2890 (s)
2-Chloro-5-hydroxybenzaldehyde[c]	Satd. soln. in CCl_4[d]	8	2752	2866 (s) [2823 (vw)]
2-Chloro-5-hydroxybenzaldehyde	0.011 g. + 1 cc. $CHCl_3$	1.4	2760	2900
2,4-Dichlorobenzaldehyde	0.05 g. + 1 cc. CCl_4	0.2	2765 (13)	2875 (26)
	0.01 g. + 1 cc. CCl_4	1.4	2765 (10)	2875 (23)
2,4-Dihydroxybenzaldehyde	0.006 g. + 1 cc. $CHCl_3$	1.4	2765 (34)[e]	
2-Nitro-5-fluorobenzaldehyde	0.08 g. + 1 cc. $CHCl_3$	0.1	2755 (13)	2890 (31) [2650 (12)]
2-Nitro-5-bromobenzaldehyde	0.21 g. + 1 cc. $CHCl_3$	0.2	2750 (4)	2890 (9)
1-Naphthaldehyde	0.046 g. + 1 cc. CCl_4	0.1	2730 (34)	2830 (20)
2-Naphthaldehyde	0.10 g. + 1 cc. CCl_4	0.1	2720 (33)	2815 (36)
3-Chlorobenzaldehyde	0.09 g. + 1 cc. CCl_4	0.1	2720 (24)	2810 (32)
3-Bromobenzaldehyde	0.11 g. + 1 cc. CCl_4	0.1	2720 (31)	2820 (40)
3-Iodobenzaldehyde	0.039 g. + 1 cc. CCl_4	0.2	2720 (25)	2820 (31)
3-Hydroxybenzaldehyde	0.015 g. + 1 cc. $CHCl_3$	1.4	2730 (22)	2820 (34)
3-Hydroxybenzaldehyde[b]	Satd. soln. in CCl_4[f]	8	2723	2812
4-Chlorobenzaldehyde	0.032 g. + 1 cc. $CHCl_3$	0.2	2735 (27)	2830 (38)

[a] Molar absorbancy index, liter mole^{-1} cm.$^{-1}$, given in parentheses after each frequency where available.
[b] Measured with fluorite prism.
[c] Measured with lithium fluoride prism.
[d] Concentration <0.005 g./cc.
[e] 2890-cm.$^{-1}$ band probably masked by nearby chloroform band.
[f] Concentration less than 0.002 g./cc.

involves the formylic hydrogen atom can exist show their first aldehydic C—H frequency in the usual region of 2720 to 2745 cm.$^{-1}$ In this respect salicylaldehyde should be considered as a "normal" benzaldehyde, as the hydrogen bond between the more acidic O—H group and the stronger proton-accepting group C=O (bigger partial negative charge) is no doubt more powerful than that between the formyl C—H group and the OH acceptor. Hence structure I rather than II represents the true configuration of salicylaldehyde (*2*).



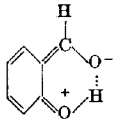
I II

In this configuration the C—H bond is unaffected by hydrogen bonding. The case of 2-hydroxy-3-methoxybenzaldehyde, which also absorbs at about 2740 cm.$^{-1}$ (*21*), is analogous. The somewhat higher frequency of these aldehydes relative to other unbonded benzaldehydes—e.g., 3-hydroxybenzaldehyde, 2730 cm.$^{-1}$; 1-naphthaldehyde, 2730 cm.$^{-1}$—seems to be due to polar resonating structures like III,

Table II. Infrared Absorption of Various Benzaldehydes

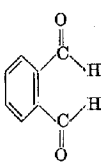
Material	Bands[a], Cm.$^{-1}$
Salicylaldehyde	885(0.42), 1029(0.11), 1150 (0.38), 1181 (0.25), 1199 (0.55), 1228(0.42), 1283(0.92), 1320(0.19), 1340(0.15), 1382(0.32), 1462(0.46), 1477(0.43), 1583(0.38), 1621(0.52), 1666(1.04), 2745(0.06), 2830(0.14), 3150(0.13)
Phthalaldehyde	859(0.56), 970(0.17), 1188(>0.8), 1272(0.44), 1305(0.23), 1389(0.10), 1455(0.16), 1489(0.14), 1576(0.43), 1594(0.50), 1688(>1.5), 2740(0.17), 2830(0.18), 3030(0.11)
2,4-Dichlorobenzaldehyde	844(0.77), 872(0.36), 1052(0.28), 1103(0.34), 1197(0.48), 1250(0.32), 1283(0.29), 1377(0.52), 1466(0.30), 1584(0.90), 1697(1.02), 2765(0.07), 2875(0.15), 3100(0.09)
2,4-Dihydroxybenzaldehyde	975(0.41), 1127(1.1), 1330(1.3), 1390(0.52), 1461(0.75), 1631(>2.0), 2765(0.20)[b]
2-Nitro-5-fluorobenzaldehyde[c]	845, 892(0.56), 967(0.23), 1074 (0.06), 1133(0.08), 1151(0.41), 1253(0.08), 1484(0.6), 1530(>2.0), 1602(1.01), 1701(>1.5), 2650(0.055), 2755(0.06), 2890(0.135)
2-Naphthaldehyde	860(0.43), 884(0.16), 897(0.17), 952(0.11), 970(0.09), 1118(0.47), 1171(0.50), 1203(0.15), 1262(0.27), 1341(0.31), 1400(0.08), 1463(0.24), 1576(0.12), 1599(0.20), 1627(0.38), 1698(1.08), 2720(0.19), 2815(0.21), 3020(0.16)
3-Chlorobenzaldehyde	871(0.20), 900(0.20), 1070(0.14), 1196(0.82), 1279(0.14), 1383(0.22), 1438(0.22), 1470(0.20), 1573(0.30), 1593(0.27), 1705(1.05), 2720(0.14), 2810(0.19), 3080(0.07)
3-Bromobenzaldehyde	852(0.19), 880(0.17), 900(0.06), 1068(0.20), 1192(0.9), 1272(0.30), 1381(0.26), 1430(0.23), 1468(0.14), 1573(0.45), 1705(1.3), 2720(0.17), 2820(0.215), 3080(0.10)
3-Iodobenzaldehyde	841(0.12), 867(0.08), 888(0.06), 1001(0.06), 1058(0.10), 1168(0.10), 1190(0.65), 1380(0.14), 1430(0.10), 1464(0.06), 1484(0.06), 1572(0.23), 1593(0.07), 1710(0.77), 2720(0.08), 2820(0.10), 3075(0.03)
4-Chlorobenzaldehyde	833(0.86), 954(0.07), 1016(0.21), 1046(0.07), 1095(0.51), 1167(0.27), 1283(0.18), 1297(0.21), 1315(0.12), 1389(0.21), 1421(0.08), 1460(0.12), 1489(0.20), 1576(0.50), 1592(0.68), 1704(1.1), 2600(0.11), 2735(0.12), 2830(0.17)

[a] Absorbances (not molar absorbancy indexes) added in parentheses after each frequency.
[b] OH band is too broad to be measured.
[c] Not measured between 1280 and 1470 cm.

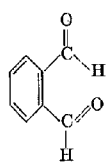


III

which contribute to the actual structure of these aldehydes. Such structures would be expected to strengthen the double bond character of the $C_{ar}-C_{formyl}$ bond, thereby increasing the $C_{formyl}-H$ frequency [CR_1R_2 = CHR_3 absorbs at 3010 to 3040 cm.$^{-1}$, $CHR_1R_2R_3$ at 2890 (*6*, pp. 13, 31)]. Phthalaldehyde also must be considered as an unbonded benzaldehyde, since as Braude and Sondheimer have shown (*7*), even in the case of *o*-tolualdehyde some steric overlap exists between the carbonyl group and the methyl group in that conformation in which these groups are neighbors one to the other. An appreciable steric interaction would therefore be expected, even in that conformation in which only one of the oxygen atoms is the neighbor of the other formylic group. The increase in steric interaction in this case (relative to that in *o*-tolualdehyde) would be expected on the basis of the planarity of the aldehydic group and the polarity of the $C_{formyl}-H$ bond (*26*), which is probably reflected in a somewhat bigger C—H distance. As the C—H. . .O=C hydrogen bond is a weak one, it is probably not strong enough to overcome this steric hindrance and IV rather than V must be taken as the structure of phthalaldehyde.



IV



V

This structure leaves the formyl groups unbonded. The value of 2740 cm.$^{-1}$ for phthalaldehyde is therefore in good agreement with what could be expected, assuming that the cause of the increase in the C—H frequencies in the case of the anomalous benzaldehydes is a hydrogen bond affecting their formylic C—H groups. If it is, however, assumed that the cause is electronic in nature, it is difficult to see why phthalaldehyde, which is fully analogous to *o*-nitrobenzaldehyde, absorbs normally while the latter does not (*21*). The very small rise in the phthalaldehyde frequency, which is still well inside the region of the unbonded benzaldehydes, seems to be due to the mutual negative effect of the formyl groups which tends to suppress the polarization of the carbonyl groups, thereby strengthening the C—H bond.

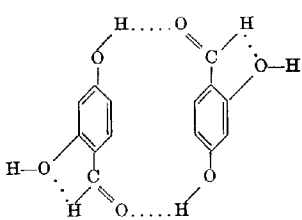
It can further be seen from Table I that in all the benzaldehydes in which a hydrogen bond involving the C_{formyl}—H bond can exist, this C—H frequency rises to the region of 2747 to 2765 cm.$^{-1}$, as could be expected on the basis of the above assumption and in good agreement with previous work (*21*). Thus there exist two near, still definitely separate, regions for bonded and nonbonded C—H frequencies.

The case of *o*-bromobenzaldehyde is of special interest, since if it is assumed that steric effects are the cause of the rise in the frequency of the anomalous benzaldehydes, *o*-bromobenzaldehyde should show this effect even more clearly than *o*-chlorobenzaldehyde because of the greater volume of the bromine atom. Still *o*-bromobenzaldehyde shows the lowest frequency of all the anomalous benzaldehydes (2747 cm.$^{-1}$; calcium fluoride prism) while *o*-chlorobenzaldehyde showed this band at about 2760 cm.$^{-1}$ when measured with a sodium chloride prism (*21*) and at 2752 cm.$^{-1}$ when measured with a lithium fluoride prism (see below). This fact is, however, understandable on the basis of the hydrogen bond formation assumption, because although the (O—H. . .Br) hydrogen bond in *o*-bromophenol is slightly stronger than that of *o*-chlorophenol (*20*), the effect of the CH. . .Br bond on the $C_{formyl}-H$ frequency would be expected to be weaker than that of the CH. . .Cl bond in the case of ortho-substituted benzaldehydes. The reason for this expectation is that the C_{ar}—Br distance is greater than that of the C_{ar}—Cl and therefore the angle formed by the —H. . .Br hydrogen bond with the C—H valency direction is here less acute than the C—H. . .Cl angle [the hydrogen bond in *o*-halogenobenzaldehydes is directed not to the center of the halogen atom but to a point nearer to the aromatic ring; hence these angles are acute (*21*)]. As a result, the effect of the CH. . .Br hydrogen bond is weaker (*21*).

Thus neither the steric nor the electronic effect of the ortho substituent can cause the increase of the C—H frequency in the anomalous benzaldehydes and only the hydrogen bond assumption is in accordance with observed facts. This assumption was also partially verified by the failure of *o*-nitrobenzaldehyde to color red a nonpolar solution of rhodamine B, as do all hydrogen donors, including the corresponding meta and para isomers (*19*). This fact was attributed to the nonavailability in the ortho isomer of bonding hydrogen atoms because of chelation between the formylic hydrogen atom and the neighboring nitro group (*19*). Additional evidence of the existence of hydrogen bonds involving the aldehydic hydrogen atom, of both inter- and intra-molecular character, in aldehydes in general and particularly in nitrobenzaldehydes was supplied recently from refractive-index measurements (*3*). There is a similar case, of intramolecular hydrogen bonding of a C—H group, in cyclo-octanone and cyclodecanone (*23*). The existence of an internal hydrogen bond in the anomalous benzaldehydes seems thus to be well substantiated.

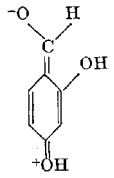
The value of about 2750 cm.$^{-1}$ for the C—H frequency of 2-chloro-3-hydroxybenzaldehyde is worth mentioning because in this case, as in all the *o*-halogenophenols (*23*), another hydrogen bond probably exists, between the phenolic OH group and the chlorine atom. However, the increase in C—H frequency because of this ortho substituent is not changed materially, as can be judged from comparison of this value with that of *o*-chlorobenzaldehyde (2752 cm.$^{-1}$). This is not surprising, as other such instances are known, where a proton acceptor forms two hydrogen bonds. Thus, in the dimer of salicylic acid one oxygen atom accepts two protons to form two hydrogen bonds (*20*). The cases of 1,8-dihydroxyanthraquinone and 2,2′-dihydroxybenzophenone (*20*) are similar.

The value of 2765 cm.$^{-1}$ for 2,4-dihydroxybenzaldehyde, in contradistinction to the value of 2744 cm.$^{-1}$ for salicylaldehyde, shows that here the formylic CH group is no longer free, but is also involved in a hydrogen bond, probably in a dimeric structure such as VI:



VI

This conclusion is supported by the fact that while the C=O group of salicylaldehyde absorbs at 1666 cm.$^{-1}$, that of 2,4-dihydroxybenzaldehyde absorbs at 1631 cm.$^{-1}$. If here also a structure similar to III is the dominant polar structure, there is no reason why this aldehyde should absorb at so much lower than salicylaldehyde. It must be assumed therefore that the actual structure of 2,4-dihydroxybenzaldehyde has an appreciable contribution from a polar form, in which the positive charge is on the 4-hydroxy group, such as VII.



VII

Such a structure would be expected to stabilize by dimerization to VI with the added energy of stabilization obtained from the two CH. . .O hydrogen bonds formed by this dimerization. The fact that this aldehyde melts at 135° C., while phenol melts at 41° C. and salicylaldehyde melts at −7° C., also seems to indicate that it tends to form stable dimers.

The molar absorbancy index of the first C—H band is usually smaller in the bonded benzaldehydes than in the unbonded benzaldehydes (Table I). Thus, all the values for this constant in the bonded aldehydes measured so far fall in the region of 4 to 34 liter mole^{-1} cm.$^{-1}$, with all but one (8) falling in the narrower region of 4 to 15 liter mole^{-1} cm.$^{-1}$; while the values for the nonbonded benzaldehydes fall in the region of 9 to 40 liter mole^{-1} cm.$^{-1}$, with all but four (*14*) falling in the region of 15 to 40 liter mole^{-1} cm.$^{-1}$. The same tendency can also be traced in the results for the second C—H frequency, at about 2820 cm.$^{-1}$, but here the effect is less marked and the values are more scattered. This phenomenon is not clear, but might be connected with a reduction of the partial positive charge on the hydrogen atom as a result of the hydrogen bond directed at an acute angle to the C—H direction.

Finally, the results obtained for a dilute solution of 2,4-dichlorobenzaldehyde show that, taking into account the uncertainty in the thickness of the individual cells (up to about 20%) and the experimental error, the C—H band frequency and intensity of an internally bonded benzaldehyde remain more or less constant on dilution, as is to be expected.

2820-Cm.$^{-1}$ Frequency. Wherever the 2730-cm.$^{-1}$ band rises, there is also a parallel rise in the 2820-cm.$^{-1}$ band to about 2860 cm.$^{-1}$ (*21*). This can be seen in Table I. In fact, this band is even more sensitive to hydrogen bonding than the 2730-cm.$^{-1}$ band, as is apparent from the case of *o*-bromobenzaldehyde, where the 2730-cm.$^{-1}$ band is only about 17 cm.$^{-1}$ higher than usual, while the 2820-cm.$^{-1}$ band is about 40 cm.$^{-1}$ higher.

The discrepancy between the values of this frequency in the cases of 2-chloro-3- and 2-chloro-5-hydroxybenzaldehyde as found in carbon tetrachloride solution (2860, 2866 cm.$^{-1}$) and those found in chloroform solution (2890, 2900 cm.$^{-1}$) seems to be due, at least in part, to the poor resolving power in this region of the sodium chloride prism (which was used for the measurements in the chloroform solution), coupled with the effect of the very strong nearby band of chloroform itself.

This 2820-cm.$^{-1}$ band was assigned (*21*), in analogy to Thompson and Harris' assignment of a similar band of acetaldehyde (*25*), to the overtone of the C_{formyl}—H bending frequency (in acetaldehyde vapor 1405 cm.$^{-1}$). As can be seen from Table II, in benzaldehydes this band appears steadily at about 1380 to 1390 cm.$^{-1}$ This region is also in agreement with the results of Kohlrausch and Koeppl (*15*), who found a Raman band at about 1390 cm.$^{-1}$ in the spectra of all the aldehydes they measured. If the above assignment is correct, this band should therefore appear at about 2770 cm.$^{-1}$, and not 2820 cm.$^{-1}$ as it does in the case of the benzaldehydes. Even if it is assumed that the original C—H stretching frequency has here a value of about 2770 cm.$^{-1}$, bringing about a Fermi resonance which "kicks apart" the two frequencies to 2730 and 2820 cm.$^{-1}$, it is still difficult to explain the behavior of the bonded benzaldehydes with this assignment. This behavior is strange, because as can be seen from Table II the 1380 to 1390-cm.$^{-1}$ band is not affected appreciably by the hydrogen bond—e.g., 4-chlorobenzaldehyde, 1389 cm.$^{-1}$; 2,4-dihydroxybenzaldehyde, 1390 cm.$^{-1}$—and its overtone should therefore occur in the bonded aldehydes at the same frequency—i.e., at about 2770 cm.$^{-1}$. Hence either the two frequencies of 2730 and 2820 cm.$^{-1}$ should remain, here also, normal (if there is no increase in the original C—H frequency in the bonded benzaldehydes), or else (if this frequency is somewhat higher, there is definitely no possibility that such a weak hydrogen bond would cause a big shift of the C—H stretching frequency) they should cease to interact appreciably and should absorb in the neighborhood of 2760 and 2830 cm.$^{-1}$ but not as high as found (about 2870 cm.$^{-1}$).

It seems therefore more reasonable to assume that the 2820-cm.$^{-1}$ band is a combination band formed by the 1380- to 1390-cm.$^{-1}$ frequency and the frequency which appears steadily at about 1455 to 1470-cm.$^{-1}$ in the spectra of all the aldehydes of Table II and many aromatic compounds (*13*). This latter band is due to some ring vibration (*14*). If these two frequencies are added together, the values given in Table III are obtained.

Thus if a usual, small negative unharmonicity term is applied, the calculated values of the 2820-cm.$^{-1}$ frequency agree well with the observed values except for the bonded aldehyde (2,4-dichlorobenzaldehyde). It may be assumed, however, that in the bonded aldehydes the original frequency of about 2730 cm.$^{-1}$ rises to about 2800 cm.$^{-1}$ and then interacts with the combination band at about 2820 cm.$^{-1}$ (Fermi resonance). This interaction would bring about a kicking apart of the original frequencies to the vicinity of 2760 and 2860 cm.$^{-1}$, respectively, as found.

The 2820-cm.$^{-1}$ band probably also incorporates in various cases other combination bands which add to its intensity. In 3-chlorobenzaldehyde, for example, these may be 1383 + 1438 = 2821 cm.$^{-1}$; 1573 + 1279 = 2852 cm.$^{-1}$.

The band at 2800 cm.$^{-1}$ in acetaldehyde observed by Thompson and Harris (*25*) can also be attributed to a combination of the 1440- and 1370-cm.$^{-1}$ frequencies.

In a recent paper Eggers and Lingren (*10*) report the measurements of light and monodeuterated benzaldehyde, *p*-chlorobenzaldehyde, piperonaldehyde, and butyraldehyde. In the deuterated aldehydes the 2730- and 2820-cm.$^{-1}$ frequencies [their somewhat lower values for these bands in light aldehydes

Table III. Sum of 1380- and 1460-Cm.$^{-1}$ Frequencies in Different Aldehydes

Material	Sum, Cm.$^{-1}$	Frequency Observed, Cm.$^{-1}$
Salicylaldehyde	2844	2830
Phthalaldehyde	2844	2830
2,4-Dichlorobenzaldehyde	2843	2870
2,4-Dihydroxybenzaldehyde	2851	...
2-Naphthaldehyde	2804, 2863[a] (2834)	2815
3-Chlorobenzaldehyde	2853	2810
3-Bromobenzaldehyde	2849	2820
3-Iodobenzaldehyde	2844	2820
4-Chlorobenzaldehyde	2849	2830

[a] There are two bands here which may be taken as the shifted 1380-cm.$^{-1}$ band: one at 1341, the other at 1400 cm.$^{-1}$ Both of the possible sums are therefore given and a mean value of 2834 cm.$^{-1}$ is probably the best to consider.

seem to be inaccurate (*8, 22,* and Tables I, II, and IV)] disappear and new bands are observed at 2082, 2035, and 1040 (weak?) in benzaldehyde; 2095, 2045 and 1080 (shoulder) in *p*-chlorobenzaldehyde; 2070, 2025, and 1029 (shoulder) in piperonaldehyde and at 2180 (weak), 2060, and 1095 cm.$^{-1}$ in butyraldehyde.

These authors assume that: (1) one of the 2700 to 2800 frequencies is a formylic C—H bending overtone; (2) it is in Fermi resonance with the C—H stretching fundamental; (3) the two frequencies at 2000 to 2100 cm.$^{-1}$ are their analogs in the C—D series; and (4) the 1029 to 1095-cm.$^{-1}$ band is the C—D bending fundamental. Assumptions 1 and 2 were considered above and rejected; assumption 3, although acceptable at first glance, does not seem to be really sound. Because the 2180-cm.$^{-1}$ band in butyraldehyde is weak, almost exactly double the 1095-cm.$^{-1}$ band and removed from the 2060-cm.$^{-1}$ frequency it can be taken as sure that practically no Fermi resonance exists between the two vibrations. Hence the value of the original C—D stretching frequency in butanal is also about 2060 cm.$^{-1}$ The corresponding frequency in light benzaldehyde is 10 to 20 cm.$^{-1}$ higher than in butanal (*21*); the original C—D stretching frequency in benzaldehyde-*d* must therefore be at about 2070 to 2080 cm.$^{-1}$. This means that here again there is no Fermi resonance between the two frequencies (at 2082 and 2035 cm.$^{-1}$), as there is no marked change in the observed C—D stretching frequency. (There is, of course, no possibility that the original frequencies of these bands are both at about 2070 cm.$^{-1}$ because the higher band would then be expected to be much higher then 2082 cm.$^{-1}$).

A similar argument can be advanced in connection with the bands of *p*-chlorobenzaldehyde-d_1 (at 2095 and 2045 cm.$^{-1}$), which should have a still higher C—D stretching frequency, the corresponding C—H frequency being 5 to 10 cm.$^{-1}$ higher than in benzaldehyde (Table I). Such a resonance is excluded in this case also because the C—D bending fundamental appears here at 1080 cm.$^{-1}$, which, if such a resonance existed, should have its resonating overtone above 2160 and not at 2095 cm.$^{-1}$ Piperonaldehyde is similar to *p*-chlorobenzaldehyde; its 2070-cm.$^{-1}$ band must therefore also be due to a nonresonating C—D stretching vibration as the 2095-cm.$^{-1}$ band and not to a resonating vibration which would imply an unusually low value for the original C—D stretching vibration. The band which appears sometimes at 2025 to 2045 cm.$^{-1}$ may be due to some combination frequency such as 1232 + 790 cm.$^{-1}$ in heavy benzaldehyde and 1220 + 880 cm.$^{-1}$ in *p*-chlorobenzaldehyde [these values are given by Eggers and Lingren (*10*) for strong bands of the heavy aldehydes].

These authors also questioned the previous tentative assignment of the 1300-cm.$^{-1}$ band to an out of the plane bending of the C—H group (*21*), on the ground that many bands are shifted upon deuteration of aldehydes; hence the actual vibrations are mixtures of group frequencies and no such simple assignment is justified. However, although this band seems to be shifted from 1289 to 1232 cm.$^{-1}$ in benzaldehyde and from 1297 to 1220 cm.$^{-1}$ in *p*-chlorobenzaldehyde (57 and 77 cm.$^{-1}$, respectively), other bands in this region are shifted by much smaller amounts—e.g., 753 to 733, 698 to 685, and 934 to 923. It appears therefore justifiable to attribute an out of the plane C_{formyl}—H bending character to this mode of vibration, which is affected by the deuteration much more than most of the other vibrations.

FURTHER EXPERIMENTS

Badger and Bauer (*4*) have shown that a difference of about 35 cm.$^{-1}$ in the fundamental O—H stretching frequency due to its hydrogen bonding ($\Delta\nu/\nu$ = 0.01) corresponds approximately to an energy difference of about 1 kcal. per mole. This regularity seems to hold true also for *o*-halophenols —e.g., *o*-bromophenol in carbon tetrachloride solution—although here the hydrogen bond forms an obtuse angle with the O—H direction and therefore only part of it would seem to be active in reducing the O—H frequency. That this regularity holds in this case can be seen by dividing the observed difference in frequency between the bonded *cis*-phenol and the unbonded *trans*-phenol, which is about 75 cm.$^{-1}$, over the calculated difference in free energy, which is about 2.1 kcal. per mole (*18*). If the same regularity applies also to the increase of the C_{formyl}—H stretching frequency in ortho-substituted benzaldehydes, it is possible to calculate the approximate amounts of the trans forms in their carbon tetrachloride solution from its increase over the unbonded frequency, according to the equation: $N = N_o\, e^{-E/RT}$, where N = number of trans molecules, N_o = number of cis molecules, and E = difference in free energy between the trans form and the cis form. This difference is then equal to $\Delta\nu/(2730 \times 0.01)$ kcal. per mole. If we take for $\Delta\nu$ the observed value (*21*) in the case of *o*-nitrobenzaldehyde—i.e., 2760 − 2730 cm.$^{-1}$ = 30 cm.$^{-1}$—we get for E the value of 1 kcal. per mole and $N = N_o\, e^{-1000/2 \cdot 300} = 0.19\ N_o$. This means that about 15% of the *o*-nitrobenzaldehyde which is in the carbon tetrachloride solution is then in the unbonded trans form. If, however, we take a $\Delta\nu$ of about 70 cm.$^{-1}$, in accordance with above assumption of the occurrence of Fermi resonance here, we get for E a value of 2.5 kcal. per mole, $N = N_o\, e^{-2500/600} = 0.015\ N_o$ and only about 1% is then "free." In any case there has to be a definite part of the aldehyde which absorbs at the normal frequency of about 2730 cm.$^{-1}$ It was hoped that if the bonded aldehydes were measured under the higher resolving power of a lithium fluoride prism, they would show this band. The following aldehydes were therefore measured under the resolution of a lithium fluoride or calcium fluoride prism (Table IV).

As Table IV shows, no such band was observed, possibly because the amount of the trans form is too low for its band to be discerned from the near and much stronger band of the cis form (*9*), especially if the Fermi resonance assumption is correct. It is also possible that the energy difference between the cis and trans isomers is much greater than calculated above, because the angle which the hydrogen bond forms with the C—H direction is not very acute and therefore only a small part of the hydrogen bond is reflected in the increase of the C—H frequency.

The values of 2730 and 2807 cm.$^{-1}$ for the C—H frequencies of benzaldehyde are in good agreement with the 2732 and 2808 cm.$^{-1}$ given in the literature (*8*).

Benzaldehyde was also measured in solution in various solvents in order to see whether it changes its C_{formyl}—H stretching frequency appreciably with the nature of the solvent by forming stronger intermolecular hydrogen bonds with solvents that can act as strong

Table IV. Aldehydic C—H Frequencies under Higher Resolution

Material	Concentration in CCl_4	Cell Thickness, Mm.	Prism	Bands, Cm.$^{-1}$
Benzaldehyde	10%	0.1	LiF	2690, 2730 (strong), 2807 (strong), 2846
o-Chlorobenzaldehyde	20%	0.05	LiF	2752, 2860 (strong)
o-Nitrobenzaldehyde	0.003 g./cc.	8	CaF_2	2760, 2893 (strong)
p-Anisaldehyde	0.005 g./cc.	8	CaF_2	2724 (strong), 2796, 2821, 2835 (strong)

proton acceptors. The following results were obtained (with a sodium chloride prism).

In carbon tetrachloride	2730 cm.$^{-1}$
In carbon disulfide	2730 cm.$^{-1}$
In nitrobenzene	2735 cm.$^{-1}$
In *o*-dichlorobenzene	2740 cm.$^{-1}$
(Undiluted[a])	2737 cm.$^{-1}$

[a] Measured with lithium fluoride prism.

Cinnamaldehyde did not show a big change in its C—H frequency on changing its solvent from carbon tetrachloride (2730 cm.$^{-1}$) to carbon disulfide (2715 cm.$^{-1}$). 2-Naphthaldehyde showed its C—H band in anisole or carbon tetrachloride at 2720 cm.$^{-1}$ and in benzene at 2710 cm.$^{-1}$. It seems therefore that the intermolecular hydrogen bonding in aldehydes, which no doubt exists (*1*, *3*), is not effective in changing their C—H frequencies materially (*3*), possibly because it is not strong enough to bring the bonded groups so near one to the other (against the van der Waals repulsion) as to effect an appreciable change of the C—H frequency.

The small changes sometimes observed in changing the solvent are probably due to usual solvent effects (*27*) and could be correlated neither with its polarizability nor with its dielectric constant.

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Quantitative Infrared Analyses of Mixtures of Isomeric or Closely Related Substances

Mixtures of Chlorinated Insecticides

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▶ Differential analysis, an accepted technique in ultraviolet spectrophotometry, is here applied to the infrared analysis of organic mixtures. Methods are presented for determining the active pesticide content of commercial BHC, lindane, DDT, TDE, and Ovex. Binary mixtures of these insecticides have also been analyzed and the procedures are described. The results of these analyses indicate that the active pesticide content can be determined to an accuracy within 2% of the abundance. The methods used in these determinations illustrate the principle of solvent and solute compensation.

DURING the commercial preparation of many insecticides, inactive isomers and closely related substances are formed, together with the active compounds. Thus technical hexachlorocyclohexane (BHC) usually contains five isomers, together with more highly chlorinated substances (*10*, *17*, *20*, *23*, *28*), while technical dichlorodiphenyltrichloroethane (DDT) is a mixture of the *p,p'*- and *o,p'*-isomers of DDT and other chlorophenyl compounds (*8*). Technical dichlorodiphenyldichloroethane (TDE) contains both the *p,p'*- and *o,p'*-isomers of TDE; but lindane and Ovex are relatively pure γ-BHC and *p*-chlorophenyl-*p*-chlorobenzene sulfonate, respectively. Commercial insecticides prepared from these technical products frequently also contain emulsifying agents, or wetting agents. With increasing knowledge of the specific action of a pesticide, different pesticides are often mixed to achieve selective insect control. Thus the commercial insecticide is a complex mixture of substances, and the efficacy of the product depends upon the amount of each present.

This paper describes methods using double-beam infrared spectrophotometry to determine the percentage of active constituents in commercial insecticides as dusts containing BHC

Table I. Analytical and Interfering Peaks

(Analytical peaks underlined. Frequencies expressed as cm.$^{-1}$)

p,p'-DDT	o,p'-DDT	p,p'-TDE	o,p'-TDE	α-BHC	δ-BHC	γ-BHC	Ovex
...	...	1015	...	...	...	...	1154
1015	1033,1015	...	1040,1015	...	1032	...	1015
893	888	...	...	926	926	910	...
807	...	800	...	...	...	...	...
777,770	772	765	772	786,774	768	776	769
...	750	755	748	...	...	...	741
685	...	...	685	692	...	686	662

lindane, DDT, TDE, Ovex, or binary mixtures of these compounds.

The γ isomer content of BHC has been measured by a variety of methods, including partition chromatography (*10, 26*), polarography (*15*), with improved modifications (*9, 30, 31*), isotope dilution using chlorine-36 (*5*) or carbon-14 (*13*), cryoscopy (*3*), dehydrohalogenation (*18*), or infrared absorption (*7, 20, 21, 25, 33*). The chromatographic (*10*) and infrared (*7*) methods have been preferred (*29*), although the isotope dilution method is gaining popularity (*14*).

Although the chromatographic methods give good recoveries, the infrared spectra of the recovered γ fractions contain at least three isomers (*29*). The γ isomer content of lindane has been determined by polarographic (*31*), differential refractometer (*12*), and infrared methods (*16*). For the determination of the *p,p'*-isomer content of DDT, crystallization techniques (*2*), dehydrohalogenation (*34*), specific color reactions (*27*), and infrared absorption (*8, 11*) have been used. Mixtures of BHC and DDT have been analyzed by total chlorine and labile chlorine assay (*32*) and by separating the BHC from the DDT by paper chromatography (*22*). DDT and TDE mixtures have been analyzed by chromatographic techniques (*1*). Infrared absorption methods have not been reported for these mixtures.

The single-beam infrared spectrophotometers used previously for the estimation of γ-BHC (*7, 20, 21, 25, 33*) and *p,p'*-DDT (*8, 11*) have marked disadvantages for quantitative analysis, because the experimental precision depends upon a knowledge of the absorption at the selected analytical peaks of each of the components of the mixture, and this information is rarely available. Furthermore, as the solvent correction should be a small proportion of the total extinction coefficient, thin cells and high solute concentrations are necessary. Solute concentration depends upon the solvent used, and solvents vary considerably in their infrared absorption properties. A high solute concentration in a satisfactory solvent is frequently difficult to obtain. A compromise must be reached between solvent thickness and solute concentration, with the result that in single-beam operation weak peaks (requiring thick cells and high concentrations) must be ignored.

With a double-beam instrument and a variable-length cell the effect of solvent absorption can be eliminated by compensating at a solvent peak, and the upper limits of cell thickness and solute concentration are dictated by the energy level at the detector. In the analysis of mixtures solvent compensation is a great advantage, but an even greater advantage results if interference from the other components of the mixture can be eliminated entirely from the desired analytical peaks (*19*). The compensation technique makes it possible to use any absorption peak of a compound to determine the concentration of that compound in a mixture, but if the peak frequency corresponds to that of a solvent peak, or to a strong peak of one of the other components, the energy level can be so reduced that the analytical precision is effected. The compensation method, by comparing prepared solutions of pure insecticides with a mixture containing the same insecticide, does not suffer from the limitations attributed to previous infrared methods, (*26*) because by suitable adjustment of the variable-length cell thickness, the true background adsorption, caused by possible interfering substances, can be determined.

APPARATUS AND REFERENCE COMPOUNDS

A Perkin-Elmer Model 21 double-beam infrared spectrophotometer, linear in wave number, was used for all determinations. The instrument was equipped with sodium chloride lenses, windows, and prism.

Cells. One Perkin-Elmer fixed-thickness cell (1 mm.) was used and it was compensated with a Perkin-Elmer variable-space cell. The fixed-thickness cell had a length equal to a reading of 1.00 mm. on the vernier of the variable-length cell. Both cells had sodium chloride windows.

Carbon Disulfide. Analytical reagent grade carbon disulfide, used as solvent in all experiments, was spectroscopically similar to pure carbon disulfide used by other workers.

REFERENCE COMPOUNDS

	Melting Point, ° C.
α-BHC	159–159.5
δ-BHC	139–139.5
γ-BHC	113–113.5
p,p'-DDT	110–110.5
p,p'-TDE	110
Ovex	87–88
o,p'-DDT	74
o,p'-TDE	77–78. Sample prepared from technical TDE (*6*)
BHC	Commercial grade
Lindane	Commercial Gammexane
DDT	Ether-soluble extract from commercial 50% dust
TDE	Ether-soluble extract from commercial product
Ovex	Ether-soluble extract from commercial dust

The spectra of these pure compounds, as solutions were compared where possible with those already published.

PREPARATION OF SAMPLE SOLUTIONS

The sample was carefully weighed into a 5-ml. graduated cylinder (with a polyethylene stopper) and made up to 5 ml. with carbon disulfide at room temperature (about 20° C.), and the cylinder was placed in a dark cupboard until required. This was a necessary precaution, as the solvent deteriorates on standing in the light. Solvent loss from polyethylene-stoppered cylinders was much less than from ground glass-stoppered cylinders. After each filling of the cell, the volume remaining in the cylinder was measured and noted, allowing correction to be made for the small solvent loss that sometimes occurred when the solutions were kept several weeks. Compensating solutions were prepared in 10-ml. stoppered cylinders in a similar manner. Throughout this investigation concentrations of insecticides were calculated on a weight-volume basis.

RECORDING SPECTRA

With the appropriate instrumental settings for qualitative or quantitative work, the amplifier balance, instrument zero, and 100% point were adjusted with no cells in the light beams. In the quantitative determinations the amplifier balance was altered to give a 0.5 to 1.0% upscale drift at the zero position (*24*). The analytical accuracy depends upon the precision of the zero setting

and the upscale drift ensures that "zero" is set with a small definite energy in each beam, rather than with practically zero energy.

The 1.00-mm. fixed-thickness cell filled with the sample solution was placed in the sample beam and the variable-length cell (initial thickness 1.00 mm.), filled with solvent or compensating solution, was placed in the compensating beam. Transmittance graphs were traced with the slit appropriate to the spectral region and at a scale of 10 cm. per 100 $cm.^{-1}$. For each mixture a preliminary survey spectrum was recorded to classify the sample and to detect any gross impurity or contaminant.

In calculating the extinction coefficient of a particular peak, the I_0 value was determined by the normal base-line technique.

CONDITIONS FOR QUANTITATIVE ANALYSES

Absorption Peaks. Carbon disulfide solutions of the pure compounds present in the mixtures were prepared and from the qualitative survey spectra of the 1400- to 650-$cm.^{-1}$ region (cells 1.0 mm. thick, concentration 0.5% w./v.) the absorption peaks suitable for quantitative work were selected (Table I). Peaks between 860 and 835 $cm.^{-1}$ were ignored because the solvent absorption in this region resulted in low energy conditions. The absorption peak frequencies of the other compounds examined that would interfere with the suggested analytical peaks or base lines, are also listed in Table I.

Although interference may not occur at the actual peak, interference on the shoulders can affect the base line, and extinction coefficients calculated therefrom are in error if the base line itself does not obey Beer's law (*25*). It is therefore evident from Table I that Ovex can be determined at 1154 $cm.^{-1}$ in mixtures of any or all of the other components, but that in the measurements of γ-BHC in mixtures allowance must be made for interference at the 910-$cm.^{-1}$ peak caused by *p,p'*- DDT, α-BHC, or δ-BHC.

Instrument Settings. Instrumental conditions have received scant attention in the chemical literature, yet for a particular compound the maximum extinction coefficient of a peak varies from instrument to instrument (*11*). The significance and amount of variation have not been stated. In this investigation attempts were made to determine the effect of the instrumental settings upon the extinction coefficient of the 1015-$cm.^{-1}$ peak of *p,p*-DDT. A solution of *p,p'*-DDT in carbon disulfide (concentration 0.431% w./v.) in a 1-mm. cell was compensated with the required thickness of carbon disulfide, and the spectrum measured in the region 1040 to 990 $cm.^{-1}$. Spectra for quantitative analysis must be free from random variations (electronic noise), and this limits the electronic gain which may be used and thus the power available to energize the recording servo-motor. By imposing a sluggish response characteristic upon the amplifier, high electronic gain can be tolerated, but the scanning speed must then be greatly reduced. With a fixed source current (0.3 ampere) and a fixed slit program, amplifier damping (response) and amplifier gain were simultaneously increased, while speed of wave length scan was decreased. The extinction coefficient reached a maximum value with highest gain and damping together with the slowest scanning speed.

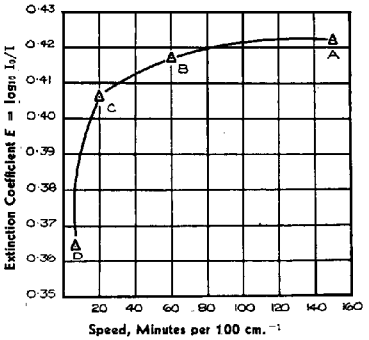

Figure 1. Effect of speed upon extinction coefficient *E*

Condition	Response	Gain	Slit Width, μ	Scan Speed, Min./100 $Cm.^{-1}$
A	4/4	8–8½	98	150
B	4/4	8–8½	98	60
C	3/3	7	105	20
D	2/2	6¼	120	6

With these settings, various fixed ("manual") slits were tried and an additional series of graphs was recorded. Thus the combination of fixed slit width, amplifier damping, amplifier gain, and scanning speed giving the maximum extinction coefficient was determined. As the optimum scanning speed found (150 minutes per 100 $cm.^{-1}$) was too slow for this project, an investigation was made of the effect on the extinction coefficient of higher scanning speeds, at the same time adjusting slit widths, gain, and damping, to give maximum extinctions at each of these speeds. The results are shown in Figure 1.

In this investigation the speed, 60 minutes per 100 $cm.^{-1}$, was used in all quantitative runs. The quantitative conditions were therefore:

Response	4.4
Gain	8–8½
Scanning speed	60 min./100 $cm.^{-1}$
Slit	Manual (see below)
Source	0.3 amp.
Stray-light filter	Automatically inserted at 1072 $cm.^{-1}$ [4]

For the qualitative survey spectra instrumental conditions were:

Table II. Slit Widths and Base Lines for Selected Analytical Peaks

Compound	Peak Frequency, $Cm.^{-1}$	Base Line, $Cm.^{-1}$	Slit Width[a], μ
Ovex	1154	1164 to 1130	73
Ovex	1015	1040 to 990	98[b]
p,p'-DDT	1015	1040 to 990	98[b]
p,p'-TDE	1015	1040 to 990	98[b]
op'-DDT	1015	1040 to 990	98[b]
op'-TDE	1015	1040 to 990	98[b]
γ-BHC	910	920 to 895	145
p,p'-TDE	765	778 to 759	200
p,p'-TDE	755	760 to 750	200
o,p'-DDT	750	758 to 738	210
o,p'-TDE	685	694 to 672	395

[a] True slit width corrected for slit error.
[b] Where extinction coefficient, *E*, is greater than 0.6, slit of 110μ was used.

Table III. Instrument Reproducibility

Concentration of *p,p'*-DDT	0.2050	0.4310
Mean extinction coefficient (ten determinations)	0.194	0.417
Range of extinction coefficients	0.191–0.196	0.408–0.425
Standard deviation, σ[a]	0.002	0.005
Variation coefficient[b]	1.1	1.2
Standard deviation, σ, expressed as variation in peak transmittance, %	0.3	0.4

[a] Standard deviation, σ, $\sqrt{\frac{\Sigma(x_1 - x)^2}{n - 1}}$.

[b] Variation coefficient $= \frac{\sigma \times 100}{\text{mean extinction coefficient}}$.

Response	1.1
Gain	6
Scanning speed	2 min./100 cm.$^{-1}$
Slit	960 Automatic
Source	0.3 amp.
Stray-light filter	Automatically inserted at 1072 cm.$^{-1}$

The extinction coefficient of a particular peak is also dependent upon the slit width—narrow slits result in low energy and wide slits in poor resolution. The ideal slit widths (giving the maximum extinction coefficients) for the various compounds were determined under quantitative instrumental conditions, and these and the base lines used in calculating the extinction coefficients are listed in Table II.

The instrumental reproducibility was determined over one day under quantitative conditions, from replicate transmittance graphs of the 1015-cm.$^{-1}$ peak of *p,p'*-DDT. Two concentrations were prepared, and the results of ten measurements for each concentration are given in Table III.

The variation coefficients are somewhat greater than the short-term relative standard deviations found for a similar instrument by other authors (*4*)—viz., 0.8%.

COMPENSATION TECHNIQUES

After the concentration of a constituent, *A*, of a mixture had been determined at a particular frequency, λ_1, a compensating solution containing the same concentration of a pure sample of *A* is placed in the variable-length cell and the peak at λ_1 retraced. If compensation is correct, the graph will record a continuous background between the shoulders of λ_1—i.e., between the two points from which the base line is normally drawn. Overcompensation will be revealed by a peak minimum at λ_1, and under compensation by the presence of a small peak at λ_1. In either case the length of the compensating cell is suitably adjusted until at λ_1 the absorption of the mixture is eliminated. By tracing a spectral region containing other prominent peaks (λ_2, λ_3, etc.) of *A*, the compensation at these peaks can be checked. Any overcompensation at λ_2, λ_3, etc., indicates the presence of interference at the original peak λ_1, used in calculating the concentration of *A*. By adjusting the variable-length cell thickness until this overcompensation is removed, the true absorption due to *A* is eliminated from the trace and the interference at λ_1 can be measured. The true concentration of *A* in the mixture can be calculated from the corrected extinction coefficient or from the variable-length-cell thickness and the concentration of *A* in that cell. The foregoing assumes that a relatively free peak can be used for the original determination of *A*, but if this not possible the content of *A* can be assumed and the procedure similarly followed.

Table IV. Specific Extinction Coefficients for Reference Compounds

Compound	Frequency, Cm.$^{-1}$	Mean *k* value[a]	Range of *k* Value	Std. Dev.	Variation Coefficient, %
Ovex	1154	0.692	0.650–0.721	0.020	2.9
	1015	1.019	1.006–1.042	0.012	1.2
p,p'-DDT	1015	0.943	0.915–0.967	0.018	1.9
p,p'-TDE	1015	0.868	0.835–0.894	0.022	2.5
γ-BHC	910	0.233	0.223–0.239	0.004	1.8
p,p'-TDE	765	0.666	0.640–0.700	0.015	2.2
	755	0.395	0.369–0.429	0.021	5.2[b]
o,p'-DDT	1015	0.480	...	...	...
o,p'-TDE	1015	0.497	...	...	...
o,p'-DDT	750	1.328	...	...	...
o,p'-TDE	748	1.012	...	...	...
o,p'-TDE	686	0.144	...	...	...

[a] *k* (specific extinction coefficient) = E/cl where E = extinction coefficient—i.e., $\log_{10} I_0/I$; C = concentration, grams per liter; l = cell thickness, cm.
[b] This peak used for approximate values only.

METHODS OF ANALYSIS

Isolation of Pesticides. The commercial insecticides in the form of dusts were separately extracted with diethyl ether, the ether was removed, and the soluble extracts were recovered and weighed. These extracts were used in all subsequent work.

γ-BHC Content of BHC. A solution of BHC (concentration 3 to 5% w./v. in carbon disulfide) was prepared, compensated with a solution of α-BHC (concentration 70% of BHC concentration) and the spectral region 930 to 920 cm.$^{-1}$ traced under quantitative γ-BHC conditions. β-BHC is insoluble in carbon disulfide, and in the determination of γ-BHC only α-BHC and δ-BHC need be considered. The thickness of the variable-length cell was then adjusted until there was no inflection at 926 cm.$^{-1}$ on the graph, and from the cell thickness the concentration of α-BHC in the sample was calculated. A solution of α-BHC of this concentration was prepared, the variable-length cells (thickness 1.0 mm.) were refilled, compensation was confirmed, and the region 930 to 890 cm.$^{-1}$ was recorded under quantitative γ-BHC conditions. From the base line drawn from 920 to 895 cm.$^{-1}$ the extinction coefficient of the 910-cm.$^{-1}$ peak was calculated, and hence the concentration of γ-BHC in the sample determined using the specific extinction coefficient of γ-BHC at 910 cm.$^{-1}$ (Table IV). To confirm the analytical results, a solution containing both α-BHC and γ-BHC in the analyzed proportions was prepared and placed in the compensating cell, and the regions 930 to 890 cm.$^{-1}$ and 700 to 670 cm.$^{-1}$ were traced under quantitative conditions. The γ-BHC peaks at 910 and 686 cm.$^{-1}$ should be absent. In the above analysis it was not necessary to compensate for the low δ-BHC content.

γ-BHC Content of Lindane. One and 2% sample solutions were compensated with carbon disulfide and the γ-BHC concentrations calculated from the extinction coefficients of the 910 cm.$^{-1}$ peaks (region 930 to 890 cm.$^{-1}$). Compensating solutions containing these concentrations of γ-BHC were prepared and the graphs repeated.

***p,p'*-DDT Content of Technical DDT.** A 0.5% solution was prepared and compensated with carbon disulfide, and the *o,p'*-DDT content calculated from the extinction coefficient of the 750-cm.$^{-1}$ peak (region traced 760 to 735 cm.$^{-1}$). During this determination if TDE was present in a concentration greater than 0.025% w./v., a peak was detected at 755 cm.$^{-1}$

A compensating solution containing this determined concentration of *o,p'*-DDT was placed in the variable-length cell and the compensation checked by retracing the 755- to 745-cm.$^{-1}$ region. If necessary, the cell thickness was adjusted to give true compensation. The spectral range 1040 to 990 cm.$^{-1}$ was then recorded with this *o,p'*-DDT compensation, and from the extinction coefficient of *p,p'*-DDT at 1015 cm.$^{-1}$ (Table IV) the concentration of *p,p'*-DDT was calculated. A further compensating solution containing the analyzed concentration of both *o,p'*-DDT and *p,p'*-DDT was prepared and spectral regions 760 to 735 cm.$^{-1}$ and 1040 to 990 cm.$^{-1}$ were repeated. If DDX [1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene] was present in the sample, overcompensation was apparent at 775 cm.$^{-1}$

***p,p'*-TDE Content of TDE.** The analysis of TDE is essentially similar to that of DDT—i.e., *o,p'*-TDE is first measured with solvent compensation, a compensating solution of the required concentration of *o,p'*-TDE is prepared, and the *p,p'*-TDE content is measured at 1015, 765, and 755 cm.$^{-1}$. The weak characteristic *o,p'*-TDE peak, 686 cm.$^{-1}$, requires a solution of 2.0% concentration, whereas the concentration of *p,p'*-TDE was measured with a 0.5% solution. The spectral regions recorded were 700 to 670 cm.$^{-1}$ for *o,p'*-TDE, and 1040 cm. to 980 cm.$^{-1}$ and 782 to 742 cm.$^{-1}$ for *p,p'*-TDE. Before the *p,p'*-TDE concentration was measured, the accuracy of the *o,p'*-

TDE compensation was checked by tracing the 755- to 745-cm.$^{-1}$ region. *o,p'*-TDE has a strong peak at 748 cm.$^{-1}$. A compensating solution containing the determined amounts of both *o,p'*- and *p,p'*-TDE was prepared and the graphs were repeated to detect any extraneous absorption.

Ovex Content of Commercial Ovotran. A 0.5% sample solution with solvent compensation was analyzed by tracing the 1170- to 1130-cm.$^{-1}$ and 1040- to 980-cm.$^{-1}$ portions of the spectra. From the extinction coefficients and the appropriate specific extinction coefficients at 1154- and 1015- cm.$^{-1}$ (Table IV) the Ovex content was determined. A compensating solution containing this concentration of Ovex was prepared and the graphs were repeated.

The 1154-cm.$^{-1}$ peak given by the sulfonate group and bis-(*p*-chlorophenyl) sulfone absorbing at 1163 cm.$^{-1}$ could affect the base line, but the presence of this substance would not reinforce the 1154-cm.$^{-1}$ peak. The slope of the base line is such that the presence of small amounts of bis-(*p*-chlorophenyl) sulfone would have little effect on the Ovex assay. The 1015-cm.$^{-1}$ peak is characteristic of a *p*-chlorophenyl) and at this peak bis-(*p*-chlorophenyl sulfone) and *p*-chlorophenol would interfere. However, by determining Ovex at 1154-cm.$^{-1}$ and then compensating for this analyzed concentration, a graph of any interfering substances that may be present will be given and if necessary additional Ovex peaks, perhaps 1202 or 1083 cm.$^{-1}$ used.

Binary Mixtures. In all cases the analyses should be confirmed by tracing the graph of the original solution compensated with a solution of the same thickness containing the determined concentration of the active constituents.

Mixtures Containing Ovex and BHC, Lindane, DDT, or TDE. Mixtures containing Ovex were analyzed as for commercial Ovex—i.e., the transmittance graph of an 0.5% sample solution compensated with solvent, was recorded in the 1170- to 1130-cm.$^{-1}$ region, and the Ovex content determined. If the Ovex contents were found to be low, the sample concentrations were increased to give extinction coefficients at 1154 cm.$^{-1}$ greater than 0.2. The transmittance graphs were then repeated. A compensating solution containing the analyzed concentrations of Ovex was prepared and the Ovex compensation confirmed at 1154 and 741 cm.$^{-1}$. The compensated solutions were then analyzed as for commercial BHC, lindane, DDT or TDE. In the measurement of *p,p'*-DDT or *p,p'*-TDE at 1015 cm.$^{-1}$ if the total uncompensated extinction coefficient, *E*, was greater than 0.6, a wider slit and slower scanning speed were required (slit 110 microns, speed 150 minutes per 100 cm.$^{-1}$).

Mixtures Containing BHC and Lindane, DDT, or TDE. A sample solution (concentration 3- to 5%) was prepared, compensated approximately with a solution of α-BHC (at a concentration about 70% of sample weight), and the spectral region 930 to 920 cm.$^{-1}$ was traced under quantitative γ-BHC conditions (Table II). By adjusting the thickness of the variable-length cell the α-BHC peak at 926 cm.$^{-1}$ was eliminated and the α-BHC concentration in the sample was calculated. A further α-BHC solution was prepared and the compensation with this solution confirmed by retracting the 930- to 920-cm.$^{-1}$ region. With the α-BHC adequately compensated, the mixtures become virtually lindane, lindane-DDT, or lindane-TDE.

The γ-BHC content of BHC-lindane or BHC-TDE can be measured at 920 cm.$^{-1}$ once the α-BHC is compensated, but *p,p'*-DDT affects the base line at this peak. Fortunately, γ-BHC does not affect the determination of *o,p'*-DDT at 750 cm.$^{-1}$, so with α compensation the BHC-DDT mixture is analyzed as a crude DDT—i.e., *o,p'*- at 750 cm.$^{-1}$—and with α-BHC and *o,p'*-DDT compensation the *p,p'*-DDT at 1015 cm.$^{-1}$. A compensating solution containing both α-BHC and *p,p'*-DDT was then prepared, and γ-BHC measured at 910 cm.$^{-1}$. In determining the *p,p'*-TDE it was necessary to prepare a solution containing the analyzed concentration of α-BHC and γ-BHC, so that the *o,p'*-TDE content could be determined at 686 cm.$^{-1}$. A very slow scanning speed (150 minutes per 100 cm.$^{-1}$) was essential, and depending upon the relative concentrations of α-BHC, γ-BHC, and *o,p'*-TDE, it was sometimes necessary to increase the slit width. At low concentrations of TDE it was not possible to measure the *o,p'*-TDE content accurately, even with these precautions, and it was necessary to assume an *o,p'*-TDE concentration and adjust it to compensate the 748-cm.$^{-1}$ peak. Additional compensating solutions containing both α-BHC and the determined concentration of *o,p'*-TDE were prepared, the compensation was confirmed by tracing the 755- to 745-cm.$^{-1}$ region, and the *p,p'*-TDE was measured at 1015 cm.$^{-1}$

Mixtures Containing Lindane and DDT or TDE. One per cent solutions of these mixtures were analyzed as described above for BHC–DDT and BHC–TDE, except that α-BHC compensation was not necessary. The determination of *o,p'*-TDE is difficult in the presence of high concentration of γ-BHC.

DDT–TDE Mixture. With a 0.7 % solution of the mixture in the sample beam, and a 0.4% *p,p'*-DDT solution in the compensating beam, a qualitative graph of the 796- to 740-cm.$^{-1}$ region was recorded. The *p,p'*-DDT content of the mixture was overcompensated as intended, and the normal 777-cm.$^{-1}$ *p,p'*-DDT peak appeared as a minimum. The thickness of the variable-length cell was then adjusted until under qualitative conditions there was no change in the slope of the graph from 790 to 774 cm.$^{-1}$—i.e., no peak or minimum at 777 cm.$^{-1}$ The spectral range 796 to 740 cm.$^{-1}$ was then retraced under quantitative *p,p'*-TDE conditions with the variable-length cell (thickness 1.00 mm.) filled with a newly prepared *p,p'*-DDT compensating solution, the concentration being calculated from previous compensation experiments. From this trace the *p,p'*-DDT compensation can be confirmed, and the *p,p'*-TDE content calculated from the 765-cm.$^{-1}$ peak. A further compensating solution containing the determined amounts of both *p,p'*-DDT and *p,p'*-TDE was prepared and the 796- to 740-cm.$^{-1}$ trace repeated.

Results. From the measured extinction coefficient of a particular peak of a mixture, and the specific extinction coefficient of the pure compound at that peak, the concentration of the compound in the mixture can be calculated. The accuracy with which the specific extinction coefficient can be measured is therefore a major factor in determining the over-all accuracy of the results that could be obtained. The specific extinction coefficients found for the pure insecticides are given in Table IV. The results in Table IV indicate that an accuracy within $\pm 2\%$ is attainable.

The active isomers present in the mixtures are separately listed; thus the γ-BHC contents of BHC and BHC mixtures appear in Table V, whereas the *p,p'*-DDT contents of the BHC-DDT mixtures are listed in Table VIII with the *p,p'*-DDT content of DDT. For each analysis the mixture is considered as a technical insecticide adulterated with another insecticide, which is identified in the tables as a diluent. In Table V DDT is the diluent of a BHC-DDT mixture, whereas in Table VIII BHC is the diluent of the same BHC-DDT mixture. In each case the active-isomer content was calculated as a percentage of the technical insecticide as defined above, so that the results—for example, γ-BHC in BHC or BHC mixtures—are strictly comparable. With efficient compensation the analysis of mixtures becomes the simple assay of a particular insecticide. Thus it is permissible to average all the results found and derive a standard deviation from these results.

γ-BHC Content of BHC and BHC Mixtures. Lindane and Lindane Mixture. Previous workers (*7*, *33*) have measured the γ-BHC concentra-

tion in BHC at the 845- or 686-cm.$^{-1}$ peaks and have ignored the weak peak at 910 cm.$^{-1}$. Carbon disulfide absorbs at 856 cm.$^{-1}$, so with 1.0-mm. cells this solvent is unsatisfactory if the 845-cm.$^{-1}$ peak is to be accurately measured (*21*). While the potassium bromide region has been proposed for measuring the γ-BHC content of technical BHC (*21*), no particular advantage accrued, using this peak for mixtures. The 483-cm.$^{-1}$ γ peak is a particularly broad peak, and marked base-line interference resulted from the presence of α-BHC, *p,p'*-DDT, or *o,p'*-DDT. α-BHC has a major peak at 692 cm.$^{-1}$, and with the high concentration of α-BHC in technical BHC, this peak interferes considerably with the γ-BHC peak at 686 cm.$^{-1}$. With α-BHC compensation this latter peak can be used, but the results that have been obtained are not entirely satisfactory, and under extreme low energy conditions have been fallacious. γ-BHC was measured at the 910-cm.$^{-1}$ peak. The γ-BHC contents of BHC and BHC mixtures are listed in Table V, and only solvent and α-BHC were compensated in analyzing BHC. The δ isomer of BHC does not materially affect the 922-cm.$^{-1}$ shoulder of the 910-cm.$^{-1}$ peak.

Analyses of lindane and lindane mixtures are similarly tabulated in Table V. BHC-lindane mixtures have also been analyzed, and the results confirm those already found for γ in BHC and γ in lindane (Table VI).

p,p'-DDT Content of DDT and DDT Mixtures. The measurement of the *o,p'*-DDT contents of DDT mixtures at the 750-cm.$^{-1}$ peak has not been previously described, although the 1037-cm.$^{-1}$ peak has been used (*11*). While the determination of the *o,p'*-content need not be highly accurate, because compensation is always subsequently checked, the results in Table VII indicate a reasonable accuracy.

In mixtures of DDT and TDE, *o,p'*-DDT was not successfully measured if the TDE content was greater than the DDT content.

Table VIII lists the percentage of *p,p'*-DDT found in DDT and the DDT mixtures when the solutions were adequately compensated and the *p,p'*-DDT was measured at 1015 cm.$^{-1}$. The 807-cm.$^{-1}$ peak used previously (*11*) for the measurement of *p,p'*-DDT is unique to *p,p'*-DDT, but the base line drawn between the adjacent minima does not represent the true base line. For this reason the basic requirement, that the base line obeys Beer's law (*25*), is not satisfied, and this peak is not recommended.

p,p'-TDE Content of TDE and TDE Mixtures. The measurement of *o,p'*-TDE at the weak 686-cm.$^{-1}$ peak gives an approximate rather than accurate value of the *o,p'*-TDE content, but from this result compensating solutions can be prepared and the compensation can be checked at the much stronger 748-cm.$^{-1}$ peak. Table IX summarizes the results obtained.

It was not possible to determine *o,p'*-TDE in the presence of high concentrations of BHC or lindane. The percentages of *p,p'*-TDE found in the mixtures are listed in Table X.

Ovex Content of Commercial Ovotran and Ovotran Mixtures. The results obtained in the determination of Ovex are summarized in Table XI.

DISCUSSION

Double-beam instruments have not

Table V. γ-BHC Content of Mixtures

(Measurements at 910 cm.$^{-1}$)

Components of Mixture	BHC Concn., %	Diluent Concn., %	Compensation Conditions	γ-BHC, %
BHC	2.690	...	CS_2, α-BHC	10.3
	3.114	...	CS_2, α-BHC	10.0
	4.106	...	CS_2, α-BHC	9.5
BHC and TDE	2.280	0.441	CS_2, α-BHC	9.6
	5.329	0.264	CS_2, α-BHC	9.6
BHC and DDT	2.477	0.562	CS_2, α-BHC, *p,p'*-DDT	9.4
	4.885	0.206	CS_2, α-BHC *p,p'*-DDT	9.7
BHC and Ovex	2.757	0.463	CS_2, α-BHC, Ovex	9.7
	4.199	0.277	CS_2, α-BHC, Ovex	9.6
			Mean	9.7
			Standard deviation, σ	0.3
			Variation coefficient	3.0
	Lindane Concn., %			
Lindane	0.440	...	CS_2	97.9
	1.036	...	CS_2	98.6
	1.163	...	CS_2	98.3
	1.998	...	CS_2	97.7
Lindane and Ovex	0.271	0.340	CS_2, Ovex	101.0
	0.620	0.310	CS_2, Ovex	99.5
Lindane and TDE	0.175	0.369	CS_2	99.5
	0.431	0.172	CS_2	100.5
Lindane and DDT	0.342	0.577	CS_2, *p,p'*-DDT	100.7
	0.414	0.247	CS_2, *p,p'*-DDT	99.8
			Mean	99.3
			Standard deviation, σ	1.2
			Variation coefficient	1.2

Table VI. γ-BHC Content of BHC and Lindane Mixtures

Mixture	Lindane, %	BHC, %	γ BHC Content, Calcd. from Table V, %	γ BHC Found, %	Recovery, %
A	0.276	1.426	0.412	0.420	101.7
B	0.355	0.937	0.443	0.448	101.0

Table VII. *o,p'*-DDT Content of DDT and DDT Mixtures

(Measurements at 750 cm.$^{-1}$)

Components of Mixture	DDT Concn., %	Diluent Concn., %	Compensation Conditions	*o,p'*-DDT in DDT, %
DDT	0.375	...	CS_2	17.5
	0.456	...	CS_2	17.6
	0.539	...	CS_2	17.9
DDT and BHC	0.206	4.885	CS_2, α-BHC	18.6
	0.562	2.477	CS_2, α-BHC	17.3
DDT and Ovotran	0.433	0.214	CS_2, Ovex	18.0
	0.308	0.449	CS_2, Ovex	16.3
DDT and lindane	0.247	0.414	CS_2	16.3
	0.577	0.342	CS_2	15.5
DDT and TDE	0.543	0.244	CS_2, *p,p'*-DDT	17.6
			Mean	17.4
			Standard deviation σ	0.8
			Variation coefficient	4.6

Table VIII. *p,p'*-DDT Content of DDT and DDT Mixtures

(Measured at 1015 cm.$^{-1}$)

Components of Mixture	DDT Concn., %	Diluent Concn., %	Compensation Conditions	*p,p'*-DDT in DDT, %
DDT	0.240	...	CS_2, *o,p'*-DDT	82.3
	0.399	...	CS_2, *o,p'*-DDT	82.2
	0.456	...	CS_2, *o,p'*-DDT	81.7
DDT and BHC	0.259	4.820	CS_2, *o,p'*-DDT and α-BHC	77.9
	0.563	2.477	CS_2, *o,p'*-DDT and α-BHC	78.6
DDT and lindane	0.247	0.414	CS_2, *o,p'*-DDT	81.8
	0.577	0.342	CS_2, *o,p'*-DDT	78.7
DDT and Ovex	0.308	0.458	CS_2, *o,p'*-DDT and Ovex	78.3
	0.433	0.214	CS_2, *o,p'*-DDT and Ovex	82.0
DDT and TDE	0.307	0.433	By compensation[a]	80.3
	0.543	0.244		80.1
			Mean.[a]	80.4
			Standard deviation, σ	1.6
			Variation coefficient	2.0

[a] Calculated from concentration of *p,p'*-DDT and variable-length-cell thickness (see text).

Table IX. *o,p'*-TDE Content of TDE and TDE Mixtures

(Measured at 686 cm.$^{-1}$)

Components of Mixture	TDE Concn., %	Diluent Concn., %	Compensation Conditions	*o,p'*-TDE in TDE, %
TDE	1.548	...	CS_2	20.2
	1.734	...	CS_2	22.9
	2.002	...	CS_2	22.0
TDE and lindane	1.033	2.376	CS_2, γ-BHC	...[a]
	2.376	1.033	CS_2, γ-BHC	21.2
TDE and Ovex	1.064	1.988	CS_2, Ovex	24.0
	1.969	1.091	CS_2, Ovex	21.4
TDE and DDT	0.810	1.766	CS_2, *p,p'*-DDT	15.2[b]
	1.270	0.904	CS_2, *p,p'*-DDT	19.7
			Mean	21.6
			Standard deviation, σ	1.2
			Variation coefficient	5.5

[a] Extreme low energy conditions.
[b] Not included in mean.

Table X. *p,p'*-TDE Content of TDE and TDE Mixtures

(Measurements at 765 cm.$^{-1}$ unless otherwise stated)

Components of Mixture	TDE Concn., %	Diluent Concn., %	Compensation Conditions	*p,p'*-TDE in Crude TDE, %
TDE	0.332	...	CS_2, *o,p'*-TDE	71.3
	0.429	...	CS_2, *o,p'*-TDE	68.0
	0.574	...	CS_2, *o,p'*-TDE	69.1
TDE and ovotran	0.271	0.540	CS_2, *o,p'*-TDE and Ovex	72.8
	0.556	0.278	CS_2, *o,p'*-TDE and Ovex	67.3
TDE and BHC	0.264	5.329	CS_2, *o,p'*-TDE α-BHC, γ-BHC	71.0[a]
	0.441	2.278	CS_2, *o,p'*-TDE α-BHC, γ-BHC	68.6[a]
TDE and lindane	0.178	0.431	CS_2, *o,p'*-TDE and γ-BHC	68.0
	0.369	0.175	CS_2, *o,p'*-TDE and γ-BHC	67.7
TDE and DDT	0.244	0.543	CS_2, *p,p'*-DDT	65.0[b]
	0.433	0.307	CS_2, *p,p'*-DDT	68.2
			Mean	69.2
			Standard deviation σ	1.9
			Variation coefficient	2.7

[a] Calculated from extinction coefficient at 1015 cm.$^{-1}$, making allowance for impurity absorbing at 1015 cm.$^{-1}$
[b] Not included in average.

been extensively used for quantitative analysis, and there have been no reports of their use in the analysis of insecticide mixtures. Although the inherent reproducibility of the Model 21 is less than that of the Model 12 (*4*), the advantages of double-beam operation used in conjunction with a variable-length cell more than compensate for this defect. By the techniques described, it is possible to demonstrate that the result obtained from the determination of a particular pesticide in a mixture is, within the limits of the experiment, the true result. This is achieved by preparing a solution containing the determined concentration of the pure pesticide and recording the graph of the mixture compensated with this solution. The results given in this paper have in the main been confirmed by this method. The limit to this compensation technique is the difficulty of precisely preparing a multicomponent compensating solution, but it is hoped to overcome this problem by using two or more compensating cells in the compensating beam.

In selecting the analytical absorption peak to be measured, a peak unique to the compound is preferable, more for maintaining a reasonable energy transmittance through the sample, than because of the complicated assumptions necessary to determine the true result (*7, 20, 25*). Although it is possible to compensate any number of interferences in a double-beam instrument, if the energy in both beams is too greatly reduced, the balance point becomes vague, and analytical precision is lost.

The principle of differential spectrophotometry has been applied here to the analysis of insecticide mixtures, but it has much wider possibilities. The experiments conducted to establish the optimum operating conditions and the reproducibility, and the description of compensation techniques are applicable to the analysis of any solvent-soluble organic mixture. Given pure samples of the substances sought, a 1-mm. fixed thickness cell, a variable-length cell, and a double-beam infrared spectrophotometer, such mixtures can be analyzed and the desired substance determined to an accuracy within about 2% of the abundance. This is achieved by eliminating from the graph of the mixture all the peaks characteristic of the desired substance. The resultant graph is the graph of all the other components of the mixture and inspection of this graph indicates the background absorption of these components at the desired analytical frequencies. In this manner analytical peaks can be chosen to coincide with positions of nil absorption in the background, or the background absorption at these frequencies can be used as a correction.

Table XI. Ovex Content of Commercial Ovotran and Ovotran Mixtures

(Measurement at 1154 cm.$^{-1}$)

Components of Mixture	Ovotran Concn., %	Diluent Concn., %	Compensation Conditions	Ovex Content of Commercial Ovotran, %
Ovotran	0.253	...	CS_2	94.9
	0.351	...	CS_2	96.0
	0.355	...	CS_2	98.5
Ovotran and BHC	0.277	4.200	CS_2	100.2
	0.463	2.757	CS_2	99.5
Ovotran and lindane	0.340	0.271	CS_2	94.5
	0.310	0.620	CS_2	94.7
Ovotran and DDT	0.214	0.423	CS_2	97.1
	0.449	0.309	CS_2	99.3
Ovotran and TDE	0.278	0.556	CS_2	98.7
	0.540	0.271	CS_2	95.1
			Mean	97.1
			Standard deviation, σ	2.4
			Variation coefficient	2.5

ACKNOWLEDGMENTS

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Determination of Traces of Ketone in a Carbinol by Differential Infrared Analysis

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▶ A simple infrared analysis designed for determining small amounts of ketone is described. The differential method is employed as a means of increasing sensitivity to the extent that ketones in the range from 0.1 to 1.0% may be determined with an accuracy of ±0.02%, and with a precision of ±0.01%. Amounts as low as 0.03% can be detected. The analytical band employed is the intense absorption at 5.93 microns, which is characteristic of the carbonyl stretching vibration.

THE DETECTION and determination of small amounts of carbonyl compounds is a problem of general interest. Classical chemical methods are often time-consuming and may require considerable sample preparation (*4, 6*). In these laboratories it was found desirable to determine small amounts of an intermediate, ethyl chlorovinyl ketone, which might occur in β-chlorovinylethinylethylcarbinol [a nonbarbiturate hypnotic, ethchlorvynol (Placidyl)]. Differential analysis by infrared absorption provided a simple and precise method for this investigation. The method appears to be generally applicable to the determination of carbonyl impurities.

Differential analysis, an approach to the determination of trace components, is especially adapted to the use of double-beam instruments. The solvent and pure major component are placed in the reference beam, with the solvent and test sample in the sample beam. The resulting tracing, under ideal

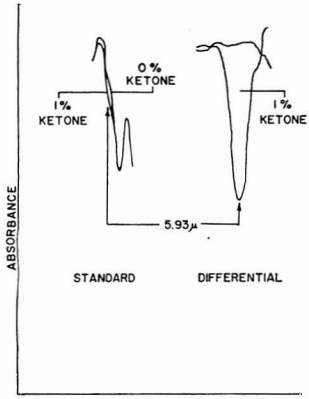

Figure 1. Comparison of standard and differential techniques in detecting presence of ketone in ethchlorvynol

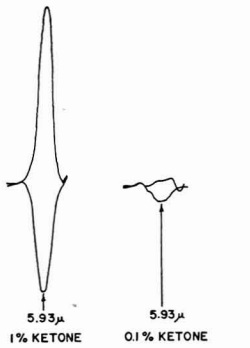

Figure 2. Differential determination of ketone in ethchlorvynol at two levels

conditions, is a composite spectrum of the impurities present in the sample. Robinson (*7*) and McDonald (*5*) describe and evaluate the differential method, and discuss the techniques involved. Subsequent publications by Bard, Porro, and Rees (*1*) on the determination of catechol and resorcinol in hydroquinone at the 0.1% level and by Freeman (*3*) on the determination of allethrolone in allethrin in the range from 0.05 to 2.5%, illustrate the method as applied to specific analyses.

EXPERIMENTAL

The qualitative spectra on the pure ethchlorvynol and the ethyl chlorovinyl ketone were obtained using a Perkin-Elmer Model 21 double-beam infrared spectrophotometer. This instrument was also used for all quantitative measurements.

The strong band at 5.93 microns, which is due to the C=O stretching vibration, was chosen as the most typical of the ketone. A working curve was plotted of absorbance *vs.* concentration in the range from 0.01 to 1.0% of the ketone in ethchlorvynol. The relationship was found to be linear throughout the range, with no individual variation of more than 0.01% from the mean value.

Procedure. Transfer a standard solution of 25% ethchlorvynol in chloroform to each of two 0.50-mm. sodium chloride cells. Place the cells in position, and with the source set at full energy, slits at 0.165 mm., wave length at 5.93 microns, and pen adjusted to rest at 0.250 absorbance unit on the paper, set the gain to give a single 3% overshoot in response to a 20% deflection introduced into the sample beam. Now scan the interval from 5.6 to 6.1 microns at a rate not exceeding 3 minutes per micron. Fill the sample cell with a 25% solution of the test sample and superimpose a scan from 5.6 to 6.1 microns. Reverse the solutions but not the cells and scan again from 5.6 to 6.1 microns, superimposing this scan over the two preceding runs.

Measure the total absorption at 5.93 microns and refer this value to a previously established working curve. Identity may be established by comparing the absorption band contours to a standard.

Table I. Results of Analyses on Synthetic Blends

Sample	Ethyl Chlorovinyl Ketone, % Known	Found
1	1.0	0.997, 0.982
2	0.9	0.916, 0.911
3	0.75	0.756, 0.759
4	0.50	0.505, 0.508
5	0.20	0.207, 0.210
6	0.10	0.092, 0.107

DISCUSSION

By the conventional approach (Figure 1), in which the reference cell contains only solvent, it is difficult to distinguish pure ethchlorvynol from that containing 1% of ketone. The differential approach, however, achieves a surprisingly sharp separation of the ketone absorption at 5.93 microns. Figure 2 shows typical determinations for ketone at 1.0 and 0.1% levels.

Table I shows the results obtained on several synthetic mixtures of known ketone content in ethchlorvynol. The duplicate results at each concentration represent one synthetic blend. Results on the synthetic mixtures show that the accuracy in all cases is better than ±0.02% and that the precision in all cases is better than ±0.01%.

The importance of instrumental operating conditions, particularly those factors employed to compensate for the large loss of energy in differential analysis, such as increasing slit widths and energy to source, are well explained elsewhere (*5*, *7*). However, it should be emphasized that, in order to obtain high precision, it is necessary to duplicate gain or sensitivity carefully, and to limit the rate of scan as described.

It is not necessary to match the cells exactly, because in making this determination, differences in cell thickness are eliminated by running the unknown in the sample cell against the solvent plus major component in the blank cell, and then reversing solutions to the opposite cells. This operation simultaneously expands the scale by a factor of 2.

Inasmuch as the differential method is much more subject to error due to small amounts of interfering substances than is the standard approach, typical trace materials were added to ethchlorvynol of known ketone content to determine what effect these might have on the determination. None of these added substances introduced an error of greater than 0.1%. The effects of interferences in this determination can be greatly reduced by noting the deviation of the pen from an absorbance of 0.250 unit at the start of the scan, and subtracting this amount from the total absorption at 5.93 microns. This type of correction assumes linearity of interference throughout the scan and is in effect, applying the theory of the base line measurement.

In differential analysis, Beroza (*2*) stressed the advisability of using a reference standard as close to the composition of the unknown as possible. This is advisable in many cases, but no effort was made to match composition of the unknown in this instance, because variation in the major component amounts to no more than 5% and the interferences encountered in the trace impurities are not serious.

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X-ray Absorption Edge Spectrometry as an Analytical Tool

Determination of Molybdenum and Zinc

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▶ By using the continuous x-ray radiation from a copper x-ray diffraction tube, in combination with a modern x-ray spectrometer, it was possible to make x-ray absorption measurements on materials as a function of monochromatic wave length. By making measurements near, and on each side of, an absorption edge, a specific elemental scheme of analysis is possible for the element characterized by the edge. The method is independent of the other atoms present, specific and nondestructive. At the higher concentrations, the method is preferred to x-ray fluorescence because of the absence of a matrix effect. It is not as sensitive as fluorescence at the trace level. Calibration equations and methods of measurement are presented for the determination of molybdenum and zinc. The absorption technique has been successfully used for determining molybdenum and zinc in liquid hydrocarbons, lead and bromine in engine combustion chamber deposits, and molybdenum, nickel, and platinum in solids.

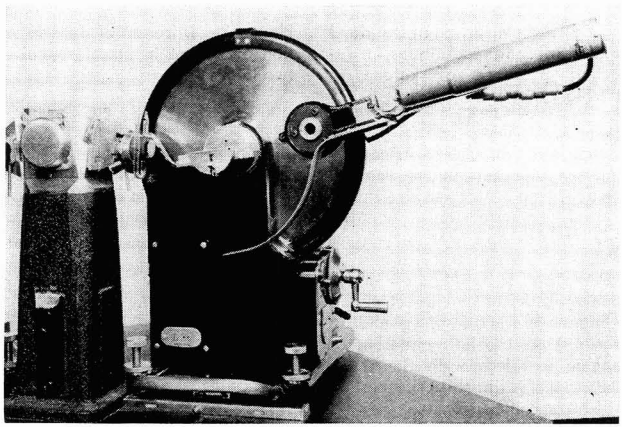

Figure 1. Apparatus for making x-ray absorption edge measurements on liquids

RECENT IMPROVEMENTS in x-ray equipment for elemental analysis have mainly been applicable to fluorescence. However, the determination of a set of working curves for the analysis of a multicomponent system by means of x-ray fluorescence is tedious and time-consuming, because there is a matrix effect—i.e., the response of an element depends not only on its own concentration but also on the other elements present. This is not a particular hardship if the samples to be analyzed are all of the same type—for example, production samples. But in a research analytical laboratory, all kinds of samples must be analyzed. In some cases, wet chemical methods are used because this is more economical than establishing the x-ray fluorescent working curves for the system.

A scheme of analysis, based on the discontinuity in the x-ray mass absorption coefficient at an edge, is known to be free of any matrix effect. This method was first developed by Glocker and Frohnmayer (*4*) over 30 years ago. Liebhafsky (*6–11*) has written several reviews covering the latest work in this field. Liebhafsky and Winslow (*12*) have written the most recent review. Hughes and Hochgesang (*5*) have demonstrated the feasibility of the method in the determination of tetraethyllead in gasolines. Ferro and Galotto (*3*) have used absorption spectrometry across the L_{III} absorption edge of lead for determining tetraethyllead in gasolines. The author has applied the absorption edge technique, using a modern x-ray goniometer, and this paper is a report of his initial work using this method.

EXPERIMENTAL APPARATUS AND TECHNIQUE

The x-ray source used was a copper target x-ray tube. By mounting a single crystal of lithium fluoride at the center of the goniometer of a North American Philips x-ray diffractometer, the diffractometer becomes an x-ray spectrometer; and measurements may be made at continuously variable wave lengths. Ordinarily, the characteristic copper K_α x-ray is used to determine diffraction patterns of powdered materials. However, every x-ray tube also generates a continuous background of x-rays up to the maximum energy of the x-ray tube voltage. Exploratory measurements showed that there was sufficient x-ray intensity of the continuous background wave lengths to be useful. The resolution of the spectrometer was such that the width at half maximum of the copper $K_{\alpha 1}$ x-ray was 0.06° to 0.07° of 2θ, which corresponds to 0.14% of the energy of the copper x-ray.

Figure 1 shows the arrangement used to make absorption edge measurements on liquids containing molybdenum.

The liquid sample holder was constructed by cementing two beryllium windows, 0.010 inch thick, to a glass cell made from tubing approximately

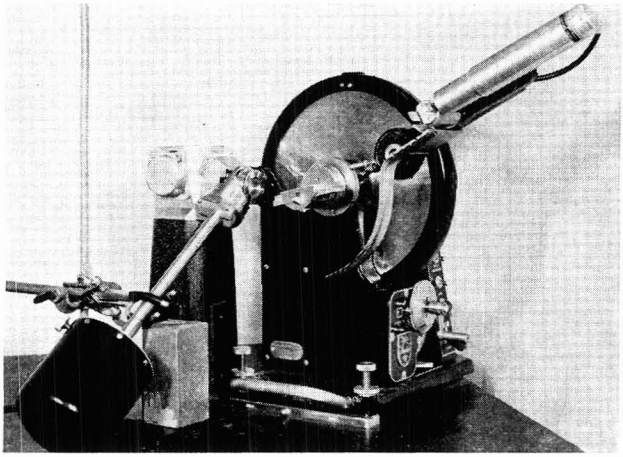

Figure 2. Apparatus for making x-ray absorption edge measurements on solids

0.75 inch in diameter and 0.62 inch long. A similar cell was constructed for making measurements on liquids containing zinc, except it was only 0.16 inch long to compensate for the increase in absorption at the higher wave length. The liquid sample holder was made to fit the holder of the usually used divergent slit. The ordinary Soller collimators were retained behind the sample and behind the detector slit. During the measurements a piece of brass sheet 1 mm. thick was mounted vertically to shield the operator from scattered radiation.

Figure 2 shows the experimental arrangements used to make x-ray absorption edge measurements on solids.

In analyzing hydroformer catalyst (8 to 10% MoO_3), the catalyst is ground in a mechanical mixer and grinder for at least an hour, and then 1 gram of solid is pelleted into a 1-inch briquet, which is then placed in the rotating sample holder shown in Figure 2. The use of the holder tends to reduce the effect of inhomogeneities in the sample.

The rotating holder is commercially available and is manufactured for mounting in the center of the diffractometer, so that powder samples may be rotated during the recording of x-ray diffractions patterns. It was easily adapted to absorption measurements.

The sample, either liquid or solid, can be placed in the x-ray beam either between the x-ray tube and the analyzing crystal or between the analyzing crystal and the detector. The former arrangement was used, as it was felt that under such conditions it would be much less probable that any of the scattered radiation would reach the detector.

Figure 3 shows the x-ray absorption spectrum of a liquid hydrocarbon containing molybdenum. The intensity of the transmitted beam is shown as a function of x-ray wave length. A sudden change in absorption occurs at 0.620 A., the K absorption edge of molybdenum. Each element has its own characteristic K absorption edge. The wave length at which a sudden change in absorption occurs can thus be used to identify an element present in a sample, and the magnitude of the change can be used to determine the amount of the particular element present.

THEORETICAL CONSIDERATIONS

The fundamental transmittance equation for monochromatic, collimated x-rays is

$$I/I_0 = \exp(-\mu t) = \exp(-\mu_M G) \quad (1)$$

where I is the intensity of the beam after passing through the sample, I_o is the intensity of the beam with the sample removed, μ is the linear absorption coefficient, t is the thickness in centimeters, ρ is the density of the sample in grams per cubic centimeter, $\mu_M = \mu/\rho$ is the mass absorption coefficient, and $G = \rho t$ in the mass thickness of the sample in grams per square centimeter.

If transmittance measurements are made on each side of an absorption edge and extrapolated to the edge, there results

$$\ln (I_0/I)' = \mu_M' G \quad (2)$$

and

$$\ln (I_0/I)'' = \mu_M'' G \quad (3)$$

and, if there is no discontinuity in the primary beam,

$$\ln (I''/I') = (\mu_M' - \mu_M'')G \quad (4)$$

where $(I_0/I)'$ is the extrapolated value on one side of the edge and $(I_0/I)''$ is the extrapolated value on the other side of the edge. μ_M' and μ_M'' are the two extrapolated mass absorption coefficients of the sample at the edge.

The mass absorption coefficient of any material is given by

$$\mu_M = \mu_{M_1} W_1 + \mu_{M_2} W_2 + \mu_{M_3} W_3 + \ldots \quad (5)$$

where μ_{M_1} is the mass absorption coefficient of element 1 and W_1 is its weight fraction; μ_{M_2} is the mass absorption coefficient of element 2 and W_2 is its weight fraction; and so on for all the elements present. Because only one element, say 1, has a change in mass absorption coefficient at the edge, there results

$$\mu_M' = \mu_{M1}' W_1 + \mu_{M_2} W_2 + \mu_{M_3} W_3 + \ldots \quad (6)$$

$$\mu_M'' = \mu_{M1}'' W_1 + \mu_{M2} W_2 + \mu_{M_3} W_3 + \ldots \quad (7)$$

and

$$(\mu_M' - \mu_M'') = (\mu_{M_1}' - \mu_{M_1}'')W_1$$

and the following relationship is finally obtained (*6*):

$$\ln I''/I' = (\mu_{M_1}' - \mu_{M_1}'')W_1 G \quad (8)$$

Then the important result is obtained that the logarithm of the ratio of beam intensities on the two sides of an absorption edge depends only upon the change in mass absorption coefficient of the element characterized by this edge and on the amount of the characteristic element in the beam. The ratio of transmittances at the edge is independent of the other elements present—i.e., there is no matrix effect. This result gives the absorption edge technique an advantage over x-ray fluorescence analysis in some cases.

Instead of making a series of measurements and extrapolating to the edge, it was decided for the sake of speed to make only a single attenuation measurement on each side of the edge. The theoretical equation for this case is derived in the following manner (*8*):

We have

$$\ln I_0'/I' = \mu_M' G \quad (9)$$

and

$$\ln I_0''/I'' = \mu_M'' G \quad (10)$$

where the primes and double primes distinguish measurements on each side of the edge.

$$\mu_M' = \mu_{M_1}' W_1 + \mu_{M_2}' W_2 + \mu_{M_3}' W_3 + \ldots \quad (11)$$

$$\mu_M'' = \mu_{M_1}'' W_1 + \mu_{M_2}'' W_2 + \mu_{M_3}'' W_3 + \ldots \quad (12)$$

It is assumed that only element 1 has a discontinuity in absorption between the two measurements, and that

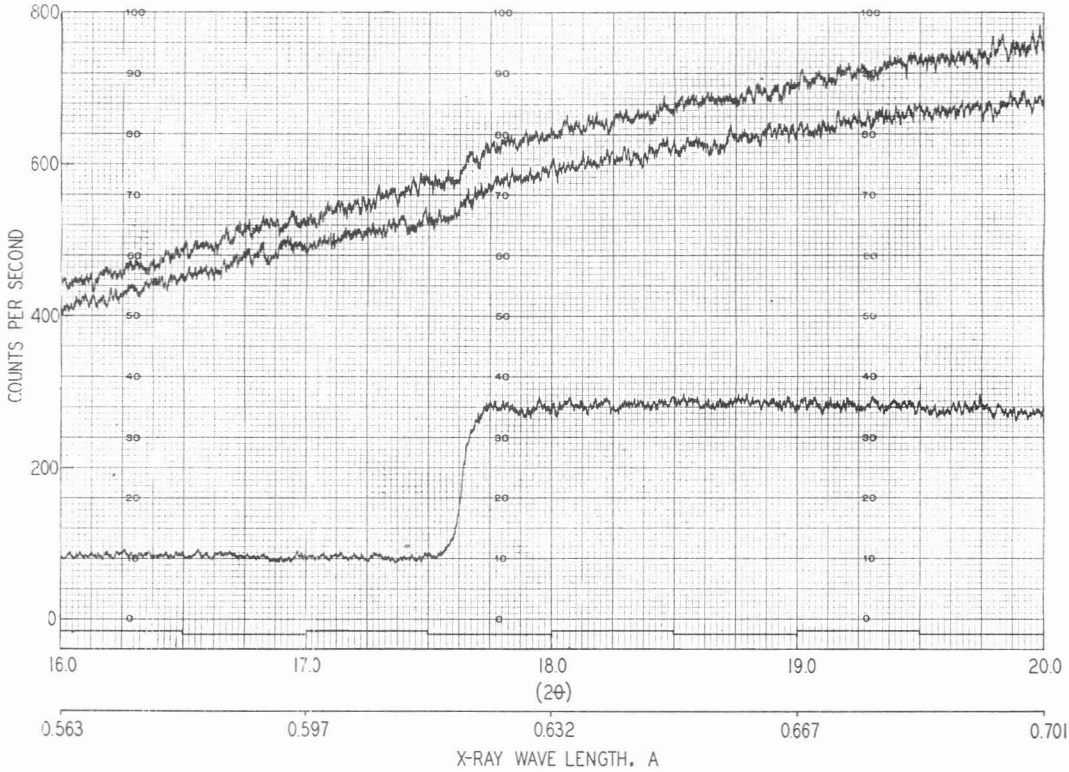

Figure 3. X-ray absorption spectra of hydrocarbon containing molybdenum

Liquid sample cell. 1.13 grams of Mo per 100 cc. Liquid path length 1.605 cm. 35.0 kvp. 11.0 ma. LiF crystal. No divergence slit, 0.003-inch detector slit, 1/4 ° scatter slit. Scale 16. Multiplier 1. Time 4. Scanning rate 1/8 ° (2 θ) per minute

Upper. Primary x-ray beam *Center.* Empty cell with Be windows *Lower.* Filled sample cell

the mass absorption coefficients of all other atoms change by the same factor when the measurement is changed from one side of the edge to the other. That is, it is assumed that

$$\mu''_{M_2}/\mu'_{M_2} = \mu''_{M_3}/\mu'_{M_3} \text{ etc.} = k = (\lambda''/\lambda')^a \quad (13)$$

where λ' and λ'' refer to the two wave lengths at which the measurements are made and a is a constant. Then

$$\ln I'_0/I' = (\mu'_{M_1}W_1 + \mu'_{M_2}W_2 + \mu'_{M_3}W_3 + \ldots)G \quad (14)$$

$$\ln I''_0/I'' = (\mu''_{M_1}W_1 + k\mu'_{M_2}W_2 + k\mu'_{M_3}W_3 + \ldots)G \quad (15)$$

Multiplying the first of these equations through by k and then subtracting, there results

$$\ln I''_0/I'' - k \ln I'_0/I' = (\mu''_{M_1} - k\mu'_{M_1})W_1G \quad (16)$$

or

$$W_1 = \frac{1}{(\mu''_{M_1} - k\mu'_{M_1})G}(\ln I''_0/I'' - k \ln I'_0/I') \quad (17)$$

In so far as all elements do not have exactly the same value of k, or a, this method is not completely independent of the other elements present. However, for practical purposes the results are independent of the matrix.

EXPERIMENTAL RESULTS

The experimental instrument conditions are listed in Table I. During the molybdenum determinations, the x-ray tube was operated at a peak voltage of 35.0 kv.; during the zinc determinations, the peak voltage was 17.0 kv. Under these conditions, it was impossible to generate harmonics of the characteristic absorption edge. This ensured that when the analyzing crystal was set to reflect at the edge, a single monochromatic beam would be detected, no $\lambda/2$ wave lengths being generated in the x-ray tube.

The detector was an argon-filled, chlorine-quenched Geiger tube. The in-

Table I. Experimental Conditions

	Molybdenum	Zinc
X-ray voltage, kvp.	35.0	17.0
Detector slit, inch	0.003	0.003
Scatter slit	1/30°	1/4°
2θ values	17.00° and 18.25°	36.75° and 37.25°
Liquid cell path length, cm.	1.605	0.403
Monochromator	LiF	LiF

tensities were measured by scaling for 64 seconds with the mechanical register on a scale of 64, giving counts per second directly on the mechanical register. This was usually repeated three times on each intensity to be measured. The total number of counts was always greater than 10,000. All counting rates were corrected for coincidence losses by means of the relation (*2*)

$$x/y = \exp \tau x \qquad (18)$$

where x is the true counting rate, y is the observed counting rate, and τ is the effective dead time. The effective dead time was determined with a special device constructed for this purpose (*1*). The effective dead time was 265 microseconds during the molybdenum measurements and 200 to 300 microseconds during the zinc measurements. The results for the zinc determinations indicated there was some variation of dead time with count rate. This was taken into account in the corrections.

Table II. Ratio of Mass Absorption Coefficient at 17.00° (2θ) to Coefficient at 18.25° for Various Pure Substances

Material	$= \left(\frac{\lambda_{17.00}{}^{a}}{\lambda_{18.25}}\right)$ k	a
Graphite	0.90 ± 0.03	1.5 ± 0.5
Wax	0.90 ± 0.03	1.5 ± 0.5
Iso-octane	0.89 ± 0.03	1.7 ± 0.5
Mixed xylenes	0.94 ± 0.03	0.9 ± 0.5
Water	0.86 ± 0.01	2.2 ± 0.2
Alumina	0.82 ± 0.02	2.9 ± 0.4
Sulfur	0.83 ± 0.01	2.7 ± 0.2
Calcium oxide	0.82 ± 0.02	2.9 ± 0.4
Zinc	0.81 ± 0.01	3.0 ± 0.2
Lead oxide + starch	0.84 ± 0.01	2.5 ± 0.2

If the sample does not contain molybdenum or zinc,

$$\ln I_0''/I'' - k \ln I_0'/I' = 0 \qquad (19)$$

and

$$k = \frac{\ln I_0''/I''}{\ln I_0'/I'} \qquad (20)$$

By making measurements on pure materials on each side of the edge, k, and thus a, may be determined.

Table II gives the values of k for various pure substances determined experimentally across the molybdenum k edge. The measurements on liquids were corrected for the attenuation of the beryllium windows. The uncertainty for each k value was calculated assuming the uncertainty in intensity measurements was 1%. The values of a, in Table II, were calculated from the defining formula, $k = (\lambda''/\lambda')^a$. From the k values listed, $k = 0.91$ was selected for measurements on molybdenum in liquid hydrocarbons and $k = 0.83$ for measurements on molybdenum in alumina-type catalysts —e.g., hydroformer catalyst.

In the case of the zinc measurements, the two angles were 36.75° and 37.25°, which were closer to the edge and k was, therefore, closer to unity. A k value of 0.98 was used for the zinc measurements.

Using the liquid sample cells, measurements were made on various concentrations of molybdenum and zinc in liquid hydrocarbons. In each case, the starting material was a sample of a hydrocarbon-soluble compound that had been analyzed by chemical methods. The material was diluted to measured volumes with mixed xylenes. Corrections were made for the absorption of the beryllium windows. Equation 17 was adapted to measurements on liquids by letting

X = grams of molybdenum or zinc per 100 cc. in liquid hydrocarbon
t = liquid path length, cm.

$$X = W_1 G \times 100/t \qquad (21)$$

or

$$X = \frac{100 \times 2.303}{(\mu''_{M1} - k\mu'_{M1})t} (\log_{10} I_0''/I'' - k \log_{10} I_0'/I') \qquad (22)$$

From the measurements on each sample of liquid and the known composition, X, a value of

$$\frac{100 \times 2.303}{(\mu''_{M1} - k\mu'_{M1})t} = \frac{X}{(\log_{10} I_0''/I'') - k(\log_{10} I_0'/I')} \qquad (23)$$

was calculated. The details of a sample calculation are given in Table III.

A background correction of 0.6 count per second was applied to each measurement.

In Table III

$I°$ is initial beam intensity
I is residual beam intensity after passing through sample and two beryllium windows of sample cell
I_0 is residual beam intensity after passing through two beryllium windows of empty sample cell
$I/I°$ is transmittance of sample and two beryllium windows of sample cell
$I_0/I°$ is transmittance of empty sample cell

The absorbance of the sample ($\log_{10} I_0/I$) is calculated from I_0/I, which is obtained by dividing the above $I_0/I°$ by $I/I°$. While the measurements could be made in such a manner that it would not be necessary to determine the initial beam intensity, $I°$, we prefer to repeat the transmittance measurements several times on the sample and the two beryllium windows. We then independently determine the transmittance of the two beryllium windows. This allows duplicate measurements to be made without emptying the sample cell between measurements.

The values of

$$\frac{100 \times 2.303}{(\mu''_{M1} - k\mu'_{M1})t}$$

calculated from the measurements on each liquid, were weighted according to the size of X and averaged. The average is 2.330 for the molybdenum determinations and 2.427 for the zinc. The calibration equations then are

$$X, \text{ g. Mo/100 cc.} = 2.330 (\log_{10} I_0/I)_{17.00} - 0.91 (\log_{10} I_0/I)_{18.25} \qquad (24)$$

$$X, \text{ g. Zn/100 cc.} = 2.427 (\log_{10} I_0/I)_{36.75} - 0.98 (\log_{10} I_0/I)_{37.25} \qquad (25)$$

The observed and calculated values from the calibration curves are compared in Table IV.

Table III. Transmittance Measurements on Liquid Molybdenum Standard

(X = 1.125 grams of Mo per 100 cc., 35.0 kvp., 20 ma., detector slit 0.003 inch, no divergency slit, 1/30° scatter slit)

	2θ 18.25	17.0
I corrected, c./s.	155.4	41.65
$I°$ corrected, c./s.	408.9	302.3
$I°/I$	2.631	7.258
$I°/I_0$	1.103	1.080
I_0/I	2.385	6.720
$\log_{10} I_0/I$	0.3775	0.8274
$0.91 (\log_{10} I_0/I)_{18.25} =$		0.3435
$(\log_{10} I_0/I)_{17.00} - 0.91 (\log_{10} I_0/I_{18.25} =$		0.4839
$\frac{X}{(\log_{10} I_0/I)_{17.00} - 0.91 (\log_{10} I_0/I)_{18.25}} =$		2.325

The results in Table IV indicate that molybdenum and zinc can be determined to an uncertainty of the order of 0.01 to 0.02 gram per 100 cc. If measurements are made at concentrations of 1 gram per 100 cc., molybdenum and zinc can be determined to about 1% or 2% of the amount present.

Table IV. Comparison of Observed and Calculated Molybdenum and Zinc Content of Standard Solutions

X, Obsd.	X, Calcd.	X, Obsd. − X, Calcd.
	Molybdenum	
0.000	0.016	−0.016
0.274	0.273	+0.001
0.541	0.549	−0.008
0.855	0.846	+0.009
1.125	1.127	−0.002
	Zinc	
0.000	−0.007	+0.007
0.250	0.235	+0.015
0.499	0.482	+0.017
0.750	0.765	−0.015
1.002	1.012	−0.010
1.253	1.244	+0.009
1.253	1.263	−0.010
1.253	1.260	−0.007

After the molybdenum calibration curve was established, the mass absorption coefficient of pure molybdenum was determined using foils 0.001 and 0.003 inch thick. The samples were disks 1 inch in diameter, which were obtained by placing several thicknesses of foil between two pieces of brass in a lathe. With pressure from the tail stock, it was possible to machine the foils to 1-inch diameter. The mass thickness in grams per square centimeter was calculated from the weight and measured diameter of the disks. Measurements were made using two different Geiger tubes, one argon-filled and the other krypton-filled. Effective dead times were determined for each tube. The absorption values obtained are given in Table V. The values listed should be accurate to better than 1%. Substituting the values of μ_M from Table V in Equation 23 and using a value of k of 0.91 as before, there results

Table V. Mass Absorption Coefficients of Molybdenum

2θ	λ, A.	μ_M
17.00	0.597	74.2
18.25	0.640	13.78

$$\frac{100 \times 2.303}{(\mu''_{M1} - k\mu'_{M1})t} = 2.327 \qquad (26)$$

This is to be compared with the value of 2.330 based on the original wet chemical analysis of the most concentrated molybdenum solution.

The application of this absorption technique to the analysis of solids is illustrated by the results given in Table VI on the determination of molybdenum in cobalt-molybdenum-alumina catalysts. Measurements were made on 1 gram of material in 1-inch briquets. After the absorption measurements, the whole pellets were dissolved and analyzed by wet chemical methods. The absorption results are about 4% lower than the wet chemical results. The cause of the discrepancy is not known. The x-ray results are based upon metallic molybdenum, while the wet chemical method was checked against a National Bureau of Standards molybdenum standard of calcium molybdate. The wet chemical method gave 34.9% molybdenum, while the NBS certified value was 35.3%.

Table VI. Comparison of Absorption Edge and Wet Chemical Molybdenum Analyses of Cobalt-Molybdenum-Alumina Catalysts

Sample No.	Weight %, MoO_3 X-ray absorption	Wet chemical	Ratio
1	10.6	11.1	1.05
2	11.7	12.0	1.03
3	11.4	11.9	1.04
4	11.4	11.8	1.04

The absorption edge technique has been used to determine lead and bromine in engine combustion chamber deposits. It is necessary to mix these samples with a binder because of the small sample required. Starch has usually been used, but graphite has the advantage of being very transparent to x-rays. The accuracy of the binder technique was checked on the NBS standard sample of calcium molybdate. The standard sample was diluted to 20 weight % with starch, mixed in a mechanical mixer and grinder, and pressed into 1-inch-diameter pellets. Based on nine determinations on three different 1-gram pellets, the molybdenum was determined as 34.8 weight %, with an average deviation of 0.5%. The certified value from the National Bureau of Standards is 35.3%.

In order to increase the number of analyses and their sensitivity, the Geiger tube detector has recently been replaced with a scintillation counter, and a second goniometer has been installed on the top of the x-ray power supply. X-ray absorption edge analyses can now be carried out and x-ray diffraction patterns obtained at the same time. X-ray intensities are determined with an Atomic Instrument Model 1070A Multiscaler in combination with an Atomic Model 510 pulse height analyzer. The pulse height analyzer allows the x-ray tube to be operated at higher voltages, thus getting higher intensities, and consequently higher sensitivity, because the harmonics reflected by the lithium fluoride crystal are discriminated out electronically.

The results presented show that the x-ray absorption edge technique provides a useful means of elemental analysis. At high concentration levels, it is to be preferred over x-ray fluorescence because of the lack of a matrix effect. It is not as sensitive as x-ray fluorescence, however. If an x-ray laboratory has modern diffraction equipment, absorption edge analyses can be added more economically than can fluorescence. Because transmittances are measured, the x-ray intensity does not need to be adjusted to exactly the same value for each analysis.

The absorption technique is inherently more accurate than the emission spectrograph because of the greater stability of x-ray sources compared to spectrographic sources. With equipment at present available, each element must be determined separately, while with the spectrograph several elements can be determined with a single excitation. The elements of lowest atomic number are very difficult to determine by x-rays but offer no appreciable difficulty with the spectrograph.

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Determination of Trace Amounts of Arsenic in Petroleum Distillates

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▶ **Arsenic in petroleum fractions can be determined by a reflectometric method in concentrations as low as 1 p.p.b. In principle, the method depends upon wet oxidation, followed by isolation of the arsenic on paper as the colored reaction product between arsine and mercuric bromide. The intensity of the resulting colored complex is evaluated quantitatively with a spectrophotometer fitted with a diffuse reflectance assembly. Recoveries of 90% or better were obtained in most cases on samples containing from 0.1 to 0.5 γ of arsenic. The standard deviation is within the range of 0.020 to 0.025 γ of arsenic. Interference by most of the common metals, except antimony above 100 γ, is not a problem.**

WITH THE DEVELOPMENT of catalytic reforming processes, it has become necessary to devise and refine analytical techniques for the determination of certain trace elements that may affect the activity of the catalyst. Important among the contaminating constituents when platinum catalysts are used is arsenic, which may be introduced during the crude oil production operations and carried through in the distillate fractions prepared as charge stocks for reforming. Depending upon the arsenic content of the charge, the catalyst may become deactivated to an extent where it is no longer feasible or economical to maintain operations, necessitating replacement of the catalyst inventory at considerable cost. For this reason, an accurate and reliable method for the determination of arsenic in concentrations of the order of a few parts per billion is needed as a control for the quality of reforming charge stocks.

Numerous methods have been described for the determination of low concentrations of arsenic in various materials. The preferred colorimetric procedure appears to be one based on the formation of molybdenum blue (*3, 5, 8–10, 12*). Although the method is simple and reproducible, it necessitates the use of large sample sizes when applied in the part per billion range. A more sensitive test for arsenic is the familiar Gutzeit reaction which has been used by a number of investigators (*4, 7, 11–13*). This method depends upon the evolution of arsine and its reaction with mercuric bromide to form a colored complex. Satterlee and Blodgett (*11*) described an ultramicro procedure whereby the reaction is concentrated by vacuum filtration through a gaged area of the sensitized medium. The method uses photographic reference scales adaptable to either visual or densitometric evaluation of the spot reactions. Jay and Dickson (*6*) simplified the Satterlee and Blodgett procedure and applied it successfully to petroleum products. Although the latter method has been used with limited success at this laboratory, it retains the undesirable feature of a visual comparison of test disks with standards.

The task of extending the application of the Gutzeit reaction to trace amounts of arsenic using impregnated strips, strings, or disks together with visual comparison embraces many factors. These include such difficulties as the appraisal of stains due to the unequal distribution of the arsenic–mercuric bromide complex and the occasional intensification of color in localized areas. The necessity of analyzing standards with each set of samples to compensate for operational variables unnecessarily complicates the analysis.

The method described here is a modification of that of Jay and Dickson (*6*). Use of their technique led to incomplete recovery of arsenic with the relatively large sample sizes involved. An improved digestion procedure is described which allows for more intimate contact between sample and acids and yields quantitative recovery of arsenic. A Beckman Model DU spectrophotometer equipped with a diffuse reflectance attachment is used to evaluate the intensity of the colored spot.

APPARATUS

The digestion apparatus consists of a three-necked, round-bottomed borosilicate flask of 500-ml. capacity, fitted with a precision bore bearing stirring unit (Ace Glass Co., Inc.) and two reflux condensers.

The evolution apparatus consists of a 250-ml. borosilicate Erlenmeyer flask connected to a hydrogen sulfide trap by means of a ground-glass joint. The trap is fabricated from a 75-mm. section of 11-mm. glass tubing having a 24/40 ⅀ inner ground-glass joint at one end and a 14/35 ⅀ joint at the other end.

Filter clamp assembly (*6*) fabricated from Plexiglas and fitted with two Teflon gaskets.

A 550-watt electric heater fitted with a Transite top having a hole 3 inches in diameter and connected to a variable transformer.

Open end manometer fitted with a bleeder valve.

Vacuum pump.

Water bath maintained at 50° ± 1° C., fitted with a suitable wire mesh rack for supporting the evolution flasks.

Beckman DU spectrophotometer equipped with the diffuse reflectance attachment. The accessory is fitted with masking plates fabricated from 1/32-inch stainless steel having a centered 1/4-inch hole reamed to ±0.000. The disks are cut to fit the carriage receptacles to ±0.001 inch and are sprayed with optical flat black paint.

REAGENTS AND SOLUTIONS

Isopropyl alcohol, 99%.

Hydrogen peroxide, 30%.

Glass wool.

Distilled water.

Ammonium oxalate, saturated aqueous solution.

Lead acetate solution prepared by dissolving 17.5 grams of lead acetate trihydrate in water containing 5 ml. of glacial acetic acid and diluting to 100 ml.

Mercuric bromide solution prepared by dissolving 2.5 grams of mercuric bromide in 95% ethyl alcohol and diluting to 25 ml. The solution is stored in a borosilicate, glass-stoppered, amber bottle.

Nitric acid, specific gravity, 1.42, arsenic content less than 0.0000001%.

Sulfuric acid, specific gravity, 1.84, arsenic content less than 0.0000001%.

Potassium iodide solution, 15%.

Filter disks prepared by cutting Schleicher and Schuell No. 575 filter paper into disks approximately 5/8 inch in diameter.

Sensitized filter disks. Prepare a supply of disks, sufficient for 1 week, as follows. Place the filter disks in a small amber jar and cover with 25 ml. of

mercuric bromide solution. Evacuate for 1 hour at approximately 0.5 atm. The disks may be safely kept for 1 week if protected from bright light and the atmosphere.

Standard arsenic solution, prepared by dissolving 1.320 grams of primary standard arsenous oxide in 25 ml. of sodium hydroxide solution (250 grams per liter) and diluting to 1 liter with water. For calibration purposes, the stock solution is diluted 1 to 10,000 with water.

Stannous chloride solution, 40% in hydrochloric acid (specific gravity, 1.19).

Sulfuric acid, 1 to 1.

Zinc metal. Screen 20-mesh zinc, containing less than 0.00001% arsenic, through a 20- and then a 30-mesh sieve. Transfer the zinc remaining on the 30-mesh screen to a beaker and cover with water. Activate it by placing the beaker on a hot plate, adding 50 ml. of concentrated hydrochloric acid, and stirring until the zinc becomes bright in appearance. Decant the acid solution and wash the pickled zinc with hot water until the washings react neutral to litmus paper. Store the activated zinc under distilled water. Just prior to use transfer the zinc to a small Büchner funnel, rinse with several small portions of acetone, and dry on the funnel with vacuum.

EXPERIMENTAL

Seasoning of Glassware. New digestion equipment is usually contaminated with small amounts of arsenic and must be seasoned with hot acids as follows.

Place 50 ml. of concentrated sulfuric acid and 10 ml. of nitric acid in each digestion flask and attach the water condensers and stirrer units. Place the flasks on heaters and reflux for 4 hours. Remove the condensers and allow the seasoning to proceed for an additional 3 hours. After the flasks are cool, discard the acid and thoroughly rinse the digestion equipment with water.

Calibration Curve. Pipet 1, 3, and 5 ml. of the diluted arsenic standard into separate evolution flasks; add 5 ml. of distilled water to a fourth flask as a blank. To each flask add 35 ml. of distilled water, 25 ml. of 1 to 1 sulfuric acid, and 20 ml. of isopropyl alcohol. Place the flasks in the constant temperature bath and allow to equilibrate. Add 5 ml. of potassium iodide to each flask and allow to equilibrate again. Finally, pipet 1 ml. of stannous chloride solution into each flask. Insert a loose plug of glass wool into the lower half of each absorption tube and moisten with lead acetate solution. Insert a loose plug of cotton in the upper half of the tube and in the tapered portion of the Plexiglas assembly. Blot a sensitized disk between filter paper, air dry, place between the two Teflon gaskets, and assemble the apparatus. Introduce 3 grams of zinc into the flasks, then immediately attach the hydrogen sulfide absorption tubes and clamp assemblies. Adjust the vacuum to 190 ± 5 mm. of mercury and allow the reaction to proceed for 30 minutes. With a 2-mm. slit width, measure the reflectance of the test spot against a clean, unsensitized disk at 400 mμ. Plot the reflectance readings, expressed as absorbance, *vs.* total micrograms of arsenic.

Procedure. Introduce a weighed amount of sample, equivalent to 0.1 to 0.5 γ of arsenic, into a seasoned digestion flask. Add 25 ml. of concentrated sulfuric acid and assemble the apparatus. Add approximately 5 ml. of nitric acid and stir the contents vigorously for 30 minutes. Then apply heat, gradually increasing the input while allowing the digestion to proceed for 2.5 hours. Add 1-ml. portions of nitric acid whenever the mixture begins to darken. Remove one condenser from the flask and continue the addition of small increments of nitric acid as darkening occurs, until the lighter nonoxidized fraction is completely volatilized. At this point add 2 ml. of 30% hydrogen peroxide to each flask, followed by nitric acid if darkening occurs. Repeat until the acid remains colorless or light amber.

After the flasks have cooled to room temperature, add 75 ml. of water and 10 ml. of ammonium oxalate solution. Heat the flasks until dense white fumes of sulfur trioxide are evolved. Then cool the digestion mixtures to room temperature and transfer into separate evolution flasks. If the concentration of arsenic in the sample is suspected of being less than 2 p.p.b., pipet 1 ml. of the diluted standard arsenic solution into the evolution flasks containing the sample. Prepare a spot reaction as described for calibration, beginning with the addition of 20 ml. of isopropyl alcohol. Convert the reflectance measurements to micrograms of arsenic by means of the calibration curve, correcting for added arsenic if necessary.

Determine the magnitude of the method blank, which consists of arsenic inherently present in the reagents as well as in the glassware, in the same way. If the value exceeds 0.025 γ of arsenic it is indicative of contamination of the reagents, improper seasoning of glassware, or both.

DISCUSSION

A sample size should be chosen so that the total arsenic content falls between 0.1 and 0.5 γ. The maximum size sample which can be handled conveniently is approximately 40 grams. Based on the recommended maximum sample size, the lower limit of the method without the addition of the standard arsenic solution is approximately 2 p.p.b.

Digestion Studies. In the search for a method sensitive to about 1 p.p.b. of arsenic, several digestion procedures were tried. One method involved the extraction of arsenic from petroleum distillates with sodium hypochlorite and 72% sulfuric acid, followed by oxidation of the extract with nitric acid and hydrogen peroxide. The evolved arsine was reacted on mercuric halide–impregnated paper strips and evaluated visually. The procedure, although simple and rapid, was unsatisfactory from the standpoint of recovery of arsenic.

The next digestion study embraced a wet oxidation method (*6*). Specifically, the technique consisted of oxidizing the sample with sulfuric and nitric acids and hydrogen peroxide in a Kjeldahl flask fitted with a water condenser. The digestion was simple to perform but, when applied to standards prepared from triphenylarsine and iso-octane, was erratic and gave low results (Table I). However, the data indicated that increasing amounts of arsenic were recovered as the sample size was diminished. This suggested that more intimate contact between sample and acid was necessary to determine arsenic concentrations of the order of a few parts per billion. To obtain this intimate contact, the digestion mixtures

Table I. Arsenic Recovery with Nonstirred Mixtures

Sample, Grams	Arsenic, P.P.B. Added	Found	Av. Recovery, %
20	41	25 25	61
15	71	25 30 35 40	46
10	115	70 24 60	44
5	163	86 100	57
2	371	340 390 300 306	90

Table II. Effect of Glassware Seasoning

(Triphenylarsine–iso-octane blends)

Arsenic Added P.p.b.	Arsenic Added γ	Arsenic Found, γ Before seasoning	Arsenic Found, γ After seasoning[a]
5.2	0.126	0.286	0.120
		0.170	0.101
		0.163	0.130
		0.191	0.124
		0.172	0.104
		0.150	0.116
13	0.227	0.242	0.222
		0.310	0.197
		0.232	0.193
		0.266	0.213
15	0.264	0.363	0.249
		0.346	0.240
		0.267	0.257

[a] Std. dev., 0.021 γ.

Table III. Arsenic Recovery with Stirred Digestion Mixtures

Sample	Arsenic Added, P.p.b.	Arsenic Added, γ	Arsenic Found, γ
Percolated naphtha	1.1	0.048	0.027
			0.034
			0.033
			0.024
			0.026
			0.034
	3.1	0.115	0.089
			0.116
			0.109
			0.107
			0.082
			0.098
	5.3	0.196	0.190
			0.156
			0.146
			0.198
			0.216
			0.189
Base naphtha (contained 20 p.p.b. of arsenic; sample taken was equivalent to 0.180 γ arsenic)	6.2	0.229 (0.409, total)	0.402
			0.438
			0.368
			0.432

Std. dev., 0.025 γ.

were *stirred* in a 500-ml. flask fitted with a precision bore stirrer unit to minimize possible loss of volatile arsenic compounds. When this apparatus was first tested, high results for arsenic were obtained. Further study showed that the glassware had to be well seasoned before use (Table II).

A number of determinations were carried out on a naphtha that was previously percolated through silica gel to remove arsenic compounds. This percolate was then blended with known amounts of triphenylarsine in the range from 1 to 5 p.p.b. of arsenic. The arsenic content was also established for the base naphtha and triphenylarsine was then added. Table III shows the results obtained on the base and percolated naphthas.

As the presence of coke or carbon in the digestion mixture enhances the formation of volatile, reduced valence arsenic compounds, the oxidation must not be allowed to proceed beyond incipient darkening before nitric acid is added. Experimental work demonstrated that, after a preliminary treatment with the sulfuric and nitric acids, the lighter fractions of the sample may be allowed to escape without loss of arsenic. When analyzing samples with an arsenic content less than 10 p.p.b., it is imperative that the method blank be checked periodically.

Color Reaction Area. To evaluate a spot reaction reflectometrically the light beam must be incident upon a fixed area of the colored product. If the spot area is constant, it need not be known; if the area varies, its value must be determined. Therefore, to standardize the exposed reactant surface and to make certain that the measurements were due entirely to the intensity of the spot reaction, masking plates were fabricated with a precisely drilled circular hole in the center, slightly smaller than the diameter of the test spot.

Type of Paper. The make of filter paper does not appear to be unduly critical as far as reflectance measurements are concerned. Each type of paper offers certain advantages and disadvantages in producing uniform spots. Schleicher and Schuell No. 575 filter paper, a thin, hardened, smooth textured paper, permits a spot reaction with minimum diffusion. The color reaction produced with this paper appears to be more intense than with Whatman No. 50 or similar grade papers. Coarse grained or soft papers should be avoided for obvious reasons.

Unsensitized filter disks may occasionally vary in reflectance, presumably because of variations in surface texture or accidental discoloration of the paper stock. For this reason, the reference disk used to adjust the spectrophotometer for 100% reflectance must be a representative one. To determine if the disk meets this criterion, several clean, unsensitized paper disks, selected at random, are compared for reflectance. If the difference in reflectance is not greater than that equivalent to 0.01 absorbance unit, the disks are considered suitable for use. If the differential reflectance is greater than this value, the disks should be discarded and a different lot of the paper employed.

Spectral Curve. An absorption spectrum of the arsenic–mercuric bromide complex was determined between 350 and 600 mμ using a spot reaction equivalent to 0.5γ of arsenic. Although no sharp peaks were found, a slight plateau occurred at 400 mμ. All subsequent measurements were made at this wave length using a 2-mm. slit width and normal sensitivity.

Evolution of Arsine. The variables governing the intensity of the arsenic spot reaction must be critically controlled if accurate results are to be obtained. These variables include the concentration of sulfuric acid, weight and mesh size of the zinc, regulation of vacuum, temperature of the evolution mixture, and impregnation of the test disks.

Several of these factors have been thoroughly investigated by other workers and their conclusions were directly employed in this method. These include concentration of sulfuric acid (*4*), weight of zinc (*1*, *4*), and impregnation of the test disks (*2*, *4*).

A study was made to determine the effect of several brands of zinc and mesh size on arsenic recovery. Two makes of granular zinc labeled as 10- and 20-mesh and a zinc produced electrolytically were investigated. For uniformity of particle size, which is related to surface area, the granular zinc was screened through a No. 20 and No. 30 sieve. The zinc collected on the No. 20 screen and on the No. 30 screen were reserved for subsequent testing. As indicated in Figure 1, the 20-mesh granular zinc appears to be the most active, and the electrolytic the least. This fact has been corroborated by Cassil (*2*). The evolution studies were made using a bath maintained at 20° C. and pressures approaching 0.5 atm. Occasional erratic staining and low recovery of arsenic suggested that not only was incomplete arsine evolution occurring but that perhaps other factors were affecting reproducibility.

Because the method was an adaptation of a vacuum spot-filtration technique pressure might be a determinative factor. Table IV shows data obtained by careful regulation of pressure in the evolution system on a series of arsenic standards. The pressure

Table IV. Effect of Pressure on Evolution of Arsine at 20° C.

Pressure, Mm.	Arsenic, γ Added	Arsenic, γ Recovered
190	0.10	0.108, 0.059
190	0.30	0.245, 0.270
190	0.50	0.463, 0.418
380	0.10	0.079, 0.076
380	0.30	0.248, 0.251
380	0.50	0.413, 0.415

studies were conducted with 20-mesh zinc and a bath at 20° C. This experiment demonstrated that consistent recovery of arsine was not attained. During the course of this work it was found that the test spot area was uniformly stained when the pressure in the reaction flask was evenly controlled.

Table V. Reproducibility of Calibration Data at 50° C. and 190-Mm. Pressure

Arsenic Added, γ	Absorbance 1st week	2nd week	4th week
0.10	0.082	0.080	0.078
0.30	0.237	0.238	0.239
0.50	0.387	0.389	0.391

Because of the incomplete evolution at 20° C. and the reported tendency for marginate diffusion of the spot reaction in the presence of excessive water vapor at high bath temperatures (*11*), a median temperature of 50° C. was selected. The water vapor produced at this temperature was effectively removed as condensate in the lead acetate absorption tube, the final traces being trapped by cotton placed in the filtration assembly. Furthermore, this temperature seemed to provide complete evolution of the arsine at a pressure of 190 mm. Analytical data (Table V) show that consistent reflectance values were obtained over a period of 1 month on three arsenic standards.

Calibration Curve. The fact that the arsenic–mercuric bromide reaction gives a complex which follows a straight line function is advantageous in that fewer points are required to define the standard calibration curve. The linearity of the curve applies only to amounts of arsenic less than about 0.5 γ. A lack of reagent is apparently not responsible for the curvature, as the mercuric bromide is present in large excess (approximately 1000 γ per disk). It seems more likely that the proportion of arsenic combined at or near the surface of the disk approaches a maximum intensification resulting in a leveling off of the curve for larger amounts of arsenic.

Loss of arsenic through the disk does not occur within the recommended concentration range, as shown by a lack of color on a second disk in the filtration assembly.

Other Variables. The spot reactions are stable for 1 to 1.5 hours without significant change, although a gradual change in the shade of the color may be noted if the disk is not protected from laboratory fumes. For precise work, the disk should be measured as soon as possible following the evolution step. Direct sunlight or strong artificial light have a bleaching effect on the reaction product.

Of the possible interfering constituents, antimony should be considered in the analysis of samples having a petroleum origin. When varying amounts of antimony were mixed with known concentrations of arsenic, the results indicated that about 100 γ of antimony can be tolerated when 0.50 γ of arsenic is present. The removal of antimony by the hydrobromic acid technique (*11*) was not investigated, because the antimony content of naphtha charge stocks is not expected to exceed the 100-γ limit.

Hydrogen sulfide interference is eliminated by the use of lead acetate. Some metallic salts tend to increase the rate of arsine evolution; this effect is usually not serious, however. Residual hydrocarbons tend to inhibit the rate of evolution and should be absent.

The ACS specification for reagent grade sulfuric and nitric acids is not more than 0.000003% arsenic. Therefore, the limit of 0.0000001% arsenic specified for these acids in this procedure might appear to be unrealistic. However, tests performed on Baker & Adamson sulfuric and nitric acids for over 1 year have showed that they never exceeded the specified lower limit of arsenic content.

Strict adherence to the details set forth in this method is absolutely necessary for accurate results. On the other hand, the procedure is relatively simple and not particularly tedious to carry out. Six determinations are easily completed by one operator in 8 hours.

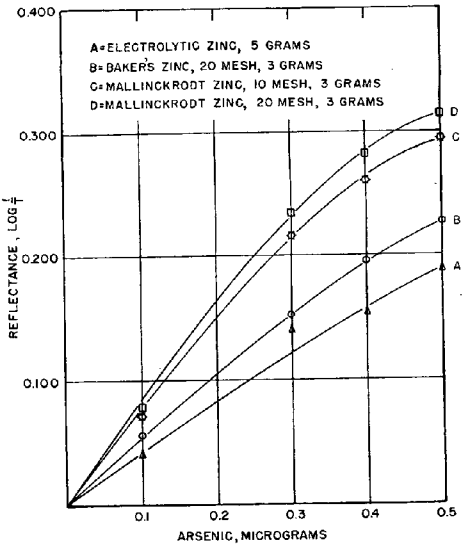

Figure 1. Effect of mesh size and type of zinc on arsenic recovery

Table VI. Reproducibility of Method

Arsenic Found, P.P.B. Gasoline	Naphtha
2.2, 2.0	19, 21
4.1, 4.4	17, 17
1.7, 1.4	19, 17
7.9, 7.3	16, 15
1.0, 1.0	23, 24
6.0, 6.3	21, 22
0.9, 0.8	21, 22
7.7, 7.5	22, 22
1.2, 1.0	19, 20
6.9, 6.5	24, 25
Std. dev., p.p.b. 0.2	0.8

ACCURACY AND PRECISION

A statistical study of the recommended procedure was made by applying it to a series of synthetic samples ranging from 1 to 26 p.p.b. of arsenic. The standard deviations obtained from this study are shown in Tables II and III. The calculations indicate the precision to be within the range of 0.020 to 0.025 γ of arsenic. Accuracy of 90% or better is obtained on samples containing from 0.1 to 0.5 γ of arsenic.

Listed in Table VI are some randomly selected results obtained for 10 gasolines and naphthas submitted for the determination of arsenic. Although the data cannot be reliably compared with the other indicated results, it is

believed the accuracy is of the same order of magnitude as that of the synthetic samples.

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Hydrocarbons in the 116° to 126° C. Fraction of Petroleum

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▶ Twenty-two hydrocarbons were found in the fraction of petroleum normally boiling between 116° and 126° C. This fraction of petroleum constitutes approximately 5.37% by volume of the representative petroleum which has been under investigation for many years by the American Petroleum Institute Research Project 6. These compounds were concentrated by extended use of the fractionating processes of regular and azeotropic distillation. Identification of the compounds was made by mass and infrared spectrometric examination, together with measurements of the simple physical properties. The names and estimated relative amounts by volume of the twenty-two compounds, given in order of decreasing amount in the petroleum, are as follows: *n*-octane, 35.5; 2-methylheptane, 16.7; 1,*cis*-3-dimethylcyclohexane, 11.8; 1,*trans*-2-dimethylcyclohexane, 5.8; 3-methylheptane, 5.6; 1,*trans*-4-dimethylcyclohexane, 4.5; 4-methylheptane, 3.7; 1,*trans*-2-ethylcyclopentane, 2.5; 3,4-dimethylhexane, 2.4; 1-methyl-*trans*-3-ethylcyclopentane plus 1-methyl-*cis*-3-ethylcyclopentane, 2.3; 3-ethylhexane, 1.7; 1,*cis*-4-dimethylcyclohexane, 1.6; 1,*trans*-3-dimethylcyclohexane, 1.3; 1,*cis*-2,*trans*-3-trimethylcyclopentane, 1.3; 1,1-dimethylcyclohexane, 1.2; 1,1,*cis*-2,*trans*-4-tetramethylcyclopentane, 0.8; 1-methyl-1-ethylcyclopentane, 0.6; 3-methyl-3-ethylpentane, 0.3; 1,*cis*-2,*trans*-4-trimethylcyclopentane, 0.2; cycloheptane, 0.15; 2,2,5-trimethylhexane, 0.03. In addition, trace amounts of an unidentified bicycloparaffin occur in the distillate fraction boiling near 124.5° C.

As PART of the continuing work of the American Petroleum Institute Research Project 6 on the composition of its representative petroleum (*7*), analysis has been completed of the hydrocarbons in that fraction of this petroleum normally boiling between 116° and 126° C., which constitutes approximately 5.37% by volume of the original petroleum. An early investigation (*4*) revealed the presence of four hydrocarbons in this fraction: 2-methylheptane at 117.65° C.; 1,*cis*-3-dimethylcyclohexane at 120.09° C.; 1,*trans*-2-dimethylcyclohexane at 124.45° C.; and *n*-octane at 125.66° C. The present investigation of this portion, using distillation equipment of much greater separating efficiency than was earlier available, in conjunction with the modern spectroscopic methods of analysis has shown that it contains 22 hydrocarbons. The details of the analysis of the adjacent lower boiling portion are given in (*3*), and those of the adjacent higher boiling portion in (*2*).

PROCEDURE

All of the paraffin-cycloparaffin material remaining from the earlier investigation (*4*) was combined and distilled at 725 mm. of mercury. Further processing of this material by distillation (regular, azeotropic, and at reduced pressure) was designed to concentrate the individual hydrocarbons so that they could be identified with reasonable certainty. The apparatus and procedures used in the distillations are described in (*3*). These distillation operations required the repeated blending of portions of distillate to produce charges for redistillation. A total of 55 charges was prepared and distilled. The amounts of the individual compounds were computed from the results of spectrographic analyses (principally mass spectra, with some infrared spectra) made for us on a total of 57 intermediate and final fractions by the Research Laboratory of the Humble Oil and Refining Co. In addition to the spectroscopic analyses, the purity of several compounds was determined from measurements of the freezing point. The details of these operations are given in a report of the American Petroleum Institute Research Project 6, entitled "Hydrocarbons in the 116° to 126° C. Fraction of Petroleum", dated March 31, 1952, which is available from the Petroleum Research Laboratory, Carnegie Institute of Technology, Pittsburgh, Pa.

The results of the distillation of the starting material are given in Figure 1. In this figure, the solid lines refer to the distillation at 725 mm. of mercury, and the dashed lines refer to the redistillation of a portion of the material at 30.5 mm. of mercury. It will be noted that the distillation at 30.5 mm. of mercury was very effective in separating the re-

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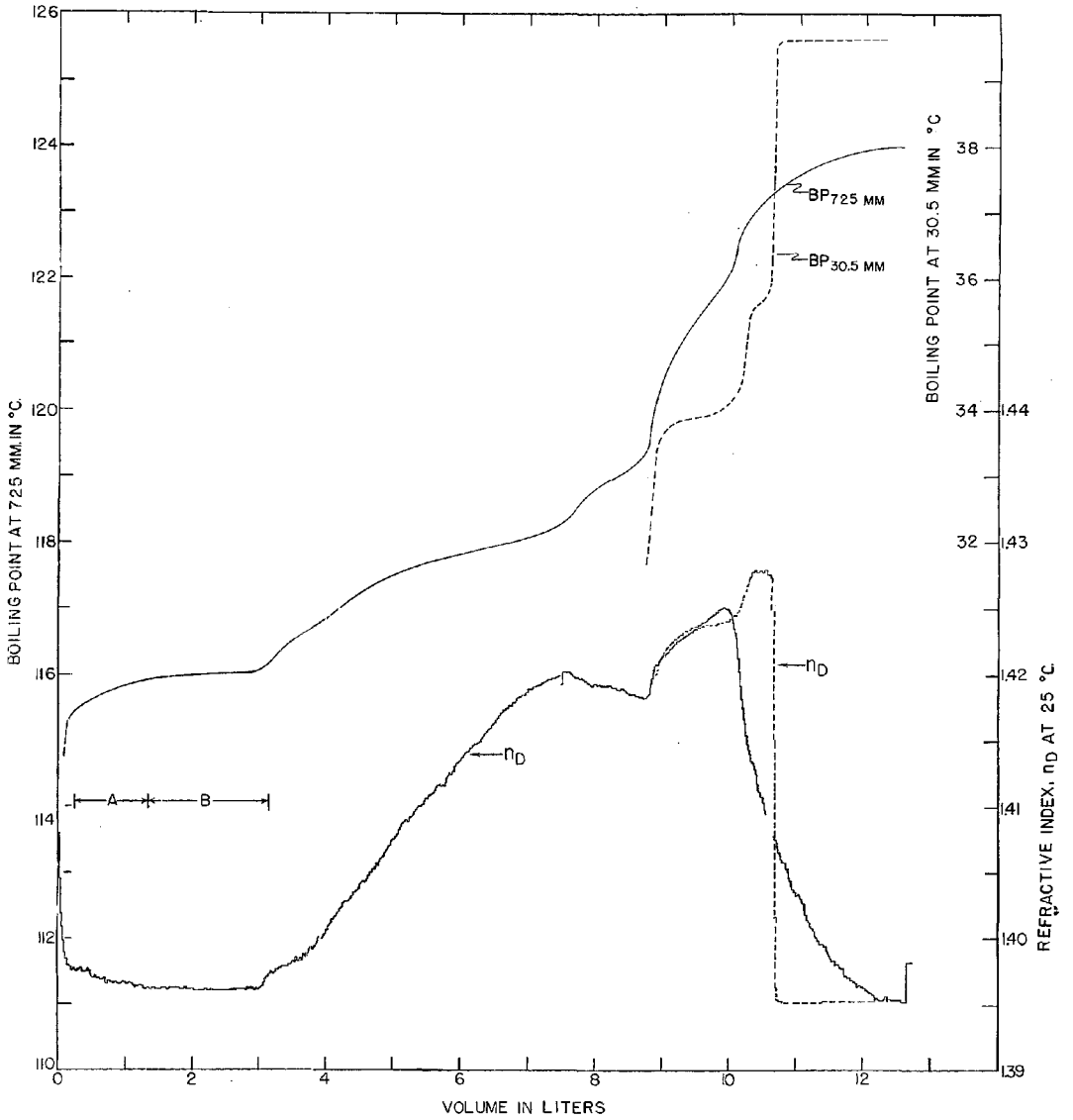

Figure 1. Results of distillation of 116° to 126° C. fraction of petroleum

Upper and lower solid lines give, respectively, boiling point and refractive index of distillate, with respect to its volume, obtained in regular distillation at 725 mm. Hg. Upper and lower broken lines near right side of figure give, respectively, boiling point and refractive index of distillate, with respect to its volume, obtained in distillation at 30.5 mm. Hg of indicated portion of material.

maining *n*-octane from the cycloparaffins which immediately precede it in normal boiling point.

The methods employed for the separation of the individual hydrocarbons are illustrated for one compound, cycloheptane, in Figures 2 and 3. The material from the preliminary distillation normally boiling in the range from 117.0° to 117.8° C. (portions *A* and *B*, Figure 1) were distilled azeotropically with ethyl alcohol to give the results shown in Parts I and II respectively, of Figure 2. The refractive indices of portions IA and IIA from this distillation were higher than those of the alkyl cyclopentanes and alkyl cyclohexanes which boil at or near this temperature and it was suspected that this material contained cycloheptane which normally boils at 118.79° C. (*6*). The further concentration of this material by azeotropic distillation is shown in Figure 3. The refractive index, n_D at 25° C., of the material near the end of the distillate (portion *B*, Figure 3) was 1.4400 which may be compared with the value 1.4424 for cycloheptane (*6*). The molecular weight of portion *B*, determined by the freezing point method using *o*-xylene as a solvent, was found to be 98.0 ± 1.4 which is to be compared with the value 98.18 for pure cycloheptane. The other

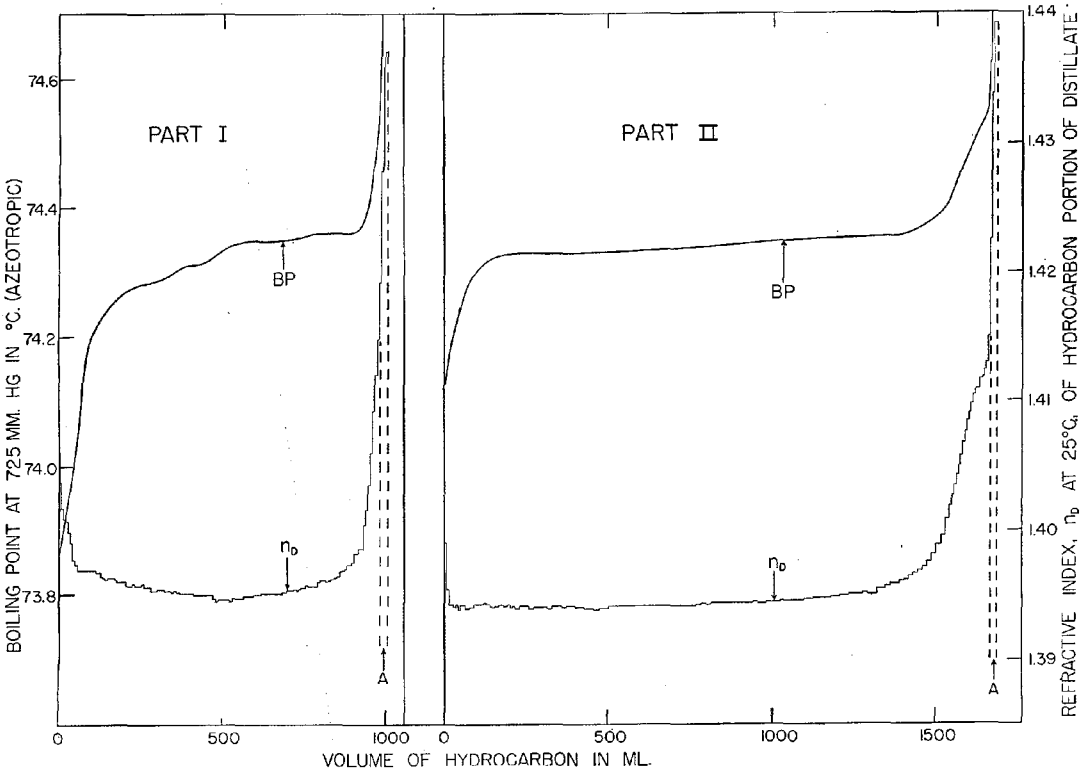

Figure 2. Results of azeotropic distillation of portions A and B from Figure 1

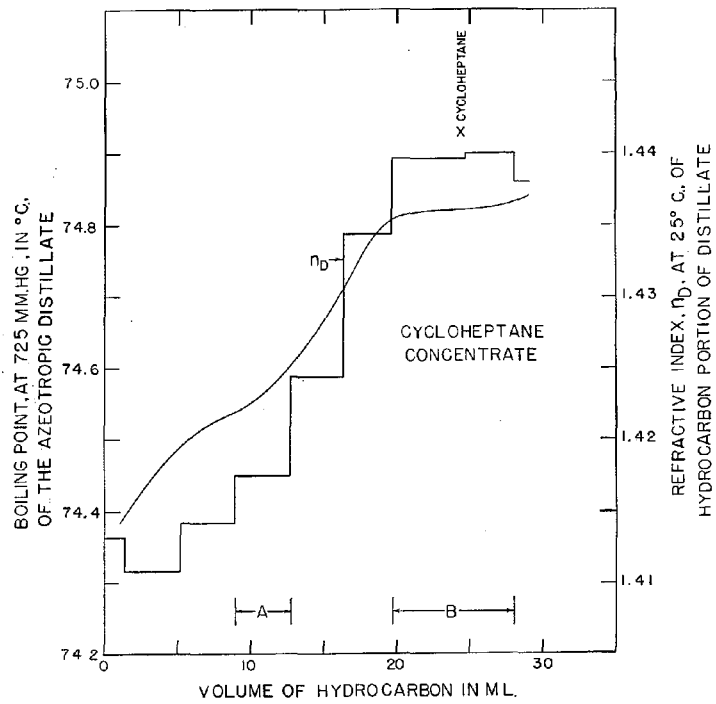

Figure 3. Results of the azeotropic distillation of portion A from Figure 2

Table I. Summary of the 22 Hydrocarbons Found in the 116° to 126° C. Fraction of Petroleum

Component[a]	Formula	Boiling Point of Pure Compound at 1 Atm., ° C.	Highest Concentration Isolated,[b] Mole %	Estimated Relative Amount by Volume in 116° to 126° C. Fraction	Amount in Original Crude Petroleum,[c] Percentage by Volume
1, *cis*-2,*trans*-4-Trimethylcyclopentane	C_8H_{16}	116.73	84	0.2	0.01
1,*cis*-2,*trans*-3-Trimethylcyclopentane	C_8H_{16}	117.5	90	1.3	0.07
2-Methylheptane	C_8H_{18}	117.65	85	16.7	0.90
4-Methylheptane	C_8H_{18}	117.71	34	3.7	0.20
3,4-Dimethylhexane	C_8H_{18}	117.72	40	2.4	0.13
3-Methyl-3-ethylpentane	C_8H_{18}	118.26	6	0.3	0.02
3-Ethylhexane	C_8H_{18}	118.53	43	1.7	0.09
Cycloheptane	C_7H_{14}	118.79	90	0.15	0.01
3-Methylheptane	C_8H_{18}	118.92	98; 97[b]	5.6	0.30
1,*trans*-4-Dimethylcyclohexane	C_8H_{16}	119.35	75	4.5	0.25
1,1-Dimethylcyclohexane	C_8H_{16}	119.54	84	1.2	0.06
1,*cis*-3-Dimethylcyclohexane	C_8H_{16}	120.09	84; 85[b]	11.8	0.63
1-Methyl-*trans*-3-ethylcyclopentane	C_8H_{16}	120.8	57	2.3	0.12
1-Methyl-*cis*-3-ethylcyclopentane	C_8H_{16}	121.4			
1-Methyl-*trans*-2-ethylcyclopentane	C_8H_{16}	121.2	74	2.5	0.14
1-Methyl-1-ethylcyclopentane	C_8H_{16}	121.52	65	0.6	0.03
1,1,*cis*-3,*trans*-4-Tetramethylcyclopentane	C_9H_{18}	121.6	83	0.8	0.04
1,*trans*-2-Dimethylcyclohexane	C_8H_{16}	123.42	98; 96[b]	5.8	0.31
2,2,5-Trimethylhexane	C_9H_{20}	124.08	11	0.03	0.002
1,*cis*-4-Dimethylcyclohexane	C_8H_{16}	124.32	76	1.6	0.09
1,*trans*-3-Dimethylcyclohexane	C_8H_{16}	124.45	49	1.3	0.07
n-Octane	C_8H_{18}	125.66	99.2	35.5	1.9
Total				100.0	5.372

[a] 2,3-Dimethylhexane at 115.61° C. and 2-methyl-3-ethylpentane at 115.65° C., which were isolated previously from the 108° to 116° C. (*3*), are also present in this fraction. Records have been adjusted to account for this additional quantity of these constituents (*7*). In addition, trace amounts of an unidentified bicycloparaffin occur in the distillate fractions boiling near 124.5° C.

[b] These values were determined from measurements of freezing points. All other values in this column were determined from spectral data.

[c] Amount of given component in gasoline fraction, 40° to 180° C., is about three times value given in this column.

hydrocarbons which are possible in this region contain eight carbon atoms per molecule and have the following molecular weights: bicycloparaffins, 110.19; monocycloparaffins, 112.21; and paraffins, 114.22. The material was subsequently identified as cycloheptane by spectroscopic analysis and estimated to have a purity near 90%. In this petroleum the cycloheptane was associated principally with 2-methylheptane; 4-methylheptane; and 3,4-dimethylhexane, and was located in distillate fractions boiling slightly more than 1° C. below the boiling point of pure cycloheptane. A displacement of the boiling point of cycloparaffins in petroleum distillates is not unusual and may be attributed to the nonideality of dilute solutions of cycloparaffins in paraffins (*5*).

RESULTS OBTAINED

Table I summarizes the information regarding the twenty-two hydrocarbons found in the 116° to 126° C. fraction of petroleum. Bell (*1*), using somewhat similar methods, has reported the presence of 17 hydrocarbons in the corresponding fraction of an East Texas petroleum. The data in Table I lead to the following conclusions. (*a*) The material is composed almost entirely of five types of hydrocarbons in the following relative amounts by volume: normal paraffins, 36; branched paraffins, 30; alkyl cyclopentanes, 8; alkyl cyclohexanes, 26; and cycloheptane, 0.15. (*b*) In addition to the twenty-two hydrocarbons found, the only other hydrocarbons of the paraffin, alkyl cyclopentane, and alkyl cyclohexane classes normally boiling in the range 116° to 126° C. are the following (*6*), which are believed to be present only in trace amounts: 1,1,-3,3-tetramethylcyclopentane at 117.96° C.; 1,*cis*-2,*cis*-4-trimethylcyclopentane at 118° C.; 2,2,4,4-tetramethylpentane at 122.28° C.; and 1,*cis*-2,*cis*-3-trimethylcyclopentane at 123° C. (*c*) The singly branched, doubly branched, and triply branched paraffins are present in this gasoline fraction in relative amounts which have magnitudes as follows: 100, 10, and 0.1, respectively. (*d*) Cycloheptane is present in very small amounts. This is the first time that this compound or any of its derivatives has been identified in petroleum. (*e*) A bicycloparaffin boiling near 124.5° C. is present in trace amounts. This is the lowest boiling bicycloparaffin to be detected in petroleum.

ACKNOWLEDGMENT

Grateful acknowledgment is made to the Research Laboratory of the Humble Oil and Refining Co., Baytown, Tex., for the spectrographic analyses reported in this paper.

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Purification, Purity, and Freezing Points of 20 API Standard and API Research Hydrocarbons

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▶ The purification and determination of freezing points and purity are described for the following 20 hydrocarbons of the API Standard and API Research series: 2,2-dimethyl-3-ethylpentane; 2,4-dimethyl-3-ethylpentane; cycloheptane; 3-methyl-1-hexene; 4-methyl-*trans*-2-hexene; 2,4-dimethyl-1-pentene; 3,3-dimethyl-1-pentene; 3,4-dimethyl-*cis*-2-pentene; 3,4-dimethyl-*trans*-2-pentene; 2-methyl-*trans*-3-heptene; 2,5-dimethyl-*trans*-3-hexene; 2-methyl-3-ethyl-1-pentene; 3-methyl-1,2-butadiene; 1-methylcyclopentene; 1-methylcyclohexene; 1-ethylcyclohexene; cyclohexylcyclohexane; cyclohexylbenzene; 3-cyclopentyl-1-propene (allylcyclopentane); and phenylbenzene (biphenyl).

This investigation is a continuation of the work of producing highly purified hydrocarbons of the API Standard and Research series (*1*, *5–10*). This paper describes the purification and determination of purity and freezing points of 20 hydrocarbons, which include two paraffins, one cycloparaffin, nine monoolefins, one diolefin, three cyclo-olefins, one dicycloparaffin, one cycloparaffin-aromatic, one cycloparaffin-olefin, and one dinuclear aromatic. The final lots of material labeled API Standard are sealed in vacuum in glass ampoules and made available as API Standard samples of hydrocarbons by the Carnegie Institute of Technology. The material labeled API Research is made available in appropriate small lots through the American Petroleum Institute Research Project 44 for loan to qualified investigators for the measurement of needed physical, thermodynamic, and spectral properties.

Table I gives the names of the 20 compounds, the laboratories providing the starting material, details concerning the first and succeeding distillations, the character of the plot of the freezing point of the hydrocarbon part of the distillate as a function of its volume, and the volumes of the final lots of API Standard and Research material.

The procedures followed in the process of purification and determination of purity were the same as described in previous papers (*2*, *5–10*) except that phenylbenzene (biphenyl) was purified using the process of zone melting (*3*). Details of the distillation apparatus and operations have been described (*4*, *11*).

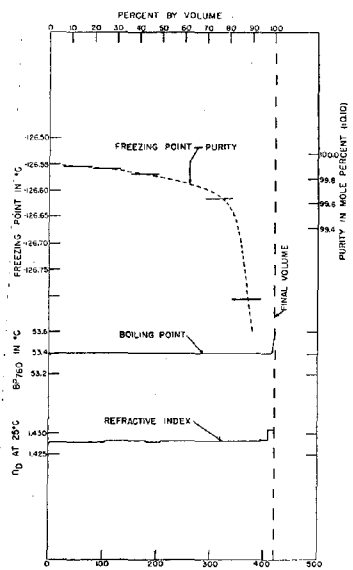

Figure 1. Results of azeotropic distillation of 1-methylcyclopentene with methanol

Figures 1, 2, and 3 show graphically the results of some typical distillations. They represent the cases where the purest material is, respectively, largely in the forepart of the distillation, in the middle of the distillation, and in the after part of the distillation. In each figure plots are given for refractive index, boiling point, freezing point, and purity as a function of the volume of the hydrocarbon part of the distillate.

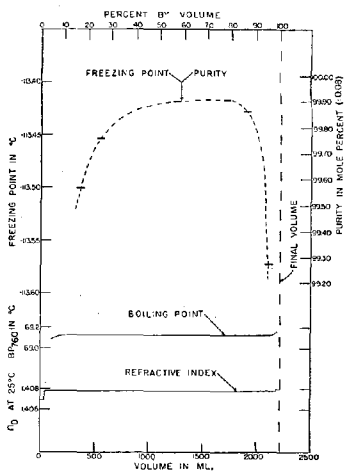

Figure 2. Results of azeotropic distillation of 3,4-dimethyl-*trans*-2-pentene with ethyl alcohol

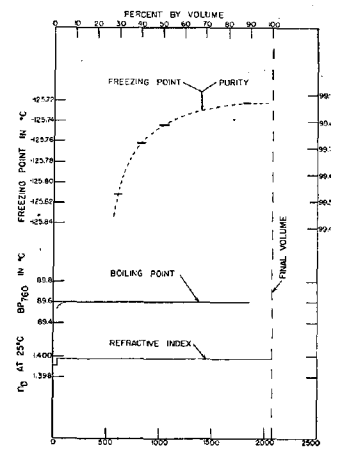

Figure 3. Results of regular distillation of 4-methyl-*trans*-2-hexene

Table I. Purification of 20 API Standard and API Research Hydrocarbons

Compound	Laboratory[a] Providing Starting Material	Hydrocarbon Charged for Distillation: Vol., liters	Hydrocarbon Charged for Distillation: Purity, mole %	Distillation[b]: Kind[c]	Distillation[b]: Azeotrope-forming substance[d]	Distillation[b]: Amt. hydrocarbon in azeotropic distillate[e], vol. %	Distillation[b]: No. of equivalent theoretical plates in distilling column[b]	Distillation[b]: Rate of collection of distillate, ml./hour	Distillation[b]: Time of distillation, hours	Distillation[b]: Location of purest material in distillate[f]	Volume of Selected Sample: API Standard, ml.	Volume of Selected Sample: API Research, ml.
2,2-Dimethyl-3-ethylpentane	NBS Auto. Sec.	1.05	...	Reg. and Azeo.[g]	... Me Cell	.. 61	200 200	4 4	336 144	A M	560	165
2,4-Dimethyl-3-ethylpentane	NBS Auto. Sec.	0.68	99.3±0.2	Reg. and Azeo.[g]	... Me Cell	.. 63	200 200	3 4	336 264	M F	425	85
Cycloheptane	APIRP45	2.99	99.97±0.01	Reg.	...	..	200	4	864	A	...	...
		2.38	99.988±0.008	Azeo.	Me Cell	64	200	4	1224	A	1075	330
3-Methyl-1-hexene	APIRP45	4.18	...	Reg.	...	..	200	4	1224	M	...	...
		2.60	...	Azeo.	Ethyl alcohol	69	200	4	1032	M	1150	340
4-Methyl-*trans*-2-hexene	APIRP45	5.60	95.4±0.3	Reg.	...	..	200	7	1104	M	...	...
		2.90	98.0±0.2	Azeo.	Ethyl alcohol	67	200	7	792	M	...	...
		2.15	99.6±0.1	Reg. and Azeo.[g]	... Ethyl alcohol	.. 67	200 200	7 7	432 192	A F	950	210
2,4-Dimethyl-1-pentene	APIRP45	3.42[h]	98.9±0.2	Reg.	...	..	200	7	672	M	...	...
		3.44[h]	98.9±0.2	Reg.	...	..	200	7	624	M	...	...
		2.65	[i]98.8±0.2	Azeo.	Ethyl alcohol	73	200	7	600	M	...	...
		2.30[j]	[i]98.0±0.2	Azeo.	Ethyl alcohol	73	200	7	600	M	...	...
		2.70[k]	99.72±0.12	Azeo.	Ethyl alcohol	73	200	7	768	M	1085	325
3,3-Dimethyl-1-pentene	APIRP45	5.93	99.59±0.12	Reg.	...	..	200	4	1584	A	...	...
		4.38	99.78±0.10	Azeo.	Ethyl alcohol	75	200	4	1920	A	...	...
		3.50	99.86±0.08	Reg.	...	..	200	7	600	A	1430	360
3,4-Dimethyl-*cis*-2-pentene	APIRP45	3.45	99.64±0.12	Reg. and Azeo.[l]	... Me Cell	.. 86	200 200	7 4	... 1440	.. M	... ...	... ...
		1.35	98.2±0.1	Azeo.	Ethyl alcohol	62	200	4	624	M	...	...
		1.86[k]	99.90±0.07	Azeo.	Ethyl alcohol	62	200	4	912	M	1175	360
3,4-Dimethyl-*trans*-2-pentene	APIRP45	3.18[h]	96.8±0.02	Reg.	...	..	200	7	504	M	...	...
		2.31[k]	99.44±0.12	Azeo.	Ethyl alcohol	64	200	7	672	M	945	245
2-Methyl-*trans*-3-heptene	APIRP45	3.78	99.75±0.08	Azeo.	Me Cell	77	200	4	1440	A	1170	345

[a] Abbreviations represent APIRP45, American Petroleum Institute Research Project 45, Ohio State University, Columbus, Ohio; APIRP6, American Petroleum Institute Research Project 6, Carnegie Institute of Technology, Pittsburgh, Pa.; NBS Auto. Sec., Automotive Section, National Bureau of Standards, Washington, D. C.; Penn State, Hydrocarbon Laboratory, Pennsylvania State University, University Park, Pa.; Standard (Indiana), Standard Oil Co. of Indiana, Whiting, Ind.

[b] See (*4*) and (*11*) for further details.

[c] Azeo, azeotropic; Reg., regular.

[d] Me Cell, methyl Cellosolve, ethylene glycol monomethyl ether; DPrG, dipropylene glycol.

[e] Approximate value obtained from actual volume of hydrocarbon recovered by extracting azeotrope-forming substance with water in separatory funnels.

[f] Designations refer to general location of purest material in hydrocarbon part of distillate as a function of its volume. F, fore or front of distillate; M, middle part of distillate; A, after part of distillate.

[g] Residue from regular distillation distilled azeotropically in order to recover it as distillate.

[h] One of two similar distillations.

[i] Purity of this material is lower than original because of rearrangement during regular distillation.

[j] Material from first distillation above.

[k] Material having substantially same composition from each of preceding two distillations.

[l] When half complete regular distillation was changed to azeotropic distillation.

[m] Second lot of 1-methylcyclopentene.

(*Continued on page 363*)

Table I. Purification of 20 API Standard and API Research Hydrocarbons (Continued)

Compound	Laboratory[a] Providing Starting Material	Hydrocarbon Charged for Distillation: Vol., liters	Hydrocarbon Charged for Distillation: Purity, mole %	Distillation[b]: Kind[c]	Azeotrope-forming substance[d]	Amt. hydrocarbon in azeotropic distillate,[e] vol. %	No. of equivalent theoretical plates in distilling column[b]	Rate of collection of distillate, ml./hour	Time of distillation, hours	Location of purest material in distillate[f]	Volume of Selected Sample: API Standard, ml.	Volume of Selected Sample: API Research, ml.
2,5-Dimethyl-*trans*-3-hexene	APIRP45	4.17	99.75±0.08	Azeo.	Me Cell	76	200	4	1725	M	...	...
		2.92	99.85±0.06	Azeo.	Me Cell	76	200	7	528	M	1200	355
2-Methyl-3-ethyl-1-pentene	APIRP45	2.88	99.80±0.08	Azeo.	Me Cell	78	200	4	1080	M	1160	340
3-Methyl-1,2-butadiene	APIRP45	3.65	93.6±0.2	Reg.	...	..	200	7	624	M	1170	325
1-Methylcyclopentene	Penn State Standard (Indiana)[m]	2.30	97.8±0.2	Reg.	...	..	135	4	696	M	...	...
		1.81	97.9±0.2	Reg. and Azeo.[g]	...	..	200	4	504	A	...	...
		...	...	Azeo.	Methanol	60	200	4	192	F	...	...
		0.47	...	Azeo.	Methanol	60	200	4	336	F	...	...
		2.03[n]	99.32±0.14	Azeo.	Methanol	60	200	7	408	M	850	200
1-Methylcyclohexene	Penn State	3.00	...	Reg. and Azeo.[g]	... Ethyl alcohol	.. 30	200 200	4 4	670 314	A F	750	195
1-Ethylcyclohexene	APIRP45	2.80[o]	...	Azeo.	Me Cell	53	200	7	1320	M	...	...
		2.84[o]	...	Azeo.	Me Cell	53	200	7	1008	M	...	...
		2.50[o]	...	Azeo.	Me Cell	53	200	7	936	M	...	...
		2.50[o]	...	Azeo.	Me Cell	53	200	7	1008	M	...	...
		2.64[p]	...	Azeo.	Me Cell	53	200	7	840	M	...	...
		1.87	96.4±0.2	Azeo.	Me Cell	53	200	7	840	M	...	...
		*1.20	97.8±0.2	Azeo.	Me Cell	53	200	9	336	F	...	...
		2.70[p]	...	Azeo.	Me Cell	53	200	9	696	M	...	...
		1.90	...	Azeo.	Me Cell	53	200	4	1344	M	...	...
		*1.01	98.2±0.1	Azeo.	Me Cell	53	200	9	336	F	...	...
		1.62[q]	99.49±0.12	Azeo.	Me Cell	53	200	4	1296	M	850	220
Cyclohexylcyclohexane	APIRP6[r]	7.62	...	Reg.	...	..	150	5	1656	A	...	...
		3.65	99.98±0.01	Azeo.	DPrG	58	150	5	1320	M	1525	480
Cyclohexylbenzene	APIRP6[r]	7.52	...	Reg.	...	..	150	5	1872	M	...	...
		3.60	99.89±0.07	Azeo.	DPrG	58	150	5	1200	M	1500	380
3-Cyclopentyl-1-propene (Allylcyclopentane)	APIRP45	2.22	99.84±0.11	Azeo.	Me Cell	62	200	7	576	A	1170	350
Phenylbenzene (Biphenyl)	APIRP6[r]	5.40	97.8±0.02	Zone melting[s]	...	..	...	...	...	..	1200	400

[n] Material from each of previous three distillations.
[o] One of four similar distillations.
[p] Material having substantially same composition from two of four similar distillations (see [o]).
[q] Material having substantially the same composition from two distillations above which are marked with (*).
[r] Obtained by purchase of commercially available material.
[s] Purified by fractionation by zone melting.

As emphasized in the previous reports, the blending of fractions of distillate for the preparation of material of the highest purity can be done safely only on the basis of the freezing points.

Table II gives the following information for the compounds measured: the kind of time-temperature curves, whether freezing or melting, used to determine the freezing point; the freezing point of the actual sample; the calculated value of the freezing point for zero impurity; the value of the cryoscopic constant, determined from the lowering of the freezing point on the addition of a known amount of a suitable impurity (*2*, *4*); and the calculated amount of impurity in the API Standard and Research materials.

ACKNOWLEDGMENT

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Table II. Freezing Points and Purity of 20 API Standard and API Research Hydrocarbons

Compound	Kind of Time-Temp. Observations Used to Determine Freezing Point[a]	Freezing Point of Actual Material in Air at 1 Atm., °C. API Standard	Freezing Point of Actual Material in Air at 1 Atm., °C. API Research	Freezing Point for Zero Impurity in Air at 1 Atm., °C.	Cryoscopic Constant[a] A, Mole Fraction/Deg.	Calculated Amount of Impurity in Actual Material,[b] Mole % API Standard	Calculated Amount of Impurity in Actual Material,[b] Mole % API Research
2,2-Dimethyl-3-ethylpentane	M	−99.523	−99.511	−99.490±0.020	0.0407	0.13±0.08	0.08±0.08
2,4-Dimethyl-3-ethylpentane	M	−122.418	−122.396	−122.36± 0.03	0.0381	0.22±0.11	0.14±0.11
Cycloheptane	M	−8.124	−8.124	−8.100±0.020	0.0033	0.008±0.007	0.008±0.007
3-Methyl-1-hexene	..	...	...	...	...	[c](0.20±0.15)	[c](0.15±0.10)
4-Methyl-*trans*-2-hexene	M	−125.737	−125.723	−125.690±0.020	0.0388	0.18±0.08	0.13±0.08
2,4-Dimethyl-1-pentene	M	−124.087	−124.086	−124.060±0.020	0.0471	0.13±0.09	0.12±0.09
3,3-Dimethyl-1-pentene	M	−134.403	−134.402	−134.380±0.010	0.0466	0.11±0.05	0.10±0.05
3,4-Dimethyl-*cis*-2-pentene	M	−124.253	−124.251	−124.235±0.015	0.0477	0.09±0.07	0.08±0.07
3,4-Dimethyl-*trans*-2-pentene	M	−113.422	−113.417	−113.395±0.020	0.0412	0.11±0.08	0.09±0.08
2-Methyl-*trans*-3-heptene	M	−107.553	−107.552	−107.520±0.015	0.0483	0.16±0.07	0.15±0.07
2,5-Dimethyl-*trans*-3-hexene	M	−95.222	−95.220	−95.200±0.015	0.0424	0.09±0.06	0.08±0.06
2-Methyl-3-ethyl-1-pentene	M	−112.948	−112.948	−112.900±0.020	0.0396	0.19±0.08	0.19±0.08
3-Methyl-1,2-butadiene	M	−113.635	−113.635	−113.625±0.010	0.0368	0.04±0.04	0.04±0.04
1-Methylcyclopentene	M	−126.562	−126.556	−126.530±0.020	0.0427	0.14±0.08	0.11±0.08
1-Methylcyclohexene[d]	M	−120.441 (I)	−120.433 (I)	−120.400±0.020 (I) −125.96± 0.03 (II) (u)	0.0427 ...	0.18±0.08 ...	0.14±0.08 ...
1-Ethylcyclohexene	M	−110.000	−109.984	−109.960±0.020	0.0430	0.17±0.09	0.10±0.09
Cyclohexylcyclohexane	M	3.649	3.651	3.670±0.010	0.0110	0.023±0.008	0.021±0.008
Cyclohexylbenzene	M	7.040	7.042	7.070±0.020	0.0245	0.07±0.05	0.07±0.05
3-Cyclopentyl-1-propene (allylcyclopentane)	M	−110.695	−110.688	−110.670±0.020	0.0538	0.13±0.11	0.10±0.10
Phenylbenzene (biphenyl)	M	68.961	68.964	68.970±0.010	0.0192	0.02±0.02	0.01±0.01

[a] M, melting. See (*2*) and (*4*) for experimental details and definition of cryoscopic constant.
[b] Values in this column, except as otherwise noted, were calculated as described in (*2*) and (*4*) using the values of cryoscopic constants and freezing points for zero impurity given in previous columns.
[c] Estimated by analogy with isomers subjected to similar purification.
[d] This hydrocarbon has more than one crystalline form. Forms indicated are labeled I and II in order of decreasing temperature of freezing point. Forms other than I will be, at their respective freezing points, in metastable equilibrium with the undercooled liquid, but will be unstable with respect to transition to some other solid form at the same temperature and pressure (1 atm.). Such metastable forms are indicated by (u) following Roman numeral.

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Flame Photometric Estimation of Copper

Application to Plant Tissue

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▶ Copper in plant tissue is extracted from a 0.1*N* hydrochloric acid solution with a chloroform-kerosine solution of dithizone and is determined by flame photometer in this organic mixture. The results obtained by this rapid procedure compare reasonably well with those obtained by the AOAC method.

For several years copper has been determined flame photometrically in the agronomy laboratories of the Kentucky Agricultural Experiment Station.

The copper is extracted with dithizone in chloroform or carbon tetrachloride, along with other heavy metals, from a pH 8.5 ammonium citrate solution of the plant ash. The chloroform is evaporated and the dithizone destroyed with perchloric acid. The residue is then made to 1 ml. and the copper is determined on a Beckman DU spectrophotometer with flame and photomultiplier attachments.

This procedure works very well, but it is rather tedious, and the use of small volumes—necessary because the most sensitive reliable standardization that could be obtained was from 0 to 50 p.p.m. of copper—tends to decrease accuracy.

Dean and Lady (*3*) extracted iron from aqueous solution into an organic phase and determined the iron directly with the flame photometer. This work suggested that other elements could be determined in a similar manner. Dean and Lady have recently reported a procedure for determining copper by flame photometer in a chloroform extract containing the copper as copper salicylaldoxime (*4*). This gives a more sensitive determination of copper than the procedure reported here, but it requires a more exacting instrumental process.

The above work indicated the feasibility of determining copper directly in the dithizone solution. The flame photometer was found much less sensitive to copper in carbon tetrachloride and much more sensitive to copper in chloroform than in water, but chloroform did not give satisfactory atomization. Wide fluctuation of the galvanometer needle seemed to be due to erratic atomization, perhaps caused by evaporation of the chloroform in the capillary tube. For this reason, other solvents and mixtures of chloroform with other solvents were tried. The best solvent found was a 1 to 1 mixture of chloroform and ordinary kerosine. Higher proportions of kerosine gave a steadier flame, but the 1 to 1 mixture was used in order to have the organic phase settle to the bottom of the separatory funnel. The optimum dithizone concentration in the organic solvent was next determined. High dithizone concentrations adversely affected the steadiness of the flame. A dithizone concentration of 0.5 gram per liter was selected, because it has little effect on the flame but is high enough so that 10 ml. of the solvent completely extracts 200 γ of copper from 200 ml. of 0.1*N* hydrochloric acid. The acidity of the solution extracted was not critical; the amount of copper extracted was essentially the same with 1*N*, 0.1*N* or 0.01*N* hydrochloric acid. The presence of 0.1*N* perchloric acid had no effect on the extraction.

REAGENTS

Standard Copper Solution. A 50 p.p.m. stock solution is prepared by dissolving 0.982 gram of clear uneffloresced crystals of copper sulfate pentahydrate in water, adding enough hydrochloric acid to make the final acidity 0.1*N*, and diluting to 500 ml. This solution is stored in a tightly sealed polyethylene bottle.

Extractant. One gram of dithizone is dissolved in 1 liter of chloroform. One part of this solution is mixed with 1 part of kerosine just prior to use. The chloroform and the kerosine should be checked for copper contamination. Reagent grade chloroform was used in this study, but less expensive grades might have been satisfactory. The kerosine was obtained from an oil station, and was free from appreciable copper contamination.

Hydrochloric acid, redistilled 6*N*.
Nitric acid, redistilled.
Perchloric acid, reagent grade.

EQUIPMENT

Flame Photometer. A Beckman Model DU spectrophotometer with flame and photomultiplier attachments was used. The exact settings depend somewhat upon the instrument, but those used in this study were:

Selector switch	0.1
Sensitivity	2
Zero suppression	1
Phototube	Blue
Slit	0.3
H_2 pressure	2.5
O_2 pressure	20.0
Wave length	324.8

Hot Plate. A steam or low temperature electric hot plate maintaining a temperature of near 100° C. is used for the preliminary digestion. An electric or gas hot plate capable of boiling perchloric acid is used for the final digestion, under a hood designed for perchloric acid digestions.

PROCEDURE

Preparation of Standard Curve. Aliquots of the standard copper solution sufficient to give 0, 50, 100, 150, and 200 γ of copper are added to 200-ml. portions of 0.1*N* hydrochloric acid in 500-ml. separatory funnels. A 10-ml. aliquot of the chloroform-kerosine solution of dithizone is then added and the copper is extracted into the organic phase. Although practically all of the copper goes into the organic phase, the extraction is rather slow. Each funnel is vigorously shaken for 3 minutes or for several shorter intervals. A small piece of filter paper is inserted into the stem of each funnel, to remove any water droplets that may be trapped in the bore of the stopcock. The organic phase containing the copper is left in the funnel until the flame photometer is warmed up and ready for use. Then approximately 2 ml. of the organic phase from each funnel is placed in a sample cup, which is quickly covered. The instrument is set at 0 with the zero standard and at 100 with the 200-γ standard, and the other standards are quickly run. Another aliquot of the organic phase in each funnel is then placed in a clean sample cup and the procedure is repeated. It is not advisable to go back to check a previously run sample, because chloroform may evaporate. If it is necessary to check a sample again, a fresh aliquot

in a clean sample cup should be used. The working curve obtained is nearly straight and remains fairly constant.

Analysis of Plant Tissue. A sample of plant tissue (ordinarily 5 grams) containing less than 200 γ of copper is placed in a 400-ml. beaker and treated with 40 ml. of redistilled nitric acid. If the plant tissue is low in copper or only small samples are available, it may be advisible to standardize with smaller quantities of copper than those given above. After foaming has stopped, the beaker is covered, placed on the low temperature hot plate, and left until the contents are dry. This can conveniently be overnight. If a large amount of charred material remains, the treatment is repeated; if not, 20 ml. of nitric acid and 5 ml. of perchloric acid are added, and the beaker is placed on the high temperature hot plate and taken to near dryness. If charring appears when the perchloric acid starts to boil, the beaker is removed from the hot plate and more nitric acid is added. Once the perchloric acid has started to boil, the cover glass is removed from the beaker to hasten removal of excess acid. When the acid has practically all evaporated, the beaker is removed from the hot plate; when it has cooled, 20 ml. of 1*N* hydrochloric acid is added, the cover is replaced, and the solution is brought to a boil on the hot plate. The beaker is then removed, 100 ml. of water is added, and the contents are transferred to a 500-ml. separatory funnel. The volume in the separatory funnel is made to approximately 200 ml. and the copper is extracted from the solution.

Table I. Values for Working Curve in Flame Photometer Determination of Copper Complexed with Dithizone in Chloroform-Kerosine Mixture

Standard No.	Cu in 10 Ml., γ	Dial Reading
1	0	0
2	50	30
3	100	57
4	150	79
5	200	100

The copper in the organic phase is determined on the flame photometer as with the standards. A blank is taken through the entire procedure as a check on possible contamination. Zero- and 200-γ standards are taken through the extraction and used to standardize the flame photometer before the samples are run. It is not necessary to use a complete set of standards with each group of samples, for the shape of the working curve is relatively constant. This curve should be checked occasionally.

Chemical Analysis. Chemical analyses were performed by the AOAC carbamate method (*1*).

RESULTS

The working curve values obtained with standard solutions are shown in Table I. The working curve shows a slight curvature, which may be assumed to be the result of self-absorption of the copper radiation (*2*). Palladium, platinum, silver, and mercury are also extracted by dithizone from 0.1*N* hydrochloric acid, but none is ordinarily present in very large quantities in plant tissue. Mercury might be present in appreciable quantities if a mercury-containing fungicide had been applied to the plants. Mercury has a weak line at 235.7 mμ and might interfere. Zero- and 200-γ copper standards were taken through the procedure with and without 200 γ of added mercury. The mercury did not affect the results. As a further check on the procedure, the recovery of copper added to various plant samples and to a synthetic ash was determined (Table II). The synthetic ash was compounded from Johnson-Matthey Specpure chemicals and was intended to represent the ash from 5 grams of "average" plant tissue. The satisfactory recovery of added copper from both plant samples and synthetic ash indicates that the copper is being uniformly extracted and that no plant constituent is affecting the extraction of the copper or its determination in the extract.

Table II. Recovery of Added Copper

Material Taken, Grams	Copper Found, γ	Recovery of Added Copper, γ
1 Rye forage	110	
1 Rye forage + 40 γ copper	153	43
2.5 Tobacco	23	
2.5 Tobacco + 40 γ copper	62	39
2.5 Pasture herbage	29	
2.5 Pasture herbage + 20 γ copper	49	20
2.5 Pasture herbage + 50 γ copper	81	52
0.5 Synthetic ash	0	
0.5 Synthetic ash + 50 γ copper	51	51

Four plant samples having copper concentrations of 6 to 110 p.p.m. were analyzed in quadruplicate by the proposed method and in duplicate by the AOAC method (Table III). The methods gave good agreement and variation between replicate samples was only a little larger with the flame photometric method. The greatest variation between replicate samples in the flame photometric procedure was about 10% and the average variation from the mean was 2.1%

Table III. Comparison of Flame Photometric and Spectrophotometric Procedures

Material Taken, Grams	Copper Found, γ: Flame photometric	Copper Found, γ: Spectrophotometric
5 Tung leaf	28	31
	29	30
	29	
	28	
2.5 Pasture herbage	29	31
	29	29
	27	
	30	
2.5 Tobacco	22	22
	23	24
	23	
	23	
1 Rye forage	110	108
	112	112
	116	
	110	

DISCUSSION

The flame photometric method for the determination of copper in plant tissues is easy and rapid, and is relatively trouble-free. Only one reagent, the extracting solution, must be quantitatively measured. Caution is required only in preventing evaporation losses of the solvent from the time it is taken from the separatory funnel until it is atomized in the flame. This loss is easily avoided by working rapidly at this point. The procedure should find considerable use in analyzing plant tissue, if the highest possible accuracy is not necessary. It should be easily adaptable to the analysis of other materials, where the quantity of copper is in the given or perhaps a slightly lower range.

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Extrapolation Plot for Photometric Titration of Weak Bases in Aqueous and Nonaqueous Systems

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▶ **A method is described for plotting photometric titration data in a way that permits linear extrapolation of information obtained beyond the end point to give the stoichiometric value. Data are given illustrating the application of the procedure to titration of extremely weak bases in water and to titration of practically nonbasic compounds such as urea and amides in acetic acid. The procedure permits acid-base titration with much greater accuracy than do visual systems. Many systems which do not yield suitable results by potentiometric means are amenable to this technique because of its inherently greater sensitivity.**

TWO TYPES of linear plots have been discussed previously (*4*) for the determination of indicator titration end points by photometric methods. These plots, based on equilibria involving both the indicator and the weak base or acid undergoing titration, permitted accurate extrapolation to the stoichiometric end point from regions prior to the end point.

This work is concerned with a third type of linear plot obtained by use of relatively weakly basic indicators, such that the changes in the indicator color occur primarily after a basic sample has been titrated with an acidic titrant. The extrapolations to the stoichiometric end points in these cases are from regions well past the end points. The Type III plots are particularly suited for titration in water of weak bases such as pyridine, aniline, acetates, benzoates, and the like, which are very difficult to titrate visually or potentiometrically. The method is also applicable to titration, in the presence of indicators, of extremely weak bases (urea, certain amides) in nonaqueous systems such as acetic acid.

THEORY

Titrations in Aqueous Systems.

For systems containing a weak indicator, one can expect the acid-base ratio of the indicator to change linearly beyond the end point with the amount of strong acid or base added. For example, if a weak base, B, is being titrated in water with a strong acid, the system will contain essentially two acidic species, BH^+ and H_3O^+. If, for a weakly basic indicator, the equilibrium

$$BH^+ + I \rightleftharpoons B + IH^+$$

is far to the left, the concentration of BH^+ will have very little effect on eliciting a color change. Beyond the end point, however, when the concentration of H_3O^+ becomes appreciable, the equilibrium

$$H_3O^+ + I \rightleftharpoons H_2O + IH^+$$

comes into play. The constant for the above equilibrium can be written as

$$K = \frac{(I)(H_3O^+)}{(IH^+)} = K_I \qquad (1)$$

or

$$H_3O^+ = K_I \frac{IH^+}{I} \qquad (2)$$

If the amount of excess acid present in the system is made to equal $X - S$, where X is the total amount of the standard acid added and S is the volume of the acid required to convert stoichiometrically the amount of base B originally present to its conjugate form (BH^+), the resulting equation is

$$X - S = K \frac{IH^+}{I} V \qquad (3)$$

where V is the total volume of solution contained in the titration vessel. Thus, a plot of ratio $\frac{IH^+}{I}$ *vs.* X will yield a straight line with an intercept corresponding to S.

To obtain analytically useful forms of the plot from the titration of weak bases, the indicator should be sufficiently weak so that the observed color change is that defined by Equation 2. For titrations of weak bases with pK_b

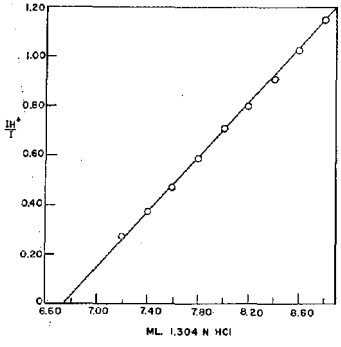

Figure 1. Type III plot for titration of aniline in water with 1.304*N* hydrochloric acid using Metanil Yellow indicator

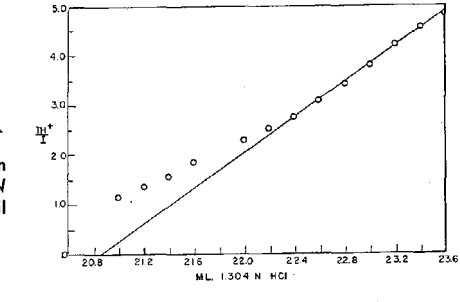

Figure 2. Titration of sodium citrate in water with 1.304*N* hydrochloric acid using Metanil Yellow indicator

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Table I. Determination of Weak Bases from Photometric Data by Titration in Water and Acetic Acid

Base	Indicator	Base, Mg. Taken	Base, Mg. Found[b]	Precision, mg.[c]
Weak Bases with 1.304*N* Hydrochloric Acid in Water				
Sodium acetate	Metanil Yellow	522.4	523.1	0.5
Sodium benzoate[a]	Metanil Yellow	573.1	572.3	0.5
Aniline[a]	Metanil Yellow	639.5	637.9	5.8
Pyridine	Metanil Yellow	694.0	694.4	2.0
Very Weak Bases with 0.1034*N* Perchloric Acid in Acetic Acid				
Urea	Sudan III	65.6	65.3	0.1
Antipyrine	Sudan III	200.1	199.7	0.2
N-Methylpyrrolidone	Sudan III	88.9	87.8	2.0

[a] Ethyl alcohol–water, 1 to 1, used as solvent.
[b] Average of three.
[c] Standard deviation.

values of 9 or less, indicators with pK_I values of 1.5 to 2.0 and strong mineral acids of moderate concentration (0.5 to 2.0*N*) have been found useful. For the titration of weaker bases, correspondingly weaker indicators and considerably stronger acid should be used.

The Type III plots are closely related to the absorbance-volume plots of Goddu and Hume (*2, 3*). As Equation 3 shows, a plot of the volume of the titrant added against IH^+, as determined by the absorbance of the solution (it is assumed that the base form of the indicator does not absorb), would be approximately linear if the extent of the conversion of the indicator to its acid color were kept very small. Direct absorbance plots, however, would have been of little value in the systems investigated here, as they would have led to highly curved lines. Furthermore, the direct plot of absorbance is of little use for indicators whose base form absorbs significantly at the wave length employed for the photometric measurements.

Titrations in Acetic Acid Systems. Several indicators which have been recently evaluated for the acetic acid system are so weakly basic that they exhibit a color change only in the presence of a strong acid such as perchloric acid (*4*). The equilibrium corresponding to this color change can be written as

$$I + HClO_4 \rightleftharpoons I.HClO_4$$

and the corresponding equilibrium constant is

$$K = \frac{(I.HClO_4)}{(I)(HClO_4)} \quad (4)$$

By rearranging the above equation into the form

$$HClO_4 = K\frac{(I.HClO_4)}{I} \quad (5)$$

where $I.HClO_4$ = the acid form of the indicator resulting from interaction with $HClO_4$

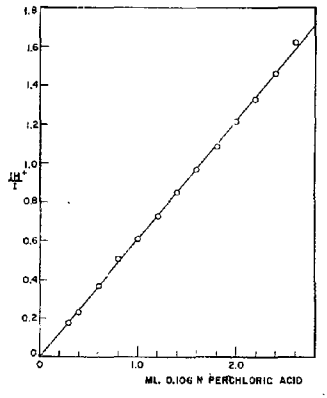

Figure 3. Blank titration of 25 ml. of glacial acetic acid with 0.106*N* perchloric acid using Sudan III indicator

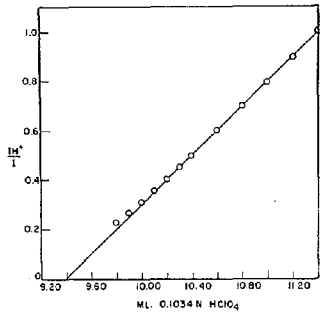

Figure 4. Type III plot for titration of urea in acetic acid with 0.1034*N* perchloric acid using Sudan III indicator

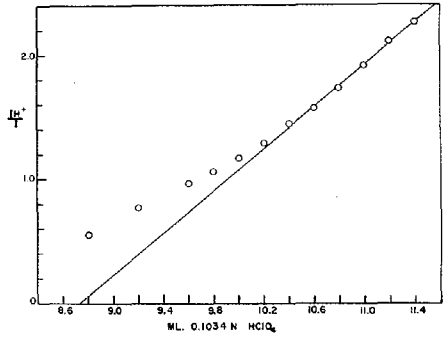

Figure 5. Titration of *N*-methylpyrrolidone in acetic acid with 0.1034*N* perchloric acid using Sudan III indicator

I = the base form of the indicator

a plot of ratio $\frac{(I.HClO_4)}{I}$ against the volume of added perchloric acid will yield a straight line with an intercept corresponding to the stoichiometric end point of the titration. In order to obtain linearity of such plots, the following equilibrium must be far to the left:

$$I + BH^+ClO_4^- \rightleftharpoons IH^+ClO_4^- + B$$

where B = the base being titrated
$BHClO_4$ = the perchlorate salt of the base

In cases where equilibrium between the indicator and the base perchlorate is significant, curvature of such plots can be expected, especially near the end point.

EXPERIMENTAL

Reagents and Chemicals. Hydrochloric acid solution, 1.3*N* in water, standardized against sodium carbonate.

Perchloric acid solution in acetic acid, 0.1*N*, prepared according to Fritz (*1*) and standardized against triphenylguanidine.

Acetic acid, ACS reagent grade, glacial.

Sodium acetate, ACS reagent grade, anhydrous.

Sodium benzoate, USP.

Aniline, ACS reagent grade, redistilled.

Pyridine, ACS reagent grade, redistilled.

Urea, recrystallized from ethyl alcohol–water mixture and dried under vacuum.

Antipyrine, recrystallized from ethyl alcohol–water mixture and dried under vacuum.

N-Methylpyrrolidone, General Aniline and Film Corp., fractionally redistilled (50-plate Oldershaw) under reduced pressure.

Metanil Yellow, Eastman Kodak.

Sudan III, National Aniline Division, Allied Chemical and Dye Corp.

Procedure. The experimental procedures used were essentially the same as previously described (*5*), except that the absorbance measurements were made using a Bausch & Lomb Spectronic 20 colorimeter.

Indicator acid-base ratios were calculated from the indicator absorbance data by means of the equation

$$\frac{IH^+}{I} = \frac{A - A_b}{A_a - A} \qquad (6)$$

where A_a = absorbance of pure acid form of indicator
A = absorbance during titration
A_b = absorbance of pure base form of indicator

The absorbance of the pure acid form of the indicator was obtained at the end of the titration after a large excess of acid had been added to the system. The absorbance of the pure base form of the indicator, if significant at the wave length used, was obtained at the beginning of the titration before the addition of standard acid. The wave lengths used in these titrations corresponded to the absorption peaks of the acid forms of the indicators.

TYPICAL RESULTS

Titrations in Aqueous Systems. In Figure 1 is shown a typical plot obtained during photometric titration of aniline in water with 1.3*N* hydrochloric acid in the presence of Metanil Yellow. Values of $\frac{IH^+}{I}$, as computed from the absorbance data obtained at 530 mμ, were plotted against the volume of standard acid added, giving a straight line, the intercept of which corresponded to the stoichiometric end point. Similar linear plots were obtained for the titrations of sodium acetate, sodium benzoate and pyridine. The results of titrations of these weak bases (Table I) show that precisions of the order of a few tenths of a per cent were obtained in most instances. It is questionable whether any other titrimetric procedure is inherently capable of yielding superior results for these same systems.

Titrations of sodium citrate (pK_b for dihydrogen citrate = 10.9) and glycine (pK_b = 11.6) in the presence of Metanil Yellow gave plots which approached linearity only at relatively high values of $\frac{IH^+}{I}$. Accurate extrapolations to the end points in these cases are, as may be expected, somewhat difficult. Figure 2 is a typical plot obtained for sodium citrate. Extrapolation of the linear portion of the curve permitted estimation of the amount of citrate present to about 2%. The curve obtained in the case of glycine did not permit simple extrapolation. Better plots for these compounds may be expected by using a weaker indicator and a somewhat more concentrated standard acid solution.

Titrations in Acetic Acid. Sudan III, which behaves as an extremely weak indicator in acetic acid (*5*), gave Type III plots for bases even as weak as urea. It was thought that the small amounts of water commonly present in acetic acid might interfere with the titration of weak bases by this method. However, a blank titration in acetic acid containing about 0.2% water gave a Type III plot (Figure 3) which was linear and passed through the origin upon extrapolation, indicating that no detectable titratable impurities were present in the solvent.

A standardized solution of perchloric acid in acetic acid containing Sudan III indicator was used to titrate several very weak bases in acetic acid by the method described. Linear plots of $\frac{IH^+}{I}$ *vs.* the volume of perchloric acid added were obtained for urea and antipyrine, both extremely weak bases. A typical plot obtained for the titration of urea is shown in Figure 4, the intercept representing the end point. Absorbance measurements were made at 615 mμ. The data in Table I show that these compounds were titrated with a precision of about 0.1%. A similar plot for the titration of *N*-methylpyrrolidone, however, did not approach linearity until relatively high indicator acid-base ratios were reached, making accurate extrapolation to the end point somewhat difficult. Figure 5 shows, however, that it was possible to extrapolate the linear portion of the curve, permitting estimation of the amount of *N*-methylpyrrolidone present to about ±2%.

ACKNOWLEDGMENT

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Determination of Strontium by X-Ray Fluorescence Spectrometry

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▶ A precise, rapid, and reliable x-ray fluorescence spectrometric method has been developed for the determination of strontium. This method is applicable to the analysis of a wide variety of earth substances. Yttrium is used as an internal standard. Calcium does not interfere. Only 100 mg. of sample is required, and a single determination can be made in 30 minutes. In the analysis of impure anhydrite, the standard deviation of the method is 0.005% strontium over the concentration range 0.005 to 0.4%.

MANY oil-bearing formations are largely composed of sediments containing calcium minerals, particularly the carbonates and the sulfates. The strontium-calcium ratio in these sediments would be of value in geological investigations. In the past, it has been impractical to study the significance of this ratio, as a reliable method for determining strontium in the presence of high calcium concentrations has been unavailable. The expressed need for a rapid and reliable method for the determination of strontium in impure anhydrite promoted the development of the method described in this paper.

Various chemical and instrumental methods have been proposed for the determination of strontium in the presence of other alkaline earths (*3, 8, 11, 12*). These methods are excessively time-consuming and unreliable when applied to the analysis of earth substances. Accordingly, the applicability of x-ray fluorescence spectrometry was investigated.

The principles of x-ray fluorescence spectrometry have been discussed fully in papers by members of the Naval Research Laboratories (*4, 5, 9, 10*). Although x-ray fluorescence is being used rather widely for the analysis of metals and alloys, relatively few applications to mineral analysis have appeared in the literature. Such applications can be found in papers by members of the U.S. Geological Survey and the U.S. Bureau of Mines (*1, 2, 6, 7*).

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INSTRUMENTATION

Spectrometer. A North American Philips x-ray fluorescence spectrometer attachment Type 52157-A was used in conjunction with the basic x-ray diffraction source unit. Collimation in this type of spectrometer is accomplished with an open source collimator and a 4-inch receiving collimator consisting of 0.001-inch-thick flat nickel plates spaced 0.005 inch apart. A Machlett OEG-50 tungsten target tube was used as the source of primary x-rays. The voltage and current supplied to the tube were regulated to ±0.25% and ±0.1%, respectively. An argon-halogen filled Geiger tube was used as the detector. All intensity measurements were made in air.

Analyzer Crystal. A lithium fluoride analyzer crystal was chosen because of its great reflectivity. It was mounted in a holder which could be positioned reproducibly in the instrument.

Sample Holder. The sample holder was made of 2S-O aluminum. Its scattering power is low and its fluorescence spectra cannot be detected with conventional instrumentation.

The holder was made to fit into the sample tray supplied with the instrument. It was 5.08 cm. long, 3.49 cm. wide, and 1.27 cm. deep. An area 22.2 by 34.8 mm. was exposed to the primary beam. The powdered sample was packed into a circular recess 17.5 mm. in diameter and 0.397 mm. ($^1/_{64}$ inch) deep.

Stirring Apparatus. This was made from an 8-ounce, screw-cap bottle and a stirring blade powered with an Eastern stirrer Model 1. The stirring blade is a modification of the one supplied with the Eastern stirrer. One to 2 grams of dry material can be homogenized in this apparatus in about 10 minutes. A small egg beater-like attachment was used for homogenizing 200 to 300 mg. of material.

INTERNAL STANDARD

In order to relate the intensity of fluorescent radiation to the concentration of the emitting element it is necessary to correct for absorption by the matrix. The most practical way to apply a systematic correction is by the use of an internal standard technique (*1*).

A suitable internal standard is an element which is not present in the sample and which has an excitation voltage close to that of the element to be determined. Yttrium was selected as the internal standard for the determination of strontium. The spectral

Table I. Spectral Characteristics of Strontium and Yttrium

Element	Atomic No.	Critical Voltage, Kv.	K_α Line, A.	K_β Line, A.	K-Absorption Edge, A.	Bragg Angle (2θ), Lithium Fluoride Crystal	
						K_α	K_β
Strontium	38	16.1	0.877	0.783	0.770	25.16	22.42
Yttrium	39	17.0	0.831	0.740	0.727	23.82	21.18

Table II. Calibration Data for Determination of Strontium in Anhydrite

Strontium Concn., %	Measurement of Y K_α at 2θ = 23.82°, Time (Sec.) for 6400 Counts				Measurement of Sr K_α at 2θ = 25.16°, Time (Sec.) for 6400 Counts				Intensity Ratio,[a] Sr K_α/Y K_α
	1	2	3	Av.	1	2	3	Av.	
0.0000	41.2	40.8	41.2	41.1	80.7	80.5	79.2	80.1	0.5131
0.1000	40.1	40.4	39.8	40.1	60.4	61.2	60.3	60.6	0.6617
0.2000	40.2	40.6	39.8	40.2	49.5	49.2	50.2	49.6	0.8105
0.3000	40.3	40.0	39.8	40.0	41.7	41.1	42.0	41.6	0.9615
0.4000	42.4	42.6	42.2	42.4	38.8	39.3	38.3	38.8	1.093

[a] $\frac{\text{Intensity Sr } K_\alpha}{\text{intensity Y } K_\alpha} = \frac{\text{counts per sec. at Sr } K_\alpha \text{ peak}}{\text{counts per sec. at Y } K_\alpha \text{ peak}} = \frac{\text{time for 6400 counts at Y } K_\alpha \text{ peak}}{\text{time for 6400 counts at Sr } K_\alpha \text{ peak}}$

characteristics of yttrium and strontium are given in Table I. The K_α line of each element was chosen for intensity measurements and the ratio of the intensity of strontium K_α to yttrium K_α was taken as a measure of the strontium content of the sample.

The excitation conditions chosen for the work presented in this paper were 45 kv. and 30 ma.

EXPERIMENTAL

The approximate concentration of the strontium in each sample of impure anhydrite was first determined by placing the powdered samples in the x-ray spectrometer and scanning in the region of the strontium K_α lines, 20° to 27° (2θ), using the lithium fluoride crystal. This preliminary scan also served to detect possible interferences. Standard samples covering the necessary concentration range were then prepared with strontium carbonate and yttrium oxide, with a synthetic anhydrite as the base material.

Standard Reagents. The strontium carbonate used was Specpure J.M. 91, obtained from the Jarrell-Ash Co. A yttrium oxide, 99% pure, was used as the internal standard; this was purchased from Research Chemicals, Inc., Burbank, Calif.

The synthetic anhydrite was prepared from Baker's analyzed, ACS reagent grade (low in alkalies) calcium carbonate. The carbonate was converted to the sulfate by dissolving it in concentrated hydrochloric acid and precipitating the calcium with sulfuric acid. The precipitate was washed, dried, and heated at 350° C. for 24 hours. An x-ray diffraction pattern showed that the calcium sulfate was in the anhydrite form. No strontium was detected in the anhydrite by x-ray fluorescence analysis.

Preparation of Calibration Standards. The preliminary scans of the impure anhydrite samples indicated that these samples contained from 0.0 to 0.3% strontium. Accordingly, standards containing 0.000, 0.100, 0.200, 0.300, and 0.400% strontium were prepared by mixing the required amount of strontium carbonate and synthetic anhydrite. A matrix mix containing 0.400% yttrium was also made from yttrium oxide and the anhydrite. Calibration standards were prepared by mixing equal weights of the strontium standards and the matrix mix. All materials were ground to 200 mesh before mixing.

The yttrium oxide and the synthetic anhydrite were homogenized by stirring in an Osterizer for 3 hours. The strontium standards and the calibration standards were mixed in the stirring apparatus.

Construction of Calibration Curve. A calibration curve was constructed by plotting the intensity ratio of the yttrium K_α line against the weight per cent of strontium in the strontium standards. The intensity ratio was obtained by measuring the time for 19,200 counts at the peak of each line and dividing the time for the yttrium count by the time for the strontium count. Three counts of 6400 each were made for each line, and the average count was used in calculating the intensity ratio. No corrections for background or dead time of the Geiger tube were made. The calibration curve is strictly empirical.

A working curve was constructed at the same time the samples were run. Typical data used in the construction of the curve are shown in Table II. A least-squares treatment of all the data was made and an analytical expression for the per cent strontium was derived:

$$\text{Strontium, \%} = 0.6920\ (\text{intensity ratio Sr } K_\alpha/\text{Y } K_\alpha) - 0.3602 \qquad (1)$$

PROCEDURE

Grind the entire sample to pass through a 200-mesh screen. Place 100.0 mg. of the sample in a 5-ml. beaker. Add an equal weight of matrix mix. Stir the dry mixture with the Eastern stirrer for 5 to 10 minutes, and place the powder in the sample holder. Place the sample in position in the instrument and scan from 20° to 27° (2θ). If no interferences are found, measure the time for 19,200 counts at the yttrium and strontium peaks under exactly the same conditions used for the standard samples. Calculate the intensity ratio, and read the per cent strontium from the calibration curve.

DISCUSSION

Table III shows the effect of mesh size of the sample on the intensity of strontium and yttrium radiation. As the particle size decreases, the intensity increases. But the gain in sensitivity obtained in going from 200- to 400-mesh samples is not justified because of the increase in time required to prepare the finer samples.

The increase in the ratios of strontium to yttrium intensities with decreasing particle size cannot be explained by variations in the density of the packings. Adler and Axelrod (*1*) and Mortimore, Romans, and Tews (*13*) attribute this type of variation to particle size effects and indicate that the variation is due to a lack of complete intimacy between the internal standard and the sample. The latter investigators (*13*) found that in the case of columbium and tantalum ores complete intimacy could be obtained only by chemical treatment of the ores. Adler and Axelrod (*1*) found that a grinding technique seemed to allow analyses to be made with an accuracy of about 5% in the worst cases investigated by them. The accuracy with which the intensity ratio is determined is the limiting factor in the accuracy of the determination.

Matrix. With the internal standard technique the matrix composition is not critical. However, the best choice of matrix material is one which approximates the composition of the samples to be analyzed. Since the samples were anhydrite, a strontium-free anhydrite was used as the matrix for the standards and as a diluent for the samples.

Mixing. The effectiveness of the mixing procedure was tested by measuring the fluorescent intensity of an element in different aliquots of a material.

The matrix mix and a standard were chosen for study. The yttrium distribution in the matrix mix and the strontium distribution in the standard were determined on five separate portions of each material. Each portion was counted

Table III. Variation of Intensity Ratio with Particle Size[a]

Particle Size, Mesh	I_{Sr}, c./s.	I_Y, c./s.	I_{Sr}/I_Y
−100	281.9	247.1	1.14
−200	329.0	284.4	1.16
−325	331.6	278.3	1.19
−400	352.6	288.3	1.22

[a] Data obtained with anhydrite sample containing 0.5% strontium and 0.5% yttrium at 45 kv. and 30 ma. A 12,800-fixed count was taken in each case.

Table IV. Replicate Yttrium Determination on Various Portions of Matrix Mix

Time, Sec. for 6400 Counts at Y K_α Peak				
1	2	3	4	5
		Undisturbed Sample		
28.9	28.3	28.5	29.0	28.1
28.3	28.2	28.1	28.0	28.3
28.1	28.0	28.5	29.0	28.5
27.9	28.0	28.6	28.3	28.0
28.2	27.8	29.0	28.6	28.4
		Repacked Sample		
29.4	28.0	29.5	28.6	28.4
29.0	28.2	29.5	28.6	28.2
28.9	28.4	27.8	28.1	27.9
28.7	28.0	28.7	28.4	28.1
29.5	28.0	27.3	28.5	28.4

in two ways. First, it was placed in the instrument and five fixed counts were made without disturbing the sample. Second, the sample was removed from the instrument, poured out of the sample holder, repacked, and placed back in the instrument for counting. One fixed count was taken. Then the sample was removed, repacked, and returned to the instrument for another count until a total of five counts on repacked samples had been made.

In this way the variance due to the distribution of the element in the material, the variance due to packing, and the replicate variance are separated. Table IV presents the data obtained for yttrium in the matrix mix. The actual quantity measured was the time in seconds required to accumulate 6400 counts at the yttrium K_α peak. Table V shows the analysis of variance from the data in Table IV. None of the F values are significant at the 99% confidence level (*14*). Thus, at this confidence level there is no evidence of a difference among the various sample portions. Therefore, it is concluded that the matrix mix is homogeneous and the mixing procedure is adequate. A similar experiment performed with the strontium standard led to the same conclusion.

Interferences. The internal standard technique is valid only if the various elements in the matrix of the sample affect the reference line and the analytical line in exactly the same way. If either line is selectively absorbed or enhanced by a matrix element, the intensity ratio is not a true measure of the analytical element. In order for an element to cause selective absorption or enhancement, it must have either an absorption edge between the two emission lines being measured or a strong emission line between the absorption edges of the elements involved (*1*). In the case of the line pair, strontium K_α and yttrium K_α, only four elements can cause this type of interference: krypton, niobium, uranium, and thorium.

Elements which give rise to emission lines falling so close to the analytical or reference line that they cannot be resolved also cause interference. In the determination of strontium, rubidium is the most likely interference of this type.

Precision. The precision of the analytical procedure was estimated by two different methods. The first estimate was based upon the deviations of the calibration points from the fitted straight line. Estimate of the standard deviation (based on 3 × 6400 or 19,200 counts for each line intensity) equals

$$\sqrt{\frac{\Sigma d^2}{n-2}} = 0.0049 \qquad (2)$$

Table V. Analysis of Variance from Data in Table IV

Source of Variance	Sum of Squares	Degrees of Freedom	Variance	F
Aliquots	24.3	4	6.10	3.51[a]
Packing	2.4	1	2.40	1.40[b]
Interaction	15.0	4	3.75	2.16[a]
Replicates	69.7	40	1.74	
Experimental	41.7	9		
Total	111.4	49		

[a] To be significant at 99% confidence level, F must be 3.83.
[b] To be significant at 99% confidence level, F must be 7.31.

Table VI. Statistical Treatment of Data Obtained on Standard Samples Used in Preparation of Working Curve for Determination of Strontium in Anhydrite

Strontium in Sample, %	Intensity Ratio, Sr K_α/Y K_α	Calcd. Strontium Content, %	Difference between Chemical and X-Ray Methods, d
0.0000	0.5131	−0.0051	0.0051
0.1000	0.6617	0.0977	0.0023
0.2000	0.8105	0.2007	−0.0007
0.3000	0.9615	0.3052	−0.0052
0.4000	1.093	0.3962	0.0038

The second estimate was obtained from replicate determinations of the strontium in a sample. Data for these methods are shown in Tables VI and VII.

Reproducibility of Calibration Curve. The calibration data obtained on various days showed a greater variance than the variance of the method for any one day with a particular calibration curve. The data from a set of standards obtained on five different days are compared in Table VIII. The values shown in terms of per cent strontium are higher than the standard deviation of 0.005% strontium for the method. This indicates that for the best possible precision a calibration curve should be prepared at the same time the samples are run.

Accuracy. An estimate of the accuracy of the anhydride analysis was made by determining the strontium in National Bureau of Standards' sample 1A, argillaceous limestone. The average of five determinations by the x-ray fluorescence procedure showed standard sample 1A contained 0.197% strontium instead of the 0.10% strontium indicated on the National Bureau of Standards' certificate of analysis. A similar discrepancy has also been found by Diamond (*8*), who reported a strontium content of 0.19%. An optical emission spectrographic determination of the strontium in sample 1A gave a value of 0.17%. The values obtained by the three methods agree to within the limits of error for each method.

Table VII. Determination of Precision

Sample Designation	Strontium, %
6987	0.1908
	0.2010
	0.1888
	0.1984
	0.1910
	Av. 0.1940
	Std. dev. 0.0054
NBS, 1A	0.1948
	0.1992
	0.2020
	0.1934
	0.1936
	Av. 0.1966
	Std. dev. 0.0038

Table VIII. Comparison of Calibration Obtained on Various Dates

Strontium Content, %	Intensity Ratio on Various Dates					Standard Deviation	
	June 16[a]	June 23	June 27	June 29	July 18	Intensity ratio	Sr, %
0.0000	0.5131	0.5444	0.4969	0.5220	0.5348	0.0185	0.0068
0.1000	0.6617	0.6575	0.6742	0.6541	0.6667	0.0079	0.0057
0.2000	0.8105	0.8220	0.8084	0.8062	0.8101	0.0057	0.0042
0.3000	0.9615	0.9574	0.9737	0.9648	0.9882	0.0122	0.0084
0.4000	1.093	1.068	1.081	1.065	1.069	0.0061	0.0081

[a] Actual counting data are shown in Table IV.

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Determination of Protactinium by Gamma Spectrometry

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▶ **Gamma spectrometry was used to determine protactinium in concentrations of less than 1 p.p.m. in uranium residues. It was found extremely sensitive for the detection of protactinium in any sample, residue, or container. Qualitatively, protactinium was identified by its 27-, 95-, and 300-k.e.v. peaks. After removal of interfering activities, the magnitude of the 300-k.e.v. peak was used as a quantitative measure of the protactinium content. Calibration was achieved with standard protactinium samples. The interfering isotopes were removed by coprecipitation with thorium fluoride carrier, whereas the protactinium remained in solution as a fluoride complex.**

ONE of the most difficult problems in the separation of protactinium from uranium residues is that of control analysis (*8*). In a review of the chemistry of protactinium (*6*), Miles stated that protactinium has been noted for the ease with which it disappears during processing.

Common radiochemical methods based on differential growth or decay (*5*) are not practical because of the relatively long half lives of protactinium-231 and its first daughter, actinium-227 (34,300 and 22 years, respectively). Direct alpha counting (*4*) is complicated by the presence of other alpha-emitting elements, such as ionium and polonium, which are always present in protactinium sources. This complication is alleviated by use of an alpha pulse height analyzer. However, because of the high absorption of alpha radiation by extraneous materials, a complete chemical separation is required and unknown quantities of protactinium may be lost during the analysis. Katzin, Van Winkle, and Sedlet (*4*) have reported a procedure for the analysis of ore residues for protactinium using a chemical separation procedure with a reported yield of only 50 to 60%.

The distribution of protactinium in chemical separations can be followed by use of beta-active protactinium-233 as a tracer (*6*). The principal complication is the lack of assurance of isotopic exchange between the tracer and the protactinium in the sample. If the exchange is incomplete, the result will be erroneous.

The foregoing discussion shows the need for a more direct method of control analysis for protactinium. The use of gamma pulse height analysis was found to be very satisfactory. Qualitatively, protactinium can be identified by the presence of its 27-, 95-, and 300-k.e.v. peaks. After removal of interfering activities by precipitation on thorium fluoride carrier the magnitude of the 300-k.e.v. peak was used as a quantitative measure of the protactinium content in uranium residues with the accuracy within ±5%. Gamma spectrometry is extremely valuable for following the distribution of protactinium during processing. Since absorption by extraneous materials is not serious for gamma radiation, the protactinium could be detected in precipitates, on filter papers, in solutions, on ion exchange resin, and even on beaker and platinum crucible walls. At no time during processing did the protactinium permanently disappear, for its presence could be detected in any sample, residue, or container.

INSTRUMENTATION

The instrumentation used for the analytical work consisted of a single-channel pulse height analyzer, Atomic Instrument Co., Model 510; a preamplifier, Radiation Counter Laboratory, Mark 15, Model Ala; a linear amplifier, Radiation Counter Laboratory, Mark 15, Model Al; an Atomic Instrument Co. scaler with a 1-microsecond input strip (Model 118), a 5-microsecond decade scaling strip (Model 108B), two 40-microsecond decade scaling strips (Model 109B), and with a 3000-volt positive grounded RF power supply; a photomultiplier tube, Du Mont No. 6292; and a 2-inch thallium activated sodium iodide well crystal.

The photomultiplier tube and crystal were covered with an aluminum light shield which was surrounded by lead bricks.

Measurements were made with a 1-volt channel width. For convenience, the analyzer was so calibrated that the gamma peaks occurred at a base line setting nearly numerically equal to the gamma energy in kilovolts. This was accomplished by placing a cesium-137 standard (660-k.e.v. peak) in the well of the crystal, and adjusting the gain so that the photopeak occurred close to a base line setting of 660. The photo-

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peak for a second standard, such as mercury-203 (280-k.e.v. peak) was found, and base-line setting was plotted against gamma energy in kilovolts. The resulting calibration curve was a straight line through the origin.

The gamma pulse height analyzer had an upper limit counting rate of 25,000 counts per minute at the highest peak. This limit was set to avoid overloading the photomultiplier tube, which would result in poor resolution and possible damage to the tube. For best quantitative results a counting rate half this large is preferable.

Protactinium-231 has gamma peaks of 27, 95, 294, and 323 k.e.v. (*2*). Resolution of the instrument was insufficient to separate the latter two peaks, thus giving rise to a single peak at about 300 k.e.v. The gamma spectrum for pure protactinium is shown in Figure 1. A pure sample is indicated by the absence of other peaks except those attributable to Compton electrons (*1*), and by the relative heights of the three protactinium photopeaks.

METHOD

Direct gamma spectrum analysis of complex mixtures containing a low concentration of protactinium relative to other radioactive constituents can only serve as a means of qualitative detection of protactinium by the presence of the 27-, 95-, and 300-k.e.v. peaks. However, a quantitative determination can be obtained if the interfering activities are separated. The separation is accomplished chemically by dissolving the sample in a mixture of hydrochloric and hydrofluoric acids, thereby complexing the protactinium with fluoride (*3*). The addition of a few milligrams of thorium to the solution then causes the precipitation of thorium fluoride which carries the thorium isotopes and their interfering daughters. The solution contains the protactinium. The spectrum of the fluoride shown in Figure 2 is typical for the interfering thorium isotopes and their daughter activities. The peaks at about 68 and 95 k.e.v. are probably due to thorium-230 and thorium-227, respectively, while the slight peak at 270 k.e.v. is probably due to radium-223. The 27- and 300-k.e.v. peaks of protactinium are not evident. When the filtrate from the thorium fluoride precipitation was evaporated to dryness its gamma spectrum was typical of protactinium with the usual peaks at 27, 95, and 300 k.e.v. The spectrum obtained from this residue is also shown in Figure 2. This spectrum resembles that of pure protactinium shown in Figure 1. The separation of thorium is indicated by the absence of a 68-k.e.v. peak. Some variation in relative peak heights from that shown in Figure 1 results because of greater internal absorption in the residue of the lower energy gamma rays than in pure protactinium and because of the presence of some residual impurities which add to the 95-k.e.v. peak. However, the absorption at 300 k.e.v. is negligible and the residual impurities amount to less than 5% of the activity at 300 k.e.v.

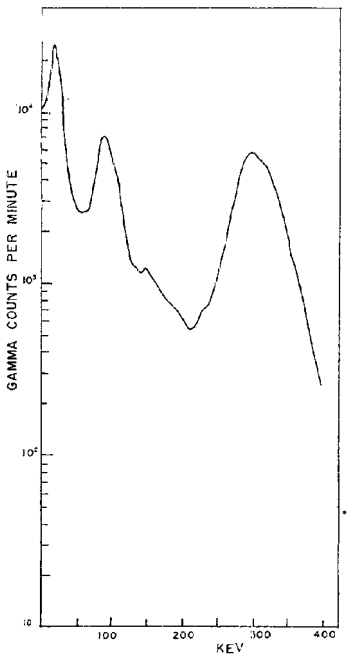

Figure 1. Gamma spectrum of protactinium-231

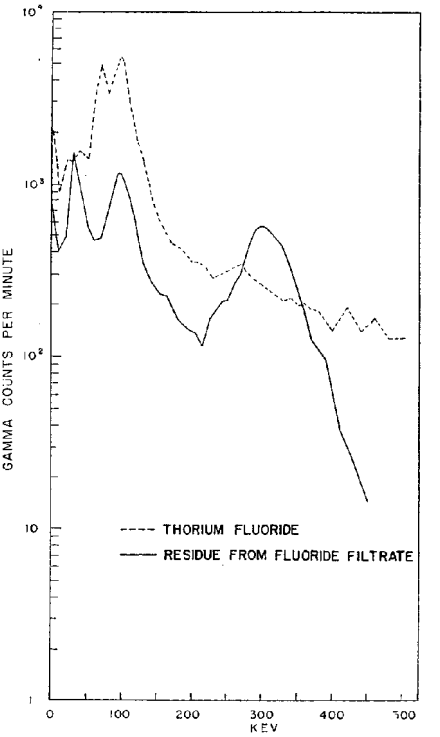

Figure 2. Gamma spectra of thorium fluoride and residue from fluoride filtrate

RECOMMENDED PROCEDURE

Weigh about 5 grams of the uranium residue into a small bottle 0.5 inch in diameter and 2 inches in height (a 2-dram vial is convenient to use). Insert the bottle into the crystal well of the gamma spectrometer and count the sample for at least 10 minutes at 300 k.e.v. Correct the count for the background of the instrument. Transfer the sample to a beaker with a small quantity of water. Add 100 ml. of 9*N* hydrochloric acid and 1 to 2 ml. of 48% hydrofluoric acid. Heat until the sample dissolves. Additional hydrofluoric acid may be necessary. Cool the solution to room temperature. Add slowly with stirring 10 ml. of a thorium solution (1 mg. of thorium per ml. of 1*N* hydrochloric acid solution). Allow the mixture to stand for 5 minutes. Add an additional milliliter of 48% hydrofluoric acid, stir the mixture, and allow to stand for 5 minutes. Add a second 10 mg. of thorium carrier, stir the mixture, and again allow it to stand for 5 minutes. Filter the thorium fluoride. Transfer the fluoride and paper to the bottle used to count the original sample. Insert the bottle into the crystal well and gamma count at 300 k.e.v. for at

Table I. Analyses of a Uranium Residue for Protactinium

	I	II
Weight of residue taken, grams	5.09	5.28
Activity taken, c./m. at 300 k.e.v.	2382	2435
Activity in first fluoride precipitate, c./m. at 300 k.e.v.	579	601
Activity in second fluoride precipitate, c./m. at 300 k.e.v.	16	4
Activity due to Pa, c./m. at 300 k.e.v.	1787	1830
Per cent activity due to Pa	75.0	75.2
Weight of Pa, γ	1.37	1.40
Pa concentration in residue, p.p.m.	0.27	0.27

Standard: 2155 c./m. at 300 k.e.v./1.65 γ Pa.

Table II. Efficiency of Analytical Procedure Utilizing Gamma Spectrometry

	I	II
Weight of uranium ore residue taken, grams	5.65	20.0
Calculated Pa content, γ	1.53	5.40
Pa recovered, c./m. at 300 k.e.v.	2020	6845
Pa recovered, γ	1.55	5.24
Deviation between calculated and recovered Pa, %	+1	−3

Standard: 2155 c./m. at 300 k.e.v./1.65 γ Pa.

least 10 minutes. Correct the counts for the background of the instrument. Make a blank determination to correct for the activity of the thorium carrier. Obtain the protactinium count by subtracting the gamma count in the fluoride from that in the original sample. Count a standard protactinium sample at 300 k.e.v. and calculate the protactinium concentration in the uranium residue.

The protactinium was usually determined by difference rather than by direct count because of the difficulty in preparing a counting sample from the thorium fluoride filtrate. The filtrate has a volume of about 125 ml. and contains all the dissolved salts in the sample. Evaporation of this solution into a small vial as a routine procedure is tedious.

It is necessary that the protactinium be complexed with fluoride prior to the addition of the thorium, otherwise part of the protactinium will be carried by the thorium fluoride (*3*). But thorium fluoride precipitated in this manner would be an inefficient carrier for even the thorium and other extraneous activities. The difficulty is overcome by precipitating the fluoride in strongly acid solution, a medium in which thorium fluoride forms rather slowly. It is possible to mix the thorium into the solution prior to its complete precipitation and, thus, improve its carrying properties. The additional hydrofluoric acid added after the thorium aids in the quantitative precipitation of the thorium fluoride due to mass action.

The intensity of the 300-k.e.v. peak was usually determined in preference to the 27- and 95-k.e.v. peaks because absorption in the samples or containers and interferences from other radioactive elements were less pronounced.

The standard samples were prepared from a protactinium solution of known concentration. Aliquots were diluted to 2 ml. in a vial. The protactinium from which the standards were prepared was obtained from the stock of Brookhaven National Laboratory and was shown to be pure by both alpha and gamma pulse height analysis.

RESULTS AND DISCUSSION

A residue obtained from the processing of pitchblende for uranium was analyzed for protactinium using the thorium fluoride separation procedure. To demonstrate the completeness of removal of thorium and its daughters, the separation procedure was repeated on the filtrate. The results of two such determinations on the same uranium residue are shown in Table I. As only a very slight count was obtained on the second fluoride precipitate, it was assumed that the recommended procedure removed essentially all the extraneous activities. The activity in each sample due to protactinium was then determined by difference. The results for the two samples showed excellent agreement. The percentage of the gamma activity at 300 k.e.v. due to protactinium was 75%. The weight of protactinium in 5 grams of residue was about 1.4 γ, corresponding to a protactinium concentration of 0.27 p.p.m.

The protactinium in the uranium ore residue was separated and purified by a procedure which results in high chemical purity as well as radiochemical purity (*7*). This process involves the solution of the ore residue in dilute hydrochloric acid, precipitation of protactinium on a silicate carrier, and final purification of protactinium by an ion exchange procedure. The radiochemical purity of protactinium obtained by this process has been established by gamma spectrum analysis, alpha spectrum analysis, and alpha range analysis. A determination by gamma spectrum analysis of the loss of protactinium at each step in the procedure shows the total recovery to be in the range of 95 to 97%. Table II shows a comparison between the actual amount of protactinium recovered by the purification process and that predicted by the analysis of the ore residue. It can be seen that the method is accurate to within at least ±5%. In some cases it may be considerably more accurate. For example, the loss of protactinium in the silicate carrier step of the chemical purification method (*7*) was determined by gamma spectrum analysis to be no more than 3%. The same silicate carrier step was also evaluated using beta-active protactinium-233 as a tracer, and again the loss of protactinium was no more than 3%. Gamma spectrometry in this example had an accuracy of better than ±1%.

ACKNOWLEDGMENT

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Spectrophotometric Determination of Trypsin and Trypsin Inhibitors

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▶ A simple and rapid spectrophotometric method for the determination of trypsin and trypsin inhibitors is based on the determination of acid production by means of changes in absorption of indicators. When a recording spectrophotometer is employed, the enzyme activities may be obtained directly from the slopes of the transmittance-time curves plotted by the recorder. Amounts of trypsin from 1 to 15 γ can be determined in a substrate-buffer-indicator solution of 0.013M p - toluenesulfonylarginine methyl ester, 0.01M tris(hydroxymethyl)aminomethane (pH 8.2), and 0.01% *m*-nitrophenol.

During studies on egg proteins in this laboratory, it became desirable to develop a simple and rapid assay for trypsin and trypsin inhibitors. Current methods utilized the esterase or amidase activity of trypsin on synthetic esters or amides (*2, 4, 7–9*). The activity was determined by titrating the acid produced or by utilizing the difference in ultraviolet absorption spectra between the substrate and its split products. A much simpler method seemed possible by photometric determination of the acid produced through color changes of an acid-base indicator. The current availability of spectrophotometers equipped with automatic recording devices would make possible the determination of enzyme activity simply by comparing the slopes of transmittance-time curves plotted by the instrument.

APPARATUS AND MATERIALS

A Beckman Model DU spectrophotometer equipped with photomultiplier attachment was used for all spectrophotometric determinations. It was connected to a Bristol's Dynamaster recording potentiometer through a Beckman energy recording adapter, Model 5800. The chart speed was 1 inch per minute. The spectrophotometer cell compartment was also furnished with thermospacers through which water from a thermostated bath was circulated. A constant temperature (37° C.) was thus maintained during all determinations. An auxiliary constant temperature bath maintained all reagents at the same temperature as that of the cell compartment.

Potentiometric titrations were performed using a Beckman Model G pH meter (*2, 7, 8*). Stirring was accomplished by bubbling nitrogen through the solution.

The trypsin was a twice recrystallized product containing approximately 50% magnesium sulfate as purchased from the Nutritional Biochemical Corp., St. Louis, Mo. It contained 37.9% trypsin as calculated on a nitrogen content for pure trypsin of 16.13% (*6*). Standard solutions of trypsin were prepared on the basis of this nitrogen content and were made up daily in 0.002*N* acetic acid.

Ovomucoid was prepared by the method of Lineweaver and Murray (*5*) and activities were reported on a dry basis corrected to a nitrogen content of 13.3% nitrogen (*5*). The soybean trypsin inhibitor, preparation S15314, was purchased from Worthington Biochemical Corp., Freehold, N. J. Egg white was blended (*10*) and diluted in distilled water (*2*) for assay.

The substrate, *p*-toluenesulfonylarginine methyl ester, was synthesized essentially according to the method of Bergmann, Fruton, and Pollok (*1*). The buffer, tris(hydroxymethyl)aminomethane (Sigma 121, lot 125–130, Sigma Chemical Co., St. Louis, Mo.), was dissolved in water and adjusted to pH 8.2 with hydrochloric acid. Indicators used were as follows: *m*-nitrophenol, Eastman White Label, No. 1340; phenol red (phenolsulfonephthalein) Eastman, Lot No. 11; *m*-cresol purple, Fisher Scientific Co.; cresol red; brilliant yellow; and neutral red. The last three indicators were from National Aniline Division, Allied Chemical and Dye Corp.

EXPERIMENTAL METHODS AND RESULTS

Development of Method. It was necessary to consider the following interrelated factors to obtain a linear change in transmittance over the desired range of change in pH:

pH optimum of the enzyme and the suitable range of change in pH.

The buffer and its concentration.

The concentrations of substrate and enzyme.

The indicators and the proper wave lengths and concentrations thereof.

The over-all operating conditions, including volumes of reagents, order of addition, reaction time, and the like.

Schwert and coworkers (*8*) reported that the maximum rate of hydrolysis of *p*-toluenesulfonylarginine methyl ester by trypsin at 25° C. occurred at a pH range of 7.9 to 8.4. Buffers were examined which should give an approximately linear change in pH with addition of acid over this range. Substrate concentrations as previously described (0.01 to 0.02*M*) (*2, 8*) and 0.01*M* tris buffer, pH 8.2, were found suitable. Indicators were then chosen which would give an approximately linear change in transmittance with change in pH in the buffer selected. Confirmation of these linear relationships was obtained by potentiometric titration of the buffer with acid and by measuring the transmittance of the indicator-buffer solution at several pH values. *m*-Nitrophenol at 395 mμ and phenol red at 440, 520, or 565 mμ were the most satisfactory of the indicators studied. The wave lengths indicated were at absorption maxima, with the exception of 520 mμ for phenol red. *m*-Nitrophenol (0.01%) gave a linear change over a greater range in pH than did phenol red and was, therefore, used.

The procedure finally adopted for the assay of trypsin was as follows. A 1-ml. portion of enzyme solution, containing 1 to 15 γ, was placed in the cuvette of the spectrophotometer, and 2 ml. of substrate-buffer-indicator solution [0.02*M p*-toluenesulfonylarginine methyl ester, 0.015*M* tris(hydroxymethyl)-aminomethane at pH 8.2, and 0.015% *m*-nitrophenol, respectively] was added. The recording was started 2 to 3 seconds after this addition. Adequate mixing of solutions was accomplished by blowing the substrate-buffer-indicator solution into the cuvette using a fine-tipped pipet. Direct estimation of enzyme activities was possible by comparison of the slopes of the linear portions of the plotted curves.

Trypsin Assays. A series of experiments was performed using various concentrations of trypsin. Figure 1 presents the curves obtained in one experiment at the concentrations of trypsin indicated. In all cases, the curves were linear for at least 60 seconds. When the slopes of these curves were then plotted against concentrations of trypsin, a linear relationship was ob-

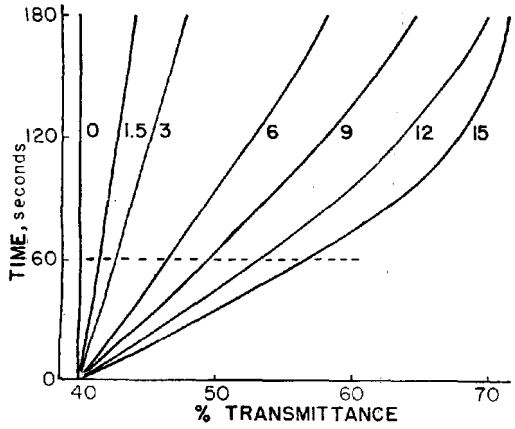

Figure 1. Reproduction of curves plotted by recorder for trypsin assay using *m*-nitrophenol at 395 mμ

Figures on curves represent micrograms of trypsin; slopes determined below dotted line

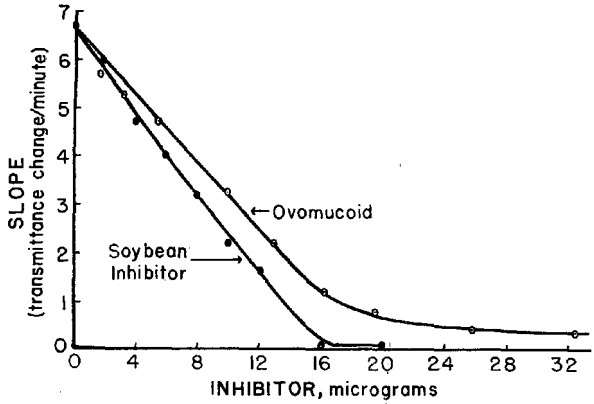

Figure 2. Curves for trypsin inhibitor assay

Each concentration of inhibitor assayed in presence of 15 γ of trypsin

Table I. Comparison of Methods for Determination of Trypsin and Ovomucoid

Material Added, γ		Method		
Trypsin	Ovomucoid	Spectrophotometric, % T/minute	Spot plate, seconds[a]	Titration, ml./minute[b] × 10^{-2}
0		0	>1200	<0.1
3		1.43	153	0.63
6		2.70	64	1.52
9		4.00	42	3.13
12		5.50	33	3.85
15		6.67	24	4.17
15	3.2	5.25	31	4.00
15	6.5	4.70	41	3.45
15	9.7	3.25	58	1.39
15	13.0	2.20	130	0.83
15	16.2	1.20	500	0.62
Weight ratio, ovomucoid to trypsin		1.16	1.2	1.30

[a] Seconds required for indicator to turn from red to yellow.
[b] Milliliters of 0.1N sodium hydroxide added per minute.

tained. The pH changed less than 0.3 unit per minute at the highest enzyme concentrations.

Amounts as low as 1.0 γ of trypsin may be detected by this method (Figure 1). Other experiments performed at lower buffer concentration (0.005M) increased the sensitivity to trypsin, and a more concentrated solution of buffer (0.03M) made it possible to determine an amount of trypsin as high as 50 γ.

Inhibitor Assays. The inhibitors tested included ovomucoid, soybean trypsin inhibitor, and crude egg white. In the assay for inhibitors, the method for trypsin was used, except that the inhibitor (3 to 15 γ) was mixed with the trypsin (15 γ) prior to addition of the substrate-buffer-indicator solution. With ovomucoid, it appeared desirable to neutralize the enzyme-inhibitor mixture before adding the substrate solution. This reduced the variability of the assay. Figure 2 represents the results of one of many experiments with ovomucoid and soybean trypsin inhibitor. If the curves are extrapolated from the initial linear portions to intercept the abscissa, the theoretical amount of inhibitor needed to inhibit completely 15 γ of trypsin is obtain d. The calculated ratios of the weights of inhibitor to trypsin were 0.96 for the soybean trypsin inhibitor and 1.16 for ovomucoid. Soybean trypsin inhibitor gave practically complete inhibition at the higher concentrations while ovomucoid did not. These observations are in agreement with previous reports (*4*, *5*). Blended egg white was found to contain 12.7% ovomucoid on a dry weight basis, as previously reported (*3*).

When calcium chloride (0.02M) was added to the substrate-buffer-indicator solution, the activity of trypsin was increased approximately 25% over the activity of trypsin without added calcium. However, the calcium also shifted the inhibitor curve of ovomucoid to a similar degree and the ratio of weight of ovomucoid to weight of trypsin therefore remained essentially unaffected at the lower levels of inhibition (less than 50%) (*4*). Calcium should be included in all determinations where contamination with appreciable amounts of calcium is suspected.

Spot Plate Estimations. Approximate estimations can be made on spot plates with the same reagents used in the spectrophotometric determination. For this purpose, phenol red was used instead of *m*-nitrophenol, because the change in color of phenol red was easier to observe visually. In this procedure the activities of the enzyme were related to the time required for the color to change from red to yellow. Standard curves were prepared with 0.01 to 0.1 ml. of solutions of trypsin containing 150 γ per ml. and 0.2 ml. of a sub-

strate-buffer-indicator solution [0.02M p-toluenesulfonylarginine methyl ester, 0.015M tris(hydroxymethyl)aminomethane at pH 8.2, and 0.002% phenol red]. Comparative results using the spot plate method are presented in Table I.

Acid Titrations. As a standard method of comparison, potentiometric titrations were performed essentially by the procedure of previous investigators (*2, 8*). Solutions were the same except for the use of 0.005M tris(hydroxymethyl)aminomethane in place of phosphate buffer.

Comparative Results. Table I gives a direct comparison of the spectrophotometric, spot plate, and conventional titrimetric determinations of trypsin and ovomucoid, as well as a comparative value of the ratio of ovomucoid to trypsin. The agreement is good for the three independent methods.

DISCUSSION AND CONCLUSIONS

The described spectrophotometric method for the determination of trypsin and trypsin inhibitors is rapid and very simple, supplies permanent records and allows for direct determination of activities from the slopes of the recorded curves. It is superior to the titration method for these reasons, and also because of greater accuracy and reproducibility. The method should be adaptable for determinations of other enzymes which produce changes in pH. Indicators and buffers, however, would need to be appropriately selected for each particular case. The spectrophotometric method for determination of acid production can be adapted for use with instruments not connected to recording devices. This would require manual recording of the changes in transmittance with time, which also seems superior to the titrimetric procedure.

The quantitative relationships of trypsin and inhibitors found in this study are comparable to those previously reported (*4, 5*). On a molar basis the ratios are approximately unity. The optimum working range for trypsin was 1 to 15 γ and for the inhibitors, as ovomucoid or the soybean trypsin inhibitor, 3 to 12 γ. The apparent reproducibility was $\pm 5\%$. Amounts of enzymes and inhibitors that could be determined were, of course, directly dependent upon the concentration of the buffer.

The spot plate method should be useful in supplying approximate values during studies involving fractionation, inactivation of inhibitors, and the like.

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Ultraviolet Absorptiometric Determination of Boron in Aqueous Medium Using Chromotropic Acid

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▶ **Boric acid causes changes in the ultraviolet absorption spectrum of chromotropic acid in aqueous solution of sufficient magnitude to be of analytical importance. An absorptiometric method was developed for determining boron in the range 0.1 to 2.4 p.p.m. by measuring the decrease in the absorbancy of chromotropic acid caused by the addition of boric acid. Measurements were made at 316.5 mμ, the point of maximum change, with solutions adjusted to a pH of approximately 7.**

BORON is one of the few common elements whose analytical chemistry leaves much to be desired, particularly in the field of spectrophotometry. The majority of spectrophotometric or colorimetric methods reported for boron involve the use of concentrated sulfuric acid, in which solvent numerous organic reagents give color changes in the presence of boron (*3, 10, 12*). 1,2,5,8 - Tetrahydroxyanthraquinone, known commonly as quinalizarin, is a widely used reagent in this class, and is the basis of the tentative ("first action") AOAC method for the determination of boron in soils (*6*).

The most widely used method not requiring the use of concentrated sulfuric acid is that involving the use of 1,7 - bis - (4 - hydroxy - 3 - methoxyphenyl) - 1,6 - heptadiene - 3,5 - dione, otherwise known as curcumin. The curcumin method depends upon the formation of a colored product upon evaporation to dryness of a mixture of boric acid, oxalic acid, and curcumin. This colored product is usually extracted into 95% ethyl alcohol. Numerous variables, particularly the evaporation temperature, must be carefully controlled for reproducible results. However, the curcumin method is used frequently in plant and soil analysis (*4,8*), and has recently been applied to the determination of boron in semiconductor materials (*7*).

Boric acid forms colorless complexes with a number of polyhydroxy compounds in aqueous medium (*2*). The use of glycerol or mannitol in the alkalimetric titration of boric acid is a practical utilization of these colorless complexes. Many of the compounds reported to form these complexes with boric acid absorb ultraviolet radiant energy. It appeared that the addition of boric acid to solutions containing these polyhydroxy compounds would result in changes in their ultraviolet absorption spectra which might have some analytical value.

Andress and Topf (*1*) have described the changes which occur in the ultraviolet spectrum of an aqueous solution of disodium 4,5-dihydroxy-2,7-naphthalenedisulfonate upon the addition of

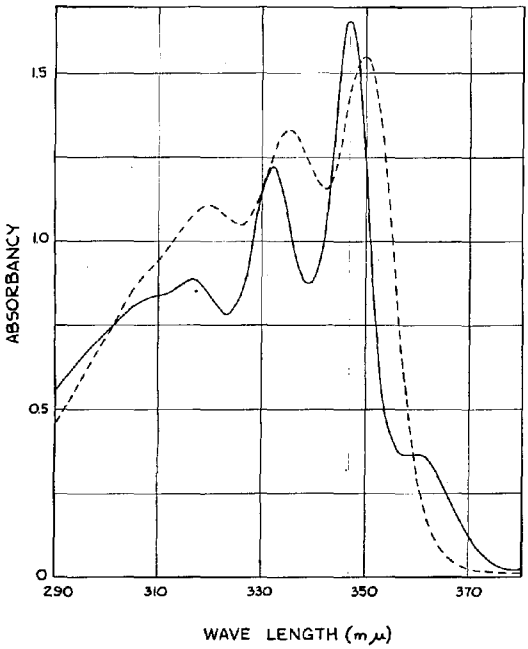


Figure 1. Effect of boric acid on absorption spectrum of chromotropic acid in aqueous solution in pH range 4 to 5

——— 0.00014*M* solution of chromotropic acid, pH 5.1
– – – – Solution 0.00014*M* in chromotropic acid and 0.04*M* in boric acid, pH 4.1

boric acid. The parent acid of this salt is commonly known as chromotropic acid. The contribution of the present investigation was the development of a simple, rapid absorptiometric method for the determination of boron in aqueous solution, utilizing this effect of boric acid on the ultraviolet spectrum of disodium chromotropate.

EXPERIMENTAL WORK

Apparatus. All absorption spectra were obtained on a Cary Model 10-11M recording spectrophotometer, using matched 1-cm. quartz absorption cells. The slit control dial was kept at position 1 for the spectra to give a spectral band width of approximately 1 A.

The Cary absorption cells fitted with tapered necks for ground-glass stoppers, intended for use with volatile solvents, proved useful in working with the solutions of high salt content (0.4*M* in sodium acetate). The solutions were transferred to the absorption cells by means of 50-ml. beakers. Any liquid adhering to the outside of the cells was conveniently removed by dipping the cell in distilled water while holding the cell by the neck during this rinsing procedure. These details are mentioned here because of the difficulties encountered using the conventional cylindrical cells without necks, which are difficult to hold and to clean when a thin film of concentrated salt solution evaporates on the outside of the cell.

Reagents. The boric acid used was a C.P. Baker's analyzed product. The sodium acetate trihydrate was a Baker and Adamson N. F. grade. The disodium 4,5-dihydroxy-2,7-naphthalenedisulfonate was obtained as a practical grade dihydrated salt from Eastman Organic Chemicals. Any further references to chromotropic acid or its solutions imply this dihydrated disodium salt as the starting material.

Preparation of Stock Solutions. Aqueous 0.00028*M* stock solutions of the disodium chromotropate were prepared both by dilution of 50-ml. aliquots of 0.0028*M* solutions, and by direct weighing of 0.056-gram portions of the reagent, with subsequent dissolution in distilled water and dilution to 500 ml. The 0.00028*M* solutions have a pH between 4.5 and 5.0, depending upon the pH of the distilled water used in their preparation. After a few hours' standing in the daylight, these solutions develop a slight yellow color. This coloration can be avoided by storing the solution in a dark amber bottle, and keeping this bottle in the dark when not in use. The 0.00028*M* solutions stored in this manner showed no signs of deterioration 6 days after preparation, and they were used throughout this period in the analytical work. The 0.0028*M* solution, on the other hand, has a slight yellow tint upon preparation, which darkens to a definite yellow after a few days' storage in a dark amber bottle. Consequently, the 0.0028*M* solution should be prepared fresh whenever the 0.00028*M* solution is to be made from it by dilution.

The 2*M* solution of sodium acetate trihydrate used to adjust the pH was filtered to remove insoluble matter. Sodium acetate proved more convenient for adjusting the pH than sodium hydroxide, and in addition gave the system some buffer capacity. Stock solutions prepared from different bottles of sodium acetate trihydrate, of the same grade and from the same manufacturer, buffered the system to different pH's. Consequently, each lot of sodium acetate solution prepared should be checked to see that 10 ml. of the solution in a final volume of 50 ml. will adjust the pH of the system to between 6.8 and 7.0. If necessary, a small amount of 0.5*N* sodium hydroxide should be added to the sodium acetate stock solution to bring its pH adjusting capacity into the desired range.

The standard 0.002*M* boric acid solution, containing 0.022 mg. of boron per ml., was prepared by suitable dilution of a 0.02*M* stock solution. Because of the report (*5*) that the species present in boric acid solutions changes upon standing, three different 0.02*M* solutions were prepared during this work. No differences in behavior toward the chromotropic acid system were detected, however, between the freshly prepared solutions and the solutions which had stood for a few weeks.

PRELIMINARY STUDIES

In absolute ethyl alcohol solution 0.25*M* boric acid causes only slight changes in the absorption spectrum of a 0.0001*M* solution of chromotropic acid.

In aqueous medium at a pH of 4 to 5, boric acid causes large changes in the absorption spectrum of chromotropic acid, shifting the peaks to longer wave lengths and causing a large increase in absorption in the 350- to 360-mμ region. Figure 1 shows the effect of 0.04*M* boric acid on the ultraviolet spectrum of chromotropic acid in this pH range. The acidic boron complex formed, as well as the excess boric acid not involved in complex formation, lowers the pH of the solution. However, hydrochloric acid, added to solutions of chromotropic acid in sufficient quantity to lower the pH to 3.5 and 0.8, causes negligible changes at the absorption maxima of the reagent, and only small changes at

the absorption minima. This indicates that the large changes caused by boric acid are not due merely to a pH effect.

The aqueous chromotropic acid system is much more sensitive to boric acid in the pH range 7 to 10 than in the range 4 to 5. This increased sensitivity is due both to the formation of larger amounts of the complex for a given amount of boric acid, and to a larger absorption change for a given amount of complex. Figure 2 shows the effect of 0.002 and 0.00028*M* boric acid on the spectrum of chromotropic acid at a pH of approximately 7. Boric acid causes a decrease in the absorption of the reagent in the 355- to 380-mμ region, with the point of maximum change occurring at 361.5 mμ. The spectra of the reagent at pH 7.6, 9.0, and 10.0 are not much different from that given in Figure 2 for the reagent at pH 6.9, with only small differences at the 360- and 346-mμ peaks. However, to ensure reproducible conditions for the formation of the complex, which is dependent upon pH, the mixtures of chromotropic and boric acids were adjusted to a pH of 6.8 to 7.0 by the addition of 2*M* sodium acetate.

Calibration Procedure at pH 7. Solutions for the calibration curve for this system were prepared by adding 25 ml. of the 0.00028*M* solution of the reagent and 10 ml. of the 2*M* sodium acetate solution to various aliquots of the standard 0.002*M* boric acid solution, followed by dilution to 50 ml. with distilled water. The reagent and buffer solutions were added by pipet, and in the order indicated. Figure 3 shows the plot of the absorbancy decrease at 361.5 mμ *vs.* the boron content of the standard solutions prepared as described above. Each point on the curve is the average of two values, obtained on two separate days using different stock solutions of boric acid, sodium acetate, and reagent. The reagent stock solutions used for this calibration work were prepared by dilution of 0.0028*M* solutions.

The absorbancy decreases used for the calibration curve were obtained on the Cary instrument. With the standard solutions in the reference cell and a reagent blank (reagent plus buffer) in the sample cell, differential-type spectra are obtained for the solutions containing boron, wherein the absorbancy decreases appear as sharp peaks rising above the base line. After the solutions have been placed in the proper absorption cell, the instrument is balanced or zeroed at 400 mμ, where neither the blank nor solutions containing boron show any absorption, before scanning down scale to 350 mμ. Figure 4 shows the differential spectra in the 350- to 390-mμ region given by the same solutions used to obtain the conventional spectra of Figure 2. Although the concentrations of boron which give the spectra of Figure 4 are above the upper limit of calibration, the differential spectra for these concentrations are shown for comparison.

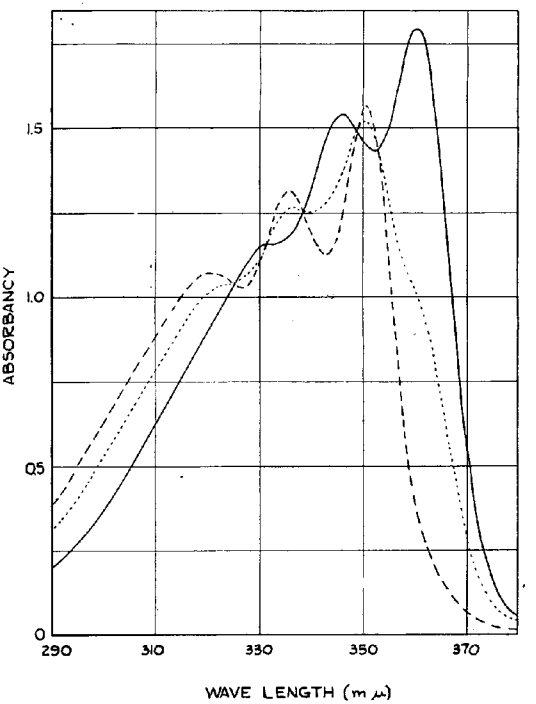


Figure 2. Effect of boric acid on absorption spectrum of chromotropic acid in aqueous solution at pH 7

All solutions 0.4*M* in sodium acetate and 0.00014*M* in chromotropic acid
——— No boric acid added, pH 6.9
. . . . 0.00028*M* in boric acid, pH 7.0
– – – – 0.002*M* in boric acid, pH 7.0

The reagent blank is placed in the sample cell, rather than in its usual position in the reference cell, because of the decrease in the absorption of the reagent being measured in this system. The intense radiant energy source and photomultiplier detector system of the Cary instrument enable slit widths of 0.25 mm. or lower to be used in obtaining these differential spectra. Because of the critical setting of the wave length (eliminated on the Cary because of the scanning procedure) and the necessity of zeroing the instrument on highly absorbing solutions, other manual spectrophotometers at hand were not considered for this work. However, instruments such as Beckman Models B and DU, for which photomultiplier attachments are available, could probably be used if sufficient care is exercised in setting the wave length dial.

Stability of System. The mixtures of chromotropic acid and sodium acetate, with or without boric acid, develop pink to red hues after a few minutes' standing in strong sunlight. Because of this light sensitivity, exposure to daylight was kept at a minimum during the preparation of the samples. The reagent and sodium acetate solutions were added to two or three samples at a time; the samples were diluted to volume, mixed, and placed in a convenient laboratory cabinet while other samples were being prepared and until the group was ready to be run on the instrument. No sign of color was observed when this procedure was followed, and the system has been shown to be stable for at least 18 hours if unnecessary exposure to light is avoided. Stability of the solutions for longer periods was not investigated.

As no color was observed in solutions which had stood in the fluorescent light of the laboratory at night, precautions such as those mentioned above were not so necessary for samples run at this time, although prolonged exposure to the fluorescent lights was also avoided.

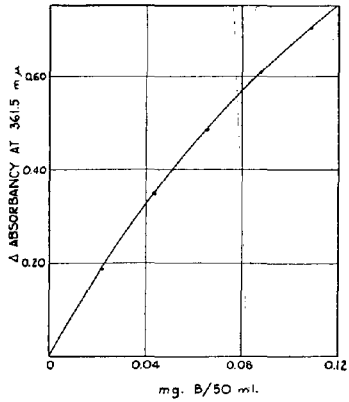


Figure 3. Calibration curve for chromotropic acid system in aqueous solution in pH range 6.8 to 7.0

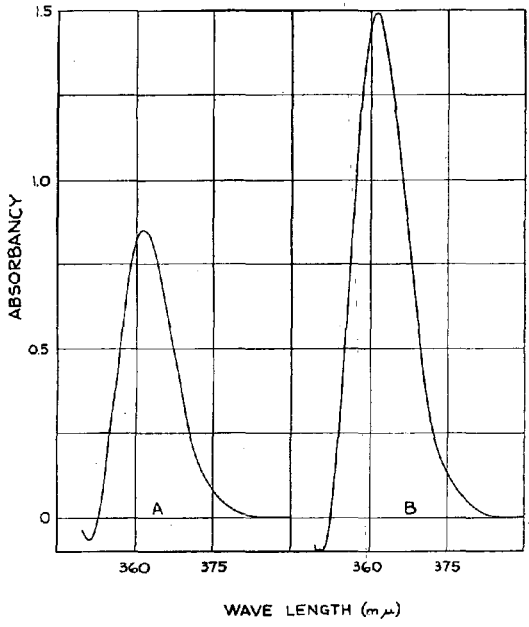


Figure 4. Differential type spectra of same solutions used to obtain spectra in Figure 2

A. Solution 0.00028*M* in boric acid
B. Solution 0.002*M* in boric acid

Table I. Results of Accuracy and Precision Tests on Chromotropic Acid Method for Boron

(All concentrations expressed as mg. of boron/50 ml.)

Range of Boron Concn.	No. of Individual Concn.[a]	No. of Separate Detn.	Deviations from Known Values	
			Range	Average
0.009–0.043	11	16	−0.002–+0.002	0.0011
0.048–0.087	11	24	−0.006–+0.007	0.0028
0.091–0.115	6	23	−0.007–+0.010	0.0044

[a] Number of different boron levels investigated within each concentration range.

The intense ultraviolet source of the instrument also appeared to have a slight effect on the system. Because of this, a fresh portion of the sample solution should be placed in the reference cell if a repeat run on the same sample is desired. The same portion of the reagent blank can be used in the sample cell for obtaining a number of differential spectra, as the reagent blank appears to be relatively insensitive to the ultraviolet source. For a rather large series of samples, however, it is recommended that a fresh portion of the reagent blank be placed in the sample cell for every four or five samples run on the instrument.

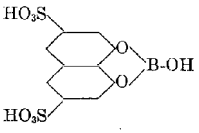
Accuracy and Precision. A number of samples of varying boron contents within the calibration limit were run over a 22-day period to test the accuracy and precision of the proposed method. These test samples were prepared in the same manner as the solutions used for calibration—that is, by addition of the reagent and sodium acetate solutions to various aliquots of the standard boric acid solution. The majority of boron concentrations involved were different from those used for the calibration curve. Differential-type spectra were obtained in the 400- to 350-mμ region for these test solutions, using a freshly prepared reagent blank for each group of samples. The absorbancy decreases obtained in this manner were referred to the calibration curve in Figure 3 to arrive at the boron content of the solutions. The samples were run in numerous groups of four to seven during the 22-day period, using 14 different 0.00028*M* stock solutions of the reagent. Twelve of these were prepared by weighing out individual 0.056-gram portions of the reagent, the other two by dilution of 0.0028*M* stock solutions. Results of this study are summarized in Table I.

DISCUSSION

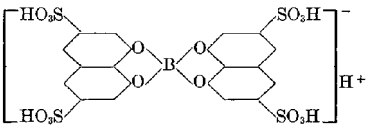
The reaction of boric acid with chromotropic acid is apparently an equilibrium reaction resulting in the formation of a complex, and is shifted toward the formation of greater quantities of the complex by further additions of boric acid. The plot of the absorbancy change (increase or decrease) *vs.* the boron concentration at both a pH of 7 and in the range 4 to 5 is not a straight line, and approaches a logarithmic relationship. The spectrum in Figure 2 given by the mixture containing a 0.002*M* concentration of boric acid is very similar to the spectrum in Figure 1 of the mixture containing a 0.04*M* concentration of boric acid. Conversion of the reagent into the complex is nearly complete in these two mixtures (as indicated by a "leveling off" of the absorbancy changes at approximately these

concentrations), and the fact that they give similar spectra suggests that the spectrum of the complex is not affected by pH changes in the range 4 to 7. However, the amount of complex formed is dependent upon pH, 20 times as much boric acid being required in the pH range 4 to 5 as at pH 7 to form approximately the same amount of complex.

Nothing could be concluded from the experimental work as to the nature of the complex present in the mixtures of chromotropic and boric acids. The generally accepted structures for complexes of boric acid with polyhydroxy compounds such as chromotropic acid are given below. Both complexes could be present in the mixtures, and contribute to the observed absorbancy changes. The proton released in the formation of the Type II complex would account for the lowering of the pH noted in Figure 1.



Type I



Type II

Reagent stock solutions prepared by dissolving 0.056-gram portions of the reagent in 500 ml. of distilled water did not give a very reproducible system for the higher boron concentrations investigated. Varying absorbancy decreases were observed with different stock solutions for the same amount of boron present. This lack of reproducibility among reagent stock solutions is believed due in part to errors involved in weighing such a small amount of reagent, coupled with the fact that the reagent itself was an impure, practical grade material. The preparation of a concentrated stock solution, using a large weight of the reagent, followed by dilution to the concentration desired, is in effect taking an average of many small portions of the solid reagent. Stock solutions prepared in this manner would be expected to lead to a more reproducible system as far as the quantity of reagent is concerned. Alternative paths to the same end of greater reproducibility would be to obtain a better grade reagent, or to purify the practical grade material by suitable recrystallization techniques.

Although the light sensitivity of the system is an inherent disadvantage of the method, it causes no great inconvenience in processing the samples, if the proper precautions are taken. The proposed chromotropic acid method is definitely less tedious and time-consuming than methods used at present. It is applicable to a wider range of boron concentrations, but, conversely, does not have the sensitivity of many of the established methods. However, utilization of 5-cm. absorption cells would probably increase the sensitivity of the chromotropic acid method to the point where it would be comparable to other methods.

Welcher (*11*) states that titanium, magnesium, aluminum, nitrous acid, chromates, dichromates, and numerous other oxidizing agents give colored products with chromotropic acid. Yoe and Sarver (*13*) reported that zirconium, uranium, and iron can be determined using the colored systems which chromotropic acid gives with these elements. Vanadium also gives a colored system with this reagent (*9*). Because of these interferences, and the possibility of separating boron by distillation as methyl borate, the effect of extraneous ions on the chromotropic acid system was not investigated.

It is known, however, that silicon causes interference in the method. Low results obtained using a certain lot of 2*M* sodium acetate solution were traced to the presence of silicon in this solution. The silicon originated from the 0.5*N* sodium hydroxide solution which had been added to the sodium acetate solution to adjust its buffering capacity. The 0.5*N* alkali had been stored in a borosilicate glass bottle for some time, and contained appreciable quantities of silicon leached from the bottle. Silicon does not affect the shape of the differential spectra in any way other than reducing their intensity. This element apparently, through some unknown mechanism, affects the equilibrium between chromotropic acid, boric acid, and the complex with the result that less complex is formed.

The results given in Table I indicate that the proposed method is sufficiently accurate and precise to be used for the determination of boron present in quantities ranging from 0.005 to 0.12 mg. per 50 ml. (0.1 to 2.4 p.p.m.). Boron concentrations higher than the calibration limit in Figure 3 can be determined by this method, particularly if a Cary recording spectrophotometer is used, which can easily measure absorbancies up to 1.50 units. However, because of the parabolic nature of the calibration curve, the system is inherently less accurate and precise at these higher boron levels, where relatively large differences in boron concentration give only small differences in absorbancy. The purity of the chromotropic acid used also becomes increasingly critical where large quantities of boron are to be determined. Because of the loss in accuracy and precision, and because of the difficulties encountered in preparing reproducible stock solutions of the reagent, the system was calibrated only up to 0.12 mg. of boron per 50 ml.

RECOMMENDED PROCEDURE

Adjust the aqueous solution containing boron, obtained by suitable previous preparative treatment of the sample, to a pH between 4.5 and 6.5. The volume of this solution should be 15 ml. or less after the pH adjustment. Add 25 ml. of a 0.00028*M* solution of disodium chromotropate and 10 ml. of 2*M* sodium acetate, dilute to 50 ml., and mix. Avoid exposure to strong light after mixing. Place the sample in the reference cell, and the blank (reagent plus buffer) in the sample cell. Zero the Cary instrument at 400 mμ, and then scan down scale to 350 mμ. Obtain the boron content of the sample by referring the absorbancy of the differential peak at 361.5 mμ to a calibration curve, previously constructed by carrying samples of known boron content through the same procedure.

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Esso Lamp Method for Sulfur

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▶ An integrated and rapid lamp method for determination of total sulfur in petroleum has been developed and cooperatively tested. Modified finishing methods consist of the following: conductometric for nonleaded samples, nephelometric for leaded or nonleaded samples in the range of 1 p.p.m. to 0.06%, and gravimetric which avoids the conventional digestion and ignition steps. The cooperative testing was among four Esso Research and affiliated laboratories and involved 12 plant-type samples. The reproducibility for conductometric and gravimetric finishing showed a standard deviation which varied from 0.0008 to 0.015 over the sulfur concentration range of 0.01 to 1.0%. The reproducibility for nephelometric finishing varied from 1.1 to 12 over the range of 5 to 500 p.p.m.

NUMEROUS variations of the lamp method for determination of sulfur in petroleum products have appeared in the literature. In general, these methods have emphasized one particular phase, whereas the object of this report is to make available an integrated method. Most of the techniques employed are well known but the task of knitting them together required certain innovations to produce a method that is adaptable to routine testing in a petroleum laboratory. A realistic production figure is 20 samples per man-day.

The use of hydrogen peroxide as an absorbent of the combustion products is currently a part of an ASTM method (*1*) as is also the synthetic oxygen–carbon dioxide combustion gas. The unique absorber system and conductivity method of finishing have been described (*4*). The nephelometric finishing technique is one of relatively long standing (*7*), whereas the use of Naphthol Yellow S for inducing large particle precipitates has been recently described (*2*).

REAGENTS

Hydrogen Peroxide, acid-free 30% reagent grade. Dilute 1 to 10 with distilled water just before use.

Solution A. Alcohol, Glycerol. Add 3 volumes of redistilled glycerol to 6 of redistilled 95% ethyl alcohol and 1 of distilled water.

Solution B. Alcohol, Glycerol, Acid. Same as Solution A but with 3*N* hydrochloric acid substituted for the water.

Solution C. Alcohol, Glycerol, Acid, Peroxide. Add one volume of solution B to 5 of 3% hydrogen peroxide.

Barium Chloride. The 20–30 mesh product available from Hellige, Inc., New York, N. Y., as Catalog No. 8042, seems to give the best results.

Naphthol Yellow S. Dissolve 0.4 gram of Naphthol Yellow S in 1 liter of distilled water. Dissolve 20 grams of barium chloride dihydrate in 1 liter of dilute hydrochloric acid (25 ml. of concentrated hydrochloric acid per liter of distilled water). Pour the Naphthol Yellow S solution into the acid barium chloride and allow to stand 24 hr. before using.

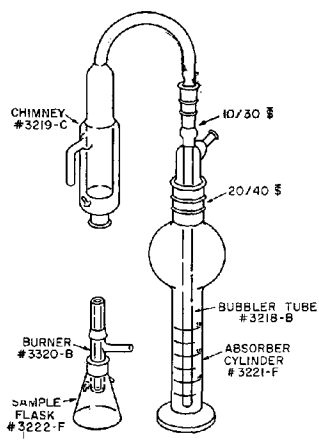

Figure 1. Lamp sulfur burner and absorber assembly

Standard Sulfuric Acid. Prepare 0.1249*N* sulfuric acid. One milliliter of this solution is equivalent to 2.0 mg. of sulfur. Further dilute for the preparation of additional standard solutions such that 1 ml. of each will contain 0.2, 0.02, and 0.005 mg. of sulfur.

APPARATUS

Lamp Assembly. The complete burner and absorber assembly are shown in Figure 1 and are obtainable from the Kontes Glass Co., Vineland, N. J., under the designations indicated. The burner and flask are in accord with ASTM specifications (*1*) as is also the chimney, with the exception of the substitution of a ⅋ 10/30 joint. The carburetion-type burner is procurable as Drawing No. 2881-F (*6*) and the low volatility burner as Drawing No. 3282-F (*6*). A blast type gas burner is described in the ASTM method (*1*).

Combustion Gas Metering Assembly. The assembly shown in Figure 2 is suitable for at least 10 lamp units. The steam-jacketed carbon dioxide heater is satisfactory for maintaining a constant pressure without overheating but is only one of several adaptions that can be devised. Rotameters should be selected to provide a maximum gas volume of 6 cu. feet per hour per lamp, of which approximately 35% is oxygen. An auxiliary manifold is shown from which a controlled amount of carbon dioxide can be obtained for mixing with the combustion gas to each lamp in order to lean the oxygen content and thus obtain better burning of the more aromatic type samples. Automatic shutoff valves and an alarm are shown as safety features in case of pressure drop from a depleted cylinder.

Manifold to Lamp Connections. In setting up a bank of ten lamps various means can be devised to support them conveniently and permit connections to be made with the manifolds. The essential connections for a single lamp are shown in Figure 3. Each manifold must be connected to a water bubbler-type pressure regulator. Eight to 10 inches of water is sufficient.

Nephelometer. Use a Coleman Model 7 Photo-Nephelometer with an 80-unit Coleman Nephelos standard and filter. This latter item can be made by obtaining an Eastman Kodak ND-2 (neutral density) photographic filter, all glass type, and mounting it in a Coleman filter holder.

Conductance Bridge and Cell. The conductance bridge supplied by Arthur H. Thomas Co., Catalog No. 3965, can be used in conjunction with the micro-type conductivity cell, Catalog No. 3997. When ordering it is essential to specify bright platinum electrodes.

Glass Fiber Filter Paper. H. Reeve Angel & Co., New York, N. Y., supplies a glass fiber filter for use in the usual No. 3 Gooch crucible. The 2.1-cm. size is listed as Catalog No. X934-AH.

Calibration of Nephelometer. Follow the supplier's directions for use of the instrument by the null method. Insert the 80 Nephelos standard and turn the Bal knob to give a scale reading of 40. Adjust Std. knob to bring the galvanometer to zero. Use this method to check instrument drift frequently. It is also suggested that the opaque end of the filter holder be inserted in the filter slot in order to darken the photocell for zero adjustment.

Accurately pipet suitable amounts of the standard sulfuric acid solutions into the lamp assembly absorber cylinder such that a range of 0.01 to 0.10 mg. of sulfur is covered for calibration without the filter and 0.10 to 0.50 mg. for calibration with the filter. Add 5 ml. of Solution B, dilute to 30 ml. with distilled water, and mix well. Add by means of a suitable scoop approximately 0.2 gram of the Hellige barium chloride crystals. Close the cylinder with a glass stopper and immediately mix for 1 minute by tilting the cylinder to permit flow from end to end. Allow to stand 3 minutes and then pour into a selected cuvette. Place in the immersion well and read after an elapsed time of exactly 5 minutes from the addition of the barium chloride.

Repeat to obtain a blank reading by omitting the standard acid. Subtract the blank reading from the above readings and plot this instrument reading against sulfur concentration. Figure 4 is representative of a typical nephelometric calibration.

Calibration of Conductivity Bridge. Operate in accord with supplier's instructions. Fire polish a heavy ring on the skirt of the conductivity electrode, without restricting the opening, in order to prevent chipping when in use. If the skirt becomes even slightly chipped, it must be recalibrated.

Add suitable volumes of the standard sulfuric acid solutions to the absorber cylinders to cover the range of 0.005 to 14.0 mg. of sulfur. Add 2 ml. of 30% hydrogen peroxide and make up to 25 ml. with distilled water. Stopper, shake thoroughly, and place in a constant temperature water bath held at ±0.1° C. When temperature equilibrium has been obtained, insert the conductivity electrode and move it in and out of the solution several times and obtain an approximate reading. Take a final and exact reading immediately after the electrode has again been moved in and out several times. Figure 5 illustrates a representative conductivity calibration curve. Hydrogen peroxide of sufficient purity is available, so a conductivity blank is not necessary.

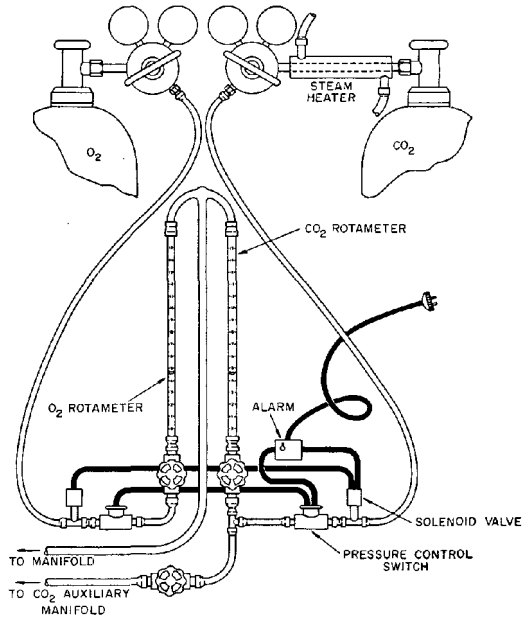

Figure 2. Assembly for metering and mixing oxygen–carbon dioxide combustion gas

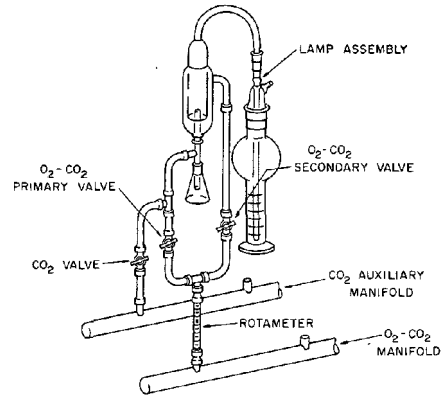

Figure 3. Schematic arrangement of manifold to lamp assembly connections

METHOD

The schematic diagram, shown in Figure 6, provides an over-all description of the operations involved in the method. The upper part shows the burner types for use with various samples and the lower part indicates the finishing methods and the circumstances under which they are applicable.

Burning Operation. Place 20 ml. of the 3% hydrogen peroxide solution in each absorber cylinder. Wick sufficient burners by drawing through two doubled strands of cotton wicking and cut flush with the top of the burner. Place 3 to 6 grams of sample in each flask and insert the wick and

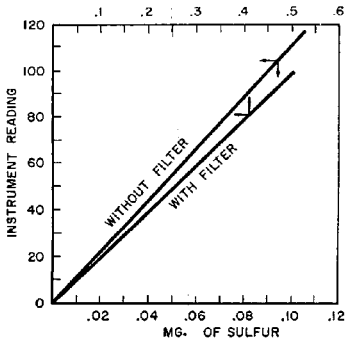

Figure 4. Typical nephelometric calibration curves

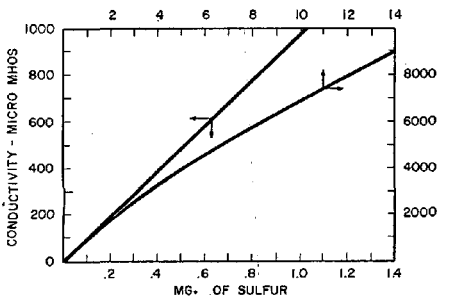

Figure 5. Typical conductometric calibration curves

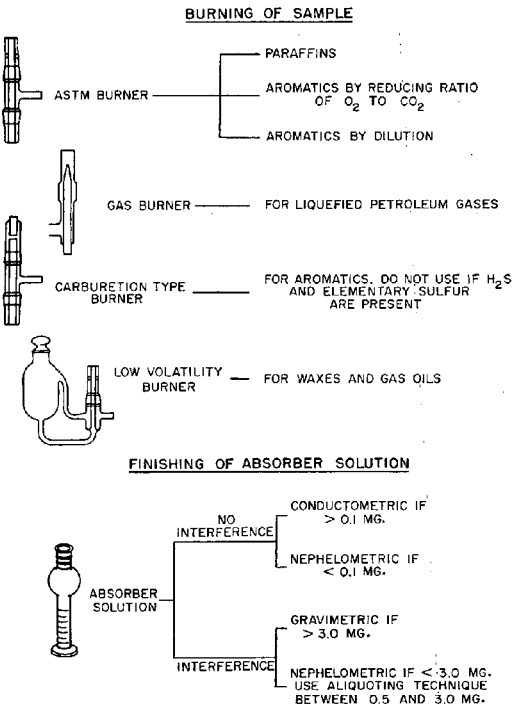

Figure 6. Schematic diagram of operations in lamp sulfur test

burner. Immediately weigh, light, and establish in place on the lamp assembly.

A certain amount of practice will be required before successful lighting can be accomplished. However, if the oxygen to carbon dioxide ratio is first adjusted to give optimum burning of paraffinic samples, most samples can be lighted and established on the bank without smoking. Final adjustments of the gas volumes can then be made. If the top of the flame tends to reach up or shows evidence of smoking, the addition of carbon dioxide from the auxiliary manifold will reduce it to normal behavior. However, with pure aromatics it is necessary either to dilute with a paraffinic hydrocarbon such as isooctane or use the carburetion-type burner.

Continue burning until the sample is depleted and the flame is about to go out. This is essential if the sample contains either hydrogen sulfide or elementary-type sulfur. Through the use of sulfur-35 it has been shown that elementary sulfur concentrates at the tip of the wick but if allowed to burn to dryness recoveries of 90% can be realized. The hydrogen sulfide is flushed out of the sample more rapidly than the sample is consumed. Lower the burner, blow out the remaining flame, and immediately reweigh.

Finishing Operation. CONDUCTOMETRIC. Samples not containing tetraethyllead or acid-forming constituents other than sulfur, that are to be finished conductometrically, must be purged with carbon dioxide–free air for 5 minutes. Partially lift out the absorber tube and rinse down both the inside and outside with an amount of water necessary to bring the volume to 25 ml. It is not necessary to rinse the chimney, since work with sulfur-35 as a radioactive tracer has shown that with nonleaded samples there is a negligible amount of sulfur remaining. Mix well and thermostat. Determine the conductivity. If the measurement indicates the presence of more than 0.1 mg. of sulfur, then it can be used to determine the actual sulfur content of the sample. If the indicated content is more than 10 mg., it is advisable to dilute with sufficient

3% hydrogen peroxide to bring the reading into a more accurate range.

NEPHELOMETRIC. If the above absorber solution was found to contain less than 0.1 mg. of sulfur, remove the conductivity cell after freeing it of as much of the solution as possible. Add 5 ml. of Solution B and mix. Filter completely through a dry No. 42 Whatman filter paper into another clean dry cylinder but do not wash the paper. About 28 ml. will be collected, to which add 0.2 gram of the barium chloride. Complete nephelometrically as was done in the calibration step. The instrument must be adjusted with the Nephelos standard in the same manner as the calibration was made.

For all nephelometric work a blank must be determined; this should include the passing of the oxygen–carbon dioxide mixture through the hydrogen peroxide, filtering, etc., as for a sample. A satisfactory blank is of the order of 0.01 mg. of sulfur.

NEPHELOMETRIC FINISHING OF LEADED SAMPLES. Remove the chimney and rinse down both the inside and outside of the absorber tube with 5 ml. of Solution A. Dissolve the white deposit in the chimney with 2 ml. of 1*N* hydrochloric acid and add to the absorber. Rinse the chimney with 1-ml. portions of water until the absorber solution is 30 ml.

If less than 0.5 mg. of sulfur is known to be in this solution, proceed to filter through No. 42 Whatman and thus continue to complete the nephelometric finishing.

If the sulfur content of the filtrate is not known or is known to contain more than 0.5 mg., remove a 5-ml. aliquot and make up to 30 ml. in another absorber with Solution C. Filter through No. 42 Whatman and complete the nephelometric finishing. If an acceptable reading is not obtained, repeat with a larger aliquot. Thus, if a 5-gram sample containing tetraethyllead is burned, sulfur contents up to 0.01% can be determined nephelometrically without aliquoting and up to 0.06% with aliquoting. Such nephelometric finishing is relatively fast.

GRAVIMETRIC. If the 5-ml. aliquot described above contains more sulfur than can be determined nephelometrically, the sample can still be salvaged for gravimetric finishing by adding all the portions, including washings of the filter paper, to a 600-ml. beaker. If gravimetric finishing is contemplated from the start, it is preferable to burn at least a 6-gram sample. Rinse the contents of the absorber into a 600-ml. beaker. Dissolve the white deposit in the chimney with 5 to 10 ml. of 1*N* hydrochloric acid and add the acid and subsequent rinsings to the beaker. Dilute to 200 ml. and filter if it appears necessary. Heat the solution to boiling and add 100 ml. of the Naphthol Yellow S–barium chloride solution. Permit the solution to stand until it reaches room temperature.

Prepare a filter by placing two glass fiber filter pads in a clean dry Gooch crucible. Best results are obtained by heating for 30 minutes in a 230° F. oven. Cool and weigh. With gentle suction (about 7 inches of mercury below atmospheric) filter the contents of the beaker and wash until free of chloride. Heat at 230° F. to constant weight.

All calculations are obvious and have been omitted. Likewise, the application of the method to liquefied gases, gaseous and low volatility samples is not described. Wax samples are easily burned with the low volatility burner, if the assembly is heated with an infrared lamp (*5*).

Table I. State of Quality Control of Various Laboratories on Practice Samples

Laboratory	No. of Tests	Average	High	Low	95% Confidence Level
Sample[a] for conductometric finishing. Theoretical S concn., 0.103%					
A	37	0.101	0.105	0.098	±0.0035
B	28	0.104	0.105	0.099	±0.0032
C	15	0.099	0.103	0.096	±0.0044
D	19	0.102	0.107	0.098	±0.0044
Sample[a] for Nephelometric Finishing. Theoretical S concn., 12 P.P.M.					
A	41	12	14	10	±2.0
B	10	11	13	10	±1.7
C	18	11	15	8	±3.4
D	12	11.5	13	10	±2.6

[a] Iso-octane plus thiophene.

COOPERATIVE TESTING

Four Esso Research and affiliated laboratories participated in the coop-

Table II. Cooperative Test Results

No.	Sample	Lab A			Lab B			Lab C			Lab D			Average
		Sulfur, P.P.M.												
	Nephelometric[b]													
1	Heavy cat. naphtha	193	196	196	211	221	215	215	215	212	211	203	208	208
2	Hydroformate	4	3	3	2	2	3	4	3	1	1	1	1	2.3
3	Leaded motor gasoline[c]	486	497	486	467	475	501	503	477	479	505	480	475	485
4	Aviation gasoline	27	24	28	24	28	28	32	30	31	31	25	27	28
5	Heavy virgin naphtha + 45 p.p.m. S added	51	49	50	46	44	48	48	49	49	50	45	47	48
	Conductometric	Sulfur, Wt. %												
6	Virgin naphtha	0.0110	0.0120	0.0120	0.0107	0.0101	0.0102	0.0098	0.0103	0.0102	0.0113	0.0105	0.0104	0.0107
7	Commercial kerosine	0.0270	0.0330	0.0280	0.0284	0.0276	0.0294	0.0264	0.0282	0.0271	0.0286	0.0274	0.0276	0.0280
8	Iso-octane + 0.1934% S added	0.191	0.187	0.186	0.1962	0.1885	0.1908	0.184	0.189	0.188	0.189	0.195	0.187	0.1893
9	Coker naphtha	0.129	0.125	0.128	0.1258	0.1291	0.1240	0.124	0.131	0.134	0.139	0.135	0.135	0.1299
10	Heavy cat. naphtha[c]	0.321	0.326	0.311	0.306	0.300	0.314	0.297	0.316	0.321	0.313	0.307	0.300	0.311
11	Coker naphtha[c]	0.905	0.910	0.916	0.900	0.912	0.907	0.900	0.910	0.940	0.926	0.919	0.895	0.912
	Gravimetric													
12	Iso-octane + 0.072% S added	0.066	0.064	0.068	0.0686	0.0695	0.0709	0.0664	0.0615	0.0660	0.068	0.068	0.069	0.0672
13	Leaded motor gasoline[c]	0.043	0.040	0.042	0.0437	0.0436	0.0459	0.0415	0.0371	0.040	0.042	0.044	0.043	0.0422
14	Heavy cat. naphtha[c]	0.303[a]	0.313	0.317	0.319	0.322	0.317	0.304	0.309	0.307	0.300	0.298	0.302	0.310
15	Coker naphtha[c]	0.910	0.932	0.931	0.927	0.895	0.915	0.913	0.918	0.911	0.929	0.922	0.927	0.919

[a] Value corrected to 0.315% S on basis of Dixon's test.
[b] Nephelometric portion of program was repeated.
[c] Samples 3 and 13, 10 and 14, 11 and 15 are the same.

Table III. Summary of Statistical Evaluation of Lamp Sulfur Cooperative Program

Sample	Sulfur, P.P.M.	Repeatability[a]	Reproducibility[a]
Nephelometric			
Heavy catalytic naphtha	208	±3	±10
Hydroformate	2.3	1.2	1.2
Leaded motor gasoline	485	14	14
Aviation gasoline	28	2.7	2.7
Desulfurized virgin naphtha + 45 p.p.m. S	48	2.3	2.3
	Wt. %		
Conductometric			
Virgin naphtha	0.0107	0.00043	0.0008
Commercial kerosine	0.028	0.0017	0.0017
Iso-octane + 0.1934% S	0.1893	0.0035	0.0035
Coker naphtha	0.1299	0.005	0.005
Heavy catalytic naphtha	0.311	0.0092	0.0092
Coker naphtha	0.912	0.0124	0.0124
Gravimetric			
Iso-octane + 0.072% S + TEL	0.0672	0.0026	0.0026
Leaded motor gasoline	0.042	0.0023	0.0023
Heavy catalytic naphtha	0.310	0.0023	0.0088
Coker naphtha	0.919	0.011	0.011

[a] Standard deviation.

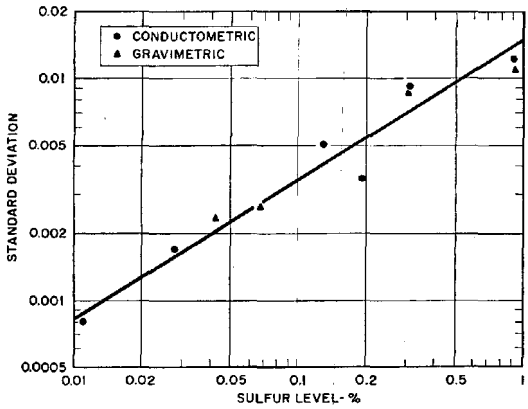

Figure 7. Reproducibility when conductometrically and gravimetrically finished

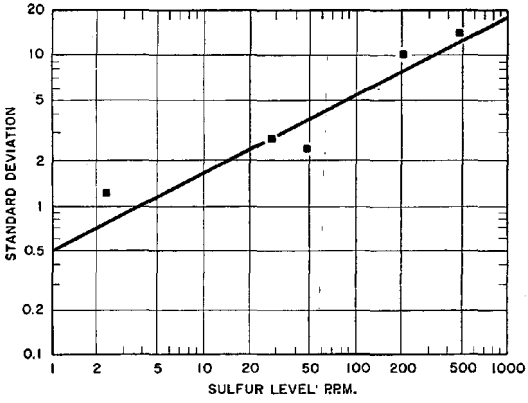

Figure 8. Reproducibility when nephelometrically finished

erative testing of the lamp sulfur method described above. However, before these laboratories undertook the actual testing, each demonstrated that it was in a satisfactory state of quality control by applying the method to known practice samples. The data shown in Table I were so obtained. In addition to determining the fitness of each laboratory, the work also demonstrated the variation from the true sulfur content which the method would give when ideal samples were tested in different laboratories. Thus for conductometric finishing at the 0.103% sulfur level, the average of all four laboratories showed a bias of −0.0014 and for nephelometric finishing at the 12 p.p.m. level the bias was −0.6 p.p.m.

Following this demonstration, 15 plant-type samples were supplied to the laboratories. Each sample was submitted in three bottles. These bottles were identified with an unrelated number and the finishing method that should be applied. The laboratory supervisor was informed which numbers to test in a single day, so that each sample would be tested singly on three different days. Contents of each of the 45 bottles were tested once. Table II describes the samples, with the results reported by the four laboratories. These data have been statistically evaluated by a method essentially the same as one developed in the Esso Laboratories (*3*) and are shown on Table III.

The standard deviation of the reproducibility results for gravimetric and conductometric finishing have been plotted against the sulfur level in Figure 7. From this curve the following sulfur levels and corresponding expected reproducibility values have been taken:

Sulfur Level, %	Standard Deviation of Reproducibility
0.010	±0.0008
0.050	0.0022
0.100	0.0035
0.500	0.0098
1.000	0.015

To translate these figures into a practical application, the 0.100% level may be considered, which is a common specification limit for sulfur in motor gasoline. The data indicate that a refinery must maintain a 0.093% test limit in order to be 95% confident that any given shipment will not exceed 0.100% sulfur.

For nephelometric finishing the reproducibility results have been plotted against the sulfur level in Figure 8.

These data do not conform to a straight-line relationship as well as those of Figure 7. However, in the lower sulfur ranges more scattering would be expected. From this curve the following sulfur levels and expected nephelometric reproducibility values have been taken.

Sulfur Level, P.P.M.	Standard Deviation of Reproducibility
5	± 1.1
10	1.6
50	3.7
100	5.3
500	12.0

To extend the above data further it is found that at the 10 p.p.m. level a result falling in the range of 6.8 to 13.2 can be expected 95% of the time. Four of the five samples finished nephelometrically and eight of the ten samples finished conductometrically or gravimetrically have the same deviation for both repeatability and reproducibility.

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Remote Control Determination of Corrosion Products and Additives in Homogeneous Reactor Fuel

Application of Ion Exchange

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▶ Ion exchange techniques have been applied to remote control separation of the most important metal corrosion products from uranyl sulfate solution in homogeneous reactor fuel. In most cases, the metal in the effluent from the column is collected in a calibrated polarographic cell sample holder, in which the effluent can be evaporated to dryness, redissolved, and diluted to volume with the proper supporting electrolyte. Adsorption characteristics of the corrosion products with respect to anion and cation exchange resins, and procedure outlines for determination of aluminum, nickel, cobalt, chromium, iron, manganese, copper, and zirconium are given in tabular form.

DURING operation of the homogeneous reactor, uranyl sulfate solution at high temperature and pressure is in contact with stainless steel, zirconium alloy, and other alloys which are integral parts of the reactor. Because of high temperature, radiation, and the acidic nature of the fuel, a certain amount of corrosion takes place. Initially, the fuel consists of 0.005*M* copper sulfate solution that contains 10 grams of uranium, as uranyl sulfate, per liter. Copper is used to catalyze the recombination of hydrogen and oxygen produced by decomposition of water in the fuel during operation of the reactor. As corrosion takes place, nickel, manganese, cobalt, iron, chromium, zirconium, aluminum, and other metals may enter the solution. Analytical determination of the corrosion products gives an indication of the stability of the metallic components of the reactor. Many of the methods now in use for the determination of these elements require separation techniques involving multiple solvent extractions or precipitations. Although these methods are effective for a particular element, they are impractical where remote control manipulation is required.

There are several prerequisites to the development of analytical methods for use in remote control analytical facilities. The methods must be simplified as much as possible, in order to minimize the handling of equipment by the master-slave type manipulator or by remotely controlled electrical and mechanical devices. It is desirable to combine as many steps as possible by constructing unitized separation and analytical apparatus, whereby complete determinations may be carried out with three or four simple manipulations.

An ion exchange column is particularly well suited to remote control analytical methods, as it requires no shaking or stirring. Simple attachments to the outlet of the column make possible the collection of effluents or eluates in the vessel that is to be used in the final step of the determination, thereby eliminating or minimizing transfers of solutions. For example, transfer of a solution from a narrow-necked volumetric flask to a polarographic cell requires expert handling of the master-slave manipulator. This step in a given polarographic analysis may be eliminated by collecting the effluent from the column directly in a polarographic cell, reducing the volume by placing the cell in a cylindrical heater, then adding reagents to the cell to provide a supporting electrolyte for polarographic determination. This principle is illustrated in Figure 1.

The ion exchange column may be prepared outside the remote facility and admitted to the facility by means of an access port. The amount of ion exchange resin required for an analysis can be predetermined from experience with nonradioactive test-loop samples. The amount of sample taken from the reactor is always the same, and the amount required for the individual determination is easily estimated. Kraus and Nelson (*3*) have determined the adsorption coefficients of all the ions considered herein from equilibration studies of radioactive tracer solutions with various ion exchange resins. The adsorption characteristics of the corrosion products with respect to anion and cation resins are given in Table I. The ion exchange column is discussed with respect to column size, resin volume, flow rates, and other factors in an earlier paper (*2*).

REAGENTS AND APPARATUS

ION EXCHANGE RESINS. Dowex 1

Table I. Adsorption Characteristics of Corrosion Products and Additives Found in Homogeneous Reactor Fuel

Dowex 1 Anion Resin						Dowex 50 Cation Resin, Hydrogen Form	
Chloride Form				Sulfate Form			
Adsorbed from 9*M* HCl	Not adsorbed from 9*M* HCl	Adsorbed from 4.5*M* HCl	Not adsorbed from 4.5*M* HCl	Adsorbed from 0.3*M* H_2SO_4	Not adsorbed from 0.3*M* H_2SO_4	Adsorbed from dil. H_2SO_4[a]	Not adsorbed from dil. H_2SO_4[a]
U(VI)	Al(III)	U(VI)	Al(III)	U(VI)	Cu(II)	U(VI)	Cr(VI)
Cu(II)	Ni(II)	Cu(II)	Ni(II)	Sn(II and IV)	Fe(II and III)	Cu(II)	
Fe(II and III)	Cr(III)	Fe(III)	Cr(III)	Cr(III and VI)	Ni(II)	Fe(II and III)	
Sn(II and IV)	Ti(IV)	Sn(II and IV)	Ti(III)	Ti(IV)	Co(II)	Sn(II and IV)	
Co(II)	Mn(II)	Cr(VI)	Co(II)		Al(III)	Co(II)	
Zr(IV)			Zr(IV)		Zr(IV)	Zr(IV)	
Cr(VI)			Mn(II)		Mn(II)	Al(III)	
			Fe(II)			Ni(II)	
						Cr(III)	
						Ti(IV)	
						Mn(II)	

[a] $<1M$ H_2SO_4.

Table II. Procedure Outlines for Remote Control Separation and Determination of Corrosion Products in Homogeneous Reactor Fuel

1 Ion to Be Determined	2 Pretreatment of Sample before Separation	3 Ion Exchange Resin	4 Column Vols. of Rinse	5 Effluent Collected in	6 Treatment of Effluent	7 Analytical Method
Al(III)	Adjust HCl concn. to 9*M*	Dowex 1 (10% DVB) chloride-form, anion	2, 9*M* HCl	50-ml. vol. flask	Evap. to dryness, add HCl and pyridine. Add aluminon-buffer reagent. Heat flask in boiling water 5 min. Cool, dil. to vol. Measure absorbance *vs.* distilled water	Spectrophotometric (*2*) 525 mμ
Ni(II) Co(II)	Same as Al	Same as Al	2, 4*M* HCl	Polarographic cell	Evap. to dryness. Cool, add HCl and pyridine to give 0.1*M* pyridine, 0.1*M* pyridinium chloride when diluted to 5 ml.	Polarographic (*5*) $E^1/_2$ −0.78 v. *vs.* S.C.E. for Ni and −1.07 v. for Co
Cr(VI)	Add slight excess $KMnO_4$ and heat to boiling. Cool	Dowex 50 (8% DVB) hydrogen-form, cation	2, H_2O	Polarographic cell containing 3 NaOH pellets	Dilute to 10 ml.	Polarographic (*6*) $E^1/_2$ −0.85 v. *vs.* S.C.E.
Fe(Total)	Adjust H_2SO_4 concn. to 0.3*M*. Dip coil made from $^1/_4$ inch wide heavy Cd foil into 10% HNO_3, then distilled H_2O. Then dip coil into sample and stir 15 min. with magnetic stirrer to remove copper. Reoxidize U and Fe with slight excess of $KMnO_4$	Dowex 1 (10% DVB) sulfate-form, anion	2, 0.3*M* H_2SO_4	Polarographic cell containing 1.47 g. citric acid	Dilute to 10 ml.	Polarographic (*4*) $E^1/_2$ −0.15 v. *vs.* S.C.E.
Mn(II)	Same as Al	Same as Al	Same as Al	Polarographic cell	Evap. to dryness. Cool. Dissolve residue in 5 ml. 1*N* KCN	Polarographic (*9*) $E^1/_2$ −1.33 v. *vs.* S.C.E.
Zr(IV)	Same as Al	Dowex 1 (10% DVB) chloride-form, anion	2,9*M* HCl. Discard effluent, then 2, 4.5*M*-HCl	Beaker	Evap. to dryness, transfer to 10-ml volumetric flask. Add 7 drops concd. HCl, 1 ml. 20% $NH_2OH.HCl$ and 1 ml. 0.2% Thoron reagent. Dilute to 10 ml. Measure absorbance *vs.* reagents	Spectrophotometric (*1*) 555 mμ
Cu(II)	Pipet 2.5 ml. 0.5*M* Versenate solution into polarographic cell. Add sample and dilute to 5 ml. with water	Ion-exchange separation not required				Polarographic (*8*) $E^1/_2$ −0.55 v. *vs.* S.C.E.

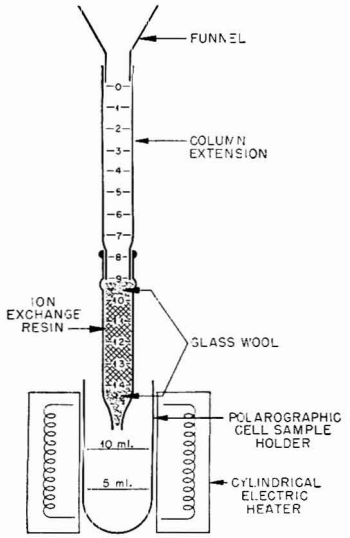

Figure 1. Apparatus for remote control separation and collection of corrosion products from homogeneous reactor fuel

(10% DVB) 50- to 100-mesh, chloride-form anion exchange resin and Dowex 50 (8% DVB) 50- to 100-mesh hydrogen-form cation exchange resin, Dow Chemical Co., Midland, Mich.

VERSENATE SOLUTION, 0.5*M* for Copper Determination. Weigh 18.6 grams of reagent grade disodium (ethylenedinitrilo) tetraacetate into a 150-ml. beaker. Cover the Versene with distilled water, and insert pH electrodes into the solution. Add sodium carbonate until the Versenate solution has a pH of 7.0. Add sodium hydroxide to a pH of 9.0. Transfer the Versenate solution to a 100-ml. volumetric flask and dilute to the mark with water.

METAL STANDARDS. Prepare the aluminum and zirconium standards according to the methods of Horton (*1*, *2*). Prepare the other metal standards in a conventional manner from reagent grade sulfate or chloride salts.

ALUMINON BUFFER AND THORON REAGENTS. Prepare aluminon buffer reagent for aluminum according to Luke and Braun (*7*), and Thoron reagent by the method of Horton (*1*).

Apparatus. The ion exchange column and attachments are shown in Figure 1.

In Figure 2 there is a bank of polarographic cells.

The dropping mercury capillary is connected to a mercury header by means of a glass tee and Tygon tubing. Mercury is pumped by compressed air from the mercury storage flask to a mercury reservoir (not shown), which is set at a height to give the desired drop time. A 50-ml. test tube serves as the saturated calomel electrode, which is connected to the cell by means of an agar-salt bridge contained in Tygon tubing with glass tips. Nitrogen for deoxygenation of the sample is brought into the cell by means of a glass-tipped Tygon tube (not shown). The microburet on the right is used for addition of standard solutions to the cells.

Ion Exchange Resins. A. CHLORIDE-FORM ANION EXCHANGE RESIN. Place a plug of glass wool in the bottom of a large glass tube with a tapered tip. Fill the tube with a slurry of Dowex 1 (10% DVB) 50- to 100-mesh resin and 9*M* hydrochloric acid. Estimate the volume of the column, and pass 10 column volumes of 9*M* hydrochloric acid through the resin. Store the resin in a glass container and use as needed.

B. SULFATE-FORM ANION EXCHANGE RESIN. Use a tube similar to the one in A. Fill with a slurry of untreated Dowex 1 (10% DVB) 50- to 100-mesh chloride-form resin and 1.0*M* sodium sulfate solution. Pass 1.0*M* sodium sulfate through the column until a negative test for chloride is obtained with silver nitrate solution. Pass 0.3*M* sulfuric acid through the resin until the pH of the effluent is the same as that of the influent. Use Accutint test paper.

C. HYDROGEN-FORM CATION EXCHANGE RESIN. Use a tube similar to the one in A. Fill the tube with a slurry of Dowex 50 (8% DVB) 50- to 100-mesh hydrogen-form resin and distilled water and wash with 10 column volumes of 6*M* sulfuric acid and then with 10 column volumes of water.

Preparation of Ion Exchange Column. Place a plug of glass wool in the bottom of the column (see Figure 1). Fill the column with a slurry of the desired resin and the proper acid (see columns 2 and 3 of Table II) to a volume of 4.0 ml. of wet resin. Place a plug of glass wool on top of the resin body to prevent disturbance of the resin when solutions are added to the column.

General Procedure. Procedures for remote control separation of the most important ions in Table I from uranyl sulfate solution and the analytical determination of these ions are given in Table II.

Place the proper collector under the tip of the column. Transfer the sample as prepared in column 2 to the resin column. When the sample has almost drained from the resin column, attach the column extension and add rinse or eluent according to column 4. When the rinse has almost drained from the resin column, remove the collecting vessel and treat according to columns 6 and 7.

RESULTS AND DISCUSSION

Most of the procedures are polarographic. Polarography is ideal for remote control analytical work, because the effluent or eluate from the sample can be collected and treated directly in the sample holder of the cell and only the cell plus the electrodes need be placed behind the barricade of the remote facility. Thus, manipulation and volumetric errors are held to a minimum.

The results of the determination of the major corrosion products and additives in homogeneous reactor fuel are given in Table III. After separation of the metals by the procedures given, interferences are eliminated. Although copper is not separated from uranium or corrosion products, no interferences have been encountered. The minimum concentrations (micrograms per milliliter) of the metals in homogeneous reactor fuel that can be determined by the procedures given herein are: aluminum, 5; nickel, 10; chromium, 10; iron, 20; zirconium, 10; copper, 5; cobalt, 10; and manganese, 10.

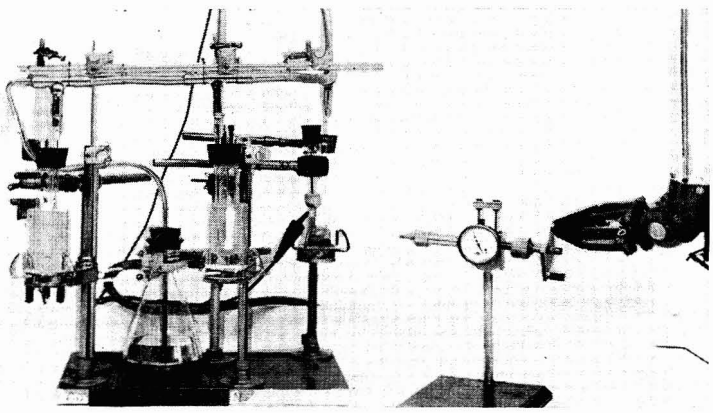

Figure 2. Polarographic cells

Table III. Determination of Corrosion Products and Additives in Synthetic Homogeneous Reactor Fuel

Composition of Synthetic Fuel. U(VI), 40 g./liter; Al(III), 50 mg./liter; Ni(II), 100 mg./liter; Fe(III), 100 mg./liter; Zr(IV), 100 mg./liter; Cu(II), 100 mg./liter; Cr(III), 50 mg./liter.

Ion	Synthetic Fuel Taken, Ml.	Ion Found, γ	Diff., γ	Rel. Std. Dev., %
Al(III)	1.0	49.5	−0.5	3.2
	0.6	30.4	+0.4	
	0.4	19.8	−0.2	
	0.2	9.4	−0.6	
Ni(II)	1.0	100.2	+0.2	2.3
		101.0	+1.0	
	0.2	21.1	+1.1	
		20.4	+0.4	
Fe(III)	1.0	98.0	−2.0	0.7
		99.0	−1.0	
	0.2	19.5	−0.5	
		19.5	−0.5	
Zr(IV)	0.62	59.3	−2.7	1.8
	0.30	29.5	−0.5	
	0.25	24.5	−0.5	
	0.29	29.0	...	
Cu(II)	1.0	100.1	+0.1	1.6
		100.0	...	
	0.1	9.8	−0.2	
		10.2	+0.2	
Cr(III)	0.4	19.9	−0.1	2.5
		20.2	+0.2	
		19.6	−0.4	
		20.8	+0.8	

ACKNOWLEDGMENT

The polarographic cells for use in the High Radiation Level Analytical Facility were designed by W. L. Maddox of this laboratory.

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Determination of Mercury in Urine

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▶ **A rapid and specific method for the determination of mercury in urine is needed for use in diagnosis of chronic mercury poisoning. The method presented utilizes a catalytic hydrogen peroxide oxidation of the sample, followed by the mercury microprocedure of Polley and Miller, which depends on the reaction: $Hg^{++} + R_2Hg \rightarrow 2RHg^+$. The RHg^+ is determined by the simple dithizone reaction for organic mercurials. As little as 1 γ per 100 ml. of sample may be determined. The procedure may also be applied to very dilute solutions of mercury.**

THE DETERMINATION OF MERCURY in urine is commonly used in the diagnosis of chronic mercury poisoning (*2*) in persons exposed during manufacture or use of metallic mercury or its compounds. The usual procedure for the determination of mercury in urine is: to absorb the mercury on copper, precipitate mercuric sulfide with another sulfide as a carrier, or extract directly with a solution of dithizone in chloroform, after oxidation (*4*). The proposed method is based on oxidation of the sample with hydrogen peroxide, followed by microdetermination of mercury by the method of Polley and Miller (*3*).

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REAGENTS

Hydrogen peroxide, 50%, technical grade, available from the Buffalo Electrochemical Co., Vancouver, Wash.

Catalyst mixture. Hydrochloric acid, 6*N*, conforming to ACS specifications, containing 1 gram of ferric chloride hexahydrate and 2 grams of chromium potassium sulfate per 100 ml. of solution.

Peroxide test reagent. Ten grams of titanyl sulfate are shaken several hours with 50 ml. of water and 20 grams of sulfuric acid, and centrifuged to obtain a clear solution (*5*).

Acetic acid, glacial, ACS grade, and 0.3*N*.

Accutint indicator paper No. 60.

Ammonium hydroxide, ACS grade, diluted with 2 volumes of redistilled water.

Chloroform, redistilled.

Ditolyl mercury. Twenty milligrams of ditolyl mercury (Eastman Kodak) are dissolved by refluxing in 200 ml. of neutral redistilled absolute ethyl alcohol. This solution is stable at least 6 weeks if stored in a dark bottle. Chilling causes undesirable crystal separation.

Dithizone solution. One hundred milligrams of Eastman Kodak White Label diphenylthiocarbazone are dissolved in 100 ml. of chloroform and stored under refrigeration. Dilution of 3 ml. with 90 ml. of chloroform gives a working range of 0 to 8 γ of mercury.

Standard mercury solution. ACS grade mercuric chloride is dissolved in water at the rate of 0.1354 gram per 100 ml. of 1*N* sulfuric acid. For daily use

the stock solution is diluted at the rate of 1.00 ml. per 100 ml.

PREPARATION OF SAMPLE

A sample of urine not exceeding 100 ml. is measured into a 300-ml. or larger flask with a ground-glass joint. The catalyst mixture is added at the rate of 10 ml., and hydrogen peroxide at the rate of 11 to 12 ml. per 100 ml. of sample. The neck of the flask is washed down and connected to a rapidly flowing condenser. The flask is placed on an actively boiling steam bath. Oxidation takes place with warming and the solution becomes pale green. When all the hydrogen peroxide has reacted (no more tiny bubbles of gas appear in the liquid), the heating is continued 30 minutes. Excessive heating is avoided. All the hydrogen peroxide must be decomposed at this point.

DETERMINATION

The cold digest is tested by placing a few drops of the peroxide test reagent on a spot plate and adding a drop of the digested sample with a clean stirring rod. There should be no yellow or orange color, which would indicate the presence of peroxide. The digest or aliquot is filtered through glass fiber filter paper or a medium-porosity sintered-glass filter. In the absence of bismuth or silver, the filtration may be omitted. The digest is then transferred to a 250-ml. separatory funnel and 1 ml. of glacial acetic acid is added. Dilute ammonia is added to adjust the pH to 3.0 to 3.5, as determined by Accutint paper as an outside indicator. One milliliter of the ditolyl mercury reagent is added and the funnel is shaken vigorously 10 to 15 times. After 1 minute 10 ml. of chloroform are added and the separatory funnel is shaken vigorously for 1 minute. In samples where emulsions may form because of separation of slightly soluble iron compounds, 1 gram of citric acid may be added prior to pH adjustment. The aqueous and chloroform layers are allowed to separate and the chloroform layer is drained into a second small separatory funnel containing 25 ml. of 0.3*N* acetic acid. One milliliter of the diluted dithizone is added and the separatory funnel is shaken for 30 seconds. The chloroform layer is transferred to a 10-ml. volumetric flask and made to volume with chloroform. The percentage transmittance of the unreacted dithizone is measured with a Beckman spectrophotometer or an Evelyn colorimeter at 620 mμ. The amount of mercury in the sample is determined from a curve prepared from known amounts of mercury, using all reagents. From 0 to 8 γ of mercury may be determined.

DISCUSSION

The use of hydrogen peroxide in the digestion of urine for the determination of mercury under different conditions was reported by d'Hoore (*1*). In the present investigation the addition of acid greatly improved the speed of the digestion and recovery of added mercury. The addition of 0.5 ml. of 12*N* hydrochloric acid per 100 ml. of sample prevented foaming at the start of the reaction. However, the solution turned alkaline during the digestion, and the mercury was converted to a form that would not react with the ditolyl mercury to form two molecules of tolyl mercuric chloride. With the catalytic mixture described, good recovery of added mercury was obtained when the acidity of the sample was from 4 to 13 ml. of 12*N* hydrochloric acid per 100 ml. of sample. With less acid (1 to 3 ml.) recovery of added mercury may be poor; with more acid the digestion was slower and occasionally the chloroform extract had a yellow color (Table I).

Table I. Effect of Acidity on Recovery of Mercury from Urine

12*N* HCl added, ml./100-ml. sample	0.5	1.0	2.0	3.0	4.0	5.0	7.0	10.0	13
Hg recovered, %	<20	Variable	Variable	89	98	99	101	97	103

A number of metal ions are reported to catalyze the decomposition of hydrogen peroxide (*5*). Of the catalysts tried, iron alone or with chromium and/or vanadate, or vanadate alone, or with copper, molybdate, or selenate appeared to be satisfactory. Copper, tungstate, molybdate, uranyl, arsenate, chloroplatinate, nickel, cobalt, manganese, tin, chromium, selenate, or titanium ions alone are unsatisfactory.

Of the suitable catalysts, the iron-chromium mixture was chosen over vanadium alone or in combination because it caused a more rapid oxidation, had a greater acid range, and did not form yellow chloroform extracts. Vanadate has been reported to give a brown color in the presence of hydrogen peroxide (*5*). However, the disappearance of the brown color in the digest and the appearance of pure green could not be taken as evidence of the absence of hydrogen peroxide. The hydrogen peroxide had to be added to the warmed sample containing the vanadium catalyst, as a precipitate formed if the peroxide was added cold.

The speed of digestion can be varied by the amount of iron in the catalyst mixture. The catalyst described will give a complete digestion of a 100-ml. sample in about 2 hours. Large amounts of iron in the catalyst appeared to decompose the hydrogen peroxide without simultaneous digestion of the sample. In testing for the absence of peroxide, the peroxide test reagent was better than starch-iodide paper.

The chloroform used was U.S.P. grade which had been shaken with lime and redistilled. If chloroform used for a mercury analysis is to be reused, it must be recovered by a special procedure. The method of Sandell (*4*) gave a redistilled product contaminated with small but variable amounts of ionic mercury. Used chloroform is recovered in this laboratory by shaking the waste chloroform containing dithizone and mercury compounds with approximately 2% of its volume of concentrated sulfuric acid until the acid layer is light brown. Three or four extractions are usually needed. Then the chloroform is washed with water once, followed by extraction with 2 to 3% of its volume of 30% technical sodium chloride solution. The resulting chloroform is shaken with lime (10 to 20 grams), allowed to stand 2 to 24 hours, then filtered and redistilled from glass.

Table II. Recovery of Mercury Added to Urine

Hg Added as	Hg Added, γ	Av. Hg Recovered, γ	Max. Deviation, γ
C_2H_5HgCl	4.24	4.21	±0.32
	3.03	2.80	±0.43
C_6H_5HgOAc	4.77	4.52	±0.77
	3.34	3.23	±0.36
	1.91	2.08	±0.54
$HgCl_2$	0	0.28	±0.60
	1	1.35	±0.80
	2	1.91	±0.20
	3	3.04	±0.38
	5	4.95	±0.30

The procedure of Polley and Miller for mercury was modified slightly. Use of ammonia in the neutralization step made unnecessary the tedious purification of sodium acetate reagent. Ammonia causes a greater temperature rise in the solution during neutralization, but with the larger volume this presented no problem. The use of permanganate to destroy the last traces of peroxide was both unnecessary and detrimental in this digest. Even when the excess was destroyed by hydroxylamine hydrochloride, high results ensued. This appeared to be due to extraction by the chloroform of some material that slowly oxidized the dithizone to a yellow product. The dithizone fades when the hydrogen peroxide is incompletely reduced in the digestion. Ascorbic acid, hydrazine or hydroxylamine sulfate, ethylenediamine, sulfur dioxide, arsenic trioxide, 2,2′-thiodiethanol, ammonium sulfamate, sodium nitrite, or hydrogen bromide, either singly or combined with

the iron-chelating materials sodium pyrophosphate, gluconic acid, or sodium fluoride, either interfered or failed to prevent fading in the presence of peroxide.

One thousand micrograms of silver, cobalt, nickel, copper, zinc, cadmium, lead, manganese, and bismuth were added singly as soluble salts to urine. Only with silver and bismuth was there any evidence of interference. This was removed by filtration through a glass fiber filter in a Gooch crucible. Filtration through paper is not recommended. No attempt was made to determine the maximum amount of metal ion tolerated in the procedure. (Ethylenedinitrilo)-tetraacetic acid (ethylenediaminetetraacetic acid), in amounts up to 0.19 gram, does not interfere when added to the sample prior to digestion. However, larger amounts, 0.38 to 0.75 gram, slow the oxidation markedly, and the digest may be lavender or yellowish. The acidity becomes more critical, possibly owing to chelation of the oxidation catalyst. A total of 5 to 9 ml. of 12*N* hydrochloric acid gave satisfactory results.

The recovery of mercury added to urine (Table II) is satisfactory whether the mercury is added as mercuric chloride, ethyl mercuric chloride, or phenyl mercuric acetate.

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Preparation and Analysis of Carbon-14–Labeled Cyanide

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▶ **Detailed directions, based on the method of Sixma and associates, are given for the preparation of sodium cyanide-C^{14} from barium carbonate-C^{14} in 90 to 95% yield. Simple, reliable methods are presented for the analysis of carbon-14–labeled alkali cyanide and barium carbonate. The labeled cyanide is analyzed for total radioactivity, radioactivity in the form of cyanide, and specific radioactivity. The procedures are based on reaction of the cyanide with a reducing sugar, and measurement of fixed carbon-14 in either formamide or alkaline ethylene glycol solution by means of a proportional counter. Carbon dioxide, formed by acid treatment of barium carbonate-C^{14} or by wet oxidation of carbon-14–labeled cyanide, is absorbed in alkaline ethylene glycol; the glycol solution is then counted directly in a proportional counter. The absorption and counting of carbon dioxide in alkaline ethylene glycol can be applied to the analysis of carbon-14–labeled materials in general. When carbonate is decomposed by acid, the last traces of labeled carbon dioxide are swept from the apparatus by the addition and slow decomposition of ethylene carbonate.**

With the rapidly increasing application of compounds labeled with carbon-14 to chemical and biological problems, the need for simple, reliable methods for the preparation and radioassay of commonly used carbon-14 labeled reagents becomes urgent.

Of the methods reported for the preparation of carbon-14–labeled alkali cyanide, that of Sixma and associates (*10*) appears most simple and efficient. It gives potassium cyanide-C^{14} in good yield by the ignition of barium carbonate-C^{14} with ammonium chloride and metallic potassium at 640° C. As experimental difficulties have arisen in some laboratories, detailed directions, based on the Sixma method, are given here for the preparation of carbon-14–labeled alkali cyanide.

The radioassay of carbon-14–labeled compounds can be performed routinely by a simple procedure of counting in solution with a windowless, gas-flow, proportional counter (*8*, *9*). The method is successful for measuring carbon-14 in nonvolatile compounds that are soluble in a suitable counting solvent. A material that does not meet these requirements must be converted chemically to a nonvolatile, soluble form before this convenient volumetric technique can be applied. This paper describes simple methods for the radioassay of carbon-14–labeled alkali cyanide, barium carbonate, and certain other materials.

In addition to excess alkali, carbon-14–labeled cyanide usually contains both acid-volatile and nonvolatile radioactive contaminants. Hence, the radioassay of carbon-14–labeled cyanide requires determination of total radioactivity, radioactivity in the form of cyanide, and specific radioactivity of the cyanide. Incidental to these determinations is the measurement of the acid-volatile and nonvolatile contaminants.

Two methods were developed for the measurement of total radioactivity.

In the first method, the cyanide in a suitable aliquot is fixed by the addition of an excess of glucose in highly alkaline solution. The solution is then diluted with alkaline ethylene glycol and counted with a windowless, gas-flow, proportional counter adapted for the assay of liquids (*9*). In the second method, the cyanide is oxidized by alkaline potassium permanganate and the resulting carbonate is converted to carbon dioxide (*1*, *11*). The reaction is conducted in this laboratory in a specially designed apparatus in which the carbon dioxide is absorbed in alkaline ethylene glycol. The glycol solution is then counted by the procedure already described.

The radioactivity in the form of cyanide is measured by fixing the cyanide in a small aliquot of solution with an excess of glucose, then removing volatile contaminants, counting the reaction mixture in formamide solution, and correcting for nonvolatile radioactive contaminants. To obtain complete reaction between the carbon-14–labeled cyanide and the sugar, it is necessary to maintain reasonably high

concentrations of both sugar and cyanide. Hence the concentration of cyanide in highly dilute solutions must be increased by the addition of the nonradioactive form.

The specific radioactivity of the cyanide is determined by the reaction of an excess of the reagent with a small known quantity of D-glucose. Degradation of the glucose is prevented by use of a suitable buffer, and the conditions are such that the sugar combines quantitatively. After volatilization of the excess cyanide and acid-volatile impurities, the residue is counted in formamide; a correction is made for nonvolatile impurities. The net count per millimole of the sugar is proportional to the specific radioactivity of the cyanide.

The absorption and counting of carbon dioxide in alkaline ethylene glycol can be applied to the analysis of carbon-14–labeled materials in general. Details are given for the analysis of labeled barium carbonate.

EXPERIMENTAL PROCEDURES

Preparation of Sodium Cyanide-C^{14} from Barium Carbonate-C^{14} (*10*). A Vycor glass tube (Corning Glass Works, Corning, N. Y.), 13 mm. (outside diameter) × 300 mm., is sealed at one end and charged with 100 mg. each of barium carbonate-C^{14} and finely powdered ammonium chloride; the materials are then pulverized together by means of a long glass rod. The amount of ammonium chloride is twice that prescribed by Sixma and associates (*10*). The tube is doubly constricted at about 200 mm. from the closed end and connected by rubber to a glass T-tube as shown in Figure 1. A gentle stream of nitrogen is introduced into the tube through arm *A* by means of a capillary tube. The nitrogen supply is then connected to arm *B* of the T-tube, the capillary tube is removed, and about 1 gram of metallic potassium is added through arm *A* in the following manner:

Potassium, from a supply stored under mineral oil, is transferred to a test tube, 32 × 300 mm., and washed three times with heptane. It is then melted under a layer of heptane in an electrically heated oil bath, kept at about 100° C. A capillary pipet is made from 8-mm. glass tubing, and is of suitable length to pass through the T-tube of Figure 1 and into the ignition tube below the constrictions. About 1.2 ml. of clean, molten potassium is drawn into the pipet by means of a 2-ml. rubber bulb. After the potassium has solidified, the pipet is inserted into the apparatus of Figure 1, with the tip extending below the constrictions in the tube. With nitrogen flowing through arm *B*, the upper part of the ignition tube is heated with a small flame until the potassium melts and flows from the pipet. Then the pipet is withdrawn, the flow of nitrogen is stopped, arm *A* is closed, and the tube is evacuated through arm *B*. Occluded heptane and volatile matter are removed by gently heating the ignition tube with a small flame. After the potassium has melted, and the evolution of gas subsided, the tube (still under vacuum) is sealed at the upper constriction; it is gently reheated to melt the potassium, and the contents are mixed in the lower part of the tube by careful shaking. The tube is then placed horizontally in a stainless steel shield, heated for 1 hour at 640° C. in an electric oven, and allowed to cool to room temperature in the oven. Finally, the tube is cautiously opened at the constriction.

Considerable pressure develops in the tube; hence, it should be opened behind a screen to protect the operator in case it shatters. Excessive pressure can be avoided by use of smaller samples or larger tubes, but the size of tube and sample described here have been satisfactory for the preparations done in this laboratory.

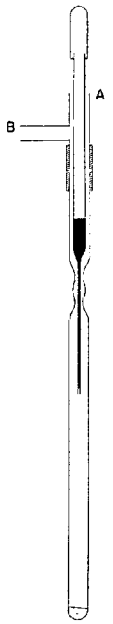

Figure 1. Apparatus for adding potassium to ignition tube

The tube is immediately placed in the apparatus of Figure 2; a slow stream of nitrogen is passed through tube *A*. The excess potassium is decomposed by cautious addition, first, of ethyl alcohol, and then, of water through tube *B*. The reaction takes place smoothly, and there is no difficulty, provided a positive pressure of nitrogen is maintained. When the evolution of hydrogen is complete, the reaction mixture is transferred with a capillary pipet to a 250-ml. round-bottomed flask; the total volume, including washings, is about 100 ml.

Most of the ethyl alcohol is removed by vacuum distillation, and the flask containing the aqueous solution is then connected to the all-glass distillation apparatus of *Figure 3*. The previously weighed receiver, *B*, containing 5 ml. of 0.2*N* carbonate-free sodium hydroxide is immersed in an ice bath. The cyanide solution is acidified by adding about 10 ml. of 6*N* sulfuric acid through the dropping funnel and is then boiled until about 25 ml. of distillate has been collected. Samples of the distillate are taken for radioassay by the procedures given in this article. The tube containing the distillate is sealed at the constriction, and the sealed ampoule and excess glass are weighed together. The gain over the previous weight, plus the weight of the samples taken for assay, gives the total weight of the cyanide solution. The solution can be stored for several months at low temperatures

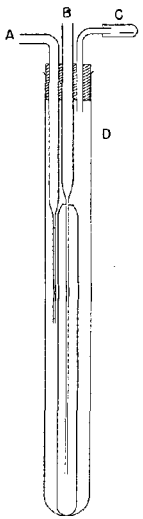

Figure 2. Apparatus for decomposing excess potassium

A. Nitrogen inlet, 8-mm. tubing
B. Tube for solvent addition, 8-mm. tubing
C. Bunsen flutter valve (rubber bulb with small slit)
D. 38 × 300 mm. test tube

without appreciable hydrolysis, but for longer periods of storage the product should be lyophilized. If the material is to be lyophilized, 5 ml. of 0.3*N* sodium hydroxide (instead of 0.2*N*) should be used in receiver *B*. A trap immersed in liquid nitrogen should be employed, and the condensate checked for radioactivity by means of the procedure described below. It is then either discarded or retained for recovery of labeled material. Considerable radioactivity may be found in the condensate if insufficient alkali has been used.

Preparations in this laboratory have given radiochemical yields of carbon-14–labeled cyanide of 90 to 95%. The products have been used in various procedures for the synthesis of carbon-14–labeled carbohydrates (*4*, *5*, *7*) and for the study of carbohydrate structure (*3*, *6*).

Measurement of Total Radioactivity by Direct Count. An aliquot of 10 to 100 μl. of a solution of carbon-14–labeled cyanide is diluted to 1 ml. with 0.1*M* nonradioactive sodium cyanide by means of a dilution pipet. The nonradioactive cyanide is used to increase the concentration to about 0.1*M* because the reaction does not go to completion at extremely low cyanide concentrations. If necessary, a second dilution with nonradioactive 0.1*M* sodium cyanide is made to reduce the radioactivity to 5 to 100 μc. per ml. A 100-μl. sample of the cyanide solution is transferred with a delivery pipet to a weighed, 5-ml. volumetric flask containing approximately 200 μl. of 6*N* aqueous potassium hydroxide and 50 mg. of D-glucose; the flask is stoppered and stored for 16 to 24 hours at room temperature. The solution is then diluted to 5 ml. with a 3*N* ethylene glycol solution of potassium hydroxide. (There is some darkening of the solution, but this does not interfere with the determination.) The flask is weighed and the density of the solution is determined. Then 1 ml. of the thoroughly mixed solution is transferred to the cell of a proportional counter adapted for the assay of solutions (*9*), and counted to a probable statistical error of not more than 1% (10,000 counts). For all carbon-14–labeled materials, the product of counts per second (corrected for background), density, and calibration factor gives the microcuries of carbon-14 per milliliter. (The calibration factor for the instrument and the cell is obtained from the count of a solution of known density and known carbon-14 content.) The total radioactivity of the original cyanide solution is the product of the radioactivity per milliliter and the proper dilution factor.

Measurement of Total Radioactivity by Oxidation of Sample with Alkaline Potassium Permanganate. The apparatus for this determination (Figure 4) includes a 100-ml. reaction flask, *A*, connected by stopcock *C* to a vacuum pump and to receiving flask *B*; funnel *D* is used for introducing reagents. *B* is a weighed, 10-ml. round-bottomed volumetric flask containing about 3 ml. of 3*N* potassium hydroxide in ethylene glycol. At the beginning of the determination 2 ml. of 6*N* aqueous potassium hydroxide is pipetted into *A*, together with the cyanide solution to be analyzed (preferably having an activity of from 1 to 20 μc.) and 0.5 ml. of 0.2*M* nonradioactive sodium cyanide. The mixture is frozen in liquid nitrogen, and 150 mg. of crystalline potassium permanganate is added. The flask is connected to the transfer apparatus of Figure 4 and evacuated while still immersed in the liquid nitrogen. The reaction side of the system is closed off with the central stopcock, and the flask is allowed to come to room temperature, with swirling to prevent bumping.

After 15 minutes at room temperature the flask is heated intermittently, with swirling, until the neck becomes warm with condensate. After a reaction period of about 30 minutes, receiver *B* is evacuated. Then the contents of flask *A* are frozen, stopcock *C* is turned to connect *A* and *B*, and about 5 ml. of 50% sulfuric acid is added to *A* through funnel *D* without the introduction of air. The mixture in *A* is allowed to thaw, with swirling to prevent bumping. When evolution of gas has ceased, the mixture in *A* is boiled gently for a few minutes. After about 15 minutes, a saturated solution of oxalic acid in 50% sulfuric acid is added gradually in small portions from funnel *D* until the solution in *A* becomes colorless. This provides a flush of nonradioactive carbon dioxide. This operation should require about 10 minutes and should be performed with gentle shaking of the flask. *A* is heated again, and *B* is finally frozen in liquid nitrogen. After about 10 minutes, to allow for complete transfer of carbon dioxide, the receiver is closed off, allowed to warm to room temperature, and shaken gently to facilitate absorption of carbon dioxide. The receiver is removed from the apparatus, and ethylene glycol is added to the volume mark. Then the flask and contents are weighed and the density of the solution is calculated. The solution is counted and the radioactivity is calculated as described previously.

Measurement of Carbon-14 Present as Cyanide. A 100-μl. sample of a solution of carbon-14–labeled cyanide (previously made approximately 0.1*M* with respect to cyanide) is transferred quantitatively to a constricted test tube, 13 × 100 mm., containing about 10 mg. of D-glucose. The tube is immediately sealed in a flame. After 24 hours or more at room temperature, the tip of the tube is opened. A few drops of 10% formic acid are introduced with a capillary pipet, and the solution is evaporated to dryness under a jet of air while the tube is kept in a bath at 60 to 70° C. The evaporation process is repeated three times, with the addition of about 0.2 ml. of water each time. Finally, the residue is dissolved in 1 ml. of formamide, and the solution is counted in the usual manner.

The count represents carbon-14–labeled cyanide and any contaminants not volatilized with aqueous formic acid. In order to determine nonvolatile contamination, an aliquot of the cyanide solution is carried through the process described immediately above, but the addition of glucose is omitted. The radioactivity, which is due to nonvolatile contaminants, is deducted from that obtained in the preceding step, to give the amount of carbon-14 present as cyanide.

The difference between the total radioactivity and that of the cyanide before correction for nonvolatile contaminants is the amount of acid-volatile contamination.

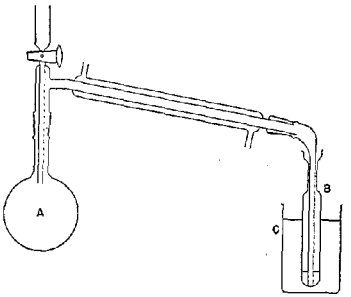

Figure 3. Distillation apparatus

A. 250-ml. 24/40 ∓ flask containing cyanide solution
B. Constricted test tube containing sodium hydroxide
C. Ice bath

Measurement of Specific Radioactivity of Carbon-14–Labeled Cyanide. The specific radioactivity of the cyanide is determined by measuring the amount of carbon-14–labeled cyanide fixed by a known amount of D-glucose. The conditions must be adjusted according to the concentration of the cyanide. At least 4 moles of cyanide must be used per mole of glucose. Preparations of cyanide-C^{14} ordinarily

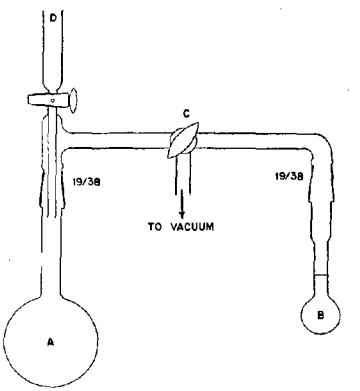

Figure 4. Apparatus for oxidation of carbon-14–labeled cyanide, and for transfer and absorption of $C^{14}O_2$

A. 100-ml. flask
B. Round-bottomed 10-ml. volumetric flask
C. Three-way stopcock
D. Addition funnel

contain an approximately known excess of alkali hydroxide. To protect the glucose from alkaline degradation, the reaction mixture is buffered with ammonium chloride; an excess of 30% over all of the alkali metal ions present is recommended. The cyanide solution to be analyzed should be adjusted, preferably to contain 0.003 to 0.010 mmole in a volume of 50 μl.

Table I. Radioassay of Carbon-14–Labeled Sodium Cyanide

Sample	Total, Mc.	Cyanide, Mc.	Nonvolatile, Mc.	Volatile, Mc.
1	4.34	4.02	0.08	0.24
	4.29	3.96	0.08	0.25
	4.32	3.98	0.08	0.26
2	2.93	2.53	0.00	0.40
	2.94	2.58	0.00	0.36
	2.94	2.54	0.00	0.40
3	42.4	41.4	0.00	1.0
	41.9	40.9	0.00	1.0
	42.4	41.4	0.00	1.0
4	4.63	4.50	0.00	0.13
		4.52	0.00	0.11
5	4.45	4.38	0.00	0.07
		4.40	0.00	0.05

A 20-μl. aliquot of 0.05M glucose solution (0.18 mg. of glucose), containing the requisite amount of ammonium chloride (see above) for the analysis, is pipetted into a constricted test tube, 13 × 100 mm.; this is followed by 50 μl. of the cyanide solution. The tube is sealed in a flame and stored at 55° C. After one day (or a longer period for more dilute cyanide) the tube is opened and the excess cyanide is removed by repeated evaporation in the presence of formic acid and water. The residue is counted in 1 ml. of formamide. The count is corrected for nonvolatile contaminants by means of a blank determination on a second aliquot of the cyanide solution, treated similarly, but without the addition of glucose.

$$\text{Specific radioactivity} = \frac{\text{cps.} \times \text{density} \times \text{calibration factor}}{\text{mmoles glucose}} = \mu\text{c/mmole cyanide}$$

Determination of Carbon-14 in Barium Carbonate-C^{14}. For this determination, the apparatus of Figure 4 is employed. A weighed sample of less than 50 mg. of barium carbonate-C^{14} (or an aliquot of a carbonate solution) with an activity of 1 to 20 μc, and 1 ml. of water are placed in flask A; 3 ml. of 3N potassium hydroxide in ethylene glycol is placed in flask B. The contents of A are frozen in liquid nitrogen, and the system is evacuated, with swirling to prevent bumping of the ethylene glycol solution. Stopcock C is turned so as to cut off the vacuum pump and to connect A and B. About 5 ml. of concentrated sulfuric acid is added to flask A through funnel D. The liquid nitrogen bath is removed and flask A is warmed to room temperature, and then heated to boiling, with gentle swirling. After 5 to 10 minutes, heating is discontinued and a freshly prepared solution of about 0.1 gram of ethylene carbonate in 1 ml. of concentrated sulfuric acid is carefully introduced into flask A through funnel D. The mixture is heated for 10 minutes to provide a nonradioactive carbon dioxide flush by partial hydrolysis of the ethylene carbonate. Finally, receiver B is cooled in liquid nitrogen for 10 minutes, after which stopcock C is turned to close off flask B. The flask is allowed to come to room temperature and the solution is diluted to the volume mark with ethylene glycol. The flask and contents are weighed, and the density of the solution is calculated. The solution is counted in the usual manner, and the radioactivity is calculated from the relationship: Cps. × density × conversion factor = μc. of C^{14} in sample.

DISCUSSION OF RESULTS

The methods presented here have given consistently satisfactory results for the analysis of numerous samples of carbon-14–labeled cyanide. The values for total radioactivity of cyanide solutions obtained by direct count are slightly higher than those obtained after oxidation of the material with alkaline permanganate. In a typical case, duplicate determinations by direct count showed 2.15 and 2.14 mc. in comparison with 2.09 and 2.10 mc. by the permanganate method. The direct count method is simpler than the permanganate method and is considered preferable.

Table I gives some results obtained for three samples of commercial carbon-14–labeled cyanide and two samples (Nos. 4 and 5) prepared in this laboratory. For some samples of commercial sodium cyanide-C^{14} the volatile contaminant (presumably carbon dioxide arising from sodium carbonate-C^{14}) was as high as 20%. Nonvolatile contamination is usually low, but preparations should nevertheless be checked for its presence before use.

Table II gives duplicate determinations of specific radioactivity after various reaction times. The results show that under the prescribed conditions, the reproducibility is satisfactory for reaction times of 22 to 45 hours. The procedure is described for use with solutions having approximately the concentrations given. With more dilute solutions, the proportion of cyanide and the reaction time must be increased.

Table II. Determination of Specific Radioactivity of Sodium Cyanide-C^{14}

Detn.	Reaction Time, Hours	Specific Activity, μc./Mmole
1	22	270
2	22	269
3	33	269
4	33	269
5	45	270
6	45	270

Quantities of reactants. D-glucose, 0.00100 mmole; cyanide, 0.0055 mmole. Total volume 70 μl.

Check determinations of a sample of barium carbonate-C^{14} having a radioactivity of 0.670 μc per mg. [as determined by a precision method (*2*)], gave 0.669 and 0.666 μc. per mg. In all cases, the reproducibility of the results was satisfactory. The methods require relatively large samples but are well suited for the assay of reagents used for synthetic work.

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3-Hydroxy-1-*p*-sulfonatophenyl-3-phenyltriazine as a Colorimetric Reagent for Palladium

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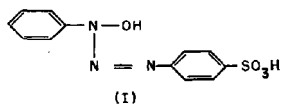
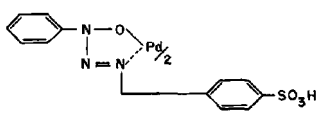
▶ 3-Hydroxy-1-*p*-sulfonatophenyl-3-phenyltriazine is an excellent reagent for the colorimetric estimation of palladium, either as such or in the presence of nickel(II), copper(II), iron(III), cobalt(II), silver, and especially members of the platinum group—platinum(IV), rhodium(IV), iridium(III), and ruthenium(III). Apart from its other highly desirable qualities, such as solubility of the reagent and the complex in water, almost instantaneous development of color and stability for 24 hours in a wide range of temperature, wide range of permissible pH (1.7 to 4.4), and easy adjustment using mineral acid alone, the reagent has great tolerance for the members of the platinum group and is superior to other palladium reagents used so far for this purpose.

THE USE of 3-hydroxy-1,3-diphenyltriazine as a gravimetric reagent for palladium has been described (*7*). Its high selectivity toward palladium and its fairly high sensitivity suggested that the sulfonic acid derivative of such a compound, either as such or in the form of its sodium salt (both soluble in water) should prove useful as a colorimetric reagent for palladium. 3 - Hydroxy - 1 - *p* - sulfonatophenyl-3-phenyltriazine (I)



(I)

has proved to be an excellent reagent for the colorimetric estimation of palladium as such or in the presence of other elements, especially the members of the platinum group. The soluble palladium complex formed by it may be best represented by the chelate structure (II).



(II)

In many respects, it is superior to other known palladium reagents for the colorimetric estimation of palladium.

3 - Hydroxy - 1 - *p* - sulfonatophenyl-3 - phenyltriazine posesses many of the properties of an ideal colorimetric reagent.

It is stable toward heat, light, and air, and can be preserved indefinitely at room temperature.

It is soluble in water, and the aqueous solution remains practically unaltered up to 48 hours.

The palladium complex is soluble in water.

Color development with palladium is almost instantaneous. Full development takes place in less than 5 minutes and the intensity remains unaltered, even after 24 hours.

Color development is not sensitive to pH variation, but remains steady between pH 1.7 and 4.4. pH can be adjusted with mineral acid alone. Color intensity is also unaffected by variation in temperature from 25° to 85° C. Full development of color takes place without any undue excess of reagent.

Color development follows Beer's law.

The reagent and its complex have reasonably well separated absorption peaks, so that excess reagent does not interfere in the photometric estimation.

Color development is very highly selective. There is no interference from most foreign ions, including other members of the platinum group.

EXPERIMENTAL

Preparation of Reagent. Because of the soluble nature of the reagent, the following procedure should be strictly followed, to avoid possible loss.

Sulfanilic acid (8.6 grams) was dissolved in 40 ml. of 5% sodium hydroxide solution and 3.5 grams of sodium nitrite was added. To this solution crushed ice was added and the reaction mixture was slowly poured into 20 ml. of concentrated hydrochloric acid, also containing crushed ice, with mechanical stirring. The diazonium salt thus obtained was then slowly added under mechanical stirring to 5.4 grams of phenylhydroxylamine dissolved in 125 ml. of 20% v./v. alcohol and containing a sufficient quantity of crushed ice. Small portions of sodium acetate (50 grams in 100 ml. of water) were added occasionally to the reaction mixture to prevent it from becoming too acidic. The temperature during the entire course of reaction was kept at about 0° C. The precipitated sulfonic acid derivative was filtered under suction, washed twice with a small amount of ice water, and crystallized from a large volume of alcohol. It was obtained as 5 grams of light green crystals, actually the sodium salt of the sulfonic acid, which had no sharp melting point. It started decomposing at 157° C.

Analysis. Found: C, 43.8; H, 4.0; N, 12.42; S, 10.48%. ($C_{12}H_{10}N_3O$)SO_3-$Na.H_2O$ requires: C, 43.3; H, 3.9; N, 12.32; S, 9.9%.

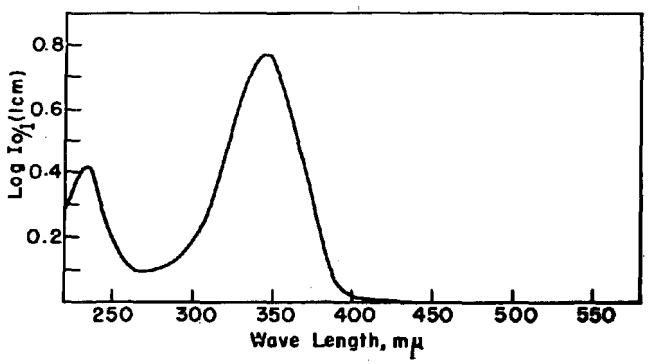


Figure 1. Spectral characteristics of reagent

9.3 p.p.m. of sulfonic reagent in water

Solubility, 2.9 grams per 100 ml. of water at 23° C.

Spectral Characteristics. In aqueous solution, λ_{max} = 235, 345 mμ; log E = 4.1782, 4.4405 (Figure 1).

Reagent solution, 0.1% w./v. solution in distilled water.

Standard Palladium Solutions. Approximately 0.5 gram of palladium chloride was dissolved in a little water containing 3 ml. of concentrated hydrochloric acid and the volume was made to 500 ml. The palladium content was determined by using dimethylglyoxime and 3-hydroxy-1,3-diphenyltriazine (developed by the authors) and was found to be 0.6216 gram of palladium per liter. When 159.9 ml. of this solution was made to 1 liter, it contained 100 γ of palladium per ml. This stock solution was further diluted 10 times, so that 1 ml. of the diluted solution contained 10 γ of palladium.

Solution of Diverse Ions. The reagent grade soluble salts, usually chloride, nitrate, or sulfate, were employed for the preparation of the solutions of inorganic ions. The solutions were diluted in such a way that they contained 1 mg. of the metallic element per ml.

Instruments. A Beckman pH meter, Model H-2, was used for pH measurements. Absorbance measurements were made with a Beckman quartz spectrophotometer, Model DU, using 1.00-cm. quartz cells for the ultraviolet region and Corex cells for the visible region. Visual color comparisons were made in 50-ml. Nessler tubes of the standard type.

Absorption Curves for Palladium Complex and Reagent. Twenty milliliters of palladium solution containing 10 p.p.m. of palladium was pipetted into a 100-ml. measuring flask, and 0.1 ml. of 1*N* hydrochloric acid was added, so that the pH after dilution was about 3.0. Then 5 ml. of 0.1% w./v. aqueous reagent solution was added and after a little shaking the volume was made to 100 ml. The resulting solution contained 2 p.p.m. of palladium.

A blank solution was prepared by pipetting 5 ml. of the reagent solution into a 100-ml. flask and making the volume up to the mark. There was no difference in the blank solution prepared as given above and that prepared by adding 0.1 ml. of hydrochloric acid to bring the pH to about 3.0. Hence addition of the acid in the blank solution was not considered imperative.

Figure 2 gives the absorbance curves of the palladium complex with a "reagent-blank solution" and reagent solution with water as blank. The absorbance of the reagent solution falls sharply after 390 mμ and is almost negligible at 430 mμ. The absorption peak of the palladium complex, in this region, is at 413 mμ, which is also true for the palladium complex of the parent reagent, 3-hydroxy-1,3-diphenyltriazine (7). However, as there is no substantial fall in absorbance up to 420 mμ, where the interference by the reagent is also decreased, 420 mμ was considered a suitable wave length for the palladium estimations; 430 mμ can also be used with advantage, as at this wave length interference by the reagent is almost negligible and so water can be used as a blank.

Effect of pH. Solutions used for studying the effect of pH on color reaction were prepared as directed above, except that different amounts of 1*N* hydrochloric acid, 1*N* sulfuric acid, or 1*N* nitric acid, and 10% w./v. sodium acetate or sodium potassium tartrate were added, so that the final pH values ranged from 1.2 to 6.5. When 1*N* hydrochloric or 1*N* sulfuric acid was used for adjusting pH, the range of constant maximum absorbance was between 2.5 and 4.4. At pH below 2.5, there was a slight turbidity, resulting in lower absorbance. However, when nitric acid was used, the pH range increased and was between 1.7 and 4.4 (Figure 3). pH can be better adjusted by using acid alone without sodium acetate or sodium potassium tartrate, as these buffering agents give turbidity when used in higher concentrations. Hence in the estimations, only 1*N* nitric acid was used for the adjustment of pH, which was kept between 2.5 and 3.0 to increase the specificity of the reagent

Reagent Concentration and Mole Ratio. A series of solutions was prepared in which the mole ratio of palladium to reagent was from 1:1 to 1:15. pH was adjusted to about 2.5 by using 0.2 ml. of 1*N* nitric acid. Absorbance was measured for each solution after about 5 minutes at 430 mμ, using water as blank. Figure 4 shows the effect of moles of reagent per mole of palladium on absorbance. The fact that there is no sharp peak indicates that the complex is appreciably dissociated in solution. Full color development is ensured at 1 to 8 ratio of palladium to reagent.

Rate of Reaction and Stability of Complex. The color formation of palladium complex with the reagent was almost instantaneous and the color was very stable. There was no difference in the absorbance taken after 5 minutes and after 24 hours.

Table I. Tolerance of Diverse Ions

Ion	Added as	Limiting Concn., P.P.M.
Ni(II)	Chloride	10
Cu(II)	Sulfate	3
Fe(II)	Sulfate	2
Fe(III)	Sulfate	20
Co(II)	Sulfate	2
Rh(IV)	Sulfate	10
Ir(III)	Chloride	30
Pt(IV)	Chloride	40
Ru(III)	Chloride	1
Ag(I)[a]	Nitrate	75

[a] For testing tolerance of silver, a solution of palladium nitrate was used.

Table II. Properties of 3-Hydroxy-1-*p*-sulfonatophenyl-3-phenyltriazine, *p*-Nitrosodiphenylamine, *p*-Nitrosodiethyl, and Dimethylaniline

Properties	*p*-Nitroso-diphenylamine	*p*-Nitroso-diethyl or Methylaniline	3-Hydroxy-1-*p*-sulfonatophenyl-3-phenyltriazine
Solubility	Insol. in water, moderately sol. in alcohol	Slightly sol. in water, sol. in alcohol	Sol. in water
Time of maximum color intensity	30 min.	5 min.	5 min.
Stability of color	1 to 2 hours	4 hours	24 hours or more
pH	2.0 to 2.1	4.0 to 5.0	1.7 to 4.4
Use of buffer	Absolutely essential	Absolutely essential	Not necessary
Temp. effect	Must keep within 5° C.	Slight difference at 20° C. variation	No difference up to 60° C. variation
Concn. of sodium chloride permissible	0.03*M*	0.05*M*	0.025*M*
Tolerance of diverse ions, p.p.m.			
Ni(II)	20	15	10
Cu(II)	50	3	3
Fe(II)		Data not given	2
Fe(III)	30	2	20
Co(II)	10	10	2
Rh(IV)	1	1	10
Ir(III)	1	1	30
Pt(IV)	20	20	40
Ru(III)	...	Data not given	1
Ag(I)	200	200	75
Au(III)	1	0.5	0

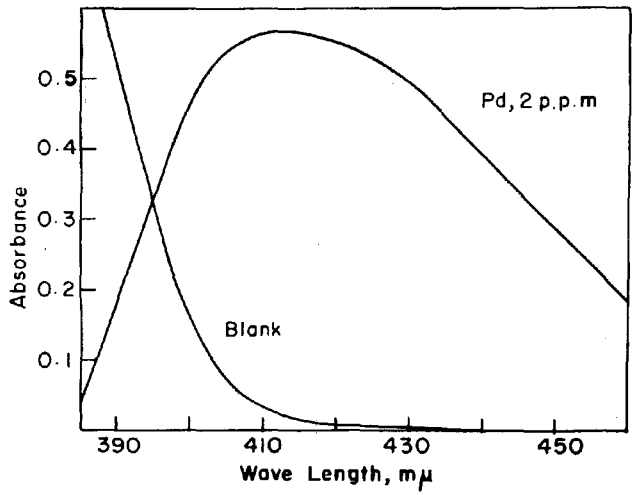


Figure 2. Absorbance curves

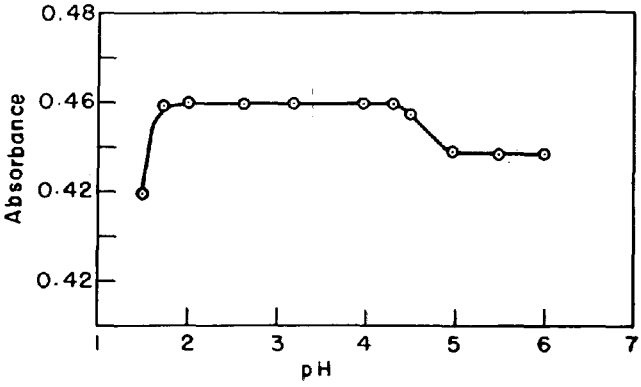


Figure 3. Effect of pH

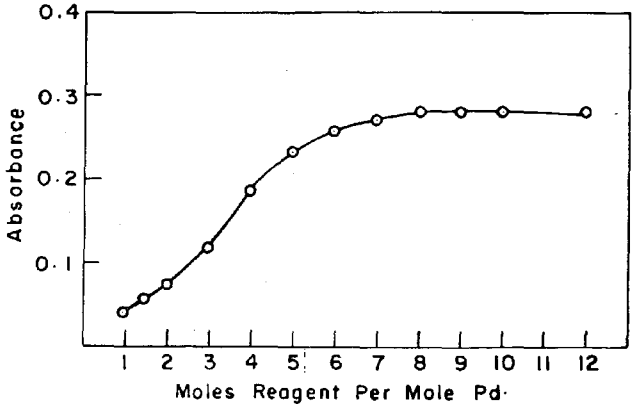


Figure 4. Effect of reagent concentration

Beer's Law. Aliquot portions of standard palladium solution were pipetted into 100-ml. flasks, so that the palladium concentration finally corresponds to 0.2 to 6 p.p.m., and 0.2 ml. of 1*N* nitric acid and 10 ml. of the reagent solution were added. The volume was made up to the mark and the absorbance was measured after about 5 minutes both at 420 mμ using reagent as blank and at 430 mμ using water as blank. Beer's law is obeyed in both cases. A 420 mμ the concentration range is from 0.2 to 5 p.p.m. and at 430 mμ it is from 0.4 to 6 p.p.m. of palladium. Larger amounts of palladium can be estimated at higher wave lengths—i.e., 435 to 450 mμ.

Effect of Temperature on Reaction. Two 10-ml. samples of standard palladium solution containing 10 p.p.m. of palladium were taken in two beakers. To one, 0.2 ml. of 1*N* nitric acid and to the other 0.1 ml. of 1*N* hydrochloric acid was added, followed by 5 ml. of the reagent solution in each case. Then 50 ml. of water was added to each beaker and the contents were kept for heating on a water bath. Initial temperature was 25° C. The beakers with the contents were heated to 85° C. and maintained at the temperature for about 10 minutes. After cooling, the contents were separately transferred into 100-ml. flasks and volume was made up to the mark. The blank reagent solution was treated simultaneously in a similar way. Absorbance was measured at 420 mμ. There was no difference in the absorbance at 25° and 85° C., and the use of hydrochloric acid or nitric acid for the adjustment of pH made no difference.

It can, therefore, be concluded that there is no change in color intensity over a temperature range of 25° to 85° C.

Sensitivity of Reaction. Fifty-milliliter solutions containing 0.5 ml. of the reagent solution, 0.1*N* hydrochloric acid, and 0.02 to 0.10 p.p.m. of palladium solution were prepared in Nessler's cylinders. The 0.05-p.p.m. palladium solution was easily distinguishable from a blank. Thus the sensitivity of the color reaction could be taken to be 1 part of palladium in 20,000,000 parts of solution.

Spot plate sensitivity was determined by taking 0.05 ml. of standard palladium solution to a depression of a white porcelain spot plate, and adding 0.05 ml. of 0.1*N* hydrochloric acid and 0.05 ml. of 0.05% reagent solution; 0.05 γ of palladium in 0.15 ml. of solution could be distinctly detected.

Effect of Adding Neutral Salts (sodium chloride used). Ten milliliters of standard palladium solution containing 10 p.p.m. was pipetted into a 100-ml. flask and 1*N* hydrochloric or 1*N* nitric acid was added for pH ad-

justment. Different quantities of 5% sodium chloride solution, followed by 5 ml. of reagent solution, were added. The permissible concentration was found to be 0.025*M*. There was no appreciable difference between hydrochloric and nitric acid.

Tolerance of Diverse Ions. Ten milliliters of standard palladium solution containing 10 p.p.m. of palladium was pipetted into a 100-ml. flask and the diverse ion solution was added. The pH was adjusted by adding 0.2 ml. of 1*N* nitric acid, 5 ml. of reagent solution was added, and the volume was made to 100 ml. The final solution contained 1 p.p.m. of palladium. The absorbance was measured after 5 to 10 minutes at 420 mμ, using reagent as blank, and also sometimes at 430 mμ using water as blank.

An ion was considered to interfere if the resulting solution differed by 0.005 in absorbance from that containing only palladium without any diverse ion. Table I summarizes the tolerance of the diverse ions as parts per million of ions.

Iron(III) reacts with the reagent at low pH; it shows a peak at about 400 mμ and interferes with the palladium estimation. This interference, however, was completely eliminated by masking it with 1 ml. of 5% sodium fluoride.

The color of iridium chloride was bleached by addition of the reagent, but this created no interference.

Gold(III) is reduced by the reagent to the metallic state and hence interferes. Lead salts also created some interference in the estimation of palladium, which is difficult to explain.

In examining the tolerance of various diverse ions, only those ions which normally occur with palladium or are usually present in the important alloys of palladium have been employed. Considering the specificity of the reagent at low pH, it is certain that palladium could be estimated in the presence of many other elements without interference, but the actual experiments were not carried out.

DISCUSSION

For a fuller understanding of the qualities of 3-hydroxy-1-*p*-sulfonatophenyl-3-phenyltriazine as a colorimetric reagent a comparison with other reagents (*1–6, 8, 9*) is desirable. The majority of these do not possess many of the desirable properties required by a colorimetric reagent. In certain cases the pH adjustment is very rigid and the range allowed is almost unworkable; in others, the colored complex has to be extracted with solvent before taking absorbance. Very often, the colored complexes are stable over only a short period and very susceptible to temperature variation. Above all, in many cases, neither the reagents nor the complexes formed by them are soluble in water. For these reasons, comparison is confined to *p*-nitrosodiphenylamine and *p*-nitrosodialkylaniline, which are currently favored for this purpose. These reagents have been developed by Yoe and Overholser (*8, 9*). The comparative data are given in Table II.

In many respects 3-hydroxy-1-*p*-sulfonatophenyl-3-phenyltriazine is very superior to other known reagents. The exceptional stability of its palladium complex is mainly due to the fact that it is an inner complex and not a coordination complex, as are most of the other reagents discussed.

Difficulties of partial salting out of the complex in the presence of an excess of neutral salts could possibly be counteracted by introducing a second sulfonic group in the reagent. Such a compound, as well as its complexes will be much more soluble in water and may prove more useful as a colorimetric reagent.

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Use of Ion Exchange Resins for Determination of Uranium in Ores and Solutions

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▶ The separation of uranium from the ions interfering with its analysis is accomplished by the adsorption of the uranium(VI) sulfate complex on a quaternary ammonium anion exchange resin. Interference of such ions as iron(III) and vanadium(V) is avoided by their preferential reduction with sulfurous acid so that they, as well as other cations, are not retained by the resin. Uranium is eluted for analysis by dilute perchloric acid. The method is applicable to both solutions and ores.

THE USE of ion exchange resins of the quaternary ammonium type to separate uranium(VI) from the ions that interfere in its determination was first described at the International Conference on the Peaceful Uses of Atomic Energy (*4*). As a result of recent declassification of some U. S. Atomic Energy Commission reports (*2, 3*) it is now possible to disclose the complete experimental procedure for this separation.

In the interim since the Geneva Conference a method has been published for the determination of uranium in solution, similarly based on the adsorption of uranium on an ion exchange resin (*1*). The present procedure is somewhat simpler in the reagents required and has been adapted to the analysis of ores.

ORE SOLUTION

Two methods for the opening of uranium-bearing ores were investigated in

conjunction with the ion exchange separation. The first is the standard digestion with hydrofluoric and nitric acids, with subsequent evaporation to dryness followed by a sodium carbonate fusion (*5*). The carbonate melt is dissolved in 5% sulfuric acid to form a solution for the separation. A second method for routine analysis, designed to eliminate the need for hood facilities and platinum vessels, involves an oxidative leach with an acidic manganese(IV) oxide system. This procedure is given in detail below. Other workers (*7*), using the authors' separation procedure, have recommended solution of the ore by treatment with 12*M* hydrochloric acid plus 16*M* nitric acid followed by fuming with sulfuric acid to produce a suitable uranium solution for the column influent.

Procedure. Weigh out samples of ore estimated to contain an amount of uranium oxide less than 100 mg. but sufficient to be detected by the chosen method of analysis. Add 20 ml. of 20% by volume sulfuric acid and 2 grams of manganese(IV) oxide. Heat the mixture to boiling. Allow to cool to room temperature. Dilute with approximately 50 ml. of water. Adjust to a pH between 1.0 and 1.5 by the dropwise addition of 20% sodium hydroxide. Filter through fine-pore filter paper using two 10-ml. portions of water to wash the residue on the paper.

ION EXCHANGE SEPARATION

The separation of uranium from interfering ions depends on the fact that the sulfate complex of uranium(VI) is quantitatively retained by quaternary ammonium anion exchange resins while all cationic constituents of the solution pass through the column. Interference of certain metals such as iron(III) and vanadium(V), which are also retained by the resin, is eliminated by their preferential reduction with sulfurous acid prior to the passage of the solution through the ion exchange bed. The uranium is eluted from the resin with dilute perchloric acid to form a solution suitable for either colorimetric or volumetric determination.

Apparatus. Tubes 0.5 inch in diameter with high-porosity sintered glass filter disks fused to the lower end are used to contain the resin. The rate of flow of solutions through the tube is regulated by a screw clamp on rubber tubing below the filter. Small separatory funnels are attached to the top of the column to feed the sample and reagents.

Procedure. Convert a portion of quaternary ammonium anion exchange resin (Amberlite XE-117, Type 2) of mesh size 40 to 60 (U. S. screens) to the sulfate form by treating a column of it with 10% sulfuric acid, using 3 volumes per volume of resin. Rinse the acid-treated resin with deionized water until the effluent is neutral to methyl red. Drain the resin so prepared free of excess water and store in a bottle. A 5-ml. portion of this resin is used for a single analysis. The resin is loaded into the filter tube and the bed so formed is backwashed with enough water to free it of air. After the resin has settled the excess water is drained off to within 1 cm. of the top of the bed prior to the passage of the sample through the bed.

Add 5 drops of 0.1% methylene blue to the partially neutralized (pH 1.0 to 1.5) solution from the dissolved sodium carbonate melt or from the filtered manganese(IV) oxide leach. Add 6% sulfurous acid dropwise until the methylene blue is decolorized and then add a 5-ml. excess. Pass the reduced sample through the resin bed at a rate not exceeding 2 ml. per minute. Wash the sample container with two 10-ml. portions of water, passing the washing through the resin bed at the same flow rate. Elute the uranium with 50 ml. of 1*M* perchloric acid. Determine the uranium content of the perchloric acid fraction colorimetrically by the standard sodium hydroxide–hydrogen peroxide method (*5*) or volumetrically (*6*). For colorimetric analysis standard uranium solutions containing perchloric acid should be used in establishing the curve.

INTERFERING IONS

Materials which would interfere in the ion exchange separation of uranium are of two types:

Ions which will compete with the uranium complex for resin sites and, hence, which may cause loss of the uranium into the sample effluent;

Anions which are retained by the resin and may subsequently be eluted by the perchloric acid to interfere in the analysis.

Table I. Effect of Interfering Anions on Uranium Retention

Added Anion	Molarity Added Ion	Mg. Uranium/Ml. Influent	Mg. Uranium/Ml. Effluent
Cl^-	0.5	10.7	2.3
Cl^-	0.1	10.7	<0.002
$H_2PO_4^-$	0.15	10.7	[a]
$H_2PO_4^-$	0.15	2.1	<0.002
NO_3^-	0.5	10.7	5.5
NO_3^-	0.1	10.7	1.1
NO_3^-	0.01	10.0	<0.002
HSO_4^-	0.3	5.6	<0.002
HSO_4^-	0.15	5.6	<0.002

[a] Precipitation in influent.

Potentially all anions give interference of the first type. However, when uranium(VI) solutions of known concentrations containing various concentrations of the common anions were run through the above procedure using 10-ml. resin beds and 50-ml. sample volumes, only the presence of chloride (>0.1*M*) and nitrate (>0.01*M*) caused leakage of the uranium into the sample effluent (Table I). Therefore, if the corresponding acids are used in dissolving the ore it is necessary to remove the major portion of the chloride and nitrate by fuming with sulfuric acid prior to the separation, but it is not necessary that precautions be taken to remove the last traces of these ions.

Anions that compete with the uranium complex for resin sites and, when subsequently eluted, also interfere with the uranium analysis are a more serious problem. Potentially the most likely possibilities in this respect are iron(III) and vanadium(V). Iron, when present in the trivalent state, results in fallacious high uranium results. When reduced to the divalent ion by an excess of sulfurous acid, however, it no longer interferes with the uranium analysis. Vanadium(V) interferes primarily by causing leakage of uranium into the sample effluent, as its presence results in little color enhancement under the alkaline conditions of the colorimetric analysis. Vanadium interference may be largely eliminated by diluting the influent. As this increases the time of analysis, a more practical solution is the use of an excess of sulfurous acid, which is required when iron is present. By reducing the vanadium to the cationic form it is possible to separate it completely from the uranium, so that vanadium may be determined in the sample effluent.

Possible interference by copper, cobalt, and molybdenum(VI) have also been specifically checked by experiments similar to those described above. Neither copper nor cobalt interfered in the procedure. Molybdenum(VI), in the absence of reducing agent or in the presence of an excess of sulfurous acid, did not interfere. When it was only partially reduced, however, uranium determinations were low.

RESULTS

This method was developed for the analysis of uranium in the leachates and has been used routinely in this laboratory for that purpose for over 3 years. As a result it was originally checked with solutions of known composition. However, a limited number of ores have also been analyzed to check its applicability. Typical results are compared with the results obtained at the Atomic Energy Commission's New Brunswick Laboratory in Table II. Agreement of the results of the ore opening with hydrofluoric-nitric acid leach followed by sodium carbonate fusion with those of the standard method are naturally better than those of the rapid oxidative leach, particularly where the amount of uranium in the ore is

Table II. Analyses of Ores by Different Methods

Ore Type	Method A[a] Sample wt.	Mg. U_3O_8[b] per sample	% U_3O_8	Method B[a], % U_3O_8	Method C[a], % U_3O_8
Pitchblende	3.06	12	0.34	0.39	0.41
Carnotite	4.02	12	0.32	0.31	0.34
Fe,Al silicate	10.06	5.0	0.035	0.020	0.05
Carnotite	2.51	17.5	0.73	0.65	0.68
Pitchblende	2.00	67	3.35	3.31	3.36
Phosphate	4.85	1.5	0.067	0.025	0.029
	7.97	4.0	0.052		
Carnotite	11.06	21	0.20	0.19	0.18
	6.37	12	0.18		
Carnotite	10.23	11	0.14	0.11	0.11
	17.66	19	0.11		

[a] A, manganese dioxide–sulfuric acid.
B, hydrofluoric-nitric acid, followed by sodium carbonate fusion; ion exchange separation.
C, standard opening and separation of New Brunswick Laboratory.
[b] Estimate from New Brunswick analysis.

small. This laboratory has not performed sufficient ore analyses to calculate the over-all accuracy of the method starting with the ore. On solutions an accuracy of ±2% of the uranium content is expected in routine analysis.

DISCUSSION

Although a definite procedure has been specified, the method outlined may be adapted to materials of a wide range of uranium contents by varying the sample size and the size of the resin bed. Because the resin serves to concentrate as well as to separate the uranium, materials of low uranium content may be analyzed by increasing the sample size or by decreasing the volume of resin and of the perchloric acid used in the elution. Similarly, larger amounts of uranium may be taken for analysis if the volume of resin and of eluting agent are increased, keeping them in the same ratio as those recommended in the above procedure. The ion exchange separation has been used for routine control, not only in this laboratory but also in the uranium purification plants in South Africa since 1954. Workers at the University of Nevada have also recently reported (7) its use in the assay of over 3000 ore samples. An experienced analyst is able to perform 20 separations per day, starting with the solution of the ore.

ACKNOWLEDGMENT

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Use of Thymol–Sulfuric Acid Reaction for Determination of Carbohydrates in Biological Material

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▷ Different absorption curves were given by nearly all sugars when they were made to react with thymol in the presence of strong sulfuric acid. Glycosidic linkages did not affect the reaction either qualitatively or quantitatively. The presence of protein had a slight effect on absorption curves, but this appeared to be due to the reaction of sulfuric acid with protein and did not involve thymol. The reaction, when used to estimate the carbohydrate bound to protein in the sera of a number of patients, correlates closely with an accepted method.

THE REACTION of thymol with carbohydrates in the presence of strong sulfuric acid, as described by Udransky (4), has been modified for use as a method for the estimation of blood sugar by Alonzo and Bruna (1) and by Schmor (2). As more specific methods are needed for the determination of carbohydrate in the presence of protein and other biological material the following work was undertaken to investigate this reaction.

REAGENTS AND APPARATUS

Thymol reagent, USP, 10% in absolute ethyl alcohol.

Sulfuric acid solution, 77% by volume. Add 770 ml. of concentrated sulfuric acid (Du Pont reagent grade, specific gravity, 1.84 at 15° C.), to 230 ml. of distilled water.

An American Optical Model 1A rapid scanning spectrophotometer and Beckman DU spectrophotometer were used for the work involving absorption curves. A Coleman Model 14 spectrophotometer was used for quantitative colorimetric work.

PROCEDURE

To 1 cc. of sugar solution in a 15-ml. glass-stoppered test tube, 7 cc. of 77% sulfuric acid was added at room tem-

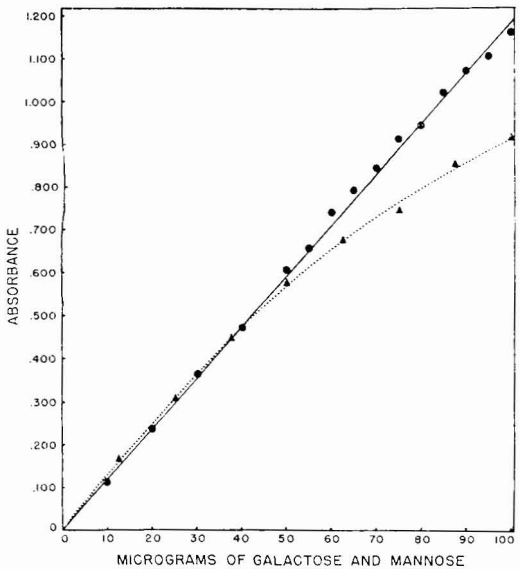

Figure 1. Standard sugar curves for determination of serum glycoprotein

Equimolar mixture of mannose and galactose used to prepare standard solutions
———Beckman Model DU
.....Coleman 14

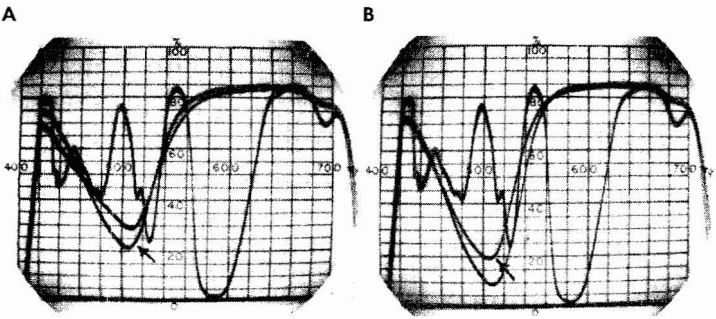

Figure 2. Absorption curves after reaction with thymol–sulfuric acid reagent

Glucose curves indicated by arrows
A. Galactose and glucose
B. Glucose and fructose

perature. The tube was left at room temperature for 10 minutes without mixing, and was then placed in an ice bath for 15 minutes. From pipets 0.1 ml. of the thymol solution and 0.9 ml. of water were added; the contents of the tubes were mixed by inversion and the tubes placed in a boiling water bath for 20 minutes. The tubes were removed from the water bath and immediately placed in an ice bath, where they were left for 5 minutes. They were then kept for 25 minutes at room temperature before readings were taken in the spectrophotometer.

Serum protein samples were prepared by diluting human serum with five times the volume of 0.9% saline. Aliquots of 0.2 ml. were pipetted dropwise into glass-stoppered 15-ml. test tubes containing 10 ml. of absolute ethyl alcohol. The stoppers were inserted and the contents of each tube mixed by inversion of the tube. The stoppers and sides of the tubes were washed down with 5 ml. of ethyl alcohol. The precipitate was centrifuged, washed by suspension in 10 ml. of absolute ethyl alcohol, and centrifuged again. The alcohol was drained off and the precipitate suspended in 1 ml. of distilled water. The mixture was subjected to the procedure described above for sugar solutions.

DISCUSSION

Heating Time. Solutions of an equimolar mixture of galactose and mannose and suspensions of serum samples prepared as described above were subjected to the color reaction with thymol and sulfuric acid, in which the reaction time was varied between 10 and 30 minutes. No qualitative differences in the curves were noted at different times.

As might be predicted, the maximum color development occurred more slowly in the serum protein samples than in the sugar solutions; however, optimum color development occurred in 20 minutes for both. The absorptions of the reaction complex from either pure sugar solutions or from serum protein were not decreased when heated for 25 minutes.

Effect of Sugar Concentration. The reaction follows the Lambert-Beer law reasonably well in the 10- to 100-γ range when the Beckman DU spectrophotometer is used, but not when the Coleman 14 is used (Figure 1). This necessitates the preparation of a concentration curve when the Coleman 14 is used. In work involving the quantitative detection of unknowns such a curve was used and two standards at different concentrations were run with each set of unknowns.

Absorption Curves of Different Sugars. Curves of several monosaccharides are shown in Figure 2. These curves, made with the AO scanning spectrophotometer, are superimposed photographically for easier comparison. A didymium calibration curve appears in both photographs.

The wave length of maximum absorbance and relative absorption of a number of sugars or sugar derivatives are given in Table I.

Curves of aldohexoses, ketohexoses, pentoses and methyl pentoses all differ from each other. Absorption curves of the aldohexoses, galactose, mannose, and glucose differ slightly from each other, as do those of the ketohexoses, fructose, and sorbose and those of the methyl pentoses, fucose, and rhamnose. The curves of the pentoses, ribose, and arabinose, however, are nearly identical qualitatively. These data indicate that pentoses are degraded to a common derivative in the reaction. However, the curves of the pentoses are not identical with that obtained with furfural. Apparently the reaction with pentoses is more complicated than a simple degradation to furfural followed by coupling with thymol. Glucuronic lactone gives a curve similar to that of furfural.

Table I. Wave Length of Maximum Absorbance and Relative Absorptions of Sugar-Thymol–Sulfuric Acid Reaction Mixtures

Determined on Beckman Model DU spectrophotometer

Sugar	Max. Absorbance, Mμ[a]	Relative Absorption
Glucose	509	1.00
Mannose	513	1.14
Galactose	511–12	0.76
Fructose	512–13	1.65
Sorbose	512–13	1.18
Arabinose	493–4	1.15
Ribose	494–5	1.18
Fucose	503–4	0.50
Rhamnose	504	1.46
Galaheptose	494–6	0.39
Glucoheptose	495–9	0.44
Glucuronic acid lactone	491	0.85
Furfural	491	4.00
Glycogen	509–10	0.98
Inulin	513	1.63
Turanose	513	1.38
Raffinose	512	1.13

[a] All samples read against reagent blank.

Effect of Glycoside Linkage. The effect of the glycoside linkage was studied by comparing the glucose absorption curve with those of glucose-1-phosphate, salicin (suligenin β-D-glucoside), trehalose (α-D-glucosido-α-D-glucoside), cellobiose [4-(β-D-glucosido)-D-glucose], and glycogen. Inulin curves were compared with fructose; lactose was compared with an equimolar mixture of galactose and glucose; and turanose was compared with a mixture of glucose and fructose. In all cases, the absorption curves derived from the complexes are those predictable from the monosaccharide components (Figure 3).

Effect of Protein. Colorimetric reactions for sugars are usually influenced by the presence of protein. An absorption curve of the thymol reaction with a serum protein sample precipitated as described above is shown in Figure 4. Human serum protein is known to contain galactose, mannose, glucosamine, and fucose. Consequently, the serum curve was compared with a mixture of 25 γ of mannose, 25 γ of galactose, 5 γ of fucose, and 30 γ of glucosamine. As compared to the simple sugar curve, the protein curve absorbs more in the 420- to 440-mμ range of the curve. Absorption maxima are the same, however. Hydrolysis of serum protein (with 4*N* hydrochloric acid for 4.5 hours) before carrying out the reaction resulted in only a slight change of the curve. Addition of crystalline pepsin (which contains very little carbohydrate) to the sugar solution results in absorption curves similar to serum protein curves; however, crystalline bovine albumin had less effect. As the absorption curve obtained when sulfuric acid reacts with protein (in the absence of thymol) has some absorption at 420 mμ (Figure 4), most of the effect is apparently due to the reaction of sulfuric acid with protein, and does not involve the reaction with thymol.

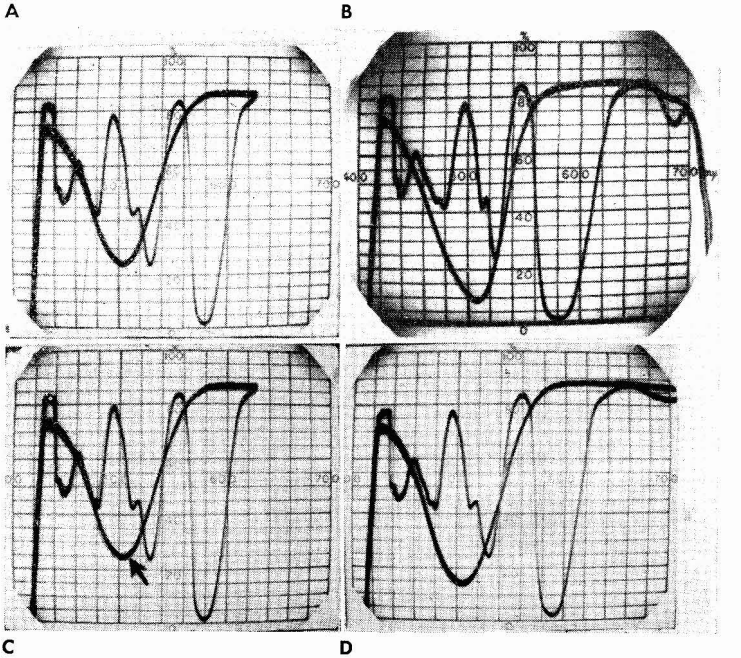

Figure 3. Effect of glycosidic linkage on sugar after reaction with thymol–sulfuric acid

Absorption curves of complexes superimposed on monosaccharide component; all curves made at concentration of 50 γ of hexose or equivalent
A. Glucose-1-phosphate, glucose
B. Inulin, fructose
C. Glycogen, glucose
D. Turanose, equimolar mixture of glucose and fructose

Determination of Serum Glycoprotein. The thymol method was used as described above for the estimation of serum glycoprotein on a series of 22 human serum samples. An analysis by the tryptophan method of Shetlar, Foster, and Everett (*3*) was made on the same samples. The following results indicate good agreement between the methods.

Method	No. of Sera	Range, Mg. %
Tryptophan	22	122 to 248
Thymol	22	125 to 257

Method	Max. Diff., Mg. %	Correlation Coefficient
Tryptophan Thymol	22	0.971

By statistical methods, the difference between averages by the two methods was not significant, and the correlation coefficient between the two methods was highly significant.

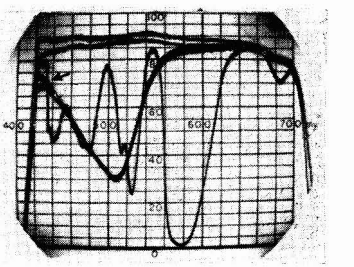

Figure 4. Effect of protein on sugar-thymol–sulfuric acid reaction

All curves made with AO scanning spectrophotometer
Curve indicated by arrow: Reaction carried out on mixture of 25 γ of galactose, 25 γ of mannose, 5 γ of fucose, and 30 γ of glucosamine
Upper curve: Blank of same sugars without thymol
Curve next to top: Serum protein blank without thymol
Last curve: Reaction carried out on serum protein

The use of thymol–sulfuric acid appears to have an advantage over tryptophan for quantitative work with biological samples, in that protein has less influence on the absorption curve.

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Estimating Total Absolute Activity of Small Radioactive Precipitates on Filter Paper

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▶ **The total absolute activity of a small amount of a radioactive precipitate on filter paper, containing a simple, low-energy beta emitter, is estimated from the counts obtained from both sides of the paper. By means of a chart based on exponential precipitate distribution, a relationship between the two counting rates and the total activity (counts per minute) is obtained. This relationship is dependent upon the product of the absorption coefficient and the thickness of the paper (including precipitate). For purposes of counting, a close geometry is stipulated—e.g., a windowless flow counter. Total activity is converted to total absolute activity (d.p.m.) by multiplying by a geometrical factor. This factor is the ratio of 4π to the solid angle subtended by the sensitive volume of the counter based on average precipitate position, including a correction factor for radiations absorbed by the walls of the counter. For radiocarbon precipitates, an accuracy within 10% of the absolute value is expected.**

WHEN a small amount of a precipitate containing a simple, low-energy beta emitter is filtered on paper, the material becomes embedded within the paper and the activity appearing at the surface is reduced by absorption of radiations by the paper and precipitate. This article shows that the total activity (activity which would have been observed in the absence of any absorption losses) can be obtained from the ratios of the observed activities of the top and the bottom of the paper. A chart showing this relationship (Figure 1) is based on exponential precipitate distribution and close-geometry conditions of counting. As shown by Suttle and Libby (*5*), activities obtained under close-geometry conditions can conveniently be converted to absolute activities.

CLOSE-GEOMETRY AND ABSOLUTE ACTIVITY

For close-geometry conditions, the absolute specific radioactivity was shown to be related to the observed activity of either an infinite or a finite thickness of precipitate (*5*). This relationship is based on the values for the absorption coefficient, the surface area of the sample, and the factor G. G is the ratio of 4π to the solid angle subtended by the sensitive volume of the counter from the point source on the precipitate being considered. These same arguments apply also to close-geometry placement of filter paper samples in end-window positions, as well as to the cylindrical placement used by Suttle and Libby in their screen-wall counter. In this case the filter paper with precipitate represents a finite sample thickness. In end-window placement, the geometrical factor, G, can be considered from a standpoint of average precipitate position, with a correction for the radiation absorbed by the walls of the counter. To convert total activity counts per minute as obtained from Figure 1, to total absolute activity (disintegrations per minute), we need only to multiply the total activity obtained by the value of G. In a 2π counter, G would be expected to be only slightly greater than 2.

EXPONENTIAL DISTRIBUTION

In a filtered precipitate the particles tend to be concentrated on top of the paper, the concentration (expressed as activity per unit thickness of paper) decreasing with increasing depth. Such a distribution of particles can readily be envisaged as exponential. This mathematical relationship satisfies a large number of precipitate distributions, while a given proportionality constant will define a particular distribution.

In an exponential distribution of precipitate:

$$-\frac{dc}{dl} = kc$$

and

$$c = c_0 e^{-kl} \qquad (1)$$

where c = activity per unit thickness at depth l which would be observed if there were no absorption of beta rays
c_0 = activity per unit thickness at zero depth
k = proportionality constant, sq. cm. per mg.
l = depth within filter paper, mg. per sq. cm.

If we consider a sample of filtered precipitate with an exponential distribution, the total activity can be considered to be the sum of the activities of an infinite number of infinitesimally thin layers.

Using Equation 1, the total activity, z, is as follows:

$$z = c_0 \int_0^g e^{-kl}\, dl = \frac{c_0}{k}(1 - e^{-kg}) \qquad (2)$$

where g = total thickness of paper with precipitate, mg per sq. cm.

Numerous authors (*1–5*) have shown the applicability of self-absorption equations relating measured activity to total activity in solid radioactive samples. These relationships are based on exponential beta-ray absorption for homogeneous samples where the activity from the top is measured.

For exponential precipitate distribution, and exponential absorption of beta rays, the measured activity, x, from the top side of filter paper becomes:

$$x = c_0 \int_0^g e^{-kl} e^{-\mu l}\, dl =$$

$$c_0 \int_0^g e^{-(\mu+k)l}\, dl =$$

$$\frac{c_0}{\mu + k}(1 - e^{-(\mu+k)g}) \quad (3)$$

where μ = absorption coefficient for beta rays in filter paper with precipitate, sq. cm. per mg.

As the distance measured from the bottom is $g - l$, the measured activity, y, on the bottom side is:

$$y = c_0 \int_0^g e^{-kl-\mu(g-l)}\, dl =$$

$$\frac{c_0}{\mu - k}(e^{-kg} - e^{-\mu g}) \quad (4)$$

Limiting Conditions. In the preceding equations k can vary from zero to infinity. By substituting these two values of k, we obtain equations for activity ratios at the limiting conditions.

Equation 2 can be expanded:

$$z = \frac{c_0}{k}\left[1 - \left(1 - kg + \frac{(kg)^2}{2!} - \frac{(kg)^3}{3!} + \ldots\right)\right] = c_0 g\left(1 - \frac{kg}{2} + \frac{(kg)^2}{6} - \ldots\right)$$

As $k \to 0$, we approach the limiting equations:

$$z = c_0 g$$

$$x = \frac{c_0}{\mu}(1 - e^{-\mu g})$$

$$y = \frac{c_0}{\mu}(1 - e^{-\mu g})$$

and

$$x = y$$

Combining equations for z and y:

$$\frac{z}{y} = \frac{\mu g}{1 - e^{-\mu g}} \quad (5)$$

Substituting $x = y$, and rearranging, result in an equation for self-absorption similar to the one given by Cook and Duncan (*2*):

$$\frac{z}{g} = \frac{\mu x}{1 - e^{-\mu g}}$$

Thus, when $k \to 0$, the ratio $x/y = 1$, the precipitate is distributed uniformly throughout the paper, and the well-known equations for self-absorption become applicable.

As $k \to \infty$, Equations 2, 3, and 4 yield the following limiting relationships:

$$\frac{x}{y} = e^{\mu g} \quad (6)$$

$$\frac{z}{y} = e^{\mu g} \quad (7)$$

and

$$z = x$$

Thus, when $k \to \infty$, the entire precipitate is in an infinitesimally thin layer on top of the paper, $x/y = z/y = e^{\mu g}$, and the measured activity on top is the total activity.

Real Conditions. For real precipitates k is neither zero nor infinite; but from Equations 3 and 4 we obtain an equation for the activity ratio, x/y, as a function of k.

$$\frac{x}{y} = \frac{(\mu - k)(1 - e^{-(\mu+k)g})}{(\mu + k)(e^{-kg} - e^{-\mu g})} \quad (8)$$

Similarly, by combining Equations 2, 3, and 4 we obtain an equation for z/y:

$$\frac{z}{y} = \frac{2(1 - e^{-kg})\left(\frac{x}{y}\right)}{1 - e^{-(\mu+k)g} + (e^{-\mu g} - e^{-kg})\left(\frac{x}{y}\right)} \quad (9)$$

By substituting arbitrary values of k, other than zero or infinity, in Equations 8 and 9 we can obtain values of x/y and z/y and construct a table showing these relationships for a given μg value. Table I shows some k values and the corresponding x/y and z/y ratios for $\mu g = 2.24$ ($\mu = 0.28$ for radiocarbon and $g = 8$ for a typical filter paper).

Similar tables can be prepared for other μg values; and the activity ratios can be used to plot z/y as a function of x/y. Thus, a value of z/y can be obtained for any measured ratio of x/y.

APPROXIMATE RELATIONSHIP

Although an assumed exponential distribution appears to be a reasonable type of precipitate distribution for obtaining an estimate of the total activity, setting up a table for each of the various thicknesses and absorption coefficients encountered might become somewhat laborious. A simpler method of calculation would be more attractive.

Table I. Tabulation for Equation 9

($\mu g = 2.24$)

k	x/y	z/y
0	1	2.51
0.2	1.71	3.35
0.3	2.17	3.85
0.5	3.19	4.90
0.7	4.17	5.82
1.0	5.30	6.78
1.6	6.60	7.75
2.0	7.09	8.08
3.0	7.79	8.52
5.0	8.40	8.87
10.0	8.88	9.13
∞	9.39	9.39

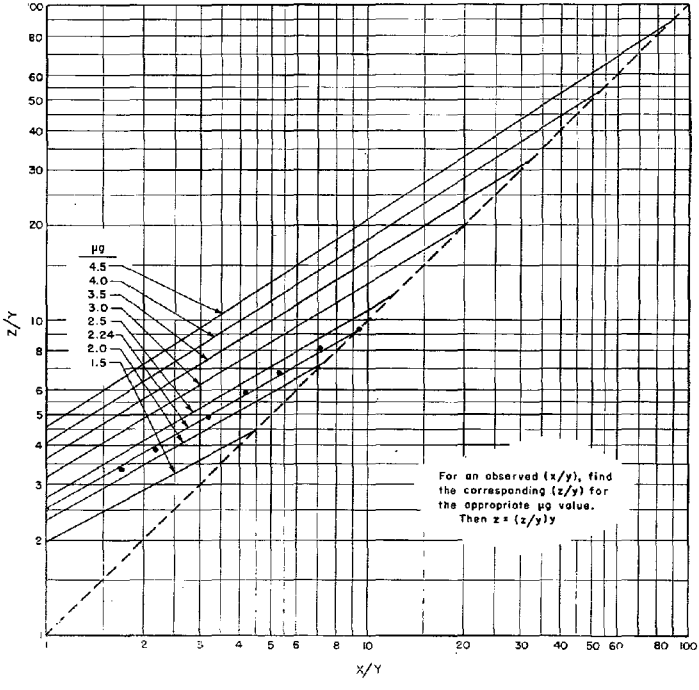

Figure 1. Chart for obtaining total activity, z, from observed activities, x and y

Plotted points are from Table I

Assuming a generalized exponential distribution of precipitate, we can construct an approximating curve through two particular points. The obvious points of choice are the limiting ones—i.e., those corresponding to $k = 0$ and $k = \infty$. Plotting z/y as ordinate and x/y as abscissa, we determine from Equations 5, 6, and 7 that the two points are $(1, \mu g/1-e^{-\mu g})$ and $(e^{\mu g}, e^{\mu g})$; but in order to connect these points by a curve it is necessary to have an equation which expresses z/y as a function of x/y.

The characteristics of filter paper are such that a usable precipitate will approximate a distribution that behaves as if all of the precipitate were concentrated in a thin layer. In an equation based on a hypothetical thin-layer precipitate in which the precipitate position is such that the measured x/y ratio is satisfied,

$$x = ze^{-\mu}$$

$$y = ze^{-\mu(g-t)}$$

Multiplying these two equations and rearranging, we can write:

$$\frac{z}{y} = \left(\frac{e^{\mu g}x}{y}\right)^{1/2} \qquad (10)$$

This equation holds only for thin-layer concentrations of precipitates; however, when the x/y ratio of a real precipitate approaches its maximum, $e^{\mu g}$, the precipitate concentration approaches a thin-layer concentration and the equation holds for that particular x/y ratio. For other x/y ratios Equation 10 is an approximation, the degree of uncertainty in z/y increasing with decreasing x/y values.

The desired relationship between z/y and x/y, satisfying the two fixed points, is now indicated by the form of Equation 10. However, in order to satisfy both limiting conditions, $k = 0$ and $k = \infty$, x/y must have some degree of independence from the exponent.

The required conditions are satisfied by the equation:

$$\frac{z}{y} = \frac{x}{y}\left(\frac{e^{\mu g}y}{x}\right)^{b} \qquad (11)$$

A solution for b can be found by substituting the value of z/y when x/y is unity. From Equations 5 and 11,

$$\frac{z}{y} = \frac{\mu g}{1 - e - \mu g} = e^{\mu g b}$$

Then,

$$b = \frac{1}{\mu g} \ln \frac{\mu g}{1 - e - \mu g} \qquad (12)$$

Equations 10 and 11 are identical when $\mu g = 0$, as

$$\lim_{\mu g \to 0} \frac{1}{\mu g} \ln \frac{\mu g}{1 - e - \mu g} = \frac{1}{2}$$

However, as $\mu g \neq 0$, b is slightly less than $1/2$, and Equation 11 gives z/y values somewhat lower than Equation 10, which is as expected.

Equations 11 and 12 can be combined to give an equation which may be plotted to give a straight line on logarithmic paper, through the two previously fixed points:

$$\ln\left(\frac{z}{y}\right) = \left(\frac{\mu g - \ln \frac{\mu g}{1 - e - \mu g}}{\mu g}\right) \ln\left(\frac{x}{y}\right) + \ln \frac{\mu g}{1 - e - \mu g} \qquad (13)$$

DISCUSSION

Agreement between Equation 9, exponential distribution equation, and Equation 13, logarithmic equation, can be seen from the curve in Figure 1. The points on the curve for $\mu g = 2.24$ were obtained from Equation 9. The curve itself was constructed by drawing a straight line between the points $(1, \mu g/1-e^{-\mu g})$ and $(e^{\mu g}, e^{\mu g})$. Similar curves were constructed by this method for μg values of 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, and 4.5. The dotted line in Figure 1 represents the curve when $z/y = x/y$—i.e., the z/y ratios one would get if $x = z$.

The use of these curves is illustrated by an example: The absorption coefficient for beta rays from carbon-14 in filter paper is 0.28 sq. cm. per mg. The absorption coefficient of the precipitate alone is 0.29 sq. cm. per mg. Consequently, the absorption coefficient for a small amount of precipitate in filter paper is assigned the value of 0.28, the same as for the paper alone. The thickness of the paper containing the precipitate is 8 mg. per sq. cm.; this gives a value of 2.24 for μg. If the top side of the paper has a measured activity of 120 counts per minute and the bottom side an activity of 40, x/y equals 3. From the curve in Figure 1, when μg equals 2.24, an x/y ratio of 3 gives a z/y ratio of about 4.8. Since y is 40, $z = (4.8)(40) = 192$ counts per minute. This represents the total activity of the precipitate, which is greater by 60% than the activity which one would have obtained by taking the top measurement as representative of the total activity.

In the example cited, the relatively low x/y ratio (max. = 9.4) may be indicative of a slight loss of precipitate. For an exponential distribution of precipitate, the value of k, as obtained from Table I, is 0.46. The fraction of precipitate lost would then be e^{-kg}, or 0.025—i.e., about 2.5% of the precipitate has not been retained by the filter paper.

The derivations in this article assume thin-layer concentrations of precipitate in which self-absorption corrections are negligible. A real precipitate, however, may have an appreciable thickness of precipitate concentrated above the paper. If the precipitate is sufficiently large to be removable, it can be counted independently, and the paper counted on both sides for total remaining activity. If it is not of sufficient thickness to be removable, it may be of interest to know how thick it can be without introducing serious errors. Two examples are given, and the total activities based on these distributions are compared to the total activities obtained by the use of Figure 1.

If we consider distributions in which all of the precipitate is on top, the bottom of the precipitate (or top of the paper) will have the same activity as the top of the precipitate, x. Activity at the bottom of the paper will be reduced by the thickness of the filter paper. Then,

$$\frac{x}{y} = e^{\mu g_1} \qquad (14)$$

$$\frac{y}{z} = \frac{\mu g_1 e^{\mu g_2}}{1 - e^{\mu g_1}} \qquad (15)$$

where g_1 = thickness of paper, mg. per sq. cm.
g_2 = thickness of precipitate, mg. per sq. cm.
$g = g_1 + g_2$

In an exaggerated example, if the thickness of a radiocarbon-tagged precipitate is the same as the thickness of the filter paper, and all the precipitate is concentrated on top, a thickness of 8 for the precipitate and 8 for the paper gives a total thickness of 16 mg. per sq. cm. with $\mu = 0.28$, $\mu g_1 = 2.24$, $\mu g_2 = 2.24$, and $\mu g = 4.48$. From Equations 14 and 15, the x/y ratio is calculated to be 9.4, and the z/y ratio 23.6. From Figure 1, if x/y is 9.4, z/y is 20.0; and the curve gives a value differing by about 15% from the value based on the hypothetical distribution.

Smaller precipitates, of the size with which this article deals, would range up to 15 mg. For a planchet 1 inch in diameter, this is equivalent to about 3 mg. per sq. cm. Taking a typical value of 2 mg. per sq. cm. for the precipitate, g is 10 mg. per sq. cm, and μg becomes 2.80. In this example x/y is calculated to be 9.4 and z/y 12.3. By interpolation between $\mu g = 2.5$ and $\mu g = 3.0$ in Figure 1, z/y is found to be 11.7. These z/y values differ by only about 5%.

The examples cited are indicative of deviations from the curves if a precipitate of appreciable thickness is concentrated solely on top of the paper. Experimentally, this is the unusual case, as shown by the x/y ratios. In general, any variation of precipitate distribution from the assumed distribution for the curve in Figure 1 will introduce minor uncertainties in the results. Although the true distribution is not

known, the deviation of the results from the correct results, while not calculable, will generally be less than in the extreme example cited. The magnitude of the z/y uncertainty for a given precipitate distribution is also influenced by the nature of the beta rays, weaker beta rays introducing a greater degree of uncertainty. On the other hand, rather pronounced shifts in precipitate distribution from an assumed distribution cause only minor deviations in results. From this it is evident that, even though a precipitate distribution differs from that represented by the curves in Figure 1, the results obtained are not expected to differ by much from the results that would have been obtained if the true distribution had been known. A z/y value obtained for a precipitate containing radiocarbon, for example, is expected to differ by less than 10% from the true value.

CONCLUSION

The proposed method allows estimation of the total activity of precipitates on filter paper from the measured activities of both sides of the paper. The absorption coefficient, μ, for the beta rays and the thickness, g, for the paper with precipitate must be known. Close-geometry conditions of counting must be used, in which the filter paper is close to the counter.

A family of curves parametric in μg (Figure 1) serve to define z/y as a function of x/y, where z is the total activity and x and y are the measured activities from the top and bottom of the filter paper, respectively.

Total activity, z, can be converted to absolute total activity (d.p.m.) by multiplying by the geometrical factor of the counting setup. This is the ratio of 4π to the solid angle subtended by the sensitive volume of the counter, based on average precipitate position and corrected for radiations absorbed by the walls of the counter. For an efficient flow counter this factor is expected to be approximately equal to 2.

In the case of small radiocarbon-tagged precipitates, the total absolute activities obtained by means of Figure 1 are expected to differ by less than 10% from the absolute values.

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Application of Thermal Diffusion to Separation of Aliphatic Alcohols and Fatty Acids from Their Mixtures

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▶ Although separation of mixtures by thermal diffusion is often very effective —for instance, in mixtures of paraffin hydrocarbons—very little or no separation was found in alcohols and fatty acids. This failure is attributed to hydrogen bonding, which obscures structural differences and prevents their separation.

IN CONNECTION with the possible application of thermal diffusion to the analysis of complex mixtures of fatty acids and their derivatives, such as those encountered in vegetable-oil technology, binary mixtures of the lower aliphatic alcohols and fatty acids were studied.

APPARATUS AND PROCEDURE

The stainless steel thermal diffusion column used in this work was similar in design to the one described by Jones and Milberger (*4*).

The fractionating section was 6 feet in length, with an annular space of 0.0115 in. and an annular volume of 22.5 ml. The inner surface was water-cooled, while the outer one was heated electrically. In order to check the efficiency of the column, the separation of a series of binary paraffin hydrocarbon mixtures was studied (Table I). As was to be expected from *n*-paraffin hydrocarbon mixtures, separation increased with increasing differences of the molecular weights between the two components of the mixture. The per cent separation is given for a 48-hour run in each case and is close to the equilibrium value for this column.

RESULTS

In Table I the density values are quoted from API Research Project 44 (*1*). The values of final composition listed were determined from experimental plots of refractive index *vs.* volume fraction, which in most cases were nearly straight lines.

Alcohol Mixtures. No such regularity was found with binary mixtures of the lower aliphatic alcohols. The

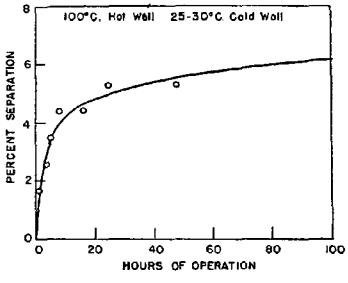

Figure 1. Effect of time on separation of 50 volume % mixture of propionic acid in butyric acid

Table I. Separation of 50 Volume % Binary Mixtures

(Duration of runs, 48 hours)

Components	Density, 25° C.	Hot Wall, ° C.	Cold Wall, ° C.	Final Composition Top 10%	Final Composition Bottom 10%	% Separation[a]
Hydrocarbons						
n-Heptane	0.6795	90	24	55.0	37.0	18.2
n-Octane	0.6985			45.0	63.0	
n-Heptane	0.6795	90	25	68.5	21.5	46.9
n-Decane	0.7262			31.5	78.5	
n-Heptane	0.6795	90	25	93.5	7.0	86.7
n-Hexadecane	0.7700			6.5	93.0	
n-Octane	0.6985	115	26	61.5	25.5	35.6
n-Decane	0.7262			38.5	74.5	
n-Octane	0.6985	115	26	93.0	6.5	86.3
n-Hexadecane	0.7700			7.0	93.5	
n-Decane	0.7262	115	30	83.5	11.0	72.7
n-Hexadecane	0.7700			16.5	89.0	
Alcohols[b]						
Methanol	0.7865		50.0	49.5		0.6
Ethyl alcohol	0.7851		50.0	50.5		
Methanol	0.7865		51.5	49.0		2.3
n-Propyl alcohol	0.7994		48.5	51.0		
Methanol	0.7865		50.0	47.5		2.7
Isopropyl alcohol	0.7808		50.0	52.5		
Methanol	0.7865		56.0	40.0		15.4
tert-Butyl alcohol	0.7807		44.0	60.0		
Ethyl alcohol	0.7851		51.0	49.0		1.7
n-Propyl alcohol	0.7994		49.0	51.0		
Ethyl alcohol	0.7851		46.0	50.0		2.6
Isopropyl alcohol	0.7808		54.0	50.0		
Ethyl alcohol	0.7851		51.0	49.0		1.6
n-Butyl alcohol	0.8057		49.0	51.0		
Ethyl alcohol	0.7851		52.0	45.0		7.0
tert-Butyl alcohol	0.7807		48.0	55.0		
n-Propyl alcohol	0.7994		50.0	50.0		0.0
Isopropyl alcohol	0.7808		50.0	50.0		
n-Propyl alcohol	0.7994		50.0	50.0		0.0
tert-Butyl alcohol	0.7807		50.0	50.0		
n-Butyl alcohol	0.8057		51.5	45.5		4.9
tert-Butyl alcohol	0.7807		48.5	54.5		
Fatty Acids[c]						
Propionic	0.9880		51.5	46.5		5.3
Butyric	0.9532		48.5	53.5		
Propionic	0.9880		51.5	47.5		3.7
Valeric	0.9345		48.5	52.5		
Propionic	0.9880		53.5	44.5		9.2
Caproic	0.9230		46.5	55.5		
Propionic	0.9880		51.0	45.5		5.5
Enanthic	0.9137		49.0	54.5		
Propionic	0.9880		52.5	47.0		5.6
Caprylic	0.9066		48.0	54.0		
Propionic	0.9880		52.0	46.0		6.1
Pelargonic	0.9017		48.0	54.0		

[a] $\frac{\Delta n_D \text{ (between top and bottom fractions)} \times 100}{\Delta n_D \text{ (between pure compounds)}} = \%$ separation.

[b] Hot wall temp. 50° C., except for *n*-butyl-*tert*-butyl, which is 75° C. Cold wall temp. 23° C.

[c] Hot wall temp. 100° C. Cold wall temp. 27–30° C.

largest per cent separation was only 15%, observed with a 50:50 mixture of methanol and *tert*-butyl alcohol. Hydrogen bonding in alcohols is very strong (*3*). As alcohols can form two hydrogen bonds per molecule, polymers will consist of molecular chains. As far as thermal diffusion is concerned, all *n*-alcohols therefore appeared to have a more or less identical structure and significant separation was obtained only between methanol and *tert*-butyl alcohol, showing the widest variation in structure. The importance of structural difference in alcohols in facilitating separation is also shown in the system benzyl alcohol—ethylene glycol, where Jones and Milberger found a 29% separation (*4*). No separation occurred in a 50:50 mixture of *n*-propyl with isopropyl alcohol and with *tert*-butyl alcohol, respectively, although it might have been expected to take place.

A ternary mixture consisting of equal percentages by volume of methyl, ethyl, and *tert*-butyl alcohols was investigated in order to find out whether ethyl alcohol might improve separation of the other two components. No such effect was observed, and separation occurred as if ethyl alcohol was not present.

Fatty Acid Mixtures. The experimental results giving the separation of butyric, valeric, caproic, enanthic, caprylic, and pelargonic acids with respect to propionic acid are also shown in Table I. All acids had been purified in a highly efficient fractionating column. Hydrogen bonding is again evident, as only small separation occurred. The irregularity up to caproic might be attributed to the differences between odd- and even-numbered acids, which is also shown by differences in melting points. Beyond caproic acid, differences between even- and odd-numbered acids appear to become negligible as far as thermal diffusion behavior is concerned.

In all pairs of fatty acids investigated, the acid having a lower density migrated to the bottom because of thermal diffusion and thus opposed the purely thermal density gradient. This situation may result in the so-called forgotten effect (*2*), an example of which is given by Jones and Milberger (*4*), where it is shown that this results in a reversal of the direction of concentration. The propionic–butyric acid mixture was selected to test whether the forgotten effect was influencing the separation of the acids. As shown in Figure 1, equilibrium was approached normally.

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Characteristics of Stationary Mercury Electrode

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▶ An investigation was made of the usefulness, sensitivity, reproducibility, and characteristics of a stationary mercury electrode of small diameter which is used in conjunction with a glass tube stirrer. A modified cell design simplifies the manipulations of the electrode. The behavior of benzil, 1,3-dinitrobenzene, maleic acid, oxalic acid, bromide ion, thiosulfate ion, uranyl ion, and ions of cadmium, cobalt, copper, lead, antimony, stannous tin, and thallium was determined. Some success was achieved in eliminating anomalous peaks. Half-wave potentials and diffusion currents were reported. The lower limit of concentration was about 0.5×10^{-6}M. A precision within about 0.4% was observed in some cases. The half-wave potentials determined with this electrode were generally more negative than the corresponding potentials obtained with the dropping mercury electrode. The diffusion currents were two to four times greater than those obtained with the dropping mercury electrode.

ONE of the recent innovations in polarography involves the use of a stationary mercury electrode of small diameter employing controlled stirring, which was reported by Arthur and associates (*1*). The polarographic waves obtained with such an electrode are usually smooth and maxima are frequently absent where they would be observed with the dropping mercury electrode.

Marple and Rogers (*3*) determined trace amounts of lead using a mercury-plated platinum electrode which was stirred with a glass tube. The waves obtained were very uneven. Rosie and Cooke (*6*) used a 3-sq. cm. mercury pool with stirred solutions to increase greatly the sensitivity of the polarographic method. This investigation is concerned with the general behavior of the electrode arrangement reported by Arthur and associates (*1*).

APPARATUS AND MATERIALS

The electrolysis cell used in this work was similar to the one described by Arthur and associates (*1*), except for a few modifications. An exploded picture of the cell is shown in Figure 1.

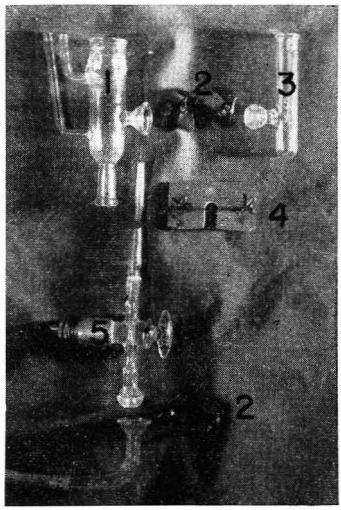

Figure 1. Exploded view of stationary mercury electrode cell

1. Body of cell
2. Ball and socket clamp
3. Reference electrode cell
4. Special tapered joint clamp
5. Stationary mercury electrode tube

A spherical joint was added below the stopcock to facilitate the cleaning and waxing of the electrode tube. The electrode tube itself extended 4 cm. up into the cell body and was of glass tubing 2.8 mm. in inside diameter, coated with ceresin wax. The two side tubes were added to facilitate degassing. The bottom end of the electrode tube was connected to a mercury leveling bulb, which was held by a leveling bulb support equipped with a screw for fine adjustment. This arrangement made the job of positioning the mercury column much easier.

A small custom-fitting water bath constructed of plate glass and polymethylmethacrylate plastic contained the body of the cell and the saturated calomel electrode. Water at 25° ± 0.2° C. was supplied to the small water bath from a large bath by a circulating pump. The support rods for the small water bath and cell were fastened to a heavy wooden base at the bottom and secured to a laboratory bench at the top to keep vibration at a minimum.

The 3/8-inch brass stirrer shaft was machined to fit closely by insertion into a 6.3-cm. length of glass tubing with a 0.74-cm. inside diameter. A 4.5-cm. length of 3-cm.-diameter brass rod served as a bushing for the shaft. The stirrer speed was controlled by reference to a Stewart-Warner portable tachometer connected to the top of the stirring motor shaft by a flexible rubber tube.

The water used for all purposes was either double-distilled or distilled water passed through an ion exchange column. The tank nitrogen was passed through a trap containing an alkaline pyrogallol solution before entering the cell. All reagents were reagent grade.

All polarograms were recorded on a Sargent Model XII polarograph and voltages were determined with a Central Scientific Co. Model 83411 potentiometer. The resistance across the cell was measured with a Wheatstone bridge, audiogenerator, and oscilloscope arrangement suggested by Pesce, Knesbach, and Ladisch (*5*).

PROCEDURE

Seventeen milliliters of the solution were placed in the cell and nitrogen was passed through the cell for 15 minutes. The mercury in the electrode tube was lowered a few millimeters below the top of the electrode tube for a few minutes just at the end of the degassing period. At the end of the degassing period the mercury column was raised, so that it was flush with the top of the electrode tube. The stirrer tube was centered over the electrode tube and lowered so that it extended 5 mm. down over the electrode. In most cases at least one unrecorded run was made to "condition" the electrode. After each run several drops of mercury were flushed out of the glass tube. The stirrer speed was maintained at 600 ± 50 r.p.m. The half-wave potentials were corrected for the voltage drop across the cell.

RESULTS AND DISCUSSION

Cations. Polarograms of eight different cations were recorded. The half-wave potentials, microamperes per millimole per liter, and diffusion currents are summarized in Table I. The half-wave potentials for lead and cadmium agree well with the values reported by Arthur and associates (*1*). The diffusion currents reported here are about twice as great as those of Arthur *et al.* because a larger electrode tube was used.

Bismuth produces a smooth, well formed wave over the entire concentration range. When calibration curves were constructed for bismuth and cad-

mium, straight lines resulted when the diffusion currents and concentrations were plotted on logarithmic scales. The concentration range investigated in each case was from 0.0005 to 0.01m*M*. A slight deviation from linearity occurred at the lowest concentrations. In ammoniacal solutions the cadmium wave rose from the start with no apparent residual current line. The half-wave potential of cobalt in 1*M* potassium thiocyanate was more positive than the dropping electrode values while the potentials in other media were more negative. This seems to support the report by Arthur *et al.* (*1*) of erratic behavior for reduction products not soluble in mercury. The waves obtained with cobalt were in general poorly formed. Peaks were observed which were not entirely eliminated by the addition of gelatin, gum arabic, or glycine. The waves obtained with lead were well formed.

A number of waves obtained with antimony in 1*N* hydrochloric acid showed peaks which could be eliminated by making the solution 0.1% in gum arabic. Glycine also was fairly effective. Gelatin, thymol, and methylene blue were ineffective. Waves covering the same voltage range and showing the different effect of 0.01 and 0.1% gum arabic are illustrated in Figure 2.

Stannous ion in 1*N* hydrochloric acid produced a wave with a maximum with a dropping mercury electrode, but no maximum was observed with the stationary mercury electrode. The half-wave potential for 1m*M* thallium in 1*M* potassium nitrate was 0.037 volt more negative than the corresponding dropping electrode value, which is in contrast to the results of Rosie and Cooke (*6*) for the stirred mercury pool electrode. These authors reported a shift of 0.01 volt in the positive direction. At the lower concentrations, the residual current line of the thallium wave was somewhat inclined. The waves obtained with the uranyl ion in 2*M* hydroxylamine hydrochloride were rather drawn out at the polarization rate (65 mv. per minute) employed.

Anions. Anodic polarograms of potassium bromide and sodium thiosulfate were made (Table II). All polarograms made of the bromide ion in 0.1*M* potassium nitrate produced peaks, probably due to the formation of mercurous bromide on the surface of the mercury. None of the maximum suppressors tried affected the peaks.

The thiosulfate ion in 0.1*M* potassium nitrate gives two waves and the half-wave potential is very much dependent on the concentration, not only for the stationary electrode, but also for the dropping electrode. The second wave obtained with the dropping mercury electrode is small and rather flattened, and disappeared below 0.001*M*. With the stationary mercury electrode the second wave produced a peak which did not have the same form on all waves and was not proportional to concentration. The peak became more pronounced as the sensitivity of the instrument was increased with decreasing concentration of the solution. The first wave was nicely formed. Two typical waves are shown in Figure 3.

Table I. Half-Wave Potentials and Diffusion Currents for Cations

Concn., Mmoles/L.	i_d, μa. SME	i_d, μa. DME	i_d^a/C	$E_{1/2}$ *vs.* S.C.E. SME	$E_{1/2}$ *vs.* S.C.E. DME
		Bi^{+++} in 1*N* HCl			
1.0	33.1	7.15	33.1	−0.114	−0.0966
0.1	3.23		32.3	−0.110	
0.01	0.297		29.7	−0.110	
0.008	0.238		29.7		
0.004	0.135		33.7		
0.002	0.0795		39.8		
0.001	0.0318		31.8	−0.106	
0.0005	0.0212	N.W.[b]	42.4		
		Cd^{++} in 0.1*M* KCl, 0.01% Gelatin			
2.0	38.7		19.4	−0.630	−0.607
1.0	19.9	4.85	19.9	−0.628	
0.1	1.80		18.0	−0.615	
0.01	0.196		19.6	−0.609	
0.005	0.102		20.4		
0.002	0.0423		21.1	−0.606	−0.600
0.001	0.0212		21.2		
0.0005	0.0106	N.W.[b]	21.2		
		Co^{++} in 1*M* KCNS			
1.0	26.5	10.8	26.5	−1.011	−1.058
		Co^{++} in 0.1*M* KCl			
1.0	27.5	12.4	27.5	−1.443	−1.345
		$[Co(NH_3)_4CO_3]^+$ in 1*M* NH_4OH + 1*M* NH_4Cl			
1.0	9.74	3.24	9.74	−0.378	−0.288
	27.05	8.75	27.05	−1.319	−1.264
		Pb^{++} in 0.1*M* KCl, 0.01% Gelatin			
2.0	48.5		24.3	−0.420	
1.0	23.3	5.80	23.3	−0.418	−0.396
0.5	12.0		24.0	−0.416	
0.1	2.49		24.9	−0.410	
0.01	0.244		24.4	−0.408	
0.004	0.0955		23.9		
0.002	0.0487		24.4		
0.001	0.0318	N.W.[b]	31.8	−0.404	
0.0005	0.0159	N.W.[b]	31.8		
		Sn^{++} in 1*N* HCl			
1.0	33.9	12.7	33.9	−0.163	−0.148
0.1	3.40		34.0	−0.158	
0.01	0.339	0.136	33.9	−0.156	−0.147
0.001	0.0318		31.8	−0.154	
		Sn^{++} in 1*N* HCl			
1.0	17.2	8.72	17.2	−0.476	−0.462
0.1	1.48	0.648	14.8	−0.472	−0.468
		Tl^+ in 1*M* KNO_3			
1.0	18.0	6.37	18.0	−0.511	−0.474
0.1	1.75	0.69	17.5	−0.500	
0.01	0.185		18.5	−0.503	
0.008	0.150		18.7	−0.500	
0.004	0.0720		18.0	−0.499	
0.002	0.0370		18.5	−0.501	
0.001	0.0185		18.5	−0.502	
		UO_2^{++} in 2*M* Hydroxylamine Hydrochloride			
1.0	9.27	4.13	9.27	−0.219	−0.214
0.1	0.932		9.32	−0.229	
0.01	0.0901		9.01	−0.221	
0.005	0.0398		7.95		

[a] Microamperes per millimole per liter.
[b] No visible wave.

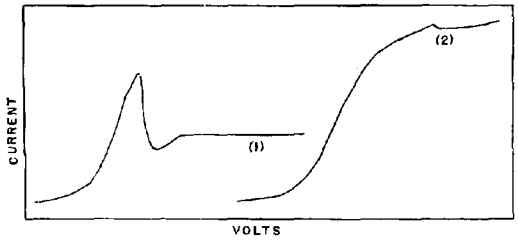

Figure 2. Waves of antimony in 1N hydrochloric acid
1. Gum arabic added, 0.01%
2. Gum arabic added, 0.1%

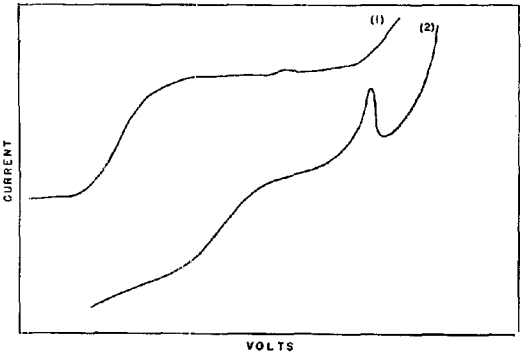

Figure 3. Waves of thiosulfate in 0.1M potassium nitrate
1. 1 mM
2. 0.01mM

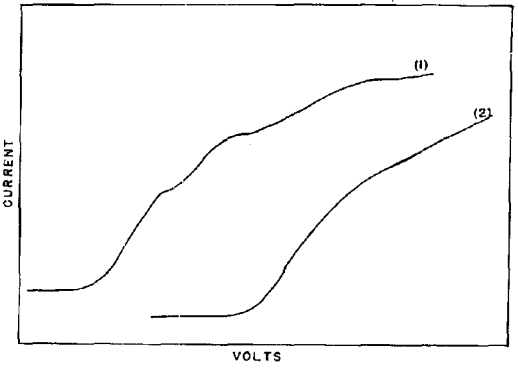

Figure 4. Waves of 1,3-dinitrobenzene and maleic acid
1. 1 mM 1,3-dinitrobenzene
2. 1 mM maleic acid in 1N HNO_3 in 10% acetone, buffer pH 8.2

Organic Compounds. Polarograms were made of benzil in 0.2N hydrochloric acid, 1,3-dinitrobenzene in buffer of pH 8.2, maleic acid in 0.1N hydrochloric acid and 1N nitric acid, and oxalic acid in three different supporting electrolytes. The half-wave potentials and diffusion current data are given in Table III. The solvent for benzil was 75% ethyl alcohol, which softened the coating on the electrode, and often caused it to be stripped from the electrode. Runs made with a freshly waxed electrode were very satisfactory. A very small peak was observed on the residual current line with the stationary electrode.

1,3-Dinitrobenzene produced two waves with the dropping mercury electrode, but three waves were observed with the stationary mercury electrode in basic media. The third wave became less distinct with decreasing concentration. Pearson (*4*) indicated three half-wave potentials at a pH of 2.5, but the first two waves were rather coalesced. Marple and Rogers (*2*) reported three waves with the stationary mercury-plated platinum electrode; in stirred solutions the waves of 1,3-dinitrobenzene were very flat and overlapped. A typical wave obtained with the stationary mercury electrode is shown in Figure 4.

The limiting current line of the maleic acid waves were inclined at an angle of about 30°, which increased the difficulty of half-wave determination. A maleic acid wave is shown in Figure 4. No usable waves were obtained with oxalic acid. A fairly sharp peak was observed in 0.1M ammonium chloride containing a buffer of pH 5.0. The oxalic acid wave obtained with the dropping mercury electrode had a steep limiting current plateau, closely followed by the hydrogen wave. In the same supporting electrolytes, 0.2M potassium acid phthalate and 0.05M tetramethyl ammonium bromide, using the stationary electrode, the large hydrogen wave completely obscured the oxalic acid wave.

Solution Containing Two Ions. The results of the determination of lead and cadmium in the same solution are given in Table IV. When gelatin was present, the lead wave had a very flat limiting plateau and the cadmium wave had a slightly inclined or rounded limiting plateau. The effect was reversed when gelatin was not present (Figure 5). Other than the effect noted, the discharge of the lead ion previous to the cadmium ion discharge has no significant effect on the diffusion current of the second wave. The diffusion current values agree very well with the values found for the solutions of the individual ions. The ratio of the cadmium diffusion current to the lead diffusion current obtained with the stationary electrode was very close to the ratio obtained with the dropping electrode.

Standard Addition. Some standard addition experiments were run using solutions of copper, cadmium, and lead, with the mercury meniscus about 1 mm. above the top of the glass tube. The waves obtained with this arrangement were not so smooth and nicely formed as the waves obtained with the mercury flush with the top of the tube. The smallest error found was about 1% and the average error was

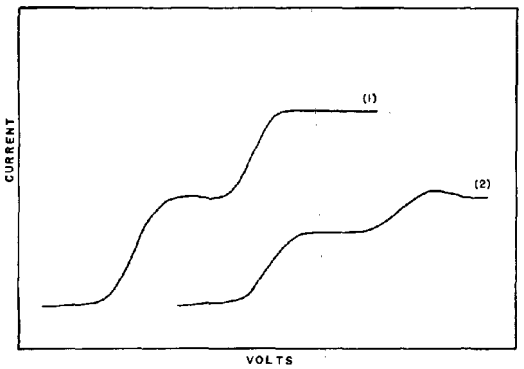

Figure 5. Waves of lead and cadmium in same solution, 0.1M potassium chloride

1. 1mM, no gelatin present
2. 0.1 mM, 0.01% gelatin present

Table II. Half-Wave Potentials and Diffusion Currents for Anions

Concn., Mmoles/L.	i_d, μa. SME	i_d, μa. DME	i_d/C[a]	$E_{1/2}$ vs. S.C.E. SME	$E_{1/2}$ vs. S.C.E. DME
		Br^- in 0.1M KNO_3			
1.0	14.82[b]	4.34	14.82	+0.0981[c]	+0.101
	9.80[b]	2.96	9.80	+0.170[c]	+0.215
		$S_2O_3^{--}$ in 0.1M KNO_3			
1.0	12.4	5.3	12.4	−0.163	−0.167
	0.159[b]	0.742	0.16	0.151[c]	0.125
0.1	1.23	0.642	12.3	−0.140	−0.147
	0.212[b]	N.W.[d]	2.12	0.058[c]	N.W.[d]
0.01	0.138	0.0754	13.8	−0.113	−0.0638
	0.148[b]	N.W.[d]	14.8	0.0752[c]	N.W.[d]
0.001	0.0118	N.W.[d]	11.8	−0.095	N.W.[d]
	0.0795[b]	N.W.[d]	79.5	0.0715[c]	N.W.[d]

[a] Microamperes per millimole per liter.
[b] Peak heights.
[c] Half-peak potentials.
[d] No visible wave.

Table III. Half-Wave Potentials and Diffusion Currents of Organic Compounds

Concn., Mmoles/L.	i_d, μa. SME	i_d, μa. DME	i_d/C[a]	$E_{1/2}$ vs. S.C.E. SME	$E_{1/2}$ vs. S.C.E. DME
		Benzil in 75% Ethyl Alcohol, 0.2N HCl			
1.0	15.2	6.35	15.2	−0.441	−0.280
0.1	1.51		15.1	−0.439	
		1,3-Dinitrobenzene in 10% Acetone, Buffer pH 8.2			
1.0	37.3	19.3	37.3	−0.600	−0.497
	20.1	16.8	20.1	−0.761	−0.667
	21.4		21.4	−1.070	
0.1	3.42		34.3	−0.590	
	2.11		21.1	−0.751	
	2.05		20.5	−1.030	
0.01	0.371		37.1	−0.588	
	0.225		22.5	−0.752	
	0.199		19.9	−1.024	
0.001	0.0635		63.5	−0.587	
	0.0423		42.3	−0.745	
	0.0318		31.8	−1.011	
		Maleic Acid in 0.1N HCl			
1.0	12.2	8.04	12.2	−0.878	−0.645
		Maleic Acid in 1N HNO_3			
1.0	10.9	7.94	10.9	−0.754	−0.584

[a] Microamperes per millimole per liter.

about 6%. The diffusion currents obtained in these experiments were about 75% greater than those obtained with the mercury flush with the top of the tube.

Mercury Leveling Method. An experiment was made to determine whether the diffusion current was affected by lowering the mercury below the top of the tube. The average current obtained by lowering the mercury was 6.5% higher than that obtained with the mercury flush with the top of the glass electrode tube. There was less deviation of the diffusion current with the mercury at top level

Table IV. Data for Determination of Lead and Cadmium in Same Solution

(Supporting electrolyte, 0.1M KCl)

Concn., Mmoles/L.	i_d, μa. Pb^{++}	i_d, μa. Cd^{++}	Ratio
	Stationary Mercury Electrode		
1.0	21.5	18.9	0.878
0.5	11.1	9.43	0.850
0.25	6.60	5.77	0.875
0.1	2.18	1.81	0.830
0.02	0.376	0.328	0.872
	Dropping Mercury Electrode		
1.0	9.70	8.47	0.874

Table V. Effect of Leveling Mercury by Raising or Lowering

(Solution used, 0.75 × 10^{-3}M, $Na_2S_2O_3$ in 0.1M KNO_3)

Run	i_d, μa.	Deviation
	Mercury at Tube Top	
1	9.13	0.41
2	9.67	0.13
3	9.80	0.26
Av.	9.54	0.27
	Mercury below Tube Top	
1	10.02	0.14
2	10.87	0.71
3	10.02	0.14
4	9.82	0.34
Av.	10.16	0.33

(Table V). The diffusion current decreased about 2.8% when four successive runs were made without flushing mercury from the electrode.

Linearity and Precision. The results obtained with cadmium showed that with renewal of the mercury surface a precision of 0.36% may be obtained. The diffusion currents obtained for most ions show a good linear relationship. The results for thallium were within 1.9% of linear. Greater deviations were observed at the lower limit of concentration.

General. For substances that produce well formed waves, the lower limit of concentration was in the vicinity of $0.5 \times 10^{-6} M$. Good waves were obtained with the ions of bismuth, cadmium, lead, thallium, stannous tin and thiosulfate, and 1,3-dinitrobenzene. Moderately good waves were obtained with antimony ion, uranyl ion, benzil, and maleic acid. Very poor waves were obtained with cobaltous ion and bromide ion. Oxalic acid did not produce a usable wave. Anomalous peaks or humps sometimes occurred, which were extremely difficult to suppress. The electrode was two to four times more sensitive than the dropping mercury electrode. The half-wave potentials determined for the stationary mercury electrode were, in general, more negative than the corresponding potentials determined with the dropping mercury electrode. Over a wide concentration range, the half-wave potentials shift to less negative values as the solutions are made more dilute, in agreement with the observations of Arthur *et al.* (*1*). The cations studied gave half-wave potentials averaging about 0.019 volt more negative, the anions about 0.004 volt more negative, and the organic compounds about 0.17 volt more negative than the dropping mercury potentials.

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Determination of Trace Amounts of Copper

Application of the Bathocuproine Reagent to Pulp, Paper, and Pulping Liquors

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▶ **The present work is concerned with the determination of copper in pulp, paper, and pulping liquors in the range of 0.1 to 40 p.p.m. A critical survey of the literature has been made and a method based on the use of a sensitive and specific colorimetric reagent, 2,9-dimethyl - 4,7 - diphenyl - 1,10 - phenanthroline, is recommended. Using this reagent, copper can be accurately determined on a 1-gram sample. The wet combustion technique using nitric and perchloric acids is recommended, as results based on dry ashing of organic matter were variable. Measurement of the colored complex is made at a wave length of 479 mμ.**

A SEARCH of the literature reveals a profusion of methods for the determination of trace amounts of copper in materials of biological origin. Interest in this determination is manifested especially by dairy and agricultural chemists, pathologists, and others concerned with the catalytic effects of this metal. Small amounts of copper (less than 1 p.p.m.) are known to promote the formation of rancidity of dairy products and vegetable fats, and contamination must be guarded against during processing, packaging, and storage. With the present surplus of dairy products, the problem of stability on storage has assumed major economic proportions.

One source of copper contamination lies in the paper or board used as a packaging medium. Wood itself has a certain residual copper content, usually less than 1 p.p.m., depending upon the species and soil conditions during tree growth. In the manufacture of paper, however, copper is picked up during cooking and beating, and especially as the sheet is formed on the fourdrinier wire. As a result, paper and paperboard may contain upwards of 20 p.p.m. of copper. In the manufacture of specialty papers—i.e., butter wrap and ice cream cartons—the copper content must be carefully controlled, and the tolerance for this metal is very low in the case of papers used for photographic purposes. Furthermore, in studying the corrosion of digesters it is sometimes of interest to determine the copper content of pulping liquors containing a high concentration of carbohydrates.

The present paper reports a critical survey of analytical methods for determining trace amounts of copper in the presence of organic matter. Emphasis was placed on the determination of copper in pulp, paper, and pulping liquors in the range of 0.1 to 40 p.p.m. A comparison has been made of the wet-combustion and the dry-ashing techniques for destruction of the organic matter. The recommended method is presumably applicable to milk, plants, fertilizer, and organs of medical and pathological interest.

SURVEY OF LITERATURE

The potassium ethyl xanthate method (*49*) and that worked out by Biazzo (*6*) were among the first to meet the needs of the dairy chemists. Close control of pH is essential in the xanthate method, and Conn *et al.* (*15*) encountered difficulty due to turbidity following color development. The Biazzo method consists of ashing the sample, adding pyridine and potassium thio-

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cyanate to the solution, and extracting the copper complex with chloroform. Drabkin and Waggoner (*17*) recommend the addition of pyrophosphate to obviate the interference of iron(III). Subsequent workers (*2, 19, 22, 43*) have modified this method for application to various biological materials, but there remain the principal drawbacks of lack of sensitivity and specificity.

Titration with nitrosochromotropic acid was suggested by Cherbuliez and Ansbacher (*10*), following separation of copper as the sulfide, but the color change at the end point left something to be desired (*45*). Several workers (*11, 30*) have reported on the pink color formed in the presence of copper upon the addition of dimethylglyoxime, pyridine, ammonium persulfate, and silver nitrate. This method is very sensitive but does not lend itself to quantitative work. In 1930, Richardson (*41*) proposed a method based on the color formed with potassium ferrocyanide following separation of copper as the sulfide, but the procedure has not met widespread acceptance.

Callan and Henderson (*9*) pioneered in the application of sodium diethyldithiocarbamate to the determination of copper, and Williams (*52*) compared the carbamate and xanthate methods. Since the early 1930's a great deal of work has been done in an effort to obviate interferences and increase the sensitivity of carbamate methods. Conn *et al.* (*15*) give an excellent review of the literature up to 1935, and recommend the carbamate method following separation of copper as the sulfide. A number of refinements have been reported in the intervening years; these include a preliminary separation with diphenylthiocarbazone (*50*), use of thiosulfocarbamate (*33*), complexation of iron using 2,2′-bipyridine (*38*) or citrate (*18*), separation of copper with dithizone (*4, 25, 39*), and use of dihydroxyethyldithiocarbamic acid (*7*). As late as 1950, however, Beeson and Gregory (*5*) recommended further study of the existing carbamate methods before adoption of one of these as an official method of the Association of Official Agricultural Chemists. In 1953, Forster (*20*) modified the method of Sedivec and Vasak (*44*) by using the ammonium salt of (ethylenedinitrilo)tetraacetic acid (EDTA) to complex iron, manganese, nickel, and zinc; the copper-carbamate complex was extracted with carbon tetrachloride. Even more recently, Cluley (*12*) has suggested the use of the diethylammonium salt of diethyldithiocarbamic acid and EDTA in the copper determination. Abbott and Polhill (*1*) reported that very low blanks were obtained when zinc dibenzyldithiocarbamate was used as a colorimetric reagent for copper. Complexing agents were not required when this reagent was used.

Several authors (*13, 29, 35, 40*) have determined trace amounts of copper polarographically after destruction of organic matter or extraction of soluble copper salts with dilute acid. None of these methods were applicable to the determination of 1 γ or less of copper in the presence of iron and other reducible cations.

Various other methods have been proposed and the applicability of several was studied in the present work. An iodometric method (*46*) was not adaptable for amounts of copper in the microgram range. Methods based on color formation with dithizone (*8, 26, 37*) suffer from lack of specificity, and have not met with widespread acceptance. The use of *o*-dianisidine (*34*) and rubeanic acid (*31*) appeared deserving of further study. Methods based on the blue copper complex with tetraethylenepentaamine (*16, 32*) are not sufficiently sensitive for the present purpose. Procedures based on the use of 2,2′-biquinoline (*23, 27, 28*) were studied. A promising method for determining copper in pulp and paper by Wetlesen and Gran (*51*) was evaluated; this method employs Cuprizone, biscyclohexanonedioxaldihydrazone, as the colorimetric reagent. Gran (*24*) has recently described a sensitive method for copper based on reaction with oxalyldihydrazide in the presence of acetaldehyde. The specificity of this reaction and the solubility of the copper compound in organic solvents have not been studied.

Much of the work reported above has been adapted from a few basic methods. Smith was the first to make a fundamental study of the copper(I) complexes with substituted 1,10-phenanthrolines, and as an outgrowth of this study predicted the requirements for chelation of the cuprous ion and postulated the effect of substitution on sensitivity and selectivity of the colorimetric methods based thereon. In two recent papers (*47, 48*) Smith and his coworkers have described the analytical applications of two new reagents, 2,9-dimethyl-1,10-phenanthroline, known as neocuproine, and 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline, known as bathocuproine. Gahler (*21*) has applied neocuproine to the determination of copper in steel. Bathocuproine has only recently become available commercially, and was evaluated in the course of the present work.

Several authors have reported difficulty in recovering added copper in using a dry-ashing technique (*3, 36*). Comrie (*14*) added magnesium nitrate to the organic matter prior to ashing in order to increase the bulk of the ash. He maintained that silica absorbs copper irreversibly and recoveries were low for this reason. Bailey and McHargue (*3*) reported that use of platinum crucibles led to interference with the carbamate method. Most proponents of the dry-ashing technique have used temperatures below 500° C. (or 465° C. in some cases), but recoveries of copper were variable and the time required for ashing was excessive under these conditions. In view of the disagreement in the literature, wet-combustion and dry-ashing techniques have been compared.

METHODS STUDIED

Besides sensitivity and specificity, a widely applicable method should not require techniques or equipment beyond the means of the ordinary control laboratory. Colorimetric measurements are ideally suited for the determination of trace metals, and this study has been limited to such methods. In so doing, it is not felt that any very promising method has been excluded from consideration.

The following colorimetric methods have been studied:

Dithizone (*42*)
Biazzo method (thiocyanate and pyridine) (*6*)
p-Anisidine
1,5-Diphenylcarbohydrazide
Di-2-naphthylthiocarbazone
Cuprizone, biscyclohexanonedioxaldihydrazone (*51*)
Rubeanic acid (*31*)
2,2′-Biquinoline (*27*)
Neocuproine, 2,9-dimethyl-1,10-phenanthroline (*21*)
Bathocuproine, 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (*48*)
Diethylammonium salt of diethyldithiocarbamic acid and EDTA (*12*)
Zinc dibenzyldithiocarbamate (*1*)

Several methods were eliminated from further consideration after preliminary experiments. Dithizone forms colored compounds with a number of metals besides copper, and contamination by zinc and mercury is especially troublesome. The extraction step requires a fairly long period of shaking and metal dithizonates are unstable in the presence of light. Extraction of the copper dithizonate may afford a convenient means of separation, but the method based on use of dithizone alone is unsuitable.

The Biazzo method was studied with respect to molar absorptivity of the colored complex, interference of other cations, stability of the complex, and effect of pH on extraction. The method was fairly specific for copper and reproducible results were obtained. However, at least 10 γ of copper were required for accurate measurement, and this meant the digestion of large amounts of organic matter for samples containing less than 1 p.p.m. of copper. For safe and rapid removal of organic matter using nitric and perchloric acids, it is advisable to use a 1-gram sample.

Table I. Comparison of Various Methods for Determining Copper

Method	Solvent Used for Extraction	pH Range for Extraction	Wave Length of Absorption Peak, mμ	Sensitivity[a]	Effect of Foreign Ions	Stability of Color	Remarks
Neocuproine	$CHCl_3$-EtOH	2.3–9.5	457	0.095[b]	No interference from 56 metals (*21*)	4 days	
Biquinoline	Isoamyl alcohol	4–6	540	0.095[b]	Hg caused ppt.	...	
Biazzo	$CHCl_3$	2–10	405	0.050[c]	Fe(III) up to 5 mg. permissible	24 hr.	Citrate used to complex Fe(III)
Diethylammonium salt of diethyldithiocarbamic acid plus EDTA	$CHCl_3$	Excess NH_4OH	435	0.230[b]	No interference from Ni, Mn, Hg, or Fe below 7 mg.	7 days	
Cuprizone	None	6.9–9.9	606	0.095[d]	None (*51*)	3 hr.	Ammonium citrate used to complex Fe and Ni
Bathocuproine	1-Hexanol	4–10	479	0.330[e]	No interference encountered	100 hr.	Specific for Cu(I) (*47*)
Zinc dibenzyldithiocarbamate	CCl_4	Less than 2	435	0.515[c]	No interference from Fe, Ni, Hg, or Mn	24 hr.	No complexing agent required (*1*)

[a] Measured absorbance for 10 γ of copper following solvent extraction; 1-cm. path length.
[b] Final volume 10 ml.
[c] Final volume 5 ml.
[d] Final volume of aqueous solution 25 ml.
[e] Final volume 6 ml.

In the case of pulping liquors a 10-ml. aliquot is suitable. Given these limitations, the Biazzo method was insufficiently sensitive for the present purpose.

A cursory examination was made of *p*-anisidine, 1,5-diphenylcarbohydrazide, and di-2-naphthylthiocarbazone as colorimetric reagents for copper. The copper-anisidine complex was extractable from an alkaline solution with carbon tetrachloride, but pH control for complete extraction was so critical as to render the method valueless for routine control purposes. 1,5-Diphenylcarbohydrazide reacts with a number of metals to form colored compounds. The rose-colored copper complex is extractable into chloroform from an ammoniacal solution, but turbidity in the chloroform layer often prevents spectrophotometric measurement. Extraction from acid solution gave the same results. In view of the number of metals known to react with this reagent, further work was abandoned. Di-2-naphthylthiocarbazone is somewhat more selective than dithizone in its reaction with mercury, but copper is known to react under similar conditions. Measurement of the absorption curve of the copper-naphthylthiocarbazonate failed to show a peak in the visible region, and the method was not investigated further.

Cuprizone (biscyclohexanonedioxaldihydrazone) has been recommended by Wetlesen and Gran (*51*) for the determination of copper in pulp and paper. These authors reported that the copper(II) complex with cuprizone has a molecular extinction coefficient of 17,120 at a wave length of 606 mμ, but the chelate is insoluble in all common organic solvents. Because the complex cannot be extracted from the aqueous phase, the method is relatively insensitive and a 10-gram sample of pulp is required. In Table I are shown comparative data for a number of colorimetric reagents for copper.

Use of rubeanic acid was ruled out because of the insolubility of the green copper compounds in common organic solvents.

2,2′-Biquinoline and neocuproine (2,9-dimethyl-1,10-phenanthroline) have about the same sensitivity and selectivity in the copper determination. The copper chelates of these two reagents were extractable in the pH range of 4 to 6, but below 5 γ of copper, blank corrections become significant and these methods are inapplicable below 1 γ of copper. Hence, they were abandoned for want of sensitivity.

Of the many reagents tested, three were found suitable for the present determination: the diethylammonium salt of diethyldithiocarbamic acid using EDTA as a complexing agent, zinc dibenzyldithiocarbamate, and bathocuproine (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline). The carbamate method using EDTA is less sensitive than either of the other two, because a large volume of chloroform is necessary for complete removal of the copper complex from the aqueous phase. The carbamate complex has limited solubility in chloroform, and upon cooling a cloudiness often developed in the chloroform layer. This cloudiness also occurred when impure EDTA was used to sequester other metals. There is little difference between the three methods as regards speed, necessity for pH control, and stability of the colored complex.

The zinc salt of dibenzyldithiocarbamic acid offers several advantages as a reagent for copper. First, the copper complex is formed in acid solution and accordingly the blank is lower than when ammonia is used to neutralize the acid digestion mixture. The reagent is fairly specific for copper, although macro amounts of nickel and mercury interfere. The precision of results using this method left something to be desired, however, and the bathocuproine method is recommended for general use. For the sake of completeness, all three procedures are given in detail.

APPARATUS AND REAGENTS

Ammonium hydroxide solution, C.P.

Nitric acid, redistilled reagent (G. F. Smith Chemical Co.).

Perchloric acid, 72%, double-vacuum-distilled (G. F. Smith Chemical Co.).

Bathocuproine, 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (G. F. Smith Chemical Co.), 0.01*M* solution in 1-hexanol.

1-Hexanol, redistilled.

Hydroxylamine hydrochloride solution, 10% aqueous.

Distilled water. Redistill from all-glass apparatus.

Congo Red indicator paper.

Standard copper solution. Dissolve a weighed amount of clean copper wire in nitric acid and dilute to the required volume with distilled water.

Beckman Model DU spectrophotometer with matched 1-cm. Corex cells.

If the alternative procedures are used, the following additional chemicals are required:

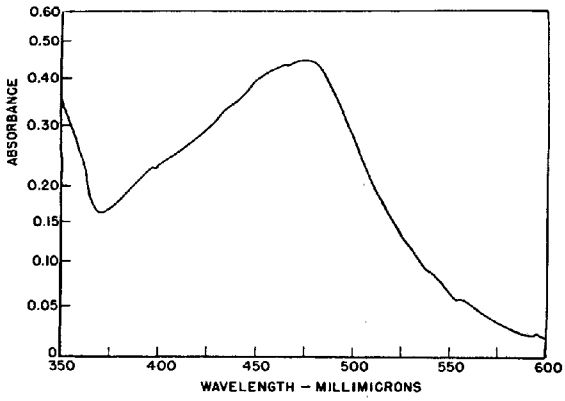

Figure 1. Absorption curve for copper(I)–2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline complex

Concn. 12 γ Cu in 6 ml. 1-hexanol. 1.0-cm. path length

(Ethylenedinitrilo)tetraacetic acid disodium salt (TitraVer powder, No. 204, Hach Chemical Co., Ames, Iowa). Dissolve 50 grams of the salt in 1 liter of distilled water.

Diethyldithiocarbamic acid diethylammonium salt (Eastman No. 2635). Dissolve 10 grams in 1 liter of chloroform.

Chloroform, C.P.

Zinc dibenzyldithiocarbamate, 0.05% in carbon tetrachloride.

Sodium sulfite, 5% in water.

Carbon tetrachloride, C.P.

RECOMMENDED PROCEDURE

Wet Digestion. Weigh accurately a sample of the appropriate size (1 gram in the case of pulp and paper, 10 ml. for pulping liquors) and transfer the sample to a 100-ml. Kjeldahl flask. Add 20 ml. of nitric acid, 5 ml. of 72% perchloric acid, and an Alundum boiling chip. Warm the solution with a Bunsen burner until most of the carbonaceous matter has been oxidized, and continue heating until all nitric acid has been removed and strong fumes of perchloric acid are evolved. Cool the solution and dilute to 25 ml. with redistilled water. Boil the solution for several minutes to destroy oxides of chlorine.

Bathocuproine Method. Add a small piece of Congo Red paper to the flask and neutralize the solution with concentrated ammonium hydroxide solution, adding 10 drops in excess. Cool the solution and transfer to a 100-ml. separatory funnel. Pipet the following reagents into the solution in the order given: 2 ml. of hydroxylamine hydrochloride solution, 1.0 ml. of 1-hexanol solution of bathocuproine and 5.0 ml. of 1-hexanol. Shake the solution for 2 minutes and then allow the layers to separate for 5 minutes. Discard the aqueous layer and transfer the hexanol solution with a pipet to the absorption cell. Measure the absorbance of the solution at a wave length of 479 mμ *vs.* a reference solution carried through the digestion and color development steps. The absorption curve is given in Figure 1.

ALTERNATIVE PROCEDURES

Carbamate Method Using EDTA. Digest the sample as directed above, and transfer the resultant solution, previously cooled, to a 100-ml. separatory funnel. Add 20 ml. of (ethylenedinitrilo)tetraacetic acid solution and dilute to about 60 ml. with distilled water. Neutralize the solution to Congo Red paper with ammonium hydroxide solution and add 5 ml. in excess. Cool the solution if necessary and add 10.0 ml. of carbamate solution from a pipet. Shake the solution for 2 minutes and then allow the layers to separate. Draw off the chloroform layer through a glass wool plug in the stem of the separatory funnel, and transfer directly to the absorption cell. Measure the absorbance of the chloroform solution at a wave length of 435 mμ *vs.* a blank solution carried through the entire procedure.

Zinc Dibenzyldithiocarbamate Method. Digest the sample as above, and add 5 ml. of sodium sulfite solution to the cool, dilute acid solution. Transfer the solution to a 100-ml. separatory funnel and add 5.0 ml. of zinc dibenzyldithiocarbamate reagent solution. Shake well for 1 minute, and then draw off sufficient carbon tetrachloride solution to fill an absorption cell. Measure the absorbance of this solution at a wave length of 435 mμ against a blank solution carried through the same procedure.

DISCUSSION

1,10-Phenanthroline and its substituted analogs form stable chelates with iron(II), copper(I), zinc(II), and cadmium(II), among the more common metals. These chelates are readily extractable with organic solvents, and in the case of iron and copper, they form the basis of sensitive colorimetric methods of determination. The ferrous-1,10-phenanthroline complexes are the most widely used, and a rather large number of ferroin-type oxidation-reduction indicators are known. Copper(I) reacts with 1,10-phenanthroline to form a colorless complex in acid but not in neutral or basic media. Introduction of methyl groups into the phenanthroline nucleus in the 2,9- positions serves to inhibit chelation with iron(II) and metals other than copper(I), because of steric requirements. Furthermore, the copper(I)–2,9-dimethyl-1,10-phenanthroline complex absorbs in the visible region, thus making possible a sensitive colorimetric determination.

As a result of fundamental studies by Smith and his coworkers, the sensitivity of neocuproine as a reagent for copper has been enhanced by the addition of phenyl groups in the 4,7- positions of the phenanthroline nucleus. The resultant compound, bathocuproine, retains the specificity of neocuproine for copper(I) and as a reagent for copper leaves little to be desired. The properties of the copper(I)–2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline system may be summarized as follows: The chelate is readily extractable into hexanol with a single extraction, pH control is not critical, other metals do not form colored complexes under the reaction conditions specified, stability of the chelate is not a problem, and measurement of light absorption can be made in the visible region. The only drawback to this reagent is its cost, but in the present work one gram of bathocuproine sufficed for over 200 determinations.

A comparison of the results obtained by the wet-combustion and dry-ashing techniques is shown in Table II. Dry-ashing temperatures were held below 500° C. and porcelain crucibles were used. The resultant ash was then leached with nitric and hydrochloric acids. Erratic results were obtained using the dry-ashing technique, and use of platinum ware failed to improve the precision. The poor results are explained either on the basis of volatilization of copper at 500° C. or irreversible absorption of copper on the porcelain surface, but in any case concordant results were not obtainable by this technique. Accordingly, the wet-combustion method using nitric and perchloric acids was studied and found applicable to the present problem. Excellent precision was obtained, blanks were low and reproducible, and recovery of added copper was quantitative. Using a 1-gram sample of pulp or paper, the digestion proceeded smoothly and without violent reaction. Some frothing was observed in the case of liquor samples, but only a slightly

Table II. Dry-Ashing and Wet-Combustion Methods Applied to Paper Samples

Sample	Copper Content, P.P.M. Dry ashing	Wet combustion
Unwaxed glassine	23.2	20.6
	25.4	20.5
	23.3	20.7
Av.	24.0	20.6
Waxed glassine	14.5	14.5
	14.4	14.0
	15.4	14.5
Av.	14.8	14.3
Bleached greaseproof	21.4	17.1
	37.2	17.7
	24.1	17.7
Av.	27.6	17.5
Margarine carton	6.29	8.84
	7.05	8.22
	8.55	8.43
Av.	7.30	8.50
Ice cream carton 1	20.5	20.4
	17.8	21.0
	17.8	21.1
Av.	18.7	20.8
Ice cream carton 2	4.76	8.91
	6.49	8.75
	5.35	9.14
Av.	5.53	8.93

Table III. Determination of Copper in Pulp, Paper, and Pulping Liquors by Various Methods

(Wet combustion used)

Sample	Copper Content, P.P.M. Carbamate plus EDTA	Zinc dibenzyldithiocarbamate	Bathocuproine
Butter wrap	4.21	4.46	4.05
	4.03	4.33	4.16
	4.26	4.31	4.09
Av.	4.17	4.37	4.10
Lard Pak	19.1	20.3	19.2
	18.5	19.9	19.4
	18.7	20.2	19.1
Av.	18.8	20.1	19.2
Bleached sulfite pulp	3.59	3.56	3.31
	3.67	3.59	3.29
	3.54	3.56	3.34
Av.	3.60	3.57	3.31
Bleached kraft pulp	1.64	1.10	0.96
	1.06	1.06	0.97
	1.21	1.08	0.95
Av.	1.30	1.08	0.96
Spent semichemical liquor	0.29	0.21	0.20
	0.29	0.19	0.21
	0.26	0.19	0.20
Av.	0.28	0.20	0.20
Spent sulfite liquor	0.21		0.24
	0.24		0.25
	0.26		0.25
Av.	0.23		0.25

Table IV. Recovery of Copper Added to Pulp, Paper, and Pulping Liquor Samples

(Wet combustion used)

Sample	Residual Copper, γ	Copper Added, γ	Total Copper Present, γ	Copper Found, γ	Recovery, %
Diethylammonium Salt of Diethyldithiocarbamic Acid Plus EDTA					
Lard Pak A	21.6	10.1	31.7	31.0	97.8
	21.5	10.1	31.6	31.5	99.7
Butter wrap	8.17	10.1	18.27	18.0	98.5
	8.04	10.1	18.14	17.8	98.1
Zinc Dibenzyldithiocarbamate					
Butter wrap	4.68	4.2	8.88	8.8	99.2
	4.70	10.5	15.2	15.4	101.3
Lark Pak	6.8	4.2	11.0	10.7	97.3
	8.6	4.2	12.8	12.7	99.2
Bleached sulfite pulp	7.18	4.2	11.38	11.4	100.2
	7.10	4.2	11.30	12.2	108.0
Semichemical liquor	2.1	4.2	6.3	6.2	98.0
	2.1	4.2	6.3	6.3	100.0
Bathocuproine					
Bleached sulfite pulp	3.67	11.4	15.07	14.95	99.2
	4.94	11.4	16.34	16.35	100.1
	6.53	11.4	17.93	17.95	100.1
Spent semichemical liquor	2.09	11.4	13.49	13.45	99.7
	2.09	11.4	13.49	13.65	101.2
	2.09	11.4	13.49	13.65	101.2
Lark Pak	6.1	11.4	17.5	17.2	98.3
	6.0	11.4	17.4	17.0	97.7
	5.8	11.4	17.2	17.1	99.4
Butter wrap	4.2	11.2	15.4	15.1	98.0
	5.0	11.2	16.2	16.1	99.4
	5.9	11.2	17.1	16.9	98.8

longer period of digestion was required in these cases.

NOTES AND PRECAUTIONS

Because of the sensitivity of the recommended methods, contamination from copper must be guarded against at every stage. All glassware should be rinsed with 1 to 1 nitric acid immediately prior to use, with a final rinse with redistilled water. Ordinary water from a Barnstead or similar still contains a small but measurable amount of copper, and for this reason redistillation from an all-glass apparatus is necessary for accurate work. All reagent solutions should be extracted with bathocuproine in hexanol (or carbamate in chloroform or carbon tetrachloride if the alternative procedures are used) to remove residual copper.

Turbidity in the absorption cells may be removed by gentle warming from the finger tips.

During the wet-digestion step the usual precautions governing the use of perchloric acid should be observed. In the present work no difficulty was encountered in the digestion of over 500 samples by this method.

All three methods can be modified to enable colorimetric measurements with filter photometers or grating spectrophotometers.

The colored complex obeys Beer's law at a wave length of 479 mμ within the concentration range of 0 to 30 γ of copper in 16 ml. of hexanol.

Tables III and IV show comparative data for the determination of copper by the three methods.

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Reactions of Arsenic(III) and Arsenic(V) with Thioacetamide in Acid Solutions

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▷ The rates of precipitation of arsenic(III) sulfide by thioacetamide have been measured in solutions having pH values from 3.78 to 1 and found to follow quantitatively the calculated rates of hydrolysis of the thioacetamide to hydrogen sulfide. Qualitative experiments with solutions of pH 4 to 6 have produced no evidence of a change of mechanism to a direct reaction, such as was observed in the case of lead. Studies of the reaction of arsenic(V) with thioacetamide have shown that initially arsenic(V) is reduced to arsenic(III) with formation of sulfur. The rate of reduction is first order with respect to the concentration of arsenic(V), thioacetamide, and hydrogen ion. The hydrogen sulfide resulting from hydrolysis of the thioacetamide reacts initially with arsenic(V) to form thioarsenic acids, and then with arsenic(III) to produce arsenious sulfide.

THE results of studies of the acid-catalyzed hydrolysis of thioacetamide and of the precipitation of lead sulfide by thioacetamide in acid solutions were reported in a previous paper (*6*). The precipitation of lead sulfide by thioacetamide proceeded through hydrolysis of the latter in solutions with hydrogen ion concentrations greater than about $10^{-3}M$. However, in solutions having pH values from 5.1 to 3.4, the precipitation proceeded at a rate appreciably greater than and independent of the rate of hydrolysis. This paper reports studies of the reactions of arsenic(III) and (V) with thioacetamide in acid solutions.

REACTIONS OF ARSENIC(III) WITH THIOACETAMIDE

Reagents. Solutions of thioacetamide, sodium thiosulfate, potassium iodate, and hydrochloric acid were prepared as described by Swift and Butler (*6*).

Considerable variation in the quality of commercial thioacetamide has been noted, even in separate samples from a single source, and anomalous results in reaction rates and precipitation effects have been obtained from some specimens. Material used consisted of white crystals, which could be dissolved completely in water to give a $1VF$ (volume formal) solution (or left only a residue amounting to not more than 1 mg. from 100 ml. of such solution), and had a melting point range of 111.0° to 113.2° C.

A stock solution of arsenious acid, $0.10VF$, was prepared by dissolving a weighed portion of arsenious oxide in hot water; the concentration was checked iodometrically.

Sodium formate–formic acid buffer solutions were prepared from sodium hydroxide and 90% formic acid and the pH values were checked with a Beckman Model G pH meter.

Apparatus. The reaction apparatus was that used in the study of the hydrolysis of thioacetamide (*6*).

Rate Measurements. The reaction solutions were prepared by mixing accurately measured volumes of the stock solutions of thioacetamide, arsenic(III), and hydrochloric acid or sodium formate–formic acid buffer and diluting to 100 ml.

The reaction solution was heated to 90° ± 1° C. and maintained at that temperature by a constant temperature bath. Samples of the reaction solution were forced by air pressure at timed intervals through the sintered-glass tube. Any excess of reaction solution which remained in the sintered-glass tube after the removal of a sample was forced back into the main body of the solution by a low pressure flow of nitrogen. Nitro-

Table I. Comparison of Measured and Calculated Concentrations of Arsenic(III) at Timed Intervals

(Calculations are based upon the second-order hydrolysis constant, $k = 0.21$ liter mole^{-1} minute^{-1}. All solutions were $0.10VF$ in thioacetamide; $T = 90°$ C.)

aH^+	As(III) Concn.	Time, Hours			
		0	1	1.5	2
1.6×10^{-4}	Measd.	0.0099	0.0097	...	0.0095
	Calcd.	...	0.0098	...	0.0096
9.3×10^{-4}	Measd.	0.0099	0.0089	0.0086	0.0084
	Calcd.	...	0.0091	0.0087	0.0083

aH^+	As(III) Concn.	Time, Minutes							
		0	3	5	6	9	10	15	20
9×10^{-3}	Measd.	0.0099	...	0.0089	...	...	0.0083	0.0081	0.0076
	Calcd.	...	...	0.0093	...	...	0.0086	0.0080	0.0074
8×10^{-2}	Measd.	0.0099	0.0062	...	0.0045	0	...	...	...
	Calcd.	...	0.0065	...	0.0032	0[a]	...	...	...

[a] Calculated to be completely precipitated in 8.8 minutes.

gen was not bubbled through the reaction solution during the period of the run.

The samples were cooled immediately in an ice bath to quench the reaction; then about 0.01 gram of finely divided dry paper pulp was added and the mixture was centrifuged. This procedure was necessary because some arsenic(III) sulfide from the reaction tube passed through the sintered glass and could be removed by centrifugation only if paper pulp was present. A 10.00-ml. portion of the centrifugate was withdrawn by pipet and to this was added 1 ml. of $6VF$ hydrochloric acid. This acidified solution was heated in boiling water for 5 minutes.

The resulting precipitate of arsenic(III) sulfide was removed by centrifugation and the arsenic was estimated by the following procedure (7). The precipitate was dissolved in 1 ml. of $6VF$ sodium hydroxide and bromine water was added until an excess was indicated by a slight yellow color. The solution was heated in boiling water for 2 minutes, after which 3 ml. of water were added, followed by $6VF$ hydrochloric acid until a permanent bromine color appeared. Sodium hydroxide solution was added dropwise until the bromine color was discharged. Two milliliters of $6VF$ formic acid were added and the solution was heated in a water bath for 4 minutes. The solution was cooled, swept with carbon dioxide for 3 minutes, and washed into a 125-ml. flask which contained about 0.5 gram of potassium iodide in 0.5 ml. of water. Hydrochloric acid, $12VF$, was swept with carbon dioxide and a volume equal to that of the arsenic–potassium iodide solution was added. The resulting solution was cooled and titrated with standard sodium thiosulfate solution to the disappearance of the iodine color. Confirmatory determinations demonstrated that this procedure gave results accurate to within 1% with the quantities involved.

In the precipitation experiments made with solutions $6VF$ in hydrochloric acid, 10-ml. portions of the reaction solution were prepared from $12VF$ hydrochloric acid, the arsenious acid, and the thioacetamide solutions. These solutions were placed in tightly stoppered tubes which were suspended in a constant temperature bath of the desired temperature. The tubes were removed from the bath after timed periods, cooled in an ice bath, and centrifuged. A large excess of thioacetamide was added to the centrifugate, which was then heated to 90° C. in order to test for completeness of precipitation of the arsenic.

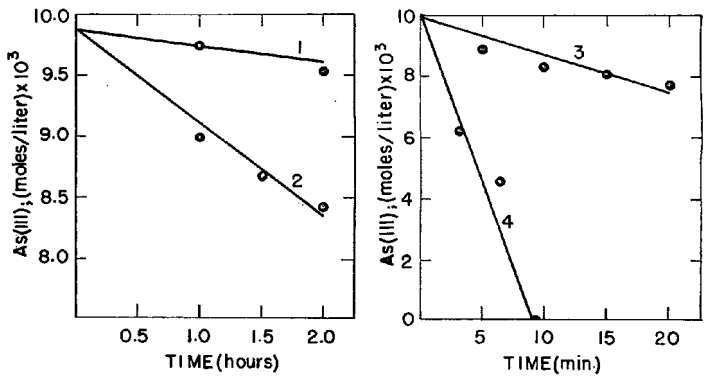

Figure 1. Calculated and measured concentrations of arsenic(III) vs. time at various hydrogen ion activities

Solid lines are calculated values.
Thioacetamide, $0.10VF$, 90° C.
Hydrogen ion activities
1. 1.6×10^{-4}
2. 9.3×10^{-4}
3. 9×10^{-3}
4. 8×10^{-2}

Data and Discussion. Precipitation of Arsenic(III) from Dilute Acid Solutions. The results of a series of precipitation experiments made in solutions with pH values ranging from approximately 1 to 4 are shown in Table I. There the analytically found arsenic(III) concentrations at various times are compared with arsenic(III) concentrations calculated upon the assumption that the hydrolysis of thioacetamide is unaffected by the arsenic(III) in solution, and that the hydrogen sulfide from the hydrolysis is removed quantitatively by precipitation of arsenic(III) as sulfide. The agreement between the analytical and calculated concentrations is within the limits of experimental error. In Figure 1 are plotted the data of Table I; there is no significant trend in measured concentrations. It appears that precipitation of arsenic(III) sulfide by thioacetamide at pH 1 to 4 proceeds through the hydrolysis of thioacetamide and there is no evidence of a direct reaction such as was found with lead(II).

In preliminary experiments a stream of one to two bubbles of nitrogen a

second was passed through the reaction solution during the reaction period. When this was done, the arsenic was precipitated more slowly than was calculated from the rate of hydrolysis. In precipitation of lead sulfide by hydrolysis of thioacetamide, such slow sweeping had a negligible effect upon the rate of formation of lead sulfide. This gives qualitative indication that the more acidic arsenic(III) is less rapidly precipitated by the hydrogen sulfide than is lead(II).

In solutions with pH values greater than about 4, the solubility of arsenic(III) sulfide increases rapidly with pH (*4*). The acid concentration at which the solubility commences to increase is a function of the total sulfide ($H_2S + HS^- + S^{--}$) concentration, and varies from about 10^{-4} for solutions saturated with hydrogen sulfide to 5×10^{-6} for solutions containing no added sulfide.

Quantitative rate measurements were not made in solutions having pH values above 4 because of the uncertainties resulting from the increasing solubility of the arsenic(III) sulfide and the very low rate of hydrolysis of thioacetamide. However, qualitative comparisons of the rates of precipitation of arsenic by thioacetamide in buffered solutions showed that rate of formation of precipitate continued to decrease as the pH was increased from 4 to 6.

Thus, unlike lead(II), arsenic(III) does not show a change of mechanism in this pH range in its reactions with thioacetamide to form the sulfide. Qualitative experiments have shown that other basic cations such as silver(I), cadmium(II), and copper(II), display evidence of a direct reaction with thioacetamide in the same pH range as does lead. However, antimony(III) is similar in its behavior to arsenic(III). Further work is being done in an effort to establish a general basis for this difference in behavior.

Precipitation of Arsenic(III) Sulfide from 6VF Hydrochloric Acid. Frequently arsenic is separated from other elements by precipitation with hydrogen sulfide from approximately 6*VF* hydrochloric acid solutions. Qualitative experiments were made to determine if the precipitation by thioacetamide is hydrolysis-controlled under such conditions.

The activity coefficient of 6*VF* hydrochloric acid is calculated from the data of Randall and Young (*3*) to be 4.3. With this information and the previously determined effect of temperature it is possible to obtain by extrapolation the rate of hydrolysis of thioacetamide at this acid concentration and at various temperatures.

Thus, if a solution is initially 0.03*VF* in thioacetamide, 6*VF* in hydrochloric acid, and 0.01*VF* in arsenic(III), and if the hydrogen sulfide reacts quantitatively with the arsenic as fast as it is formed, one can calculate by assuming an average thioacetamide concentration that at 40° C. approximately 10 minutes would be required for the production by hydrolysis of a quantity of hydrogen sulfide equivalent to the arsenic.

Experimentally, when several identical solutions of the above concentrations were maintained at 40° C. for various periods of time, the precipitation was incomplete after 8 minutes but complete after 10 minutes. Similar experiments were conducted at 20° C. with solutions 0.1*VF* in thioacetamide, 6*VF* in hydrochloric acid, and 0.01*VF* in arsenic(III). The calculated time was 16 to 17 minutes, and only a trace of arsenic remained in solution after 25 minutes. Thus, even with the approximations involved, the calculated time for complete precipitation was within 50% of the measured time.

These experiments, besides indicating that the precipitation of arsenic(III) sulfide by thioacetamide is hydrolysis-controlled even in 6*VF* hydrochloric acid, show that the hydrolysis of thioacetamide can be caused to proceed at room temperature at a significant rate by the use of suitable acid concentrations. Statements in the literature have implied that elevated temperatures are essential for the hydrolysis, and the effect of pH upon the hydrolysis rate has been overlooked.

Effect of Chloride Ion. Arcand (*1*) has shown that in 0.1*VF* hydrochloric acid the ratio of arsenious dihydroxychloride to arsenious acid is 9×10^{-4}. This ratio increases to about 33 in 6*VF* hydrochloric acid. Thus, in the quantitative rate measurements reported above, chloride-containing species of arsenic(III) were not significant, but in the qualitative experiments in 6*VF* hydrochloric acid there was a change of predominant species to the dihydroxychloride. There was no apparent change in the rate of precipitation of the sulfide at the higher acid concentration; this indicates that both of the above mentioned arsenic(III) species react with hydrogen sulfide rapidly as compared with the rate with which hydrogen sulfide is produced by hydrolysis.

Analytical Applications. These results make possible the calculation of the time required for quantitative precipitation of arsenic(III) as the sulfide by thioacetamide under various conditions. For example, with 100 ml. of a solution 0.10*VF* in thioacetamide, and containing 300 mg. of arsenic(III), the approximate times required for complete precipitation at various hydrogen ion activities and temperatures are given in Table II.

These calculated values show the importance of control of the temperature and acid concentration if arsenic(III) is to be quantitatively precipitated. Such calculations are invalid if the solution is boiled or an inert gas passed through it; under such conditions the hydrogen sulfide can be expelled from the solution so rapidly that no or only partial precipitation is obtained.

Table II. Calculated Time Required for Quantitative Precipitation of 300 Mg. of Arsenic(III) as Sulfide by Thioacetamide

(Volume, 100 ml.; thioacetamide, 0.10*VF*)

	Time, Minutes		
aH^+	90° C.	70° C.	60° C.
1	3	15	30
0.3	10	50	100
0.1	30	150	300

Thioacetamide has been extensively used as a substitute for hydrogen sulfide gas for the precipitation of the conventional hydrogen sulfide group elements from hot solutions approximately 0.3*F* in hydrochloric acid. The time and the thioacetamide required to obtain quantitative precipitation can be minimized by beginning precipitation in a small volume of solution and then diluting to the desired final volume and acid concentration. During the pretreatment the concentrations of thioacetamide and acid are correspondingly greater and advantage is taken of the fact that the rate of hydrolysis of the thioacetamide to give hydrogen sulfide is first order with respect to the concentrations of both the thioacetamide and the acid. When the solution is diluted to the final volume, thus decreasing the acid concentration, the dissolved hydrogen sulfide precipitates the more soluble sulfides, such as cadmium and lead. The solution should not be boiled during this pretreatment or hydrogen sulfide will be lost. The solution should be warmed, the container stoppered, and then heated in a bath of boiling water for the necessary time.

REACTIONS OF ARSENIC(V) WITH THIOACETAMIDE

Reagents. Solutions of arsenic(V) were prepared from reagent grade arsenic pentoxide and standardized by the following procedure:

A measured portion of the solution was swept for 3 minutes with a stream of carbon dioxide and then washed into a 125-ml. flask which contained 0.5 gram of potassium iodide dissolved in 1 to 2 ml. of water. The solution was cooled as an equal volume of 12*VF* hydrochloric acid previously swept with carbon dioxide was added. The resulting solution was titrated with standard sodium thiosulfate to the disappearance of the io-

dine color. The average deviation of four determinations by this procedure was 0.7 part per hundred. Experiments showed that the arsenic(V) solutions contained less than 0.1% of arsenic(III).

Magnesium ammonium nitrate reagent, 0.5*VF* in magnesium nitrate, 3*VF* in ammonium nitrate, and 0.2*VF* in ammonium hydroxide, was prepared from reagent grade chemicals.

Apparatus. The same apparatus was used as in the experiments with arsenic(III).

Procedure. The rate of reduction of arsenic(V) by thioacetamide was measured in the following way:

The reaction solution was prepared from the standard solutions of arsenic(V), hydrochloric acid, thioacetamide, sodium chloride, and water. The sodium chloride was added to maintain the chloride concentration constant at 0.1*VF*, except in certain qualitative experiments made in more concentrated hydrochloric acid. The reaction solution was placed in a heating bath and, when the desired temperature was reached, samples of the solution were taken at timed intervals. The samples were cooled rapidly in an ice bath and centrifuged to remove the suspended sulfur which passed through the sintered-glass plug in the sampling tube.

Two 5-ml. portions of the sample were taken by pipet and analyzed separately for arsenic(V). To each portion were added 2 ml. of 3*VF* ammonium chloride, 1 ml. of 6 *VF* ammonium hydroxide, and 2 ml. of the magnesium ammonium nitrate reagent. The solution was stirred vigorously and then allowed to stand for at least 2 hours. The tendency for magnesium ammonium arsenate to form supersaturated solutions made this latter treatment necessary. The precipitate was removed by centrifugation and washed twice with 2-ml. portions of the magnesium ammonium nitrate reagent. The centrifugate was removed in each case by a drawn glass capillary attached to an aspirator. The precipitate was dissolved in 5 ml. of 0.6*VF* hydrochloric acid, the resulting solution was swept for 3 minutes with carbon dioxide, and the arsenic(V) was determined iodometrically as described above.

Confirmatory experiments demonstrated that at least 99% of the arsenic(V) present was precipitated as magnesium ammonium arsenate by this procedure in the absence of thioacetamide; the possible effect of thioacetamide is discussed below.

Data and Discussion. Qualitative experiments showed that in solutions 0.3 to 1*VF* in hydrochloric acid, arsenic was precipitated appreciably faster by thioacetamide than by saturation with hydrogen sulfide under the same conditions. A white precipitate of sulfur was observed first; the quantity of white sulfur formed before appearance of any yellow precipitate indicated that the reduction of arsenic(V) was proceeding to a considerable extent before precipitation of a sulfide.

On the basis of these observations, quantitative measurements were made to determine the rate and order of the reaction involved in the reduction of arsenic(V) by thioacetamide.

Effect of Hydrogen Ion Concentration. Rate measurements were made on solutions initially 0.0100*VF* in arsenic(V), 0.100*VF* in thioacetamide, 0.10*VF* in chloride ion, and 0.0100 to 0.100*VF* in hydrochloric acid. In Figure 2 the arsenic(V) concentration is plotted logarithmically against the time for runs at various hydrochloric acid concentrations. The linearity of the plots indicates that the reduction reaction is first order with respect to the concentration of arsenic(V), and the rate constants shown in Table III indicate a first-order hydrogen ion dependence.

The range of acid concentrations considered was extended by qualitative experiments with solutions from 0.1 to 4*VF* in hydrochloric acid. The time required for the first appearance of a yellow sulfide in 0.1*VF* hydrochloric

Table III. Effect of Hydrochloric Acid Concentration upon Rate of Reduction of Arsenic(V) by Thioacetamide (TAA)

(Calculated constants for the expression $-\frac{d[\mathrm{As(V)}]}{dt} = k\,[\mathrm{TAA}][\mathrm{As(V)}][\mathrm{H^+}]$.) Initial concentrations. 0.100*VF* CH_3CSNH_2, 0.010*VF* As(V). 0.10*VF* total chloride. T = 90° C.

[HCl], Mole/Liter	$[As(V)]_1$, Mole/Liter	$[As(V)]_2$, Mole/Liter	Time, Interval, Min.	k, Liter²/Mole² Min.
0.0100	0.010	0.0037	20	50
0.020	0.010	0.0047	10	38
0.040	0.010	0.0040	6	38
0.080	0.010	0.0031	4.5	33
0.100	0.010	0.0036	3	34
			Av.*	36

* Does not include k for 0.010*F* HCl.

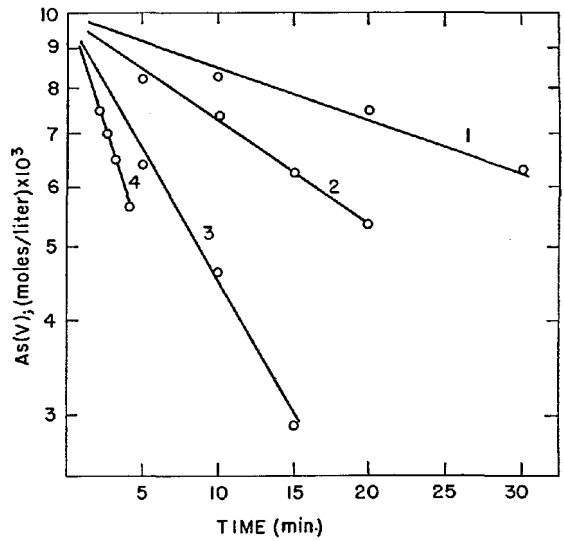

Figure 2. Effect of acid concentration on rate of reduction of arsenic(V)

Thioacetamide, 0.100*VF*; total chloride 0.10*VF*; 90° C.
Hydrochloric acid concentrations

1. 0.010*VF*
2. 0.020*VF*
3. 0.040*VF*
4. 0.080*VF*
5. 0.100*VF*

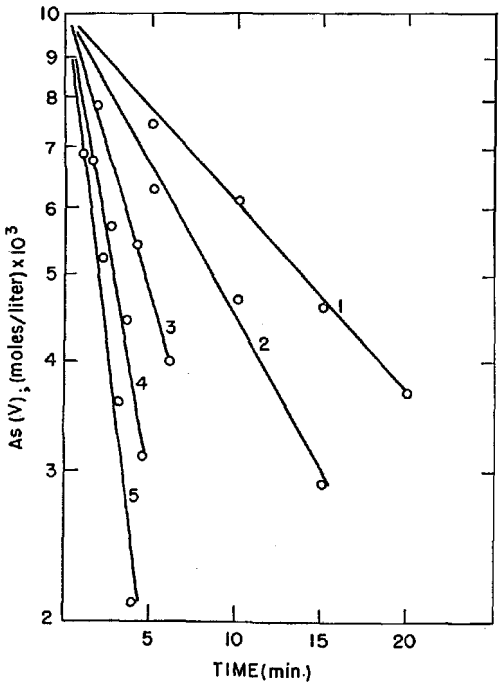

Figure 3. Effect of thioacetamide concentration on rate of reduction of arsenic(V)

Hydrochloric acid, 0.02*VF*; sodium chloride, 0.08*VF*; 90° C. Thioacetamide concentrations

1. 0.025*VF*
2. 0.050*VF*
3. 0.100*VF*
4. 0.200*VF*

tion; the extrapolated and observed values for several acid concentrations are shown in Table IV. The agreement indicates a first-order dependence upon the hydrogen ion activity through this range.

Qualitative experiments in the range pH 3.4 to 4.5 indicated that the dependence of the rate upon the hydrogen ion concentration was less than first order. Subsequent quantitative measurements yielded the rate constants shown in Table V when first-order and half-order hydrogen ion dependence were assumed.

A distinct trend is apparent even with the half-order assumption. A change in rate is to be expected as the hydrogen ion concentration is decreased below about $10^{-2}M$, since there is a change in the predominant arsenic(V) species. The first acid constant of arsenic acid is given as 5.6×10^{-3} (*5*); therefore the calculated ratio of arsenic acid to dihydrogen arsenate decreases from about 2 at pH 2 to 0.07 at pH 3.4. No further experiments were made in this low range of hydrogen ion concentrations, as analytically such precipitations are of little importance. At pH 2 the third-order rate constant is already above its value at higher acid concentrations (Table II). This is in agreement with the above discussion of the species involved, as at pH 2 about 33% of the arsenic(V) exists as dihydrogen arsenate.

Effect of Thioacetamide Concentration. In another series of experiments the initial concentrations of arsenic(V), acid, and chloride ion were kept constant while various thioacetamide concentrations were used. The results of these experiments, plotted in Figure 3, again demonstrate the first-order dependence of the reaction upon arsenic(V) concentration. Calculations made from the plots, presented in Table VI, indicate a first-order dependence of the reduction reaction upon the concentration of thioacetamide.

Mechanism of Reduction. The apparent first-order dependence of the rate of reduction upon the arsenic(V)

Table IV. Effect of Acid Concentration upon Time Required for Initial Sulfide Precipitation at 90° C.

[Initial concentrations. 0.1*VF* thioacetamide, 0.01*VF* arsenic(V)]

HCl, Moles/Liter	Calcd. Time, Sec.	Observed Time, Sec.
0.5	36	45
1.0	18	20–30
2.0	9	10–12
4.0	3	5

Table V. Calculated Rate Constants

For expressions $-\frac{d[\text{As(V)}]}{dt} = k_a[\text{H}^+][\text{TAA}][\text{As(V)}]$ and $-\frac{d[\text{As(V)}]}{dt} = k_b[\text{H}^+]^{1/2}[\text{TAA}][\text{As(V)}]$ in solutions having pH values from 3.4 to 4.5

pH	k_a	k_b
3.4	146	2.9
4.0	358	3.6
4.5	932	5.0

acid at 90° C. was approximately 180 seconds when the initial concentrations of thioacetamide and arsenic(V) were 0.1*VF* and 0.01*VF*, respectively. If it is assumed that dependence upon acid concentration is first order, and that quantitative reduction precedes sulfide precipitation, estimated times for the first formation of the sulfide can be obtained by extrapola-

Table VI. Effect of Thioacetamide Concentration upon Rate of Reduction of Arsenic(V)

Calculated constants for expression $-\frac{d[\text{As(V)}]}{dt} = k\,[\text{TAA}][\text{As(V)}][\text{H}^+]$. Initial concentrations. 0.02*VF* HCl, 0.010*VF* As(V), 0.08*VF* NaCl, T = 90° C.

$[CH_2CSNH_2]$, Mole/Liter	$[As(V)]_1$, Mole/Liter	$[As(V)]_2$, Mole/Liter	Time Interval, Min.	k, Liter²/Mole² Min.
0.025	0.010	0.0062	30	34
0.050	0.010	0.0053	20	32
0.100	0.010	0.0047	10	38
0.200	0.0074	0.0056	2	35
			Av.	35

concentration indicates that the reduction of arsenic(V) by thioacetamide is independent of the rate of hydrolysis of thioacetamide. Moreover, no yellow sulfide formed until the measured arsenic(V) concentration had dropped to less than 10% of its original value. These observations led to experiments designed to give information regarding the mechanism of the reduction of arsenic(V) and its subsequent precipitation.

Rate of arsenic(III) sulfide precipitation corresponds to the rate of hydrolysis of the thioacetamide. Moreover, even very slow sweeping of the solution with an inert gas caused a decrease in the rate of precipitation of the sulfide. Thus, it should be possible to prepare an acid solution which contains arsenic(III) and thioacetamide, heat this to a specified reaction temperature, sweep it vigorously with an inert gas to remove hydrogen sulfide, and thus prevent precipitation of the sulfide. Such an experiment was performed with a solution $0.01VF$ in hydrochloric acid, $0.10VF$ in thioacetamide, and $0.01VF$ in arsenic(III) at 90° C. No precipitate was obtained in 10 minutes when the solution was swept with a rapid stream of nitrogen, but immediate precipitation occurred when the flow of gas was stopped.

On the other hand, vigorous sweeping of the reaction solution with nitrogen should have no effect upon the rate of reduction of arsenic(V) by thioacetamide, if the reduction reaction depends not upon hydrolysis of the latter, but upon a direct reaction. Experiments showed that in solutions $0.02VF$ in hydrochloric acid, $0.10VF$ in thioacetamide, and $0.01VF$ in arsenic(V) the reduction of arsenic(V) proceeds during the first half of the reaction at a rate which is independent of whether or not the solution is swept with nitrogen. Thus a direct reaction is indicated.

Effect of Thioarsenic Acids. In the rate determinations after about half of the arsenic(V) initially present had been reduced, the arsenic(V) concentration decreased somewhat more rapidly than would be estimated by extrapolation of the straight-line portion of the semilogarithmic plot. It is recognized that arsenic(V) is reduced to some extent by hydrogen sulfide in acid solution (*2*) and that thioarsenic acids such as H_3AsO_2S are formed prior to the reduction; therefore the possibility that hydrogen sulfide from hydrolysis of the thioacetamide reacts with arsenic(V) was considered. If thioarsenic acids are formed, a significant difference should be observed in the rate at which hydrogen sulfide can be swept from an acid solution of thioacetamide containing arsenic(V) and that from a solution free of arsenic.

Table VII. Effect of Hydrogen Sulfide upon Arsenic Acid Solution

Solution	A	B	C
Composition, *VF*	*0.10 TAA*[a] 0.010 HCl	*0.010 As(V)* 0.010 HCl	*0.4* $CdCl_2$ 6 NH_4OH
Volume, ml.	20.0	20.0	15
Temperature, ° C.	90 ± 2	25	25
	Time, Min.		
Experiment I	5		
H_2S formed (calcd), 0.02 mmole			
Found by analysis, mmole		As(V) 0.19	Sulfide 0.01
Experiment II	30		
H_2S formed (calcd.), 0.12 mmole			
Found by analysis, mmole		As(V) 0.19	Sulfide 0.08

[a] Thioacetamide.

In experiments with solutions $0.02VF$ in hydrochloric acid and $0.10VF$ in thioacetamide, the rate at which hydrogen sulfide could be removed from the solution was found to be decreased by approximately 90% if the solution was also $0.01VF$ in arsenic(V). This observation, coupled with the apparent increase in the rate of reduction of arsenic(V) after the reaction was about half complete, indicates that hydrogen sulfide from the hydrolysis of thioacetamide reacts with the arsenic(V) in solution to form complex thioarsenic acids. This reaction is slow relative to the direct reaction between arsenic(V) and thioacetamide during the first half of the reduction, but becomes significant as the rate of the direct reaction decreases because of decreased arsenic(V) concentration and increased total hydrogen sulfide concentration.

It appeared of interest to determine whether the thioarsenic acids, once formed, would remain as such and whether during precipitation of magnesium ammonium arsenate the thioacid was reconverted to oxygen acid and sulfide.

Experiments were conducted in which a slow stream of air was drawn consecutively through three solutions (Table VII). In each experiment the total quantity of hydrogen sulfide formed by hydrolysis was calculated, and the quantity collected in solution C was determined iodometrically (*6*). Thus the quantity of hydrogen sulfide retained by solution B was obtained by difference. Immediately after the reaction was stopped, portions of solution B were analyzed by the magnesium ammonium arsenate procedure described above. In neither experiment was there a perceptible precipitate in solution B at the conclusion of the reaction period, but, in both cases there were precipitates of sulfur in remaining portions of solution B after approximately 1 hour. Subsequent boiling of the solutions did not cause the formation of additional precipitate.

In Experiment I all of the sulfide produced is accounted for, 0.01 mmole having passed into solution C and 0.01 mmole retained in B. In Experiment II 0.03 mmole of the sulfide produced is not accounted for in the analyses. A possible cause of this discrepancy is the formation of thioarsenic acids containing more than one atom of sulfur.

These and previous experiments show that the rate of formation of the thioarsenic acid from hydrogen sulfide is relatively fast compared to the subsequent decomposition of these acids. Pentapositive arsenic, present as a thio compound at the start of a magnesium ammonium arsenate precipitation, may not be quantitatively precipitated.

Effect of Chloride Concentration. In all the quantitative rate experiments discussed, the chloride concentration was maintained at $0.1VF$, in order that variations due to chloride complexes of arsenic(V) would not cause uncertainties in the results. As a check on the magnitude of the chloride effect, a rate determination was made in chloride-free perchloric acid solution, initially $0.10VF$ in thioacetamide, $0.010VF$ in arsenic(V), and $0.020VF$ in perchloric acid. The rate constant obtained from this experiment was 35 liter^2 mole^{-2} min.$^{-1}$, which is in good agreement with the values obtained from experiments made in chloride solutions. This indicates that at the acid concentration involved, chloride does not have a significant effect upon the rate of reduction of arsenic(V) by thioacetamide.

Effect of Temperature. A single rate measurement was made at 70° C. to determine whether the reduction of arsenic(V) by thioacetamide shows a normal temperature dependence. The third-order rate constant obtained at 70° C. is 6.3 which indicates an energy of activation of about 20 kcal. per mole. This is near the values obtained earlier in this study for other thioacetamide reactions.

Analytical Applications. In comparing the precipitation of arsenic(V) as sulfide by hydrogen sulfide and by thio-

acetamide, studies of the former method (*5*) have shown that if 50 ml. of solution, 0.6*VF* in hydrochloric acid, which contains 500 mg. of arsenic(V) is heated almost to boiling, saturated with hydrogen sulfide, diluted to 100 ml. and cooled, resaturated with hydrogen sulfide, and heated to 100° C. in a pressure bottle, quantitative precipitation of the arsenic will require at least 30 minutes in the pressure bottle.

By contrast it is calculated from the present study that in 100 ml. of solution, 0.3*VF* in hydrochloric acid and 0.5*VF* in thioacetamide, 500 mg. of arsenic(V) should be 99.9% reduced at 90° C. in approximately 1 minute and that sufficient hydrogen sulfide should be produced to precipitate the arsenic completely in about 6 minutes. (This allows for the loss by volatilization of 10 to 20% of the hydrogen sulfide from the relatively concentrated thioacetamide solution.) In experiments with a similar solution the arsenic was completely precipitated after 6 minutes under the above conditions. The time would be reduced to about 3 minutes if a pretreatment in 0.6*VF* hydrochloric acid were used.

Thioacetamide has been used at the California Institute of Technology in a recently developed system of elemental analysis as the precipitant for a sulfide group which included pentapositive arsenic. Much time is saved by the change from hydrogen sulfide, and the method has been consistently satisfactory in student use.

This investigation has emphasized again that reactions of thioacetamide and inorganic ions do not consist only of hydrolysis to give hydrogen sulfide, which then reacts with the inorganic ions. Studies of the reactions of thioacetamide with other inorganic ions are being continued.

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Potentiometric Determination of Mercaptans in Presence of Elemental Sulfur

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▶ In the potentiometric titration of mercaptans in gasoline using alcoholic silver nitrate, low results and poorly defined titration curves may be obtained with samples having a mercaptan–elemental sulfur ratio greater than 1 to 1, and when the sample remains in the alkaline alcoholic titration solvent for an appreciable time before the titration. Experimental results indicate that the poor results can be attributed to the presence of inorganic polysulfides whose formation is related to the amount of elemental sulfur and the mercaptan types. Because the postulated reaction for the formation of the inorganic polysulfide is promoted by the alkalinity of the solvent, a less alkaline solvent was employed and more accurate results were obtained. The reaction of elemental sulfur with *n*-butyl, *tert*-butyl, and phenyl mercaptans has been studied and a probable mechanism for the steps in this reaction has been postulated. Some evidence is presented for the existence of the $(RSS)^-$ ion, which is produced as an intermediate in the reaction.

THE POTENTIOMETRIC METHOD of Tamele and Ryland (*11*) for determining mercaptans (thiols) in gasoline is accurate, reliable, and uncomplicated in absence of elemental sulfur and hydrogen sulfide. Even in the presence of these materials, the determination can still be accurate for most samples, if the analyst is aware that certain precautions must be taken. This paper describes some of the complications encountered when elemental sulfur is present and recommends a slight modification, to minimize these difficulties.

The original method consists of potentiometrically titrating the sample dissolved in alcohol, containing 0.1*N* sodium acetate to buffer the solution, with an alcoholic solution of silver nitrate using a silver sulfide indicator electrode *vs.* a mercury–sodium acetate half cell. More recently a glass electrode has been employed as the reference electrode (*8*) in place of the mercury–sodium acetate half cell. External calomel cells have also been successfully used. These cells are electrically connected to the solution by an agar-saturated potassium nitrate bridge to avoid contaminating the solution with chloride ions from the calomel cell. In the presence of hydrogen sulfide and/or elemental sulfur erroneous results may be obtained unless the titration curve is correctly interpreted. When hydrogen sulfide is present, the initial potential between the silver electrode and the solution is approximately −0.7 volt (see Figure 1). As the silver ion is added to the solution, silver sulfide is precipitated and after all of the sulfide ion has reacted, the potential breaks sharply to the voltage characteristic of the particular mercaptan present. This voltage is influenced largely by the solubility product of the silver mercaptide in the solvent. For *n*-butyl mercaptan it is about −0.35 volt. Upon continued addition of silver ion to the solution, silver mercaptide begins to precipitate and finally another sharp "break" in voltage is observed, which corresponds to the end point of the mercaptan titration.

The presence of hydrogen sulfide presents no difficulty, as the volume of silver nitrate used to reach the first

break in voltage is taken as that required for the sulfide ion, and the volume of silver nitrate from that point to the last break in voltage is taken as that required for the mercaptan sulfur. In calculating the sulfide sulfur content, 0.016 is taken as the milliequivalent weight; 0.032 is taken to calculate the mercaptan sulfur content. When elemental sulfur is present in the sample with a mercaptan, a reaction occurs which is accelerated when the sample is placed in an alkaline titrating solvent. One of the products of the reaction is the sulfide ion or some material which reacts with silver ion to yield a sharp break in potential in the region of the sulfide break. Thus, when sulfur is present with the mercaptan, the titration characteristics of the sample are the same as though hydrogen sulfide were present. As the analyst does not know whether hydrogen sulfide or elemental sulfur is present, he must treat the sample to remove one or the other of these materials before a correct interpretation of the titration curve can be made.

Contact with mercury to remove elemental sulfur is not satisfactory, as this treatment partially removes the mercaptans, as shown by Schindler, Ayers, and Henderson (*10*). This finding was confirmed in this laboratory. The other alternative, removal of hydrogen sulfide by washing with acidified cadmium salts (*2, 5*), has been recommended by Davies and Armstrong (*3*).

This leaves elemental sulfur in the sample, which will react with the mercaptan in the alkaline solvent to yield two titration breaks. In calculating the mercaptan content, however, the first break is ignored and the total volume of silver nitrate used in titrating to the second break is employed with 0.032 as the milliequivalent weight.

Davies and Armstrong (*3*), in presenting the stoichiometric relationship between the elemental sulfur and the mercaptan, postulate the formation of an intermediate, sodium alkyl disulfide, which reacts with silver ion to produce silver sulfide and an organic trisulfide.

$$2S + 2RSNa \rightarrow 2RSSNa \quad (1)$$

$$2RSSNa + 2Ag^+ \rightarrow Ag_2S + R_2S_4 \quad (2)$$

At the first break, the titration corresponds to the reaction shown in Equation 2, while the second break corresponds to the reaction of silver ion with the remainder of the mercaptan sulfur.

$$Ag^+ + RSH \rightarrow RSAg + H^+ \quad (3)$$

This interpretation indicates two facts of analytical importance: (1) that the mercaptan content of a sample can be determined even though elemental sulfur is present, and (2) that elemental sulfur itself can be determined by measurement of the first break. In both cases 0.032 is used as the milliequivalent weight.

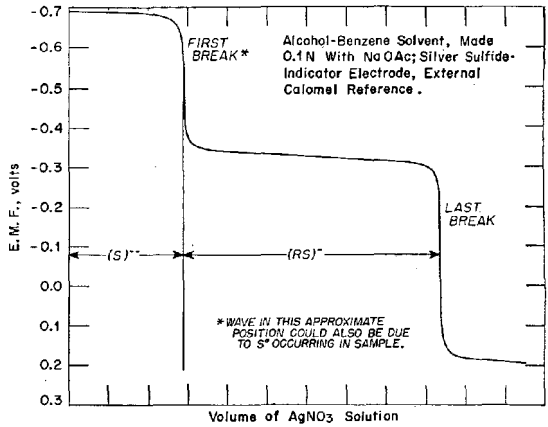

Figure 1. Typical titration curve of hydrogen sulfide and mercaptan with silver nitrate

In using this method with synthetic samples of many types it was found that the mercaptan content in many cases was low, and often the first break did not correlate with the amount of elemental sulfur present.

Consequently, an investigation was undertaken to explain some of the difficulties and to determine the conditions which would yield complete recoveries of mercaptans in various types of refinery samples. The practice of taking the sum of both breaks to obtain the mercaptan content is satisfactory for certain types of samples, but in other types when the ratio of mercaptan sulfur to elemental sulfur is less than 1 to 1, the titration must commence immediately after the sample is placed in the titration solvent; even after 5 minutes noticeably lower results are obtained (first part of Table I).

This effect may be largely eliminated by the use of a more acidic type of solvent. In the course of this work there was some experimental evidence to indicate the existence of the monoalkyd disulfide ion, $(RSS)^-$, as postulated by Davies and Armstrong (*3*). This ion is difficult to detect because its silver salt decomposes readily, the ion degrades to yield a sulfide ion on standing even in air-free systems, and the potential produced by this ion *vs.* the silver sulfide electrode is very nearly the same as that produced by the sulfide ion. Consequently, a break between these is not readily apparent. The stoichiometry of the reaction of mercaptide and elemental sulfur is not always that shown in Equation 2, as there is a difference in the degree to which various organic radicals retain sulfur atoms in forming organic polysulfides. Consequently, the practice of determining the sulfur content from the first break should be discouraged, except under highly controlled conditions, when the type of mercaptan is known and the mercaptan–elemental sulfur ratio is high.

METHOD, APPARATUS, AND MATERIALS USED

The procedure followed in carrying out these determinations was essentially that described by Tamele and Ryland (*11*), with the following exceptions.

A Recordomatic titrator (Precision Scientific Co.) was used. The silver sulfide–glass electrode system described by Lykken and Tuemmler (*8*) was employed for most of the experimental work; in certain cases an external calomel cell was substituted for the glass electrode. The exceptions are indicated. Calomel and glass cells are equal in performance in their application, although the initial voltages and the magnitude of the voltage breaks may differ slightly.

The mercaptans used were obtained from Eastman Kodak Co. (Distillation Products Industries), White Label grade. The synthetic blends were prepared by dissolving the mercaptan in isopropyl alcohol and using the actual mercaptan sulfur content as obtained from a potentiometric determination.

In preparing the titration solvents, a mixture of methanol, isopropyl alcohol, and benzene was substituted for ethyl alcohol, originally recommended by Tamele and Ryland, because this mixture could dissolve larger quantities of hydrocarbon samples. These materials were purified by silica gel percolation. Two titration solvents were used. The "regular" solvent was prepared by dissolving 13.7 grams of sodium acetate trihydrate in 20 ml. of distilled water and adding to it a mixture of 400 ml. of methanol and 400 ml. of isopropyl alcohol. Sufficient benzene was added to

Table I. Effect of Time and Sulfur Content upon Accuracy of Potentiometric Mercaptan Determination

Sample Mercaptan plus Rhombic Sulfur	Solvent	S Present in Sample, Mg.		Approx. Ratio, SH:S	Time, Min.	Volume of Titrant[a]			Total Mercaptan Found	
		Mercaptan	Elemental			Apparent sulfide	Apparent mercaptan	Total	Mg.	%
n-Amyl	Regular	2.47	0	Blank	5	..	7.70	7.70	2.47	100.0
					120	..	7.41	7.41	2.37	96.0
		2.47	5.04	1:2	5	6.32	0	6.32	2.02	82.0
					30	4.21	0	4.21	1.35	54.7
					60	3.00	0	3.00	0.96	38.9
					120	1.69	0	1.69	0.54	21.9
		2.47	2.52	1:1	5	7.17	0	7.17	2.30	93.0
					30	6.55	0	6.55	2.10	85.0
					60	6.12	0	6.12	1.96	79.4
					120	4.59	0	4.59	1.47	59.6
		2.47	0.81	3:1	5	4.47	3.18	7.65	2.45	99.3
					30	3.16	4.33	7.49	2.40	97.3
					60	5.06	2.40	7.46	2.39	96.8
					120	2.67	4.44	7.01	2.28	91.0
	Acidic	2.47	0	Blank	5	0.0	7.70	7.70	2.47	100.0
					120	0.0	7.70	7.70	2.47	100.0
		2.47	4.520	1:2	5	7.72	0.0	7.72	2.48	100.4
					30	7.50	0.0	7.50	2.40	97.2
					60	7.25	0.0	7.25	2.33	94.3
					120	6.50	0.0	6.50	2.09	84.6
		2.47	2.58	1:1	5	7.71	0.0	7.70	2.47	100.0
					30	7.65	0.0	7.65	2.45	99.2
					60	7.35	0.0	7.35	2.36	95.5
					120	6.95	0.0	6.95	2.23	90.3
		2.47	0.774	3:1	5	3.55[b]	4.18	7.73	2.48	100.4
					30	2.51[b]	5.21	7.72	2.48	100.4
					60	2.68[b]	5.04	7.72	2.48	100.4
					120	2.58[b]	4.87	7.45	2.40	97.2
Phenyl	Acidic	3.11	0	Blank	5	..	9.70	9.70	3.11	100.0
					120	..	8.92	8.92	2.86	92.0
		3.11	6.05	1:2	5	9.82	0.0	9.82	3.15	101.3
					30	9.63	0.0	9.63	3.09	99.4
					60	9.04	0.0	9.04	2.90	93.2
					120	8.48	0.0	8.48	2.72	87.5
		3.11	3.03	1:1	5	9.88	0.0	9.88	3.17	101.9
					30	9.54	0.0	9.54	3.06	98.4
					60	9.20	0.0	9.20	2.95	94.9
					120	8.48	0.0	8.48	2.72	87.5
		3.11	1.01	3:1	5	5.10	4.70	9.80	3.14	101.0
					30	4.50	4.90	9.40	3.01	96.8
					60	4.88	4.32	9.20	2.95	94.9
					120	4.0	4.58	8.58	2.75	88.4
tert-Butyl	Acidic	3.25	0	Blank	0	..	10.12	10.13	3.25	100.0
					120	..	10.06	10.09	3.24	99.7
		3.25	6.05	1:2	5	0.0	10.19	10.19	3.27	100.6
					30	7.18	3.01	10.19	3.27	100.6
					60	6.90	3.11	10.05	3.22	99.1
					120	6.50	2.95	9.45	3.03	93.2
		3.25	3.03	1:1	5	0.0	10.40	10.40	3.33	102.5
					30	4.05	6.15	10.20	3.27	100.6
					60	3.85	6.25	10.10	3.24	99.7
					120	3.68	6.26	9.94	3.19	95.7
		3.25	1.01	3:1	5	0.0	10.32	10.32	3.31	101.8
					30	0.0	10.18	10.18	3.26	100.3
					60	0.60	9.60	10.10	3.24	99.7
					120	1.02	8.91	9.93	3.18	97.8
		3.19	12.6	1:4	5	9.77	0	9.77	3.13	98.2
					30	9.71	0	9.71	3.11	97.5
					60	9.65	0	9.65	3.09	96.9
					120	8.44	0	8.44	2.71	84.8

[a] Alcoholic 0.01*N* $AgNO_3$.
[b] Poor breaks.

bring volume to 1 liter. The "acidic" solvent was prepared by dissolving 13.7 grams of sodium acetate trihydrate and 6 ml. of glacial acetic acid in 500 ml. of methanol and diluting to 1 liter with benzene. Although isopropyl alcohol and a small amount of water were used in the regular solvent employed in this particular study, these are not essential and a simple regular solvent of methanol, benzene, and sodium acetate would be equivalent.

TITRATION OF MERCAPTANS IN PRESENCE OF ELEMENTAL SULFUR

The first series of experiments was designed to establish a base case and to demonstrate the difficulties that may arise under extreme conditions with the alkaline (regular) solvent. In this experiment, a series of synthetic samples was prepared containing *n*-amyl mercaptan and elemental sulfur in different mole ratios. A portion of each of these blends was dissolved in the alkaline titration solvent and titrated 5, 30, 60, and 120 minutes after being put into solution (first portion of Table I). The volume of silver nitrate required to reach the first and last breaks are shown together with the total mercaptan recovery as calculated by taking the total silver nitrate titer and using 0.032 as the milliequivalent weight. As the ratio of elemental sulfur to mercaptan sulfur and the length of time of standing in the alkaline titration solvent increase, the poorer are the recoveries. As long as elemental sulfur was equal to or greater than the mercaptan sulfur content, all of the titratable material was found at the sulfide break. When mercaptan sulfur was in excess, two breaks were obtained. In the solutions exposed to air the correlation of the first break with the amount of elemental sulfur appears to be erratic.

After a series of preliminary experiments it was observed that the poor results were related in some way to the alkalinity of the solution. It was then decided to employ a less basic solvent to carry out the titration. It could not be too acidic because of the possibility of losing the mercaptans of low molecular weight or increasing the solubility of the silver mercaptides. The solvent selected was the same as used in the polarographic determination of elemental sulfur and other sulfur compounds (*7*) whose preparation has been described.

Various blends of the three different types of mercaptans with elemental sulfur were prepared, which varied from a mercaptan sulfur–elemental sulfur ratio of 1:4 to 3:1. Portions of each of these blends were placed in the acidic titrating solvent and the titration was started 5, 30, 60, and 120 minutes after the addition of the sample to the solvent. The volumes of 0.01*N* silver nitrate solution required to reach both the first and last break were recorded. The mercaptans employed in this series were *n*-amyl mercaptan, phenyl mercaptan, and *tert*-butyl mercaptan.

From these data shown in Table I it may be seen that the acidic solvent minimizes the errors obtained even under the drastic conditions of high elemental sulfur content and exposure to air oxidation. Although the acidic solvent gives better results for the mercaptan content, it is not recommended in determining elemental sulfur.

The foregoing experiments were carried out in open titration vessels, because open vessel titrations are commonly employed in many service laboratories. Initially, it was thought that the poor recoveries were entirely due to the oxidation of sodium sulfide to sodium polysulfide and sodium thiosulfate. These latter two materials also titrate with silver nitrate in the regular solvent but yield poorly defined curves which may confuse the interpretation. Furthermore, sodium polysulfide does not react with silver nitrate in the same stoichiometric ratio as does sodium sulfide. This was illustrated by titrating a freshly prepared solution of sodium sulfide with silver nitrate in the regular solvent and comparing the results with those obtained when the same solution with added sulfur (1 to 1 mole ratio) was titrated 5, 30, 60, and 120 minutes after standing. Table II shows that in presence of elemental sulfur the sulfide recovery is low and becomes lower as time of standing increases.

Table II. Reaction of Sodium Sulfide and Elemental Sulfur in Alkaline Solvent

Solvent. Regular
Sample. Sodium sulfide and rhombic sulfur added to 100 ml. of titration solvent

Sample Composition, Mg.		Reaction Time,	Sulfide Sulfur Found	
Sulfide sulfur	Elemental sulfur	Min.	Mg.	%
2.24	0	5	2.24	100
		120	2.17	96.9
2.24	2.52	5	1.28	57.1
		30	0.70	31.2
		60	0.59	26.3
		120	0.32	14.3

TITRATION IN ABSENCE OF AIR

In order to determine whether oxidation was the only cause of the poor recoveries, a series of experiments was conducted under air-free conditions. As may be seen in Table III, even in the absence of air, the mercaptan recoveries are low when an excess of sulfur is present and losses become greater as the length of time increases. The losses in air however, were greater.

While the elimination of air from the titration did not produce complete recoveries, it minimized the formation of sodium polysulfide and sodium thiosulfate and hence allowed more information to be obtained from the titration curves. Sodium polysulfide and sodium thiosulfate produce a series of breaks in both titration solvents which obscure other end points and make interpretations of the intermediate portions (as reported in Table I) of the titration curve extremely doubtful.

Table III. Effect of Exclusion of Air upon Potentiometric Determination of Mercaptan

Titration solvent. Regular, 50–50 volume % isopropyl alcohol–benzene made 0.1*N* with sodium acetate
Sample. 3.975 mg. of *n*-amyl mercaptan sulfur + 8.028 mg. of rhombic sulfur (approximate ratio SH:S = 1:2)
Solutions blown with nitrogen before mixing and blanketed after mixing and during storage period

Time of Standing, Min.	Mercaptan Recovered, % In air[a]	In nitrogen
5	82.0	90.0
30	54.7	83.1
60	38.9	75.7
120	21.9	69.7

[a] Data taken from Table I.

Synthetic solutions of a mercaptan and elemental sulfur (3 to 1 mole ratio) were prepared in a nitrogen atmosphere and allowed to stand in the regular solvent for 5, 30, 60, and 120 minutes before titration under a nitrogen blanket. The compounds used were phenyl, *n*-butyl, and *tert*-butyl mercaptans (Table IV). In Figure 2 titration curves for only the *tert*-butyl mercaptan mixtures are shown. The titration breaks of the remaining two mercaptans, while showing similar characteristics, were not as well defined as *tert*-butyl mercaptan. The lack of resolution of these curves is probably due to the presence of the polysulfide ion, which can form in this solution even in absence of air.

From Figure 2 it may be seen that the 5-minute titration curve possessed only two breaks; one with an end point in the vicinity of −0.43 volt and the other at −0.05 volt. On standing 30 minutes an additional break is visible at

Table IV. Effect of Mercaptan Type and Storage Time upon Accuracy of Potentiometric Mercaptan Determination

Air-free system
Regular titration solvent
Ag_2S–ext. calomel electrodes
Approximate mercaptan–elemental sulfur ratio, 3:1

Sample	Length of Standing, Min.	Volume of 0.01*N* $AgNO_3$ Ml. to Titrate 1 (S)$^{--}$	2 (RSS)$^{-}$[a]	3 1 + 2	4 (RS)$^{-}$	5 3 + 4	Total Sulfur Found Mg.	%
tC_4SH + S° (4.42 mg. mercaptan sulfur, 1.535 mg. elemental sulfur)	5	0.00	3.23	3.23	10.46	13.69	4.39	99.3
	30	0.36	2.58	2.94	10.76	13.70	4.39	99.3
	60	0.80	1.60	2.40	10.90	13.30	4.26	96.5
	120	1.02	0.90	1.92	11.03	12.95	4.15	93.9
nC_4SH + S° (5.00 mg. mercaptan sulfur, 1.616 mg. elemental sulfur)	5	0.00	5.78	5.78	9.76	15.54	4.98	99.6
	30	2.15[b]	3.67	5.82	9.99	15.81	5.06	101.2
	60	3.30[b]	2.38	5.68	9.27	14.95	4.79	95.9
	120	4.30[b]	1.72	6.02	8.98	15.00	4.81	96.1
ϕSH + S° (6.18 mg. mercaptan sulfur, 2.046 mg. elemental sulfur)	5	[c]	[c]	11.55	7.30	18.85	6.04	97.8
	30	[c]	[c]	12.03	5.27	17.30	5.54	89.7
	60	7.52	2.48	10.00	6.24	16.24	5.20	84.2
	120	7.02	2.29	9.31	6.92	16.23	5.20	84.2

[a] Material titrated by intermediate break, postulated to be $(RSS)^-$.
[b] Poor break, making values for $(S)^{--}$ and $(RSS)^-$ somewhat doubtful.
[c] Very poor break, making values for $(S)^{--}$ and $(RSS)^-$ unreliable.

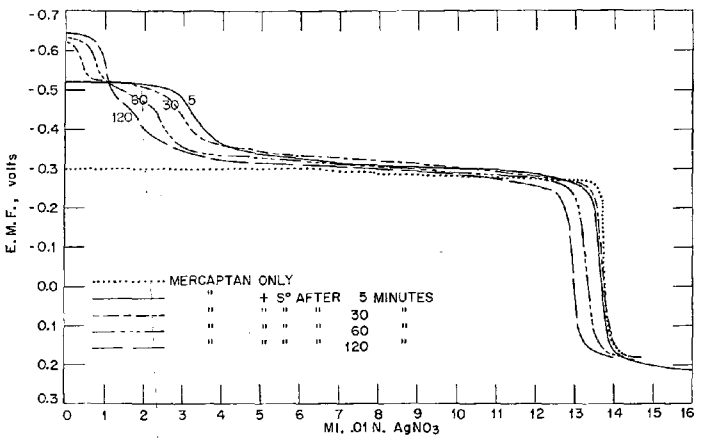

Figure 2. Titration curve of *tert*-butyl mercaptan in presence of sulfur

Regular titration solvent
Silver sulfide–calomel electrodes
Titrant, 0.001*N* silver nitrate
Mercaptan–elemental sulfur ratio, 3 to 1
In absence of air

−0.57 volt; at 60 minutes the material, causing the break which occurs at about −0.57 volt, has increased, and at 120 minutes its concentration has increased still further. Concurrent to this change the concentration of the material causing the second break (approximately −0.43 volt) is decreasing. This same trend may be observed in the data (Table IV) for the other two mercaptans.

In order to determine if the sulfide ion were involved in this change, a portion of the *tert*-butyl mercaptan and sulfur mixture that had been allowed to stand 120 minutes was purged with nitrogen for 10 minutes and hydrogen sulfide was identified in the effluent. The titration curve of this purged sample reflected a substantial lowering of the titer required to reach the first break. Purging of a portion of the sample that had only stood 5 minutes revealed no significant loss of hydrogen sulfide and no essential change in the amount of titer to reach the break at −0.43 volt. It thus appears that the first break is caused by the presence of sulfide ion and the intermediate break is caused by some compound which cannot be removed from the solution by 10-minute nitrogen blowing. The sulfide was not present immediately, but required some time to form. These facts tend to support the Davies and Armstrong (*3*) postulation that sulfur plus a mercaptide yields $(RSS)^-$, and to indicate that this $(RSS)^-$ ion will decompose to a sulfide on standing even in absence of air.

INTERPRETATION OF EXPERIMENTAL RESULTS

Chemistry Involved. Not too much is definitely known about the reaction of elemental sulfur and mercaptans in an alkaline solution. In a comprehensive review of the chemistry of mercaptans, all that Malisoff, Marks, and Hess (*9*) have to say about it is that the reaction is "surmised" to be

$$2\,RSH + 2S \rightarrow R_2S_3 + H_2S \qquad (4)$$

Faragher, Morrell, and Monroe (*5*) note that elemental sulfur, mercaptans, and sodium hydroxide react to form sodium sulfide and an organic disulfide. Holmberg (*6*) states that ethyl mercaptan dissolved in an alkaline solution produces ethyl disulfide when sulfur is added.

The reaction presented by Davies and Armstrong (Equations 1 and 2) is of interest, as it can serve as a starting point to interpret certain experimental observations herein reported.

Equation 1 may be written in the ionic form

$$(RS)^- + S° \rightarrow (RSS)^- \qquad (1')$$

This ion may react with silver ion to form the unstable silver compound

$$(RSS)^- + Ag^+ \rightarrow AgSSR \qquad (5)$$

which decomposes

$$2\,AgSSR \rightarrow Ag_2S + RSSSR \qquad (6)$$

The sum of Equations 1′, 5, and 6 is

$$2(RS)^- + 2S° + 2Ag^+ \rightarrow Ag_2S + RSSSR \qquad (7)$$

which is identical with the sum of Equations 1 and 2.

However, if a solution is allowed to stand (without the silver ion and in absence of air) sulfide ions are produced, as was shown experimentally. This reaction could be represented

$$2(RSS)^- \rightarrow (S)^{--} + RSSSR \quad (8)$$

As shown experimentally (Table II), this sulfide ion could combine with elemental sulfur

$$(S)^{--} + S^\circ \rightarrow (SS)^{--} \quad (9)$$

to form a polysulfide ion (disulfide ion in this case) and if there is a sufficient excess of elemental sulfur the trisulfide or tetrasulfide ion probably forms. It is not known which of the polysulfide ions, $(SS)^{--}$, $(SSS)^{--}$, or $(SSSS)^{--}$, yields poorer recoveries. Presumably the poorer recoveries are associated with the higher polysulfides, and each ionic specie could yield a slightly different titration break with silver ion.

Another mechanism by which the existence of polysulfide ions in the mixtures can be postulated is as follows. The monoalkyl disulfide ion, $(RSS)^-$, may be able to add elemental sulfur to form a monoalkyl trisulfide ion

$$(RSS)^- + S^\circ \rightarrow (RSSS)^- \quad (10)$$

This material on standing could decompose

$$(RSSS)^- \rightarrow (SS)^{--} + (RS)^+ \quad (11)$$

The $(RS)^+$ could combine with some of the negative ions

$$(RS)^+ + (RSS)^- \rightarrow RSSSR \quad (12)$$

These mechanisms are largely speculative, as there is no physical evidence proving the existence of the $(RS)^+$ and $(RSS)^-$ ions. However, some experimental data support the validity of Equations 7 and 8, as polarograms of mercaptan sulfur and elemental sulfur obtained with the acidic solvent indicated the presence of organic polysulfides. A trisulfide was actually identified as the product in Equation 7, using the polarographic techniques described by Karchmer and Walker (*7*), when *n*-butyl mercaptan and elemental sulfur were used.

These equations indicate possible routes by which polysulfide ions could form in the solutions, as it was shown that the presence of polysulfide ions is related to the poor recoveries. In the presence of air the polysulfide would further oxidize to thiosulfate and complicate the titration still more.

Equation 9 is accelerated in presence of a base; hence in the more acidic solvent, one may expect a decrease in the amount of polysulfide ion produced, which is consistent with the fact that in the acidic solvent better recoveries are obtained. By analogy the reaction shown in Equation 10 could be minimized in the acidic solvent.

In order to determine whether the existence of polysulfide ions was dependent upon the presence of mercaptans, elemental sulfur without any mercaptan was allowed to stand in the regular solvent 5, 30, 60, and 120 minutes before titration with silver nitrate. Although a discoloration of the solution was observed after the addition of a small amount of silver nitrate, the potential observed in all cases was lower than +0.2 volt, which is more positive than the final potential obtained for the mercaptan titration. Thus in 2 hours the reaction of the elemental sulfur with the regular solvent would not be a significant factor. The results of this experiment were essentially the same when it was repeated in presence of ammonium hydroxide which was added to minimize the formation of silver oxide.

Effect of Organic Substituent of Mercaptans. In this work there was some experimental evidence of differences in the behavior of the various mercaptans. It was suspected that the stoichiometry of the reaction shown in Equation 7 may not be the same for all mercaptan types. For example, Eby (*4*) showed that tertiary mercaptans could form stable tetrasulfides in presence of an alkaline sulfide solution, whereas *n*-butyl tetrasulfide was less stable. Work by Karchmer and Walker (*7*) and Birch, Cullum, and Dean (*1*) has indicated a difference in the degree to which various organic radicals in polysulfides retain sulfur atoms. Thus the end product in Equation 7 would not necessarily be a trisulfide and the amount of sulfur consumed could be more or less than indicated. When R is a normal alkyl, Equation 7 is essentially correct and the organic trisulfide is formed; however, when R is a tertiary alkyl group, such as *tert*-amyl or *tert*-butyl, the reaction could be

$$2(tC_4S)^- + 3S^\circ + 2Ag^+ \rightarrow tC_4SSSStC_4 + Ag_2S \quad (13)$$

when R is a phenyl group the over-all reaction may be

$$2(\phi S)^- + S^\circ + 2Ag^+ \rightarrow \phi SS\phi + Ag_2S \quad (14)$$

This is supported by data in Table IV, which show that when a 3 to 1 ratio of *tert*-butyl mercaptan sulfur to elemental sulfur is used, the amount of unreacted mercaptan present (after 5 minutes) equals 76.5% (10.46 ml. out of a total of 13.69 ml. of silver nitrate used). According to Equation 13, when 9 moles of mercaptan are used with 3 moles of elemental sulfur, only 2 moles of the mercaptan should be converted to a sulfide, leaving 7/9 or 77.8% of mercaptan unreacted, which is in close agreement with the experimental data. With *n*-amyl mercaptan, which reacts as shown in Equation 7, 1 mole of mercaptan should react with 1 mole of elemental sulfur; thus at a 3 to 1 mercaptan–elemental sulfur ratio, 2 of the 3 moles of mercaptan should be unreacted for a theoretical 66.7%. From Table IV it may be computed that at the end of 5 minutes, 9.76/15.54 or 62.8% of mercaptans remained. By this same line of reasoning the amount of excess phenyl mercaptan sulfur remaining when 3 to 1 mixtures are used should be 1 mole out of 3 or 33.4%, according to Equation 14. The data in Table IV for phenyl mercaptan indicate that 7.30/18.85 or 38.8% of unreacted mercaptan sulfur was present at the end of 5 minutes. While the agreements in the latter two cases were not as good as that obtained with the *tert*-butyl mercaptan, the trend is nevertheless apparent.

From the differences in the observed reactions of elemental sulfur with various mercaptans certain deductions may be made. As di-*tert*-butyl tetrasulfide is a reasonably stable compound, its formation uses the excess elemental sulfur which would otherwise promote the formation of inorganic polysulfides deleterious to the titration. As the di-*n*-butyl tetrasulfide is less stable than the di-*n*-butyl trisulfide and the diphenyl trisulfide is less stable than the diphenyl disulfide, increasing amounts of inorganic polysulfides may form with the same mercaptan to elemental sulfur ratio as one progresses from *tert*-butyl to *n*-butyl to phenyl. It would also follow that the so-called unstable organic polysulfides could react with other mercaptans.

CONCLUSION

In the potentiometric titration of mercaptan sulfur with silver nitrate using the regular solvent (alcohol-benzene containing sodium acetate) the presence of elemental sulfur in the sample in a mole ratio greater than 1 to 1 causes low results if the sample remains for significant periods of time in the titration solvent before the titration is begun. It is believed that the alkalinity promotes the formation of inorganic polysulfides which cause the low results and poorly defined titration curves. In presence of air the deleterious effects produced by excessive amounts of sulfur are accentuated. The behavior of several mercaptans in the presence of elemental sulfur has been studied in a more acidic solvent and it was shown that reasonable recoveries could be obtained even in presence of

air under rather extreme conditions of sulfur content and times of standing.

The routine use of this acidic-type solvent is recommended only for mercaptan samples known to contain a relatively large amount of elemental sulfur. For samples containing little or no elemental sulfur the regular titration solvent may be satisfactorily used. In fact, the use of the more basic solvent is mandatory when it is desired to determine both hydrogen sulfide and mercaptans on a sample which does not contain elemental sulfur. It is recommended that a nitrogen blanket be used with both solvents as a precautionary measure.

In the experimental studies there were indications that the initial product of the reaction of elemental sulfur and mercaptan sulfur was the monoalkyl disulfide ion, $(RSS)^-$, which decomposed to the sulfide ion on standing. Additional standing time in presence of excess elemental sulfur promoted the formation of inorganic polysulfides. In studies showing the effect of the organic substituent groups of the mercaptan it was indicated that phenyl, *n*-butyl, and *tert*-butyl groups tended to form decreasing amounts of inorganic polysulfides in that order.

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Quantitative Infrared Analysis of Apatite Mixtures

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▷ The quantitative determination of fluorapatite in hydroxyapatite is difficult, if not impossible, by conventional chemical and even crystallographic methods. Two infrared methods for accomplishing this determination involve spectral differences in the 16- to 18-micron region. One employs a base-line technique, and the other is an area-measurement method. The two methods yield results with average errors of 1.5 and 1.2% fluorapatite in the sample, respectively.

With the innovation of the pressed disk technique simultaneously by Stimson and O'Donnell (*8*) and Schiedt and Reinwein (*7*), a new approach to the infrared study of solids was opened. Nujol had formerly been used as the common mulling agent, but it is seldom satisfactory for quantitative work. However, quantitative work may be more feasible with the newer pressed disk technique.

Infrared spectrophotometry has been applied to the study of apatites in a few instances. Posner and Duyckaerts (*6*), Pobeguin (*5*), and Underwood, Toribara, and Newman (*10*) have studied bone and tooth enamels, which consist in large part of apatites, but with particular emphasis upon the carbonate content. The spectra of a variety of compounds containing combined phosphorus and calcium or magnesium have been studied in the 2- to 15-micron range by several investigators (*1–4*). These studies have led to the identification of certain absorption bands as due to P-O, P-OH, P-F, and P-Cl linkages. However, the 15- to 24-micron range has not been investigated for bands due to these compounds and linkages.

In conjunction with some studies of the effects of fluoride treatment upon the structure of dental enamel, it became desirable to devise a quantitative procedure for the analysis of apatite mixtures. Of particular interest is the determination of fluorapatite in the presence of dental enamel, which is essentially hydroxyapatite. This determination is difficult, if not impossible, by conventional chemical methods; it is not expected that any crystallographic method could possibly detect less than 17% fluorapatite in dental enamel (*9*). This paper presents a quantitative infrared method for the determination of fluorapatite in the presence of hydroxyapatite.

EXPERIMENTAL METHODS

A Perkin-Elmer Model 21 infrared spectrophotometer was used with sodium chloride and potassium bromide prism interchange units to provide complete coverage of the 2- to 24-micron region. All but preliminary work was done in the "KBr region," 11 to 24 microns. Pressed disk specimens, about $1 \times 1/4$ inch, were formed in a homemade evacuatable die under a pressure corresponding to 40,000 pounds per square inch. Potassium iodide was used as the matrix material rather than the more conventional potassium bromide, because the former has an index of refraction closer to that of the apatites and it absorbs water less readily.

Standard samples were prepared with known amounts of the two apatites. The fluorapatite was a naturally occurring one. The hydroxyapatite was actually powdered dental enamel, which may have contained a slight trace of fluorapatite, but this material may be considered to be hydroxyapatite in studying the portions of the infrared spectra included in all figures in this paper. Each synthetic mixture was ground in an agate mortar for 1 or 2 hours and then mixed with previously ground and dried potassium iodide for a few minutes' further grinding. Each sample contained 0.8 mg. of the apatite mixture and 40 mg. of potassium iodide.

The apparent transmittance of each sample was normally low, but it was increased adequately without noticeably enhancing random noise by placing a metal screen in the path of the reference beam.

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EXPERIMENTAL RESULTS

Spectra. Preliminary study of the complete infrared absorption spectra from 2 to 24 microns revealed that the best possibility for basing an analytical determination lay in the longer wave length region. Absorption spectra of the fluorapatite and the hydroxyapatite in the "KBr region" are shown in Figure 1. In Figure 2 are shown, expanded and superimposed, the 16- to 18-micron portions of both spectra. It appears that the substances may be distinguished from each other by the shift of the 17.5-micron fluorapatite band to 17.8 microns for hydroxyapatite, with an accompanying difference in the relative shapes of the 16.6- and 17.5 to 17.8-micron bands.

Base-Line Method. On a double-beam instrument, the effect of random scattering by the specimen is not cancelled out, so the transmittance apart from specific absorbances of the sample does not equal 100%. In the base-line technique, which is intended to correct for this scattering effect, absorbances are measured from a line connecting the transmittances on both sides of the absorption band to be measured, Figure 3. The absorbance, A, at any particular wave length is related to the indicated quantities, I and I_0, by the equation,

$$A = \log \frac{I_0}{I}$$

Twenty-one synthetic mixtures containing from 0 to 100% fluorapatite were prepared, and their spectra were obtained in this region. The absorbances were measured at 17.8 and 16.6 microns, and the ratio of absorbances was plotted against composition of the sample (Figure 4). Considering the error of each sample as the difference between its known per cent fluorapatite and its per cent fluorapatite as read from this calibration curve, the average error is about 1.5% fluorapatite in the sample. Other ratios were calculated of absorbances measured at other wave lengths in this region. Some provided results as good as the ratio used in Figure 4,

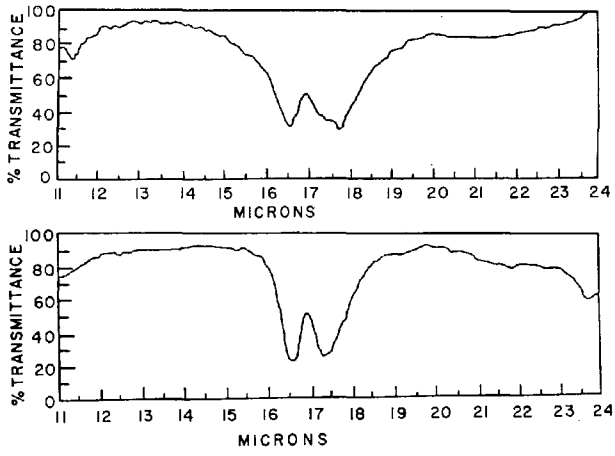

Figure 1. Spectra of hydroxyapatite (top) and fluorapatite (bottom)

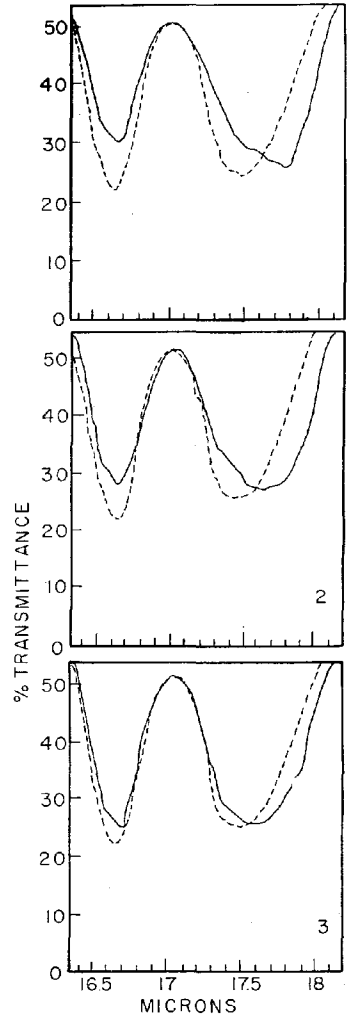

Figure 2. Enlarged portions of spectra

1. Hydroxyapatite (solid line) and fluorapatite (broken line)
2. Mixture of 75% hydroxyapatite and 25% fluorapatite (solid line) and fluorapatite (broken line)
3. Mixture of 50% hydroxyapatite and 50% fluorapatite (solid line) and fluorapatite (broken line)

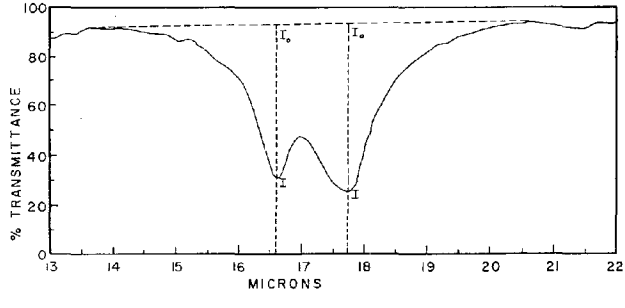

Figure 3. Spectrum diagram showing base-line measurements

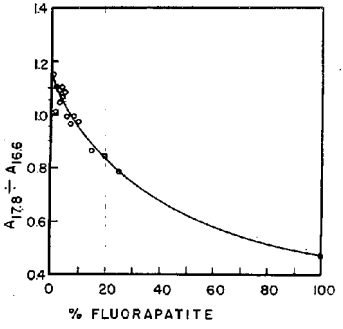

Figure 4. Calibration curve for base-line method

others were worse, but none were better. This base-line method is empirical, but it makes possible the determination of fluorapatite in the presence of hydroxyapatite (dental enamel) with an average error of about 1.5% fluorapatite in the sample. Pure fluorapatite is only about 3.8 weight % fluoride, and the data of Figure 4 and elsewhere in this paper are expressed as per cent fluorapatite, not as per cent fluoride.

Area-Measurement Method. When an attempt was made to use the base-line method for samples containing calcium fluoride along with the apatites, considerable difficulty was encountered. Calcium fluoride exhibits a very broad absorption band which overlaps this portion of the spectrum sufficiently to make it impossible to locate the base line. Therefore, it became necessary to devise another quantitative method not requiring location of the base line. A method based upon area measurement was devised.

In Figure 5 are shown superimposed spectra from 16 to 19 microns of fluorapatite and hydroxyapatite (dental enamel). A horizontal line is drawn tangent to the intersection of the 16.6- and 17.5- to 17.8-micron bands, and the two enclosed areas are marked *A* and *B*. It is evident that the ratio of *B* to *A* decreases as the ratio of fluorapatite to hydroxyapatite increases.

Spectra were obtained of synthetic apatite mixtures, from which areas *A* and *B* were measured with a compensating polarimeter. The observed ratios of *B* to *A* are plotted against percentage fluorapatite in the mixture in Figure 6. The average deviation of repeated measurements on a given disk was found to correspond to 0.8% fluorapatite in the sample. From the data on the known samples and the compositions as read on the calibration curve, the average error for a fluorapatite determination is found to be about 1.2% fluorapatite in the sample.

In the course of this work, many data were collected to check the reproducibility at each step in the procedure, including (1) preparation of replicate powdered mixtures; (2) preparation of replicate disks from a given powdered mixture; and (3) preparation of replicate spectra from a given disk. Under the conditions used, both steps 2 and 3 seemed to limit the accuracy of the over-all method. With improved apparatus for pressing the disks, perhaps only step 3 would remain as the limiting factor.

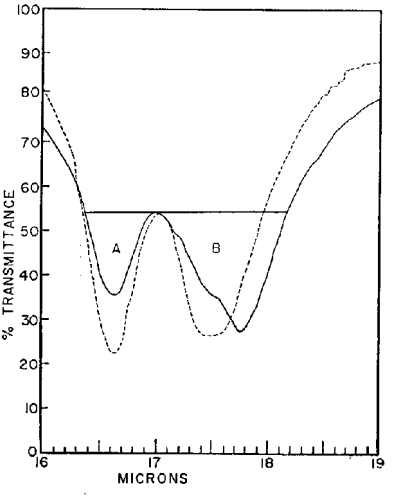

Figure 5. Spectrum showing areas for area-measurement method

—— Hydroxyapatite
- - - - Fluorapatite

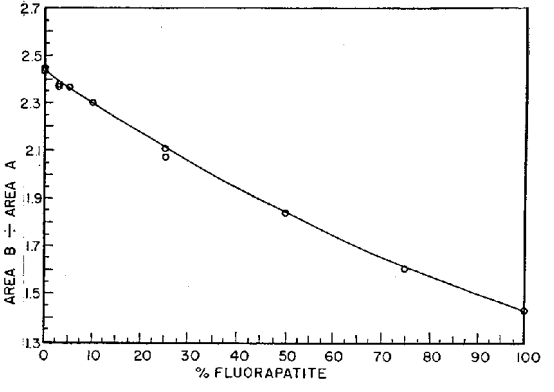

Figure 6. Calibration curve for area-measurement method

CONCLUSIONS

Two infrared procedures for the determination of fluorapatite in hydroxyapatite are based upon spectral differences in the 16- to 18-micron region. In the base-line method, the average error is about 1.5% fluorapatite in the sample. Individual wave lengths must be located accurately, and the method fails if any additional component is present which makes location of the base line difficult. In the area-measurement method, the average error is about 1.2% fluorapatite in the sample. More time is required than in the other method, but this one does not require accurately locating either wave lengths or base line. Empirical calibration is required, with fixed instrumental operating conditions, in both methods. The accuracy of each method is far better

than that of any other method which has been reported for this determination.

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Determination of Milligram Quantities of Thiosulfate by a Clock Reaction

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▶ **The oxidation of p-phenylenediamine by ferric chloride (with the formation of Lauth's violet in the presence of sulfide) is inhibited by the presence of thiosulfate. A well-marked induction period results which is proportional to the amount of thiosulfate present. This clock reaction is almost specific for thiosulfate and can be used as a rapid and precise analytical method for small quantities of thiosulfate in the presence of other sulfur acids. An example is given of its application to the analysis of black liquor from kraft sulfate pulp.**

THE FORMATION of Lauth's violet or methylene blue by treating sulfide solutions in acid with ferric chloride and *p*-phenylenediamine, or the *N,N'*-dimethyl derivative thereof, is the basis of an established method for the absorptiometric determination of traces of sulfide (*1-3*). When a ferric salt is added to a mixture of *p*-phenylenediamine and sulfide, a blue color appears at once or, if sulfide is absent, the solution rapidly becomes orange-yellow. Experiments in these laboratories showed that the presence of even traces of thiosulfate in the solution delayed the formation of color, either orange or blue, and that the color finally appeared with great rapidity after an induction period of as much as 0.5 hour or more, thus giving a beautiful clock reaction with a well-defined end point. As the induction period was a function of the thiosulfate concentration, the use of the reaction as an analytical procedure became apparent. Normally such a technique would have little to recommend it, but in view of the formidable analytical difficulties encountered in the precise and rapid determination of small quantities of thiosulfate in the presence of most other substances, the method was further investigated.

In the course of a study, which is not to be reported here in detail, it became clear that the oxidation of *p*-phenylenediamine by ferric iron proceeds by a chain reaction mechanism for which the thiosulfate acts as a chain breaker. The comparatively slow direct reaction of *p*-phenylenediamine with ferric ions takes place in the presence of thiosulfuric acid until the free radical first formed has used up the thiosulfate, after which the chain reaction sets in and all the remaining amine is oxidized in a comparatively few seconds. Hydrogen sulfide is without effect on the course of this reaction, but, if present, the deep blue Lauth's violet dye appears as an end product and greatly improves the detection of the onset of the chain reaction. This can then be observed with a precision of better than 1 second after an induction period of many minutes.

The induction period is a complex function of reagent concentrations and is sensitive to temperature. A wide variety of conditions can be used as the basis of an analytical method; the general illustration of the effect of variables given here enables the analyst to choose conditions suitable for any particular problem. A full description is given of one possible procedure which is considered about the optimum for a

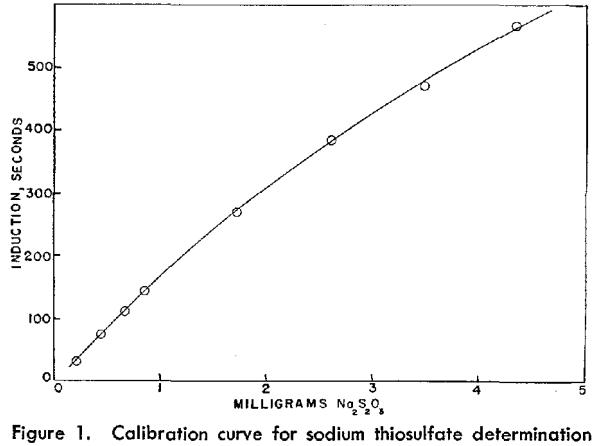

Figure 1. Calibration curve for sodium thiosulfate determination

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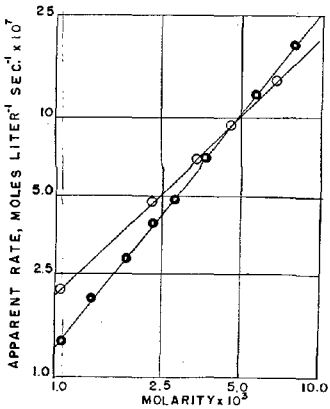

Figure 2. Change of apparent rate with reagent concentration

1M Cl^-, 0.175M H^+, 4 × 10^{-5}M thiosulfate Reagent not being varied held at 4.55 × 10^{-3}M
○ p-Phenylendiamine, slope 1.0
● Ferric Iron, slope 1.3

general analytical method and which has been applied to the analysis of black liquor from kraft sulfate pulp.

SUGGESTED METHOD

Reagents. Base Electrolyte. Dissolve 40 grams of sodium chloride in water, add 30.0 ml. of concentrated hydrochloric acid, (specific gravity, 1.18), and dilute to 1 liter.

p-Phenylenediamine Solution. This chemical should be recrystallized once from ethyl alcohol if dark colored. Dilute 2.5 grams of amine and 6.0 ml. of concentrated hydrochloric acid to 100 ml. with water.

Ferric Chloride Solution. Prepare as a 4.0% solution in water, using a good quality of anhydrous ferric chloride.

Hydrogen Sulfide Solution. Prepare a saturated aqueous solution fresh daily.

Procedure. The bottles containing the reagents were allowed to come to temperature in a water bath thermostated at 25° ±0.5° C. The clock reaction was carried out in a 100-ml. beaker which was placed on a white tile in the water bath. Base electrolyte, 25.0 ml., was added to the beaker followed by the sample dissolved in x ml. of water. The pH of the sample solution, unless initially neutral and unbuffered, was first adjusted to about pH 3.3 to 3.6 using xylene cyanol screened methyl orange (gray tint). Next 1.00 ml. of *p*-phenylenediamine solution was added from a pipet, followed by $(23-x)$ ml. of water and 1 to 2 drops of hydrogen sulfide solution. The solution was stirred and allowed to reach a constant temperature between 24° and 26° C., which was noted to 0.1° C. by a thermometer graduated in 0.1° C., with the bulb immersed in the solution in the beaker. By blowing from a calibrated pipet, 1.00 ml. of ferric chloride solution was rapidly added to the beaker. The solutions were mixed and a stop watch was started. The induction period, I_t seconds, was then recorded as the time from adding the ferric solution to the moment when the blue color rapidly appeared. This time was corrected to 25.0° C.by the formula

$$I_{25} = I_t[1 - 0.14\,(25-t)]$$

which applied accurately for temperatures, t° C., not more than 0.5° to 1° C. removed from 25.0° C.

The amount of thiosulfate was then read from a calibration curve (such as Figure 1) prepared by using standard sodium thiosulfate solutions. To eliminate the necessity for exact reproducibility of reagent and base electrolyte solution concentrations, it is best to recalibrate each time new solutions are prepared. This need not be done at all thiosulfate levels, however, as the percentage change in induction time found at one level of thiosulfate concentration applies at all others. Once this is determined at a suitable level the whole curve may be reconstructed by calculation.

EFFECT OF VARIABLES

Amine and Ferric Ion Concentrations. Figure 2 is a plot of the apparent rate of thiosulfate destruction *vs.* the molarity of *p*-phenylenediamine, with all other variables held constant at the values shown. If the thiosulfate were used up at a uniform speed, the rate would be given by the concentration of thiosulfate ion divided by the induction time. There is reason to believe, however, that the reaction does not necessarily proceed at a uniform velocity throughout the induction period, so the term apparent rate is used. The data in Figure 2, plotted on a log-log scale, show that the rate varies with the first power of the *p*-phenylenediamine concentration.

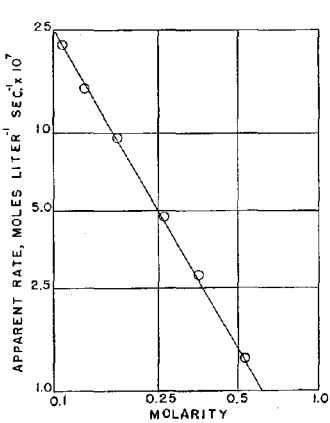

Figure 3. Change of apparent rate with acidity

1M Cl^-, 4 × 10^{-5}M thiosulfate, ferric iron and *p*-phenylenediamine each 4.55 × 10^{-3}M Slope, −1.8

Figure 2 also shows the effect of ferric ion concentration when the amine concentration is held constant. Here the rate is proportional to the oxidant concentration raised to a power significantly greater than unity. An increase in either amine or ferric ion concentration decreases the induction period observed with a given amount of thiosulfate.

Acid Concentration. Figure 3 shows that the rate is inversely proportional to a power of the hydrogen ion concentation—i.e., the induction period increases with an increase of acidity. The exponent, −1.8, is significantly greater than −2, illustrating the complexity of the reaction, but at least part of the acid effect is thought to be the result of a suppression of the concentration of free, unionized amine, which is probably the reacting species.

Temperature. This effect is very marked. The Arrhenius plot of log (apparent rate) against $1/K$ is shown in Figure 4. The energy of activation of about 28 kcal. probably varies very little with reagent concentration and other variables. Temperature control to better than 0.1° C. is necessary for a 1% precision in the induction period. It is more convenient to note the temperature during measurement and correct the induction period than to attempt exact thermostatic control.

Chloride Concentration. The rate of reaction is very slow in the complete absence of chloride, indicating that a ferrichloride complex is probably the oxidant. The effect of chloride is shown by the family of curves in Figure 5. For the experiments illustrated by curves 1, 2, and 3 the reagents were added as perchlorates and only chloride was varied. Not only does the apparent rate vary with the amount of chloride present, but it also depends on the quantity of thiosulfate. Thus, a linear relationship between induction period and thiosulfate concentration cannot be expected. However, when the chloride concentration exceeds a few tenths molar, the deviation from linearity is not great over a wide range of thiosulfate concentration, as shown by a calibration curve such as Figure 1 where the chloride is about 0.5M. The closest approach to linearity is obtained with about 0.2M chloride. However, this is dangerously near the region where the chloride concentration becomes critical, and a somewhat higher concentration is recommended. With a concentration of 1M or greater, the rate increases very rapidly with small amounts of thiosulfate and restricts the use of the method. The curves shown in Figure 5 are only intended to be in-

dicative of the general effect of chloride. The reproducibility with very low chloride concentrations was poor, but the anomalous position of curve 2 has been confirmed.

Interferences. The induction time will vary somewhat if any electrolyte is added in sufficient amounts, but in many cases the effect is undoubtedly one of ionic strength and is not very great. For the best results a calibration should be made in the presence of whatever electrolyte is present in the sample being analyzed, the sample weight being chosen so that between about 0.25 and 5 mg. of sodium thiosulfate is added per 50 ml. of solution.

In general, little or no interference is found with ions of the alkali metals or alkaline earth metals present to the extent of 1 to 2 grams in 50 ml. Similarly, nitrate and perchlorate anions do not interfere and chloride only inasmuch as the velocity is increased somewhat (Figure 5).

Oxidizing or reducing agents which react rapidly with *p*-phenylenediamine or ferric chloride must be absent or present only in amounts of 10% or less of the equivalent weight of the reagent which they attack. Similarly, substances reacting with thiosulfate cannot be tolerated—e.g., iodine—although the very low concentration of thiosulfate present "protects" it to some extent. For example, decomposition with acid is only noticed after 30 minutes or more in 0.15*M* hydrochloric acid, although it is almost immediate when concentrated thiosulfate solutions are allowed to stand in an acid of this strength.

Although the search for interfering ions has not been exhaustive, Table I indicates the tolerable limit of some common substances with this method. In some cases larger amounts could possibly be removed chemically without destroying the thiosulfate—for example, sulfite can be eliminated by bubbling air through a weakly acid solution. It should be possible to precipitate sulfide by silver, although this was not tested. The limits given do not imply that no interference occurs, but that the effect on the induction period is not too great and can be overcome by a suitable calibration in the presence of the ion concerned. Where very serious interference is noted with metal ions such as copper(II), the behavior can be attributed to complexing of the thiosulfate; however, the action is of no value as an indirect method for analysis of the metal concerned.

A brief search for other chain break-

Table I. Interference of Foreign Ions

Ion	Max. Wt. in 50 Ml.	Limit of Detection of Sodium Thiosulfate, %
Na^+, K^+, Ca^{++} Ba^{++}, Mg^{++}, NH_4^+, Cl^-, NO_3^-, ClO_4^-	Little interference up to 1 to 2 grams	0.01
SO_4^{--}	0.2 gram without special calibration	0.125
	1 gram with calibration	0.02
Br^-	0.5 gram	0.05
SO_3^{--}	0.005 gram direct	5
	0.2 gram if 5-ml. sample is acidified to pH 3.5 and SO_2 removed by bubbling air for 10 to 15 minutes	0.125
S^{--}	0.02 gram	1.25
Thionic acids	At least 0.1 gram	0.05 (about)
I^-	0.025 gram	1
Ag^+	0.15 gram. Sulfide and chloride precipitate; end of induction period shown by orange color	0.015
Zn^{++}, Ni^{++}, Mn^{++}	0.5 gram	0.05
Sn^{+4}	0.1 gram	0.25
Fe^{++}	0.02 gram	1.25
Hg^{++}	Interferes at all levels	
Cu^{++}	Interferes at all levels	

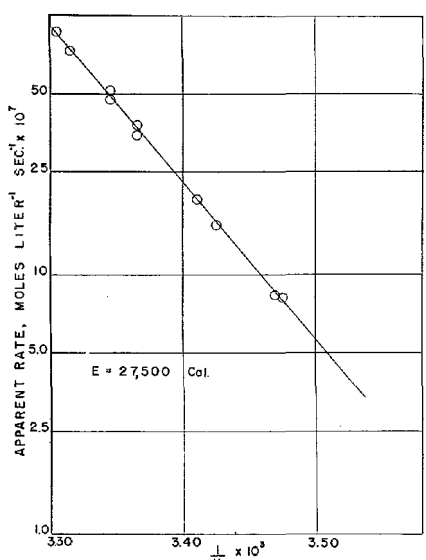

Figure 4. Arrhenius plot of apparent rates

0.2M Cl^-, 0.2M H^+, 4 × 10^{-5}M thiosulfate, 4.8 × 10^{-3}M ferric iron, 3.1 × 10^{-3}M *p*-phenylenediamine

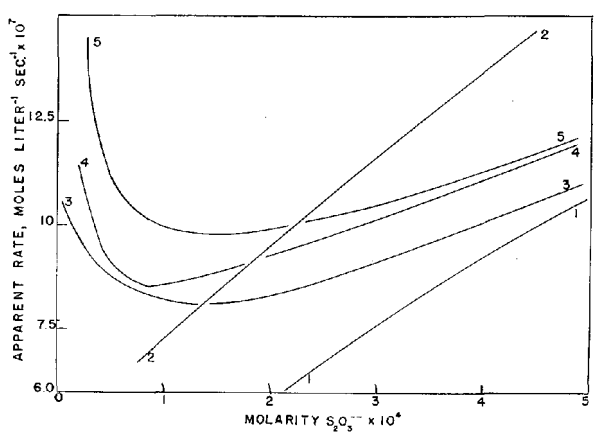

Figure 5. Effect of chloride ion and thiosulfate concentration on apparent rate

0.15M H^+, ferric iron and *p*-phenylenediamine each 4.55 × 10^{-3}M

1. No chloride
2. 0.05M chloride
3. 0.2M chloride
4. 0.5M chloride
5. 1.0M chloride

Table II. Determination of Thiosulfate in Black Liquor

Sodium Thiosulfate, Grams per Liter		
Added to 0.5 ml. of black liquor	Found	Recovered
None	1.6, 1.9, 1.75 Av. 1.75	...
2.0	3.7, 3.9	2.05
3.0	4.7	2.95
4.0	5.65	3.9
6.0	7.3	5.55
8.0	9.5	7.75

ers that might be expected to behave like thiosulfate and give a clock reaction indicates that the well-defined behavior found with thiosulfate ion (or perhaps the undissociated acid) is not common. Thiocarbamates, aminophenols, and the thionic acids had no effect when present in 50- to 100-fold excess over the thiosulfate concentration. Ascorbic acid could be present to the extent of 0.05 gram in 50 ml. before any delay in color formation was noted. Even then, an ill-defined end point was observed. Hydroquinone gave an induction period, but again with a poor end point, and the time was not simply related to the hydroquinone concentration. No delay at all was found with less than about 0.5 mg. and 2.5 mg. gave only the same induction period as 0.3 mg. of thiosulfate.

DISCUSSION

The method can detect between about 0.25 and 5 mg. of sodium thiosulfate with a precision around 1% in favorable instances. A considerable latitude is possible, however, by changing the conditions suitably, although any new procedure should be given a thorough testing. An application of the technique to 0.5-ml. samples of black liquor from sulfate pulp is shown in Table II. The calibration curve, Figure 1, was used directly and the sample was given no preliminary treatment. Black liquor contains, in addition to some 2 to 10 grams per liter of sodium sulfide, large amounts of alkali lignins and other organic substances such as mercaptan, and several grams per liter of sodium sulfite, sulfate, carbonate, and hydroxide. A rapid determination of thiosulfate in such a mixture by any existing techniques is very difficult.

ACKNOWLEDGMENT

The authors wish to thank Margaret Daw for assistance in the earlier stages of the investigation.

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Routine Determination of Nitrogen in the Microgram Range with Sealed Tube Digestion and Direct Nesslerization

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▶A procedure for 1- to 16-γ quantities of nitrogen was developed for routine analyses of small amounts of protein in studies of poliomyelitis virus purification. This was achieved by digesting each sample in a sealed tube, mixing the digest with Nessler's reagent with the aid of magnetic stirring, and reading the resulting colored complex photometrically.

FOR the routine determination of the quantity of protein or nucleoprotein present in various fractions obtained in the purification of poliomyelitis viruses (*5*) a simple procedure for nitrogen determination was developed. Because of the limited amount of material available for analysis, it was necessary to determine 1- to 16-γ quantities of nitrogen. The procedure developed incorporated the advantages of sealed tube digestion with sulfuric acid—namely, complete decomposition of organic material with total recovery of protein and nucleic acid nitrogen without catalyst—and protection from contamination by atmospheric ammonia (*1, 2*). The critical step in color development, the mixing of the reagent with the diluted digest, was accomplished with the aid of a magnetic stirrer.

REAGENTS

Digesting Acid. Reagent grade sulfuric acid was mixed with an equal volume of ammonia-free distilled water.

Nessler's reagent was prepared according to Koch and McMeekin (*3*), except that the final normality of sodium hydroxide was increased to 2.3 to compensate for the quantity of digesting acid used.

PROCEDURE

Each liquid sample was pipetted into a 10 × 75 mm. borosilicate glass test tube and 20 μl. of digesting acid was added. Excess water was driven off in an oven at 100° to 110° C. The tubes were then sealed in a gas-oxygen flame, encased in individual brass tubes, and placed upright in a muffle furnace preheated to 450° C. Ten minutes were allowed for the samples to attain digestion temperature, and the furnace was held at 450° ± 10° C. for 30 minutes. After cooling, the tubes were removed from the brass containers and centrifuged to collect the digest at the bottom. The tubes were scored near the top and opened with the aid of a molten end of a glass rod. To each tube were added a 3 × 10 mm. glass-covered stirring bar and 1.00 ml. of water. The tube was held vertically by a clamp directly over the magnetic stirrer. The contents were mixed, and with continued stirring 0.20 ml. of Nessler's reagent was added rapidly. Each tube was allowed to stand 20 minutes or more for full color development. The absorbance was measured in a 10 × 75 mm. cuvette in the Coleman Junior spectrophotometer at 480 mμ. The corresponding quantity of nitrogen was estimated from a standard curve which had been prepared by applying the same procedure to aliquots

Table I. Results of Duplicate Analyses of Aliquots of a Standard Tryptophan Solution

(466 mg./l.)

Aliquot, μl.	N, γ	
	Theoretical	Found
15	0.96	1.0, 1.0
50	3.2	3.1, 3.1
75	4.8	4.8, 4.7
100	6.4	6.3, 6.2
200	12.8	12.9, 12.9
250	16.0	16.2, 16.4

Mean recovery 100 ± 3%.

of an ammonium chloride solution of known concentration.

RESULTS AND DISCUSSION

In routine use, samples were run in duplicate, with appropriate blanks included in each set of analyses. In addition, ammonium chloride standards were included at frequent intervals. To check the reliability of the method the nitrogen content of aliquots of a standard tryptophan solution was determined (Table I). For duplicate samples the precision was considered wholly adequate for the purpose. When greater accuracy was required, the more precise diffusion-titration technique (*2*) was employed. The method of Levy (*4*) for the determination of microgram quantities of nitrogen with Nessler's reagent, although more precise than the present procedure, requires special vessels and considerable attention and technical skill.

Digestion in sealed tubes at elevated temperatures obviated the use of a catalyst for complete recovery of nitrogen of refractory substances such as tryptophan. Sealed tubes also offered protection from contamination, but while the tubes were open protection from atmospheric ammonia was necessary. Additional sulfuric acid was added to ensure complete digestion of samples containing excessive quantities of organic matter or of anions of volatile acids—e.g., chloride. On the other hand, a large excess of acid was to be avoided, because of formation of a red precipitate and incomplete color development upon mixing with the reagent.

At the wave length selected, 480 mμ, a linear relation between ammonia concentration and absorbance was found over the range from 0 to 10 γ of nitrogen, and a slight departure from linearity above 10 γ. Greater sensitivity could be obtained at shorter wave lengths, but at the expense of greater uncertainty in the blanks. It is suggested that microcuvettes and smaller volumes of all materials be used as a means of extending the method to the submicrogram range.

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Microdesalter for Qualitative Paper Chromatography of Amino Acids

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▷ A microdesalter has been developed to remove interfering inorganic ions from biological samples spotted on filter paper for chromatography of amino acids. The instrument is an adaptation of an electrolytic macrodesalter. The advantages of this technique are the speed of the operation and the small size of the sample required for desalting.

HIGH concentrations of inorganic salts cause severe interference in obtaining good paper chromatograms of amino acids and sugars. "Tailing," "crescent spots," and retardation of R_f values are some of the observed effects caused by inorganic ions. In order to remove the salts prior to chromatography, several methods have been described (*2*): ion exchange for amino acids and sugars, electrodialysis, and pyridine extraction for sugars.

Ion exchange methods for the removal of inorganic ions cause some loss of the amino acids because of their amphoteric character. This method also results in the formation of artifacts during the desalting of sugars (*5*).

The electrodialysis desalter, first described by Consden and coworkers (*3*) and modified by others (*1, 4, 7*), has seemed to be the most satisfactory technique for desalting. This method is based on the electrolysis of inorganic ions, using a flowing mercury cathode and a platinum anode; the anode is separated from the solutions by a dialysis membrane. The disadvantages of this technique are the relatively large volume of solution (1 to 10 ml.), the length of time for one desalting procedure (1 to 3 hours), and the partial conversion of arginine to ornithine.

This electrolytic desalter has now been modified in order to handle a large number of samples containing micro amounts of amino acids.

The principle of the microdesalter is that the desalting is carried out directly on the filter paper, which is then developed by suitable paper chromatographic techniques. This procedure permits the rapid desalting (1 to 5 minutes) of small volumes of solutions 5 to 25 μl.). The original sample, therefore, does not have to be diluted and reconcentrated as in other methods (*2*). The actual desalting proceeds by electrolysis as in the original technique (*3*). However, the water lift pump for the mercury at the cathode has been replaced by a gravity pump.

APPARATUS

Figure 1 is a simplified drawing of the microdesalter, and Figure 2 is a photograph. The anode cell, *A*, from a commercial desalter (Research Equipment

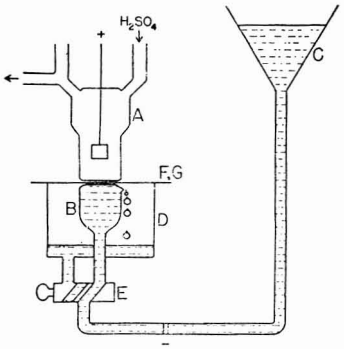

Figure 1. Drawing of microdesalter

A. Anode cell
B. Cathode
C. Leveling bulb
D. Plastic dish
E. Three-way stopcock
F. Platform
G. Location of filter paper

Corp., Oakland, Calif., Model X-100) is fastened to a buret clamp which is held to the vertical support by a swivel Castaloy clamp holder (Fisher Scientific Co.). The bottom of the anode is covered with a piece of dialyzing cellophane tubing, held in position with a rubber band. The setscrew is loosened in order to permit free pivoting movement.

The cathode, *B*, consists of a thistle tube, 1 inch in diameter, with a small lip on the upper rim. This lip is made by heating the glass rim and making a slight indentation with a carbon shaper. The bottom of the thistle tube is cut to a length of 1.5 inches and is fitted through the middle of a 3-inch round plastic dish, *D*, where it is glued in position. The thistle tube outlet is connected by means of a three-way stopcock, *E*, to a leveling bulb, *C*, and an outlet (0.25-inch diameter) in the bottom of *D*. The leveling bulb is supported on a ring clamp which is placed about 3 inches above the rim of the thistle tube, *B*. The leveling bulb, *C*, is now filled with mercury. Stopcock *E* is turned to position 1 during the operation of the desalter, allowing the mercury to flow into the thistle tube and slowly spilling from the lip into dish *D*. Position 2 of the stopcock serves as a means of replenishing mercury in the leveling bulb by holding it for a few moments below *E*, closing the stopcock, and replacing *C* in the clamp. A platform, *F*, made of clear plastic, is flush with the top of *B* and aids to support the filter paper during desalting.

The instrument is connected to a suitable, constant-voltage power supply fitted with a voltmeter and a milliammeter—e.g., Heathkit, Variable Power Supply, 0 to 500 volts, direct current, 0 to 200 ma. The anode cell is filled with 1% sulfuric acid, and during the operation of the desalter a constant trickle of the acid through the anode cell is maintained.

EXPERIMENTAL PROCEDURE

The sample to be desalted is spotted by means of a micropipet on the filter paper. The spot size may vary from 2.5 to 25 μl., depending on the sensitivity of the detection and the concentration of the compounds in the original sample. While the spot is still moist, the paper is quickly placed in position between the anode and cathode (*G*, Figure 1). If it is not possible to desalt the sample immediately after spotting, the spot may be allowed to dry, but must be moistened with about 5 μl. of water just prior to desalting, taking care that the moistened area encompasses the area of the originally applied spot.

The voltage is now turned on at its minimum position (less than 10 volts), and the stocpcock is turned slowly to position 1 until the needle on the ammeter indicates the flow of current. The amperage should not go above 40 ma. Because of the heat evolved at higher current readings, rupturing of the anode cellophane membrane has been observed. A black deposit, probably mercury(II) oxide, is also formed on the paper at above 50 ma.; this deposit seems to interfere in subsequent chromatographic development. The voltage is slowly increased using a manual control, until 30 volts direct current has been reached. When the current has dropped to less than 15 ma. at 30 volts direct current the desalting is completed. Five microliters of 1% sodium chloride are desalted in 2 minutes; 25 μl. of urine are desalted in about 5 minutes. The same length of time is required for spinach sap which is deproteinized with trichloroacetic acid.

After desalting, the filter paper is removed, and the spot, which has retained its original shape and size, is dried at 40° C. The paper is now ready for solvent development.

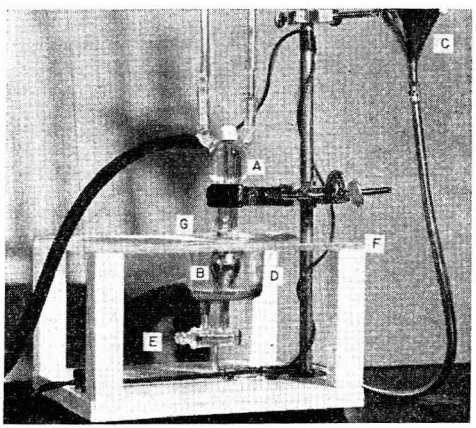

Figure 2. Microdesalter

RESULTS AND DISCUSSION

Removal of Inorganic Ions. To test the efficiency of the microdesalter, the disappearance of sulfur-35–labeled sodium sulfate after desalting was used as a criterion. Five microliters of 0.5% sodium sulfate containing about 1.50 μc. were spotted on several disks of Whatman No. 1 filter paper. Each disk was desalted for a different time period at 30 volts direct current. The radioactivity was measured by counting the paper directly with a thin end window Geiger-Müller probe (Technical Associates, Burbank, Calif.) connected to a Berkeley decimal G.-M. scaler. The results in Table I indicate that less than 1% of the initial sodium sulfate remained on the paper after 2 minutes' desalting.

Table I. Loss of Sulfur-35–Labeled Sodium Sulfate after Desalting[a]

Time of Desalting, Seconds	Counts per Minute
0	43,694
30	15,926
60	3,410
120	359

[a] 30 volts d. c.

Recovery of Amino Acids after Desalting. Initial attempts were made to measure the recovery of amino acids after desalting by the elution method and subsequent ninhydrin color development (*9*). The observed results were not consistent; they sometimes indicated a "gain" in concentration after desalting and other times were not reproducible. One possible explanation for this lack of precision is the interference of sulfate ions in the development of the ninhydrin color. The sulfate ions would diffuse onto the paper from the anode compartment. As the amino acids were not chromatographed for this study, the sulfate ions would be eluted together with the amino acids.

A more direct method for the analysis of the concentration change of the amino acids due to desalting was developed.

The alga *Chlorella pyrenoidosa* was grown for 1 week in an atmosphere of carbon-14–labeled carbon dioxide. The cells were harvested by centrifugation, and the water-soluble compounds were extracted with 70% ethyl alcohol. The residue, consisting mainly of protein, was hydrolyzed for 24 hours with 6*N* hydrochloric acid. The hydrolyzate was evaporated repeatedly on a steam bath and the dry residue was dissolved in 1.0 ml. of 10% 2-propanol. The protein hydrolyzate was spotted on two sheets of Whatman No. 1 filter paper by depositing 50 μl. 20 times on each sheet of filter paper. One sheet was developed by descending paper chromatography using liquid phenol-water (100:20) in an atmosphere of ammonium hydroxide; the other sheet was developed in a like manner with 1-butanol–acetic acid–water (4 to 1.5, v./v.) [(*4*), Chap. V].

After solvent development, the chromatograms were exposed to x-ray film (Du Pont 507, single emulsion) for 1 week. Radioactive regions were cut out parallel to the line of application and were identified by markers of known amino acids. These markers (2.5 μl. of 10 *mM* solutions) were chromatographed with the same solvents on parallel sheets of filter paper. The markers were visualized by dipping the papers into 0.2% ninhydrin in acetone and heating them at 75° C. for 15 minutes (*2*). The radioactive regions were eluted with water by the descending technique. No attempt was made to determine the specific activity of the carbon-14–labeled amino acids.

Twenty five microliters of each carbon-14 amino acid were spotted on a Whatman No. 1 filter paper disk. The radioactivity was determined on the paper as described previously. The original spot was then wetted with 25 μl. of distilled water, and the amino acid was desalted for 1 to 2 minutes until the current reading had dropped below 20 ma. The paper was dried in an oven at 60° C. and was counted again.

The results of these experiments are summarized in Table II. Losses of amino acids, probably due to diffusion and electrolysis, as indicated by the loss of radioactivity, were as high as 50% (valine). Aspartic acid, alanine, and glycine were recovered from 90 to 96%. These findings could be compared with losses of 5 to 30% in the macrodesalter (*8*). For quantitative estimation of the amino acids by paper chromatography, desalting the unknown as well as standard solutions may be recommended.

Table II. Recovery of Carbon-14–Labeled Amino Acids after Desalting

Amino Acid	Counts per Minute		Recovery, %
	Initial	Final	
Alanine	1,158	1,075	92.83
Aspartic acid	2,450	2,351	95.96
Cystine	773	569	73.60
Glutamic acid	2,989	2,463	82.40
Glycine	524	471	89.88
Isoleucine	916	597	65.17
Leucine	1,347	780	57.91
Lysine	1,364	1,119	82.04
Serine	484	374	77.27
Threonine	1,102	783	71.05
Tyrosine	1,266	1,003	79.23
Valine	1,103	555	50.32

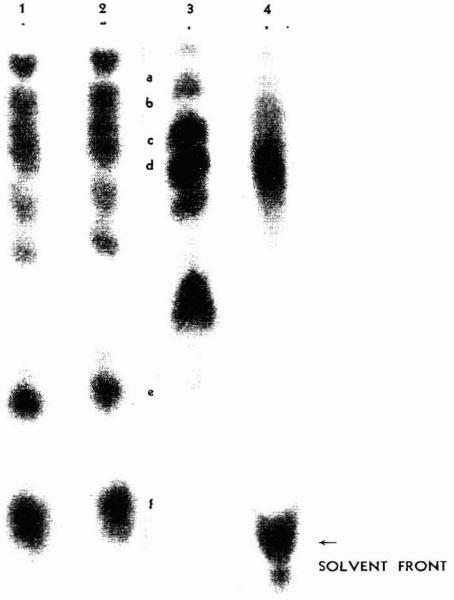

Figure 3. Descending, one-dimensional chromatograms of deproteinized spinach sap

Solvent. 1-butanol–acetic acid–water (4 to 1.5, v./v.)
1, 2. 5 mM standard solution, amino acids
3. Desalted sample
4. Untreated sample
→ "Salt effect"
a. Cystine, glutathione
b. Lysine
c. Histidine
d. Arginine
e. Valine and methionine
f. Phenylalanine, leucine, isoleucine

Paper Chromatography of Desalted samples. For qualitative analysis, a chromatogram for each standard amino acid which was desalted was prepared with 1-butanol–acetic acid–water (4:1:5:v./v.) by the descending technique. The colors were developed by dipping the chromatograms into 0.2% ninhydrin in acetone and heating them at 75° C. for 20 minutes. Methionine was partially converted to methionine sulfoxide, as evidenced by one-dimensional paper chromatography. This oxidation may be due to a reaction with hydrogen peroxide formed by the reduction of oxygen at the mercury electrode (*6*). Arginine was not converted to ornithine, as in the macrodesalter (*8*).

To study the effect of desalting by the micromethod, paper chromatograms of several biological samples were run with and without desalting. Figure 3 illustrates one-dimensional chromatograms of deproteinized spinach sap. The untreated sample (Strip 4) has streaked badly, and no single spots are discernible in the lower R_f region. A cresent spot has formed in the phenylalanine region. The desalted sample (Strip 3) compares favorably with a synthetic mixture of the amino acids (Strips 1 and 2).

Two-dimensional chromatograms of human urine that was not desalted showed several ninhydrin-positive artifacts. A large yellow region was also formed which would mask aspartic acid, if present, and caused the spots due to serine and taurine to be misshapen. The solvents used were liquid phenol-water (100:20) and collidine-lutidine-water-diethylamine (100:100:100:3) (*2*).

No appreciable loss of amino acids was observed by visual inspection, but the chromatograms were greatly improved after the desalting procedure.

ACKNOWLEDGMENT

The microdesalter was constructed by Earl W. Winter and Albert Emanuel, whose efforts are gratefully acknowledged.

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Determination of Ozone and Other Oxidants in Air

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▶ **Ehmert modified the method of Crabtree and Kemp for the determination of ozone in air to eliminate the need for a correction factor for the volatilization of iodine. The ozone was absorbed in a potassium iodide solution containing a measured excess of sodium thiosulfate, which was back-titrated with iodine. A further modification is now made by titrating with 0.001*N* potassium iodate to an amperometric end point. The apparatus for detection of the end point consists of a calomel electrode, a platinum electrode, and a sensitive galvanometer. No batteries or resistors are required. On triplicate analyses of eight samples ranging from 15 to 25 parts of ozone per hundred million parts of air, by volume, a standard deviation of 1.45 was found. By varying the concentrations of the solutions, samples containing from 2 to 10,000 p.p.h.m. of ozone can be analyzed.**

THE TERM "OXIDANT" is used here to mean anything that will oxidize potassium iodide in aqueous solution buffered at pH 7. This definition was used by Littman and Benoliel, who pointed out that the oxidation of potassium iodide under these conditions is not specific for ozone but also responds to oxides of nitrogen and some organic hydroperoxides (*6*). Ozonides and free halogens also liberate iodine in the solution. The amount of iodine formed by oxides of nitrogen has been stated to range from 2% (*4*) to 80% (*7*) of the amount expected from Reaction 1.

$$NO_2 + 2H^+ + 2I^- \rightarrow I_2 + H_2O + NO \quad (1)$$

Effenberger stated that 80% of the oxidant in air is ozone (*2*). Crabtree and Kemp stated that three to four times as much nitrogen dioxide as is normally found in air will not interfere in the determination of ozone (*1*). In studying the aging of rubber, many of the ozone determinations are made on the contents of test chambers containing artificially produced ozone concentrations of 25 to 50 p.p.h.m., where the interference of other oxidants is probably small.

Crabtree and Kemp absorbed ozone in 20% potassium iodide solution, where it forms iodine.

$$O_3 + 2KI + H_2O \rightarrow I_2 + O_2 + 2KOH \quad (2)$$

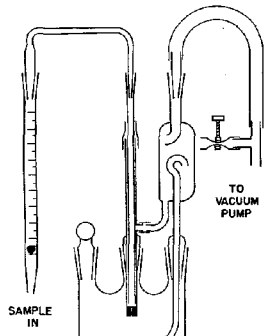

Figure 1. Assembly of glassware for sampling, originally used by Crabtree and Kemp (*1*)

The solution must be buffered at pH 7 or higher to prevent the formation of more than 1 mole of iodine per mole of ozone (*8*).

$$4O_3 + 10KI + 10H^+ \rightarrow 5I_2 + H_2O_2 + 4H_2O + 3O_2 + 10K^+ \quad (3)$$

They found that bubbling a large sample of air through the solution resulted in the loss of some iodine by volatilization, and applied a 10% correction factor. As this correction factor is dependent on the rate of flow and the volume of the solution, it is desirable to use a method that does not require correction. Ehmert used a potassium iodide solution containing a measured excess of sodium thiosulfate and then back-titrated the excess (*3*).

$$I_2 + 2Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI \quad (4)$$

The iodine reacts as soon as it is formed and no volatility correction is needed.

As potassium iodate is sufficiently pure to be used as a primary standard and its solutions are stable, it was proposed to use the technique of Ehmert, then to acidify the solution and back-titrate with 0.001*N* potassium iodate solution.

$$KIO_3 + 5KI + 6H^+ \rightarrow 3I_2 + 3H_2O + 6K^+ \quad (5)$$

Knowles and Lowden described a circuit for amperometric end point detection, which requires simpler apparatus and is more sensitive than the dead-stop end point (*5*). The amperometric circuit was therefore adopted. The starch end point is not sensitive enough for titrations with 0.001*N* solutions.

EXPERIMENTAL WORK

Apparatus. The sampling flask was the same as that used by Crabtree and Kemp, except that the Woulff bottle was replaced by a 1-liter round-bottomed flask (Figure 1). The design of the spray jet is critical and is described in detail by Crabtree and Kemp (*1*).

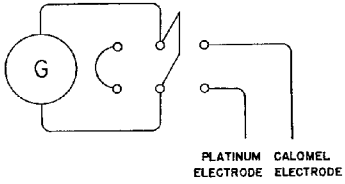

Figure 2. Circuit for amperometric end point detection

Rotameter-type flowmeter such as Fischer and Porter No. 2-F-1/4-16-5, having a range of 3 to 7 liters of air per minute.

Source of vacuum capable of maintaining a sampling flow rate of 5 liters per minute. A water aspirator will serve.

Ten-milliliter buret, graduated to 0.05 ml.

Calomel reference electrode, Beckman No. 1170.

Platinum thimble indicator electrode, Beckman No. 1271. It is important that the platinum electrode have a surface area of at least 1.5 sq. cm. If a small area such as a short platinum wire is used, the circuit will lack sensitivity.

Galvanometer with sensitivity of at least 0.05 μa. per mm.

Magnetic stirrer.

Reagents. To prepare the buffer solution, dissolve 1.8 grams of disodium hydrogen phosphate and 1.7 grams of potassium dihydrogen phosphate in 1 liter of water.

Potassium iodate standard solution, 0.00100*N*. Dissolve 0.0357 gram of potassium iodate in 1 liter of water.

Sodium thiosulfate solution, 0.001*N*. Dissolve 0.25 gram of sodium thiosulfate decahydrate and 0.1 gram of sodium carbonate in 1 liter of water.

Sulfuric acid, 2 *N*

Potassium iodide.

The sampling flask and all tubing for conducting the sample to the flask were made of glass. Ground joints without lubrication were used wherever possible. Where this was impractical, joints were made by butting glass tubing together and covering the joint with a sleeve of plastic tubing. Contact of the sample with rubber or other organic material should be avoided, to prevent consumption of ozone.

To test the stability of sodium thiosulfate during sampling, 70 ml. of buffer solution and 5 ml. of sodium thiosulfate solution were placed in the sampling flask and 125 liters of air were drawn through the solution. Titration showed that no decomposition had occurred.

The galvanometer and electrodes were connected in series through a double-pole double-throw switch as shown in Figure 2.

RECOMMENDED PROCEDURE

Place 70 ml. of buffer solution, 1 gram of potassium iodide, 5 ml. of sodium thiosulfate solution measured with a pipet, and 10 ml. of 2*N* sulfuric acid in a beaker. The galvanometer will settle down to a steady reading within a few seconds after the reagents are mixed. The speed of stirring should be constant during a titration but need not be duplicated from one titration to another. Titrate with potassium iodate solution until a permanent galvanometer deflection of 5 mm. is obtained. This is taken as the end point. Repeat the titration and average the values. As the sodium thiosulfate solution changes strength, a new blank value must be established each day.

Place in the sampling flask 70 ml. of buffer solution, 1 gram of potassium iodide, and 5 ml. of sodium thiosulfate solution. Draw about 125 liters of sample through the flask at a rate of about 5 liters per minute, adjusting the rate with the pinch clamp, and note the exact flow rate and time. Empty the solution into a beaker, add 10 ml. of 2*N* sulfuric acid, and titrate.

The flow rate must be great enough to keep the flask filled with a fine mist. The sample should be large enough so that the blank and sample titrations differ by at least 1 ml. If the sampling is continued so long or the ozone content of the sample is so high that the thiosulfate is exhausted, the determination must be repeated. In sampling a chamber with an ozone concentration of 10,000 p.p.h.m., this difficulty was overcome by using 0.1*N* sodium thiosulfate and potassium iodate solutions. The other reagents were used without modification.

CALCULATIONS

From Reaction 2 it can be seen that 1 mole of ozone forms 2 equivalents of iodine.

$$Z = \frac{(A - B) \times N \times 11.21 \times 760 \times T \times 10^5}{F \times t \times P \times 273}$$

where A = ml. of potassium iodate solution required to titrate the blank

B = ml. of potassium iodate solution required to titrate the solution after sampling

N = normality of potassium iodate solution

T = temperature, degrees Kelvin

F = sample flow rate, liters per minute

t = sampling time, minutes

P = atmospheric pressure, mm.

Z = oxidant concentration, parts of ozone per hundred million parts of air by volume.

Table I. Analysis of Ozone Samples

Sample No.	Ozone Found, P.P.H.M.	Average
1	15.5, 15.3, 14.7	15.2
2	18.6, 20.5, 21.5	20.2
3	19.2, 17.5, 13.8	16.8
4	24.6, 23.6, 23.2	23.8
5	18.3, 18.7, 16.5	17.8
6	20.9, 20.1, 19.4	20.1
7	18.8, 21.3, 22.4	20.8
8	25.2, 23.9, 23.2	24.1

RESULTS

Triplicate analyses of air in an ozone test chamber on eight different days are shown in Table I. The standard deviation is 1.45 p.p.h.m.

ACKNOWLEDGMENT

The author wishes to thank the Goodyear Tire and Rubber Co. for permission to publish this paper.

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Rapid Routine Method for Determination of Uranium in Ores

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▶ A rapid method for the determination of uranium in ores permits detection of as little as 0.01% uranium oxide with a precision of ±0.005%. The uranium is separated from all commonly occurring interfering ions by adsorption on an anion exchange resin. After elution with perchloric acid, the uranium is determined colorimetrically by the sodium hydroxide–hydrogen peroxide method. Refinements in operating techniques and apparatus have made the method well suited for routine application at minimum cost.

BECAUSE of the increased interest in uranium, a rapid, routine method of analysis was needed that could be used successfully on a large variety of ore types having a wide concentration range of uranium. In an associated laboratory in which a research project on beneficiation of low-grade ores is being conducted, a large number of test samples of all grades must be analyzed each day. A standard volumetric procedure (*7*) using a lead reductor and ceric sulfate after a cupferron separation of impurities was used until recently. Although satisfactory in most cases, the volumetric method was rather time consuming and required a moderate number of reruns. In addition it gave erratic results for some ores, particularly a Canadian pitchblende ore.

The most promising new approach to uranium analysis involves an ion exchange separation (*1–4*) which utilizes the adsorption of uranium as a sulfate complex on an anion exchange resin, followed by elution with 1*M* perchloric or hydrochloric acid. This effects a separation from nearly all interfering ions, especially if the iron and vanadium are reduced with sulfurous acid. After elution the uranium is determined colorimetrically by the sodium hydroxide–hydrogen peroxide method (*6*). Fisher (*2–4*) obtained excellent results in the analysis of solutions and good results in the analysis of a limited number of ores. The methods recommended for the solution of the ores were treatment with nitric and hydrofluoric acids followed by fusion with sodium carbonate or treatment with manganese dioxide and sulfuric acid.

This investigation was planned to extend the work of Fisher (*3*) to routine ore analyses. The principal objective was to adapt the method to a larger scale and to extend the range to very low-grade material. Preliminary colorimetric determinations with a filter photometer proved unsatisfactory because of the band width of the filters available. However, experiments with the Bausch & Lomb Spectronic 20 colorimeter proved most successful. By proper choice of wave lengths and cell size, the range was extended to include the limits from 0.5 to 40 mg. of uranium oxide. This permits determination of uranium in low-grade materials containing from 0.01% (5-gram sample) down to 0.005% (10-gram sample).

APPARATUS AND REAGENTS

Spectrophotometers. A Beckman Model DU quartz spectrophotometer with 1-cm. silica cells was used for the developmental work. A Bausch & Lomb Spectronic 20 colorimeter with 0.5- and 1-inch cells was used for the colorimetric measurements.

pH Meters. A Beckman Model G and a Beckman Model N were used for pH control.

Columns. To facilitate the handling of a larger number of samples, a special column was made (Figure 1). This design was advantageous in keeping the liquid level above the resin bed, in providing for easier control of drop rate, and in facilitating the loading and washing of the columns.

Resin. The resin is a quaternary ammonium, anion exchange type and is a modification of Amberlite IRA-400, consisting of 40- to 60-mesh beads and purchased under the name Amberlite XE-117, Type 2. Other similar resins such as Dowex 2 (*1*) can be used. Before use the resin is converted batchwise to the sulfate form by treating it for 20 minutes with three times its volume of 10% sulfuric acid. The treated resin is then washed several times by decantation with distilled water. A final 50-ml. wash is given the resin in the column. No difficulties have been encountered in storing the sulfate form of the resin under distilled water for several weeks. Because the resin is inexpensive, it may be discarded after use.

Standard Uranium Solutions. Two uranyl sulfate standard solutions were prepared by fuming 1.790 and 3.580 grams of uranyl nitrate with 10 ml. of 6*N* sulfuric acid and diluting to 1 liter. These solutions contain approximately 1 and 2 mg. of uranium oxide per ml., respectively, and were standardized in two ways: by passing through a lead reductor and titrating with standard ceric sulfate; and gravimetrically by precipitation with ammonia and ignition to uranium oxide.

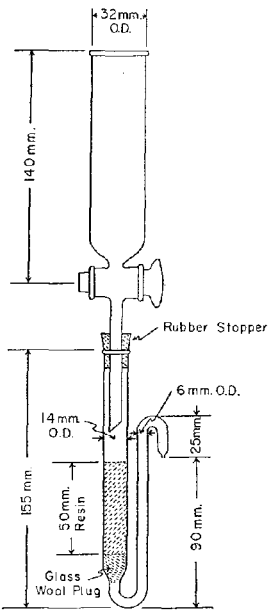

Figure 1. Ion exchange apparatus

All other chemicals were of reagent grade except manganese dioxide, in which case both the technical and reagent grades were used.

PROCEDURE

An appropriate weight of sample containing 0.5 to 40 mg. of uranium

[1] Present address, Shell Oil Co., Martinez, Calif.

oxide is treated on a hot plate for about 10 minutes with 15 to 30 ml. of 12*M* hydrochloric acid. Then 5 to 10 ml. of 16*M* nitric acid is added and heating continued until acid-soluble minerals are dissolved. The samples are then fumed strongly with 5 to 10 ml. of 9*M* sulfuric acid. The larger volume of acid in each case represents the amount used for 5-gram samples. After the samples are fumed, the residues are taken up in 50 ml. of water and boiled gently for 5 to 10 minutes. The solutions are then filtered and washed with hot water. For samples containing appreciable phosphate the washing is done with hot 0.1*N* sulfuric acid instead of hot water. The solutions are then adjusted to a pH of 1.0 to 1.5 with 6*M* sodium hydroxide. An estimation of the amount of iron present can be made in this step and an appropriate amount of 6% sulfurous acid (10 to 30 ml.) added to effect complete reduction of the iron. After allowing the solutions to stand about 10 minutes, they are passed through the ion exchange columns at a rate of about 1 drop per second to adsorb the uranium. The beakers and dropping funnels are then rinsed three times with hot water, after which the resin is washed with 50 ml. of hot water.

The uranium is then eluted into 100-ml. volumetric flasks with 50 ml. of 1*M* perchloric acid which has been heated to boiling. The elution rate is again maintained at 1 drop per second. Under these conditions the temperature of the resin bed varies from 60° to 70° C. After elution the resin is allowed to stand 10 minutes in contact with the last of the eluent. The columns are then blown out with air to remove all possible eluent. A 25-ml. portion of 6*M* sodium hydroxide is added to each flask and the solutions are diluted to within a few milliliters of final volume. After they cool to 20° C., 1 ml. of 30% hydrogen peroxide is added to the samples and they are diluted to volume. After color development absorbances are determined at either 380 or 420 mμ in 0.5- or 1-inch cells, the choice depending on the uranium content. If the sample is known to contain appreciable quantities of phosphate and iron, the solutions should be allowed to stand 30 minutes and filtered on a dry, retentive paper prior to reading the absorbances. The blank used for the determinations contains 50 ml. of 1*M* perchloric acid, 25 ml. of 6*M* sodium hydroxide, and 1 ml. of 30% hydrogen peroxide diluted to 100 ml.

DEVELOPMENT OF METHOD

Stability of Color and Selection of Wave Length. To determine the most suitable wave length, the absorbance curve for the peroxide complex of uranium was determined with the Beckman Model DU spectrophotometer for a solution containing 10 mg. of uranium oxide in 100 ml. of solution. The blank contained the same concentration of sodium hydroxide and hydrogen peroxide as the uranium solution. The absorbance curve of the peroxide blank was also determined against distilled water. These solutions were then stored for a period of 72 hours and the absorbance curves redetermined. Results of these investigations are illustrated in Figure 2. Maximum stability occurs at 420 mμ, which was selected for determining higher concentrations of uranium. The curves show the possibility of peroxide interference at wave lengths below 370 mμ. Because a colorimeter with a 20-mμ band pass was to be used for the determinations, 380 mμ was selected as the wave length to be used for lower concentrations of uranium.

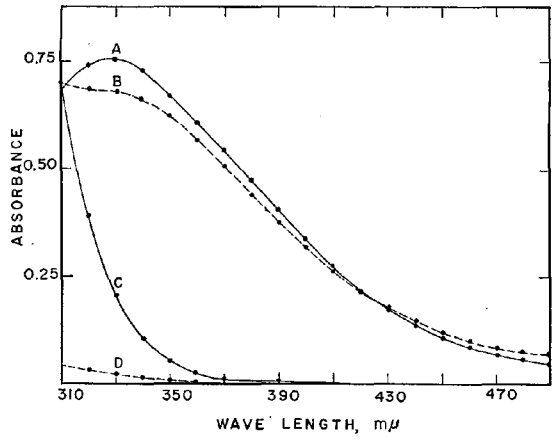

Figure 2. Absorption spectra

A. Uranium peroxide complex vs. peroxide blank
B. Same as *A* after 72 hours
C. Peroxide blank vs. distilled water
D. Same as *C* after 72 hours

Effect of Hot and Cold Elution and Blowing Out of Columns. As this procedure was designed to be a rapid, routine method, the need for determining the maximum efficiency of elution with a minimum amount of eluting agent is evident. Because it was desirable to keep the volume of eluting agent at 50 ml., a study was made of the effect of using a hot elution technique, as well as the effect of blowing out the columns. Results of this investigation are illustrated in Figure 3. Although 100% efficiency in elution is never obtained with 50 ml. of eluting agent, the hot elution technique, including blowing out the columns, comes the closest to reaching this goal.

Standard Curves. The elution efficiency with 50 ml. of eluting agent is not quite 100%; therefore, maximum accuracy in the actual analysis of samples can be obtained only if standard curves are prepared using the same techniques as in the analysis. The curves were prepared from standard uranium solutions that had been adsorbed on the resin from a solution containing 0.1*M* sodium sulfate (*2*) and 10 ml. of 6% sulfurous acid in 100 ml. The pH was controlled between 1.0 and 1.5 with 3*M* sulfuric acid. The standards were then eluted and the color was developed as for samples. Absorbances were determined in 0.5- and 1-inch cells at 380 and 420 mμ.

Flow Rates during Adsorption and Elution. The flow rate during the adsorption step does not appear to be critical up to 2 drops per second. However, an appreciable decrease in efficiency of elution is noted at 2 drops per second, particularly with cold elution. The decrease in efficiency is much less with hot elution. Maximum reproducibility is obtained with hot elution and a flow rate of 1 drop per second for both adsorption and elution.

Solution of Ores. The methods recommended by Fisher (*3*) for dissolving the ore samples include an oxidizing leach with sulfuric acid and manganese dioxide, a nitric-hydrofluoric acid leach, and a sodium carbonate fusion. The fusion was ruled out as unsuitable for routine analysis. The use of hydrochloric acid followed by nitric acid and final fuming with sulfuric acid (aqua regia method) was investigated thoroughly. Comparative results on standard samples using the manganese dioxide–sulfuric acid and aqua regia dissolving techniques (Table I) show that the manganese dioxide method gives slightly higher values, particularly when the technical grade reagent is used. The samples also filtered slowly. A comparison of results obtained by the aqua regia method with two cell sizes and at

two wave lengths for a series of analyzed samples is reported in Table II.

The Atomic Energy Commission samples referred to in Tables I and II are a series of ores from the Colorado Plateau. The reported uranium content of these samples is a statistical average obtained from the results of several independent laboratories. These samples contain several minerals, including carnotite, tyuyamunite, autunite, torbernite, and schoepite.

The aqua regia method was successful in dissolving all of the ores encountered in this research. However, some uranium-containing minerals, such as monazite, columbite, and tantalite, are not completely dissolved by aqua regia. In such cases the analyst must resort to other methods including the classic nitric-hydrofluoric acid leach followed by a sodium carbonate fusion.

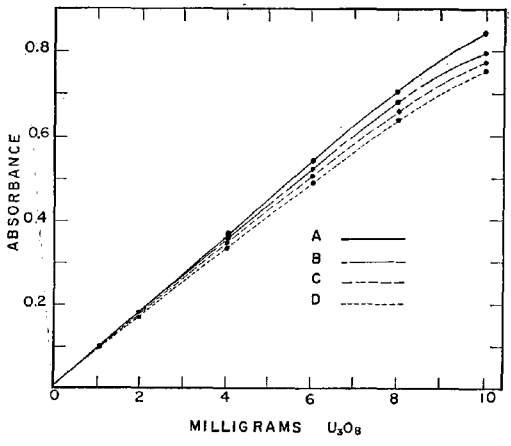

Figure 3. Absorbance studies of elution efficiency of 50 ml. of 1M perchloric acid

A. Reference curve prepared from standard uranium solutions not adsorbed or eluted
B. Recoveries using hot elution
C. Recoveries using cold elution
D. Recoveries using hot elution but not blowing out column

Table I. Comparison of Solution Methods

(1-gram samples used)

	Method of Sample Solution to Obtain % U_3O_8[a]						
	Manganese Dioxide						
	Technical		C.P.		Aqua Regia		% U_3O_8 Reported for Sample
Sample No.	380 mμ	420 mμ	380 mμ	420 mμ	380 mμ	420 mμ	
AEC No. 9	0.158	0.170	...	...	0.105	0.111	0.109
AEC No. 10	0.465	0.468	0.434	0.440	0.395	0.390	0.399
AEC No. 11	0.830	0.825	0.770	0.760	0.755	0.750	0.754
AEC No. 12	...	...	0.600	0.610	0.525	0.513	0.528
AEC No. 13	0.40	0.41	...	...	0.34	0.33	0.346

[a] Uranium percentages are averages of duplicates.

INTERFERENCES

Previous workers (*1–3*) showed that iron, vanadium, chromium, and molybdenum did not interfere after reduction with sulfurous acid. Nitrates, chlorides, and perchlorates, however, were shown to interfere unless present in low concentrations. Because the procedure utilizes an anion exchange resin, all metal ions that do not form anionic complexes can be excluded from the list of interfering ions. Other metal ions that were investigated during this work and produced no interference were cerium and thorium.

One type of interference that was not expected involved samples containing organic carbon. Examples were ores from Temple Mountain, Utah, and Lonesome Pete Claim No. 2, South Dakota, as well as flotation concentrates in which sulfonated petroleum oils had been used as flotation reagents. The organic material is not completely removed by the aqua regia method and imparts to the solutions a brown color that is partially adsorbed by the resin and eluted by the perchloric acid. Ignition of the samples prior to solution is the most satisfactory method of eliminating this interference. Organic carbon can also be removed by the use of perchloric acid in the solution step followed by strong fuming with sulfuric acid. However, because of the danger of incomplete removal of all perchlorates before adsorption, this procedure is not recommended. Removal of the carbon

Table II. Standard Samples Run by Routine Procedure

Sample	% U_3O_8 Reported for Sample	Grams of Sample	Av. % U_3O_8 Found				Over-all Av. % U_3O_8	Dev. of Av. from Reported %
			1-Inch Cells		0.5-Inch Cells			
			380 mμ	420 mμ	380 mμ	420 mμ		
New Brunswick No. 3 Pitchblende	3.36	0.25	3.32	3.36	3.28	3.30	3.32	−0.040
AEC No. 9	0.109	2.5	0.105	0.111	...	...	0.108	−0.001
AEC No. 10	0.399	1.0	0.395	0.385	0.380	0.385	0.386	−0.013
AEC No. 11	0.754	1.0	0.755	0.750	0.745	0.740	0.748	−0.006
AEC No. 12	0.528	1.0	0.525	0.510	0.513	0.515	0.516	−0.012
AEC No. 13	0.346	1.0	0.340	0.330	0.315	0.350	0.334	−0.012
AEC No. 8	0.064	5.0	0.060	0.065	...	...	0.063	−0.001
New Brunswick No. 5 (carnotite)	0.110	5.0	0.118	0.118	0.114	0.116	0.117	+0.007
Lonesome Pete[a] Claim No. 2	0.330	5.0	...	0.340	0.330	0.330	0.333	+0.003

[a] Filtered prior to reading absorbance.

by evaporation of the perchloric acid eluate to fumes is also possible, but this procedure gives slightly low results, indicating some type of uranium loss due to the organic material.

Sill and Peterson (7) reported that samples containing appreciable amounts of calcium tend to give low results when they are fumed with sulfuric acid because of coprecipitation of uranium with the calcium sulfate. This was verified in this laboratory on samples containing 20% calcium carbonate and 0.2% uranium oxide. The loss due to coprecipitation, however, was less than 0.01% uranium oxide.

Because nitrates and chlorides cause leakage of uranium in the adsorption step, several tests were conducted to determine whether double fuming with sulfuric acid might be necessary when 10-gram samples were used. In all cases single fuming was found sufficient.

The presence of appreciable amounts of phosphate and iron presented a problem. Because of the decrease in the oxidation potential of the iron system in the presence of phosphate, the anionic complexes of iron(III) with phosphate or sulfate are not completely reduced by the sulfurous acid. As a result some iron is adsorbed by the resin as an anionic complex and eluted along with the uranium. Similar observations have been reported by Holroyd and Salmon (5). When the eluate is made basic prior to color development, iron(III) hydroxide is formed in a near colloidal condition which imparts a yellow to orange coloration to the solutions, giving high readings on the colorimeter. This interference starts to become troublesome in samples that contain 250 mg. of iron when combined with phosphorus pentoxide in excess of 250 mg. Stronger reducing agents such as hypophosphorus acid completely reduced the iron and eliminated it from the eluate, but low uranium values resulted, probably due to partial reduction of uranium to the +4 oxidation state. When hot reduction with sulfurous acid was attempted, some ferric phosphate precipitated and plugged the columns. In addition some iron was still present in the eluate. At present the only method of eliminating this interference involves filtering of the solutions as described in the procedure.

CONCLUSIONS

The ion exchange method described has been successfully applied to a routine determination of uranium in primary and secondary uranium minerals. It has been used on a wide variety of ores for 1 year with excellent results; the total number of assays run now exceeds 3000. In the adoption of this method considerable analysis time has been saved, with a significant extension of ore types that can be analyzed. About 20 analyses can be made per man per day with a minimum of equipment and at relatively low cost. An additional benefit with the use of this method has been an extension of the lower concentration limits that could be adequately handled. In order to effect these improvements, refinements in operating techniques and apparatus together with the elimination of common troublesome interferences was realized. Duplicate analyses on 0.5- to 10-gram samples consistently agree to about 0.005% uranium oxide on material with a total uranium content as low as 0.01%.

ACKNOWLEDGMENT

The authors wish to express their appreciation to John N. Butler and his staff at the Mackay School of Mines for their suggestions and encouragement, and for early adoption of the procedure in routine analyses for uranium.

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Stability of Sodium Tetraphenylboron Solutions

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▶ **Water solutions of sodium tetraphenylboron have been prepared which remained stable for several weeks, even though stored in colorless containers at laboratory temperature (26° C.) in diffused light. When a solution of sodium tetraphenylboron in water is brought to a pH above 7 (ca. 8 or 9) by the addition of sodium hydroxide, the sodium tetraphenylboron remains stable for at least several weeks, as shown by ultraviolet transmittance spectra.**

SINCE the announcement of Wittig and his coworkers (3–6) that the sodium tetraphenylboron complex, $B(C_6H_5)_4^-$, forms potassium and rubidium salts which are low in water solubility, many reports have described the application of this useful reagent as its sodium salt. Gloss (1) has published a good review of the literature.

Water solutions of sodium tetraphenylboron undergo slow changes, with loss of precipitating power for potassium ion, the appearance of a turbidity, and the development of a phenolic odor. Such a solution, if it is to be relied on as an analytical reagent, must be stabilized against decomposition. Gloss and Olson (2) discuss the stability of such solutions and recommend that sodium tetraphenylboron solution be adjusted to a pH near 5 (normally attained after treatment and clarification by alumina) and that the solutions be stored at or below room temperature.

This paper reports further studies on the stability of water solutions of sodium tetraphenylboron through measurements of their ultraviolet absorption. Water solutions of this compound (water as reference) absorb greatly at wave lengths below 280 to 290 mμ and this absorption can serve as an excellent guide to the stability of this substance.

MATERIALS AND APPARATUS

Sodium tetraphenylboron, prepared by Henry Lee, St. Louis, Mo.

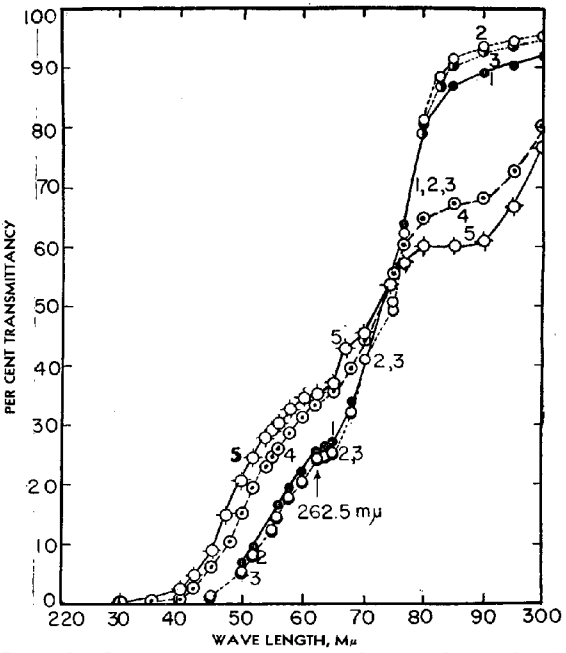

Figure 1. Per cent transmittance as a function of wave length (λ) for water solutions of sodium tetraphenylboron ($NaΦ_4B$).

Solutions were originally from same stock (about 0.01M) diluted 1 to 10 (M = *ca.* 0.001M). Each transmittance curve was determined on 0.001M solutions diluted 2:5. During transmittance measurements each solution was at pH approximately 9

1. ● ———Solution of sodium tetraphenylboron, pH = 6.7, 30 minutes after preparation
2. ○ - - - -Solution of sodium tetraphenylboron, pH = 10.5, 30 minutes after preparation
3. ◑ - - - -Solution 2, pH = 10.5, 24 hours later
4. ○ - - - -Solution 1, pH = 6.7, 24 hours later
5. ◎ ———Solution 1, pH = 6.7, 48 hours later

All other chemicals were of the best purity commercially available.

A Beckman DU quartz spectrophotometer was employed for the ultraviolet measurements.

PROCEDURE

Solid sodium tetraphenylboron was dissolved in water, allowed to stand overnight and filtered, and its ultraviolet transmittance spectrum was determined between 220 and 300 mμ. Solutions of 10^{-4}, 10^{-3}, and $10^{-2}M$ sodium tetraphenylboron in water were prepared, stored under normal laboratory conditions, and also in the dark at 5° to 10° C. Tests were periodically made to determine the precipitating power of the solutions by their reaction with potassium chloride. Measurements of pH were made both with pH paper and with the glass electrode.

In order to test for stabilizing influence of high pH values a fresh stock solution (about $10^{-2}M$) was divided into two parts: part 1 (pH = 6.7) and part 2 (pH made 10.5 by the addition of sodium hydroxide). Each of the parts was diluted 1 to 10 (giving a concentration of $10^{-3}M$). Parts 1 and 2 were allowed to stand at room temperature in diffused light and were periodically checked by ultraviolet transmittance. Before transmittance curves were determined, samples of the $10^{-3}M$ solutions were diluted 2 to 5 and brought to a pH of 9. Transmittancies were determined on these solutions. Results are plotted in Figure 1.

RESULTS AND CONCLUSIONS

In Figure 1 are given ultraviolet transmittance curves which prove that elevated pH values stabilize a water solution of sodium tetraphenylboron. The caption to Figure 1 is self-explanatory. A stock solution of sodium tetraphenylboron (approximately 10^{-2} M) was prepared and divided into two parts: part 1 (pH = 6.7) and part 2 (pH made 10.5). Stock solutions were diluted 1 to 10, giving ($10^{-3}M$), and were then diluted 2 to 5 and brought to a pH of 9 by addition of sodium hydroxide before ultraviolet transmittances were taken. Transmittance curves 1 and 2 (Figure 1) were determined 30 minutes after the solutions were prepared; curve 1 for part 1 (pH = 6.7), and curve 2 for part 2 (pH = 10.5). Curve 3 was determined on part 2 (pH = 10.5), 24 hours later. Curves 2 and 3 are essentially the same over the range 240 to 300 mμ. Curves 4 and 5 are for part 1, 24 and 48 hours, respectively, after the data for curve 1 were taken. Curves 4 and 5 show a greater change than does curve 1, from the composition represented by curve 2. Part 2 was tested periodically for 3 weeks and the transmittance curve obtained was essentially the same as that of curve 2 or 3. Part 1 continued to change in transmittance values and its pH slowly rose. The rate of change slowed down as the pH rose. Both parts 1 and 2 showed precipitation with $10^{-3}M$ potassium chloride.

Sodium tetraphenylboron ($10^{-3}M$) can be used as a precipitant for potassium, but soon loses its power for precipitation if not stabilized at high pH values.

Stabilization at pH values of between 8 and 9 with sodium hydroxide does not render sodium tetraphenylboron solutions unusable for precipitation and determination of potassium. The sodium ion introduced as sodium hydroxide does not exceed the sodium ion introduced with the compound itself. As an example, 1 ml. of 0.1N sodium hydroxide was employed in bringing 100 ml. of $10^{-3}M$ sodium tetraphenylboron to a pH of 9. Calculations show that the total concentration of sodium in the final solution is $2 \times 10^{-3}M$, one half of which arises from the sodium tetraphenylboron itself. The pH of this solution should rise to 9, even if the sodium tetraphenylboron did not hydrolyze at all. The total sodium in the stabilized solutions at high pH is never high enough to interfere with the use of the reagent in precipitating reactions. Of course, one may precipitate with the reagent from acid solutions, since the concentration of base is never high in the stabilized solution.

Sodium tetraphenylboron solutions covering a concentration range of 4×10^{-4} to $4 \times 10^{-5}M$ and at a pH of 10.5, have been shown to obey Beer's law strictly at 240, 250, 262.5, and 272 mμ. These solutions remained essentially unchanged for at least 1 week.

Solutions of approximately 0.01M sodium tetraphenylboron show pH values, when first made, of 6.5 to 6.7 and do not change composition rapidly because of their relatively high pH value. Bringing the 0.01M or 0.001M

solution to a pH of about 8 or above will cause it to retain its composition and its precipitating power over a period of several weeks. No additional effect on the stability was noticed on storing the solutions at elevated pH values in either clear soft glass or clear borosilicate glass containers.

By measuring the change in absorbance (ultraviolet) when potassium tetraphenylboron is precipitated with an excess of sodium tetraphenylboron and the precipitate is filtered away, the author has been able to establish a method for the quantitative estimation of potassium ion.

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Separation and Determination of Radiocerium by Liquid-Liquid Extraction

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▶ Because of the need for a rapid and effective method for determining radiocerium in fission products, which would give good decontamination from transuranium elements, the liquid-liquid extraction of cerium(IV) with 2-thenoyltrifluoroacetone was studied. A method for both tracer and macro levels was developed for the separation of cerium from many other elements, based on extraction into 0.5*M* 2-thenoyltrifluoroacetone–xylene from a 1*N* sulfuric acid solution containing potassium dichromate and sodium bromate. Yields are approximately 80%, with an average deviation of about ±2%. The procedure offers a fast, safe, and simple method for radiocerium with or without carrier and for the purification of radioactive or inactive cerium.

DETERMINATION of radiocerium by the method of Hume, Ballou, and Glendenin (*1*) is tedious, requires about 3 hours, and is not readily adaptable to remote control when high levels of radioactivity are present. The more recent hexone extraction method (*3*) is considerably faster and applicable to remote control. However, special precautions are required to avoid the hexone–nitric acid hazard, and thorium, uranium, and neptunium must be removed prior to extraction of the cerium.

A liquid-liquid extraction method for the purification and/or determination of radiocerium may be used with or without cerium carrier. In principle the cerium(IV) ion forms a stable chelate complex with 2-thenoyltrifluoroacetone (TTA) in 1*N* nitric acid. In its simplest form, assuming no other cerium(IV) complexing in the aqueous phase, the over-all reaction may be written as:

$$Ce_A^{+4} + 4HT_X \rightleftharpoons CeT_{4X} + 4H_A^+$$

where HT is the enol form of TTA and CeT_4 is the cerium(IV) chelate. Subscripts *A* and *X* refer to the aqueous and xylene phases, respectively.

The mechanism of the reaction involves hydrogen replacement and coordinate bonding. Both the 2-thenoyltrifluoroacetone and the cerium chelate have negligible solubility in the aqueous acid solutions but are soluble in xylene.

PROCEDURES

Cerium-144 Tracer. Pipet a suitable aliquot, free of fluoride, chloride, and phosphate ions, into an extraction vessel (preferably a 125-ml. separatory funnel) and adjust the solution to approximately 1*N* in nitric acid, 0.1*M* in potassium dichromate, and 0.1*M* in sulfuric acid (or to 1*N* in sulfuric acid and 0.1*M* in potassium dichromate). Mix the reagents and allow to stand for 5 minutes at room temperature. Add an equal volume of 0.5*M* 2-thenoyltrifluoroacetone–xylene and mix the phases thoroughly for 10 minutes with a glass-paddle stirrer driven by a high-speed motor. After the phases have separated, draw off the aqueous phase and discard. Wash the organic phase by mixing with an equal volume of 0.1*M* sulfuric acid–0.1*M* potassium dichromate for 3 minutes. Discard the aqueous phase and strip the organic phase by mixing with an equal volume of 10*N* nitric acid for 1 minute. Discard the organic phase and use an aliquot of the aqueous strip phase for the radioactivity measurement.

Cerium-144 with Carrier Solution. PREPARATION OF CARRIER SOLUTION (*1*). Dissolve 31 grams of cerium(III) nitrate hexahydrate in distilled water and dilute to 1 liter. To standardize, pipet 5-ml. aliquots of the solution into 50-ml. centrifuge tubes. To each add 1 ml. of 6*N* nitric acid and 15 ml. of water. Heat just to boiling and add 15 ml. of saturated oxalic acid with stirring. Cool in an ice bath for 10 minutes with occasional stirring.

Wash a fine, sintered-glass crucible by passing through 5 ml. of distilled water, three 5-ml. portions of 95% ethyl alcohol, and three 5-ml. portions of anhydrous ether. Put the crucible in a vacuum desiccator without desiccant and apply vacuum for 2 minutes. Flush out the ether vapors by releasing the vacuum, then pump out again. Release, evacuate for 2 minutes, flush as before, release, and then evacuate for 2 minutes. Release the vacuum and weigh the crucible.

Filter the oxalate precipitate through the crucible. Wash and dry the crucible and contents in exactly the same way as the crucible was treated. Weigh the precipitate as $Ce_2(C_2O_4)_3 \cdot 10H_2O$.

STANDARD METHOD. Pipet a suitable aliquot of sample, free of fluoride, chloride, and phosphate ions, into a 50-ml. Lusteroid centrifuge tube and adjust the aqueous solution to a concentration of 1*N* in sulfuric acid, 0.1*M* in potassium dichromate, and 0.2*M* in sodium bromate, with 0.8 mg. per ml. of cerium carrier. After swirling to mix the reagents, place the solutions in an ice bath for 5 minutes. Add an equal volume of 0.5*M* 2-thenoyltrifluoroacetone–xylene to the aqueous solution and extract for 10 minutes with the paddle stirrer. Rinse the stirrer with acetone after each extraction, then with distilled water. Centrifuge the solutions for 0.5 minute in a clinical centrifuge and remove the aqueous phase with a transfer pipet or micropipet attached by rubber tubing to a vacuum trap. By using mild suction and squeezing the tubing until the

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pipet is at the bottom of the tube, the aqueous phase can be withdrawn with a negligible loss of the organic phase. Wash the pipet after each use by dipping the tip into acetone and applying suction. Then wash down the sides of the centrifuge tube with several milliliters of distilled water and centrifuge again for 0.5 minute.

Again remove the aqueous layer by suction, taking care to remove a minimum of the organic phase. Carefully decant the organic phase into a clean Lusteroid centrifuge tube, add to it an equal volume of 1*N* sulfuric acid, and mix the phases thoroughly for 3 minutes. Centrifuge for 0.5 minute and remove the aqueous wash solution by suction. Again wash the sides of the tube with several milliliters of distilled water and centrifuge for 0.5 minute. Withdraw the aqueous solution and carefully decant the organic phase into a clean Lusteroid tube as described above. Add an equal volume of 10*N* nitric acid, and, after thoroughly agitating the phases for 1 minute, centrifuge for 0.5 minute and draw off the organic phase. Rinse the sides of the centrifuge tube with several milliliters of xylene, centrifuge for 0.5 minute, and remove the xylene by suction. If desired, an aliquot of the aqueous layer may now be decanted for counting, as in the above procedure. Add concentrated ammonium hydroxide to precipitate cerium(III) hydroxide and centrifuge for 2 minutes. Discard the supernatant solution and wash the precipitate well with 10 ml. of distilled water, then centrifuge again for 2 minutes. Again decant the supernatant liquid and dissolve the cerium(III) hydroxide in 1 ml. of 6*N* hydrochloric acid.

Dilute to approximately 20 ml., heat the solution to boiling, and add 15 ml. of saturated oxalic acid. Then place the solution in an ice bath for 10 minutes. Filter the precipitate through a tared filter paper in a Hirsch funnel and wash as for the preparation of the cerium carrier solution. Weigh as $Ce_2(C_2O_4)_3 \cdot 10H_2O$. Prepare for either beta or gamma counting, which is performed 3 hours after the final chemical separation to allow praseodymium-144 to grow in to saturation.

EXPERIMENTAL

Variables in Tracer Method. To study the effect of time, the procedure given for tracer cerium was used, except that the original aqueous phase was adjusted to 1*N* nitric acid–0.1*M* potassium dichromate, and the extraction time was varied. The following results show that substantial equilibrium was reached in 10 minutes:

Minutes	Cerium-144 Extracted, %
2	82.1
5	96.6
10	98.2
30	97.9

It is surprising that the dichromate ion will oxidize cerium(III) to cerium(IV) to the extent shown above, because the standard oxidation potential (E_0) of the cerous-ceric couple is higher (more negative by the Latimer convention) than that of the dichromate-chromic couple. Such a phenomenon has also been observed by Pitzer (*6*). The oxidation may take place because the relatively small number of cerium atoms involved with cerium-144 tracer, together with their removal from the aqueous phase, may cause the reaction to go in the direction Ce(III) → Ce(IV). Effectively, the cerous-ceric couple potential is probably lowered relative to the dichromate-chromic potential.

With carrier-free cerium-144 tracer, sodium bromate could not be used in the system as an oxidant because a very slight scum of undetermined nature, containing >90% of tracer, usually appeared at the interface. It was established that this tracer cerium behavior was due to the copresence of sodium bromate and xylene. If it is not known whether carrier cerium is present or not, a small amount of cerium carrier should be added (~0.5 mg. per ml.) and the macro method used.

Experiments showed that variation in nitric acid acidity during dichromate oxidation had negligible effect in cerium tracer work. The procedure described above was used, and the acidity was adjusted after the 5-minute oxidation period. The use of less than 1*N* nitric acid is inadvisable, because of inadequate decontamination from other elements.

HNO_3, *N*	Cerium-144 Extracted, %
0.5	98.5
1.0	98.0
1.5	96.4
2.0	92.5

Washing the organic phase containing the cerium-144 tracer with dilute nitric acid containing 0.1*M* potassium dichromate resulted in severe losses. A wash solution consisting of an equal volume of 1*N* sulfuric acid resulted in 5 to 10% losses of cerium-144 tracer, whereas a wash solution of 1*N* sulfuric acid–0.1*M* potassium dichromate gave losses of only 0.7% in 3 minutes. A wash solution consisting of an equal volume of 0.1*M* potassium dichromate and 0.1*M* sulfuric acid gave losses of only 0.1%. The presence of an oxidant in the wash phase was necessary to prevent excessive losses in carrier-free cerium tracer work. If the initial aqueous solution was made 0.1*M* in sulfuric acid, the yield of cerium-144 tracer was increased from about 98 to 99%. The cerium-144 tracer was readily stripped quantitatively from the organic phase into an equal volume of 10*N* nitric acid.

Typical decontamination results are shown in Table I. These values are the average of duplicates.

Table I. Decontamination from Various Elements in Cerium Tracer Method

Element	Amount Added, C.P.M.	Found in Strip Phase, %
Eu-152-154	3.1×10^7	0.04
Ru-106	2.8×10^7	0.2
Zr-95–Nb-95	5.9×10^7	0.01[a]
I-131	6.2×10^7	0.1[b]
Pa-233	6.6×10^6	0.1[a]
Pu-239	6.0×10^5	0.5

[a] 10*N* nitric acid strip solution washed for 5 minutes with an equal volume of 0.5*M* TTA–xylene.

[b] Iodine removed by extraction into carbon tetrachloride after addition of 20 mg. of potassium iodide carrier.

Variables with Carrier Cerium. Effect of Reagent Concentration, Time, Temperature, and Acidity. When more than traces of inactive carrier cerium are present or when the solution may contain unknown impurities, it is necessary to add carrier cerium (~1 mg. per ml.) and determine the extraction yield by oxalate precipitation (*1*). As expected from the oxidation potentials involved, experiments showed that dichromate ion failed to oxidize cerium(III) carrier appreciably. Table II shows the effect of varying the concentration of cerium carrier added, using the tracer procedure described above.

Table II. Effect of Varying Concentration of Cerium Carrier on Extraction of Cerium-144

Cerium Carrier Added, γ	Cerium Extracted, %
0	99.0
8	99.0
80	33.3
800	8.6

Sodium bromate was the most convenient reagent for the oxidation of macroquantities of cerium. As the presence of dilute sulfuric acid in the aqueous solution stabilized the ceric ion, it was decided to study the more important variables in a sulfuric acid system.

In the cerium distribution experiments each initial aqueous phase contained 8×10^5 gamma counts per minute of cerium-144 in 12-ml. vol-

Table III. Effect of Variables on Extraction of Macroquantities of Cerium into 0.5M 2-Thenoyltrifluoroacetone–Xylene

Aqueous Phase		Cerium Extracted, %	
		Oxidation Temperature	
H_2SO_4, N	HNO_3, N	5 Min. at room temp. (24° C.)	5 Min. in ice bath
1	1	60.3	74.0
0.5	..	66.9	79.9
1		90.2	92.2
		Oxidant Concentration	
H_2SO_4, N	$K_2Cr_2O_7$, M	$NaBrO_3$, M	
1	..	0.6	86.9
1	0.1	0.6	91.8
1	0.05	0.2	91.6
1	0.25	0.2	93.9
		Sulfuric Acid Concentration	
H_2SO_4, N			
1			94.3
2			74.4
3			53.2

umes. All counting was done in a well-type scintillation counter having a sodium iodide crystal (thallium-activated). Table III indicates that the oxidation is somewhat improved when performed in an ice bath. The presence of concentrations of sodium bromate greater than 0.2M does not appear necessary in the standard method (Table III). Potassium dichromate seems to give slightly greater yields of cerium. However, it is not mandatory to use oxidation in an ice bath or potassium dichromate in an analytical radiochemical method where a yield correction is applied.

Sulfuric acid concentrations less than approximately 1N gave lower yields of cerium, presumably because of a decrease in sulfate stabilization of cerium(IV) and an increasing tendency of cerium to hydrolyze at low aciditics. A sulfuric acid concentration higher than approximately 1N affects the cerium distribution adversely because the ceric chelate becomes less stable and sulfate ion competition for the ceric ion is more pronounced (Table III). Because the chelation of cerium(IV) is highly selective from many other ions in 1N sulfuric acid, subsequent experiments were made at this concentration.

Based on the following data, a 10-minute extraction period was selected for subsequent work:

Minutes	Cerium Extracted, %
5	63.0
10	94.6
15	95.5
20	97.3

Two 10-minute extractions are recommended for approximately quantitative recovery of the cerium, but for radiochemical analytical purposes where a yield correction is applied, one extraction is adequate. Use of a double volume of the organic phase did not increase the yields appreciably.

The following data indicate that sulfate stabilization of cerium(IV) is important to effect a good yield and that moderate concentrations of nitric acid can be tolerated.

Aqueous Phase Acidity		Cerium Extracted, %
HNO_3, N	H_2SO_4, N	
1	..	47.2
0.8	1	86.7
0.4	1	82.2

A solution of cerium, originally contained in nitric acid, should be adjusted to approximately 1N sulfuric acid–0.5N (or less) nitric acid before performing the extraction.

At cerium concentrations greater than approximately 1 mg. per ml. in the aqueous phase, the extraction behavior was somewhat erratic, although yields were still adequate for an analytical radiochemical method (Table IV). The decreasing extractability of cerium with increasing concentration at constant acidity may be an indication of the hydrolytic polymerization of cerium(IV) to form nonextractable species. Such a phenomenon for zirconium (2, 4, 5) has been observed in this type of system. In cerium carrier work the aqueous phase after the initial extraction was usually slightly cloudy, and a small amount of precipitate settled out within a few hours. However, the organic phases were clear and cerium yields were always satisfactory.

Selection of Wash Solution. Equal volumes of 0.5M 2-thenoyltrifluoroacetone–xylene containing the cerium(IV) chelate prepared by the standard method were washed for 3 minutes by mixing intimately with the following various wash solutions:

	Cerium Loss, %
1N H_2SO_4–0.1M $K_2Cr_2O_7$–0.2M $NaBRO_3$	0.5
1N H_2SO_4–0.2M $NaBrO_3$	0.6
1N H_2SO_4	0.6
Distilled water	<0.03

Less than 0.1% cerium washed out of the organic phase in 1-minute distilled water wash after standing overnight, indicating that the cerium(IV) chelate is rather stable in the 0.5M 2-thenoyltrifluoroacetone–xylene phase. However, the standard wash solution selected was 1N sulfuric acid, because it gave more efficient decontamination from other elements. The use of oxidants in the wash phase did not appear necessary under the conditions used in cerium carrier experiments.

Table IV. Effect of Cerium Carrier on Extraction of Cerium into 0.5M 2-Thenoyltrifluoroacetone–Xylene

Cerium Carrier, Mg./Ml.	Cerium Extracted, %
0	99.0
0.4	94.5
0.8	92.1
1.7	82.6
2.5	77.1
4.8	61.0

Stripping of Cerium Carrier. Equal volumes of 10N nitric acid were used to strip the cerium from the organic phase. The data given below indicate the ease of removing the cerium from the organic phase with 10N nitric acid.

Minutes	Volume of Stripping Solution	Cerium Stripped, %
1	Equal	>99.9
3	Equal	>99.9
1	One half	>99.9

Even smaller volumes of 10N nitric acid stripping agent may be used. The standard stripping solution selected was an equal volume of 10N nitric acid with a 1-minute agitation period. A nitric acid concentration of 8N was

also effective, requiring only a few seconds longer than 10*N* nitric acid. The main criterion in stripping the cerium carrier is decolorization of the organic phase. Actually, a 1-minute agitation with an equal volume of 10*N* nitric acid is a several-fold excess.

Comparable concentrations of hydrochloric acid and sulfuric acid, or dilute hydrofluoric acid and various reducing agents, may be used to strip the cerium. Nitric acid was selected as the standard stripping solution because occasionally it may be desirable to re-extract the strip phase to give more effective decontamination from zirconium, protactinium, and iron.

Table V. Effect of Hydrochloric Acid Concentration on Extraction of Cerium(IV) Carrier

Aqueous Phase		Cerium Extracted, %
H_2SO_4, *N*	HCl, *N*	
1	0.05	10.2
1	0.5	5.2
1	1	1.7

EFFECTS OF HYDROCHLORIC ACID. Though chloride ion is known to greatly accelerate the reduction of cerium(IV), several experiments were performed in the presence of varying amounts of hydrochloric acid to determine the degree of interference. Table V indicates that even 0.05*N* hydrochloric acid interferes severely with the oxidation of cerium(III). Hydrochloric acid (or chloride ion) should be removed or destroyed prior to the oxidation of cerium. One convenient method would be to precipitate cerous hydroxide when carrier is present, wash thoroughly, dissolve the precipitate in sulfuric acid, and then use the standard method.

DECONTAMINATION FROM OTHER ELEMENTS

Elements found in fission product solutions were tested for degree of separation from cerium. Each pure element was studied individually in the standard procedure and the analyses were performed in quadruplicate.

The standard procedure was used. Appropriate reagents were added and mixed before placing the solution in the ice bath. After completion of the procedure, the strip phase was analyzed for the appropriate element. In several instances (Table VI) the strip phase was washed for 5 minutes with an equal volume of 0.5*M* 2-thenoyltrifluoroacetone–xylene to achieve increased decontamination.

The alkalies and alkaline earths were not tested because they do not form chelates in 1*N* acid with 0.5*M* 2-thenoyltrifluoroacetone–xylene. The trivalent actinides, americium and curium, behave similarly to europium(III) in this system. Decontamination appeared to be essentially the same whether cerium carrier (0.8 mg. per ml.) was present or absent. The decontamination results may, therefore, be regarded as conservative if an additional oxalate precipitation is performed in the radiochemical analytical method.

Table VI. Decontamination of Carrier Cerium from Various Elements

Element	Amount Added, Counts/Min.	Found in Strip Phase, %	
			After one wash
Eu(152–154)	1.2×10^7 (γ)	0.0004	..
Nb-95	2.3×10^6 (γ)	0.07	..
Pa-233	1.2×10^6 (γ)	1.16	0.09
Zr-95	1.8×10^7 (γ)	0.33	0.02
Ru-106	4.5×10^5 (γ)	0.35	..
U-233	3.8×10^5 (α)	0.01	..
Th-232	1750 (mg.)	0.002	..
Np-239	3.7×10^6 (γ)	0.03	..
Pu-239	6.2×10^5 (α)	0.02[a]	..
I-131	1.9×10^7 (γ)	0.02[b]	..
		<0.001[c]	
Sb-124	6.6×10^5 (γ)	0.05	..
Fe-59	4.6×10^5 (γ)	0.70	0.04

[a] The 5-minute oxidation should be performed in boiling water bath to effect good decontamination. About 63% of plutonium followed through when ice bath oxidation was used. Small amounts of plutonium may be allowed to follow through the method, if beta or gamma counting of cerium is to be done. Use of sodium bromate gives excellent decontamination from iodine, due to formation of nonextractable iodate.
[b] No KI carrier.
[c] 1.6 mg. per ml. of KI.

APPLICATIONS

The method discussed offers a rapid purification technique for radioactive or inactive cerium, and may occasionally prove useful for the elimination of cerium interference in other work. In carrier-free cerium tracer work, the technique may be used to estimate fission product cerium in an all-extraction method. For instance, radiocerium may be counted directly in an aliquot of the strip solution. This technique is often adequate in process control–type work.

The carrier method offers a fast, safe, and simple technique for the determination of radiocerium. Yields average approximately 80% and the precision is within about 2%. The method requires about 1 hour. Although separatory funnels or other extraction vessels may be used, 50-ml. Lusteroid centrifuge tubes were used in this work. They are inexpensive enough to discard after use, and eliminate much washing of glassware as well.

During the course of this work, it was observed that approximately 700 γ of cerium per ml. of 0.5*M* 2-thenoyltrifluoroacetone–xylene gave a very dark reddish-brown color. Upon dilution it was possible to detect a few tenths of a microgram of cerium visually. Instrumentally, the sensitivity could be extended considerably. The color was stable for several weeks.

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Estimation of Sodium Hyponitrite in the Presence of Sodium Nitrite, Sodium Nitrate, and Sodium Carbonate

Action of Nitrogen Tetroxide or Dioxide on Hyponitrites

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▶ Experiments conducted in this laboratory indicate that when sodium hyponitrite reacts with nitrogen tetroxide at a low temperature the only solid product of the oxidation is sodium nitrate. Because liquid nitrogen tetroxide dissolved in low concentration in an organic solvent, such as carbon tetrachloride or chloroform, does not react with sodium nitrite, sodium nitrate, or sodium carbonate, it is thus possible to determine sodium hyponitrite in their presence. Details of the low temperature procedure are given.

SODIUM hyponitrite is oxidized ultimately to sodium nitrate by the action of nitrogen tetroxide through several intermediate reactions (*1*). Nitrogen tetroxide reacts with sodium carbonate dried at 600° C. on prolonged contact. The ultimate product of reaction is nitrate and the percentage conversion only 10.2% in 19 hours (*2*).

Sodium hyponitrite, though isosteric with sodium carbonate, was found to react vigorously even with chlorine and bromine. When a solution of chlorine or bromine in dry carbon tetrachloride was added to dry sodium hyponitrite, vigorous reaction took place with evolution of heat and gas. The reaction was carried out at 0° C. The residue, after being dried with dry ether and dissolved in water, gave a distinct test for chlorate in admixture with chloride or bromate in admixture with bromide, but both hypochlorite and hypobromite were absent. Dry sodium carbonate under similar conditions was stable, even on prolonged contact. If sodium hyponitrite reacts in the same way as sodium carbonate with nitrogen tetroxide the reaction should proceed completely as follows:

$$Na_2N_2O_2 + 2N_2O_4 \longrightarrow 2NaNO_3 + N_2O + N_2O_3 \quad (1)$$

No nitrogen should be formed and the amount of nitrous oxide formed should be the same as the amount of nitrogen trioxide formed. An experiment carried out in vacuum (*9*) at 0° C. revealed the presence of nitrogen, nitrous oxide, nitrogen trioxide, and nitrogen tetroxide in the gas mixture and only sodium nitrate in the solid residue. Thus, it is clear that the reaction is not as simple as shown by Equation 1.

Nitrogen tetroxide or dioxide does not react with sodium nitrite below 140° C. (*3, 4*). These experiments were also performed by the author in vacuum, and the same conclusion was reached from the analysis of the solid residue and gaseous products.

Liquid nitrogen tetroxide diluted with organic solvent at a much lower concentration than in experiments cited in the literature has been found to be inert toward sodium nitrite, sodium nitrate, or sodium carbonate. However, it reacts with sodium hyponitrite which may exist in different forms (*7*), giving undoubtedly only one solid product, sodium nitrate, at low temperature.

$$Na_2N_2O_2 \xrightarrow{N_2O_4} 2NaNO_3$$

EXPERIMENTAL

Materials. Sodium hyponitrite was prepared according to Partington and Shah (*11*). Its purity was established by methods worked out by Oza and coworkers (*6, 8, 10*). Sodium nitrite, sodium nitrate, and sodium carbonate were extra pure analytical reagent chemicals. Anhydrous sodium carbonate was dried at 900° F. (482° C.) in an electric furnace for 2 hours, kept in a desiccator over phosphorus pentoxide, and analyzed. All organic solvents used in experiments were redistilled over phosphorus pentoxide. Nitrogen tetroxide (or dioxide) was prepared and sealed in tubes as described by Oza, Oza, and Thaker (*9*). These tubes (Figure 1, *A*), containing liquid nitrogen tetroxide, were made specially and sealed at the end, so that they could be broken easily in the tube containing organic solvent.

Procedure. About 20 ml. of carbon tetrachloride or chloroform was placed in a tube (Figure 1, *B*) and cooled to approximately 0° C. by an ice-water mixture. A tube, *A*, containing nitrogen tetroxide (approximately 0.5 gram) was inserted in tube *B* and placed as shown in Figure 1 in order to facilitate its breaking in the solvent. This assembly was kept in the bath for about

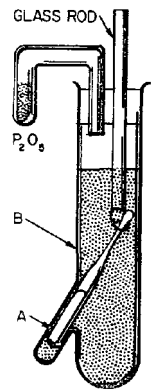

Figure 1. Assembly for determination of sodium hyponitrite by reaction with nitrogen tetroxide at low temperature

15 minutes. Tube *A* was then broken; nitrogen tetroxide was released slowly (the temperature being kept low) and allowed to dissolve in the organic solvent. The tube was shaken carefully in the bath to make the solution homogeneous.

Sodium hyponitrite was placed in a dry test tube and weighed. This tube was placed in the same bath to attain low temperature. Approximately 5 ml. of the previously prepared solution of nitrogen tetroxide in organic solvent was added to this hyponitrite and the solution and solid mixture were shaken carefully and allowed to remain in the bath for 10 to 15 minutes. Effervescence indicated the escape of some gas; this ceased within 2 or 3 minutes, but the test tube was allowed to remain at low temperature for 10 or 15 minutes.

The liquid was then carefully removed by decantation and the residue washed repeatedly with pure, cold solvent until the final washing gave no color, even at 50° C. The residue was then washed with dry ether, dried in hot air at about 70° C., and weighed. The residue was then dissolved in water

and analyzed qualitatively for hyponitrite ion, nitrite ion, and nitrate ion. Hyponitrite and nitrite were absent, and only nitrate was found. The material was then analyzed quantitatively for nitrate (*5*).

The experiments were conducted in a similar manner with pure sodium nitrite, sodium nitrate, and sodium carbonate, and in admixture with the hyponitrite. Carbonate and nitrite in aqueous solution were determined by standard methods.

RESULTS

Experiments 1, 2, and 3 (Table I) were carried out with sodium hyponitrite alone, and the nitrate formed was determined by a standard method (*5*). In experiments 4, 5, and 6 the nitrate formed from hyponitrite and that from the sodium nitrate taken were determined (column 4). Total sodium nitrate formed from hyponitrite was calculated (column 5), and the weight thus obtained, if substracted from the weight of residue formed (column 3), was in close agreement with the amount of nitrate taken. In Experiments 7, 8, 9, and 10, only sodium hyponitrite reacted with nitrogen tetroxide or dioxide; sodium carbonate remained unaffected. Chemical determination of nitrate formed gave results in close agreement with those calculated for its formation from hyponitrite only. In Experiments 11, 12, 13, and 14, the nitrite content of the residue was estimated by a standard method. The nitrite was converted to nitrate by standard permanganate solution, and this along with the other formed from hyponitrite was then determined by the standard method. These results are given in column 4.

Table I. Estimation of Sodium Hyponitrite[a]

No.	Substance Taken	Gram	Weight of Residue, Gram	Nitrate (Total) Found by Chemical Estimation, Gram	Nitrate (Calcd. from Hyponitrite Taken), Gram
1	$Na_2N_2O_2$	0.0525	0.0843	0.0841	0.0842
2		0.0735	0.1182	0.1190	0.1179
3		0.0852	0.1370	0.1368	0.1367
4	$Na_2N_2O_2 + NaNO_3$	0.025 + 0.020	0.0600	0.0602	0.03999
5		0.0352 + 0.030	0.0864	0.0865	0.05644
6		0.0485 + 0.040	0.1177	0.1178	0.07773
7	$Na_2N_2O_2 + Na_2CO_3$	0.0387 + 0.0412	0.1040	0.06210	0.06205
8		0.0465 + 0.0525	0.1268	0.07460	0.07457
9		0.0565 + 0.0425	0.1330	0.0907	0.09067
10		0.0156 + 0.0415	0.0665	0.0250	0.02501
11	$Na_2N_2O_2 + NaNO_2$	0.0210 + 0.025	0.0587	0.06448	0.03368
12		0.0280 + 0.045	0.0899	0.1005	0.04490
13		0.0325 + 0.045	0.0963	0.1070	0.05122
14		0.0415 + 0.050	0.1164	0.1285	0.06655

[a] Solvent used to dissolve nitrogen tetroxide was chloroform

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Sensitive Photometric Technique for Determination of Organophosphorus Compounds

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▶ A brief examination was made of a technique for determining small amounts of organophosphorus materials, which involves initial reduction to a phosphine with a solution of lithium aluminum hydride. The phosphine vapors generated are allowed to react with either silver nitrate or gold chloride on paper. A qualitative estimation may be made visually, or a more precise determination can be made by photometric examination of the test papers. Of the several compounds tested, organocompounds could be detected in microgram amounts, while inorganic materials produced but small response.

CETYL PHOSPHATE and benzenephosphonous acid can be reduced with lithium aluminum hydride in boiling diethyl ether solution. Karrer and Jucker (*5*) and Weil, Prijs, and Erlenmeyer (*8*) have recently published work on these experiments, in which they obtained phosphine and phenylphosphine, respectively, and swept it out of the reaction system with a stream of nitrogen. Similar reductions have been effected by Horvat and Furst (*4*) and by Freedman and Doak (*2*). These observations suggested an analytical method based on reduction with lithium aluminum hydride and subsequent determination of the phosphines.

The Gutzeit test (*3, 6, 9*) for detection of arsine or phosphine, which is based upon the ability of these vapors to react with silver ions or with other metal ions to produce a colored product, appeared suitable for detection of the phosphines produced. The procedure finally developed involved reduction

of the phosphorus compound in a suitable solvent by addition of an ether solution of lithium aluminum hydride. The phosphine produced was passed through silver nitrate–treated indicator paper using air as sweeping gas. The intensity of the test was measured by photometric examination of the test spots.

EXPERIMENTAL

Lithium Aluminum Hydride Reducing Solution. Ethers, particularly diethyl ether, are generally recommended for this reducing agent, although a number of other solvents can be used including the cyclic ether, tetrahydrofuran, and *N*-ethylmorpholine (*1*). Qualitative experiments with various solvents indicated that diethyl ether or a 1 to 1 mixture with *n*-butyl ether was suitable for the phosphine reduction test. No test could be obtained using diisopropyl ether, dioxane, or Carbitol (monoethyl ether of ethylene glycol) as solvents. Tetrahydrofuran solutions were moderately active when first prepared but lost their activity rapidly. *N*-Ethylmorpholine solutions gave a high blank test. The phosphorus compounds could not be reduced to phosphines by adding solid lithium aluminum hydride to an ether solution of the test sample, perhaps because of a different course of reaction with the solid reagent while it is dissolving.

In the present study the reducing solution was prepared by adding 25 to 50 mg. of the solid hydride to 3 ml. of 1 to 1 ethyl ether and di-*n*-butyl ether and agitating gently for 1 or 2 minutes to assist solution. A small amount of material always remained undissolved. The solution was prepared fresh for each experiment.

Indicator Papers. The Gutzeit test for arsine or phosphine is normally performed by allowing the vapor to come in contact with a paper which has been moistened with an aqueous solution of a silver, copper, or mercuric salt (*3*). A gold chloride solution in ethyl alcohol has also recently been suggested (*6*). In tests with 0.1*M* solutions, silver nitrate in 95% ethyl alcohol was as effective as gold chloride–ethyl alcohol, and the former test paper was selected. With silver the test spots were yellow to brown, while gold produced lavender spots.

The paper was in the form of strips cut from a roll of paper tape (Hurlbut Paper Co. South Lee, Mass.). A medium porosity filter paper worked equally well. When ethyl alcohol was used as solvent for the indicator salts, the test paper dried more rapidly to a constant absorbance than if water solutions were used. However, it was necessary to keep the paper moist with water during the test to obtain the best sensitivity. The moisture content was maintained by passing the test stream through a sintered-glass plate water bubbler. The solubility of phosphine in water is low enough so that apparently very little was retained in the bubbler. When a number of tests were to be performed, several papers were prepared beforehand by moistening them with the silver solution and superficially drying them in a dark chamber through which a moist stream of air (water bubbler) was passed. Alternatively the paper strips can be dried individually by allowing ethyl alcohol to evaporate for about 0.5 minute before placing the strip in the photometer test cell.

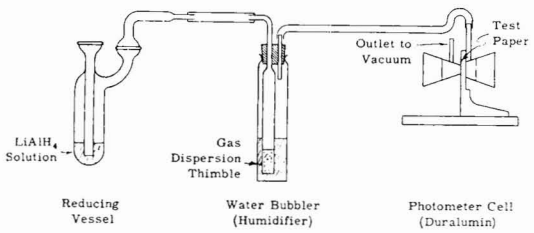

Figure 1. Apparatus for reduction of phosphorus compounds to a phosphine

Scale, 1/4 size

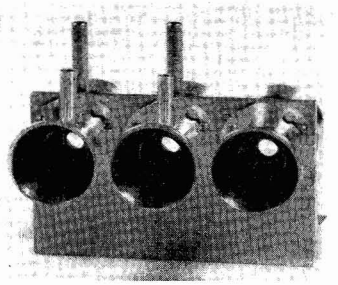

Figure 2. Cell assembly for phosphine paper test

Photometric Examination. To permit quantitative measurement of the extent of color produced by reaction of the phosphine on paper, a Fisher AC Model Electrophotometer was equipped with a Duralumin photometer cell assembly (Figures 1 and 2) especially constructed for the purpose, thus converting the instrument into a paper densitometer. The test cell had a gas inlet and outlet such that the intensity of light transmitted through the paper could be measured continuously. The additional two cells of the three-cell assembly were for a simultaneous blank test, if desired, and a standard reference paper. The glass windows of the cells were cemented into place with an Epon (registered trade-mark) adhesive. The test papers were held tightly between two halves of the cell and against an O-ring by a spring mechanism, an area 5 mm. in diameter being exposed to the gas stream. In order to prevent reduction of the gold or silver detection agent in the test paper by the Duralumin alloy, the portion of the cell in contact with the paper was coated with an amine-cured Epon varnish (*7*). To obtain greater sensitivity in the measurement, the potentiometer of the instrument was replaced by a Beckman Helipot, and an external galvanometer having a sensitivity of 0.03 μamp. per mm. was used. It was necessary to reduce the intensity of the reference light beam to approximately that of the light transmitted through the test papers. This was done by interposing a small piece of filter paper between the light source and the reference photocell.

A red filter (625 mμ) was used in all the tests because it was felt that this light would minimize photoreduction of gold or silver salts used as impregnants for the test papers. However, later experiments showed that yellow, green, or blue filters give a greater response with the gold chloride test paper, yet do not produce unduly high blanks.

Procedure. One milliliter of a solution of the test sample in dibutyl phthalate was placed in the reducing vessel (Figure 1). Dibutyl phthalate was used in these experiments because of its solvent power, although a greater response could be obtained with some of these materials in the test with ether solutions, possibly because ethers are less reactive with the hydride. The silver nitrate test paper was inserted into the photometric test cell and the vacuum line connected to the outlet of the test cell, so as to draw humidified air through the paper at the rate of 100 cc. per minute. The Helipot reading was recorded after it became constant (1 to 2 minutes). The outlet of the reducing vessel containing the sample was then connected to the humidifier, with the gas flow rate still at about 100 cc. per minute, and 3 ml. of the lithium aluminum hydride reducing solution was added slowly through the opening of the dip tube in the reducing vessel. The

Table I. Results of Phosphine Tests

No.	Compound	Phosphorus Present, γ	Decrease in Transmittance,[a] %	Remarks[b]
1	Blank	...	3	
2	Tributyl phosphate (11.6% P)	0.23	14	
		2.3	69	
3	Diisopropyl methyl phosphonate	0.34	5	
		3.4	24	
		34	88	
	$[(CH_3)_2CHO]_2\overset{O}{\overset{\|}{P}}-CH_3$ (17.2% P)			
4	Diethyl chlorophosphonate	0.36	23	
		3.6	90	
	$(C_2H_5O)_2\overset{O}{\overset{\|}{P}}-Cl$ (18.0% P)			
5	Parathion			
	$(C_2H_5O)_2\overset{S}{\overset{\|}{P}}-OC_6H_4NO_2$ (10.7% P)	2.6	8	
		6.4	15, 16[c]	
		12.8	28	
		25.7	49, 48[c]	
6	H_3PO_4, 85% aqueous	0.8 mg.	4	Suspension in DBP
7	KH_2PO_4	2.1 mg.	6	No DBP present
8	P_2O_5	2.2 mg.	3	Suspension in DBP
		2.2 mg.	33, 26	No DBP present

[a] Equivalent to per cent decrease in Helipot reading.
[b] DBP, dibutyl phthalate.
[c] Dilute sodium hydroxide scrubber used in exit line.

reaction vessel was not heated during the test. After about 5 minutes the Helipot reading attained a nearly constant value, indicating that generation of phosphine was essentially finished. Actually the major portion of the phosphine produced was usually evolved in 2 or 3 minutes.

Blank tests were performed in exactly the same way except that a phosphorus compound was not added to the dibutylphthalate.

RESULTS

Several phosphorus compounds were subjected to the reduction test using 1 ml. of dibutylphthalate as the solvent; the results are summarized in Table I. These materials gave phosphine tests of varying intensity, indicating that the particular groups attached to the phosphorus atoms have considerable effect upon the yield of phosphine obtained. In No. 3 methylphosphine would be expected, which may not behave the same as phosphine in the Gutzeit test. The greatest response was found with diethyl chlorophosphonate, in which case the equivalent of 0.2 γ of phosphorus was detected. No tests were made with known amounts of phosphine to determine the lower limit of the test. However, some idea of the limit of the Gutzeit test is indicated by the work of Mokranjac and Rašajski (*6*), in which 0.05 γ of arsine, equivalent to 0.02 γ of phosphine, was detected.

In the test obtained with Parathion it was suspected that hydrogen sulfide, which interferes with the silver nitrate paper test for phosphine, may have been produced. However, when dilute sodium hydroxide solution was used in the humidifier instead of water, a procedure which removed hydrogen sulfide quantitatively from the product gas stream, the result was unchanged. Therefore, the test obtained was due entirely to phosphine.

A few inorganic compounds were also tested. With phosphoric acid and potassium dihydrogen phosphate little or no effect was obtained beyond that observed in a blank test. The amount of lithium aluminum hydride employed was in large excess to the small amount of water present in the 85% phosphoric acid sample. Phosphoric anhydride gave no test for phosphine when it was suspended in dibutyl phthalate. However, this compound produced an appreciable amount of phosphine when the hydride solution was added directly to the dry sample.

ACKNOWLEDGMENT

The authors are indebted to C. J. Penther and W. H. Husing for assistance in modifying the Fisher colorimeter to serve as a paper densitometer.

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Tests for Aluminum and Hydroxytriphenylmethane Dyes

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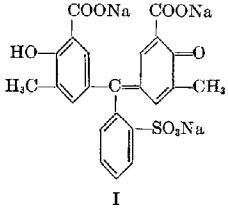
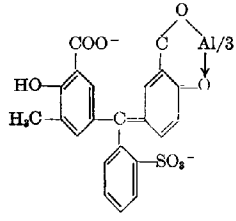
▶ **The chelate compounds produced in neutral or basic media by the action of aluminum ions with hydroxytriphenylmethane dyes are decomposed but slowly by hydrochloric or sulfuric acid (up to 1 to 1) and excess dye or dye acid can be removed by extraction with ether. Tests for aluminum ions can be based on these findings. As little as 0.1 γ of aluminum can be detected if Chrome-fast pure blue B is used as the reagent. The color reaction can be employed for the specific detection of alumina and also for traces of aluminum in water and silicates. The acid resistance of their aluminum compounds makes possible a spot test for hydroxytriphenylmethane dyes; the limits of detection are from 0.1 to 5 γ.**

EEGRIWE (*1*) described a very sensitive test for aluminum in which a violet color is produced by adding Eriochromecyanine (I) and alkali to an aluminum solution and then acidifying with dilute acetic acid. This color reaction is likewise the basis for colorimetric methods for the determination of aluminum (*6, 9, 11*). According to Millner (*5*), the colored product contains three equivalents of the dye radical per atom of aluminum. [In the literature cited here and likewise the work of Sandell (*7*) the water-soluble aluminum-Erichromecyanine compound is designated as a color lake. This expression, taken from dye chemistry for products derived from the action of metallic mordants and dyes, should be reserved for nonstoichiometrically defined adsorption compounds of dyes and metal oxides, hydrous oxides, or hydroxides, in gel or hydrosol form (*3*).] This indicates a chelate compound in which the aluminum is a constituent of a colored anion, as shown in the possible formulation (Ia):



I

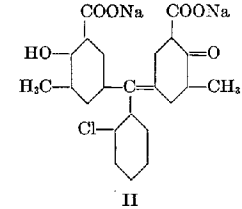


Ia

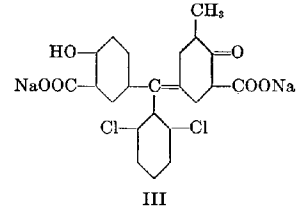
Although the Eegriwe color reaction can be accomplished with a drop or two of an aluminum salt solution, it has not been adopted in spot test analysis because of insufficient selectivity. In studies to overcome this fault it was found that the aluminum-Eriochromecyanine product formed in an alkaline medium has considerable resistance to strong hydrochloric acid, even though the color reaction does not occur at this degree of acidity. This fact was shown by the following trials, in which the reactants at like concentrations were brought together in a different order and the unused dye (or its acid) was then extracted with ether: (*a*) 1 ml. of 0.1% aluminum sulfate plus 1 ml. of 1% dye solution plus 1 ml. of 1% sodium hydroxide + 3 drops of concentated hydrochloric acid and (*b*) 1 ml. of 0.1% aluminum sulfate plus 1 ml. of 1% sodium hydroxide plus 3 drops of concentrated hydrochloric acid plus 1 ml. of dye solution.

If *a* and *b* are shaken with 1 or 2 ml. of ether, the latter becomes yellow, and the water layer is intensely red in *a* but only pale pink in *b*. The color in *b* is not due to a slight amount of the aluminum-dye product but is the consequence of incomplete extraction of the excess dye acid from the water layer. A blank with water yielded this same pink. The red water layer obtained in *a*, after the extraction with ether, gradually loses its color because the strong hydrochloric acid slowly decomposes the aluminum-Eriochromecyanine product. It is likely that during the decomposition there is transient production of the ionic species $[AlAc_2]^+$ and $[AlAc]^{++}$, in which Ac denotes an inner complex bound anion of the dye.

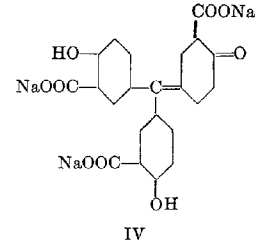
Since the dye acid is not extracted quantitatively by ether from an acidic solution, a comparison blank is required if very small amounts of aluminum are being sought. This slight drawback led the authors to try other hydroxytriphenylmethane dyes of analogous structure, since in accord with the accepted concept of group action in organic reagents (*2*), such other dyes could be expected to function likewise as color reagents for aluminum under the prescribed conditions. This expectation was confirmed with Eriochromeazurole (II), Chrome-fast pure blue B (III), Aurintricarboxylic acid(IV), Naphthochrome green(V), Chromeazurole S, and chromate blue.



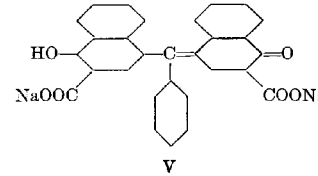
II



III

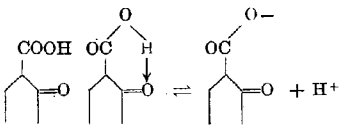


IV



V

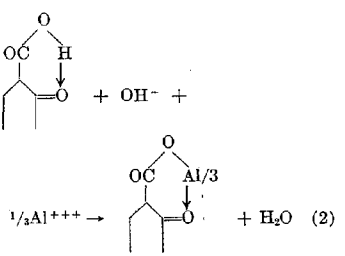
All these dyes come on the market as their water-soluble alkali salts, and addition of acid to their solutions liberates the corresponding dye acid, which is not soluble in water in most cases. Group A of these acids is probably responsible for the chelate binding of the aluminum as shown in Ia. The reason that no color reactions are observed when solutions of aluminum salts acidified with mineral acids are treated with alkali salts of these dyes or with alcohol or dioxane solutions of the respective dye acids may be that A is present in the nonreactive hydrogen-chelate form, A′, which is in pH-dependent equilibrium with the reactive anion, A^2. Adequate concentrations of A^2 are produced from this equilibrium



A A^1 A^2 (1)

when the system is neutralized or made basic.

On this basis the formation of the acid - resistant aluminum salts of hydroxytriphenylmethane dyes can be formulated:



(2)

Of the dyes that have been tried, the most worthy of recommendation are: Chrome-fast pure blue B (III) and Naphthochrome green G (IV): their dye acids can be quantitatively extracted with ether. The respective aluminum salts are magenta and blue-green. If magnesium oxide is used as hydrogen acceptor and sulfuric acid is employed for the acidification, prior to the extraction with ether, the only interfering cation is chromium (III), and this difficulty is easily overcome by preliminary oxidation to chromate.

If the solution is made alkaline with ammonium hydroxide, cobalt(II) ions act like aluminum ions but with lower sensitivity. Likewise, if ammonium hydroxide and hydrochloric acid are used, zirconium(IV) ions interfere by yielding a red color. The destruction of this red zirconium compound by sulfuric acid is probably due to the production of complex zirconium-sulfate ions.

DETECTION OF ALUMINUM

A 5% water solution of either Chrome-fast pure blue B or Naphthochrome green G can be used in the following procedure. The positive response of the test is indicated by a red or blue-green color, respectively.

Procedure. The test is conducted in a micro test tube. A drop of the solution being tested is treated with slight additions of powdered magnesium oxide until a slight excess remains. One drop of the dye solution is introduced, the mixture is swirled, and after about a minute is made acid with a drop of 1 to 1 sulfuric acid. If no more than small amounts of aluminum are present, the excess red dye acid is precipitated, but considerable quantities of aluminum consume the dye completely. The mixture is shaken with 10 to 15 drops of ether. Any superfluous dye acid passes into the ether, and the water layer is magenta, pink, or green, depending on the dye used and the quantity of aluminum present.

Limit of detection, 0.1 γ of aluminum. Dilution limit, 1 to 500,000.

This procedure revealed: 2 γ of aluminum in the presence of 1000 γ of beryllium, zirconium, copper, nickel, cobalt; 0.2 γ of aluminum in the presence of 1000 γ of zinc or manganese.

Five micrograms of aluminum were detected in the presence of 2500 γ of iron or 500 γ of titanium. If the trivalent iron is reduced to the divalent state by means of concentrated thioglycolic acid prior to introducing the magnesium oxide, as little as 0.5 γ of aluminum can be detected in the presence of 2500 γ of iron.

Gallium, scandium, and lanthanum salts show no reaction at all.

When chromium is present, 1 drop of the neutral or slightly acid test solution is treated with a drop of 5*N* sodium hydroxide and a drop of 30% hydrogen peroxide and the mixture is kept for 10 minutes in a boiling water bath. The mixture is cooled and then a drop of the 5% dye solution and a drop of 1 to 1 sulfuric acid are added. The mixture is then shaken with 10 to 15 drops of ether and the water layer is examined for a red or orange color. It is possible in this way to detect 5 γ of aluminum in the presence of 1000 γ of chromium.

Even better limiting ratios can be obtained by using the following procedure.

The chromium is oxidized in a larger volume of liquid, the solution is acidified with a drop of concentrated hydrochloric acid, a drop of 2% ferric chloride solution is introduced, and the mixture is made basic with ammonium hydroxide. The mixed precipitate $[Al(OH)_3 + Fe(OH)_3]$ is separated from the chromate solution by centrifugation and washed free of chromate with water. The purified precipitate is treated with a drop of concentrated thioglycolic acid (80%) and then dissolved in a drop of 1 to 1 hydrochloric acid. A drop of the dye solution is added, the mixture is made basic with ammonium hydroxide, and enough hydrochloric or sulfuric acid is added to bring about solution. The excess dye acid is taken up in ether as before.

DETECTION OF ALUMINUM IN EXTREME DILUTION

The collector action of ferric hydroxide, as just described, can be employed for the detection of slight amounts of aluminum.

Procedure. Twenty milliliters of the test solution are treated with a drop of 1% ferric solution (chloride or sulfate) and an excess of ammonium hydroxide is added. The suspension is centrifuged, in portions, in a conical tube (capacity 5 to 7 ml.). The mixed precipitate on the bottom of the tube is dissolved in a mixture of equal volumes of 2*N* hydrochloric acid and concentrated thioglycolic acid. A drop of the fast pure blue B solution is introduced and the solution is made alkaline with ammonium hydroxide. Finally, the system is acidified with 1 to 1 hydrochloric acid and shaken with ether. The water layer is pink if aluminum is present.

This procedure revealed as little as 0.4 γ of aluminum. This corresponds to a dilution of 1 to 50,000,000.

However, the aluminum in sea water did not respond to this procedure. The most likely reason for this failure is that the aluminum is not present in the ionogenic form, but as a constituent of colloidal clays. The usual content of aluminum in sea water is 0.16 to 1.8 mg. per liter (*8*).

DETECTION OF ALUMINUM IN SILICATES

Aluminum bound in silicates must be converted to the ionogenic state before the present color reaction can be used successfully. The usual tedious fuming with hydrofluoric and sulfuric acids in platinum can be advantageously replaced by fusion with zinc chloride. The resulting superheated hydrogen chloride rapidly decomposes the silicate, and the metals previously bound to silica are released as chlorides (*4*).

Procedure. The pulverized sample (less than 1 mg. suffices) is fused with zinc chloride in a small borosilicate or hard-glass test tube and kept molten for about 2 minutes. The cooled mass is taken up in the smallest feasible volume of dilute hydrochloric acid. One drop of the fast pure blue B solution and enough ammonium hydroxide to make the system alkaline are added. One drop of 1 to 1 hydrochloric acid is added and the shaking with ether is conducted as described previously. The water layer will be pink or red if the silicate contained aluminum.

A very few minutes were sufficient to reveal alumium in clay.

DETECTION OF ALUMINA

The color reaction described under detection of aluminum will reveal

the presence of alumina in insoluble materials if the specimen is decomposed by fusion with alkali bisulfate (pyrosulfate) or potassium persulfate (*10*). A much quicker procedure is to moisten the powder sample with an ether solution of the fast pure blue B; a red-violet color appears almost at once. Beryllium oxide yields a deep blue under these conditions. These two colored products, which are probably adsorption complexes of the dye (*3*), can be readily distinguished from each other, because only the alumina adsorbate is stable against dilute mineral acids.

The following procedure is specific for alumina and for its differentiation from other acid-resistant colorless metal oxides. Alkaline earth carbonates are tinted blue-violet by the dye solution, but the colored products are decomposed by acids. Because carbonates consume the reagent, it is advisable to remove them by digestion of the sample with dilute acid before proceeding with the actual test.

Reagent. One milliliter of a 1% water solution of Chrome fast pure blue B is acidified with dilute hydrochloric acid. The precipitated dye acid is taken into solution by means of 10 ml. of ether.

Procedure. A micro test tube is used. A little of the powdered sample is moistened with a drop or two of the ether solution of the dye acid. Alumina will assume an intense blue-violet color. The ether is driven off, the residue is treated with 2 drops of 1 to 1 acetic acid, and the mixture is shaken with ether to remove the excess dye acid. The solid retains its color if alumina is present.

This procedure is suitable for testing the ignition residues of organic aluminum preparations used in medicine and for technical purposes. The ash obtained from paper and leather can also be tested for aluminum by this procedure.

DETECTION OF HYDROXYTRIPHENYLMETHANE DYES

The color reaction of hydroxytriphenylmethane dyes with aluminum offers the possibility of detecting dyes of this kind (compare the introductory discussion). Aqueous solutions of their water-soluble alkali salts or ether solutions of the dye acids may be tested.

Procedure. The test is made in a micro test tube. A drop of a 5% solution of aluminum sulfate is treated with a drop of the water or ether test solution, and a drop of ammonium hydroxide is then added. If necessary, the ether is expelled from the basic solution by heating. A drop of 1 to 1 hydrochloric acid is introduced. A color that persists even after extraction with ether indicates the presence of a hydroxytriphenylmethane dye.

The procedure revealed the following amount of the various dyes, which yield the respective colors in the water layer:

0.1 γ	Chrome-fast pure blue B	(violet)
0.1 γ	Eriochromecyanine	(violet)
2.5 γ	Naphthochrome green G	(blue-green)
1 γ	Naphthochrome violet R	(violet)
5 γ	Aurintricarboxylic acid	(red)

If this test is being applied to a mixture of dyes, it is well to digest the sample with acid and to apply the test to the ether extract of the mixed dye acids.

ACKNOWLEDGMENT

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MEETING REPORTS

Society for Analytical Chemistry

AT THE annual general meeting of the Physical Methods Group held on November 28 in London, a paper on "Optical Rotations in the Study of Organic Structures" by W. Klyne, Post Graduate Medical School, was presented and discussed.

The use of optical rotations in the investigation of molecular structure depends on two principles. (1) In compounds which have two or more asymmetric centers, the contribution of each center is more or less independent of the other centers (many exceptions). (2) The effect of substitution on the contribution of a center is less, the farther away from the center that substitution takes place.

The limitations which must be observed in applying these ideas to structural problems were discussed. An adequate series of analogies is necessary if conclusions are to be reliable; the use of false analogies has led on occasion to serious errors.

Rotational evidence has been helpful in elucidating the structures of many natural products. Rotations may be of particular value in stereochemical problems and above all in the assignment of absolute configurations; the importance of these in biochemical problems has hardly received its due measure of interest until recently.

The recent introduction of commercial spectropolarimeters, which measure rotations over the wave length range 250 to 700 mμ, makes it possible to determine rotatory dispersion curves easily. These curves promise to be of much greater value than "monochromatic" rotations as an aid in the determination of organic structures.

The fundamental theory of optical rotations was not considered, as the empirical approach seemed more profitable at present. All values discussed were molecular rotations (M) = (α) × mol. wt./100.

At a meeting of the Scottish Section, held at the University of Glasgow in Glasgow December 10 Edgar Rentoul discussed "Problems and Techniques in Forensic Analysis."

He dealt with the role of analysis in forensic work and expressed concern at the increasing specialization of analysis, tending to place particular determinations in the hands of a limited number of individuals, ultimately rendering independent checks almost impossible. The determination of various poisons was reviewed, special reference being made to arsenic; irradiation techniques show clearly the distribution of arsenic along a single hair. The uses of modern apparatus in forensic analysis and possible future applications were described. Some of the problems associated with blood and blood stains were enumerated, the benzidine test being regarded as presumptive evidence, requiring corroboration. Dangers associated with some of the newer insecticides were also mentioned. Medicolegal aspects of urine analysis in cases charged under Section 15 of the Road Traffic Act were described.

At the second annual general meeting of the Western Section, held December 15 at Newport, R. C. Chirnside, General Electric Co., Wembley, discussed

the "Coordination of Analytical Techniques in Industrial Research."

The properties and behavior of materials depend very largely on their chemical constitution and physical structure. In a research organization, and in technical industry generally, it is therefore of paramount importance to have available the proper facilities for determining the composition, structure, and other attributes of all materials of interest. In fact, modern industry relies at almost every stage of research, development, and production on the results of what has veen called broadly chemical analysis of materials used in, or in conjunction with, the product.

It is now becoming more widely appreciated that "chemical analysis" goes further than determining the percentage composition of a sample, usually expressed in some rather arbitrary way. It refers to all those techniques and investigations which contribute to our knowledge of the structure and composition of materials. This conception of analysis implies that the analyst may find himself as a individual in a team of specialists, some of whom would have no place in the traditional chemistry laboratory. This in turn creates a situation in which some over-all coordination of the various analytical techniques is established and maintained.

CRYSTALLOGRAPHIC DATA

153. Uranium Tetrabromide, UBr_4

R. M. DOUGLASS and EUGENE STARITZKY, *The University of California, Los Alamos Scientific Laboratory, Los Alamos, N. M.*

Crystals of uranium tetrabromide were prepared by J. F. Suttle of the University of New Mexico and this laboratory by reaction of uranium dioxide with a tenfold excess of carbon tetrabromide at 175° C. and subliming off of the uranium tetrabromide thus formed. Quantitative chemical analysis gave, by weight, 43.57% uranium and 56.10% bromine, to be compared with 42.69 and 57.31%, respectively, calculated for UBr_4.

Crystal Morphology

System. Monoclinic.
Habit. Elongated parallel to *b*, with {101}, {10$\bar{1}$}, and {011}.
Cleavage. {001} prominent.

X-Ray Diffraction Data

Diffraction Symbol. $2/mC\text{-}/\text{-}$.
Cell Dimensions. $a_0 = 10.92 \pm 0.02$ A., $b_0 = 8.69 \pm 0.03$ A., $c_0 = 7.05 \pm 0.01$ A., $\beta = 93.9 \pm 0.1°$; cell volume 667 A.^{3}; $a:b:c = 1.26:1:0.81$.
Formula Weights per Cell. 4.
Formula Weight. 557.73.
Density. 5.55 grams per cc. (calculated; weight of unit atomic weight 1.6602 $\times 10^{-24}$ gram); 5.35 [measured (*1*)].

Absorption Spectrum

Band maxima in millimicrons and relative intensities as viewed with Zeiss prism microspectrometer eyepiece

Parallel to *X*	Parallel to *Y*	Parallel to *Z*
685 m		675 vs
670 vs	665 vs, wide	660 s, wide
645 m	650 m	
	640 m	
		632 w
	626 ms	
600 m, wide	600 vw	598 mw
583 vw		
563 w	560 w	558 vw
526 w	523 w	523 m
512 s		512 w
		495 mw

Optical Properties

Refractive Indices (5893 A.). $n_X = 1.86$, $n_Y = 2.02$, $n_Z = 2.06$; geometric mean 1.98. Lorentz-Lorenz refraction 49.6 cc.
Optic Orientation. $Z = b$; $X \wedge c$ = small.
Optic Axial Angle (5893 A.). $2V_X = 51 \pm 3°$.
Color. Brown.

Partial Powder X-Ray Diffraction Pattern of Uranium Tetrabromide

hkl	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]
001	7.03	6.99	10
200	5.45	5.48	3
111	4.78	4.77	2
20$\bar{1}$	4.45	4.48	3
020	4.35	4.36	5
021	3.70	3.70	1
220	3.40	3.41	2
20$\bar{2}$	3.05	3.06	7
221	3.01	3.02	3
311	2.95	2.96	6
202	2.87	2.86	<1
130	2.80	2.82	2
022	2.73	2.75	1
40$\bar{1}$	2.60	2.60	3
31$\bar{2}$	2.51	2.51	2
312	2.35	2.36	3
420	2.31	2.32	<1
330	2.26	2.28	1
113	2.25	2.25	4
40$\bar{2}$	2.23	2.22	2
13$\bar{2}$	2.21		
20$\bar{3}$	2.21		
113	2.187	2.187	5
33$\bar{1}$	2.182		
331	2.129	2.133	2
402	2.085	2.081	4
041	2.076		
023	2.063		
51$\bar{1}$	2.061	2.035	<1
240	2.018		
511	1.987	1.998	2
42$\bar{2}$	1.982	1.975	1
22$\bar{3}$	1.968		

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; $\lambda(CuK_\alpha) = 1.5418$ A.
[b] Relative peak intensities above background from densitometer measurements.

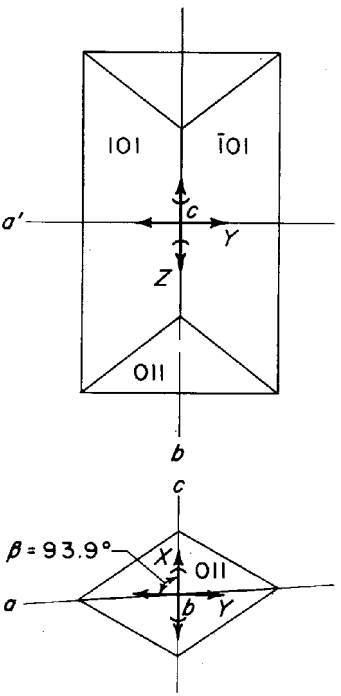

Figure 1. Orthographic projections of crystal of uranium tetrabromide parallel to c and to b

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Work done under auspices of Atomic Energy Commission. Contributions of Crystallographic data for this section should be sent to Walter C. McCrone, 500 E. 33rd St., Chicago 16, Ill.

Buret Arrangement for Some Special Titrations

Wolfgang J. Kirsten, Pharmacia Research Laboratories, Uppsala, Sweden

A BURET arrangement has been developed which is suitable for titrations with solutions which cause stopcock freezing, or which must be kept under inert gases or protected against moisture or carbon dioxide, such as Karl Fischer, titanous trichloride, and nonaqueous solutions. For titrations with potassium methoxide in benzene-methanol solution, modified stopcocks are used with the arrangement described.

When not in use, the buret is as shown in Figure 1, with stopcock S in position I, Figure 2. When titrations are to be carried out, gas is made to enter (either from a hand pump or from a source of inert gas) through opening A, stopcock S being turned counterclockwise to position II. The gas passes in and causes an overpressure in the buret bottle. Stopcock S is turned further counterclockwise to position III. The gas in the buret passes now out through tube B, Figure 1, and capillary R, and the solution from the bottle passes up through tube C into the buret. The function of capillary R is to prevent solution from coming up too quickly, and it thus provides for a foam-free, smooth filling of the buret. When the buret is filled, stopcock S is turned counterclockwise again back to position I. The excess solution in the buret now passes down through tube C into the bottle and the titration can begin.

The arrangement seems to be foolproof. It is easy to remember always to turn the stopcock counterclockwise, but if it should be turned in the wrong direction, nothing disastrous results. It is not necessary to have the source of inert gas attached to the buret when it stands idle. With stopcock S in position I, the buret and bottle are under a slight overpressure and are hermetically sealed from the atmosphere. When titrations are to be carried out, the source of inert gas is attached at A, stopcock S is turned to position IV, and gas is passed through for a while to expel atmospheric contaminants from the connections. Stopcock S is then turned to a suitable position for use.

Figure 2, V, shows the stopcock arrangement suitable for titrations with titanous trichloride or similar solutions; the arrangement in Figure 2, VI, is suitable for Karl Fischer titrations or those in nonaqueous solutions with potassium methoxide.

When the arrangement was used for titrations with potassium methoxide in benzene-methanol solution, some troubles occurred with stopcock D, Figure 1. The solution in the buret and bottle, under a slight overpressure of nitrogen, dissolves more gas than it does under atmospheric pressure. As soon as the solution passes out into the Teflon part of the stopcock, the pressure diminishes. The excess of dissolved gas is liberated and accumulates in the hollow part of the stopcock above the buret tip. When this part is filled with gas, bubbles pass down through the tip at irregular intervals and cause volumetric errors. The stopcock was, therefore, modified in the following manner.

The hole in the Teflon body was drilled

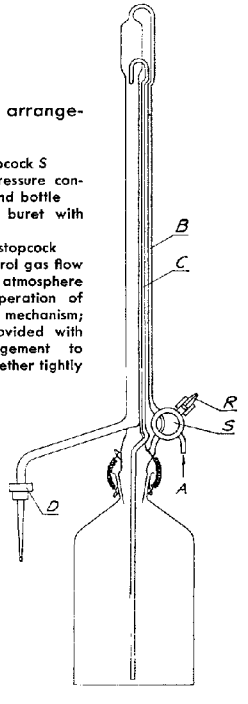


Figure 1. Buret arrangement

A. Side tube of stopcock S
B. Tube for gas pressure control in buret and bottle
C. Tube for filling buret with solution
D. Ultramax Teflon stopcock
R. Capillary to control gas flow from buret to atmosphere
S. Stopcock for operation of buret filling mechanism; should be provided with spring arrangement to hold parts together tightly

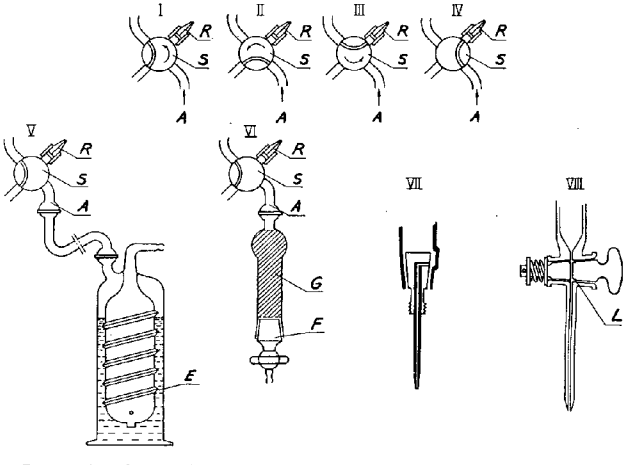


Figure 2. Stopcock positions and special modifications for buret

I, II, III, IV. Positions of stopcock S
V. Arrangement for titanous trichloride titrations
VI. Arrangement for Karl Fischer titrations and titrations in nonaqueous solution
VII. Modified Ultramax stopcock
VIII. Glass stopcock with Teflon inner part; ground-glass surface polished with ferric oxide
A. Side tube of stopcock S
E. Oxygen-absorbing solution
F. Ground joint sealed with Krönig's glass cement
G. Water- and carbon dioxide–absorbing reagents
L. Scores in Teflon part, 0.5 mm. across, 0.5 mm. deep, 2 mm. long, which connect through bore also in case the Teflon body might float during use

out to be cylindrical up to the side channel. A narrow glass capillary, which fitted tightly into the hole and which had a score in the upper end, was forced into the hole in such a manner that the score connected the bore of the capillary and the side channel of the Teflon body. The stopcock is shown in Figure 2, VII. There are no more hollow parts in the stopcock, and any liberated gases pass immediately down through the buret tip. Because the green plastic of the Ultramax stopcock was attacked by the potassium methoxide–benzene-methanol solution, it had to be replaced with Teflon. Another possibility is to use an ordinary glass stopcock provided with an inner part of Teflon. This arrangement works well, provided that the stopcock used is small and that its inner part is held tightly in the outer part with a strong spring arrangement (Figure 2, VIII).

ACKNOWLEDGMENT

The author is indebted to Pharmacia Ltd., for the permission to publish this paper. Stopcock VIII was constructed in cooperation with Paul Wildén and Lars Magnusson, Pharmacia Ltd. The apparatus is now available from Messrs. R. Grave, Malmskillnads gatan 48 C, Stockholm.

Titration Flask

Ira Kukin, Gulf Research & Development Co., Pittsburgh 30, Pa.

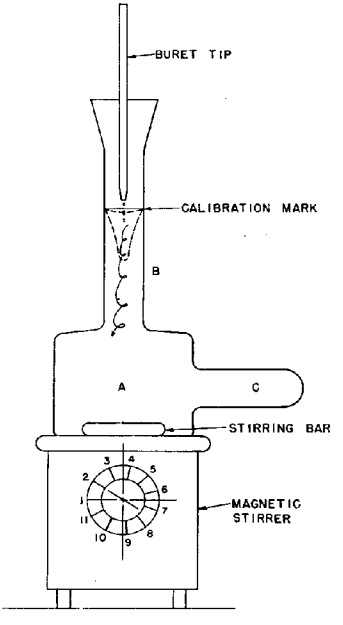
THE FLASK shown in the diagram has been used to detect visual colorimetric end points, particularly with dark colored solutions which could not otherwise be titrated readily by indicator methods.

Thus, in the determination of oils of acid number between 0.0 and 0.35, a 20-gram sample was weighed in the flask and diluted with a suitable solvent (1 to 1 benzene–isopropyl alcohol) to the 120-ml. calibration mark on the neck, *B*, of the flask, and indicator solution (0.25 ml. of 1% *p*-naphtholbenzein in methanol) was added. The solution was titrated with dilute (0.03*N*) alcoholic sodium hydroxide contained in a 5-ml. Koch microburet. The solution in the neck of the flask was readily stirred with a magnetic stirrer. The path of descending droplets of titrating solution is shown by the arrow. The broken line shows the vortex created by the stirring.

The bulk of the mixture to be titrated and the diluting solvent are contained in the base of the flask, *A;* only a small portion is contained in the elongated neck, *B*, and side arm, *C*, which are made of tubing 19 mm. in outside diameter. If the bulk solution is too dark to permit a sharp visual end point to be detected in it, color changes can still be recognized by watching the solution contained in the elongated sections. In particular, near the end point, the change in color of the descending drops of titrating solution in the neck of the flask is in marked contrast to the background color of the solution. Because color changes first occur in the neck of the flask, the end point can be approached rapidly, without danger of overshooting.

Side arm *C*, which extends out about 1.5 inches, is convenient for comparing the color with a standard, as the side arm of the flask containing the solution being titrated can be placed alongside the side arm of the standard. The side arm may also serve as a double check of the end point, as it is the last portion of the flask to show the color change brought about by the drops of titrating solution which must first traverse the bulk section *A*. *A* is 2.25 inches in diameter for 100- and 120-ml. flasks.

The flask is very convenient for determining small amounts of material present in a mixture, particularly when



Titration flask

the initial dark color or opacity of the solution obscures the visual end point. Although such solutions can often be titrated potentiometrically, the simplicity and rapidity of an indicator method are desirable. Thus, known amounts of naphthenic acid were blended with a series of distillate oils of NPA colors ranging from 1- to 8-; the acid content of the blends ranging from 0.15 to 0.90 acid number. The blends were titrated potentiometrically, as well as colorimetrically with the new flask. The average deviation of the results between the two methods was ±0.03 neutralization number. Two of the five samples of oil, having NPA colors of 7- and 8-, were too dark for accurate titration by the ASTM color-indicator method, D 974-55 T. In the range of 0.0 to 0.5 acid number, a repeatability standard deviation of 0.005 acid number was obtained when titrations were made in the new flask; 11 of the 44 samples evaluated had NPA colors between 6- and 8-.

The flask can be used to extend the range of some titrations to lower concentrations. Thus, ASTM method D 664 for the titration of acids potentiometrically is inapplicable to materials having a neutralization number less than 0.05; no pronounced inflection point usually is found in such cases. The end point then must be obtained from a predetermined e.m.f. value, which results in errors that may exceed ±0.04 neutralization number. When these solutions were titrated colorimetrically in the new flask, this difficulty was not present; the dark colored oils were easily titrated.

The flask has been compared with other titration flasks—i.e., where a small side arm of narrow tubing attached to the base is used to detect the end point of dark colored solutions. The big advantage of the flask described is that it permits one to detect the color change from the droplets of titrating solution as they descend in the narrow neck of the flask.

Flasks of 100- and 120-ml. capacities (to calibration mark) were most convenient in this work. A liquid level of at least 1 inch should be present in the neck of the flask after the sample has been diluted with a suitable solvent. The sample size is chosen so that no more than 10 ml. of titrating solution will be necessary; this avoids exceeding the over-all capacity of the flask.

The opening of the flask is wide enough to accommodate the tip of the buret and an inlet for nitrogen when it is necessary to flush the solution or blanket it with an inert atmosphere.

The flask should prove useful in facilitating rapid and precise titrations, particularly for those not trained or accustomed to detecting end points in solutions where the initial dark color or opacity obscures the visual end point.

Simple Centrifugal Filtration Assembly for Preparation of Solid Samples for Radioassay

Felix Bronner and Nils A. Jernberg, Rockefeller Institute for Medical Research, New York 21, N. Y.

CENTRIFUGAL devices at present available for the preparation of solid samples for radioassay (*2*, *3*) merely separate the precipitate from the supernate, which is then removed in a separate step. The apparatus described here (Figure 1) employs centrifugal forces for simultaneous separation and filtration and thus yields in one operation a precipitate ready for counting.

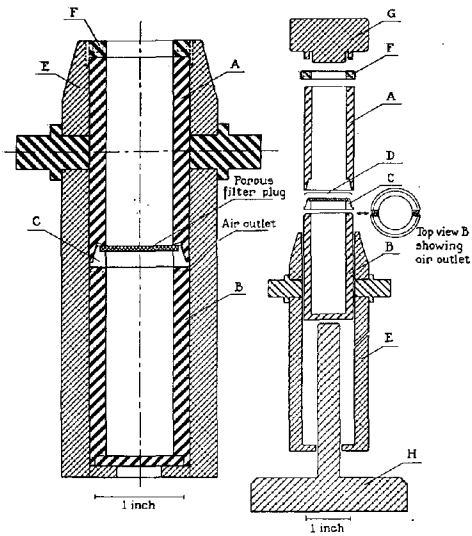


Figure 1. Cross-sectional view and diagrammatic exploded view of centrifugal filtration assembly

Diagonal shading: Stainless steel
Cross-hatching: aluminum

The use of centrifugal filtration not only permits the easy handling of many samples at once (up to 16 samples in a conventional centrifuge equipped with a 16-place head), but also speeds operation in those cases where suction filtration (*4*) would normally be very slow (as when calcium oxalate is precipitated from serum which has not previously been deproteinized). At the same time it minimizes the danger of losing some precipitate in siphoning off the supernate, as may easily occur in existing centrifugal devices.

CONSTRUCTION DETAILS

The apparatus (Figure 1) consists of a polished, stainless steel filter tube in two parts, *A* and *B*, between which a porous planchet holder, *C*, and a circle of filter paper, *D*, are held. The filter tube rests inside a polished aluminum carrier, *E*, where it is kept tightly in place by a stainless steel locknut, *F*, screwed in with the aid of tool *G*. Tightness of fit is further assured by the edge of the lower end of *A*. This edge presses against the filter paper at an angle of 20°. The paper thus acts also as a gasket. The tapered end of *A* and the corresponding taper (80°) of the stainless steel planchet holder, *C*, facilitate removal of the filter paper when the apparatus is taken apart with the aid of device *H*.

The apparatus can be constructed from commercially available materials at an approximate cost of $60. Tolerances of the filter tube and planchet holder were held at ±0.003 inch. The porous metal of the planchet holder is available as porous stainless steel sheets, composition 18-8, Type 304 (Micro Metallic Corp., Glen Cove, N. Y.; 3/32 inch thick; mean pore opening, 10 microns).

PROCEDURE

The apparatus has been used in this laboratory for the preparation of calcium-45–labeled calcium oxalate samples.

After overnight precipitation (*1*), the samples are transferred quantitatively to the centrifugal filtration assembly. Before transfer the filter paper (Schleicher and Schuell, No. 589, red ribbon, 30-mm. diameter) is prepared with fuller's earth suspension. [Ten grams of Florida fuller's earth (Floridin Co., Warren, Pa., 60–100 mesh) is suspended in 1000 ml. of distilled water and filtered through glass wool; 1.5 ml. of filtrate is used per circle of filter paper.] Centrifugation follows for 5 minutes at 150 to 175 g's. The use of centrifugal force in excess of 175 g's may yield a cloudy filtrate. If the filtrate is cloudy, it is reprocessed.

After centrifugation, the locknut is removed and the inside assembly is pushed out with the aid of *H*. The filter paper is then transferred to a holder for counting. Samples with a volume in excess of the 15-ml. capacity of the upper chamber, *A*, can be prepared by successive centrifugation. Before the thin layer of precipitate is counted, it is coated with 4 drops of collodion (0.5% in acetone). This keeps the samples from flaking and makes it possible to store them longer. Ordinary washing after use has kept the apparatus free from contamination.

RESULTS

A series of duplicate samples was prepared by both suction and centrifugal filtration. Differences in the counts obtained on duplicate samples were not statistically significant, and the two methods are therefore comparable. In a further test, a standard prepared by suction filtration counted 1163 counts per minute (standard deviation 24, six samples), while the same standard prepared by centrifugal filtration counted 1179 (standard deviation 37, four samples).

The apparatus also has been used successfully for the isolation of sulfur-35–labeled barium sulfate precipitates.

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WORK carried out during the tenure by Felix Bronner of a research fellowship of the Arthritis and Rheumatism Foundation, supplemented by a grant from the Helen Hay Whitney Foundation. This aid is gratefully acknowledged.

THREE MODELS OF THE

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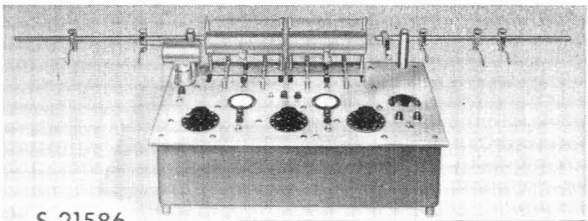

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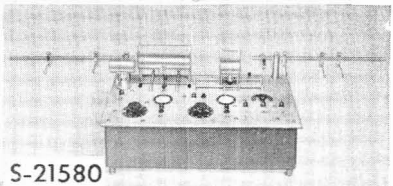

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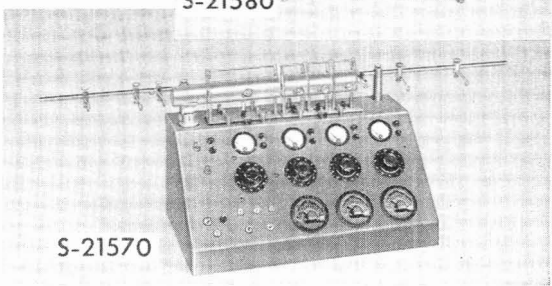

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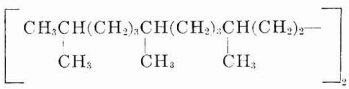

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This is *Squalane*. (Note the "a".) We hereby announce our readiness to sell it as Eastman 7311 at $15.60 per 100 grams. *Squalane* is hydrogenated *Squalene* (Eastman P6966, note the "e"). We can distill squalene in our unique molecular stills from the oil found in the gigantic, oily liver of the mighty but leisure-loving basking shark. *Squalene* is being added to at least one brand of cattle feed on the strength of certain findings by the manufacturer about cholesterol and sex hormones. The merest *soupçon* of it in dog food is said to bring utter bliss to the canine palate.

(We once read a book about how a man went broke fishing for basking sharks. We also know a man who sells us oil from another kind of shark that has a liver even richer in squalene than basking shark liver. We doubt, though, that is why the first fellow went broke. Squalene is to be found in sweat. This is a statement about human sebum, not an aphorism. Squalene is also found in olive oil but not in other cheaper vegetable oils. Some fakers found out about that once. But we digress.)

The latest is that *Squalane* has a contribution to make to gas chromatography, which is booming. This is an analytical technique whereby a volatile sample mixture is swept by an inert gas through an adsorbing column and resolved by virtue of the different times it takes each component to make its way through against the adsorption forces. *Squalane* is reported (*Anal. Chem. 28*,303, March '56) to modify the adsorbing characteristics of a commercial carbon black in a manner that shuffles the order of emergence from what it is with other adsorbents, thus providing a good fix on the proportions of each different C_5, C_6, and C_7 saturated hydrocarbon present. One of our own plants tried it out and forthwith contributed further to the burgeoning art by discovering that *Squalane* is very good at separating hydrocarbons from oxygen-bearing compounds close to them in physical properties. They found, for example, that n-heptane emerges later than n-butanol, even though n-butanol is the higher boiling substance.

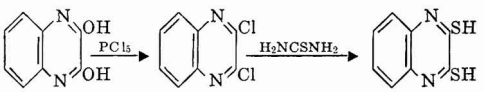
Will we reveal more about this? Will a certain series of experiments now in progress with *Squalane* at a certain well known medical school turn out to be as interesting as the preliminary results promise? Wait for the next gripping chapter, if any.

A nickel test

In ammoniacal solution, *2,3-Quinoxalinedithiol* forms a dark red complex with nickel. We have decided for this reason to make and sell it as Eastman 7317. Soon, doubtless, somebody will publish a procedure employing this as a more sensitive and/or more convenient and/or more foolproof reagent for nickel. More than what? It is better not to ask. Today man gives vent to the fires that burn within him by inventing a more ( ) test for nickel. (There are few galleons to plunder, no hairy mammoths to hunt down.)

We made this compound from *2,3-Dihydroxyquinoxaline* (Eastman 6232), which is useful in precipitating barium, calcium, and strontium from solution and then distinguishing between them. (We can furnish an abstract on that.) The *2,3-Dihydroxyquinoxaline* we make by condensing o-phenylenediamine with oxalic acid, not from quinoxaline itself. To make *Quinoxaline* (Eastman 7094), we use glyoxal, which is the simplest possible dialdehyde instead of oxalic acid, the simplest dibasic acid. (In truth, we don't directly use that green gas glyoxal, the simplest colored organic substance, but handle it as sodium glyoxal bisulfite.) *Quinoxaline* is an intermediate for antispasmodic compounds.

To replace the oxygens of *2,3-Dihydroxyquinoxaline* with the sulfurs of *2,3-Quinoxalinedithiol*, we choose for yield and quality this route:



The intermediate *2,3-Dichloroquinoxaline* we can offer as Eastman 7300. Maybe it's good for starting a fire of creativity.

Neither *Squalene*, *Squalane*, nor *2,3-Quinoxalinedithiol* is going to pay for the cost of this ad. What we want are thousands of chemists each using a copy of *Eastman Organic Chemicals, List No. 40*, to order a few grams of one of some 3500 other organics we stock. *That's* what makes the wheels go 'round. Do you have your copy? *Distillation Products Industries*, Eastman Organic Chemicals Department, Rochester 3, N. Y.

DPi

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Also...vitamins A and E in bulk...distilled monoglycerides

Distillation Products Industries *is a division of* **Eastman Kodak Company**

For your convenience — short "refreshers" on last month's ads. Follow directions on page 75 A to obtain free information on products and services described

EQUIPMENT AND INSTRUMENTS

Analysis and Control Instruments. Circle indicated numbers for complete information, applications, specifications and features of the following analysis and process control instruments: 6A-1 and 6A-2 for leak dectector bulletins; 6A-3 for Titrilog; 6A-4 and 6A-5 for mass spectrometer bulletins; 7A-1 and 7A-2 for chromatograph bulletins; and 7A-3 for moisture monitor bulletin. Consolidated Electrodynamics Corp., 300 No. Sierra Madre Villa, Pasadena, Calif. **6A-1 to 5, 7A-1, 2, 3**

Analyzers. Bulletin available providing complete descriptions, including sample curves, of all Cary instruments for analytical research and process stream control. Bulletin includes information on recording and Raman spectrophotometers, electrometers, and infrared and ultraviolet analyzers. Applied Physics Corp., 362 W. Colorado St., Pasadena 1, Calif. **73A**

Balances. Circle 10A for complete file on modern Mettler balances and see company's booth (No. 61) at Pittsburgh Conference. Mettler Instrument Corp., Hightstown, N. J. **10A**

Balances. Detailed information available on company's complete stock of analytical balances. Selection includes the Sartorius "Selecta Rapid," the Becker model "AB-2," the "Mikrowa," the Ainsworth "Right-A-Weigh," the Sartorius "Projecta Rapid," and the Voland model 100. Harshaw Scientific, Div. of Harshaw Chemical Co., 1945 East 97th St., Cleveland 6, Ohio **18A**

Balances. Brochure available on the new Harvard trip balance. Balance features undivided tare beam and poise which makes possible direct reading of net value. Tare capacity: 160 gram. Ohaus Scale Corp., 1050 Commerce Ave., Union, N. J. **55A-3**

Balances. Catalog available on company's balances and weights. Weights are available in rhodium plated bronze, brunton metal, or stainless steel. Wm. Ainsworth & Sons, Inc., 2151 Lawrence St., Denver 5, Colo. **83A**

Blenders. New catalog available on twin-shell blenders. Yoke model is cited as being ideal for small scale batch blending. Yoke arrangement fits standard four or eight quart blender frame, permits two different blends at once. The Patterson-Kelley Co., Inc., 2320 Hanson St., East Stroudsburg, Pa. **84A**

Burettes, Automatic. Available is special apparatus catalog, "Custom-Made Laboratory Glassware." Includes information on five automatic burettes made of Pyrex brand glass No. 7740. Corning Glass Works, 69-2 Crystal St., Corning, N. Y. **82A**

Burners. Bulletin available on company's "Flame Master," a universal laboratory gas burner with general purpose assemblage. Adapts to any gaseous fuel ranging from 150 to 3000 B.t.u. Flame sizes from the smallest micro to 2" diameter. New York Laboratory Supply Co., Inc., 76 Varick St., N. Y. 13, N. Y. **27A**

Cells, Glass Absorption. Circle 81A-3 for complete information on Klett glass absorption cells. Klett Manufacturing Co., 177 East 87th St., N. Y., N. Y. **81A-3**

Centrifuges. Circle 66A-1 for complete information on company's model SBV centrifuge that accommodates 11 horizontal swinging heads, 15 angle heads, 2 multispeed attachment heads, and 4 basket style heads. Speeds up to 5600 r.p.m. Circle 66A-2 for model V centrifuge that accommodates 14 horizontal swinging heads, 16 angle heads, 2 multi-speed attachment heads, and 4 basket style heads. Speeds up to 5100 r.p.m. International Equipment Co., 1284 Soldiers Field Rd., Boston 35, Mass. **66A-1, 2**

Centrifuges. Circle indicated numbers for bulletins on the following "Servall" centrifuges. Superspeed angle centrifuges (15,500 rpm, 400 ml), 72A-2; medium angle centrifuges (5,000 rpm), 72A-3; and large angle centrifuges (3,500 rpm), 72A-4. Ivan Sorvall, Inc., Norwalk, Conn. **72A-2,3,4**

Centrifuges. Circle 78A-2 for catalog on the new "Kern Rotofix" centrifuge. 3,000 rpm four speed motor. Circle 78A-3 for information on "Eternabrand and Shellbach" graduated cylinders. Kern Laboratory Supply Co., 8639 Venice Blvd., Los Angeles 34, Calif. **78A-2,3**

Chromatographs. Bulletin available on the Beckman gas chromatograph cited as being the only low-cost, precision-engineered chromatographic instrument designed for routine gas analysis. Scientific Instruments Div., Beckman Instruments, Inc., 2500 Fullerton Rd., Fullerton, Calif. **3A**

Combustion Analysis Equipment. Circle 80A for catalog on apparatus for combustion, carbon and sulfur analysis. Includes details on determinators, sulfur titrators, furnaces, and accessories. Laboratory Equipment Corp., 3002 Hilltop Rd., St. Joseph, Mich. **80A**

Combustion Tubes. Circle 89A-1 for price list on company's complete line of tubes, available in a wide variety of sizes and shapes, designed for extreme high temperature service. Coors Porcelain Co., Golden, Colo. **89A-1**

Comparators. Complete information available on lightweight, portable Taylor comparators cited as providing accurate determinations of pH, chlorine, bromine, phosphate, QAC, nitrate. W. A. Taylor and Co., 7306 York Rd., Baltimore 4, Md. **86A**

Cylinders, Stainless Steel. Bulletin available on Hoke stainless steel cylinders for fixed or liquified gas sampling applications. For low or high pressure service up to 1800 psi. 32 stock sizes from 10 milliliters to one gallon. Hoke Inc., 137 S. Dean St., Englewood, N. J. **44A-2**

Densitometers. Bulletin available on company's photoelectric instrument for the rapid and convenient evaluation of strips and sheets of filter paper in partition chromatography and paper electrophoresis. Photovolt Corp., 95 Madison Ave., N. Y. 16, N. Y. **31A-2**

(Continued on page 62 A)

Determination Apparatus. Circle 57A for catalog providing full description and photos of all Labconco protein, fat and fiber apparatus. Catalog includes Kjeldahl apparatus for protein determination, micro-Kjeldahl digestor, crude fiber condenser, and Goldfisch fat extractors. Laboratory Construction Co., 1109 Holmes St., Kansas City, Mo. **57A**

Distilled Water Equipment. Bulletin available describing company's tin-lined piping, fitting, valves, and faucets for distilled water distribution systems. Barnstead Still & Demineralizer Co., 9 Lanesville Terrace, Boston 31, Mass. **44A-1**

Electroanalysis Apparatus. Bulletin available on company's ultra-speed electro-analyzer for swift or volume determinations. Example: provides copper assay in 8 minutes. Eberbach Corp., Ann Arbor, Mich. **54A**

Filters, Interference. Bulletin available on company's monochromatic interference filters that isolate narrow regions of the spectrum and provide high transmission. Farrand Optical Co., Inc., Bronx Blvd. & East 238th St., N. Y. 70, N. Y. **98A-1**

Fluorimeters. Klett fluorimeters are designed for the rapid and accurate determination of thiamin, riboflavin, and other substances which fluoresce in solution. Klett Manufacturing Co., 179 East 87th St., N. Y., N. Y. **48A-2**

Fluorimeters. Bulletin available on line-operated multiplier fluorescence meter possessing high-sensitivity for measurement of low concentrations. Photovolt Corp., 95 Madison Ave., N. Y. 15, N. Y. **31A-3**

Fraction Collectors. Bulletin available on the new "Rinco" fraction collector for the automatic collection of fractions by timed-flow or volumetric method. Collects 237 fractions. Neither mercury nor current contacts sample. Aloe Scientific Div., A. S. Aloe Co., 5655 Kingsbury, St. Louis 12, Mo. **47A**

Freeze-Drying Apparatus. Circle 105A-1 for details on company's new "Centri-Freeze" cited as being an extremely versatile, fully automatic, completely self-contained freeze-drying laboratory on wheels. Samples may be placed in unfrozen. Circle 105A-2 for details on company's "Roto-Freeze" drying apparatus. E. Machlett & Son, 220 East 23rd St., N. Y. 10, N. Y. **105A-1,2**

Furnaces. Bulletin available on compact laboratory pot crucible furnace. Laboratory Equipment Div., Lindberg Engineering Co., 2440 W. Hubbard St., Chicago 12, Ill. **45A-2**

Furnaces. Bulletin available on laboratory furnace with temperature range to 2600°. Transformer provides 48 steps of adjustment. Pyrometer and ammeter mounted. Ceramic muffle forms the heating chamber. Inside dimensions: 4″ × 3″ × 7″. Hevi Duty Electric Co., Milwaukee 1, Wis. **63A-2**

Furnaces and Titrators. Company's improved "H-F" induction units (circle 99A-2 for details) and automatic titrators for the determination of sulfur in petroleum and metal products (circle 99A-3 for details) will be on display in Booth No. 20 at the Pittsburgh Conference. Laboratory Equipment Div., Lindberg Engineering Co., 2440 W. Hubbard St., Chicago 12, Ill. **99A-2,3**

Fused Quartz Ware. Bulletin available on "Vitreosil" tubing, crucibles, dishes, trays, muffles, pots, retorts, tanks, electric immersion heaters, ball & socket joints, standard taper joints, and graded seals. Thermal American Fused Quartz Co., Inc., 18–20 Salem St., Dover, N. J. **58A**

(Continued on page 63 A)

Glass Tubing Cutters. Hot-wire-type unit cuts glass tubing bottles or jars up to 3″ in diameter. 6″ × 4½″ × 9″. Operates on 115 volts, 50 or 60 cycle A.C. W. M. Welch Scientific Co., 1515 Sedgwick St., Chicago 10, Ill. **63A-1**

Glassware. Circle 34A-1 for 200-page catalog on the complete Corning line. Circle 34A-2 for company's "Custom Catalog CA2." Corning Glass Works, 72-2 Crystal St., Corning, N. Y. **34A-1,2**

Glassworking Lathe. Catalog available on glassworking lab-lathe. Specifications: self-centering sleeve grip chuck; length between chucks 36″; total chuck capacity 0″ to 14″; radial clearance 8″. Bethlehem Apparatus Co., Inc., Hellertown, Pa. **55A-1**

Glassworking Manual. Circle 55A-2 for copy of "Glass Blowing on the Glass Lathe." Bethlehem Apparatus Co., Inc., Hellertown, Pa. **55A-2**

Grinders. Unit grinds KBr pellets and Nujol mulls, mixes powders and grinds samples to homogeneous powders automatically. Spex Industries, Inc., 205-02 Jamaica Ave., Hollis 23, N. Y. **104A-1**

Hot Plates. Complete data available on company's hot plate with cast aluminum 6″ × 6″ top plate that heats to 700° F in 17 minutes. Thermostat holds temperature within 5°, provides stepless control. Thermo Electric Mfg. Co., 478 Huff St., Dubuque, Iowa **87A-1**

Hot Plates. Information available on magnetic-stirring hot plate rated at 750 watts and measuring 6½″ square. Operates on 115 volts, 60 cycle. Hammertone finish. Standard Scientific Supply Corp., 808 Broadway, N. Y. 3, N. Y. **88A-3**

Infrared Analysis Accessories. Circle 65A for complete data and specifications on accessories to the model 21 double beam recording infrared spectrophotometer that extend the instrument's versatility. Instrument Div., Perkin-Elmer Corp., Norwalk, Conn. **65A**

Laboratories, Miniature. Available is new supplement describing many more individual components, plus new assemblies for distillation, small-scale reaction work, and a new magnetic semi-automatic head. Ace Glass Inc., Vineland, N. J. **25A**

Laboratory Instruments. Circle indicated numbers for complete information on the following instruments: new model vapor fractometer with reproducible gas and liquid system accessory 64A-1; double beam model 21 infrared spectrophotometer, 64A-2; a report (including bibliography and results) on the application of infrared for trace analysis, 64A-3; and a quarterly publication, "Instrument News," 64A-4. Instrument Div., Perkin-Elmer Corp., 800 Main Ave., Norwalk, Conn. **64A-1 to 4**

Laboratory Mills. Information available on the new standard model No. 3 Wiley laboratory mill for the preparation of a wide variety of materials for laboratory analysis. Mill features harder cutting edges on the knives, quieter performance with less vibration, enclosed moving parts and latest U.L. approved wiring. Arthur H. Thomas Co., Vine St. at 3rd, Philadelphia, Pa. **74A**

Laboratoryware. Laboratoryware brochure available. Contains information on 1-piece corrosion-resistant polyethylene cup sink drain cited as being strong, resilient, impermeable and non-oxidizing. 3″ × 6″ standard oval shape. American Agile Corp., P. O. Box 168, Bedford, Ohio **41A**

(*Continued on page 64 A*)

Product Capsules

Melting Point Apparatus. Detailed information available on the Kofler micro hot stage, an electrically heated micro melting point apparatus with stage calibrated thermometers. Arthur H. Thomas Co., Box 779, Philadelphia 5, Pa. **92A-1**

Microscopes, Electron. Company's latest electron microscopes are cited as providing magnification and resolution higher than ever before possible and as including many advanced engineering features. Complete information available. Dept. O-11, Bldg. 15-1, Radio Corp. of America, Camden, N. J. **50A**

Microscopes, Polarizing. Detailed specifications available on the Carl Zeiss polarizing microscopes (GF and KF). Both the binocular and monocular types feature the inclined-tube system. Carl Zeiss, Inc., 485 Fifth Ave., N. Y. 17, N. Y. **56A**

Moisture Balances. Company's automatic, portable, infrared moisture balance permanently records moisture readings of granular solids, pastes, liquids, waxes or anything else that can be dried by heat. Complete information available. Scientific Products, Div. of American Hospital Supply Corp., 2020 Ridge Ave., Evanston, Ill. **2nd Cover**

Needle Valves. Circle 62A for "Needle Valve Special Glassware Bulletin." Provides information on Manostat glass and teflon needle valves for use in combination with Pyrex brand glass. The Emil Greiner Co., 20–26 N. Moore St., N. Y. 13, N. Y. **62A**

Nefluoro-Photometers. Illustrated how-to booklet available on company's 3-purpose nefluoro-photometer that combines in one instrument a colorimeter, nephelometer and fluorometer for rapid and precise determination of a wide variety of organic, inorganic, synthetic and biological materials. Fisher Scientific Co., 100 Fisher Bldg., Pittsburgh 19, Pa. **95A**

Nephelometers. Bulletin available that provides complete data on company's photo-nephelometers and accessories used to measure haze and turbidity in liquids, at extremely low concentrations. Coleman Instruments, Inc., 318 Madison St., Maywood, Ill. **53A-2**

Ovens. Brochure available on new controlled humidity and temperature oven for testing materials under controlled environmental conditions. Range: room to 100° C ± ½° C. 20% to 100% RH within 5% RH. The Electric Hotpack Co., Inc., 5047 Cottman St., Phila. 35, Pa. **17A**

(Continued on page 65 A)

Product Capsules

Ovens. Pamphlet available on the Blue-M "power-o-matic" mechanical convection oven. Temperature range: to 316° C. Control: ±½° C or less. Minimum wattage is automatically modulated. Blue M Electric Co., 138th and Chatham St., Blue Island, Ill. **49A**

Ovens. Bulletin, "Despatch Laboratory Ovens," available. 8 models feature air velocity control and precision indicating control. Despatch Oven Co., 341 Despatch Bldg., Minneapolis 14, Minn. **89A-3**

pH Meters. Data file available on the push-button Beckman "Zeromatic" pH meter. Instrument features no-drip circuit and mirror-backed scale. Reproducible to .02 pH. Scientific Instruments Div., Beckman Instruments, Inc., 2500 Fullerton Rd., Fullerton, Calif. **16A-1**

pH Meters. Bulletin available on company's complete line of battery and line-operated pH meters incorporating modern electronic tubes and circuits. Fully stabilized for wide range of line voltage fluctuations. Photovolt Corp., 95 Madison Ave., N. Y. 16, N. Y. **31A-1**

Photochemical Equipment. Circle 22A-1 for complete information on company's utility model quartz lamp, 22A-2 for information on company's laboratory photochemical reaction equipment, and 22A-3 for 16-page brochure, "Photosensitization," a review that details facts on photochlorination, oxidation, etc. Hanovia Chemical & Mfg. Co., 100 Chestnut St., Newark 5, N. J. **22A-1,2,3**

Photometers. Information available on the new Brice-Phoenix Universal light scattering photometer that measures absolute turbidity, dissymmetry, and depolarization. Phoenix Precision Instrument Co., 3805 N. 5th St., Phila. 40, Pa. **89A-2**

Phototubes, Multiplier. Circle 14A for complete information on DuMont multiplier phototubes available in a wide selection of sizes and electrica characteristics for every photoelectronic need. Industrial Tube Sales, Allen B. Du Mont Laboratories, Inc., 2 Main Ave., Passaic, N. J. **14A**

Phototubes, Multiplier. Information available on the new RCA 14-stage multiplier phototube for investigations involving low-level light sources. Tube features fast response, high current gain, relative freedom from after-pulses, and small spread in electron-transit time. Tube Div., Radio Corp. of America, Harrison, N. J. **20A**

(Continued on page 66 A)

Product Capsules

Pipettes and Burettes. Company states that every Kimble pipette and burette is individually retested, precision ground, thoroughly annealed, and inspected in a field of polarized light. Owens-Illinois, Kimble Glass Div., Toledo 1, Ohio **3-1 Cover**

Pipettes. Bulletin available on company's pipetter for dispensing liquids accurately and reproducibly. Accuracy cited as being better than 1%. Dispensing range 2 to 50 ml. Laboratory Glass & Instruments Corp., 514 W. 147th St., N. Y. 31, N. Y. **78A-1**

Power Supplies. Complete information available on company's two types of stabilized power supplies for (1) multiplier phototubes and similar high voltage applications and (2) for general purpose laboratory applications. Leeds & Northrup Co., 4906 Stenton Ave., Phila. 44, Pa. **90A**

Presses, Hydraulic. Catalog available on Carver laboratory presses. Unit is used for forming KBr pellets, dehydrating, pressing out oils, filtering thick fluids, determining fatty acids, etc. Available in 10 and 20-ton models. Fred S. Carver, Inc., 54 River Rd., Summit, N. J. **28A**

Presses, Hydraulic. Bulletin available on press used for making KBr pellets for infrared spectroscope analysis. 20-ton capacity. Bench mounted, hand operated. Loomis Engineering & Manufacturing Co., 133 So. 14th St., Newark 7, N. J. **26A**

Pressure Reaction Apparatus. Specifications available on company's "Series 4500" stirrer type pressure reaction apparatus. Maximum operating pressure is 1000 psig at 350° C. One or two liter bombs operate in 1500 watt electric heater with variable voltage temperature control. Parr Instrument Co., Moline, Ill. **99A-4**

Pumps. Catalog available on pumps that use steel fingers in sequence to force material through tubing. Pumps liquids, gases, and slurries without corrosion or contamination. Sigmamotor, Inc., 500 Vernon St., Middleport, N. Y. **99A-1**

Pumps & Stirrers. Company's midget pumps and laboratory stirrers are available in a variety of powers, capacities, and specifications. Circle 81A-1 for midget pump and 81A-2 for laboratory stirrer bulletins that provide performance charts, standard models, and prices. Eastern Industries, Inc., 100 Skiff St., Hamden 14, Conn. **81A-1,2**

(*Continued on page 67 A*)

Product Capsules

Pumps, Bottle. Bulletin available on company's "Ventgard" bottle pump that pumps out distilled water while permitting only purified air to enter. Filters particulate matter as small as 0.2 micron. Barnstead Still & Demineralizer Co., 9 Lanesville Terrace, Boston 31, Mass. **45A-1**

Pumps, Vacuum. Booklet, "Planning the High Vacuum System," available. Contains valuable information, pumping speed curves and illustrations together with full details about all ten models of Cenco high vacuum pumps. Central Scientific Co., 1708 Irving Park Rd., Chicago 13, Ill. **43A**

Refractometers. Complete data available on the Abbe-3L refractometer that features front, horizontal loading, fixed prism assembly, "Duospeed" control knob, instant-reading precision scale graduated to .0005, quick estimates to .0001. Bausch & Lomb Optical Co., 60914 St. Paul St., Rochester 2, N. Y. **4th Cover**

Spectra, Infrared. Information available on standard infrared spectra atlas composed of 10,600 spectra of usable organic compounds. Sadtler Research Laboratories, 1517 Vine St., Phila. 2, Pa. **72A-1**

Spectrometers. Bulletin available providing general data on company's "Tri-Carb" liquid scintillation spectrometer. Instrument makes possible the labeling of end products at or below the level of natural radiocarbon. Packard Instrument Co., P. O. Box 428, La Grange, Ill. **61A**

Spectrometers, N-M-R. Information available on company's high resolution nuclear magnetic resonance spectrometer now with a new "Super Stabilizer." Applications include quantitative and qualitative analyses of functional groups, hydrogen bonding studies, following molecular rearrangements and structural studies of natural products. Instrument Div., Varian Associates, Palo Alto 4, Calif. **12A**

Spectrophotometers. Circle 98A-2 for booklet, "Coleman Tools for Science." Contains discussion of analysis with light, plus complete description of all Coleman instruments including the "Universal" spectrophotometer cited as being fast, versatile and exact. Coleman Instruments, Inc., Maywood, Ill. **98A-2**

Spectrophotometers, Infrared. Data file available on the Beckman IR-4 spectrophotometer that may be operated double or single-beam. Instrument features a double monochromator design, and push-button control for routine work. Scientific Instruments Div., Beckman Instruments, Inc., Fullerton, Calif. **93A**

(*Continued on page 68 A*)

Product Capsules

Spectrophotometers, Recording. Circle 29A-1 for information on recording spectrophotometer that helps set color tolerances for production runs. Circle 29A-2 for information on company's tristimulus integrator, an automatic computer that provides numerical values for 3 factors: hue, vividness, and lightness. Circle 29A-3 for periodical "Spectrophotometry Digest" that provides the latest news on color measurement. General Electric Co., Schenectady 5, N. Y. **29A-1,2,3**

Spectrophotometers, Recording. Complete information available on the new KM-1 table-top double-beam infrared recording spectrophotometer designed especially for routine analyses. Baird-Atomic, Inc., 33 University Rd., Cambridge 38, Mass. **76A**

Stopcocks. Circle 101A for catalog describing the full line of company's products with the new "Lab-Crest" stopcock that is conventional in design, requires no lubrication, and provides no product contamination. Fischer & Porter Co., 1516 County Line Rd., Hatboro, Pa. **101A**

Sulfur Determinators. 16-page Dietert-Detroit catalog illustrates and describes both sulfur and carbon determinators and the complete line of accessory equipment and supplies. Circle 32A for copy. Harry W. Dietert Co., 9330 Roselawn, Detroit 4, Mich. **32A**

Titration Equipment. Bulletins available on instruments for all electrometric titrations. Includes information on the standard, automatic, high-frequency, amperometric, and coulomatic titrimeters. Fisher Scientific Co., 100 Fisher Bldg., Pittsburgh 19, Pa. **70A-1**

Titration Equipment. Catalog available. Provides full information on company's automatic electrometric titration apparatus using the second derivative method of H. V. Malmstadt. For use in all titrations usually as classified as potentiometric. Push-button start, automatic shut-off. E. H. Sargent & Co., 4647 W. Foster Ave., Chicago 30, Ill **75A**

Titration Equipment. Titrator bulletin available. Describes low cost "Waco" titrator for Karl Fischer moisture determinations featuring no-dip, ball joint Pyrex glassware, drain flask, and magnetic stirrer. Wilkens-Anderson Co., 4525 W. Division St., Chicago 51, Ill. **69A**

Tubing, Plastic. Tygon flexible plastic tubing is cited as being chemically resistant, glass-clear, tough, non-con-

(*Continued on page 70 A*)

Lab-Hyam

GENERATOR

(Type CFH35)

by LINDBERG

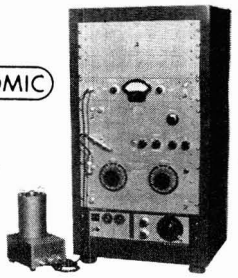
This practical, low CFH generator can produce economically dissociated ammonia atmosphere for use in laboratory furnaces and even used for small production runs. This generator is sold only through Laboratory Supply Dealers. For detailed information request Bulletin No. 1059 from your dealer or write us direct.

LINDBERG Laboratory Equipment Division
LINDBERG ENGINEERING COMPANY
2440 West Hubbard Street, Chicago 12, Illinois
Circle No. 69 A-1 on Readers' Service Card, page 75 A

BETTER ANALYSIS . . .

With

BAIRD ATOMIC



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The Model 513 provides a complete system for quantitative and qualitative analysis of the energy spectra of gamma-emitting isotopes. Especially designed for chemical analysis . . . physical research . . . biological tracing . . . medical investigation.

Features:

- Adjustable "window" that counts only pulses within pre-selected segments of energy spectrum.
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- May also be used for integral counting.
- Permits exploration of the spectrum in successive segments.

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Circle No. 69 A-2 on Readers' Service Card, page 75 A

For fluorometric analyses, assay and identification of chemical constituents . . . in the
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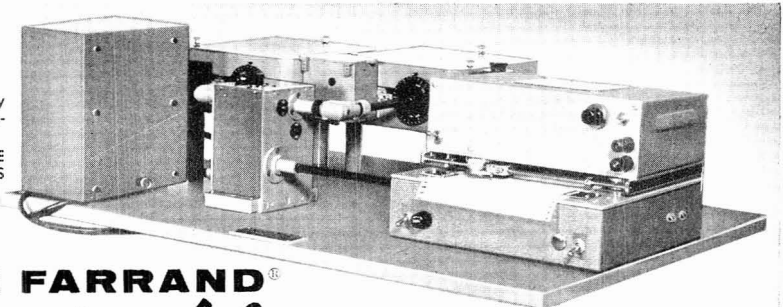

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FOR MANUAL OPERATION, FOR RECORDING OR FOR OSCILLOSCOPE PRESENTATION

The Farrand Spectrofluorometer is designed for determining the optimum wavelength for activating Organic Compounds and the optimum wavelength for measuring their emitted fluorescence spectra.

The instrument is simple to operate, and the efficiency of the optical and detector system affords great sensitivity and response even when using capillary or micro volumes of highly diluted solutions.

It is not of a console-type construction, and therefore can be serviced readily without dismantling.

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For further information, circle number 69 A-3 on Readers' Service Card, page 75 A

Product Capsules

taminating, non-toxic, seamless, and easy to handle. U. S. Stoneware, Akron 9, Ohio **96A**

Ultracentrifuges. Full details available on the automatic, vacuum, refrigerated Spinco Model L preparative ultracentrifuge that applies forces up to 144,000 times gravity on 162 ml of material at a maximum speed of 40,000 rpm. Unit is self-contained. Spinco Div., Beckman Instruments, Inc., Belmont 7, Calif. **97A**

Viscosimeters. Circle 88A-1 for bulletin on the Hoeppler viscosimeter that operates on the falling ball principle. Circle 88A-2 for bulletin on the Ubbelohde viscosimeter that operates on the principle of the suspended level. Fish-Schurman Corp., 72 Portman Rd., New Rochelle, N. Y. **88A-1,2**

Voltage Regulators. Details available on the Curtiss-Wright line regulator that provides fast recovery time (less than $^1/_{50}$th cycle, or 330 microseconds) plus the ability to reduce typical power line distortion to less than 0.3%. Capacity is 1.4 KVA. Electronics Div., Curtiss-Wright Corp., Carlstadt, N. J. **100A**

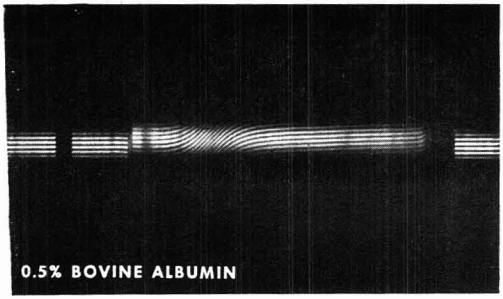
Washers, Laboratory. Bulletin available that provides complete descriptive literature on company's glassware washer and dryer. Automatic, it accommodates over 90% of all laboratory glassware, company states. The Chemical Rubber Co., 2310 Superior Ave., Cleveland 14, Ohio **104A-2**

Water Bath Heaters. Complete data available on unit that regulates, heats, stirs and circulates. Sensitive within ±0.05° C. Can circulate 1 liter per minute to external instruments. Has 1000-watt tubular immersion heater. Weight: $8^1/_2$ lbs. Case dimensions: $5^1/_2'' \times 4^1/_2'' \times 3^1/_2''$. Arthur S. LaPine and Co., 6001 S. Knox Ave., Chicago 29, Ill. **103A**

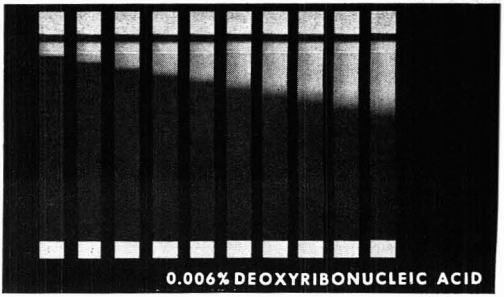
CHEMICALS AND MATERIALS

Research and reagent chemicals go hand in hand with the latest improved materials to help the technologist analyze properties and create new applications. Information available from the following sources will help to improve existing techniques and open up new and profitable avenues of investigation. See page 75 A for directions on obtaining free information on the following items.

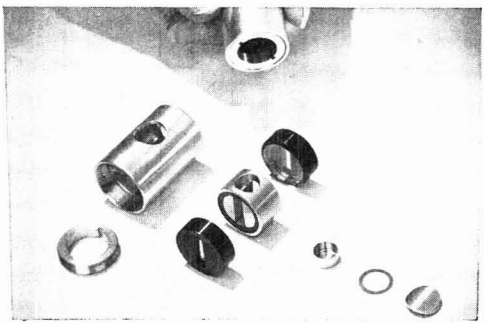
(Continued on page 72 A)



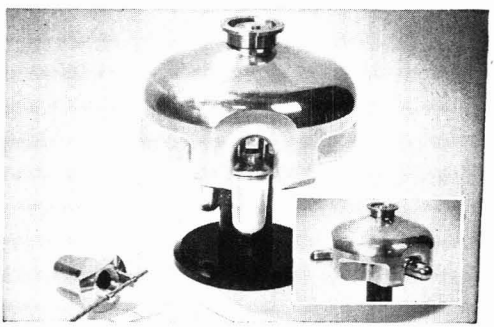
RAYLEIGH INTERFERENCE FRINGES provide high-sensitivity measurement of solute concentration at any level in analytical cell. Used for determining molecular weights—both high and low—by the equilibrium method, as well as for measuring sedimentation velocity by the centrifugal transport of material past a given level in the cell.



ULTRAVIOLET ABSORPTION method gives very high sensitivity with respect to concentration rather than concentration gradient, as in schlieren techniques. Newly-designed optical system is particularly valuable for such materials as nucleoproteins and other ultraviolet-absorbing substances exhibiting diffuse or inhomogeneous boundaries.



SYNTHETIC-BOUNDARY cell permits establishment of an immediately-measurable boundary by a layering technique. This extends sedimentation-rate measurements from the previous practical lower molecular-weight limit of 10,000, downward to 500.



40,000-rpm SWINGING-BUCKET rotors make preparative operations most closely comparable with analytical, give most accurate estimation of sedimentation by sampling. Boundaries sediment normally and undergo minimum reorientation. Quartz inserts permit spectrography.

Advancements in Ultracentrifugation

New developments, constantly introduced, have greatly extended the usefulness of the Analytical Ultracentrifuge. They are typical of the continuing research by Spinco in high-speed centrifugal force for use in medicine, pharmaceuticals, chemicals, petroleum, food, plastics, agriculture, atomic energy, metallurgy, and material testing.

Your inquiry is invited for further information on these or other developments which may provide solutions to your problems in physical research. Write Spinco Division, Beckman Instruments, Inc., Belmont 7, California.

spinco Beckman® / *Spinco Division*

For further information, circle number 71 A on Readers' Service Card, page 75 A

Product Capsules

Adsorbents. A hard, porous, white granular synthetic adsorbent, produced in a variety of mesh classifications, "Florisil" is being used in the most difficult separations of organic compounds, company states. Floridin Co., P.O. Box 989, Tallahassee, Fla. **85A**

Buffer Solutions. Information available on handy pH buffer kit that consists of three 500 ml polyethylene dispensers and 9 packages of powdered buffer salts, sealed in moisture proof envelopes. Analytical Measurements, Inc., 585 Main St., Chatham, N. J. **87A-2**

Chemicals, Reagent. New catalog available. A reference guide, it lists more than 1,300 "Baker Analyzed" reagents and other high-purity laboratory chemicals. Has new listings important in new procedures. J. T. Baker Chemical Co., Phillipsburg, N. J. **35A**

Filter Papers. Circle 21A-1 for analysis kit sampler with large assortment of 11 cm circles in wide selection of grades. Several quantitative papers included in sampler are also available in sheets or strips for chromatography and electrophoresis. Carl Schleicher & Schuell Co., Keene, N. H. **21A-1**

Filter Papers. Circle 59A for samples of synthetic fiber papers made of dynel, teflon, glass, polyvinylchloride, zein, nylon, and cellulose acetate. H. Reeve Angel & Co., Inc., 52 Duane St., N. Y. 7, N. Y. **59A**

Isobutyronitrile. A reactive intermediate, isobutyronitrile is now available in commercial quantities. Boiling range: 100°–105° C. Specific gravity: 20° C/20° C–0.7690–0.7720. APHA color: 20 max. Water: 0.8% max. Aldehydes (as carbonyl): 1.0% max. Circle 33A for sample. Eastman Chemical Products, Inc., Kingsport, Tenn. **33A**

Laboratory Chemicals. Circle indicated numbers for complete information on the following Baker & Adamson laboratory chemicals: inorganic reagents, 8A-1; "C.P." acids, 8A-2; radiochemicals, 8A-3; rare earths, 8A-4; organic chemicals, 8A-5; and biological stains & indicators, 8A-6. General Chemical Div., Allied Chemical & Dye Corp., 40 Rector St., N. Y. 6, N. Y. **8A-1 to 6**

Lithium Compounds. Lithium metal, lithium hydride, lithium hydroxide and lithium carbonate are the basis for both experimental and commercial studies as polymerization catalysts in the manufacture of certain plastics, polymers and resins. Circle 24A for general information. Lithium Corp. of America, Inc., 2525 Rand Tower, Minneapolis 2, Minn. **24A**

SAVE TWO-THIRDS—A large Eastern University cut its replacement costs by two-thirds after it switched to PYREX brand No. 3046 graduated cylinders.

Test at large university shows how to erase two-thirds of your graduated cylinder breakage

When one of the largest schools of technology in this country decided to find out how true are the claims we make for PYREX brand graduated cylinders, it came up with this result:

Just one PYREX *cylinder ends up in the "broken glass" pail for every three of the leading competitive brand.*

The university based its test on the cylinder-breaking ability of two large groups of chemistry students. One group used only PYREX No. 3046 cylinders for a full semester. The other group used only the competitive brand.

Why do you get such low breakage?

No magic involved—just forty-and-then-some years of know-how that's built into the design of the PYREX No. 3046 cylinder and the glass it's made of.

These design features, for example: Reinforced bead strengthens cylinder, helps prevent breakage if cylinder tips over.

Hexagonal base—a Corning first—with extra base width to resist tipping. Also prevents rolling when you set cylinder on its side.

Permanent graduations

You also get LIFETIME RED graduations on No. 3046 cylinders. Etched right into the glass through a permanent layer of red, they can't wear off.

No. 3046 cylinders are available in sizes 10 thru 250 ml. For more information on these and other PYREX volumetric ware, consult your Laboratory Supply Dealer or your Laboratory Glassware Catalog LP36. If you don't have this catalog of Corning glassware, we'll be glad to send you a copy.

CORNING GLASS WORKS
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Corning means research in Glass

PYREX® **laboratory ware**
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For further information, circle number 74 A on Readers' Service Card, page 75 A

ADVERTISED PRODUCTS INDEX

EQUIPMENT AND INSTRUMENTS

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These handy cards will bring information of value to chemists, engineers, and other technologists in laboratory, pilot plant, and production

Circle desired items: Numbers on the adjoining tear-out card are keyed to items in the NEW PRODUCTS, NEW CHEMICALS, and MANUFACTURERS' LITERATURE sections on the immediately following pages. For further information on any new product or service, or for a copy of literature listed in MANUFACTURERS' LITERATURE, circle the corresponding number and mail card. For information about any of the products listed in the ADVERTISED PRODUCTS INDEX, or in the PRODUCTS CAPSULES section, the reader should write directly to the company concerned.

See page 78 A for New Products, 84 A for New Chemicals and 86 A for Manufacturers' Literature

ANALYTICAL CHEMISTRY

Readers' Information Service

March 1957—Valid through August 1957

New Products

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Manufacturers' Literature

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Position_____

Company_____

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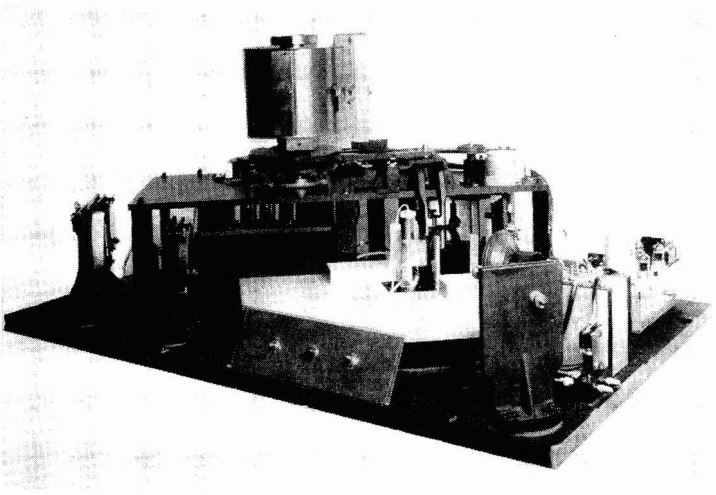


Baird-Atomic KM-1 spectrophotometer

Low Price IR Spectrophotometer Features Expandability

FURTHERING the trend to make infrared available to the chemist for routine analyses, Baird-Atomic, Inc. has announced its reasonably-priced model double-beam infrared spectrophotometer. This table-top instrument is designed not only to make reliable infrared analysis possible at a moderate cost, but features expandability which will provide versatility approaching that of their more expensive Model 4-55.

In developing the KM-1, Baird-Atomic has kept many of the larger instrument's basic features. One of these is the large prism optical system which incorporates an 80-square-centimeter NaCl prism. This, the company says, assures optimum performance in minimum operating time and allows use of auxiliary prisms to extend the KM-1's range. The chart size is kept the same as in the 4-55, namely $5 \times 17\frac{1}{2}$ inches, allowing continuity in accumulating a chart library where both instruments are used to supplement each other.

Baird-Atomic considers the principle of expandability very important in the design of the KM-1. Therefore, several provisions are made for addition of components which, when incorporated, will make the KM-1 approach the more complex model in versatility, if not convenience, says the company. The researcher interested in differential analysis can add fixed slit mechanism, a variable scan drive, and an a.c. bias. A narrow slit program device can be added for high resolution work, as well as a recycling mechanism for kinetic studies.

To make this infrared instrument simple and compact, company engineers eliminated the general console structure, cell walls, linkages between cams and drums, several versatility features, and convenient front panel controls. In the new model, sampling space is at the front of the instrument. The recorder drum is placed on top of, and concentric with, the cams, thus eliminating an entire series of bearings, worm wheels, and other connecting linkages. The electronics have been redesigned so that the amplifier, power supply, battery, and battery charger are repackaged in a small unit. Total number of tubes in the entire instrument was reduced to eight. With the substitution of a permanently sealed bolometer for the evacuated bolometer, the vacuum pump was eliminated. The scan drive was reduced to a synchronous motor with the overdrive operation provided by rotating the drum-cam combination by hand.

A company spokesman reports that this rugged instrument represents the best of Baird-Atomic's long experience in infrared, combined with new ideas and new features carefully executed in design. The manufacturer's feeling is that low initial cost, good basic performance, compatible chart size, plus expandability, will make the KM-1 attractive to the budget-limited laboratory, the laboratory needing a second instrument, and the university laboratory. **1**

Miniature TV Camera for Research

A streamlined TV camera, small enough to fit in the palm of the hand, is now available, says the Majestic International Sales Corp. The miniature camera, made in West Germany by Grundig, measures only $5\frac{1}{4}$ inches in length and 2 inches in diameter. The camera is equipped with a variety of lenses and mirror attachments. Tiny spring-mounted wheels permit the camera to examine remote, cramped locations.

It can be moved back and forth in tight spaces by drawing or pushing the thin perlon cable which contains remote control and power wires. Viewing of the picture can be done at whatever distance from the camera is necessary.

Indicated possible applications are: remote monitoring of gages and instruments, viewing close-ups of reactions and experiments from a safe distance, and examination of areas not accessible to the human eye. **2**

Miniature TV camera

Ultramicrobalance Receives Major Improvements

According to Microtech Services Co., its Rodder Model E quartz torsion ultramicrobalance has recently undergone several major improvements. With a 200 mg. load on each pan, the sensitivity has been increased to 0.05 microgram. Both the case and loading chambers have increased insulation to minimize drift resulting from local temperature changes. Another improvement listed by the company is more convenient loading for pans, capillaries, and tares provided with a redesigned pan holder which will give rather than break. **3**

Old-Line German Glassware Again Available in U. S.

Kern Laboratory Supply Co. announces the re-entry to this country of Jena and Haldenwanger glassware after an absence of a number of years. Both lines of laboratoryware are manufactured in West Germany and are imported exclusively by Kern. Said to contain many modern improvements, both lines of the German glassware are now available for distribution by Kern through laboratory supply houses. **4**

Filtration Units

The physical quality of water used in the laboratory techniques can be improved by removal of infinitesimal particles with a MIK filtration unit, claims Heico, Inc. Best results cannot always be obtained with chemically pure water due to sub-microscopic matter, Heico says; therefore, the new filtration units have been developed to insure physical purity.

Specific models of the MIK filtration unit are available for analytical laboratories, chemical industries, nucleonics, and photography. **5**

Freezing Point Apparatus

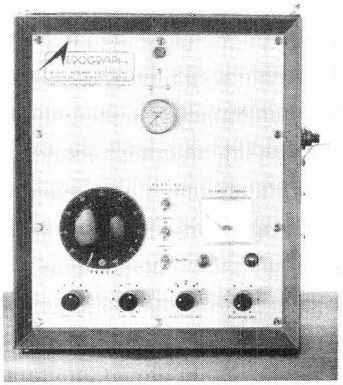
A new series of nitrogen cryostats, available for both laboratory and military applications, is now offered by the Perkin-Elmer Corp. These units are said to differ from conventional open flask coolers in that they are completely closed recirculating systems. Danger of spilling or loss by evaporation is eliminated, and the cooling heads can be operated vertically, horizontally, or at any other desired angle.

The Model 147, designed for laboratory use, consists of a miniature cooling head and a compressor-regulator assembly. The cooling head utilizes the Joule-Thompson effect and the principle of regenerative cooling. Compressed nitrogen is passed through a miniature heat exchanger where it is cooled by nitrogen returning from an expansion nozzle. After the expanded gas is recovered, it is returned to the compressor for recirculation.

Operating from a nominal 117-volt a.c. power supply, the Model 147 cryostat is suitable for cooling infrared detectors, for cooling traps in vacuum systems, and for similar applications, the company says. **6**

Gas-Liquid Chromatography Apparatus

The Aerograph, manufactured by Wilkens Instrument & Research, Inc., is designed to make gas-liquid chromatography economical for the school and independent researcher. According to the company, the instrument features rapid and accurate resolution of volatile compounds in samples ranging from 2 to 100 mg.



Aerograph Model A-90

Compounds with boiling points ranging from 20° to 400° C. may be separated qualitatively and collected quantitatively, says the company. Samples are injected with a 1/4 ml. syringe through a special silicone rubber gasket, and may be separated and collected without the formation of azeotropes in minutes, claims Wilkens.

Two models of the instrument are available, each occupying only 1.1 square feet of bench space. The Model A-100 is supplied with a strip chart

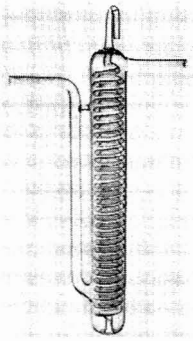
For further information, see coupon on page 75 A ▶

recorder: Model A-90 comes without a recorder but may be connected to recording equipment if desired. **7**

New Condensation Apparatus

A new type of condensation trap for efficiently trapping volatile compounds is announced by the California Scientific Glass Co. The company says it is designed for use where quantitative condensation is required in the vacuum manipulation of hydrocarbons and other volatile compounds. Completely made of glass, the product can also be used as a vacuum system fore trap and as a collector in air pollution analyses.

When the trap is immersed, coolant fills the inner glass spiral and surrounds



New condensation trap

the outer surface of the trap. The company claims this inner spiral provides 50% greater cold surface for condensation with the same external dimensions of conventional traps.

It is available in sizes to fit pint and quart Dewar flasks; larger or special sizes can be obtained on special order. **8**

Portable Photometer Available

A new photometer is available to users of light measurement equipment, announces Eldorado Electronics Co. The PH-200 Universal photomultiplier photometer is said to feature high sensitivity and accuracy, low drift, flexibility of operation, and modest cost. It is also claimed to be the first photometer capable of utilizing any commercially available photomultiplier or photoelectric tube.

Zero and dark-current adjustments as well as decade and continuously variable

PH-200 Universal photometer

sensitivity controls are included. Provision is also made for oscilloscope and graphic recorder readout, thus broadening the possible uses of the instrument.

The PH-200 is completely self-contained and portable; it incorporates an electronic power supply and uses no batteries. **9**

Push-Button pH Meter

An automatic push-button instrument with drift-free amplifier, the Zeromatic pH meter announced by Beckman Instruments, Inc. can be adapted for potentiometer recorder output, automatic temperature compensation, and Karl Fischer titrations.

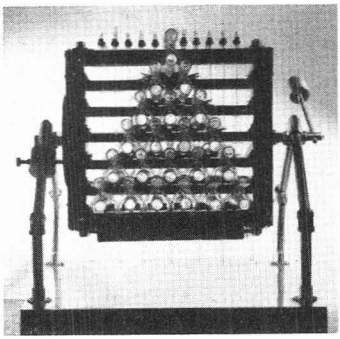
The new instrument is line-operated and features automatic correction for electronic zero drift and push button

control. It has a 0 to 14 pH scale range and two millivolt ranges—700–0–700 and the extended 0 to 1400 millivolt range. According to the company, pH readings are accurate to 0.1 pH unit and reproducible to 0.02 pH.

The compact Zeromatic is left connected to the 115-volt power source at all times on Standby, and is ready to operate at the touch of a button. Temperature compensation and millivolt range selection are also button controlled. Connections for a standard potentiometer recorder and for an automatic temperature compensator are provided on the rear panel. **10**

Three-Phase Counter-Current Apparatus

Laboratory Glass & Instruments Corp. announces the availability of its apparatus for counter-current distribution using three-phase solvent system combinations. The instrument is essentially based on the design of the individual glass cell in which mixing and decanting take place and on the resulting interconnection of phase distributions.



Labglass counter-current extractor

It is furnished for either manual or robot shaking; an automatic fraction collector is available as an accessory.

The company says the apparatus functions as a complete laboratory, its chief advantages being greater efficiency of separation, more exact criteria for purity, and higher resolution than that obtainable with two phase systems. **11**

Peristaltic Action Pump for Gases or Liquids

A new pump, developed at the National Institutes of Health, is being manufactured by the American Instrument Co. The Aminco peristaltic action pump is said to pump liquid or gas

For further information, see coupon on page 75 A ▶

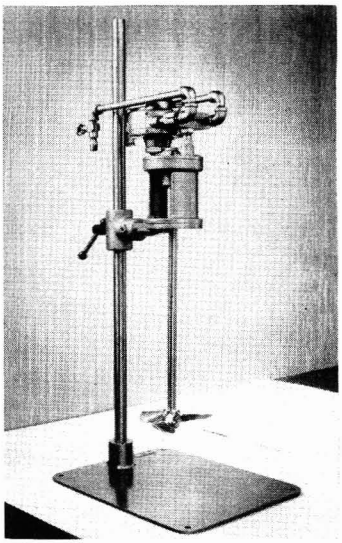
through rubber or plastic tubing, and is claimed to serve as an excellent micrometering device.

The basis of the pump consists of a series of rollers placed at right angles to a length of flexible tubing. When the tubing is filled with fluid and the rollers set in motion, all rotating and moving in the same direction, pumping results and a flow of liquid occurs in the direction of the movement.

The company feels that the flow range of the pump against high head pressures, its small size, and freedom from contamination make it adaptable for a great many applications. **12**

Heavy Duty Laboratory Agitator

Designed for mixing viscous materials in small batches, a new bench type agitator is available from the Eclipse Air Brush Co. Known as the DL Pneumix, the agitator is recommended for handling adhesives, glues, unthinned resins, and similar materials.

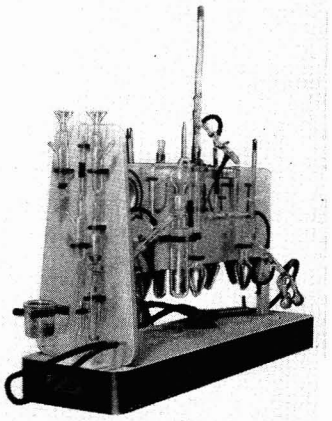


The DL Pneumix cannot overheat or burn out, even if stalled, says the manufacturer. It utilizes an explosion-proof ½ hp. air motor with 8:1 gear reduction; speed of rotation is controlled by a hand throttle. **13**

Compact Semimicro Set

The Arthur F. Smith Co. is now offering its Quickfit semimicro organic preparation set. The miniature laboratory is mounted on a board, eliminating additional supports and clamps.

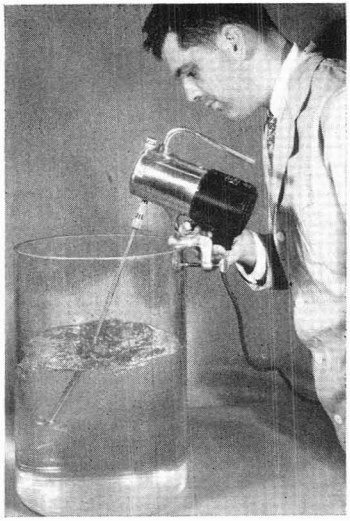
The set consists of over 60 components comprising 14 basic assemblies. The company claims any number of varia-



tions are possible because the parts are interchangeable.

Having a total weight of 14 pounds, the entire set can be packed in a carrying case and easily transported. **14**

▶ For further information, see coupon on page 75 A



Mix all fluids
at any speed with this mixer

If you could have only *one* mixer in your lab, you'd be very safe in choosing this one.

You can mix or stir *any* fluids with it, up to 4,000 centipoises viscosity.

You can mix at *any* speed from 100 to 1,600 RPM, with stepless speed control.

And you can run the mixer 24 hours a day, seven days a week if you need to.

The friction drive in this LIGHTNIN Model F never needs oiling. At low speeds, it provides extra torque for mixing thick liquids. Drive and motor are fully enclosed, safe with inflammables. Your choice of rugged bronze tank clamp or ring-stand clamp for easy mounting; removable 304 stainless steel or Monel shaft and propeller. Supplied for 110-volt or for 220-volt 50/60-cycle 1-phase. *Fully guaranteed.*

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Circle No. 83 A-1 on Readers' Service Card, page 75 A

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SUPERSPEED ANGLE CENTRIFUGE
15,500 rpm (31,000 x G) 400 ml

Internationally known as "the work-horse of the modern research laboratory."

Sealed Rotor. Portable — separate transformer for remote control. Operable in cold rooms, fume hoods, and under routine conditions. **Write For Bulletin AC-31S**

MEDIUM ANGLE CENTRIFUGES
5,000 rpm (3,500 x G)

Type M: Twenty-Seven 15 ml tubes.
Type SP: Six 75 ml tubes.
Type SP/X: Ten 50 ml tubes and Five 15 ml tubes. **Write For Bulletin AC-31M**
Small Angle Centrifuges "A" and "XL" also available.

LARGE ANGLE CENTRIFUGES
3,500 rpm (2,500 x G)

Compact, space-saving instruments. Maximum capacity: 2,000 ml. Rotors accommodate 500 ml, 250 ml bottles; 150 ml tubes. **Write For Bulletin AC-31G**

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For further information, circle numbers 83 A-2, 83 A-3, 83 A-4 on Readers' Service Card, page 75 A

Parr

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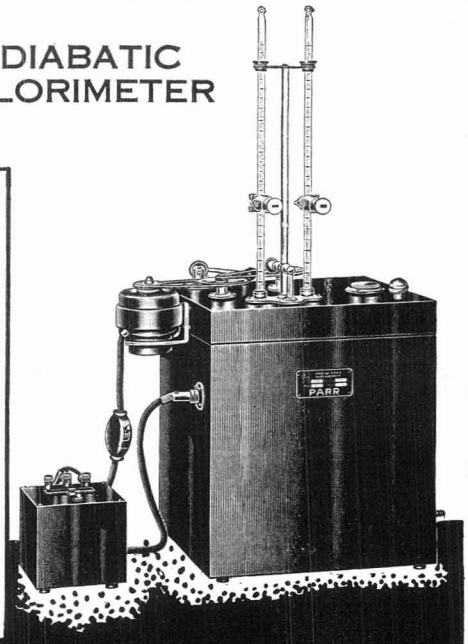
Parr adiabatic oxygen Bomb calorimeters are designed for highest precision in calorific measurements, yet permit rapid tests with minimum calculations.

No heat radiation corrections required. Has Parr self-sealing oxygen combustion bomb, either single valve or double valve style.

Ask your Parr dealer for complete details or write direct to the factory for Spec. 1200.



PARR INSTRUMENT COMPANY
MOLINE, ILLINOIS

For further information, circle number 83 A-5 on Readers' Service card, page 75 A

Sodium Analysis Simplified

A new reagent for detection and determination of the sodium ion is offered by the Monroe Chemical Co. It is said to facilitate simple and reliable analysis, qualitative and quantitative, of the sodium ion in the presence of other ions generally considered contaminants.

Designated Reeve's Sodium Reagent, it consists of a buffered aqueous-alcoholic solution of α-methoxyphenylacetic acid. In the presence of the sodium ion, the solution deposits an insoluble sodium acid salt.

Qualitative analysis involves observation of the precipitate; quantitative determination is based upon the deposit of sodium as the acid salt followed by acidimetric titration of the washed precipitate.

The company claims the following features for its sodium reagent as compared to others available: greater selectivity; simple and less time-consuming procedures for qualitative and quantitative analyses; comparable sensitivity; and comparable cost. **15**

New Organic Monomer

Experimental and pilot plant quantities of glycidyl methacrylate (GMA) are offered by the DuPont Co. The monomer is said to be a convenient means of introducing epoxide groups into vinyl polymers or vinyl groups into condensation polymers.

A light-colored, almost odorless liquid, GMA may be polymerized or copolymerized through the double bond and further reacted through the epoxide, says DuPont. Or, the oxirane ring may be first polymerized leaving the vinyl group available for crosslinking.

Another property pointed out by the manufacturer is that GMA will polymerize thermally, with ultraviolet light, or with the usual free radical or ionic polymerization catalysts. Thus, copolymers containing any desired amount of functional groups may be made by varying the proportion of GMA in the monomer charge.

DuPont feels GMA has promising applications in the following fields: chemical, dyeing and fine chemicals, leather, paint, plastics, and rubber. **16**

Chemical Detector Crayons

Aromil Chemical Co. is producing sensitive crayons for the detection of phosgene, hydrogen cyanide, cyanogen bromide, and lewisite. The crayons write on paper, wood, or any other suitable surface, and the resulting mark will reportedly turn a distinctive color when exposed to very low concentrations of the appropriate gas.

These new crayons are designed to provide a simple, inexpensive means for determining contaminated areas, detecting and locating leaks, and are said to be good for hundreds of tests.

Aromil claims they are highly sensitive. The phosgene detector crayon, for example, is capable of detecting 1 p.p.m. of the toxic gas in less than one minute.

The company warns that the crayons will deteriorate slowly with time and recommends that they be discarded after six months. **17**

Hydrocarbon, Nitrogen Standards

The American Petroleum Institute announces the addition of three hydrocarbons and three organic nitrogen compounds to its list of standard samples. The newly available compounds are:

5-Methyl-trans-2-hexene
Ethylidenecyclopentane
Ethylidenecyclohexane
Pyridine
Pyrrole
Pyrrolidine

Standard samples come in 5-ml. quantities, vacuum sealed in special borosilicate glass ampoules with "break-off" tip. Purity of the hydrocarbons has been evaluated from freezing point measurements; purity of the nitrogen compounds, from calorimetric as well as freezing point measurements. **18**

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MANUFACTURERS' LITERATURE

Electrophoresis Apparatus. Four-page catalog provides information on apparatus and accessory instruments for paper electrophoresis, featuring a migration chamber, power supply, and power distribution unit. Research Specialties Co. (*Cat. 254*) **19**

Synthetic Paraffin. Brochure relates background and development of Paraflint, new synthetic paraffin. The high melting point hydrocarbon wax has properties which make it useful as a modifier for petroleum waxes, as a chemical raw material, or as an intermediate for chemical processing. Moore & Munger. **20**

Glass Products. Eight-page catalog describes apparatus for the wet laboratory, including fractionating-distillation columns, still heads, product condenser-receivers, and other accessories. Glass Engineering Laboratories. **21**

Temperature Controls. Bulletin contains data on operation and specifications, giving temperature ranges, tube sizes, and accuracy of Model L-1S temperature control. Burling Instrument Co. (*Bull. No. 106*) **22**

Plasticizers. Bulletin gives methods of preparation and individual properties of several different epoxy fatty acid ester plasticizers. Becco Chemical Division, Food Machinery and Chemical Corp. (*Bull. No. 80*) **23**

Laboratory Apparatus. New catalog comprises 700 pages of text, and lists over 20,000 items of chemical laboratory apparatus. Griffin & George Ltd. (*Cat. 56S*) **24**

Thermometers and Hydrometers. Twenty-four page booklet provides information on ASTM, general and specific purpose thermometers and hydrometers for measurement of temperature and specific gravity. Includes complete specifications and prices. Central Scientific Co. (*Bull. No. 40*) **25**

Succinic Acid. Sixteen-page bulletin contains description, physical properties, specifications, shipping, handling, and storage data on succinic acid and its derivatives. Also includes list of references. The Borden Co. (*Bull. M-4a*) **26**

Magnetizers. Data sheet lists available magnetizers, demagnetizers, and laboratory magnets, featuring new self-contained unit. F. W. Shrader Co. (*Cat. Sheet M-11*) **27**

Low Temperature Apparatus. Catalog of 16 pages describes equipment for applications in low temperature field. Hofman Laboratories, Inc. **28**

Gloss Recorder. Company newsletter features description and illustrations of automatic 75° gloss recorder for gloss measurement of paper, fabrics, plastics, metals, and other materials. Gardner Laboratory, Inc. (*Newsletter No. 19*) **29**

Laboratory Magnet. Data sheet covers new horizontally-rotating version of electromagnet for the laboratory. Instrument Division, Varian Associates. **30**

Coumarin. Booklet contains solubility tables for coumarin, synthetic aroma-fixing and odor-masking agent. Organic Chemicals Division, Monsanto Chemical Co. **31**

Refractometers. Eight-page bulletin illustrates and describes 8 new refractometers for use in laboratory, pilot plant, and process control. Phoenix Precision Instrument Co. (*Bull. R 1000*) **32**

Inedible Fats. Illustrated booklet discusses bleaching of inedible fats with chlorine dioxide. Olin Mathieson Chemical Corp. **33**

Autotransformers. Twenty-four page bulletin gives specifications, dimension drawings, and photographs for all models of complete line of adjustable autotransformers. General Radio Co. (*Buil. 0*) **34**

Ceramics. Catalog contains descriptive and engineering data on full company line of ceramics, including commercial white porcelain, Steatite, and 5606 Refractory. Star Porcelain Co. (*Cat. 57*) **35**

Chemical Developments. First issue of quarterly publication, "Ideas in Development," contains articles on oleyl amines, alpha-sulfoalkyl acids, and ethochemicals. Chemical Division, Armour and Co. (*Vol. 1, No. 1*) **36**

Glass. New brochure explains manufacturing methods, applications, and the history of glass. Includes charts, drawings, and photographs. Corning Glass Works. **37**

Nuclear Equipment. Catalog provides technical information to aid in selection of nuclear instruments for the laboratory. Nucleonic Corp. of America. (*Cat. No. 103*) **38**

Glassware Washers. Data sheet illustrates and describes portable washer for cleaning different sizes and shapes of bottles, test tubes, flasks, burets, and other laboratory glassware. Arthur S. LaPine & Co. **39**

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MANUFACTURERS' LITERATURE

Molybdenum Disulfide. Two-page bulletin covers physical, thermodynamic, electrical, magnetic, and chemical properties, preparation and uses of molybdenum disulfide. Climax Molybdenum Co. (*Bull. Cdb-5*) **40**

Laboratory Equipment. Bulletin features new chromatographic equipment available, as well as complete line of sectional laboratory furniture. Also includes other new laboratory apparatus, such as flask heater, pH meter, extractor, and timer. Schaar and Co. **41**

Integrating Counter. Bulletin provides details on remote integrating counter for use with penumatic transmission systems. Bristol Co. (*Bull. No. A128*) **42**

Chromatography, Electrophoresis Apparatus. Six-page booklet lists apparatus necessary for one and two dimensional paper separations and describes an instrument for electrophoresis measurements. Includes information on dimensions and prices. Central Scientific Co. (*Bull. No. 80*) **43**

Polyester for Urethane Foams. Technical service bulletin contains comprehensive analysis of Witco Fomrez 50, new polyester for the production of flexible urethane foams. Witco Chemical Co. (*Bull. No. E-10*) **44**

Combustion-Testing and Air-Measurement Instruments. Two-page bulletin describes and illustrates gas pressure manometers, oil flow graduates, air velocity meters, recording thermometers, pressure point testers, and others. General Scientific Equipment Co. (*Bull. No. 138*) **45**

Ceramic Ware. Bulletin lists available items in company line of ceramic laboratoryware, including combustion boats, tubes, crucibles, incinerating dishes and custom-made ware. Laboratory Equipment Corp. **46**

Plastic Laboratory Supplies. Sixteen-page catalog presents full line of laboratory products made of polyethylene, polyurethane, polyvinyl, and nylon. Palo Laboratory Supplies, Inc. (*Cat. E956*) **47**

Electrophoresis Apparatus. Four-page brochure discusses specifications, operation, and applications of continuous-flow paper electrophoresis instrument (Model CP). Spinco Division, Beckman Instruments, Inc. (*Form 4-CP*) **48**

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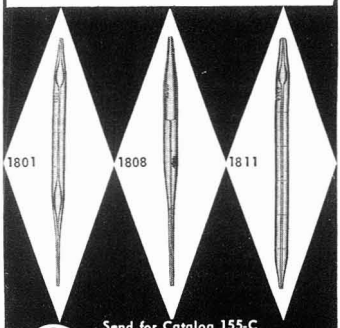

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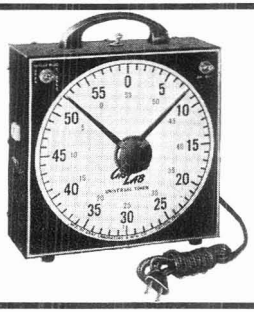

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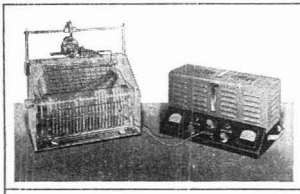

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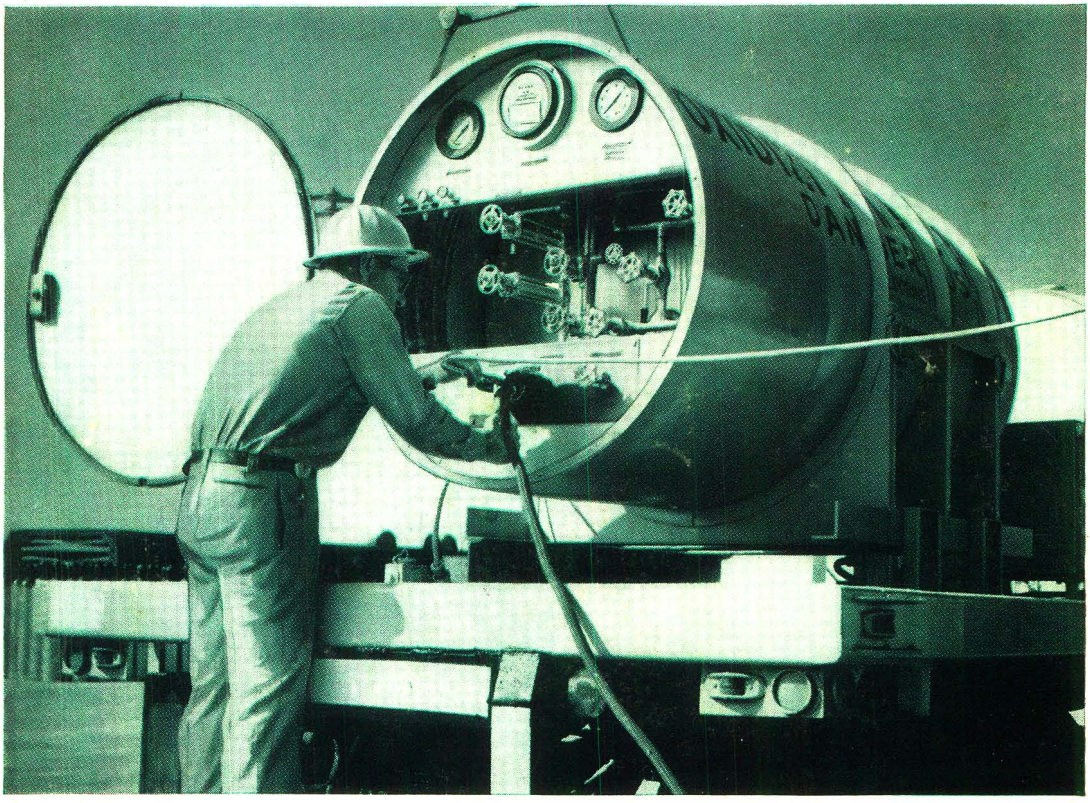

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