INDUSTRIAL AND ENGINEERING CHEMISTRY

ANALYTICAL EDITION

VOLUME 18, NUMBER 3 | **ISSUED MARCH 29, 1946** | **CONSECUTIVE NUMBER 6**

EDITOR: WALTER J. MURPHY

Assistant to Editor: N. A. PARKINSON | Associate Editor: LAWRENCE T. HALLETT | Contributing Editor: R. H. MÜLLER

Assistant Editors

Manuscript Editing: G. GLADYS GORDON | Manuscript Reviewing: STELLA ANDERSON | Make-up: CHARLOTTE C. SAYRE

Advisory Board

R. P. CHAPMAN, J. R. CHURCHILL, B. L. CLARKE, T. R. CUNNINGHAM, G. E. F. LUNDELL, M. G. MELLON, R. H. MÜLLER, B. L. OSER, H. H. WILLARD

BIBLIOTEKA POLITECHNIKA ŚLĄSKA

Boiling Range of Chlorinated Hydrocarbons . . . . . . Dwight Williams 157

Amperometric Titration of Mercaptans with Silver Nitrate I. M. Kolthoff and W. E. Harris 161

Determination of Tungsten in Silicate Rocks . E. B. Sandell 163

Equations of Thixotropic Breakdown for Rotational Viscometer . . . Henry Green and Ruth N. Weltmann 167

Measurement of Small-Angle X-Ray Scattering . . . . M. H. Jellinek, Ernest Solomon, and I. Fankuchen 172

Polarographic Determination of Acetaldehyde . . . . . . Philip J. Elving and Emile Rutner 176

Determination of Beryllium in Ores . . . Mary H. Fletcher, Charles E. White, and Milton S. Sheftel 179

Determination of Inorganic Phosphorus in Plant Materials . . W. A. Pons, Jr., and John D. Guthrie 184

Quantitative Determination of Ergosterol, Cholesterol, and 7-Dehydrocholesterol . . . . Frances W. Lamb, Alexander Mueller, and George W. Beach 187

New Method for Determination of Manganese . . . . . . James J. Lingane and Robert Karplus 191

Fractional Separation of Hafnium and Zirconium by Means of Triethylphosphate . H. H. Willard and Harry Freund 195

Determination of Tocopherol in Plant Tissue . . . . Monroe E. Wall and Edward G. Kelley 198

Method of Evaluating Metal Cleaners . . . . Samuel Spring, Howard I. Forman, and Louise F. Peale 201

Fluorometric Attachment for Beckman Spectrophotometer Mary H. Fletcher, Charles E. White, and Milton S. Sheftel 204

MICROCHEMISTRY

Spectrophotometric Determination of Traces of Nickel . . O. R. Alexander, Edith M. Godar, and N. J. Linde 206

Amperometric Microtitration of Very Dilute Chromate Solutions . . . . I. M. Kolthoff and D. R. May 208

Rapid Estimation of Rubber in Guayule Latex Dispersions . . . . . R. T. Whittenberger and B. A. Brice 209

Modified All-Dithizone Method for Determination of Traces of Copper . S. L. Morrison and Harriet L. Paige 211

NOTES ON ANALYTICAL PROCEDURES

Simple Automatic Control for Vacuum Systems . . . . J. J. Spadaro, H. L. E. Vix, and E. A. Gastrock 214

Absorption Spectrum of Antimony Trichloride–Ergosterol Reaction Product . . . . Alexander Mueller 214

Large-Size Laboratory Soxhlet Extractor . . . . Ralph Salkin and Irving Allan Kaye 215

BOOK REVIEWS 216

Instrumentation in Analysis . . R. H. Müller (Advt. Sect.) 29

The American Chemical Society assumes no responsibility for the statements and opinions advanced by contributors to its publications. Views expressed in the editorials are those of the editors and do not necessarily represent the official position of the American Chemical Society.

We acknowledge with thanks the action of J. T. Baker Chemical Co. in releasing the front cover of this issue for editorial purposes.

Copyright 1946 by American Chemical Society.

37,200 copies of this issue printed.

Published by the American Chemical Society at Easton, Pa. Editorial Headquarters: 1155 16th Street, N. W., Washington 6, D. C., telephone, Republic 5301, cable, Jiechem (Washington). New York Editorial Branch: 60 East 42nd Street, New York 17, N. Y., telephone, Murray Hill 2-4662. Chicago Editorial Branch: Room 819, 25 E. Jackson Blvd., Chicago 4, Ill., telephone, Wabash 7376. Business Office: American Chemical Society, 1155 16th Street, N. W., Washington 6, D. C. Advertising Office: 332 West 42nd Street, New York 18, N. Y., telephone, Bryant 9-4430.

Entered as second-class matter at the Post Office at Easton, Pa., under the Act of March 3, 1879, as 24 times a year—Industrial Edition monthly on the 1st, Analytical Edition monthly on the 15th. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized July 13, 1918.

Remittances and orders for subscriptions and for single copies, notices of changes of address and new professional connections, and claims for missing numbers should be sent to the American Chemical Society, 1155 16th Street, N. W., Washington 6, D. C. Changes of address for the Industrial Edition must be received on or before the 18th of the preceding month and for the Analytical Edition not later than the 30th of the preceding month. Claims for missing numbers will not be allowed (1) if received more than 60 days from date of issue (owing to delivery hazards, no claims can be honored from subscribers in Continental Europe, Asia, or the Pacific Islands other than Hawaii), (2) if loss was due to failure of notice of change of address to be received before the dates specified in the preceding sentence, or (3) if the reason for claim is "missing from files".

Annual subscriptions—Industrial Edition and Analytical Edition sold only as a unit, members $3.00, nonmembers $4.00. Postage to countries not in the Pan-American Union $2.25; Canadian postage $0.75. Single copies—current issues, Industrial Edition $0.75, Analytical Edition $0.50; back numbers, Industrial Edition $0.80, Analytical Edition prices on request; special rates to members.

The American Chemical Society also publishes *Chemical and Engineering News*, *Chemical Abstracts*, and *Journal of the American Chemical Society*. Rates on request.

Available...

GRAPHIC REPRESENTATION OF THE COMPLETE SCHEME FOR THE SEPARATION AND IDENTIFICATION OF THE COMMON ELEMENTS

Compliments of MERCK & CO., Inc. MANUFACTURING CHEMISTS RAHWAY, N. J.

BASIC CONSTITUENTS

★ The NEW Qualitative Analysis Chart. *Revised 1945.*

★ More accurate results through the use of Merck Laboratory Chemicals.

THE Merck line of Reagents and C. P. Chemicals, as well as those chemicals suitable for industrial research, educational, and routine plant laboratory uses, is comprehensive. C.P. and Reagent mineral acids and Ammonia Water are of highest purity, and are indicated wherever those acids are used.

The use of Reagent grade chemicals in plant operations is constantly increasing. If, in your experimental work, you find the need of a chemical of special purity, or one made to meet your individual specifications, our technical and manufacturing facilities are well adapted to the production of such custom-made chemicals.

MERCK & CO., Inc., RAHWAY, N. J.

Manufacturing Chemists

Please send me the following charts:

......Revised Qualitative Analysis Chart

......Periodic Chart of the Elements

......Sensitivity Chart

Name..

Company....................................

Position.....................................

Street..

City.............................State.......

IND. ENG.-3-46

Results in a Hurry

THE CONSOLIDATED 12 Equation ELECTRICAL COMPUTER

Model 30-103

A NEW TOOL FOR RAPID, ACCURATE CALCULATION OF SIMULTANEOUS LINEAR EQUATIONS

QUICKER ANSWERS

Computation time 1/5 to 1/10 that required by mechanical calculators.

EASY TO OPERATE

Short operation training period.

EASY TO INSTALL AND MAINTAIN

Operates from lighting circuit.

Write for new descriptive bulletin.

CONSOLIDATED ENGINEERING CORPORATION

620 NORTH LAKE AVENUE • PASADENA 4, CALIFORNIA

Manufacturers of Mass Spectrometers, Vibration and Strain Analysis and Recording Equipment

OF THE ESTABLISHED STANDARDS . . .

upon which our lives have been built, only a few have been left unchanged by the war. Among these is the uniform dependable purity of Mallinckrodt Analytical Reagents. Send for the Mallinckrodt Analytical Reagent catalog together with any specific information desired on Mallinckrodt chemicals to fit your specialized operations.

Always Specify Reagents In Manufacturer's Original Packages

Uniform • *Dependable* • *Purity*

CHEMICAL WORKS

Mallinckrodt Street, St. Louis 7, Mo.
72 Gold Street, New York 8, N. Y.

Chicago • Philadelphia • Los Angeles • Montreal

THE *Royal* FAMILY OF LABORATORY FURNACES

Here are furnaces, the like of which laboratory technicians have never seen—vastly improved in both function and appearance. Engineered and built by the leaders in developing and manufacturing industrial heat-treating equipment, they are the last word in efficient and practical laboratory furnaces.

The Lindberg Box Furnace designed for fast and accurate metallurgical tests and chemical analyses heats up to 2000° F. Has built-in transformer; "Stepless" Input Controller and Indicating Pyrometer are contained in a separate metal case.

The Lindberg Pot-Crucible Furnace does salt or lead bath immersion tempering, hardening and annealing, cyaniding and aluminum heat-treating. On the other hand, as a crucible furnace, it is used for determining critical points of steel, melting base metals, thermocouple calibrations and other necessary laboratory operations for heats up to 2000° F.

The Lindberg Combustion Tube Furnace is designed for fast carbon and sulphur determinations using the volumetric method of analysis and for gravimetric type analyses for carbon determinations of all alloy steels including stainless and heat-resisting steels. Heats up to 2500° F.

The Lindberg Hot Plates are for precise, controlled all-round laboratory use for temperatures up to 950° F. Built-in input control provides exact temperature selection.

These furnaces can be secured from your laboratory equipment dealer. See him today for full information.

LINDBERG ENGINEERING COMPANY
2450 WEST HUBBARD STREET · CHICAGO 12, ILLINOIS

LINDBERG

WELL KNOWN THROUGHOUT THE WORLD AS LEADERS IN DEVELOPING AND MANUFACTURING INDUSTRIAL HEAT TREATING EQUIPMENT

SOLD EXCLUSIVELY THROUGH LABORATORY EQUIPMENT DEALERS

DU PONT

Announces

HEXACHLOROCYCLOHEXANE

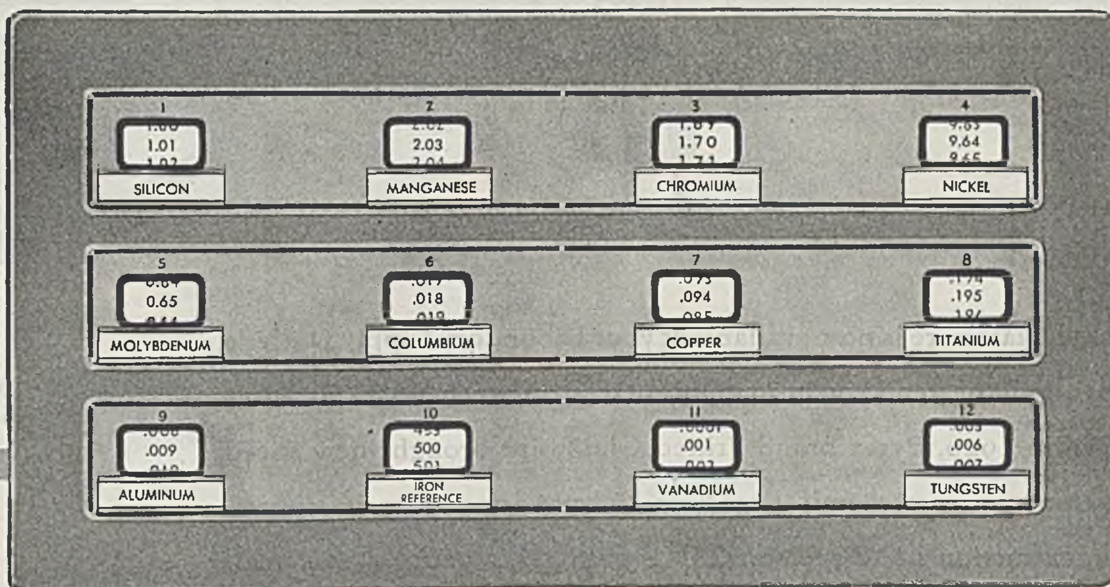
Another Forward Development in Insecticides

HEXACHLOROCYCLOHEXANE is a new and outstanding insecticide. In the annual Hurter Memorial Lecture, delivered on March 8, 1945, by Dr. R. E. Slade, Research Controller of Imperial Chemical Industries, he stated, "There has now come to light what promises to be one of science's important contributions to the welfare of man." Dr. Slade referred to Hexachlorocyclohexane which was developed as an insecticide by Imperial Chemical Industries under the following additional names: benzene hexachloride, 666, and Gammexane.

Du Pont has confirmed the British records of efficiency of this remarkable insecticide in its own laboratories, and is privileged to announce it will have Hexachlorocyclohexane available this season in moderate quantities for experimental use. Inquiries from research institutions and qualified investigators are invited, and on request a copy of Dr. Slade's lecture will be mailed as soon as it is available for distribution. Grasselli Chemicals Department, E. I. du Pont de Nemours & Company, (Inc.), Wilmington 98, Delaware.

BETTER THINGS FOR BETTER LIVING
...THROUGH CHEMISTRY

Chemical Analysis at a GLANCE



within 45 SECONDS after Your Sample is placed in the Quantometer

★ The Quantometer consists of a spectrometer and a recording console. The spectrometer measures photo-electrically the spectral energy of the various metallic elements in an electrically excited sample.

The Quantometer will analyze eleven elements in forty-five seconds. The actual chemical composition, element by element, is shown on visual recorders. Thus, preliminary or final analyses are immediately available.

The costly "wait-periods" for analytical returns are eliminated and "off-specification" rejections are minimized.

A Quantometer-equipped laboratory may be staffed with only one or two operators.

The Quantometer will save man hours and materials. Details of this remarkable development in the field of analytical instruments are now available. Recommendations as to specific applications can be made.

Your inquiry is invited.

A.R.L. • DIETERT

APPLIED RESEARCH LABORATORIES
4336 SAN FERNANDO RD., GLENDALE 4, CALIF.

HARRY W. DIETERT CO.
9330 ROSELAWN AVE., DETROIT 4, MICH.

Good News for Laboratories About

PYREX FRITTED GLASSWARE
BRAND

"Pyrex" Fritted Glassware is now available at your Laboratory Supply Dealer's. He can provide all the items you need in this newest aid to chemical filtration.

Introduced in 1940, Pyrex brand Fritted Glassware brought new speed, retentivity, freedom from chemical reaction and precision porosity control to filtration, extraction and dispersion. Glass particles of uniform size, made from Pyrex brand Chemical Glass No. 774, are fritted by a special process and sealed into transparent bodies—cells, beakers, crucibles, tubes, etc.—fabricated from the same glass. No flux or glazing material is used.

Five porosities, from ultra fine to extra coarse, cover practically every possible requirement. Filtration, extraction and dispersion are completely visible at all times and the glass—both disc and body—is not affected by solutions which destroy other filtering media. Long life is assured since Pyrex Fritted Ware is readily cleaned with water or chemicals—may be heated in an electric furnace to 115°C. Pyrex Fritted Glassware is described in Part III of Catalog LP 24.

Consult your regular laboratory supply dealer for delivery data

"Pyrex" is a registered trade-mark and indicates manufacture by

CORNING GLASS WORKS • CORNING, NEW YORK

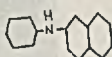
CORNING
means
Research in Glass

BALANCED FOR ALL-AROUND USE

PYREX brand LABORATORY GLASSWARE

B. F. Goodrich Chemical Company

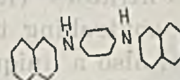
has available for sale these organic chemicals



Phenyl B Naphthyl Amine

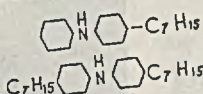
Distilled—Available in commercial quantities
M. P. 107°
Purity 99.5%

Commercial—Available in commercial quantities
M. P. 106°
Purity 98.0%



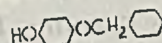
Di B Naphthyl p Phenylene Diamine

Available in commercial quantities
M. P. 230° C
Purity 98%



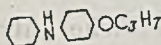
Mixed Mono-and Diheptyl Diphenyl Amines

Available in commercial quantities
Distillation range—145-245 (3.0 mm)
Purity 98%



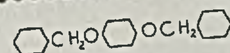
Monobenzyl Ether of Hydroquinone

Available in commercial quantities
M. P. 113°
Purity 90%



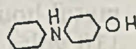
Isopropoxy Diphenyl Amine

Available in commercial quantities
M. P. 78°
Purity 92% min.



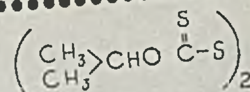
Dibenzyl Ether of Hydroquinone

Available in Pilot Plant quantities
M. P. 119°
Purity 85%



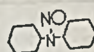
p Hydroxy Diphenyl Amine

Available in commercial quantities
M. P. 15°
Purity 92%



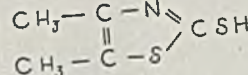
Di Isopropyl Dixanthogen

Available in commercial quantities
M. P. 52°
Purity 98%



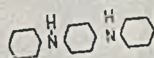
N-Nitroso Diphenyl Amine

Available in commercial quantities
M. P. 62°
Purity 97%



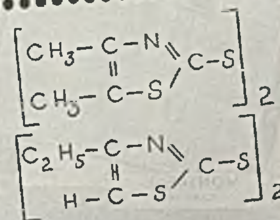
Mixed Ethyl and Dimethyl Mercaptothiazoles

Available in commercial quantities
M. P. 136-153°
Purity Approximately 85% dimethyl and 15% ethyl mercaptothiazoles



Diphenyl p Phenylene Diamine

Available in commercial quantities
M. P. 144°
Purity 92%



Mixed Aliphatic Thiazyl Disulfides

Available in commercial quantities
Liquid

For additional information please write B. F. Goodrich Chemical Company, Department CA-3, Rose Building, Cleveland 15, Ohio.

B. F. Goodrich Chemical Company

A DIVISION OF THE B. F. GOODRICH COMPANY

Modern Appliances for Analytical Chemistry

Fisher Filtrators

Fisher Filtrators (Reg. U.S. Pat. Off.) have means for controlling the vacuum and collecting the filtrate, also a unique double-walled porcelain funnel with slotted inner wall.

Filtrators can be operated in series, all connected to an Airejector, and each can be separately connected or disconnected from the vacuum.

Fisher Filtrator, Low Form (illustrated), with funnel and glass bell. **Each, $15.75**

Fisher Filtrator, High Form, with funnel and cylinder for use with volumetric flasks. **Each, $15.75**

Any number of Fisher Filtrators can be assembled in series, for convenient operation.

Fisher Infra-Rediators

Analyses are speeded when charring of filter papers is done with the Duplex Infra-Rediator.

Fisher Infra-Rediators (Reg. U.S. Pat. Off.) save valuable time because they employ infra red lamps that evaporate solutions, char filter paper, dry pipettes, etc., in as little as one-third the time required by ordinary methods.

The **Fisher Infra-Rediator** is furnished with convenient support stand upon which the lamps in a metal housing, are attached. Complete for use with 110 volts A.C. or D.C. **$23.00**

The **Fisher Duplex Infra-Rediator** (illustrated), has two heating units (four lamps) with support and a metal plate perforated to hold dishes or 14 crucibles. For 110 volts, A.C. or D.C. **$41.00**

Available with Other Modern Laboratory Appliances and High Purity Reagents from:

MONTREAL CANADA

NEW YORK, N. Y.

PITTSBURGH, PA.

ST. LOUIS, MO.

Manufacturers—Distributors

FISHER SCIENTIFIC CO.

EIMER AND AMEND

717 Forbes St., **Pittsburgh (19), Pa.**
2109 Locust St., **St. Louis (3), Mo.**

Greenwich and Morton Streets
New York (14), New York

In Canada: **Fisher Scientific Co., Ltd.,** 904 St. James Street, **Montreal, Quebec**

KIMBLE LABORATORY GLASSWARE

To meet the varied and exacting requirements of Research, Analysis and Control in Science and Industry.

For Assurance

Consult Leading Laboratory Supply Houses Throughout the United States and Canada for Kimble Laboratory Glassware to Meet YOUR Needs.

The Visible Guarantee of Invisible Quality

KIMBLE GLASS COMPANY . . . VINELAND, N. J.

NEW YORK • CHICAGO • PHILADELPHIA • DETROIT • BOSTON • INDIANAPOLIS • SAN FRANCISCO

AGAIN AVAILABLE!

THE HOEPPLER VISCOSIMETER

For Viscosity Measurements by the Falling Ball Method

Unavailable for the past several years, the Hoeppler Falling Ball Viscosimeter is now obtainable of domestic manufacture in the precision model as illustrated.

With this instrument, absolute viscosities of gases and liquids can be determined with a greater degree of accuracy (0.1% to 0.5%) and over a wider range (0.01 to 1,000,000 centipoises) than with other types of viscosimeters. Applications cover a range from gases to viscous liquids which will hardly pour through a 16 mm. opening. Measurements are free from manipulative variables. Accurate results may be obtained even by unskilled operator and measurements repeated as often as desired without replacing or transferring sample, making it particularly well suited for routine and industrial use as well as for precise scientific research. A sample of 30 ml. only is required.

The absolute viscosity is determined by measuring the time of fall of an accurately made ball of glass or steel through the accurately calibrated distance between two marks on the glass tube. The balls and glass tube are of optical precision, being accurate to within 0.0005 mm. and the center of gravity of each ball coincides with its geometrical center. The replaceable glass tube has a coefficient of expansion identical with that of the glass balls and is of non-corrosive heat-resistant glass with heavy walls.

The glass tube is mounted in a water jacket at an angle of 10° from the vertical, the minimum inclination which insures a straight path of fall without lateral oscillations and resultingly constant area for fluid passage throughout the period of descent. The tube is filled with the sample and closed with the appropriately calibrated ball submerged in the sample by means of a special capillary plug which permits inversion without introduction of air into the column, so that as many determinations can be made as desired. Measurements are then made by accurately timing the fall of the ball through the distance between the two marks on the glass tube, the time interval being multiplied by a factor, thus obtaining absolute viscosity directly in centipoises.

The instrument is equipped with a special levelling stand, allowing free inversion of the tube and jacket unit while maintaining a 10° tube angle. Provision is made for the maintenance of a constant temperature by inlet and outlet water connections but for all ordinary samples accurate determinations may be made without heating, a temperature higher than 20°C. seldom being required.

17350—HOEPPLER VISCOSIMETER. Supplied with a set of six balls, consisting of two glass balls and four balls of corrosion-resistant steel alloy in velvet-lined case; ball gage; thermometer reading from 19° to 21°C. in 0.02° divisions; one ball tweezer; two gold-plated stoppers; one camel hair brush; one cleaning piston; one glass rod; one cleaning brush; one chamois skin; and one calibration certificate. In polished wood instrument case with lock...$275.00

WILL CORPORATION

ROCHESTER 3, N. Y. **NEW YORK 12, N. Y.**

BUFFALO APPARATUS CORP., BUFFALO 5, N. Y.

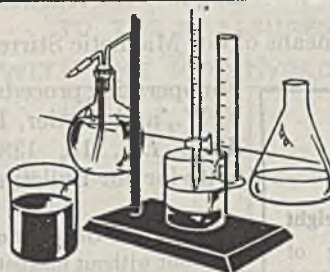
LABORATORY APPARATUS AND CHEMICALS

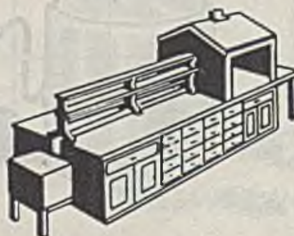
When you need LABORATORY CHEMICALS OR APPARATUS

HARSHAW SCIENTIFIC

Can Meet Your *Requirements*

More than 25,000 items are carried in stock by Harshaw Scientific Division of The Harshaw Chemical Co. • Your requirements can be filled, whether you need chemicals and apparatus for a single experiment, or to furnish a complete laboratory. • Branch offices and stocks are maintained in convenient locations to help you obtain your requirements within a short time. • Send your order, with confidence, to the Harshaw Scientific office nearest to you — Cleveland, Cincinnati, Detroit, or Philadelphia.





HARSHAW SCIENTIFIC
DIVISION OF THE HARSHAW CHEMICAL CO.
CLEVELAND 6, OHIO

STOCKS ALSO CARRIED IN CINCINNATI · DETROIT · PHILADELPHIA

Now

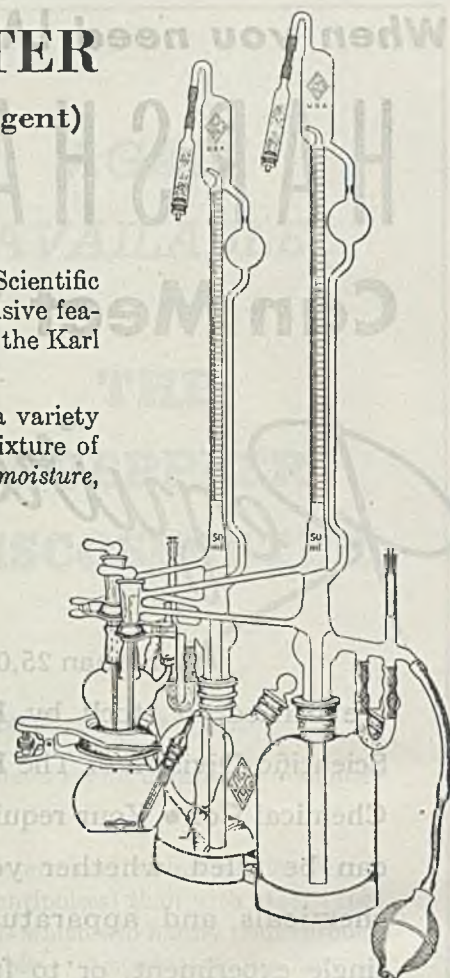
The MOISTURIMETER

(for use with the Karl Fischer Reagent)

by Scientific Glass

DEVELOPED by Louis Sattler of Brooklyn College and William Geyer of Scientific Glass Apparatus Company, this new apparatus has a number of exclusive features which make it the most desirable instrument for moisture analysis using the Karl Fischer Reagent.

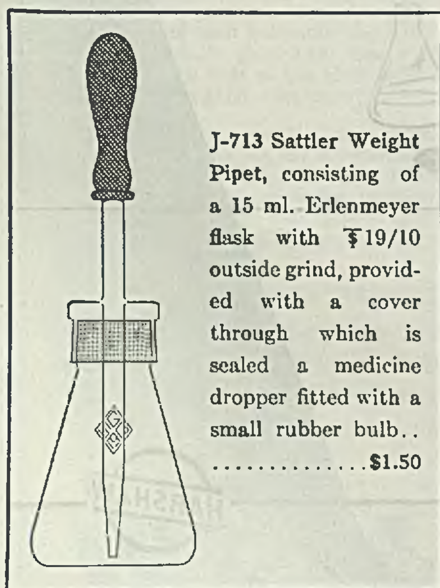
This reagent is widely used for the quantitative determination of water in a variety of products. It consists of a solution of iodine and sulfur dioxide in a mixture of anhydrous methanol and pyridine. *It is extremely sensitive to atmospheric moisture, and consequently it is imperative that titrations with it should be carried out in a completely closed system*, especially in those cases where minute quantities of water are being determined. Furthermore, in view of the fact that *the reaction mixture must be stirred during the titration*, an extra difficulty is thereby introduced into the design of a suitable apparatus.



J-710
(PATENT PENDING)

The Sattler-Geyer Moisturimeter overcomes the problems of exclusion of atmospheric moisture and mechanical stirring in a closed system. It consists of two 50-ml. automatic burets which are so modified that the lower ends of the barrels of the Fresenius stopcocks are enlarged and formed into the outer parts of semiball joints. The titration vessel is a flat-bottom extraction flask of 250-ml. capacity provided with a mouth, which is a male semiball joint, and a sidetube through which go the mutually insulated platinum electrodes sealed into a tube with a male joint and connected to the electro titration apparatus. The flask is covered with a bell-shaped dome, the lower part of which is a female semiball joint. From the top of the dome project two male semiball joints set at sufficiently differing heights to permit free rotation of the buret stopcocks. In addition, the dome is provided with a checkvalve to permit venting of the apparatus whenever liquid is being admitted into the flask from the burets. The male joints fit into the burets which are provided with stopcock plugs with tips extending into the reaction flask.

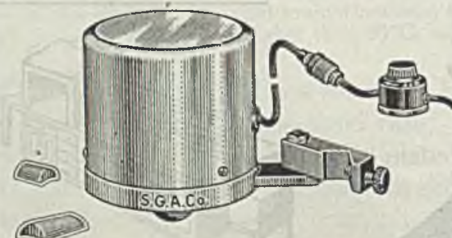
Stirring is accomplished by means of our Magnetic Stirrer, J-711.



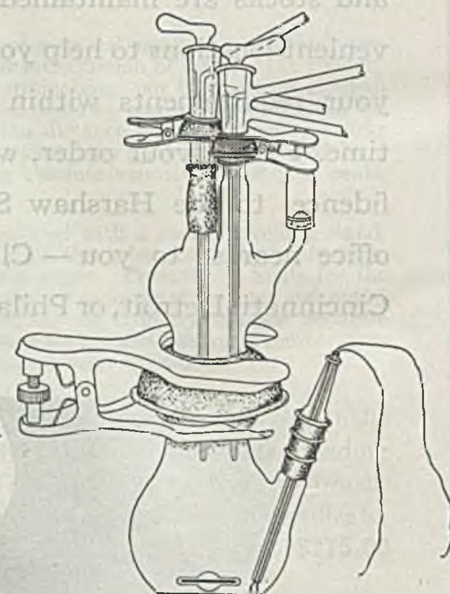
J-713 Sattler Weight Pipet, consisting of a 15 ml. Erlenmeyer flask with Ŧ19/10 outside grind, provided with a cover through which is sealed a medicine dropper fitted with a small rubber bulb.. ....**$1.50**

For operating procedure, see Zerban, F. W., and Sattler, Louis, *I & E C Anal. Ed.*, 18, 138–9 (1946), or write for our Bulletin, 710.

J-710 Sattler-Geyer Moisturimeter complete but without magnetic stirrer. .**$65.00**



J-711 Magnetic Stirrer.........**$32.35**



(Close up of Titration Vessel)

J-712 Karl Fischer Reagent. Complete set sufficient to prepare one qt. Fischer Reagent and one qt. water in Methanol Standard.............................per set **$10.00**

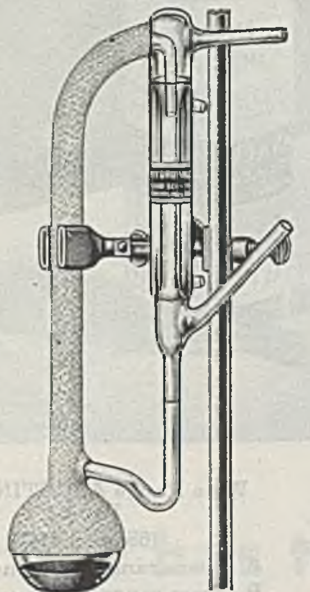
SCIENTIFIC GLASS APPARATUS COMPANY
BLOOMFIELD, N.J.

Introducing

HIGH VACUUM with WATER

TODD SCIENTIFIC SPECIFICATIONS

A vacuum of 0.008 mm. of mercury (99.9989% evacuation) is rapidly produced with the calibrated TODD HYDRO-VAC PUMP,* a specially designed all glass mercury diffusion pump, when used in series with an ordinary water aspirator pump capable of producing 25 mm. of vacuum or better. No supporting mechanical pump is necessary.



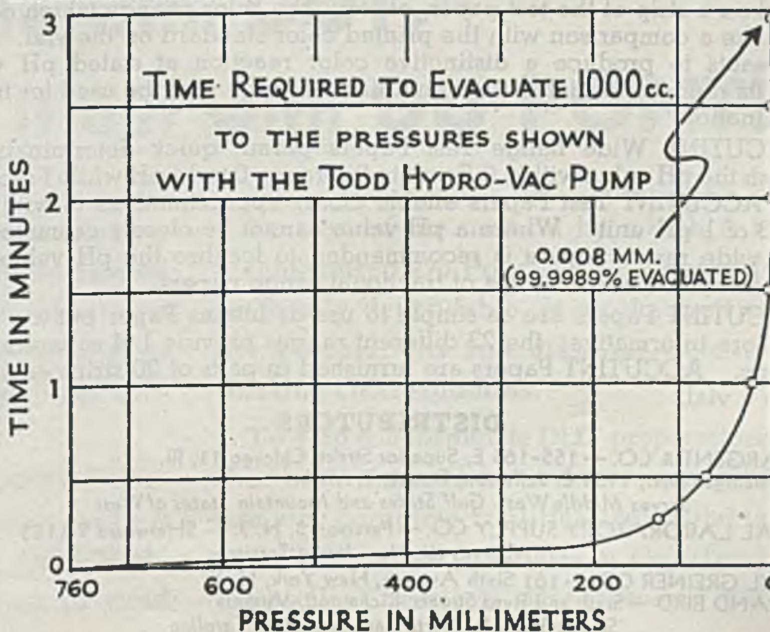
TODD HYDRO-VAC PUMP*

The TODD HYDRO-VAC PUMP is designed to eliminate serious disadvantages inherent in the ordinary piston type vacuum pump, such as is generally used by chemical laboratories for the evacuation of distilling apparatus. Since this highly efficient mercury diffusion pump operates on the diffusion principle, it is not damaged by the presence of non-condensable vapors, water, or other corrosive vapors which are frequently evolved during distillation processes. In addition, this pump is a much less expensive piece of apparatus, is more portable, and occupies less table space than a mechanical pump.

FEATURES:

1. Vacuum of 0.008 mm. of Hg readily obtainable.
2. Not damaged by non-condensable vapors, water, or other corrosive vapors.
3. Rapid evacuation of apparatuses.
4. Entirely automatic operation.
5. Calibrated for maximum vacuum.
6. Highly efficient due to special tapered jet construction.
7. Compact and sturdy single piece construction with Pyrex brand glass.
8. Readily cleaned with nitric acid.
9. Inexpensive.
10. Never wears out.

EXPERIMENTAL DATA:



RECOMMENDED FOR

Vacuum distillations
Vacuum fractionations
Vacuum extractions
Vacuum sublimations
Vacuum pyrolyses
Drying apparatuses of the Abderhalden type
Molecular still distillations
Drying high boiling liquids in bulk
And many other vacuum operations in chemical, physical and biological laboratories.

TODD HYDRO-VAC PUMP

as shown above, calibrated but without insulation and mercury. Complete operating instructions included. Height, 13 inches . . . $25

* The name "HYDRO-VAC" is registered in the United States Patent Office, and designates a product manufactured and distributed solely by the

TODD SCIENTIFIC COMPANY

Designers and manufacturers of special scientific laboratory apparatus.

SPRINGFIELD, PA.

Hydrogen Ion Concentrations CAN NOW BE MEASURED WITHIN 0.1 pH WITH ACCUTINT TEST PAPERS

Simple—

No Instrument Required

Can Be Employed Anywhere

Color Scale on Each Vial

Master Color Chart

Graphic Correlation

ACCUTINT Test Papers are simple, rapid and accurate means of making hydrogen ion determinations. Merely place a drop of the solution being examined on a strip of the test paper, observe the color change which occurs, then make a comparison with the printed color standard on the vial. Each strip reacts to produce a distinctive color reaction at stated pH values within its range or indicates that an adjacent range must be used for further determination.

ACCUTINT Wide Range Test Papers permit quick determination to establish the pH value within 0.5 unit in the range 0 to 14 pH while Fractional Range ACCUTINT Test Papers enable closer approximations to within 0.1 and 0.3 of 1 pH unit. Where a pH value cannot be closely estimated, the use of wide range papers is recommended to localize the pH value sufficiently for quick, effective use of fractional range papers.

ACCUTINT Papers are as simple to use as Litmus Paper but are many times more informative: the 23 different ranges provide 144 separate color reactions. ACCUTINT Papers are furnished in pads of 20 strips each, five pads to a vial.

DISTRIBUTORS

E. H. SARGENT & CO. — 155–165 E. Superior Street, Chicago 11, Ill.
Michigan Div., 1959 E. Jefferson, Detroit 7, Mich.
Serves Middle West, Gulf States and Mountain States of West

GENERAL LABORATORY SUPPLY CO. — Paterson 3, N. J. — SHerwood 2-1123
Serves the New Jersey Territory

THE EMIL GREINER CO. — 161 Sixth Avenue, New York, N. Y.

PHIPPS AND BIRD — Sixth and Byrd Streets, Richmond, Virginia
Serves Virginia, North Carolina, South Carolina

STANDARD SCIENTIFIC SUPPLY CORP. — 34 W. Fourth Street, New York, N. Y.

WILL CORP. — Rochester, New York

ALL other territory served by:

ANACHEMIA NEW YORK — 70 East 45th Street, New York 17, N. Y.
MUrray Hill 6-0753

Wide Range ACCUTINT Test Papers
(65c per Vial)

A covers range 0 through 5 pH
B covers range 1 through 12 pH
C covers range 9 through 14 pH

Packed in vial, 5 pads, 100 strips
(Please specify ranges desired)

Fractional Range ACCUTINT Test Papers
(65c per Vial)

No.	Fractional Range
10	0.0 through 1.2
20	0.8 through 2.4
30	1.3 through 3.3
40	1.4 through 3.0
50	1.7 through 3.3
60	2.7 through 4.7
70	3.9 through 5.4
80	5.0 through 6.6
90	5.2 through 6.9
100	5.3 through 7.0
110	6.1 through 7.4
120	6.9 through 8.4
130	7.2 through 8.8
140	7.3 through 8.8
150	8.4 through 9.4
160	8.9 through 10.0
170	9.1 through 10.4
180	10.1 through 12.0
190	10.7 through 14.0
200	12.4 through 14.0

Packed in vial, 5 pads, 100 strips (Please order by Range Numbers)

Master ACCUTINT Color Chart, 21 x 10¾", correlates by value and graphically all 144 pH values, per chart $3.25.

1 complete set of ACCUTINT Papers, comprising 23 vials (3 wide ranges and 20 fractional ranges) and an ACCUTINT Master Chart, $18.20.

ANACHEMIA 70 E. 45 St., NEW YORK 17, N.Y.

CHEMICAL SPECIALTIES

If it has to do with DDT, work it out with DU PONT!

MANUFACTURERS who have been thinking of using DDT in their products are invited to come to Du Pont for technical service. Experience gained as a major supplier of DDT to the armed forces may be of considerable value to you.

That experience plus Du Pont's continuing laboratory and field studies make it possible to supply you with DDT Technical that always measures up to rigid requirements.

Because of the method by which it is manufactured, Du Pont DDT Technical is uniformly dependable. It works well in dry preparations and dissolves quickly, forming clear solutions.

To help you formulate DDT preparations that have a sound basis for commercial use, our complete facilities are available. E. I. du Pont de Nemours & Co. (Inc.), Grasselli Chemicals Department, Wilmington 98, Delaware.

BETTER THINGS FOR BETTER LIVING
...THROUGH CHEMISTRY

DU PONT DDT *Technical*

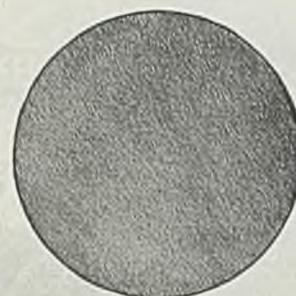
A. H. T. CO. SPECIFICATION

QUALITATIVE FILTER PAPER

Suitable for analytical purposes other than those requiring the use of an acid washed paper

5160. Fig. 1.
Showing label.

5160. Fig. 2.
Showing method of packaging.



5160. Fig. 3.
Showing embossed surface.

QUALITATIVE FILTER PAPER, A. H. T. Co. Specification. A white paper with embossed surface, made of carefully controlled 100% rag stock of uniform texture.

Suitable for analytical purposes other than those requiring the use of an acid washed paper, or one which completely retains fine precipitates such as Barium Sulfate. Weight of ash is exceptionally low for unwashed filter paper, i.e. approximately 0.0006 grams per 110 mm circle, as determined by improved tests recently developed by the U. S. Bureau of Standards. See *Research Paper RP1653, Journal of Research of the National (U. S.) Bureau of Standards, Vol. 34, No. 5 (May, 1945), pp. 453 to 458.*

Wet bursting strength, surface hardness and filtering speed all have been considered carefully in the manufacture to provide a paper which is useful both in educational and in industrial laboratories.

5160. Filter Paper, Qualitative, A. H. T. Co. Specification, as above described. Sizes up to and including 380 mm diameter are packed in convenient cardboard carton containing 100 sheets.

Diameter, mm.	75	90	100	110	125	150	180
Per pack of 100	.14	.18	.19	.20	.23	.29	.38
Code Word	*Foxau*	*Foxcq*	*Foxdo*	*Foxem*	*Foxgi*	*Foxie*	*Foxka*

Diameter, mm.	200	250	330	380	450	500	600
Per pack of 100	.45	.75	1.17	1.68	2.11	2.47	3.25
Code Word	*Foxnt*	*Foxrl*	*Foxth*	*Foxyx*	*Foybr*	*Foycp*	*Foyel*

10% discount in lots of 100 packs } or more,
20% " " " 500 " } one size or assorted

ARTHUR H. THOMAS COMPANY

RETAIL—WHOLESALE—EXPORT

LABORATORY APPARATUS AND REAGENTS

WEST WASHINGTON SQUARE, PHILADELPHIA 5, PA., U.S.A.

Cable Address "Balance" Philadelphia

INDUSTRIAL AND ENGINEERING CHEMISTRY
PUBLISHED BY
THE AMERICAN CHEMICAL SOCIETY
WALTER J. MURPHY, EDITOR
Analytical Edition

Determination of the Boiling Range of Chlorinated Hydrocarbons

DWIGHT WILLIAMS, Research Department, Westvaco Chlorine Products Corporation, South Charleston, W. Va.

A study of the factors that affect precision in the determination of distillation temperatures of chlorinated hydrocarbons showed that thermometer calibration errors cause some variation. The effect of variations in atmospheric conditions, such as the ambient temperature and drafts, is appreciable. The rate of distillation is important in some cases. The most important single factor is superheating of vapor. Because, over limited ranges, distillation temperatures are linear functions of the composition, it is possible to determine the composition of most binary mixtures and some ternary mixtures by this method.

SEVERAL years ago this corporation initiated a study of the determination of boiling ranges of relatively pure commercial solvents such as carbon tetrachloride and trichloroethylene. A study of the effect of numerous impurities on the boiling range of pure solvents showed that, over limited ranges, distillation temperatures were linear functions of the composition. Thus, it is possible to determine the composition of most binary mixtures and some ternary mixtures very simply from distillation temperatures.

A study of the precision of the determination of distillation temperatures showed that it was possible to estimate relatively small concentrations of impurities from such data. However, the precision was far poorer than the precision with which a mercury-in-glass thermometer may be read. This led to a study of the factors which affect precision. Thermometer calibration errors caused some variation. A relatively simple piece of equipment was built for calibrating boiling range thermometers. The effect of variations in atmospheric conditions, such as the ambient temperature and drafts, was studied and was shown to be appreciable, at least under extreme conditions. The rate of distillation was shown to be important in the case of some solvents, but not others.

The most important single factor contributing to lack of precision is probably superheating of the vapor. While no satisfactory solution to this problem has been found, it is still under investigation and it is planned to make this the subject of a subsequent paper. It is believed that the present paper will prove of some interest, since methods of this type are in general use for testing the purity of fluids.

APPARATUS

The shield, condenser, distilling flask, and graduate are those specified in A.S.T.M. Designation D86-38 for the determination of the boiling range of gasoline. The following apparatus differs from that specified in the A.S.T.M. method:

HEATER. The heater sold under the trade name Ful-Kontrol is used. The upper refractory is 1.9 cm. (0.75 inch) thick and has a 2.5-cm. (1-inch) hole in the center. The refractory is used plane side up.

THERMOMETER. Precision grade, length 40 cm. (16 inches), scale 23.8 cm. (9.5 inches), immersion 10 cm. (4 inches) including bulb 1.9 cm. (0.75 inch) in length. Scale starts 2.5 cm. (1 inch) above line of immersion, expansion chamber above bulb and above column, distance from bottom of bulb to top of lower expansion chamber not over 3.8 cm. (1.5 inches), scale 40° C. in 0.1° C. divisions. Thermometers conforming to these specifications and covering any desired range may be purchased from Taylor Instrument Companies on special order. The thermometers are calibrated at the boiling point of each fluid with which they are used, according to the procedure described below.

THERMOMETER READER. Central Scientific Company Catalog 19,520 or its equivalent.

PROCEDURE

The heater is clamped into the position in which it is to be used during the distillation. A tight connection is made by means of a cork between the vapor tube of the flask and the condenser tube. The flask is adjusted so that its outlet extends into the condenser tube 2.5 to 5 cm. (1 to 2 inches). The thermometer, provided with a cork, is tightly fitted into the flask, so that it is in the middle of the neck of the flask and the top of the expansion chamber is level with the inside of the bottom of the outlet tube at its junction with the neck of the flask. The immersion mark should be close to the bottom of the cork. Water at approximately room temperature is allowed to circulate through the bath.

The heater is turned on long enough before starting the distillation to maintain a constant rate of distillation. The heater must be turned on at the voltage at which it is to be used for at least an hour, or for a shorter time at a higher voltage, before starting the distillation. The voltage required to give a constant rate of distillation of 5 to 6 ml. per minute is determined empirically for each fluid.

A 100-ml. sample, which is approximately at room temperature, is measured in a dry, 100-ml. graduated cylinder and transferred to the dry distillation flask. The thermometer is placed in the charged flask and the flask is fitted to the condenser. The flask must be carefully fitted onto the hole in the refractory of the heater. The graduated cylinder is placed without drying under the outlet of the condenser.

The temperature is read and the time recorded when the first drop falls into the graduated receiver. The receiver is then moved so that the tip of the condenser touches the side of the receiver. The temperature is read when the volume in the graduate reaches 5 ml. and 95 ml., and when the distilling flask just goes dry. All temperature readings are made by means of the thermometer reader and are recorded to the nearest 0.01° C. The time is recorded at the dry point. The time between the first drop and the dry point should be between 17 and 20 minutes.

The barometer and the temperature alongside are read during the course of the distillation. The pressure is corrected for brass scale as follows:

$$P_0 = P_t - (t \times P_t \times 0.000160)$$

where P_0 is the barometric pressure in millimeters of mercury at 0° C. and P_t is the pressure at temperature t. The distillation temperatures are corrected to 760-mm. pressure as follows:

$$T_{760} = T_{obs.} + dT/dP\ (760 - P_0)$$

where T_{760} and $T_{obs.}$ are the corrected and observed distillation temperatures, respectively.

These formulas may be simplified in a given laboratory in the

Table I. Precision of Boiling Range of Carbon Tetrachloride under Best Conditions

Test No.	Temperature, ° C. First drop	5 ml.	95 ml.	Dry	Range
1	76.58	76.71	76.80	76.89	0.31
2	76.58	76.73	76.80	76.88	0.30
3	76.56	76.72	76.80	76.90	0.34
4	76.58	76.73	76.80	76.90	0.32
5	76.56	76.71	76.79	76.84	0.28
6	76.58	76.72	76.80	76.87	0.29
7	76.53	76.69	76.78	76.87	0.34
8	76.59	76.72	76.79	76.86	0.27
9	76.57	76.72	76.79	76.87	0.30
10	76.56	76.72	76.79	76.83	0.27
Av.	76.57	76.72	76.80	76.87	0.30
σ_1 of group	±0.016	±0.011	±0.008	±0.022	±0.024
LU_1 of method	±0.054	±0.036	±0.024	±0.072	±0.081

interest of expediting routine work. The following simplified formulas are used in this laboratory:

$$P_0 = P_t - (t \times 0.122)$$

$$T_{760} = T_{obs.} + f\,(760 - P_0)$$

The factor f is 0.044° C. per millimeter for carbon tetrachloride.

Finally, the thermometer calibration correction is added.

MATERIALS

With few exceptions the materials used in this work were the normal commercial products. It was assumed that a product of sufficient purity was being used when the specific gravity and the boiling point agreed closely with the accepted value found in the literature. In case the normal commercial product was not sufficiently pure, a selected product was obtained or the material was purified by rectification in the laboratory.

EXPERIMENTAL

Precision. The precision of the boiling range method as applied to a number of fluids was determined according to the procedure outlined by Moran (3). Data for a typical solvent, commercial carbon tetrachloride, are shown in Tables I and II. As was pointed out by Moran, the high ratios of LU_2:LU_1 (limit of uncertainty under routine conditions to limit of uncertainty under the best conditions) indicates a method having considerable personal and seasonal variations. Nevertheless, these data are useful in estimating the precision within which impurities in carbon tetrachloride and other solvents may be determined from boiling range data.

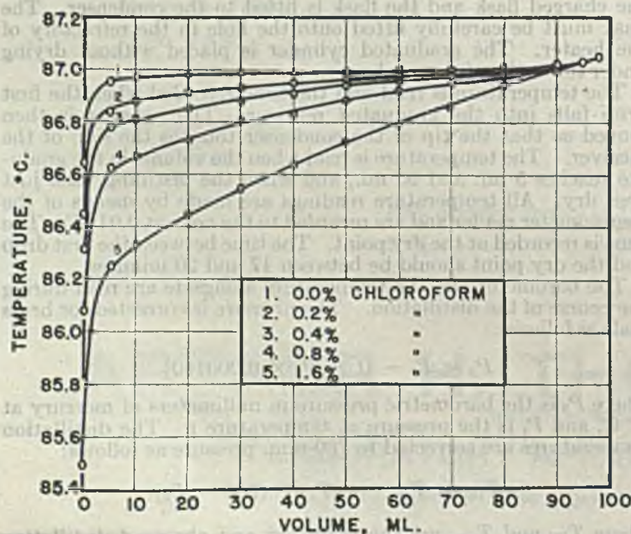


Figure 1. Effect of Chloroform on Boiling Range of Trichloroethylene

Effect of Impurities. The boiling ranges of trichloroethylene to which known amounts of chloroform have been added are shown in Figure 1. These data are typical of those obtained for binary mixtures. By plotting the temperature of the 5-ml. point against concentration it can be shown that the chloroform concentration over the range 0 to 16 volume per cent is given by:

$$c = 2.39\,(86.93 - t)$$

where c is the concentration in volume per cent and t is the temperature in ° C. The LU_1 of the 5-ml. temperature for trichloroethylene is ±0.036° C. and the LU_2 ±0.11° C. Thus, under the best conditions, it would be possible to determine the concentration of chloroform in trichloroethylene within ±0.09%, while under routine conditions the concentration of this impurity could be determined within ±0.26%.

Data for the calculation of a number of impurities in different solvents are summarized in Table III. The carbon tetrachloride which was used in obtaining these data was the commercial product, since the boiling range data showed this product to be pure within the precision of the method. The trichloroethylene, perchloroethylene (tetrachloroethylene), and acetylene tetrachloride (1,1,2,2-tetrachloroethane) were rectified to obtain materials having narrow boiling ranges. The purities of the trichloroethylene and perchloroethylene were comparable to that of the carbon tetrachloride. The acetylene tetrachloride was somewhat less pure, the boiling range from the 5-ml. point to the dry point being 0.8° C.

The concentrations of all low-boiling impurities were calculated from the temperature at the 5-ml. point, while the concentrations of high-boiling impurities were calculated from the temperature at the dry point. Low-boiling impurities have a somewhat greater effect on the first-drop temperature than on the temperature at the 5-ml. point, but the precision of the latter temperature is greater than that of the former, and in general more than compensates for the lower slope of the temperature-composition curve. For example, the LU_2 of the first-drop temperature of trichloroethylene is three times that of the LU_2 of the 5-ml. point. In determining chloroform in trichloroethylene, the slope of the temperature-composition curve, expressed in degrees per per cent, is twice as large for the first drop as for the 5-ml. point. In the determination of ethylene dichloride (1,2-dichloroethane) in trichloroethylene it is 1.4 times as great. It is seen that the increase in slope does not compensate for the reduced precision. A further indication of the relative precision is a greater scatter-

Table II. Precision of Boiling Range of Carbon Tetrachloride under Routine Conditions

Date	Analyst	Temperature, ° C. First drop	5 ml.	95 ml.	Dry	Range
Jan.	1	76.60	76.72	76.83	76.91	0.31
	1	76.56	76.78	76.84	76.96	0.40
Feb.	2	76.64	76.79	76.88	76.99	0.35
	2	76.64	76.81	76.90	77.00	0.36
March	1	76.63	76.75	76.83	76.90	0.27
	1	76.63	76.75	76.87	76.94	0.31
April	3	76.57	76.68	76.79	76.86	0.29
	3	76.56	76.68	76.78	76.86	0.30
May	1	76.52	76.79	76.89	77.01	0.49
	1	76.43	76.78	76.89	76.99	0.56
	1	76.58	76.76	76.83	76.90	0.32
June	3	76.61	76.78	76.85	76.90	0.29
	3	76.61	76.79	76.85	76.90	0.30
July	1	76.54	76.81	76.87	76.94	0.40
	1	76.55	76.80	76.87	76.92	0.37
Aug.	4	76.60	76.78	76.85	76.92	0.32
	4	76.55	76.77	76.82	76.90	0.35
Sept.	4	76.59	76.77	76.83	76.88	0.29
	4	76.58	76.76	76.82	76.89	0.31
Oct.	5	76.55	76.71	76.77	76.83	0.28
	5	76.64	76.76	76.86	76.88	0.24
Nov.	5	76.60	76.73	76.80	76.86	0.26
	5	76.62	76.77	76.82	76.88	0.26
Dec.	6	76.50	76.76	76.85	76.92	0.42
	6	76.60	76.75	76.81	76.88	0.28
	Av.	76.58	76.76	76.84	76.91	0.33
	σ_2 of group	±0.05	±0.035	±0.035	±0.05	±0.075
	LU_2 of group	±0.15	±0.11	±0.11	±0.15	±0.23
	Ratio LU_2:LU_1	3.0	2.8	5.5	2.1	2.9

Table III. Calculation of Impurities in Solvents from Boiling Range Data

Substance	Impurity	Equation	Range[a] %	LU_1 ° C.	LU_2 ° C.
CCl_4	(1,2) $C_2H_4Cl_2$	$c = 8\,(76.72 - t)$	0 to 2	±0.036	±0.11
CCl_4	C_2HCl_3	$c = 2.56\,(t - 76.87)$	0 to 1	±0.072	±0.15
CCl_4	C_2Cl_4	$c = 0.077\,(t - 76.87)$	0 to 1.6	±0.072	±0.15
CCl_4	C_2Cl_6	$c = 0.031\,(t - 76.87)$	0 to 0.02	±0.072	±0.15
C_2HCl_3	$CHCl_3$	$c = 2.39\,(86.93 - t)$	0 to 1.6	±0.036	±0.11
C_2HCl_3	(1,2) $C_2H_4Cl_2$	$c = 5.71\,(86.93 - t)$	0 to 4	±0.036	±0.11
C_2HCl_3	C_2Cl_4	$c = 0.187\,(t - 87.05)$	0 to 1	±0.087	±0.17
C_2Cl_4	CCl_4	$c = 1.26\,(121.08 - t)$	0 to 1	±0.02	...
C_2Cl_4	C_2HCl_3	$c = 1.70\,(121.08 - t)$	0 to 4	±0.02	...
C_2Cl_4	C_2HCl_5	$c = 0.140\,(t - 121.34)$	0 to 3	±0.05	...
C_2Cl_4	C_2Cl_6	$c = 0.069\,(t - 121.34)$	0 to 2	±0.05	...
(1,1,2,2) $C_2H_2Cl_4$	(1,2) $C_2H_4Cl_2$	$c = 0.71\,(145.5 - t)$	0 to 2	...	...
(1,1,2,2) $C_2H_2Cl_4$	C_2HCl_5	$c = 1.33\,(t - 146.3)$	0 to 8	...	...
(1,1,2,2) $C_2H_2Cl_4$	C_2Cl_6	$c = 0.22\,(t - 146.3)$	0 to 4	...	...

[a] Liquid impurities in volume %; solids in weight %.

ing of the points when the temperature-composition curve is plotted for the first drop than when it is obtained for the 5-ml. point. The precision of the measurement of the dry-point temperature is normally slightly poorer than that of the 95-ml. temperature, but this slight decrease in precision only partially offsets the greater slope in the temperature-composition curve.

The ranges over which linear equations apply are shown in Table III. For the most part, linear equations applied over the entire range tested and presumably over even wider ranges.

In general, one impurity, if present in significant amounts, affects the entire boiling range. Thus, the boiling range method is generally applicable only for the analysis of binary mixtures. For example, the presence of chloroform, ethylene dichloride, or acetylene tetrachloride in carbon tetrachloride prevents the quantitative determination of any of the others. Chloroform or ethylene dichloride may, on the other hand, be present in trichloroethylene without interfering with the determination of high-boiling impurities. Ethylene dichloride does not interfere with the recovery of either pentachloroethane or hexachloroethane from acetylene tetrachloride, nor do either of the latter interfere with the former over the ranges given in Table III.

In general, commercial products contain both low-boiling and high-boiling impurities and the identity of the impurities may not be known. While the boiling range cannot be used for either the qualitative or the quantitative analysis of such fluids, it is useful in indicating the presence of both low-boiling and high-boiling impurities. Further, an indication of the difference between the boiling point of the main component and that of the impurity can be obtained from the rate of change of temperature with respect to the volume. Possibly the most useful application of the method is in the study of intermediates. It may be applied to supplement specific gravity and refractive index data for the analysis of complex mixtures. As is shown in Table II, very small concentrations of impurities can be detected from boiling-range data in case of compounds which have a substantial difference in boiling points and even when the difference in boiling points is relatively small the method is reasonably sensitive. The smallest difference in boiling points is that between trichloroethylene and ethylene dichloride (4° C.). The data show that ethylene dichloride may be detected in trichloroethylene in concentrations as small as 0.5%. Where a wide difference exists between the boiling points, as in the case of hexachloroethane in carbon tetrachloride, concentrations as small as 0.005% may be detected.

Calibration of Thermometers. A coaxial constant-temperature bath similar to that used by the National Bureau of Standards (*1*) was constructed for the calibration of thermometers. The bath consisted of a 25 × 45 cm. (10 × 18 inch) Pyrex jar. The sides were covered with a 3.8-cm. (1.5-inch) layer of Fiberglas insulation and the bottom with a 5-cm. (2-inch) layer of magnesia. The cover consisted of two circles of 0.6-cm. (0.25-inch) Transite bolted together, one circle fitting inside and one outside the glass jar. The cover was split for convenience and slots were cut in it for insertion of the stirrer, heater, and thermometers. The inside cylinder was a 7.5-cm. (3-inch) metal tube. The heat input was controlled by means of a Variac. A temperature of 85° C. could be maintained with a heat input of about 80 watts.

The standard thermometers were purchased from Taylor Instrument Companies, Rochester, N. Y., under the following specifications: precision grade, length 45 cm. (18 inches), total immersion, with ice point, expansion chamber at top of column, length of scale 30 cm. (12 inches), range 40° C. in 0.1° C. divisions. The manufacturer supplied a laboratory test certificate with each thermometer at the ice point and each 5° C. The ice points were checked prior to use. These thermometers were subsequently calibrated by the Bureau of Standards.

The following procedure was used for the calibration: The bath was adjusted to the temperature at which the calibrations were to be made. The standard thermometers were placed in the bath with 2.5° C. emergent stem measured to the center of the cover. The distillation thermometers (several were calibrated simultaneously) were adjusted to their immersion marks. The heat input to the bath was adjusted by means of the Variac, so that the temperature rose 0.1° to 0.2° C. during the time required to make a set of readings, which was about 10 minutes. The thermometers were then read in rotation beginning with the standard. Two laboratory-grade thermometers, which were used to measure the stem temperatures of the standard and distillation thermometers, respectively, were then read, after which the series was read in the reverse order. This cycle was repeated five times, making a total of ten readings for each thermometer. The average of the ten readings was calculated for each thermometer. This average for the standard was corrected for emergent stem (about 0.03° C.) and for the calibration correction given by the manufacturer. The difference between this temperature and the average temperature calculated for the distillation thermometer is the calibration correction for the latter.

Thermometers were calibrated at the boiling points of the fluids with which they were to be used. The maximum calibration error observed was 0.17° C. The precisions of the calibrations were checked under actual working conditions by making distillation ranges. The distillation ranges for carbon tetrachloride, using calibrated thermometers, are shown in Table IV. Slight differences are indicated between the several calibrated thermometers. These data were taken over a period of several weeks by one person. The precision of the entire series falls between the LU_1 and LU_2 values given in Tables I and II. A precision of this order of magnitude is to be expected under these conditions. It is therefore improbable that there is any significant difference between the distillation temperatures given by the calibrated thermometers.

Variations in the emergent stem temperature will cause significant errors in apparent distillation temperatures. The temperature of the midpoint of the emergent stem during the calibration of thermometers at the boiling point of trichloroethylene was approximately 10° C. above that of the room. The temperature of the midpoint of the emergent stem during distillation varied from 10° to 20° C. above that of the room. Seasonal

Table IV. Distillation Ranges of Carbon Tetrachloride Using Calibrated Thermometers

Thermometer No.	Calibration Correction, ° C.	Temperature, ° C.			
		First drop	5 ml.	95 ml.	Dry
1,977,258	−0.09	76.42	76.61	76.70	76.77
	−0.09	76.41	76.61	76.70	76.73
2,172,778	−0.17	76.41	76.62	76.70	76.76
	−0.17	76.41	76.61	76.67	76.74
1,977,259	−0.09	76.39	76.61	76.69	76.77
	−0.09	76.41	76.60	76.69	76.78
2,284,595	−0.13	76.43	76.65	76.73	76.76
	−0.13	76.44	76.65	76.72	76.77
	−0.13	76.47	76.66	76.72	76.77
5,677	−0.05	76.44	76.65	76.73	76.79
	−0.05	76.45	76.65	76.72	76.80
	−0.05	76.36	76.66	76.74	76.83
5,678	−0.02	76.43	76.65	76.71	76.79
	−0.02	76.40	76.64	76.72	76.81
5,679	−0.08	76.43	76.68	76.74	76.83
	−0.08	76.43	76.66	76.75	76.83
5,680	−0.05	76.39	76.67	76.74	76.82
	−0.05	76.40	76.67	76.74	76.83

variations in room temperature of 15° C. are not uncommon. Thus, the temperature of the emergent stem during use may differ by 25° C. from that during calibration. The emergent stem of the particular thermometer which was used for this work (range 70° to 110° C.) measured about 20° C. during the distillation of trichloroethylene. Thus, errors up to 0.08° C. may result from this cause. If the top of the scale were used, this error would be twice this value. For the highest precision both the calibration and the distillation temperature must be corrected to the same emergent stem temperature. Since, however, this correction is subject to considerable uncertainty, the most precise results would be obtained by using a thermometer with a shorter range, say 10° C.

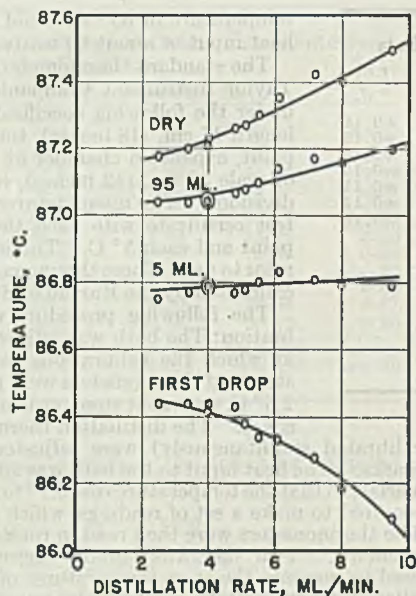


Figure 2. Effect of Rate on Distillation Temperature of Trichloroethane

Effect of Conditions. From Figure 2 it is seen that the rate of distillation has a marked effect on the distillation temperatures of trichloroethylene. It was found that the rate has a significant but less pronounced effect on the distillation temperatures of perchloroethylene, but it has no detectable effect on the distillation temperatures of carbon tetrachloride. Thus it appears that the rate has a marked effect on some fluids and none whatever on others. In general it is desirable to maintain the rate as nearly constant as possible. It is considered practical to maintain the rate constant within a range of about 1 ml. per minute. A rate of 5 to 6 ml. per minute was chosen in order to complete the analysis as rapidly as possible. To ensure that this rate is maintained, it is necessary that the time of the first drop and the dry point be recorded. No attempt is made to control the time between the application of heat and the first drop, since this is automatically controlled by the heat input required to maintain the desired rate.

The shield which is specified in the A.S.T.M. method for the distillation of gasoline was normally used during this work. The temperature inside the shield was substantially above that of the room, owing to heat radiated from the distillation heater. Calculation showed that the efficiency of the heater was only about 8%, the remaining 92% of the heat serving to raise the temperature of the surrounding atmosphere.

To exaggerate this effect a cover of asbestos paper was provided for the shield. Thus, the heater and flask were completely surrounded by the shield. The distillation thermometer protruded through a hole in the cover. An auxiliary thermometer to measure the temperature of the space inside the shield also extended through the cover. The bulb of this thermometer was about 10 cm. (4 inches) from the heater. This thermometer registered a temperature of 60° to 70° C. during the time the distillation was in progress. This additional shielding raised the distillation temperature of trichloroethylene at the 95-ml. and dry points by 4° and 8° C., respectively.

In an effort to reduce this effect, the preceding experiment was repeated except that the neck of the distillation flask was insulated by wrapping it with asbestos wicking. This relatively inefficient method of insulation reduced the effect on the temperature at 95-ml. point and the dry point to 2° and 5° C., respectively.

The effect of drafts was greatly exaggerated by removing the shield and directing a fan on the distillation flask. This procedure reduced the rate of distillation to 2 ml. per minute in spite of the fact that the heat input was more than doubled. Except for the high dry point, the distillation temperatures were not affected. This experiment was repeated except that the insulated flask described above was used. A normal boiling range was obtained under these conditions.

From these data it appears extremely doubtful whether the drafts which are normally present in the laboratory will have any significant effect on the boiling range of relatively pure liquids such as those tested in this work, even though the A.S.T.M. shield were omitted. Superheating of the vapors by the radiant energy lost from the heater occurs to a substantial degree and is magnified by any device which encloses the distillation flask within the same space occupied by the heater. While it cannot be definitely concluded that the shield is harmful, it appears extremely doubtful whether it serves any useful purpose. It appears probable that, if the elimination of the effect of atmospheric conditions is necessary, it should be done by insulating the flask and not by enclosing the flask and heater within a shield.

The author recognizes that this and perhaps other conclusions drawn from this work are contrary to commonly accepted beliefs and are apt to arouse controversy. Such conclusions cannot be considered as final, but if they serve to stimulate further investigation their purpose will have been served.

Since the most serious error is caused by the very low efficiency of the heater, the most promising method for improving the procedure would involve the design of a more efficient heater. Some work has been done along this line but a satisfactory solution has not been obtained to date. Formed-in-place heaters similar to those described by Krantz and Hufferd (2) were built to fit a 100-ml. distillation flask. Heaters of this design 6.25 cm. (1.5 inches) in diameter appear to have entirely eliminated superheating. Heaters of larger diameter cause superheating. That superheating is eliminated is shown by the following facts:

Using formed-in-place heaters, the rate has no effect on the distillation temperatures of trichloroethylene and the range of distillation temperatures from the first drop to the dry point is less than that which was obtained at the lowest rate at which the Ful-Kontrol heater was used. The Ful-Kontrol heater causes superheating of methylene chloride of as much as 10° C. near the end of the distillation, but there was no indication of superheating of methylene chloride when using a 3.75-cm. (1.5-inch) formed-in-place heater. While this type of heater appears to eliminate superheating entirely, it is difficult to construct and has a short life in the small size required for this work. Other types of heaters are being tested, but none has been found to date which is considered entirely satisfactory. This work is continuing and it is anticipated that a heater will eventually be found which is generally satisfactory. It is planned to make this the subject of a subsequent paper.

ACKNOWLEDGMENT

The author wishes to express his appreciation to G. F. Foy, J. E. George, F. D. Heindel, and C. C. Meeker for the preparation of most of the experimental data and to R. F. Moran, chief chemist, and members of the Control Laboratory staff for furnishing the precision data under routine conditions.

LITERATURE CITED

(1) Busse, Johanna, "Temperature, Its Measurement and Control in Science and Industry", p. 242, New York, Reinhold Publishing Corp., 1941.
(2) Krantz, H. A., and Hufferd, R. W., Ind. Eng. Chem., Anal. Ed., 12, 752–3 (1940).
(3) Moran, R. F., *Ibid.*, 15, 361–4 (1943).

Amperometric Titration of Mercaptans with Silver Nitrate

Using the Rotating Platinum Electrode

I. M. KOLTHOFF AND W. E. HARRIS, School of Chemistry, University of Minnesota, Minneapolis, Minn.

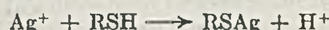
THE rotating platinum wire electrode has been introduced as an indicator electrode in amperometric titrations (2) by Laitinen and Kolthoff (3). The performance of an amperometric titration with the rotating platinum electrode as indicator electrode becomes especially simple, when no e.m.f. needs to be applied to the cell consisting of the indicator electrode and the reference electrode.

The rotating electrode is placed in the solution to be titrated, electrolytic connection is made with the reference electrode, and the current which flows through the cell during the titration is read on a microammeter. The diffusion current of the substance titrated or of the reagent is measured at the potential of the rotating electrode. For example, in the titration of a mercaptan with silver nitrate using the saturated calomel electrode as a reference electrode, the current is zero until the end point. After the end point, when there is an excess of silver in the solution, the diffusion current of silver is measured with the microammeter, this diffusion current being proportional to the concentration of silver ions. When the current readings during the titration are plotted against the volume of reagent added, two straight lines are obtained, which intersect at the end point (see Figure 2). In the example under consideration, the current before the end point is practically zero and is equal to the residual current of the medium.

If the titration is carried out in ammoniacal medium as in the titration of mercaptans with silver in the presence of chloride, the potential of the saturated calomel electrode is not negative enough to yield the diffusion current of the amino-silver ions. Therefore, another reference electrode, which is more negative than the saturated calomel electrode, but not sufficiently negative to give cathodic currents of oxygen, has been used.

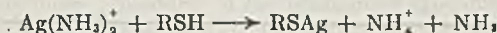
In the present paper the amperometric titration of very small amounts of primary, secondary, and tertiary mercaptans with silver nitrate is described. In the absence of chloride, this titration can be carried out in acid or neutral medium with the saturated calomel electrode as reference electrode. Only the procedure in ammoniacal medium is presented here, since much chloride and little bromide do not interfere in the presence of much ammonia. The titration in the presence of ammonia is as simple as that in neutral or acid medium.

The reaction equation is:



$$Ag^+ + RSH \longrightarrow RSAg + H^+$$

or



$$Ag(NH_3)_2^+ + RSH \longrightarrow RSAg + NH_4^+ + NH_3$$

RSH denotes a mercaptan.

APPARATUS AND MATERIALS

The apparatus and the circuit are shown in Figure 1.

A rotating platinum wire electrode, *A*, about 6 to 8 mm. long and 0.5 mm. in diameter, sealed in 6-mm. soft glass tubing, serves as indicator electrode. Since the titrations can be completed within a short time, an ordinary motor can be used to rotate the electrode. A synchronous motor is not necessary in titration work. For amperometric titration a simple rotating electrode can be made by replacing the shaft of an ordinary cone drive motor, *H*, with a short length of brass tubing (developed in this laboratory by D. G. Weiblen). The 6-mm. glass tubing with the electrode is fastened inside the brass tubing. Electrical contact is then readily made by dipping a wire in the mercury inside the glass tubing.

A reference electrode, *F*, is used which has a potential of −0.23 volt against the saturated calomel electrode. The electrolyte solution for the reference half-cell is prepared by dissolving 4.2 grams of potassium iodide and 1.3 grams of mercuric iodide in 100 ml. of saturated potassium chloride solution. A layer of mercury serves as the electrode.

Electrical connection between the reference and indicator electrodes is made by means of a salt bridge, *E*, consisting of about 60 cm. (2 feet) of 6-mm. (inside diameter) soft rubber tubing filled with saturated potassium chloride solution (1). The rubber tubing is connected with a short length of glass tubing, *D*, filled with a gel of 3% agar and 30% potassium chloride. At the end of the glass tube, a coarsely sintered glass disk may be inserted, if desired. For further protection of the solution from contamination with iodide, the glass tube, *B*, having an agar or a fine sintered-glass plug at its end, may be interposed. The electrolyte solution, *C*, inside *B* can be easily rinsed out and replaced with fresh electrolyte whenever it has been in use long enough to become contaminated with iodide. It is essential that all sources of high resistance, such as air bubbles, be eliminated from the salt bridge.

To complete the circuit the two half-cells are short-circuited through a microammeter, *G*. A Weston Electrical Instrument Corporation, Newark, microammeter (Model 430) has been used in the present work. Instead of the microammeter, other current-indicating devices may be used, such as a pointer galvanometer with sensitivity of 0.25 microampere per division on the attached scale.

The mercaptans tested were analyzed for mercaptan sulfur content by the potentiometric method of Tamele and Ryland (5). Sources of the various mercaptans were:

n-Dodecyl	Organic Chemical Division, University of Minnesota
Commercial primary C_{12}	U. S. Rubber Co.
Commercial tertiary C_{12}	Sharples Chemicals, Inc.
Cyclopentyl	C. S. Marvel, University of Illinois

PROCEDURE

In a 250-ml. beaker dilute a sample of mercaptan containing about 5 mg. of mercaptan sulfur to 100 ml. with 95% ethanol. Make the solution about 0.25 *M* in ammonia and 0.01 to 0.1 *M* with some noninterfering electrolyte such as ammonium nitrate. Immerse the end of the salt bridge and the rotating platinum electrode in this solution. Titrate with aqueous 0.005 *M* silver nitrate.

Make 2 or 3 readings of the microammeter before the end point. As long as the silver nitrate is not in excess the current is very small or zero. After the end point the deflection of the ammeter corresponds to the diffusion current of the excess of silver. When

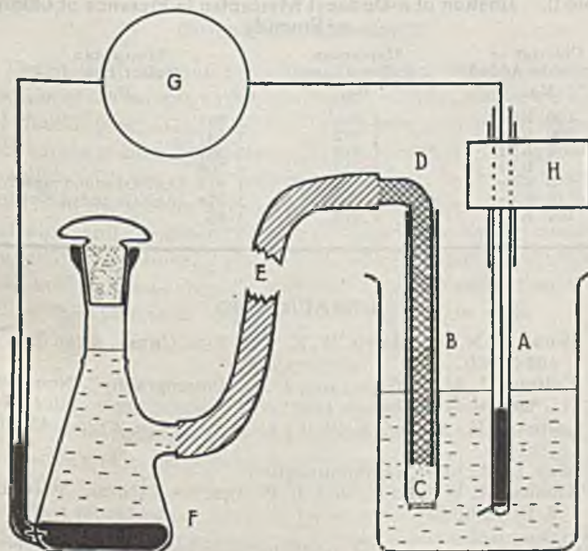


Figure 1. Apparatus for Amperometric Titration

Table I. Amperometric Titrations of Mercaptans

Mercaptan Used	Method of Taking Sample	Mercaptan Sulfur Present	Mercaptan Sulfur Found
		Mg	*Mg.*
n-Dodecyl	Weighing	5.04	5.075
		5.48	5.490
	Dilution of standard solution	1.942	1.945, 1.943
		7.77	7.77, 7.80
		1.93	1.89, 1.93
Commercial primary C_{12}	Weighing	5.26	5.249
		5.01	5.006
Commercial tertiary C_{12}	Weighing	6.47	6.456
		6.94	6.944
Cyclopentyl	Dilution of standard solution	3.047	3.051

the ammeter indicates the end point is passed add two or three small increments of silver nitrate; read the current after each addition. If the electrode becomes sluggish, clean, by wiping with a piece of cloth or between two fingers. Plot the readings of the microammeter against the volume of silver nitrate added. Draw two straight lines (*A* and *B*, Figure 2); the point of intersection, *C*, corresponds to the end point.

A titration carried out by an experienced operator requires no more than 2 minutes.

RESULTS OF TITRATION

Table I shows the results obtained with various mercaptans. The figures of mercaptan sulfur present are based upon the results of potentiometric titrations of large samples. With 5 mg. or more of mercaptan sulfur the amperometric titrations were reproducible within 0.2%.

The current readings obtained during the titration of 1.942 mg. of *n*-dodecyl mercaptan sulfur are shown in Figure 2. The method has been applied to a large number of other mercaptans with satisfactory end-point determinations in all cases. Among mercaptans that have been titrated are: primary C_4, C_6, C_8, C_{10}, C_{14}, C_{16}, and C_{18}; secondary C_8 and C_{12}; tertiary C_4, C_6, C_7, C_8, C_{10}, C_{14}, and C_{16} as well as thiols with other functional groups.

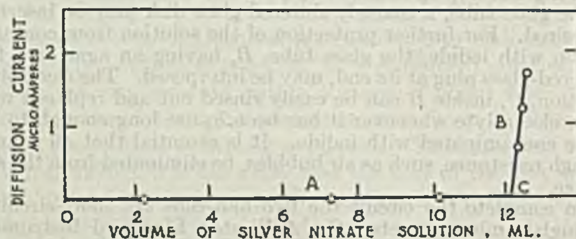


Figure 2. Amperometric Titration of 1.942 Mg. of Mercaptan Sulfur with 0.00495 *N* Silver Nitrate

NOTE. An indicator electrode may become insensitive or erratic after long use or when titrating large amounts of mercaptan. As suggested by May (4) full sensitivity can be restored by wiping the electrode with a piece of cloth or even between two fingers. In the most troublesome cases the following procedure is recommended in determining the end point.

After the addition of the first excess of silver nitrate as indicated by the first slight deflection of the ammeter stop the rotating electrode, wipe, and start again. Read the current immediately; add small increment of silver nitrate. Stop the rotating electrode, wipe, and continue as before. Read the current immediately after starting the electrode. Repeat the above operations 3 to 4 times. The end point is obtained from the readings in the manner previously described.

Thorough cleaning of the electrode with concentrated nitric acid is usually necessary only after it has been used for several hundred titrations. When a new or freshly cleaned electrode is placed in the ammoniacal mercaptan solution and the cell is short-circuited, a large current of 20 to 30 microamperes may be observed. This current decreases rapidly and is practically zero after waiting for 5 to 10 minutes.

Infrequently the glass in the region of the glass-to-platinum seal may become very slightly cracked. In such case a new electrode must be prepared.

In some cases much suspended material in solution interferes mechanically with the current readings. The use of an electrode of the design shown in Figure 3 eliminates interferences of this nature.

INTERFERENCES

Cyanide in ammoniacal medium forms a stable complex with silver ions and interferes in the titration. Other ions, like iodide and sulfide, which form insoluble silver salts in ammoniacal medium, also interfere. It is a simple matter to separate the mercaptan from interfering ions by shaking the mercaptan out in ether or some other suitable organic solvent.

Large amounts of chloride and small amounts of bromide do not interfere in the procedure (Table II).

Figure 3. Rotating Platinum Electrode

SUMMARY

A simple, rapid, and accurate amperometric titration method for the routine determination of primary, secondary, and tertiary mercaptans with silver nitrate is described, using the rotating platinum wire electrode as indicator electrode. The apparatus required is simple and is available in most laboratories.

Amounts of mercaptan sulfur as small as 0.2 mg. in 100 ml. of ethanol can be determined with an accuracy of 1 or 2%. Amounts greater than 2 mg. per 100 ml. can be determined with an accuracy and precision of at least 0.3%. The time required for performance of the entire titration need not be greater than 2 minutes. In ammonical medium, large amounts of chloride and small amounts of bromide do not interfere. Large amounts of bromide, as well as cyanide and other ions which yield insoluble silver salts in ammoniacal medium, interfere.

Table II. Titration of *n*-Dodecyl Mercaptan in Presence of Chloride or Bromide

Chloride or Bromide Added	Mercaptan Sulfur Taken	Mercaptan Sulfur Found
Mg.	*Mg.*	*Mg.*
100 KCl	1.942	1.941
300 KCl	1.942	1.947
1 KBr	1.313	1.307
2 KBr	1.313	1.306
5 KBr	1.313	1.413 ($AgNO_3$ added rapidly)
10 KBr	1.313	1.378 ($AgNO_3$ added slowly)
100 KBr	1.313	1.85

LITERATURE CITED

(1) Hume, D. N., and Harris, W. E., IND. ENG. CHEM., ANAL. ED., 15, 465 (1943).
(2) Kolthoff, I. M., and Lingane, J. J., "Polarography", New York, Interscience Publishers, 1941.
(3) Laitinen, H. A., and Kolthoff, I. M., *J. Phys. Chem.*, 45, 1079 (1941).
(4) May, D. R., private communication.
(5) Tamele, M. W., and Ryland, L. B., IND. ENG. CHEM., ANAL. ED., 8, 16 (1936).

THIS investigation was carried out under the sponsorship of the Office of Rubber Reserve, Reconstruction Finance Corporation, in connection with the Government Synthetic Rubber Program.

Determination of Tungsten in Silicate Rocks

E. B. SANDELL, School of Chemistry, University of Minnesota, Minneapolis, Minn.

A colorimetric method is described which is suitable for determining tungsten in silicic and mediosilicic rocks for geochemical purposes. As little as 0.5 p.p.m. of tungsten can be detected when a 1-gram sample is taken. The method involves separation of tungsten from iron and titanium by sodium hydroxide precipitation, elimination of molybdenum as sulfide with antimonic sulfide as collector, and final determination of tungsten with stannous chloride and potassium thiocyanate. The yellow thiocyanate of tungsten in a lower valence state is extracted with a small volume of ether.

BECAUSE of the difficulty of determining the minute amounts of tungsten occurring in the common rocks of the earth's crust, relatively little is known concerning its abundance and distribution. Up to now tungsten has been determined by x-ray or optical spectrography after concentration by chemical methods. Von Hevesy and Hobbie (*5*), to whom most of our knowledge of the abundance of tungsten is due, applied the x-ray spectrographic method to samples ranging in weight from 150 to 270 grams. Wilson and Fieldes (*14*) used an optical spectrographic method for the determination of tungsten in schist. In their procedure tungsten was first concentrated by coprecipitation with titanium by the joint use of tannin, antipyrine, and cinchonine.

It has been found possible to work out a colorimetric method for tungsten, which gives satisfactory results when applied to silicic and intermediate igneous rocks. With subsilicic rocks that contain much iron and titanium the results are low, approximately one half of the tungsten being lost in the separations. A sample weighing 0.8 to 1 gram is used. As little as 0.5 part per million of tungsten can be detected. The method has been used in a study of the abundance of tungsten in a series of igneous rocks from North America and other parts of the world. The amount of the element found in these samples was of the order of a few parts per million, much less than the 69 p.p.m. reported for a composite of central European rocks (*5*).

Two colorimetric reagents come into consideration for the determination of minute amounts of tungsten—namely, thiocyanate in conjunction with a reducing agent such as stannous chloride, and toluene-3,4-dithiol (*7*). Since the latter reagent has so far been used only qualitatively and semiquantitatively, and is difficult to obtain, it was decided to use thiocyanate for the present purpose.

OUTLINE OF THE METHOD

The method developed involves four main steps: (1) decomposition of the sample with hydrofluoric, sulfuric, and nitric acids; (2) double precipitation of iron, titanium, and other elements with excess sodium hydroxide; (3) precipitation of molybdenum as sulfide with antimonic sulfide as collector, in the acidified filtrate from the sodium hydroxide precipitate after the addition of tartrate; and (4) determination of tungsten in the filtrate from the sulfide precipitate by the addition of thiocyanate, hydrochloric acid, and stannous chloride, followed by ether extraction of the yellow thiocyanate of tungsten in a lower valence state.

DISCUSSION

The important sources of error in the method lie in the coprecipitation of tungsten with the precipitate produced by sodium hydroxide and in the possible incomplete removal of molybdenum as sulfide. In the analysis of intermediate and subsilicic rocks the effect of vanadium must be taken into account.

SEPARATION OF TUNGSTEN FROM IRON AND OTHER ELEMENTS. When extremely small quantities of tungsten are to be determined by the thiocyanate–stannous chloride method, appreciable amounts of iron and titanium must be absent. These elements, as well as others incidentally, are separated from tungsten as tungstate by sodium hydroxide precipitation (cf. *11*). In the present case it is necessary to make this precipitation under rather unfavorable conditions as concerns the coprecipitation of tungsten, in that the volumes must be kept small and only a limited excess of sodium hydroxide may be used in order to avoid difficulties later in the procedure. A few preliminary experiments were run to find the approximate amount of tungsten carried down by the hydroxides of ferric iron, titanium, calcium (carbonate), magnesium, and manganese under conditions similar to those that would exist in the determination of tungsten in a silicate rock if a single sodium hydroxide precipitation were made.

In these experiments, 10 ml. of neutral or slightly acid solution containing 5 micrograms of tungsten and the specified amount of metal as chloride (Ca, Mn) or as sulfate (Fe, Ti, Mg) were added dropwise to 15 ml. of hot 10% sodium hydroxide solution. The precipitate was filtered off on a sintered-glass filter crucible and washed with 5 ml. of water. The filtrate was acidified and treated with an excess of hydrochloric acid and stannous chloride to form the yellow tungsten complex, which was extracted with ethyl ether. Comparison was made against a similar ether extract obtained by adding 5 micrograms of tungsten to the filtrate from a like portion of metal solution which had been added to sodium hydroxide. This method of preparation of the standards largely eliminates the effect of any traces of molybdenum possibly present. In the case of calcium, the solution containing tungsten was evaporated to fumes with a slight excess of sulfuric acid to test the recovery of tungsten from slightly soluble calcium sulfate.

The results obtained (Table I) show that there is little tendency for tungsten to be retained by the calcium, magnesium, or manganese precipitate, but that it is coprecipitated with iron and titanium. The loss of tungsten in the sodium hydroxide precipitation is borne out by the results obtained in the application of the method, as finally worked out, to various types of rocks. In spite of the double sodium hydroxide precipitation which is called for in the procedure, recovery of tungsten is not complete when much iron and titanium is present. Useful results (approximately 80% recovery of tungsten) can still be obtained with a sample containing 6% total iron oxides, 0.9% titanium dioxide, 3% magnesia, and 5 or 6% calcium oxide as well as 0.25% phosphorus pentoxide. In other words, the method can be applied to a typical diorite.

As the amount of iron and titanium in the sample increases the loss of tungsten becomes greater, so that only about one half of the tungsten (ca. 4 p.p.m. added) is recovered from a diabase containing 13% total iron oxides and 1.75% titanium dioxide. The proposed method cannot therefore be applied to subsilicic rocks without modification. Presumably better results can be obtained with such rocks by preparing a solution containing approximately the same amount of iron, titanium, etc., as the sample being analyzed and adding an amount of tungsten comparable to that expected in the sample, and then carrying this standard through the procedure. The need for a complete analysis of the specimen

Table I. Recovery of Tungsten in Sodium Hydroxide Precipitation of Iron, Titanium, and Other Elements

(In each case 5.0γ of tungsten were added as tungstate)

Elements Present	W Found
Gram	γ
0.1 CaO	5.0
0.1 MgO	5.0
0.025 MnO	5.3
0.1 Fe_2O_3	4.2
0.1 Fe_2O_3, 0.12 Al_2O_3	4.4
0.015 TiO_2	4.0
0.015 TiO_2, 0.015 P_2O_5	4.0

would be only a slight objection, since the method would usually be applied to analyzed samples. However, the precision probably would not be very good, especially since very low tungsten contents are to be expected with subsilicic rocks, and the method would become considerably more laborious.

With granitic rocks it appears that a single sodium hydroxide precipitation gives good results, but a double precipitation is recommended.

The possibility of precipitating tungsten with α-benzoinoxime, after the addition of molybdenum as a collector, was considered as a possible method for the isolation of tungsten. However, the introduction of molybdenum, which must subsequently be carefully removed before the colorimetric thiocyanate method for tungsten can be applied, is an undesirable feature of such a procedure. Moreover, doubt has been expressed regarding the completeness of precipitation of tungsten by this method (*3*).

Separation of Molybdenum as Sulfide. Molybdenum accompanies tungsten into the filtrate in the sodium hydroxide separation. Before tungsten can be determined by the thiocyanate method, molybdenum must first be removed, since under the conditions it gives a stronger color than does tungsten. The separation of the two elements must be so complete that less than 0.1 microgram of molybdenum will remain with the tungsten. The classical method for the separation of tungsten and molybdenum involves sulfide precipitation of the latter from a dilute mineral acid solution containing tartrate. As is well known, complete precipitation of molybdenum is difficult even with macro amounts (*12*) and would be entirely hopeless for micro amounts unless resort can be made to mixed crystal formation between molybdenum trisulfide and another sulfide possessing slight solubility in acid solution. Extrapolation of known ionic radii (Goldschmidt's)

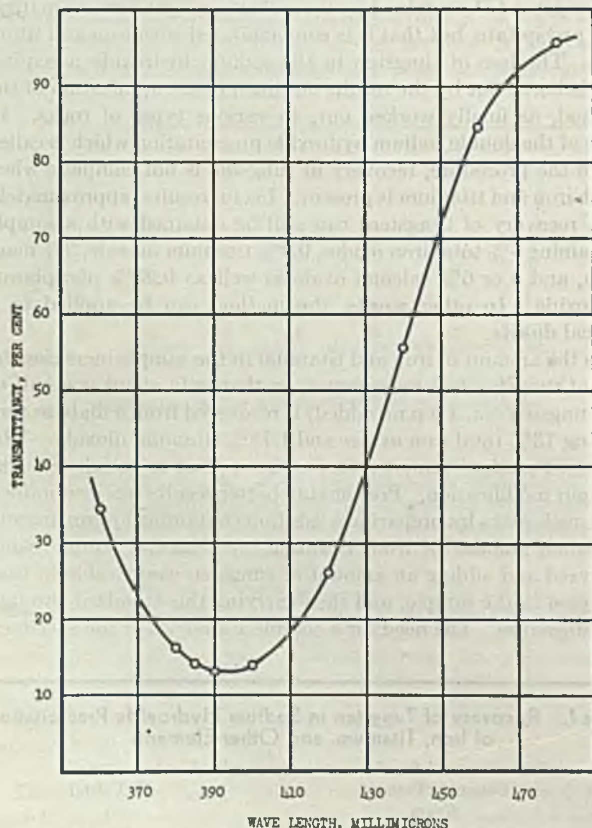


Figure 1. Transmission Curve of Tungsten-Thiocyanate Complex in Ethyl Ether

40 p.p.m. of tungsten, 1-cm. cell
Curve constructed from data obtained by B. Warshowsky using Coleman Model 11 spectrophotometer with 35-millimicron slit width. Color developed by Feigl's method (2) and complex extracted after 2 hours

in the series of elements from rubidium to molybdenum on the one hand, and of the elements from silver to antimony on the other, leads to the conclusion that sexivalent molybdenum and quinquevalent antimony should have similar radii. Experiment showed that antimony pentasulfide was actually a good collector for molybdenum. Trivalent antimony cannot be used in place of the quinquevalent. Moreover, copper sulfide, which has been recommended as a collector for molybdenum, is not suitable for the purpose. These findings are in accord with the experimentally determined or the estimated ionic radii (Å.): Mo^{++++++}, ca. 0.5 (estimated); Sb^{+++++}, 0.50 (estimated); Sb^{+++}, 0.90; Cu^{++}, 0.83. (In the nonionic sulfides the radii will be different from these values for the ions, but it may plausibly be assumed that the similarity or disparity will be preserved in passing from the ionic type of crystal to the nonionic.)

Preliminary experiments showed that a single hydrogen sulfide precipitation in 25 ml. of hot solution containing 20 micrograms of molybdenum, 1 mg. of quinquevalent antimony, 2 grams of sodium sulfate, and ca. 0.5 ml. of 6 *N* sulfuric acid, left from 0.1 to 0.3 microgram of molybdenum in solution as found by colorimetric examination of the filtrate and washings by the thiocyanate method. When the filtrate from the molybdenum sulfide precipitate was boiled to expel hydrogen sulfide, a small amount of bromine water added, and the hydrogen sulfide precipitation repeated after the addition of 1 mg. of quinquevalent antimony, less than 0.1 microgram (perhaps less than 0.05 microgram) of molybdenum was found in the filtrate. Thus it appears that one hydrogen sulfide precipitation with antimony as collector does not remove the molybdenum with sufficient completeness for the purpose, but that a second precipitation reduces its concentration to a negligible value.

In applying this separation of tungsten and molybdenum to silicate rocks, the effect of phosphorus must be considered. The precipitation of molybdenum becomes more difficult when phosphate is present because of the greater ease of reduction of molybdenum by hydrogen sulfide (*12*). It is not advisable to make the hydrogen sulfide precipitation from an initially hot solution when appreciable amounts of phosphorus are present, as is the case with basic rocks. Hydrogen sulfide should be passed into a cold solution to minimize the reduction of molybdenum to molybdenum blue. The colloidal suspension of the sulfides may then be heated to hasten coagulation. This procedure satisfactorily separated the molybdenum from a diabase containing 0.25% of phosphorus pentoxide. It is believed that two precipitations with hydrogen sulfide as described in the procedure below are sufficient to remove with satisfactory completeness the quantities of molybdenum likely to be encountered in igneous rocks. The maximum amount of molybdenum found in a series of 22 American rocks was 0.0007%, or 7 micrograms in a 1-gram sample (*10*). The presence of even less than 5 micrograms of molybdenum in the antimony sulfide precipitate (1 mg. of antimony) is made evident by a pronounced change in color of the precipitate from orange to brownish.

The second antimony sulfide precipitates obtained from some rock samples of intermediate silica content which contained about 1 p.p.m. of molybdenum were examined for molybdenum by dissolving in sodium hydroxide, adding excess bromine and warming, and finally treating with thiocyanate and stannous chloride in excess hydrochloric acid, followed by extraction with a small volume of ethyl acetate. In some cases no molybdenum could be detected, in others 0.1 to 0.2 microgram was found.

Determination of Tungsten. After concentration of the filtrate from the last sulfide precipitate to expel hydrogen sulfide and to reduce the volume to a convenient size, the solution is extracted with ethyl ether to remove traces of a foreign coloring substance (not identified but possibly from the bromine) which imparts a faint brownish coloration to the solvent. The extracted solution is treated with potassium thiocyanate and stannous chloride in strong hydrochloric acid to form the thiocyanate com-

Table II. Determination of Tungsten in Silicate Rocks

Sample	Sample Weight	W Present	W Added	W Found	W Recovered
	Gram	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*
Synthetic silicic rock[a]	1	0.7	3.0	3.3	2.6
Synthetic silicic rock[a]	1	0.7	5.0	6.4	5.7
Synthetic silicic rock[a,b]	1	0.7	10.0	9.0	8.3
Synthetic silicic rock[a]	1	0.7	10.0	10.6	9.9
Synthetic silicic rock (10 p.p.m. Mo)[a,b]	1	0.7	10.0	12.7	12.0
Synthetic silicic rock (10 p.p.m. Mo)[a]	1	0.7	10.0	10.8	10.1
Synthetic silicic rock (15 p.p.m. Mo, 300 p.p.m. V_2O_3)[a]	1	0.7	5.0	6.2	5.5
Synthetic silicic rock (20 p.p.m. Mo)[a]	1	0.7	0	1.2	(0.5)
Granodiorite	1	1.1	2.0	3.2	2.1
Granodiorite[a]	1	1.1	3.0	3.5	2.4
Granodiorite	1	1.1	3.0	3.7	2.6
Granodiorite (11 p.p.m. Mo)[a]	1	1.1	0	1.2	(0.1)
Quartz monzonite	0.8	1.5	2.5	3.5	2.0
Diorite A	0.8	1.6[c]	3.7	4.6	3.0
Diorite B	0.8	0.9[c]	3.7	3.8	2.9
Diabase	0.8	1.0[c]	3.7	2.9	1.9
Diabase	0.8	1.0[c]	6.2	5.0	4.0

[a] Single NaOH precipitation instead of double as in others.
[b] One H_2S precipitation instead of two as in others.
[c] Not corrected for vanadium.

plex of tungsten in a lower valence state (*2*, *4*). It is not necessary to add these reagents to an initially basic solution as in previous procedures for determination of tungsten by the thiocyanate method. This colorimetric method for tungsten has not been studied in a systematic manner but it is known that a high acid concentration is necessary and that the color develops slowly at room temperature, so that the solution should be allowed to stand for an hour. Beer's law is obeyed. The color reaches its maximum intensity more rapidly if the solution is warmed (*8*), but too high a temperature or too long a period of heating results in the formation of a small amount of stannous sulfide. In the present work the colored compound was allowed to develop at room temperature.

The tungsten complex is then extracted with a small volume of ether. Without extraction the determination would be impossible because of the very minute amounts of tungsten involved. Moreover, the aqueous solution may show a slight coloration due to vanadium and possibly other substances. The tungsten content of the ether extract is found by comparison against a series of standards, or against a single standard by varying the height of the column of solution in a narrow tube. The visual comparison is preferred to a photometric measurement of color intensity, because of the very faint color obtained with the quantities of tungsten normally present in igneous rocks. The visual comparison can readily be made in small glass tubes having a cross-sectional area of 1 sq. cm. Under the conditions described below 0.3 microgram of tungsten imparts a faint but definite coloration to the ether in such a tube—i.e., the sensitivity of the reaction is 0.3 microgram of tungsten per square centimeter. The photometric sensitivity may be expressed in terms of the quantity of tungsten in a column of ether solution having a cross-sectional area of 1 sq. cm. which will produce a barely measurable extinction at the wave length of maximum absorption. The transmission curve of the tungsten thiocyanate complex in ether, obtained under conditions similar to, but not identical with, those of the procedure below is shown in Figure 1. It is evident from this curve that at approximately 390 mμ, an extinction of 0.001 corresponds to about 0.05 microgram of tungsten in a column of solution having a cross section of 1 sq. cm. This is the photometric sensitivity based on 0.001 as the smallest extinction ($\log I_0/I$) that can be measured. In other words, visual comparison is roughly as sensitive as photometric measurement in an absorption cell of 1-cm. thickness when the volume of the solution is 5 ml.

Only one ether extraction of the aqueous solution is made. The partition coefficient of the tungsten complex is such that the latter is concentrated in the ether even when the volume of the aqueous phase is much greater. Any error due to unextracted tungsten is cancelled by treating the standard solution in the same manner. The salt solution used to prepare the aqueous standard solution is treated with hydrogen sulfide to remove any traces of molybdenum that may be present. The error in comparing a solution containing 1 microgram of tungsten with a standard of similar concentration may amount to ±0.3 microgram, but is less on the average.

Comparatively few elements will survive the sodium hydroxide and hydrogen sulfide precipitations and find their way into the final aqueous solution. Chief among these, which may have an effect on the determination of tungsten, are phosphorus and vanadium. Phosphate (10 mg. of phosphorus pentoxide) was found to have no effect. Borate also did not interfere. Vanadium, however, produces a coloration similar to that given by tungsten under the conditions of the determination. Hoffman and Lundell (*6*) have already observed that vanadium interferes to a slight extent in the analogous determination of molybdenum with thiocyanate (ethyl ether as extractant) by giving a color about $^1/_{200}$ as strong as molybdenum. In the procedure described below 500 micrograms of vanadium sesquioxide produce as much color as 1 ± 0.2 microgram of tungsten. The hue of the vanadium compound is somewhat more brown than that of the tungsten complex in ether solution, but the two are not readily distinguishable at low concentrations. The amount of vanadium in silicic rocks is too small to affect the determination of tungsten by a detectable quantity. In intermediate rocks it is possible that enough vanadium may at times be present to give an appreciable positive error. Thus a sample containing 0.03% of vanadium sesquioxide would show an approximate apparent tungsten content of 0.6 p.p.m.

Although a vanadium oxide content of 0.03% is not likely to be encountered in rocks for which the proposed method is intended, the possibility of error from the presence of vanadium must be borne in mind. The vanadium content of the sample can be determined on a separate portion (*9*). Then, if the quantity of vanadium in the sample warrants, the equivalent amount of vanadate can be added to the standards to compensate the color produced in the sample solution. The possibility of determining vanadium directly in the sample solution, prior to the addition of thiocyanate and stannous chloride, by adding a little hydrogen peroxide was considered, but this does not seem to be a desirable procedure because of the common presence of a trace of iron, and possibly of titanium, in this solution.

Table III. Composition of Samples in Table II

	SiO_2	Al_2O_3	Fe_2O_3	FeO	MgO	CaO	Na_2O	K_2O	TiO_2	P_2O_5	MnO
Synthetic silicic rock	77	17	2.1	...	1.3	2.2	...	...	0.2	0.1	0.1
Granodiorite	68.2	15.2	0.6	3.3	0.8	2.0	3.4	5.1	0.67	0.23	0.08
Quartz monzonite	65.6	16.6	1.3	2.5	1.1	3.4	4.1	3.7	0.57	0.17	0.08
Diorite A	62.6	16.3	1.3	4.0	2.7	5.3	3.5	2.6	0.89	0.21	0.08
Diorite B	61.5	15.5	2.5	3.6	3.1	5.7	3.4	1.8	0.88	0.26	0.14
Diabase	52.7	14.5	7.4	5.6	3.7	8.0	3.2	1.1	1.76	0.25	0.24

With the exception of vanadium, no element that can be present is known to give a coloration with thiocyanate and stannous chloride in the final solution obtained according to the procedure described below. Any rhenium escaping separation in the preliminary treatment would produce a color resembling that of molybdenum in the final solution, but since the amount of rhenium in igneous rocks is of the order of 0.001 p.p.m., its effect can be left out of account. Columbium and tantalum are without effect (*13*). Uranium in small amount was found to impart no color to the ether phase. Titanium in small amount together with phosphate gave no color. Fluoride in comparatively large amounts tends to decrease the tungsten color intensity, but it will not be present.

A small amount of iron is always present in the filtrate from the sodium hydroxide precipitation, apparently in colloidal form. Frequently more iron is present in the filtrate from a silicic rock than in that from a subsilicic one. The iron thus introduced imparts a yellowish color to the acid tartrate solution but does not interfere in the determination of tungsten. One milligram of Fe_2O_3 intentionally added produced no coloration in the ether. However, in some of the determinations the ether solution of the tungsten complex darkened slightly on long standing. This darkening, with a change in hue to brownish, may possibly be due to the presence of iron. No error results if the color comparison is made immediately after extraction. It was observed that the color of an old extract could be restored to the original hue and intensity by adding a few drops of 10% stannous chloride in concentrated hydrochloric acid to the tube together with an equal volume of water and shaking well.

Platinum, introduced in traces as a result of attack in the decomposition, might be expected to interfere by being reduced to chloroplatinous acid, soluble in ether with a yellow color, if not completely removed as sulfide. The possibility of error from this source was investigated by carrying a sample of granodiorite through the procedure as described below, except that thiocyanate was omitted in the final colorimetric determination. The ether extract was not perceptibly colored; it could not be distinguished from a blank. The tungsten complex was formed when thiocyanate was added to the extracted aqueous solution.

When the tungsten content of the sample is greater than 2 p.p.m., the hue of the ether extract offers considerable evidence that the coloration is actually due to tungsten and not to some other substance. The tungsten complex has a characteristic pure yellow color easily distinguishable from the amber of the molybdenum thiocyanate.

The possibility of using ethyl acetate instead of ether as an extractant for the tungsten thiocyanate complex was investigated, but it was found that the former solvent was less suitable because it was more strongly colored by vanadium (and molybdenum) than was ethyl ether under the conditions of the determination. It is possible that pure isopropyl ether can be used in place of the more volatile ethyl ether. Isopropyl ether has been used by Cunningham (*1*) as an extractant for the tungsten thiocyanate complex in procedures for the determination of tungsten in low-grade ores and tailings.

No tungsten was found in the reagents.

SPECIAL SOLUTIONS

Sodium Hydroxide, 10 grams in 100 ml. of water.

Tartaric Acid, 50 grams in 100 ml. of solution.

Antimony Pentachloride, 0.5% antimony in 4 *N* hydrochloric acid. This solution may be prepared by adding saturated potassium bromate solution to a hot antimony trichloride solution containing 0.5% antimony in 4 *N* hydrochloric acid until the color of bromine appears.

Potassium Thiocyanate, 10 grams in 100 ml. of water.

Stannous Chloride, 5 grams of the dihydrate in 100 ml. of concentrated hydrochloric acid. The solution should not be older than a month.

Ethyl Ether. Only the reagent grade product should be used. The best quality ether need not be subjected to any purification procedure before use.

Salt Solution for Standards. Dissolve 50 grams of sodium hydroxide and 10.0 grams of tartaric acid in 250 ml. of water and add to the cold solution 225 ml. of 6 *N* sulfuric acid and 10 ml. of antimony pentachloride solution. Pass a rapid stream of hydrogen sulfide into this solution, cooled to room temperature, for 0.5 hour. Allow the mixture to stand overnight and filter off the precipitate on a retentive paper.

Standard Tungsten Solution, 100 micrograms of tungsten per milliliter. Prepare by dissolving pure sodium tungstate dihydrate in water and diluting to volume $\left(\frac{Na_2WO_4.2H_2O}{W} = 1.79\right)$.

COLOR COMPARISON TUBES

These should have a capacity of 5 ml. with the dimensions 1.2 × 8 cm., and should be provided with glass stoppers. A narrow strip of millimeter graph paper is glued to the back of each tube to serve as a scale for reading the height of the liquid column. The paper strip should be waterproofed with paraffin or other suitable material.

PROCEDURE

Transfer 1.0 gram of 100-mesh silicic rock or 0.8 gram of mediosilicic rock to a platinum dish and add 5 ml. of 6 *N* sulfuric acid, 2 ml. of concentrated nitric acid, and 5 ml. of 48% hydrofluoric acid. Evaporate to dryness and heat the residue until fumes of sulfuric acid cease to come off. Add 1 ml. of 6 *N* sulfuric acid and a few milliliters of water to the dish and warm with stirring to bring as much as possible of the salts into solution. Then evaporate to dryness and fume off the excess of sulfuric acid at approximately 350° C. Add 1 ml. of 6 *N* sulfuric acid and 10 ml. of water and digest slightly below the boiling point for 15 or 30 minutes while stirring at intervals. Disregard undissolved material.

Sodium Hydroxide Precipitation. Add the mixture dropwise with stirring to 15 ml. of hot sodium hydroxide solution in a 50-ml. beaker. Pour the liquid back into the platinum dish and digest slightly below the boiling point for about 15 minutes. Filter the mixture through a sintered-glass crucible (Jena 1G4 or equivalent), catching the filtrate in a vial or test tube hung inside the suction flask. Wash the precipitate, which has been sucked dry, with 2 or 3 ml. of water. Transfer the filtrate to a 50-ml. beaker and place on a steam bath to reduce the volume by evaporation.

By means of a stirring rod transfer most of the precipitate in the crucible to the beaker in which the precipitation was made. Clean the platinum dish with about 5 ml. of 1 to 1 hydrochloric acid and pour the solution into the crucible. Without applying suction, allow the precipitate remaining in the crucible to dissolve and then pour the solution into the beaker containing the solution of the major part of the precipitate. Now apply suction and wash the crucible with a few milliliters of water. Add the washings to the solution of the precipitate and evaporate the whole to a volume of a few drops. Dilute with 5 ml. of water and add the solution dropwise to 10 ml. of hot sodium hydroxide solution. Keep the mixture slightly below the boiling point for 10 or 15 minutes and then filter through a sintered-glass crucible. Wash with two portions of water totaling 5 ml. Combine the filtrate and washings with those from the first precipitation and evaporate the whole to a volume of 15 ml.

Hydrogen Sulfide Precipitation. Cool the evaporated solution to room temperature and add 6 *N* sulfuric acid dropwise with stirring until a slight permanent precipitate of aluminum hydroxide is formed. Then add 1.0 ml. of tartaric acid solution, 0.5 ml. of 6 *N* sulfuric acid, and 0.2 ml. of antimonic chloride solution. If necessary allow the solution to stand until it becomes clear. Cover the beaker and pass in a rapid stream of hydrogen sulfide for 10 minutes. Then heat the solution to the boiling point while continuing the passage of hydrogen sulfide, keep at the boiling point for a few minutes, remove the beaker from the heat, and pass in the gas for another 5 or 10 minutes. Stirring during this interval aids in the coagulation of the precipitate. Allow the precipitate to stand for 2 hours or preferably overnight.

Filter off the sulfide precipitate on a small (5-cm.) filter paper of fine texture and wash with a few milliliters of hydrogen sulfide water containing a drop of 6 *N* sulfuric acid. Evaporate the combined filtrate and washings to 15 ml. Cool the solution to room temperature and add 5 drops of bromine water (a yellow color should persist for a minute or two) and 0.2 ml. of antimonic chloride solution. Precipitate with hydrogen sulfide as before. Allow the precipitate to stand overnight and filter it off on a small retentive filter paper which has been washed with a little dilute (0.5 *N*) sulfuric acid. Wash the precipitate with 2 or 3 ml. of cold water containing a drop of 6 *N* sulfuric acid.

Determination of Tungsten. Evaporate the combined filtrate and washings from the second sulfide precipitation to a volume of 15 ml. in a 50-ml. beaker. At the same time evaporate 25-ml. portions of the salt solution for the standards to which have been added 1, 2, and 3 micrograms of tungsten, respectively. If the vanadium content of the sample is equal to, or greater than, 0.02% (0.03% vanadium sesquioxide), add an amount of ammonium metavanadate equivalent to the quantity of vanadium in the sample to each of the standards (see Discussion, p. 163).

Cool each solution to room temperature (20° C.), transfer to a small separatory funnel, and extract with 5 ml. of ether. Draw off the aqueous solution in each case and wash the ether layer in the funnel with 1 or 2 ml. of water, adding the latter to the extracted aqueous solution. If the ether extract from the sample solution shows an appreciable coloration (compare in a small vial against the ether from one of the standard solutions), extract the aqueous solution once more with 2 to 3 ml. of ether. Discard the ether extracts.

Treat the extracted sample and standard solutions with 1.0 ml. of potassium thiocyanate and 10 ml. of stannous chloride solution. If necessary adjust the volumes to equality by adding water until the heights of the solutions in the beakers are the same. The volume should lie between 25 and 30 ml.; if it is less than 25 ml., salt is almost certain to crystallize out. After mixing, set the solutions aside at 25° to 30° C. for an hour.

Cool the solutions to 20° C. or slightly less and transfer to separatory funnels. Extract each solution with 5.0 ml. of ether by shaking for 15 seconds. Allow the phases to separate, drain off the aqueous solutions, and run the ether layers into the color comparison tubes. If desired the funnels can be rinsed with 1 ml. of ether. Compare the tubes axially against a white background in good light. Pick out the standard having a color intensity closest to the sample. By means of a glass tube remove small volumes of ether from the standard or the sample tube, as the case requires, until the two solutions show the same color intensity. Usually it is best to note the heights of the solution being withdrawn at which it is just perceptibly darker and lighter, respectively, than the solution against which comparison is made. The intermediate height is then taken as the matching height. This operation should be carried out in a cool environment (20° C.) to avoid undue loss of ether by evaporation. Find the tungsten content of the sample from the ratio of the matching height to the original height of the solution whose depth is varied. Run a blank through all steps of the procedure.

LITERATURE CITED

(1) Cunningham, T. R., private communication.
(2) Feigl, F., and Krumholz, P., *Angew. Chem.*, 45, 674 (1932).
(3) Fischer, P., Spiers, R., and Lisan, P., IND. ENG. CHEM., ANAL. ED., 16, 608 (1944).
(4) Grimaldi, F. S., and North, V., *Ibid.*, 15, 652 (1943).
(5) Hevesy, G. von, and Hobbie, R., *Z. anorg. allgem. Chem.*, 212, 134 (1933).
(6) Hoffman, J. I., and Lundell, G. E. F., *J. Research Natl. Bur. Standards*, 23, 497 (1939).
(7) Miller, C. C., *Analyst*, 69, 109 (1944).
(8) Poluektov, N. S., *Zavodskaya Lab.*, 10, 92 (1941).
(9) Sandell, E. B., IND. ENG. CHEM., ANAL. ED., 8, 336 (1936).
(10) Sandell, E. B., and Goldich, S. S., *J. Geol.*, 51, 112 (1943).
(11) Scobie, A. G., IND. ENG. CHEM., ANAL. ED., 15, 79 (1943).
(12) Straumanis, M., and Ogrims, B., *Z. anal. Chem.*, 117, 30 (1939).
(13) Tschernichow, J. A., and Karsajewskaja, M. P., *Ibid.*, 98, 97 (1934).
(14) Wilson, S. H., and Fieldes, M., *Analyst*, 69, 12 (1944).

Equations of Thixotropic Breakdown for the Rotational Viscometer

HENRY GREEN AND RUTH N. WELTMANN

Research Laboratories, Interchemical Corporation, New York, N. Y.

Fundamental principles of the thixotropic hysteresis loop and equations of thixotropic breakdown as produced by the rotational viscometer are presented. The work has practical applications in printing and in all other types of coatings where changes in consistency may affect the quality of the manufactured product. The equations developed give the relationship between consistency and rate or duration of shear applied to the material and enable calculation of plastic viscosity and yield value for any rate of shear that is desired.

IN A previous paper (*2*) on thixotropy, it was experimentally shown (with pigment-vehicle suspensions) that breakdown can be induced in two ways—the "breakdown by time" and the "breakdown by rate of shear". The first is exemplified by applying a constant rate of shear for a sufficient time. Breakdown will continue until all bonds below a certain strength are broken and equilibrium is attained. The second way is employed when further breakdown is required. In this case, it is necessary to increase the rate of shear, making more power available; then stronger bonds are broken.

In the authors' work on thixotropy, the rotational viscometer (*1*) has been used exclusively. There are several reasons for doing this: The material under test is not lost by extrusion, but remains in the viscometer cup; hence the results of increasing breakdown can be continuously recorded; the rate of shear employed can be regulated by adjusting the r.p.m.; and the Reiner and Riwlin equation (*3*) can be applied for determining plastic viscosity and yield value.

The consistency curve obtained with the rotational viscometer is composed of an upcurve and a downcurve. These curves do not coincide when the material is thixotropic, thus forming a "hysteresis" loop, used for measuring thixotropic breakdown. It is not formed when the measurements are obtained with an extrusion viscometer.

Assuming as previously (*2*), that breakdown is proportional to the top rate of shear it has been shown that

$$e^{\frac{2U}{M}} = k/\omega^2 \qquad (1)$$

where U is the plastic viscosity, ω the top angular velocity, k a constant for the upcurve of the loop, and M a coefficient of thixotropic breakdown. An analysis (*2*) of the mathematical procedure leading to M will show that it is the loss in shearing force per cm.² per unit increase in rate of shear.

Because no action can take place in zero time, it follows that if ω is raised to $\omega + d\omega$, a time dt is involved. The coefficient M, therefore, is in some way associated with time. It is important that the exact nature of this association be understood, because the investigator would like to know if M is an attribute only of the test material, or whether its value also depends on the method of carrying out the measurement.

When a constant rate of shear is applied to a thixotropic material, the plastic viscosity decreases with the logarithm of the time. This has been shown by Weltmann (*5*), and has been expressed as:

$$B = -\frac{dU}{dt} \times t \qquad (2)$$

B is a constant and is called the "time coefficient" of thixotropic breakdown. In order to show the relation between M and time, it is necessary only to combine Equations 1 and 2. Before doing this, it should be explained more fully what is meant by "time".

If an unbroken material is placed in the viscometer and a constant shearing force applied resulting from an angular velocity ω, the material will break down with a rate B/t as given in Equation 2. If the torque at point A, Figure 1, is the initial and therefore maximum torque acquired for an angular velocity ω, then after a certain time, t, point C will be reached because the material is steadily breaking down. If fresh material of the same kind as before is now used and the upcurve, starting at point B, is made, it will be found that when the time is correctly adjusted, the curve will meet point C in exactly the same time, t, which it took when starting from A. If the time of the upcurve is adjusted too short, the curve will meet some point C': if too long, some point C''. The conclusion is that the time needed to attain a certain broken down state such as exists at C is the same regardless of the path taken—i.e., from A to C or from B to C. This means that time t in Equation 2 when going

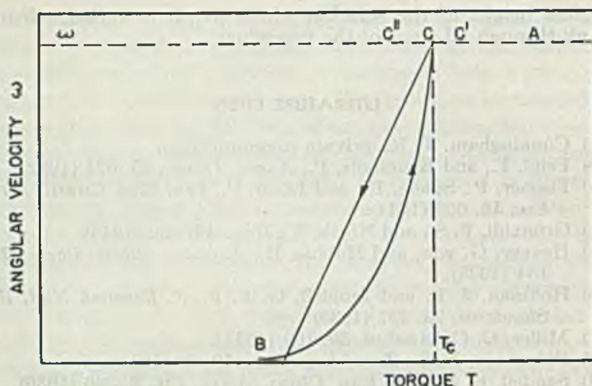


Figure 1. Schematic Flow Curve

from A to C is the same as the time of the upcurve of the hysteresis loop implied in Equation 1, when going from B to C.

In obtaining the upcurve a definite number, N, of experimental points are made. To each point is given the same amount of time (Δt). Adjacent points are separated by the same number of r.p.m. Hence $t = \Sigma(\Delta t)$ and is proportional to ω. Consequently,

$$t = R\omega \tag{3}$$

and

$$\Delta t = t/N \tag{4}$$

where R is the proportionality constant.

SYMBOLS USED IN PAPER

A. Area of hysteresis loop
A_E. Area of equilibrium hysteresis loop
B. Coefficient of thixotropic breakdown with time
C. Rotational viscometer constant for calculating f
Δt. Time element for each point on curve
f. Yield value
f', f''. Yield values at different top rates of shear
h. Depth of immersion of bob
J. Slope of the yield value curve, f vs. $(\omega - \omega_0)$
K. Proportionality constant. Independent of Δt
k. Proportionality constant. Not independent of Δt
k_0. Value of k when M_0 takes the place of M. Independent of Δt
k'. Value of k when ω_0 is finite. Not independent of Δt
k''. Proportionality constant in yield value curve, f vs. $(\omega - \omega_0)$. Independent of Δt
M. Coefficient of thixotropic breakdown for increasing ω. It contains B, which being constant permits M to be independent of time
M_0. That part of M associated only with rate of shear. Independent of Δt
N. Number of points on upcurve
ω. Angular velocity
ω_0. Angular velocity of crossing point
R. Proportionality constant between ω and t
R_b. Radius of bob
R_c. Radius of cup
RPM. Revolutions per minute at top point of curve
rpm. Revolutions per minute
$(rpm)_0$. Revolutions per minute at crossing point
S. Rotational viscometer constant for determining U
t. Total time in reaching top point of curve
t_E. Total time in reaching top point in equilibrium curve
T. Torque
T_E. Torque at top point of equilibrium curve
T_2. Torque of yield value intercept
$T_{\omega 0}$. Torque at crossing point
V. Coefficient of thixotropic breakdown when yield value intercept is not constant. Independent of Δt

INTRODUCTION OF TIME

Returning to the addition of Equations 1 and 2.

From what has been stated, it is reasonable to assume that M is composed of two factors, the one factor arising from increasing the rate of shear (expressed by an increase, $d\omega$, in the angular velocity); and the other resulting from the duration of the shearing force (equal to dt for the increase $d\omega$). Let M_0 be the part of M associated only with an increase in ω. Then making the same basic assumption as made in the previous paper, that the thixotropic loss in torque (the torque that corresponds to the loss in shearing force) is proportional to the rate of shear, it follows that

$$e^{2U_\omega/M_0} = k_0/\omega^2 \tag{5}$$

where M_0 and k_0 have values that differ from the previous M and k; and U is designated at U_ω to distinguish it from the U determined under normal conditions. From Equations 5 and 2,

$$dU_\omega = -M_0 d\ln\omega \tag{6}$$

$$dU_t = -B d\ln t \tag{7}$$

Adding gives

$$dU_\omega + dU_t = dU = -M_0 d\ln\omega - B d\ln t \tag{8}$$

where U_t is the change in U induced only by the duration of the shearing force.

Integrating gives

$$U = -M_0\ln\omega - B\ln t + \ln K^{M_0/2} \tag{9}$$

where $\ln K^{M_0/2}$ is the constant of integration. K is given the exponent $M_0/2$ so that it will be dimensionally correct. The introduction of M_0 is permissible because M_0 is independent of t and ω.

Condensing 9 gives

$$e^{2U/M_0} = K/\omega^2 t^{2B/M_0} \tag{10}$$

Our hypothesis in regard to the dual nature of M specifies that $M = M_0 + X$, where X is some unknown factor related to time. The next step is to find what X actually is. Using two points, $(U_1\omega_1)$ and $(U_2\omega_2)$, it can be shown with the aid of Equation 10 that

$$M_0 = 2(U_1 - U_2)/\ln(\omega_2^2/\omega_1^2) - B[\ln(t_2^2/t_1^2)/\ln(\omega_2^2/\omega_1^2)] \tag{11}$$

It has been previously established (2) that

$$2(U_1 - U_2)/\ln(\omega_2^2/\omega_1^2) = M \tag{12}$$

Substituting 12 in 11 and making $t = R\omega$ (see Equation 3) gives

$$M = M_0 + B \tag{13}$$

The unknown quantity, X, is therefore B, the time coefficient of thixotropic breakdown.

Substituting Equation 13 in Equation 10 gives

$$e^{2U/(M-B)} = K/\omega^2 t^{2B/(M-B)} \tag{14}$$

and

$$e^{2U/M} = K^{(M-B)/M}/\omega^{2(M-B)/M} t^{2B/M} \tag{15}$$

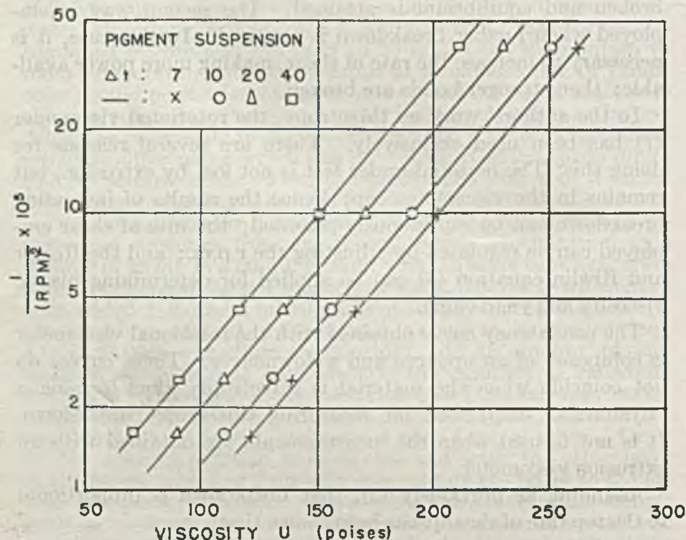


Figure 2. Experimental Data of ln($1/RPM^2$) versus U Obtained with Different Timed Flow Upcurves for One Pigment Suspension

EXPERIMENTAL PROOF OF INDEPENDENCE OF M AND (Δt)

From Equation 1 it is evident that when U is plotted against $\ln(1/\omega^2)$, a straight line results with a slope equal to $2/M$. A change in (Δt) moves the straight line parallel with itself, thus maintaining the same slope. This means that M does not vary and is independent of (Δt). The experimental evidence of this has been obtained for various pigment suspensions. The curves for one of these suspensions is shown in Figure 2.

EFFECT OF TIME ON UPCURVE AND ON AREA EQUATIONS

Plastic viscosity is

$$U = (T - T_2)S/\omega \quad (16)$$

Substituting 16 in 15 gives the equation of the upcurve:

$$T = \frac{(M-B)\omega}{2S}\ln K - \frac{(M-B)\omega}{S}\ln\omega - \frac{B\omega}{S}\ln t + T_2 \quad (17)$$

It has been shown (2) that the area of the hysteresis loop is

$$A = M\omega^2/4S \quad (18)$$

Since M is independent of (Δt), the area is not affected by any change in (Δt). This is not true of the equilibrium hysteresis loop. The equilibrium loop is made by allowing the top shearing force to act until equilibrium is attained. The downcurve is then made. Referring to Figure 3, the normal loop is $ADBA$; the equilibrium loop is $ADBCA$. Its area will be designated as A_E and the equilibrium time as t_E. The time of the upcurve, ADB, is t.

The area of the normal loop, as before, is A. Then

$$A_E = A + (T - T_E)\omega/2 \quad (19)$$

Also

$$T_E = \frac{(M-B)\omega}{2S}\ln K - \frac{(M-B)\omega}{S}\ln\omega - \frac{B\omega}{S}\ln t_E + T_2 \quad (20)$$

Subtracting Equation 20 from Equation 17 gives

$$T - T_E = \frac{B\,\omega}{S}\ln(t_E/t) \quad (21)$$

Substituting Equations 18 and 21 in Equation 19 gives

$$A_E = \frac{M\omega^2}{4S}\left(1 + \frac{2B}{M}\ln(t_E/t)\right) \quad (22)$$

Since ω, M, S, B, and t_E are constants, Equation 22 is of the form

$$A_E = a - b\ln t \quad (23)$$

Equation 23 gives a linear curve with a negative slope when A_E is plotted against $\ln t$.

In the authors' initial work on the loop area, it was impossible to determine from experimental data whether the abscissa should be t or $\ln t$. Both forms seemed to give a linear relationship with

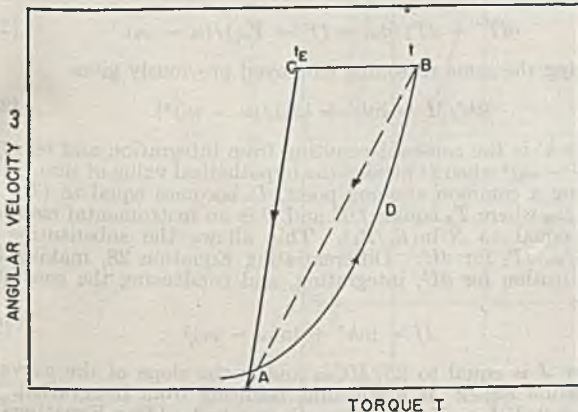


Figure 3. Schematic Equilibrium Flow Curve

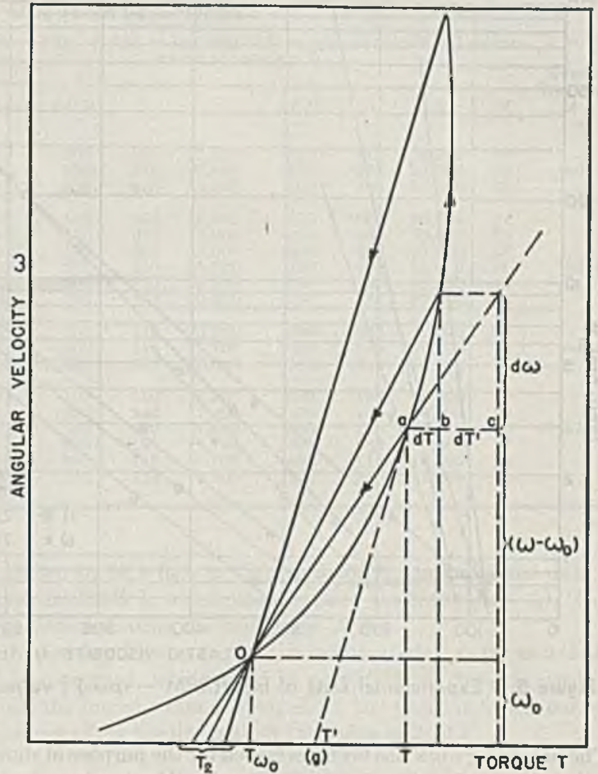


Figure 4. Schematic Flow Curve Demonstrating a Common Crossing Point at ω_0 for Different Downcurves

the loop areas. The shape of the curve was not noticeably affected by shifting from t to $\ln t$, so no decision could be made as to which expression was correct. Equation 22 was not then available as a guide; so the simpler of the two forms was selected and the abscissa was plotted as t. The experimental difficulties in obtaining a large series of equilibrium loops on a single thixotropic material are not small; consequently, the experimental points deviate somewhat from their linear position, making a double decision possible. In the original paper (2) the laboratory measurements indicated that curve A, Figure 4, made a small angle with the horizontal. Since t does not affect the area of the normal loop, it is evident that this small angle should have been zero. Subsequent data have shown that this small angle is within the limit of experimental error.

DIFFERENCE BETWEEN k AND K

When a thixotropic material breaks down, it does so in accordance with Equations 1 and 15.

Let it be assumed that breakdown can be continued until U actually becomes equal to zero. It can then be seen that k and K are equal to the squares of the theoretically highest angular velocities. These two constants do not behave alike. In Equation 1, a change in (Δt) cannot affect M or B. Such a change, therefore, must alter the value of k. This it does, as can be seen from the different intercepts of the series of curves given in Figure 2. In Equation 15, t is included; so any variation in (Δt) affects t and thus allows K to remain constant.

A direct comparison of k and K can be made. Substituting $t = R\omega$ in Equation 14; then

$$e^{2U/(M-B)} = K/(R\omega)^{2B/(M-B)}\omega^2 \quad (24)$$

and

$$e^{2U/M} = K^{(M-B)/M}/R^{2B/M}\omega^2 \quad (25)$$

Comparing Equation 25 with Equation 1 shows that

$$k = K^{(M-B)/M}/R^{2B/M} \quad (26)$$

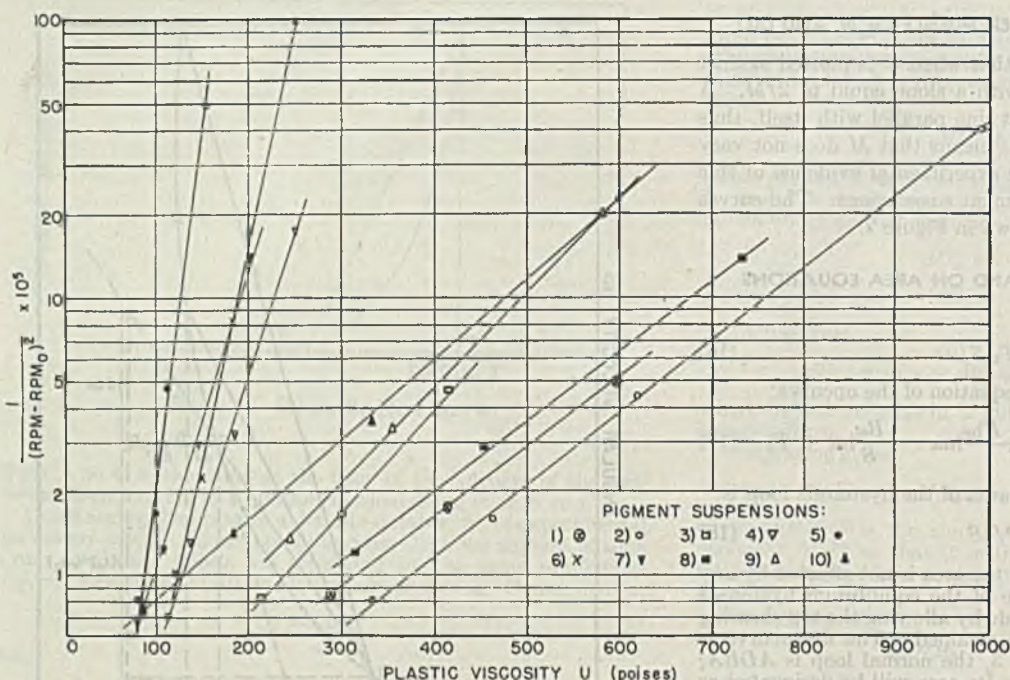


Figure 5. Experimental Data of $\ln[1/(RPM - rpm_0)^2]$ versus U for Various Pigment Suspensions

The preceding work has been developed for the purpose of showing that M and B define that property of a thixotropic material that regulates its method and extent of breakdown. Neither M nor B is a "coefficient of thixotropy", for that term refers to a property that cannot be expressed dimensionally. It falls in the same category with concepts like "toughness", "brittleness", and "hardness". On the other hand, thixotropic breakdown is a physical reaction subject to measurement. M gives the drop in plastic viscosity resulting from a unit increase in rate of shear. M has been shown in this paper to be independent of the speed at which the upcurve is made, and therefore belongs entirely to the material. It does not depend on the dimensions of the viscometer or on the method of measurement being applied. B, likewise, has been shown to be independent of the instrument and of time.

CHANGE IN YIELD VALUE INTERCEPT WITH THIXOTROPIC BREAKDOWN

In the authors' previous paper on thixotropy, the decrease in plastic viscosity resulting from thixotropic breakdown was described. No mention was made at that time of any corresponding change in the yield value intercept because such an effect was considered comparatively negligible in the materials chosen for investigation. Subsequently, a change in the intercept was described briefly by Weltmann (4), and reference made to this paper for further information. This aspect of thixotropic breakdown will now be discussed.

Since structure produces yield value, it would be expected that breakdown would decrease the size of the yield value intercept. This does not seem to happen. The size of the intercept either remains practically unaltered or else actually increases. Stirring, however, does break down the initial static yield value, but this takes place so rapidly that no record of the breakdown is shown when the viscometer is hand-operated. The yield value actually appearing in the consistency curve is a dynamically determined one. The dynamic yield value is near the point where the velocity of structural breakdown is equal to the velocity of buildup. The static yield value is that which exists before the material has been touched by any stirring device. Obviously, it is greater than the dynamic yield value.

It has been found that for heavy pigment-vehicle suspensions, breakdown with time does not materially alter the yield value intercept (5). On the other hand, breakdown by rate of shear substantially increases it. In preventing a broken down thixotropic structure from reforming, there will be used a certain portion of the energy input for that purpose. This energy ($2\pi \times$ torque), not producing flow, must appear as an intercept on the torque axis. If breakdown is then increased by raising the power, a still larger amount of energy is required to maintain the new state, and, consequently, there will be a corresponding increase in the yield value intercept. Conditions are different when additional breakdown results from prolonging the application of a constant rate of shear. In this case there is no power increase and consequently there is no extension of the yield value intercept. Also, there is no decrease in the intercept because it evidently takes as much energy to maintain a given state of breakdown as it does to produce it.

DERIVATION OF COEFFICIENT OF THIXOTROPIC BREAKDOWN, V

Previously, equations were derived for the upcurve and for the loop area where the intercept was not affected by breakdown. Those equations were derived on the assumption that loss in torque is proportional to the increase in rate of shear. In this paper equations are developed where the intercept increases with thixotropic breakdown. In order to derive these new equations certain additional assumptions must now be made. They are: all downcurves intersect at a common point O, Figure 4. The upcurve also passes through this same point. Neither of these assumptions is strictly true; nevertheless, their adoption does not introduce serious errors in the final equations, as is shown below (in table, note the constancy of V).

The differential equation is set up as before, except that $(\omega - \omega_0)$ is substituted for ω (see Figure 4). The assumption is again made that the loss in torque (dT'), due to breakdown, is proportional to the velocity gradient at radius R. The instrumental constant, S, is equal to $(1/R_b^2 - 1/R_c^2)/4\pi h$, where h is the immersed height of the bob; R_b is the radius of the bob, and R_c the radius of the cup.

From similar triangles, Figure 4, it follows that

$$(dT' + dT)/d\omega = (T - T_{\omega_0})/(\omega - \omega_0) \tag{27}$$

Using the same reasoning employed previously gives

$$2U/M = \ln k' + \ln[1/(\omega - \omega_0)^2] \tag{28}$$

where k' is the constant resulting from integration and is equal to $(\omega - \omega_0)^2$ when U attains the hypothetical value of zero. Assuming a common crossing point, U becomes equal to $(T_{\omega_0} - T_2)S/\omega_0$ where T_2 equals f/C and C is an instrumental constant and equal to $S/\ln(R_c/R_b)$. This allows the substitution of $-(S/\omega_0)dT_2$ for dU. Differentiating Equation 28, making the substitution for dU, integrating, and condensing the constants gives

$$Jf = \ln k'' + \ln(\omega - \omega_0)^2 \tag{29}$$

where J is equal to $2S/MC\omega_0$ and is the slope of the curve in Equation 32: k'' is a constant resulting from integration. If we let $2/MJ = V$, by differentiating and adding Equations 28 and 29, we get

$$V = -df/dU \tag{30}$$

or

$$V = (f_2 - f_1)/(U_1 - U_2) \tag{31}$$

V is the coefficient of thixotropic breakdown when the intercept increases with decreasing U. It is the increase in yield value per unit decrease in plastic viscosity. Its dimension is sec^{-1}.

The following equations also can be derived by the process similar to the one given in the previous work (*2*).

$$T = \frac{M(\omega - \omega_0)}{2S} \ln[k'/(\omega - \omega_0)^2] + T_{\omega_0} \tag{32}$$

$$A = M(\omega - \omega_0)^2/4S \tag{33}$$

Equations 28 and 32 can be combined with time in the same manner employed for Equations 15 and 17. Then k' is a function of time as k was shown to be in Equation 26.

It has been found experimentally that f is not affected by time when the top shearing rate or RPM is constant. Therefore, if f is plotted against t for different constant top RPM, a series of straight lines parallel to the t axis will result. This means that $f_1' - f_2' = 0$, $f_1'' - f_2'' = 0$, etc. Plotting f *vs.* $\ln(RPM - rpm_0)^2$ or against $\ln(\omega - \omega_0)^2$ for both t_1 and t_2 must give two linear coincident curves. These curves can be seen to be linear from Equation 32. They will be coincident because of the relation, $f_1' - f_2' = 0$, etc. The slope of the curve for t_1, then, will be the same as the slope of the curve for t_2, thus proving J and k'' to be independent of time. Because J and M are both independent of (Δt), V which is equal to $\frac{2}{MJ}$ is also independent of time.

Table I. Data Taken from Figures 5 and 6 to Show Constancy of V

$V = (f_2 - f_1)/(U_1 - U_2)$. RPM = top *rpm*. U = plastic viscosity (poises). f = yield value (dynes/cm.²)

Pigment Suspension	rpm_0	RPM_1	U_1	f_1	RPM_2	U_2	f_2	V	Average Deviation of V %
1	60	200	583	17,400	300	415	24,000	39	
1	60	200	583	17,400	400	298	28,500	39	±0.9
1	60	300	415	24,000	400	298	28,500	38	
2	50	100	990	12,000	200	620	25,700	37	
2	50	100	990	12,000	300	465	32,000	38	
2	50	100	990	12,000	400	335	37,000	38	
2	50	200	620	25,700	300	465	32,000	41	±3.6
2	50	200	620	25,700	400	335	37,000	40	
2	50	300	465	32,000	400	335	37,000	38	
3	55	200	416	23,700	300	302	27,000	29	
3	55	200	416	23,700	400	215	29,400	28	±1.2
3	55	300	302	27,000	500	215	29,400	28	
4	25	100	250	7,400	200	186	8,700	20	
4	25	100	250	7,400	300	139	9,500	19	
4	25	100	250	7,400	400	115	10,000	19	±5.3
4	25	200	186	8,700	300	139	9,500	17	
4	25	200	186	8,700	400	115	10,000	18	
4	25	300	139	9,500	400	115	1,000	21	

EXPERIMENTAL RESULTS

The object of the following measurements is to show that Equations 28 and 29, and consequently Equation 31, are substantially in agreement with experiment, and therefore the assumptions previously made in regard to the crossing point are not too far out of line to be of practical importance.

From Equations 28 and 29, it is evident that U *vs.* $\ln[1/(\omega - \omega_0)^2]$ and f *vs.* $\ln(\omega - \omega_0)^2$ should be both linear relationships. This is shown to be a fact in Figures 5 and 6. Some of the data are given in Table I, which also contains the ratio $(f_2 - f_1)/(U_1 - U_2)$, demonstrating the constancy of V.

In plotting Figures 5 and 6, it is immaterial, as far as $2/MJ$ or V is concerned, whether RPM or $(RPM - rpm_0)$ is used, for while the introduction of rpm_0 shifts the position of the curve, it does not affect the linearity or the value of $2/MJ$.

CONCLUSION

This paper, together with the previous work of the series (*2*), presents the fundamental principles of the thixotropic hysteresis loop, and the equations of thixotropic breakdown as produced by the rotational viscometer. It is hoped that this work will be found sufficiently complete to be useful to investigators who wish to study and measure thixotropic systems.

The practical aspect of the work depends upon the fact that industries that use materials of the pigment-vehicle type are often confronted with the problem of consistency changes arising from

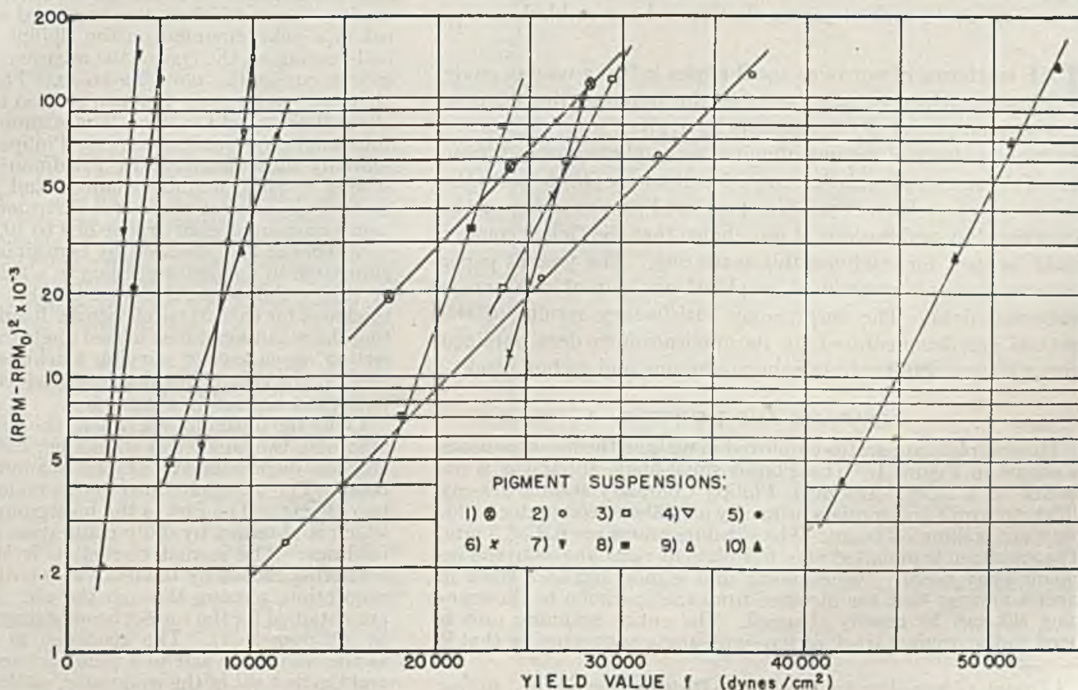


Figure 6. Experimental Data of $\ln(RPM - rpm_0)^2$ versus f for Various Pigment Suspensions

thixotropic breakdown. The plastic viscosity and yield value have one set of measurements when the substance is at rest; but these measurements change when the material is forced to flow. The magnitude of these new values will depend upon the applied rate of shear; and consequently can assume any of an infinite number of such values.

If this change to a new and unknown consistency affects the quality of the manufactured product, it is desirable to know beforehand how far thixotropic breakdown will go. If it continues too far, adjustments must be made in the original consistency, in the rate, or in the duration of shear applied to the material. The equations developed in this paper give the relationship between these factors and express them in the form of coefficients M, B, and V. They enable the investigator to calculate U and f for any rate of shear desired.

A well-known case where consistency changes can cause trouble is in the trapping of process printing inks. When printing is carried out at high rates of shear thixotropic changes can produce a reversal in the plastic viscosities of the inks. This in turn can cause a reversal in the order of trapping, so that a second-down ink, for instance, which should trap the third-down ink, is, instead, trapped by the third-down ink and pulled off the paper. Other difficulties can arise in the printing industry from thixotropic breakdown. An ink must not be too stiff to "follow the fountain". Thinning an ink down so that it can follow the fountain might result in trouble, if subsequent thixotropic breakdown reduced the plastic viscosity below that required for correct trapping. A better method might be to use an ink of suitable M, B, and f values.

Not only in printing but in any type of coating work—whether the process be carried out by means of a paint brush, a spray gun, or a roller coating machine—problems are found involving thixotropic changes. These problems might not be recognized as such, but that is because general information on the subject is still very scanty. So far industry has made little commercial use of thixotropy; however, it is highly desirable that the industrial rheologist be thoroughly acquainted with thixotropic reactions, so that he understands the effect thixotropic breakdown has on consistency measurements. Without that understanding the rheology of plastic systems can become a confusing and contradictory subject. The equations given here should help the investigator meet that situation.

ACKNOWLEDGMENT

The authors are indebted to Interchemical Corporation for permission to publish this paper and to Miss Evelyn Berezin for assistance in carrying out the laboratory work.

LITERATURE CITED

(1) Green, H., Ind. Eng. Chem., Anal. Ed., 13, 632 (1941).
(2) Green, H., and Weltmann, R. N., *Ibid.*, 15, 201 (1943).
(3) Reiner, M., and Riwlin, R., *Kolloid Z.*, 43, 1 (1927); *J. Rheol.*, 1, 5 (1929).
(4) Weltmann, R. N., Ind. Eng. Chem., Anal. Ed., 15, 424 (1943).
(5) Weltmann, R. N., *J. Applied Phys.*, 14, 343 (1943).

Measurement and Analysis of Small-Angle X-Ray Scattering

M. H. JELLINEK[1], The Polytechnic Institute of Brooklyn, Brooklyn, N. Y., ERNEST SOLOMON, Petroleum Research Division, The M. W. Kellogg Company, Jersey City, N. J., AND I. FANKUCHEN, The Polytechnic Institute of Brooklyn, Brooklyn, N. Y.

Geiger counter methods can be used effectively for the recording of small-angle x-ray scattering. A graphical method of analyzing the data is presented and the results of such an analysis are given for one specimen each of gamma-alumina and carbon black.

THE scattering of x-rays at small angles is being used to study inhomogeneities in substances in the range of 10 to 200 Å. In a previous paper (*9*) the authors have given some qualitative results of a study of gamma-alumina, using photographic film to record the scattered radiation and a densitometer (*12*) to make the measurements. In a paper (*10*) presented at a meeting of the American Physical Society, it was shown that the Geiger counter could be used for studying this scattering. The present paper presents a simple method of graphical analysis of small-angle scattering data. The surprisingly satisfactory results of the method are demonstrated by its application to data obtained with a Geiger counter from gamma-alumina and carbon black.

APPARATUS AND TECHNIQUE

The complete apparatus employed in making the measurements is shown in Figure 1. The Philips small-angle apparatus is attached to a North American Philips Company standard x-ray diffraction unit and consists primarily of a 30-cm. collimator yielding a slit collimated beam. The slit dimensions are 0.3 × 3 mm. The specimen is mounted on a flat plate at right angles to the incident x-ray beam. The scanning unit is mounted on a track in such a manner that the distance from the specimen to the scanning slit can be readily changed. The entire scanning unit is fixed to the original track of the small-angle apparatus, so that it can be rotated about the line where the incident beam and the specimen intersect. The scattered radiation is scanned by turning a micrometer screw placed 60 cm. from the specimen, causing the scanning unit to rotate about the above axis. The movable track is suspended from two hardened steel pins that bear on V's cut in a yoke mounted on the Philips standard fixed track. A ball-bearing on the end of the micrometer screw rides along a V-groove cut in the movable track. The displacements are read on a scale and vernier that can be read to 0.05 mm., equivalent to about 0.35 minute of arc. The scanning unit contains a Geiger tube made by North American Philips and the circuits are essentially those described by Friedman (*7*). The combination of scaling circuits, impulse counter, and microammeter, and integrating circuits for the latter instrument easily allow measurements over an intensity range of 1 to 10,000.

Specimens are prepared by containing the material under examination in a circular opening in a thin metal sheet by the use of thin cellulose acetate membranes. The optimum specimen thickness for each type of sample handled is determined by setting the scanning unit at a fixed angle from the main beam and inserting specimens of varying thickness in the beam. By this method the specimen thickness producing the most intense scattering can be readily obtained.

Using the optimum specimen, the measurements are made by obtaining the number of counts per unit time interval at various angular displacements of the scanning unit from the main beam. The value obtained in this manner requires correction for two effects. The first is the background count of the apparatus which is obtained by daily calibration and is subtracted from all readings. The second correction is the subtraction of the air scattering caused by the main beam, diminished by the specimen absorption, passing through the air. The air scattering values are obtained for the correct beam strength by a method suggested by Campbell (*3*). The specimen is moved from its position at the collimator exit to a point between the x-ray tube window and the first slit of the collimator, so that all radiation, other than that scattered by the specimen at essentially zero angle is elimi-

[1] Present address, Petroleum Research Division, The M. W. Kellogg Co., Jersey City, N. J.

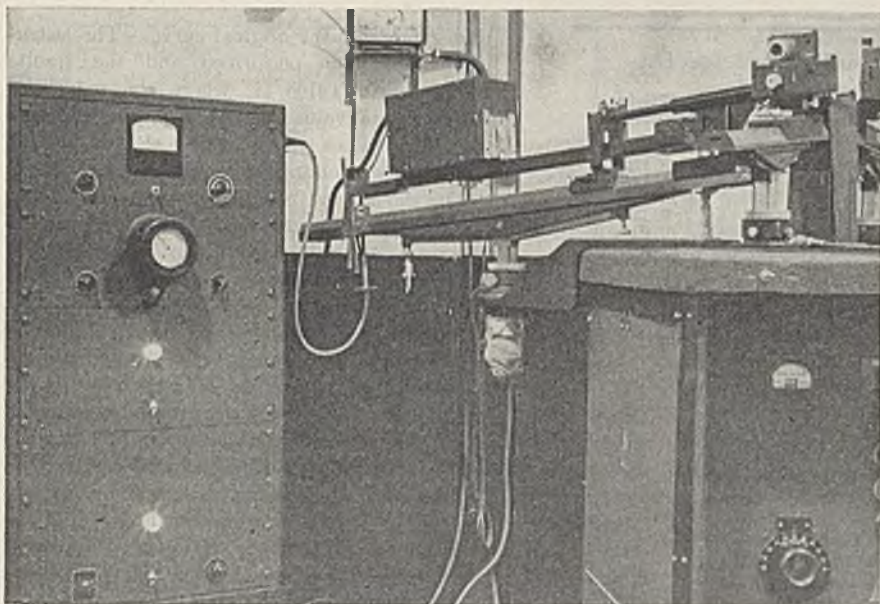


Figure 1. Diffraction Unit, Small-Angle Scatter Apparatus, Scanning Unit, and Geiger Counter

nated. Thus the air scattering can be measured directly and allowed for without the added complication of the small-angle scattering of the specimen.

THEORY

It has been shown (*2, 8, 11*) that the scattering of x-rays of wave length λ by a homogeneous assemblage of spherical particles of radius R may be given by the equation,

$$I(\theta) = CWR^3 \exp(-0.221\, k^2R^2) \quad (1)$$

where $I(\theta)$ is the intensity of scattering as a function of θ, θ is half the scattering angle, k is $(4\pi \sin \theta)/\lambda$, W is the weight of specimen doing the scattering, and C is a constant for a given material. If there is a distribution of particle sizes given by $W(R)$, where $W(R)$ is the weight fraction of radius R, it can be shown that the curve of scattered radiation is then given by

$$I(\theta) = C \int_0^{\infty} W(R)R^3 \exp(-0.221\, k^2R^2)dR \quad (2)$$

For the homogeneous case, Biscoe and Warren (*2*) suggest plotting log. $I(\theta)$ against k^2. (Equally satisfactory is a plot against $\sin^2 \theta$, $\tan^2 \theta$, or θ^2.) The more convenient plot (*9*) is one of $\log_{10} I(\theta)$ against r^2, where r is the distance in millimeters of the scanning slit from the center of the direct beam. When photographic recording was used r was a distance measured on the film. This logarithmic plot of Equation 1 yields a straight line whose slope is a single-valued function of R and whose intercept depends, among other things, on W and R^3.

When the assemblage contains a distribution of particle sizes, the logarithmic plot is no longer a straight line. Equation 2, in which $I(\theta)$ is experimentally determined and $W(R)$ is the desired function, suggests the use of Fourier transforms, and undoubtedly $W(R)$ can be calculated as the Fourier transform of some amenable analytical expression (or sum of expressions) for $I(\theta)$. In a recent note, Bauer (*1*) has discussed such an inversion, giving a method for transforming the observed small-angle scattering into a continuous distribution function. However, this method is somewhat involved and in many cases the data may not justify so detailed a treatment. The authors have been using a simple graphical method of approximating $W(R)$, not as a continuous function but rather as a set of discrete fractions. While such an interpretation and treatment of the scattering data are a distinct compromise, the results are, by empirical test, apparently useful and therefore this graphical method is here discussed.

EXPERIMENTAL RESULTS

As an example of this graphical method of analysis, the small-angle scattering from a heat-treated alumina gel was measured. Figure 2 is a plot of $\log_{10} I$ against r^2 in (mm.)^2 obtained for this alumina using filtered copper radiation of $\lambda = 1.54$ Å. and a specimen-to-slit distance of 453 mm. Figure 2 also illustrates the method of graphical reduction.

A tangent to the experimental curve is drawn at the greatest angle of scattering studied. This tangent intersects the axis of ordinates at a value K_1. The values corresponding to this tangent are then subtracted from the original curve and a new corrected curve not containing the contribution of this fraction is obtained (shown in Figure 2 by the dashed line). In a similar manner the next tangent of minimum slope is drawn to the new curve with its intercept K_2. The procedure is repeated until the final points yield a straight line of intercept K_6. In this way, there are obtained six lines, with successively larger slopes, and inter-

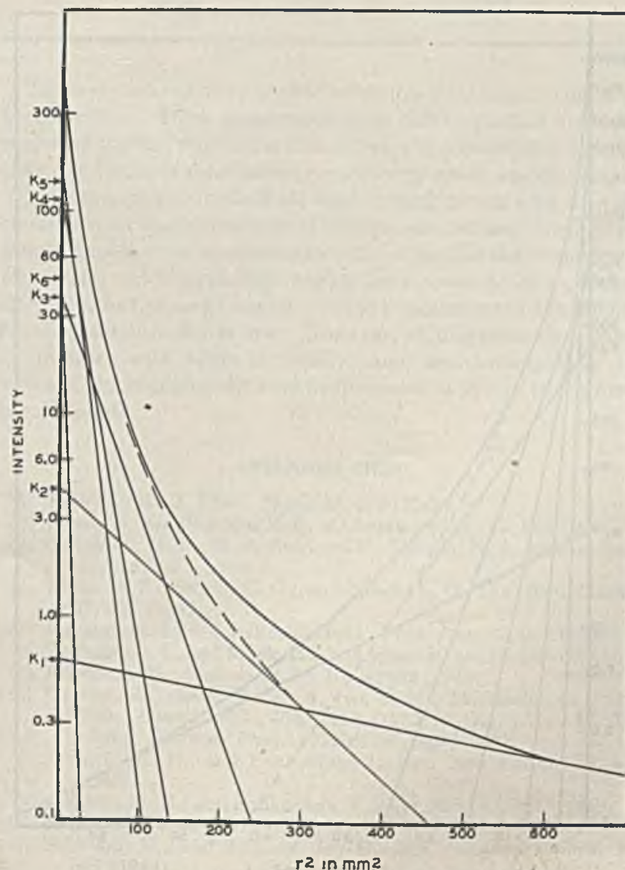


Figure 2. Plot of $\log_{10} I(\theta)$ against r^2 for Alumina Gel

Illustrating graphical breakdown into fractions

Table I. Calculation of Particle Sizes from Small-Angle Scatter Data

Ordinate Intercept		Calculation of Slope $\Delta \log I$	Δr^2	$\Delta \log I/\Delta r^2$	R	R^3	$K/R^3 \times 10^4$	Weight Proportion
K_1	0.61	0.484	630	0.000769	10	1,000	6.10	0.194
K_2	4.3	1.633	457	0.00358	22	10,600	4.06	0.129
K_3	39	2.591	240	0.0108	37	50,600	7.71	0.245
K_4	115	3.061	140	0.0219	54	157,000	7.32	0.232
K_5	140	3.146	107	0.0294	62	238,000	5.88	0.186
K_6	48	2.681	33	0.0812	103	1,092,000	0.44	0.014
						Total	31.51	1.000

Calculated surface = 350 sq. meters per gram.

cepts $K_1 \ldots K_6$. The slopes of these lines are calculated, the corresponding radii are read from an appropriate graph of slope against R on log-log paper (see *9*, Figure 3), and the weight fractions of particles of each of the radii can then be calculated. The complete calculation is outlined in Table I. This tabulation indicates the values of the intercept K's which have been shown to be proportional to $R^3W(R)$, the calculation of the slopes of the various lines, the corresponding radii, and the cubes of these values. When each K is divided by its appropriate R^3 the resulting values are proportional to the weight fraction of that size particle.

From the weights of the fractions one can compute the surface area of the specimen material. Calculations using an average particle size have been made by Elkin, Schull, and Roess (*4*). In their own studies the authors have found that for gel-based catalysts of various activities an acceptable correlation can be obtained between the surface area calculated from these fractions and catalytic activity.

It is of some interest to demonstrate how closely the graphical reduction of the experimental curve into a sum of a set of straight lines represents the original curve. This calculation has been performed and the results are given in Table II, where the values of I for certain values of r^2 are presented along with the values at the same r^2 obtained by analytically summing the straight lines.

The deviations from the experimental values are seen to be largest at the highest intensities where the curve is rising almost vertically, and hence represent the values most difficult to read from the graph.

Another application of this graphical method can be seen in the analysis of a scatter curve obtained from a sample of carbon black obtained from J. A. Hillier of the RCA Research Laboratories and thought to consist of nearly spherical uniform particles on the basis of many electron microscope pictures. Figure 3 shows the scatter curve (run at a specimen-slit distance of 235 mm.) obtained from this material. It is clear from a comparison of the shapes of the curves shown in Figures 2 and 3 that the carbon must consist of larger particles than the alumina gel. The results of the graphical analysis bear this out (Table III).

The calculated surface area of this material is 280 square meters per gram and that determined by nitrogen absorption is 450 square meters per gram. This may be compared with the surface area of the alumina gel which was calculated to be 350 square meters per gram. The actual measurement of the small-angle scatter yields a curve of scattered intensities as a function of θ and it is this curve that we analyze and convert into a particle distribution. The result can be very misleading if the total observable scattering should arise from only a small proportion of the sample. Thus, if the samples used in these analyses were heat-treated more severely, the particle distribution would shift as larger sized particles were formed. When most of these particles reached a radius of well over 100 Å. the largest part of the scattering would be very close to the main beam, but a minor amount of scatter due to the smaller particles would occur at reasonable angles and might conceivably be analyzed as due to the entire sample. This can easily be seen in Table IV, taken from a paper by Fankuchen and Mark (*6*), which shows the angles at which the scattering falls to half its value at $\theta = 0$ for various values of R.

The only evidence of this effect would be a lesser intensity at the larger angles and sometimes an increase at the lowest scattering angles reached by the authors' experimental technique. This effect has been observed (*9*) by the authors on gamma-alumina. There is naturally no foolproof guide to these effects other than experience and knowledge of the past history of the sample. Certain of these effects can even be duplicated by variations in specimen thickness and intensity of the original beam. Conse-

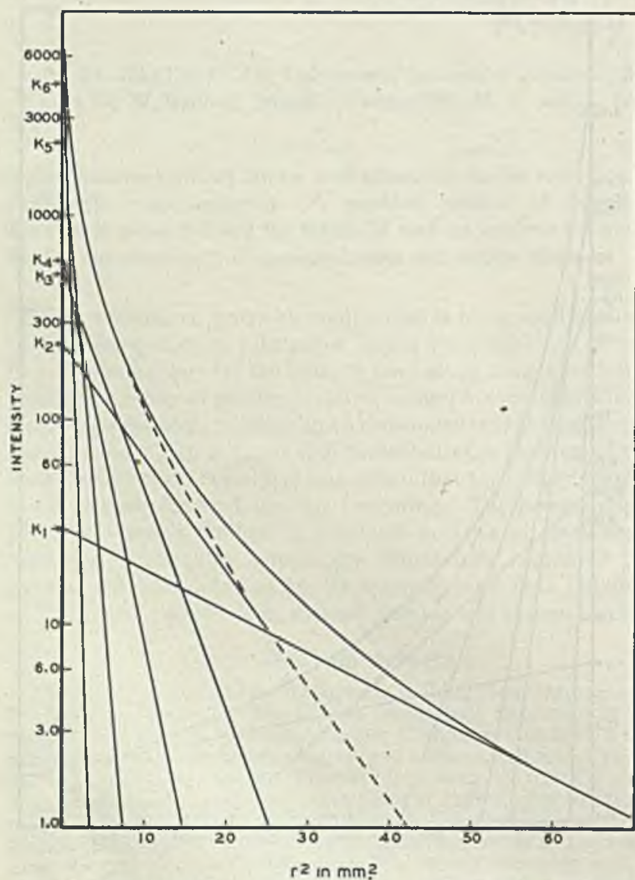


Figure 3. Plot of $\log_{10} I(\theta)$ against r^2 for Carbon Black

Illustrating graphical breakdown into fractions

Table II. Comparison of Experimental and Calculated Small-Angle Curves

r^2	I (Experimental)	I (Calculated)
5	280	245
25	80	83
50	26.5	28.8
100	6.4	6.7
150	2.75	2.71
200	1.60	1.54
300	0.74	0.74
400	0.44	0.46

Table III. Weight Distribution of Particles in Carbon Black

Radius, Å.	Weight %
29	14.7
46	29.4
63	25.7
85	12.2
130	12.1
210	5.9

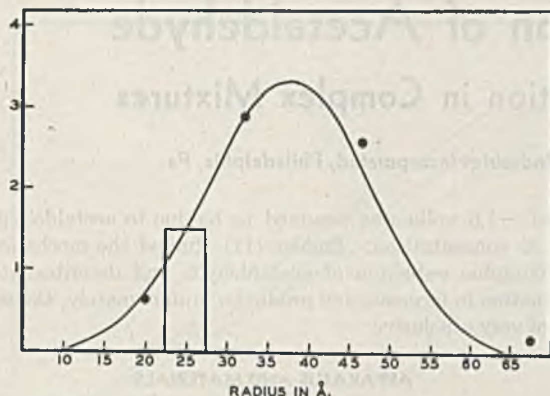


Figure 4. Assumed Distribution Curve Used in Calculation of Synthetic Small-Angle Scatter Plot

Points spotted are results of graphical analysis

quently, this graphical method of determining particle size distribution from small-angle scatter may be a useful tool when the limitations and range of failure of the method are recognized.

Having used the graphical method to evaluate two experimental curves, it may be informative to utilize the method on a synthetic curve obtained from an assumed particle distribution. This has been carried out in the following manner.

Figure 4 shows an assumed continuous distribution curve with the area under the curve equal to 100% of the material. The curve was then approximated by a series of twelve fractions taken at 5 Å. intervals from 10 to 65 Å.—that is, for the continuous curve there was substituted a series of rectangles of width 5 Å. and height such that the area of each rectangle equaled the area under the corresponding part of the continuous curve. Thus the rectangular block in Figure 4 would represent the 25 Å. particles, and would include the range from 22.5 to 27.5 Å. From these relative weights and the corresponding radii, the slope and a number proportional to the intercept were obtained. These data were then used to draw a series of straight lines of correct slope and ordinate intercept such that their sum constituted a curved line which represented the small-angle scatter of a sample containing such an original particle distribution. This curve was broken down in turn by the usual graphical method and yielded the results given in Table V.

Table IV

Radius, Å.	Angle 2 θ at Which *I* Has Fallen to Half of Initial Value, Min.
25	63.0
50	31.5
100	15.8
200	7.8
400	3.9

When these data are spotted in Figure 4, multiplied by a constant to place them in the same range as the original distribution curve, it can be seen that the breakdown of a continuous curve can yield, via this method of discontinuous evaluation, a fair approximation of the original sample. Further refinements of this method have been attempted and evaluated by drawing a continuous cumulative percentage curve and then differentiating at as many points as desirable to yield a continuous distribution function. No improvement is noted, because of the differences possible in the exact location of the cumulative curve and the arbitrary manner in which the inflection point must be chosen. Thus, we conclude that the "shot distribution" is as useful a set of values as any other that may be obtained by the graphical breakdown.

Some of the difficulties can probably be overcome by carrying out the measurements under standardized conditions such that intensities from different specimens of similarly constituted materials can be compared with one another. This will require careful control of the constancy of the x-ray beam intensity and uniformity in specimen preparation. It would then be meaningful to compare intensities from different specimens and thus by suitable calibration tell what proportion of the specimen is observable by the small-angle technique used.

CONCLUSION

At this stage of development it would be premature to take too seriously these computations of particle size distribution. However, whenever relative surfaces have been calculated based on these studies and when comparisons between these surfaces and properties based on them, such as catalytic activity, are made there has always been a reasonably good correlation.

Undoubtedly the small-angle scatter reflects an inhomogeneity in the specimen. This inhomogeneity is of the same order of magnitude as what we here like to call particle size. Perhaps in many cases, further study may show that particles in the sense in which we now think of them do not exist; the small-angle scattering merely reflecting this inhomogeneity in structure which is responsible in large part for the physical behavior of the specimen. Should this possibility be shown to exist, there nevertheless may still be a justification for continuing to talk of particles—the justification being that it works.

Table V. Analysis of Synthetic Scattering Curve

Radius, Å.	Weight %
20	10
32	46.5
46.5	41.0
67.5	2.5

There seem to be considerable possibilities for improvement experimentally. Thus, measurements at the very small and large angles at present offer some difficulties, but the authors feel that they can be made satisfactorily. At very small angles (even to 0°) the two-crystal method (*5*) used in conjunction with a Geiger counter with the sensitivity of the equipment described above should enable these measurements to be carried down to almost any angle. At intermediate angles there seems to be no serious difficulty, but at large angles reliable measurements are difficult because the intensity is low. However, at this point the change in intensity with angle is usually small and consequently the width of the scanning slit may be increased to yield a larger number of counts.

LITERATURE CITED

(1) Bauer, S. H., *J. Chem. Phys.*, 13, 450 (1945).
(2) Biscoe, J., and Warren, B. E., *J. Applied Phys.*, 13, 364 (1942).
(3) Campbell, H. N., U. S. Rubber Co., Passaic, N. J., private communication.
(4) Elkin, P. B., Schull, C. G., and Roess, L. C., Ind. Eng. Chem., 37, 327 (1945).
(5) Fankuchen, I., and Jellinek, M. H., *Phys. Rev.*, 67, 201 (1945).
(6) Fankuchen, I., and Mark, H., *J. Applied Phys.*, 15, 364 (1944).
(7) Friedman, H., *Electronics*, 18, No. 4, 132 (1945).
(8) Guinier, A., thesis (Series A, No. 1854), Université de Paris, 1939; *Compt. rend.*, 204, 1115 (1937); *Ann. phys.*, 12, 161 (1939); *J. Chem. Phys.*, 40, No. 7–8, 133 (1943).
(9) Jellinek, M. H., and Fankuchen, I., Ind. Eng. Chem., 37, 158 (1945).
(10) Jellinek, M. H., and Fankuchen, I., *Phys. Rev.*, 67, 201 (1945).
(11) Kratky, O., Sekora, A., and Treer, R., *Z. Elekt.*, 48, 587 (1942).
(12) Solomon, E., and Jellinek, M. H., *Rev. Sci. Instruments*, 15, 348 (1944).

Taken in part from a thesis submitted by M. H. Jellinek in partial fulfillment of the requirements for the degree of doctor of philosophy in chemistry at The Polytechnic Institute.

Polarographic Determination of Acetaldehyde

Application to Routine Determination in Complex Mixtures

PHILIP J. ELVING AND EMILE RUTNER, Publicker Industries Incorporated, Philadelphia, Pa.

A polarographic study has been made of acetaldehyde, and a method is described for the polarographic determination of acetaldehyde in the complex liquid mixtures and in liquefied C_4 distillation fractions obtained in the conversion of ethanol to butadiene. The method is simple, rapid, and accurate, and is superior to the titrimetric methods which are usually used.

IN CONNECTION with research and production control on the single-step process for the conversion of ethanol to butadiene it became necessary to have a rapid, simple, and accurate method for the determination of acetaldehyde. It was desired to determine acetaldehyde in the concentration range from 0.1 to 10% in alcohol denatured with varying amounts of acetaldehyde and in complex liquid mixtures which might contain C_3, C_4, C_5, and C_6 olefins and diolefins, C_2 and C_4 alcohols, diethyl ether, water, and small amounts of unknown organic compounds. It was also desired to apply the same method to the determination of acetaldehyde present in the liquefied C_4 samples obtained by the fractionation of butadiene-containing samples. The presence of acetaldehyde is due to the formation of azeotropes of it with butadiene and various butenes, and to difficulties in the separation of the C_4 fraction from other azeotropes containing acetaldehyde.

Investigation of the methods generally used for the determination of acetaldehyde showed the titrimetric hydroxylamine method (*2*, *3*) as modified to be the most accurate and reliable, although it was not entirely satisfactory. This was also found true by Russian workers (*7*, *8*) dealing with the same problem. While the hydroxylamine method could be readily applied to samples containing only small amounts of components boiling below room temperature, the titration was difficult to carry out because of the effect of the ethanol and other solvents present on the indicator. The substitution of an electrometric detection of the end point and the use of synthetic mixtures duplicating end-point conditions were unsatisfactory. The determination of acetaldehyde by the hydroxylamine method in samples containing considerable amounts of volatile material as in samples containing over 5% acetaldehyde or C_4's, or in C_4 fractionation samples, was time-consuming and tedious, owing to the necessity of carrying out the determination as a sealed tube reaction.

The difficulties attending the use of the hydroxylamine method led to a study of the polarographic method and a satisfactory solution was found. The procedure described is applicable to the complex mixtures previously indicated which were found to contain no detectable amounts of any other compound reducible at less negative potentials at the dropping mercury cathode (Figure 1). In the presence of substances reducible at different voltages from acetaldehyde the method could be applied with slight modification. In connection with this method a study was made of the polarographic behavior of acetaldehyde at various temperatures, hydrogen-ion concentrations, and acetaldehyde concentrations in order to make more extensive use of the polarographic method.

The polarographic behavior of acetaldehyde has been discussed by several workers whose results are summarized by Kolthoff and Lingane (*6*). Shikata and his co-workers (*9*, *10*) noted the polarographic determination of minute amounts of aldehydes including acetaldehyde in alcoholic beverages. The samples were dissolved in aqueous electrolyte solution, freed from dissolved oxygen by having hydrogen bubbled through them, and examined polarographically. The wave at decomposition potential −1.6 volts was assumed to be due to acetaldehyde for 10^{-4} *M* concentration. Smöler (*11*) studied the mechanism of polarographic reduction of acetaldehyde and described its determination in fermentation products; unfortunately, the results are not very conclusive.

APPARATUS AND MATERIALS

A Leeds & Northrup Electro-Chemograph and a Fisher Scientific Company Elecdropode were used for the polarographic measurements. For the constant-temperature experiments, water from a thermostat was circulated through a jacket surrounding the polarographic cell which kept the temperature in the cell constant to ±0.1° C. The drop time of the capillaries used for the analytical studies could be controlled to between 3 and 5 seconds by regulation of the mercury column. It was unnecessary to calibrate the apparatus, since variations in conditions are accounted for by use of a standard sample whenever a series of unknown samples is analyzed. If the instrument is in continuous use for several hours, a standard sample should be analyzed each hour or two as described below.

The acetaldehyde used for standardization and the preparation of synthetic mixtures was C.P. grade which was purified before use by fractionation through an efficient distilling column containing Stedman packing. Its physical constants checked those reported in the literature. The other chemicals used were of C.P. grade. The base solution used in the analytical procedure was 0.1 *M* lithium chloride. The following buffer solutions were prepared: (1) the buffer of pH 6.8 was 0.1 *M* in lithium, 0.05 *M*

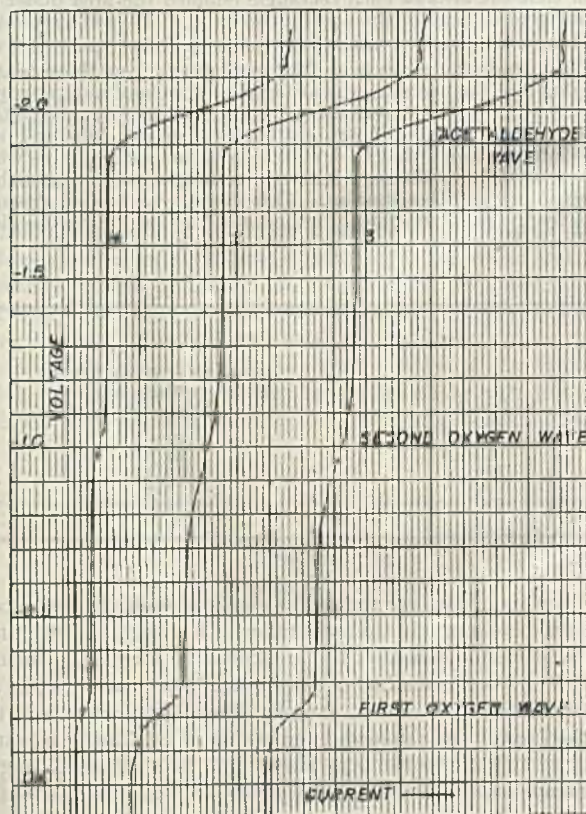


Figure 1. Current Voltage Curves (Electro-Chemograph) for Butadiene Condensates

Temperature, 25° C. Supporting electrolyte, 0.1 *N* LiCl
Concentration of condensate, 1/400
Sensitivities: (1) 1/400, (2) 1/40, (3) 1/40

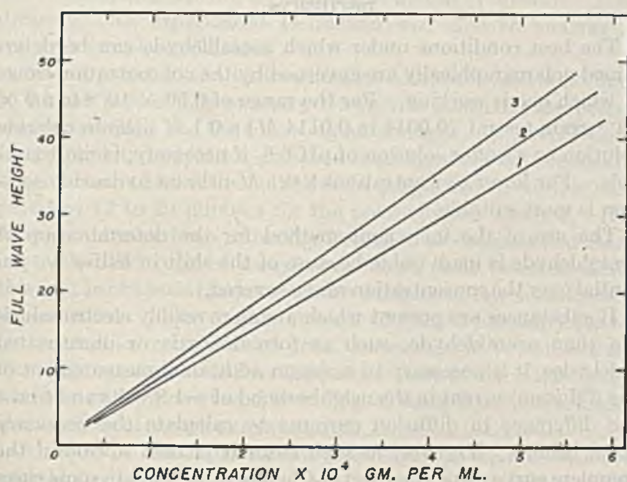


Figure 2. Effect of Temperature on Wave Height

Sensitivity calculated to 1/50. pH, 6.8
Temperature: (1) 20° C., (2) 25° C., (3) 30° C.

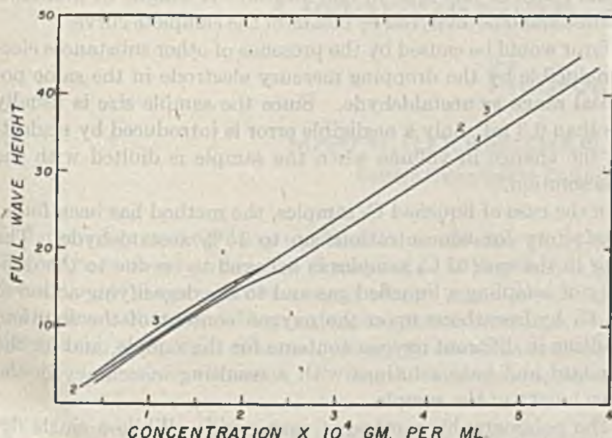


Figure 3. Effect of pH on Wave Height

Sensitivity calculated to 1/50. Temperature, 25° C.
pH: (1) 8.8, (2) 6.8, (3) 12.7

in acetate, and 0.05 M in chloride ion, and had enough acetic acid added to adjust the pH to 6.8; (2) the buffer of pH 8.8 was prepared by adjusting the pH of 0.2 M lithium hydroxide with acetic acid; (3) the solution of pH 12.7 was 0.11 M in lithium, 0.06 M in hydroxyl, and 0.05 M in chloride ion. For the study of the factors affecting the polarographic determination of acetaldehyde, ten solutions were prepared by diluting weighed samples of acetaldehyde with distilled water; these solutions had the following concentrations in grams per ml. $\times 10^{-3}$ (molar concentration in parentheses): 5.26 (0.120), 4.86 (0.110), 3.96 (0.090), 3.61 (0.082), 3.11 (0.071), 2.47 (0.056), 2.12 (0.048), 1.62 (0.037), 0.96 (0.022), 0.48 (0.011). These solutions were diluted 1 to 10 with appropriate base solutions before use. Two solutions for standardization were prepared by dissolving weighed amounts of acetaldehyde in 0.1 M lithium chloride solution; one solution contained 5.0×10^{-5} gram of acetaldehyde per ml. (0.00114 M) and the other contained 5.0×10^{-4} (0.0114 M). The latter two solutions were found to be stable for at least 3 weeks. The solution used depended on the range of concentration of the samples being analyzed.

STUDY OF TEMPERATURE, pH, AND CONCENTRATION EFFECTS

The capillary used in these studies had a drop time of 3.76 seconds per drop for a mercury column 51.3 cm. high. The weight of mercury dropped per second was 1.093 mg. The value of $m^{2/3} t^{1/6}$ is, accordingly, 1.32 mg. $^{2/3}$ sec$^{-1/2}$.

Since the effects of temperature and pH on the diffusion current are the most important factors in the polarographic determination of organic compounds, the effect of varying these conditions was studied. Polarograms were obtained at 25° C. for pH values of 6.8, 8.8, and 12.7; at pH 6.8 for temperatures of 20°, 25°, and 30° C. All wave heights were recalculated to a sensitivity of 1/50 for the Elecdropode. Half-wave potentials were referred to the saturated calomel electrode.

Diffusion Current. The temperature coefficient for the diffusion current, $(1/i)(di/dT)$, at pH 6.8 was found to be approximately 1.8% per ° C. over the range of 20° to 30° C. (Figure 2). This is in agreement with normal behavior, as the diffusion currents for most ions and molecules have temperature coefficients of 1.5 to 2.0% per ° C. (*5*).

The concentration–diffusion current curve at pH 6.8 showed a linear relation over the whole concentration range studied, while the curve at pH 8.8 showed a linear relation only over certain concentration ranges (Figure 3). The curve at pH 12.7 showed a linear relation between 2.5 and 5.5×10^{-4} gram per ml. (0.0057 to 0.0125 M). In this range the curve is identical with the curve for pH 6.8. For determining low concentrations of acetaldehyde it is best to use a base solution of high pH between 12 and 13, since the curve at pH 12.7 is linear for low concentrations in limited regions and has a large slope. It was found impractical to use a base solution of pH less than 6.8, as the interference from the hydrogen-ion wave is appreciable below this pH.

A linear relation was found between the concentration and the reading at −2.14 volts (dropping mercury electrode *vs.* pool) (Figure 4). The linearity of this relation was of the same order as that obtained for the full wave heights.

Half-Wave Potential. The effect of temperature, pH, and concentration on the half-wave potential was also observed. All half-wave measurements were made on the Elecdropode and were referred to the saturated calomel electrode. The half-wave potential is independent of temperature at pH 6.8 in the range of 20° to 30° C. with a shift of less than 0.01 volt, which is not significant as the limit of sensitivity of the instrument is about 0.005 volt. With 0.0119 M acetaldehyde solution (5.26×10^{-4} gram per ml.) potentials at pH 6.8, 8.8, and 12.7 were −1.89, −2.04, and −1.89 volts *vs.* the saturated calomel electrode at 25° C. A change in half-wave potential with concentration was noted for the concentration range studied (0.00114 to 0.0114 M acetaldehyde). The shift for tenfold change in concentration is about 0.08 volt at pH 6.8 and 25° C.; similar results were obtained using other buffer solutions.

ANALYTICAL PROCEDURE

The method is based upon the fact that the diffusion current of acetaldehyde is a linear function of concentration under the conditions used. The wave heights, galvanometer deflections, or similar measures of the diffusion currents are determined at a potential difference between the cell mercury pool and the dropping mercury electrode of −2.14 volts for the unknown samples, a standard sample containing a known amount of acetaldehyde, and the sample-free base solution. The concentration of acetaldehyde in the unknown sample is readily ascertained by a simple proportion involving the concentration of the standard sample. The removal of oxygen from the base and sample solutions is unnecessary, since the effect of oxygen is compensated for

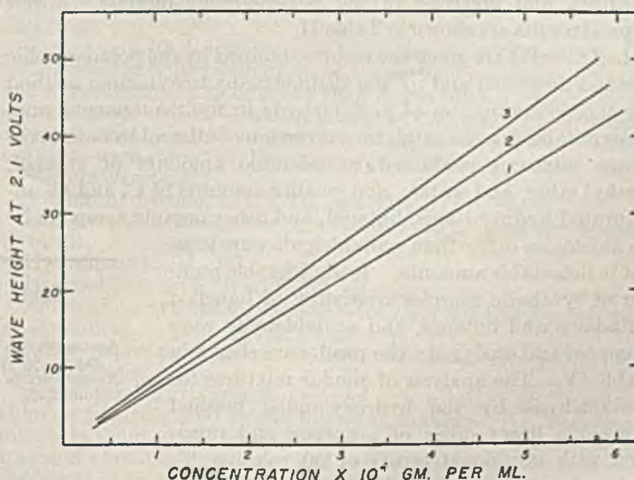


Figure 4. Polarograph Reading at −2.14 Volts (D.M.E. *vs.*, Pool)

Sensitivity calculated to 1/50. pH, 6.8
Temperature: (1) 20° C., (2) 25° C., (3) 30° C.

Table I. Determination of Acetaldehyde in Butadiene Condensate

Sample No.	Acetaldehyde Found: Using 0.1 *N* LiCl, Using full wave height	Using 0.1 *N* LiCl, Using reading at −2.14 volts	Using a Buffer of pH 6.8, Using full wave height	Using a Buffer of pH 6.8, Using reading at −2.14 volts
	%	%	%	%
5970	1.70	1.71	1.75	1.75
5486	1.61	1.60	1.64	1.65
5492	1.34	1.34	1.29	1.30

Table II. Determination of Acetaldehyde in Known Mixtures

Calcd. concn., mg./ml.	0.088	0.122	1.22
Polarographic detn., mg./ml.	0.091	0.120	1.24

in the measurement of the base solution. Polarograms of acetaldehyde and of typical samples containing acetaldehyde indicated that the use of a maxima suppressor was unnecessary.

A sample weighing 0.10 to 0.25 gram, depending on the concentration of acetaldehyde present, is taken in a sealed glass ampoule (*4*). If volatile compounds are present, the ampoule is immersed in an acetone-solid carbon dioxide or similar cooling bath for filling and sealing. The ampoule and several glass marbles or 2.5-cm. (1-inch) lengths of 7-mm. glass rod are introduced into a 400-ml. (16-ounce) citrate of magnesia bottle containing 50 ml. of base solution (300 ml. should be used for very volatile samples such as liquid C_4 hydrocarbon fractions). The bottle is stoppered and the ampoule is crushed by shaking the bottle, thus diluting the sample to 50 or 300 ml. A portion of the solution is transferred to the polarographic cell and a reading is taken of the current at a potential difference between the cell mercury pool and the dropping mercury electrode of −2.14 volts. A reading at this potential difference is on the plateau of the polarographic wave due to acetaldehyde (Figure 1). The wave height due to the reduction of acetaldehyde is calculated by subtracting the galvanometer reading of the base solution from that for the sample solution. The wave height is then compared with that obtained with a known amount of acetaldehyde in order to determine the acetaldehyde in the unknown, the concentrations being directly proportional to the wave heights.

It was thought necessary to show that no difference in results is obtained when samples of the liquid condensate obtained in the butadiene process are analyzed in 0.1 *M* lithium chloride whose pH is 6.8 instead of in a buffer solution of the same pH. Three samples of condensate were analyzed in a buffer of pH 6.8 and in 0.1 *M* lithium chloride; the results agreed well within the limit of error of the method (Table I).

ANALYTICAL RESULTS

The results obtained with known mixtures containing acetaldehyde confirmed the linearity of the diffusion current-concentration relationship and indicated the satisfactory order of accuracy and precision of the polarographic method. A few typical results are shown in Table II.

In Table III are given the results obtained by the polarographic method described and by the titrimetric hydroxylamine method for the determination of acetaldehyde in multicomponent mixtures obtained in the catalytic conversion of ethanol to butadiene. These mixtures contained considerable amounts of ethanol, diethyl ether, and water; and smaller amounts of C_4 and C_6 unsaturated hydrocarbons, butanol, and other organic compounds: no aldehydes other than acetaldehyde were present in detectable amounts. A considerable number of synthetic samples consisting of liquefied butadiene and butenes, and acetaldehyde were prepared and analyzed; the results are shown in Table IV. The analysis of similar mixtures for acetaldehyde by the hydroxylamine method showed a lower order of accuracy and precision with occasional errors of 50 relative %. Subsequently a new set of butadiene-2-butene-acetaldehyde mixtures was prepared and analyzed; the results are also shown in Table IV.

DISCUSSION

The best conditions under which acetaldehyde can be determined polarographically are governed by the concentration range in which one is working. For the range of 0.50×10^{-4} to 5.0×10^{-4} gram per ml. (0.0014 to 0.0114 *M*) a 0.1 *M* lithium chloride solution or a buffer solution of pH 6.8, if necessary, is most suitable. For lower concentrations a 0.1 *M* lithium hydroxide solution is most suitable.

The use of the increment method for the determination of acetaldehyde is inadvisable because of the shift in half-wave potential over the concentration range covered.

If substances are present which are more readily electroreducible than acetaldehyde, such as formaldehyde or unsaturated aldehydes, it is necessary to make an additional measurement of the diffusion current in the neighborhood of −1.8 volts and to use the difference in diffusion currents to calculate the necessary wave heights; however, in such cases it is best to obtain the complete curve using the Electro-Chemograph, since in some cases the reduction of one substance affects the subsequent reduction of another (*1*). If there are no interfering substances, the Elecdropode at a setting of −2.14 volts is preferred with sensitivities in the range of one fifth to one fiftieth. A sample of unknown nature should be analyzed by means of the complete curve.

Error would be caused by the presence of other substances electroreducible by the dropping mercury electrode in the same potential range as acetaldehyde. Since the sample size is usually less than 0.3 ml., only a negligible error is introduced by neglecting the change in volume when the sample is diluted with the base solution.

In the case of liquefied C_4 samples, the method has been found satisfactory for concentrations up to 15% acetaldehyde. The error in the case of C_4 samples is believed to be due to the difficulty of sampling a liquefied gas and to the degasifying action of the C_4 hydrocarbons upon the oxygen content of the solution, resulting in different oxygen contents for the sample, and for the standard and base solutions with a resulting inaccuracy in the wave height of the sample.

The polarographic method is very rapid. While a single determination takes about 20 minutes, a group of determinations takes about 12 minutes per sample plus 10 minutes for running a standard sample and the base solution. These periods of time include sampling and the necessary weighings. The method has been in constant use for over 20 months and over 4000 determinations have been performed on many types of samples in addition to those described. The method has been used successfully by laboratory technicians after several hours of train-

Table III. Determination of Acetaldehyde in Complex Mixtures

Sample No.	Acetaldehyde: Polarographic method	Acetaldehyde: Hydroxylamine method
	%	%
5967	1.03, 1.01, 1.00	1.0, 1.0
5982	0.97, 0.97	1.0, 0.9
5987	0.85, 0.86	0.9, 0.9
6507	1.12, 1.10	1.3

Table IV. Polarographic Determination of Acetaldehyde in Synthetic Mixtures of Liquefied C_4 Hydrocarbons

Acetaldehyde taken, %	0.58	0.71	0.92	1.00	1.63	2.09	3.39	5.3	6.5	8.7	10.4	13.1
Acetaldehyde found, %	0.50	0.76	1.10	0.98	1.63	2.29	3.42	5.0	6.6	8.7	9.8	13.4
	0.50	0.82	1.05	1.02	1.52	2.22	3.48	4.7	6.3	8.4	9.6	12.7
	0.52											

Butadiene-2-butene-acetaldehyde mixtures

Acetaldehyde taken, %	0.67	1.25	4.26	9.34	19.0
Acetaldehyde found, %	0.69	1.16	4.23	9.23	19.0

ing in sampling and in adjusting the manually operated Electropode. An experienced technician was able to analyze 30 research samples in 5 hours.

As compared to the hydroxylamine method which was found to be the best of the ordinary titrimetric methods, the polarographic method has the following advantages. It is very much faster; the hydroxylamine method requires about 60 minutes for the determination of acetaldehyde in a liquefied C_4 sample compared to 12 to 20 minutes for the polarographic method. It is simpler to use and can be readily taught to technicians and chemists. For the type of samples discussed it is more accurate; this fact is of special importance for samples of low acetaldehyde concentration. The minute amount of sample necessary for the polarographic method is of great advantage in research work. The only apparent disadvantage is the cost of the polarograph, which is rapidly recovered in saving of manpower if a considerable number of samples are analyzed.

A pilot-ion technique for the polarographic determination of acetaldehyde is being investigated.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of Benj. Warshowsky and Joyce Becker with the experimental work.

LITERATURE CITED

(1) Adkins, H., and Cox, F. W., *J. Am. Chem. Soc.*, 60, 1151 (1938).
(2) Bennett, A. H., and Donovan, F. K., *Analyst*, 47, 146 (1922).
(3) Brochet, A., and Cambier, R. *Compt. rend.*, 120, 449 (1895).
(4) Elving, P. J., and Ligett, W. B., Ind. Eng. Chem., Anal. Ed., 14, 449 (1942)
(5) Kolthoff, I. M., and Lingane, J. J., "Polarography", pp. 74–7, New York, Interscience Publishers, 1941.
(6) *Ibid.*, pp. 353–4.
(7) Livshitz, I. A., and Bessmertnaya, quoted in (*8*).
(8) Shaposhnikov, V. G., Makovskaya, Ya. I., and Kalinicheva, N. A., *Trudy Gosudarst. Opyt. Zavoda Sintet. Kauchuka Litera B*, 3, 118 (1934).
(9) Shikata, M., and Shoji, K., *Mem. Coll. Agr. Kyoto*, 4, 75–91 (1927).
(10) Shikata, M., and Tachi, I., *Proc. Imp. Acad.* (Tokyo), 2, 226–8 (1926), quoted in (*11*).
(11) Smöler, I., *Collection Czechoslov. Chem. Commun.*, 2, 692 (1930).

Determination of Beryllium in Ores

Fluorometric Method

MARY H. FLETCHER, CHARLES E. WHITE[1], AND MILTON S. SHEFTEL[2]
Eastern Experiment Station, Bureau of Mines, College Park, Md.

A fluorometric method using quinizarin in an alkaline citrate solution has been developed for the determination of beryllium in ores. Preparation of the sample for analysis consists essentially of fusion with sodium carbonate and borax glass, solution of the melt in hydrochloric acid, and proper dilution. Directions are given for removal of other ions when necessary. A simple visual comparator or a photoelectric instrument may be used for the measurements. The effect of changes in dye concentration and beryllium content on the characteristics of the fluorescence, the influence and removal of other ions, and the effect of light, temperature, and time are discussed. The method is particularly practicable for routine analysis since reliable results may be obtained with rapidity and ease.

THE fluorometric method described herein was developed in answer to the need for a rapid, reliable method which could be used for large numbers of beryllium determinations on both low- and high-grade materials. It has been successfully used for the past 18 months in the analysis of hundreds of samples.

Fluorescent reagents were first used in 1933 when Zermatten (*7*) found that morin reacted with beryllium in the presence of alkali to produce a yellow-green fluorescence and described a spot test based on the reaction. Sandell (*4, 5*) made a thorough study of the morin reaction for beryllium and its application to the determination of beryllium in silicates.

White and Lowe (*6*) described 1-amino-4-hydroxyanthraquinone as a less sensitive but more specific reagent than morin for beryllium. Palmes and Alford (*3*) adapted White and Lowe's qualitative method to the quantitative determination of minute amounts of beryllium in biological materials, and also proposed the use of quinizarin (1-4-dihydroxyanthraquinone), a similar reagent.

Tests of the three reagents (morin, 1-amino-4-hydroxyanthraquinone, and quinizarin) in this laboratory showed quinizarin to be the most suitable. The method devised measures the amount of beryllium present by comparison of the intensity of the fluorescence of the unknowns with that of a set of similarly prepared standards. The fluorescence is produced when an alkaline solution containing beryllium and quinizarin is exposed to ultraviolet light. The comparison may be made either directly in a simple visual comparator, or alternatively with a photoelectric fluorometer; both give about the same accuracy.

A single complete analysis can be made in about 2 hours on samples free of interfering ions; ten to fifteen determinations can be made in an 8-hour day. Samples which contain interfering ions require 1.5 to 2 days for a single determination, but even under these conditions fifteen to twenty analyses can be run in 4 days.

[1] Address, University of Maryland, College Park, Md.
[2] Present address, Gering Products Co., Kenilworth, N. J.

REAGENTS AND APPARATUS

All reagents were c.p. chemicals with the exception of the quinizarin, aerosol, and the borax glass used in the flux. All reagents were tested for fluorescence before they were used.

Fusion mixture, 3 parts of anhydrous sodium carbonate with 1 part of borax glass (technical grade such as that used in fire assaying).

Quinizarin (1,4-dihydroxyanthraquinone). Technical-grade quinizarin was boiled with decolorizing carbon in acetone, filtered, and crystallized. The dye was then recrystallized from acetone and air-dried. Stock solution, 0.29% in c.p. acetone. Working solution, stock solution diluted (1 to 10) with c.p. acetone. These solutions were stored in glass-stoppered bottles.

Sodium citrate, 5%.

Sodium hydroxide, 1, 2, 5, 10, and 20%.

Laboratory aerosol, the commercial 10% product diluted (1 to 10).

Hydrofluoric acid, 48%.

Hydrochloric acid, 10 and 30% by volume.

Ammonium hydroxide, concentrated, and a 0.1% (by volume) wash solution.

Filter paper pulp, prepared from Whatman No. 41 H in a Waring mixer.

Standard Beryllium Solutions. A solution of beryllium chloride was prepared in the following manner. c.p. beryllium nitrate in solution was treated with 8-hydroxyquinoline to remove traces of aluminum and iron, and excess 8-hydroxyquinoline was destroyed with nitric and sulfuric acids. Beryllium hydroxide was precipitated with ammonia. The precipitate was dissolved in 10% hydrochloric acid, and diluted until 1 ml. was equal to approximately 10 mg. of beryllium oxide. The solution was

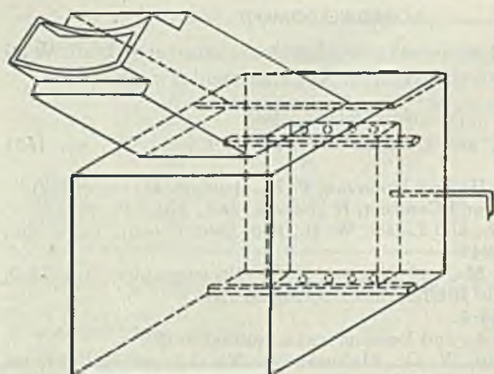


Figure 1. Front View of Fluorometric Comparator

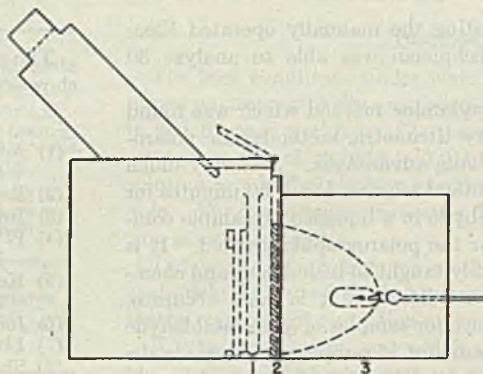


Figure 2. Side View of Fluorometric Comparator

1. Sliding test tube holder
2. No. 5874 H.R. red purple ulta filter
3. Mercury vapor lamp, Type B-H-4

standardized gravimetrically. This solution was diluted 1 to 1000 to give the working standard.

A standard beryllium solution was also prepared from a high-grade beryl of known beryllium content by the following procedure: A 500-mg. sample was fused with 10 grams of fusion mixture, the melt dissolved in 150 ml. of 1 to 1 hydrochloric acid, and diluted to 250 ml. This solution was further diluted 1 to 20 to give a working standard, 1 ml. of which contained 11.2 micrograms of beryllium oxide. The weaker solution was used almost exclusively as a standard in the analysis of unknowns. A new standard solution was prepared about once a month, as silica separated from the concentrated solution after standing some time. The diluted solutions were unaffected by standing.

EQUIPMENT. In addition to the ordinary glassware, pipets, burets, and platinum ware common to all laboratories, the following pieces of equipment were used:

Two 2-ml. automatic pipets.

Pyrex test tubes (20 × 200 mm.), calibrated at 12.5 and 25 ml. These are sold as Lewis-Benedict blood sugar tubes. When used for visual comparison, only those tubes were selected which were the same height (within ±3-mm. tolerance) from the bottom up to the 25-ml. graduation.

Optical glass cell (for photoelectric determination only), 31-mm. cube inside measurement, 1-mm. wall thickness. These may be purchased from Pyrocell Manufacturing Co., 207 East 84th St., New York 28, N. Y.

Glass standards (for photoelectric determination only). A series of glass standards was made by embedding 2.5 × 2.5 cm. squares of fluorescent glass in a horizontal position in wooden blocks which were the same size as the optical cell. The glass squares were prepared by cutting a 5-cm. (2-inch) square Corning filter No. 3750 into quarters. The faces next to the phototube were covered by a section of Corning filter No. 3480, to transmit light of the desired wave length. The sides of the block next to the phototube and the light source, respectively, were covered with brass plates. The apertures in these plates were made of such size that readings were obtained comparable to those resulting from the beryllium standards.

Visual comparator. A visual comparator similar to that used by Palmes and Alford (*3*) was built from wood and painted a flat black with a nonfluorescent paint. Figure 1 shows the light-tight comparison box, which was equipped with a sliding rack to carry four sample tubes. Ultraviolet light was admitted directly behind the test tube rack through a Corning heat-resistant red purple ultra filter No. 5874, which eliminated visible light. The fluorescence was observed through the viewing aperture in the front of the box. A hinged panel immediately above the tube rack facilitated rapid changing of unknowns and standards. The light source shown in Figure 2 was a General Electric Type B-H-4 high-pressure mercury lamp with an aluminum reflector. An auxiliary ballast transformer with a primary of 115 volts was needed for the mercury lamp. This complete light source unit may be purchased from the General Electric Vapor Lamp Co., Hoboken, N. J. All the samples analyzed during this work were matched in this comparator although many were also run photoelectrically.

Photoelectric fluorometers. The original fluorometer used in the early part of the work was built in the laboratory of one of the authors (C. E. White) according to the description of Hand (*2*), except that a galvanometer with a sensitivity of 1 scale division equivalent to 0.0004 microampere was substituted for the microammeter and a voltage regulator and a ventilating fan were used to control the lamp voltage and temperature.

All later data, including those in Table II, were taken with a Beckman spectrophotometer for which a fluorometric attachment was designed (*1*).

A Lumetron fluorescence meter could also be used.

ANALYTICAL METHOD

If the mineralogical composition and approximate beryllium content of the sample are not known, a preliminary test is made to indicate which of the procedures described below is to be followed in preparing the beryllium-bearing solution.

For the test run the sample is treated as though it contained over 1% beryllium oxide. Aliquots of 0.1 and 1.0 ml. are then used to develop the fluorescence as described under the section for the determination of beryllium oxide in solutions. Only 5 standards (0.0, 0.5, 1.0, 1.5, and 2.0 ml. of standard solution) are used for this comparison. If a photoelectric instrument is employed, no standard solutions are required; the instrument is set with a glass standard and the readings are compared with a previously prepared standard curve.

PREPARATION OF SAMPLES FOR ANALYSIS. Procedure For Ores of Beryllium Oxide Content Greater Than 1% (low in interfering ions.) Mix a 500-mg. sample with 10 grams of fusion mixture in a 100-ml. platinum dish. Fuse at 1000° C. in a muffle furnace for 3 to 5 minutes. Remove the dish from the furnace, cool somewhat, and place in a 600-ml. beaker which contains 150 ml. of 1 to 1 hydrochloric acid. When solution is complete, which usually requires about 5 minutes, remove and rinse the platinum dish. Cool the solution to room temperature, transfer to a 250-ml. volumetric flask, and add water to 250 ml. Make a test run on this solution to indicate necessary dilution, and dilute so that 1 ml. of solution is equivalent to approximately 10 micrograms of beryllium oxide. Use four aliquots which cover a range of 5 to 24 micrograms of beryllium oxide for the analysis.

PROCEDURE FOR ORES OF BERYLLIUM OXIDE CONTENT LESS THAN 1% (low in interfering ions). Moisten a 1.000-gram sample contained in a 100-ml. platinum dish with aerosol and distilled water. Add 30 to 40 ml. of hydrofluoric acid, 3 ml. of nitric acid, and 1 ml. of sulfuric acid. Evaporate to dryness on a hot plate, and ignite over a burner until the sulfur trioxide fumes are expelled. Add 10 grams of the fusion mixture and fuse in a muffle furnace. Remove from the furnace, cool several minutes, place the dish and contents in a 600-ml. beaker, add 100 ml. of 30% hydrochloric acid, and heat on a steam bath until the melt is dissolved. Columbium and tantalum, if present, will hydrolyze and separate at this point. When this occurs remove and rinse the platinum dish, boil the solution 0.5 hour, filter, and wash the precipitate well with water. In the absence of columbium and tantalum filtration is unnecessary.

If the beryllium oxide content is 0.1% or more, transfer the solution into a 250-ml. volumetric flask and make up to volume. Make a test run to determine whether dilution is necessary, and if so, dilute until 1 ml. contains approximately 10 micrograms of beryllium oxide. Use four aliquots of original or diluted solution, which contain 5 to 24 micrograms of beryllium oxide for the analysis.

When the beryllium oxide content is less than 0.1%, concentration is necessary. To do this, heat the solution until it boils and precipitate beryllium and aluminum with ammonium hydroxide at a pH of 8.0. Boil 1 minute and set aside for at least 2 hours or preferably overnight. Filter on Whatman No. 41 H paper. Wash several times with 0.1% ammonium hydroxide and once with water. Return paper and precipitate to original beaker, dissolve the precipitate in 5 ml. of concentrated hydrochloric acid and about 25 ml. of water, filter into a 100-ml. volumetric flask, wash paper well with water, and dilute the solution to 100 ml.

Make a test run to determine whether further dilution is necessary, and if so, dilute until 1 ml. contains approximately 10 micro-

grams of beryllium oxide. Use aliquots of original or diluted solution, which contain 5 to 24 micrograms of beryllium oxide for the analysis.

PROCEDURE FOR ORES WITH HIGH IRON, MANGANESE, AND MAGNESIUM CONTENT. Treat as directed in the preceding section to the step where the sample is in solution and columbium and tantalum are removed. At this point add 0.5 ml. of concentrated nitric acid and heat to boiling. Add 20% sodium hydroxide until precipitation starts, and then add at one time a volume of 20% sodium hydroxide equivalent to one half of the volume of the neutralized solution. Add about 1 gram of sodium peroxide. Boil 0.5 hour, cool 15 minutes, and filter with suction on a heavy pad of filter-paper pulp prepared from Whatman No. 41 H paper. Wash well with hot 5% sodium hydroxide and twice with water. Acidify the filtrate with hydrochloric acid, bring to a boil, and precipitate beryllium and aluminum with ammonium hydroxide at a pH of 8.0. Boil 1 minute and set aside for at least 2 hours or preferably overnight. Filter with suction on a Whatman No. 41 H filter paper and continue as directed in the preceding method.

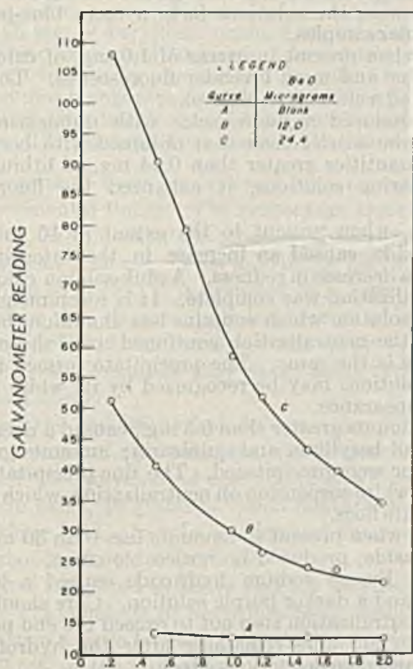


Figure 3. Effect of Dye Concentration on Fluorescence of Beryllium and Quinizarin

PROCEDURE FOR DETERMINATION OF BERYLLIUM OXIDE IN SOLUTIONS. Preparation of tubes for the development of fluorescence should follow rigidly the standard procedure because small variations in the preparative routine frequently lead to off-color solutions.

Prepare a set of standards in twenty-three calibrated tubes which contain the following volumes of standard beryl solution, or beryllium chloride solution, 1 ml. of which contains an equivalent of 10 to 12 micrograms of beryllium oxide: five blank tubes, 0.2, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, and 2.0 ml. Pipet four aliquots of each unknown sample which contain 5 to 24 micrograms of beryllium oxide into similar tubes. Add 2 ml. of 5% sodium citrate from an automatic pipet and 3 drops of quinizarin to each tube. Neutralize with 2% sodium hydroxide until the color of the solution changes to purple. Add water to 12.5-ml. mark. Add 1 ml. of 0.029% quinizarin to each tube and mix by swirling. If any of the solutions are incompletely neutralized, as indicated by a light color, add more sodium hydroxide until they match the color of the standards. Add 2 ml. of 1% sodium hydroxide from an automatic pipet, and water to 25 ml. Starting with the blank tubes, cover the end of the tubes with the thumb, and shake well to mix. Glass-stoppered containers may be used, but rubber stoppers must not be used in these tubes under any circumstances, because the action of acetone on the rubber produces a brilliant blue fluorescence. The solutions even dissolve a less brilliant blue fluorescent substance from the skin; therefore, the same thumb should be used to shake all the tubes and it should be well rinsed before starting. The blank solutions remove most of the material from the thumb, so that the standards and samples are unaffected. Should it prove necessary to make up a single tube, the mixing may be effected by pouring the solution back and forth from two test tubes, or by preceding it with four blank solutions.

All solutions should be protected from sunlight or the direct light from an electric lamp; however, it is not necessary to work in darkness.

Compare the unknown solutions with the standard solutions in the visual comparator, or make readings with a photoelectric fluorometer and calculate the percentage of beryllium oxide.

EXPERIMENTAL RESULTS AND DISCUSSION

The method was tested on four beryllium ores. These samples were analyzed for beryllium oxide by the visual fluorescent method; also, representative samples were sent to seven different laboratories experienced in beryllium analysis. The average results obtained by these seven laboratories were considered as representing most nearly the true beryllium content of the ores. The results are given in Table I, which also shows the reproducibility of the fluorescent method.

Table II shows the results of analyses by the fluorometric method on standard mixtures of known beryllium oxide content. These were prepared from the high-grade sample by dilution with beryllium-free potash feldspar. The errors on the mixture that contained 0.022% beryllium oxide, although somewhat large in a relative sense, are within acceptable limits because in practice the third figure would not be shown; with one exception, all

Table I. Determination of BeO in Analyzed Samples

Sample	BeO Found: Mean and standard deviation of mean of results from seven laboratories[a] %	Visual fluorometric method %
Beryl concentrate	11.2 ± 0.1	11.1
		11.2
		11.1
		11.2
Medium-grade beryl ore	5.51 ± 0.07	5.36
		5.51
		5.38
		5.36
Low-grade beryl ore	1.39 ± 0.05	1.38
		1.39
		1.36
		1.39
Tailing waste	0.26 ± 0.02	0.26
		0.25
		0.24
		0.26

[a] Geological Survey, Bureau of Mines, Rolla, Mo., Salt Lake City, Utah, and College Park, Md., Paul Brinton, Pasadena, Calif., Beryllium Corp. of Pennsylvania, Temple, Pa., and Ledoux and Co., New York, N. Y.

Table II. Determination of BeO in Standard Mixtures by Fluorometric[a] Method

(Comparison of visual and photoelectric methods)

BeO Present %	BeO Found: Visual method %	BeO Found: Photoelectric method %
2.24	2.28	2.31
	2.27	2.27
	2.29	2.29
	2.31	2.26
1.12	1.14	1.12
	1.12	1.15
	1.13	1.09
	1.12	1.16
0.22	0.23	0.24
	0.22	0.23
	0.24	0.23
	0.22	0.23
0.022	0.019	0.024
	0.020	0.026
	0.019	0.023
	0.016	0.020

[a] For each concentration of beryllium oxide, four different samples were carried through entire procedure.

would be reported as 0.02%. It is virtually impossible to analyze samples which contain such low amounts of beryllium oxide by gravimetric procedures. Table II also gives a comparison of results by the visual and photoelectric methods and shows them to be in close agreement. The choice of procedure need be governed only by personal preference or the availability of a photoelectric instrument.

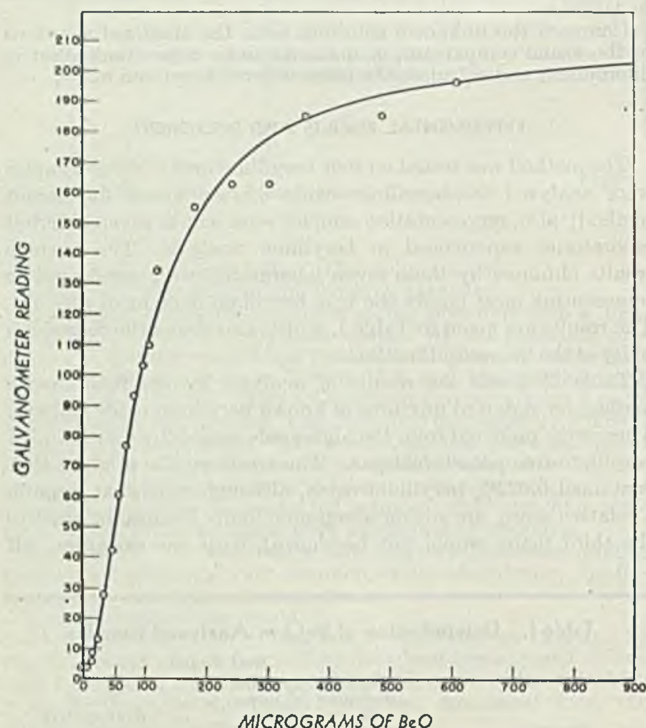


Figure 4. Effect of Beryllium Concentration on Fluorescence of Beryllium and Quinizarin

CHARACTERISTICS OF THE FLUORESCENCE. Beryllium reacts with quinizarin over a wide range of concentration and produces a fluorescent band which covers the spectral range from 5700 to 6400 Å. One milliliter of 0.029% quinizarin with 5 to 24 micrograms of beryllium oxide appeared to be the best ratio for visual comparison. Figure 3 shows the effect of changes in the dye concentration on the fluorescence and indicates the necessity for careful control of the amount of dye used.

Figure 4 shows the relation between the beryllium concentration and the fluorescence of the solution. Even though the use of the straight-line portion of the curve between 25 and 90 micrograms of beryllium oxide would seem desirable, this is not generally practical except for pure beryllium solutions when measured photoelectrically.

EFFECT OF OTHER IONS. All elements that are precipitated in an alkaline citrate solution must be removed before beryllium is determined by the fluorometric method. Among these are iron, manganese, magnesium, calcium, and silica, all of which, when present in sufficient quantities, decreased the intensity of the fluorescence. Strong oxidizing ions such as hexavalent chromium interfered through destruction of the dye. For the most part, the presence of these substances in amounts sufficient to interfere are indicated by off-color fluorescence.

The weights of the ions given in the following section refer to the amount of the various substances contained in one comparison tube.

Silica, when present to the extent of 1.2 mg., caused the fluorescence to pale, and the silica separated as a gelatinous precipitate which adhered to the walls of the tube. When 4.0 mg. or more were present the precipitate settled to the bottom of the tube. It appears that more silica can be tolerated as sodium silicate than as a complex ion with boron.

Iron, in amounts greater than 0.04 mg. of ferric oxide, produced a dull and darker red fluorescence than pure beryllium solutions. When more than 0.6 mg. of ferric oxide was present ferric hydroxide settled out. During the neutralization step, solutions which contained appreciable amounts of iron assumed a maroon and then a brownish color.

Manganese, when present in quantities greater than 0.02 mg., caused a decrease in the intensity of fluorescence. A dark-red shade characterized the color of the fluorescence when 0.2 mg. or more of manganese was present. Solutions which contained more than this had a murky appearance due to a fine suspended precipitate.

Magnesium, when present in amounts exceeding 0.06 mg. of magnesium oxide, produced a fluorescence that was sometimes brighter when first prepared but soon faded to pale lavender. Formation of a purple flocculent precipitate started at 0.1 mg. of magnesium oxide and absorbed all the dye from the solution. Excessive amounts of magnesium oxide (30 to 40 mg.), which produced a heavy precipitate of magnesium hydroxide, gave a brilliant fluorescence that could be seen in ordinary light. The presence of magnesium can be recognized during the neutralization step because the solutions have a more blue-purple color than the regular samples.

Calcium, when present in excess of 1.0 mg. of calcium oxide, caused a paler and more lavender fluorescence. The solutions themselves had a clear slate-blue color.

Lithium produced a fluorescence with quinizarin that was somewhat more scarlet than that obtained with beryllium. If present in quantities greater than 0.04 mg. of lithium oxide in beryllium-bearing solutions, it enhanced the fluorescence of beryllium.

Aluminum, when present to the extent of 10 to 60 mg. of aluminum oxide, caused an increase in the intensity of fluorescence but a decrease in redness. A pink-salmon color appeared before neutralization was complete. It is recommended in such cases that a solution which contains less aluminum be used as a control and the neutralization continued until the color of the two solutions is the same. The precipitate formed in the high aluminum solutions may be recognized by its white bulky nongelatinous appearance.

Zinc, in amounts greater than 0.5 mg., caused a decrease in the fluorescence of beryllium and quinizarin; amounts greater than 1.0 mg. of zinc were precipitated. The zinc precipitate appeared first as a fine white suspension on neutralization, which flocculated into large white flocs.

Phosphate, when present in amounts less than 30 mg. of phosphorus pentoxide, produced no noticeable effect.

Hydroxyl. Excess sodium hydroxide caused a less intense fluorescence and a darker purple solution. Care should be taken during the neutralization step not to exceed the end point.

Fluoride, in amounts remaining after the hydrofluoric acid treatment, caused no deleterious effect.

Sulfate. Sulfuric acid of the same normality as the hydrochloric acid may be substituted for the latter in the procedures.

The precautions that must be observed in almost all fluorometric analyses are applicable. For example, technical-grade acetone has a decided blue fluorescence and cannot be substituted for the C.P. reagent. All commercial alcohol on hand in this laboratory also had a blue fluorescence, although if redistilled from an all-glass apparatus it could be substituted for acetone. The dye solution and solutions prepared for comparison must be kept from all contact with rubber and grease. On short

Table III. Effect of Sodium Hydroxide Separation of 20 Mg. of Ferric Oxide on Recovery of Beryllium Oxide

Solution No.	BeO Originally Taken	BeO in Diluted Solution Present	Found	Error
	Mg.	*Micrograms/ml.*		%
1	0	0	0	...
2	0.122	1.22	0.77	−36.1
3	0.610	6.10	5.52	− 9.4
4	1.22	12.2	10.6	−13.1
5	1.83	9.15	8.74	− 4.5
6	2.44	12.2	12.4	+ 1.6
7	4.88	9.76	9.74	− 0.21
8	7.32	7.32	7.73	+ 5.6
9	9.76	9.76	10.5	+ 7.2
10	12.2	4.88	5.10	+ 4.5
11	24.4	9.76	9.91	+ 1.5
12	36.6	7.32	6.65	− 9.1
13	61.0	12.2	12.1	− 0.82
14	85.4	8.54	8.34	− 2.3

contact, acetone extracts a substance from rubber that produces a brilliant blue fluorescence. Grease, as on stopcocks, is also fluorescent and should never be used with acetone solutions and only sparingly with the other solutions. Rubber tube connections may be used for the sodium citrate and sodium hydroxide solutions.

SEPARATIONS. The composition of the beryllium ores is such that iron, manganese, and silica are frequently present in low-grade materials in amounts exceeding the tolerable limits. Silica in the gangue materials is removed by the preliminary hydrofluoric acid treatment used for all low-grade materials. The iron, manganese, and all other ions mentioned except aluminum are removed by the sodium hydroxide and ammonium hydroxide treatments. These separations were adopted after laboratory tests indicated that they could be safely employed.

Experiments were made to test the effects of the sodium hydroxide separation on the beryllium recovery (Table III). Fourteen solutions were prepared which contained the equivalent of 0 to 85 mg. of beryllium oxide, 8 mg. of aluminum oxide, 20 mg. of ferric oxide, and 10 grams of fusion mixture. These solutions were treated by the method described for samples with a high iron and manganese content, and the beryllium present was determined after treatment for the separation of iron and proper dilution. The readings were made in the visual comparator.

The results of these experiments were considered well within the experimental limits. The percentage error was always less than 10%, except when the amount of beryllium oxide present was less than 1.22 mg. This amount of beryllium oxide would represent 0.12% BeO if it occurred in a 1-gram sample; hence it is evident that the seemingly large errors which appear for the first three solutions in Table III can easily be tolerated, since in the analysis of samples they would make very little difference in the final answer.

To test the sodium hydroxide separation for larger amounts of iron, a series of nine solutions was prepared. Each solution contained the equivalent of 4.88 mg. of beryllium oxide, 8 mg. of aluminum oxide, 10 grams of fusion mixture, and 75 ml. of 30% hydrochloric acid. Iron in the form of the chloride was added in the amounts shown in Table IV.

Iron was separated as above and the beryllium content determined. The amounts of beryllium oxide found by visual comparison were a little lower than the amount present, but all were within 7.5% of the true value. The results are summarized in Table IV.

Table IV. Effect of Separation of Large Amounts of Iron on Recovery of Beryllium Oxide

Solution No.	Fe_2O_3 Added	BeO in Diluted Solution		Error
		Present	Found	
	Mg.	*Micrograms/ml.*		%
1	20	9.76	9.49	−2.8
2	30	9.76	9.31	−4.6
3	40	9.76	9.80	−0.41
4	50	9.76	9.24	−5.3
5	60	9.76	9.37	−4.0
6	80	9.76	9.31	−3.6
7	100	9.76	9.04	−7.4
8	150	9.76	9.05	−7.3
9	200	9.76	9.21	−5.6

It can be concluded from these experiments that sodium hydroxide can be used satisfactorily to separate iron from beryllium wherever it may occur in amounts sufficient to interfere with the analysis.

Should the aluminum concentration ever exceed the tolerable limits, the bulk of it may be separated as the chloride in ether by the Gooch Havens method. This procedure was tried on the synthetic sample which contained 0.22% beryllium oxide and to which aluminum was added. Excellent results were obtained and there was no loss of beryllium.

EFFECT OF LIGHT. Tubes prepared for comparison of the fluorescence must be protected from sunlight and the direct light from an electric lamp. Upon exposure to strong light, the purple color faded, first with an increase in the intensity of the fluorescence followed by complete destruction of the color and fluorescence.

EFFECT OF TEMPERATURE. Small temperature changes as experienced under normal room conditions appear to have no appreciable effect on the fluorescence of beryllium and quinizarin, and any changes which might occur are offset by preparation and comparison of standards and unknowns at the same time. Large temperature changes of 15° to 20° do affect the fluorescence. A decrease in temperature is accompanied by increase in the intensity of the fluorescence.

EFFECT OF TIME. The solutions prepared for fluorometric comparison may stand approximately 4 hours before the fluorescence is measured without introduction of an appreciable error; after longer periods of time, the purple color fades slowly and the fluorescence is increased.

Table V. Determination of Beryllium Oxide in Minerals Other Than Beryl

(Comparison of gravimetric and fluorometric methods)

Mineral	% BeO Found Gravimetric	% BeO Found Fluorometric
Chrysoberyl	16.5	17.1
Helvite	5.30	5.35
Phenacite	44.6	44.2

APPLICATION TO OTHER MINERALS

Minerals other than beryl may be analyzed for beryllium by the procedures given in this paper. Samples of phenacite, helvite, and chrysoberyl were analyzed by the gravimetric and fluorimetric methods (Table V). The agreement between the two methods was of the same order as that obtained with the standard samples. Phenacite and helvite behaved like beryl and caused no difficulty. Chrysoberyl, however, required a prolonged fusion (30 minutes) followed by solution, filtration, and a second fusion of the residue. Two fusions gave complete solution.

ACKNOWLEDGMENTS

The study reported in this paper was completed under the general direction of J. B. Zadra, chief, College Park Division, Metallurgical Branch, Bureau of Mines, and the immediate supervision of Alton Gabriel.

The authors wish to acknowledge the kind assistance of their associates in the conduct of this work. Especially are they indebted to Roscoe D. Dwiggins for the gravimetric analyses of many samples; to John P. Wintermoyer and the Drafting Unit for the drawings; to Rudolf Kudlich and his men in the shop for the construction of the visual comparator; to the Mineral Dressing Unit for the preparation of the standard samples; to the Geological Survey, the Bureau of Mines Stations at Rolla, Mo., and Salt Lake City, Utah, and the Beryllium Corporation of Pennsylvania for the analyses of the standard samples; and to Alton Gabriel and Morris Slavin for helpful comment and criticism in the preparation of this paper.

LITERATURE CITED

(1) Fletcher, M. H., White, C. E., and Sheftel, M. S., IND. ENG. CHEM., ANAL. ED., 18, 206 (1946).
(2) Hand, D. B., *Ibid.*, 11, 306 (1939).
(3) Palmes, E. D., and Alford, W. C., with Hyslop, F., Monoco, A. R., and Hairhall, L. T., U. S. Public Health Service *Bull.* 181, 10 (1945).
(4) Sandell, E. B., IND. ENG. CHEM., ANAL. ED., 12, 674 (1940).
(5) *Ibid.*, 12, 762 (1940).
(6) White, C. E., and Lowe, C. S., *Ibid.*, 13, 809 (1941).
(7) Zermatten, H. I. J., *Proc. Acad. Sci. Amsterdam*, 36, 899 (1933).

PUBLISHED by permission of the Director, Bureau of Mines, U. S. Department of the Interior.

Determination of Inorganic Phosphorus in Plant Materials

W. A. PONS, JR., AND JOHN D. GUTHRIE
Southern Regional Research Laboratory, New Orleans, La.

A method is described for the determination of inorganic phosphorus in a variety of plant materials, including those high in protein. A trichloroacetic acid extraction and colorimetric evaluation by a modification of the Berenblum and Chain procedure are utilized. The molybdenum blue complex developed in isobutyl alcohol has absorption maxima at 625–630 and 730 mμ, respectively, and is stable for 19 hours.

A METHOD for determining inorganic phosphorus in plant materials was required in investigations on the influence of the phosphorus compounds present on the preparation of oilseed proteins for industrial utilization. It was essential that the method be unaffected by large amounts of protein or by colored and turbid extracts. After consideration of available methods, a trichloroacetic acid extraction of inorganic phosphorus and colorimetric analysis by a modified Berenblum and Chain (*2*) procedure were selected for study. Certain details of the colorimetric method have been improved; the procedure has been adapted to use with either a spectrophotometer or photoelectric colorimeter; and the validity of the method has been investigated.

REAGENTS

Trichloroacetic acid solution, 0.75 *N*. Dissolve 123 grams of reagent grade acid in water, and make up to 1 liter. Make the reagent as needed, or store in a refrigerator.

Molybdate reagent. Dissolve 50 grams of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, in 400 ml. of 10 *N* sulfuric acid and 500 ml. of water, make up to 1 liter, and store in a paraffin-lined bottle.

Sulfuric acid, approximately *N*. Dilute 114 ml. of concentrated sulfuric acid to 4 liters.

Stannous chloride stock solution, 10 grams of stannous chloride hexahydrate dissolved in 25 ml. of concentrated hydrochloric acid. Store in a small glass-stoppered brown bottle.

Stannous chloride, dilute solution. Dilute 1 ml. of stock solution to 200 ml. with approximately *N* sulfuric acid just before use.

Isobutyl alcohol. Commercial grade, with a boiling range 106° to 110° C., is satisfactory.

Ethyl alcohol, 95%.

Standard phosphate solution. Recrystallize A.C.S. grade monobasic potassium phosphate three times from water, dry at 110° C., and store in a desiccator over concentrated sulfuric acid. Dissolve 4.3929 grams of dry salt in 300 ml. of water and 200 ml. of approximately *N* sulfuric acid. Add a few drops of 0.1 *N* potassium permanganate as preservative and make up to 1 liter with water. This stock solution, 1.0 mg. of phosphorus per ml., is stable. Dilutions of the stock solution are made as needed.

ANALYTICAL PROCEDURE

Weigh 1-gram samples of the ground material, 60-mesh or finer into 125-ml. glass-stoppered Erlenmeyer flasks; add exactly 50 ml. of 0.75 *N* trichloroacetic acid; and shake mechanically for 1 hour. Filter through ashless filter paper of medium retentivity, discarding the first portion of the filtrate.

Pipet aliquots containing from 0.005 to 0.045 mg. of inorganic phosphorus (usually 2 ml.) into 125-ml. separatory funnels with a mark at 20 ml. Add 5 ml. of the molybdate–sulfuric acid reagent and distilled water to the 20-ml. mark. Add 10 ml. of isobutyl alcohol and shake for 2 minutes. Discard the aqueous layer, and wash by shaking once with 10 ml. of approximately *N* sulfuric acid. Add 15 ml. of dilute stannous chloride, shake for 1 minute. then discard aqueous layer. Transfer the blue isobutyl alcohol layer to a 50-ml. volumetric flask, washing the funnel with ethyl alcohol. Make to volume with ethyl alcohol. Determine the transmission of the blue solutions against a blank containing all reagents with an Evelyn photoelectric colorimeter, using the No. 720 filter or a spectrophotometer at 730 mμ at any time from 40 minutes to 19 hours after color development.

Prepare a calibration curve by pipetting known concentrations of inorganic phosphorus in the range 0 to 0.045 mg. of phosphorus, obtained by diluting stock phosphate solution, into 125-ml. separatory funnels and developing the color exactly as outlined in the procedure. The logarithms of the per cent transmission values of the standards may be fitted to a straight line by the method of least squares and the calculations for unknowns made from the equation of this line (*7*). The calibration curve once determined for any one instrument need not be repeated, since the standard curve has been found to be reproducible.

An alternate calibration method for the Evelyn colorimeter consists of determining the K_2 constant from the logarithms of the transmission values of the standards. Values for K_2 determined with the Evelyn colorimeter (No. 720 filter) are shown in Table I. When using the Beckman spectrophotometer calculations may be made from the extinction coefficient. The extinction coefficient α at 730 mμ, $\alpha = E^{g/L}_{1\text{ cm.}}$, was found to be 523 using the Beckman spectrophotometer. The concentration of phosphorus, in mg., in the final 50-ml. volume is obtained by the equation:

$$\text{Mg. of P in 50-ml. volume} = \frac{\text{optical density}}{\alpha L} \times 50$$

where L = cell length in cm.

Table I. Calibration Data for Evelyn Colorimeter

Mg. of P in 50-Ml. Volume	Evelyn Colorimeter, 720 Filter: % transmission	Log % transmission	K_2[a]
0.005	80.2	1.9042	0.0521
0.010	64.7	1.8109	0.0529
0.015	52.2	1.7177	0.0531
0.020	41.7	1.6201	0.0526
0.025	33.8	1.5289	0.0531
0.030	27.5	1.4393	0.0535
0.035	22.5	1.3522	0.0540
0.040	18.3	1.2625	0.0542
0.045	15.0	1.1761	0.0546
			Av. 0.0533

Mg. of P = (2-log transmission) × K_2

[a] $K = \frac{\text{2-log transmission}}{\text{mg. of P in 50-ml. volume}}$; $K_2 = \frac{1}{K}$

INVESTIGATION OF EXTRACTION PROCEDURE

Most inorganic phosphorus methods for plant material utilize a dilute sulfuric or hydrochloric acid extraction of inorganic phosphorus (*3*, *6*, *11*). Alcohol acidified with hydrochloric acid has also been used (*5*). For material high in protein, however, extraction with dilute sulfuric or hydrochloric acid is unsatisfactory, since enough protein is extracted to cause a precipitate to form upon addition of ammonium molybdate in the colorimetric analysis, unless this protein is precipitated before analysis. Trichloroacetic acid was found satisfactory in the present method since, in the concentration used, it does not extract protein precipitable by the molybdate reagent and may be assumed to extract only nonprotein nitrogen.

In an experiment on this phase of the method, 1-gram samples of Skellysolve F–extracted peanut meal were extracted for 1 hour with trichloroacetic acid in which the concentration varied from 0.1 to 2.0 *N*. Within the concentration range 0.37 to 1.0 *N*, the amount of inorganic phosphorus extracted, 0.71 mg., was constant. One-gram samples of the same meal were also extracted for 1 hour with both 0.36 *N* and *N* sulfuric acid and 0.55 *N* and *N* hydrochloric acid, protein being precipitated from the filtrates by making them 0.75 *N* with respect to trichloroacetic acid before analysis. The same amount of inorganic phosphorus, 0.71 mg., extracted in each case, was equal to that extracted by trichloroacetic acid in the concentration range 0.37 to 1.0 *N*. Experience with a variety of plant materials has shown 0.75 *N* trichloroacetic acid to be satisfactory for the extraction.

Extraction time was investigated by extracting 1-gram samples of a different solvent-extracted peanut meal for periods ranging from 0.5 to 4 hours with 0.75 N trichloroacetic acid. The amount of inorganic phosphorus extracted, 0.69 mg., was practically constant over the entire time range, the only effect of increasing the time being the extraction of a little more organic phosphorus as determined by difference between the inorganic and total phosphorus contents of the extracts. The constancy of the inorganic phosphorus values with increasing extraction time is evidence that there was no conversion of organic to inorganic phosphorus during the 1-hour extraction of the plant materials investigated.

RECOVERY OF ADDED INORGANIC PHOSPHORUS AND STUDY OF INTERFERENCES

Satisfactory recovery of inorganic phosphorus added to peanut and cottonseed meals in the form of monobasic potassium phosphate, KH_2PO_4, and primary calcium phosphate, $Ca(H_2PO_4)_2.H_2O$, was obtained as shown in Table II. Satisfactory recovery of inorganic phosphorus added to highly colored trichloroacetic acid extracts of goldenrod, sweet potato, and tobacco leaves was also obtained. Tricalcium phosphate, $Ca_3(PO_4)_2$, is also soluble in 0.75 N trichloroacetic acid.

No interference due to colored extracts has been observed. Chlorophyll is not extracted by 0.75 N trichloroacetic acid. The yellow color of most plant extracts did not pass into the isobutyl alcohol and consequently was discarded with the aqueous phase. In cases where color was extracted by the isobutyl alcohol, examples being cottonseed meal, wheat straw, goldenrod, and sweet potato leaves, the extracted color had no absorption at 730 mμ and consequently did not interfere. Blanks run on all the above colored extracts, adding 5 ml. of 4 N sulfuric acid instead of the acid molybdate reagent, gave transmission values ranging from 99.5 to 100% when read against 10 ml. of isobutyl alcohol made up to 50 ml. with 95% ethyl alcohol.

With a few plant materials, notably cottonseed, turbid solutions were obtained on extraction with 0.75 N trichloroacetic acid. Shaking with isobutyl alcohol and washing once with N sulfuric acid in the method cleared these extracts. No turbidity has been encountered in the final blue solutions.

Table II. Recovery of Inorganic Phosphorus Added to Petroleum Ether–Extracted Peanut and Cottonseed Materials

Material, 0.5 Gram	Inorganic Phosphorus In sample	Added	Total	Found	Recovered
	Mg.	*Mg.*	*Mg.*	*Mg.*	%
Peanut kernels	0.378	0.500[a]	0.878	0.883	101
Peanut testa	0.180	0.500[a]	0.680	0.673	99
Cottonseed meal	0.301	0.500[a]	0.801	0.795	99
Peanut kernels	0.365	2.255[b]	2.620	2.600	99
Cottonseed meal	0.390	2.458[b]	2.848	2.800	98

[a] Added as KH_2PO_4.
[b] Added as $Ca(H_2PO_4)_2.H_2O$.

Most of the trichloroacetic acid extracts of the plant materials investigated contained reducing agents which caused a partial reduction to molybdenum blue prior to the addition of stannous chloride, the extent varying considerably with different plant materials. Although this premature reduction does not affect the precision of the method, inasmuch as a large excess of stannous chloride is used to develop the maximum molybdenum blue color, it might affect those inorganic phosphorus methods in which the concentration of reducing agent must be carefully controlled.

To test the applicability of the method in the presence of organic phosphorus compounds, 1-gram samples of solvent-extracted peanut meal were weighed, and 25 mg. of the various organic phosphorus compounds listed in Table III were added, and carried through the analytical procedure. Suitable controls for the meal alone and for each phosphorus compound were also run. The samples were allowed to stand at room temperature for 24 hours, and were then again analyzed for inorganic phosphorus.

Table III. Stability of Organic Phosphorus Compounds Added to 1-Gram Samples of Solvent-Extracted Peanut Meal under Method Conditions

Organic Phosphorus Compound Added, 25 Mg.	Inorganic Phosphorus Present: In meal	In added compound	Total	Inorganic Phosphorus Found: 1-hour extraction	24-hour extraction at 25° C.
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Na-β-glycerophosphate	...	0.000	0.000	0.000	0.000
Na-β-glycerophosphate + meal	0.723	0.000	0.723	0.723	0.745
Di-K-glucose-1-phosphate	...	0.019[a]	0.019	0.228	1.388
Di-K-glucose-1-phosphate + meal	0.723	0.019	0.742	0.915	1.083
Fructose diphosphate	...	0.015[a]	0.015	0.025	0.145
Fructose diphosphate + meal	0.723	0.015	0.738	0.750	0.883
Adenylic acid	...	0.000	0.000	0.000	0.000
Adenylic acid + meal	0.723	0.000	0.723	0.720	0.745
Peanut phytin	...	0.032	0.032	0.028	0.028
Peanut phytin + meal	0.710	0.032	0.742	0.745	0.770

[a] Calculated from inorganic phosphorus analysis by shakeout method and extrapolation to zero time.

From the results shown in Table III it is evident that glucose-1-phosphate is rapidly hydrolyzed by the 0.75 N trichloroacetic acid used in the extraction step. Fructose diphosphate is hydrolyzed very slowly, while sodium β-glycerophosphate, adenylic acid, and phytin show no hydrolysis after 24 hours in contact with the acid. Glucose-1-phosphate is said to be involved in one step of plant carbohydrate metabolism through phosphorylation; and the determination of inorganic phosphorus is frequently used to follow the progress and direction of phosphorylation, with glucose-1-phosphate as a substrate (*4, 10*).

To determine the hydrolysis of glucose-1-phosphate in the shakeout procedure, 10-mg. samples of the dipotassium salt of glucose-1-phosphate were added to a series of separatory funnels along with 5 ml. of the acid-molybdate reagent. The samples were analyzed after standing for various periods determined from the time the molybdate was added to the time at which the shaking with stannous chloride was completed. The shortest interval, 4 minutes, showed 0.07% hydrolysis of glucose-1-phosphate, 5 minutes 0.13%, 10 minutes 0.37%, 28 minutes 0.97%, and 67 minutes 2.87% hydrolysis. The 5-minute value of 0.13% hydrolysis compares favorably with that found by Hanes (*10*), who, with the method of Allen (*1*), obtained 0.4% hydrolysis of glucose-1-phosphate in 5 minutes' contact with 0.77 N perchloric acid.

No increase in inorganic phosphorus was found after the developed colored solutions were allowed to stand overnight, showing that complete separation of inorganic phosphorus from glucose-1-phosphate was obtained in the shakeout method. The shakeout method should prove valuable when it is used to follow enzymatic synthesis by measuring increase or decrease in inorganic phosphorus. An almost insignificant amount of hydrolysis of glucose-1-phosphate takes place in the approximately 5 minutes needed for analysis; and, once developed, the colorimetric readings may be taken at any time up to 19 hours after development. The time factor, which must be carefully controlled in the usual colorimetric methods when glucose-1-phosphate is present, is thereby eliminated.

Inasmuch as compounds such as glucose-1-phosphate and fructose diphosphate were found to be hydrolyzed by 0.75 N trichloroacetic acid in the extraction step, 1-gram samples of the plant materials listed in Table IV were simultaneously extracted for 24 hours at room temperature and 24 hours at 5° C., after which the extracts were analyzed for inorganic phosphorus.

Although from the results in Table IV it is evident that easily hydrolyzable organic phosphorus compounds do not appear to be present in any significant amount in the plant materials investigated, with other materials 1-hour extraction should be compared with overnight extraction to establish the absence of these compounds.

EVALUATION OF MOLYBDENUM BLUE COMPLEX

Spectrophotometric curves of the molybdenum blue complex developed by the shakeout method for 0.030 mg. of inorganic phosphorus and for extracts of peanut and cottonseed meals of approximately the same phosphorus concentration are shown in Figure 1. A Beckman quartz spectrophotometer with a 1-cm. cell was used, a reagent blank being the reference solution. All three curves show the same two points of maximum absorption at 625–630 mμ and at 730 mμ, which are characteristic. The maximum at 730 mμ is much sharper than that at 625–630 mμ, and measurements should be made at 730 mμ to obtain maximum sensitivity. That the position of the maxima is unchanged by the presence of plant extracts, as shown in Figure 1, is evidence that materials extracted from plants do not interfere with the determination by the shakeout method. In another experiment with the same concentration of phosphorus, dilution to volume with isobutyl alcohol did not change the position of the two maxima, proving that these are not due to the influence of ethyl alcohol. The use of isobutyl alcohol, however, intensifies the color and decreases transmission at 730 mμ by about 7%, which demonstrates that the ratio of isobutyl to ethyl alcohol should be kept constant in the method.

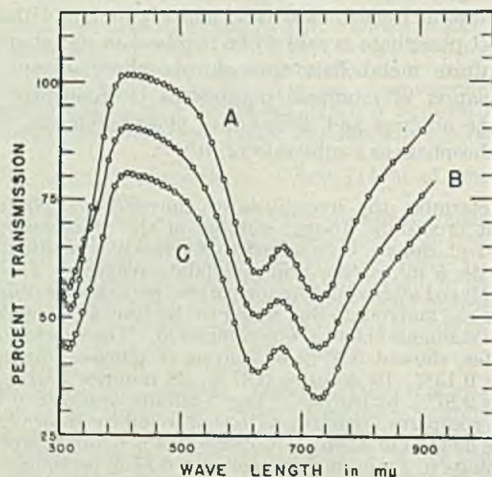


Figure 1. Spectrophotometric Curves of Molybdenum Blue Complex

A. Trichloroacetic acid extract of peanut meal, 0.029 mg. of phosphorus. Curve set off +10 units
B. Standard phosphorus solution, 0.030 mg. of phosphorus
C. Trichloroacetic acid extract of cottonseed meal, 0.031 mg. of phosphorus. Curve set off −10 units

Extraction of the molybdenum blue developed by the methods of Fontaine (*8*) and Gerritz (*9*) with 10 ml. of isobutyl alcohol produced solutions having single maxima at 780 mμ for the Fontaine method and at 800 mμ for the Gerritz method. For both procedures, the absorption maxima of the solutions prior to extraction with isobutyl alcohol were at 820 mμ. The shape of the spectrophotometric curves for each of the complexes extracted with isobutyl alcohol was similar to that of the curves for the unextracted complexes.

That the molybdenum blue developed by the shakeout method is very stable in the mixture of isobutyl and ethyl alcohol was shown when 0.005, 0.025, and 0.045 mg. of phosphorus were carried through the colorimetric procedure, placed in well-stoppered colorimeter tubes, and read in the Evelyn colorimeter (No. 720 filter) at once and at intervals up to 19 hours. The colors were found to increase slowly up to 40 minutes after development, and then remain constant for 19 hours. The transmission values of unknowns, checked from time to time after the solutions were allowed to stand overnight, have been found to be unchanged.

Table IV. Inorganic Phosphorus Content of Various Plant Materials on Dry Basis

Material	Total Phosphorus	Inorganic Phosphorus: 1-hour extraction	Inorganic Phosphorus: 24-hour extraction at 25° C.	Inorganic Phosphorus: 24-hour extraction at 5° C.
	%	%	%	%
Cottonseed meal, diethyl ether extracted	1.722	0.085	0.085	0.085
Cottonseed meal, Skellysolve B extracted	1.711	0.071	0.073	0.071
Peanut kernels, Skellysolve F extracted	0.849	0.081	0.082	0.079
Raw cotton fiber	0.028	0.015	0.015	0.015
Sweet potatoes, L-5	0.135	0.075	0.076	0.076
Jerusalem artichokes	0.385	0.083	0.085	0.085
U. S. 13 corn[a]	0.282	0.016	0.016	0.016
Milo[a]	0.274	0.016	0.018	0.017
Federation wheat[a]	0.377	0.018	0.019	0.019
Kharkof wheat[a]	0.445	0.017	....	....
Wheat straw[a]	0.165	0.118	0.122	0.119
Soybean meal, Skellysolve F extracted[b]	0.750	0.036	0.037	0.036
Phytin, crude, from peanuts[b]	14.31	0.070	....	....
Phytin, crude, from cottonseed[b]	13.84	0.061	....	....
Dialyzed peanut protein[b]	0.650	0.014	....	....
Dialyzed cottonseed protein[b]	1.164	0.013	....	....

[a] From Northern Regional Research Laboratory.
[b] Prepared by T. D. Fontaine.

PRECISION

Sixteen determinations made on the same sample of solvent-extracted peanut meal over a period of several months gave an average value of 0.0717% inorganic phosphorus with a standard deviation of ±0.0017%. Twenty-five determinations on another sample gave an average value of 0.0696% inorganic phosphorus with a standard deviation of ±0.0012%.

APPLICABILITY

The general applicability of the method is illustrated by Table IV, where the inorganic phosphorus content of a number of plant materials is reported. Total phosphorus values obtained by the reduced molybdate method of Gerritz (*9*) are also given.

ACKNOWLEDGMENT

The authors are indebted to R. T. O'Connor for the spectrophotometric curves, to E. B. Schuman for preparation of the figures, and to T. D. Fontaine for the preparation of the peanut and cottonseed meals and for a number of suggestions. The samples of glucose-1-phosphate and sodium β-glycerophosphate were obtained through the generosity of David M. Greenberg of the University of California Medical School and J. P. Remensnyder of the Heyden Chemical Corporation, respectively.

LITERATURE CITED

(1) Allen, R. J. L., *Biochem. J.*, 34B, 858 (1940).
(2) Berenblum, I., and Chain, E., *Ibid.*, 32, 295 (1938).
(3) Bertramson, B. R., *Plant Physiol.*, 17, 447 (1942).
(4) Bliss, L., "A Study of the Phosphorylase of Waxy Maize", unpublished Ph.D. thesis, Iowa State College Library, Ames, Iowa, 1943.
(5) Collison, R. C., J. Ind. Eng. Chem., 4, 606 (1912).
(6) Emmert, E. M., *Plant Physiol.*, 5, 413 (1930).
(7) Ezekiel, M., "Methods of Correlation Analysis", New York, John Wiley & Sons, 1930.
(8) Fontaine, T. D., Ind. Eng. Chem., Anal. Ed., 14, 77 (1942).
(9) Gerritz, H. W., *J. Assoc. Official Agr. Chem.*, 23, 321 (1940).
(10) Hanes, C. S., *Proc. Roy. Soc.*, B129, 174 (1940).
(11) Litynski, T., *Roczniki Chem.*, 13, 578 (1932).

Quantitative Determination of Ergosterol, Cholesterol, and 7-Dehydrocholesterol

Antimony Trichloride Method

FRANCES W. LAMB[1], ALEXANDER MUELLER, AND GEORGE W. BEACH[2]
Gelatin Products Corporation, Detroit, Mich.

Methods for the quantitative determination of ergosterol, cholesterol, and 7-dehydrocholesterol are based upon the spectrophotometric measurement of the optical densities of the characteristic absorption maxima of the reaction products resulting from the treatment of the chloroform solutions of these sterols with an antimony trichloride–acetyl chloride reagent. The absorption spectra of the reaction products of the three sterols are distinctly different. By this method it is possible to differentiate between ergosterol and 7-dehydrocholesterol. This differentiation is not possible by the direct ultraviolet spectrophotometric method. A comparison has been made of results obtained by both the direct spectrophotometric and the antimony trichloride method in the determination of ergosterol in three different types of yeast and one mold extract.

THE most reliable method for the quantitative determination of ergosterol has been the spectrophotometric measurement of its ultraviolet absorption spectrum, with its characteristic absorption maxima at wave lengths of 262, 271, 281, and 293 millimicrons. However, as shown both by Hogness, Sidwell, and Zscheile (*6*) and Huber, Ewing, and Kriger (*7*), the ultraviolet absorption spectra of ergosterol and 7-dehydrocholesterol are so nearly identical that it is impossible to differentiate between these two compounds spectrophotometrically. Accurate spectrophotometric measurements in the ultraviolet region require that the sample be highly purified, since numerous organic compounds show either general or specific absorption in this region and would thus contribute to the absorption measurements. Furthermore, a spectrophotometer with ultraviolet accessories including a satisfactory ultraviolet light source is required for the determination of ergosterol by this method. A method requiring less expensive equipment and less labor in purification of sample than required by the direct ultraviolet method is desirable.

A number of colorimetric methods employing concentrated sulfuric acid (*1, 9, 12*), acetic anhydride (*2, 5, 10, 16*), trichloroacetic acid (*14*), antimony trichloride (*5, 8, 13*), and various combinations of these reagents have been reported in the literature for the determination of ergosterol, 7-dehydrocholesterol, cholesterol, and other sterols. However, these methods as reported lack the desired specificity and sensitivity. Several of these methods were investigated by making absorption measurements of their respective colored reaction products by means of the Beckman quartz spectrophotometer Model DU. Some interesting observations, which were made on two of the above methods are reported here, since it is thought they may be of value to other investigators in this field.

In repeating the work of Petersen and Harvey (*12*) the authors have found that the colored solution produced by the treatment of ergosterol with 90% sulfuric acid has an absorption curve as shown in Figure 1 with two maxima. One maximum occurs at a wave length of 315 mμ with an $E^{1\%}_{1\text{cm.}}$ value of approximately 333 and another at 415 mμ with an $E^{1\%}_{1\text{cm.}}$ value of approximately 196. Spectrophotometric measurements show that the absorption maxima of this reaction product become stable 50 to 60 minutes after the addition of the reagent. These two maxima seem to have been overlooked by Petersen and Harvey, since they based their method of determination upon a measurement of the per cent transmission at a wave length of 550 mμ. While neither of the two observed maxima is very sharp or well defined, the $E^{1\%}_{1\text{ cm.}}$ values are high and if measurements were made at either of these wave lengths instead of at 550 mμ the sensitivity and accuracy of the method might be greatly improved.

In a study of the method described by Goldhammer and Kuen (*4*), spectrophotometric measurements were made on the blue colored reaction product produced by the treatment of ergosterol with an antimony trichloride–acetic anhydride reagent in accordance with their method. Table I shows the absorption data of a chloroform solution of the ergosterol–antimony trichloride–acetic anhydride reaction product at various time intervals. The most prominent and stable maxima are the ones occurring at a wave length of 680 mμ with an $E^{1\%}_{1\text{ cm.}}$ value of 43.6 and at 390 mμ with an $E^{1\%}_{1\text{ cm.}}$ value of 55.0. The maximum at 390 mμ was found to be sharp and well defined but the extinction value is rather low for analytical purposes, and further it has not reached its maximum intensity at the end of 50 minutes. The 680 mμ maximum is also of low intensity.

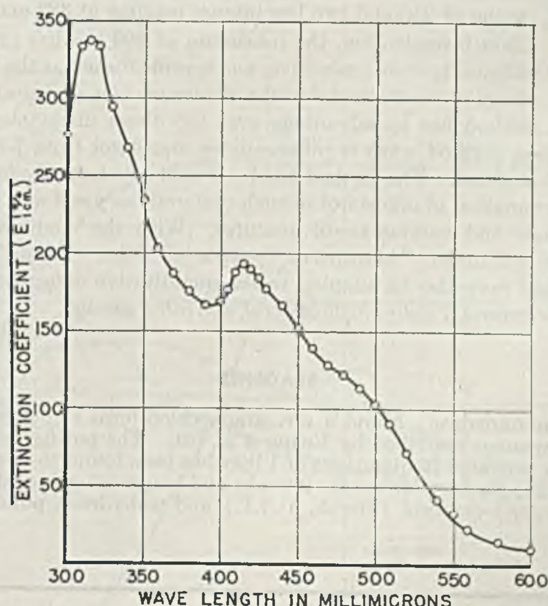


Figure 1. Absorption Spectrum of Ergosterol–Sulfuric Acid Reaction Product

The method for the determination of ergosterol which has proved most satisfactory for the authors' needs results from the treatment of ergosterol with an antimony trichloride–acetyl chloride reagent prepared according to Nield, Russell, and Zimmerli (*11*). Upon treatment of a chloroform solution of pure

[1] Present address, Ethyl Corporation, Detroit, Mich.
[2] Present address, Libby, McNeil & Libby, Chicago, Ill.

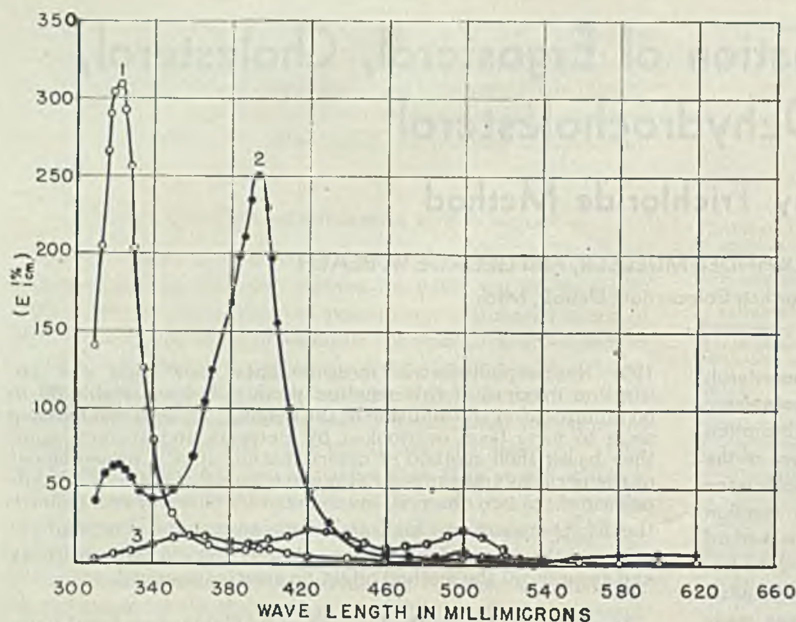


Figure 2. Absorption Spectra of Sterol–Antimony Trichloride–Acetyl Chloride Reaction Products

1. 7-Dehydrocholesterol–antimony trichloride–acetyl chloride reaction product. Measurements made 5 to 15 minutes after addition of reagent
2. Ergosterol–antimony trichloride–acetyl chloride reaction product. Measurements made 5 to 15 minutes after addition of reagent
3. Cholesterol–antimony trichloride–acetyl chloride reaction product. Measurements made 1 to 15 minutes after addition of reagent

ergosterol with this reagent a yellow color develops, which is stable at the end of 2 minutes. A reddish-yellow color is obtained on ergosterol samples which have not been purified. A chloroform solution of the colored compound was found to have an absorption spectrum as shown in curve 2 of Figure 2 with a prominent maximum at a wave length of 393 mμ with an $E^{1\%}_{1\text{ cm.}}$ value of 250 and two less intense maxima at 322 and 505 mμ. Upon investigation, the maximum at 393 mμ has proved to be sufficiently stable, sensitive, and specific for use as the basis of a quantitative method for the determination of ergosterol. This method has an advantage over the direct ultraviolet absorption method since it differentiates ergosterol from 7-dehydrocholesterol. The method has been used satisfactorily for the determination of ergosterol in such materials as yeast and plant extracts and various sterol mixtures. With the modifications described under "Absorption Spectra of Other Sterols" this method may also be adapted to the quantitative determination of cholesterol, 7-dehydrocholesterol, and other sterols.

REAGENTS

Chloroform. Merck's c.p. grade chloroform is purified in the manner described by Ewing *et al.* (*3*). The purified chloroform prepared in quantities of 1 liter has been found to be stable and satisfactory for use for 2 weeks and longer when stored over activated charcoal (Merck, U.S.P.) and anhydrous potassium carbonate. The chloroform stored in this manner is first shaken well and then filtered just prior to using. The usual qualitative tests for the presence of alcohol and phosgene are made to ensure their absence.

Antimony Trichloride–Acetyl Chloride Reagent. The antimony trichloride-acetyl chloride reagent is prepared according to the method of Nield, Russell, and Zimmerli (*11*). This reagent, containing 18 grams of antimony trichloride and 3 ml. of acetyl chloride per 100 ml. of chloroform (18% reagent), is used for the quantitative determination of both ergosterol and 7-dehydrocholesterol. For the determination of cholesterol a reagent containing 30 grams of antimony trichloride and 3 ml. of acetyl chloride per 100 ml. of chloroform (30% reagent) is prepared. In determining cholesterol the use of the 30% reagent was found to improve the reproducibility and sensitivity of the method. When pure dry chloroform and pure acetyl chloride are used very little difficulty is encountered with the reagent. It has been found to remain uncolored and satisfactory for use when stored for periods of 1 and 2 weeks in an amber bottle at room temperature.

Table I. Extinction Coefficients of Absorption Maxima of Ergosterol–Antimony Trichloride–Acetic Anhydride Reaction Product at Various Time Intervals

Wave Length, Millimicrons	$E^{1\%}_{1\text{ cm.}}$ of Absorption Maxima 1 to 5 min.	15 to 30 min.	30 to 40 min.
390	4.1	34.0	55.0
520	23.0	No max.	No max.
580	No max.	24.0	No max.
680	20.0	43.6	43.6

EXPERIMENTAL PROCEDURE

Method. Solutions containing 0.005 to 0.05% ergosterol are prepared in purified chloroform. To 1 ml. of the ergosterol solution in a 50-ml. beaker, 10 ml. of the 18% antimony trichloride-acetyl chloride reagent are added. The optical density of the absorption maximum, which develops at 393 mμ, is read on a suitable spectrophotometer exactly 5 minutes after the addition of the reagent.

A Beckman DU quartz spectrophotometer with 10.0-mm. Corex absorption cells and incandescent light source was used for all spectrophotometric measurements reported in this paper. The slit width is maintained at 0.068 to 0.072 mm. when taking readings at 393 mμ. The amount of ergosterol present in the solution is calculated from the $E^{1\%}_{1\text{ cm.}}$ value of 250 at 393 mμ for pure ergosterol.

Purification of Ergosterol and Determination of Its $E^{1\%}_{1\text{ cm.}}$ Value. In the preceding paper the extinction coefficients given at 322, 393, and 505 mμ were the results of measurements made upon commercial ergosterol. Since the ultraviolet absorption spectrum of this material conformed to the data reported in 1937 by Hogness, Sidwell, and Zscheile (*6*) it was considered to be reasonably pure ergosterol. However, when the ultraviolet absorption data of Huber, Ewing, and Kriger (*7*) were reported, it was realized that the commercial samples of ergosterol used for these observations were not 100% pure.

For this study commercial ergosterol was purified by recrystallization from acetone containing approximately 1% water. In the purification 4 to 5 grams of ergosterol were dissolved in 100 ml. of pure, acid-free acetone by heating on a 70° C. water bath. Three to 5 drops of distilled water were added to the hot solution with stirring. Upon slowly cooling to room temperature the ergosterol crystallized from the clear solution as fine, small needles. The crystals were filtered by suction and a portion dried in an Abderhalden vacuum dryer at 60° C. for 1 hour. The greater part of the crystals were redissolved in acetone and recrystallized. After each recrystallization a dried portion was used for determination of its melting point and $E^{1\%}_{1\text{ cm.}}$ values by the ultraviolet absorption and antimony trichloride methods. The data obtained are given in Table II.

These physical constants show an increase in purity of the ergosterol upon successive recrystallizations. For the eighth, ninth, and tenth recrystallizations the melting point remained constant and the ultraviolet and antimony trichloride values were constant within the limits of error for individual determinations. These findings are contrary to the statement of Huber *et al.* (*7*); "The highest values are obtained by recrystallizing

Table II. Physical Constant Data for Ergosterol Following Successive Recrystallizations

Sample	Melting Point[a], ° C.	E (1%, 1 cm.)[b] Values at Following λ: Ultraviolet 271 mμ	Ultraviolet 282 mμ	Ultraviolet 293 mμ	$SbCl_3$ 322 mμ	$SbCl_3$ 393 mμ	$SbCl_3$ 505 mμ
As received	155–164	242.8	255.3	148.2	66.7	212.1	33.3
Recrystallization							
1	162–165	269.3	285.2	162.3	69.1	239.8	20.3
2	164–166	269.7	284.1	161.8	61.3	241.3	17.8
3	164–166.5	270.4	285.9	161.4	59.9	246.1	14.9
4	164–166.5	276.0	291.6	163.1	59.2	248.2	11.4
5	164–166.5	275.0	288.1	165.4	61.3	245.3	10.2
6	164.5–167	274.5	288.3	163.6	70.0	252.0	13.2
7	165–167	276.7	289.0	167.1	64.9	249.7	12.1
8	166–167	276.1	289.1	164.9	63.6	250.4	13.1
9	166–167	275.5	287.6	164.2	60.2	249.0	13.6
10	166–167	276.9	290.8	166.5	63.5	252.2	13.1

[a] Melting points obtained with Anschütz thermometer and sealed melting point tubes.
[b] Data represent single trials and consequently do not conform with average values presented in Table III.

once only the commercially available material". This divergence in results is undoubtedly due to the difference in solvents, since these authors recrystallized their ergosterol from a benzene-ethanol solvent. From Table II it is noted that as the ergosterol is purified the antimony tricholoride maximum at 505 mμ decreases and the maximum at 393 mμ increases, while the maximum at 322 mμ fluctuates although it shows a somewhat lower value upon purification.

Over twenty individual determinations of the $E^{1\%}_{1\text{ cm.}}$ values at the 322, 393, and 505 mμ maxima were made on the purified ergosterol by the antimony trichloride method. The average $E^{1\%}_{1\text{ cm.}}$ value was found to be 62.9 at 322 mμ, 250.02 at 393 mμ, and 12.4 at 505 mμ. The individual determinations of the $E^{1\%}_{1\text{ cm.}}$ values at 393 mμ showed a maximum deviation of ±2.0% from the average. For optical density (log I_0/I) readings of 0.20 to 1.00, the ergosterol–antimony trichloride–acetyl chloride reaction product was found to obey Beer's law.

Time Rate of the Reaction. The absorption maximum of the ergosterol–antimony trichloride–acetyl chloride reaction product at 393 mμ reaches its maximum intensity in a little less than 2 minutes after the addition of the reagent and remains stable for 6 to 7 minutes. The rate of the reaction is shown graphically in curve 1 of Figure 3, in which the optical density (log I_0/I) at 393 mμ is plotted against time. The optical density readings become steady after the addition of the reagent and remain constant for 6 minutes. For this reason the procedure standardized upon in the method for determining ergosterol has been to make the spectrophotometric measurements exactly 5 minutes after the addition of the antimony trichloride–acetyl chloride reagent.

Effect of Temperature. Ergosterol determinations were made at 20°, 25.5°, and 30° C, which cover the range of ordinary laboratory temperatures. The results obtained showed no measurable variation.

Effect of Solvent Purity. The reproducibility of the reaction between ergosterol and the antimony trichloride reagent as determined by spectrophotometric measurements is very sensitive to the presence of traces of moisture and polar solvents, which if present may cause the results to vary as much as 10%. To ensure reproducible results the reagents must be carefully purified and the sample dried in a stream of warm carbon dioxide (80° to 90° C.).

Absorption Spectra of Other Sterols. Figure 2 shows the absorption spectra of the antimony trichloride–acetyl chloride reaction product with ergosterol, cholesterol, and 7-dehydrocholesterol, using the 18% reagent. All measurements were made during the 1- to 15-minute period following the addition of the antimony trichloride reagent. From these curves it is seen that the reaction products of 7-dehydrocholesterol and ergosterol have absorption spectra which are distinctly different, making it possible to differentiate the two clearly and also to determine them individually in the presence of each other. Except for the method recently reported by Sobel, Mayer, and Kramer (*15*) for the differentiation of ergosterol and 7-dehydrocholesterol by treating the sterols with glycerol dichlorohydrin in the presence of acetyl chloride, this is the only method known to the authors for the differentiation and determination of ergosterol

1. Optical density measurements with time of absorption maximum at 393 mμ of ergosterol reaction product. Concentration of ergosterol 0.002072%
2. Optical density measurements with time of absorption maximum at 322 mμ of 7-dehydrocholesterol reaction product. Concentration of 7-dehydrocholesterol 0.001875%
3. Optical density measurements with time of absorption maximum at 320 mμ of cholesterol reaction product. Concentration of cholesterol 0.0069%
4. Same as 3 except for absorption maximum at 360 mμ of cholesterol reaction product
5. Same as 3 except for absorption maximum at 420 mμ of cholesterol reaction product
6. Same as 3 except for absorption maximum at 500 mμ of cholesterol reaction product

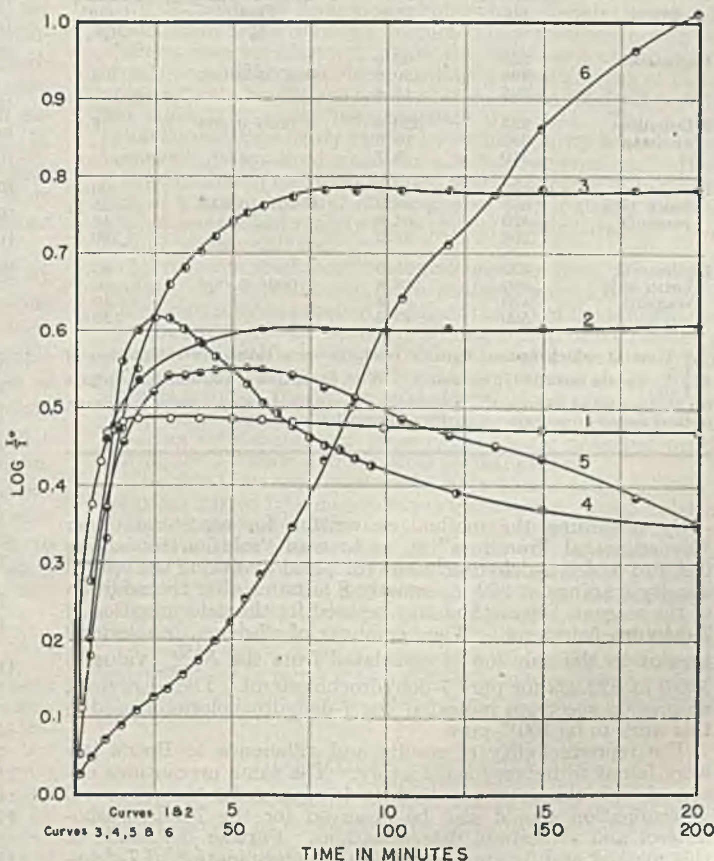


Figure 3. Time Reaction Curves

and 7-dehydrocholesterol, when present together. The absorption curve for cholesterol is of relatively low intensity. Consequently the presence of cholesterol would not interfere appreciably with the determination of either ergosterol or 7-dehydrocholesterol. The four absorption maxima of the cholesterol reaction product develop much more slowly and irregularly than the maxima for ergosterol and 7-dehydrocholesterol.

Figure 3 shows the time rate of the development of the various absorption maxima of the cholesterol, ergosterol, and 7-dehydrocholesterol reaction products, using the 18% antimony trichloride reagent. In the case of ergosterol the absorption maximum at 393 mμ is stable during 2 to 8 minutes after the addition of the reagent, and in the case of 7-dehydrocholesterol the absorption maximum at 322 mμ is stable during 6 to 15 minutes after addition of the reagent. The maximum for cholesterol at 360 mμ develops strongly during the first 20 to 25 minutes and then decreases in intensity. Of the four maxima, the one at 360 mμ was found to be the most satisfactory for the determination of cholesterol from the standpoint of sensitivity, reproducibility, time for development of maximum intensity, and adherence to Beer's law. The results obtained with the 420, 500, and 320 mμ maxima show considerable variation and the reproducibility is poor, with either the 18% or 30% reagent. A summary of the absorption maxima, corresponding $E^{1\%}_{1\text{ cm.}}$ values, optimum concentration range, and time for development of stable absorption maxima for the three sterol antimony trichloride–acetyl chloride reaction products is given in Table III. The data for cholesterol with both the 30% and 18% reagents are given.

Table III. Absorption Data for Ergosterol, Cholesterol, and 7-Dehydrocholesterol–Antimony Trichloride–Acetyl Chloride Reaction Products

Sterol	λ of Maximum mμ	$E^{1\%}_{1\text{ cm.}}$	Range of Optimum Concentration (Final) %	Reading Time[a] *Min.*
Ergosterol	322	62.9	...	...
	393	250.0	0.0005–0.005	5
	505	12.4	...	...
7-Dehydrocholesterol	322	320.0	0.0003–0.003	8
	390	10.0	...	...
	510	6.7	...	...
Cholesterol (with 18% reagent)	320	111.7	...	90
	360	99.9	0.001 –0.010	25
	420	63.9	...	40
	500	87.5	...	120
Cholesterol (with 30% reagent)	320	125.9	...	90
	360	168.8	0.0006–0.006	20
	420	53.8	...	40
	500	40.3	...	120

[a] Time at which optical density readings were taken for calculation of $E^{1\%}_{1\text{ cm.}}$ values recorded in column 3. With exception of 120-minute readings for cholesterol at 500 mμ, times stated are those for development of maximum optical density readings at various absorption maxima.

By rewording the method as written for ergosterol under "Experimental Procedure" so as to read "solution containing 0.003 to 0.03% 7-dehydrocholesterol", and by taking the optical density readings at 322 mμ exactly 8 minutes after the addition of the reagent, the method may be used for the determination of 7-dehydrocholesterol. The amount of 7-dehydrocholesterol present in the solution is calculated from the $E^{1\%}_{1\text{ cm.}}$ value of 320.0 at 322 mμ for pure 7-dehydrocholesterol. The ultraviolet absorption spectrum indicated the 7-dehydrocholesterol used in this work to be 100% pure.

The reproducibility of results and adherence to Beer's law were found to be very satisfactory. The same precautions observed with the antimony trichloride reagent for the ergosterol determination should also be observed for the 7-dehydrocholesterol and cholesterol determinations. Further discussion of this method and its application to the determination of 7-dehydrocholesterol will be given by the authors in a later paper.

Table IV. Comparison of Ergosterol Determinations in Yeast and Mold Extracts by Direct Spectrophotometric and Antimony Trichloride Methods

Sample	Calculated from Spectrophotometric Measurements 271 mμ	282 mμ	293 mμ	Antimony Trichloride Method
	Milligrams per gram			
Yeast 1	1.17	1.05	1.24	1.03
Yeast 2	2.78	2.63	2.87	2.29
Yeast 3	2.94	2.79	3.06	52.41
Mold 1	6.18	6.12	8.37	4.80

For the determination of cholesterol, it was found preferable to use a reagent containing 30 grams of antimony trichloride and 3 ml. of acetyl chloride per 100 ml. of chloroform. In determining cholesterol, 10 ml. of the 30% reagent are added to 1 ml. of a chloroform solution containing 0.006 to 0.06% cholesterol. The optical density reading at 360 mμ is taken at its maximum value, which is reached during the 20- to 25-minute period following the addition of the reagent. The amount of cholesterol is calculated from the $E^{1\%}_{1\text{ cm.}}$ value at 360 mμ for pure cholesterol. The average $E^{1\%}_{1\text{ cm.}}$ value for pure cholesterol at 360 mμ was found to be 168.8 with a maximum deviation of ± 5.0%.

DISCUSSION

The antimony trichloride method here described has been applied to the determination of ergosterol in one plant and three yeast extracts. The ultraviolet absorption data were also obtained. A comparison of the results by the two methods is tabulated in Table IV. In each case the results by the antimony trichloride method are lower than those obtained by a direct measurement of the ultraviolet absorption spectrum. This may be attributed to the fact that the direct spectrophotometric method is more liable to high results due to the presence of impurities. The variation in per cent concentration of ergosterol calculated at the maxima 271, 282, and 293 mμ also indicates the presence of impurities. It is likely that the interfering substances are contributing to the measured density values.

A thorough investigation of the application of the antimony trichloride method to natural materials has not yet been made; however, the few results presented in Table IV are indicative of its possibilities. In determining the amount of ergosterol in impure samples these data indicate the advisability of supplementing the ultraviolet absorption measurements with the antimony trichloride values in order to make a more complete evaluation of the sample.

LITERATURE CITED

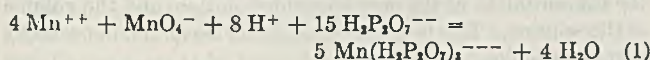
(1) Bandow, F., *Biochem. Z.*, 301, 37 (1939).
(2) Eck, J. C., and Thomas, B. H., *J. Biol. Chem.*, 128, 267 (1939).
(3) Ewing, D. T., Kingsley, G. V., Brown, R. A., and Emmett, A. D., Ind. Eng. Chem., Anal. Ed., 15, 301 (1943).
(4) Goldhammer, H., and Kuen, F. M., *Biochem. Z.*, 267, 406 (1933).
(5) Hoffmann, Wm. S., *Proc. Soc. Expt. Biol. Med.*, 43, 38 (1940).
(6) Hogness, T. R., Sidwell, A. E., and Zscheile, F. P., *J. Biol. Chem.*, 120, 239 (1937).
(7) Huber, W., Ewing, G. W., and Kriger, J., *J. Am. Chem. Soc.*, 67, 609 (1945).
(8) Kerppola, W., *Skand. Arch. Physiol.*, 56, 173 (1929).
(9) Levine, V. E., and Richman, E., *Proc. Soc. Expt. Biol. Med.*, 27, 833 (1930).
(10) Messemaecker, R., *Compt. rend.*, 190, 216 (1930).
(11) Nield, C. H., Russell, W. C., and Zimmerli, A., *J. Biol. Chem.*, 136, 73 (1940).
(12) Petersen, R. B., and Harvey, E. H., Ind. Eng. Chem., Anal. Ed., 16, 495 (1944).
(13) Raoul, Y., and Meunier, P., *Compt. rend.*, 209, 546 (1939).
(14) Rosenheim, O., *Biochem. J.*, 23, 47 (1929).
(15) Sobel, A. E., Mayer, A. M., and Kramer, Benjamin, Ind. Eng. Chem., Anal. Ed., 17, 160 (1945).
(16) Sundermann, F. W., and Rasek, J., *J. Biol. Chem.*, 118, 397 (1937).

New Method for Determination of Manganese

JAMES J. LINGANE AND ROBERT KARPLUS
Mallinckrodt Chemical Laboratory, Harvard University, Cambridge 38, Mass.

A new method for determination of manganese is based on the potentiometric titration of manganous ion with permanganate ion in neutral pyrophosphate solution. The method is applicable without any separations to a wide variety of manganiferous metallurgical products, and it yields very accurate results.

THIS paper presents a new method for the determination of manganese based on the titration of manganous ion with permanganate ion in neutral pyrophosphate solution, the +2 manganese being oxidized, and the permanganate ion reduced, to a pyrophosphate complex of the +3 state. The stoichiometry of the reaction may be represented by



$$4\,Mn^{++} + MnO_4^- + 8\,H^+ + 15\,H_2P_2O_7^{--} = 5\,Mn(H_2P_2O_7)_3^{---} + 4\,H_2O \quad (1)$$

Since the intense reddish violet color of the pyrophosphate complex of +3 manganese precludes the use of a color indicator, the titration is performed potentiometrically.

In solutions of the proper pH the titration reaction is stoichiometrically exact, and hence the titrant permanganate solution can be standardized by any of the usual procedures. The method is at least as accurate, and even less subject to interferences, then the bismuthate method, which is generally conceded to be the most reliable of existing methods for manganese (*1, 3, 4, 6, 7, 8*). In particular, even large amounts of chloride, cobalt, and chromium do not interfere with the present method as they do in most other procedures, including the bismuthate method. Practically all other elements commonly associated with manganese, such as iron, nickel, copper, molybdenum, tungsten, and uranium, are also innocuous. The method has been applied successfully, without any preliminary separations, to a wide variety of manganiferous materials, including pyrolusite ore, ferromanganese, spiegeleisen, copper-base alloys, and alloy steels, with an accuracy equal to that obtainable by the bismuthate method. In spite of the fact that the titration is performed potentiometrically, the time required for an analysis by the present method is no greater than in the bismuthate method, because no filtrations are involved.

The development of the present method was facilitated by information gleaned from the recent excellent studies by Watters and Kolthoff (*9, 10*) of the properties of the pyrophosphate complexes of +3 manganese. These authors developed polarographic and amperometric titration procedures for the determination of manganese after oxidation to the +3 manganese pyrophosphate complex by excess lead dioxide. Watters (*9*) also described a volumetric method based on oxidation with lead dioxide, removal of the excess of the latter by filtration, and subsequent titration of the +3 complex with standard ferrous solution with diphenylbenzidine indicator. These methods are subject to interference by chloride ion, chromium, vanadium, and other elements which are oxidized by lead dioxide.

GENERAL EXPERIMENTAL TECHNIQUE

Standard solutions of manganous ion in dilute sulfuric acid were prepared in two ways; some were prepared from manganous oxalate dihydrate, which had been synthesized from recrystallized potassium permanganate and oxalic acid by the method of Coltman (*2*) and air-dried, and others were prepared from Bureau of Standards manganese ore No. 25b. Weighed portions of the manganous oxalate dihydrate were decomposed by digestion with concentrated sulfuric acid in a Kjeldahl flask, and diluted to volume in calibrated volumetric flasks. The purity of the salt was checked by determining its oxalate content by the usual permanganimetric procedure, with a permanganate solution that had been standardized against sodium oxalate under exactly the same conditions. The average percentage of oxalate ion found in ten determinations was 49.21 ± 0.06, which agrees to within 1 part per 1000 with the theoretical value 49.17. Standard solutions were prepared from the manganese ore by decomposing weighed samples (dried at 120° C.) in a mixture of hydrochloric and sulfuric acids in a Kjeldahl flask, boiling down to fumes of sulfur trioxide, and finally diluting to volume.

Standard 0.02 molar potassium permanganate solutions were prepared and standardized against pure sodium oxalate by the usual procedure (*4, 6*). Some of the solutions were also standardized against anhydrous potassium ferrocyanide by potentiometric titration in 2 *N* sulfuric acid at room temperature according to the method described by Kolthoff (*5*). The potassium ferrocyanide had been recrystallized twice and dehydrated by heating to constant weight at 110° C. The titer obtained with potassium ferrocyanide agreed very well with that obtained with sodium oxalate; in a typical case a solution was found to be 0.01857 molar against sodium oxalate and 0.01858 molar against potassium ferrocyanide. Standard 0.002 molar permanganate solutions were prepared by appropriate dilution of the 0.02 molar solutions with high-quality distilled water; these dilute solutions were prepared immediately before use. In the titration of manganese by the procedure described herein the permanganate ion undergoes only a 4-electron reduction to the +3 state, and hence a 0.02 molar solution is 0.08 *N*, and 1 cc. corresponds to 4.394 mg. of manganese.

Weights and volumetric equipment were calibrated with care, and temperature corrections were applied in measuring the solutions.

All reagents were of analytical reagent quality. Sodium pyrophosphate ($Na_4P_2O_7 . 10H_2O$) was used in the form of freshly prepared solutions saturated at room temperature (ca. 12 grams per 100 cc. or 0.3 *M*). Although the hydrolysis of the pyrophosphate ion is slow in cold neutral or alkaline solutions (*11*), it is advisable not to use solutions that are more than 2 or 3 weeks old. Samples of sodium pyrophosphate (analytical reagent grade) from three different manufacturers were used, and most of them were satisfactory. However, with some of the samples erratic potentials were observed during the titrations of manganese under the conditions hereinafter described, and 0.2 to 0.3 cc. too much 0.02 molar permanganate was used. This atypical behavior was apparently caused by some impurity in the samples, because it disappeared when the salt was recrystallized. Hence, it is advisable to test each new lot of salt by using it in the titration of a known amount of manganese, and if steady potentials are not established rapidly (as is typical of this titration) the salt should be recrystallized.

All reagents used must be completely free from reducing substances. In particular the dilute sodium hydroxide solution (ca. 5 *N*) which may be required to adjust the pH of the solutions prior to titration should be tested by adding a drop of 0.02 molar permanganate to a 10-cc. sample. No green color due to manganate ion should develop. It is advisable to prepare the sodium hydroxide solution freshly as needed; solutions which have stood in rubber-stoppered bottles invariably become contaminated by reducing substances, and these cause erratic potential readings during the titration and consume permanganate.

The titrations were performed in ordinary beakers, with the solutions stirred by a motor-driven glass stirrer. Bright platinum indicator electrodes were used in combination with a saturated calomel reference electrode. The e.m.f. was measured with an ordinary potentiometer in most of the titrations. Since the potential break at the end point is very large, simple potentiometric equipment, reading to a few millivolts, suffices. A number of titrations were made rapidly and accurately with a simple high-resistance voltmeter, consisting of a Rubicon box-type galvanometer in series with a 300,000-ohm resistance and a tapping key; this instrument had a range of 1 volt and permitted readings to ±0.01 volt.

CHARACTERISTICS OF THE TITRATION

Typical potentiometric titration curves of +2 manganese with permanganate in pyrophosphate solutions of various pH values are shown in Figure 1. In all cases the amount of manganese titrated was 83.6 mg. (1.522 millimoles) as manganous sulfate,

the initial volume was 180 cc., and the total concentration of sodium pyrophosphate was approximately 0.27 *M*. The pH was adjusted by addition of dilute sulfuric acid or sodium hydroxide, and measured with a glass electrode. The titrations were made at room temperature. Air was removed from the solutions with nitrogen because it was thought that some air oxidation of the manganese might occur, but later it was established that no appreciable air oxidation takes place if the pH of the solution is 8 or smaller; titration curves obtained in the presence of air are identical in all respects with those obtained in a nitrogen atmosphere.

Although the titration can be performed successfully at all pH values between about 1 and 8, the potential break at the equivalence point is maximal when the pH is between 6 and 7. When the pH is decreased below about 6 the magnitude of the potential break decreases markedly, and at pH values above about 8 no break at all is observed at the point corresponding to oxidation of the manganese to the +3 state. At all pH values below 8 in these titrations the end point determined from the maximum of $\Delta E/\Delta V$ was at 20.50 ± 0.02 cc., which agrees exactly with the theoretical volume of 0.01857 molar permanganate required to oxidize 1.522 millimoles of manganese to the +3 state.

The diminishing potential break with decreasing pH results from the fact that the potential of the manganous-manganic couple increases more rapidly than that of the permanganate-manganic couple with decreasing pH. The large effect of pH on the potential of the manganous-manganic couple in these solutions reflects a change in the predominating species of pyrophosphate ion, and hence in the manganese complex ions, with changing pH. From the dissociation constants of pyrophosphoric acid (1.4×10^{-1}, 1.1×10^{-2}, 2.1×10^{-7}, and 4.0×10^{-10}), the predominant species is $H_2P_2O_7^{--}$ at a pH between 2.0 and 6.7, $HP_2O_7^{---}$ between pH 6.7 and 9.4, and $P_2O_7^{----}$ above pH 9.4. Watters and Kolthoff (*9, 10*) have presented evidence that the manganic complex has the formula $Mn(H_2P_2O_7)_3^{---}$ at a pH between about 2 and 5, but at a pH between 6 and 8 the complex probably contains the mono- rather than the dihydrogen pyrophosphate ion.

At a pH much above 8 the pyrophosphate complex of +3 manganese is unstable and disproportionates into hydrous manganese dioxide and +2 manganese. This disproportionation is reversible; when a solution which has been titrated to the equivalence point at a pH between 6 and 7 is made strongly alkaline with sodium hydroxide hydrous manganese dioxide precipitates, but when the mixture is acidified the precipitate dissolves and the solution again assumes the characteristic reddish violet color of the +3 manganese complex.

The effect of pH on the standard potential of the manganous-manganic couple in pyrophosphate solutions of pH 0 to 9 has been studied by Watters (*9*), and the sections of the curves before the equivalence points in Figure 1 agree with what one would predict from his data. No previous data are available for the potential of the permanganate-manganic couple in pyrophosphate solutions as a function of pH; the curves in Figure 1 indicate that the standard potential of this couple is in the neighborhood of 1.25 volts *vs.* the saturated calomel electrode at pH = 0, and hence very close to the potential of the permanganate-manganous ion couple.

The optimum pH for the titration is between 6 and 7. In practice, a 25- or 50-cc. sample of the acidic manganese solution to be analyzed is added to 150 to 300 cc. of saturated sodium pyrophosphate solution (ca. 0.3 *M*), and the pH is adjusted to a value between 6 and 7 by addition of either dilute sulfuric acid or sodium hydroxide. If the solution is colorless, or nearly so, the proper pH is easily attained by adding bromothymol blue or chlorophenol red indicator and neutralizing to the intermediate yellowish green of the former or to an orange with the latter. With highly colored solutions, such as those encountered in the analysis of nickel or chromium steels, the pH adjustment is conveniently made with the aid of indicator test papers. Since the solution is well buffered the pH adjustment is easily accomplished.

In titrations at a pH of 6 to 7 the potential of the platinum indicator electrode becomes constant quickly after each addition of permanganate solution, and a complete titration can be performed in 15 to 20 minutes. Since steady potential readings are attained quickly, the technique of titrating directly to the equivalence point potential can be applied advantageously with a consequent shortening of the titration time to 10 minutes or less. With relatively pure manganese solutions, and a concentration of sodium pyrophosphate between 0.2 and 0.3 *M*, the equivalence point potential at a pH between 6 and 7 is +0.47 ± 0.02 volt *vs.* the saturated calomel electrode. In the presence of very large amounts of other metals a slightly lower value is usually found.

At a pH of 6 to 7 the magnitude of the potential change at the equivalence point—i.e., the maximal value of $\Delta E/\Delta V$—is between 100 and 200 mv. per 0.1 cc. of permanganate solution, and it is virtually independent of the amount of manganese titrated, the concentration of the permanganate solution, and the volume of the solution. This is the expected behavior for a homogeneous titration reaction.

The effect of the concentration of pyrophosphate was studied by titrating 100 mg. of manganese at a pH of 6 in various concentrations of sodium pyrophosphate from 0.04 to 0.3 *M* in a volume of 250 cc. The titration curves obtained with concentrations of sodium pyrophosphate between 0.15 and 0.3 *M* displayed virtually identical characteristics. With 0.04 *M* sodium pyrophosphate the potentials at all points in the titration were 0.05 to 0.1 volt more positive, and the potential break at the equivalence point was somewhat smaller (120 instead of 180 mv. per 0.1 cc.) than with the larger concentrations of pyrophosphate. With 100 mg. of manganese in 250 cc. a white precipitate of

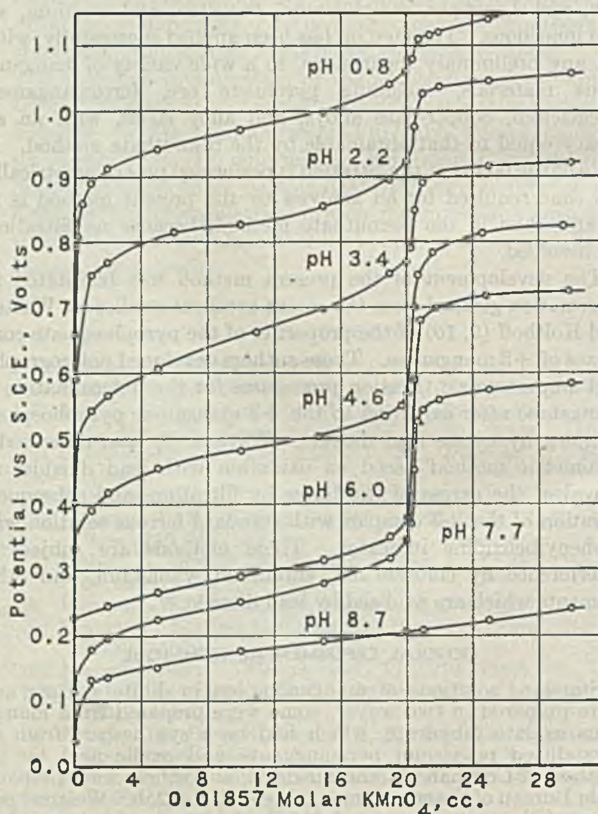


Figure 1. Influence of pH on Potentiometric Titration Curve of Manganous Ion with Permanganate Ion in Pyrophosphate Solution

manganous pyrophosphate forms if the concentration of sodium pyrophosphate is smaller than about 0.2 *M*. This precipitate dissolves completely before the equivalence point is reached, and it does not interfere with the titration.

A small blank correction was found with some samples of sodium pyrophosphate. This was determined by titrating 200-cc. samples of saturated sodium pyrophosphate alone with 0.002 molar permanganate after adjusting the pH to 6 with dilute sulfuric acid. In some cases the potential jumped sharply from about +0.2 to above +0.45 volt after adding only one drop of the 0.002 molar permanganate solution, indicating a negligibly small blank. With other samples of sodium pyrophosphate 0.10 to 0.15 cc. of 0.002 molar permanganate were required to obtain the characteristic potential break. Since at worst the blank amounts to only about 0.05 mg. of manganese, and usually is considerably less, it is small enough to be neglected when the amount of manganese titrated is greater than about 50 mg. With smaller amounts of manganese the appropriate correction should be applied.

The titration has been applied to amounts of manganese from a few milligrams up to 200 mg. in a volume of 150 to 500 cc. With relatively pure manganese solutions the accuracy of the titration appears to be limited only by the accuracy of the volumetric measurements.

STOICHIOMETRICAL EXACTNESS OF THE TITRATION REACTION

The exactness of the stoichiometry of the titration reaction was verified without reference to any standard substance in the following manner:

Samples of about 100 grams of a 0.02 molar permanganate solution were weighed, transferred to a Kjeldahl flask, acidified with 15 cc. of 4 *N* sulfuric acid, and reduced to manganous ion by dropwise addition of a fresh saturated solution of sodium sulfite. The slight excess of sulfur dioxide was expelled by boiling, after which the solution was cooled, transferred to the titration beaker, diluted to about 250 cc., and treated with 25 grams of recrystallized sodium pyrophosphate. The pH of the resulting solution was approximately 6. A sample of the original permanganate solution, 0.2 to 0.5% smaller than the theoretical one fourth of the sample that was reduced, was weighed and added to the solution. The titrations were finally completed with a very dilute permanganate solution (25 grams of the original solution diluted to 1 liter) from an ordinary buret.

The results of four such experiments are shown in Table I. The average ratio of the solution weights agrees exactly with the theoretical value of 4 predicted by Equation 1, and the average deviation from the mean is only ±0.05%.

Table I. Stoichiometry of Titration Reaction

$KMnO_4$ Solution Reduced	$KMnO_4$ Solution Required to Titrate	Ratio
Grams	*Grams*	
99.747	24.917	4.0032
99.965	25.004	3.9980
99.854	24.966	3.9996
99.843	24.969	3.9987
		Av. 4.000 ± 0.002

TITRATION OF MANGANESE IN PRESENCE OF OTHER ELEMENTS

From the conditions extant during the titration, and the fortunate circumstance that most metals form complexes with pyrophosphate that are soluble at a pH of 6 to 7, it was expected that very few elements would interfere with the determination of manganese. This has been substantiated by the series of experiments shown in Table II, in which a small amount of manganese (10 mg.) was titrated in the presence of large added amounts of those elements which more or less commonly accompany manganese. These experiments simulate conditions encountered in the determination of only 0.5 to 2% manganese in various metallurgical products and alloy steels.

Table II. Determination of Manganese in Presence of Other Elements

[10.62 mg. of manganese taken in all cases. Titrated with 0.002 molar permanganate (standardized against sodium oxalate) in 0.25 to 0.30 *M* sodium pyrophosphate at pH 6 to 7.]

No.	Addition	Volume	Mn Found	Error
	Mg.	*Cc.*	*Mg.*	%
1	None	175	10.64	+0.2
			10.63	+0.1
2	9000 KCl	175	10.62	0
			10.63	+0.1
3	1000 Fe^{+++}, 250 each Cu^{++}, Cr^{+++}, Ni^{++}, Co^{++}	400	10.66	+0.4
			10.56	−0.6
			10.58	−0.4
4	1000 Fe^{+++}, 100 each Mo^{++++++}, W^{++++++}, U^{++++++}	250	10.58	−0.4
			10.62	0
			10.62	0
5	250 Zn, 125 Al, and 50 each Mg, Cd	350	10.61	−0.1
			10.60	−0.2
			10.61	−0.1
6	1 V^{+++++}	180	10.61	−0.1
			10.63	+0.1
7	2.5 V^{++++}	180	10.60[a]	−0.2
			10.62	0
8	100 V^{+++++}	180	10.6[b]	...
9	800 Fe^{+++}, 400 V^{+++++}	300	10.6[b]	...
			10.6	...

[a] Corrected for volume of permanganate required to oxidize vanadyl ion.
[b] Titrated at pH 3 to 3.5; see text.

Since small amounts of manganese were present as an impurity in some of the salts used, particularly those of iron, cobalt, and nickel, blank determinations were run on each mixture and the appropriate correction was applied. This blank correction varied from a few hundredths up to 0.68 mg., depending on the amount and kind of metal salt.

Large amounts of chloride, ferric iron, +2 cobalt, copper, nickel, +3 chromium, +6 molybdenum, +6 tungsten, +6 uranium, zinc, aluminum, magnesium, and cadmium, have no significant effect. Nitrate, sulfate, and perchlorate ions are also harmless. The noninterference of chloride, cobalt, and chromium is a unique advantage of the present method over the classical bismuthate procedure.

Under the conditions stated, clear solutions were obtained in every case. With much larger quantities of certain metals, notably magnesium, cadmium, and aluminum, precipitates formed. Since some of these precipitates coprecipitate manganese, determinations should not be attempted in their presence.

Oxides of nitrogen interfere, and cause high results, because they are titrated by the permanganate. Hence when nitric acid is used to dissolve samples, the resulting solutions must not only be boiled thoroughly but a small amount of urea or sulfamic acid should be added to the acid solutions to remove the last traces of nitric oxide before adding the sodium pyrophosphate.

Of the elements likely to be associated with manganese, vanadium is the only one which causes difficulties, and then only when its amount is equal to or larger than the amount of manganese. When it is present originally in the +4 state vanadium is titrated to the +5 state along with the manganese. If, as is usually the case in steel analyses, the amount of vanadium is considerably less than that of the manganese the appropriate correction can be applied; an example is furnished by experiment 7 in Table II. When present in the +5 state in a pyrophosphate solution of pH 6 to 7 vanadium partially oxidizes the manganese, and is reduced to the +4 state. This causes no particular difficulty, and no error provided that the amount of vanadium is small (less than one fifth the amount of manganese), because the +4 vanadium so produced is subsequently reoxidized by the titrant permanganate solution (see Table II, experiment 6). However, the reaction between the +4 vanadium and permanganate ion is rather slow and with large amounts of vanadium a long time is required to obtain constant potentials, so that only small amounts of vanadium can be tolerated.

By working at a lower pH, and thus raising the potential of the manganous-manganic couple to a greater extent than that of the vanadyl-vanadate couple, the oxidation of manganese by +5 vanadium can be prevented. Under this condition it is possible to determine 10 mg. of manganese in the presence of as much

Table III. Determination of Manganese in Bureau of Standards Samples

(Permanganate solutions standardized against sodium oxalate)

Sample and Bureau of Standards Certificate Values	Mn Found, %
Manganese ore 25b Mn 58.35	58.35 58.37 58.33 58.29 58.35 58.38
	Av. 58.35 ± 0.02
Ferromanganese 68a Mn 80.07, C 6.83, Si 0.81, P 0.29, S 0.01	80.04 80.15 80.19 80.08
	Av. 80.12 ± 0.06
Spiegeleisen 66 Mn 19.93, Fe 73.45, C 4.05, Si 2.22, P 0.07, S 0.016, Ni 0.015, V 0.012, Ti 0.20, Cu 0.019, Cr 0.009, Co 0.01, Mo 0.005	19.97 20.01 20.00 20.00
	Av. 20.00 ± 0.01
Manganese rail steel 100 Mn 1.38, C 0.617, Si 0.19, P 0.02, S 0.02, Copper 0.12, Ni 0.15, Cr 0.18, V 0.011, Mo 0.005	1.385 1.381 1.392
	Av. 1.386 ± 0.004
Manganese bronze 62b Mn 1.28, Cu 57.40, Zn 37.97, Sn 0.97, Pb 0.27, Al 0.97, Fe 0.81, Ni 0.27, Si 0.05	1.277 1.274 1.273
	Av. 1.275 ± 0.002
Copper-nickel-chromium cast iron 115 Mn 1.01, Cu 6.44, Ni 15.89, Cr 2.17, V 0.009, Ti 0.021, Co 0.08, Si 1.60, C 2.42, P 0.113, S 0.032, Mo 0.002, and As 0.007	0.989 0.996 1.001 1.001
	Av. 0.997 ± 0.004
Chromium-vanadium steel 30d Mn 0.786, V 0.190, Cr 1.15, Cu 0.092, Ni 0.150, Mo 0.034, C 0.363, Si 0.286, P 0.031, and S 0.031	0.796 0.796 0.796
	Av. 0.796
Chromium-nickel steel 101b Mn 0.597, Ni 8.99, Cr 18.49, V 0.049, Co 0.078, Mo 0.078, Cb 0.062, Sn 0.012, Cu 0.168, C 0.069, Si 0.483, S 0.025, and P 0.017	0.60 0.63 0.61 0.60 0.66
	Av. 0.62 ± 0.02
Chromium-molybdenum steel 135 Mn 0.458, Cr 5.15, Mo 0.575, Cu 0.076, Ni 0.083, V 0.010, C 0.094, Si 0.383, P 0.017, and S 0.010	0.456 0.467 0.464
	Av. 0.462 ± 0.004

as 400 mg. of +5 vanadium, with an accuracy of about 1% (experiments 8 and 9 in Table II).

DETERMINATION OF MANGANESE IN METALLURGICAL PRODUCTS AND STEELS

To test the reliability of the present method under practical conditions, and compare its performance with other methods, a variety of samples were analyzed (Table III).

In general, a suitable weight of sample was decomposed in a Kjeldahl flask with an appropriate acid, and, after diluting and boiling thoroughly to remove gaseous reaction products, the resulting solutions were cooled and made up to volume in calibrated volumetric flasks without removing silica or the small amounts of carbon that usually remained. Whenever nitric acid was used to dissolve the samples 1 gram of urea or sulfamic acid per 250 cc. was added before finally diluting to volume, to ensure complete absence of nitric oxide. Aliquot portions (usually 50 cc.) of these solutions were pipetted into 200 to 300 cc. of saturated sodium pyrophosphate solution, and after adjusting the pH to a value between 6 and 7, the titrations were performed as already described. The titrant permanganate solutions (0.02 molar for samples 25b, 68a, and 66, and 0.002 molar for the others) were standardized against sodium oxalate.

Manganese Ore 25b. Six- to 8-gram portions of this sample, which consists mainly of pyrolusite, were dried at 120° C. and decomposed with a mixture of 100 cc. of 1 to 1 hydrochloric acid and 25 cc. of concentrated sulfuric acid, and finally diluted to 1 liter. Aliquots containing from 80 to 160 mg. of manganese were titrated. The time required for the analysis of pyrolusite ores by the present method is considerably less than that required in the bismuthate method, chiefly because hydrochloric acid can be used to dissolve the sample and no filtration is required.

Ferromanganese 68a and Spiegeleisen 66. One-gram samples of the ferromanganese and 3-gram samples of the spiegeleisen were decomposed by boiling with 50 cc. of 1 to 1 nitric acid. The solutions were finally diluted to 250 cc., and 50-cc. aliquots (120 to 160 mg. of manganese), were titrated.

Manganese Rail Steel 100 and Manganese Bronze 62b. Five-gram samples were dissolved in 50 to 100 cc. of 1 to 1 nitric acid. The solutions were diluted to 250 cc., and 50-cc. aliquots, corresponding to 12 to 14 mg. of manganese, were titrated.

Copper-Nickel-Chromium Cast Iron 115, Chromium-Vanadium Steel 30d, Chromium-Nickel Steel 101b, and Chromium-Molybdenum Steel 135. Since all these samples contain appreciable amounts of vanadium an oxidizing attack was used in preparing the solutions to make sure that vanadium would finally be present in the +5 state. Five-gram samples were dissolved in 1 to 1 nitric acid with the aid of a minimal amount of hydrochloric acid. The solutions were then boiled down to a small volume with an excess of concentrated nitric acid to reoxidize any vanadium reduced by the hydrochloric acid, and were finally diluted to 250 cc. Aliquots of 50 cc. were titrated.

SUMMARY

A new method is described for the determination of manganese based on the titration of manganous ion with permanganate ion in a pyrophosphate solution of pH 6 to 7, the +2 manganese being oxidized, and the permanganate ion reduced, to a pyrophosphate complex of the +3 state. The titration is performed potentiometrically with a platinum indicator electrode. The potential break at the equivalence point is very large and high precision is attainable. The titration reaction is stoichiometrically exact, and the titrant permanganate solution can be standardized against sodium oxalate or anhydrous potassium ferrocyanide.

Data presented demonstrate that large amounts of chloride, +3 iron, +2 cobalt, copper, nickel, +3 chromium, +6 molybdenum, +6 tungsten, +6 uranium, zinc, aluminum, magnesium, and cadmium do not interfere with the determination of manganese. Nitrate, sulfate, and perchlorate ions are also harmless. When the titration is performed at pH 6 to 7 vanadium interferes if present in large amounts, but amounts of this element up to about one fifth the amount of manganese can be tolerated. The interference of large amounts of vanadium can be circumvented by performing the titration at a lower pH (3 to 3.5).

The method has been applied without any separations to Bureau of Standards samples of manganese ore, ferromanganese, spiegeleisen, manganese bronze, and alloy steels, with results that equal in accuracy those obtainable by the bismuthate method.

LITERATURE CITED

(1) Blum, W., *J. Am. Chem. Soc.*, 34, 1395 (1912).
(2) Coltman, R. W., Ind. Eng. Chem., 16, 606 (1924).
(3) Cunningham, T. R., and Coltman, R. W., *Ibid.*, 16, 58 (1924).
(4) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis", New York, John Wiley & Sons, 1929.
(5) Kolthoff, I. M., *Rec. trav. chim.* 41, 343 (1922).
(6) Kolthoff, I. M., and Sandell, E. B., "Textbook of Quantitative Inorganic Analysis", New York, Macmillan Co., 1943.
(7) Lundell, G. E. F., *J. Am. Chem. Soc.*, 45, 2600 (1923).
(8) Lundell, G. E. F., Hoffman, J. I., and Bright, H. A. "Chemical Analysis of Iron and Steel", New York, John Wiley & Sons, 1931.
(9) Watters, J. I., Ph.D. thesis, University of Minnesota, 1943.
(10) Watters, J. I., and Kolthoff, I. M., Ind. Eng. Chem., Anal. Ed., 15, 8 (1943); 16, 187 (1944).
(11) Yost, D. M., and Russel, H., Jr., "Systematic Inorganic Chemistry", New York, Prentice-Hall, 1944.

Fractional Separation of Hafnium and Zirconium by Means of Triethylphosphate

HOBART H. WILLARD AND HARRY FREUND
University of Michigan, Ann Arbor, Mich.

This study is an extension of previous work on separations in which the precipitating ion was not added as such, but formed slowly by hydrolysis in solution. The principles set forth in the original work on the slow formation of hydroxyl ion were confirmed. When the solution is homogeneous with respect to the precipitating ions, the precipitation of a normally gelatinous product may be improved to yield a dense, granular easily filterable precipitate, and a great improvement in the efficiency of separation of difficultly separable elements can be attained. Hafnium was separated from zirconium by fractional precipitation of the ethylphosphates obtained by hydrolysis of triethylphosphate in a boiling solution of 6 *N* sulfuric acid. Data from this separation were used to develop an optimum fractionation procedure, resulting in efficient separation of hafnium from a mixture containing a relatively low percentage of hafnium.

WHEN a precipitation reaction is used for the separation of ions, the formation of a gelatinous precipitate is a serious problem. The slimy nature of the product makes filtration difficult and inefficient washing leaves adsorbed and occluded impurities. If the precipitating ion is not added as such but is formed slowly and homogeneously throughout the body of the reaction mixture, and other conditions are fulfilled, a dense and granular precipitate is formed. This precipitate is easily filtered and washed and carries down few impurities, thus affording a simple means of solving the problem. Willard and his co-workers (*3-6*) successfully carried out the separation of hydroxides and basic salts from many ions by a gradual and uniform increase in pH resulting from the hydrolysis of urea.

This paper extends the work to the formation of ions other than hydroxyl, and the hydrolysis of triethylphosphate is employed successfully in the fractional separation of hafnium and zirconium. Triethylphosphate is completely miscible with sulfuric acid solutions of zirconium and hafnium. When such a mixture is boiled, the ester is hydrolyzed in a stepwise manner and a precipitate of zirconyl or hafnyl ethyl acid phosphate is produced. By using only sufficient ester to precipitate a fraction of the combined hafnium plus zirconium, the hafnium concentrates in the precipitate.

A systematic approach to the separation of zirconium and hafnium by direct fractional precipitation with phosphoric acid was developed by Larsen, Fernelius, and Quill (*1*), in whose paper the references to the literature of the phosphate method are given. The gelatinous nature of the precipitate was overcome by rigid control of the many factors influencing the precipitation reaction. In addition, a method of opening the ore with concentrated sulfuric acid and the solution of the phosphate precipitate were described and are used extensively in the present investigation.

STARTING MATERIAL

The source of the hafnium and zirconium salts was the mineral cyrtolite, an altered zircon containing about 5% hafnia by weight. The ore was crushed, ground to pass 100-mesh, and digested with concentrated sulfuric acid (*1, 2*). The resulting sulfate solution was then used directly for fractional precipitations or purified by precipitation of the hafnium and zirconium by means of excess triethylphosphate.

METHOD OF ANALYSIS

Two analyses were usually required, one for the combined hafnia and zirconia and the other for the hafnia present in the combined oxides. Whenever possible the determination of combined oxides was run first, the oxides were returned to solution by fusing with potassium bisulfate, and the resulting solution was used for the hafnium determination.

As no simple chemical process is available that will distinguish between hafnium and zirconium, it is necessary to have recourse to some indirect method. One of the most feasible is the conversion of a mixture of two well-defined hafnium and zirconium compounds into two other compounds.

Schumb and Pittman (*2*) published a method of determining hafnium in a mixture of hafnium and zirconium oxychlorides by conversion to the normal selenites followed by ignition to the oxides. Three changes were essential before the method could be applied to the mixtures in this investigation. (1) It was necessary to convert the sulfuric acid solutions of hafnium and zirconium into the corresponding hydrochloric acid solutions. This was accomplished by a double precipitation with ammonia followed by solution in 10 ml. of 1 to 1 hydrochloric acid. (2) It was necessary to destroy the wood sugars resulting from the hydrolysis of the cellulose (filter paper), by boiling the hydrochloric acid solutions with hydrogen peroxide. Following solution in 1 to 1 hydrochloric acid, the solution was boiled with 5 ml. of 30% hydrogen peroxide, and then diluted to 200 ml. for precipitation of the selenites with 50 ml. of 20% selenious acid. (3) It was necessary to remove the silica from the ignited oxides in the combined oxides determination. This was accomplished by evaporating cautiously 10 drops of concentrated sulfuric acid and 0.5 to 1 ml. of hydrofluoric acid from the oxides. The mixed sulfates were then carefully ignited to the oxides, the final ignition being made at 950° C.

NATURE OF PRODUCT SEPARATED BY HYDROLYSIS OF TRIETHYLPHOSPHATE IN PRESENCE OF ZIRCONIUM

If the hydrolysis of triethylphosphate went to completion, zirconyl phosphate, $ZrO[H_2(PO_4)]_2$, would be precipitated. However, the stepwise hydrolysis of the ester results in the precipitation of zirconium ethyl phosphate.

A carefully purified sample was analyzed to obtain the loss on ignition and zirconium and phosphorus contents. On the basis of these results a suggested formula is $ZrO[H(C_2H_5)(PO_4)]_2.2H_2O$. A comparison of the chemical analysis and calculated analysis is made in Table I.

Near the completion of this investigation trimethylphosphate became available and because of its increased rate of hydrolysis over that of triethylphosphate, some experiments were run to compare the two esters. The results indicated that the time required for the same degree of precipitation of zirconium by trimethylphosphate was about one third that required by the triethyl compound. This means a tremendous saving in time, since the usual fractionation procedure to produce relatively pure hafnium compounds with triethylphosphate requires five to six

Table I. Results in Analysis of Zirconium Compound Precipitated by Hydrolysis of Triethylphosphate

	Calculated	Experimental
Mole ratio P/Zr	2.00	2.00
% Zr	23.20	23.12 ± 0.06
% P	15.76	15.76 ± 0.03
% loss on ignition	32.55	32 6 ± 0.1
Molecular weight	393.2	393 ± 1

Table II. Degree of Fractionation in Sulfuric, Nitric, and Hydrochloric Acid Solutions

Type Solution	Mole Fraction $HfO_2 + ZrO_2$ removed	HfO_2 removed
6 *N* sulfuric acid	0.333	0.623
6 *N* nitric acid	0.333	0.516
6 *N* hydrochloric acid	0.333	0.513

Table III. Effect of Free Acid on Fractionation of Hafnium

Normality of Sulfuric Acid	Initial Weight % HfO_2	Mole Fraction $HfO_2 + ZrO_2$ Removed	Final Weight % HfO_2
6	20.3	0.519	32.9
8	20.3	0.540	29.6
10	20.3	0.525	30.9
12	20.3	0.553	30.9
14	20.3	0.567	30.2

steps at 20 hours per step for the reaction time alone. Also, it is reasonable to expect that the product separated would be analogous to the zirconium ethyl phosphate and therefore would show about the same fractionation characteristics.

EFFECT OF ANION ON FRACTIONATION OF HAFNIUM

The usual explanation of the operation of a fractional precipitation method is based upon the slight difference in the solubility of the products. The ratio of the molecular solubilities of hafnium and zirconium phosphates in 6 *N* hydrochloric acid is only 1 to 4. Zirconium generally preponderates in the usual mixture of zirconium and hafnium, and the repressing effect of the high zirconium concentration should overbalance the slight differences in solubility. The fact that it does not suggests that relative stabilities of anionic complexes may be involved. If this is so, the fractionation should depend upon the anion present in the solution. To check this, the same fraction of the combined oxides was precipitated with triethylphosphate from 6 *N* solutions of sulfuric, nitric, and hydrochloric acids. The results, tabulated in Table II, indicate that the best results are obtained in a sulfuric acid solution.

EFFECT OF FREE ACID CONCENTRATION ON FRACTIONATION OF HAFNIUM

The effect of the free acid concentration was determined by precipitating approximately the same fraction of the combined oxides from solutions of varying sulfuric acid concentration. As shown in Table III, no noticeable improvement in the fractionation takes place as the acid concentration is varied.

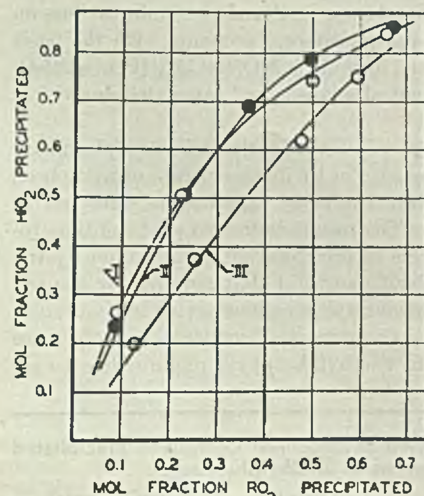


Figure 1. Comparison of Hafnia and Total Oxides Precipitated

Mole Fraction HfO_2 in Starting Material
I. 0.0753
II. 0.179
III. 0.504

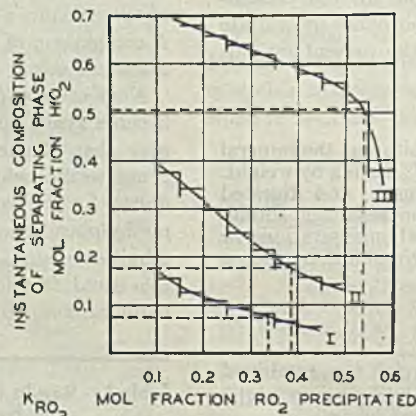


Figure 2. Relationship of Instantaneous Composition and Mole Fraction of Combined Oxides Precipitated

Initial Mole Fraction HfO_2	Optimum Cutoff K_{RO_2}
I. 0.0753	0.34
II. 0.179	0.39
III. 0.504	0.54

6 *N* sulfuric acid was selected as the most desirable reaction medium, primarily for the dense, granular precipitate produced and also because the solutions were the least corrosive.

DEVELOPMENT OF AN OPTIMUM FRACTIONATION PROCEDURE

The development of an optimum fractionation procedure depends upon the application of a suitable criterion for determining the fraction of the total hafnia plus zirconia that should be precipitated at a given step. The highest concentration of hafnia in the product and the greatest recovery of hafnia are the two most important factors to be considered. Unfortunately they are dependent upon one another. If a relatively small amount of the total hafnium plus zirconium is precipitated, the hafnium content of the precipitated phase will be considerably higher than if a larger amount of the combined oxides had been separated. Thus as the yield of hafnia recovered in the precipitated phase is increased, the hafnium concentration is decreased.

At this point a clarification of the term "instantaneous composition" is required. The chemical analysis indicates the average composition over a given interval. If this interval were continually shortened, then at the limit the composition would represent an instantaneous value. Physically it would correspond to the composition of the product separating at a given instant, if all of the previously separated product could be removed.

From the above discussion it is apparent that the instantaneous composition of the separating phase will vary from high values of per cent hafnia to low values as the fractionation progresses. Therefore, the fractionation should be stopped at the point where the instantaneous composition of the separating product is equal to the initial composition of the starting material. To carry the fractionation beyond this point would simply mean that the product was being diluted with a mixture less rich in hafnium than the starting material. Although there is no convenient way to determine experimentally the relation between the instantaneous composition and the fraction of the combined oxides precipitated, it can be deduced by graphical means from other data more easily measured. The first step is a comparison of the fraction of the hafnia precipitated with the fraction of the combined oxides precipitated for initial mixtures containing varying amounts of hafnia, somewhat analogous to the treatment of Larsen *et al.* (*1*).

The experimental work consisted in carrying out a series of single precipitations on starting solutions having the same hafnia content, in which 10 to 70 mole % of the total oxides were precipitated as the ethylphosphates. Sufficient triethylphosphate (375 ml. of ester per mole of oxides) was added to solutions of 6 *N* sulfuric acid, containing 0.1 mole per liter of combined oxides, to precipitate the desired fraction of the oxides as the ethylphosphates. A mole ratio of ester to oxides slightly greater than 2 to 1 was required because of side reactions. The mixtures were boiled for 20 hours, cooled, filtered, and washed with 2% sulfuric acid. Sodium hydroxide-sodium peroxide mixtures were used to decompose the phosphate precipitate (*1*), and make it soluble in sulfuric acid. The resulting solutions were analyzed for both combined oxides and hafnia, from which the desired data were easily calculated. These experiments were repeated for solutions whose combined oxides had different initial hafnia contents. These data are plotted in Figure 1.

For selected values of the abscissa the corresponding ordinates are read from the curves of Figure 1. Then on the basis of 1 mole of combined oxides, the moles of hafnia separated and the

Table IV. Fractionation Series Using Optimum Procedure

Fractionation step		Experimental										Predicted Composition Mole fraction HfO_2 from Fig. 4	Experimental Mole Fraction RO_2 Precipitated	Desired Mole Fraction RO_2 Precipitated from Fig. 3
		Weight of RO_2	HfO_2 Weight	Solution volume	HfO_2	Mole fraction HfO_2 precipitated	Over-all efficiency HfO_2 precipitated	ZrO_2	RO_2	Composition mole fraction HfO_2				
		Grams	*%*	*Ml.*	*Mole*			*Moles*	*Moles*					
1	Initial	215	16.0	4760	0.163			1.464	1.627	0.100				
	Final	75.3	30.3		0.1081	0.664	0.664	0.425	0.5331	0.203		0.19	0.328	0.34
2	Initial	74.1	30.3	1525	0.1063			0.418	0.5243	0.203				
	Final	29.8	53.6		0.0757	0.712	0.472	0.1120	0.1877	0.403		0.35	0.358	0.38
3	Initial	28.9	53.6	738	0.0735			0.1089	0.1824	0.403				
	Final	15.1	75.8		0.0543	0.738	0.348	0.0296	0.0839	0.647		0.57	0.460	0.49
4	Initial	14.0	75.8	435	0.0503			0.0275	0.0778	0.647				
	Final	11.65	80.0		0.0442	0.878	0.306	0.0189	0.0631	0.700		0.75	0.812	0.66
5	Initial	10.48	80.0	225	0.0397			0.0170	0.0567	0.700				
	Final	7.16	91.1		0.0309	0.779	0.238	0.0052	0.0361	0.853		0.80	0.637	0.71

increments in the moles of hafnia are determined. From these the average compositions of the separating phases corresponding to increments in the precipitation of the combined oxides are calculated and plotted in Figure 2. The instantaneous curve is drawn so that the area under the rectangles equals the area under the curve. The intersection of the instantaneous curve with the ordinate representing the initial composition gives the optimum cutoff in the fractionation. Obviously, if the curve relating the optimum cutoff and the initial composition were plotted, it would have to pass through the points 0,0 and 1,1. These data are made of more general value by putting them on a semilog plot as shown in Figure 3. Thus, for any given initial composition of combined oxides, the optimum extent of precipitation is determined from the curve. Again, considering 1 mole of combined oxides as a basis, and using the curves of Figures 1 and 3, the plot of Figure 4 relating the composition at steps $N + 1$ and N may be obtained. If the experimental data were very accurate and if rigid control could be maintained over the fractionation procedure, this curve would assist greatly in planning the entire fractionation series.

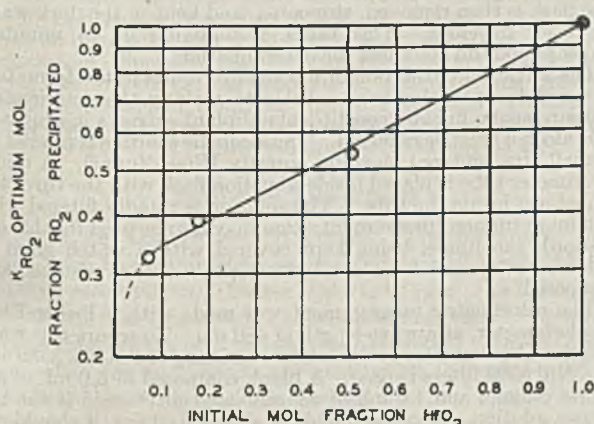


Figure 3. Comparison of Optimum Mole Fraction of Combined Oxides Precipitated and Composition of Starting Material

Using a 45° reference line, a series of steps in the fractionation process could be plotted. Unfortunately, the very small error per step adds to a very considerable cumulative error which greatly lessens its value with the data at present available.

APPLICATION OF OPTIMUM FRACTIONATION PROCEDURE TO FRACTIONATION SERIES

The procedure for using the fractionation curves consists in determining first the initial moles of combined oxides and the hafnia content of these oxides. Then using Figure 3 the desired

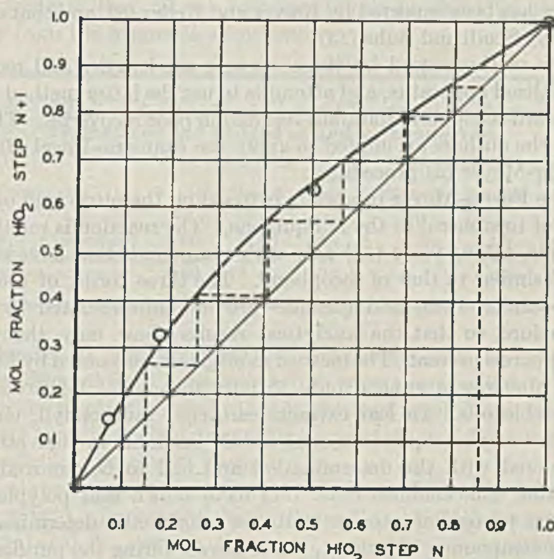


Figure 4. Comparison of Composition at Step $N + 1$ and at Step N

mole fraction of the combined oxides to be precipitated is selected and sufficient triethylphosphate is added to carry out the precipitation. The ethylphosphates are decomposed with a caustic peroxide mixture, and the acid-soluble product is dissolved in 6 N sulfuric acid and analyzed.

From the new analysis the next fractionation step may be planned as before. Figure 4 may be used to predict the composition one or two steps in advance. The data for an entire series are tabulated in Table IV. In this series 215 grams of combined oxides containing 16.0 weight % hafnia were enriched to 7.16 grams of combined oxides containing 91.1 weight % hafnia in five steps. This represents an over-all efficiency in the recovery of hafnia of 23.8%.

LITERATURE CITED

(1) Larsen, E. M., Fernelius, W. C., and Quill, L. L., Ind. Eng. Chem., Anal. Ed., 15, 512–15 (1943).
(2) Schumb, W. C., and Pittman, F. K., *Ibid.*, 14, 512–15 (1942).
(3) Sheldon, J., doctoral thesis, "Study of Ferric Hydroxide Precipitated by Urea and Its Use in Quantitative Separations", University of Michigan, 1940.
(4) Willard, H. H., and Fogg, H. C., *J. Am. Chem. Soc.*, 59, 1197 (1937).
(5) Willard, H. H., and Tang, N. K., Ind. Eng. Chem., Anal. Ed., 9, 357 (1937).
(6) Willard, H. H., and Tang, N. K., *J. Am. Chem. Soc.*, 59, 1190 (1937).

From a dissertation submitted by Harry Freund to the Graduate School of the University of Michigan in partial fulfillment of the requirements for the degree of doctor of philosophy in chemistry.

Determination of Tocopherol in Plant Tissue

MONROE E. WALL AND EDWARD G. KELLEY
Eastern Regional Research Laboratory, Philadelphia 18, Pa.

Modifications of the Emmerie-Engel and Furter-Meyer methods for the determination of tocopherol are reported, which consist essentially of the removal of substances that interfere with application to plant extracts. In most cases the results obtained by the two procedures agreed within ±5 to 10%.

DURING the course of investigations on the fat-soluble constituents of vegetable leaf meals, the authors wished to determine the tocopherol content of a number of leaf meals and extracts. A survey of the literature indicated that little work had been done with such materials. The tocopherol content of lettuce leaves has been reported by Karrer and Keller (*8*), and that of alfalfa by Scudi and Buhs (*13*).

The methods used by these workers are lengthy and require specialized apparatus, and attempts to use the latter method with standard tocopherol solutions resulted in poor recoveries. Therefore, the authors attempted to apply the Emmerie-Engel (*3*) and Furter-Meyer (*5*) procedures.

The Furter-Meyer procedure is based on the nitric acid oxidation of tocopherol to the red quinone. The reaction is relatively specific, taking place (*14*) only with compounds that have structures similar to that of tocopherol. The three forms of tocopherol—alpha, beta, and gamma—are not differentiated by this procedure, so that the analytical results show only the total tocopherols present. The method as originally presented by Furter and Meyer was applied only to pure tocopherol solutions and vegetable oils. In leaf extracts carotene, xanthophyll, chlorophyll, tocopherolquinones, and lipoids insoluble in cold ethanol interfered with the determination and had to be removed. In addition Quackenbush *et al.* (*12*) have shown that polyphenols such as pyrogallol interfere with the colorimetric determination. Such compounds, if present, are removed during the purification of the plant extract.

The Emmerie and Engel procedure is indirect. Ferric ion is reduced to the ferrous form by all three tocopherols. The ferrous ion then forms a colored complex with α,α'-dipyridyl. Any compound which can reduce iron will interfere with the method. In leaf extracts carotene, xanthophyll, chlorophyll, and certain lipoids which slowly reduce iron interfere and must be removed.

The modifications of these methods described here consist essentially of the removal of substances which interfere with their application to plant extracts.

EXTRACTION OF TOCOPHEROL

The solutions are kept in the dark except during manipulations and all manipulations are conducted in a room shielded from direct sunlight. All-glass apparatus is used throughout.

Dry Samples. A 25-gram sample of leaf meal, of 30- to 40-mesh, is exhaustively extracted in a Soxhlet with Skellysolve B for 5 to 8 hours, and the extract thus obtained is made to 250 ml. In this manner sufficient sample is obtained for duplicate determinations by both procedures.

Fresh Samples. A 10-gram fresh sample is necessary for the Emmerie-Engel procedure and a 25-gram sample for the Furter-Meyer. The material is extracted in a Waring blender using the foaming mixture of alcohol and Skellysolve B described by Moore and Ely (*9*). This procedure has been successfully used by Devlin and Mattill (*2*) to extract tocopherols from muscle tissue. The alcohol is separated and extracted with Skellysolve B as described by Wall and Kelley (*15*). The Skellysolve solution is then dried with anhydrous sodium sulfate and evaporated under vacuum and nitrogen to approximately 25 ml.

FURTER-MEYER PROCEDURE

The sample taken should contain a minimum of 0.3 mg. of tocopherol and preferably about 1.0 mg. An extract equivalent to 2.5 to 5.0 grams of dry leaf meal usually contains the necessary quantity of tocopherol. The sample dissolved in about 25 ml. of Skellysolve B is passed through a 7 × 2 cm. adsorption column consisting of three parts of Hi-Flow Supercel (Johns-Manville Corporation) and one part of activated magnesia No. 2641 (Westvaco Chlorine Products Company, Newark, Calif.) The preparation of the column and the adsorption technique are identical with those described by Wall and Kelley (*15*) for the determination of carotene. After adsorbing the extract, the adsorption column is washed with about 80 ml. of 5% acetone in Skellysolve B and finally with 20 ml. of 10% acetone in Skellysolve B. The carotene and tocopherol are eluted, chlorophyll and xanthophyll being retained on the column. The eluate is caught in a 250-ml. flat-bottomed boiling flask, ₸24/40.

The solution is evaporated in a water bath at 40° to 50° C. under vacuum and nitrogen to approximately 25 ml. To the concentrated solution are added 2.0 ml. of 85% (by volume) sulfuric acid. The flask is stoppered and vigorously shaken on a shaking machine for 3 minutes. The acid is colored deep blue by the decomposed carotene. Occasionally, if all the pigment is not removed, 2.0 ml. more of acid must be added. The stopper is removed and rinsed with Skellysolve B, and the contents of the flask are rinsed into a 250-ml. separatory funnel with Skellysolve B. The acid layer which separates is withdrawn. The Skellysolve solution is washed once with aqueous 5% sodium sulfate solution, then once with aqueous 1% potassium hydroxide, and finally with more sodium sulfate solution until the aqueous washings are practically neutral.

The solution, which should be colorless or only slightly yellow, is dried with anhydrous sodium sulfate, decanted from the sodium sulfate, and evaporated almost to dryness. The residue is transferred with 3 or 4 washes of Skellysolve B to a 50-ml. Erlenmeyer flask, ₸19/22, and the solution is again evaporated just to dryness. To the flask are added 5.0 ml. of absolute ethanol and then, while the flask is swirled, 1.0 ml. of concentrated nitric acid. The flask, attached to a small Liebig condenser by means of lightly greased interchangeable joints, is then set in a steam bath. The alcoholic solution is refluxed for exactly 3 minutes from the time the alcohol begins to boil. The flask is taken from the steam bath, and cooled with cold water while still connected to the condenser. The flask is then removed, stoppered, and kept in the dark while the color develops. This takes a minimum of 15 minutes; longer periods do not affect the determination.

The nitric acid oxidation of tocopherol results in the formation of a red tocopherolquinone. Prior to making the colorimetric measurements, lipoidal constituents of plant extracts insoluble in cold alcohol must be removed. The alcoholic solution is filtered on a small, dry, sintered, medium porosity Hirsch funnel. A micro colorimeter tube is placed inside a suction flask with the tip of the funnel just inside the tube. The solution is rapidly filtered with minimum suction (pressure filtration may also be used for this operation), the funnel being kept covered with a watch glass to minimize evaporation. The colorimeter tube is then removed and stoppered.

The colorimetric measurement was made with a Fisher Electrophotometer, at a wave length of 480 mμ. To secure this wave length a Corning filter combination consisting of one No. 3389 and two No. 4303 filters is used. A blank composed of 5.0 ml. of absolute ethanol and 1.0 ml. of concentrated nitric acid is the reference solution. Because the blank slowly darkens, it should not stand more than 0.5 hour.

A standard calibration curve, shown in Figure 1, A, was made with solutions of Merck synthetic α-tocopherol in Skellysolve B. The standard solutions were evaporated, oxidized with nitric acid, and filtered as described previously.

EMMERIE-ENGEL PROCEDURE

The best concentration range for this procedure is 0.05 to 0.20 mg. It is more convenient to purify a larger sample containing 0.2 to 1.0 mg. and then take a suitable aliquot for the final determination.

A Supercel-activated magnesia column approximately 4.0 × 2.0 cm. is prepared as described. The sample is adsorbed, eluted, and concentrated as in the Furter-Meyer procedure. After the sample is shaken for 3 minutes with 1 to 2 ml. of 85% sulfuric

Table I. $E^{1\%}_{1\ cm.}$ and 480 and 520 mμ Values for Synthetic α- and Natural α- and γ-Tocopherols

Compound	Furter-Meyer Method			Emmerie-Engel Method		
	Mg.	$E^{1\%}_{1\ cm.}$ 480 mμ		*Mg.*	$E^{1\%}_{1\ cm.}$ 420 mμ	
Synthetic α-tocopherol	1.0	15.5	15.5	0.1	238	238
	2.0	15.5		0.2	238	
Natural α-tocopherol	1.0	16.5	16.4	0.1	249	242
	2.0	16.3		0.2	234	
Natural γ-tocopherol	1.0	13.5	13.9	0.1	174	152
	2.0	14.2		0.2	130	

acid, it is transferred to a separatory funnel and treated as described above. After the last sodium sulfate wash, as much aqueous solution as possible is removed and, without drying, the purified Skellysolve solution is run into a 100-ml. volumetric flask and made to volume.

A 25-ml. aliquot is evaporated almost to dryness on a warm water bath under vacuum and nitrogen. The residue is dissolved in 10 ml. of chloroform and transferred with three chloroform washes totaling 20 to 30 ml. to a 125-ml. separatory funnel. To the chloroform solution are added 1.0 ml. of a 0.5% α,α'-dipyridyl solution in absolute ethanol and 1.0 ml. of a 0.4% ferric chloride solution in absolute ethanol. The reagents are run down the sides of the funnel to minimize mixing. The funnel is then shaken vigorously, approximately 10 ml. distilled water are added at once, and the funnel is again shaken. The stopper and sides of the funnel are then rinsed with a few milliliters of absolute alcohol. Two layers form. The lower chloroform layer is discarded; the upper aqueous alcohol layer, which contains the red ferrous dipyridyl color complex, is run into a 25-ml. volumetric flask and made to volume with absolute ethanol.

The time for color development is 10 minutes, measured from the time the reagents are first mixed with the tocopherol solution. The colorimetric measurement was made with a Fisher Electrophotometer at 520 mμ, obtained by a combination of three Corning filters, one No. 4380, one No. 4303, and one No. 3384. A blank containing 10 ml. of distilled water diluted to 25 ml. with absolute ethanol is used.

A calibration curve was made with Merck synthetic α-tocopherol in Skellysolve B solution. Suitable aliquots were evaporated, and the color was developed as described above. A ferric chloride-α,α'-dipryridyl reagent blank obtained in the same manner as the standards was determined, and the value thus obtained was deducted from the standard readings. Calibration curves obtained before and after deducting the reagent blank are shown in Figure 1, B. Either calibration curve may be used.

DISCUSSION AND RESULTS

Effect of Tocopherol Mixtures. The procedures presented were based on colorimetric standardization with Merck synthetic α-tocopherol. Baxter *et al.* (*1*) and Hove and Hove (*7*) have shown that α-tocopherol is oxidized more rapidly by ferric chloride than are the beta and gamma compounds. On the other hand, when the tocopherols are oxidized to the red *o*-quinone with silver nitrate, this order is reversed (*1*)

In view of the fact that γ-tocopherol might be present in plant tissue and that the synthetic and natural tocopherols might behave differently, the $E^{1\%}_{1\ cm.}$ and 480 and 520 mμ values for synthetic α- and natural α- and γ-tocopherols were determined by modifications of the Furter-Meyer and Emmerie-Engel procedures (Table I).

The $E^{1\%}_{1\ cm.}$ values obtained by both methods for synthetic and natural α-tocopherol agree fairly well, the result for natural tocopherol being about 5% high in both cases. As compared with synthetic α-tocopherol, the natural gamma compound gave values that averaged 10% low by the Furter-Meyer and 35% low by the Emmerie-Engel method. Therefore if γ-tocopherol were present in appreciable quantity in leaf material, the error in colorimetry alone would be serious, especially in the Emmerie-Engel determination. As is shown below, in most cases results by the two procedures were in close agreement, indicating that there was little, if any, γ-tocopherol in the products studied.

In this connection, recent papers by Hove and Hove (*7*) and Fisher (*4*) describe the determination of α-tocopherol in the presence of the beta and gamma compounds. It is possible that these procedures in conjunction with the methods described here may be used to determine the individual tocopherol compounds present in plant tissue.

Removal of Pigments. Passage of the extract through activated magnesia not only effectively removes the chlorophyll and xanthophyll but allows quantitative elution of the tocopherol, together with the carotene. Table II shows the results of adsorption experiments with synthetic α- and natural α- and γ-tocopherols. After elution with the acetone-Skellysolve B solutions described previously, the colorimetric determination was made by the Furter-Meyer method, individual calibration curves prepared from each tocopherol compound being used. The results indicate that all the tocopherols can be eluted quantitatively from the magnesia adsorbent.

Table II. Effect of Adsorption and Acid Treatment on Recovery of Synthetic α- and Natural α- and γ-Tocopherols

Compound	Adsorption			Sulfuric Acid Treatment		
	Taken	Found	Recovery	Taken	Found	Recovery
	Mg.	*Mg.*	%	*Mg.*	*Mg.*	%
Synthetic α-tocopherol	2.00	2.00	100	2.0	1.90	95.0
Natural α-tocopherol	2.00	2.03	101.0	2.0	1.95	97.5
Natural γ-tocopherol	2.00	2.05	102.5	2.0	1.90	95.0

Attempts at separating carotene from tocopherol by adsorption on Florisil from benzene solution were unsuccessful. The quantities of carotene and other lipoids are much larger in leaf extracts than in oil and animal tissue extracts, which have been successfully treated with Florisil.

Carotene is removed in both methods by 85% (by volume) sulfuric acid. Parker and McFarlane (*11*) used 85% by weight sulfuric acid to remove the small quantities of carotenoids present in oils. In order to remove the carotene quantitatively, it is necessary to work with a concentrated solution, which must be vigorously shaken with the acid. Under these circumstances the sulfuric acid rapidly decomposes the carotene, and the resultant petroleum ether solution is colorless. Tocopherol losses in this case are low. If the solution is not concentrated, prolonged shaking with the acid is required. Often much larger quantities of acid are necessary. The tocopherol losses in this case amounted to 25 to 50% with the Emmerie-Engel procedure, probably due to oxidation; they were somewhat lower with the Furter-Meyer method. Table II shows the effect of the 85% sulfuric acid treatment on the recovery of snythetic α- and natural α- and γ-tocopherols. The acid treatment was identical with the procedure described above. The colorimetric determination was made by the Furter-Meyer method, individual calibration curves being used. The results show that the acid treatment caused a maximum loss of 5%.

According to Scudi and Buhs (*13*), treatment with 85% sulfuric acid also removes tocopherolquinones, which would otherwise be included with tocopherol values by the Furter-Meyer method. The authors' observations with pure α-tocopherolquinone confirm this. Water-soluble polyphenols, if present, are removed by the water and alkali shakings subsequent to the treatment of the extract with 85% sulfuric acid.

Effect of Unknown Lipoidal Interferences. After the acid treatment, the only subsequent interference observed in the Furter-Meyer procedure came from lipoidal compounds insoluble in cold ethanol. When nitric acid is added to the ethanol containing the tocopherols, and the solution boiled, a clear red product is obtained. On cooling, however, a considerable quantity of material is usually precipitated. The solution thus

becomes turbid and colorimetric readings taken with such a solution would be erroneous. These lipoids are easily removed by filtration, leaving a clear solution. In the Emmerie-Engel procedure, several interfering substances of unknown nature are left after adsorption and acid and alkali treatments. One source of interference is a lipoid which slowly reduces iron.

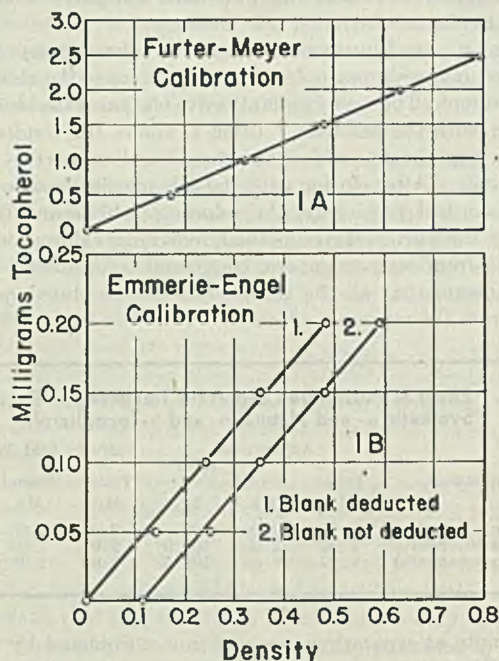


Figure 1. Calibration Curve for Determination of Tocopherol

Purified plant extracts transferred to alcohol and then reacted with the Emmerie-Engel reagents reduced the iron at a slow rate for periods up to 2 or 3 hours. Under the same circumstances pure tocopherol gave a constant reading in 10 minutes. The slow reduction of iron was not linear, for extracts containing twice the quantity of sample gave results 150 to 200% higher than the calculated results. The same effect was noted when pure tocopherol was added to a plant extract, the tocopherol recovery being 150 to 200% higher than the calculated value. The interfering lipoids may be plant sterols, for Devlin and Mattill (*2*) working with muscle extracts noted the same type of interference with the Emmerie-Engel procedure and traced the effect to cholesterol. The authors completely eliminated this source of interference by immediately transferring the colored dipyridyl complex to an aqueous medium, thus taking the lipoidal reducing substances out of contact with the iron. Pure α-tocopherol reduced the iron immediately. Experiments with pure tocopherol solutions treated in this manner gave practically quantitative recovery.

Still another type of interference was noted with certain plant extracts, particularly beet and rhubarb extracts. Recovery of tocopherol added to these extracts and carried through the whole procedure was consistently low. When tocopherol was added directly to the extract after purification of the sample, the recovery was still low. Working on the assumption that some constituent of the purified extract was forming a complex with the ferric ion, the concentration of the iron reagent was doubled (from 0.2 to 0.4%), the quantity of sample used for the final tocopherol determination being kept at a minimum. This technique resulted in greatly improved tocopherol recoveries and was adopted for all plant extracts.

Since the high blank shown in Figure 1, B, may have been due to the exposure to suffused light during the manipulation or to the higher concentration of ferric chloride, these two factors were examined. The densities of blank solutions prepared in the conventional manner were compared with those of solutions so handled that there was no exposure to light. The results in both cases were identical.

To determine the effect of ferric chloride concentration, a series of pure synthetic α-tocopherol standards was run by the conventional procedure, using in one case 1 ml. of 0.2% and in the other 1 ml. of 0.4% ferric chloride solution. The results are shown in Table III.

It is apparent that the increased blank is due to the higher ferric chloride concentration. If the blanks are deducted, the results with either concentration are in good agreement. Although a large blank is generally undesirable, it is necessary to use the high ferric chloride concentration for reasons explained above, and apparently there is no loss of accuracy.

Recovery of Tocopherol. Recovery of synthetic α-tocopherol added to plant extracts and carried through the entire procedures is shown in Table IV. In most cases recovery of tocopherol added to a wide variety of vegetable leaf extracts was satisfactory, averaging about 95% with both methods.

Recovery of tocopherol added to a plant extract is probably more a measure of the precision of the method than of its intrinsic accuracy. The fact that tocopherol is determined rather accurately by the two methods is shown by the fairly good agreement of values obtained by them on the same plant materials (Table V). With a few exceptions, the two methods give results checking within ±5 to 10%. In those few cases in which there is a large discrepancy, the values obtained by the Furter-Meyer method are probably more reliable, since this determination is based on a reaction relatively specific for tocopherol.

Only a few analyses reported in the literature compare the results obtained by the Furter-Meyer and the Emmerie-Engel procedures on biological material. Morton (*10*) quotes an analysis of an oil in which the Furter-Meyer result was 400% higher

Table III. Effect of Iron Concentration on Reagent Blanks Found with Emmerie-Engel Method

Tocopherol	0.2% Ferric Chloride: Density	0.2% Ferric Chloride: Density (blank deducted)	0.4% Ferric Chloride: Density	0.4% Ferric Chloride: Density (blank deducted)
0	0.08	0.00	0.15	0.00
50	0.21	0.13	0.27	0.12
100	0.32	0.24	0.38	0.23
200	0.55	0.47	0.61	0.46

Table IV. Recovery of α-Tocopherol Added to Plant Extracts

Dry Leaf Sample	Tocopherol in Sample	Tocopherol in Sample Plus 1.0 Mg. of Added Tocopherol: Calculated value	Observed value	Total Tocopherol Recovered
	Mg.	*Mg.*	*Mg.*	%
Furter-Meyer Method				
Alfalfa	0.47	1.47	1.38	93.8
Beet	1.19	2.19	2.04	93.0
Broccoli	1.25	2.25	2.22	98.6
Kale	0.91	1.91	1.80	94.2
Lima bean	1.79	2.79	2.68	96.0
Rhubarb	2.74	3.74	3.64	97.3
Spinach	1.29	2.29	2.24	97.8
Turnip	0.85	1.85	1.78	96.2
				Av. 95.8
Emmerie-Engel Method		Plus 62.5 γ Added Tocopherol		
	γ	γ	γ	
Alfalfa	65.0	127.5	133.0	104.3
Beet	89.0	151.5	147.0	97.0
Broccoli	100.0	162.5	149.0	91.7
Kale	97.0	159.5	144.0	90.0
Lima bean	175.0	237.5	221.0	93.0
Rhubarb	150.0	212.5	213.0	100.2
Spinach	100.0	162.5	155.0	95.3
Turnip	88.0	150.5	152.0	100.9
				Av. 96.5

POLITECHNIKI

Table V. Tocopherol Content of Vegetable Tissues

Sample	Furter-Meyer	Emmerie-Engel
	Mg. per gram	
Alfalfa leaf meal	0.23	0.26
Beet leaf meal	0.44	0.71
Broccoli leaf meal	0.39	0.42
Kale leaf meal	0.36	0.39
Lima bean leaf meal	0.72	0.70
Rhubarb leaf meal	1.24	1.20
Spinach leaf meal	0.42	0.40
Turnip leaf meal	0.34	0.30
Mash, 5% alfalfa	...	0.02
Mash, 2.5% broccoli	...	0.02
Scratch corn	...	0.00
Fresh carrot root	0.14[a]	0.13[a]
Fresh carrot top	0.79[a]	0.56[a]
Frozen spinach	0.47[a]	0.43[a]

[a] Calculated on moisture-free basis.

than the Emmerie-Engel value. Hickman *et al.* (*6*) report an analysis of a mixture of foods in which the Furter-Meyer value was 1400% higher than the Emmerie-Engel result. These extreme discrepancies are undoubtedly due to insufficient purification of the extracts prior to color measurement.

The Emmerie-Engel procedure is much more sensitive than the Furter-Meyer, and hence is more convenient for low potency samples.

SUMMARY

The Furter-Meyer and Emmerie-Engel methods for determination of tocopherol have been applied to plant extracts. In both cases, dry plant materials are extracted with Skellysolve B, and fresh materials with an ethanol-petroleum ether solution, the alcohol then being removed. It is necessary to purify the sample before the final estimation can be made. Chlorophyll and xanthophyll are separated from tocopherols by adsorption on a Supercel-activated magnesia column. Carotene and tocopherolquinones are then destroyed by treatment with 85% sulfuric acid.

In the Furter-Meyer procedure the tocopherols are finally transferred to ethanol solution, oxidized with nitric acid, and, after removal of ethanol-insoluble lipoids, determined with a photoelectric colorimeter at 480 mμ.

In the Emmerie-Engel procedure the tocopherols are finally dissolved in chloroform, and reacted with the ferric chloride-α,α'-dipyridyl reagent. The pink ferrous dipyridyl complex is immediately transferred to aqueous solution to prevent further reduction of the iron by a slowly reducing fat-soluble compound. The colorimetric determination is carried out at 520 mμ in a photoelectric colorimeter. $E_{1\,\mathrm{cm.}}^{1\%}$ and 480 and 520 mμ values obtained by the two methods with synthetic and natural α-tocopherols agreed within 5%. The values for natural γ-tocopherol were 10% lower by the Furter-Meyer method and 35% lower by the Emmerie-Engel method. All these compounds can be quantitatively eluted from a magnesia-Supercel adsorbent and show a maximum loss of 5% when shaken with 85% sulfuric acid.

Tests in which pure synthetic α-tocopherol was added to the extracts and carried through the entire procedure showed an average recovery of 95% with both methods. In most cases the results obtained by the two procedures agreed within $\pm$5 to 10%.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of Margaret E. Heller in this investigation.

LITERATURE CITED

(1) Baxter, J. G., Robeson, C. D., Taylor, J. J., and Lehman, R. W., *J. Am. Chem. Soc.*, 65, 918 (1943).
(2) Devlin, H. E., and Mattill, H. A., *J. Biol. Chem.*, 146, 123 (1942).
(3) Emmerie, A., and Engel, Chr., *Rec. trav. chim. Pays-Bas*, 57, 1351 (1938).
(4) Fisher, G. S., Ind. Eng. Chem., Anal. Ed., 17, 224 (1945).
(5) Furter, M., and Meyer, R. E., *Helv. Chim. Acta*, 22, 240 (1939).
(6) Hickman, K. C. D., Kaley, M. W., and Harris, P. L., *J. Biol. Chem.*, 152, 321 (1944).
(7) Hove, E. L., and Hove, Z., *Ibid.*, 156, 601 (1944).
(8) Karrer, P., and Keller, H., *Helv. Chim. Acta*, 21, 1161 (1938).
(9) Moore, L. A., and Ely, R., Ind. Eng. Chem., Anal. Ed., 13, 600 (1941).
(10) Morton, R. A., "Vitamins", London, Adam Hilger, 1942.
(11) Parker, W. E., and McFarlane, W. D., *Can. J. Research*, B 18, 405 (1940).
(12) Quackenbush, F. W., Gottlieb, H. L., and Steenbock, H., Ind. Eng. Chem., 33, 1276 (1941).
(13) Scudi, J. V., and Buhs, R. P., *J. Biol. Chem.*, 141, 451 (1941).
(14) Smith, L. J., *Chem. Rev.*, 27, 287 (1940).
(15) Wall, M. E., and Kelley, E. G., Ind. Eng. Chem., Anal. Ed., 15, 18 (1943).

Method of Evaluating Metal Cleaners

SAMUEL SPRING, HOWARD I. FORMAN, AND LOUISE F. PEALE, Frankford Arsenal, Philadelphia, Pa.

A quantitative method for performance evaluation of alkaline metal cleaners is described and discussed. Reproducibility is rather good. The method involves coating of metal panels with various oils by a specific dipping and drainage technique, followed by a carefully controlled cleaning and rinsing procedure. The panels are covered with a fine spray of water, which condenses as droplets on the oil-covered areas, providing a pattern that remains constant for a sufficient time for a sketch to be drawn on paper divided into 100 squares. The average value for cleaned area of 5 panels is the cleaning efficiency index. Conditions influencing results and variations in the procedure are discussed.

THE importance of having an adequate method for evaluating alkaline cleaners for the removal of contaminants from metal surfaces is generally agreed upon. Morgan and Lankler made an important contribution in this direction (*3*) in 1942 in devising a semiquantitative method that involved photographing fluorescent oil residues under ultraviolet light after a standard cleaning procedure. It was applied specifically to the removal of mineral oil by alkaline salts containing a surface-active agent of the sodium keryl benzene sulfonate type (*2*). This procedure is rather unwieldy, particularly for the control and procurement of alkaline cleaners.

The "water-break" method has been used for a long time as a criterion for evaluating metal surface cleanliness. This test is based on the ability of metal surfaces to sustain an unbroken film of water when "chemically" clean. It has not been found adequate, since the water-break pattern was observed to be dependent on the thickness of the water film. Smaller and smaller areas sustaining a complete water film were obtained as the water drained from the panel. As these areas began to reach a more or less steady state, evaporation of the water became a factor in obscuring the results. As a result of these factors, evaluations performed with the water-break test, as normally used, did not provide an adequate estimate of the efficiency of metal cleaners.

For these reasons, a method has been devised which is fairly simple and has been found capable of yielding results of good reproducibility.

PROCEDURE

Some of the modifications desirable for application to specific problems are obvious. In general, the method used at the arsenal

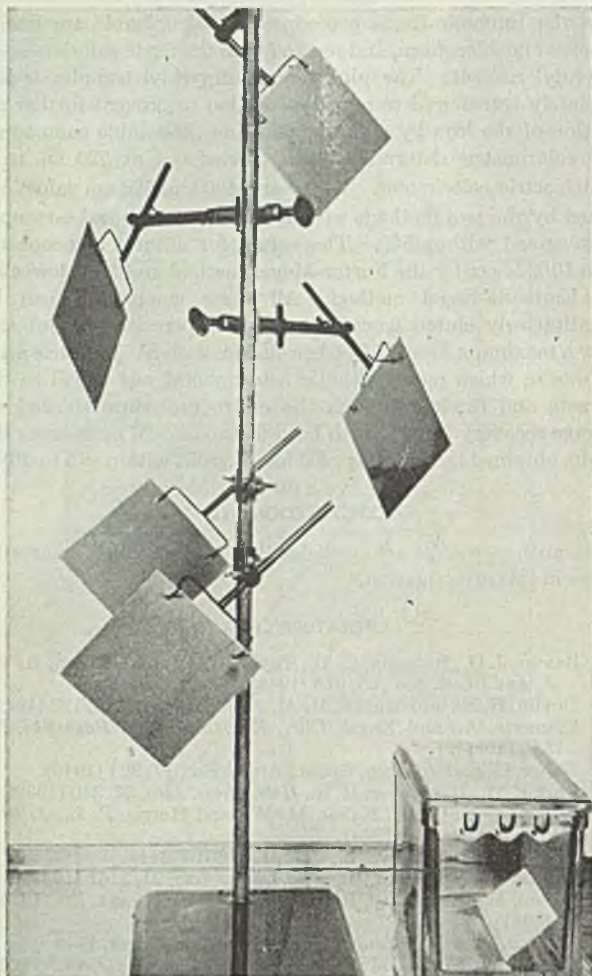


Figure 1. Coating Technique

involves coating of panels by a controlled dipping and drainage technique, followed by carefully controlled cleaning and rinsing operations. The panels are then subjected to a fine spray of water, which has the effect of sharply delineating the oil-covered regions. Quantitative estimation is made by sketching this pattern on paper divided into squares and counting the number of squares that are free of oil.

PREPARATION OF PANELS. The panels are of light-gage metal, 10 cm. (4 inches) square, of cold-rolled SAE 1010 steel. Cold-rolled brass or aluminum may also be used. Two holes are drilled in each panel to enable the insertion of a 2-pronged panel holder (Figure 1) which maintains the panel in position without the use of nuts and bolts or similar arrangements. This was found necessary because other holders trapped some of the oil, which afterwards spread and gave erroneous values. The steel panels are degreased before use with an alkaline silicate plus a synthetic detergent and rinsed with water until a complete water film is sustained. They are then pickled at room temperature, for 1 minute, in 6 N hydrochloric acid containing a small amount of wetting agent, rinsed in running cold water for a short time, given two successive rinses in hot alcohol containing 1% ammonia (70° to 80° C.), air-dried, and stored in an evacuated desiccator until used.

COATING OF PANELS. The panel is immersed in the oil to about half its height. The container is tilted so that the entire panel is covered with oil and then returned to an upright position such that the excess of oil drains from around the holes in the panel. The panel holder prongs are inserted and the panel is hung in a rack at a 45° angle (see Figure 1).

The panels are allowed to drain at 25° ± 2° C. for 1 hour. The globule of oil that remains on the bottom corner is removed. The panels should be shielded from drafts during the drainage period.

It is desirable to determine the weight of oil on individual panels at frequent intervals to ensure control over these conditions.

Two oils are used for coating panels. In one case, the coating is obtained from a hydraulic mineral oil of high viscosity index, with a viscosity at 100° F. of 470 seconds Saybolt Universal viscosity. In the other case, the coating is obtained from a solution of a sulfurized lard oil, containing approximately 12% sulfur, dissolved in toluene to the extent of 1 part of sulfurized oil to 9 parts of toluene. In the work performed with this procedure, the average weight of oil on each panel after coating with mineral oil was 0.283 gram and after coating with sulfurized fatty oil was 0.038 gram. Maximum variations were ±4% for the mineral oil and ±6% for the sulfurized fatty oil, with the great majority of the weights varying from the average by no more than 2%.

ALKALINE SALT SOLUTIONS. The alkaline salts and surface-active agents are dissolved in water to make 2 liters of solution of appropriate concentration. Solutions of 1.5 or 3.0% of alkaline cleaner are usually employed. The beaker containing the solution is heated and then placed in a constant-temperature bath in which the temperature of the solution is maintained at 60° ± 1° C.

CLEANING AND RINSING OPERATIONS. The panel holder is inserted through a cover which tends to reduce evaporation, and connected to a small motor (Figure 2). The panel is positioned in the cleaner so that at least 2.5 cm. (1 inch) of the solution are above the top of the panel, to reduce the effect of temperature changes near the surface. The motor is operated at 10 r.p.m. and the cleaning process is carried on for 5 minutes. The panel is then withdrawn and placed in a tank of running water maintained at 50° C. It is withdrawn from the water once each minute during a rinsing period of 5 minutes. The panel is then rinsed in water at room temperature for 1 minute and allowed to drain for 1 minute.

QUANTITATIVE EVALUATION. A fine spray of water is directed at the panel from a distance of approximately 60 cm. (2 feet) by means of an atomizer connected to a compressed air line. (The head of a De Vilbis bulb atomizer was attached to a compressed air line equipped with a reducing valve. A small spray gun, such as is used for spraying paint, was also employed. The air is filtered through glass wool.) The oil-covered regions are delineated by the condensation upon them of fine droplets of water, resulting in a pattern which remains constant for at least 20 minutes (Figure 3). These are then sketched on paper ruled into 100 squares (Figure 4). The number of squares covered with water (no droplets condensed) is counted for each side of the panel. The results obtained with 5 panels, resulting in 10 observations, are averaged and this is called the cleaning efficiency index. The standard deviation is determined by the formula:

$$\text{Standard deviation} = \sqrt{\frac{\Sigma d^2}{n-1}}$$

d = deviation of individual values from the mean
n = number of observations

Individual values deviating from the mean by more than three times the standard deviation are discarded. The entire determination is discarded when the standard deviation is greater than 9. A large value for the standard deviation was found to be due to

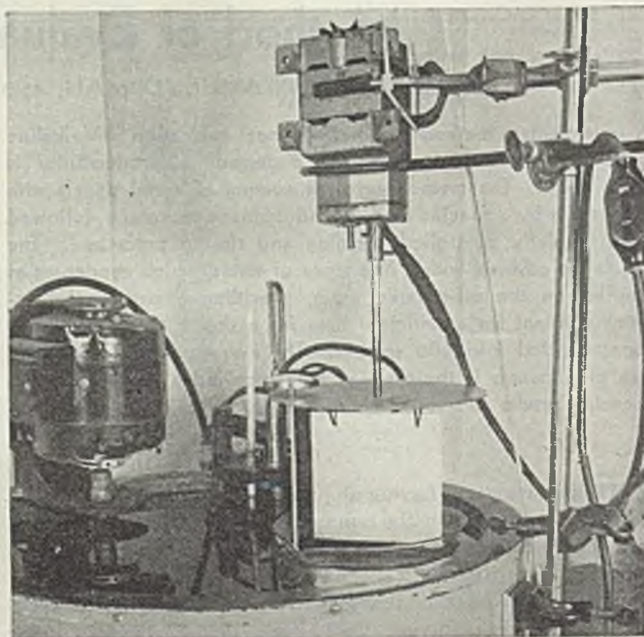


Figure 2. Assembly before Test

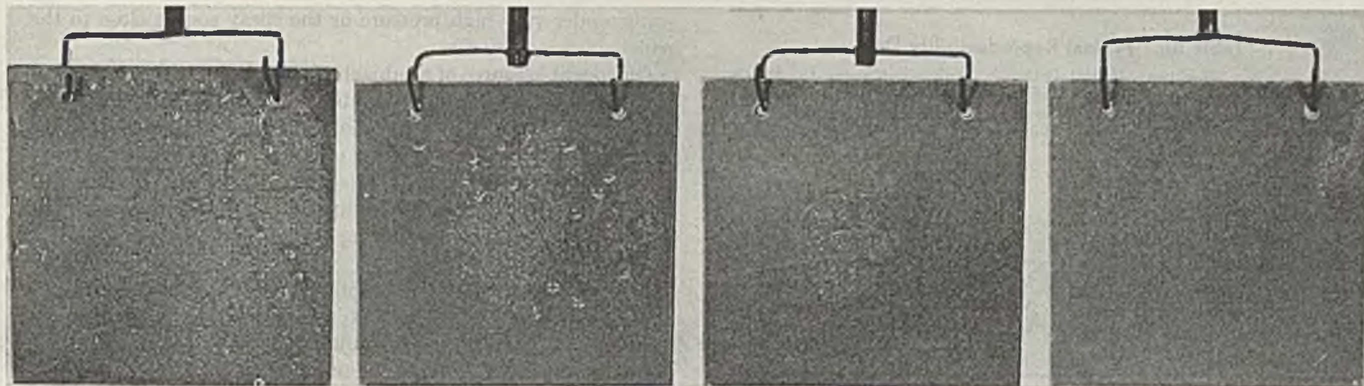


Figure 3. Test Panels

1. Poor cleaning (cleaning index 6) 2. Fair cleaning (cleaning index 73) 3. Fair to good cleaning (cleaning index 88) 4. Good cleaning (cleaning index 94)

Table I. Effect of Surface Smoothness of Aluminum Panels

Oil	Cleaning Index Aluminum (mirror finish)	Aluminum (roughened)
Light mineral oil	99	89
Heavy mineral oil in toluene (1 to 3)	97	11
Lard oil (Prime No. 1)	94	17
Sulfurized mineral oil	95	23
Sulfurized fatty-mineral oil	88	2
Sulfurized fatty base in mineral oil	84	11

variations in the temperature of the room during the drainage of oil from the panels. The frequency of this occurrence, however, is not sufficient to interfere with the usefulness of the method.

DISCUSSION

Selection of Panels. The surface condition of metal panels has a considerable bearing on cleaning efficiency. In utilizing this test to solve specific problems, therefore, panels should be selected with surfaces similar to those involved in the problem. So far as the test itself is concerned, the conditions listed above are out of the critical range for surface variations.

Coating of Panels. It was observed that the ease of removal of oil from the panels was dependent to some extent upon the oil film that was used. Thus, specific differences were observed between sulfurized fatty base oils and mineral or lard oils under certain conditions. Another very important factor was the viscosity of the oil used. It may be seen from Table II that the ease of oil removal decreased as the viscosity of the oil and the weight of oil on the panel increased. The distribution of oil on each of the panels is not uniform, since during the drainage period a greater amount of oil is concentrated on the lower portion of the panel. In a great many tests, however, it was observed that results were not consistently poorer for the lower half of the panel than for the top half, even though approximately two thirds of the total oil was on the bottom half of the panel.

One of the factors that controlled the reproducibility of results to a great extent, in utilizing the above coating procedure, was the temperature at which the excess oil was drained from the panel. In order to obviate difficulties due to this, it was found necessary to maintain the drainage temperature within ±2° C. at 25° C. during the 1-hour period. Examination of the data in Table III indicates that in this range the temperature effect is less than that due to random variables. Lower temperatures resulted in erratic results; higher temperatures in better results which were reproducible. After considerable experience with a particular set of conditions, it should be possible to apply a correction factor for various room temperatures during the hour-long drainage period.

Cleaning and Rinsing Operations. A temperature of 60° C. (140° F.) was utilized in the procedure. This temperature is somewhat lower than that recorded for most industrial cleaning operations, although it is not uncommon for the temperature of production cleaning tanks to drop to this level. Cleaning efficiency improved rapidly as the temperature of cleaning was increased above 60° C. This tended to obscure differences among cleaners and the various factors affecting cleaning.

The effect of agitation, up to 36 revolutions per minute, was found to be fairly small. Generally, the effect of concentration of alkali was considerable up to a minimal value, beyond which large increases in concentration caused relatively small improvements in cleaning efficiency. The concentration of 3% alkaline salts in the above procedure was found to be above this minimum concentration for most conditions. Lower concentrations of alkaline salts tended to accentuate differences among surface-active agents.

Table II. Effect of Viscosity of Mineral Oil on Cleaning Efficiency

Viscosity at 100° F.	Cleaning Index	Weight of Oil on Each Panel	No. of Determinations
Sec. S.U.V.		*Gram*	
970	11	0.454	2
651	42	0.378	7
456	55	0.336	6
283	95	0.265	6

Cleaner, 3% sodium orthosilicate plus 0.15% sodium keryl benzene sulfonate (40%).
Conditions, 60° C., 10 r.p.m., 5 minutes.

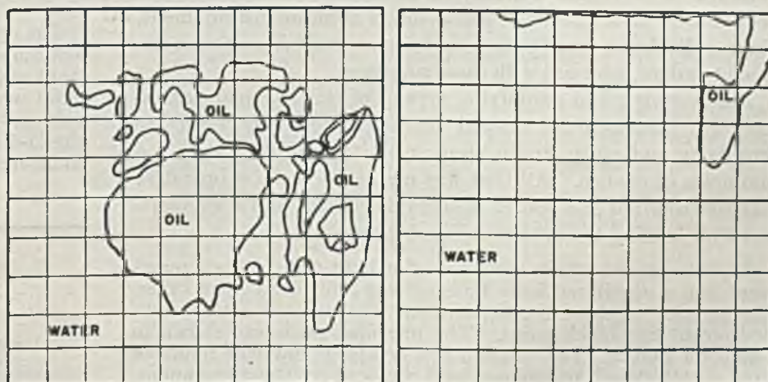


Figure 4. Cleaning Results

Left. Cleaning index 73 (fair). *Right.* Cleaning index 94 (good)

Table III. Typical Reproducibility Data

	Temp., ° C.	Medium Mineral Oil[a] Cleaning efficiency index	Standard deviation	Sulfurized Fatty Oil Base[b] Cleaning efficiency index	Standard deviation
1	25	56	6	80	9
2	25	53	5	86	8
3	23	55	5	85	5
4	24	53	3	81	6
5	24	55	5	83	6
6	26	56	8	85	6

[a] Mineral oil of high viscosity index. Viscosity 470 sec. per 100° F. S.U.V. Av. weight 0.283 gram.
[b] Sulfurized fatty oil in toluene (1 to 9). Av. weight 0.038 gram. Temperature between 23° and 27° C.
Cleaner, 3% sodium orthosilicate plus 0.15% sodium keryl benzene sulfonate (40%). Conditions, 5 minutes, 10 r.p.m., 60° C.

Variations in the rinse-water temperature were found to be not very important. In the procedure a fairly long rinsing time is provided at a fairly high temperature. Under these conditions it was found unnecessary to dip the panels in dilute acid, prior to the evaluation, to avoid "false water film continuity" (*1*) due to the presence of some of the surface-active agent. The main reason for eliminating the acid rinse was that rusting of the panel developed after this rinse and tended to obscure the patterns of oil-covered areas that were being sketched.

EVALUATION AND REPRODUCIBILITY. While the spraying of the panel with water is not extremely critical, care should be exercised to prevent its becoming drenched with a large excess of water. Two or three fairly slow passes with a fine spray of water appear to be optimum. It is also desirable to avoid having the water under very high pressure or the spray source close to the panel.

One possible source of a subjective error lies in the sketching of the pattern of oil-covered areas disclosed by the condensation of the spray of water. Comparison of the values obtained with three different operators, on a number of occasions, indicated that this error was rather small, being less than 2%. For operators who might have difficulty in this evaluation, it is possible to provide assistance in the form of a viewing screen divided into 100 squares or some similar arrangement.

Some reproducibility data, obtained on different days over a period of several months, are listed in Table III. The values for cleaning index fall well within the range to be anticipated from the magnitude of the standard deviation.

Appreciation is expressed to Lt. Col. C. H. Greenall, officer-in-charge, Maj. W. W. Culbertson, research officer, C. C. Fawcett, associate director, and E. R. Rechel, chief of the Chemical Research Section of Frankford Arsenal Laboratory, as well as the Ordnance Department, for permission to publish this paper. Special thanks are due J. W. Mitchell for his helpful review of the paper.

LITERATURE CITED

(1) Harris, J. C., *A.S.T.M. Bull.*, 136, 39 (1945).
(2) Morgan, O., and Lankler, J. G., IND. ENG. CHEM., 34, 1158 (1942).
(3) Morgan, O., and Lankler, J. G., IND. ENG. CHEM., ANAL. ED., 14, 725 (1942).

PRESENTED at the spring meeting of the Philadelphia Section and the Meeting-in-Print of the AMERICAN CHEMICAL SOCIETY, Division of Analytical and Micro Chemistry, 1945.

Fluorometric Attachment for the Beckman Spectrophotometer

MARY H. FLETCHER, CHARLES E. WHITE[1], AND MILTON S. SHEFTEL[2]
Eastern Experiment Station, Bureau of Mines, College Park, Md.

A fluorometric attachment for the Beckman spectrophotometer for use in the measurement of fluorescence in solutions consists of a light-tight cell compartment equipped with suitable filters and a lamp housing. It uses the receiving and amplifying systems of the Beckman instrument. General Electric B-H-4 mercury lamp is light source. Lamp emission is controlled by Sola constant-voltage transformer No. 30,852 and by proper ventilation of lamp housing.

MEASUREMENT of the fluorescence of solutions as an analytical procedure has been confined primarily to the field of vitamin chemistry, and for this reason, most commercial fluorometers have been designed for use with the brilliantly fluorescing solutions encountered in this field. For weakly fluorescing solutions, such as those encountered in the determination of beryllium (*2*), the commercial instruments at hand proved to be insufficiently sensitive for an accurate measurement. Since a Beckman spectrophotometer was available, it was decided to adapt it for use with these solutions.

The receiving and amplifying system of the Beckman instrument (*1*) possessed the desired characteristics with respect to sensitivity and range, and consequently it could be used with a minimum of change. All that was needed to make it operative was the addition of a source of ultraviolet light and a compartment for holding the optical cell and filters.

An attachment which consisted of a light-tight cell compartment and a ventilated lamp housing was built from sheet brass. The interior of the cell compartment was painted with a nonfluorescent flat black paint. The principal parts are shown in Figures 1 and 2. The original cell compartment was removed from the Beckman instrument and replaced by the attachment, the cell compartment of which was bolted to the original phototube housing. The arrangement is shown in Figure 1, in which 1 is the lamp housing, 2 the cell compartment, 3 the phototube housing, 4 the Beckman spectrophotometer, 5 the ventilating fan, and 6 the ventilating louvers. The ventilating louvers occupy two of the outer adjacent sides and the top of the lamp housing section. An opening is provided on the third side to permit a connection with the fan. Figure 2 is a plan view (covers removed) of the fluorometric attachment and shows the relative positions of the various parts of the instrument.

The light source, a General Electric B-H-4 mercury lamp, was chosen because previous experience had shown it to be generally satisfactory. Another possible light source, the hydrogen discharge tube furnished with the Beckman instrument, was tried on the beryllium-quinizarin solutions and proved unsatisfactory because of the very low intensity of the exciting wave lengths. On the other hand, the B-H-4 lamp because of its strong emission at 3650 Å. is an excellent light source and provides an intense radiation of the proper wave lengths for an efficient excitation of fluorescence in solutions of the type under consideration.

The emission of the mercury lamp is influenced by the voltage and ambient temperature of the lamp; therefore, to ensure constant emission, both voltage and temperature regulation on the lamp were found necessary. For the former, a Sola constant-voltage transformer No. 30,852 specifically designed for use with the H-4 lamp was employed. For the latter, an Eastman darkroom ventilating fan, Model A, was used.

Table I. Stability

Input Voltage	Reading for 22.4 Micrograms of BeO in 25 Ml. of Solution	Reading for 11.2 Micrograms of BeO in 25 Ml. of Solution
110	98.0	46.8
120	101.4	49.0
130	103.8	50.0
Spread, 20 volts	5.8 scale divisions	3.2 scale divisions

[1] Address, University of Maryland, College Park, Md.
[2] Present address, Gering Products Co., Kenilworth, N. J.

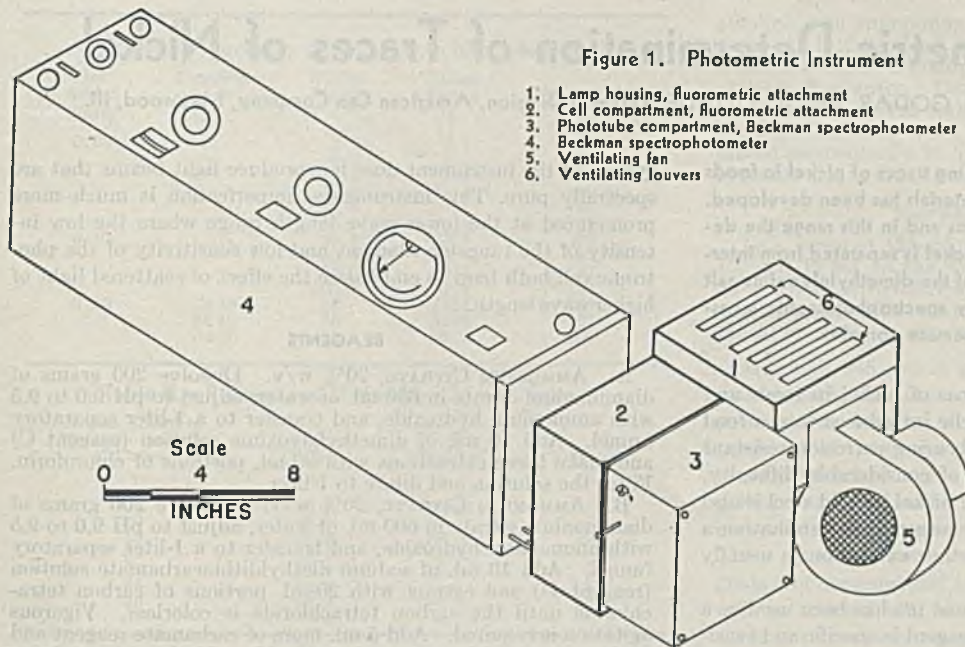


Figure 1. Photometric Instrument

1. Lamp housing, fluorometric attachment
2. Cell compartment, fluorometric attachment
3. Phototube compartment, Beckman spectrophotometer
4. Beckman spectrophotometer
5. Ventilating fan
6. Ventilating louvers

The stability of the instrument accompanying large changes in input voltage is illustrated by Table I. These data were obtained by varying the input voltage to the Sola transformer by means of an autotransformer connected to the line, and noting the change in the phototube response to two of the beryllium-quinizarin standards.

In the case of the stronger solution, a difference of 5.8 scale divisions resulted from a change of 20 volts. This change would correspond to 0.81 microgram of beryllium oxide (value obtained from working curve, micrograms of BeO versus Beckman scale reading), which is 3.6% of the amount measured, or an error of 0.18% per volt. For the weaker solution, the difference of 3.2 scale divisions corresponds to 0.90 microgram of beryllium oxide, which is 8.1% of the amount present, or an error of 0.4% per volt. These figures are given merely to show the performance of the setup under extreme conditions and do not represent the results obtained in normal operation. There was no measurable lag between voltage change and change in reading.

Proper ventilation of the lamp housing provides satisfactory control for the lamp temperature, and equilibrium is generally reached after a half-hour warm-up period.

In all fluorometers certain problems of light filtration exist. Visible light should be excluded from the cell compartment, and ultraviolet light should be excluded from the phototube, yet the fluorescent light must pass freely to the phototube.

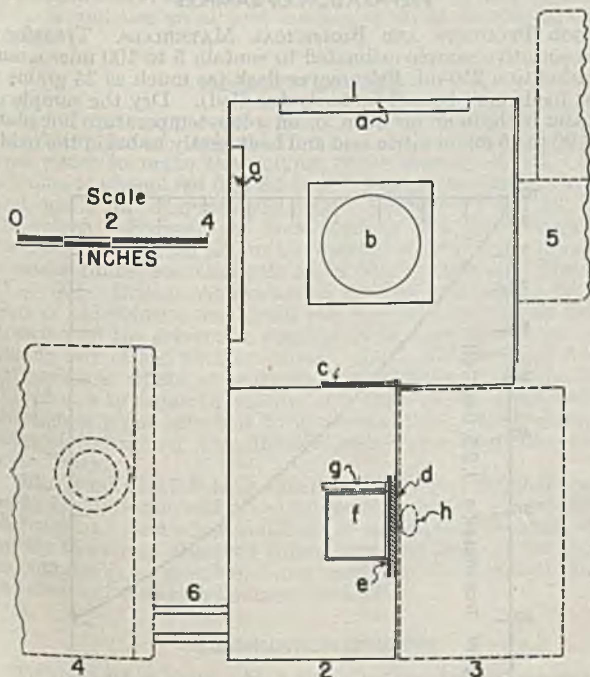


Figure 2. Fluorometric Attachment

1. Lamp housing
   a. Louvers
   b. B-H-4 mercury lamp
   c. Filter for absorbing visible light
2. Cell compartment
   d. Filter for absorbing ultraviolet light
   e. Brass diaphragm
   f. Optical cell
   g. Guide for optical cell
3. Phototube housing
   h. Phototube
4. Beckman spectrophotometer
5. Fan
6. Electrical cables

In the present case the combination of filters chosen (Corning Nos. 5874 and 3486), together with the blue sensitive phototube, have the characteristics necessary to permit measurement of the fluorescence. The primary filter (Corning 5874) eliminates all light lying between 4100 and 7000 Å. from the exciting radiation; and the secondary filter (Corning 3486) absorbs all light of a wave length less than 5000 Å. but has a high transmission in the region 5700 to 6400 Å. The phototube is insensitive to light with a wave length greater than 7000 Å. Hence, since the fluorescent radiation is the only light transmitted which affects the phototube, it alone is measured.

The attachment herein described was designed for a particular purpose, the measurement of a weak fluorescence of 5700 to 6400 Å. In this case, the optical glass cell used to contain the solutions was a 31-mm. cube, inside measurement, with a wall thickness of 1 mm. Cells of this type may be purchased from Pyrocell Manufacturing Co., 207 East 84th St., New York 28, N. Y.

Comparative data on the performance of the instrument are given elsewhere (3). It can be adapted for use in the measurement of other wave lengths and stronger fluorescence by changing the sensitivity setting of the Beckman instrument, by the proper selection of filters, by varying the size of the aperture in the brass diaphram, and by the use of other light sources. The attachment should prove useful to any laboratory that has a Beckman spectrophotometer in its possession.

ACKNOWLEDGMENTS

The study reported in this paper was under the general direction of J. B. Zadra, chief, College Park Division, Metallurgical Branch, U. S. Bureau of Mines. The writers are indebted to Alton Gabriel and Morris Slavin for assistance in the writing of this paper, to Rudolf Kudlich and his men in the shop for the construction of the attachment, to John P. Wintermoyer and Rebecca Bland for the drawings, and to Stephen L. Windes for assistance with the electrical units.

LITERATURE CITED

(1) Cary, H. H., and Beckman, A. O., *J. Optical Soc. Am.*, 31, 682–9 (1941).
(2) Fletcher, M. H., White, C. E., and Sheftel, M. S., Ind. Eng. Chem., Anal. Ed., 18, 179 (1946).
(3) *Ibid.*, Table II.

Published by permission of the Director, Bureau of Mines, U. S. Department of the Interior.

Spectrophotometric Determination of Traces of Nickel

O. R. ALEXANDER, EDITH M. GODAR, AND N. J. LINDE, Research Division, American Can Company, Maywood, Ill.

A rapid and precise method of determining traces of nickel in foods and biological materials and in ferrous materials has been developed. The optimum range is 5 to 80 micrograms and in this range the determinations are accurate to ±5%. Nickel is separated from interfering elements by a solvent extraction of the dimethylglyoxime salt of divalent nickel, and is determined by spectrophotometric measurement of the yellow diethyldithiocarbamate complex.

THE accurate determination of traces of nickel in foods and biological material, such as might be introduced into a food product through contact with nickel-bearing corrosion-resistant equipment, is an analytical problem of considerable difficulty. The determination of small amounts of nickel in mild steel is also a time-consuming procedure, since for accurate determination a preliminary separation by means of ether extraction is usually required.

The potassium salt of dithiooxalic acid (*6*) has been used as a colorimetric reagent for nickel. The reagent is specific and sensitive, but the reagent itself as well as the nickel complex is unstable. The literature contains a number of references to the use of dimethylglyoxime as a colorimetric reagent for the determination of nickel (*4, 5*). This reaction between dimethylglyoxime and nickelic (IV) ion is somewhat empirical, and the results depend upon adherence to a closely defined operational procedure. A number of other reagents have been proposed, but have little to recommend them for general analytical purposes (*1, 3*).

The method described herein employs two reactions of nickel, both of which have been previously reported. Nickel is first separated by means of a chloroform extraction of the divalent nickel salt of dimethylglyoxime (*5*). After decomposition with dilute hydrochloric acid, and extraction of the nickel from the chloroform, the nickel is converted to the diethyldithiocarbamate complex and extracted with isoamyl alcohol. The nickel is determined in this solution by spectrophotometric measurement of the concentration of the yellow-green nickel complex. This reaction has been widely recognized, but has not to the authors' knowledge been used to determine nickel (*2*).

DISCUSSION OF METHOD

Dimethylglyoxime has long been regarded as one of the most specific organic precipitants available in trace metal analysis. This specificity as a precipitant also characterizes the chloroform extraction described in this report. According to available literature references to the use of sodium diethyldithiocarbamate (*2*) the metals giving colored precipitates soluble in organic solvents are nickel, copper, cobalt, bismuth, and iron. Iron does not react in ammoniacal citrate solutions and nickel is separated completely from the other potential interfering elements by the glyoxime extraction. Copper if present in high concentrations may accompany the nickel to some extent, but may easily be removed from the chloroform solution by an intermediate wash with dilute ammonia.

The nickel dithiocarbamate complex absorbs most strongly in the very edge of the visible spectrum, and since the eye does not adequately resolve minor differences in the concentration of the faintly colored solutions the use of a spectrophotometer is almost mandatory. This low visual sensitivity probably explains in part why the reagent has not been used previously. The data presented in this report were obtained with a Coleman Model 11 Universal spectrophotometer. The curves relating concentration to the logarithm of the transmission are curved not because the solutions fail to obey Beer's law, but because the optical system of the instrument does not produce light beams that are spectrally pure. This instrumental imperfection is much more pronounced at the lower wave length range where the low intensity of the tungsten filament and low sensitivity of the photronic cell both tend to emphasize the effect of scattered light of higher wave length.

REAGENTS

A. Ammonium Citrate, 20% w/v. Dissolve 200 grams of diammonium citrate in 600 ml. of water, adjust to pH 9.0 to 9.5 with ammonium hydroxide, and transfer to a 1-liter separatory funnel. Add 10 ml. of dimethylglyoxime solution (reagent C) and make three extractions with 30-ml. portions of chloroform. Filter the solution and dilute to 1 liter.

B. Ammonium Citrate, 20% w/v. Dissolve 200 grams of diammonium citrate in 600 ml. of water, adjust to pH 9.0 to 9.5 with ammonium hydroxide, and transfer to a 1-liter separatory funnel. Add 10 ml. of sodium diethyldithiocarbamate solution (reagent D) and extract with 20-ml. portions of carbon tetrachloride until the carbon tetrachloride is colorless. Vigorous agitation is required. Add 5 ml. more of carbamate reagent and again extract with carbon tetrachloride. If the solvent layer is colorless the extraction is complete; if it remains yellow, add more carbamate and continue the extraction.

C. Dimethylglyoxime, 0.1%. Dissolve 0.25 gram of reagent grade reagent in 50 ml. of 95% ethanol and dilute to 250 ml.

D. Sodium Diethyldithiocarbamate, 0.2%. Dissolve 1 gram in 100 ml. of distilled water, filter, and dilute to 500 ml.

E. Hydrochloric Acid, 0.5 *N*. Dilute 40 ml. of hydrochloric acid (sp. gr. 1.18) to 1 liter with redistilled water.

F. Nickel Standard. Dissolve 0.500 gram of pure nickel in 20 ml. of (1 + 1) nitric acid and dilute to 1 liter. Prepare a working standard by diluting 10 ml. of this solution to 1 liter with 0.5 *N* hydrochloric acid. One milliliter of the working standard contains 5 micrograms of nickel.

PREPARATION OF SAMPLES

Food Products and Biological Materials. Transfer a representative sample estimated to contain 5 to 100 micrograms of nickel to a 250-ml. Erlenmeyer flask (as much as 25 grams of most foods may be satisfactorily handled). Dry the sample on a steam bath, in an air oven, or on a low-temperature hot plate. Add 20 to 25 ml. of nitric acid and heat gently to begin the oxida-

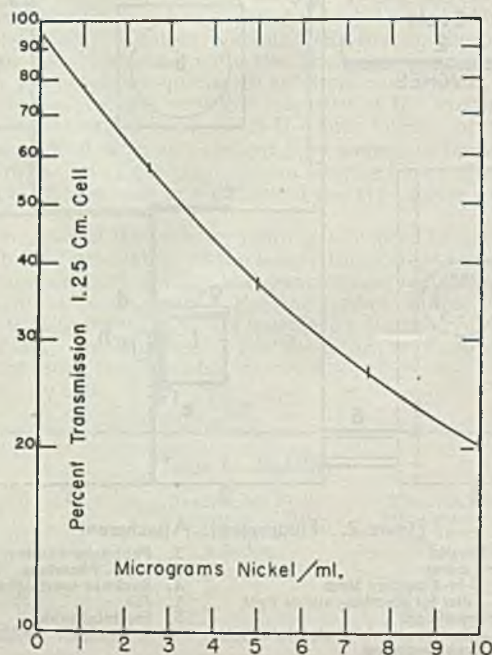


Figure 1

Table I. Recovery of Nickel from Iron Solutions

Iron Added	Impurity Added	Nickel Added	Nickel Found	Recovery
Gram	*Mg.*	γ	γ	%
0.20	...	2.5	2.7	108
		5.0	4.9	98
		7.5	7.9	105
		10.0	10.0	100
		12.5	12.1	97
		15.0	14.8	99
0.10	...	25.0	25.7	103
		50.0	48.6	97
		75.0	72.3	97
		100.0	97.1	97
0.20	Co 10	25	25.2	101
	Bi 10	25	25.6	102
	Cu 10	25	25.1	100

Table II. Recovery of Nickel from Tomato Catsup

Nickel Added to 10-Gram Samples of Catsup	Nickel Found	Nickel Present	Recovery
γ	γ	γ	%
0	16.5	(16.5)	...
0	16.5	(16.5)	...
10	26.9	26.5	102
25	40.0	41.5	94
50	66.0	66.5	99
65	80.0	81.5	98
80	94.4	96.5	98

tion. When the initial reaction subsides, add 5 ml. of sulfuric acid and continue heating. Add more nitric acid in small increments as the mixture darkens and begins to char. When fumes of sulfur trioxide are evolved with no further charring or darkening, add 1 ml. of perchloric acid and continue heating until the perchloric acid has been volatilized and sulfuric acid is refluxing freely. Allow to cool and dilute with 20 to 25 ml. of distilled water.

STEELS. Dissolve 0.200 gram of steel in a small covered beaker with 15 ml. of nitric acid (1 + 2). After the first vigorous reaction subsides, add 10 ml. of perchloric acid and heat slowly until the perchloric acid is refluxing freely and solution of the steel is complete. Cast iron samples may require the addition of more nitric acid to complete the oxidation and solution of refractory carbides. Cool the digest and dilute with water. If the nickel content of the steel is expected to run over 0.04%, dilute to volume and use an aliquot containing 20 to 80 micrograms of nickel.

ISOLATION OF NICKEL

Transfer the prepared sample, or a suitable aliquot, to a 125-ml. separatory funnel, and add 10 ml. of citrate buffer (reagent A) and water to make the volume approximately 40 ml. Make alkaline to phenol red (pH 8.5 to 9.0) with ammonium hydroxide, and add 5 ml. of dimethylglyoxime (reagent C) and 10 ml. of chloroform. Stopper the flask securely and shake vigorously for 1 minute. Drain all but a few drops of the solvent layer into a second funnel containing 25 ml. of dilute ammonium hydroxide (1 + 50). Repeat the extraction with an additional 5-ml. portion of chloroform, and drain this too into the second funnel, drawing off the solvent as completely as possible without introducing any of the aqueous phase. Shake the combined solvent extracts and dilute ammonium hydroxide for 1 minute, allow the phases to separate cleanly, and draw off the aqueous layer through a pipet attached to a vacuum line. Wash down the sides of the funnel with distilled water, and again remove the aqueous layer.

Add 25 ml. of 0.5 N hydrochloric acid (reagent E), shake vigorously for 1 minute, and allow to separate. Drain off and discard the solvent. Add a few milliliters of carbon tetrachloride, shake briefly to extract dissolved chloroform, and draw off the solvent as completely as possible, being careful to dislodge and remove the drop which usually floats on the surface.

DETERMINATION OF NICKEL

To the acid solution add 5 ml. of citrate buffer (reagent B). (The two citrate solutions should not be used interchangeably. If solution B, which contains residual carbamate, were used in the first extraction, copper, cobalt, lead, zinc, cadmium, bismuth, and possibly other metals would be partially extracted with the nickel. If solution A were used in the final extraction, impurities in the citrate such as copper and cobalt which are not removed by the dimethylglyoxime extraction would contaminate the final extract.) Add a small piece of litmus paper and make slightly alkaline with ammonium hydroxide, adding 8 to 10 drops in excess. Add 10.0 ml. of isoamyl alcohol and 5 ml. of carbamate solution (reagent D), stopper the funnel, and shake vigorously for at least 2 minutes. Draw off the aqueous phase, transfer the solvent layer to the spectrophotometer cell, and determine the transmission at a wave length of 385 mu. The cell may be placed momentarily in hot water in the event that the solution becomes cloudy due to separation of dissolved water.

PREPARATION OF STANDARDS

To a series of 125-ml. separatory funnels, add 0, 1, 2, 3, 5, 7, 10, and 15 ml. of the working standard solution, and dilute to 25 ± 0.5 ml. with 0.5 N hydrochloric acid. Treat these standards exactly as described under the preceding section, beginning with the addition of the citrate buffer (reagent B).

Although 385 mu is given as the wave length of minimum transmission, the actual value may vary somewhat from one instrument to another, depending upon the width and spectral purity of the wave-length band isolated. Figure 1 shows an average standard curve prepared from data obtained over a period of several weeks. The vertical lines intercepted by the curve indicate the spread of some 10 to 12 values in each case. These standards were not all prepared with the same lot of reagents and consequently the data reflect variations in reagents as well as instrumental and manipulative errors. Although this curve is reproducible over relatively short periods of time, some variation may be expected due to the aging of the tungsten filament bulb and the accompanying change in spectral emission.

The sensitivity and precision of the instrumentation could be materially increased through the use of a spectrophotometer giving greater dispersion and more nearly monochromatic light. Somewhat greater sensitivity and precision would also be gained if it were possible to use spectrophotometer cells designed to permit transmission measurements on small volumes of solution at longer cell lengths.

Table III. Recovery of Nickel from Evaporated Milk

Nickel Added to 10-Gram Samples of Evaporated Milk	Nickel Found	Nickel Present	Recovery
γ	γ	γ	%
0	0.2	(0.2)	...
0	0.2	(0.2)	...
0	0.3	(0.2)	...
5	5.2	5.2	100
10	10.3	10.2	99
15	15.4	15.2	101
30	30.3	30.2	100
50	49.8	50.2	99

RECOVERY DETERMINATIONS

Known amounts of nickel were added to aliquots of a nickel-free iron solution containing 0.10 and 0.20 gram of iron, to 10-gram samples of tomato catsup, and to 10-gram samples of evaporated milk. These samples then were subjected to the procedure described. The data presented in Tables I, II, and III show that satisfactory recovery of added nickel is obtained, even in the presence of relatively large amounts of potentially interfering elements.

In order to test further the application of the method, a number of Bureau of Standards steel samples were analyzed for nickel

Table IV. Determination of Nickel in Bureau of Standards Samples

N.B.S. Sample No.	Reported Nickel Content of Standard Samples: Certified value	Reported Nickel Content of Standard Samples: Range of results of collaborating analysts	Nickel Found
10 d	0.002	0.001–0.004	0.0054
8 g	0.011	0.010–0.013	0.0099
55 a	0.019	0.019–0.020	0.0186
15 d	0.045	0.022–0.026	0.0233
11 e	0.036	....	0.0446
74	0.227	0.034–0.039	0.0363
20 d	0.152	0.217–0.239	0.231
21 c		0.144–0.17	0.149

by the method described. The results of these determinations (Table IV) are in very good agreement with the certified value, when the variation in the values reported to the Bureau of Standards by the collaborating analysts is taken into consideration.

SUMMARY

A colorimetric micromethod for nickel is described which is applicable to the determination of traces of nickel in foods and biological materials as well as in carbon steels. It is based upon the isolation of nickel by solvent extraction of the dimethylglyoxime complex and the subsequent determination by means of sodium diethyldithiocarbamate. Recovery determinations and analyses of standard samples indicate that nickel may be determined with satisfactory precision when present in amounts of 1 microgram or greater.

LITERATURE CITED

(1) Denigès, G., *Bull. soc. pharm. Bordeaux*, 20, 101 (1932).
(2) Holland, E. B., and Ritchie, W. S., *J. Assoc. Official Agr. Chem.*, 22, 333 (1939).
(3) Lindt, T. V., *Z. anal. Chem.*, 53, 165 (1914).
(4) Murray, W. M., Jr., and Ashley, S. E. Q., Ind. Eng. Chem., Anal. Ed., 10, 1 (1938).
(5) Sandell, E. B., and Perlich, R. W., *Ibid.*, 11, 309 (1939).
(6) Yoe, H. J., and Wirsing, F. H., *J. Am. Chem. Soc.*, 54, 1866 (1932).

Amperometric Microtitration of Very Dilute Chromate Solutions

Using the Rotating Platinum Electrode

I. M. KOLTHOFF AND D. R. MAY[1], School of Chemistry, University of Minnesota, Minneapolis, Minn.

Chromate (chromic acid) can be titrated rapidly by amperometric titration with ferrous iron, using the rotating platinum wire microelectrode as the indicator electrode. The potential of the indicator electrode is maintained at 1.0 volt vs. the saturated calomel electrode which is used as the reference electrode. The method is accurate and precise to within 0.5% at concentrations as small as 1 to 2 × 10^{-4} *M* chromate. The method can also be used in the reverse titration of traces of ferrous iron with dichromate.

CHROMATE in very dilute acid solutions can be accurately determined by amperometric titration with ferrous sulfate, using a rotating platinum electrode as indicator electrode. This method does not involve an indicator correction; this latter is appreciable when very dilute solutions are titrated. The principles and technique of amperometric titrations with the rotating platinum wire microelectrode have been discussed and described by Laitinen and Kolthoff (*1*, *2*). The method described here for chromate involves the titration of an acidified chromate solution with a ferrous iron solution. The end point is found graphically as the point of intersection of the "residual current" line before the end point and of the anodic diffusion current line of the ferrous iron after the end point. Under the experimental conditions used in this work the residual current was zero.

The anodic oxidation of ferrous iron to ferric gives a well-defined diffusion current at a rotating platinum microelectrode. Current-voltage measurements using 5 × 10^{-5} *M* ferrous ammonium sulfate in 0.1 *M* perchloric acid are shown in curve 1, Figure 1. In curve 2, the residual current of 0.1 *M* perchloric acid solution is shown. At a potential of +1.0 volt of the rotating electrode, as measured against a saturated calomel reference electrode, proportionality was found between the concentration of the ferrous ammonium sulfate and the diffusion current, as may be seen in Table I.

At potentials of +1.0 volt no current is obtained by electrode reactions with Cr^{++++++}, Cr^{+++}, or Fe^{+++}. Thus when an acid solution of chromate is titrated with a ferrous ammonium sulfate solution no current is observed at a rotating platinum electrode potential of 1.0 volt until all the chromate has been reduced and the solution contains an excess of ferrous iron. By measuring the current after adding successive amounts of ferrous solution and obtaining two or more values in the presence of an excess of ferrous solution the end point is found by graphical extrapolation to zero current. The volume of ferrous solution at that point is equal to that at the equivalence point. Typical titrations are shown in Figure 2.

[1] Present address, American Cyanamid Co., Stamford, Conn.

PROCEDURE

The chromate solution to be titrated is placed in a beaker of suitable size and a rotating platinum microelectrode and salt bridge are placed in position. A saturated calomel electrode is used as an outside reference electrode. If the solution is not already acid, sufficient acid is added to give a concentration of 0.1 *M* (perchloric, hydrochloric, sulfuric, or nitric acid may be used). A 1-volt difference of potential is applied across the platinum (anode) and saturated calomel electrodes, using a salt bridge of low resistance. A 0.01 *N* standard ferrous ammonium sulfate, 0.05 *M* in sulfuric acid, is added to the chromate solution from a microburet until a current is observed on the microammeter or other suitable current-indicating instrument. The sides of the beaker are rinsed with distilled water and the diffusion current of

Table I. Diffusion Currents of Ferrous Ammonium Sulfate Solutions in 0.1 M Perchloric Acid Solution

(Rotating platinum electrode. Potential + 1.0 volt *vs.* saturated calomel electrode)

Molar Concentration of Ferrous Iron × 10^5	Diffusion Current, Microamperes	i_d/c × 10^5
10	8.9	0.89
5	4.5	0.90
2	1.8	0.90
1	0.9	0.90

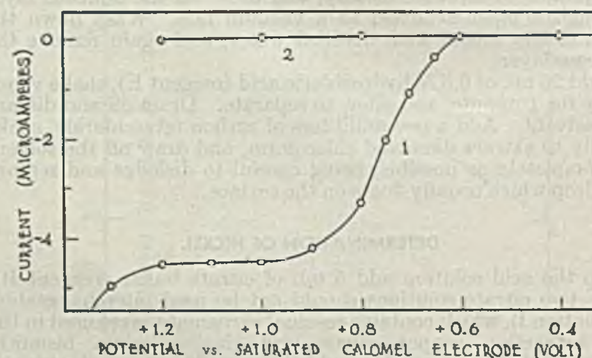


Figure 1. Current-Voltage Curve of 5 × 10^{-5} M Ferrous Ammonium Sulfate in 0.1 M Perchloric Acid

1. 5 × 10^{-5} *M* ferrous ammonium sulfate in 0.1 *M* perchloric acid
2. 0.1 *M* perchloric acid

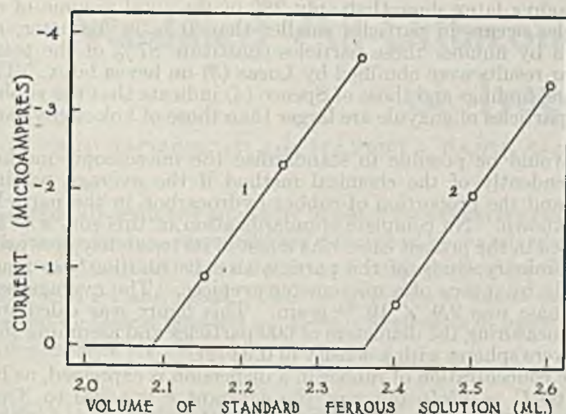


Figure 2. Amperometric Titration of Potassium Chromate in 0.1 *M* Perchloric Acid with 0.00962 *N* Ferrous Ammonium Sulfate

1. 50 ml. of 1.333×10^{-4} *M* chromate
2. 50 ml. of 1.510×10^{-4} *M* chromate

the ferrous iron is measured. The current is measured again after the addition of 0.2 ml. more of ferrous solution. One or two more readings are made after successive addition of more ferrous iron. On graph paper, points representing the microamperes (ordinate) and volume of ferrous solution (abscissa) are plotted. These points lie on a straight line. The volume of ferrous solution at zero current is obtained by drawing a line through these points. For the most accurate work the current should be corrected to compensate for dilution (*2*).

Several dilute chromate solutions were analyzed in this manner. The ferrous solution used was prepared by diluting a 0.098 *N* ferrous ammonium sulfate solution tenfold. The diluted solution was standardized against potassium chromate, using sodium diphenylamine sulfonate as indicator and correcting for the indicator blank. The concentration found was 0.00961 *N*. In Table II are the results obtained in the amperometric titration of dilute chromate solutions with 0.0096 *N* ferrous ammonium sulfate solution. The solutions were 0.1 *N* in perchloric acid.

Table II. Amperometric Titration of 50 Ml. of Dilute Chromate Solutions

Molar Concentration of Chromate $\times 10^4$	Volume of 0.01 *N* Ferrous Solution, Ml.	Calculated Molar Concentration of Chromate Solutions $\times 10^4$	Error, %
1.00	1.560	0.999	−0.1
1.333	2.086	1.336	+0.2
1.510	2.358	1.511	+0.1
1.667	2.603	1.668	+0.1
1.667	2.590	1.660	−0.4
2.000	3.122	2.000	0.0
2.000	3.120	1.999	−0.1

The rapid method, which is accurate and precise to 0.5% even at concentrations as small as 1 to 2×10^{-4} *M* chromate, can also be used for the reverse titration of traces of ferrous iron with dichromate. The current decreases continuously during the titration and becomes zero at the end point. Again the exact location of the end point is found graphically.

LITERATURE CITED

(1) Kolthoff, I. M., and Lingane, J. J., "Polarography", New York, Interscience Publishers, 1941.
(2) Laitinen, H. A., and Kolthoff, I. M., *J. Phys. Chem.*, 45, 1079 (1941).

From part of a thesis submitted by D. R. May to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the doctor's degree (1944).

Rapid Estimation of Rubber in Guayule Latex Dispersions

R. T. WHITTENBERGER AND B. A. BRICE, Eastern Regional Research Laboratory, Philadelphia 18, Pa.

SPENCE (*4*) has shown that the rubber of guayule (*Parthenium argentatum* Gray) is recoverable as a latex which yields a product of high purity and superior quality. His observations on the quality of the latex rubber have been confirmed recently by Clark and Place (*1*). The occurrence of the latex in small separate cells rather than in a continuous duct system makes necessary a fine comminution of the shrub, and as a result the dilute dispersions initially obtained are highly contaminated with nonrubber plant and soil fragments. A microscopic method for the rapid estimation of rubber in these dispersions has been developed.

The microscopic method, which consists essentially in the identification and counting of rubber latex particles in a small volume of dispersion, is advantageous in several respects. It requires a minimum amount of equipment and is rapid, taking from 10 to 20 minutes for a determination. A rapid method is important because of the instability of some dispersions and the need of knowing the approximate concentration of dispersed rubber during a recovery experiment. Furthermore, only a small quantity of dispersion, as little as one drop, is required for the microscopic method, and it is possible to distinguish between dispersed and partly agglomerated latex in the samples. In contrast, the chemical methods of analysis (*5, 7*) are time-consuming, do not distinguish between dispersed and partly agglomerated latex, and require larger samples and more extensive equipment.

STANDARDIZATION AND PROCEDURE

The method is standardized by making counts on a number of guayule latex dispersions of known rubber content, as determined by duplicate analyses by the tetrabromide method (*7*). In this method, the dispersions are extracted in a Waring Blendor with benzene, and rubber hydrocarbon is then determined gravimetrically by precipitation as the tetrabromide. Only dispersions free from agglomerated or partly agglomerated rubber particles should be used for the standardization. Since some dispersions are unstable, the microscopic counts should be made at the time the chemical analyses are begun.

A small sample (usually 1 ml.) of a dispersion of known rubber content is mixed with neutral distilled water to give a known dilution in which the rubber latex particles can be accurately counted in a Petroff-Hausser bacteria counter. Probably a hemacytometer would serve as well, although such a chamber was not tested in the present case. Owing to the tendency of the latex particles to accumulate at the surface on standing, the dispersion must be thoroughly agitated, not only just before sampling for dilution but also after dilution, immediately before mounting in the counter. A dilution which gives 2 to 5 recognizable latex particles per square (1/20,000 cu. mm.) has been found most satisfactory. To ensure a uniform film of dispersion for the mount, the usual precautions must be taken, such as carefully adjusting the cover glass and blotting the excess liquid.

For one determination, all identifiable rubber latex particles, irrespective of size (usually 0.4 to 3.5μ in diameter), in 36 squares in each of two mounts are counted. If agreement between the number of particles counted in the two mounts is not within 5%, counting should be continued on a third mount, or on an additional number of mounts until such agreement is obtained. The count is readily made with a magnification of approximately 800 to 900 diameters, such as is obtained with a 4-mm. objective and 20× ocular. Each particle must be brought into clear focus for identification; those too small for identification should not be counted. Since all flow of particles must be eliminated during counting, it is sometimes necessary to readjust the cover glass

Table I. Standardization of Microscopic Method for Estimation of Rubber in Guayule Crude Latex Dispersions

(N, average number of latex particles per square; D, dilution factor; C_0, concentration of rubber in original dispersion as determined by chemical method; $k = C_0/ND$; C, concentration of rubber in original dispersion calculated from relation $C = 1.85\ ND$.)

Original Plants			Extracted Latex Dispersions					
						Rubber		
Description[a]	Resin[b], %	Rubber[b], %	N	D	k	Microscopic, C	Chemical, C_0	Difference, %
						Mg./100 ml.		
YW	...	3.3	3.00	10	1.77	56	53	+5.7
YX	5.9	8.2	2.42	25	1.87	112	113	−0.9
YX	...	7.3	2.87	25	1.87	133	134	−0.7
YX	6.0	8.3	3.67	25	1.84	170	169	+0.6
YX	5.9	8.2	4.08	25	1.93	189	197	−4.1
YX	...	7.3	2.75	50	1.79	254	246	+3.3
YW	6.8	3.6	2.75	50	1.99	254	273	−7.0
YW	6.8	3.6	3.42	50	1.93	316	330	−4.2
YX	5.4	5.2	2.08	100	1.74	385	361	+6.7
MW	9.0	15.0	2.17	100	1.94	401	421	−4.8
YX	5.6	7.7	2.79	100	1.87	516	521	−1.0
YX	5.4	8.7	3.50	100	1.81	648	632	+2.5
YX	6.8	8.6	3.54	100	1.96	655	694	−5.6
YX	...	8.0	3.92	100	1.82	725	715	+1.4
YX	...	8.0	4.25	100	1.83	786	779	+0.9
MW	9.0	15.0	4.83	100	1.89	894	911	−1.9
YX	5.4	8.7	2.67	200	1.78	988	947	+4.3
YX	...	8.0	2.58	1000	1.74	4773	4501	+6.0
				Av.	1.85			±3.4

[a] Y, 2-year old plants; M, 12-year old plants. X, defoliated plants; W, whole or nondefoliated plants. Data in first three columns indicate range in applicability of method rather than any relationship between original rubber content and amount of rubber extracted.
[b] Moisture-free basis.

carefully. Often slight pressure on the margin of the cover glass will suffice. The predominantly spherical rubber latex particles can readily be distinguished from the variously shaped and colored extraneous nonrubber matter, such as cell-wall fragments, protoplasmic granules, chloroplasts, soap particles, bacteria, and silt. If difficulty is experienced initially in identifying the rubber particles, a portion of them may be stained on a separate slide with a rubber stain (*6*). Staining, however, coagulates the particles.

Standardization of the method is illustrated by Table I. The observed average number of particles per square, N, in the diluted dispersion, the dilution factor D, and the rubber content of the undiluted dispersion, C_0, the latter determined by the chemical method of analysis (*7*), are shown in columns 4, 5, and 8. Constant k in column 6 is the ratio of the concentration of rubber in the diluted dispersion (C_0/D) to the average number of particles per square, or $k = C_0/ND$. The average value of k for a number of dispersions, as shown in the table, was 1.85. Thus the standardization of the method for the guayule latex dispersions is expressed by the relation $C = 1.85\ ND$.

The quantity of rubber in an unknown dispersion is determined microscopically by counting the particles in an appropriate dilution of the dispersion in a manner identical with that employed during standardization. The concentration of rubber in the original dispersion, in mg. per 100 ml., is calculated by the equation $C = 1.85\ ND$.

DISCUSSION

The concentration of rubber calculated from the microscopic count (column 7) using the relation $C = 1.85\ ND$ and the concentration determined by the chemical method (column 8) are tabulated in Table I. The average per cent difference between the two methods (±3.4) is sufficiently small to establish the validity of the microscopic method. In practice, in some cases where the results obtained by the two methods differed rather widely, a repeated chemical analysis showed the results of the original chemical analysis to be in error. In other cases, differences in results between the two methods may be due to the fact that the chemical method, unlike the microscopic, does not differentiate between dispersed and agglomerated rubber.

The error produced by failure to count the submicroscopic rubber particles is negligible, since these particles, although numerous, constitute only an insignificant fraction of the total rubber. Studies in this laboratory of electron micrographs of kok-saghyz latex show that only 2% of the total volume of all particles occurs in particles smaller than 0.3μ in diameter, although by number these particles constitute 87% of the total. Similar results were obtained by Lucas (*3*) on hevea latex. The authors' findings and those of Spence (*4*) indicate that the rubber latex particles of guayule are larger than those of kok-saghyz and hevea.

It would be possible to standardize the microscopic method independently of the chemical method if the average particle mass and the proportion of rubber hydrocarbon in the particles were known. No complete standardization of this sort was attempted in the present case. As a test of its feasibility, however, a preliminary study of the particle size distribution was made visually by means of a micrometer eyepiece. The average particle mass was 9.9×10^{-13} gram. This figure was calculated after measuring the diameters of 600 particles and assuming that they were spheres with a density of 0.92 (*2*).

The concentration of rubber in a dispersion is expressed, as before, by $C = kND$, but now the constant k is equal to Apm, where m is the average particle mass, p is the proportion of rubber hydrocarbon in the particles, and A is a constant 2×10^{12} dependent only on the units and on the volume used for determining N. In the present case the value calculated for k is 1.98 if p is assumed to be unity (100% rubber hydrocarbon), or 1.73 if p is assumed to be 0.875, a value postulated by Kemp (*2*) for hevea particles. These values of k are in good agreement with the value 1.85 found by standardization in terms of the chemical method. The results indicate that an accurate standardization could be carried out which would be independent of any chemical method except for a determination of p.

The wide applicability of the microscopic method to various types of guayule latex dispersions is illustrated by the data of Table I and by subsequent analysis of many samples, in which frequent comparisons with the chemical method were made. Dispersions examined included those extracted in various aqueous media from 2- and 12-year old plants, from whole and defoliated shrub, from fresh and stored plants, and from plants of both high and low resin and rubber content. Agreement with the chemical method under these varying conditions indicated that the rubber latex particles were readily distinguishable from any other particles present, and further that the size distribution and composition of these rubber particles were remarkably constant. On storage after extraction, however, the particles may change in size and possibly composition.

The microscopic method of analysis has been employed in various types of research on guayule. By means of small-scale experiments in which pieces of living guayule tissue were ground in a mortar in anticoagulant solutions and the resulting latex dispersions were analyzed microscopically, significant information on the distribution of latex in the plant and on the effect of pH, enzymes, soaking, temperature, surface active agents, storage, etc., on latex extractions has been obtained. The microscopic method has been applied also in evaluating the effectiveness of each of a series of steps in large-scale latex recovery experiments. With appropriate standardization and with suitable extraction of latex, the method may be applied as a rapid quantitative test for rubber in selection, breeding, and recovery work on other rubber-bearing plants. The data of Lucas (*3*), showing that about 90% of the rubber in hevea latex occurs in particles 0.4 μ or larger in diameter, suggest that the method may be useful also in the rapid analysis of hevea latex.

ACKNOWLEDGMENT

Credit is due C. O. Willits of this laboratory for the chemical analyses.

LITERATURE CITED

(1) Clark, F. E., and Place, W. F. L., *India Rubber World*, 112, 67 (1945).
(2) Kemp, A. R., IND. ENG. CHEM., 30, 154 (1938).
(3) Lucas, F. F., *Ibid.*, 30, 146 (1938).
(4) Spence, D., U. S. Patent 2,119,030 (1938).
(5) Traub, H. P. (in preparation, U. S. Dept. Agr., *Tech. Bull.*).
(6) Whittenberger, R. T., *Stain Tech.*, 19, 93 (1944).
(7) Willits, C. O., Swain, M. L., and Ogg, C. L. (in preparation).

NATURAL Rubber from Domestic Sources, Paper 12.

Modified All-Dithizone Method for Determination of Traces of Copper

S. L. MORRISON[1] AND HARRIET L. PAIGE, Seventh Service Command Medical Laboratory, Fort Omaha 11, Neb.

A modified all-dithizone procedure in which the copper is extracted in Mojonnier fat-extraction flasks and determined by the mixed color method is described. Interference and contamination by likely metallic ions are eliminated. Data on recoveries of added copper to several biological products are given. Recoveries of better than ±0.2 microgram are obtained.

DURING the examination of dried whole and skim milk for copper content according to army specifications, the need for a rapid, reproducible method adaptable for routine use was recognized. Various procedures based upon the use of sodium diethyldithiocarbamate (*5*, *6*) have been recommended. However, since nickel, cobalt, and bismuth interfere, these methods leave much to be desired. In other methods (*3*, *4*, *8*, *9*), which combine the desirable features of dithizone and sodium diethyldithiocarbamate, the copper is separated from the nickel, cobalt, and part of the bismuth by extraction from acid solution with dithizone. Only copper and part of the bismuth will be carried into the dithizone layer. The dithizonate complexes are destroyed by ashing and the copper is determined finally with the carbamate reagent. Bismuth interferes, causing high results. Greenleaf (*4*), however, eliminated interference from bismuth by extraction with acidified potassium iodide prior to destruction of the dithizonate.

Bendix and Grabenstetter (*1*) describe an all-dithizone method in which the copper is extracted by means of dithizone at a pH of 2.3, along with gold, platinum, palladium, silver, mercury, bismuth, and stannous tin. No other metals react with dithizone at this pH. Gold, platinum, and palladium are not considered because of their rarity; acid digestion oxidizes stannous tin to the stannic state, in which it does not form complexes with dithizone; silver, mercury, and bismuth are removed as complex iodides by extraction with 2% acidic potassium iodide. The excess dithizone is then removed from the carbon tetrachloride solution with an ammonia wash, and the copper determined colorimetrically as the violet-colored keto copper dithizonate.

In the authors' experience this method, though practicable, presents two difficulties: (1) the transfer through three separatory funnels without the hazard of contamination, particularly by stopcock lubricant, and (2) the fact that the ammonia wash changes variable portions of the violet-colored keto copper dithizonate into the yellowish-brown enol tautomer. In this observation the authors have confirmed Sandell's (*7*) earlier reference.

In the method herein presented copper is extracted with dithizone at a pH of 2.3 in a Mojonnier fat-extraction flask or a container of similar design. This flask is so designed that 20 ml. of the heavier carbon tetrachloride solution of dithizone remain in the bulb of the flask when any aqueous solution is poured off above it. The obvious advantage of such a flask in this particular procedure lies in the fact that after each extraction it is the aqueous layer that is discarded. Therefore all extractions can be made in one flask and the reactive dithizone is safely protected from air-, glass-, or stopcock-borne contamination. The carbon tetrachloride layer is then shaken with 2% acidic potassium iodide solution, the copper remaining in the carbon tetrachloride layer as the dithizonate, while the other metals are extracted into the aqueous layer as iodide complexes and discarded. The concentration of copper is then measured in a spectrophotometer by the mixed color method at the wave length of maximum absorption of keto copper dithizonate. The use of the mixed color method of measurement obviates the necessity for removing uncombined dithizone with ammonia, thereby eliminating the possibility of copper loss through enol formation (*7*).

[1] Present address, 33 North Genesee St., Waukegan, Ill.

APPARATUS

A spectrophotometer, Coleman, model 6, with cuvettes, No. 6-504B, 19 × 150 mm.

Mojonnier fat-extraction flasks fitted with ground-glass stoppers.

A mechanical shaker, averaging 275 oscillations per minute.

Glassware, cleaned with concentrated nitric acid, and well rinsed with tap, distilled, and redistilled, metal-free water.

REAGENTS

Water, redistilled from Pyrex, for making all solutions.

C.P. nitric acid, redistilled from Pyrex.

C.P. sulfuric acid. Since different lots of sulfuric acid are contaminated by varying microquantities of copper, a large volume of pooled acid is advisable.

C.P. concentrated ammonium hydroxide.

Cresol red indicator solution, 0.02 gram per 100 ml. of redistilled water.

Buffer reagent, pH 2.3. Dissolve 8.3 grams of C.P. anhydrous disodium hydrogen phosphate and 38 grams of C.P. citric acid in redistilled water. Purify by shaking in a separatory funnel with successive portions of the concentrated solution of dithizone, until there is no noticeable change in the color of the dithizone. Wash with C.P. carbon tetrachloride until the washings are water-clear. Transfer to a 250-ml. volumetric flask and dilute to volume with redistilled water; 2 ml. of this reagent will buffer 25 ml. of water at pH 2.3.

Concentrated solution of dithizone (diphenylthiocarbazone), 15 mg. per liter. Dissolve 15 mg. of dithizone in 1 liter of C.P. carbon tetrachloride. This can best be accomplished by first dissolving it in about 10 ml., then diluting to 1 liter. Store the solution in a black-painted bottle in the refrigerator until ready to use.

Dilute dithizone solution, 7.5 mg. per liter. Make a 1 to 1 dilution of the concentrated dithizone with C.P. carbon tetrachloride. Since this solution is extremely unstable, prepare fresh daily and keep in refrigerator unless actually in use. At all times during the determination, shield the solution from direct sunlight.

Two difficulties are encountered in preparation of dithizone solutions. Generally, dithizone as manufactured by Eastman Kodak Co. is satisfactory for use; however, the commercial product may be purified, according to the method outlined by Sandell (*7*). Occasional lots of reagent grade carbon tetrachloride are unfit for use because of the presence of decomposition products, such as phosgene and free chlorine. Methods of reclaiming carbon tetrachloride have not been devised; therefore, a bad lot should be discarded.

The practice of "stripping" the dithizone solution is recommended as a simple daily procedure for determining whether suitable for use. This is performed by shaking 10 ml. of concentrated dithizone solution with 25 ml. of 1 to 200 C.P. ammonium hydroxide; if satisfactory, the heavier carbon tetrachloride layer will be water-clear, or at most, slightly tinged with yellow.

Acidic potassium iodide reagent, 2%. Dissolve 10 grams of C.P. potassium iodide in 450 ml. of redistilled water, acidify with 5 ml. of 1 *N* hydrochloric acid, and add 0.1 *N* sodium thiosulfate, dropwise, to expel any yellow color of free iodine. Wash with dithizone and carbon tetrachloride as in the preparation of the buffer reagent, dilute to 500 ml. with redistilled water, and add a few milliliters of C.P. carbon tetrachloride as a preservative. Store in the refrigerator, inspecting daily for decomposition of the iodide, and adjusting, if necessary, with a drop or two of thiosulfate.

Standard copper solution, stock, 1 mg. of copper per ml. Dissolve 0.5000 gram of electrolytic sheet copper in 20 ml. of 6 *N* nitric acid, and evaporate almost to dryness. Add 2 to 3 drops of glacial acetic acid and transfer the solution quantitatively to a 500-ml. volumetric flask, diluting to volume with redistilled water.

Working standard copper solution, 1 microgram per ml.

Table I. Recovery of Copper from Solutions Containing Other Metallic Ions

Copper Added	Other Metal Added	Copper Recovered		Error
		Without KI shaking	With KI shaking	
Micrograms	*Micrograms*	*Micrograms*	*Micrograms*	*Micrograms*
	Fe^{+++}			
5.0	50.0	....	4.85	−0.15
5.0	50.0	....	4.90	−0.10
5.0	1000.0	....	4.95	−0.05
5.0	1000.0	....	5.00	0.0
	Sn^{++++}			
4.0	50.0	....	4.10	+0.10
4.0	100.0	....	3.90	−0.10
4.0	200.0	....	3.95	−0.05
5.0	500.0	....	5.00	0.0
5.0	500.0	....	4.80	−0.20
	Pb^{++}			
3.0	30.0	....	3.0	0.0
4.75	1000.0	....	4.80	+0.05
4.75	1000.0	....	4.80	+0.05
6.0	200.0	....	6.0	0.0
5.0	100.0	5.10	5.05	+0.05
5.0	100.0	5.20	5.10	+0.10
	Zn^{++}			
5.0	100.0	5.10	5.00	0.0
5.0	100.0	4.95	4.85	−0.15
3.0	200.0	....	3.00	0.0
4.0	200.0	....	3.90	−0.10
	Cd^{++}			
3.0	200.0	....	3.15	+0.15
4.0	200.0	....	4.05	+0.05
5.0	100.0	5.05	5.00	0.0
	Co^{++}			
5.0	100.0	5.00	5.00	0.0
5.0	200.0	5.00	5.05	+0.05
	Ni^{++}			
5.0	100.0	5.00	4.90	−0.10
5.0	125.0	4.90	5.15	+0.15
	Mn^{++}			
5.0	100.0	5.10	5.00	0.0
5.0	200.0	5.05	4.95	−0.05
	Bi^{+++}			
5.0	50.0	5.10	5.10	+0.10
5.0	500.0	....	5.15	+0.15
5.0	200.0	5.00	5.15	+0.15
	Hg^{++}			
5.0	20.0	....	5.00	0.0
5.0	20.0	....	4.80	−0.20
5.0	30.0	....	4.25	−0.75
5.0	30.0	....	4.20	−0.80
	Ag^{+}			
5.0	20.0	....	4.90	−0.10
5.0	20.0	....	4.95	−0.05
5.0	30.0	....	3.50	−1.50
5.0	30.0	....	4.0	−1.00

Dilute exactly 10 ml. of the concentrated standard to 100 ml. After mixing, dilute exactly 10 ml. of the intermediate solution to 1000 ml. to prepare the working standard, containing 1 microgram per ml.

PREPARATION OF SAMPLE

Weigh and transfer to a 500-ml. Kjeldahl flask a portion of well-mixed food or biological material containing less than 70 micrograms of copper. Add 10 to 15 ml. of c.p. nitric acid, redistilled, and 2 short lengths (about 1.25 cm., 0.5 inch) of copper-free, thin-walled glass tubing to prevent superheating. Add exactly 10 ml. of c.p. sulfuric acid. Heat the contents of the flask over a direct flame, maintaining oxidizing conditions at all times by the dropwise addition of nitric acid. Oxidation is complete when there is no perceptible darkening of the fluid upon the appearance of thick white fumes of sulfur trioxide. The final solution should be colorless, or at most straw-colored. Continue heating about 5 minutes after this stage is reached to assure complete digestion.

Cool the flask, add 50 ml. of redistilled water, and resume heating until sulfur trioxide fumes appear. Repeat the dilution and evaporation in order to remove all oxides of nitrogen. Caution must be exerted at this point to avoid superheating, especially when digesting milk. Use of a hot flame and constant shaking until boiling has begun are helpful. The reappearance of a precipitate is a signal to resume shaking. Transfer the contents of the flask to a 100-ml. volumetric flask, and dilute to volume with redistilled water. Conduct a blank, using 10 ml. of sulfuric acid, and approximately the same quantity of nitric acid as in the sample.

When digesting substances which foam, it is more satisfactory to carry out the heating for some time without the addition of the sulfuric acid. When the oxidation seems to be almost complete, cool the mixture, add 10 ml. of sulfuric acid, and carry the digestion to completion.

PROCEDURE

Pipet a suitable aliquot of the sample solution (containing less than 7 micrograms of copper) into a Mojonnier extraction flask and add sufficient redistilled water to make a volume of 25 ml. Add 3 drops of cresol red solution and bring the contents of the flask to the yellow color of the indicator range by dropwise addition of c.p. concentrated ammonium hydroxide. Add 2 ml. of the buffer reagent and exactly 20 ml. of the dilute dithizone solution, shake for 10 minutes, and discard the aqueous layer. Add 10 ml. of acidic potassium iodide solution to the flask and shake for 2 minutes. Discard the aqueous layer, and draw off the last 2 or 3 drops with the aid of a pipet bent slightly at the tip. Transfer to a cuvette and determine the transmittance or optical density of the solution in the spectrophotometer at 520 millimicrons, with a blank determination, which has been treated in the same manner as the sample, set at 100% transmittance or 0 optical density. Determine the concentration of copper from a previously prepared calibration curve.

PREPARATION OF CALIBRATION CURVE

Pipette 1-, 2-, 3-, 4-, 5-, and 6-ml. quantities of copper standard (containing 1 microgram of copper per ml.) into six extraction flasks, with a seventh to serve as a blank. Add 5 ml. of 10% sulfuric acid to each of the seven, and add sufficient redistilled water to make a volume of 25 ml. Continue as with the unknown sample and determine the transmittance or optical density in the spectrophotometer at 520 millimicrons. Plot the transmittance, optical density, or log transmittance value against the known concentration of copper in each sample.

EXPERIMENTAL FINDINGS

Recovery of Copper from Solutions Containing Contaminating Metals. Known amounts of copper and other metals whose effects as interfering substances were to be tested were placed in glass-stoppered Mojonnier fat-extraction flasks, 5 ml. of 10% sulfuric acid were added, and the volume was made up to 25 ml. with redistilled water. The procedure described above for copper was then followed. The amount of copper recovered in each case was read from a calibration curve prepared with known amounts of copper.

Table I describes the results obtained upon the addition of metals whose presence is likely in food products or biological material. Overwhelming amounts of lead, zinc, cadmium, cobalt, nickel, and manganese do not influence the quantitative extraction of copper, since these metals do not react with dithizone at the pH chosen. This fact is demonstrated by the quantitative recovery of copper from solutions containing the above-mentioned contaminants when the extraction with acidic potassium iodide is omitted.

Stannous tin and ferrous iron, two possible interfering substances, are of no consequence since the method of sample preparation oxidizes them to their higher valences in which they are not reactive with dithizone.

Although previous workers (*1*, *10*) have said that small amounts of bismuth are extracted at a pH of 2.3 and must be removed by

Table II. Recovery of Copper from Solutions of Varying pH

pH of Solution	pH of Solution after Addition of Buffer	Cu added	Cu Recovered	Error
		Micrograms	*Micrograms*	*Microgram*
1.6	1.8	5.0	5.10	+0.10
1.6	1.8	5.0	4.90	−0.10
1.8	2.0	5.0	4.80	−0.20
1.8	2.0	5.0	4.95	−0.05
2.3	2.4	5.0	4.95	−0.05
2.3	2.4	5.0	4.90	−0.10
6.8	2.8	5.0	4.90	−0.10
6.8	2.8	5.0	5.00	0.0

Table III. Effect of Dithizone Strength upon Recovery of Copper

Dithizone Concentration	Copper Added	Copper Recovered	Error
Mg./l. CCl_4	*Micrograms*	*Micrograms*	*Microgram*
7.0	5.0	5.05	+0.05
7.0	5.0	4.85	−0.15
7.0	5.0	4.95	−0.05
7.5	5.0	5.0	0.0
7.5	5.0	5.0	0.0
7.5	5.0	5.05	+0.05
8.0	5.0	4.85	−0.15
8.0	5.0	5.05	+0.05
8.0	5.0	5.05	+0.05

potassium iodide extraction, this fact has not been confirmed in the present study. Experimental findings shown in Table I indicate that bismuth is not extracted at this pH.

Silver and mercury, however, are extracted more readily than copper at the pH chosen. For this reason, the method as outlined above will tolerate the presence of only 20 micrograms of either of these two metals. However, since the dithizonates of both of these metals are yellow, their interference is easily detected.

EFFECT OF SMALL VARIATIONS IN EXTRACTION pH. Known amounts of copper were placed in Mojonnier fat-extraction flasks, acid and water added to make volumes of 25 ml., and the pH values were varied from 1.6 to 6.8. These pH values were selected because they correspond to the faint pink color on both the acid and alkaline side of the desired yellow color of cresol red indicator. Furthermore they correspond to the maximum error likely to be encountered in pH adjustment during routine analyses.

Table II shows (1) the buffering capacity of the citric acid-phosphate buffer, and (2) that small variations in pH have no effect upon the quantitative extraction of copper when the shaking time is 10 minutes.

EFFECT OF VARIATIONS IN SHAKING TIME. The authors' findings in general corroborate those of Bendix and Grabenstetter (*1*) in that in most cases all copper is extracted in 6 minutes. However, because of small individual variations it was decided that a 10-minute shaking time was a safer routine practice.

EFFECT OF SMALL VARIATIONS IN DITHIZONE STRENGTH. Recovery of known amounts of copper was determined in the usual manner, except that the dithizone solution concentration was varied to a slight extent in order to determine its effect upon the transmittance. Clifford (*2*) has pointed out, relative to the determination of lead by the mixed color method, that since the amount of metal present is based upon the measurement of the light absorption of the red phase of the color mixture at a wave length where the absorption of the green dithizone is small, variations in the concentration of free dithizone must be considerable to influence the results appreciably. Table III points out that this statement is just as true in the determination of copper, and that recovery of copper, despite small variations in dithizone strength, is within the limits of error claimed for the method.

APPLICATION OF METHOD. Samples of several biological products were analyzed for copper. Recoveries of added copper were made from samples of dried milk, dried eggs, and whole blood. Results of some of the dried milks were checked by the Bendix and Grabenstetter method (*1*). Table IV demonstrates that copper recoveries from biological products by the modified all-dithizone method are satisfactory, and that agreement between this method and the method of Bendix and Grabenstetter is good.

SUMMARY AND CONCLUSIONS

The chemical literature has been studied for the purpose of developing a quantitative, reproducible, rapid method for the determination of copper in biological products. It was decided that the principles embodied in the method of Bendix and Grabenstetter possessed the soundest chemical basis. Experience with the above-mentioned method pointed out that it could be advantageously modified. The authors' innovations consist of a change in the strength of the dithizone solution, the use of the mixed instead of the mono color system for the final determination of copper, and the replacement of separatory funnels with Mojonnier fat-extraction flasks for all extractions. The net effect of these changes is to make the determination more consistently quantitative, more reproducible, and more adaptable for routine use in the hands of relatively inexperienced technicians. Furthermore, by its simplicity the modified method readily lends itself to multiple determinations, a weighty factor in the routine analytical laboratory.

Studies upon the effect of interfering substances show that the method is specific for copper. Interference from overwhelming amounts of other possible metallic ions is shown to be nonexistent, with the exception of silver and mercury, whose dithizonates are easily detectable visually.

Experimental data indicate that pH is not a critical factor if a pH of 2 is approximated, and that a shaking period of 10 minutes allows a fair margin of safety for quantitative extraction of copper from the aqueous solution. Further studies demonstrate that small deviations in dithizone strength have no effect upon the quantitative copper recovery, since the concentration of copper is measured at a wave length where the absorption of the dithizone itself is slight.

Recoveries of added copper from various biological products are excellent, values of better than ±0.2 microgram being obtained.

Table IV. Analyses of Biological Products

Biological Product	Copper Content: Modified dithizone method	Copper Content: Bendix and Grabenstetter method	Cu Added	Cu Recovered	Error
	P.p.m.	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*
Dried whole and skim milks	0.98	...	2.0	3.00	+0.02
	0.98	...	2.0	2.80	−0.18
	0.98	...	3.0	3.90	−0.08
	0.98	...	3.0	4.15	+0.17
	0.98	...	4.0	4.80	−0.18
	0.98	...	4.0	5.00	+0.02
	2.39	2.42	...	...	...
	1.34	1.31	...	...	...
	1.30	1.46	...	...	...
	0.59	0.64	...	...	...
	0.58	0.52	...	...	...
	0.99	1.11	...	...	...
	1.34	1.40	...	...	...
	0.45	0.45	...	...	...
Turkey fat	0.30	...	...	...	...
	0.38	...	...	...	...
	0.16	...	...	...	...
	0.64	...	...	...	...
	0.23	...	...	...	...
Dried whole eggs	5.35	...	2.0	7.40	+0.05
	5.35	...	2.0	7.40	+0.05
	5.35	...	4.0	9.30	−0.05
	5.35	...	4.0	9.20	−0.15
Horse blood	0.88	...	1.50	2.50	+0.12
	0.88	...	1.50	2.24	−0.14
	0.80	...	0.50	1.35	+0.05
	0.80	...	0.50	1.23	−0.07
	0.80	...	1.00	1.78	−0.02
	0.80	...	1.00	1.75	−0.05

LITERATURE CITED

(1) Bendix, G. H., and Grabenstetter, D., IND. ENG. CHEM., ANAL. ED., 15, 649 (1943).
(2) Clifford, P. A., *J. Assoc. Official Agr. Chem.*, 21, 695 (1938).
(3) Greenleaf, C. A., *Ibid.*, 24, 337 (1941).
(4) *Ibid.*, 25, 385 (1942).
(5) Hetrick, J. H., and Tracy, P. H., *J. Milk Tech.*, 8, 5 (1945).
(6) Menefee, S. G., *J. Dairy Sci.*, 28, 243 (1945).
(7) Sandell, E. B., "Colorimetric Determination of Traces of Metals", pp. 84, 89, 218, New York, Interscience Publishers, 1944.
(8) Sylvester, N. D., and Lampitt, L. H., *Analyst*, 60, 376 (1935).
(9) Wichmann, H. J., *J. Assoc. Official Agr. Chem.*, 19, 212 (1936).
(10) *Ibid.*, 25, 378 (1942).

NOTES ON ANALYTICAL PROCEDURES

Simple Automatic Control for Vacuum Systems

J. J. SPADARO, H. L. E. VIX,
AND E. A. GASTROCK
Southern Regional Research Laboratory, New Orleans, La.

A MODIFICATION of a commercial type of Cartesian manostat was so constructed as to bleed air to the system rather than throttle or modulate the vacuum supply. No electrical connections are required and the unit can thus be safely used with systems handling inflammable solvents under conditions where manostats as described by Munch (*1*) might be undesirable.

OPERATION. This modified manostat is illustrated in Figure 1. The valve unit is adjusted so that the rubber disk, *E*, rests on the orifice when the lower end of the wire is approximately 1 mm. from the top of the floating bell. Stopcocks *B* and *H* remain open until the vacuum approaches 10 mm. of the desired reduced pressure. *B* is then closed, while *H* remains partially open. The exact reduced pressure desired is obtained by adjusting the rubber disk on the wire. Functioning of the manostat is indicated by the continuous and rapid axial modulation of the valve unit.

The operation is produced as follows: Since equal pressures must be maintained in the mercury-sealed chamber, *K*, and in chamber *J* leading to the system, a change of pressure in *J* causes an upward or downward displacement of mercury in this chamber, thereby raising or lowering the floating bell, *D*. This in turn opens or closes orifice *G*, thus maintaining the equilibrium in the two chambers.

A 2-mm. orifice controls reduced pressures down to 200 mm. of mercury (absolute) and a 1-mm. orifice controls pressures between 220 and 30 mm. The reduced pressure in a system can be automatically controlled to within ±0.25 mm. of mercury by proper adjustments of the orifice, valve unit, and petcock *B*. After proper adjustments are made the pressure can be controlled for an indefinite period of time with no drifting of pressure within the system.

Two modified manostats as described have been successfully used in the Southern Regional Research Laboratory for several months.

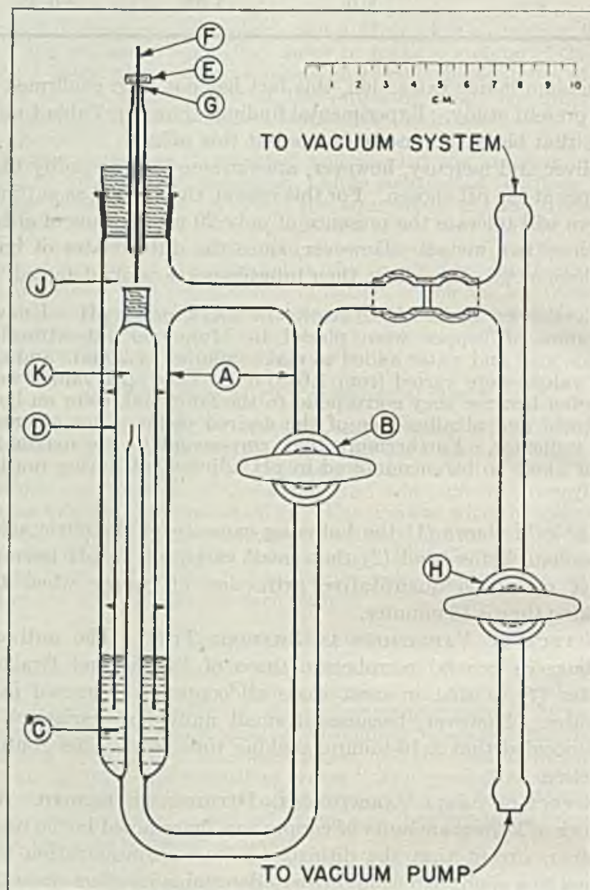


Figure 1. Modified Manostat

A. Main body of manostat
B. Stopcock
C. Section for mercury seal
D. Floating bell
Valve unit consists of adjustable rubber disk, *E*, attached to a rigid noncorrosive wire, *F*, free to move axially through orifice *G*.

LITERATURE CITED

(1) Munch, R. H., *J. Chem. Education*, 9, 1275 (1932).

Absorption Spectrum of the Antimony Trichloride–Ergosterol Reaction Product

ALEXANDER MUELLER, Gelatin Products Corporation, Detroit, Mich.

DURING the course of investigating the antimony trichloride absorption spectrum of vitamin D at 500 mμ, according to the method described by Nield, Russell, and Zimmerli (*3*), it was observed that solutions of the antimony trichloride reaction products of irradiated ergosterol exhibit an absorption band at 393 mμ as well as at 500 mμ. Further investigation of the absorption spectra of the antimony trichloride reaction products of pure samples of calciferol (vitamin D_2) obtained from Winthrop Chemical Co. and of pure ergosterol obtained from Standard Brands, Inc., indicates the absorption maximum at 393 mμ to be due to ergosterol. The absorption maximum at 393 mμ is sharp and well defined, having a molecular extinction coefficient of 9122 and an $E^{1\%}_{1\ \text{cm.}}$ value of 230.0 ± 3% (average of 20 trials). Two additional absorption maxima were observed at 322 mμ and 510 mμ with $E^{1\%}_{1\ \text{cm.}}$ values of 68.0 and 25.0, respectively. These measurements were performed on a sample of ergosterol which was shown to be 100% pure by a measurement of its ultraviolet absorption spectrum.

The absorption maximum at 393 mμ was found to be stable for the period from 2 to 10 minutes following preparation of the antimony trichloride–ergosterol reaction product, making it possible to make very satisfactory quantitative measurements of its intensity. The absorption spectrum of the antimony trichloride–calciferol reaction product shows no absorption bands

in the 300 to 400 mμ region. These results indicate that a measurement of the absorption maximum at 393 mμ of the antimony trichloride reaction product of irradiated ergosterol should offer a satisfactory method for the quantitative determination of the ergosterol present. The absorption maxima at 322 and 510 mμ are of low intensity and not satisfactory for quantitative measurement, but are helpful in the qualitative identification of ergosterol. A more detailed report on a spectrophotometric method for the quantitative determination of ergosterol in the presence of vitamins D, cholesterol, and 7-dehydrocholesterol based upon the observation is reported in this issue (*2*).

The spectrophotometric measurements were made with a Beckman Model DU spectrophotometer and incandescent light source. The details of the procedure used in carrying out the antimony trichloride reaction are the same as described by Ewing, Kingsley, Brown, and Emmett (*1*).

LITERATURE CITED

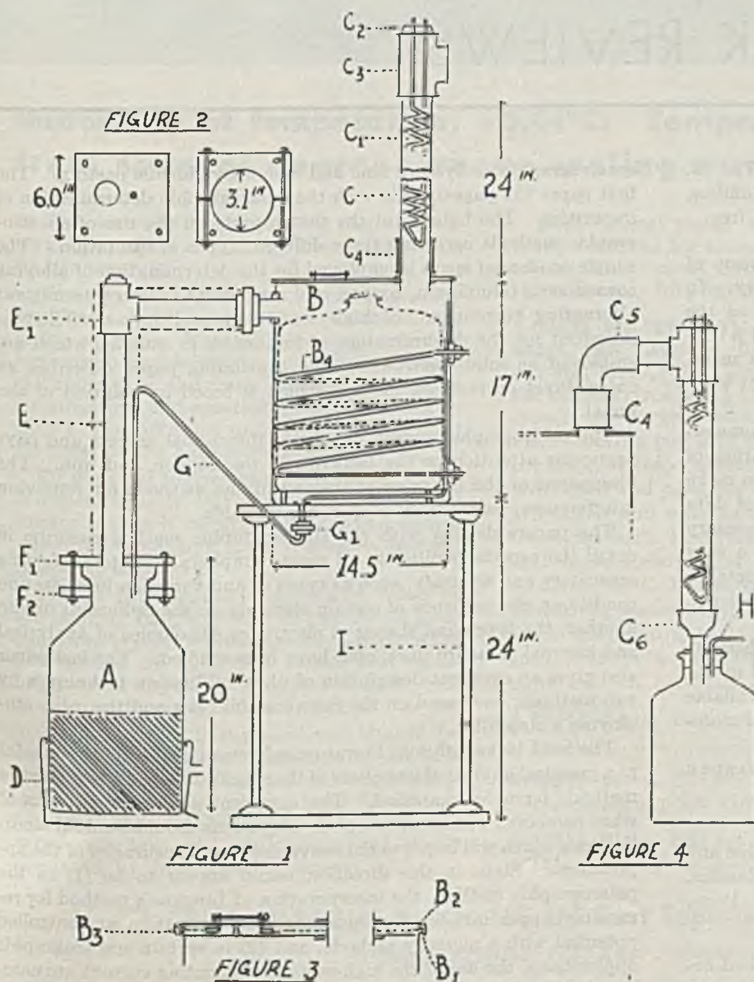
(1) Ewing, D. T., Kingsley, G. V., Brown, R. A., and Emmett, A. D., Ind. Eng. Chem., Anal. Ed., 15, 301 (1943).
(2) Lamb, F. W., Mueller, A., and Beach, G. W., *Ibid.*, 18, 187 (1946).
(3) Nield, C. H., Russell, W. C., and Zimmerli, A., *J. Biol. Chem.*, 136, 73 (1940).

Large-Size Laboratory Soxhlet Extractor

RALPH SALKIN AND IRVING ALLAN KAYE[1]
Research Laboratory, Endo Products, Inc.
Richmond Hill 18, N. Y.

IN AN investigation involving the extraction of plant lipids, the need arose for an apparatus which could efficiently extract 25 to 30 pounds of finely ground milkweed seeds. Although numerous extractors of different capacities have been described in the chemical literature, it was felt that the type described by Rapp, Woodmansee and McHargue (*1*) was most satisfactory. This extractor can be used for a Soxhlet-type of extraction with hot solvent. In preparing a smaller model of this apparatus, several simplifications in fabrication were introduced, enabling it to be constructed from materials readily available in most research laboratories and manufacturing plants.

[1] Present address, Brooklyn College, Brooklyn, N. Y.



Figures 1, 2, 3, and 4. Diagram of Laboratory Soxhlet Extractor, Showing Details of Construction and Dimensions

CONSTRUCTION OF EXTRACTOR

The extractor is shown in Figure 1. The boiler, *A*, consists of a 5-gallon wide-mouthed Pyrex bottle set in a steam bath, *D*, of appropriate size. The vapor tube, *E*, made of 1.0-inch brass pipe and fittings, with a union adjacent to the extraction chamber, *B*, to facilitate dismantling, is attached to *A* by means of an adapter, *F*. It is advisable to lag the vapor tube, *E*, with 1.0-inch asbestos pipe covering.

The upper part of the adaptor, F_1, consists of a 6-inch square (Figure 2) of 0.25-inch sheet brass having a hole tapped with a 1-inch I.P.S. It consists of a hole for the siphon tube, *G*, and four corner holes for bolts. The bottom half of the adaptor, F_2, is made of 1-inch wood, the inside hole being lined with sheet cork. The details are given in the drawing. A vapor-tight seal is obtained by use of either an asbestos or sheet cork gasket in conjunction with any vegetable-base pipe-joint compound. A sound cork, in conjunction with the pipe-joint compound, may be substituted for the adaptor.

The siphon tube, *G*, is made of 0.31-inch outside diameter copper tubing which is soldered into F_1, and coupled with the extraction chamber, *B*, by means of a 0.125-inch union, G_1. The diameter of *G* for the particular application was found to be important. Tubing of larger diameter was apparently inadequate to provide proper siphon action, the solvent merely spilling over as rapidly as it was condensed. The optimum diameter appears to depend on several operational variables—e.g., rate of distillation, temperature of condensate, etc. A sharp bend is important in facilitating siphoning.

B is made from a sheet-metal batch can of about 12-gallon capacity, the type used in shipping bulk chemicals. It has a beaded top edge, B_1 (Figure 3), and recessed cover, B_2, containing a gasket. The cover and can were coupled in a solvent-tight seal by means of a metal ring (furnished as an integral part of the can), B_3. The best type of gasket was found to be Johns-Manville style No. 322$^3/_{16}$-inch packing. After repeated use, it showed no signs of wear.

B is provided with an internal steam coil, B_4, made of 0.31-inch outside diameter copper tubing (about 6 to 7 coils are adequate). This coil serves

a dual purpose, enabling the extraction to be performed at elevated temperatures and facilitating the drying of the extracted material in the final recovery of the solvent. The tubing is attached to B by inserting two 1-inch lengths of 0.5-inch running thread through holes reamed in the side of the can and securing them in place by lock-nuts and fiber washers of appropriate size. The precoiled tubing is placed in the extraction chamber, the ends of the tubing passing through the nipples and being held in place inside the nipples by asbestos tape. Solder is then poured into the remaining space. The openings for the siphon tube on the bottom of the can and for the condenser on the top of the can are made in a similar manner, except that no solder is needed.

A breather tube, to facilitate siphoning, is made of L-shaped copper tubing 0.25 inch in outside diameter, which extends from the siphon tube along the bottom and up the side of the can above the siphoning level.

The material to be extracted is placed in a strong canvas bag with a pull-cord top. The bag fits snugly into the can and is placed on a circular piece of 1.0-inch mesh which is raised slightly from the bottom of the can to facilitate siphoning.

In constructing the stand, I, for the extraction chamber, 0.5-inch pipe is used.

The condenser coil, C_1 is made of 0.25-inch outside diameter copper tubing tightly wound, the ends projecting through a 2.0-inch brass plug, C_2, and soldered in place. C_2 is screwed into a 2.0-inch tee, C_3, which is attached to a 24-inch length of 2.0-inch pipe. The latter is attached to the extraction chamber by means of a 2.0-inch brass coupling, C_4.

To adapt this apparatus for downward distillation, C is dismantled, a 2.0-inch street ell, C_5 (Figure 4), is screwed into C_4, a 2.0-inch nipple is screwed into the street ell, and the condenser is screwed to this nipple as shown. To facilitate collection of the recovered solvent, the pipe size of the condenser is reduced to 0.75 inch by means of a reducing coupling and a 6.0-inch nipple, C_6, which passes through a sound stopper into a wide-mouthed receiver. This stopper also bears an L-shaped piece of tubing, H, as illustrated. If it is desired to dry the extracted material completely and remove all but traces of solvent from the extracted oil, steam may be passed through the coil of the extractor chamber and through the steam bath, D.

A capillary may be inserted through the tee at the top of the vapor tube, E, and gentle suction applied at H for more efficient removal of solvent.

If the material to be extracted is intended for food use and copper and brass may lead to possible future deterioration, block tin tubing may be substituted for copper and Monel fittings used in place of brass.

CONCLUSION

The apparatus described is efficient, giving values that closely approximate quantitative determinations in the conventional all-glass apparatus. The over-all solvent recovery is good, varying from 80 to 90%, dependent on the solvent.

LITERATURE CITED

(1) Rapp, K. E., Woodmansee, C. W., and McHargue, J. S., Ind. Eng. Chem., Anal. Ed., 15, 351 (1943).

BOOK REVIEWS

Colorimetry for Chemists. *M. G. Mellon.* 120 pages. The G. Frederick Smith Chemical Co., 867 McKinley Ave., Columbus, Ohio, 1945. Price, heavy board back, $1.00; paper cover, free.

In this 120-page booklet, the author discusses the application of colorimetry in its broader sense to the problems of chemistry. In addition to the chemist's common concept of colorimetry as the measurement of the concentration of a colored constituent, or a constituent which can be transformed into a colored form, the measurement and use of such color quality factors as the dominant wave length, purity, and brightness are described in some detail.

The distinguishing features and limitations of typical commercially available instruments are briefly discussed. Recognition of the limitations of instrumentation has prompted the author to make a very timely plea for more uniformity in the presentation of data for publication, especially for the inclusion of information necessary to the critical evaluation of the data. The author includes a very brief section on the interpretation of transmission and reflectance curves in terms of the corresponding I.C.I. tristimulus coefficients, luminance, dominant wave length, and purity.

This publication should hold considerable interest for chemists planning colorimetric studies; it should aid the experienced in obtaining even more useful and valuable information from available equipment; and it should serve as a guide in the preparation of material for publication in the chemical literature.

O. R. Alexander

Polarographic and Spectrographic Analysis of High Purity Zinc and Zinc Alloys for Die Casting. *A. S. Nickelson* and *J. E. B. Randles.* ix + 117 pages. H. M. Stationery Office, London, 1945. Price, 5 s.

This book, which is composed of four papers, gives a detailed account of the experimental work carried out from 1941 to 1944 by the British Standards Institution Panels in connection with its commission to prepare and recommend methods for the polarographic and spectrographic analysis of zinc and zinc alloys for die casting. The first paper (32 pages) deals with the polarographic determination of impurities. The balance of the text reports on the use of spectrographic methods involving three different types of excitation. The simple condensed spark is employed for the determination of alloying constituents (aluminum, copper, and magnesium). An intermittent alternating current arc of high sensitivity and reproducibility is described for the determination of impurities in samples which are employed as solid electrodes. The concluding paper describes an oxide–direct current arc method which is based on solution of the metal.

The polarographic section disscusses theoretical aspects and pays particular attention to the behavior of tin, copper, and iron. The discussions of the behavior of tin and of the methods for removing interferences, particularly copper, are valuable.

The papers dealing with the spectrographic method describe in detail the aspects familiar to all spectrographers. Factors affecting sensitivity and accuracy, such as types of and variation in excitation conditions, the influence of certain elements on the radiations due to another, the types and shapes of electrodes, the choice of analytical and internal standard lines, etc., have been studied. The last paper also gives an excellent description of plate calibration techniques by two methods, one based on the Schwarzschild law and the other employing a step filter.

The book lacks sufficient literature references, but will prove useful as a practical guide in the analysis of the specific materials to which the methods have been applied. The experiences which are reported when combined with more recent developments should result in modifications which will improve the convenience and accuracies of the applications. Steps in this direction would appear to be (1) in the polarographic method, the incorporation of Lingane's method for removing copper interference by electrolytic separation at controlled potential with a mercury cathode, and (2) in certain spectrographic applications, the use of the high-voltage alternating current arc combined with photometry based on plate calibration and background correction as employed in American practice.

J. Cholak

Uniformity of temperature, ±0.01°C. Temperature range from several degrees above cooling water to 60°C.

● This water bath is applicable to all laboratory operations requiring close regulation of temperature.

The central circulating and heating tower applies heat at the point of most rapid circulation of water. This efficient application of heat combined with the complete and rapid circulation of the bath medium eliminates stratification and creation of permanent gradients. Temperature uniformity throughout all areas of the bath is within ±0.01°C.

Of the total surface area of the bath which is approximately 190 square inches, only 15 square inches is occupied by control equipment. All remaining space is entirely free for insertion of vessels.

Except for the inlet and outlet tubes of the cooling coil, the wall of the Pyrex glass container is free of obstructions. Unrestricted observation of immersed vessels and measurement of capillary rise, liquid flow, etc., can be made easily and accurately.

A cooling coil is supplied and should be used when operating temperatures are less than 3°C above average room temperature. A compact constant water level device attached to the wall of the container permits rapid overflow and replacement of water displaced by immersed apparatus.

Both central tower and cooling coil are easily removed for cleaning Pyrex glass container. The central circulating and heating tower merely lifts out of the bath.

S-84805 WATER BATH—Constant Temperature, Full Visibility, 0.01°, Sargent, (Patent No. 2,037,993) Consisting of container, the central circulating and heating unit with motor, the Sargent mercurial thermoregulator, a cooling coil and constant level device, the Sargent zero current relay unit complete with cord and plug for connection to standard outlet and with cable for connection to the water bath. Temperature range from several degrees above that of the cooling water to about 60°C. Diameter, 16 inches; height, 10 inches; water depth, 9½ inches; temperature uniformity, ±0.01°C. For operation from 115 volt, 60 cycle single phase circuits **$150.00**

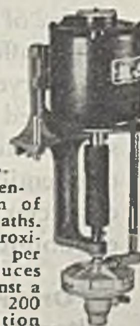
S-84815 Ditto. But for 115 volt D.C. circuits **$155.00**

S-84825 Ditto. But for 230 volt, 60 cycle, single phase circuits....... **$150.00**

S-84835 Ditto. But for 230 volt, D.C. circuits....... **$155.00**

ACCESSORIES

S-84847 CONSTANT HEAD DEVICE. A simple, inexpensive unit to provide constant rate of flow of water passing through the cooling coil of a bath, thus minimizing variations in temperature uniformity caused by fluctuation in pressure of cooling water. Made entirely of Pyrex glass.
Each **$6.00**



S-71565 PUMP —Water Circulating. For circulating temperature controlling water from bath through water jacketed apparatus. Designed for suspension from rim of Sargent water baths. Flow rate, approximately 4 liters per minute. Produces circulation against a water head of 200 cm. For operation from 115 volt, A.C. 60 cycle circuits.
Each **$45.00**

E. H. SARGENT & CO., 155-165 East Superior Street, Chicago 11, Illinois
Michigan Division: 1959 East Jefferson, Detroit 7, Michigan

SARGENT
SCIENTIFIC LABORATORY SUPPLIES

For the most exacting clarification processes

CELITE* 521

Specially purified, virtually iron-free

THE finest natural filtering medium (specially selected diatomaceous silica) is processed in the J-M Laboratory, acid-treated and calcined . . . to produce Celite 521, a pure commercial filter aid for the most exacting clarification procedures.

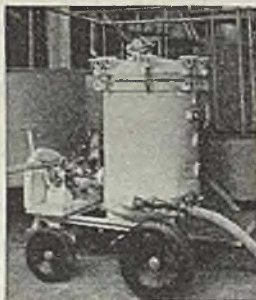
Free of soluble iron and other impurities which might affect filtrate quality, Johns-Manville Celite 521 serves a wide variety of industrial purposes. It is used exclusively by many makers of distilled spirits, liquors, cordials, etc., and in the filtration of chemicals, pharmaceuticals and foods. In each case clarification is better, speedier and more economical.

Other Special Celite Filter Aids for laboratory and commercial use:

Filter-Cel, Laboratory Standard, for the standardization of filtration procedure.

Celite Analytical Filter Aid, for analytical filtrations of the highest type.

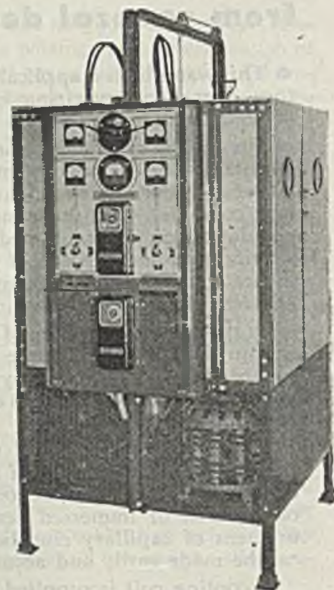
For complete information write Johns-Manville, 22 East 40th Street, New York 16, N.Y.



*Reg. U. S. Pat. Off.

BRING THE WEATHER RIGHT INTO YOUR LABORATORY WITH THE . . .

WEATHER-OMETER



● Accelerated weathering of fabrics, finishes, paints, rubber, plastics, and hundreds of other products used in outdoor service can be conducted in the laboratory with the Weather-Ometer. The deteriorating effects of outdoor exposure can be reproduced in the Weather-Ometer by controllable cycles of light, water spray, selected temperatures and humidity.

A few days in the Weather-Ometer equals years of outdoor service. Operation is fully automatic, the Weather-Ometer can be run continuously without attention 24 hours a day.

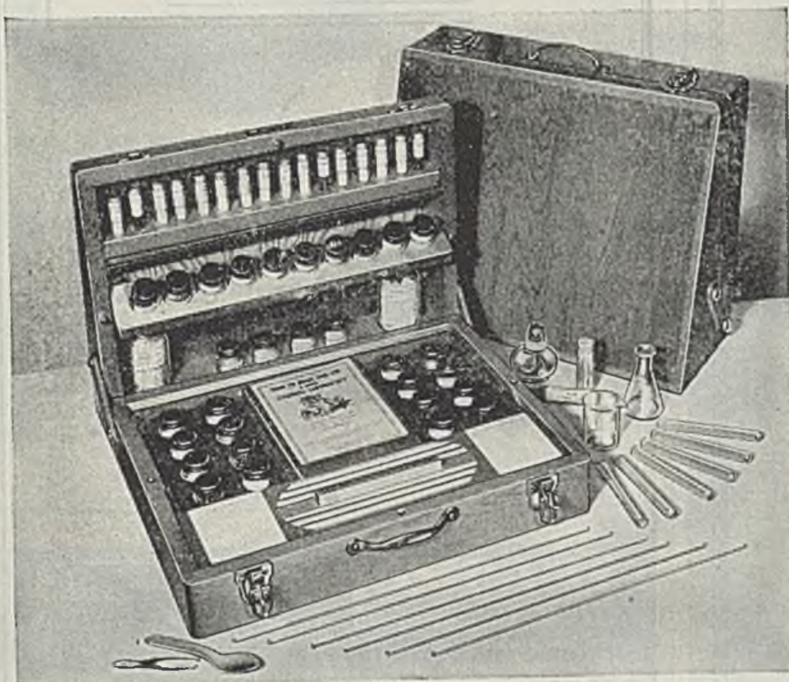
Used all over the world { **Atlas Weather-Ometers**, **Launder-Ometers**, **Fade-Ometers**

SALES OFFICES IN PRINCIPLE CITIES IN U. S. A., AND LONDON

ATLAS ELECTRIC DEVICES CO.

361 West Superior Street, Chicago 10, Illinois

ECONOMICAL LABORATORY FACILITIES FOR CHEMISTRY STUDENTS



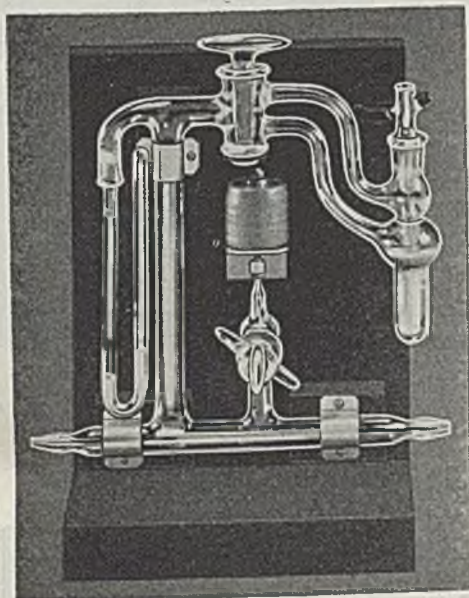
• This portable kit is designed to provide maximum laboratory facilities for students of qualitative, general, organic and inorganic chemistry. The single precision-made unit contains glassware, burner, chemicals and a special built-in, waterproof, chemical-resistant laboratory table. Kits are outfitted to fulfill specific requirements for organic and inorganic chemical applications of individual schools and universities. Chemicals used in Kit Corporation portable laboratories are manufactured by Merck & Company, and are available for refills at nominal costs.

• Kit Corporation also manufactures a high quality junior laboratory kit which offers complete essential materials for basic experiments.

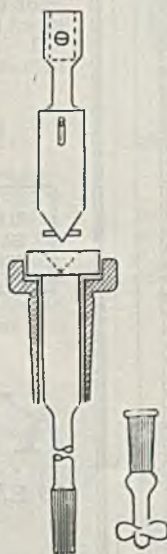
• Write for further information, or request one of these kits on approval.

KIT *Corporation*

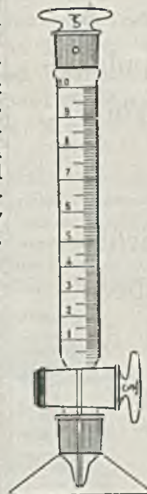
212TH ST. AND JAMAICA AVE.
QUEENS VILLAGE, N. Y.



Cat. #4400 Vacuum Regulator Smith-Nester type for maintaining constant pressure. Special stopcocks simplify operation. Floating electrical contact improves pressure constancy and eliminates fouling of mercury in monostat.



Cat. #4550 Stirrer, Smith-Uhrig type for laboratory use over a wide range of temperatures and pressures from 0.25 mm to 800 mm or more of mercury. All "Pyrex" construction except for universal swivel joint.



Cat. #778 Burette, Weighing, D. M. Smith for weighing out samples of volatile organic liquids, made with standard ground joints and stopcocks, Capacity 2; 10 and 30 ml.

We have fifty new laboratory items in preparation, carefully designed to improve laboratory efficiency. A new bulletin describing this equipment will be available soon and will be sent upon request.

We specialize in High Vacuum Stopcocks with hollow plugs, Diffusion Pumps, McLeod Gauges, Geiger-Muller Counters, Special Vacuum Distilling Apparatus, etc. Our precision grinding is unexcelled. We are in a position to give expert advice on the design of laboratory glassware of all types.

ECK & KREBS
131 West 24th St.
New York 11, N. Y.

WHATMAN

Filter Paper No. 42

for Fine Precipitates

For such difficult precipitates as Barium Sulfate, Metastannic Acid and other compounds of similiar particle size, WHATMAN Filter Paper No. 42 has long been a favorite. A list of the users of this popular grade reads like a "Who's Who" in analytical chemistry.

Such preference is richly deserved for No. 42 has been serving analysts faithfully for years.

Samples cheerfully supplied.

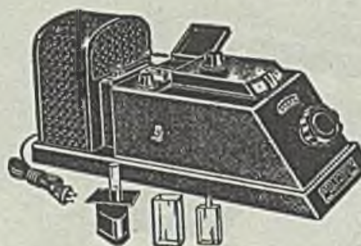
H. REEVE ANGEL & CO., INC.
7-11 Spruce St. New York 7, N. Y.

WHATMAN
High Grade FILTER PAPERS

Klett .... Photometers

Klett-Summerson

Photoelectric Glass Cell Colorimeter



No. 900-3

The Klett Fluorimeter

No. 2070

Designed for the rapid and accurate determination of thiamin, riboflavin, and other substances which fluoresce in solution. The sensitivity and stability are such that it has been found particularly useful in determining very small amounts of these substances.

KLETT SCIENTIFIC PRODUCTS
ELECTROPHORESIS APPARATUS • BIO-COLORIMETERS
GLASS ABSORPTION CELLS • COLORIMETER NEPHELOMETERS • GLASS STANDARDS • KLETT REAGENTS

Klett Manufacturing Co.
179 EAST 87TH STREET NEW YORK, N. Y.

For Your Lab!

- Stepless heat control instead of the usual three-heat switch.
- Accurate temperature control over the entire surface.
- 110-950° F range—both higher and lower than is usually available.
- Modern, streamlined, easy to clean.
- Highest quality materials and workmanship throughout.
- Choice of three sizes.

*LINDBERG HOT PLATES

with STEPLESS Temperature Control

Cat. No.	5690/5	5690/10	5690/15
Model	H-1	H-2	H-3
Size	10x12"	12x20"	12x30"
Watts	1000	2000	3000
Current	110 or 220 v. 60 cycle, A.C.	110 or 220 v. 60 cycle, A.C.	220 v. 60 cycle, A.C
Price	$50.00	$75.00	$100.00

SCHAAR & COMPANY

Complete Laboratory Equipment

748 W. LEXINGTON STREET CHICAGO 7

DETERMINATION OF TUNGSTEN

The LaMotte Chemical Products Company announces a new organic compound developed by John H. Yoe and A. Lechter Jones, University of Virginia (Reference — Industrial and Engineering Chemistry, Analytical Edition, Vol. 16, p. 45–48, 1944), as a reagent for the gravimetric determination of tungsten. Procedures have been devised for its use in the determination of tungsten ores and alloys. Determinations of tungsten with the new reagent are equivalent in accuracy to the standard cinchonine method.

DETERMINATION OF IRON

A new sensitive stable, and widely applicable reagent, developed by John H. Yoe and A. Lechter Jones, University of Virginia (Reference — Industrial and Engineering Chemistry, Analytical Edition, Vol. 16, p. 111–115, 1944), for the colorimetric determination of ferric iron. The reagent may be used in either acid or alkaline medium. In alkaline medium it is sensitive to one part of iron in 200,000,000 parts of solution. In acid solution the sensitivity is one part in 30,000,000.

Write for further information and prices.

If you do not have the LaMotte "ABC of pH Control," a complimentary copy will be sent without obligation upon request.

LaMOTTE CHEMICAL PRODUCTS COMPANY

Dept. F., Towson 4, Md.

Back Again!

VARI-SPEED STIRRERS

The only laboratory stirrer combining positive gear drive with built-in electric governor, instead of a rheostat for speed control without loss of power at low speeds.

• Increased Torque at Low Speeds • Electric Governor for Speed Control • Built-in Reduction Gears, No Slippage • Speed Unaffected by Voltage Fluctuations • Powerful, Continuous-Duty Motor Rated 1/15 H. P., Underwriters' Approved • Sturdy Motor With Plus Features • Utmost Mounting Flexibility.

No. M-38353 (above). "Vari-Speed" Stirrer. On metal base with 5½" dia. rubber disc to prevent sliding of glassware. Rubber feet prevent marring of table tops. Stirrer height adjustable to place chuck 5½" to 10½" from base. Distance between mounting rod and stirrer rod 4". Includes ½ x 6¾" mounting rod, ¼" chuck, glass stirring rod 6" long, cord and plug. For 115 Volts. A. C. or D. C.................. $36.00

No. M-38351 (left) "Vari-Speed" Stirrer. On semi-circular cast iron support stand, with ½" x 24" nickel plated rod and 90° support clamp. Radius of base 6½". Includes ½" x 6 mounting rod, ¼" chuck, glass stirring rod 6" long, cord and plug. For 115 Volts. A. C. or D. C.................. $36.00

No. M-38356 (right) "Vari-Speed" Stirrer, less base. With mounting rod ½" x 6¾"; ¼" chuck; glass stirring rod 6" long, and 6 ft. rubber covered cord with plug. For 115 Volts. A. C. or D. C.................. $30.00

ORDER NOW!

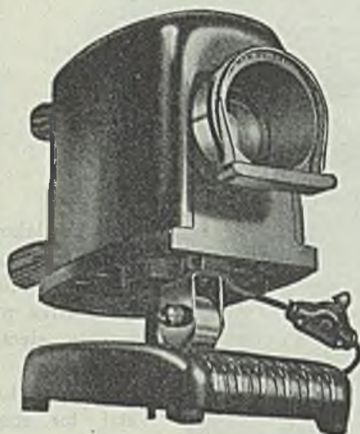
ARTHUR S. LaPINE & COMPANY

LABORATORY APPARATUS & REAGENTS • INDUSTRIAL CHEMICALS

109 W. Hubbard St. Chicago 10, Illinois

1000 FOOT CANDLES AT 12" DISTANCE

The "LAB-SPOTLIGHT"



A scientifically designed light source for bright or dark field microscopy, photography and many other "lab" uses.

Takes readily obtainable type G-16½ bulb having 100 watt rating and 200 hour life.

Provided with Fresnel lens (step-down prisms) which refracts light output to lens center at high intensity, absorbs heat to such an extent that light beam is cool as close as 11" to the lens and eliminates excessive reds and yellows to give light of "daylight" quality. Additional correction of light beam may be made by the use of filters which can be placed in front of the lens and secured by a built-in spring clip.

Lamp housing is of molded Bakelite, well ventilated and provided with cool plastic handle in rear for tilting. Lens may be racked in or out by means of a rotating knob* at rear of housing to obtain either "spot" or "flood" light. Further diffusion is made by the use of diffusing filters.

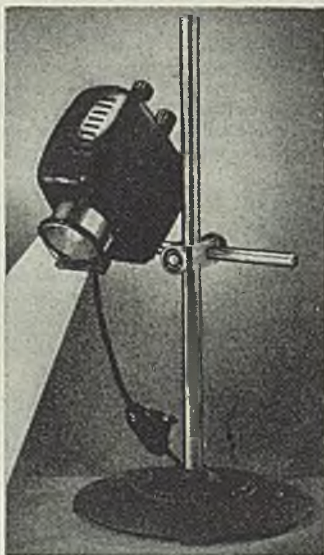
* Current Desk Models supplied with finger-grip adjustment.

DESK MODEL

has heavy metal base 4¼ x 5⅝" with inclination joint for tilting, and "non-scratch" feet. Complete with switch and 100 watt bulb. Overall height about 7" Complete with bulb . . . **$13.75**

SUPPORT MODEL

Has heavy metal base 8" dia., support rod ⅝ x 15", extension rod ½ x 5", junction clamp with plastic locking knob. Swivel mount permits light direction at any angle. Complete with switch and 100 watt bulb . . . **$19.75**



ACCESSORIES

White diffusing filter . $1.00
Blue diffusing filter . . $1.00
Heat-absorbing lens . $1.50
Clear white lens $1.50
Slit plate, for Kahn or agglutination test readings . . . . $ .50
Extra bulb, Type G-16½, 100 watts . . $1.10
Extra support stand . . $6.00

THE CHEMICAL RUBBER CO.

Scientific Equipment, Analytical Chemicals, Laboratory Supplies
HANDBOOK OF CHEMISTRY AND PHYSICS

2310 Superior Ave. N. E. • Cleveland 14, Ohio

365-T *Blaisdell* "LABORATORY" HEAT-RESISTING MADE IN U.S.A.

PULL NICK

To Sharpen—
NICK with string
PULL the paper
*(U. S. Pat. No. 1,756,953)

Mark Laboratory Vessels with the Heat-resistant

*Blaisdell "LABORATORY" MARKING PENCIL

Clearly and easily indicates contents of glass beakers, test tubes, retorts and all laboratory equipment. Markings are runproof up to 300° C. *Note:* The special process used in paper encasing these pencils seals in the ingredients, preventing *oxidation.* Write us for free sample mentioning this publication and order a supply from your dealer.

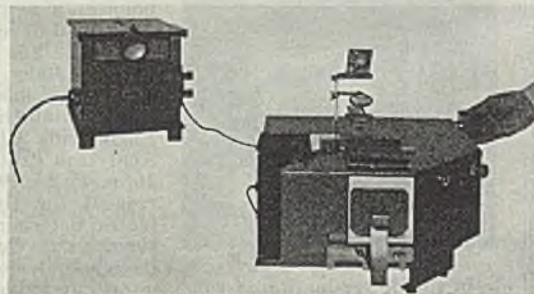
365-T Red 370-T Yellow
368-T Blue 373-T Black

*Reg. U. S. Pat. Off.

Blaisdell PENCIL COMPANY, EST. 1893—PHILADELPHIA 44, PA.

HUNTER MULTIPURPOSE REFLECTOMETER

A high-precision instrument used for reflectance, color, gloss, and transmission measurement



Hunter Multipurpose Reflectometer with galvanometer

OVER 50 APPLICATIONS

WIDELY USED FOR:

1. Testing paints for reflectance, hiding power, color difference, whiteness, yellowing, fading, etc.
2. Determining detergent power of soaps.
3. To indicate lightness of surfaces.
4. To measure color and whiteness of dry powders, pigments, pastes and liquids.
5. To classify materials for gloss.
6. To indicate shininess of surfaces.
7. To measure luminous transmission.
8. To determine resistance of surfaces to weathering, wear and abrasion.

SEND FOR LITERATURE

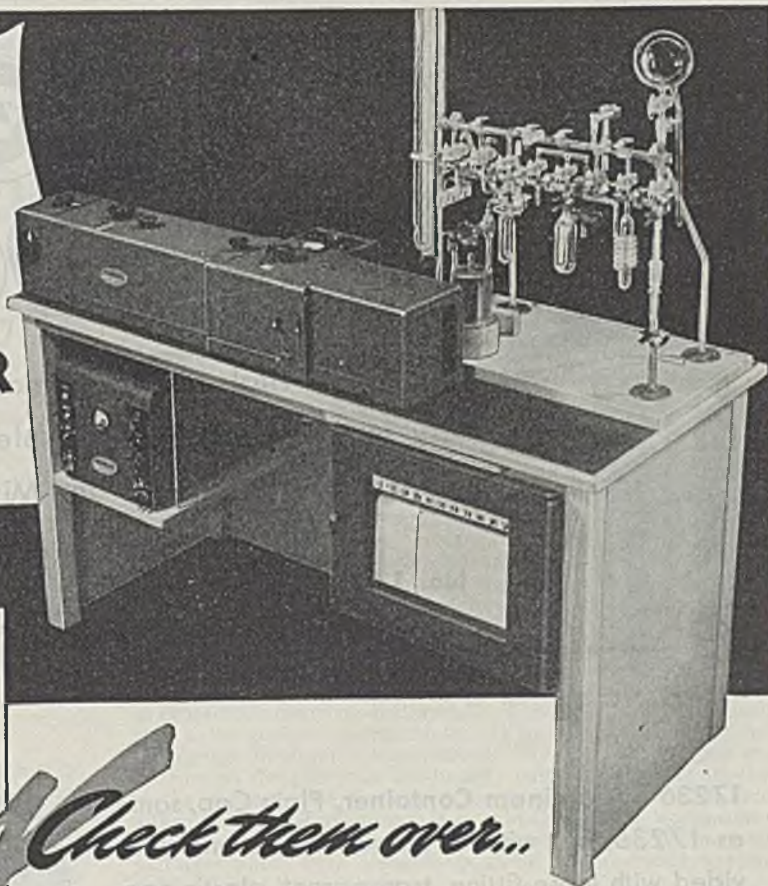
HENRY A. GARDNER LABORATORY, INC.

4723 Elm Street
Bethesda 14, Maryland

What Are the Advancements That Make

THE BECKMAN IR-2 INFRARED SPECTROPHOTOMETER

so Outstanding?



ONLY THE BECKMAN IR-2 COMBINES SUCH FAR-REACHING ADVANCEMENTS AS THESE . . .

HERMETICALLY SEALED
No costly air-conditioning required. Unaffected by humidity or atmospheric contamination.

INTEGRAL AMPLIFIER
No extra amplifier needed for recording. IR-2 can be direct-connected to standard recorder.

DIRECT-READING SCALES
Wavelength scale reads directly without curves, charts or computations. Percent Transmission scale reads directly.

CONSTANT RADIATION
Photoelectronic regulator maintains radiation constant within 0.1%.

ZERO DRIFT ELIMINATED
Greater accuracy; simpler, faster operation.

FALSE ENERGY ELIMINATED
Negligible stray light effects assure increased accuracy.

GALVANOMETERS ELIMINATED
Beam-modulation, bolometer and electronic amplifier eliminate vibration, temperature and non-linearity difficulties associated with galvanometers.

TEMPERATURE CORRECTIONS ELIMINATED
Entire instrument thermostated.

UNUSUAL VERSATILITY
Light sources, cell holders and other elements are interchangeable for a wide range of applications on gases, liquids and solids.

The above are only a few of many IR-2 innovations. Write for full details.

Check them over...

THE BACKGROUND of experience behind this Beckman instrument is unmatched. From the first-hand experience, user suggestions and the practical knowledge gained in proving infrared equipment for industrial use, National Technical Laboratories has developed the new Model IR-2. It was Beckman infrared equipment, developed in close cooperation with leading petroleum scientists, that made possible the development of the first accurate, analytical methods for hydrocarbon analysis which contributed so much to the success of the butadiene and aviation gasoline industries. Today, in these and other industries throughout the world, there are large numbers of Beckman Infrared Spectrophotometers in use—a fact which has contributed immeasurably to the "know how" behind every Beckman instrument.

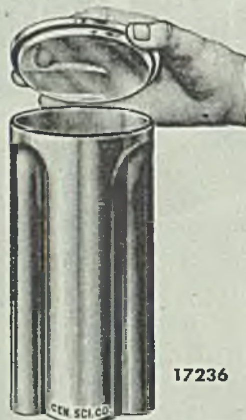
It is the continuing aim of National Technical Laboratories to produce instruments which combine the best previous achievement with unique developments which further extend the application of instruments in industry. The IR-2 Spectrophotometer again attains this goal. It integrates well-known basic principles of optics with new developments in electronic circuit design, many of which originated in our own laboratories in the production of thousands of scientific instruments. The result is an Infrared Spectrophotometer with increased convenience and versatility that greatly extend the usefulness of infrared methods.

There is no other instrument like the Beckman IR-2. Check over the unique features outlined at left. They represent design advancements which mean greater versatility, higher accuracy, more speed and convenience in all types of, infrared analytical work. A careful comparison with other infrared equipment will verify how much more the Beckman IR-2 provides.

After checking over the features outlined here let our technical staff further explain them and other important advantages. Your inquiry will bring you complete information. Beckman Instruments, National Technical Laboratories, South Pasadena, California.

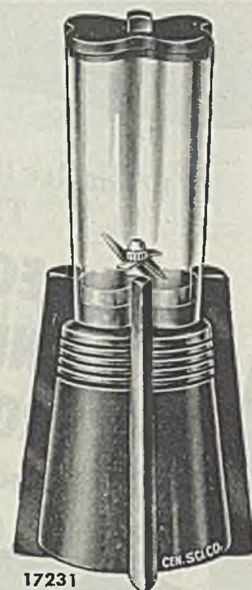
BECKMAN INSTRUMENTS CONTROL MODERN INDUSTRIES

The WARING BLENDOR



17236

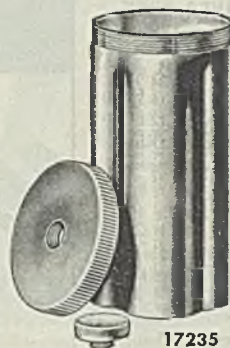
Dozens of vital, new uses make it a necessity in every laboratory. Blends, Comminutes, Pulps, Macerates and Mixes Materials.



17231

No. 17231—Waring Blendor with Standard Glass Jar. **Each $30.00**

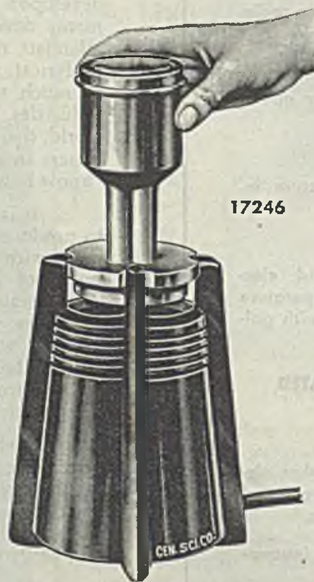
17235—Aluminum Container, Full capacity 1000 ml, Screw Cap for use on Waring Blendors for preparation of sterile biological products. Made of aluminum alloy with alumilite finish. Fitted with air-tight, screw-cap cover with threaded sampling plug in center. With blending assembly. **Each $14.50**


17235

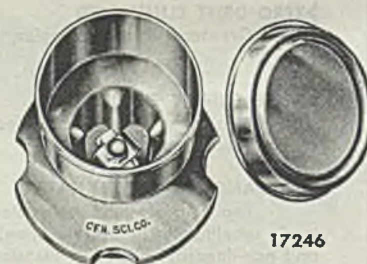
17236—Aluminum Container, Plain Cap, same as 17235, but without screw threads and provided with loose-fitting, transparent, plastic cap. **Each $10.50**

17244

17246—Monel Metal Container, Semi-Micro, with friction-fit monel cover and small size blending assembly. **Each $18.50**



17246



17246

Top view of No. 17246 Monel Semi-Micro Container showing blades of blending assembly and constricted neck and small volume of lower portion of container.

17244—Glass Container, Small Size, Full Capacity 500 ml; minimum, 30 ml. Made with metal screw cap and small size blending assembly for use on Waring Blendors. **Each $6.80**

All prices are subject to Federal Excise Tax

CENTRAL SCIENTIFIC COMPANY

Scientific CENCO *Apparatus*

New York BOSTON San Francisco CHICAGO Los Angeles TORONTO Montreal

Current Developments in

INSTRUMENTATION IN ANALYSIS

Discussed by Ralph H. Müller

THE three R's of instrumentation, reading, 'riting, and 'rithmetic, are fundamental topics and they present, we believe, some timely questions. More accurately they are concerned with indication, recording, and computing. The analyst is constantly concerned with each of these three operations. In some of the newer instruments which have been devised for his work, considerable elaboration in technique has been necessary, in order to provide greater precision, speed of operation, and convenience. It is not difficult to understand why elegant means of indication, recording, or computing can be built into complicated instruments, the intrinsic costs of which are high. It is rather with relatively simple devices where improvements would be very welcome.

Indication

The ideal instrumental indication is bold, legible, requires no interpolation, and reads the desired quantity directly. The professional man will part with scale divisions and verniers with great reluctance. Industrial measuring techniques incline more and more to the limit principle. A typical example is the "go–no go" type of dimensional gage in which a specimen is acceptable if it falls between the limits set by the extremes of the gage. The criterion in this case is the tactile sense of the inspector. In more elaborate forms, the answer is given in terms of signal lamps—red, green, or white—signifying over- or undersize, or acceptable. A physical measurement of high order has been accomplished, but the unmistakable interpretation can be made by a high-grade moron.

Instrument reading has reached a dangerous limit and all-time high in the cockpit of the large airliner or bomber. The psychologists have already been drafted for this problem and have restored some sanity to the process. They have found that a pilot is primarily concerned with the "essential correctness or acceptability" of a given condition and only with its numerical value if something is wrong. Commander Hibbard [*Instruments*, **18**, 760 (1945)] has stated that under battle fatigue, a pilot has been known to set his course by the indicated value of engine temperature, because that value happened to coincide numerically with the original compass heading!

It has been said that one of the great advantages of photoelectric photometers is the elimination of eyestrain and fatigue, but on numerous occasions we have been properly rebuked by research students who pointed out that squinting at a microammeter needle for hours at a time is just as swift and certain a means of becoming cross-eyed. Thus, whatever gains have been made in the mechanization of this optical procedure, such as objectivity and greater precision, the elimination of fatigue is not one of them. Complete mechanization, yielding bold, unambiguous results is the only justifiable excuse for the change.

The null-indicator, of which the lamp and scale galvanometer is a common example, is a case in point. It is very unlikely that the use of complex electronic attachments would be justifiable in order to make its use more convenient, but a few pieces of colored glass or cellophane in place of the millimeter scale, enable one to secure the advantages of the red-green-white criterion of acceptability. In the same sense, polaroid sheet in combination with phase-retarding films of selenite or ordinary cellophane, affords another simple substitute. In general, the whole field of optical projection in connection with null indicators looks very promising. From what has been done during the war, it is clearly evident that complex optical assemblies for this purpose can be molded in one unit from colorless plastics and at very low cost.

We are not too certain that small servo-mechanisms are ruled out of consideration even for simple indicating devices. A large variety of these devices can be borrowed from the aviation industry. The only drawback to their immediate utilization in the plant or laboratory is their use of 24- to 28-volt power supplies, but it is likely that widespread use would justify redesign to suit the more common power sources.

The cathode-ray oscillograph continues to be our most versatile indicator. It can present an almost unlimited wealth of detail at writing speeds in excess of 50,000 inches per second. Its use is no longer restricted to recurrent phenomena because the improvement in long persistence screens and beam intensification permit the convenient observation of transients and low-speed phenomena. DuMont has recently announced the Type 5SP tube which contains two independent gun and deflection systems in one envelope. This allows the simultaneous observation and comparison of two related or entirely independent phenomena. Heretofore this operation has required complex electronic switching circuits. In passing, we recall the successful application of cathode-ray tubes to the inspection of metallurgical samples for minor variations in physical properties and chemical composition at practically conveyor-belt speed! This is getting dangerously close to the analyst's prerogatives. At best we have not scratched the surface in possible applications. We hope the analyst and instrument designer will assiduously cultivate this big brother of the vacuum tube, because it can perform many of his more difficult tasks. Furthermore it is unlikely that the communication industries alone can retain the economic and low-cost advantages which were brought about by the enormous wartime production of these tubes.

We cannot evade the feeling that the laboratory will ultimately share with industry and commerce the equivalent convenience afforded by digit-type Telechron clocks, gasoline pumps which indicate gallons, price plus tax, and weighing machines which print the answer on a ticket. The chemist will wish to establish his own tolerance limits, and indeed vary them to suit the conditions. Beyond this, the result is merely good or bad.

Recording

The automatic recording of data is becoming increasingly common in analytical instruments. The incentive to do so is naturally greater in industrial laboratories, but the advantages are not restricted to routine work. Recording is useful in the interests of speed, use of nontechnical personnel, permanence of the record, and a degree of detail which is often lacking in manual operation. We believe we are correct in recalling that the first indications that aromatics of small, but economically significant amount, were present in mid-continent crudes, was established by automatic distillation records. The complexity of a typical infrared absorption curve is another example in which apparently insignificant detail might be missed by manual operation.

The true incentive for the improvement of recorders has really come from problems of the above type. The old style recorder with intermittent "feeler" action was adequate for 24-hour records of temperature, pressure, and other variables. The newer problem requires much higher recording speed. The Leeds & Northrup Speedomax was the first answer to this requirement. It scored its first triumphs in steel mills but now is serving in many laboratory applications. Others like the Brown Elektronik recorder accomplish the same result. One important adjunct to these devices has been the vibrator or resonant chopper element which will convert small D.C. potentials from low impedance sources into A.C. suitable for amplification. This has eliminated the ancient "bug" of amplifying thermocouple or thermopile e.m.f.'s, and it has been possible by careful design to bring their sensitivity down to noise levels of the order of 10^{-8} to 10^{-9} volt.

Modern problems in indication and recording will bring the analyst into repeated contact with the study of servo-mechanisms.

Servo-Mechanisms

A servo-mechanism may be required to follow the deflections of a mirror galvanometer, or on a large scale to maintain the guns of a battleship on target independent of the roll, pitch, or yaw of

Calibrated FACTS on WEARABILITY

Yours in Advance with the Taber Abraser

No need for guesswork in the testing of your own or competitor's products for wearability or resistance to surface abrasion. The Taber Abraser gives you the facts with scientific precision—yet the procedure is as practical as it is accurate. Eliminates time-consuming, expensive performance tests. Used by hundreds of manufacturers and research laboratories for testing surface wearing qualities of paints, lacquer, plastics, leather, rubber, paper, electrotypes, etc.

Write: Full details on the Taber Abraser and the Taber wear testing method described in new handbook. Write for your free copy.

TABER INSTRUMENT CORPORATION
111 Goundry Street North Tonawanda, N. Y.

The Taber Test Proves What Wears Best!

No Technical Training Required to Make Accurate DETERMINATIONS with a PHOTOVOLT REFLECTION and GLOSS METER

Designed to measure both diffuse reflection (whiteness, brightness, color) and specular reflection (shininess, gloss)

- Paper brightness tests
- Detergency tests
- Paint gloss tests, and many others
- Line—or battery—operated models
- Twelve interchangeable search units

For solid surfaces of every description, for powders, pastes and opaque liquids. To the basic instrument can be attached any of a number of search units, each engineered especially for one or more specific purposes. A variety of color filters is also available, including those for ICI Tristimulus values.

SCHAAR & COMPANY
Complete Laboratory Equipment
748 W. LEXINGTON STREET CHICAGO 7

Instrumentation

the vessel. Regardless of application, most systems are of the closed-loop servo-type. In essence, the input or primary information is fed into a data-transmission unit, and after amplification, is fed to a servo-motor. The latter orients the output indicator (pointer or gun turret). The output element returns to the data-transmission unit a signal which is proportional to its present position. As a consequence of this feedback, the driving element is constantly exposed to a signal which is proportional to the difference of the two quantities. Under ideal conditions, this condition will approach zero at balance. The complete behavior of a simple system is expressed by a second-order differential equation, the first term of which represents the energy required to accelerate the system, the second term, the energy required to overcome the internal friction of the mechanism. The third term accounts for any disturbing torques applied to the system. The theory of such systems is treated in two recent books: "Fundamental Theory of Servomechanisms", by L. A. MacColl, D. Van Nostrand Co., 1945, and "Automatic Control Engineering", by E. S. Smith, McGraw-Hill, 1944.

Unfortunately there is no completely convenient reference work for the potential user of servo-mechanisms. The expert can refer to the above sources. The analyst and instrument designer could profit handsomely from a treatise intermediate between these and the service manuals of the "oil it here and oil it there" variety. We happen to know someone who could do the job, and we hope to be successful in inducing him to write a comprehensive article for these pages.

We predict great activity in this field, particularly in instrument servos of all classes. The results will be of great interest and utility to the analyst, although he may not be concerned with the details of operation.

Computations

Frequently the results of a measurement, or readings from a recorder, require extensive computation which may involve more time than the actual measurement. This may well become a bottleneck in an otherwise satisfactory method. In some cases, the problem can, and has been, referred back to the instrument for solution. An example is the provision of logarithmic cams in the recording color analyzer to compute extinction values or even log-extinction values from the measured transmission data. Consequently, what we discuss here does not refer necessarily to isolated computers, but suggests their incorporation wherever possible in the instrument itself. Every scientist or engineer is a slide rule or log table addict, and adding machines and punched card sorters are common office accessories. In addition, the use of specially shaped cams or linkages is common enough in various machines. Beyond this, it may be stated that electrical, electronic, or electro-mechanical elements are available for handling the following mathematical functions or operations: addition, subtraction, multiplication, division, logarithmic, powers or roots of a variable, trigonometric, conic sections, differentiation, integration, reciprocals, counting, coincidence (in time), sorting or selection, discrimination of relative magnitude, solution of simultaneous equations, statistical averaging.

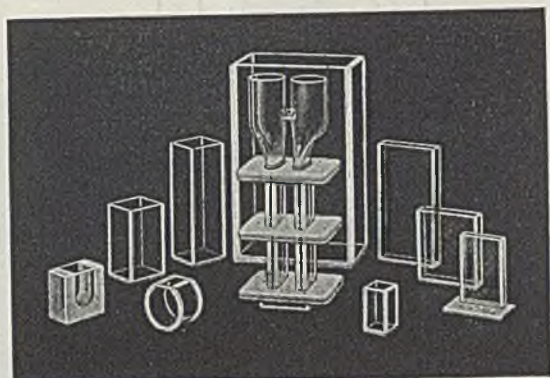
Once more, the war effort has furnished improved components which may be used in these problems. Synchros and control transformers provide accurate means for handling data involving trigonometric functions. Potentiometers of extended range and higher precision have been developed and in some cases with tapers which will accommodate nonlinear functions over a limited range. Electron tubes are widely used in computing mechanisms. One very elaborate installation uses 18,000 tubes. In general, a complex computer will employ a principle akin to the closed-loop servo, in which output and input are constantly intercompared. Any vacuum tubes serving in the process are consequently functioning primarily to amplify residuals and no particular dependence is placed upon their reproducibility.

Lord Kelvin once said that "the human mind is never performing its highest function when it is doing the work of a calculating machine". The same may be said of the analyst and his chores. At present, under the compulsion of industry's pace, we are in a stage of extensive mechanization. That process cannot be stayed, however much the classical analyst bemoans the intrusion of the physicist and engineer upon our sacred domain. It is to be hoped, rather, that it will afford the analyst more time and better tools to investigate those obscure and neglected phenomena which, when developed, will be the analytical chemistry of tomorrow.

GLASS ABSORPTION CELLS....

OF FINE QUALITY

made by Klett



★ Sole manufacturer in the United States of fused Electrophoresis Cells. ★

Makers of complete Electrophoresis Apparatus

Fused in an electric furnace with cement that is acid, alkali and solvent resistant.

Optical Flat Walls. Many stock sizes are available. Special sizes made to order.

KLETT SCIENTIFIC PRODUCTS

KLETT-SUMMERSON PHOTOELECTRIC COLORIMETERS • COLORIMETERS • NEPHELOMETERS • FLUORIMETERS • BIO-COLORIMETERS • COMPARATORS • ELECTROPHORESIS APPARATUS • GLASS STANDARDS • GLASS CELLS • KLETT REAGENTS

Klett MANUFACTURING CO., 177 East 87th Street, New York, N. Y.

Durability is a Feature of Welch Pumps

Welch Rotary Oil-Sealed Vacuum Pumps are precisely machined to insure smooth, efficient operation. Before being placed in stock, every pump undergoes a long, continuous "run-in" period, so that each moving part will fit perfectly into the other. Surfaces of rotor and stator are ground to within 1/10,000 inch.

HIGH VACUUM—.05 Micron (.00005 mm. Hg.) GUARANTEED

Double-Seal prevents diffusion of exhaust gases back into intake side, increasing vacuum.

FREE AIR CAPACITY—33.4 Liters Per Minute

Double-vane system evacuates crescent-shaped air spaces in the two rotor units twice each minute at comparatively low speed of 300 R.P.M.

OIL REQUIRED 650 ml. Duo-Seal Oil

Only a small fraction of the oil circulates in the pump—a distinct advantage in out-gassing the oil. Positive oil seal prevents oil from backing up into the exhaust line.

Write for FREE 32-page booklet on Welch Duo-Seal Pumps and Vacuum Technique

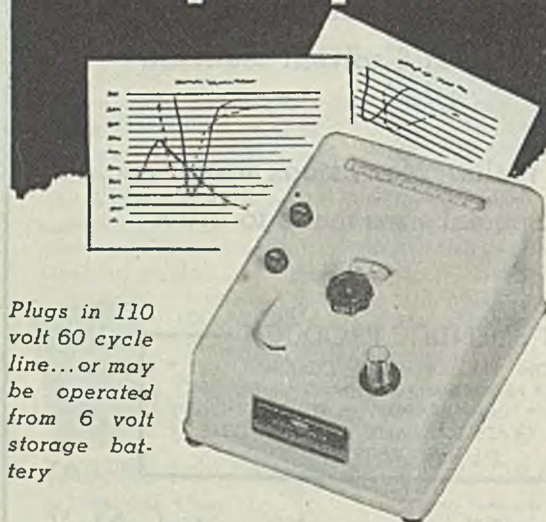
No. 1405-H

DUO-SEAL VACUUM PUMP, Motor Driven. Vacuum .05 micron—free air capacity of 33.4 liters per minute........................$140.00
With larger motor giving 57 liters free air capacity per minute and vacuum of 0.1 micron.....$155.00

W. M. Welch Scientific Company
1515 Sedgwick St., Dept. A Established 1880 Chicago 10, Illinois, U.S.A.

"...speeded up our work three times"

the New Coleman JUNIOR Spectrophotometer



Plugs in 110 volt 60 cycle line... or may be operated from 6 volt storage battery

This simplified, stream-lined spectrophotometer, used in hundreds of U. S. Army and Navy laboratories is now ready for general distribution!

The JUNIOR is a true Spectrophotometer so extra filters are not required . . . any wave band is available from 400 to 700 mu . . . at the turn of the one selector knob! It utilizes the same Wood's diffraction grating and substantially the same optical system as the UNIVERSAL Spectrophotometer . . . and includes direct reading, double galvanometer scale! Accepts test tubes from 10 mm. to 1" diameter!

Coleman Spectrophotometers replace all filter photoelectric colorimeters as ANY band is available with turn of ONE knob!

TURNING ONE KNOB permits selection of any color light band required.

We send exact procedures with each instrument for hundreds of colorimetric determinations.

Write Dept. 1E3 for new steel and water procedures, including the popular Sober's Combined Method for Iron and Steel analysis.

WILKENS-ANDERSON CO.
111 NORTH CANAL STREET • CHICAGO 6, ILLINOIS

COORS PORCELAIN COMPANY
GOLDEN, COLORADO

PHYSICAL CONSTANTS OF HYDROCARBONS

by Gustav Egloff
Director of Research, Universal Oil Products Co., Chicago

American Chemical Society Monograph No. 78

Volume III — Mononuclear Aromatic Compounds
674 pages $15.00

We are particularly glad to announce the publication of the third volume of this exhaustive catalog of hydrocarbon compounds and their properties.

Volume III contains the structural formulas and complete data on the boiling point, melting point, density, and refractive index of the benzene series of hydrocarbons. This highly important group of substances forms the very core of organic chemistry. Knowledge of their physical properties is, therefore, indispensable in all branches of industrial research; it is especially necessary for those in the coal tar and petroleum fields. Every technical library and organic research chemist will find particular need for this volume of the series.

EARLIER VOLUMES

Volume I — Paraffins, Olefins, Acetylenes, and Other Aliphatic Hydrocarbons 416 pages $9.00

Volume II — Cyclanes, Cyclenes, Cyclynes, and Other Alicyclic Hydrocarbons 608 pages $12.00

REINHOLD PUBLISHING CORP. 330 W. 42nd Street New York 18, N. Y.

FINE ANALYTICAL FILTERPAPERS

SINCE EIGHTEEN FIFTY-SIX

CARL SCHLEICHER & SCHUELL CO.

Plant and Laboratories: SOUTH LEE, MASS.
Executive Offices: 116-118 WEST 14th STREET
NEW YORK, N. Y.

PALO-MYERS
Tygon Tubing No. 7449

A new synthetic flexible tubing—Better than rubber, not just a substitute.

Stands heat and cold (−50°F to +225°F)

Completely immune to the oxidizing effect of light and air. It will withstand repeated flexing and exposure to oxidizing chemicals for long periods without danger of embrittlement or cracking.

Stands steam sterilization, useful for gas, air and water connections (non toxic).

Write for Price—Stating Sizes and Quantity Required

Other Laboratory apparatus and supplies available for immediate delivery.

Balances, Centrifuges, Ovens, Microscopes, Water Stills, pH Equipment, Glass and Porcelain Ware Laboratory Hardware.

PALO-MYERS INC.

81 Reade Street New York 7, N. Y.

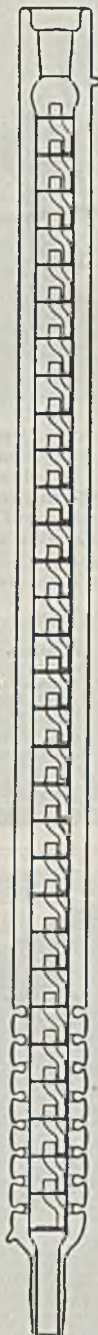
LABORATORY DISTILLATION EQUIPMENT

The Oldershaw Perforated Plate Fractionating Column (Shell Design), is now being manufactured under rigid quality control. A majority of the leading petroleum and chemical company laboratories have been equipped by us during the past year.

A true bubble plate column, constructed entirely of Pyrex glass, each plate has 80 accurately drilled holes, with weir and drain tube for control and direction of the refluxing liquid.

The superior vacuum jacket, with guaranteed lasting heat insulation is an integral part of the column, and eliminates the need for outside heating. Internal type expansion bellows give full protection against thermal change breakage up to 450°F. at the top of the column.

The qualities of high efficiency, fast rate of throughput and reproducible results, with great flexibility in use, make the Oldershaw Column especially valuable in control, analytical and research laboratories.



CROSS SECTION
30 PLATE COLUMN

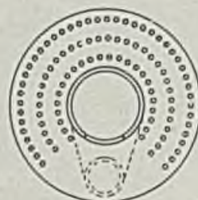


PLATE — top view

Side View

We are also making Automatic Still Heads, Receivers, Kettles, Timers, etc., with advanced features of design and construction.

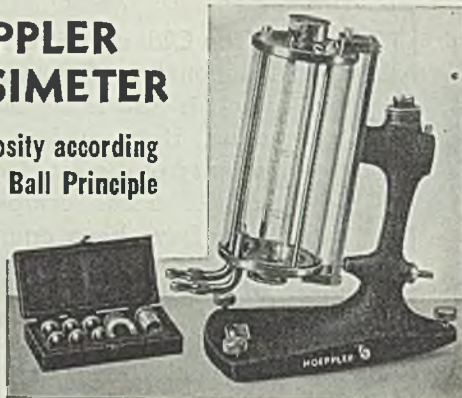
Your inquiries, giving particular problems and needs will be appreciated and we will gladly make recommendations.

GLASS ENGINEERING LABORATORIES
SAN CARLOS, CALIFORNIA

Greater Accuracy in Absolute Units

HOEPPLER VISCOSIMETER

Absolute viscosity according to the Falling Ball Principle



For determining the absolute viscosity of gases, liquids, oils, plastic, syrups, or viscous tars. Direct readings in centipoises (or centistokes). From 0.01 to 1,000,000 centipoises. Accuracy: 0.1% to 0.5%. Difference in viscosity between distilled and tap water can be measured.

The falling time of the ball multiplied by other given factors shows the absolute viscosity in centipoises. Small sample (30 cc) required. Results consistent and reproducible.

Write today for new Bulletin HV-303.

Order direct or from leading laboratory supply dealers.

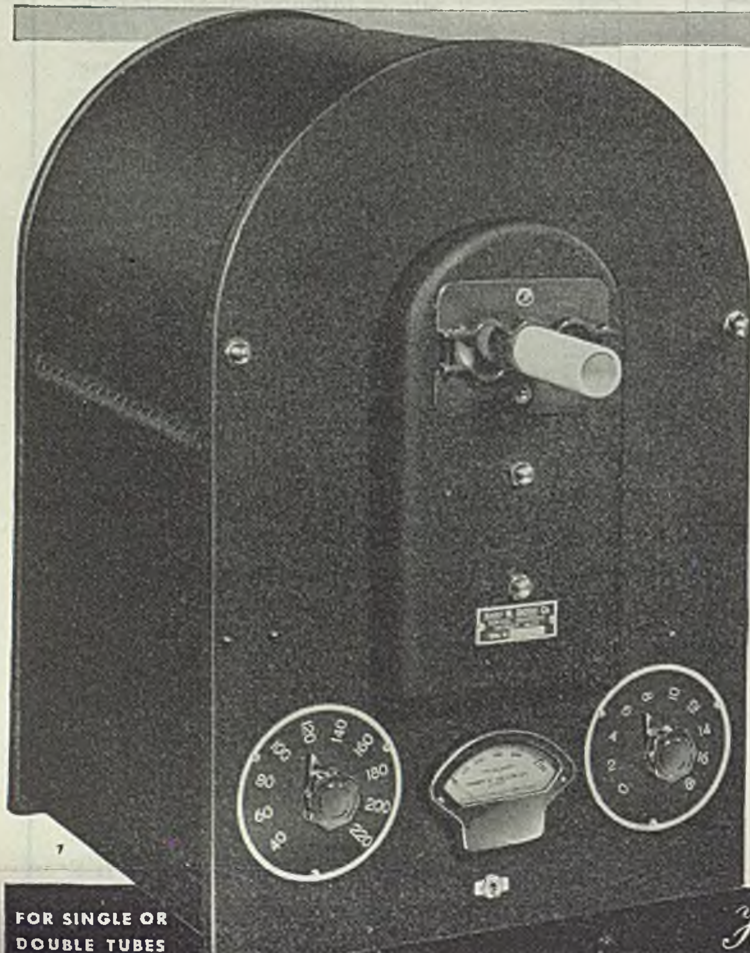
FISH-SCHURMAN CORPORATION
230 East 45th Street, New York 17, N. Y.

Fish-Schurman

This instrument retains all of the advantages of the well established Hercules method without any of its disadvantages. Range extended to cover from 75 to 1,000,000 centipoises Constant temperature jacket built-in. Automatic timer can be furnished upon request. Write for bulletin No. 451.

U. S. PATENT NO 2,252,572 LICENSED UNDER HERCULES POWDER CO PATENTS

TECH LABORATORIES
337 CENTRAL AVE., JERSEY CITY 7, N. J.



Use VARITEMP FURNACE for high temperature combustion of ferrous and non-ferrous metal samples

The Varitemp Furnace is the original leader of all modern high temperature furnaces. It was the first and is beyond comparison the best furnace unit for rapid carbon and sulfur determination by high combustion methods. The continuous operation temperature range is up to 2750° Fahr.

A complete laboratory furnace unit, in either single or double tube models, at an economical price. Single Tube Models $177.00. Double Tube Models $230.00

Write today for descriptive leaflet and quotation. Fast delivery assured

Harry W. Dietert Co.
9330 ROSELAWN AVE. DETROIT 4, MICH.

Laboratory Ware
OF QUALITY AND UTILITY

PLATINUM

CRUCIBLES
DISHES
WIRE
FOIL
TUBING
SPONGE
BLACK
CHLORIDES
SALTS AND
SOLUTIONS

SEND for our
Catalogue IE-17

THE AMERICAN PLATINUM WORKS
231 NEW JERSEY R.R. AVE., NEWARK 5, N. J.
PRECIOUS METALS SINCE 1875

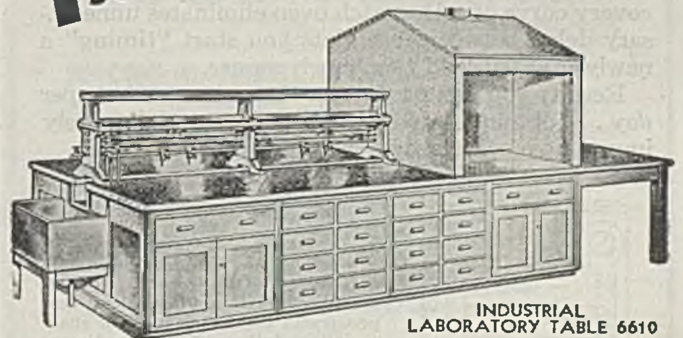
FOR BETTER
pH Control
there is nothing like
NON-FADING GLASS COLOR STANDARDS

Permanent reliability of Hellige Glass Color Standards, accuracy of color comparison, simplicity of the technique, and compactness of the apparatus are exclusive features of Hellige Comparators not found in any similar outfits.

WRITE FOR BULLETIN No. 602

HELLIGE
INCORPORATED
3718 NORTHERN BLVD., LONG ISLAND CITY 1, N.Y.

1st FOR QUALITY



INDUSTRIAL LABORATORY TABLE 6610

FOR MORE THAN 50 YEARS

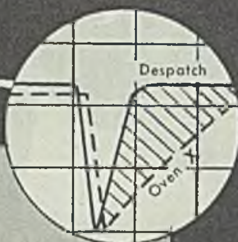
Peterson laboratory furniture has come through the past half century with a reputation for quality and integrity which, to shrewd buyers, has been the keynote for sound satisfaction.

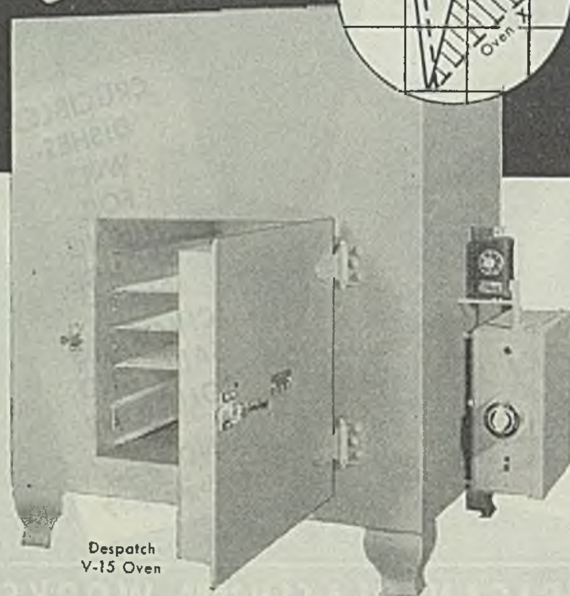
Today, Peterson furniture receives 1st consideration from some of the nation's largest users who have specified "Peterson" for one decade after another.

Let Peterson Experts help you plan any changes or additions you may require. .... No obligation is incurred.

LEONARD PETERSON & CO., Inc.
1222-34 FULLERTON AVE. CHICAGO 14, U. S. A.

HEAT RECOVERY IS *faster*





Despatch V-15 Oven

in a DESPATCH LABORATORY OVEN

Here's a feature technicians appreciate in a Despatch Laboratory Oven. Fast, accurate heat recovery *within 2 to 8 minutes* after loading a cold charge . . . and without overshooting or heat lag!

As the graph suggests, the nearly-vertical heat recovery curve of a Despatch oven eliminates unnecessary delay between tests, lets you start "timing" a newly-inserted load that much sooner.

Result: you can easily run through *more tests per day* . . . obtain needed data faster . . . and definitely improve your laboratory work-schedule.

Why This Speed is Possible

Powerful high volume fan drives air through heating elements, down sidewall duct and horizontally through chamber. Fast, uniform heat-flow penetrates every inch of shelf space . . . allows full use of shelf load-areas . . . provides over 100 air changes per minute (on smaller models).

TYPICAL FEATURES

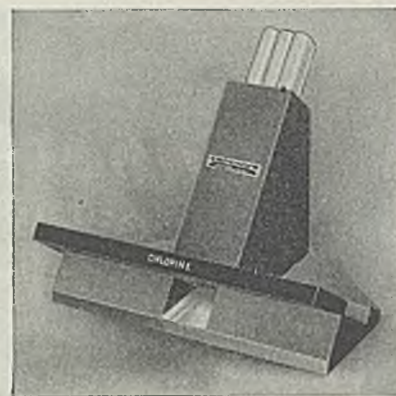
RUGGED BODY: Very sturdy and compact; 3" insulation throughout; attractive baked enamel exterior. Looks fine in any laboratory. **EFFICIENT HEATING SYSTEM:** Despatch cross-flow forced draft; electric or gas-fired heater. **WIDE RANGE:** From 35° to 260°C.; one of the most versatile ovens available.

ACCURATE CONTROL: Efficient thermostat with ±1°C. sensitivity; also handy 3-heat switch for added economy. **MODELS:** One or 2-door with removable mesh shelves; sizes (inside) 13"x13"x13" to 37"x25"x37". **WRITE FOR ILLUSTRATED BULLETIN 105. Gives full details about performance, new applications and new features.**

SEE YOUR LOCAL DEALER or WRITE DIRECT

DESPATCH OVEN COMPANY MINNEAPOLIS 14 CHICAGO 1

Here's Your WATER ANALYZER *Laboratory!*



This small, portable Taylor Water Analyzer will outperform many a far more costly apparatus. Gives quick, *accurate* determinations of pH, color, total iron, manganese, ammonia, silica, chlorine, nitrogen, nitrates, nitrites, copper and fluorides. All color standards are enclosed in plastic slides . . . making it possible to obtain a complete set of color standards at a fraction of the cost of fragile and expensive Nessler tube standards. Constructed for durability, the Taylor Water Analyzer should last a lifetime . . . and all color standards carry an UNLIMITED GUARANTEE AGAINST FADING. Complete with any one slide . . . $18.00. Extra slides . . . each, $8.00.

See your dealer, or write direct for new 88-page book, "Modern pH and Chlorine Control" . . . it's FREE!

W. A. TAYLOR AND CO.
7302 YORK RD. • BALTIMORE-4, MD.

TEMCO ELECTRIC FURNACE

MODEL GRP

FOR HEAT TREATING SMALL TOOLS AND PARTS, TESTING AND LABORATORY WORK.

FAST HEATING—1500° F. in 30 min. Temperature controlled by four point switch set for 500°, 1000°, 1500° and 1850° F. These settings easily changed by adjusting bands on rheostat coils. Dependable indicating pyrometer is calibrated in both Fahrenheit and Centigrade scales.

UNIFORM TEMPERATURE—Heating element of highest quality nickel-chromium alloy forms a continuous coil and is embedded in sides, top and bottom of muffle chamber. This construction assures uniform temperature and protects element from damage and oxidation. Complete muffle unit is replaceable.

PORTABLE—Body is aluminum. Total weight, 17 lbs. Muffle chamber, 4" x 3¾" x 3¾"; furnace overall, 9¼" x 13½" x 10½". Supplied with attachment cord. Operates on A.C. and D.C., 115 or 230 V. (*be sure to specify voltage*). Max. power consumption 1200 watts.
Model GRP complete, 115 V............$42.50; 230 V............$47.50

See your supply house or write

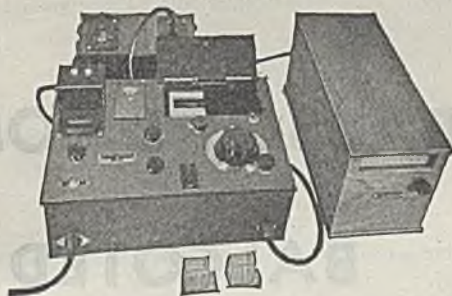
THERMO ELECTRIC MFG. CO., 478 W. Locust St., Dubuque, Ia.

LUMETRON

Photoelectric COLORIMETER and FLUORESCENCE METER

A high precision instrument of unusual flexibility for all tests involving . . .

TRANSMISSION
FLUORESCENCE
TURBIDITY
U. V. ABSORPTION



Mod. 402EF for determination of Vit. B_1 and B_2

Also

Colorimeters
Reflection Meters
Glossmeters
Densitometers
Smoke Meters
Vitamin A Meters
Continuous-Flow Colorimeters

Write for Literature *Prompt Delivery*

PHOTOVOLT CORP.

95 Madison Ave. New York 16, N. Y.

INDEX TO ADVERTISERS

American Platinum Works.......... 35
Anachemia, Ltd.......... 18
Angel & Co., Inc., H. Reeve.......... 24
Atlas Electric Devices Co.......... 22
Baker & Adamson.......... 40
Baker Chemical Co., J. T.......... 2
Bausch & Lomb Optical Co.......... 38
Blaisdell Pencil Co.......... 26
Central Scientific Co.......... 28
Chemical Rubber Co.......... 26
Consolidated Engineering Corp.......... 5
Coors Porcelain Co.......... 32
Corning Glass Works.......... 10
Despatch Oven Co.......... 36
Dietert Co., Harry W.......... 9:34
Dupont de Nemours & Co., Inc., E. I.......... 8:19
Eck & Krebs.......... 23
Eimer & Amend.......... 12
Fish-Schurman Corp.......... 34
Fisher Scientific Co.......... 12
Gardner Laboratory, Inc., Henry A.......... 26
Glass Engineering Laboratories.......... 33
Goodrich Chemical Co., B. F.......... 11
Harshaw Chemical Co.......... 15
Hellige Inc.......... 35
Johns-Manville Corp.......... 22
Kimble Glass Co.......... 13
Kit Corp.......... 23
Klett Manufacturing Co.......... 24:31
LaMotte Chemical Products Co.......... 25
LaPine & Co., Arthur S.......... 25
Lindberg Engineering Co.......... 7
Mallinckrodt Chemical Works.......... 6
Merck & Co., Inc.......... 4
Microchemical Specialties Co.......... 37
National Technical Laboratories.......... 27
Palo-Myers Inc.......... 33
Peterson & Co., Inc., Leonard.......... 35
Photovolt Corp.......... 37
Precision Scientific Co.......... 39
Reinhold Publishing Corp.......... 32
Sargent & Co., E. H.......... 21
Schaar & Co.......... 25:30
Schleicher & Schuell Co., Carl.......... 33
Scientific Glass Apparatus Co.......... 16
Taber Instrument Corp.......... 30
Taylor & Co., W. A.......... 36
Tech Laboratories.......... 34
Thermo Electric Mfg. Co.......... 36
Thomas Co., Arthur H.......... 20
Todd Scientific Co.......... 17
Welch Scientific Co., W. M.......... 31
Wilkens-Anderson Co.......... 32
Will Corp.......... 14

INTRODUCING A NEW LINE OF MICROCHEMICAL APPARATUS

MICROCHEMICAL SPECIALTIES CO.
Announces

THE KIRK-TYPE DROP-SCALE ANALYTICAL EQUIPMENT

For the first time many new items designed by Dr. Paul L. Kirk of the University of California are available to microchemists the world over.

In addition to the well known Kirk-type apparatus such as the one-piece all glass Micro-Kjeldahl still, ultra-micro or drop scale burette and pipettes, we are exclusively offering, many new items developed during World War II which are now being released for the first time.

Some of the items which we manufacture for direct sale to your laboratory are:

CAPILLARY BURETTE—

capacity ranging from 0.035 ml to 0.10 ml. (your choice) reads to nearest 10^{-5} ml.

CAPILLARY PIPETTES—

drop-scale wash out pipettes range from 0.001 ml. to .500 ml. Available in several types including self filling, transfer, and measuring types.

ULTRAMICRO CENTRIFUGE—

operates at 20,000 R.P.M. on laboratory source of compressed air. Clear blood serum in 3 minutes.

DROP STIRRER—

electric stirrer which stirs a drop. Used in drop scale titrations.

MICRO-KJELDAHL STILL—

distills samples in 8 minutes and washes itself out. Has no equal for research or in the industrial laboratory.

MICRO-KJELDAHL DIGESTION RACK—

Draft proof—with "Misco" burner. Acclaimed by microchemists.

MICRO MANIPULATORS—

for precision control, used in microchemical transfers, biological separations, microscopical manipulation of all types.

SYPHILIS MICROTEST UNIT—

a precipitation test (Kahn type) which requires only a drop of blood. "Results" in 20 minutes.

PORTABLE DROP-SCALE UNIT—

A complete self-contained analytical drop-scale laboratory, contains pipettes, burettes, reagents, fluorescent lighting, outlets for gas, air, water, vacuum, electricity, all within fingertip reach. Maximum size of unit is 2′ x 3′ x 1½′.

RESPIROMETER—

measures the respiration of small bits of tissue, a single pupae, amoeba, etc.

Our objective in building equipment is to combine simplicity with accuracy. Each item is designed with the thought in mind that convenience permits exact manipulative technique.

We are pleased to serve your needs.

CATALOG FURNISHED UPON REQUEST

MICROCHEMICAL SPECIALTIES CO.

MICRO CHEMICAL APPARATUS
DROP-SCALE APPARATUS
SPECIAL CLINICAL APPARATUS
CONSULTING
GLASS BLOWING
PRECISION MACHINE WORK
PRODUCTION GLASS WORK
MICRO MANIPULATORS

1834 UNIVERSITY AVENUE • BERKELEY 3, CALIFORNIA
THORNWALL 1282

Comparative Photomicrographs of Cast Iron
Left Field, Taken with uncoated 4mm., 0.95 N.A. Apochromat — Flare 20%
Right Field, Taken with Balcoted 4mm., 0.95 N.A. Apochromat — Flare 4.9%

BAUSCH & LOMB BALCOTED METALLURGICAL OBJECTIVES

Bausch & Lomb now offers the metallurgist the advantage of Balcote surfaced objectives for his metallurgical microscopes. This revolutionizing lens surface treatment applied to metallurgical objectives provides improved image contrast and shorter photographic exposures. "Flare" is decreased; light transmission is increased.

This is of particular advantage where the metallurgist must work with specimens of low reflectivity and long tone range. For complete information write Bausch & Lomb Optical Co., Rochester 2, N. Y.

BAUSCH & LOMB
ESTABLISHED 1853