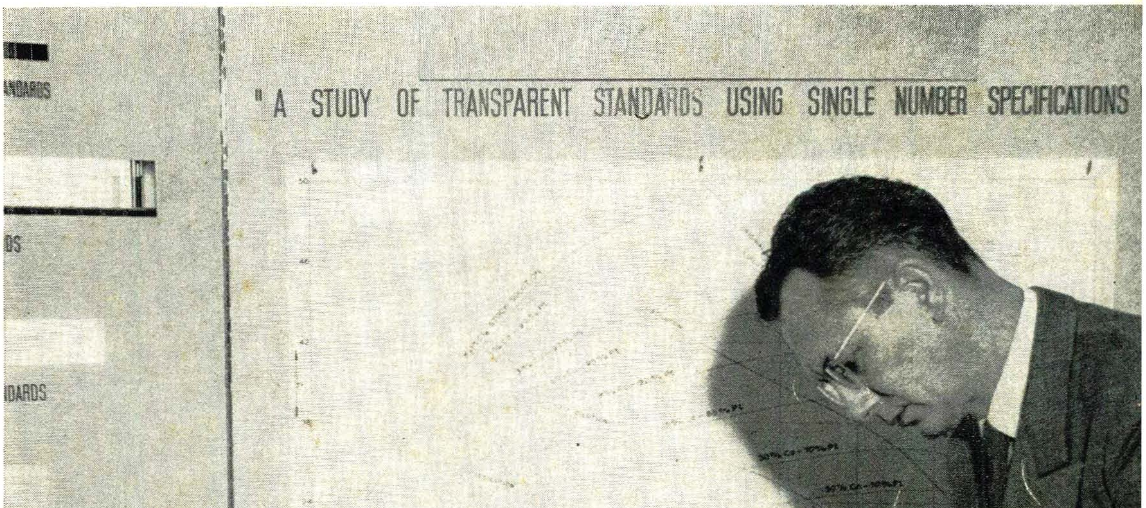
DECEMBER 1952

ANALYTICAL CHEMISTRY



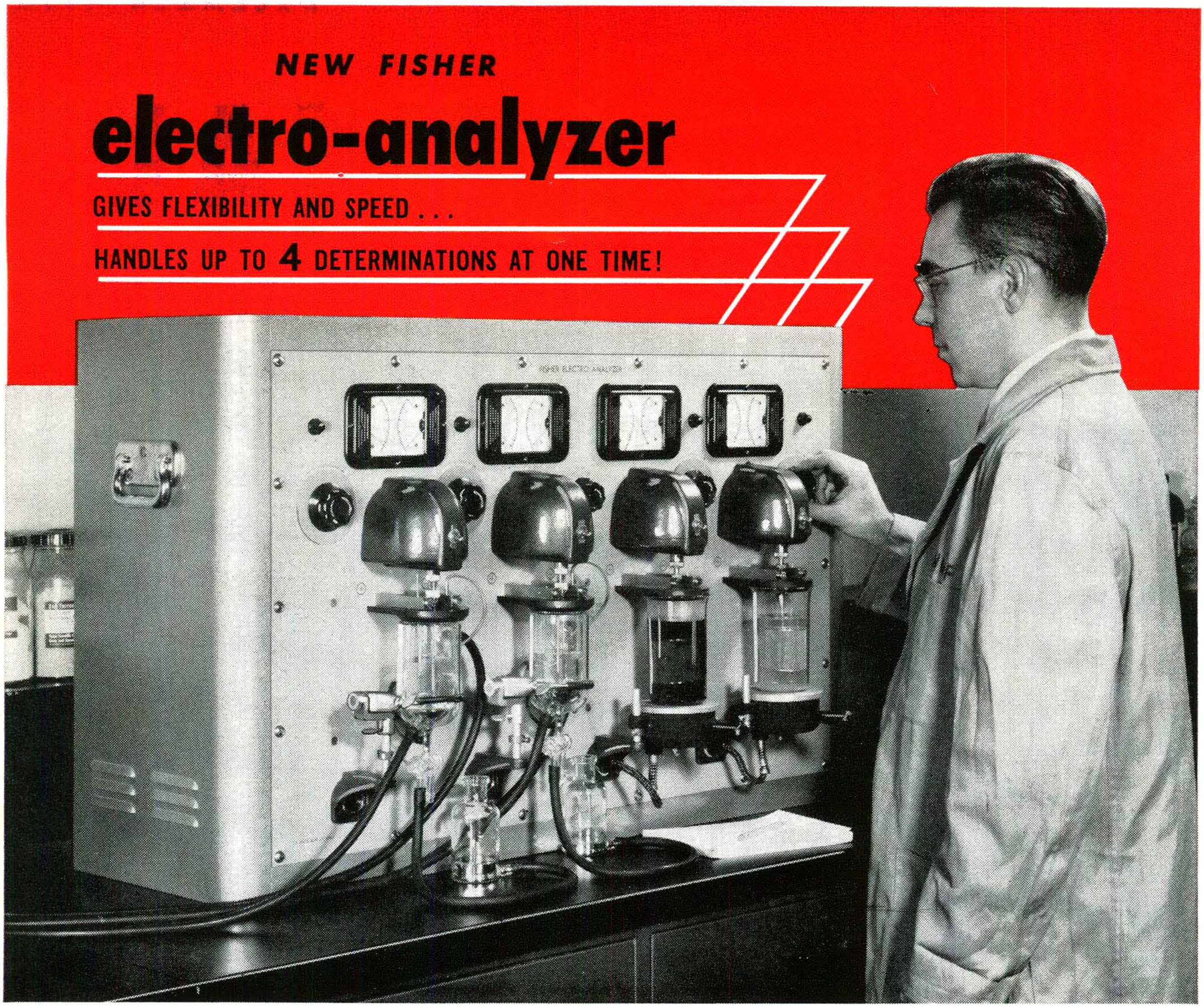
Modern analytical chemistry calls for the development of special instruments such as this photometer at the Hercules Powder Co.

NEW FISHER

electro-analyzer

GIVES FLEXIBILITY AND SPEED . . .

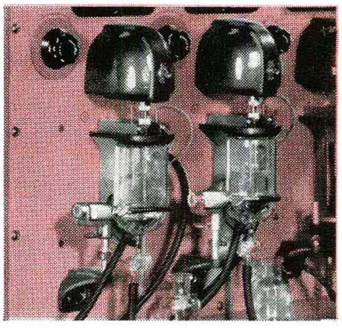
HANDLES UP TO 4 DETERMINATIONS AT ONE TIME!



Quantitative determination of metallic elements in solutions is provided at heretofore unobtainable speed, flexibility and convenience with the new Fisher Electro-Analyzer. Gold, silver, platinum, cadmium, zinc, lead, nickel, copper, tin, antimony and mercury can all be determined with this apparatus in a fraction of the time required to make the same determinations by the usual chemical methods . . . and it is particularly well suited for the analysis of brass, bronze, amalgams and similar alloys in solutions.

It handles up to four determinations at one time since each station is equipped with its own independent components, making possible, for the first time, a complete flexibility of schedule. Each unit has its own stirring motor, with two sizes of chucks that accommodate all types of stirrers and stirring-electrodes. The beaker supports are removable to permit the use of sample-heaters. It is an efficient source of power (10 amperes and 10 volts) for a variety of laboratory tasks such as electrolytic polishing, electroplating and battery charging.

The Electro-Analyzer is entirely housed in a single sturdy, compact case handsomely finished in light gray. The front panel is durable gray Formica, on which are mounted all electrical outlets, controls and stirring motors. These are all clearly labelled in permanent black letters. Available in Two-Spindle Model and Four-Spindle Model.



At two left hand spindles, mercury cathode cells remove interfering elements.

At two right hand spindles, heaters bring other samples to optimum temperatures.

IMMEDIATE DELIVERY FROM STOCK

Write today for complete details

Complete stocks of laboratory instruments, apparatus, reagent chemicals, furniture, and supplies at: 717 Forbes St., Pittsburgh 19, Pa.—635 Greenwich St., New York 14, N. Y.—2850 S. Jefferson Ave., St. Louis 18, Mo.—7722 Woodbury Dr., Silver Spring, Md.—904 St. James, Montreal, P.Q., Canada—245 Carlaw Ave., Toronto 8, P.O., Canada.

FISHER SCIENTIFIC

America's Largest Manufacturer-Distributor of Laboratory Appliances and Reagent Chemicals

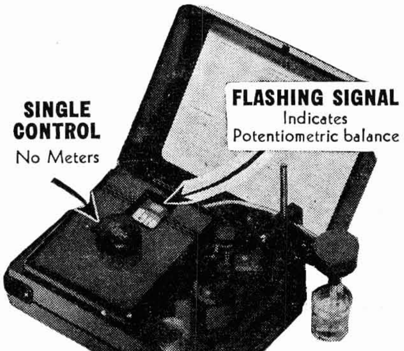
ANALYTICAL CHEMISTRY

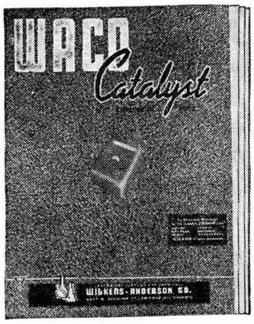
Volume 24, No. 12

Issued December 18, 1952

This Month's Cover. Modern analysis calls on many professions and skills in solving the ever more complex problems of science and industry. This is illustrated by our front cover photograph showing R. H. Osborn of the Physics Division, Experiment Station, Hercules Powder Co., with a specially developed general purpose photometer. Color is a very important property of many products, especially at Hercules. There analytical chemists faced with color problems found that there were no suitable instruments available for their solution. Working together, a team of chemists and physicists not only had to work out analytical methods but also had to design the photometer shown. It has been very useful in a variety of color problems and is used in grading many products.

(Continued on page 4A)





CONTENTS, continued

NOTES, *Continued*

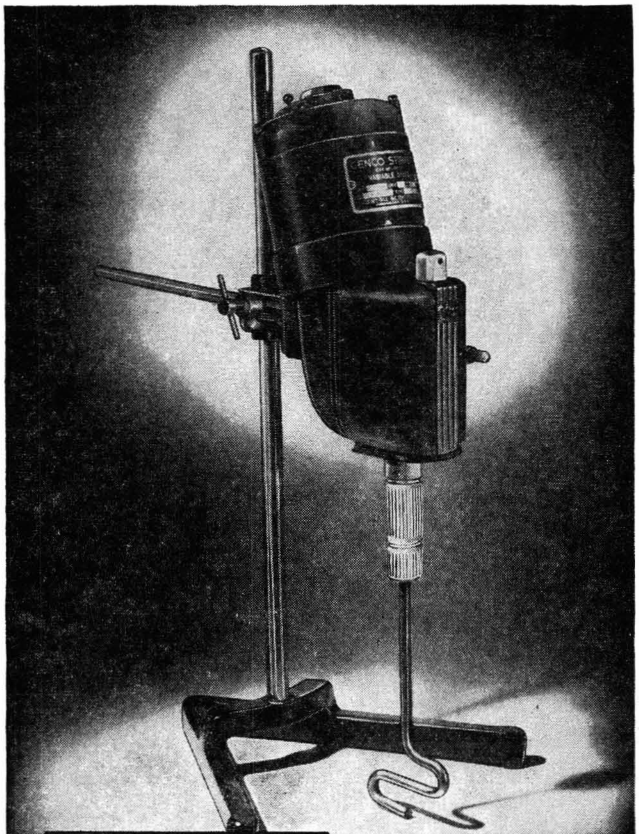
CRYSTALLOGRAPHIC DATA

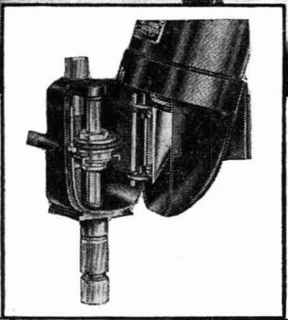
AIDS FOR THE ANALYST

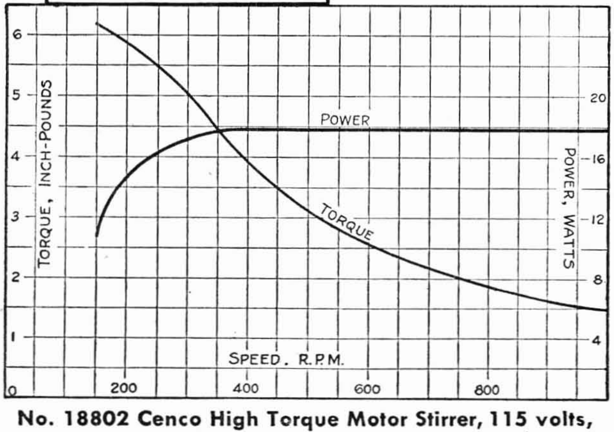
The American Chemical Society assumes no responsibility for the statements and opinions advanced by contributors to its publications. Views expressed in the editorials are those of the editors and do not necessarily represent the official position of the American Chemical Society. Published by the American Chemical Society, from 20th and Northampton Sts., Easton, Pa. Executive Offices, Editorial Headquarters, and Circulation Department, 1155 Sixteenth St., N.W., Washington 6, D. C. Advertising Office: 330 West 42nd St., New York 36, N. Y. Branch Editorial Offices: Chicago 1, Ill., 86 East Randolph St.; Houston 2, Tex., 623 West Bldg.; New York 17, N. Y., 60 East 42nd St.; San Francisco 2, Calif., 760 Market St.; London, Bush House, Aldwych. Entered as second-class matter at the Post Office at Easton, Pa., U.S.A., February 18, 1948, under the Act of March 3, 1879, as 12 times a year monthly on the 15th. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of Oct. 3, 1917, authorized July 13, 1918. Annual subscriptions: members $3.50, nonmembers $4.00. Postage to countries not in the Pan-American Union $1.20. Canadian postage $0.35. Combination rate for Analytical Chemistry and *Industrial and Engineering Chemistry:* members $7.00, nonmembers $8.00. Postage for the combination: countries not in the Pan-American Union $3.90, Canadian postage $1.10. Single copies: current issues, members $0.40, nonmembers $0.50; back numbers, prices on request, special rates to members. Claims for missing numbers will not be allowed if received more than 60 days from date of mailing plus time normally required for postal delivery of journal and claim. No claims allowed from subscribers in Central Europe, Asia, or the Pacific islands other than Hawaii, or because of failure to notify the Circulation Department of a change of address, or because copy is "missing from files."

CHANGE OF ADDRESS: Notify Circulation Department, American Chemical Society, 1155 Sixteenth St., N.W., Washington 6, D. C. Such notification should include both old and new addresses and postal zone number, if any. The American Chemical Society also publishes: *Chemical and Engineering News, Industrial and Engineering Chemistry, Chemical Abstracts, Journal of the American Chemical Society,* and the *Journal of Physical Chemistry.* Rates on request.


Baker
1 LB. (453.6g.)
Hydrofluoric Acid
'Baker Analyzed' REAGENT
MEETS A. C. S. SPECIFICATIONS
J.T. BAKER CHEMICAL CO., PHILLIPSBURG, N.J.

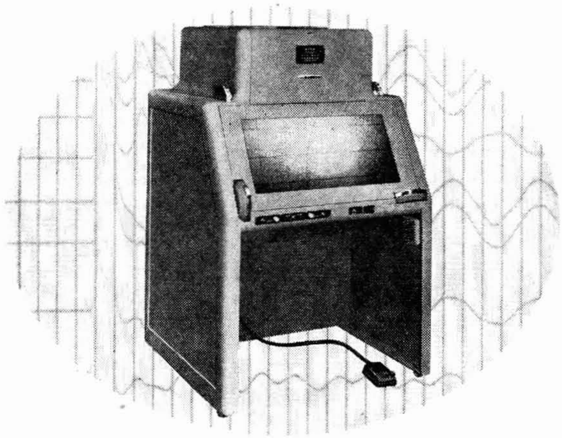





6
5
4
3
2
1
0
TORQUE, INCH-POUNDS
POWER
TORQUE
20
16
12
8
4
POWER, WATTS
SPEED, R.P.M.
200
400
600
800

Speed up your data analysis

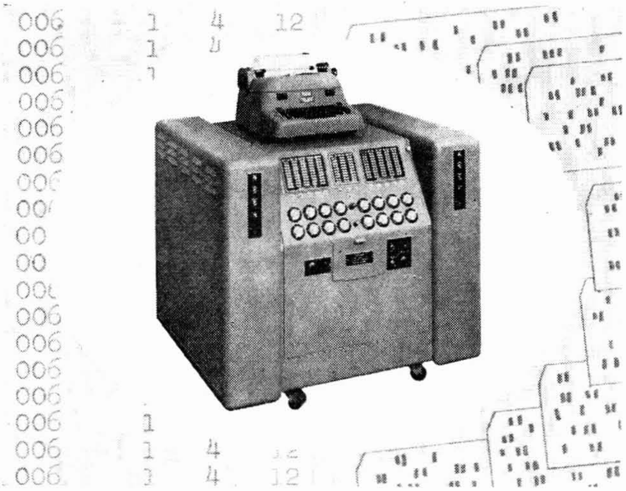
These Telecomputing Instruments measure, record, plot at automatic speeds:



Today you can reduce and analyze film and oscillograph data faster than ever before. Telecomputing Instruments, in conjunction with electronic computing equipment, have made this possible.

The following sequence of automatic data analysis is typical:

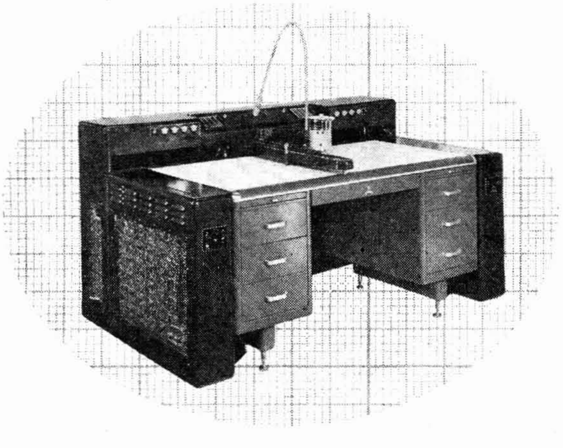
The Universal Telereader measures records ranging from 16 and 35 mm film to 12″ oscillograph records. Speed: up to 50 measurements per minute.



The Telecordex records the Telereader measurements in decimal form electronically on its own electric typewriter, transmits the data to an IBM Summary Punch for card punching.

The IBM card punch receives the data from the Telecordex, punches it and continues the cycle.

All necessary calculations, including linear and non-linear calibrations, are performed on IBM or other electronic computers.



The Teleplotter plots the data electronically from IBM cards or a manual keyboard. Speed: up to 70 points per minute.

Technical bulletins on the Universal Telereader, Telecordex and Teleplotter will be mailed you upon your request. Coupon below is for your convenience.

Mr. Preston W. Simms, Engineering, Dept. AC-12
Telecomputing Corporation, Burbank, California
Dear Sir: Please send me:

☐ Universal Telereader Technical Bulletin TC 101
☐ Telecordex Technical Bulletin TC 102
☐ Teleplotter Technical Bulletin TC 103

Name

Company

Street Address

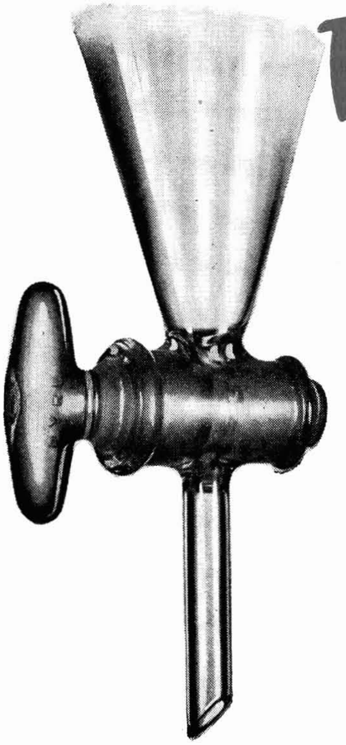
City and State

TELECOMPUTING
Corporation

Burbank, California

Electronic and mechanical engineers can build their future with Telecomputing. Write for information.


Mallinckrodt
ANALYTICAL REAGENT
7581
1 LB.
MALLINCKRODT CHEMICAL WORKS
SAINT LOUIS MONTREAL PHILADELPHIA NEW YORK



FRACTIONATION PROBLEMS?

here's help for you . . .

THIS tireless servant in your laboratory will automatically collect any number (up to 200) of rigidly controlled samples of predetermined fluid volumes. Each collected sample may comprise any number of drops from one to four hundred. As each separate sampling is completed, the carriage automatically advances to repeat identical collections consecutively until the whole fractionation has been made.

All you have to do is to set it up for the conditions of the chosen experiment, short or long, *and then leave it alone.* The machine will plod along without attention hour after hour, all day (and night) long. When the job's done, it will shut itself off.

The Technicon Fraction Collector has been thoroughly tested in actual laboratory installations, where it has proven invaluable to busy research staffs. We shall be happy to send you details.

Ref. Chromatography of Amino Acids on Starch Columns—
W. H. Stein and S. Moore, Jrnl. Biol. Chem. 176, 337,(1948)

saves time and labor

relieves laboratory staff of the fretful and time-consuming chore of fraction-cutting: releases workers for other duties.

triples work output

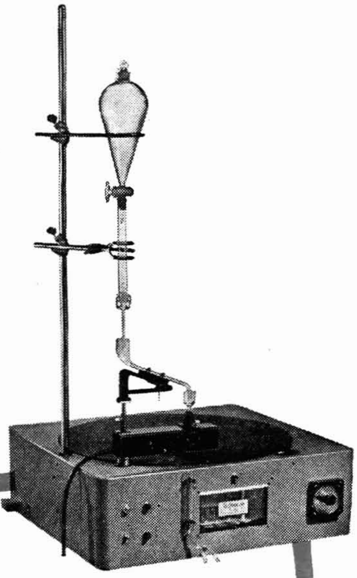
you can run it twenty-four hours a day, continuously, to triple fractionation output as compared with an 8 hour laboratory day.

gives greater resolution

by collecting a large number of small fractions, rather than a few gross ones, you'll get more data from a given fractionation, e.g. a chromatogram, or a fractional distillation.

assures accuracy

because the possibility of human error is automatically ruled out. Now fraction-collection becomes a straightforward mechanical procedure.



TECHNICON CHROMATOGRAPHY CORP.,
215 East 149th Street,
New York 51, N. Y.

Send me detailed information
on the Technicon Automatic Fraction Collector.

Name..

Address..State..................

City..

technicon

automatic
fraction
collector

MODEL PR-1

Improved Model
International Refrigerated Centrifuge

The New International Model PR-1 Portable Refrigerated Centrifuge embodies the many time-proven features found in previous models and, in addition, incorporates important engineering improvements. The result is superior performance in the laboratory without increase in cost.

A new type of temperature controller enables the user to pre-select the operating temperature within the Centrifuge chamber. Temperature is controlled within ±1° C. and indicated on a calibrated dial.

An autotransformer speed controller is provided with alternating current models, eliminating heating associated with the rheostat type of controller, and providing uniform, stepless speed control throughout the entire range.

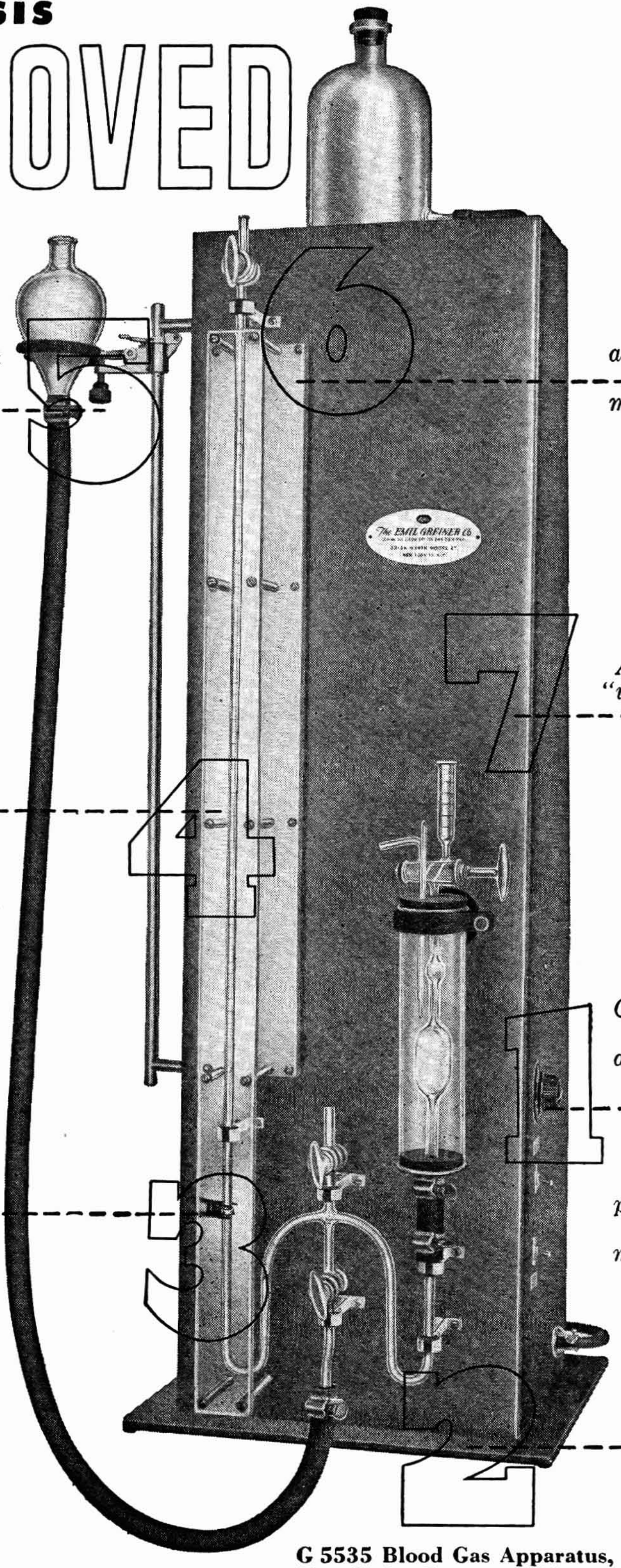
A new hinged panel provides easy access to all instruments and electric controls, thus facilitating maintenance.

A hermetically sealed unit is substituted for the open type compressor heretofore used. This eliminates troublesome vibration caused by the action of the compressor and minimizes the possibility of gas leakage.

Interchangeable accessories are available in wider variety, enhancing the usefulness of the Centrifuge.

Send today for new descriptive Bulletin RC-1952 containing complete details and current prices.

INTERNATIONAL EQUIPMENT COMPANY
1284 SOLDIERS FIELD ROAD, BOSTON 35, MASS.


5
6
7
4
1
3
2

the analyst's column

THE U. S. Army's Far East Criminal Investigation Laboratory (FECIL) in Tokyo was host on July 23 to the second meeting of Japanese narcotic analysts, which consisted of 18 representatives from the following institutions: Tokyo University, Yokohama Medical College, National Hygienic Laboratory of the Ministry of Welfare, Tokyo Metropolitan Hygienie Laboratory, National Rural Police Scientific Crime Detection Laboratory, Tokyo Metropolitan Police Department Laboratory, Yokohama City Police Laboratory, Sankyo Pharmaceutical Co., Ltd., and Takeda Pharmaceutical Co., Ltd.

In welcoming representatives of the other laboratories, Martin B. Williams, chief chemist, FECIL, pointed out the desirability of a permanent organization of narcotic analysts as a medium for the exchange of technical information and for the coordination of research being conducted in the various laboratories. Numai's paper on diacetylmorphine (heroin) summarized the many tests currently used for the identification and assay of heroin. Pfc. M. S. Dobro pointed out the potential advantages of chromatography over the usual chemical methods in the identification of minute quantities (as small as 10 micrograms) of narcotics and in the separation of mixtures of narcotics. Numai also reported a new test for cocaine and certain related compounds. Major G. R. Bird traced the development of the science of criminalistics from the time of Bertillon to the present-day usage of such instruments as the spectrograph, electron microscope, and x-ray diffractometer.

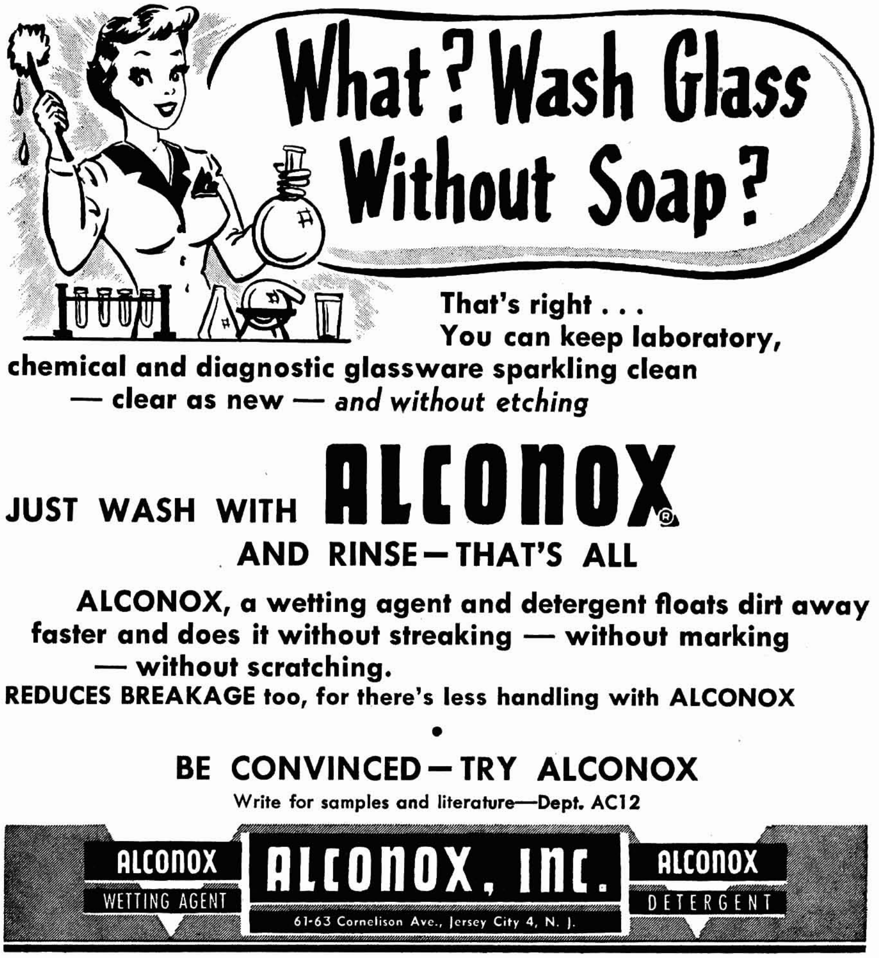
During the business meeting, organization of the Japan Narcotic Analysts' Association was discussed, and plans were formulated for the creation of this association at a meeting in September.

New Test for Identification of Cocaine

TETSUTARO NUMAI
Far East Criminal Investigation Laboratory, Tokyo, Japan

According to the author's investigation concerning the differentiation of cocaine from other local anesthetics as reported by

(*Continued on page 15 A*)



VACUUM VERSATILITY

for Research and Production

—RCA VACUUM UNIT

HERE IS A DEPENDABLE vacuum equipment—offering the flexibility you must have for research applications as well as the speed and convenience you need for production work.

MEETS VARIED REQUIREMENTS OF RESEARCH

Providing a ready means of performing any studies at reduced pressures or in special atmospheres, the RCA Vacuum Unit gives the research scientist a truly flexible tool to meet a variety of vacuum problems. It may also be used for "shadowing" specimens for light and electron microscopy.

APPLIES COATING WITH PRODUCTION SPEED

The RCA Vacuum Unit combines operating speed (only 7 minutes to 0.1 micron Hg) with electro-magnetic valving which eliminates heating and cooling delays of thermal pumps—permits immediate reopening, fast recycling. Completely interlocked for safety and equipped with 3 pairs of filament supports and an eighteen-inch bell-jar, the RCA Vacuum Unit is widely used for coating lenses, crystals, and plastics.

AVAILABLE FROM STOCK

RCA Vacuum Units are quantity produced for stock, eliminating the delays normally associated with custom-built equipment.

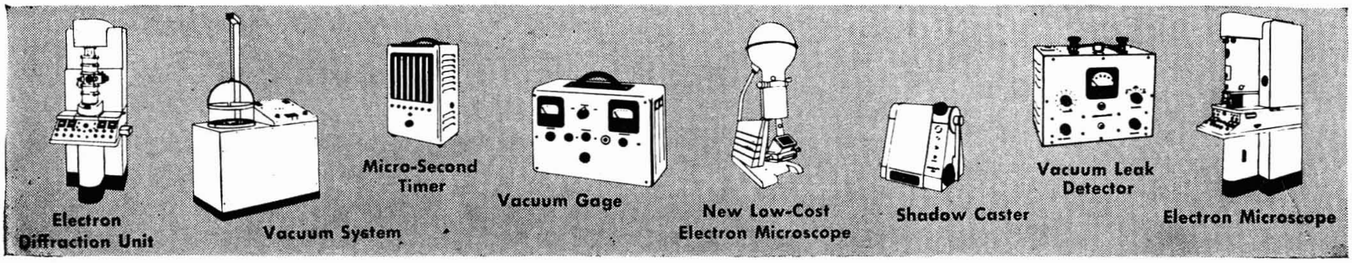
NATION-WIDE SERVICE

Complete maintenance contracts for RCA Vacuum Equipment—covering installation, regularly scheduled check-ups, emergency service, and parts replacement—are available through the RCA Service Company, a nation-wide organization of highly skilled electronic specialists.

For INFORMATION on RCA vacuum systems—or any member of the RCA Scientific Instrument family—write today to Scientific Instruments, Dept. 11L, Radio Corporation of America, Camden, N. J.

SCIENTIFIC INSTRUMENTS
RADIO CORPORATION of AMERICA
ENGINEERING PRODUCTS DEPARTMENT, CAMDEN, N.J.

In Canada: RCA VICTOR Company Limited, Montreal



Ekkert [*Magyar Gyógyszerésztud. Társaság Értesitöje*, **8**, 269 (1932), using Thienes and Haley's "Clinical Toxicology," 2nd ed., p. 306, 1949], a test by chloramine T reagent was applicable, but a secondary test using 10% "formalin" and sulfuric acid was not applicable, owing to the fact that the color of reaction was barely perceptible.

A new semimicro analytical test for the identification of cocaine and other like substances was devised by the author, in which Marquis' reagent (formaldehyde sulfuric acid) was warmed with the substance on a steam bath and the reaction product was examined under ultraviolet light. A striking orange-yellow colored fluorescence shown by cocaine or tropacocaine and very different fluorescences by procaine tetracaine and other local anesthetics and compounds of other groups are reported in this paper.

Procedure. Take 1 to 2 mg. of sample (solid) in a white porcelain evaporating dish and add in the cold 0.5 ml. of freshly prepared Marquis' reagent. Watch the color and then warm on the steam bath for 10 minutes (usually, less, but special cases sometimes require up to 30 minutes). Transfer the reaction product to a small clean test tube and examine the color of fluorescence under ultraviolet light.

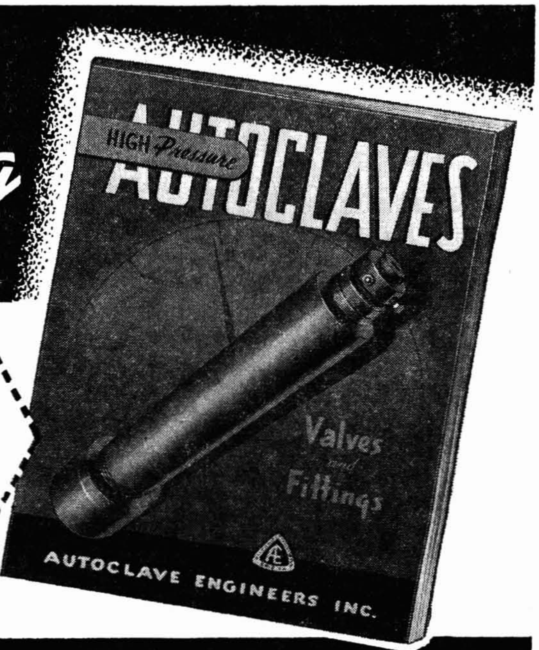
Experiment

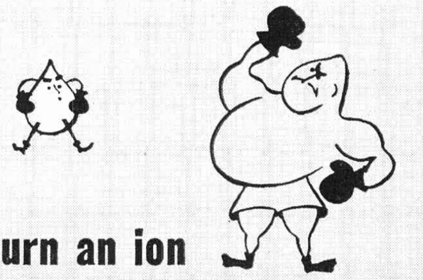
Substance	Fluorescence after Being Warmed
Marquis' reagent only	Gray
Cocaine hydrochloride	Orange yellow
Tropacocaine hydrochloride	Orange yellow
Benzoic acid	Orange yellow
Benzaldehyde	Orange yellow
p-Aminobenzoic acid	Whitish violet
Ethyl aminobenzoate	Whitish violet
Procaine hydrochloride (Novocaine hydrochloride)	Whitish violet
Tetracaine hydrochloride (Pantocaine hydrochloride or Pontocaine hydrochloride)	Whitish violet
Aminocaine hydrochloride (Tutocaine hydrochloride)	Greenish yellow
Percaine hydrochloride	Greenish yellow
Ephedrine sulfate	Deep pink
Atropine sulfate	Turbid red
Yohimbine hydrochloride	Green
Phthalic anhydride	Whitish violet

The orange-yellow fluorescence which is given by cocaine, tropacocaine, or benzoic acid in the new test is considered to be based upon the formation of benzoin. In an investigation of the mechanism of of the above reaction, experiments conducted in this laboratory showed that benzoic acid, benzaldehyde, and benzoin yielded an orange-yellow fluorescence similar to that obtained from cocaine or tropococain, while para-substituted derivatives of benzoic acid (such as *p*-aminobenzoic acid, procaine, and tetracaine) did not yield this characteristic orange-yellow fluorescence.

The sensitivity (lower limit of detection) of cocaine or tropacocaine hydrochloride by the new test lies near 0.3 to 0.5 mg.

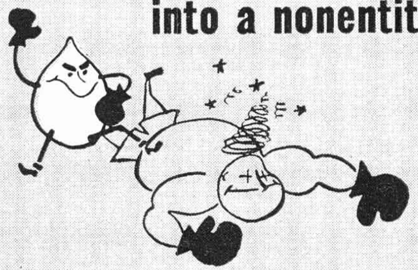
Associate Editor





how to turn an ion

into a nonentity . . .



. . . . Versene*

CHEMISTRY OF CHELATION

To turn "somebody" into "nobody" you remove him from his environment and prevent him from exerting further influence on it. Essentially, this is what you do when you use Versene* and the Chemistry of Chelation to make metallic ions lose their chemical identities. When metal ions form a complex compound with Versene, they become so strongly attached to the Versene that they lose their identity and can no longer interfere with or influence their environment in any way.

CHELATION IS THE KEY

In other words, Chelation and the Versenes* (powerful Chelating Agents) may well be the solution to your chemical problem — especially if it involves metallic ion contamination in any kind of liquid formulation.

GUARANTEED QUALITY

Made only under processes originated, developed and patented by F. C. Bersworth, the Versenes* are exceptionally stable at elevated temperatures and all pH's. Quality standards of manufacture are so high that uniform complexing power is guaranteed in both sample and carload quantities. Find out now how these most powerful Chelating Agents can help you. Write Dept. V for Technical Bulletin #2 and samples. Chemical counsel available.

*Trade Mark Registered.

VERSENE WATER TEST KIT. Tells total hardness in 2 minutes. Accurate to 1 grain per gal. Complete with instructions $5.00 postpaid.

"Chemistry's most precise chemicals"

KEY TO CHELATION

BERSWORTH CHEMICAL CO.
FRAMINGHAM, MASSACHUSETTS

WAREHOUSE STOCKS
Providence Agent: George Mann & Co., Inc., 251 Fox Point Boulevard, Providence, R. I.
W. Coast Agent: Griffin Chemical Co., San Francisco, Los Angeles
Midwest Agent: Kraft Chemical Co., Inc., 917 W. 18th Street, Chicago
Wasatch Chemical Co., Salt Lake City, Utah
Southwest Agents: Barada & Page, Inc., Dallas, Houston, Kansas City, etc.
Chas S. Tanner Co., 1815 Liberty Life Bldg., Charlotte, North Carolina
Siegel Chemical Co., Brooklyn, N. Y.

for more uniform heat control

BLUE 'M' LINE

Mechanical Convection
UTILITY OVENS

Full control of all heating factors . . . insures results and saves time in procedures involving baking, heating, drying or sterilizing.

Selective Wattage . . . the Power Selector Switch controls heat electrically to within 1/2°C.

Adjustable air speed . . . velocity is controlled from 50 to 250 feet per minute with about 50 air changes per minute.

Symetrical Heating . . . Horizontal Air Flow The special Modella heating element is distributed over the entire bottom of the oven, and air is introduced horizontally at various levels, to provide maximum uniformity of temperature distribution.

Stainless steel interiors . . . heavy gauge steel gray finished exterior, heavy duty ball bearing motor . . . Blue M Ovens are completely wired and solidly built throughout. Guaranteed for 5 years.

Made in two temperature ranges
35°C to 180°C and 35°C to 288°C
8 sizes in each range

For complete information see your local dealer or write for Bulletin

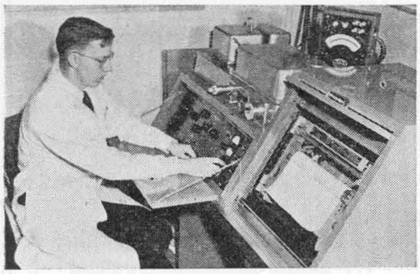
BLUE 'M' LINE

BLUE M ELECTRIC COMPANY
306 - 308 West 69th Street, Chicago, Illinois

THE PERKIN-ELMER INSTRUMENT DIGEST

A condensation of some articles in the Fall issue of THE PERKIN-ELMER *INSTRUMENT NEWS*, a publication of The Perkin-Elmer Corporation, manufacturers of scientific instruments—Infrared Spectrometers, Tiselius Electrophoresis Apparatus, Monochromators, Flame Photometers, Continuous Infrared Analysers, Amplifiers, Astronomical Equipment, Thermocouples, Lenses, Crystal Optics, Special Designs for the Government.
For further information, write The Perkin-Elmer Corp., Norwalk, Conn.
Southern Regional Office: Lee Circle Building, New Orleans, La.

Norwalk, Conn. December, 1952 Vol. 4, No. 1



Model 12C Spectrometer at Merck & Co.

Infrared Determination Of Deuterium in Water

The importance of deuterium in chemical, biochemical and process development tracing studies as well as in isotope dilution assay methods may now be regarded as well established. Any improvement in reliability and precision of method, whereby deuterium can be determined in water, naturally is of primary interest to investigators in these fields.

A method is described in detail in the Fall 1952 issue of INSTRUMENT NEWS that can be carried out directly on liquid water samples as small as 10 mg. It covers a concentration range of 0-5 percent.

- **Deuterium Shift**—The spectrophotometry is made possible by the magnitude of the shift in wavelength (of the stretching frequency) which accompanies the substitutes of a deuterium atom for one of the hydrogen atoms of water. This shift from 2.8 microns to 4.0 microns (for the fundamental) corresponds to a factor approximately equal to $\sqrt{2}$ and is in accord with fundamental theory for a doubling of the mass of the atom involved. The intensity of the O-D band can be measured spectrophotometrically and thus provides a determination of the deuterium content of the water sample.

About two years ago, an infrared spectrophotometric technique for the determination of deuterium in water based on the principle outlined above was described. Extensive experience with the method revealed that it suffered from a lack of adequate reproducibility (precision). This was found to be due to an unexpectedly large temperature coefficient of the absorbance of the O-D band at 3.98 microns. This was eliminated by the construction of a thermostatted absorption cell. It is now possible to obtain deuterium assay values in the region of 2 percent with a precision of ±.01 units, *i.e.*, ±1 percent.

From an article by N. R. Trenner and R. W. Walker in the current INSTRUMENT NEWS.

CONTINUOUS ANALYSER PROGRAM APPROACHING FINAL STAGES

The continuous analyser program, which has been under way for some time at Perkin-Elmer, is nearing final stages with the completion of two different types of instruments.

The TRI-NON® Analyser (basically a *Tri*ple Beam, *Non*-Dispersion, Selective Detector, Infrared Analyser) is a highly stable and sensitive instrument. It is well suited for the common class of problems where there is considerable interference between the component of interest and the other components in the stream.

The BICHROMATOR® Analyser (*i.e.*, two color or wavelength analyser) records the ratio of any two wavelengths of radiation. With the proper choice of prism materials and detectors it may be used in virtually any region of the spectrum for continuous analysis of either liquids or gases. It may be used wherever single point analysis is possible on a laboratory spectrometer.

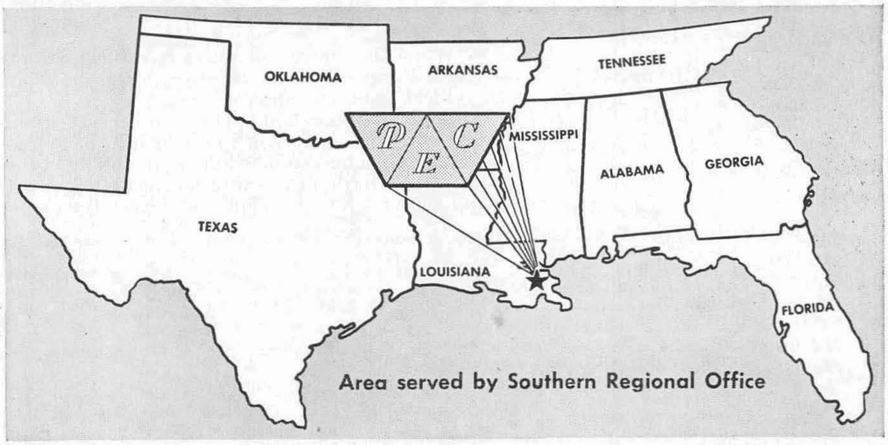
It is interesting to note that the electronic components in the two instruments from the preamplifier are identical, thus, simplifying field servicing in *multi-unit* installations.

The next issue of INSTRUMENT NEWS will carry further details on the analysers.

Our approach to process control instrumentation will be to provide a complete solution to the problem using whatever instrumentation is necessary rather than sell two different analysers and let the user decide between them.

We are now ready to start considering your control problems. Questionnaire data sheets are available from us to describe your applications completely. Your problems will be given our careful attention.

Perkin-Elmer Opens New Southern Regional Office



Area served by Southern Regional Office

A Perkin-Elmer sales and service office was opened in New Orleans' Lee Circle Building. Headed by Seymour G. Linsley, the new office will provide sales and service assistance to present and potential users of Perkin-Elmer equipment in the south and southwest.

An important feature of the office is the laboratory-showroom where operating models of all Perkin-Elmer instruments are installed and sample infrared spectra will be run. Repair and service facilities will also be available from the New Orleans office.

Visitors to New Orleans are invited to inspect our new office. Trained personnel will be happy to assist with your infrared and analytical problems. Working models of Perkin-Elmer instruments are on display.

Receive 8-page Instrument News

Write: The Perkin-Elmer Corporation,
800 Main Avenue, Norwalk, Connecticut

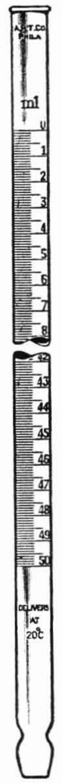
Featured in the Fall Issue are:

INFRARED DETERMINATION OF DEUTERIUM IN LIQUID WATER
Article by N. R. Trenner and R. W. Walker

"BIG GLASS"
Fabricating large optical pieces

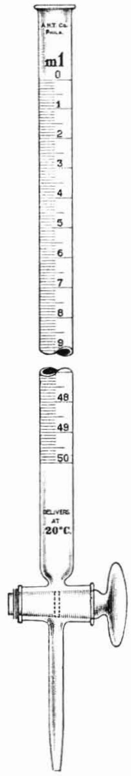
INFRARED DETERMINATION OF FIVE C_{10} AROMATICS
Monsanto Case History

(ADVERTISEMENT)



A.H.T. Co. PHILA.
ml
0
1
2
3
4
5
6
7
8
42
43
44
45
46
47
48
49
50
DELIVERS AT 20°C

2400.



A.H.T. Co. PHILA.
ml
0
1
2
3
4
5
6
7
8
9
48
49
50
DELIVERS AT 20°C

2404.

ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

New Advisory Board Members

WE ARE pleased to report that Wayne W. Hilty, James J. Lingane, and Vernon A. Stenger have accepted four-year appointments (1953–56) to the Analytical Advisory Board.

Mr. Hilty, assistant head of the analytical department, Eli Lilly and Co., literally started his professional training at birth, for much of his childhood was spent in his father's drugstore in Pandora, Ohio. He received his collegiate training in pharmacy at Ohio State University, graduating with a B.S. degree in 1936.

Mr. Hilty has been a very active and enthusiastic worker in the American Chemical Society vineyard. He has held all the major offices of the Indiana Section; has played a prominent role in Council affairs for several years, including membership on such important committees as the Local Section Councilor Representation Committee, Professional Relations and Status Committee, Constitution and Bylaws Committee. At present, Mr. Hilty is a member of the powerful Council Policy Committee.

Analytical Chemistry has published many papers by Hilty, most of them coauthored with Eli Lilly associates. He authored the first review article in Analytical Chemistry on "Pharmaceuticals and Natural Drugs" (1949). Since 1950, the review has been coauthored with M. M. March.

James J. Lingane received his B.S. and Ph.D. at the University of Minnesota and, of course, was closely associated with I. M. Kolthoff. He was an instructor in physical chemistry at Minnesota for two years (1938–39), and then joined the University of California, at Berkeley, as an instructor in analytical chemistry. He was called to Harvard in 1941. His present rank is that of professor of chemistry.

Dr. Lingane is the author of nearly 90 papers in the general field of analytical chemistry and electrochemistry, with special emphasis on polarographic analysis and analytical methods based on controlled potential electrolysis. He is coauthor with Kolthoff of "Polarography," the second edition of which has just been released by the publisher. Dr. Lingane is an associate editor of *JACS*.

The third new member to be appointed is Vernon A. Stenger, and again the Kolthoff influence is noted. Dr. Stenger did his undergraduate work at the University of Denver, graduating in 1929, the year in which the Analytical Edition of Industrial and Engineering Chemistry was established.

After a year with Eastman Kodak he became a teaching assistant at Minnesota. He studied for his Ph.D. under Kolthoff, receiving his doctorate in 1933. The following two years were spent at the Northwest Institute of Research at Minnesota.

Early in 1935, Dr. Stenger joined the Dow Chemical Co. staff at Midland, Mich., as an analytical research chemist. His special interests include volumetric and trace analysis of rare elements and bromine compounds, and the analysis of magnesium alloys.

The retiring members of the board are: H. A. Laitinen, University of Illinois, C. J. Rodden, U. S. Atomic Energy Commission, and J. W. Stillman of E. I. du Pont de Nemours & Co., Inc., Wilmington, Del. The editorial staff extends to these men heartfelt thanks for the many and varied services performed for the journal and the profession of analytical chemistry.

The year 1952 marks the 10th anniversary of an active advisory board. It is most appropriate at this time to remind our readers that the men who have served have contributed substantially to the improvement and growth of the journal during the past decade.

The anniversary was celebrated at a special meeting held in the Washington headquarters on November 5, at which time the members were given the opportunity of seeing at firsthand the editorial operations of three of the Society's publications.

The editors extend an invitation to readers visiting Washington a cordial invitation to visit with us and to see, as did the members of the advisory board, how Analytical Chemistry becomes a reality 12 times a year.

Analytical Methods for Industrial Wastes

Papers presented before the Division of Water, Sewage, and Sanitation Chemistry, Symposium on Analytical Methods for Industrial Wastes, at the 121st Meeting of the AMERICAN CHEMICAL SOCIETY, Milwaukee, Wis.

Systematic Analysis of Organic Industrial Wastes

HARRY BRAUS, F. M. MIDDLETON, AND C. C. RUCHHOFT
Environmental Health Center, Public Health Service, Cincinnati, Ohio

QUALITATIVE and quantitative characterization of the organic constituents of industrial wastes is important in the solution of problems of stream pollution abatement. Specifically, complete or even partial resolution of organic wastes would aid in: determination of actual loads of pollution, studies of methods of treatment, isolation and identification of materials toxic to animal life, studies of persistence of identified waste components in waters, studies of recoverable waste components, studies of causes of tastes and odors in drinking water, and studies of the applicability of the oxygen consumed and biochemical oxygen demand tests to wastes and waste components. If practical and economical programs for abatement of organic industrial waste pollution are to be developed, knowledge of the composition of the individual wastes concerned is essential.

Braus *et al.* (*1*) described a method for the recovery and identification of minute quantities of organic chemicals in drinking waters and drinking water sources. This work suggested that existing analytical procedures could be applied directly to the examination of organic industrial wastes.

Studies were carried out on five wastes of diverse origin and various degrees of complexity: the gravity oil separator effluent from a petroleum refinery, alkaline butadiene wash water from a

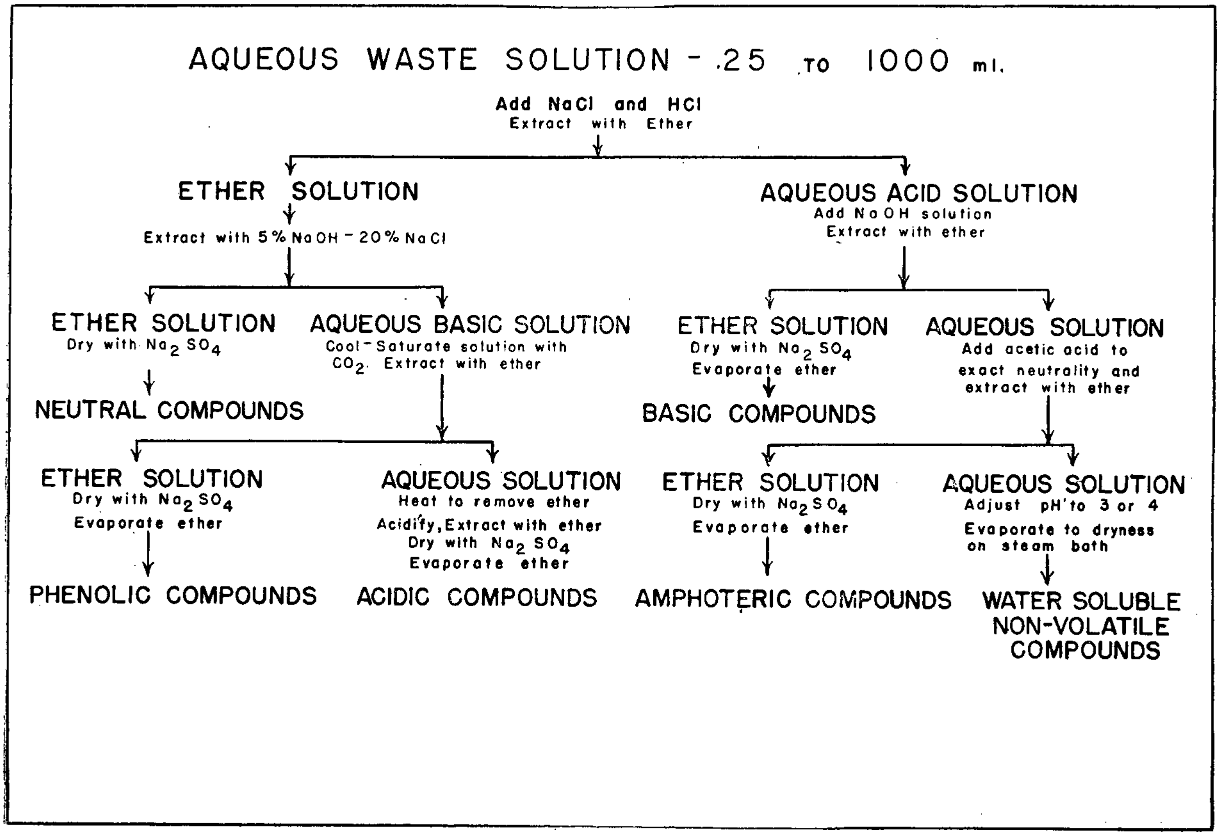


Figure 1. Schematic Separation of Organic Compounds

synthetic rubber plant, the effluent from a naval stores hydrogenation plant, the ammonia-still waste liquor from a coke plant, and the effluent from the manufacture of a synthetic explosive stabilizer. The analytical results suggest a semisystematic procedure for the direct examination of organic industrial waste concentrates and waste effluents. The general scheme of analysis, modified according to the method of Shriner and Fuson (*2*), is shown diagrammatically in Figure 1.

GENERAL PROCEDURE

The waste to be analyzed may be a dilute suspension and/or solution of organic materials in water, which is typical of most waste effluents, or a concentrate of organic material obtained by the use of the active carbon filter.

From 25 to 1000 ml. of waste, depending on the quantity of organic material present, is taken for analysis. The exact quantity of waste chosen will depend on the recovery of sufficient material for subsequent examination and may entail a preliminary run. The aqueous waste sample is saturated with sodium chloride and made acid with dilute hydrochloric acid. The water phase should contain approximately 5% acid. The solution is then extracted with diethyl ether. The quantity of ether used per extraction and the number of extractions depend upon the quantity of waste taken for analysis. The quantity of ether is kept small and a number of extractions are made to facilitate the recovery of the material in subsequent operations.

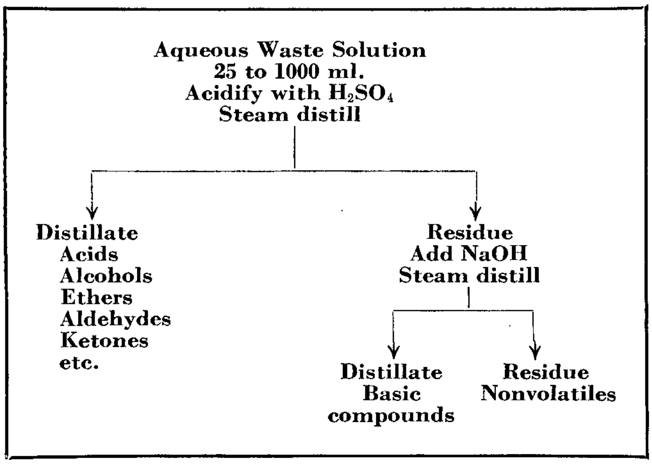


Figure 2. Schematic Separation of Volatile Organic Materials

The ether layers are combined and washed with a small quantity of 5% hydrochloric acid, which is added to the original aqueous solution. If, at this point, the ether volume is large, it is carefully distilled to a volume of 20 to 50 ml. The ether solution now contains the neutral, phenolic, acidic, and some amphoteric compounds. The combined ether layers from the hydrochloric acid extraction are now extracted with three 30-ml. portions of 5% sodium hydroxide solution. The ether layer is separated and dried over anhydrous sodium sulfate and the ether is distilled. The residue remaining is composed of neutral compounds. The sodium hydroxide solution is cooled, saturated with carbon dioxide, and then extracted with several portions of ether. The ether layers are combined and dried over anhydrous sodium sulfate and the ether is distilled. The residue is composed of phenolic compounds. The aqueous solution remaining after carbonation is gently heated to remove dissolved ether, cooled, acidified, and extracted with several portions of ether. After drying, the ether is distilled. The residue is composed of organic acidic compounds.

The original aqueous solution remaining after the first ether extraction is made alkaline with sodium hydroxide and extracted with several 50-ml. portions of ether. The combined ether layers are dried over sodium sulfate and distilled. The residue is composed of organic basic compounds. The remaining aqueous solution may now contain certain amphoteric compounds and nonvolatile water-soluble components such as polybasic acids, polyhydroxy compounds, and sulfonic acids. The solution is made exactly neutral with acetic acid and extracted with several portions of ether. The ether is dried as usual (over anhydrous sodium sulfate) and distilled. The amphoteric compounds are found in the residue.

There has long been a need for a scheme of systematic analysis for the identification and estimation of the constituents in organic industrial wastes. These wastes may render water unfit for drinking or recreational use, create toxic conditions, cause taste and odor in drinking water supplies, and cause depletion of oxygen from the receiving water supply. A systematic method for the analysis of organic industrial wastes based on the separation of classes of organic compounds by virtue of differences in solubility has been applied to five diverse industrial wastes. Separations and analysis are accomplished by the use of liquid-liquid extraction, distillation, infrared examination, and other optical methods. The scheme of analysis presented will be helpful in determining the organic constituents of waste effluents. This knowledge is essential for practical and economical solutions of stream pollution abatement.

The aqueous solution remaining after all extractions is adjusted to a pH of 3.0 with dilute sulfuric acid and evaporated to dryness on the steam bath. The residue is composed of inorganic salts and nonvolatile water-soluble compounds. The material is ground to a fine powder and mixed to produce homogeneity. A sample of this material may be subjected to an elemental analysis for carbon or the char test may be applied. If considerable organic material is present, knowledge of the origin of the waste may help to ascertain the identity of these materials. Generally, polybasic acids may be extracted with hot alcohol. Some polyhydroxy compounds may be extracted with pyridine. A recent paper by Tink and Neish (*4*) indicates the feasibility of extracting polyhydroxy compounds from dilute aqueous solutions by cyclic acetal formation, employing *n*-butyraldehyde in the presence of dilute acid.

When volatile solvents miscible with both ether and water are present in large quantities in the original waste, a preliminary scheme of examination may be necessary. Figure 2 shows this scheme diagrammatically.

From 25 to 1000 ml. of the waste is acidified with sulfuric acid and steam distilled. All steam-volatile, neutral, and acidic compounds are collected in the distillate. An aliquot of the distillate may be titrated with dilute sodium hydroxide to the phenolphthalein end point for the determination of organic acidity. The remainder is salted out with potassium carbonate and extracted with ether. The residue is made alkaline with sodium hydroxide and again steam distilled. Volatile basic compounds are collected in the distillate. These may be salted out with potassium carbonate and extracted with ether. The organic basicity is determined by appropriate titration, depending upon the types of amines present. The residue containing the nonvolatile materials is taken care of in the general procedure as outlined in Figure 1.

After group separation, according to solubility, has been accomplished, various other organic techniques may be employed. Infrared analysis may be applied to individual groups or fractions derived from these groups. Fractional distillation with and without reduced pressure may be used. Steam distillation and extraction with or without chemical change are often useful techniques. Examples of the application of these methods to five industrial organic wastes are shown below.

APPLICATIONS OF ANALYTICAL TECHNIQUES

Gravity Oil Separator Effluent from Petroleum Refinery. The sample for analysis was obtained by the use of the active carbon filter technique (*1*). A total of 7489 gallons of oil separator effluent was passed through the carbon filter. The total organic extract weighed 369 grams. Elemental analysis of this mixture gave the following results: carbon, 84.9; hydrogen, 11.2; nitrogen, 0.13; sulfur, 1.2; oxygen by difference, 2.57; ash, 0%.

The high percentage of carbon is indicative of hydrocarbon material. The relatively high percentage of hydrogen indicates aliphatic and/or alkylated aromatic hydrocarbons. By the use of the bromine and permanganate tests on the neutral fraction and distillates thereof, lesser amounts of unsaturated hydrocarbons were also shown to be present. Organic sulfur which is present in this waste might serve as a tracer element in determining pollution of adjacent water supplies. Concentration of organic sulfur compounds at various points along a body of water by the use of active carbon filters has been studied in relation to this waste.

The extract from the refinery waste effluent was separated into acidic, basic, phenolic, and neutral groups in accordance with the scheme outlined. The quantitative recoveries of these materials are: neutral compounds, 84.5; phenolic compounds, 3.73; acidic compounds, 0.15; basic compounds, 0.44; materials unrecovered, 11.18%. It will be noted that the neutral group of compounds was present in the greatest quantity. A substantial amount of phenolics was present in this waste, though small in comparison to the neutral group. The materials unrecovered represent amphoteric compounds, ether-insoluble material, and materials lost by manipulation.

Table I. Properties of Fractional Distillates of Oil Separator Effluent Extract

Fraction No.	Refractive Index, 25° C.	Boiling Point Range, 10 Mm. Hg, ° C.	Odor
1	1.4738	....	Mercaptan
2	....	38	Mercaptan
3	1.4738	40	Hydrocarbon
4	1.4782	40.5–49.5	Hydrocarbon
5	1.4870	50	Naphthalenic
6	1.4938	50.5–51	Naphthalenic
7	1.4953	51.5–60	Hydrocarbon
8	1.4960	60.5–66	Hydrocarbon
9	1.4970	66.5–70	Hydrocarbon
10	1.4911	70.5–76	Acrid

Table II. Characteristics of Organic Layer of Naval Stores Waste

Fraction No.	Boiling Point Range, 30 Mm. Hg, ° C.	Refractive Index, 25° C.	% by Weight	Compound Type
1	40–69	1.4408	6.43	Hydrocarbon
2	70–74	1.4442	36.40	Hydrocarbon
3	102–108	1.4710	2.1	Alcoholic carbonyl
4	109–120	.....	5.78	Alcoholic carbonyl
5	124–130	1.4781	0.78	Alcoholic carbonyl
6	135–145	1.4811	1.44	Alcoholic carbonyl
7	150–170	1.4812	1.10	Alcoholic carbonyl
8	170–210	1.5081	19.80	Resin oil (acid)
9	Residue		26.20	Resin (acid)

A sample of the oil separator effluent extract was distilled under reduced pressure in a Penn-State semimicro fractionating column. A strong odor of sulfureted gases was noted during the distillation. All the fractions recovered had extremely obnoxious and nauseating odors. The mixture consisted of compounds of very similar boiling point range which were difficult to separate. Table I shows the boiling point range and some of the physical properties of the fractions distilled.

The refinery extract and certain of the group constituents of the extract were examined for infrared absorption characteristics on a Baird infrared double beam recording spectrophotometer. Figures 3, 4, and 5, respectively, show the spectrograms of the total refinery effluent extract, the neutral group, and distilled fraction 2 as shown in Table I. Figures 3 and 4 show that the gross material is composed largely of aliphatic and alkylated aromatic hydrocarbons. The major aromatic constituents of fraction 2 as shown in Figure 5 are *o*-xylene, *m*-xylene, and *p*-xylene (cf. API Catalog Nos. 310, 311, and 312). Infrared studies of the various fractions shown in Table I have been made; they appear to be largely higher boiling hydrocarbons.

Alkaline Butadiene Wash Water from a Synthetic Rubber Plant. This waste arises from the alkaline washing of butadiene prior to its processing. Butadiene as delivered to the plant contains an inhibitor largely to prevent the formation of peroxides and to inhibit internal polymerization while it is in storage. The inhibitor, which is usually phenolic in nature, is removed by washing with a 10% solution of sodium hydroxide. The spent caustic is pumped to open tanks where residual butadiene flashes off. The waste is then discharged to the plant sewer at a maximum controlled flow of 2 gallons per minute.

A 1-gallon sample of this waste was collected and a 1-liter portion was analyzed. Group separation by the scheme shown in Figure 1 resulted in material recovered as follows: neutral compounds, 176; phenolic compounds, 586; acidic compounds, 10,400; basic compounds, 20 p.p.m.

The organic acids, which are present in the largest amount, probably arise as an impurity formed in the butadiene. Infrared analysis shows these acids to be largely aliphatic in character. Aliphatic acids are relatively innocuous in so far as taste and odor are concerned but contribute a high biochemical oxygen demand. They are generally easily attacked biologically and should cause little if any difficulty when discharged into a stream with adequate dilution. Little is known of the toxicity of these acids to the flora and fauna of streams. This method of group separation makes available material for studies of toxicity to fish and other aquatic life.

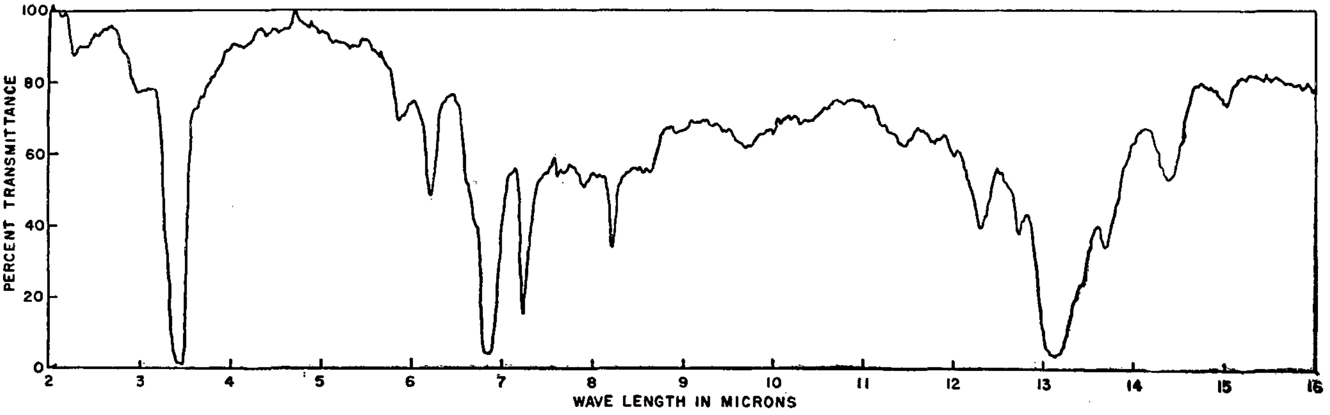


Figure 3. Infrared Spectrogram of Extract from Oil Separator Effluent

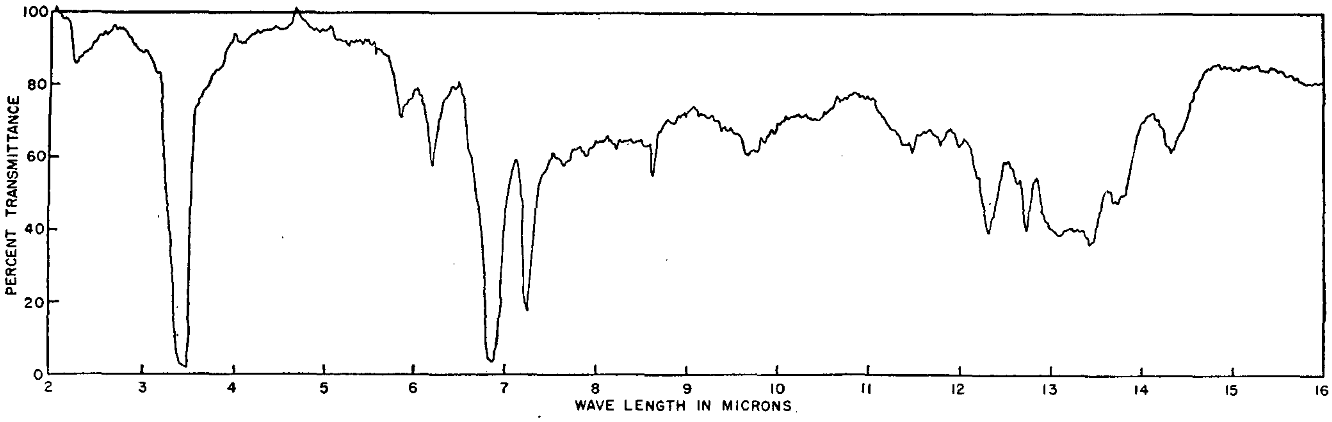


Figure 4. Infrared Spectrogram of Neutral Group from Oil Separator Effluent

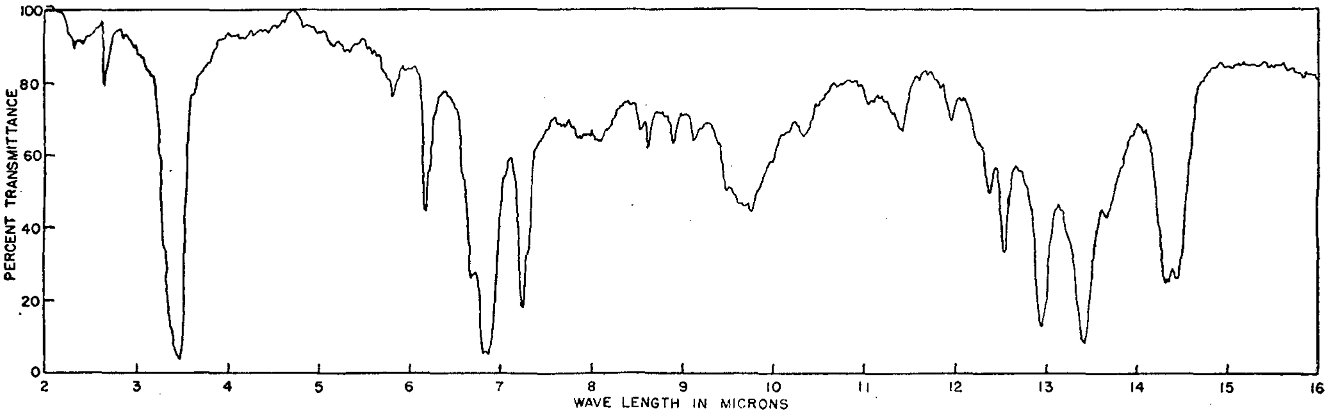


Figure 5. Infrared Spectrogram of Distilled Fraction No. 2 from Oil Separator Effluent

The phenolic fraction was difficult to work with because of its tarry consistency and a suitable sample for infrared analysis could not be obtained. However, with ferric chloride it gave the blue-green color typical of catechol-type compounds. Phenol determinations using the Gibbs and aminoantipyrine methods on the waste gave negative responses, as did a sample of pure *tert*-butylcatechol (T.B.C.). Consequently, it is assumed that *tert*-butylcatechol is the major if not the only phenolic represented in the 586 p.p.m. shown in this group.

Table III. Concentrations of Materials Recovered from Ammonia Still Waste with and without Salt

Group	Concentration Found, P.P.M. With salt	Concentration Found, P.P.M. Without salt
Phenolic	140	132
Acidic	38	29
Basic	58	25
Neutral	62	55

A series of threshold taste and odor studies was run on pure *tert*-butylcatechol and the phenolic fraction recovered from the waste. Neither gave an appreciable taste or odor in the concentrations that might be encountered in waters used for drinking. It is concluded that *tert*-butylcatechol and its chlorinated derivatives which were also tested would not contribute appreciably to taste and odor problems in water.

Sollman (*3*) found that *tert*-butylcatechol was highly toxic to goldfish. This may indicate that high concentrations of *tert*-butylcatechol discharged into a stream might be cause for concern. That *tert*-butylcatechol gave no response to the biochemical oxygen demand test may be due to the toxicity of this compound on the microfauna associated with this test. The possibility also exists that adaptive organisms for *tert*-butylcatechol were not present during the time of this test.

Neutral and amine compounds were present in small quantities in this waste. These should present little or no problem if discharged in small amounts.

Effluent from a Naval Stores Hydrogenation Plant. A waste obtained for analysis from a naval stores hydrogenation plant consisted of an organic layer floating on top of a water layer. The organic layer weighed 1200 grams, and the water layer 173 grams. The waste (organic layer) had an oxygen consumed value of 1,400,000 p.p.m. and a 5-day B.O.D. of 730,000 p.p.m. Group separation and subsequent examination indicated the waste to be composed largely of neutral compounds, abietic acid, and hydroabietic acids. Phenols and amines were present in negligible quantities.

A portion of the organic layer was steam distilled. Terpene and hydroterpene solvents which were steam distillable accounted for 49% of the total weight. The nondistillable residue composed of resin and resin oils amounted to 51% of the organic layer. Another portion of the organic layer was carefully distilled under reduced pressure and separated into nine fairly distinct fractions. Table II shows the results of this distillation, the refractive index of the fractions, and the type of compounds separated.

Infrared examination indicated fractions 1 and 2 were largely hydrocarbons, presumably terpenes, and constituted 42.8% of the mixture. The spectrograms of fractions 3 to 7 indicated that the material was terpene alcohols and/or carbonyl compounds, which comprise only 11.2% of the waste. More exact identification of these fractions can be made if warranted. The remaining

46.0% of the mixture was composed of resin oils, resins, and probably hydrogenation products thereof. This study indicates that recoverable materials are present in this waste. Treatment for recovery is indicated where direct discharge of the untreated waste is prohibited.

Ammonia-Still Waste Liquor from a Coke Plant. The waste liquor from a coke-oven ammonia still was subjected to group analysis. Table III shows the results obtained on two 500-ml. portions of the sample, one of which was saturated with salt. The addition of salt increases the efficiency of recovery, especially in the amine fractions. The distribution ratio of nitrogen-base compounds, which are present in this waste, is favorably altered by the presence of salt; hence the increased recovery.

Phenols represent the largest single organic component in this waste. As the phenol determination has long been recognized as an important index of this waste pollutant, the Gibbs and aminoantipyrine determinations for phenol were made for comparative purposes. A phenol concentration of 82 and 139 p.p.m. was found by the Gibbs and aminoantipyrine methods, respectively, as compared to 140 p.p.m. by the method of liquid-liquid solvent extraction described above. The comparative results indicate good agreement between the aminoantipyrine method and the organic group separation method, whereas the lower result by the Gibbs method may represent only simple phenols.

Infrared examination of the neutral group, which is highly odorous, indicates the probable presence of organic cyanide compounds. Very little is known concerning the presence of such compounds in this waste. Hydrocarbon material is also indicated in this group. In general, this group is of complex composition and work is under way in this laboratory to determine the constituents of the waste, using countercurrent extraction and distribution and chromatographic adsorption techniques.

Effluent from Manufacture of a Synthetic Explosive Stabilizer. A 5-gallon sample of waste from the manufacture of Centralite was submitted to the laboratory for analysis. Centralite, an explosive stabilizer, is *N,N'*-diethylcarbanilide, prepared by reaction of a mixture of monoethylaniline and diethylaniline with phosgene.

The sample of waste was blue-green in color and had an amine odor. The density of the solution was 0.9950 at 20° C. and the pH of the solution was 9.7. One liter of waste was extracted with ether according to the scheme presented above, and separated into four main groups. The quantities of the materials obtained are: neutral compounds, 49.8; phenolic compounds, 14.6; acidic compounds, 10.6; basic compounds, 427 p.p.m.

The basic compounds were shown to be present in the largest amount and were also the most obnoxious from the standpoint of odor, in both quality and intensity. Direct infrared examination of the mixture of basic components indicates the presence of primary and/or secondary amines, together with tertiary amines. These amines appear to be aromatic in character.

Chemical examination of this basic fraction substantiates the presence of all three types of amines. The mixture of amines heated with solid potassium hydroxide in chloroform yielded carbylamine, which is a positive test for a primary amine. Benzenesulfonyl chloride in alkaline solution was used to indicate the presence and effect separation of the amines. These tests indicated that the mixture consisted of small amounts of primary amine, a large amount of secondary amine, and still larger quantities of tertiary amine. By comparison of the infrared spectra of the known amines, the mixture was shown to be composed of aniline, monoethylaniline, and diethylaniline.

Examination of the neutral fraction, an oil which crystallized spontaneously, by infrared absorption indicated Centralite. The displacement of the carbonyl group above 6 microns is typical of a substituted urea. Comparison with the spectra of pure Centralite confirms the conclusion that the neutral group is largely Centralite. The neutral group possesses an obnoxious odor and does contribute to the odor of the waste. It is interesting to note that carbanilide has no odor, while if the two hydrogens are replaced by ethyl groups an odorous product results. This may be explained by the fact that hydrogen bonding occurs in the carbanilide molecule and is impossible in the completely substituted urea. The acidic and phenolic compounds are present in small quantities and have small odor intensities. It is believed these compounds contribute little or no odor to the composite waste.

When discharged untreated into sewers, this waste gives rise to an obnoxious odor which seeps through the sewer manholes and outlets to contaminate the atmosphere in the area. If the waste is acidified the odor is greatly reduced, as the amines are converted to nonvolatile salts. Acid treatment, however, is not recommended because of damage to sewers. As an alternative method the waste was treated with varying amounts of activated carbon to determine whether the odor could be reduced in the normal alkaline wash. It was found that approximately 3 grams of carbon per liter reduced the threshold odor of the waste from 2500 to 16.

SUMMARY

An approach to a systematic method for the analysis of organic industrial wastes and waste effluents is based on the separation of groups of organic compounds by virtue of differences in solubility. Liquid-liquid extraction is employed. Saturation of the aqueous phase with sodium chloride increases the efficiency of recovery. Further separation and identification are achieved by the use of distillation, infrared analysis, and other organic analytical techniques.

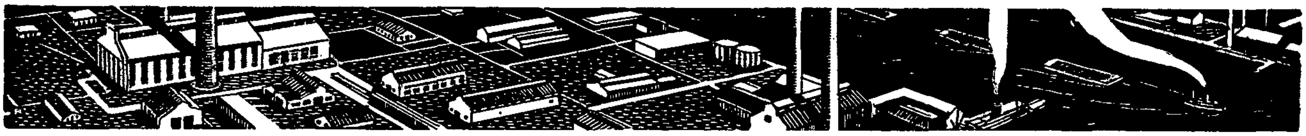
These methods have been applied to five organic wastes representative of the petroleum industry, synthetic rubber industry, naval stores, coke and steel industry, and a synthetic chemicals plant.

With the procedure described the neutral, phenolic, acidic, and basic components were separated and estimated on all of these wastes. This separation and study of the organic components of a waste are important in determining possible treatment and pollution abatement procedures and for solving taste and odor problems in water purification.

LITERATURE CITED

(1) Braus, Harry, Middleton, F. M., and Walton, Graham, Anal. Chem., 23, 1160 (1951).
(2) Shriner, R. L., and Fuson, R. C., "Systematic Identification of Organic Compounds," 2nd ed., p. 252, New York, John Wiley & Sons, 1940.
(3) Sollman, T., *J. Gen. Physiol.*, 32, 671 (1948–49).
(4) Tink, R. R., and Neish, A. C., *Can. J. Technol.*, **29**, 243 (1951).

Received for review May 28, 1952. Accepted June 21, 1952.



Determination of Pyridine and Pyridine-Base Compounds

In River Water and Industrial Wastes

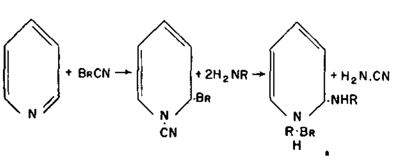
R. C. KRONER, M. B. ETTINGER, AND W. ALLAN MOORE
Environmental Health Center, Public Health Service, Cincinnati, Ohio

Trace quantities of pyridine and structurally related pyridine bases may be present in surface waters as a result of discharges from coking operations and petroleum processing. As an aid to the study of the persistence and taste- and odor-producing properties of these compounds, a sensitive, quantitative method of measurement was required. The method requires an initial distillation to remove turbidity and possible interfering compounds and may also be used for concentrating the sample. After dilution to the proper concentration, the sample is buffered with sodium acetate. Benzidine hydrochloride and cyanogen bromide are added and the reaction is carried out in a layer of butyl alcohol. The color reaction is complete in about 90 minutes. Factors affecting the reaction, such as time, temperature, concentration of reagent, and pH, are discussed. The reaction is sensitive to 0.005 p.p.m. of pyridine and can be used in the range from 0.005 to 1.0 p.p.m. Other pyridine derivatives that react quantitatively under the same conditions are 1-, 2-, and 3-picoline, 2,4- and 2,6-lutidine, and nicotinic acid.

PYRIDINE and related pyridine bases are known to be present in the wastes from by-product coking operations and some oil refining processes. This study of the determination of pyridine and some of its derivatives was designed to provide means for the determination of the small amounts of such materials in certain wastes or for the detection of trace amounts of such materials in surface waters. For the intended use, a reaction capable of detecting as little as 0.01 p.p.m. of pyridine or pyridine base in a 100-ml. sample would be satisfactory.

The Koenig (*7*) reaction has been used with many modifications for the detection of some of the compounds containing the pyridine ring structure. The reaction consists essentially of the addition of a cyanogen halide (usually cyanogen bromide) to the pyridine base and subsequent reaction of the compound formed with a suitable aromatic amine.

The reaction has been discussed by Migrdichian (*10*), Karrer (*5*), and Waisman and Elvehjem (*13*). Migrdichian (*10*) represents the reaction by the following equations:



Various investigators have proposed the use of a number of different aromatic amines together with cyanogen bromide to detect pyridine-type compounds. Tallantyre (*11*) used aniline as the coupling agent but obtained a sensitivity of only 1 part in 350,000. Tapia Freses *et al.* (*12*) linked pyridine with acetophenone but did not study the reaction extensively. Alekseev (*1*) employed benzidine in the determination of pyridine in aqueous ammonia solutions and further proposed the use of isoamyl alcohol for extracting the resultant dye. Other investigators have proposed 2-naphthylamine (*3*), *p*-methylaminophenol (*2*), and *p*-aminoacetophenone (*4, 6, 14*) in the Koenig reaction.

Various investigators have used the acetate ion for increasing the sensitivity of the reaction. McCormack and Smith (*8*) used potassium acetate as an intensifier in the determination of nicotine (using the Koenig reaction) and obtained a maximum color intensity at a concentration of 0.24 gram of potassium acetate per 100 ml. This was in accord with Markwood's (*9*) value of 0.2 gram of potassium acetate per 100 ml. On the other hand, von Euler (*3*) and Bandier and Hald (*2*) found that the acetate ion interfered in the reaction, while Kodicek (*6*) reported that the acetate ion had no effect on the color formation. Waisman and Elvehjem (*13*) suggested that the use of different amines influences the results obtained.

The effect of light has been reported to be an important factor in the reaction (*2, 4*). Waisman and Elvehjem (*13*) suggested that the stability of color in conjunction with light was an important consideration only when amines of high molecular weight are used as a coupling agent.

As a starting point for this investigation, benzidine was chosen as the aromatic amine to be used. Previous investigations by this laboratory, utilizing the Koenig reaction for the detection of cyanides, had indicated benzidine to have a better combination of sensitivity and stability than a number of the other amines proposed. The use of cyanogen bromide was suggested by its ease of preparation.

Preliminary investigation showed that the concentration of benzidine and cyanogen bromide had a definite influence on the amount of color produced, while the order of addition of reagents was of no consequence. The details for the preparation of these solutions are given under "Preparation of Reagents."

Exploratory work indicated that the reaction did not proceed at pH values below 4.0 and that colors formed faded very rapidly at pH values in excess of 10.0. A satisfactory amount of color was produced in the pH range of 7.0 to 8.0 using a bicarbonate buffer. However, the behavior of the yellow-orange color which resulted was unpredictable, sometimes fading to pink before the reaction was complete.

It was, therefore, decided to investigate the method of Alekseev (*1*). This procedure employs the same reagents already under consideration but carried out the reactions in a two-phase system. The final reaction, which yields a red coloration, apparently takes place in an isoamyl alcohol phase floating on the aqueous phase. While Alekseev's technique possessed the desired characteristics, difficulty was encountered in obtaining isoamyl alcohol of the required purity.

A number of different brands of isoamyl alcohol were secured and used in the reaction but in all cases there was color formation in reagent blanks. Moreover, the isoamyl alcohol resisted all efforts to remove the color-producing impurities. As an alternative, *n*-butyl alcohol was substituted for the isoamyl alcohol with considerably improved success in the matter of colorless blanks and stability of color.

The adoption of *n*-butyl alcohol as a component of the reaction mixture resulted in stabilizing and sensitizing the reaction. The final reaction takes place in the alcohol layer. If the reaction is carried out in aqueous solution and is subsequently extracted with *n*-butyl alcohol, less color results. Apparently the butyl alcohol extracts intermediate products and then affords a superior medium for the progress of the reaction.

FACTORS AFFECTING THE REACTION

Effect of pH. The change from an aqueous system to a two-phase alcohol-water system necessitated a resurvey of the pH requirements for maximum color development. At this point, it was observed that the color in samples buffered with solutions containing acetate ions was more intense than samples of comparable pH but lacking the presence of acetate ion.

To establish the pH range most suitable for the reaction, a series of samples was prepared containing 0.10 p.p.m. of pyridine and 0.50 gram of sodium acetate per 100 ml., the acetate concentration being based on preliminary experimentation. After addition of benzidine, the pH of the aqueous samples was adjusted, using small amounts of dilute hydrochloric acid or dilute sodium hydroxide to produce a pH range from 3.4 to 11.3. The reaction was then carried out in a two-phase system using *n*-butyl alcohol and cyanogen bromide. The samples were well shaken and left to age for 4 hours, after which a portion of the colored alcohol layer was carefully pipetted off and the absorbancy measured in the spectrophotometer.

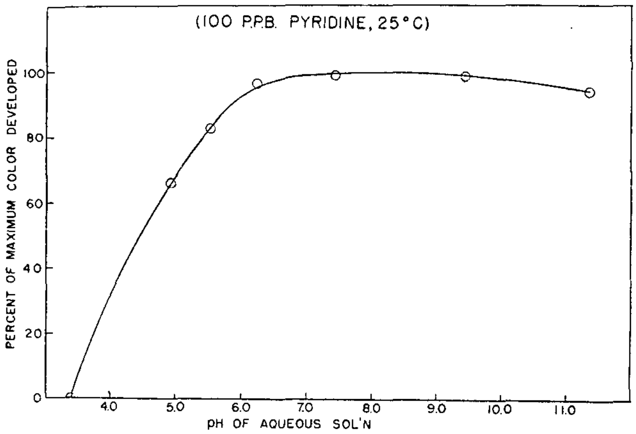


Figure 1. Effect of pH on Color Formation

The data, presented graphically in Figure 1, indicate that little or no color develops at a pH of 3.4, and the amount of color produced by the reaction is fairly constant from pH 6.2 to 11.3.

Not shown graphically is a slight tendency of the reaction color to fade more rapidly at pH levels in excess of 9.0. Furthermore, the presence of hydrochloric acid in the benzidine solution may cause lowering of the pH upon addition of this reagent. Because of the buffering capacity of sodium acetate, a rough pH control between 6.8 to 8.0 is easily made with indicating pH paper.

Effect of Sodium Acetate. The effect of sodium acetate on color formation was determined by adding sodium acetate in various amounts to similar samples of pyridine. Benzidine was then added and the pH adjusted to 6.8 to 8.0 with dilute sodium hydroxide, if too little sodium acetate was used to yield a solution with the desired pH. The reaction was completed with cyanogen bromide in the two-phase water–butyl alcohol system.

The data from three similar experiments indicate that:

The amount of color formed by pyridine in the complete absence of sodium acetate is variable and unpredictable.

The quantity of color formed is greater in the presence of sodium acetate than in its absence at comparable hydrogen ion concentrations.

Maximum color formation takes place in the range between 0.2 and 0.8 gram of sodium acetate in 100 ml. of aqueous sample (Figure 2).

The color intensity begins to decrease at about 0.8 gram of sodium acetate per 100 ml. of aqueous sample, and gradually decreases until a complete inhibition of color formation is reached at a concentration of about 3.0 gram per 100 ml. of sample (Figure 2).

In the case of this procedure, color may be intensified or decreased by sodium acetate, depending upon its concentration.

Table I. Effect of Amount of Reagents on Absorbancy per 1.0 Cm. at 520 Millimicrons

(100-ml. samples, reaction carried out at room temperature, readings on butyl alcohol extract)

Benzidine, Ml.	Cyanogen Bromide, Ml. 5.0	7.5	10.0	20.0[a]
1.0	0.340	0.354	0.342	0.342
2.0	0.369	0.409	0.424	0.314
4.0	0.410	0.415	0.461	0.455
8.0[b]	0.200	0.265	0.333	0.209

[a] Above 10 ml. of cyanogen bromide fumes are very evident.
[b] Above 4.0 ml. of benzidine precipitate forms and color fades.

Sodium acetate in the amount of 0.6 gram per 100 ml. of sample causes optimum intensification of the color formed, and also serves to raise the pH of the reaction mixture to the desired pH range. Accordingly, 4.0 ml. of 15% sodium acetate solution (0.6 gram) was selected as a suitable quantity of the chemical to provide both color intensification and pH control.

Experiments were also conducted to determine whether the sodium ion or the acetate ion was the sensitizing agent in the procedure. It was found that no substantial difference in the quantity of color resulted, regardless of the amount of sodium chloride present between the limits of 0 and 1.0 gram. At the same time, comparable samples containing sodium acetate and sodium chloride together showed color in greater concentration than samples containing only sodium chloride. Apparently, the acetate radical is the intensifying agent in the reaction.

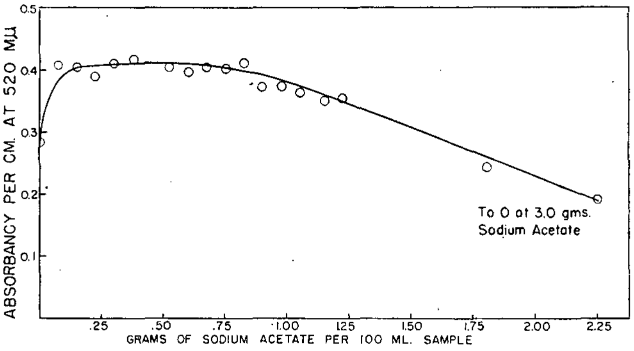


Figure 2. Effect of Sodium Acetate on Color Formation

100 p.p.b. of pyridine at pH 7.1

Effect of Benzidine and Cyanogen Bromide Concentration. A systematic study of the benzidine and cyanogen bromide requirements was next undertaken. Pyridine samples (100 ml.) at a concentration of 0.1 p.p.m. were prepared, the amount of benzidine was held constant, and the quantity of cyanogen bromide was varied. The experiment was repeated several times using a larger amount of benzidine in each series. In all cases, the previously determined figures for pH and sodium acetate concentration were used and the reaction carried out in the butyl alcohol phase.

The spectrophotometric readings, given in Table I, show that maximum color development occurs with addition of 4.0 ml. of 2% benzidine solution. However, in this range of concentration a precipitate of benzidine occasionally forms in the sample, which hinders the separation of the alcohol-water layers and apparently retards or deteriorates the formation of color.

When higher concentrations of cyanogen bromide are used, an increase in the intensity of color formed is apparent. Use of a minimum quantity of cyanogen bromide, however, is desirable in view of the hazardous nature of the solution. Experience shows that quantities of reagent that furnish linear results, provide sufficient sensitivity, and are practical for routine analysis are 2.0

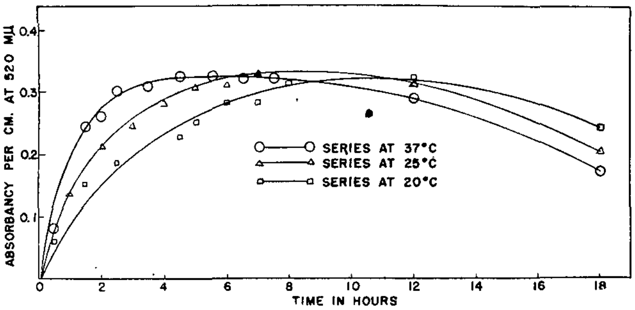


Figure 3. Effect of Time and Temperature on Color Formation

100 p.p.b. of pyridine, pH 7.1, reactions carried out in darkness

ml. of the benzidine solution and 5.0 ml. of cyanogen bromide. As no substantial difference is caused by the order in which the reagents are added, the cyanogen bromide should be added last to minimize fumes of the chemical from open vessels.

Effect of Time and Temperature. Investigation of the effect of time and temperature on the production of color with the reagents selected was next undertaken. Three series of samples were prepared containing 0.1 p.p.m. of pyridine. Series 1 was placed in a constant temperature room at 20° C., series 2 was treated similarly at 37° C., and series 3 was held at room temperature of 25° C. At measured time intervals samples were removed and the absorbancy was determined.

The data, shown in Figure 3, indicate that with an increase in temperature the reaction reaches a maximum color development in a shorter period of time, although the maximum color formed is approximately the same regardless of temperature. Maximum color development takes place in about 4 hours at 37° C., while a temperature of 20° C. requires 10 hours, but in all cases the maximum color persists unchanged for several hours.

At a room temperature of 25° C. between 6 and 8 hours are required for complete color development. After 4 hours the reaction is approximately 90% complete and further color development is very slow. Hence, good results can be obtained in routine analysis if the optical density of the developed color is read after 4 hours. At any stage of the reaction the amount of color apparently is proportional to the concentration of pyridine. This permits determinations to be made with a fair degree of accuracy based on readings of the samples after a relatively short storage period, provided that mechanical operations do not introduce a substantial time differential in the observations.

Because of the effect of time and temperature on color development, standard solutions and portions of samples to be analyzed should be set up simultaneously and under the same conditions. From the standpoint of accuracy and sensitivity, it is preferable to compare the colors after complete color formation and stabilization have taken place.

Effect of Light. During studies on the effect of time and temperature on the development of color, it was concurrently determined that (Figure 4):

> Samples exposed to strong sunlight at room temperature reached a maximum of color formation in a shorter period of time than samples stored in the dark.
>
> The maximum color formed in the light-exposed samples was less than the maximum of similar samples not exposed to strong light.
>
> Samples developed in strong sunlight exhibit a tendency to fade more rapidly than samples stored in the dark.

In addition, in direct sunlight, without pyridine or pyridine-base compounds present, the reagents alone will develop a light brown color unlike the red color resulting in the presence of pyridine. In several cases, where linear series of pyridine samples were prepared, discrepant results were noted on light-exposed samples, whereas similar series reacted in total darkness exhibited good linear properties. When the reaction was carried out in diffused light the formation of the brown color or fading was less pronounced. To secure maximum accuracy for the pyridine measurement, samples should be stored in the dark during the required reaction time.

Reactions of Other Pyridine-Base Compounds. This reaction is not specific for pyridine alone. Other pyridine-base compounds will also react under the conditions stipulated. Various investigators differ regarding the specificity of the reaction for different pyridine bases, probably because of the variety of conditions and reagents used in the application of the Koenig reaction.

Table II lists a few of the compounds that have been examined. Because of the difficulty of purchasing or preparing chemically pure compounds of the pyridine series, no really decisive study of the different pyridine-type compounds that will enter into the reactions has been made.

Specificity of Reaction for Pyridine Bases. The specificity of the procedure was given a limited investigation by testing the reactivity of representative compounds having various functional organic groups. The groups included were phenols, amines, aldehydes, ketones, and one nitrile.

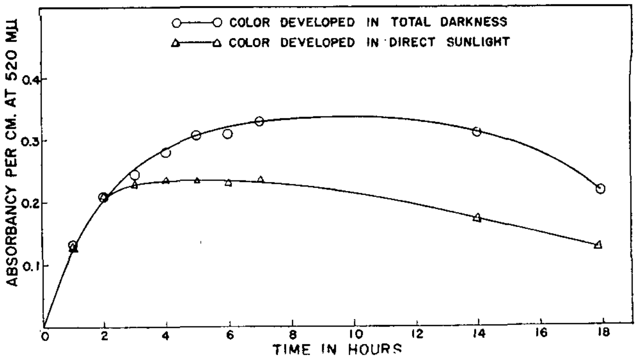


Figure 4. Effect of Light on Color Formation

100 p.p.b. of pyridine, pH 7.1, 25° C.

The data (Table III) indicate that a number of the phenols are apparently reactive in concentrations of about 500 p.p.m., and that 1-naphthol is reactive at a concentration of about 100 p.p.m. In both cases, reactivity may be due to trace impurities in the original compounds. However, because of the relatively large quantities required to produce color formation and because of the conditions prescribed under "Preparation of Samples," no interference in the pyridine-base measurement should be encountered from phenols.

1-Naphthylamine and furfural show a greater tendency to react with the pyridine-base reagents than the previously mentioned phenols. While the screening procedure proposed will not re-

Table II. Reactivity of Various Pyridine Bases

Compound	Reaction	Approximate Sensitivity, P.P.M.	Color	Wave Length at Maximum Absorption, Mμ
Pyridine	+	0.005	Red	520
Quinoline	−	....	....	...
Quinaldine	−	...	....	...
1-Picoline	+[a]	1.0	Orange-red	510
2-Picoline	+	0.05	Red	520
3-Picoline	+	0.1	Red	555
Picolinic acid	−	....	....	...
1-Aminopyridine	−	....	....	...
Nicotinic acid	+[b]	10	Yellow	No maximum in visible range
2,4-Lutidine	+[a]	1.0	Orange-yellow	480
2,6-Lutidine	+[a]	1.0	Orange-yellow	480

[a] Reaction questionable, color may be due to impurities.

[b] Substantial amounts of color develop in both alcohol and water phase.

Table III. Specificity of Reaction

Compound	Reaction	Approximate Sensitivity, P.P.M.	Color
Phenol	+	<500 >5	Pink
m-Cresol	+	<500 >5	Yellow
o-Cresol	+	<500 >5	Pink
Pentachlorophenol	+	<500 >10	Yellow
2,4,6-Trichlorophenol	−	...	....
Thymol	−	...	....
1-Naphthol	+	100	Orange-pink
Aniline	−	...	....
1-Naphthylamine	+	10	Pink
Tyrosine	−	...	....
Benzaldehyde	−	...	....
Furfural	+	1.0	Red-pink
Formaldehyde	−	...	....
Acetophenone	−	...	....
Acetone	−	...	....
Acetonitrile	−	...	....

move these materials prior to the final reaction, no interference is expected in the normal area of application of the procedure.

Aromatic amines may interfere with the procedure by competition with benzidine in the reaction. In the presence of substantial amounts of benzidine the amount of such side reaction would be expected to be small.

DETERMINATION OF PYRIDINE AND RELATED BASES

Recommended Procedure for Preparation of Sample. So far, the procedure described has been applied only to polluted aerobic river waters. For this work, simple distillation, after addition of sufficient sodium hydroxide to produce a pH in excess of 10, has sufficed. Pyridine in trace quantities distills over readily. About 98% of the pyridine distills over in the first 20% of the distillate and complete recovery is obtained in the first 40% of the distillate. The rate of distillation of other pyridine bases has not been investigated. However, quantitative recovery of 2-picoline and 3-picoline has resulted from the distillatory purification procedure described in detail below.

The properties which will permit separation of pyridine bases from more complicated waste solutions are obvious. Most of the pyridine-base compounds are volatile from alkaline solutions and their salts are not volatile from highly acid solutions. These materials may be recovered from wastes or river waters by simple distillation or steam distillation from neutral or alkaline aqueous solutions. Moreover, many interfering organic materials may be extracted by organic solvents from aqueous solutions containing substantial amounts of free mineral acids without extracting the pyridine bases.

A universally applicable procedure for preliminary preparation of samples for analysis cannot be given at this time. The following procedure has been applied satisfactorily to prepare samples of polluted river waters for determination of pyridine and 2- and 3-picoline.

Take a 500-ml. portion of the original sample containing a mixture of pyridine bases and add sodium hydroxide until the pH is above 10. Transfer the material to an all-glass distilling apparatus (Pyrex Catalog No. 3360) or to a Kjeldahl distilling apparatus. Add several boiling chips to prevent bumping and a pea-sized portion of silicone grease to prevent excessive foaming when the solution boils. Distill over 450 ml. Stop the distillation, add 50 ml. of distilled water, and continue the distillation until 500 ml. of purified sample have been collected.

Preparation of Reagents. STANDARD PYRIDINE SOLUTION. Dissolve 1.0 gram of C.P. pyridine in distilled water and make up to 1 liter. Dilute 5.0 ml. of the stock solution to 500 ml. with distilled water. This solution contains 10 p.p.m. of pyridine.

BENZIDINE HYDROCHLORIDE SOLUTION, C.P. Dissolve 2.0 grams of C.P. benzidine hydrochloride in 100 ml. of distilled water by stirring and add 2 or 3 drops of concentrated hydrochloric acid to complete solution of the benzidine crystals. Filter if necessary.

CYANOGEN BROMIDE SOLUTION. Dissolve 1.0 gram of potassium cyanide in 100 ml. of distilled water and add bromine to the solution dropwise until the reddish brown color of excess bromine is apparent. Remove the excess bromine by adding a solution of 10% potassium cyanide dropwise until the bromine color is discharged.

All of the free bromine must be removed from the solution; otherwise an intense side reaction takes place between the reagents themselves, making any analytical determination impossible.

To prevent the presence of free bromine, the following procedure is recommended: Make up a 0.1% solution of indigo carmine (indigo disulfonate) and place 2 drops of the solution on a spot plate. Add 1.0 ml. of the cyanogen bromide to the indicator. If bromine is present in excess, the dye will be decolorized immediately to a pale yellow. When the bromine is removed by a small excess of potassium cyanide, the addition of 1.0 ml. of cyanogen bromide to 2 drops of the indicator will not change the original blue color. The solution is then ready for use and is stable for 2 or 3 weeks.

Prepare the cyanogen bromide under a well ventilated hood and avoid inhalation of the fumes during preparation and use. Store in a glass-stoppered bottle.

SODIUM ACETATE. A 15% solution of the C.P. chemical in distilled water is used.

n-BUTYL ALCOHOL, C.P. Run a blank determination on every batch of untested butyl alcohol. If a positive blank is obtained, the *n*-butyl alcohol may be purified by washing. Using a separatory funnel, wash each liter of contaminated alcohol with three successive portions of 5% hydrochloric acid. Follow with three washings using successive 100-ml. portions of distilled water. Mallinckrodt's *n*-butyl alcohol has shown consistent absence of interfering materials.

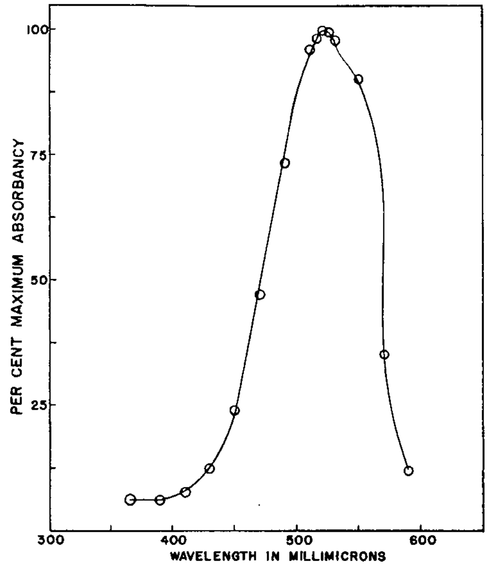


Figure 5. Absorbancy Characteristics of Color Produced from Pyridine at pH 7.1

Apparatus. Beckman Model B spectrophotometer equipped with 1.0-cm. cells.

Procedure. Pipet off an aliquot portion of the purified sample of such size as to contain between 0.001 and 0.02 mg. of pyridine bases as "pyridine."

Dilute the sample portion to 100-ml. with distilled water, using a glass-stoppered bottle to contain the solution.

Prepare a series of standards, ranging from 0.01 to 0.2 p.p.m. of pyridine, and a blank of 100-ml. volume and treat in parallel with the sample. For routine work one standard at 0.1 p.p.m. is sufficient.

Add 4.0 ml. of 15% sodium acetate and 2.0 ml. of the benzidine reagent. Shake well.

Check the pH of the solution with indicating pH paper and adjust to 6.8 to 8.0 if necessary.

Add 25 ml. of *n*-butyl alcohol and 5.0 ml. of cyanogen bromide. Shake well and store the samples in the dark for 3.5 to 4.0 hours at normal room temperature.

Pipet off very carefully about 5.0 ml. of the colored butyl alcohol layer. Avoid inclusion of any water droplets during the pipetting procedure; otherwise the sample will not be clear and will be unfit for spectrophotometric reading. Place the pipetted sample in a 1.0-cm. cell and read the absorbancy at 520 mμ (Figure 5) against the similarly prepared blank extract.

Compute the pyridine content of the sample on a proportional basis, using the standards to determine the relation between color and pyridine concentration.

Evaluation of Results. If the sample analyzed contains one known pyridine base, analysis for the pyridine-base content yields a well defined quantity, but when the sample contains a mixture of pyridine bases of unknown proportions the measurement is less definite in terms of total concentration. When the reaction is carried out as specified, the amount of color produced per unit weight of pyridine base is a maximum in the case of pyridine. Also, those pyridine-base compounds which are known to react in this procedure possess maximum absorbancies close to that of pyridine (Table II). Hence, when a mixture of pyridine bases is examined and the specific constituents are not definitely known, the results should be reported as pyridine. When so reported, the value is indicative of the minimum amount of pyridine base material which could be present.

SUMMARY

Because of the presence of pyridine-base compounds in by-product coke and oil refinery wastes, a sensitive method for their detection is desirable.

Pyridine, picolines, lutidines, and nicotinic acid give colored reaction products by the Koenig reaction suitable for their spectrophotometric determination. Phenol, *o*- and *m*-cresol, 1-naphthol, 1-naphthylamine, and furfural interfere.

Factors affecting the determination of pyridine, such as pH, temperature, solvent, time, amounts of reagents, and effect of sunlight, were studied.

The maximum color formation took place in the pH range of 6.2 to 11.3 when the reaction was allowed to proceed in the dark. Sodium acetate was used not only to act as a buffer but also to sensitize the reaction. An increase in temperature caused maximum color formation in a shorter period of time. However, it was found that consistent results could be obtained at room temperature with a reaction time of 4 hours. The separation of pyridine and related compounds from interfering materials was effected by distillation from an alkaline solution.

The method was found to be sensitive to 5 parts per billion of pyridine.

LITERATURE CITED

(1) Alekseev, R. I., *Zavodskaya Lab.*, **8**, 807–9 (1939).
(2) Bandier, E., and Hald, J., *Biochem. J.*, **33**, 264 (1939).
(3) Euler, H. von, Schlenk, F., Heiwinkel, H., and Hogberg, B., *Z. physiol. Chem.*, **256**, 208 (1938).
(4) Harris, L. J., and Raymond, W. D., *Biochem. J.*, **33**, 2037 (1939).
(5) Karrer, P., "Organic Chemistry," 3rd ed., p. 786, New York, Elsevier Publishing Co., 1947.
(6) Kodicek, E., *Biochem J.*, **34**, 712 (1940).
(7) Koenig, W., *J. prakt. Chem.*, **69**, 105 (1904).
(8) McCormack, W. E., and Smith, H., Ind. Eng. Chem., Anal. Ed., **18**, 508 (1946).
(9) Markwood, L. N., *J. Assoc. Offic. Agr. Chemists*, **22**, 427 (1939).
(10) Migrdichian, V., "Chemistry of Organic Cyanogen Compounds," American Chemical Society Monograph Series, No. **105**, p. 110, New York, Reinhold Publishing Corp., 1947
(11) Tallantyre, S. B., *J. Soc. Chem. Ind.*, **49**, 466 (1930).
(12) Tapia Freses, A., Sanchez Moreno Tira, C., and Canales, Cockburn, J., *Actas y trabajos congr. peruano quim.*, **2**, I, 337 (1943).
(13) Waisman, H. A., and Elvehjem, C. A., Ind. Eng. Chem., Anal. Ed., **13**, 221 (1941).
(14) Wollish, E. G., Kuhnis, G. P., and Price, R. T., *Ibid.*, **21**, 1412 (1949).

Received for review May 28, 1952. Accepted September 24, 1952.

Study of Chromium Toxicity by Several Oxygen Demand Tests

R. S. INGOLS and E. S. KIRKPATRICK
State Engineering Experiment Station, Georgia Institute of Technology, Atlanta, Ga.

The toxicity of chromium in its various forms has been studied, using three techniques for determining B.O.D. The data indicate that the toxic level of chromium is controlled by several variable factors, including the presence or absence of oxygen, the valence of the chromium, the type of organism (autotrophic *vs.* heterotrophic), and the amount of organic matter present (rate of metabolism). A possible mechanism for the chromate ion toxicity under anaerobic conditions is discussed, and it is suggested that the mechanism for chromic ion toxicity is simply one of inert, small particle interference.

THE presence of chromium compounds in many industrial effluents discharging to sewage treatment plants and rivers makes it important to understand the factors involved in the toxicity of such compounds toward either the microorganisms in the sewage treatment plant or the aquatic plants and animals of the river. The Research Committee (*17*) of the Federation of Sewage and Industrial Wastes Associations has recently reviewed the literature on the toxicity of chromium as part of a larger study on the toxicity of industrial wastes. According to this review, chromium has been reported to have about 40% toxicity at a concentration of only 1 p.p.m. under some experimental conditions (*14*), while under other conditions (*4*) a concentration of 5000 p.p.m. or more was required for approximately the same toxicity. The data reported in the present paper indicate that bacteria can tolerate high concentrations of chromium where large amounts of organic matter are present, as in sludge digestion. This may be one factor responsible for the large divergence in reported toxic levels. Some authors (*4, 15, 20*) indicate that the valence of the chromium is not important in defining the toxicity limits, while others (*5, 6, 13, 14, 16, 18*) claim that there is a difference in the toxicity of the tri- and hexavalent chromium.

Krieger and Moore (*14*) report that both trivalent and hexavalent chromium are toxic at 1 or 2 p.p.m. in the dilution B.O.D. test, but that the trivalent form is generally more toxic. Dawson and Jenkins (*6*) indicate that trivalent chromium is more toxic to activated sludge than the hexavalent chromium. However, the concentration for obvious toxicity to activated sludge is approximately 10 times that for a definite toxic reaction in the dilution

B.O.D. test reported by Krieger and Moore (*14*). Jenkins and Hewitt (*11*) indicate that 10 p.p.m. of chromium as chromate may reduce nitrification by 50% in a trickling filter while altering the amount of carbonaceous oxidation only slightly. When 100 p.p.m. of chromium was used, both nitrification and carbonaceous oxidation were effected but the effluent was still passable. In a study of the B.O.D. test it was indicated that chromates may be reduced when added to full-strength sewage and left during a 5-day incubation period. These same authors (*13*) have indicated that the addition of 10 p.p.m. of chromate to sewage in an activated sludge pilot plant eliminated nitrification without greatly impairing carbonaceous oxidation. In further work they (*12*) reported similar results in a study of the effect of chromate in the B.O.D. test.

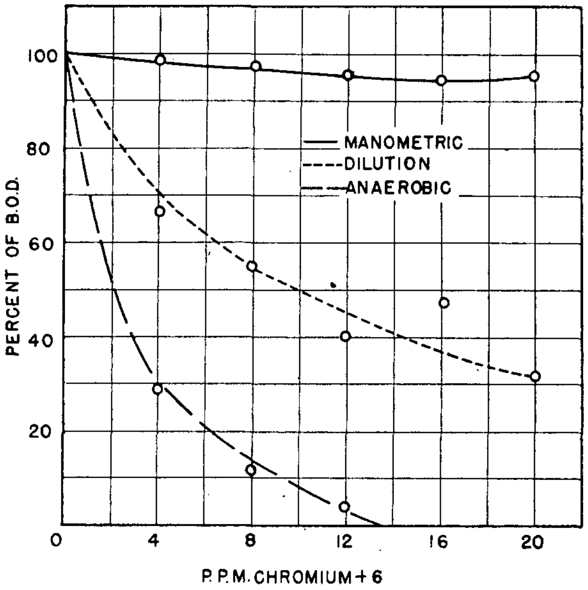


Figure 1. Interference or Toxicity of Various Concentrations of Chromic Ion on B.O.D. Values

Coburn (*5*) indicates that trivalent chromium is very toxic in sludge digestion tanks in spite of its insolubility at sludge digestion pH values. He states that hexavalent chromium is so soluble that it will not appear in the digestion tank, going off, instead, in the sewage. However, he intimates that it would be toxic in the digester if present. On the other hand, Barnes and Braidech (*4*) found that 5000 p.p.m. of trivalent chromium and 10,000 p.p.m. of hexavalent chromium could be tolerated in the digestion tanks, although the organisms produced only 41 and 68%, respectively, of the normal amount of gas. Southgate (*18*) agrees with Barnes and Braidech (*4*) that trivalent chromium has very little effect on digestion. However, it has been reported (*16*) that the presence of 1 p.p.m. of hexavalent chromium seriously interferes with digestion.

In unpublished work on sludge digestion, the senior author found hexavalent chromium highly toxic and trivalent chromium of low toxicity when gas production was used as the measure of toxicity. However, it is not known whether the chromate ion can act as a hydrogen acceptor in the anaerobic oxidation of organic matter in the same way as nitrate acts in the anaerobic digestion of paper pulp (*19*). Methane-producing bacteria must operate in a medium which has a very low redox potential (*3*), while nitrates are normally reduced at redox potentials higher than the E_0 of methylene blue on the basis of the relative stability test. It has been pointed out (*11–13*) that chromates retard nitrate ion formation, but there is no information concerning the possibility that chromates may retard nitrate reduction by microorganisms. Jenkins and Hewitt (*11*) have pointed out slight reduction of chromates in sewage over several days, but they obtained no information on the redox potential under which this occurred or the mechanism of its reduction.

The review of the literature indicated so much confusion in assigning a value for the toxic level of chromium that it was considered necessary to choose experimental techniques that could use the same organisms under various environmental conditions. The classical dilution B.O.D. test (*2*) has a very limited concentration range, but it would serve as the technique for measuring toxicity at low organic matter concentration under aerobic conditions. The Sierp or manometric B.O.D. test as recently developed (*7*, *8*) offers the choice of a very wide range of organic matter concentration as well as a choice of oxygen concentrations. The use of nitrates as the source of oxygen added to full-strength sewage would permit the study of the rate of oxygen uptake by a sewage sample under anaerobic conditions. Allen (*1*) found that highly oxygenated substances were especially toxic to methylene blue reduction in the relative stability test. Because chromates carry a high content of oxygen, the relative stability test should provide the means of studying the relative toxicity of chromic versus chromate ion, and indicate the fate of the oxygen in the chromate ion. The recording apparatus for studying relative stability of sewage developed in this laboratory (*10*) was used in this study.

Thus, these three B.O.D. tests have permitted the study of the toxicity of chromic and chromate ions under aerobic and anaerobic conditions, under various organic matter concentrations, and under varying oxygen tensions.

METHODS OF STUDY

Three methods or procedures were used in the study of the toxicity of tri- and hexavalent chromium. Two of these were carried out under aerobic conditions and the other was conducted in the absence of free oxygen.

The first of the aerobic tests was the classical dilution B.O.D., which was modified by the addition of equal amounts of chromium ion into two bottles, one containing only dilution water and the other containing dilution water and sewage. The dissolved oxygen content (total iodine titration) of both bottles was determined at the end of the standard 5-day, 20° C. incubation period, and the difference was taken as the B.O.D. of the sample.

The other aerobic test was carried out in the Sierp apparatus, using the procedure as outlined by Falk and Rudolfs (*7*) and as modified by Gelman and Heukelekian (*8*). Various concentrations of chromium were introduced directly into a full-strength sample of sewage, and the amount of oxygen required by the sample was measured directly. This method proved particularly satisfactory, in measuring both the rate of oxygen utilization and the total amount of oxygen used at any given time. The pH of the sample was taken at the end of incubation, as the authors believe that a pH of 8 provides an excellent indication of adequate carbon dioxide absorption, while an extremely high pH indicates contamination of the sample by some of the carbon dioxide absorbent. Where the Heukelekian modification is to be used, it is strongly recommended that a pH value be taken of all samples incubated.

The procedure used in the anaerobic or nitrate test was that described by Ingols (*10*). This is essentially a modified relative stability test in which the amount of combined oxygen available to anaerobic bacteria is constant in all samples at the beginning of the run. A limited amount of nitrate was added to the full strength of sewage in order to get an end point in the control within 16 to 24 hours. A constant amount of available oxygen was provided by the use of a given amount of sodium nitrate with the trivalent chromium; then, with the hexavalent chromium, the total oxygen from the nitrate plus chromate was generally kept constant. Thus, four bottles were set up for one run with the nitrate and chromate solution placed in the bottles before the sewage was added. The nitrate and chromate solutions were made up so that each contained the same amount of combined oxygen per milliliter. The control contained nitrate while the other three contained the same total volume (generally 4.0 ml.) of nitrate plus chromate solutions before the sewage was added. Frequently, the dye was added to the sewage before it was added to the individual bottles. The time required for the reduction of the nitrate or chromate was taken as the point at which the methylene blue started to lose color as discussed in the original article (*10*). The relative toxicities of different concentrations of chromium were indicated by the increase in time taken for different samples to decolorize. Even in the control, the test with certain sewage samples did not give B.O.D. values which were numerically comparable with the dilution B.O.D. values. However, in a study of reproducibility, replicates have been shown to give excellent agreement. Thus, the relative B.O.D. values and the relative toxicity from the chromium ions are considered valid.

The sewage samples used in the study were entirely domestic and were taken from a local sewage treatment plant at the same time each week in order to obtain as much uniformity of character as possible. The mean B.O.D. of the settled waste was about 200 p.p.m., and values varied very little from this figure during the time covered by the studies reported here.

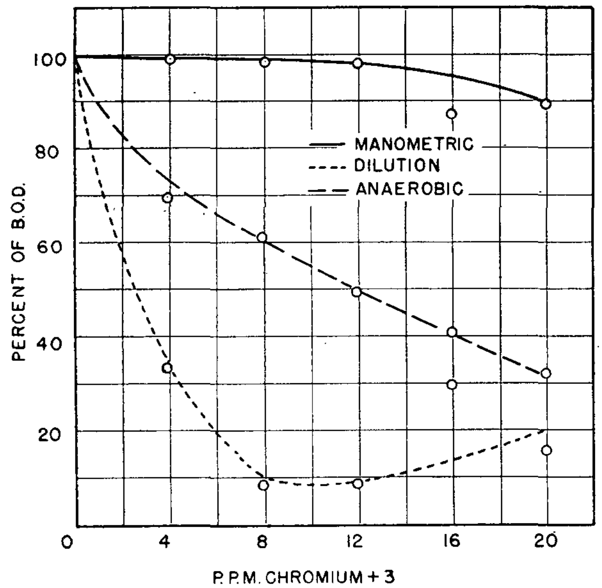


Figure 2. Interference or Toxicity of Various Concentrations of Chromate Ion on B.O.D. Values

At times the sewage was supplemented with various known organic substances for the dual purpose of giving a wider range of values with which to work in the aerobic tests and for supplying an adequate amount or specific type of food in the anaerobic procedure.

RESULTS

Many preliminary runs with the various B.O.D. techniques were set up in order to determine the range of toxicities that could be expected for the different techniques with the two chromium valencies. The results shown in Figures 1 and 2 were all obtained from one sample of sewage, but they are typical. The data for the trivalent chromium in Figure 1 show that in the manometric oxygen technique even 20 p.p.m. of chromium had very little effect. This was also true for the rates of oxygen utilization and the 5-day B.O.D. values, except for an initial heightened lag in some samples where the control also showed some lag. The data also showed that the values from the dilution B.O.D. technique were lower than those where nitrates served as the source of oxygen.

The data in Figure 2 show that there was no toxicity at the oxygen concentrations resulting from saturation with pure oxygen, while the hexavalent chromium was very toxic under anaerobic conditions. At first it was thought that a concentration of 4 p.p.m. of chromium (as chromate) was much more toxic than is reported in Figure 2 with nitrate reduction, until it was noted that there was no chromate left in the sample after incubation at these concentrations. This would confirm the observation of Jenkins and Hewitt (*11*) concerning the reduction of chromate when it had been added to undiluted sewage and left for several days. Thus, when the chromate oxygen was added to the available nitrate oxygen, the B.O.D. value showed a much lower apparent toxic effect. When higher amounts of chromate were used, the time of reduction was increased but the chromate was eventually reduced. When a sample was set up in duplicate, 20 p.p.m. of chromium was completely reduced, yet the methylene blue in a replicate was not reduced in another 20 days. A test of a portion of the sample used to determine chromates indicated that the organisms remaining were capable of oxidizing the organic matter present under aerobic conditions. A sample of sterile sewage gave no change in the chromate concentration over a period of 20 days.

Because the dilution and manometric techniques vary both concentration and oxygen tension under aerobic conditions, the manometric technique was varied to use air on one series of tests in comparison with oxygen. The results, as shown in Table I, indicated that under aerobic conditions different oxygen tension had no effect on toxicity.

Table I. Effect of Oxygen Tension upon Toxicity of Both Oxidized and Reduced Forms of Chromium in Manometric B.O.D. Procedure

	Chromium Valence			
	+3		+6	
	Manometric B.O.D. Values, P.P.M.			
Concn., P.P.M.	Air	Oxygen	Air	Oxygen
0	280	250	265	250
10	225	225	225	225
15	240	240	235	250
20	250	250	210	210

The results, as shown in Table II, indicated that a given amount of chromium produced an approximately constant amount of B.O.D. reduction. When this amount of B.O.D. was related to the total B.O.D., the percentage of the reduction was less at the higher B.O.D. values.

DISCUSSION

An understanding of the mechanism of the toxic reaction would make it much easier to determine a reasonable toxic level for any substance. Thus, this discussion is devoted largely to attempting to develop a concept of the mechanism of chromium toxicity from data in the literature and those given in this paper.

It is now known that mercury, a heavy metal, forms a tight bond with the sulfhydryl radical of essential enzymes and is therefore not only toxic but lethal at very low concentrations.

Table II. Effect of Varying Organic Matter Concentrations upon Toxicity of 20 P.P.M. of Chromium in Manometric Procedure Using Pure Oxygen

	B.O.D. Values			
			Reduction	
Sample	Control, p.p.m.	Chromium present, p.p.m.	P.p.m.	%
Control	265	175	90	34
Plus 500 p.p.m. dextrose	690	570	120	18
Plus 500 p.p.m. peptone	690	560	130	19
Plus 500 p.p.m. dextrose 500 p.p.m. peptone	1090	1000	90	8

Chromium, however, is not a heavy metal; its atomic weight is less than iron, which is a normal constituent of many organisms. It has such a strong affinity for proteins that it is now widely used as a tanning agent in the production of leather. Some workers with chromium accept this idea of the reaction between the chromic ions and protein as the proper explanation of the toxicity of the chromium. This would not necessarily mean the attachment of chromium at a specific or highly specialized group on the bacterial protein. When a peptone and sugar were compared as the food, in one experiment, the data showed that chromium was equally toxic toward the oxygen demand with each type of food. Because chromium will not form a complex with sugar as it can with proteins, such as peptone, it would appear that the chromium does not exert its toxic influence through a reaction with the food of the microorganisms.

A second consideration of the toxic level of the chromic ion, at least under anaerobic conditions, was the possible necessity for the bacteria to differentiate between iron and the chromic ion in the formation of new hemin enzymes. Were this true, additional ferrous ions should reduce the toxicity of the chromic ion. The authors' experimental results were negative.

As a third consideration, it would appear that the trivalent chromium could interfere with the bacteria itself in some nonspecific fashion. Heukelekian (*9*) indicates that many of the light metals show that chromium and the other metals are more toxic at a lower organic matter concentration, as is shown by the authors' data and by the individual reports in the literature (*6*, *14*). The authors interpret these data to mean that these medium sized particles (between sodium and mercury) exert their toxic influence by some nonspecific mechanical interference with metabolism. This would mean that a search for a specific toxic level would be very tedious, at best.

While the toxic effect of the chromium in the chromate ion is slightly lower than that from the same chromic ion concentration under aerobic conditions, the data show that the toxic level of the chromate is also dependent upon the food concentration. This would indicate that the mechanism of the chromate ion toxicity under aerobic conditions may be the same as that for the chromic ion. However, the very high relative toxicity of the chromate ion under anaerobic conditions indicates that there may be a more specific mechanism under anaerobic conditions than has been suggested as the mechanism for the chromic ion or for the chromate ion under aerobic conditions.

On the basis that chromates are apparently toxic to both the oxidation of ammonia and reduction of nitrates, an explanation considered the possibility that the chromates were interfering with the enzyme systems responsible for nitrification because of a similarity on the spatial configuration of the chromate and nitrate ions. However, nitrate ion atoms are in a single plane, while chromate ion atoms form a tetrahedron. The sulfate ion is also a tetrahedron and would be even more likely to cause interference than the chromate ion because of its size. Since the sulfate ion fails to cause interference in nitrate metabolism, it does not seem likely that spatial configuration is the cause of chromate toxicity.

When it is maintained at pH 7.0 and 20° C., the chromate ion apparently has a redox potential similar to that of the nitrate ion. Thus, it is concluded that it interferes with the nitrate ion metabolism because of this similarity in redox potentials. This hypothesis implies that chromates pass into the cell and do not react with any particular cellular substance as does the mercuric ion. It further assumes that the chromates are reduced under those conditions and by those organisms which reduce nitrates.

The last assumption immediately raises the question of the possible production of chromate ions by the enzymatic oxidation of chromic ions. The occurrence of such oxidative transformation has not been observed except in possibly trace amounts over a period of 6 weeks in three series of experiments. However, chromium in its trivalent form is not a free metal ion but is highly hydrated in the center of a Werner complex, while the nitrogen in ammonia is water-free and the ammonia is nonionized. The hydrogen of the ammonia is involved in chemosynthetic action, while the chromic ion has no hydrogen.

The question has been raised as to whether the chromates are reduced within the bacterial cell or are reduced by the chemical reaction of a bacterial by-product outside the cell. This is obviously very difficult to answer, but a large number of experiments have been carried out to gain a better understanding of the situation. The free sulfhydryl radical of cysteine can reduce chromate at pH 8.0. Thus, chromates flowing in a stream over sludge beds may be reduced even in the presence of dissolved oxygen. This condition has been found in a small stream receiving an industrial waste containing both organic matter and chromates. It is realized that the mechanism whereby chromates are reduced under anaerobic conditions may be the result of the production of by-product sulfhydryl radicals from organic sulfur-bearing compounds. In order to evaluate the necessity of organic sulfhydryl radical production, methionine (with a thio-ether group) was added to a sample of sewage and its stimulation to chromate reduction was compared with the stimulation from a similar amount of dextrose. The dextrose and methionine required the same time for reducing the chromate and methylene blue, indicating that dextrose was as good a hydrogen donor as a sulfhydryl-bearing organic compound. It has been observed that the dye is decolorized after the chromate is reduced. In order for the bacteria to produce organic sulfhydryl radicals such as mercaptans (thiols), it is understood by the authors that the bacteria would have to be operating in a medium with a redox potential less than the E_0 of methylene blue. If the methylene blue color persisted, one would assume that the bacteria were operating in a medium of higher potential than is normal for sulfhydryl production.

Methionine, cystine, sodium nitrite, and sodium sulfide are all possible by-products of anaerobic metabolism, but they do not cause direct reduction of chromate at pH 8.0; this does not facilitate reduction of chromate under the same anaerobic condition, possibly because the excess sulfide is too toxic for the bacteria.

Because of the similarity in the redox potential of nitrate formation and carbonaceous oxidation, it may be that the chromate also interferes somewhat in carbonaceous oxidation by the similarity of the chromate potential to the carbonaceous oxidations. The general similarity of the toxicity of chromate and chromic ions under aerobic conditions does not lend much weight to this idea.

LITERATURE CITED

(1) Allen, L. A., *Appl. Bacteriol.*, **2**, 26 (1949).
(2) Am. Public Health Assoc., New York, "Standard Methods for the Analyses of Water and Sewage," 9th ed., 1946.
(3) Barker, H. A., *Arch. Mikrobiol.*, **7**, 420 (1936).
(4) Barnes, G. E., and Braidech, M. M., *Eng. News Record*, **129**, 496 (1942).
(5) Coburn, S. E., *Sewage Works J.*, **21**, 522 (1949).
(6) Dawson, P. S. S., and Jenkins, S. H., *Sewage and Ind. Wastes*, **22**, 490 (1950).
(7) Falk, L. L., and Rudolfs, W., *Sewage Works J.*, **19**, 1000 (1947).
(8) Gelman, I., and Heukelekian, H., *Sewage and Ind. Wastes*, **23**, 1267 (1951).
(9) Heukelekian, H., private communication.
(10) Ingols, R. S., *Sewage Works J.*, **21**, 984 (1949).
(11) Jenkins, S. H., and Hewitt, C. H., *J. Soc. Chem. Ind.*, **59**, 41 (1940).
(12) Jenkins, S. H., and Hewitt, C. H., *Nature*, **147**, 239 (1941).
(13) Jenkins, S. H., and Hewitt, C. H., *Surveyor*, **101**, 211 (1942).
(14) Krieger, H. S., and Moore, W. A., "Determination of the Toxicity of Trivalent and Hexavalent Chromium on Oxygen Utilization of Sewage," U. S. Pub. Health Service, January 1949.
(15) Laboon, J. F., *Sewage Works J.*, **21**, 197 (1949).
(16) *Public Works*, **73**, No. 11, 32 (1942).
(17) Rudolfs, W., *et al.*, *Sewage and Ind. Wastes*, **22**, 1157 (1950).
(18) Southgate, B. A., "Treatment and Disposal of Industrial Wastes," London, England, H. M. Stationery Office, 1948.
(19) Straub, C. P., *Sewage Works J.*, **16**, 30 (1944).
(20) Wise, W. S., *Ibid.*, **17**, 338 (1945).

RECEIVED for review May 14, 1952. Accepted November 5, 1952.

Determination of Residual Chlorine in Metal Finishing Wastes

HENRY C. MARKS AND NOEL S. CHAMBERLIN
Wallace & Tiernan Co., Inc., Belleville, N. J.

Methods for determining residual chlorine in water and sewage are frequently not suitable for industrial wastes. In each case it is necessary to examine the applicability of known methods and perhaps to devise modifications. The present discussion concerns experiences with several types of plating wastes, showing how the usual methods can be modified to meet particular situations. When it is unnecessary to distinguish between free available chlorine and combined available chlorine in the treatment of cyanide wastes, any of the usual methods for residual chlorine in water are suitable. For distinguishing between the two forms of available chlorine, only an amperometric titration procedure properly modified is satisfactory. In wastes containing chromate it is necessary to resort to an amperometric titration procedure which is carried out within certain definite experimental restrictions. Of the other heavy metals likely to be encountered in the plating wastes, cuprous ion, silver ion, and high concentrations of cupric ion interfere with the amperometric titration.

IN THE expanding program of industrial waste treatment, chlorine plays its important part. Frequently it is used as a sanitary measure on the final effluent from a waste treated with another chemical agent or a biological or physical process. In other cases chlorine itself may be the actual destructive or modifying agent. As usual, reliable analytical control is necessary to obtain the desired result and the most economic use of chemicals. This frequently means that the residual be controlled to a given concentration of free available chlorine as contrasted to chloramine.

The methods for determining residual chlorine in water or sewage are not necessarily satisfactory for this purpose. There may be adverse conditions and interfering substances utterly unlike those normally encountered. Where such conditions are known or suspected, it is necessary to examine the applicability of the usual methods carefully and to devise proper modifications. The object of this discussion is to present typical examples of special conditions and the possibilities of modification of available methods to meet these conditions.

CYANIDE WASTES

The treatment of cyanide wastes from plating or metal-treating operations is an example wherein chlorine is used as the actual destroying or modifying agent. The highly toxic and objectionable nature of the waste product makes it very important to carry the chlorination to the proper point. Careful control and good control methods are necessary to ensure complete treatment of the toxic material at all times and to make the most economical use of the chemicals used.

The problem of determining residual chlorine in this waste varies with the exact composition of the waste being treated and the desired result. Whether or not it is necessary to determine free available chlorine separately depends on whether heavy metals are present and on whether the cyanide is being completely destroyed or merely converted to cyanate.

When the waste is free of heavy metal cyanides and where it is required only to convert cyanide to cyanate, only a chloramine residual of sufficient magnitude is necessary at the end of the treatment. In this case, control of chlorination consists merely in determination of total residual chlorine with no attempt to determine free available chlorine separately. Any of the usual residual chlorine tests may be applied without particular complication. The *o*-tolidine method, the amperometric titration (*2–4*), or the ordinary iodometric titration can be used.

If the waste contains heavy metal cyanides, or even if these are absent but the cyanide has to be completely destroyed, more careful control is required. To ensure the desired end it is necessary to have a definite residual of free available chlorine after the treatment is completed. Otherwise, there is no certainty that all the cyanate will be destroyed and that all the insoluble cyanides will be oxidized. This means that a method suitable for distinguishing free available chlorine from combined chlorine must be used. The two methods now in general use for this purpose are the OTA method (*1*) and the amperometric titration with phenylarsene oxide (*4*).

There is a serious limitation on the use of the OTA test for this purpose. A sizable fraction of the combined chlorine reacts with *o*-tolidine as rapidly as free available chlorine. It is not feasible to apply a correction factor, as Moore suggests for water (*5*). For one thing, the speed of reaction of combined chlorine with the *o*-tolidine reagent depends on the relative proportions of monochloramine and dichloramine. Table I shows the results of tests with these two substances at several temperatures. In each case the particular chloramine was made in solution at a concentration of 100 p.p.m. The proper amount to give concentrations of the order of 1 to 3 p.p.m. was then added to distilled water at the right temperature just before starting the OTA procedure. Colors were read in a comparator with 26-ml. tubes, using a 15-ml. sample and 0.5 ml. of *o*-tolidine solution. The arsenite was added as quickly as possible, which is estimated to be 5 to 7 seconds. The values given are averages of several determinations. There is essential agreement with Moore's data which apply to periods of either 3 or 20 seconds between addition of *o*-tolidine and arsenite. Just as with iodide ion, dichloramine reacts much less rapidly with *o*-tolidine than does monochloramine.

Table I. Errors in OTA Test

Temp., ° C.	Percentage Error Monochloramine	 Dichloramine
10	16	8
15	22	12
25	39	27

In the complete destruction of cyanide wastes dichloramine is always present and the total combined chlorine may amount to as much as 25 times that of the free chlorine. Not only is the percentage error somewhat indefinite, but it is of the same magnitude as the value being determined even if the sample is cooled. As neither cooling nor use of a correction factor overcomes the difficulty, the OTA method is not widely used for this purpose.

The amperometric titration with phenylarsene oxide is free of this fault. High concentrations of chloramine in either form give no titration in the absence of iodide ion and in the vicinity of pH 7. Under these conditions the free available chlorine or hypochlorous acid can be titrated without interference.

Modification of the usual procedure is required because the total residual often is extremely high. Since the electrodes are usually designed for sensitivity at low concentrations, exposure to very high concentrations of available chlorine causes a film-type polarization which reverses very slowly. This is avoided by diluting with demand-free water until the solution titrated contains no more than 10 p.p.m. of available chlorine. Too much dilution naturally reduces the precision of the free chlorine determination. A free chlorine content of as low as 0.2 p.p.m. can be determined satisfactorily. At the end of chlorination the total chlorine may be as high as 500 p.p.m. A fiftyfold dilution means that chlorination would have to be carried far enough to give 10 p.p.m. of free available chlorine in the waste. Where the total chlorine is less, the analytical method will permit adequate control with correspondingly less chlorine. This is important, because to obtain 10 p.p.m. of free available chlorine requires the addition of a much greater quantity.

Another modification is necessary when the ratio of combined to free chlorine is as high as this. Even after dilution, the current produced by the titrating cell may be so great that the microammeter will not read on scale even at the end point in the free chlorine titration. Recent experiments show that dichloramine is particularly active, producing four to five times as much current as monochloramine. It is possible to titrate free chlorine in the presence of up to 25 p.p.m. of monochloramine, but the dichloramine must be less than 5 p.p.m. The remedy is to apply opposing voltage to make the proper change in the zero point of the meter scale.

Because the usual amperometric titrator circuit does not permit the use of sufficient opposing voltage, an outside voltage source must be inserted. In the usual circuit, voltage obtained from a suitable winding in the agitator motor is passed through a rectifier and then impressed on the indicating microammeter in opposition to the voltage of the cell. This opposing voltage can be increased to any desired value by disconnecting the leads from the rectifier and connecting them to another source, such as a 1.5-volt dry cell. With these modifications the amperometric titration procedure is suitable for determination of free chlorine to ensure complete destruction of cyanides. The method has been proved in practice and is in common use for this purpose.

CHROME WASTES

Problems in the determination of residual chlorine in wastes arise through contamination with chromate-bearing wastes from the plating industry. Chromate may be present in a cyanide waste and complicate the selection of the proper degree of chlorination. In other instances chromate may be found in a mixed waste which is being chlorinated for sanitary or other purposes.

Chromate ion interferes with the *o*-tolidine test not only in an optical sense but also by reaction to produce the same colored oxidation product as chlorine. The color of the chromate ion itself can be readily compensated in the usual way by using as a blank a sample without *o*-tolidine. The second type of interference is the more serious. The magnitudes of the possible errors are shown in Table II. In these tests chromium was present as potassium dichromate dissolved in distilled water. In the table the column headed "Color Equivalent" shows the effect of the chromate color itself with no *o*-tolidine added. In determining the flash and maximum *o*-tolidine readings, the chromate color was compensated for by placing some of the acidified sample in the proper position in the comparator. Even 20 p.p.m. of chromium as chromate produces appreciable error. This error will be important in any case where the chromium concentration is at least two to three times that of the residual chlorine.

Table II. Effect of Chromate on *o*-Tolidine Test

(Parts per million)

Chromate	Color Equivalent	Flash Reading	Max. Reading
100	0.50	0.50	2
50	0.30	0.15	2
20	0.12	0.07	0.6

Table III. Amperometric Titration in Presence of Chromate

pH	Chromate, P.P.M.	PAO, Ml.
4	0	0.97
4	100	0.97
4	0	0.98
4	100	0.97
7	0	0.96
7	100	0.93
7	0	0.92
7	100	0.91

The amperometric titration method as applied to residual chlorine might be expected to include other oxidizing agents as well. Since chromate is a fairly powerful oxidizing agent, it was necessary to conduct a series of experiments to determine whether proper conditions could be found to determine residual chlorine in the presence of chromate. It was immediately found that below pH 4 chromate ion poisoned the platinum electrode with loss of sensitivity. At higher pH values the electrode is not affected in this way nor is there any change in the current flowing through the cell with moderate concentrations of chromate. Other experiments showed that chromate does not liberate iodine at the concentrations of potassium iodide used in the chlorine determination as long as the pH is 4 or above. In a typical experiment 5 ml. of 5% potassium iodide solution was added to 1 liter of water buffered at pH 4. At various intervals 200-ml. solutions were titrated amperometrically with phenylarsene oxide. Even after 15 minutes no titratable iodine was found. In the same way it was demonstrated that chromate ion is not titrated with phenylarsene oxide in the absence of potassium iodide in this pH range.

Conforming to these requirements, a series of titrations of low concentrations of free iodine with and without added chromate was performed. The proper amount of stock iodine solution was added to a quantity of distilled water buffered at the desired pH, and 200-ml. aliquots of these solutions were then titrated with 0.00564 *N* phenylarsene oxide solution amperometrically, 100 p.p.m. of chromium as chromate being added to alternate aliquots. The results at pH 4 and 7 are given in Table III.

The absence of any effect means that the amperometric titration for hypochlorous acid, monochloramine, and dichloramine separately according to the usual procedure can be performed with no difficulty in the presence of as much as 100 p.p.m. of chromium. It is safe to conclude that chromate will have no effect either on the electrode or on the phenylarsene oxide during the titration for hypochlorous acid at pH 7 in the absence of iodide. Likewise, there will be no interference in the determination of monochloramine and dichloramine in the presence of potassium iodide at pH values of 7 and 4, respectively.

The amperometric titration procedure as modified for sewage cannot be used in the presence of chromate. While electrode poisoning may be avoided by maintaining the pH above 4, serious error arises from another source. The excess phenylarsene oxide reacts with chromate to an appreciable extent before it can be titrated with standard iodine. When in accordance with the regular procedure 5 ml. of 0.00564 *N* phenylarsene oxide and 1 ml. of 5% potassium iodide solution were added to 200 ml. of water containing 100 p.p.m. of chromium at pH 4, there was a loss of 20% of the phenylarsene oxide in 4 minutes, 35% in 10 minutes, and 67% in 30 minutes. Since several minutes may elapse between addition of phenylarsene oxide and titration of the excess, the method cannot be relied upon even as an approximation.

In general, any procedure where an appreciable concentration of phenylarsene oxide does not have to remain unchanged in the presence of chromate will give satisfactory results, provided the necessary conditions are fulfilled. This means that the amperometric titration will be satisfactory in the presence of chromate wherever there is no interference from organic matter. It is believed that most wastes containing chromate will meet this requirement and can be analyzed by the direct titration. There may be instances where chromate gets into sewage and remains in the presence of organic matter for sufficient length of time to cause difficulty. As these instances are probably rare, the amperometric titration procedure is the method of choice.

HEAVY METAL IONS

The effect of various metallic ions on the amperometric titration procedure is of particular importance because they frequently are present where this procedure is to be used. A series of the most common metallic ions was tested for their effect in two ways. First, the relation between concentration of metal ion and current through the polarized cell was determined at pH values of 2, 4, and 7. In addition, electrode poisoning was detected by measuring the increase in current through the cell per unit concentration of iodine at pH 7 both before and after exposure of the cell to the metal ion.

Cadmium, trivalent chromium, divalent nickel, and zinc in concentrations up to 1000 p.p.m. had no immediate effect on the current flowing nor did they show any tendency to poison the electrode. Cuprous copper as the complex cyanide increased the current at pH 2, 500 p.p.m. of copper giving an increase approximately equal to that given by 1 p.p.m. of iodine. This concentration of copper showed a definite poisoning effect on the electrode at all three pH values. Cupric ion increased the current flowing at all pH values, 500 p.p.m. of copper being equivalent to somewhat less than 1 p.p.m. of iodine. At pH 4 and 7, 500 p.p.m. of copper showed a slight poisoning tendency on the electrode.

Silver as the complex cyanide did not give an increase in cell current at pH 7 nor did it show any poisoning effect at this pH. At pH 2 the complex ion was apparently decomposed, since there were both an increase in current and a poisoning effect. This conclusion is based partly on the result with silver ion, where as little as 5 p.p.m. gave an enormous increase in current at pH 4 and 7 and almost as much increase at pH 2. There was complete poisoning of the electrode at all pH values. It is concluded that the amperometric titration cannot give reliable results in the presence of cuprous ion and silver ion. Fortunately, neither is likely to be present in sufficient concentration once available chlorine persists in the solution. Cupric ion increases the current, so that at high concentrations measures have to be taken to compensate for this current. The poisoning effect of cupric ion probably would not interfere too seriously unless there was continuous exposure to high concentrations.

SUMMARY

The applicability of a given method for determination of residual chlorine in cyanide wastes depends on the detailed composition of the waste and the desired result. When heavy metal cyanides are absent and cyanide is to be converted to cyanate it is unnecessary to distinguish between free available chlorine and combined chlorine. The *o*-tolidine method, amperometric titration, and iodometric titration are all satisfactory.

When heavy metal cyanides are present or simple cyanide is to be completely destroyed, a definite concentration of free available chlorine is necessary. The amperometric titration with certain modifications is the method of choice.

Chromate ion interferes with the *o*-tolidine method, so that in chromate-bearing wastes the amperometric titration is used for determination of residual chlorine.

Of the heavy metal ions present in plating wastes, cuprous ion, silver ion, and high concentrations of cupric ion interfere with the amperometric titration of residual chlorine.

LITERATURE CITED

(1) Gilcreas, F. W., and Hallinan, F. J., *J. Am. Water Works Assoc.*, **36**, 1343–8 (1944).
(2) Mahan, W. A., *Water & Sewage Works*, **96**, 171–4 (1949).
(3) Marks, H. C., and Glass, J. R., *J. Am. Water Works Assoc.*, **34**, 1227–40 (1942).
(4) Marks, H. C., Williams, D. B., and Glasgow, G. U., *Ibid.*, **43**, 201–7 (1951).
(5) Moore, E. W., *Water & Sewage Works*, **98**, 130–6 (1951).

RECEIVED for review May 14, 1952. Accepted October 30, 1952.

Determination of the B.O.D. of Sewage and Industrial Wastes with the Polarograph

ARTHUR W. BUSCH AND CLAIR N. SAWYER
Sedgwick Laboratories of Sanitary Science, Massachusetts Institute of Technology, Cambridge, Mass.

THIS paper reports the results of an investigation to determine the practicability of using the dropping mercury electrode (polarograph) for dissolved oxygen measurements in B.O.D. (biochemical oxygen demand) studies.

Recent studies by Ruchhoft and his colleagues (*13–15*), Gotaas (*4*), and the Committee on Sanitary Engineering of the National Research Council (*2*) have shown great variations in the velocity constant of deoxygenation rates in polluted waters, thus indicating the need for the actual determination of deoxygenation rates for proper evaluation of B.O.D. data and stream conditions. Such determinations are usually based on daily B.O.D. values obtained over a 7-day period, and, therefore, multiply considerably the number of dissolved oxygen measurements required especially when replicate samples are set to obtain statistically reliable data. Under such conditions, use of the Winkler (*1*) method for measuring dissolved oxygen represents a laborious and time-consuming procedure. Furthermore, analysis of some industrial wastes requires special modifications of the Winkler procedure which add to the labor and time involved.

Application of the polarograph to dissolved oxygen measurements in B.O.D. studies requires control of two factors of prime importance:

1. Elimination of residual current determinations which require much more time than does the Winkler test.
2. Elimination of the addition of chemical reagents which preclude subsequent use of the Winkler test on the same samples to obtain calibration and/or correlation data.

The polarographic method of chemical analysis, developed by Heyrovský (*8*), is based on an interpretation of the current-voltage curves that are obtained when solutions of electroreducible or electro-oxidizable substances are electrolyzed, with one elec-

This investigation was made to determine whether the polarograph can be used as a labor-saving device for measurement of dissolved oxygen in B.O.D. determinations. The method, when using an applied voltage of −1.6 volts, was found to have an order of accuracy comparable to the standard Winkler technique. Residual current measurements were found unnecessary in the case of all the substrates and wastes studied at the concentration levels used in routine B.O.D. studies. The total elapsed time per determination averaged 75 seconds, which is approximately a 50% saving in time over that needed in the Winkler test when large numbers of samples are involved. In instances where radioactive samples are under study, the method will greatly reduce radiation hazards through elimination of sample overflow and need for titration.

trode consisting of mercury falling dropwise from a capillary glass tube of very fine bore. The recent appearance in the technical literature of articles by a number of authors (*5, 6, 10–12, 16*) indicates gradual acceptance of the polarographic determination of dissolved oxygen as a valuable analytical method in the field of sanitary engineering. However, as far as could be ascertained from a survey of the literature, no attempt has been made to apply dissolved oxygen measurements obtained with the polarograph to B.O.D. studies.

Substrates used in this work were a synthetic sewage of highly reproducible character (Table I) and a series of industrial wastes of widely varying composition—i.e., kier liquor, waste sulfite liquor, and formaldehyde, furfural, rag-rope, and tannery wastes. All samples subjected to the incubation test were seeded with 2 ml. of domestic sewage collected from the Ward Street pumping station in Boston.

The apparatus used in this work was a Fisher Electropode. A dropping mercury electrode, a Beckman No. 270 calomel reference electrode, and a thermometer were fitted into a special Lucite holder in such a manner that all elements could be inserted into the neck of a standard B.O.D. bottle. The flared mouth of the B.O.D. bottle fitted into an annular recess on the under side of the Lucite holder which served to center the electrodes. The dropping mercury electrode extended about 1.5 inches below the surface of the liquid when observations were taken. The mercury was allowed to fall into the bottle and did not interfere with the subsequent Winkler test for dissolved oxygen. Inclusion of a small receptacle to collect the mercury drops, so as to remove them from the bottle when the electrode assembly is withdrawn, might make successive readings on the same sample possible. Such a technique would reduce greatly the number of samples required in B.O.D. analysis where *k* and *L* values are being determined.

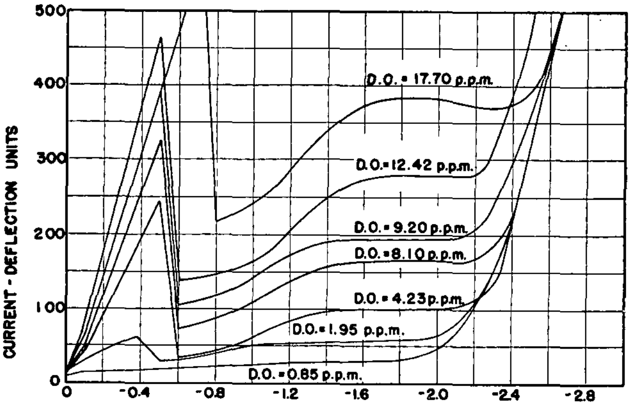


Figure 1. Current Voltage Curves for Dropping Mercury Electrode

Table I. Synthetic Sewage Formula

Constituent	Amount, Gram/Liter
Glucose	0.075
Starch	0.075
Peptone	0.075
Soap	0.0375

5-day B.O.D. approximately 200 p.p.m.

SELECTION OF OPERATING POTENTIAL

The proper potential at which to operate was selected by plotting current-voltage curves for the substrates in use. The potential was varied in increments of 0.1 volt over a range of 0 to 3 volts versus the calomel electrode. A series of samples of varying dissolved oxygen content was analyzed. Analyses of samples containing sufficient potassium chloride to make the solution 0.1 *N* were conducted, as well as studies of samples containing organic dye maxima suppressors. Current-voltage curves were obtained by plotting galvanometer deflection values against applied voltage.

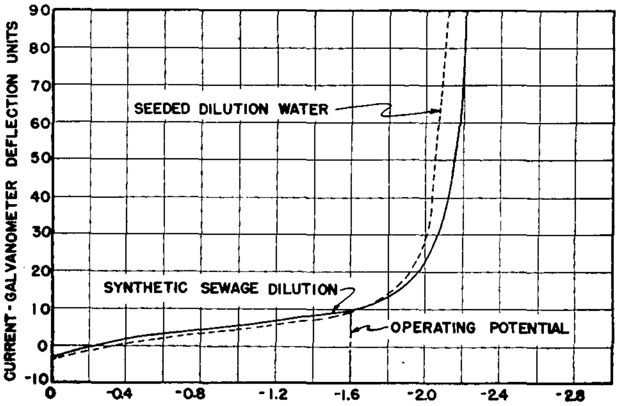


Figure 2. Residual Current Curves for Dropping Mercury Electrode

Both potassium chloride and the dyes suppressed the first wave maxima completely. Utilization of either substance made possible the operation of the polarograph at potentials of the first wave of cathodic oxygen discharge. However, the use of these substances was not considered permissible in B.O.D. work for a number of reasons:

1. Use of a dilution water made 0.1 *N* with potassium chloride reduced the solubility of oxygen significantly.
2. The presence of such a salt concentration resulted in an inhibiting action of variable nature on the rate of biochemical oxygen utilization.
3. The use of organic dye maxima suppressors in the dilution water was unacceptable because of their high B.O.D. characteristics.
4. The addition of either electrolyte or dye to the individual sample immediately before analysis was a return to time-consuming dosing and mixing procedures which are objectionable in the Winkler test and rendered questionable the results of analyses obtained by the Winkler procedure on the same samples.

Table II. Residual Current Values for Dilutions of Various Substrates at 1.6 Volts

Substrate	Incubation Time, Days 0	3	4	6
	Galvanometer Deflection Units			
Kier liquor	17	18	15	16
Waste sulfite liquor	17	18.5	14.5	15
Tannery waste	17	17	14.5	15
Rag-rope wastes	16.5	16.5	16	15.5
Formaldehyde waste	16	16.5	15.5	16
Furfural waste	18.5	18.0	14.5	16
Standard dilution water	18	17	14	16
Double salts dilution water	18	17.5	15	16.5
Domestic sewage	17	18.5	16	16

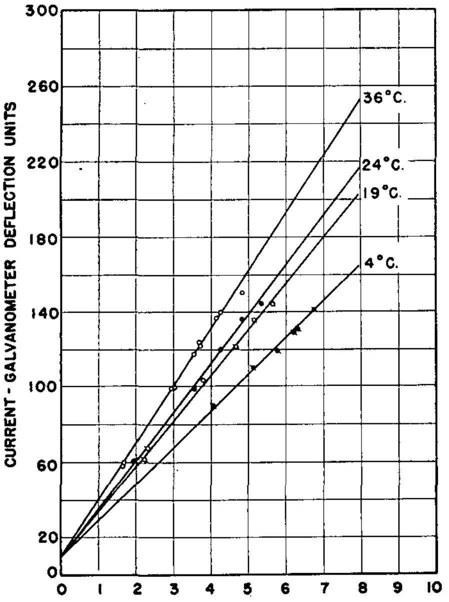


Figure 3. Effect of Temperature on Galvanometer Deflection

Fortunately, the excellently defined plateau of the second wave of oxygen discharge offered an alternative choice in the selection of the potential to be used. Rand and Heukelekian (*12*) object to the use of potentials related to the second wave because of the tendency of the wave to shift with dissolved oxygen concentration. However, Figure 1 shows the voltage selected for these studies (1.6 volts) to be applicable to dissolved oxygen concentrations up to at least 9.20 p.p.m., which is higher than levels normally encountered in B.O.D. work. Furthermore, the data indicate a potential of 1.8 volts would be satisfactory for dissolved oxygen concentrations as high as 18 p.p.m.

RESIDUAL CURRENT

A knowledge of the limited concentration of organic and inorganic substances which are added to standard dilution water in B.O.D. work makes it seem unlikely that such concentrations would have a significant effect on the residual current. However, because glucose has been quantitatively measured with the polarograph and other substances, such as castile soap and potassium chloride, have been used as maxima suppressors, it was deemed necessary to study this factor.

Extensive measurements of the residual current of seeded dilution water and some of the same water containing synthetic sewage were made at various applied potentials. The results are given in Figure 2 and show very little effect due to the added sewage at potentials up to 1.8 volts. Additional studies were made with routine dilutions of several industrial wastes at the selected potential of 1.6 volts. These studies were conducted at 0, 3, 4, and 6 days of incubation. The results presented in Table II show that none of the substrates affected the residual current values appreciably. The maximum variation for a given day was 2 deflection units, which was later found to correspond to 0.08 p.p.m. of oxygen, or a variation of ±0.04 p.p.m., which was well within the standard deviation for the instrument. The residual current appears to vary considerably with the capillary used; consequently, the data of Figure 2 and Table II may not be compared directly as the experiments were performed with different capillaries.

From these studies it was concluded that the residual current in samples set for B.O.D. analysis usually can be considered a constant and, if calibration curves include it, no correction for residual current need be made. This point should be checked when working with samples whose composition or behavior is not known.

EFFECT OF SAMPLE TEMPERATURE

The diffusion current obtained for any given concentration of a reducible substance increases as the temperature increases. It is therefore necessary to perform all polarographic analyses at the calibration temperature or to ascertain the effect of sample temperature on the diffusion current.

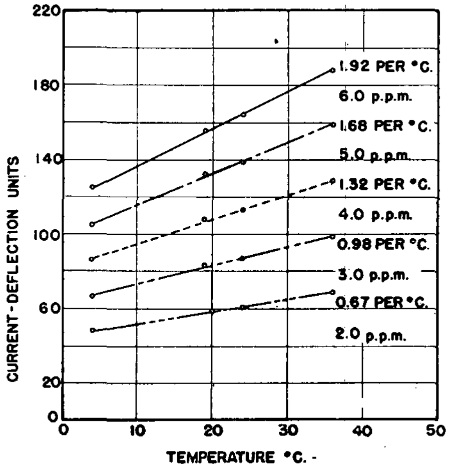


Figure 4. Effect of Temperature on Galvanometer Deflection at Constant Dissolved Oxygen Levels

The technique developed for determining this effect involved the setting up of a number of replicate samples identical to those used for B.O.D. measurements. The use of nitrogen to displace some of the dissolved oxygen in the samples enabled measurement of results over a wide range of oxygen concentrations to be made in a short time. Four temperatures of incubation were used, varying from 3° to 37° C. Three replicate samples were incubated for each dissolved oxygen concentration at the various temperatures. When the samples had reached constant temperature, each was analyzed by the dropping mercury electrode and by the Winkler procedure. Dissolved oxygen values plotted as abscissa versus galvanometer deflections yielded, in effect, calibration curves for four temperatures of incubation as shown in Figure 3. From these curves, data were taken at constant dissolved oxygen concentrations, and galvanometer deflections were plotted against temperature as abscissa (Figure 4). The slopes of the family of curves thus obtained were then plotted against galvanometer deflections read at 20° C. The slope of the resulting curve, Figure 5, represents the effect of temperature of galvanometer deflections at 20° C.—i.e., 1.28% per degree. A similar study made on blank samples yielded a correction of 1.22% per degree centigrade. The mean value of 1.25% was used in all subsequent studies.

Table III. Statistical Evaluation of Data Obtained by Dropping Mercury Electrode and Winkler Procedures in Dissolved Oxygen Analysis of B.O.D. Samples

(n = 50)

Method of Analysis	0-Day Dissolved Oxygen: Mean, $\bar{x}$, p.p.m.	Std. dev., σx, p.p.m.	% error, $\sigma x/\bar{x}$	5-Day Dissolved Oxygen: Mean, $\bar{x}$, p.p.m.	Std. dev., σx, p.p.m.	% error, $\sigma x/\bar{x}$
Electrode	8.75	0.068	0.774	4.49	0.094	2.10
Winkler	8.75	0.047	0.537	4.48	0.117	2.60

CALIBRATION PROCEDURE

One hundred and twenty samples were used for a calibration run. Twenty samples—6 blanks and 14 replicate B.O.D. samples—were analyzed each day for incubation periods of 0 to 5 days. Each sample was analyzed by the polarograph and by the azide modification of the Winkler procedure (*1*). This modification was used in all subsequent chemical analyses. Such a method of calibration gave due weight to possible variations in residual current occurring in the incubated samples and resulted in a progressive depletion of dissolved oxygen. The data accumulated during this procedure were treated as 120 individual results by the method of least squares.

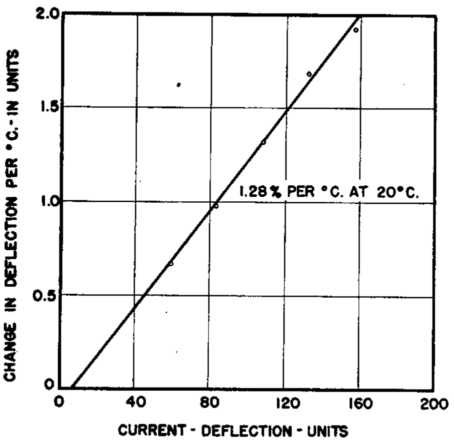


Figure 5. Absolute Effect of Temperature on Galvanometer Deflection at 20° C.

The resultant curve, Figure 6, conformed to the equation:

$$C = K_{20}[1 - 0.0125(T - 20)][D - D_0]$$

wherein

C = dissolved oxygen concentration, p.p.m.
T = sample temperature, degrees centigrade
D = galvanometer deflection
D_0 = galvanometer deflection due to residual current

CORRELATION OF B.O.D. RESULTS

Correlation studies of dissolved oxygen and B.O.D. results which were obtained with the dropping mercury electrode (D.M.E.) with results secured by the Winkler procedure consisted of two phases:

1. Statistical evaluation of dissolved oxygen data obtained in analysis of B.O.D. samples.
2. Comparison of parameter values obtained by the two methods of analysis during a series of experimental B.O.D. runs.

The first phase of the investigation was accomplished by setting up 100 identical B.O.D. samples containing a diluted synthetic sewage seeded with 2 ml. of domestic sewage per liter. Fifty of these samples were analyzed immediately by both the dropping mercury electrode and the Winkler procedures. The remaining 50 samples were incubated at 20° C. for 5 days, after which time they were subjected to the dual analysis technique. The mean, standard deviation from the mean (σ) and the per cent error were calculated for each method of analysis for each of the two groups of 50 samples. The tabulated results of this phase of the correlation studies (Table III) show that the precision of the polarographic analysis is comparable to that of the Winkler, and, because the test was performed on a substrate known to be free of Winkler interferences, the accuracy of data obtained with the dropping mercury electrode is acceptable, considering the Winkler test as a primary standard of dissolved oxygen measurement.

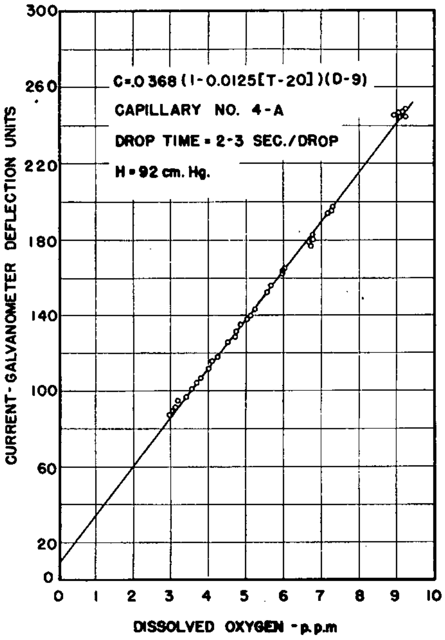


Figure 6. Calibration Curve for Dropping Mercury Electrode at 20° C.

The second phase of the correlation studies involved a series of experimental B.O.D. runs. Each value of daily B.O.D. was computed from the average of three blank samples and seven replicate synthetic sewage dilutions which had been inoculated with 2 ml. of domestic sewage per liter for seeding purposes. A summary of the data is given in Table IV.

Table IV. B.O.D. Data for Synthetic Sewage

Run	Method[a]	Daily B.O.D., P.P.M.: 1	2	3	4	5	k	L
1	W	95	116	157	190	188	0.161	234
	E	100	122	166	197	202	0.160	247
2	W	94	107	157	186	194	0.138	261
	E	104	121	165	194	202	0.109	279
3	W	99	142	163	182	187	0.310	193
	E	101	143	168	185	189	0.308	198
4	W	90	120	164	178	185	0.218	206
	E	89	118	158	174	184	0.220	201
5	W	92	140	162	162	182	0.373	178
	E	91	140	164	161	181	0.370	177
6	W	97	115	129	146	164	0.304	160
	E	99	112	128	144	166	0.262	166
7	W	96	107	121	152	158	0.187	180
	E	97	110	129	156	156	0.209	183
8	W	92	110	124	128	147	0.539	133
	E	90	110	120	130	150	0.480	136
9	W	100	125	164	188	208	0.184	236
	E	104	130	164	189	206	0.187	232
10	W	98	128	156	192	195	0.179	230
	E	102	132	154	192	192	0.212	216
11	W	96	125	164	182	197	0.209	216
	E	104	130	168	184	197	0.241	210

[a] W. Winkler.
E. Dropping mercury electrode.

Inspection of Winkler and dropping mercury electrode data for individual runs reveals excellent agreement of 5-day B.O.D. values. Velocity constants and ultimate first-stage B.O.D. results computed from daily B.O.D. data (*19*) are also in suffi-

Table V. Dissolved Oxygen and B.O.D. Data for Industrial Wastes

($n = 25$)

Sample	Method	Dilution, %	0-Day, P.P.M.		5-Day, P.P.M.		Depletion, P.P.M.	B.O.D., P.P.M.	$\frac{E - W}{W}$, %
			D.O.	σ	D.O.	σ			
Kier liquor		0.148							
	Electrode		8.97	0.121	2.58	0.045	6.39	4,320	0
	Winkler		8.82	0.090	2.43	0.045	6.39	4,320	
Rag-rope		0.030							
	Electrode		8.61	0.045	1.86	0.084	6.75	22,800	2.47
	Winkler		8.41	0.039	1.82	0.079	6.59	22,250	
Waste sulfite		0.021							
	Electrode		8.85	0.027	2.79	0.089	6.06	29,250	3.0
	Winkler		8.51	0.050	2.62	0.057	5.89	28,400	
Furfural		0.0018							
	Electrode		8.68	0.046	3.28	0.077	5.30	296,500	−3.89
	Winkler		8.72	0.050	3.25	0.079	5.47	308,500	
Formaldehyde		0.178							
	Electrode		8.83	0.046	3.27	0.040	5.56	3,135	3.3
	Winkler		8.63	0.036	3.25	0.049	5.38	3,035	
Tannery waste		0.089							
	Electrode		8.12	0.027	2.19	0.055	5.93	6,680	8.8
	Winkler		7.22	0.066	1.77	0.073	5.45	6,140	
Blanks		...							
	Electrode		8.71	0.055	8.56	0.058	...	...	...
	Winkler		8.71	0.040	8.53	0.071			

ciently close agreement to demonstrate clearly the validity of polarographic analysis in a single run.

INDUSTRIAL WASTE STUDIES

Polarographic analyses for dissolved oxygen in industrial wastes have heretofore involved laborious residual current determinations, and, in the concentrations studied, have been subject to interferences sometimes almost as extensive as those encountered with the Winkler test (*12*, *15*). However, no data on dilutions of industrial wastes applicable to incubation studies have been presented in the literature.

Table VI. Replication of Dissolved Oxygen Analyses in Presence of Industrial Wastes

($n = 10$)

Sample	Dilution, %	Electrode D.O., P.P.M.	Winkler D.O., P.P.M.
Group A			
Blanks		8.91	8.91
Kier liquor	0.148	8.94	8.88
Rag-rope	0.030	8.96	8.74
Tannery	0.089	8.91	8.29
Group B			
Blanks		8.78	8.77
Formaldehyde	0.178	8.79	8.74
Furfural	0.0018	8.76	8.74
Waste sulfite	0.021	8.64	8.39

Six industrial wastes of widely varying characteristics were selected for study. Table V shows results of 5-day B.O.D. determinations using 50 replicate samples of each waste. Twenty-five replicates were analyzed at 0 days and 25 after 5 days of incubation at 20° C. The calibration curve developed for synthetic sewage was used for all polarographic analyses. Only 0- and 5-day analyses were performed, as the samples tested at these times represented the maximum and minimum concentrations of organic matter. Of the six substrates analyzed, only the tannery waste results deviated from the Winkler by more than 5%. This deviation was to be expected, as indicated by Sheets (*17*).

In order to determine which of the variations between the Winkler and the polarographic results were due to the respective methods of analysis, comparison studies were conducted (Table VI). Samples were set up in dilutions applicable to B.O.D. determinations. As preliminary dilutions of the wastes were aerated to satisfy any possible immediate oxygen demand, and all bottles were filled with the same dilution water, all samples contained an equivalent amount of dissolved oxygen. Seeding materials were not introduced, thus eliminating another possible variable. Results showed that of the six wastes studied, only sulfite waste liquor interfered with the polarographic method of analysis, while tannery waste and waste sulfite liquor clearly produced low results in the Winkler analysis. These results substantiate the findings of Seaman and Allen (*16*), Sheets (*17*), and Theriault and McNamee (*18*).

In general, the dropping mercury electrode results showed a higher degree of replication between substrates than did the Winkler results. This fact leads to the postulation that the variations in the B.O.D. values of Table V may be due more to interferences with the Winkler test than to inaccuracies of the polarographic analysis.

SUMMARY

The polarographic method of dissolved oxygen analysis in B.O.D. determinations has been shown to be applicable to sewage and industrial wastes. A single potential technique eliminating residual current measurements and using only one calibration curve for a series of substrates is practical. The precision of the dropping mercury electrodes compares favorably with that of the Winkler method and in industrial waste studies, the accuracy of polarographic analysis appears to be somewhat greater. Considerable saving in time can be accomplished by the dropping mercury electrode method, as each sample requires less than 1.5 minutes for analysis and the need for time-consuming modifications of the Winkler procedure may be eliminated.

REFERENCES

(1) Am. Pub. Health Assoc., New York, "Standard Methods for the Examination of Water and Sewage," 1946.
(2) Committee on Sanitary Engineering, National Research Council, *Sewage Works J.*, **18**, 791 (1946).
(3) Croxton, F. E., and Cowden, D. J., "Applied General Statistics," New York, Prentice Hall, Inc., 1939.
(4) Gotaas, H. B., *Sewage Works J.*, **20**, 441 (1948).
(5) Ingols, R. S., *Ibid.*, **13**, 1097 (1941).
(6) Ippen, A. T., Yoseph, R. S., and Posthill, B. N., "Continuous Measurement of Oxygen Concentration in Water during Aeration Processes," *Tech. Rept.* **3**, Public Health Project No. **R.G.-863** (1951).
(7) Keilin, Bertram, "Adsorption of Capillary Active Substances at the Dropping Mercury Electrode," Ph.D. thesis, California Institute of Technology, 1950.
(8) Kolthoff, I. M., and Lingane, J. J., "Polarography," New York, Interscience Publishers, 1941.
(9) Kolthoff, I. M., and Miller, C. S., *J. Am. Chem. Soc.*, **63**, 1013 (1941).
(10) Moore, E. J., Morris, C. J., and Okun, D. A., *Sewage Works J.*, **20**, 1041 (1948).
(11) Petering, H. C., and Daniels, F., *J. Am. Chem. Soc.*, **60**, 2796 (1938).
(12) Rand, M. C., and Heukelekian, H., *Sewage & Ind. Wastes*, **23**, 1141 (1951).
(13) Ruchhoft, C. C., *Sewage Works J.*, **13**, 542 (1941).
(14) Ruchhoft, C. C., Placak, O. R., and Ettingen, M. B., *Ibid.*, **20**, 832 (1948).
(15) Ruchhoft, C. C., Placak, O. R., Kachman, J. F., and Cabert, C. E., *Ind. Eng. Chem.*, **40**, 1290 (1948).
(16) Seaman, W., and Allen, W., *Sewage & Ind. Wastes*, **22**, 913 (1950).
(17) Sheets, W. D., *Water & Sewage Works*, **95**, 240 (1949).
(18) Theriault, E. J., and McNamee, P. D., Ind. Eng. Chem., Anal. Ed., **4**, 59 (1932).
(19) Thomas, H. A., Jr., *Sewage Works J.*, **9**, 925 (1937).

Received for review May 14, 1952. Accepted September 22, 1952.

Determination of Microquantities of Iodine in Water Solution by Amperometric Titrations

H. P. KRAMER, W. ALLAN MOORE, AND DWIGHT G. BALLINGER
Public Health Service, Environmental Health Center, Cincinnati, Ohio

The amperometric titration method for determining micro quantities of iodine has the distinct advantage of ability to determine the concentration of the germicidal entities actually present at pH levels within the practical range of water treatment practice. The effect of ammonia on iodine residuals under the conditions of this investigation was found to be negligible. The oxidation state of iodine in water solution is a function of time and pH. At a given pH, free iodine residuals diminish as a function of time. This loss in free iodine concentration is caused by the oxidation-reduction reaction of hypoiodous acid, which results in the formation of iodate and iodide ions. The selection of a system of buffers which minimized the catalytic action encountered with certain buffers and the use of chlorine-demand-free water resulted in negligible loss of free iodine in a 30-minute contact period. In order to secure precise results when utilizing the amperometric titration method, the electrodes should be sensitized by an appropriate procedure. The determination of free iodine by the amperometric method was found to be accurate to ±0.01 p.p.m. in concentrations varying from 0.2 to 2.0 p.p.m.

THE effectiveness of free chlorine and chloramine as water disinfecting agents has been evaluated for different pH values and time intervals (*1*). Neither iodine nor bromine has been investigated as extensively as has chlorine and chloramines. Both iodine and bromine have been recommended as germicidal agents for swimming pools (*5*).

The objective of this study was the selection or development of a residual iodine test in water solution suitable for the concentration range 0.2 to 2.0 p.p.m. The method was to find immediate application in an investigation carried out at the Environmental Health Center on the effectiveness of iodine as a germicide in water solution.

In the study of the bactericidal properties of chlorine and chloramines, Chang (*2*) has shown how the chemical form of chlorine changes with variations in pH.

Marks and Strandskov (*4*) have evaluated the sporicidal efficiencies of chlorine, bromine, and iodine with respect to pH and, based upon equilibrium considerations, have shown how the chemical composition of the water solution of each of the halogens varies with pH. With chlorine, the predominant compound present over the range of pH 2 to 8 is hypochlorous acid; there is no free chlorine above pH 2. With iodine the molecular entity predominates below a pH of 8 at which level significant hypoiodous acid formation occurs. These authors show that the molecular iodine has the highest sporicidal efficiency. Other investigators have shown that iodine and hypoiodous acid have germicidal properties in water solution (*7*).

Iodine undergoes certain fundamental reactions in water solution, as shown by Wyss and Strandskov (*7*). In these reactions pH is the dominating factor in determining the chemical entity present. The oxidation-reduction reaction in which both iodic acid and iodide ion are formed has been shown (*7*) to be catalyzed much more strongly by phosphate buffers than by certain other buffers.

In this study phosphate, phthalate, glycocoll, borate, and citrate buffers were investigated. Of these buffers it was found that borate and citrate had the least effect on the stability of hypoiodous acid.

Theoretically, in a water-iodine system, the relative and absolute concentrations of iodine, hypoiodous acid, iodide ion, and iodate ion determine the germicidal efficiency. A trial run was made to evaluate the germicidal effect of the iodide and iodate, and the results indicate that in the range of concentrations under investigation, these two entities were not germicidal. Accordingly, analytical methods were sought which would quantitatively measure iodine and hypoiodous acid (here called free iodine) and which would include no iodide and iodate. The method selected must be efficient in the pH range 6.5 to 9.15.

Various colorimetric methods were tried in an attempt to develop a suitable procedure. Fluorescein gave poor color differentiation with small changes in iodine concentration. Both *p*-aminodimethylaniline and tetramethyl benzidine gave excellent color development with a sensitivity of 0.05 p.p.m. within the range of 0.05 to 0.2 p.p.m. The best color development and the least color production in the blank were obtained at pH 2 for both reagents. The requirement of pH adjustment nullified the usefulness of these methods for this study.

Amperometric determination of the free iodine was investigated using a Wallace and Tiernan amperometer with phenylarsene oxide as the standard titrating solution. Marks and Glass (*3*) claim an accuracy of 0.01 p.p.m. for chlorine by this method, which is equivalent to approximately 0.04 p.p.m. of iodine.

It was found that free iodine concentrations could be determined by this method without pH adjustment over the range investigated—namely, 6.5 to 9.15. Thus the free iodine could be determined at the pH actually employed in the bactericidal tests.

Table I. Effect of Time on Free Iodine Concentration

pH	Initial Free Iodine Concn., P.P.M.	Free Iodine Concentrations, P.P.M. Elapse of time, minutes 30	60	90	120	Free Iodine Concn., P.P.M. after Adjustment
6.5	0.25	0.23	0.21	0.19	0.18	0.23
	0.60	0.58	..	0.54	0.52	0.59
	0.78	0.76	0.72	0.71	0.62	0.75
	2.59	2.55	2.47	2.41	2.41	2.52
9.15	0.25	0.25	0.22	0.18	0.18	0.24
	0.53	0.52	0.49	0.47	0.46	0.60
	0.97	0.91	0.86	0.81	0.71	0.88

The possibility of any iodine ammonia-nitrogen reaction was of interest in this study, since such a reaction would exert an iodine demand. To determine this factor, ammonia nitrogen solutions in unbuffered chlorine-demand-free water in concentrations of 0.1 and 0.2 p.p.m. were prepared prior to the addition of definite amounts of iodine. After a 2-hour interval the iodine residuals were determined amperometrically. Under the conditions of this experiment, no significant iodamine formation was evident. This fact does not preclude such formation under other conditions.

It was assumed that chlorine-demand-free water would also be iodine-demand-free because of the greater chemical activity of chlorine. This assumption appears to be justified based upon the results shown under experimental conditions. All water used in these determinations was made chlorine-demand-free by a slight modification of the method of Megregian (*6*). Sulfite addition was stopped when the chlorine residual dropped below 0.005 p.p.m. and boiling was substituted to remove the last traces.

Table II. Stability of Iodine Residuals

(30 minutes at room temperature in buffered waters)

No. of Detns.	pH	Average P.P.M. Initial	Average P.P.M. Final	Average P.P.M. Loss	Maximum Loss Shown by Any Sample in This Series
6	6.5	0.22	0.19	0.03	0.05
43	7.5	0.21	0.21	0.00	0.04
14	8.5	0.20	0.19	0.01	0.05
24	9.15	0.21	0.19	0.02	0.05
7	6.5	0.44	0.40	0.04	0.07
49	7.5	0.42	0.41	0.01	0.05
14	8.5	0.42	0.41	0.01	0.04
24	9.15	0.44	0.42	0.02	0.06
11	6.5	0.61	0.60	0.01	0.04
51	7.5	0.62	0.60	0.02	0.06
14	8.5	0.61	0.60	0.01	0.06
25	9.15	0.62	0.58	0.04	0.07
12	6.5	0.81	0.79	0.02	0.05
46	7.5	0.81	0.78	0.03	0.07
14	8.5	0.80	0.78	0.02	0.06
26	9.15	0.81	0.78	0.03	0.08
7	6.5	1.01	1.00	0.01	0.03
33	7.5	1.00	0.98	0.02	0.07
6	8.5	1.01	0.98	0.03	0.05
30	9.15	1.01	0.97	0.04	0.08
7	6.5	1.61	1.60	0.01	0.03
8	7.5	1.62	1.58	0.04	0.06
10	8.5	1.43	1.43	0.00	0.02
21	9.15	1.60	1.56	0.04	0.09
4	6.5	2.02	2.02	0.00	0.01
16	7.5	2.03	1.99	0.04	0.14
8	8.5	1.81	1.81	0.00	0.01
22	9.15	2.04	1.98	0.06	0.13

Extending the amperometric method to measurements of low concentrations of iodine with the apparatus specified, led to difficulties in obtaining reproducible results. The necessity for sensitizing the electrode to iodine was observed. Either a preliminary 0.5-hour soaking of the electrode in an approximately 2 p.p.m. iodine solution or making three to five titrations on an iodine solution before the actual titration of the sample proved successful. Furthermore, the sensitivity of the electrode could be maintained by removing the end products of the titration as soon as possible.

EFFECT OF TIME ON FREE IODINE CONCENTRATION

In order to demonstrate that the oxidation-reduction reaction exhibited by hypoiodous acid is a function of time, the authors decided to reverse this reaction after a suitable period by the addition of hydrogen ion. Results obtained from one determination, run in triplicate, at each of the iodine concentrations and pH values indicated are given in Table I. The following procedure was used:

Iodine stock solution was added to 2 liters of buffered chlorine-demand-free water. Three 100-ml. aliquots were diluted to 200 ml. with water and titrated. The average of these results was reported as initial free iodine concentration.

The amperometric determinations of free iodine were made at 30, 60, 90, and 120 minutes on the iodine solution prepared.

After the 120-minute period, 10 ml. of a 7% solution of potassium iodide were added to another aliquot.

The pH was then lowered to between 2 and 3 with 1% sulfuric acid, then raised to approximately 6 with 0.1 *N* sodium hydroxide. One milliliter of citrate buffer was then added to hold the pH at 6.5.

The result of the titration then performed was called free iodine concentration in p.p.m. after adjustment.

Table I shows an increasing loss of free iodine with increasing time. Solutions of greater concentration exhibited the greater loss. The reaction is shown to be reversible since the free iodine after the pH adjustment is approximately equal to the initial concentration.

One of the conditions required for the germicidal testing of iodine residuals was a stable pH for the period of exposure of the test organism. It was, therefore, desirable to buffer the solutions at the required pH levels with a buffer which would be nontoxic and would not catalyze the oxidation-reduction reaction of iodine in solution. Borate and citrate buffers most nearly satisfied these requirements. In the procedure, as outlined below, Palitzch borate buffer was used at 9.15, 8.5, and 7.5, using 10 ml. per liter. For pH 6.5 Sorensen's citrate buffer was used, 10 ml. being added per liter of sample. With the citrate buffer it was found necessary to make the addition subsequent to preparation of the chlorine-demand-free water to avoid the decomposition of the citrate in the presence of chlorine at high temperature. (A Beckman line-operated pH meter was used in making all pH measurements.)

PROCEDURE

Make all water used in the following procedure chlorine-demand-free by the previously mentioned method. Sensitize the electrode immediately before use by soaking in an approximately 2 p.p.m. iodine solution for about 30 minutes or by making three to five preliminary titrations on an iodine solution (1 to 2 p.p.m.).

1. Stock solution: Dissolve 0.50 gram of iodine in 300 ml. of ethyl alcohol (95%). Dilute this solution to 1000 ml. with water. Allow to stand for at least 24 hours before using to satisfy any halogen demand of the water and glassware.
2. Buffer solution, use 10 ml. per liter: Sorensen citrate buffer for pH 6.5; Palitzch borate buffer for pH 7.5, 8.5, 9.15.
3. Dilute the stock solution 1 to 500 with buffered water.
4. Pipet three 100-ml. aliquots of the diluted stock solution into titration cells and dilute each to 200 ml. with water.
5. Titrate amperometrically with an approximately 0.003 *N* phenylarsene oxide solution. (For convenience a 1 to 1 dilution of the solution furnished by Wallace and Tiernan may be used. *N* = 0.00282; 1 ml. = 0.358 mg. of iodine.)
6. Dilute and titrate the sample in the same manner as in 4 and 5.
7. Report mean result as the free iodine concentration.

Table III. Precision of Amperometric Titrations

Iodine Concn., P.P.M.	No. of Replicates	Range	Standard Deviation
0.13	10	0.02	0.01
0.20	10	0.02	0.01
0.26	10	0.02	0.01
0.52	10	0.04	0.01
1.01	10	0.04	0.01
2.06	10	0.04	0.01

STABILITY STUDY

Actual results obtained in the bacteriological experiments on free iodine concentrations of 0.2 to 2.0 p.p.m. at pH 6.5, 7.5, 8.5, and 9.15 are shown in Table II. Each determination is the mean of three replicate titrations on a sample. The means of concentrations and losses for the indicated number of determinations of each concentration and at each pH level are shown. Generally the tendency exists for the largest losses to appear at the highest pH levels. The maximum loss exhibited by an individual sample was found at the highest concentration used. The greatest mean loss was shown by the samples of highest pH and highest concentration. The stability of the iodine solutions under the conditions specified was suitable for the bacteriological studies.

PRECISION OF METHOD

To establish the precision of the amperometric titration method for the measurement of iodine in the concentration range of interest in this study, unbuffered solutions were titrated in replicate. The pH values in all cases were between 6.6 and 6.8. Table III gives the range and standard deviation for each series.

These values indicate the feasibility of the amperometric method in measuring low iodine residuals.

LITERATURE CITED

(1) Butterfield, C. T., *U. S. Pub. Health Repts.*, **63**, 934–40 (1948).
(2) Chang, S. L., *J. Am. Water Works Assoc.*, **36**, 1192 (1944).
(3) Marks, H. C., and Glass, J. R., *Ibid.*, **34**, 1227 (1942).
(4) Marks, H. C., and Strandskov, F. B., *Ann. N. Y. Acad. Sci.*, **53**, 163 (1950).
(5) Marks, H. C., and Strandskov, F. B., U. S. Patent 2,443,429 (1948).
(6) Megregian, Stephen, *U. S. Pub. Health Repts.*, **63**, 137–41 (1948).
(7) Wyss, O., and Strandskov, F. B., *Arch. Biochem.*, **6**, 261–8 (1945).

RECEIVED for review May 28, 1952. Accepted July 24, 1952.

Determination of Copper Ion by a Modified Sodium Diethyldithiocarbamate Procedure

Iron, Zinc, and Lead Interference Eliminated

C. A. NOLL AND L. D. BETZ
W. H. & L. D. Betz, Gillingham and Worth Sts., Philadelphia 24, Pa.

The American Public Health Association's method for the determination of copper ion in water involves the use of sodium diethyldithiocarbamate, to produce a yellow color suitable for colorimetric estimation. Iron interferes, and zinc and lead produce interfering turbidity. By reducing the concentration of the sodium diethyldithiocarbamate to 1% of the strength previously used and diluting the ammonium hydroxide reagent, it was found possible to eliminate these interferences. With modified procedure small amounts of copper can be determined accurately by a simple, rapid procedure, without solvent extraction.

THE present method for the determination of copper ion in water (*1*), involves the use of sodium diethyldithiocarbamate to produce a yellow color suitable for colorimetric estimation. Iron interferes with this method, and an extraction procedure with a solvent such as carbon tetrachloride is necessary when iron is present. Zinc and lead produce an interfering turbidity, and extraction with carbon tetrachloride is required in the presence of these ions. Blanks must be prepared by a similar extraction procedure.

Table I. Concentrations Tolerable without Interference

(At copper concentration = 5.0 p.p.m.)

	P.P.M.		P.P.M.
Na_2SO_4	1000	K_2CrO_4	500
Na_2SO_3	1000	Quebracho tannin	50
NaCl	1000	Chestnut tannin	50
$NaNO_2$	1000	$Na_5P_3O_{10}$	5
KNO_3	1000	Fe^{+++}	10
$CaCl_2$ as $CaCO_3$	1000	Zn^{++}	10[a]
$MgSO_4$ as $CaCO_3$	1000	Pb^{++}	2[a]

[a] Data supplied by Edward Chow, assistant public health chemist, California Department of Public Health.

Table II. Effect of 10 P.P.M. of Fe^{+++} at Low Copper Concentrations

Present, Copper as Cu, P.P.M.	Found, Copper as Cu, P.P.M.		
	Analyst A	Analyst B	Analyst C
0.0	0.00	0.02	0.00
0.1	0.09	0.07	0.10
0.25	0.26	0.23	0.23
0.50	0.50	0.48	0.52

It was thought that by reducing the concentration of the reagents employed in this test, it might be possible to reduce or eliminate the interference of iron in particular. Accordingly, tests were conducted with successive dilutions of the reagents until it was found that iron interference was eliminated. The concentration of the sodium diethyldithiocarbamate solution was reduced to 1% of the strength previously used, and the ammonium hydroxide reagent was also diluted. By these measures it was found possible to eliminate the interference due to iron, zinc, and lead. With the modified procedure, small amounts of copper can be determined accurately in the presence of these potentially interfering ions by a simple, rapid procedure, without solvent extraction.

APPARATUS

Both a Leitz-Rouy photometer and a Klett-Summerson photometer have been satisfactorily employed for this determination. Both instruments used were equipped with a logarithmic scale. Nessler tubes can also be employed with visual comparison.

REAGENTS

Ammonium hydroxide, 0.25 *N*.
Sodium diethyldithiocarbamate, 0.1 gram per liter.

PROCEDURE

With the Leitz-Rouy photometer, the 415-mμ color filter and the 20 × 20 mm. absorption cell are employed.

A "zero" reference blank is prepared by adding to a beaker 10 ml. of sample, 10 ml. of 0.25 *N* ammonium hydroxide, and 10 ml. of distilled water. This blank sample is used to set the meter pointer at zero immediately prior to test.

To a second beaker, 10 ml. of sample, 10 ml. of 0.25 *N* ammonium hydroxide, and 10 ml. of sodium diethyldithiocarbamate are added. The solution is transferred to cell and photometer is read in 1 to 5 minutes. Photometer reading is translated to parts per million of copper by reference to a standard curve calibrated under these same conditions and covering the range of 0 to 5 p.p.m. of copper as Cu.

RESULTS AND DISCUSSION

Table I shows the concentrations of ions that can be tolerated without interference with the test. Up to 10 p.p.m. of iron can be tolerated, but above this concentration a precipitate forms on the addition of the ammonium hydroxide. Below 10 p.p.m., iron is not precipitated by the ammonium hydroxide, but is held in an apparent colloidal suspension.

Table III. Effect of Varying Quantities of Test Reagents

Cu Present, P.P.M.	NH_4OH, Ml.	Thiocarbamate, Ml.	Cu Found, P.P.M.	Cu Corrected for Error in Vol., P.P.M.
3.00	10	10	3.00	..
3.00	10	11	2.92	3.02
3.00	10	9	3.16	3.05
3.00	11	10	2.94	3.04
3.00	9	10	3.14	3.04
3.00	9	9	3.24	3.02
3.00	11	11	2.87	3.06

Table II shows the effect of the presence of 10 p.p.m. of iron as Fe at low copper concentrations. Even at these low concentrations of copper, iron does not interfere.

The color produced in the test follows Beer's law. Maximum absorption is obtained at 448 mμ as determined on a Beckman Model DU spectrophotometer. No effect of temperature was found over the range of 20° to 36° C.

In fifteen tests, by three different analysts, on samples containing from 0 to 4 p.p.m. of copper, the maximum error encountered was 0.05 p.p.m.

Tests were also conducted to determine the error introduced by inaccurate measurement of the test reagents. Table III shows the data obtained with ±1 ml. variation in either and both test reagents. When the reading is corrected for the error due to the different volume, excellent checks are obtained. These results indicate that, using Nessler tubes for comparison, no error will be introduced by slight variations in the amount of reagents.

This modified method of copper determination has also been applied to a Klett-Summerson photometer employing a 420-mμ filter and 13-mm. depth test cell. The same procedure is recommended except that 5 ml. of test sample, 5 ml. of ammonium hydroxide, and 5 ml. of sodium diethyldithiocarbamate are employed. A straight-line calibration curve is obtained.

Recommended copper ranges for cells of different depths are listed below. Above 10 p.p.m. of copper, the line tends to flatten out and is no longer a straight line.

Cell Depth, Mm.	Copper Range, P.P.M.
20	0–5
10	0–10
5	0–10

This method of copper determination has been studied by Subcommittee W-9 (S-12) of the American Water Works Association and recommended for the next (tenth) edition of "Standard Methods for the Examination of Water and Sewage."

LITERATURE CITED

(1) American Public Health Association, "Standard Methods for the Examination of Water and Sewage," New York, 9th ed., pp. 47–9, 1946.

RECEIVED for review May 14, 1952. Accepted August 6, 1952.

[End of Symposium on Analytical Methods for Industrial Wastes]

High Sensitivity Recording Polarograph

MYRON T. KELLEY AND HUGH H. MILLER

Oak Ridge National Laboratory, Carbide and Carbon Chemicals Co., Division of Union Carbide and Carbon Corp., Oak Ridge, Tenn.

The polarograph described is intended to extend the useful range of polarography to the 10^{-6} *M* region, as the analysis of such dilute solutions is frequently valuable in chemical analysis of radioactive materials. The instrument described has useful current sensitivity ranges from 0.01 to 10 μa. for full scale deflection of the recorder. It is provided with a linear compensator to minimize the effect of condenser current on the recorded polarograms and also a "curve follower" which can be used to subtract the peculiar, nonlinear blank curves which are obtained at high sensitivities. The instrument has been used for the determination of the diffusion current for lead in solutions of 2×10^{-7} *M* to 2×10^{-6} *M* with an estimated precision within ±20%. The diffusion current is shown to be proportional to concentration in this concentration range, although the diffusion coefficient seems to be somewhat different than at higher concentrations.

POLAROGRAPHY is readily adaptable to the analysis of radioactive materials because of the few manipulations required. This laboratory has been interested in applying this method to highly radioactive samples where the size of the sample must be severely limited. When such is the case (as with samples less than 0.1 ml. in volume), one must either adopt micropolarographic techniques or analyze very dilute solutions with more conventional polarographic cells (1 to 10 ml. of solution). The latter system was chosen for study at this laboratory because of mechanical difficulties in handling micropolarographic equipment behind barricades used for radiation shielding.

The aim in this work was to obtain readily measurable waves at concentrations of 10^{-6} *M* or lower. The diffusion current to be expected at such concentrations is of the order of 5×10^{-3} microampere. On any of the commercially available polarographic instruments, this represents a deflection of less than 1 mm. or essentially an unmeasurable current. Attempts to increase the sensitivity of available recording instruments by increasing the resistor across which voltage drop is measured led to an increase in the dead space of instruments using a low-impedance electronic recorder of conventional type. As the advantages of the pen recording instruments with galvanometers are self-evident, a recording instrument has been used for this work.

DESCRIPTION OF INSTRUMENT

The instrument is conventional in many respects—i.e., it uses a motor-driven Helipot to supply the polarizing voltage, and the cell current is measured by recording the potential drop across a known resistor by means of a Brown recorder. However, it incorporates several features which are not found in commercial instruments.

A preamplifier is used with the Brown recorder, so that it will operate from current-measuring resistors up to 1 megohm without appreciable dead space. A recorder with a 1-mv. range for full scale is used in order to hold the potential drop in the measuring resistors to a minimum.

A damping circuit employing high resistance and relatively low capacity (10 μfd.) is used, so that the time constant is almost independent of the value of the current-measuring resistor. This also avoids the necessity of using electrolytic capacitors, which sometimes show objectionable leakage effects.

A linear compensator is incorporated similar to that proposed by Ilkovič and Semerano (2) which bucks out the so-called "condenser current." Over considerable portions of the potential range the condenser current increases almost linearly; hence an increasing potential of opposite sign applied to the recorder can remove most of the effect of this condenser current.

A device known as a "curve follower" is incorporated for the purpose of subtracting the blank due to the supporting electrolyte. Without such a device, the blank effects can sometimes completely disguise the wave due to a very small concentration of a reducible ion.

The instrument is built in three separate units connected as shown in the block diagram in Figure 1. The polarizing unit is conventional, with the exception of the "residual current compensation" mentioned.

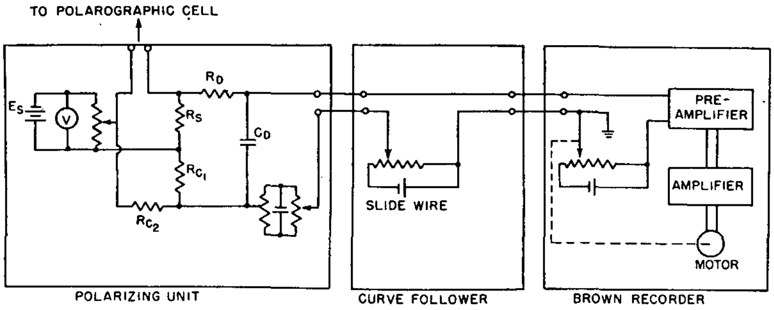


Figure 1. Block Diagram of Polarograph

Figure 1 shows the potential source, E_s, with the motor-driven voltage divider. Resistor R_s determines the current sensitivity, while resistors R_{c1} and R_{c2} determine the extent of linear compensation applied to counteract the condenser current. C_D and R_D form an RC damping circuit to reduce the magnitude of recorder oscillations, especially at high sensitivities. The block diagram shows only the slide-wire of the curve follower, which is described in more detail below. The Brown recorder is conventional except for the preamplifier.

The polarizing unit is shown in detail in Figure 2. A battery, E_1, supplies a fixed potential, measured on voltmeter V_1, to a motor-driven Helipot, R_{2A}, from which the polarizing voltage for the polarographic cell is drawn. The 10-turn Helipot, R_{2A}, is turned by a 1 r.p.m. Bodine motor, with provisions for reversing and a friction clutch to allow a quick return to zero potential. A second potential source, E_2, can be used to supply an initial potential up to 3 volts, either positive or negative. Thus the total useful voltage range is from +3 to −4.5 volts.

The current through the polarographic cell is measured by recording the potential drop in a series of standard ($\pm 1\%$) resistors, R_{11} to R_{22}. Damping is supplied by the RC combination, R_6 and C_1, with a time constant up to 20 seconds. The network between the two large electrolytic condensers, C_2 and C_3, provides a means of setting the zero of the instrument and also is useful to buck out constant currents due to causes other than the diffusion current of the ion desired. The range of R_{10} (a 200-ohm Helipot) is about 30 times the full scale deflection of the recorder. The true zero is at midscale or about 5.0 on the Helipot dial.

The remaining resistor network, consisting of R_{2B}, R_3, R_4, and R_{23} to R_{30}, constitutes a linear compensator to buck out the "condenser" current at high current sensitivities with the dropping mercury electrode. With R_4 out of the circuit (on point 1 of switch S_4) and S_3 closed, this network supplies a potential to the recorder which is opposite in sign to the normal component of cell current and increases with the increase of potential from R_{2B} (which is directly coupled to R_{2A}). The degree of compensation can be adjusted by setting R_3 (a 10-turn Helipot), or the compensation can be eliminated by opening S_3. R_4 can be shunted across three different fixed taps on R_{2B} (at 2, 4, or 6 turns) so that correction can be applied according to two different linear formulas, changing at a preselected point. To date, this feature has not proved very useful, because the blank curves do not show abrupt changes of slope which can be closely approximated by two linear curves. The curve follower has proved more useful to compensate for nonlinearity in the blanks.

The recorder used in this instrument is a Brown recorder modified by the addition of a preamplifier (Figure 3) as described by Rosenbaum and Stanton (4). The circuit of Rosenbaum and Stanton has been changed in the following respects:

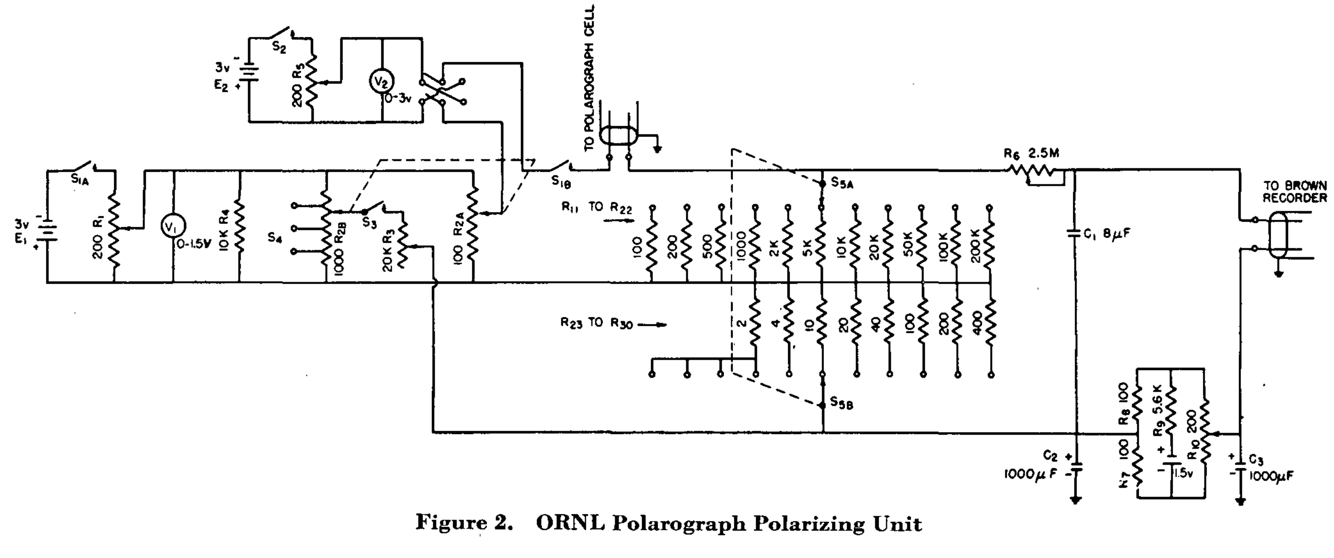


Figure 2. ORNL Polarograph Polarizing Unit

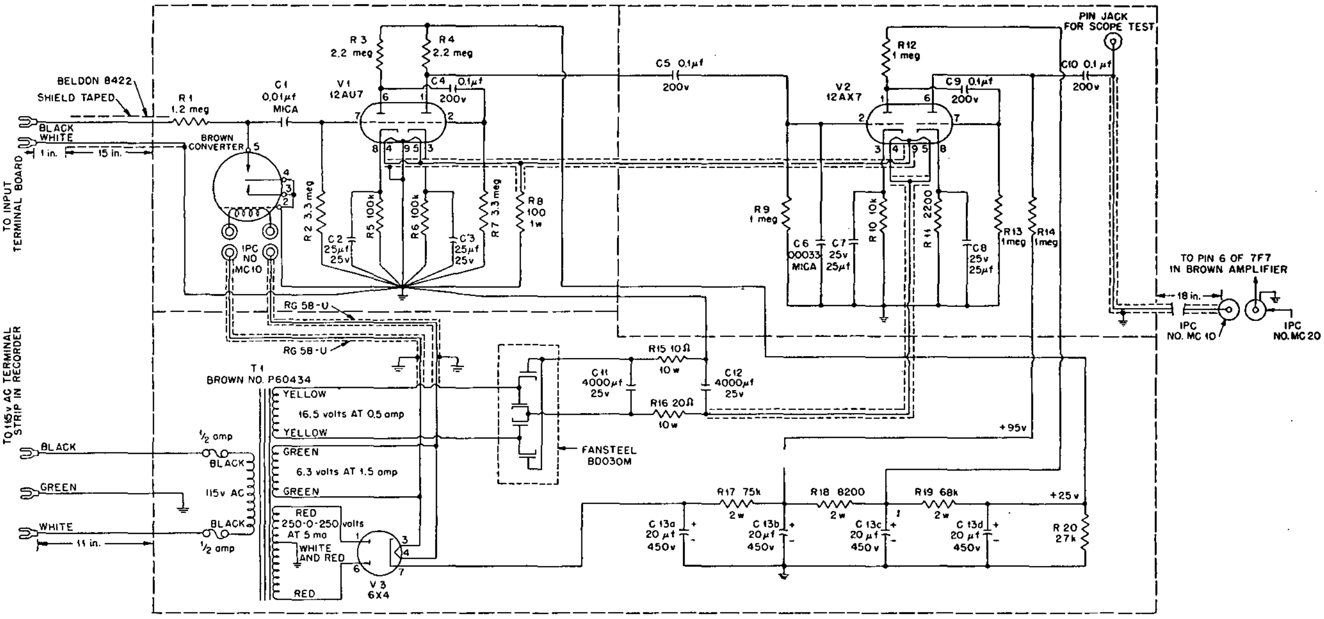


Figure 3. Preamplifier Used with Brown Recorder

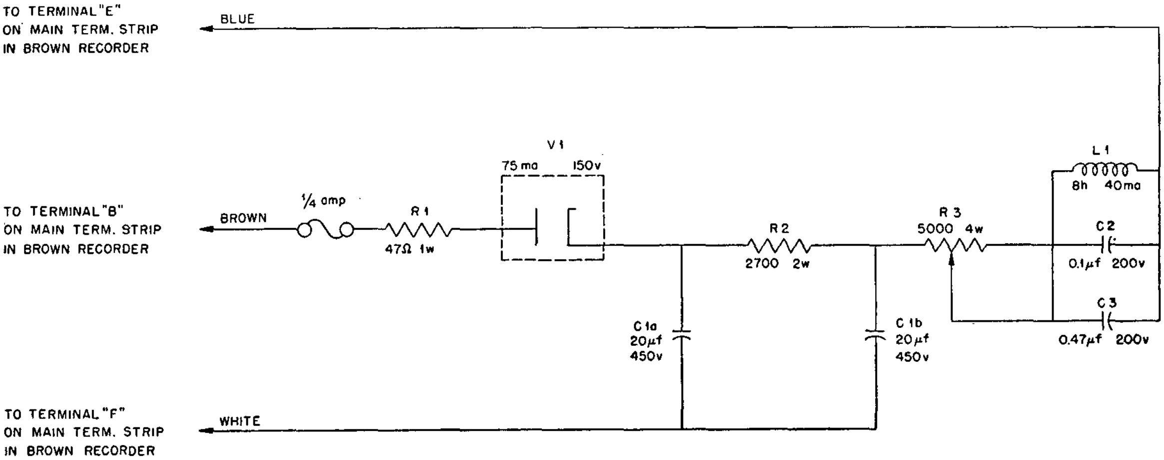


Figure 4. Balance Motor Damping Unit

The input resistor is changed to 1.2 megohms, as that is sufficiently high for this purpose and less undesirable pickup results with the lower resistor. Along with this the grid resistor of the first stage is changed to 3.3 megohms and the coupling condenser to 0.05 µfd. to maintain the same time constant as the original circuit.

A second twin-triode (12AX7) is added in the preamplifier, as it was found that the signal level after the first two stages was so low that appreciable pickup occurred in transferring to the Brown amplifier at this point. By transferring the function of the first 7F7 in the Brown amplifier to the 12AX7 in the preamplifier alternating current pickup difficulties were greatly reduced and the modifications necessary in the regular Brown amplifier were reduced to installing a plug connecting to pin 6 of the first 7F7 (which is removed).

As in any other high-gain amplifier, alternating current pickup elimination was troublesome. The two chief precautions observed were the use of a single ground point on the chassis for all grounds in the first two stages, and the use of a Brown converter (chopper) which had the connections for the driving coil entering from the top rather than through the usual pin connections in the base.

It was found that 12AU7 tubes manufactured by General Electric were less microphonic than others tried. 12AY7 tubes made by this manufacturer can be used interchangeably in the first stages.

Figure 4 shows a very simple circuit used to introduce more damping on the motor of the Brown recorder, as this was found desirable to eliminate overshoot when a high speed recorder was used.

The curve follower is used only at the higher sensitivities for the subtraction of the blank curve from that obtained on a sample. This device, first described by Coor and Smith (*1*), is constructed by modifying a Brown recorder as indicated in Figures 5 and 6.

Table I. Polarographic Diffusion Current of Lead in Very Dilute Solutions

[Dropping mercury electrode ($m^{2/3}t^{1/6} = 2.17$)]

Pb^{++} Concn., Micromoles/Liter	Diffusion Current, µa. × 10^3	i_d/C, µa./Millimole/Liter
0.24	1.33	5.54
0.48	2.83	5.90
0.72	3.56	4.94
1.20	5.44	4.53
2.39	11.48	4.80
7.2[a]	39.4	5.47
72[a]	568	7.89
720[a]	5830	8.10

[a] Run without curve follower.

The pen of the Brown recorder is replaced by a photocell unit which "observes" the chart through a microscope objective. The photocell amplifier is adjusted so that the output is zero when the photocell sees an area half black and half white. When the field is predominantly black, the output drives the unit to the right, while a predominance of white drives it to the left. The photocell unit thus will follow the right-hand edge of a broad black line. A suitable curve is derived from the polarogram obtained on a blank by tracing over the tips of the oscillations onto a sheet of unruled recorder paper by use of a light box or a similar technique. If this curve is then run through the curve follower at the same time that a polarogram is being recorded, the curve follower subtracts at each point a potential equal to the ordinate of the blank curve. In this way a blank curve which is far from linear can be made to approximate a straight line.

APPLICATION OF INSTRUMENT

Some typical blank curves are shown in Figure 7. Curve *A* shows a blank on 1 *M* potassium nitrate with no compensation of any kind. It is obvious that no polarographic waves could be measured on this sort of a curve. Curve *B* shows the same blank

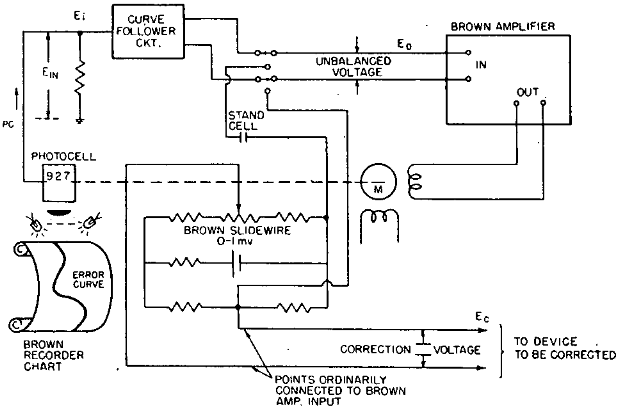


Figure 5. Block Diagram of Curve Follower

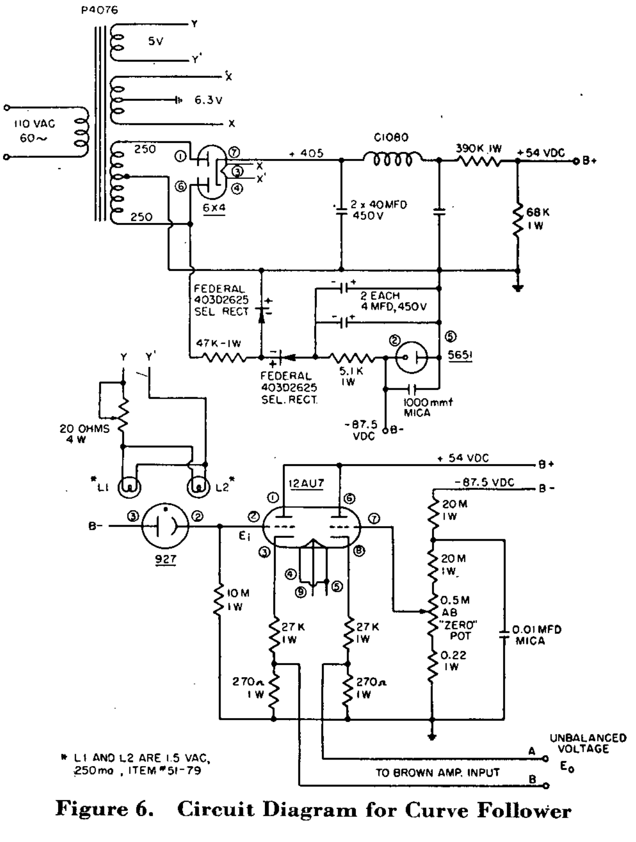


Figure 6. Circuit Diagram for Curve Follower

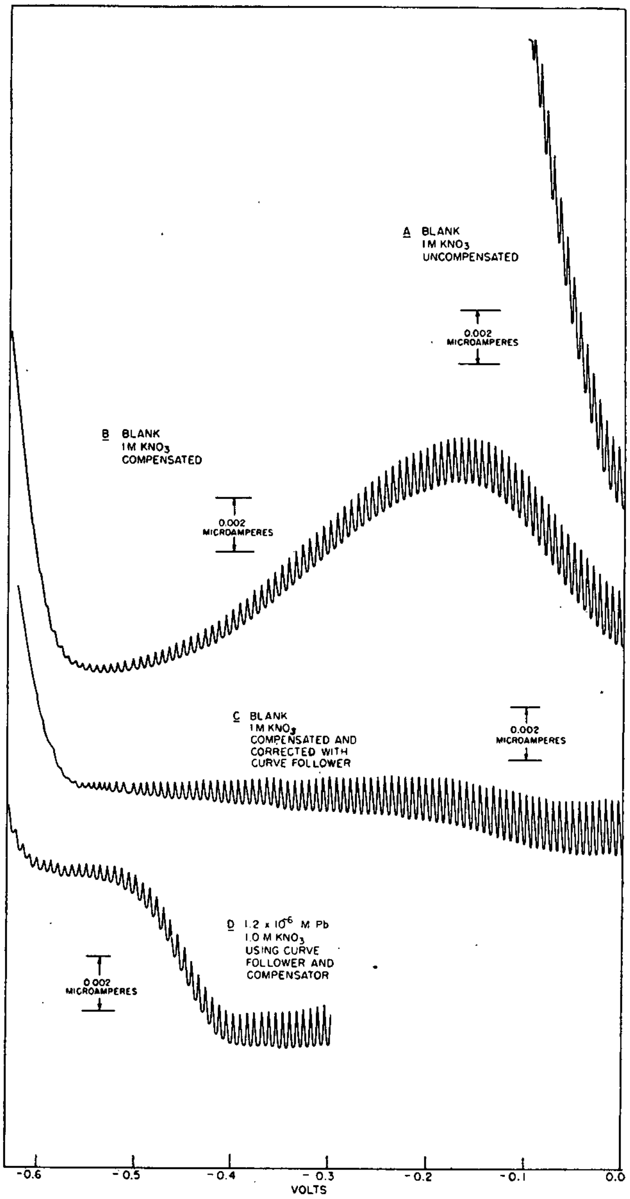


Figure 7. Polarograms Illustrating Use of Compensator and Curve Follower at Very Low Concentrations

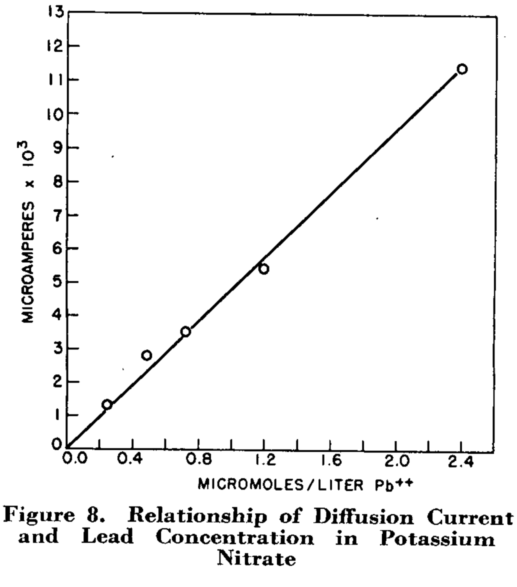


Figure 8. Relationship of Diffusion Current and Lead Concentration in Potassium Nitrate

at the appropriate setting of the residual current compensator. Here reduction waves could be measured on some linear portions of the curve, but this blank still leaves much to be desired. Curve *C* shows the result obtained when a trace derived from curve *B* is used on the curve follower. Here the blank is fairly linear from zero to a potential of −0.5 volt.

The instrument has been applied to the determination of lead in a potassium nitrate medium. As shown in Figure 7 by using the appropriate compensating curve on the curve follower, a blank curve approaching a straight line can be obtained. Using this same compensating curve, polarograms were drawn on a series of lead concentrations in 1 *M* potassium nitrate ranging from 0.2×10^{-6} *M* to 2.0×10^{-6} *M*. One of these curves is shown in Figure 7 (curve *D*) and the measured diffusion currents (measured by extrapolation to the half-wave potential) are tabulated in Table I. The same data are shown in graphical form in Figure 8.

The figures in Table I show that the diffusion current at these low concentrations is very nearly a linear function of concentration. The precision of measurement is relatively poor, but it is hoped that further study of the factors influencing polarography in these very dilute solutions will permit an improvement in precision to at least ± 10%. The figures at higher concentrations are given in Table I to show that the diffusion current constant, i_d/c, appears to change with large concentration changes but to be linear over a small range. The diffusion constant predicted by the Ilkovič equation (*3*) for this electrode was 8.14 µa. per millimole per liter, agreeing very well with 8.10 observed at 0.72×10^{-3} *M*.

LITERATURE CITED

(1) Coor, Thomas, Jr., and Smith, D. C., *Rev. Sci. Instruments*, **18**, 173–81 (1947).
(2) Ilkovič, D., and Semerano, G., *Collection Czechoslov. Chem. Communs.*, **4**, 176 (1932).
(3) Kolthoff, I. M., and Lingane, J. J., "Polarography," New York, Interscience Publishers, 1941.
(4) Rosenbaum, F. J., and Stanton, L., ANAL. CHEM., **19**, 794–8 (1947).

RECEIVED for review July 25, 1952. Accepted August 29, 1952. Presented before the Division of Analytical Chemistry at the 118th Meeting of the AMERICAN CHEMICAL SOCIETY, Chicago, Ill.

Determination of Small Amounts of Hydrocarbons in the Atmosphere

PAUL P. MADER, MERLYN W. HEDDON, ROBERT T. LOFBERG, AND RUTH H. KOEHLER
Los Angeles County Air Pollution Control District, Los Angeles, Calif.

A spectrophotometric method for determining small concentrations of hydrocarbons in the atmosphere has been developed. It employs the use of a specially built 100-cm. gas cell supplied with rock salt windows and inserted between the light source compartment and receiver compartment of a Beckman infrared spectrophotometer. Because of the large volume of the new cell, the entire gas sample can be swept into the light path of the instrument. The hydrocarbon sample is collected by a freeze-out technique, using a Shepherd sampler immersed in liquid oxygen. Based on the collection of the condensates of a 60-liter air sample, the instrument records the presence of 23 micrograms of hexane, or less than 0.1 p.p.m. of this hydrocarbon. The results obtained for known hydrocarbon concentrations are accurate and consistent. This method was successfully applied to the determination of hydrocarbons from air streams over polluted areas or inside industrial establishments.

THE studies made by the Los Angeles Air Pollution Control District show hydrocarbons to be the largest single group of gaseous and vapor phase contaminants that are being discharged into the atmosphere. Olefins, branched-chain, and perhaps other hydrocarbons represent the original raw materials which, in the presence of air oxygen, ultraviolet radiation, ozone, and oxides of nitrogen, may be converted into oxygenated products (*1–3*, *6*). These hydrocarbons in their oxidized form contribute substantially to the smog conditions prevalent in the Los Angeles area in days of low wind speeds and temperature inversion. It has been demonstrated that oxidized forms of hydrocarbons reduce the visibility (*3*, *5*) in the affected area and produce eye, nose, and throat irritation, as well as damage to leafy vegetables (*2*, *4*).

In view of the significant effects caused by the hydrocarbon oxidation products, it became desirable to find a method for the determination of small amounts of hydrocarbons in the atmosphere at various times of the day. Specific measurements were needed in air pollution investigations to establish the correlation which might exist between the absolute amounts of hydrocarbons found in the atmosphere and the harmful effects experienced by humans and observed on vegetation.

Previous methods used for determination of minute amounts of hydrocarbons are based on the combustion of the collected sample to carbon dioxide, which is absorbed in an excess of barium hydroxide. The excess of barium hydroxide is back-titrated with a standard hydrochloric acid solution. The amount of carbon dioxide developed corresponds to the hydrocarbon content of the sample. The disadvantage of this method lies in the fact that the determination is executed against a background of 300 p.p.m. of carbon dioxide in the surrounding air, while the amounts of hydrocarbons expected to be present in the atomosphere may be of the order of a few parts per million only. Any loose connection in the combustion train assembly or any air contact of the titration vessel receiving the generated carbon dioxide will lead to erroneous results. Frequent "blank" determinations must be performed. Whenever the combustion train is not in use for several days, it is necessary to heat the entire assembly for several hours to drive all accumulated carbon dioxide from the combustion tubes and the rest of the system. In spite of these inherent shortcomings, this method, laborious and time-consuming as it is, can still be used by a skillful and patient operator.

A fairly simple and straightforward method for determination

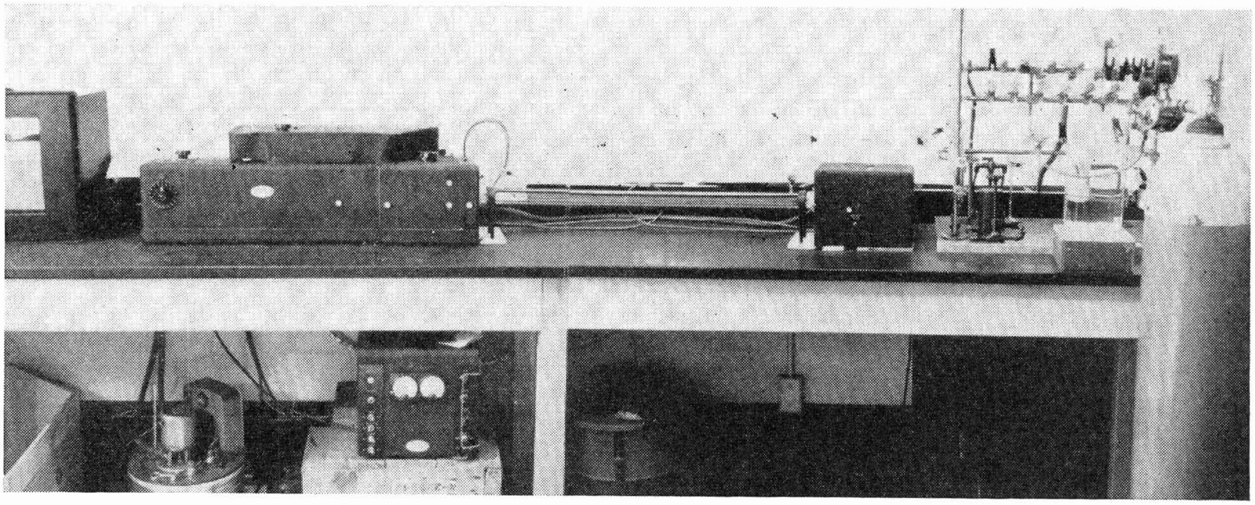


Figure 1. Beckman Infrared Spectrophotometer with New Gas Cell Inserted

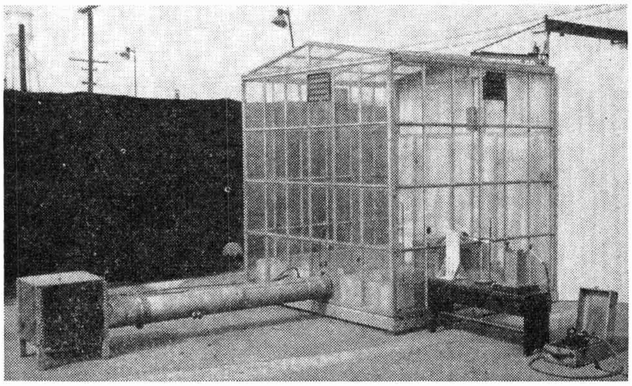


Figure 2. Plexiglas Chamber Used to Generate Known Hydrocarbon Concentrations under Dynamic Conditions

Designed and built by William P. Thompson, Earhart Plant Research Laboratories, California Institute of Technology, Pasadena, Calif., under Los Angeles County Air Pollution Research Contract

of minute quantities of hydrocarbons in the atmosphere was developed by this laboratory. It involves the use of a Beckman infrared spectrophotometer, Model IR2. In its standard form, the instrument is equipped with two 10-cm. gas cells. The contained volume of gas is usually sufficient in all cases where concentrated samples are available for analysis. In the case of analyses of air samples, however, where the total amount of the air pollutants present is very small, it was found that only a small fraction of the dilute sample entered into the gas cells, while a considerable portion stayed in the glass tubing of the gas-handling system and the remainder was left in the collector vessel used to trap the pollutants from the atmosphere. This handicap was overcome by the construction of a specially built gas cell. This cell is cylindrically shaped, 100 cm. long and 3.5 cm. in diameter; both ends are supplied with rock salt windows. Two glass tubes near the ends of the cell connect the gas cell with the gas-handling system and vacuum pump. The gas cell is inserted between the light source compartment and receiver compartment (Figure 1). Because of the large volume of the gas cell, the entire sample can now be swept completely into the gas cell with dry nitrogen. By introducing the entire gas sample instead of a fraction into the light path of the instrument, it became possible to observe absorption bands for hydrocarbons present in the atmosphere at concentrations of less than 0.1 p.p.m. by volume. Based on the collection of condensate from a 60-liter air sample, the instrument, with its special gas cell built in, will register 23 micrograms of hexane. The results obtained by this method are consistent and reproducible even for minute concentrations.

CALIBRATION OF INSTRUMENT AND SAMPLING

The method employed for the calibration of the infrared instrument with the special gas cell consisted in generating known hydrocarbon concentrations in an 8 × 8 × 7 foot Plexiglas chamber (Figure 2). Representative samples were withdrawn from the chamber by the same method applied in the atmosphere.

Before each run the house was thoroughly aired by blowing filtered air through the house for at least 20 minutes while the doors and vents of the house were left open and a mixing fan inside the house was in operation.

n-Hexane (Phillips, redistilled, with boiling point 68–69° C.) was selected as the hydrocarbon to be used for the calibration. The initial calibration of the infrared spectrophotometer was performed with a blend of gasolines comprising the products of five major oil producers in this area. When individual hydrocarbons from C_4 to C_{10} were used for the standardization, it was found that the calibration curve obtained with *n*-hexane coincided most closely with the graph obtained with the gasoline mixture. As it is desirable to recalibrate the instrument from time to time with known hydrocarbon concentrations, *n*-hexane seemed particularly suitable for this purpose because this hydrocarbon is available in a high degree of purity while the composition of gasolines and their blends changes on standing. However, these reference calibrations can be carried out with either compound, as long as material used exhibits essentially the same absorption characteristics as the hydrocarbon components tested for.

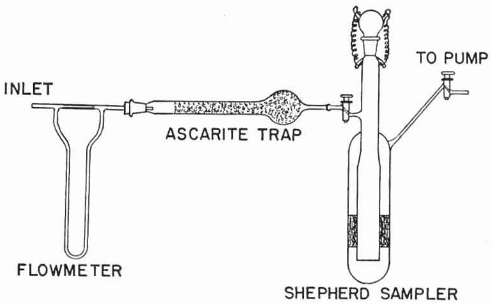


Figure 3. Freeze-Out Assembly

n-Hexane was introduced into the house from a syringe (designed and developed by Herman Roth at the University of Southern California for the Los Angeles County Air Pollution Control District, *1*) designed to dispense a liquid at a known rate. The drops from the syringe were allowed to fall on a hot plate; the liquid hexane was vaporized when it came in contact with the hot surface of the plate. The initial and final volumes of the hydrocarbon as well as the time during which it was dispensed were noted for each run. The hexane vapors were mixed with a constant flow of purified air, provided by means of a blower unit and measured by an orifice meter. Concentrations within the chamber could be varied by changing the amounts of *n*-hexane dispensed or by adjusting the flow of filtered air. Mixing inside the chamber was accomplished by means of a fan. Temperature inside the house was noted before and after each run.

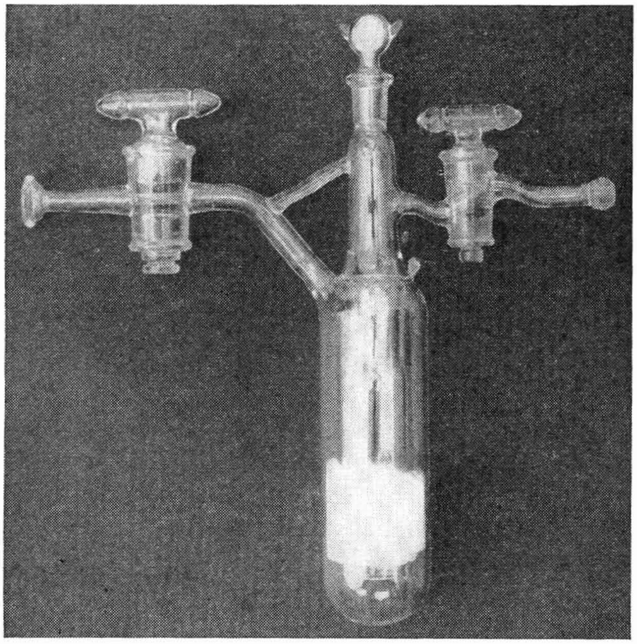


Figure 4. Shepherd Sampler for Collection of Minute Amounts of Gases and Vapors

Samples of the house air were then taken with the help of a freeze-out assembly (Figure 3). The same arrangement is used for the collection of hydrocarbons from the atmosphere. It consisted of an orifice flowmeter, calibrated to deliver 0.5, 1.0, 1.5, and 2.0 liters per minute, and a drying tube filled with Ascarite for the removal of water vapor, carbon dioxide, aldehydes, ketones, and organic acids from the incoming air-hexane mixture. The hydrocarbon components of the air stream are not affected by the Ascarite trap. The sample itself was frozen out in the Shepherd collector trap (*6*), which was immersed in a Dewar flask filled with liquid oxygen. The Shepherd sampler contained a plug of tightly wound glass wool held in place by sealed-in spokes (Figure 4). The purpose of this plug is to prevent extremely small frozen-out particles, formed when the incoming air is rapidly cooled to low temperature, from following a streamlined flow and escaping through the warm sampler exit.

Before each run it is necessary to relieve the vacuum created in the Shepherd sampler by the low temperature; this was accomplished by bleeding the traps to the atmosphere through Ascarite. "Blanks" for hydrocarbons were taken at a point in the duct connecting the blower unit and the Plexiglas chamber, after the incoming air had passed through the filtering system. The concentrated samples were maintained at liquid oxygen temperature until they were ready for analysis.

MODIFICATIONS IN USE OF IR2

A 1-meter gas cell with rock salt windows was installed (see above). The original 10-cm. gas cells, although still available to the system, were kept in blank position.

An automatic connection between the slit width, wave-length drive and gain control was installed, thereby maintaining an amplifier output of blank runs close to 100% transmittance.

All unnecessary sections of the standard gas-handling system, including the manometer, were cut out of use. The latter was accomplished by installing an additional stopcock between the mercury reservoir and the meter gas cell; thus the entire sample was swept into the cell.

A small Ascarite trap to remove possible residual moisture was attached to the gas-handling system. This trap is a small L-shaped tube with 12/5 ball and socket joints (Figure 5). Small constrictions at *A*, *C*, and *E* hold the glass wool in place.

The following method is suggested for filling this trap:

Borosilicate glass wool is packed loosely through the male joint until it reaches the constrictions at point *A*. Ascarite is added through the other end to point *B*. Another glass wool plug is now forced through the constrictions at point *C*. About half an inch of Ascarite is added up to point *D*. A final glass wool plug is placed below the constrictions at *E*. In filling this trap care should be taken to leave a free space between *D* and *E*. Any reasonably fast flow of gas will lift several or all of the particles in *D*. As the rate of flow from the Shepherd trap into the evacuated gas cells decreases, the particles return to their original level. In this way the trap also acts as a flow indicator and the necessity of using the manometer of the gas handling system is eliminated.

PROCEDURE OF ANALYSIS

The gas-handling system and gas cell were evacuated. The Shepherd sampler containing the frozen condensate was removed from the liquid oxygen and connected to the system via the Ascarite trap.

By this time the pressure of the sample had increased to about 2 atmospheres, although the sampler was still cold. This increase in pressure was relieved by allowing the vapors to flow from the sampler into the gas cell. The closed trap was then immersed in a water bath of about 50° C. to melt any ice crystals which may be observed inside the sampler. After all condensate had vaporized, the exit valve of the sampler was opened, allowing the contents to flow to the gas cell. As soon as the Ascarite trap showed that the flow into the gas cell had stopped (by the return of the Ascarite particles to the original level), the exit valve was closed and nitrogen was added through the inlet valve of the trap. By repeatedly filling the sampler with nitrogen and emptying it into the meter cell, virtually all of the concentrates of the trap were transferred to the cell. It was calculated from the dimensions of the system that a total of eight transfers swept 99.3% of the concentrate into the cell, provided the temperature of the sampler was brought up to 50° C. Both valves were then opened, and more dry nitrogen was swept through the trap and gas-handling system until atmospheric pressure was reached. Although nitrogen does not exhibit absorption in infrared, the total pressure inside the gas cell was increased to 1 atmosphere in all determinations.

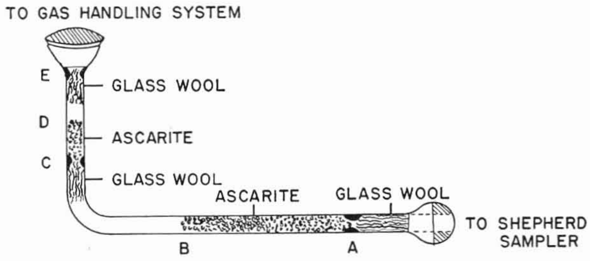


Figure 5. Apparatus for Removing Residual Moisture

At first the evacuated cell was scanned in the 3.0- to 4.0-micron region at a slow speed and a response time of 32 seconds. The same procedure was repeated after the transfer of the sample into the gas cell had been completed. The per cent transmittance of the sample was found for the carbon-hydrogen absorption band at 3.45 microns, by comparison of the results obtained for the evacuated cell and for the sample. At the beginning it was deemed advisable to run several samples in duplicate. A number of samples of the *n*-hexane–air mixtures were also collected at various sampling rates. The results obtained in all cases were consistent and therefore the collection and analysis of more than one sample at one concentration were no longer considered necessary.

The results found by these measurements are shown in Figure 6 and are tabulated in Section A of Table I.

In addition to generating known hydrocarbon concentrations under dynamic conditions, a number of *n*-hexane–air mixtures were generated under static conditions.

The method used consisted of introducing into a 226-liter Plexiglas box or a 22-liter borosilicate glass flask concentrations of hydrocarbons approximating those existing in the amosphere. After thorough mixing, representative samples were withdrawn into nine sampling tubes of known volumes. The samples were then transferred into the gas cell and the absorption at 3.45 microns was again determined as before.

The results obtained are also shown in Figure 6 and are tabulated in Sections B and C of Table I.

DISCUSSION OF FIGURE 6 AND TABLE I

Of the 21 different concentrations of *n*-hexane collected under dynamic conditions and used for the calibration of the infrared spectrophotometer, 13 results, when plotted, fall on a straight line which approaches a transmittance of 100% when the concentration of sample decreases to zero. Except for one determination, where the measured transmittance is about 3% higher than the calculated value, the deviations of the other points from the straight line are minor. The group of values obtained from samples collected under static conditions and involving lower concentrations also fall close to the same straight line. The straight-line relationship between the logarithm of per cent transmittance and parts per million of hexane used in the tests can be expressed by the following equation:

$$\text{P.p.m.}/31.03 + \log T\ \% = 2$$

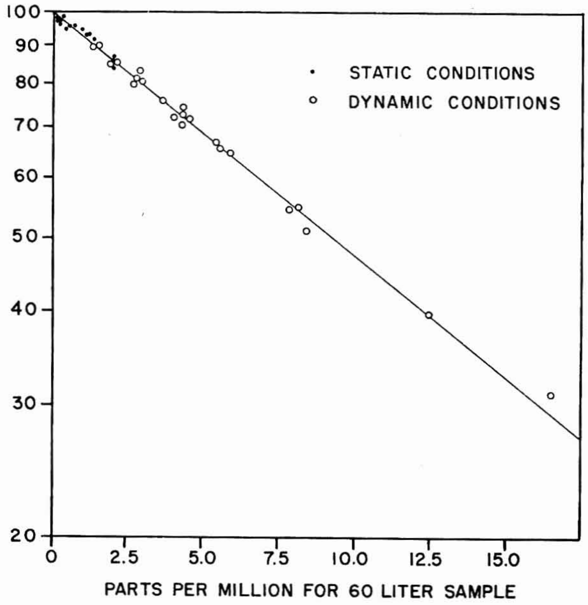


Figure 6. Measurement of Hexane in Air

On the basis of this equation the per cent transmittance can be calculated for every concentration of *n*-hexane used. The comparison between the experimentally found values and the calculated values is shown in Table I. In 13 determinations collected under dynamic conditions (Section A), the deviations of the calculated values from the experimental values were 1% transmittance or below. The average deviation between all the calculated and the found values taken under dynamic conditions is 1.2% transmittance. The results of the hydrocarbons collected under static conditions, using a 22-liter flask and a 220-liter Plexiglas box, show an average deviation of 0.7 and 1.3% transmittance, respectively, between the found and calculated values (Sections B and C of Table I).

Table I. Comparison of Calculated and Experimentally Determined Values of Hexane

Weight of Hexane, Mg.	P.P.M. in Sample Diluted to 60 Liters	% Transmittance		Deviation, %
		Found	Calculated	
Section A. Plexiglas House, 11,000 Liters				
0.276	1.38	90.0	90.2	−0.2
0.283	1.41	90.3	90.1	+0.2
0.371	1.84	85.1	87.3	−2.2
0.396	1.96	85.4	86.5	−1.1
0.536	2.71	81.7	81.8	−0.1
0.553	2.76	80.7	81.5	−0.8
0.557	2.76	80.5	81.5	−1.0
0.565	2.83	83.8	81.1	+2.7
0.742	3.68	76.4	76.1	+0.3
0.792	3.92	72.6	74.8	−2.2
0.823	4.15	70.9	73.5	−1.6
0.829	4.14	72.7	73.6	−0.9
0.848	4.24	74.9	73.0	+1.9
1.072	5.42	67.1	66.9	+0.2
1.106	5.52	65.7	66.4	−0.7
1.188	5.88	65.1	64.6	+0.5
1.584	7.84	55.0	55.9	−0.9
1.608	8.13	55.6	54.7	+0.9
1.645	8.30	51.4	54.0	−2.6
2.468	12.45	40.3	39.7	+0.6
3.290	16.60	32.8	29.2	+3.6
			Av.	1.2
Section B. Borosilicate Glass Flask, 22 Liters				
0.064	0.30	99.0	97.7	+1.3
0.106	0.50	96.0	96.4	−0.4
0.128	0.60	96.0	95.7	+0.3
0.192	0.89	94.5	93.6	+0.9
0.213	1.04	93.0	92.6	+0.4
0.256	1.34	92.0	90.5	+1.5
0.426	1.98	85.0	86.5	−0.5
0.426	1.98	86.0	86.5	+0.5
0.426	1.98	87.0	86.5	+0.4
			Av.	0.7
Section C. Plexiglas Box, 226 Liters				
0.021	0.10	98.4	99.3	−0.9
0.021	0.10	98.5	99.3	−0.8
0.041	0.19	97.5	98.7	−1.2
0.041	0.19	97.0	98.7	−1.7
0.083	0.39	95.0	97.1	−2.1
			Av.	1.3

SUMMARY

Known hydrocarbon concentrations were generated under dynamic and static conditions. Representative samples were withdrawn by two different methods and analyzed by infrared analysis. The fact that of the many samples analyzed most results, when plotted, fall on a straight line, while the rest show only minute deviations indicates that the method of collection is effective and the results of the infrared analyses are consistent and reproducible, even when very low concentrations of hydrocarbons are involved. After the calibration of the infrared instrument, the method was successfully employed for the collection and determination of smog samples. In view of the accurate results obtained, this method is recommended for the determination of trace amounts of hydrocarbons in gases and in air, as a relatively simple and reproducible spectrophotometric procedure.

LITERATURE CITED

(1) Air Pollution Control District of Los Angeles County, Technical and Administrative Report on Air Pollution Control in Los Angeles County, Annual Report, 1949–50.
(2) *Ibid.*, 1950–51.
(3) Haagen-Smit, A. J., *Ind. Eng. Chem.*, **44**, 1342 (1952).
(4) Haagen-Smit, A. J., Darley, E. F., Zaitlin, Milton, Hull, Herbert, and Noble, Wilfred, *Plant Physiol.*, **27**, 18 (1952).
(5) Mader, P. P., MacPhee, R. D., Lofberg, R. T., and Larson, G. P., *Ind. Eng. Chem.*, **44**, 1352–5 (1952).
(6) Shepherd, M., Rock, S. M., Howard, R., and Stormes, J., ANAL. CHEM., **23**, 1431 (1951).

RECEIVED January 21, 1952. Accepted September 24, 1952.

Spectrophotometric Method for Following Dichromate Oxidations

MARIO J. CARDONE AND JOHN COMPTON
Research and Development Division, Wyandotte Chemicals Corp., Wyandotte, Mich.

Studies of dichromate oxidations indicate that a spectrophotometric method might offer some advantages. The dichromate absorption band in the near ultraviolet at 349 mμ is employed, as it is free of trivalent chromium interference. Shifting of the absorption band does not occur in sulfuric acid solutions below 8 *M*. The lowering of the intensity of the absorption band due to sulfuric acid is minimized by the addition of phosphoric acid. Absorbancy index deviation plots are presented which allow determination of dichromate with an accuracy of ±0.3% in the range of 0.12 to 0.30 meq. per 100 ml. with a precision equal to that of the classical iodometric method. The method has been successfully applied in following the oxidation of several classes of organic compounds, has been shown to be extremely sensitive to oxidizable impurities, and should be of value as a test of purity of organic compounds.

ANALYTICAL methods for following the course of dichromate oxidations may be placed in two broad groups: (1) those based upon the measurement of the excess dichromate remaining after the oxidation process is complete or has been stopped and (2) those which determine the amount of oxidation end product produced. The former are of general application, whereas the latter are unique and depend upon the nature of the end product formed. The nature of the end product depends upon the material being oxidized and the oxidation reaction mechanism.

Excess dichromate has been determined by iodometric titration (*1, 28*) and by titration with ferrous iron (*3, 11, 13, 17, 20, 24–26*). Francis (*9*) determined the excess dichromate by a polarographic method. Christensen *et al.* (*5*) used the novel method of thermally decomposing the excess dichromate and measuring the volume of oxygen gas formed. Fonteyne and DeSmet (*8*) used a photometric method for the determination of ethyl alcohol and glycerol. A plot of absorbancy (of dichromate at 435 mμ) versus milligrams of compound was prepared where the initial absorbancy of the dichromate was obtained when the compound content was zero. A straight-line relationship with a negative slope was obtained. As the wave length of peak absorption was found to vary with the amount of organic compound taken, an average value had to be employed. The procedure requires a standardization plot for each substance and demands strict adherence to the conditions employed.

Methods based on the determination of end products have been almost wholly limited to the measurement of carbon dioxide (*3, 4, 16, 22, 23*). Segal *et al.* (*22*) described a steam distillation–titrimetric method for the detection of acetic acid, which could be used as a determination method, and an apparatus for measurement of carbon monoxide.

In this paper a spectrophotometric method for the determination of dichromate is presented in which the spectral characteristics of dichromate have been established. The method is of general application for use in studying organic oxidation reactions.

EXPERIMENTAL

Spectrophotometer and Its Use. A Beckman Model DU quartz spectrophotometer was used throughout this work. Matched silica or Corex 1.000-cm. cells were employed as required. After the cells had been cleansed by the customary techniques, they were checked before each use by filling them with distilled water, and the "cell correction" was determined at each wave length at which the solutions were to be read. A "background correction" on the solutions was also made by taking readings against water or solvent at a wave length at which the solution does not absorb. Both corrections were applied to all absorbancy readings. With careful technique ensuring optically clean cells, these corrections were almost always less than 0.005 absorbancy. Any large stray error such as that due to turbidity is quickly detected by the background measurements.

The spectral absorption curves were taken with distilled water in the reference cell. Measurements were made in all cases by setting the sensitivity of the instrument at the maximum specified by the manufacturer (sensitivity knob three turns from the counterclockwise limit) and adjusting the slit width accordingly in order to bring the instrument into balance. This procedure yields the smallest spectral band widths possible for the entire wave-length range covered with the instrument operating at maximum sensitivity. Thus, according to the curve of effective band width *vs.* millimicrons supplied with the instrument (*2*), the above procedure allowed the use of effective spectral band widths of 1.5 microns or less at all wave lengths.

Definition of Terms. The Beer's law expression $A_s = a_sbc$, is used as recommended by the National Bureau of Standards (*18*), where A_s is the absorbancy, a_s is the absorbance index, and b is the cell thickness in centimeters. The unit of concentration, c, employed is milliequivalents per 100 ml. and is used for its convenience in application to dichromate oxidations. Absorbance index, a_s, is then defined as absorbance units per milliequivalent (per 100 ml.) per cm.

The most convenient method of expressing the oxidizability of a compound is in terms of oxygen equivalents. Thus, the oxidation of diethylene glycol may be expressed:

$$(CH_2OHCH_2)_2O + 10[O] = 4CO_2 + 5H_2O$$

Ten oxygens are required per molecule and the oxygen equivalent is then 20. The term "oxidation number" is employed synonymously for oxygen equivalents.

Chemicals and Solutions. All of the chemicals used were the highest quality reagent grade material available and no attempt was made to purify any of these materials further.

Potassium dichromate, Bureau of Standards No. 136.

Chromic sulfate, J. T. Baker Chemical Co., specially purified grade analytical reagent.

Sulfuric acid, 99.5% analytical reagent grade.

Phosphoric acid (ortho), 85%, analytical reagent grade.

Acetic acid, Eastman Kodak White Label No. 763, 99.9% CH_3COOH.

Formic acid, Eastman Kodak White Label No. 139, 98 to 100% HCOOH. A standard solution standardized by the acidimetric method of Rosin (*21*) indicated 99.4% HCOOH.

Formaldehyde, Baker and Adamson analytical reagent, Code 1778, assay 36% by weight HCHO minimum. A standard solution standardized by the alkalimetric–hydrogen peroxide method of Rosin indicated 36.7% HCHO.

Acetaldehyde, Eastman Kodak White Label No. 468. The aldehyde content of the standard solution was determined by the bisulfite method of Kolthoff and Furman (*12*).

Glycerol, Baker and Adamson analytical reagent, Code 1782. Assay by refractive index and specific gravity indicated 95.1% glycerol.

Ethylene glycol, Eastman Kodak White Label No. 133. Assay by specific gravity indicated 100% purity.

Propylene glycol (1,2-propanediol), Eastman Kodak White Label No. 1321. Assay by specific gravity indicated 99.9% purity.

Diethyleneglycol (β,β'-dihydroxyethyl ether), Eastman Kodak White Label No. 2041. Assay by specific gravity indicated 100% purity.

Dipropylene glycol (4-oxaheptane-2,6-diol), Eastman Kodak practical grade No. 4773 which had been fractionated for use as an infrared standard. Assumed 100% purity.

Procedure. Throughout the work, the standard solutions were dispensed by pipet and the reagents such as sulfuric and phosphoric acids by buret. Precautions against possible contamination from the buret stopcock must be observed. No trouble is encountered if the stopcock is lubricated infrequently and if after an application of a very minimum of silicone grease to the stopcock, the excess is removed by filling the buret with concentrated sulfuric acid and allowing it to stand for several hours, discharging a few milliliters at a time at frequent intervals. Oxidation blanks serve as the final check. The general procedure used for measuring dichromate absorption is:

Pipet 10.0 ml. of standard potassium dichromate solution into a volumetric flask. Add the approximate volume of distilled water required and then slowly add the calculated volume of acid reagent(s), using diluted acid(s) where possible. Mix, place in a constant temperature water bath (25° C.) until cooled to temperature, remove, and adjust to volume. Transfer to the 1.000-cm. silica or Corex cells and take the absorption readings in the Beckman spectrophotometer, correcting the readings as described.

Absorption Spectra. Spectral curves for dichromate and chromium(III) are shown in Figure 1. Trivalent chromium is considered, because it is a principal reduction product of the oxidation system. The sensitivity of the dichromate absorption maximum in the near ultraviolet at 349 mμ is approximately sevenfold that of the broader maximum in the visible at 446 mμ. Lingane and Collat (*14*) place these maxima at 350 and 440 mμ for a solution which is 1.0 *M* in sulfuric and 0.7 *M* in phosphoric acid.

Chromium(III) has two broad maxima in the visible, as shown in curve 4 of Figure 1. The shape of the curve agrees well with that reported by Kasline and Mellon (*10*) for chromic sulfate in 0.12 *M* sulfuric acid, except that their maxima are placed approximately 10 mμ lower. Chromium(III) interferes measurably with dichromate absorption measurements employing the 446 mμ peak, whereas interference with the 349 mμ dichromate peak is small. Calculations from the data shown in Figure 1 show that for equivalent amounts of dichromate and chromium(III), the interference error due to chromium(III) is on the order of 7% at 446 mμ but only 0.2% at 349 mμ. Although dichromate could be determined at 446 mμ by correcting for the chromium(III) content as determined at 590 mμ, this procedure would not be as accurate as the ultraviolet determination of dichromate, because the accuracy of the final result would be dependent upon the accuracy of both the dichromate and chromium(III) determinations and the sensitivity of the chromium peak at 590 mμ is only approximately one tenth that of dichromate at 446 mμ.

Effect of Acidity. As oxidation of organic compounds by dichromate has usually been conducted in sulfuric acid medium, sometimes with phosphoric acid also present, the effect of these acids upon the dichromate absorption peak in the ultraviolet was studied.

A series of solutions containing 0.2 meq. of potassium dichromate per 100-ml. volume with various amounts of sulfuric acid and another series with various amounts of phosphoric acid were prepared and the absorbancies were determined over a range of wave lengths which covered both sides of the absorption peak. Distilled water was used in the reference cell, as it was

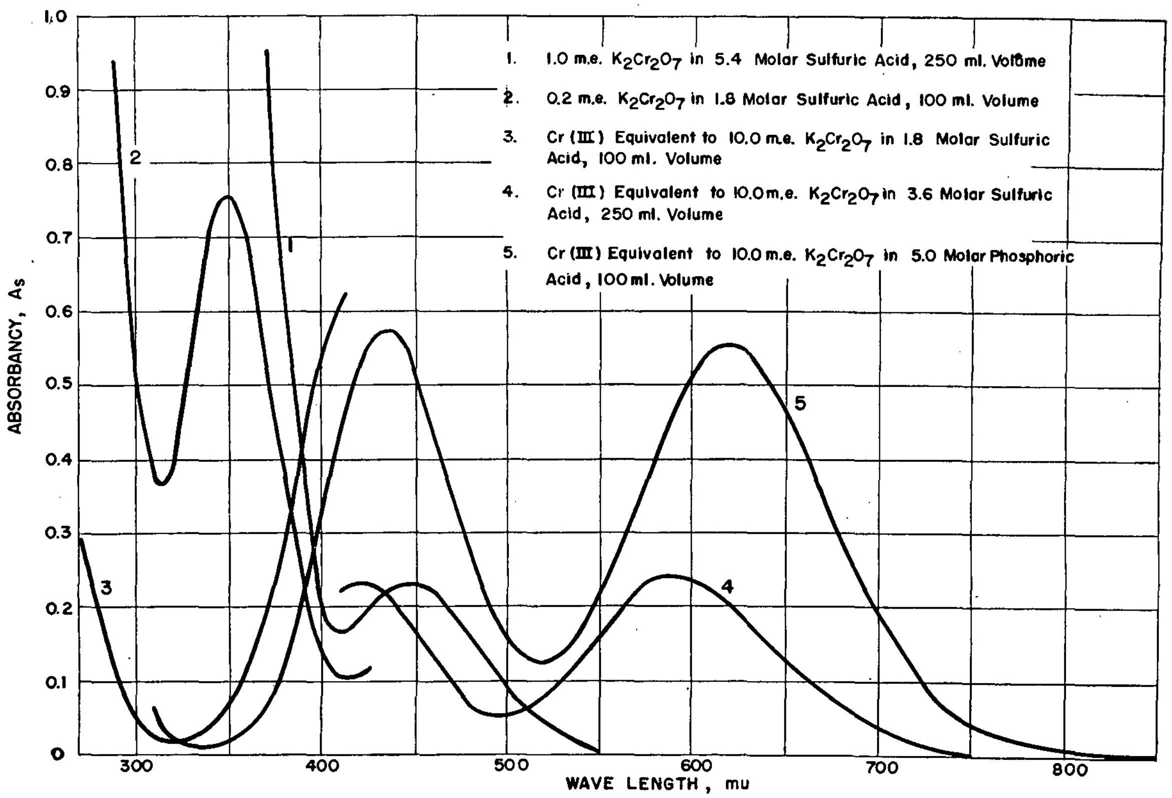


Figure 1. Absorption Spectra of Potassium Dichromate and Trivalent Chromium

found that sulfuric and phosphoric acid solutions had very low absorption.

Sulfuric acid below 8 *M* and phosphoric acid below 10.0 *M* have no significant effect upon the position of the dichromate maximum. Above these molarities, shifts toward lower wave lengths occur. The intensity of the maximum decreases with increasing sulfuric acid concentration, the rate of decrease being significant until a concentration of approximately 7 *M* is reached. No further significant change occurs until a concentration of 9 *M* is attained, at which concentration a sharp increase of intensity occurs. Phosphoric acid also decreases the intensity of the maximum, but the point of leveling off is relatively quickly attained and is a relatively broad range of acid concentration, 3 to 6 *M*, above which a further (but much slower rate of) decrease of intensity is noted.

Mixtures of Acids. Mixtures of phosphoric and sulfuric acids should behave nonadditively with respect to the over-all decrease in the intensity of the maximum. On addition of a large amount of phosphoric acid, so that the ratio of phosphoric to sulfuric acid would be high, the residual effect of sulfuric acid should be small.

In order to test this nonadditive hypothesis, several series of solutions were prepared, each containing 0.2 meq. of potassium dichromate per 100-ml. volume with various ratios of phosphoric to sulfuric acids. The sulfuric acid concentration was varied over the range of 0 to 1.80 *M* for phosphoric acid concentrations of 3.0, 4.0, 5.0, 6.0, and 7.5 *M*. Absorbancy of the solutions was read at 349 mμ against water in the reference cell.

The absorbancy of the dichromate-phosphoric acid solutions containing no sulfuric acid served as the reference base of each of the five series. The absorbancies of the dichromate-phosphoric-sulfuric acid solutions were subtracted from the absorbancy of the respective bases to obtain the absorbancy deviation due to the sulfuric acid content. The results are shown in Table I, which clearly indicates that a dichromate solution which is 5.0 *M* in phosphoric acid is the least affected by increasing sulfuric acid concentration. The solutions can be up to 0.90 *M* in sulfuric acid but are preferably kept below 0.45 *M*. Precision in the addition of the phosphoric acid was found to be not critical and an error of ±1.0 ml. of 10 *M* phosphoric acid reagent is tolerable so that no undue precautions in the addition of the phosphoric acid reagent are required.

Table I. Effect of Sulfuric Acid on Absorbancy of Dichromate–Phosphoric Acid Solutions at 349 Millimicrons

(0.2 meq. $K_2Cr_2O_7$ per 100-ml. volume, 0.35-mm. slit width, water reference)

Molarity, H_3PO_4	Molarity, Sulfuric Acid 0.45	0.90	1.35	1.80
3.0	−0.005	−0.018	−0.030	−0.035
4.0	−0.009	−0.017	−0.025	−0.026
5.0	−0.004	−0.010	−0.017	−0.032
6.0	−0.009	−0.017	−0.032	−0.030
7.5	−0.002	−0.018	−0.030	−0.035

Effect of Slit Width. Several solutions from each of the dichromate-sulfuric and dichromate–phosphoric acid series and all of the solutions of the mixed acid series were examined for any effects due to varying the slit width. The "slit width" measurements were made by reading at the wave length of maximum absorption and varying the slit width over the entire operating range of the instrument. In no case was any effect observed, the same readings being always obtained.

Stability of Solutions. All of the dichromate-acid solutions were read in the Beckman spectrophotometer as quickly as possible after preparation, then read at definite time intervals over a period of 3 hours. The dichromate–sulfuric acid and the dichromate–phosphoric acid solutions were found to be optically stable for at least 3 hours when the sulfuric acid concentration was below 4.5 *M* and the phosphoric acid concentration was below 7.5 *M*. Above these limits, the solutions became progressively less stable. All the dichromate-phosphoric–sulfuric acid solutions were found to be optically stable for 3 hours.

Spectrophotometric Constants. Whether Beer's law is valid for such dichromate-phosphoric-sulfuric solution mixtures was determined by preparing six series of solutions, each series containing, respectively, 0.02, 0.05, 0.10, 0.20, 0.25, and 0.30 meq. of potassium dichromate per 100-ml. volume. All solutions were made 5.0 *M* in phosphoric acid. The sulfuric acid concentrations for each dichromate concentration were, respectively, 0, 0.45, and 0.90 *M*. A careful check of the absorption of a 5.0 *M* phosphoric acid solution, and 5.0 *M* phosphoric acid solutions containing, respectively, 0.45 and 0.90 *M* sulfuric acid against water showed a small but measurable corrected absorbancy of 0.008 ± 0.001 unit in each case. To avoid having to employ an additional correction to the observed absorbancies, a solution 5.0 *M* in phosphoric acid and 0.90 *M* in sulfuric acid was used in the reference cell in all subsequent work. The results are shown graphically in Figure 2.

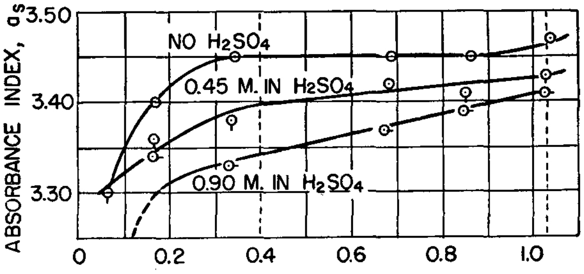


Figure 2. Effect of Sulfuric Acid Concentration upon Absorbance Index of Dichromate–5.0 *M* Phosphoric Acid Solutions at 349 mμ

Constancy of a_s indicates adherence to Beer's law. Dichromate in 5.0 *M* phosphoric acid solution obeys the law in the range of 0.35 to 1.00 absorbancy, which corresponds to a dichromate concentration range of 0.10 to 0.30 meq. per 100 ml. Linear deviations in the value of the absorbance index, a_s, in the range of 0.40 to 1.00 absorbancy (0.12 to 0.30 meq. per 100 ml.) result for solutions which are 0.45 and 0.90 *M* in sulfuric acid, the positive slope of the deviation increasing with increasing sulfuric acid concentration. As the deviations are a maximum at 0.40 absorbancy, it can be shown that if the sulfuric acid concentration is held below 0.45 *M* and its actual concentration is then disregarded, the resulting deviation in the value of a_s and the subsequent error in the dichromate concentration ($c = A_s/a_s$) using the average value of a_s would be ±0.6%. [For the authors' instrument, this average value is 3.43; Ewing and Parsons (7) discuss interchangeability of Beckman spectral data.]

For solutions up to 0.90 *M* in sulfuric acid, this approximation method is not sufficiently accurate. For this case, or whenever maximum accuracy is desired, a knowledge of the approximate sulfuric acid content is required. This may be obtained experimentally (by titration); however, in organic oxidation applications, the sulfuric acid content is arrived at with sufficient accuracy by simple calculation. When this is done, dichromate content can be ascertained to within ±0.3%, since the appropriate a_s value is then easily found within 0.01 unit by interpolation of the sulfuric acid concentration in Figure 2, obtaining a_s from the observed corrected absorbancy. The corresponding dichromate concentration is then calculated using the expression, $c = A_s/a_s$.

Effect of Phosphoric Acid upon Chromium(III). The absorption curve of a chromium(III) solution which was 5.0 *M* in phosphoric acid is shifted towards longer wave lengths and the intensity of the absorption at 349 mμ is decreased, as can be seen by reference to Figure 1, curve 5. Sulfuric acid contents up to

0.90 M caused no significant difference in the curve (not shown). The interference of chromium(III) on dichromate at 349 mμ is approximately 0.05% for equivalent amounts of dichromate and chromium(III). The absorbance index for chromium(III), a_s', at 349 mμ is 0.002 absorbance unit per equivalent dichromate milliequivalent (per 100 ml.) per cm. and may be applied in correcting the dichromate absorption at 349 mμ. This correction is negligible or very small in almost all cases; hence any deviation of the constant, a_s', from Beer's law may be safely ignored.

RECOMMENDED SPECTROPHOTOMETRIC PROCEDURE

The solutions for the determination should contain no less than 0.12 and no more than 0.30 meq. of dichromate and should contain an amount of sulfuric acid such that upon dilution to 100-ml. volume the concentration will be less than 0.45 M. In case of necessity in order to meet the dichromate concentration requirement the sulfuric acid concentration may be as high as 0.90 M.

Pipet the dichromate–sulfuric acid solution into a 100-ml. volumetric flask (room temperature) and add from a buret 50.0 ml. of 10 M phosphoric acid, waiting 15 to 30 seconds for buret drainage. Fill the flask to approximately 1 cm. from the mark, mix, and place in a constant-temperature water bath at 25° C. for at least 15 minutes. Adjust to the mark. Transfer the solutions to optically clean 1.0-cm. silica cells and read in the Beckman spectrophotometer at 349 mμ (0.30 to 0.35-mm. slit) and 850 mμ (0.03 to 0.05-mm. slit). The "cell correction" readings for the cells in the order to be used should be made beforehand at the same wave lengths. (Checks made beforehand instead of after will detect "dirty" cells.) Apply the cell correction and background correction to the observed absorbancy of the solution at 349 mμ. Calculate the approximate sulfuric acid concentration (determined experimentally where necessary). Using Figure 2, interpolate between the curves to the calculated sulfuric acid concentration and read off the a_s value at the corrected absorbancy of the solution. Calculate the concentration of dichromate, as milliequivalents of dichromate per 100-ml. volume, from the expression $c = A_s/a_s$.

In making the readings at 349 and 850 mμ, two techniques in using the Beckman spectrophotometer are possible. The first, which was the one used in obtaining spectrophotometric constants reported here, is to employ the tungsten lamp for the 850 mμ reading (red photocell) and the hydrogen lamp (blue photocell) for the 349 mμ reading. A second procedure is optional and is actually more convenient. In this case, the 349 mμ readings are also taken using the tungsten lamp together with a red-purple Corex A, Corning No. 9683 filter (supplied by the manufacturer) which allows the lamp to be used down to 320 mμ. Constants obtained by the two techniques are not quite interchangeable and must be determined by actual calibration as described. In a few trials using the second technique, no "slit effect" was observed.

INTERFERENCES

In an oxidation process end products are formed which may remain in the system unless they are sufficiently volatile to escape. The most important end product, trivalent chromium, has already been considered. Besides the end products, the compound being oxidized may be present after the oxidation process has been stopped, owing to incomplete oxidation.

The possible interference action of such compounds has been studied but only for a number of compounds of interest, as the oxidation study of any particular compound presents its own particular interference problem. Two types of interference were considered. The first is that due to the possible absorption of the interfering compound itself at 349 mμ under the conditions of the test. Absorption of 10 to 20 mg. of all of the compounds studied (as listed in Table II) was determined in 5.0 M phosphoric acid solution without and with sulfuric acid up to 0.90 M and was found to be nil in every case. The second is the resultant effect upon dichromate under the conditions due to some interaction or reaction of the compound and dichromate. In order to measure this chemical effect, it was necessary to add the standard solution of the compounds to the dichromate-phosphoric–sulfuric acid solution mixtures just before adjusting to volume. All of the solutions were 5.0 M in phosphoric acid and contained 0.2 meq. of potassium dichromate per 100-ml. volume. The sulfuric acid concentrations were 0, 0.45, and 0.90 M. After mixing, the solutions were allowed to stand for 1 hour before the absorbancy readings were taken. The results are shown in Table II.

The extent of the interference, where it occurred, was somewhat dependent upon the order of mixing of these reagents and the time of standing after preparation. The interpretation of the data is relative, as it is assumed that the compound would be compatibly present in the dichromate–sulfuric acid solution after the oxidation. For example, the aldehydes are shown to interfere seriously; yet it was impossible to add phosphoric acid to the dichromate-sulfuric-aldehyde mixture (which order of addition would have more closely approached the actual determination conditions) because the oxidation of the aldehyde proceeded too rapidly.

Table II. Chemical Interference of Organic Compounds

Substance	Mg.	Molarity, Sulfuric Acid	A_s, Cor.[a]	% A_s, Dev.	No. of Runs
Formaldehyde	1.106	...	0.647	− 6.2	3
	1.201	0.45	0.588	−13.9	2
	1.201	0.90	0.498	−26.0	2
Acetaldehyde	0.812	...	0.614	−11.0	1
	0.812	0.45	0.515	−24.6	1
	0.812	0.90	0.401	−40.4	1
Acetic acid	1.763	...	0.690	0.0	3
	1.763	0.45	0.682	0.0	3
	1.763	0.90	0.674	0.0	3
Formic acid	1.929	...	0.691	0.0	2
	1.929	0.45	0.680	− 0.4	2
	1.929	0.90	0.674	0.0	2
Ethylene glycol	0.981	...	0.692	+ 0.3	1
	0.981	0.45	0.683	0.0	1
	0.981	0.90	0.676	+ 0.4	1
Propylene glycol	1.088	...	0.691	0.0	2
	1.088	0.45	0.679	− 0.6	2
	1.088	0.90	0.664	− 1.3	2
Glycerol	0.966	...	0.691	0.0	1
	0.966	0.45	0.681	− 0.3	1
	0.966	0.90	0.673	0.0	1
Diethylene glycol	1.165	...	0.692	+ 0.3	1
	1.165	0.45	0.683	0.0	1
	1.165	0.90	0.669	− 0.6	1
Dipropylene glycol	1.069	...	0.692	+ 0.3	1
	1.069	0.45	0.677	− 0.9	1
	1.069	0.90	0.660	− 1.9	1

[a] Expected corrected optical densities are, respectively, 0.690, 0.683, and 0.673 for a "no sulfuric," 0.45 and 0.90 M sulfuric acid solutions containing 0.20 meq. of dichromate per 100-ml. volume.

Formic acid, acetic acid, glycerol, ethylene glycol, and diethylene glycol do not interfere. Propylene and dipropylene glycol interfere slightly, being significant in 0.90 M sulfuric acid solution. The authors have attributed the interference to the presence of oxidizable impurities in the reagents. This contention is supported, for example, by tests made as above using reagent grade glacial acetic acid (99.5%), which resulted in interference of the same order of magnitude as shown by propylene and dipropylene glycols.

APPLICATION

In order to test the performance of the analytical method under applied oxidation conditions in such a manner that the proper evaluation could be made, it was desirable to employ oxidation conditions which would result in oxidations as complete as possible upon compounds of known high purity. In this case, any interference due to the end products formed or the compound remaining would be a minimum which is desirable for ease of interpretation, since the immediate problem is to demonstrate the ability of the method to determine analytically the extent of an oxidation based upon the excess dichromate content.

Table III. Oxidation of Organic Compounds with Dichromate at 100° C.

Run No.	Compound	Mg. Taken[a]	H_2SO_4, Vol. %	Oxid. Time. Min.	Vol. Dil. Fact.	A_s, Cor.	a_s	Oxidation No.			No. of Runs
								Theory		Exptl.[b]	
1	Glycerol	9.656	50	30	20	0.606	3.43	CO_2	14.0	14.0	2
2		9.656	50	60	20	0.604	3.43			14.0	2
3		9.656	50	120	20	0.605	3.43			14.0	2
4	Formaldehyde	12.01	5	30	25	0.478	3.45	CO_2	4.0	3.8	3
5		12.01	5	60	25	0.469	3.45			4.0	3
6		12.01	5	120	25	0.454	3.45			4.3	1
7	Acetaldehyde	16.25	5	30	20	0.689	3.45	CO_2 CH_3COOH	10.0 2.0	2.7	3
8	Formic acid	19.29	50	30	25	0.581	3.43	CO_2	2.0	1.9	2
9		19.29	50	60	25	0.577	3.43			2.0	2
10		19.29	50	120	25	0.579	3.43			2.0	2
11	Acetic acid	17.63	50	120	20	0.854	3.44	CO_2	8.0	0.02	2
12		17.63	25	120	20	0.858	3.45			0.0	2
13		17.63	10	120	20	0.858	3.45			0.0	1
14	Ethylene glycol	9.806	50	30	20	0.594	3.43	CO_2	10.0	9.7	2
15		9.806	50	60	20	0.593	3.43			9.7	2
16	Propylene glycol	9.670	50	30	20	0.661	3.43	CO_2 CH_3COOH and CO_2	16.0 8.0	9.0	2
17		9.670	50	60	20	0.660	3.43			9.0	2
18		9.670	50	120	20	0.661	3.43			9.0	2
19	Diethylene glycol	11.65	50	30	20	0.483	3.43	CO_2	20	19.9	2
20		11.65	50	60	25	0.382	4.43			20.1	2
21		10.42	50	240	25	0.417	3.43			20.0	2
22	Dipropylene glycol	10.69	50	30	20	0.610	3.43	CO_2 CH_3COOH and CO_2	32 16.0	17.6	2
23		10.69	50	60	20	0.608	3.43			17.7	2

[a] 5.0 meq. $K_2Cr_2O_7$ taken.

[b] Corrected for blank.

The optimum oxidation conditions were selected after a careful examination of the literature (*1, 3, 5, 6, 9, 17, 28*).

Oxidation Procedure. Pipet 10.0 ml. of standard potassium dichromate solution into a standard-taper 24/40 Erlenmeyer flask. From a buret, add a calculated volume in water in order to make a final volume of 50 ml. Add 25 ml. (buret) of concentrated sulfuric acid (for concentrations of sulfuric acid other than 50% by volume, add an appropriate volume of sulfuric acid, using diluted acid where possible). Add this acid carefully and slowly, running the acid down the walls of the flask and swirling constantly. Cool under the tap to room temperature. Now add 10 ml. of a standard solution of the organic substance by pipet, swirling constantly. Immerse the flask up to the shoulder in a beaker of boiling water which rests upon a hot plate, attach a water-cooled standard taper condenser, and maintain the water bath at boiling temperature for the time specified in Table III. Rinse the condenser walls with a stream of water and then remove flask. Dilute contents of the flask immediately with water and transfer to a volumetric flask, diluting to the mark at 25° C. Take a suitable aliquot of this solution for the spectrophotometric analysis as described under "recommended procedure."

Blank. A blank run in a similar manner serves as a check on the purity of the reagents, particularly the sulfuric acid. Since the consumption of dichromate by the oxidizable impurities in the reagents is always low, 10.0 ml. of 0.1 *N* potassium dichromate is taken and the dilution and aliquot volumes are 100 and 20 ml., respectively.

Calculational Technique. The general expression for calculating oxidation number is:

Oxidation number = (meq. of $K_2Cr_2O_7$ consumed) (mol. wt. of compd.)/(mg. of compd. taken)

As applied, the expression becomes,

$$\text{Ox. No.} = [(x - y) - (A_s/a_s)(B)]\ (\text{mol. wt.})/\text{mg. compd.}$$

where x = meq. of $K_2Cr_2O_7$ taken
y = meq. of $K_2Cr_2O_7$ blank = $x - (A_s\text{, blank}/a_s\text{, blank})(B)$
B = volumetric dilution factor = dilution volume/aliquot

RESULTS AND DISCUSSION

The data illustrating the performance of the method are given in Table III. Glycerol, formic acid, ethylene glycol, and diethylene glycol are completely oxidized to carbon dioxide in 50% sulfuric acid solution. Oxidation of diethylene glycol where the oxidation number was determined by the iodometric method also gave the theoretical value. Oxidation time does not significantly affect the results.

In the case of formaldehyde and acetaldehyde, the oxidation is complete in a solution containing only 5% sulfuric acid by volume. Formaldehyde goes to carbon dioxide and acetaldehyde to acetic acid. This acid concentration is as low as would ever be employed in dichromate oxidation studies; therefore, the interference behavior of these aldehydes is of no concern, since they are incapable of existing as oxidation end products. The gradual rise in oxidation number obtained with formaldehyde with increasing oxidation time is attributed to the methanol content of the formaldehyde reagent, which is not included by the assay method employed for determining the formaldehyde content.

Acetic acid is not oxidized by dichromate under any of the variable conditions tested. This is an important point in the study of dichromate oxidation mechanisms and is somewhat controversial. The authors' results confirm those of investigators such as Polonovski (*19*) and Taufel and Wagner (*27*); however, recently Medalia (*15*) and Moore *et al.* (*17*) reported that acetic acid is slightly oxidized by dichromate in sulfuric acid solution. Thus, Moore obtained oxidation numbers of 0.13 and 0.40 in sulfuric acid solutions of 33 and 50% by volume, respectively. When these investigators obtained an oxidation number of 4.3 for the oxidation of ethyl alcohol (Medalia obtained a value of 5.0) where a value of 4.0 is expected for oxidation to acetic acid, the overoxidation was attributed to a slight oxidation of the acetic acid formed.

It is significant that oxidation number values obtained by the authors using reagent grade glacial acetic acid (99.5%) were in almost exact agreement with those of Moore and that the apparent "interference" on the determination of dichromate as mentioned in the preceding section was only indicated by the less pure reagent. Actually, the order of interference of the 99.5% acetic acid reagent in terms of oxidation number are also of the same order of magnitude as values obtained in oxidation tests.

These results lead to the conclusion that the apparent overoxidation to the acetic acid stage observed in the oxidation of propylene glycol and dipropylene glycol (and acetaldehyde) are due to the presence of oxidizable impurities. Oxidation time has no effect upon the values of the oxidation numbers obtained, which is not what one would expect if the overoxidation were the result of the partial oxidation of an end product obtained.

The values of the oxidation numbers in Table III are averages of the duplicate or triplicate runs where indicated. Analysis of the variance of the individual runs indicates that a precision measure of ±0.05 oxidation number from the average values as shown was attained. Since the individual errors in the over-all procedure such as the absorbancy readings, dilutions, and aliquoting are all less than ±0.3%, the accuracy of the method in terms of oxidation number is ±0.06 unit for a compound having an expected oxidation number of 20.

LITERATURE CITED

(1) Allen, N., Charbonnier, H. Y., and Coleman, R. M., Ind. Eng. Chem., Anal. Ed., **12**, 384–6 (1940).

(2) Beckman Instruments, Inc., South Pasadena, Calif., *Bull.* **91-G, DD 25-2.**

(3) Birtwell, C., and Ridge, B. P., *J. Textile Inst.*, **19**, 341T–8T (1928).

(4) Burtle, J., and Buswell, A. M., *Sewage Works J.*, **9**, 224–39 (1937).

(5) Christensen, B. E., Williams, R. J., and King, A. E., *J. Am. Chem. Soc.*, **59**, 293 (1937).

(6) Elkins, H. B., Storlazzi, E. D., and Hammond, J. W., *J. Ind. Hyg. Toxicol.*, **24**, 229–32 (1942).

(7) Ewing, G. W., and Parsons, T., Anal. Chem., **20**, 423–5 (1948).

(8) Fonteyne, R., and DeSmet, P., *Mikrochemie*, **13**, 289–304 (1933).

(9) Francis, C. V., Anal. Chem., **21**, 1238–9 (1949).

(10) Kasline, C. T., and Mellon, M. G., Ind. Eng. Chem., Anal. Ed., **8**, 463–5 (1936).

(11) Kettering, J. H., and Conrad, C. M., *Ibid.*, **14**, 432 (1942).

(12) Kolthoff, I. M., and Furman, N. H., "Volumetric Analysis," Vol. 11, p. 451, New York, John Wiley & Sons, 1929.

(13) Langmuir, A. C., *et al.*, *J. Ind. Eng. Chem.*, **3**, 679–86 (1911).

(14) Lingane, J. J., and Collat, J. W., Anal. Chem., **22**, 166–9 (1950).

(15) Medalia, A. I., *Ibid.*, **23**, 1318–20 (1951).

(16) Mohlman, F. W., and Edwards, G. P., Ind. Eng. Chem., Anal. Ed., **3**, 119–23 (1931).

(17) Moore, W. A., Kroner, R. C., and Ruchhoft, C. C., Anal. Chem., **21**, 953 (1949).

(18) Natl. Bur. Standards, *Letter Circ.* **LC-857** (1947).

(19) Polonovski, M., *Compt. rend.*, **178**, 576–8 (1924).

(20) Procter and Gamble Co., Chemical Division, Ind. Eng. Chem., Anal. Ed., **9**, 514–15 (1937).

(21) Rosin, J., "Reagent Chemicals and Standards," New York, D. Van Nostrand Co., 1937.

(22) Segal, L., Tripp, R. C., Tripp, V. W., and Conrad, C. M., Anal. Chem., **21**, 712–18 (1949).

(23) Seligson, D., and Seligson, H., *Ibid.*, **23**, 1877–8 (1951).

(24) Shaw, J. A., *Ibid.*, **23**, 1764–7 (1951).

(25) Shenk, W. E., and Fenwick, F., Ind. Eng. Chem., Anal. Ed., **7**, 194–7 (1935).

(26) Simon, L. J., and Guillaumin, A. J. A., *Compt. rend.*, **175**, 525–7 (1922).

(27) Taufel, K., and Wagner, C., *Z. anal. Chem.*, **67**, 16–20 (1925).

(28) Werner, H. W., and Mitchell, J. L., Ind. Eng. Chem., Anal. Ed., **15**, 375–6 (1943).

Received for review July 21, 1952. Accepted September 25, 1952. Presented at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 1952.

Resolving Normal and Isomeric Paraffins in Complex Hydrocarbon Mixtures

A Mass Spectrometric Method

HAROLD SOBCOV[1]

Research and Development Department, Socony-Vacuum Laboratories, Paulsboro, N. J.

Methods previously reported have shown how complex hydrocarbon mixtures in the gasoline and kerosene ranges may be considered to have four major mass spectrometric types and how each type may be determined quantitatively. One of these is the aliphatic paraffin type, but there has not existed any simple and rapid method of determining the individual proportions of *n*-paraffins and total isoparaffins. By an extension of the type analysis method, this further resolution is possible by mass spectrometry after a rough distillation of the sample. While the method is not rigorous and estimates of its probable accuracy are difficult to make, it has been found useful on the basis of checks made with known mixtures.

IN THE evaluation of hydrocarbon mixtures such as gasolines, it is often useful to know the concentrations of both straight-chain and branched paraffins. The adduction of *n*-paraffins by urea is a well-known chemical method for identifying this class of compounds, particularly in the gas oil or wax range. The reaction with antimony chloride forms the basis of a recently described method for distinguishing isoparaffins in a hydrocarbon mixture (*4*). These methods have the drawbacks of complexity, consumption of time, and/or lack of complete specificity. While the method described here does not fully overcome these defects, it has the additional advantage of giving information about both aliphatic paraffin types simultaneously. At present it is applied to mixtures containing paraffins predominantly of a single carbon number with small amounts of the two adjacent carbon numbers. This implies the use of a simple fractionation of the original sample by distillation.

In type analysis by mass spectrometry four classes of hydrocarbons (grouped by equality of content of carbon and hydrogen atoms) are specified by different characteristic ion classes to which they are major contributors (*3*). One of these four hydrocarbon types, the aliphatic paraffins, has the general formula C_nH_{2n+2}; and mass spectra of such compounds characteristically show major concentrations of ions having the general formula $C_nH^+_{2n+1}$. It has been found that the summation of ions, $\Sigma C_nH^+_{2n+1}$ ($n = 3,4,5,6$), is sufficiently characteristic for specifying the paraffins. This is abbreviated to $\Sigma 43$, as in terms of mass units the summation of ions at $m/e = 43, 57, 71,$ and 85 is used.

---

[1] Present address, Perkin-Elmer Corp., Norwalk, Conn.

In varying relatively small amounts the other types also contribute to Σ43 in any mixture spectrum; and during the process of solving four simultaneous equations for the primary type analysis, the portion of Σ43 due to paraffins only is determined.

In the present extension of the method use is made of a second characteristic of paraffins—viz., that the concentration of molecule or "parent" ions, $C_nH^+_{2n+2}$, is easily detectable in a mixture spectrum. Mainly heavy-isotope ions, the concentrations of which can be calculated both readily and accurately, will overlap these parent ions, which can thus be determined by difference and called *P* ions. Furthermore, an inspection of the spectra of pure paraffins reveals that the concentration of *P* ions for *n*-paraffins is relatively much higher than for isoparaffins of the same carbon number. The values for the isoparaffins vary rather widely, *P* ion concentrations tending to approach zero the greater the branching of the molecule.

Table I is a compilation of data for C_4 to C_8 paraffins, which are pertinent to the present method of analysis. These data were obtained using a Model 21-103 mass spectrometer manufactured by the Consolidated Engineering Corp. Electron catcher current was 10 μa. and magnet current was 0.75 ampere. The ion source temperature control was operated to minimize variations about the control point. Over a period of several months the 43/58 pattern ratio for *n*-butane was constant to ±0.5%.

It is important in this method that such stability be maintained because of the high temperature coefficient of parent molecule ions (*5*). The Σ43/*P* ratios listed in Table I have been constant to roughly ±2%, and they do not agree with the published data (*1*). From the latter compilation it appears that the instruments used in obtaining the spectra had consistently higher ion source temperatures, since Σ43/*P* values averaged about 20% higher than reported herein.

The reason for lack of complete specificity of the method is evident from Table I. At any carbon number an average Σ43/*P* ratio must be assumed for the isoparaffins, and the deviations from this average of the values for individual compounds can be very large. However, it is fortuitous that in most cases the large deviations from the assumed average occur for compounds having low probability of occurrence. In any event, there are available checks for gross errors generated by assumption of an average ratio for the isoparaffins.

To date the range up to and including the octanes has been studied thoroughly. Beyond this range only the *n*-paraffins and a very few isoparaffins are available in pure form, so that extrapolation of the data must be resorted to. Similar procedures are being applied to the National Bureau of Standards mass spectral data on the nonanes (*1*). However, it is planned to run representative distillate fractions containing isoparaffins of only one carbon number to arrive experimentally at average Σ43/*P* and sensitivity values.

Table I. Coefficients for the C_4–C_8 Paraffins

	Relative[a] Weight	Σ43/*P*	Sens. *P*[b] Divs./μ	Sens. Σ43[b] Divs./μ	Sens. Σ57[b] Divs./μ	Sens. Σ71[b] Divs./μ
C_4						
n-Butane	100	8.2	12.50	103	2.6	0
Isobutane	100	39.7	3.09	129	3.9	0
				Av. 116	3.2	0
C_5						
n-Pentane	100	12.2	10.33	129	15.3	0.6
Isopentane	100	24.5	4.70	119	44.0	1.5
2,2-Dimethylpropane	0	2550[c]		117	115	0.1
		Av. 24.5[d]		124	29.7	1.0
C_6						
n-Hexane	100	11.3	12.96	146	85.6	4.6
2-Methylpentane	22	37.9	4.51	183	52.4	38.5
3-Methylpentane	17	36.2		135	101	6.4
2,2-Dimethylbutane	2	5560[c]		211	135	60.3
2,3-Dimethylbutane	4	27.9		157	24.6	25.0
		Av. 36.2[d]		152	81.2	11.5
C_7						
n-Heptane	100	13.3	14.82	199	94.8	48.3
2-Methylhexane	30	34.4	4.39	216	84.6	48.8
3-Methylhexane	22	46.2		200	101	53.2
2,2-Dimethylpentane	1	2330[c]		251	167	45.2
2,3-Dimethylpentane	4	74.4		173	92.1	33.8
2,4-Dimethylpentane	3	128		229	113	25.4
3,3-Dimethylpentane	0	∞[c]		267	120	114
3-Ethylpentane	2	64.7		213	71.5	65.9
2,2,3-Trimethylbutane	0	2930[c]		221	149	38.3
		Av. 47.6[d]		202	94.2	48.4
C_8						
n-Octane	100	25.5	9.44	241	113	68.9
2-Methylheptane	47	32.6	6.38	293	116	37.2
3-Methylheptane	16	62.0		268	147	65.4
4-Methylheptane	11	46.4		275	110	89.4
2,2-Dimethylhexane	0.5	2340[c]		252	218	14.8
2,3-Dimethylhexane	4	74.6		253	100	74.3
2,4-Dimethylhexane	3	112		263	153	70.7
2,5-Dimethylhexane	3	42.5		244	123	46.5
3,3-Dimethylhexane	2	11500[c]		324	183	131
3,4-Dimethylhexane	7	65.7		209	141	48.7
3-Ethylhexane	5	79.5		270	96.2	73.8
2-Methyl-3-ethylpentane	3	117		270	88.2	65.8
3-Methyl-3-ethylpentane	1	∞[c]		311	161	118
2,2,3-Trimethylpentane	0.2	4160[c]		223	183	11.6
2,2,4-Trimethylpentane	0	3120[c]		260	212	12.3
2,3,3-Trimethylpentane	0.3	∞[c]		300	156	109
2,3,4-Trimethylpentane	0.3	430[c]		283	125	98.9
2,2,3,3-Tetramethylbutane	0	4720[c]		225	191	12.6
		Av. 50[d]		258	119	61.1

[a] Relative weights arbitrary for C_4 and C_5; for C_6, C_7, and C_8 taken from data of Rossini and Mair (*6*).
[b] Referred to *n*-butane sensitivity = 100 divs./μ at *m/e* = 43.
[c] Data for this compound not included in average.
[d] This average does not include the *n*-paraffins.

METHOD OF ANALYSIS

Consider the case of a hydrocarbon mixture in which paraffins of a single carbon number are to be resolved. While not absolutely necessary, a saturate portion of the sample (paraffins and naphthenes) is first obtained by acid treating (*2*). From the primary type analysis Σ43 due to total paraffins is known and can be written as $\Sigma 43_{n+i}$, where the subscripts *n* and *i* refer to *n*-paraffin and isoparaffin, respectively. The mixture spectrum also reveals the total paraffin parent molecule ion concentration, which can be written as P_{n+i}; in addition, the carbon number is immediately evident, so that the properly corresponding $\left(\frac{\Sigma 43}{P}\right)_n$ and $\left(\frac{\Sigma 43}{P}\right)_i$ can be chosen from Table I.

$$\text{Now } \left(\frac{\Sigma 43}{P}\right)_{n+i} = \frac{\Sigma 43_{n+i}}{P_{n+i}} = \frac{\Sigma 43_n}{P_n + P_i} + \frac{\Sigma 43_i}{P_n + P_i} = \frac{\left(\frac{\Sigma 43}{P}\right)_n}{1 + \frac{P_i}{P_n}} + \frac{\left(\frac{\Sigma 43}{P}\right)_i}{\frac{1}{\frac{P_i}{P_n}} + 1}$$

Writing Σ43/*P* as *k* for simplicity and transposing and collecting terms:

$$(k_{n+i} - k_i)\left(\frac{P_i}{P_n}\right)^2 + (2k_{n+i} - k_n - k_i)\frac{P_i}{P_n} + (k_{n+i} - k_n) = 0$$

Solving for P_i/P_n and using the physically significant positive root, we get

$$\frac{P_i}{P_n} = \frac{k_{n+i} - k_n}{k_i - k_{n+i}} \tag{1}$$

Also

$$P_i + P_n = P_{n+i} \tag{2}$$

so that P_i and P_n are determined.

If now we apply to P_n and P_i factors of sensitivity (ion current per unit pressure) as shown in Table I, partial pressures of both n-paraffin, μ_{P_n}, and isoparaffin, μ_{P_i}, are obtained. Again a weighted average must be used for the isoparaffin sensitivity factor, which is seen to be much lower than for the corresponding n-paraffin.

It appears at once that a major source of inaccuracy lies in the assumption of a weighted average for the isoparaffins. However, gross errors arising from this assumption are either overcome or detected by applying the following criteria:

$k_n \leqq k_{n+i} \leqq k_i$. Both k_n and k_{n+i} are always known and fixed; and if the assumed k_i is, say, low, then P_i will be determined high in Equation 1. The error will be compensated partially because the assumed sensitivity for P_i is also too high, since k_i and sensitivity P_i always have a converse relationship.

The total partial pressure, $\mu_n + \mu_i$, of the resolved paraffins should agree with the pressure, $\mu_{\Sigma 43n+i}$, obtained from the original type analysis for total paraffins. The Σ43 sensitivity factors listed in Table I are seen to agree fairly well for all paraffins of a single carbon number, and experience with many samples of widely varying composition has indicated a probable accuracy of ±10% for $\mu_{\Sigma 43n+i}$. This conclusion is further substantiated by comparison of sample pressures so computed with pressures observed by micromanometer.

For highest accuracy the method should be applied to samples containing paraffins having a single carbon number predominant, which implies the necessity of rough distillation procedures. For wider boiling ranges, error arises in determining the intensity of the Σ43 ions due to paraffins of each of the different carbon numbers, if more than one is present in high concentration. The simplest procedure appears to be a rapid distillation, with cuts taken at points corresponding to the n-paraffins. In any one cut there should then be paraffins of three carbon numbers with one very dominant.

In such cuts the following procedure is used where paraffins of three different carbon numbers are present. If the partial pressures of the paraffins at the different carbon numbers are μ_1, μ_2, and μ_3, we have:

$$\begin{aligned} S_1\mu_1 + S_2\mu_2 + S_3\mu_3 &= \Sigma 43 \\ S_1'\mu_1 + S_2'\mu_2 + S_3'\mu_3 &= \Sigma 57 \\ S_1''\mu_1 + S_2''\mu_2 + S_3''\mu_3 &= \Sigma 71 \end{aligned}$$

where

Σ43 = sum of paraffin ions of m/e = 43, 57, 71, and 85
Σ57 = sum of paraffin ions of m/e = 57, 71, and 85
Σ71 = sum of paraffin ions of m/e = 71, 85, and 99
S_1, S_2, S_3 = Σ43 average sensitivities of paraffins at different carbon numbers
S_1', S_2', S_3' = Σ57 average sensitivities of paraffins at different carbon numbers
S_1'', S_2'', S_3'' = Σ71 average sensitivities of paraffins at different carbon numbers

Solving these simultaneous equations determines μ_1, μ_2, and μ_3, from which is obtained $(\Sigma 43)_1 = S_1\mu_1$, etc. The parent molecule ions at each carbon number P_1, etc., are easily determined and the procedure outlined previously can now be applied to $\left(\frac{\Sigma 43}{P}\right)_1$, etc., to resolve the paraffins at each carbon number. A distribution of the paraffins by carbon number is obtained coincidentally.

In Table I the Σ43, Σ57, and Σ71 sensitivities for individual C_4 to C_8 paraffins are listed. Weighted average sensitivities for all paraffins at each carbon number are also given.

The close similarity of the average coefficients for different carbon numbers and the deviations of individual compounds from the average values for Σ57 and Σ71 coefficients may give rise to large errors when the method is applied to mixtures which do not have paraffins of a single carbon number predominant. However, the results of Table II indicate a reasonable working accuracy for mixtures which were mainly C_6 or C_7 fractions, but contained compounds of the two adjacent carbon numbers.

RESULTS AND EXAMPLES

Table II illustrates results obtained with several samples of narrow boiling range obtained from distillations of typical gasolines. The compositions of these fractions were accurately known either by synthesis with A.P.I. standard samples or by accurate mass spectrometer analysis for individual components. The probable accuracy of the method is seen to be high and certainly of use in evaluation of hydrocarbon products. The reason for preferable use of the saturate portion of the sample is also seen in Table II. A small error made in determining the Σ43 ions from paraffins only is magnified by the method of resolution, and this error is minimized particularly by removing the monoolefins.

Table II. Results of Resolution of Total Paraffins in Known C_6 to C_8 Fractions

	n-Paraffin as % of Total Paraffins[a]		
	Paraffin-Naphthene Portion		Total sample, present method
Sample	Accurate M.S. analysis	Present method	
1 (53)[b]	51	55	...
2	45	44	39
3	44	40	36
4	59	61	66
5	88	88	100
6	16	16	21
7	15	10	19
8	21	23	42

[a] For each carbon number mol. % = liquid vol. % assumed.
[b] Synthesized value.

It is difficult to establish a definite value for the error to be expected in analyses by this method. However, an idea of the effect of incorrect assumptions of average isoparaffin values can be gathered from the hypothetical examples given below.

Example I. Nearly Correct Isoparaffin Values Assumed. Consider a C_7 paraffin fraction made up of 50% n-heptane and 50% 3-methylhexane and assume 20 microns of sample entered into the instrument. For 3-methylhexane $\Sigma 43/P = 46.2$ and the sensitivity of P = 4.33 divisions per micron. From data in Table I we would have:

	Σ43, Divs.	P (m/e = 100), Divs.
10 μ nC_7	1975	148.2
10 μ $3MC_6$	2000	43.3
Totals observed in mixture spectrum	3975	191.5

$$k_{n+i} = \frac{3975}{191.5} = 20.7$$

We know k_n = 13.3 and assume k_i = 47.6

$$\frac{P_i}{P_n} = \frac{20.7 - 13.3}{47.6 - 20.7} = 0.275$$

$$P_i + P_n = 191.5$$

	Sensitivity, Divs./μ	Partial Press., μ	Mole %
P_n = 150.1	14.82	10.13	51.8
P_i = 41.4	4.39	9.43	48.2
		19.56	

Check: Σ43 = 3975 divisions. Average sensitivity Σ43 for C_7 paraffins = 202 divisions per micron.

$$\text{Total partial pressure} = \frac{3975}{202} = 19.70 \text{ (good agreement)}$$

Example II. Isoparaffin Values Approximately 100% in Error. Consider the same case as in Example I, but substitute 2,3-

dimethylpentane for the 3-methylhexane. For 2,3-dimethylpentane $\Sigma 43/P = 74.4$ and sensitivity of $P = 2.31$ divisions per micron.

	Σ43, Divs.	P ($m/e = 100$), Divs.
10 μ nC_7	1975	148.2
10 μ $2{,}3M_2C_5$	1720	23.1
Totals observed in mixture spectrum	3695	171.3

$$k_{n+i} = \frac{3695}{171.3} = 21.6$$

$$\frac{P_i}{P_n} = \frac{21.6 - 13.3}{47.6 - 21.6} = \frac{8.3}{26.0} = 0.319$$

$$P_i + P_n = 171.3$$

	Sensitivity, Divs./μ	Partial Press., μ	Mole %
$P_n = 129.9$	14.82	8.76	48.2
$P_i = 41.4$	4.39	9.43	51.8
		18.19	

CHECK: $\Sigma 43 = 3695$ divisions. Sensitivity $\Sigma 43 = 202$ divisions per micron.

Total paraffin partial pressure $= \frac{3695}{202} = 18.30$ μ (good agreement)

Example III. Isoparaffin Values in Extreme Error. Consider the same case once more with 3,3-dimethylpentane as the isoparaffin. In this case $\Sigma 43/P = \infty$ and sensitivity of $P = 0$.

	Σ43, Divs.	P ($m/e = 100$), Divs.
10 μ nC_7	1975	148.2
10 μ $3{,}3M_2C_5$	2675	0
Totals observed in mixture spectrum	4650	148.2

$$k_{n+i} = \frac{4650}{148.2} = 31.4$$

$$\frac{P_i}{P_n} = \frac{31.4 - 13.3}{47.6 - 31.4} = \frac{18.1}{16.2} = 1.118$$

$$P_i + P_n = 148.2$$

	Sensitivity, Divs./μ	Partial Press., μ	Mole %
$P_n = 70.0$	14.82	4.73	21.0
$P_i = 78.2$	4.39	17.80	79.0
		22.53	

CHECK: $\Sigma 43 = 4650$ divisions. Sensitivity $\Sigma 43 = 202$ divisions per micron.

Total paraffin partial pressure = 23.03 microns. The discrepancy in this case, while larger than for the others, would not in itself indicate the large error in the result. However, it is seen that even for the case of an unusual compound comprising 100% of the isoparaffin portion of a mixture, the analytical error that results is very much smaller than the error in the assumed isoparaffin coefficients would infer.

ACKNOWLEDGMENT

The author is appreciative of helpful consultations with F. P. Hochgesang and the assistance of Mrs. B. L. Brown in compiling the reported data.

LITERATURE CITED

(1) Am. Petroleum Inst., "Catalog of Mass Spectral Data," A.P.I. Research Project 44.
(2) Am. Soc. Testing Materials, Method D 1019.
(3) Brown, R. A., ANAL. CHEM., 23, 430 (1951).
(4) Leithe, W., *Ibid.*, 23, 493 (1951).
(5) Reese, R. M., Dibeler, V. H., and Mohler, F. L., *J. Research Natl. Bur. Standards*, 46, 79 (1951).
(6) Rossini, F. D., and Mair, B. J., A.P.I. Research Project 6, Report, June 1951.

RECEIVED for review April 29, 1952. Accepted August 15, 1952. Presented before the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 1952.

Determination of Individual Alkyl Aromatic Hydrocarbons

From Benzene through the C_{10} Aromatics by Infrared Spectrometry

R. B. WILLIAMS, S. H. HASTINGS, AND J. A. ANDERSON, JR.
Humble Oil and Refining Co., Baytown, Tex.

As a result of process research studies on segregating aromatic hydrocarbon fractions for possible use as chemical raw materials, it became necessary to determine many individual aromatics over a wide molecular weight range. Procedures involving distillation plus infrared spectrometric analysis have been developed for determining essentially all of the individual alkyl benzene hydrocarbons through the C_{10}'s. Techniques are presented for achieving the infrared analysis, including a simple method for linearizing aromatic absorptivities that is an improvement over previous methods. Composition data of particular interest to the petroleum technologist are given which cover in great detail a few typical refinery aromatic stocks of C_8, C_9, and C_{10} molecular weight range.

THE trend during the past few years toward the use of individual aromatics derived from petroleum sources as chemical raw materials has prompted the extension of existing techniques and the development of new techniques for determining these constituents. The infrared analytical method described here has been used both in exploratory characterization studies of the higher boiling aromatic stocks and in routine determinations of the C_8 and lower boiling aromatics in connection with the various phases of process research dealing with these compounds. This method now largely supersedes formerly employed routine ultraviolet procedures for benzene and toluene and for the C_8 aromatics.

Except for certain modifications in measuring absorption, the infrared method is based on commonly known multicomponent techniques. Fractional distillation, and sometimes other separations, are generally required for the higher boiling ranges. Although determinations of the various compounds are relatively straightforward in most cases, there are situations in higher boiling ranges in which the infrared method alone is inadequate for distinguishing between a few compounds, even in well separated fractions, and in these cases auxiliary analytical tools are required both for supplementary analyses and for cross checking.

A discussion of the infrared method and the presentation of analyses of aromatics in virgin naphthas and of aromatics derived from hydroforming operations are presented in the following sections.

DESCRIPTION OF METHOD

The major differences between this method and the usual liquid multicomponent scheme are in the necessity for making corrections for apparent Beer's law deviations and for eliminating wave-length shifts of the absorption bands. Kaye and Otis (*10*) mention the first difficulty but not the second. The apparent Beer's law deviations occur because the absorption bands used are very sharp and are situated in the 11.5- to 15.0-micron region where spectrometer resolution is poor. The wave-length shifts occur when the aromatics exist in different relative concentrations or in different solvents. This phenomenon evidently is due to molecular association. The difficulty of the shifting absorption bands is eliminated if the samples are diluted in the same solvent each time prior to analysis to a concentration of total aromatics of less than about 10%. In any event it is necessary to dilute the sample, as these aromatic bands are intense. The authors therefore, as did Kaye and Otis (*10*), blend all samples in carbon disulfide, which is transparent in the spectral region of interest.

The wave-length shifts for toluene in various solvents are shown in Table I. Other aromatics behave in a qualitatively similar fashion.

Table I. Peak Shift of 13.7-Micron Toluene Band in Different Solvents

Solvent	Shift, Microns (Relative to Pure Toluene)
None	0.00
n-Heptane	+0.05
Methylcyclohexane	+0.02
Benzene	−0.04
m-Xylene	−0.02
p-Xylene	−0.02
Carbon disulfide	+0.02

The correction for the apparent Beer's law deviation is straightforward and simple. It is based on the conclusion that the deviation is due principally to inadequate resolution and to a lesser extent to uncompensated stray radiation. Observations show the absorptivity deviates only as a function of the absorbance level and not of the concentration of aromatic in solvent at the low concentrations employed. For example, if both the concentration of aromatic in solvent and the length of sample cell are varied in such a way as always to maintain the absorbance at the same level, the absorptivity of the aromatic does not change. If, however, either the concentration or the cell length is varied while the other is maintained fixed, the absorptivity also varies. This is shown in Figure 1 for the case of toluene. If the deviation were due to any significant factor other than spectrometer limitations, such as, for example, molecular association (which depends on concentration), the two sets of data would not be adequately represented by a single curve. The observed effect appears to be that which one would predict from resolution limitations (*3, 12, 13*). It is thus permissible to make a correction to absorptivity deviation based on absorbance alone and to employ whatever concentrations of sample in solvent and whatever cell lengths are chosen. The process of calibration thereby becomes much simpler and the method more versatile; the method presented thus seems to be a significant improvement over that of Kaye and Otis (*10*) from these standpoints.

In order to achieve the optimum in photometric accuracy and to keep the Beer's law deviations small and the corrections thereto sufficiently linear, all absorbance measurements are restricted to the range 0.3 to 0.5. All absorptivities are then corrected to a 0.400 absorbance basis—i.e., all absorptivities are corrected to those values which would have been obtained had the absorbance been exactly 0.400.

In the case of toluene and several of the other aromatics, the absorptivity deviation is about 2% for 0.100 change in absorbance from the 0.400 level, the higher absorbances giving negative deviations, and vice versa. For benzene, whose band appears at the longest wave length of all, the comparable deviation is about 4%. The various corrections are slightly different for each compound, depending upon the spectrometer resolution (wave-length location and slit size) and the sharpness of the absorption band. The actual corrections can be made by means of the simple relation,

$$a_c = a_u\,[1 + (A - 0.400)f]$$

where

a_c is the corrected absorptivity
a_u is the uncorrected absorptivity
A is the absorbance of sample in carbon disulfide
f is the correction factor—0.2 for toluene and 0.4 for benzene, for example

The separation requirements prior to analysis depend upon the boiling range of the sample and the magnitude and types of compounds other than the aromatics in the sample. Because the absorptivities of the aromatic compounds are so much greater than those of other hydrocarbons, and the interference between aromatics is generally low even for relatively wide boiling ranges, it is evident that the separation requirements usually are not too stringent. For samples containing high concentrations of nonaromatic hydrocarbons, silica gel percolation or other similar separation is sometimes necessary to remove these otherwise minor interferers and to improve analytical accuracy. For very wide boiling ranges, distillations are required. Occasionally both types of separations are needed. Such requirements, especially of distillation, naturally become more severe for the higher boiling ranges where the number of aromatic isomers is great.

EQUIPMENT AND EXPERIMENTAL TECHNIQUE

All absorption measurements were made on a Perkin-Elmer Model 12-B infrared spectrometer having rock salt optics, operated at an amplifier gain equivalent to a full-scale recorder de-

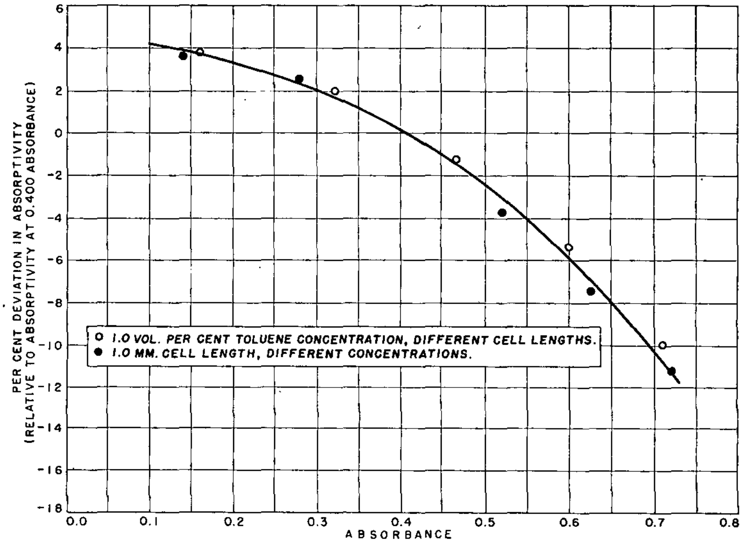


Figure 1. Deviation of Toluene Absorptivity with Absorbance

Table II. Calibration Data on Pure Compounds

Compound	Boiling Point, ° F.	Source	Purity, Mole %	Analytical Wave Length, μ	Actual Slit Width, Mm.	Corrected Absorptivity at Analytical Wave Length, Ml./G. Mm.
Benzene	176.19	Phillips Petroleum Co.	99.93	14.86	1.190	500.3
Toluene	231.12	Phillips Petroleum Co.	99.89	13.74	0.670	300.1
Ethylbenzene	277.14	Phillips Petroleum Co.	99.82	14.37	0.940	108.1
				14.05[a]	0.990	22.7
1,4-Dimethylbenzene	281.03	NBS	99.94	12.58	0.460	209.1
1,3-Dimethylbenzene	282.38	NBS	99.94	13.02	0.520	190.3
1,2-Dimethylbenzene	291.95	NBS	99.99	13.51	0.590	299.8
Isopropylbenzene	306.31	NBS	99.93	13.15	0.520	121.0
n-Propylbenzene	318.59	NBS	99.75	13.47	0.590	87.2
1-Methyl-3-ethylbenzene	322.34	NBS	99.57	12.80	0.490	51.9
1-Methyl-4-ethylbenzene	323.57	NBS	99.87	12.28	0.425	98.7
1,3-5-Trimethylbenzene	328.48	NBS	99.95	11.96	0.425	130.7
1-Methyl-2-ethylbenzene	329.27	NBS	99.33	13.27	0.520	108.0
tert-Butylbenzene	336.40	NBS	99.94	13.12	0.520	118.7
1,2,4-Trimethylbenzene	336.82	NBS	99.67	12.40	0.425	127.6
Isobutylbenzene	342.96	NBS	99.87	13.58	0.615	125.6
s-Butylbenzene	343.94	NBS	99.88	13.18	0.520	85.5
1-Methyl-3-isopropylbenzene	347.4	NBS	99.936	12.78	0.490	113.6
1,2,3-Trimethylbenzene	348.94	NBS	99.982	13.04	0.520	152.8
				14.05[a]	0.690	23.2
1-Methyl-4-isopropylbenzene	350.78	NBS	99.95	12.28	0.425	140.9
Hydrindene	352	NBS	99.94	13.33	0.520	128.7
1-Methyl-2-isopropylbenzene	352.9	Republic Chem. Co.	95 (est.)	13.21	0.520	89.0
1,3-Diethylbenzene	358.03	NBS	99.93	12.56	0.460	47.3
1-Methyl-3-*n*-propylbenzene	360	Penn State College	98.	12.83	0.490	103.0
n-Butylbenzene	361.88	NBS	99.88	13.44	0.590	61.5
				14.36[a]	0.840	98.8
1-Methyl-4-*n*-propylbenzene	362.21	Penn State College	99.6	12.46	0.490	76.0
1,2-Diethylbenzene	362.26	NBS	99.95	13.28	0.520	137.0
1,3-Dimethyl-5-ethylbenzene	362.75	NBS	99.89	11.83	0.455	100.2
1,4-Diethylbenzene	362.80	NBS	99.93	12.07	0.425	116.9
1-Methyl-2-*n*-propylbenzene	363	Penn State College	99.4	13.44	0.590	109.3
2-Methylhydrindene	367	None	...	13.55[b]	0.590	...
1-Methylhydrindene	368	None	...	13.55[b]	0.590	...
1,4-Dimethyl-2-ethylbenzene	368.44	Penn State College	99.8	12.38	0.490	95.0
1,3-Dimethyl-4-ethylbenzene	371.14	Penn State College	99.9	12.24	0.460	82.2
1,2-Dimethyl-4-ethylbenzene	373.55	Penn State College	99.6	12.20	0.460	77.9
1,3-Dimethyl-2-ethylbenzene	374.02	Penn State College	99.8	13.05	0.570	136.4
1,2-Dimethyl-3-ethylbenzene	381.04	Penn State College	99.6	13.84	0.765	38.0
1,2-4,5-Tetramethylbenzene	385	Humble	99.8	11.54	0.350	70.2
1,2,3,5-Tetramethylbenzene	388.27	NBS	99.92	11.78	0.360	90.2
5-Methylhydrindene	395	Humble	50 (est.)	12.34	0.490	...
4-Methylhydrindene	396 (est.)	Humble	50 (est.)	13.05	0.490	...
1,2,3,4-Tetramethylbenzene	401.07	Humble	95	12.44	0.490	96.4
1,2,3,4-Tetrahydronaphthalene	402.2	Penn State College	95	13.39	0.590	160
Naphthalene	424.33	NBS	99.96	12.78	0.510	477.8

[a] Alternate.

[b] Estimated approximately from spectra given in (*9*), and more precisely from measurements on samples containing these substances.

flection for about 1-microvolt input signal. The precision of measuring absorbances was such that the standard deviation at the 0.400 absorbance level was about 0.002 absorbance unit, or 0.5%, when tested over a period of several days and about 0.004 absorbance unit, or 1%, when tested over a period of several months.

Samples generally were weighed and diluted to the proper volume with carbon disulfide, the concentrations being expressed in grams per milliliter. Occasionally blends were made volumetrically. Because of the necessity for dilute solutions, the sample cell has to be fairly thick. Almost all sample measurements, and all calibration measurements, were made with the same sample cell, which was about 1.0 mm. in thickness. The thickness, or length, was measured by the method of interference fringes. At frequent intervals this was checked for possible changes, but there never was a change greater than about 1% over several months.

Absorption measurements were made from short scans, only a few hundredths of a micron in width, recorded across the desired spectral positions of the various absorption bands. The scans were kept short to reduce errors from zero drift. A lithium fluoride shutter was used for zero deflection measurements. A scan of a rock salt plate "reference" always was made along with the sample scan, either immediately before or immediately after, to avoid errors from long period changes in deflection sensitivity. Either at the beginning or end of a series of measurements on a sample, similar scans of the salt plate and of the sample cell filled with pure carbon disulfide were made to provide a more accurate correction for the sample cell absorption. The absorbance of the pure carbon disulfide with respect to the salt plate was subtracted from the absorbance of the sample diluted in carbon disulfide with respect to the salt plate. This effectively gives the absorbance of the sample with respect to carbon disulfide.

The use of the rock salt reference as an intermediate step in the absorbance determination is important in two ways. First, it saves time, allowing one to move to another analytical wave length and to change the slit without having to empty the sample cell alternately of sample and of carbon disulfide between settings. It would not be good practice to try to obtain several measurements of the sample (P's or I's) at various slit settings and wave lengths and then replace the sample with carbon disulfide and try to determine the correct corresponding reference intensity measurements (P_0's or I_0's), without some intermediate absorption reference. Second, because the pure carbon disulfide is the ultimate absorption reference, the accuracy of calibration of the various pure compounds does not depend upon changes between the relative absorption of the salt plate and sample cell over extended periods so long as there is no change during a single analysis.

CALIBRATION

All compounds used in calibration were of the highest purity available in the laboratories. Of the 39 compounds employed, 35 had purities of better than 99.5 mole % and 37 had purities of better than 99.0%. Fortunately, neither of the two compounds of lowest purity has been present in very large concentrations (above 10%) in any fraction yet analyzed. All absorptivities were corrected to a constant absorbance level basis (0.400, as already mentioned) and the absorptivities at the peaks of the major absorption bands were further corrected for purity of the spectroscopic standards by dividing the absorptivities obtained by the mole fraction purity of the respective standards. The latter correction was not applied to interference absorptivities, as the absorptivities of the impurities at these interference positions many times are of about the same level as of the standards; at a position of strong absorption, however, the probability is that the absorptivities of these impurities are far less than that of the standard, and in this case one is justified in applying a correction. It is sometimes possible to determine individual impurities if they are known, and correct for their specific con-

Table III. Relative Absorptivities, or

Compound	14.86	13.74	14.37	14.05	12.58	13.02	13.51	13.15	13.47	12.80	12.28	11.96	13.27	13.12
	Analytical Wave Length, Microns													
Benzene	**100.0**	0.9	21.6	11.2	0.4	0.8	0.6	1.1	2.0	0.8	1.3	2.6	1.3	...
Toluene	1.0	**100.0**	37.9	45.4	0.7	1.2	4.6	2.6	16.6	4.0	1.4	1.9	6.0	...
E Bz[c]	0.6	8.9	**100.0**	**100.0**	4.2	8.6	15.6	11.0	53.7	26.0	2.2	2.1	22.8	...
1,4-DM Bz	0.0	0.2	2.2	0.5	**100.0**	0.9	0.2	0.8	0.7	13.5	5.1	3.0	0.5	0.8
1,3-DM Bz	0.3	0.6	6.6	10.7	1.2	**100.0**	0.8	17.2	2.6	21.6	1.4	0.5	5.5	16.6
1,2-DM Bz	0.1	2.9	7.9	0.9	0.6	1.6	**100.0**	4.9	343.8	3.9	1.0	0.9	12.7	4.7
IsoC$_3$ Bz	0.8	1.5	40.5	111.4	0.7	0.7	2.8	**100.0**	9.6	5.4	0.9	1.6	19.3	75.8
*n*C$_3$ Bz	0.5	7.7	62.1	90.7	1.0	2.0	30.3	7.2	**100.0**	3.9	3.6	1.9	19.7	4.9
1-M-3-E Bz	0.3	1.4	26.4	52.5	10.5	11.7	2.0	11.3	6.8	**100.0**	2.9	0.8	11.6	11.9
1-M-4-E Bz	0.1	1.0	8.8	0.8	5.1	2.9	0.7	4.4	2.3	12.1	**100.0**	5.7	3.2	4.5
1,3,5-TM Bz	0.3	0.0	1.8	3.0	0.7	0.4	0.4	0.6	0.7	0.6	1.5	**100.0**	0.2	0.2
1-M-2-E Bz	0.1	18.4	12.8	1.1	6.0	4.5	10.6	22.9	36.6	14.3	2.1	0.9	**100.0**	16.8
tert-C$_4$ Bz	...	...	...	...	0.7	15.5	0.7	62.0	2.5	4.7	1.0	2.8	12.9	**100.0**
1,2,4-TM Bz	0.0	0.0	4.4	1.3	3.1	0.4	0.2	0.4	0.8	3.1	13.4	1.3	0.6	0.6
IsoC$_4$ Bz	...	...	...	...	...	...	12.9	2.6	43.7	5.4	4.3	1.5	4.6	2.0
s-C$_4$ Bz	...	...	...	...	...	...	...	59.5	12.9	7.0	2.2	2.2	28.1	41.2
1-M-3-iC$_3$ Bz	0.1	0.4		11.8	2.7	2.2	0.3	1.4	1.0	176.5	1.3	0.1	0.9	1.6
1,2,3-TM Bz	0.1	0.4	30.4	1.1	1.0	68.1	0.9	27.4	3.2	18.3	1.2	0.3	8.7	54.2
1-M-4-isoC$_3$ Bz	...	...	...	...	...	...	...	0.8	0.7	2.8	138.8	7.7	0.6	0.7
Hydrindene	0.1	4.3	9.3	1.2	0.8	3.7	26.0	16.3	89.3	5.6	1.6	1.9	87.7	13.4
1-M-2-isoC$_3$ Bz											2.0	1.5	53.1	55.3
1,3-DE Bz											4.6	1.1	7.2	7.9
1-M-3-*n*C$_3$ Bz												2.6	5.3	...
n-C$_4$ Bz												1.6	16.9	...
1-M-4-*n*-C$_3$ Bz												12.9	6.3	...
1,2-DE Bz												0.9	124.9	...
1,3-DM-5-E Bz												7.2	0.6	0.9
1,4-DE Bz												28.8	2.0	2.5
1-M-2-*n*-C$_3$ Bz												1.4	24.1	...
2-M Hydrindene														
1-M Hydrindene														
1,4-DM-2-E Bz														
1,3-DM-4-E Bz														
1,2-DM-4-E Bz														
1,3-DM-2-E Bz														
1,2-DM-3-E Bz														
1,2,4,5-TM Bz														
1,2,3,5-TM Bz														

(continued)

Compound	13.44	13.55	13.55	12.38	12.24	12.20	13.05	13.84	11.54	11.78	12.34	13.05	12.44	13.39	12.78
	Analytical Wave Length, Microns														
1,2,3-TM Bz	3.1														
1-M-4-isoC$_3$ Bz	0.6														
Hydrindene	72.4														
1-M-2-isoC$_3$ Bz	26.1														
1,3-DE Bz	4.1	x	x												
1-M-3-*n*C$_3$ Bz	8.7	xx	xx												
*n*C$_4$ Bz	56.3	x	x	2.5	3.0	3.3									
1-M-4-*n*-C$_3$ Bz	4.9	x	x	30.4	19.6	17.5	2.4	5.5	1.3	1.3					
1,2-DE Bz	18.7	x	x	6.0	5.3	4.4	8.6	25.3	3.0	0.5					
1,3-DM-5-E Bz	0.6	x	x	1.3	2.0	2.3	0.9	3.4	4.4	35.4					
1,4-DE Bz	1.7	x	x	8.5	21.3	27.9	2.6	4.2	1.0	6.4					
1-M-2-*n*-C$_3$ Bz	**100.0**	x	x	1.3	1.8	2.2	10.9	32.1	4.1	1.4					
2-M Hydrindene	xx	**100.0**	xxx	x	x	x	xx	xx	x	x					
1-M Hydrindene	xx	xxx	**100.0**	x	x	x	xx	xx	x	x					
1,4-DM-2-E Bz	1.0	xxx	xxx	**100.0**	18.9	15.3	0.5	4.6	1.2	0.0	xxx	x	46.0	x	0.2
1,3-DM-4-E Bz	0.3	x	x	11.6	**100.0**	99.2	0.6	1.2	6.0	4.9	x	x	6.1	x	0.3
1,2-DM-4-E Bz	1.6	x	x	9.7	80.8	**100.0**	1.8	10.4	4.3	0.0	x	x	6.2	x	0.6
1,3-DM-2-E Bz	11.8	x	x	1.5	1.2	1.2	**100.0**	1.7	0.0	0.0	x	xx	2.6	x	1.5
1,2-DM-3-E Bz	5.4	x	x	7.3	4.5	4.1	9.3	**100.0**	0.3	0.0	x	x	12.5	x	5.7
1,2,4,5-TM Bz	0.0	x	x	0.6	0.9	0.8	0.2	0.8	**100.0**	3.7	x	x	0.5	x	0.1
1,2,3,5-TM Bz	0.5	x	x	0.7	0.8	0.7	0.2	1.7	2.3	**100.0**	x	x	0.5	x	0.1
5-M Hydrindene				xxx	xx	xx	x	x	x	x	**100.0**	?	xx	x	x
4-M Hydrindene				?	?	?	?	?	?	?	?	**100.0**	?	?	?
1,2,3,4-TM Bz				4.2	6.0	5.0	0.6	1.6	1.3	0.5	xxx	x	**100.0**	x	0.3
1,2,3,4-Tetrahydronaph.				28.1	...	4.3	7.8	14.2	4.3	2.8	x	xx	4.2	**100.0**	0.8
Naphthalene				5.2	4.7	5.5	7.0	6.3	2.5	1.8	x	x	6.7	x	**100.0**

tributions. This has been done for *o*-cymene where the major impurities were *m*-cymene and *p*-cymene, for which accurate calibration data were available, and similarly for 1-methyl-3-*n*-propylbenzene where the impurities were 1-methyl-2-*n*-propyl benzene and 1-methyl-4-*n*-propylbenzene.

A listing of the compounds used in the infrared calibration, their source, and their purity, and a tabulation of the analytical wave lengths and key corrected absorptivities are shown in Table II. Four compounds found in the C$_{10}$ range, the methyl hydrindenes, were not available for calibration except in very impure form in the case of 4- and 5-*m*-hydrindene. These can be identified and semiquantitatively determined by means of their tabulated infrared spectra (*9*), by mass spectrometer analysis, and by other analytical methods. These supplementary methods are referred to again in a later section.

Table III lists the relative absorptivities, or absorption coefficients, on a percentage basis at each analytical wave length. The boxes shown are intended to indicate, by the data they enclose, which compounds generally can be allowed in any single fraction for the attainment of highest accuracy. They do not necessarily restrict the analysis of more complex mixtures as particular situations may warrant. The last column of Table II plus the figures in Table III constitute the complete calibration data. It can be seen from Table III that, except for a few scattered compounds, the general level of interference is low. It is essentially this factor which accounts for the relatively high accuracies obtainable with the infrared method for determination of individual aromatics.

ANALYTICAL APPLICATIONS

The infrared method has been applied to a wide variety of samples, derived both from virgin naphthas and from hydroformates. Since the composition of these types of stocks might be of as much general interest as the analytical method itself, the analytical results on the aromatic portions of a virgin naphtha, covering the boiling range from about 200° to 380° F., and of

Absorption Coefficients, Expressed in Per Cent[a] *(continued)*

Analytical Wave Length, Microns																	
12.40	13.58	13.18	12.78	13.04	14.05[b]	12.28	13.33	13.21	12.56	12.83	13.44	14.36	12.46	13.28	11.83	12.07	Compound
0.7	...	...	0.4	0.9	0.0	...	1.2										Benzene
1.1	...	...	1.9	1.6	−23.7	...	5.8										Toluene
2.8	...	...	11.9	9.1	−30.2	...	29.3										E Bz[c]
8.8	...	...	6.2	1.0	0.0	...	0.3										1,4-DM Bz
1.3	...	...	9.9	103.4	−2.6	...	3.4										1,3-DM Bz
0.9	...	...	1.8	2.3	−1.3	...	22.7										1,2-DM Bz
0.8	4.5	105.3	2.5	11.5	−23.7	...	9.2										IsoC$_3$ Bz
2.2	42.2	12.9	1.8	3.0	−23.3	...	30.7										*n*C$_3$ Bz
4.7	1.2	17.0	43.1	11.8	−19.0	...	7.8										1-M-3-E Bz
28.3	0.8	5.6	5.6	3.5	−3.0	...	2.1										1-M-4-E Bz
0.7	0.2	0.3	0.2	0.1	−0.9	1.1	0.2	0.3	1.8								1,3,5-TM Bz
2.2	46.5	51.1	6.0	6.4	−7.3	1.6	46.2	69.9	22.2								1-M-2-E Bz
0.8	6.6	59.5	1.9	24.8	...	0.8	4.0	26.2	3.0								*tert*-C$_4$ Bz
100.0	0.7	0.9	1.5	0.5	−6.0	9.1	0.5	0.9	18.5	1.4	1.1	1.6					1,2,4-TM Bz
2.5	**100.0**	3.7	2.8	1.3	...	3.3	6.1	4.0	3.8	2.9	31.7	100.0					IsoC$_4$ Bz
4.4	6.6	**100.0**	3.1	14.1	...	1.8	17.2	85.8	8.3	4.2	25.7	...					*s*-C$_4$ Bz
1.8	3.4	1.6	**100.0**	2.0	...	0.9	0.7	1.4	11.4	62.6	1.5	13.0					1-M-3-iC$_3$ Bz
1.0	1.7	27.3	6.1	**100.0**	**100.0**	0.9	4.8	18.2	4.2	10.7	5.5	1.2	1.8	6.9	0.2	0.7	1,2,3-TM Bz
13.6	0.9	1.2	1.2	0.5	...	**100.0**	0.5	1.0	8.9	1.3	1.0	0.5	11.1	0.5	1.9	6.5	1-M-4-isoC$_3$ Bz
1.3	59.6	32.9	2.4	5.8	−1.7	1.1	**100.0**	45.0	3.6	2.9	128.6	1.3	2.1	74.2	2.5	2.1	Hydrindene
1.5	13.0	96.4	1.4	20.9	...	1.4	15.5	**100.0**	6.4	3.0	46.3	3.7	4.6	43.4	1.1	1.5	1-M-2-isoC$_3$ Bz
11.1	1.1	10.5	14.8	6.4	...	3.3	5.3	9.8	**100.0**	15.6	7.3	36.3	32.9	5.8	0.8	1.6	1,3-DE Bz
3.2	10.2	10.6	37.6	6.2	...	1.4	4.4	8.4	11.0	**100.0**	15.5	53.6	8.9	4.2	1.0	1.0	1-M-3-*n*C$_3$ Bz
2.0	35.3	9.6	4.2	2.7	...	1.7	36.8	11.7	5.5	5.3	**100.0**	**100.0**	3.3	13.5	1.4	1.5	*n*-C$_4$ Bz
35.7	2.6	4.4	9.6	2.2	...	13.6	7.5	4.9	56.8	9.7	8.6	1.2	**100.0**	4.9	5.8	7.1	1-M-4-*n*-C$_3$ Bz
4.8	9.1	58.5	9.0	7.7	...	4.2	73.7	87.4	32.0	8.7	33.2	1.5	9.0	**100.0**	0.9	1.3	1,2-DE Bz
1.0	0.6	1.1	1.5	0.8	...	0.9	0.5	1.0	2.5	1.5	1.1	40.6	1.5	0.5	**100.0**	3.1	1,3-DM-5-E Bz
5.4	1.4	3.0	4.5	2.2	...	8.9	1.6	2.8	13.5	4.5	3.1	3.5	9.2	1.6	8.4	**100.0**	1,4-DE Bz
1.1	39.9	30.5	2.9	10.7	...	1.0	36.4	28.0	6.0	3.2	177.7	11.5	2.0	19.6	5.9	2.1	1-M-2-*n*-C$_3$ Bz
									x	xx	xx	x	x	xx	x	x	2-M Hydrindene
									x	xx	xx	x	x	xx	x	x	1-M Hydrindene
				0.5	...	15.3	0.8	1.0	20.4	4.9	1.8	1.3	39.2	0.5	0.8	2.6	1,4-DM-2-E Bz
				0.6	...	45.1	0.7	0.9	7.6	1.3	0.5	1.4	6.6	1.0	1.3	10.3	1,3-DM-4-E Bz
				1.8	...	1.7	1.4	2.1	7.6	2.9	2.8	1.4	6.3	1.0	2.0	7.9	1,2-DM-4-E Bz
				89.3	...	0.9	4.7	14.5	8.5	11.7	21.0	0.4	3.3	5.3	0.0	0.8	1,3-DM-2-E Bz
				8.3	...	3.1	10.6	14.8	25.4	37.2	9.6	1.3	17.1	12.7	0.9	1.4	1,2-DM-3-E Bz
								0.3	1.1	0.6	0.0	0.5	0.7	0.1	2.7	0.7	1,2,4,5-TM Bz
								0.5	1.1	0.5	0.7	1.8	0.7	0.3	43.4	0.7	1,2,3,5-TM Bz

[a] ... Data not obtained.
? No means for estimating interference.
x Low estimated interference.
xx Medium estimated interference.
xxx High estimated interference.

[b] Baseline measurements; base end points at 13.92 and 14.18μ.

[c] E. Ethyl
Bz. Benzene
DM. Dimethyl
TM. Trimethyl
DE. Diethyl

Table IV. Analysis of Aromatic Portion of Virgin Naphtha (Volume Per Cent)

	Boiling Range of Fraction, ° F.												
	204–257	257–272	272–277	277–289	289–301	301–306	306–315	315–326	326–334	334–345	345–361	361–380	Summary 204–380
	Vol. % Fraction, Based on Total Aromatics												
Compound	21.12	8.06	14.88	11.83	1.51	0.54	3.95	9.97	10.45	6.64	5.66	5.39	100.0
Toluene	100.0	0.0	0.0										21.12
E Bz	0.0	79.1	19.7	1.7									9.52
1,4-DM Bz		8.4	21.0	9.3	0.0								4.90
1,3-DM Bz		12.5	59.2	31.6	0.0								13.56
1,2-DM Bz		0.0	0.1	55.4	20.8	0.0							6.88
Iso-C$_3$ Bz				2.0	79.2	23.4	1.7	0.0					1.63
n-C$_3$ Bz				0.0	0.0	76.6	25.9	3.3	0.0				1.77
1-M-3-E Bz						0.0	51.9	23.3	0.0				4.37
1-M-4-E Bz							18.6	16.1	0.4				2.38
1,3,5-TM Bz							1.9	29.3	13.7	0.0			4.43
1-M-2-E Bz							0.0	20.0	5.6	0.0			2.58
tert-C$_4$ Bz								[a]	[a]	0.0			[a]
1,2,4-TM Bz								8.0	74.0	9.5	0.0		9.17
Iso-C$_4$ Bz								0.0	2.5	5.2	0.0		0.61
s-C$_4$ Bz									[a]	8.7	0.0		0.58
1-M-3-iso-C$_3$ Bz									[a]	17.8	8.7		1.67
1,2,3-TM Bz									3.3	35.6	8.1		3.17
1-M-4-iso-C$_3$ Bz									[a]	9.9	1.6		0.75
Hydrindene									0.5	3.0	0.0		0.25
1-M-2-iso-C$_3$ Bz										6.3	1.6		0.51
1,3-DE Bz										3.2	8.4		0.69
1-M-3-*n*C$_3$ Bz										[a]	9.7		0.55
n-C$_4$ Bz										0.8	19.5	[a]	1.16
1-M-4-*n*-C$_3$ Bz											6.7	[a]	0.38
1,2-DE Bz											3.6	3.3	0.38
1,3-DM-5E Bz											14.0	3.8	1.00
1,4-DE Bz											4.3	1.6	0.33
1-M-2-*n*-C$_3$ Bz											6.5	[a]	0.36
2-M Hydrindene 1-M Hydrindene											1.4	8.2	0.52
1,4-DM-2-E Bz											5.2	5.7	0.60
1,3-DM-4-E Bz 1,2-DM-4-E Bz											0.0	14.4	0.78
1,3-DM-2-E Bz												3.8	0.20
1,2-DM-3-E Bz												14.2	0.77
1,2,4,5-TM Bz												5.2	0.28
1,2,3,5-TM Bz												4.4	0.23
5-M Hydrindene 4-M Hydrindene												[a]	[a]
1,2,3,4-TM Bz												1.5	0.08
1,2,3,4-Tetrahydronaph.												[a]	[a]
C$_{11}$ aromatics											0.7	33.9	1.84
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.00

[a] Possible trace present.

Table V. Analysis of C_8 Fraction of Hydroformate

Compound	Weight, %
Benzene	0.0
Toluene	1.6
Ethylbenzene	19.4
p-Xylene	16.1
m-Xylene	40.9
o-Xylene	19.5
C_9 aromatics[a]	1.7
Saturates and olefins[a]	0.7
	100.0

[a] Determined by mass spectrometer analysis.

three hydroformate fractions, comprising roughly the C_8 (250° to 300° F.), C_9 (300° to 350° F.), and C_{10} (350° to 420° F.) fractions of total hydroformate are presented.

Table IV summarizes the results for a virgin naphtha for each distillation fraction analyzed and for the total naphtha. Actual analyses were performed on the fractions after they were percolated through silica gel to remove the saturates, so that the accuracies in determining the individual aromatics could be increased. The aromatic totals were normalized to those values determined on the original fractions by specific dispersion measurements. Results by specific dispersion were checked and confirmed by mass spectrometer analyses and by sulfuric acid extraction. In the boiling ranges above 350° F., mass spectrometer type analyses were employed independently to determine the total C_{11} and higher aromatics; lack of complete calibration data for these compounds made it impossible to employ infrared for their determination.

Tables V to VII give the analytical results on the aromatic portions of the three hydroformate fractions. Analyses of the C_8 and C_9 fractions were conventional, but it was necessary to employ supplementary techniques, principally the mass spectrometer, for the C_{10} fraction, both for cross check of the infrared totals and for estimating the concentrations of the methyl hydrindenes. The presence of the latter was definitely verified by several methods—infrared spectrometer, mass spectrometer, specific gravity, etc. Figure 2, which gives the boiling point (Cottrell midboiling point) and specific gravity of each distillation cut of the C_{10} hydroformate fraction, shows a sharp increase in specific gravity at the boiling points of the hydrindene homologs, further verifying their presence. The absence of Tetralin in the C_{10} hydroformate fraction can be explained on the basis that the Tetralin in the original virgin naphtha was probably completely hydroformed to naphthalene.

ANALYTICAL ACCURACY

To get an estimation of analytical accuracy one could prepare a number of synthetic blends of different relative concentrations of components and then perform enough analyses of each to give good statistical measures of precision at each concentration level. This is often too laborious, particularly for complex multicomponent schemes, and necessitates the use of scarce, expensive standards which are more effectively used if saved for future calibrations. (The effectiveness of occasional synthetics for checking calibration, however, is without question.) What one can do in principle is determine the precision of the various separate measurement or instrumental errors which contribute to the errors in determining component concentrations, and then calculate the expected errors (probable errors, standard deviations, or other statistical measures of precision) in concentration. An expression for calculating these errors is given by Tunnicliff,

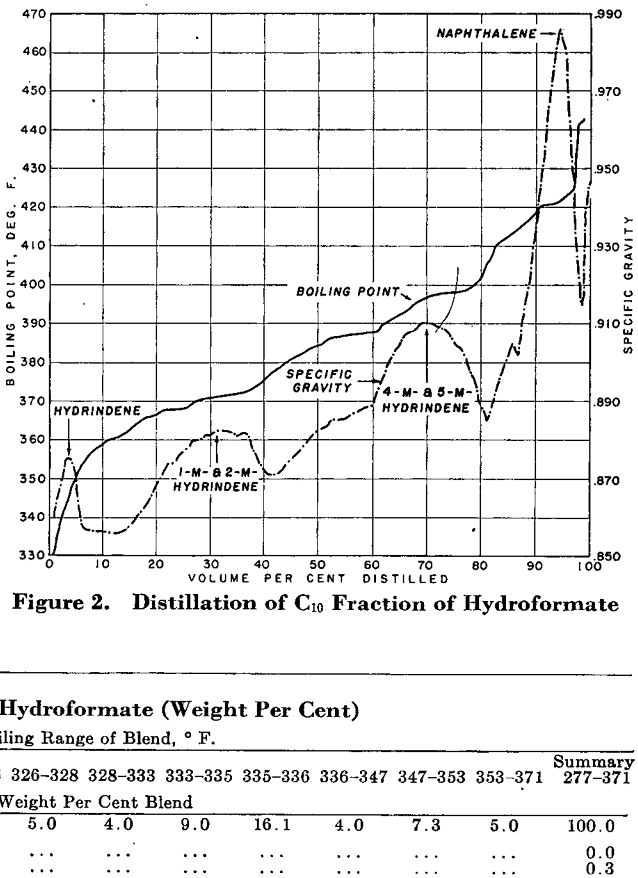


Figure 2. Distillation of C_{10} Fraction of Hydroformate

Table VI. Analysis of C_9 Fraction of Hydroformate (Weight Per Cent)

Boiling Range of Blend, ° F.	277–290	290–315	315–321	321–323	323–324	324–326	326–328	328–333	333–335	335–336	336–347	347–353	353–371	Summary 277–371
Weight Per Cent Blend														
Compound	5.9	6.8	5.9	21.0	5.0	5.0	5.0	4.0	9.0	16.1	4.0	7.3	5.0	100.0
Toluene	0.0	...	...	...	...	...	...	...	...	...	...	...	...	0.0
Ethylbenzene	4.9	...	...	...	...	...	...	...	...	...	...	...	...	0.3
p-Xylene	8.9	0.5	...	...	...	...	...	...	...	...	...	...	...	0.6
m-Xylene	26.7	0.4	...	...	...	...	...	...	...	...	...	...	...	1.6
o-Xylene	52.1	34.5	0.0	...	...	...	...	...	...	...	...	...	...	5.4
Iso-C_3-benzene	0.0	30.0	5.0	0.7	...	...	...	...	...	...	...	...	...	2.4
n-C_3-benzene	...	15.3	55.5	11.3	4.4	2.3	1.5	...	...	...	...	...	...	7.1
m-Ethyltoluene	...	1.6	29.4	54.9	38.1	23.8	11.7	7.6	...	...	...	...	...	17.4
p-Ethyltoluene	...	...	5.6	24.3	27.7	18.8	11.4	7.3	2.6	0.5	...	...	...	8.9
1,3,5-Trimethylbenzene	...	...	0.0	3.7	16.9	31.5	40.9	38.2	18.7	2.0	...	...	...	8.8
o-Ethyltoluene	...	...	...	4.5	11.9	23.1	33.5	37.4	21.2	2.1	...	...	...	8.1
tert-C_4-benzene	...	...	...	...	...	...	...	...	...	0.3	...	...	...	Trace
1,2,4-Trimethylbenzene	...	...	...	...	...	...	...	9.0	55.5	94.1	56.9	0.0	...	22.8
Iso-C_3-benzene	...	...	...	...	...	...	...	...	...	0.6	3.2	0.0	...	0.2
sec-C_4-benzene	...	...	...	...	...	...	...	...	...	...	3.4	0.0	...	0.1
m-Cymene	...	...	...	...	...	...	...	...	...	...	9.1	6.8	...	0.9
1,2,3-Trimethylbenzene	...	...	...	...	...	...	...	...	...	...	21.8	72.3	...	6.2
p-Cymene	...	...	...	...	...	...	...	...	...	...	0.6	2.5	...	0.2
Hydrindene	...	...	...	...	...	...	...	...	...	...	4.2	16.3	...	1.4
o-Cymene	...	...	...	...	...	...	...	...	...	...	0.0	1.9	...	0.1
Higher boiling aromatics	...	...	...	...	...	...	...	...	...	...	...	...	97.8	4.9
Total aromatics[a]	92.6	82.3	95.5	99.4	99.0	99.5	99.0	99.5	98.0	99.6	99.2	99.8	97.8	97.4
Saturates and olefins	7.4	17.7	4.5	0.6	1.0	0.5	1.0	0.5	2.0	0.4	0.8	0.2	2.2	2.6
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Determined by acid extraction; infrared totals normalized to these values.

Table VII. Analysis of C_{10} Fraction of Hydroformate (Weight Per Cent)

	Boiling Range of Blend, ° F.														
	IBP–355	355–363	363–368	368–373	373–381	381–387	387–389	389–396	396–399	399–406	406–413	413–421	421–422	422–450	Summary IBP–450
	Weight Per Cent Blend														
Compound	6.81	7.67	9.76	11.90	7.84	7.94	7.97	8.13	8.13	4.01	4.03	6.20	4.45	5.16	100.00
Lower boiling aromatics	3.4	...	...	...	...	...	...	...	...	...	...	...	...	...	0.2
1,2,4-Trimethylbenzene	14.7	1.2	...	...	...	...	...	...	...	...	...	...	...	...	1.1
Iso-C_4-benzene	0.0[a]	...	...	...	...	...	...	...	...	...	...	...	...	...	0.0[a]
sec-C_4-benzene	0.0[a]	...	...	...	...	...	...	...	...	...	...	...	...	...	0.0[a]
m-Cymene	4.4	0.0	...	...	...	...	...	...	...	...	...	...	...	...	0.3
1,2,3-Trimethylbenzene	24.5	5.7	...	...	...	...	...	...	...	...	...	...	...	...	2.1
p-Cymene	2.4	0.4	...	...	...	...	...	...	...	...	...	...	...	...	0.2
Hydrindene	8.8	0.0	...	...	...	...	...	...	...	...	...	...	...	...	0.6
o-Cymene	3.3	0.0	...	...	...	...	...	...	...	...	...	...	...	...	0.2
1,3-Diethylbenzene	10.0	9.7	0.0	...	...	...	...	...	...	...	...	...	...	...	1.4
1-M-3-*n*-C_3-benzene	3.3	9.3	1.3	...	...	...	...	...	...	...	...	...	...	...	1.1
1-M-4-*n*-C_3-benzene	1.5	8.0	4.0	...	...	...	...	...	...	...	...	...	...	...	1.1
n-C_4-benzene	3.5	8.7	2.4	0.0	...	...	...	...	...	...	...	...	...	...	1.1
1,3-DM-5-ethylbenzene	2.8	22.0	9.4	0.8	0.0	...	...	...	...	...	...	...	...	...	2.9
1,4-Diethylbenzene	...	4.8	1.8	0.0	...	...	...	...	...	...	...	...	...	...	0.5
1,2-Diethylbenzene	...	2.7	1.4	0.0	...	...	...	...	...	...	...	...	...	...	0.3
1-M-2-*n*-C_3-benzene	...	6.7	3.5	0.0	...	...	...	...	...	...	...	...	...	...	0.9
2-M-Hydrindene 1-M-Hydrindene[b]	...	4.2	10.5	19.3	8.3	1.1	...	...	...	...	...	...	...	...	4.4
1,4-DM-2-ethylbenzene	...	3.0	22.2	8.8	1.6	...	...	...	...	...	...	...	...	...	3.6
1,3-DM-4-ethylbenzene 1,2-DM-4-ethylbenzene	...	0.5	22.6	52.8	25.8	4.0	0.2	...	...	...	...	...	...	...	10.9
1,3-DM-2-ethylbenzene	...	...	1.1	2.5	3.3	1.2	0.4	...	...	...	...	...	...	...	0.8
1,2-DM-3-ethylbenzene	...	...	0.0	0.5	21.6	12.9	1.6	...	...	...	...	...	...	...	2.9
Durene	...	...	0.0	0.2	6.0	27.1	23.3	7.0	0.0	...	...	...	...	...	5.1
Isodurene	...	...	...	0.0	4.1	30.3	49.4	16.1	1.2	...	...	...	...	...	8.1
5-M-Hydrindene 4-M-Hydrindene[b]	...	...	...	...	...	...	3.6	33.9	30.1	11.4	8.7	5.0	0.4	...	6.6
Prehnitene[c]	...	...	...	...	...	...	...	3.4	26.5	17.1	0.0	...	...	...	3.1
Tetralin	...	...	...	...	...	...	...	...	0.0[a]	0.0[a]	...	...	...	...	0.0[a]
C_{11} alkylbenzenes[b]	...	0.9	7.2	6.3	18.1	16.9	18.1	36.7	40.3	65.5	68.3	59.2	14.2	25.1	23.0
C_{12} alkylbenzenes[b]	...	...	...	...	...	...	...	...	0.0	4.4	15.1	16.8	17.9	47.7	5.1
Naphthalene	...	...	...	...	...	...	...	...	...	...	4.5	15.6	62.7	12.1	4.5
2-M-Naphthalene[d]	...	...	...	...	...	...	...	...	...	...	...	...	0.0	7.1	0.4
1-M-Naphthalene[d]	...	...	...	...	...	...	...	...	...	...	...	...	0.0	2.0	0.1
DM-Hydrindenes Ethyl hydrindene[b]	...	...	...	...	...	...	...	...	...	...	...	...	...	Trace	Trace
Total aromatics[e]	82.6	87.8	87.4	91.2	88.8	93.5	96.6	97.1	98.1	98.4	96.6	96.6	95.2	94.0	92.6
Olefins[f]	0.5	0.9	0.2	0.2	0.2	1.1	0.7	1.1	1.0	0.7	0.7	0.7	1.2	2.4	0.8
Saturates[g]	16.9	11.3	12.4	8.6	11.0	5.4	2.7	1.8	0.9	0.9	2.7	2.7	3.6	3.6	6.6
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Possible trace, but not detectable.
[b] Determined by mass spectrometer analysis.
[c] Determined by combination of infrared and mass spectrometer analyses.
[d] Determined by ultraviolet analysis.
[e] Determined by acid absorption.
[f] Determined by bromine number.
[g] Determined by difference.

Brattain, and Zumwalt (*15*). This expression cannot be used, however, unless the simultaneous equations for determining component concentrations are available in inverse form, which is frequently not the case unless many analyses are performed using the same matrix. The expression also does not account for possible random errors in calibration data; unless the average of several calibration determinations is employed for each compound, the random errors from inaccurate calibration alone can be a major source of analytical inaccuracy. If all possible sources of error are taken into account in theoretical calculations of accuracy, the necessary expressions become too complex and unwieldy to be generally useful. With very complicated mixtures such as in the higher boiling ranges, difficulty in achieving clear-cut qualitative analyses is perhaps the major source of error. In such cases estimations of accuracy are always subject to considerable question. The authors therefore have resorted principally to the use of a few key synthetic blends for estimating accuracy in lower boiling ranges where qualitative analyses are easily obtained; in the higher boiling ranges, no estimation of accuracy is made, as there is no realistic way of doing so.

Table VIII shows experimentally obtained accuracies from single determinations on synthetic blends containing compounds through the C_8's. Table IX compares the accuracies obtained on a wide boiling fraction when the total fraction is separated into smaller fractions and when the total fraction is analyzed without prior separation. With regard to the latter, it is interesting to note that accuracies on many components from the single analysis of the total sample are good; it is thus demonstrated that in certain situations satisfactory accuracies on the majority of components can be obtained without prior distillation and at great savings in time and labor.

OTHER ANALYTICAL METHODS

Others have successfully applied the infrared spectrometer to the determination of the composition of C_8 aromatic mixtures (*2, 10*) and to a few of the C_{10} aromatics (*11*); the authors have shown that extension of the scope of application by infrared methods to include all of the aromatics from benzene through the C_{10}'s is equally feasible. It appears likely that the Raman spectrometer is similarly applicable (*4, 8, 14*), the major limitations seemingly being due to the presence of compounds which exhibit fluorescence in the higher boiling regions. Although the mass spectrometer can be successfully applied to the determination of benzene and toluene as individual constituents, it is not possible to distinguish between the isomers in any given molecular weight range above C_7 owing to the similarities of their mass spectra. The mass spectrometer is still valuable for cross-check purposes and for determining the totals of particular types of aromatics. The method which combines distillation and refractometric techniques is applicable up to relatively high boiling ranges (*5–7*), but necessitates rather precise and complex distillation equipment and large time and manpower requirements, conditions which are severe limitations in many situations. The ultraviolet procedures employed in the past for determining benzene and toluene and for determining the C_8 aromatics are not sufficiently broad in scope to allow the presence of out-of-range interferers without prohibitive loss in accuracy and are therefore not generally satisfactory for many purposes. Experience with the specialized types of lower boiling refinery stocks studied has shown that seldom does a sample containing toluene as the major constituent exist which does not also contain small but significant amounts of the C_8 aromatics, and likewise seldom are xylene samples found which do not contain a certain

Table VIII. Deviations in Analysis of Synthetic Blends

(Weight per cent, total sample basis)

	Synthetic No. 1355			Synthetic No. 1423		Synthetic No. 1431	
		Deviations in analyses			Deviations in analysis		Deviations in analysis
Compound	Synthesis	Δ_1	Δ_2	Synthesis	Δ	Synthesis	Δ
Benzene	0.00	...	...	4.6	+0.2	0.0	...
Toluene	2.04	−0.11	+0.08	14.2	−0.3	0.0	...
Ethylbenzene	19.86	+0.72	+0.38	20.1	+0.4	24.7	0.0
p-Xylene	15.76	−0.09	+0.07	19.7	−0.3	24.3	−0.3
m-Xylene	42.84	−0.24	−0.30	31.7	+0.1	39.1	+0.5
o-Xylene	19.39	−0.17	−0.12	9.6	0.0	11.8	0.0
Other	0.11	...	...	0.1	...	0.1	...
Total	100.00			100.0		100.0	
Total before normalizing		98.0	97.2		103.4		99.0

Table IX. Analyses of C_7 to C_9 Aromatic Fraction

Compound	Calcd. from Analyses of 13 Narrow Distillation Fractions	Calcd. on Total Sample without Distillation	Δ
Toluene	0.3	0.3	0.0
Ethylbenzene	0.2	0.0	−0.2
p-Xylene	0.1	0.1	0.0
m-Xylene	0.3	0.6	+0.3
o-Xylene	0.4	0.8	+0.4
Iso$_3$-benzene	0.4	1.0	+0.6
n$_3$-benzene	5.4	4.0	−1.4
m-Ethyltoluene	19.9	20.1	+0.2
p-Ethyltoluene	9.2	9.7	+0.5
1,3,5-Trimethylbenzene	8.9	9.0	+0.1
o-Ethyltoluene	8.5	7.7	−0.8
1,2,4-Trimethylbenzene	33.6	33.7	+0.1
1,2,3-Trimethylbenzene	7.3	6.7	−0.6
Hydrindene	1.2	2.0	+0.8
Total aromatics	95.7[a]	95.7[a]	
Total analytical man-hours[b]	100	8	

[a] Totals from each analysis normalized to values determined by acid extraction.
[b] Including distillation requirements.

amount of toluene and C_9 aromatics. These cases, of course, do not necessarily apply to samples derived from precise laboratory distillations. Other investigators have developed an ultraviolet method for determining C_6 to C_8 aromatics in one mixture (*15*), but admittedly not without sizable error when all components are present, and techniques in the ultraviolet for minimizing errors from background interference (*16*) have been developed. These refinements are worthy of considerable merit, but still appear to be less desirable than a procedure utilizing the infrared spectrometer, because of the generally lower level of extraneous interference and of mutual interference between the aromatic compounds with the latter instrument. For low aromatic content samples, of course, the ultraviolet methods when applicable are generally superior. As has previously been pointed out (*1*), the ultraviolet spectrometer is not suited to determining individual C_9 and heavier alkyl benzenes.

ACKNOWLEDGMENT

The authors wish to acknowledge gratefully the assistance of Theo Hines, who did most of the calibrations and performed most of the analyses reported herein, and to thank the Humble Oil and Refining Co. for permission to publish this paper.

LITERATURE CITED

(1) Coggeshall, N. D., Anal. Chem., **22**, 381 (1950).
(2) Duyckaerts, G., and Pirlot, G., *Bull. soc. chim. Belges*, **58**, 48 (1949).
(3) Eberhardt, W. H., *J. Optical Soc. Am.*, **40**, 172 (1950).
(4) Fenske, M. R., Braun, W. G., Wiegand, R. V., Quiggle, D., McCormick, R. H., and Rank, D. H., Ind. Eng. Chem., Anal. Ed., **19**, 700 (1947).
(5) Forziati, A. F., Willingham, C. B., Mair, B. J., and Rossini, F. D., *J. Research Natl. Bur. Standards*, **32**, 11 (1944).
(6) Gooding, R. M., Adams, N. G., and Rall, H. T., Ind. Eng. Chem., Anal. Ed., **18**, 2 (1946).
(7) Griswold, J., and Van Berg., C. F., *Ind. Eng. Chem.*, **38**, 170 (1946).
(8) Heigl, J. J., Black, J. F., and Dudenbostel, B. F., Jr., Anal. Chem., **21**, 554 (1949).
(9) Institute of Petroleum Research Committee, Hydrocarbon Research Group, Report XI, 1946.
(10) Kaye, W. I., and Otis, M. V., Anal. Chem., **20**, 1006 (1948).
(11) Perry, J. A., *Ibid.*, **23**, 495 (1951).
(12) Philpotts, A. R., Thain, W., and Smith, P. G., *Ibid.*, **23**, 268 (1951).
(13) Robinson, D. Z., *Ibid.*, **23**, 273 (1951).
(14) Rosenbaum, E. J., Martin, C. C., and Laur, J. L., Ind. Eng. Chem., Anal. Ed., **18**, 731 (1946).
(15) Tunnicliff, D. D., Brattain, R. R., and Zumwalt, L. R., Anal. Chem., **21**, 890 (1949).
(16) Tunnicliff, D. D., Rasmussen, R. S., and Morse, M. L., *Ibid.*, **21**, 895 (1949).

Received for review April 25, 1952. Accepted September 24, 1952. Presented before the Division of Petroleum Chemistry at the 121st Meeting of the American Chemical Society, Milwaukee, Wis.

Infrared Spectrophotometric Study of Veratrum Alkaloids

GILLES PAPINEAU-COUTURE and ROBERT A. BURLEY
Ayerst, McKenna and Harrison, Ltd., Montreal, Canada

THE recently renewed interest in the hypotensive properties of *Veratrum viride* has prompted a great deal of research in several laboratories. This research, in turn, has called for the development of suitable assay methods. A biological method based on the lowering in blood pressure in the dog has been used successfully (*4*, *5*), but is time-consuming and inconvenient for guiding the chemist in the development of an extraction process or in the isolation of active principles. A rapid chemical or physical assay method of analysis is, therefore, highly desirable.

There are several hypotensive alkaloids in *Veratrum viride*, but even highly active extracts also contain inactive ones. Consequently, the first consideration in developing an analytical procedure must be given to finding a property which is common to all the active constituents, is absent from all the nonactive ones, and lends itself to convenient measurement.

The alkaloids isolated from *Veratrum viride* may be grouped into two broad classes with respect to hypotensor action: those, such as jervine, rubijervine, isorubijervine, and veratramine which are inactive, and those such as germerine, germidine, germitrine, and neogermitrine which possess considerable activity. This last group is characterized by the fact that all its members are esters of the polyhydroxylated alkamine germine (*1*, *2*). From the analytical point of view, one of the most convenient properties of the ester group is the intense infrared absorption band, assigned to the carbonyl linkage, occurring around 1740 cm.$^{-1}$ Consequently, the possibility of determining ester alkaloids by measuring the intensity of the carbonyl absorption was investigated.

A preliminary qualitative examination of chloroform solutions of various alkaloidal extracts showed that a carbonyl band always

This work was undertaken in order to establish a rapid, convenient method of determining the amount of hypotensive ester alkaloids in extracts of *Veratrum viride*. The intensity of the infrared band due to the carbonyl double bond of the ester grouping was found to provide an index of the concentration of these alkaloids. A quantitative method was developed and used to evaluate various extraction procedures. A parallelism has been found to exist between the intensity of the carbonyl band in crude extracts and hypotensive activity in the dog.

accompanied hypotensive activity and, equally important, that this band was absent from inactive fractions. The only inactive constituent known to absorb appreciably in the same region of the spectrum is jervine, with two bands at 1630 and 1705 cm.$^{-1}$ (*6*). However, these bands are easily distinguished from the ester absorption. It was noticed that the intensity of the ester band seemed to be parallel to the biological activity. Finally, the ester absorption always consisted of a relatively sharp single peak, indicating that the various ester linkages all absorb at the same frequency, or very nearly so. Thus, the measurement of the ester absorption appeared to be a convenient quantitative method for the determination of hypotensive alkaloids from *Veratrum*.

INSTRUMENT AND METHOD

All measurements were made with a Perkin-Elmer Model 12-C instrument equipped with a sodium chloride prism, using a 0.1-mm. cell. The same cell was used for all determinations and the exact thickness was not determined.

Chloroform was the solvent used. It was purified by washing with water, drying over anhydrous sodium sulfate, and distilling.

About 10 to 20 mg. of alkaloidal product was weighed accurately and dissolved at the concentration required to give from 35 to 50% transmittance at 1736 cm.$^{-1}$ The tracings of the solvent blank and of the solution between 1600 and 1800 cm.$^{-1}$ were superimposed and the optical density of a 10% solution ($D_{10\%}$) was calculated from the measured per cent transmittance. The linearity of the Beer's law graph was checked and found satisfactory.

By comparison with a known standard, it was possible to express the results in terms of the degree of esterification instead of optical density. Protoveratrine was chosen as the standard. This triester has been characterized by Jacobs and Craig (*3*) and can be obtained in a crystalline state from *Veratrum album*. A sample melting at 270° to 272°C. was obtained by recrystallization from acetone and its $D_{10\%}$ value was determined. As this value is that of a triester, one third of it is taken as the optical density corresponding to one ester group. Thus, the "ester number" of an unknown sample is defined as the value obtained by dividing its $D_{10\%}$ by one third the $D_{10\%}$ of protoveratrine.

It is realized that the ester number thus obtained is not exactly equal to the number of ester groups per molecule, since the molecular weight is disregarded in the calculation. However, as the sample generally consists of a mixture, a relative value is all that can be obtained. On the other hand, when dealing with pure esters, it is possible to calculate the expected ester number, using the following relationship:

$$\text{Ester number} = n_s \times M_p/M_s$$

where n_s = number of ester groups in the sample
M_s = molecular weight of sample
M_p = molecular weight of standard (protoveratrine)

Comparison of Two Processes Used in Purification of Veratrum Alkaloids

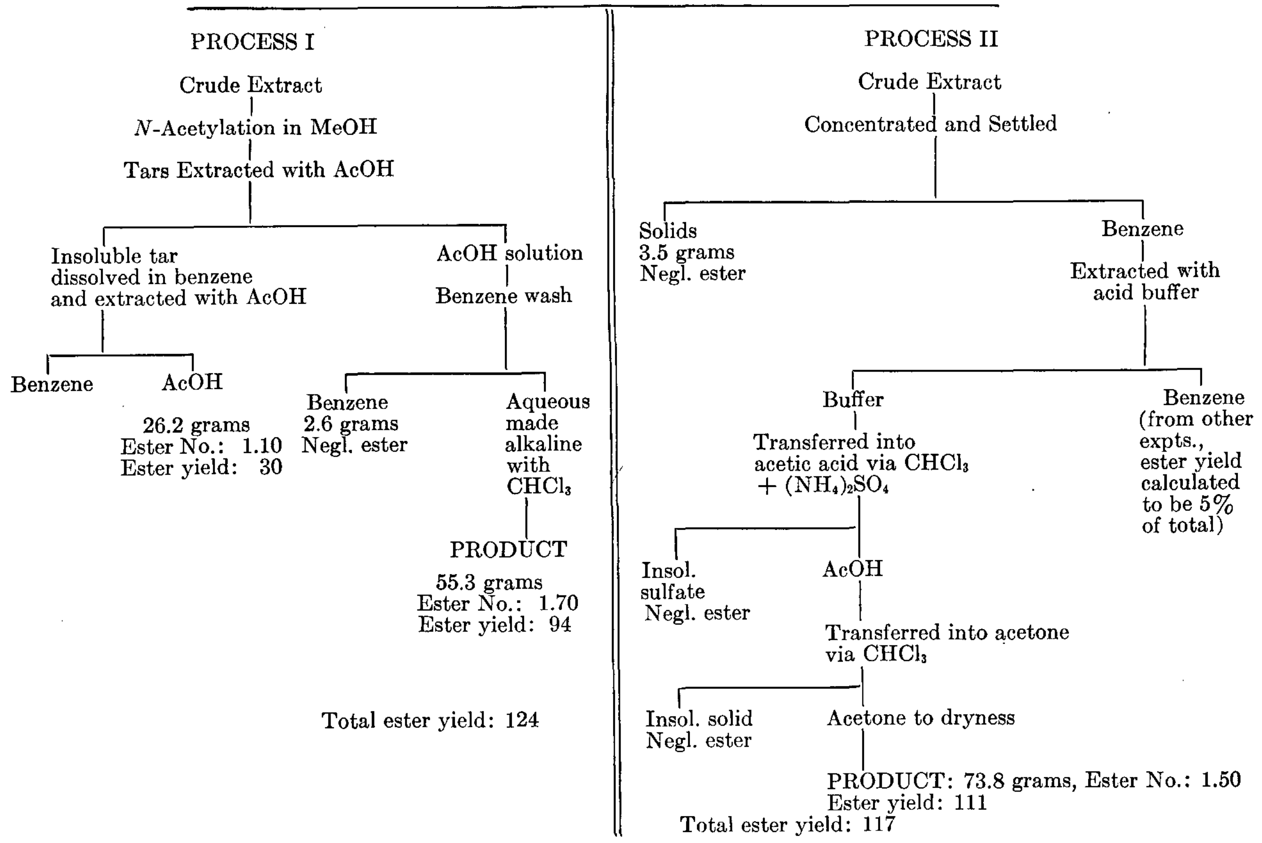


Table I. Ester Number of Various *Veratrum* Alkaloids

Alkaloid	M.W.	No. of Ester Groups	Calcd. Ester No.[a]
Protoveratrine	751	3	3.00
Germitrine	735	3	3.06
Neogermitrine	677	3	3.33
Germidine	635	2	2.37
Germerine	693	2	2.16

[a] Based on protoveratrine.

The ester numbers of several known hypotensive alkaloids have been calculated and are included in Table I.

Although the discrepancy between the calculated ester number and the actual number of ester groups may seem relatively large, the method has proved to be applicable to a wide variety of cases and has been unquestionably helpful in process development, separation, and identification of pure ester alkaloids (*7*). It has also considerably accelerated the biological assay work by permitting a fast screening of the most promising fractions (*8*, *9*).

APPLICATIONS

The infrared method of analysis has been used to follow the various extraction-purification processes used in these laboratories.

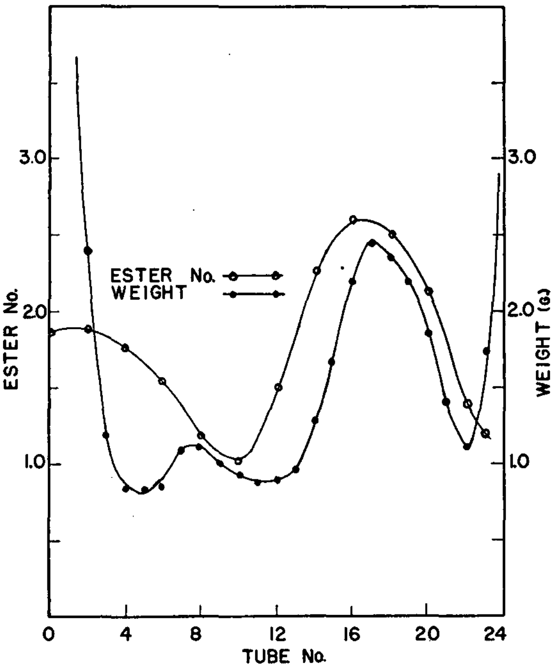


Figure 1. Countercurrent Distribution of *Veratrum* Alkaloids

pH 5.5, 24 tubes

The flowsheet on page 1919 represents two different purification schemes applied to a crude extract. The initial "crude extracts" are aliquots taken from the same batch. The ester number determination has confirmed the absence of ester alkaloids in most side fractions, such as the insoluble sulfate (consisting mainly of jervine) and the acetone-insoluble solid (consisting of rubijervine and isorubijervine). Moreover, if the two processes are compared on a weight-yield basis, Process II would be preferred. However, infrared examination shows that the product obtained by Process I has a higher relative degree of esterification which partly compensates for the lower weight, so that in both cases the ester yield (Ester No. × weight-yield) is comparable. It has also been possible to determine the losses suffered in some steps of the procedures. Finally, the total ester yields have been calculated and found to agree within about 3%.

Table II. Hypotensive Activity and Ester Number of Various *Veratrum* Alkaloids

Alkaloid	Ester No.	Relative Activity
Standard extract	1.4	1.0
Neogermitrine	3.0	4.7
Germbudine[a]	2.3	0.97
Veratetrine[a]	2.9	0.87
Isogermidine[a]	2.0	0.12

[a] New esters isolated in laboratory of Ayerst, McKenna, and Harrison, Ltd. (*7*).

Table III. Hypotensive Activity and Ester Number of *Veratrum* Extracts

Batch No.	Relative Activity	Ester No.
Standard	1.0	1.4
A	0.92	1.3
B	0.95	1.6

The present method has been especially useful when applied to the various fractions resulting from countercurrent distribution. This technique, involving distribution between benzene and phosphate buffer, has been used to great advantage to characterize an extract and to isolate pure esters (*7*). Figure 1 shows the weight and ester distribution of a purified extract having an ester number of 1.86. Although the weight curve indicates four distinct fractions, the ester curve shows that only two of them (the first and third) contain potentially active alkaloids. The peak centered around tube 8 contains a relatively high concentration of inactive material (probably isorubijervine). The ester curve suggests also that the alkaloids of the first peak are mainly diesters (ester No. 1.8), whereas the second peak, with a maximum ester number of 2.6, contains probably a high proportion of triesters.

Tubes 0, 1, and 2 were then combined and put through another countercurrent distribution (Figure 2), but, this time, the pH of the aqueous phase was adjusted to 6.5 to increase the benzene solubility of the ester alkaloids The weight curve indicates that the diester fraction has been split into two entities and the flatness of the second peak of the ester curve suggests that this frac-

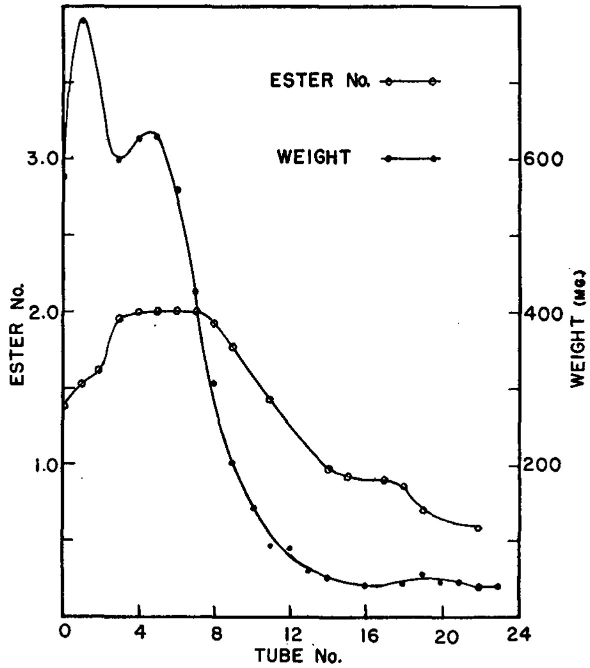


Figure 2. First Countercurrent Distribution of Diester Fractions of *Veratrum* Alkaloids

pH 6.5, 24 tubes

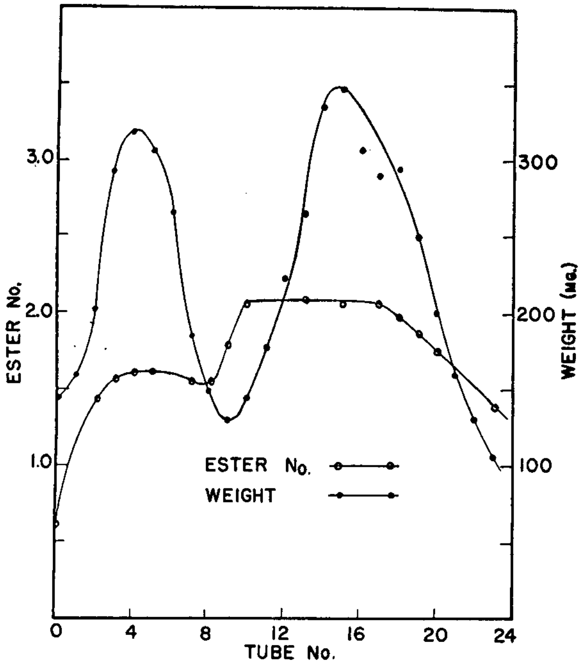


Figure 3. Second Countercurrent Distribution of Diester Fraction of *Veratrum* Alkaloids

pH 6.8, 48 tubes

tion consists of a diester of a high degree of purity. The first eight tubes were then put through a 48-tube countercurrent separation at pH 6.8 (Figure 3). The separation between the two peaks is considerably improved. However, there is no increase in the ester number of the second fraction, confirming the fact that this second fraction is probably a pure diester.

The fact that, to date, all the hypotensive alkaloids isolated from *Veratrum* are esterified is a strong indication that esterification is essential for hypotensive activity. However, it can be assumed that the nature and position of the esterifying acids are of importance in determining the degree of that pharmacological property. Consequently it cannot be expected that the respective hypotensive activity of different pure ester alkaloids is simply a function of their degree of esterification. Comparisons of biological assay data with infrared results bear out this point (Table II).

However, in dealing with extracts containing substantially all the naturally occurring ester alkaloids, a closer correlation exists between ester number and biological activity (Table III). This is probably due to the fact that the composition of the esters in the plant varies within relatively narrow limits and that the infrared assay serves to measure the "bulk concentration" of these esters. Hence, the present method can be used to estimate the biological activity of various batches produced in the plant—at least, within an order of magnitude.

ACKNOWLEDGMENT

The authors wish to express their indebtedness to W. L. Glen and G. S. Myers, who prepared and supplied all samples, and to F. R. Skelton, who supplied the pharmacological data.

LITERATURE CITED

(1) Fried, J., and Numerof, P., Abstracts, 119th Meeting, Am. Chem. Soc., 1951, p. L12.
(2) Fried, J., White, H. L., and Wintersteiner, O., *J. Am. Chem. Soc.*, 72, 4621 (1950).
(3) Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, **149**, 271 (1943).
(4) Maison, G. L., Gotz, E., and Stutzman, J. W., *J. Pharmacol. and Exptl. Therap.*, **103**, 74 (1951).
(5) Maison, G. L., and Stutzman, J. W., *Arch. intern. pharmacodynamie*, **85**, 357 (1951).
(6) Marion, L., Ramsay, D. A., and Jones, R. N., *J. Am. Chem. Soc.*, **73**, 305 (1951).
(7) Myers, G. S., Glen, W. L., Morozovitch, P., Barber, R., and Grant, G. A., *Ibid.*, **74**, 3198 (1952).
(8) Papineau-Couture, G., and Grant, G. A., *Chem. in Can.*, **4**, 37 (1952).
(9) Skelton, F. R., Beck, M., and Grant, G. A., *Federation Proc.*, **11**, 390 (March 1952).

Received for review April 25, 1952. Accepted September 17, 1952. Presented before the Pittsburgh Conference on Analytical Chemistry and Spectroscopy, March 1952.

Spectrographic Method for Analysis of Cracking Catalysts

C. W. KEY AND G. D. HOGGAN
Richfield Oil Corp., Wilmington, Calif.

THE application of emission spectroscopy to the qualitative and quantitative determination of metallic elements in materials encountered in industrial processes has been practiced in many industries for several years. The utility of this versatile analytical method will, of course, depend upon many factors, such as the physical and chemical characteristics of the material to be analyzed, the type of spectrograph and auxiliary instrumentation available, the required precision, the frequency of analyses, and countless other variables. Each problem involved is more or less specific for each industry in which spectrographic methods are applied and, in many cases, for one particular plant or process. It is therefore necessary for the spectrographer to develop a method or alter another method to suit his individual equipment and problems.

In the processing of petroleum hydrocarbons with cracking catalysts, it is necessary to analyze the catalyst periodically to determine its composition, with particular interest in foreign materials that may be introduced during their manufacture or in subsequent contact with the petroleum feed stocks or processing equipment. In some cases, contact with these foreign contaminants may considerably reduce the activity of the catalyst and thereby cause substantial economic losses. Of particular concern to this laboratory is the presence and concentration of nickel, vanadium, sodium, iron, and chromium encountered in processes which utilize silica-alumina cracking catalyst. Several classical chemical methods of analyses have been used; however, most of these are very time-consuming and, in many cases, are not sensitive enough to yield accurate results on the low concentrations normally encountered. It was therefore decided to investigate the possibilities of using a spectrographic technique. Several methods (*2*, *4*, *5*, *9*) have been reported, none of which, however, appeared to satisfy major requirements completely.

In spectrographic analyses of cracking catalysts, as with other materials, certain basic requirements must be met before satisfactory results may be obtained. The type of spectrograph and excitation source unit to be used is generally predetermined by that already in use in the laboratory; however, methods of introducing the sample, excitation, internal standard, and photometry must be chosen. The basic method should generally be applicable to more than one type of catalyst. In this laboratory, it was

This spectrographic method was developed to provide determinations of chromium, iron, nickel, sodium, and vanadium in silica-alumina cracking catalysts. It was desirable that the procedure yield greater accuracies in less analysis time than was required by conventional wet chemical methods. An investigation of spectrographic techniques disclosed that catalyst of this type could be analyzed with this instrument. A rotating disk electrode composed of powdered catalyst, lithium carbonate, graphite, and cobalt was found to provide excellent sampling areas. A high voltage spark discharge gave good reproducibility and sensitivity. The results obtained by this procedure were superior to those obtained by chemical methods. Furthermore, the analysis time was a fraction of that required for the chemical methods. Through the utilization of this spectrographic method for the analysis of cracking catalysts, better analytical control has been provided for petroleum processes which use the silica-alumina type catalyst. It has reduced the time required for an analysis, thereby enabling the analyst to devote more time to other problems. Increased production at a lower operating cost, plus an improvement in product quality, has resulted from better and more frequent analyses.

desirable that the method be equally well suited for the analysis of natural and synthetic silica-alumina catalysts of the pelleted, bead, or fluid type. With modifications in sample preparation to suit the individual catalyst, this condition has been met.

EQUIPMENT

The Applied Research Laboratories 2-meter spectrograph is used in this work. The camera is normally positioned to cover the spectral range between 2400 and 4900 A. in the first order. It is pivoted on a radius arm which allows it to be positioned along the Rowland circle to cover the 2400 to 4900 A. range on a 25-inch strip of 35-mm. Spectrum Analysis No. 2 film. The prespark and exposure times are controlled manually.

The source unit is an ARL high precision unit, which produces a range of energy between the extremes in sparklike and arclike discharges. A high voltage spark case is utilized in this procedure.

A three-tray ARL film developing machine, thermostatically maintained at 65° F. and provided with a driving motor which operates at 20 cycles per minute and a film holder to handle the 25-inch length of 35-mm. film, is used. The film is dried in a film dryer equipped with an electric fan and an infrared lamp, which provides warm air and radiant heat. The drying period of 4 minutes is controlled by a preset timer.

The spectrograms are read on the ARL projection comparator densitometer. Its light source consists of a prefocused 108-watt projection lamp, cooled by a motor-driven fan.

An ARL specimen briquetting press is utilized to compress the catalyst sample into a rotating disk electrode. The pressure used is controlled by an automatic pressure regulator preset for 100,000 pounds per square inch.

Standard spectrographic laboratory equipment, such as a high sensitivity analytical or microanalytical balance, thermostatically controlled muffle furnace, mortar and pestle, platinum dishes, etc., are also required.

INVESTIGATIONS

The Harvey semiquantitative method (*6*) was insufficiently sensitive for vanadium and sodium in the concentrations normally found in the catalysts encountered in the authors' laboratory. This indicated that any direct current arc method with comparable sensitivity would be unsatisfactory; therefore, it was necessary to develop a more sensitive method. Various types of matrices, electrodes, and discharge cases were investigated. A rotating disk electrode with lithium in the matrix and a high voltage spark discharge was found to provide the necessary sensitivity. Lithium carbonate served as a flux and a buffer, and in addition, very markedly enhanced the sensitivity of sodium and vanadium. When boric anhydride was tried in this same role, no lines for these elements were visible in the ultraviolet spectral range.

A rotating disk electrode was chosen because it provided better sampling. The use of an electrode in this form, with nonmetallic matrices, has been described (*7*, *8*). The rotating disk, consisting of a homogeneous matrix of dehydrated powdered catalyst, special purity lithium carbonate, and SP No. 1 graphite, to which cobalt has been added as the internal standard, is compressed into a disk 0.5 inch in diameter with a 0.125-inch hole in the center by the specimen briquetting press at 100,000 pounds per square inch. Satisfactory pellets were not obtained when lower pressures were used.

Because natural and synthetic catalysts have different pelleting characteristics, it was necessary to establish the required composition ratios for satisfactory pelleting of the catalysts after they had been ground to impalpable powders. The matrices selected for these catalysts were a compromise between sensitivity and optimum physical characteristics of the pellets. With a low graphite-to-catalyst ratio, which gave a higher sensitivity, the pellets disintegrated before or during the spark discharge. When a high graphite-to-catalyst ratio was used, insufficient sensitivity was obtained, but the pellets were physically satisfactory with respect to disintegration.

Because of the high degree of reproducibility achieved by the high precision source unit and the greater accuracy of spark methods for some of the elements sought, as compared to arc methods (*7*, *10*), it was considered desirable to use a high voltage spark discharge if possible. It was found that with the high voltage spark, high inductance cases gave high sensitivity but relatively poor reproducibility; conversely, low inductance cases gave low sensitivity and very good reproducibility. Hence, a compromise had to be made between reproducibility and sensitivity.

Time studies were made to secure the optimum times of exposures for the various elements sought. Reproducibility series were made to establish best operating conditions, line pairs, and the internal standard, which, in this method, is cobalt. The cobalt is introduced into the pellet along with graphite. Reproducibility curves for synthetic and natural catalysts are shown in Figures 1 and 2. Where necessary, intensity ratios for some of the elements have been multiplied by a factor in order to place the curves

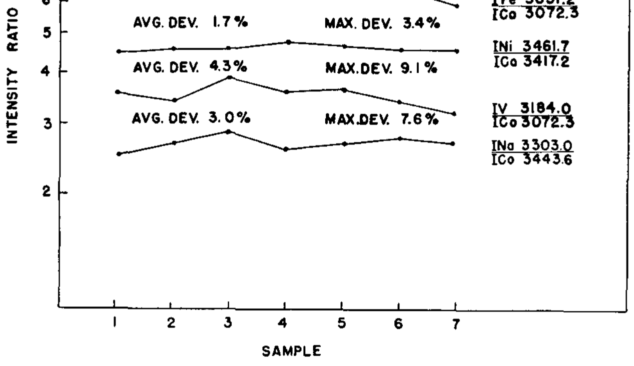


Figure 1. Reproducibility Series for Natural Clay Catalyst

in a suitable position on the graph. The curves have been included to show the good reproducibility obtained by the method when analyzing these catalysts. It was found that the high voltage spark-rotating disk method possessed better reproducibility than did a direct current arc method, and that it had comparable sensitivity to that obtained by the latter.

SAMPLE PREPARATION

The catalyst is ground, heated in a muffle furnace for 2 hours at approximately 1050° F., and stored in a desiccator as soon as sufficiently cooled. The temperature and 2-hour period were chosen in order to keep the conditions of preparation as uniform as possible for the different catalysts. As shown in Table I, different mixture ratios are used for the catalysts. The pellets of synthetic catalyst are prepared by adding a weighed amount of lithium carbonate–graphite–internal standard mixture to the necessary amount of catalyst, mixing in a mortar, and pelleting.

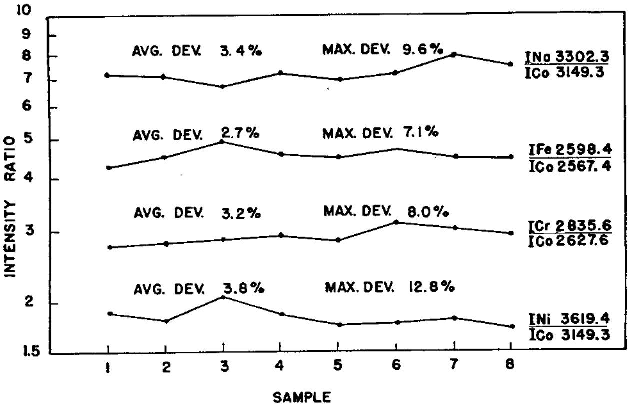


Figure 2. Reproducibility Series for Synthetic Catalyst

The procedure for natural clay catalyst varies in that the catalyst is mixed with the necessary amount of a cobalt-graphite mixture, put in a platinum dish, and blasted for 5 minutes with a Fisher burner. This heating apparently causes a change in the physical form and is necessary to ensure satisfactory pelleting. After cooling, the mixture is weighed and SP No. 1 graphite (without internal standard) is added to make up for the small amount lost during heating. Lithium carbonate is added and the whole is transferred to a mortar, thoroughly mixed, and then pelleted.

STANDARDS

The standards were prepared from new powdered catalyst of each type and from reagent grade or better chemicals, whose acceptability had been established by semiquantitative spectrographic methods. The catalyst materials were previously heated in a muffle furnace for at least 2 hours at 1050° F., cooled, and stored in a desiccator. The new catalysts had been previously analyzed by classical chemical methods and spectrographic addition procedures to determine the original concentrations of iron, sodium, nickel, vanadium, and chromium. In preparing the standards, the amount of catalyst required is weighed into a beaker and treated with a solution containing the necessary amounts of the elements desired. It is desirable to adjust the solution concentration so that the total volume of this solution is retained by the catalyst and none wets the beaker. The catalyst and added chemicals are thoroughly mixed and then slowly dried in an oven with occasional stirring. The dry mixture is transferred to a mortar and very thoroughly ground to produce a homogeneous blend and then heated in an evaporating dish in a muffle furnace at 1050° F. for 2 hours. It is stored in tightly capped bottles. The standard pellets prepared from these mixtures contain the same ratios of catalyst, lithium carbonate, and graphite as do the sample pellets.

INTERNAL STANDARD

The cobalt internal standard is introduced into the powdered graphite by adding a solution containing the required amount of cobalt. This solution is prepared by converting the pure oxide into a nitrate. It was found that better homogeneity was achieved when the cobalt was added as a solution, rather than as a powder. Here, again, the concentration of the added solution must be such that all of the solution is taken up by the graphite and none wets the beaker. The graphite-cobalt mixture is dried slowly in an oven with occasional stirring, and, when dry, is placed in a muffle furnace at 1050° F. for approximately 5 minutes to ensure complete decomposition of the nitrate into the oxide. After cooling, the mixture is transferred to a mortar and thoroughly blended. A different cobalt-graphite ratio is used for each of the catalysts. The quantities of cobalt used and the instrument operating conditions are shown in Table I.

DISCHARGE CONDITIONS

For purposes of uniformity, the same discharge conditions are used wherever possible for both types of catalysts. The pellet is placed on a solution excitation unit shaft, which is rotated at 6 r.p.m. during discharge. This stainless steel shaft must be polished before analyzing each new pellet. The counterelectrode, which in this case is the upper electrode, is a 0.25-inch graphite rod with a 120° conical tip. The gap is adjusted to 3 mm. Time studies have indicated that the exposure periods given in Table I are to be preferred. The exposure conditions, which are also included in Table I, were selected so that only a negligible background is shown. As many as 14 exposures, including standards, may be recorded on the same film strip.

DEVELOPING

The Eastman Spectrum Analysis No. 2 film is developed for 2 minutes at 65° F. in Eastman D-8 developer with constant agitation in the ARL developing machine. The film remains in the short-stop for a half-minute, in Kodak Liquid X-Ray fixer for 2 minutes, and in the wash tray for 5 minutes. Drying is accomplished in 4 minutes in the ARL dryer.

WORKING CURVES AND CALCULATIONS

Because discharge conditions have been adjusted so that background is negligible, this is ignored in reading lines on the densitometer. Working curves prepared from line pairs for the natural and synthetic catalysts are shown in Figures 3 and 4. The line pairs used for these catalysts are shown in Table II.

Chromium was of no concern in the natural clay catalysts analyzed in the authors' laboratory. Likewise, no vanadium has been found in any synthetic catalysts, either new or after use in the processing equipment; therefore, line pairs were not established for these elements in the respective catalyst.

The method has been evaluated by reproducibility series and comparisons with classical chemical methods, with the results shown in Tables III and IV.

Table I. Pellet Composition and Discharge Conditions

	Natural	Synthetic
Mixture ratio (catalyst to lithium carbonate to graphite)	1:1:1	1:1:4
Pellet weight, gram	0.930	1.00
Cobalt, mg. per pellet	0.19	1.6
High precision case		
Capacity, microfarad	0.007	0.007
Inductance, microhenrys	360	360
Current, amperes	8	8
Intensity control stand position	2/5	1/5
% Transmittance	80	100
Grating shutter setting	0.2	0.2
Prespark, seconds	90	120
Exposure, seconds	20	20

Table II. Line Pairs

Catalyst	Cr	Fe	Ni	Na	
Synthetic	$\frac{\text{Cr } 2835.6}{\text{Co } 2627.6}$	$\frac{\text{Fe } 2598.4}{\text{Co } 2567.3}$	$\frac{\text{Ni } 3619.4}{\text{Co } 3149.3}$	$\frac{\text{Na } 3302.3}{\text{Co } 3149.3}$	
Natural clay		$\frac{\text{Fe } 3031.2}{\text{Co } 3072.3}$	$\frac{\text{Ni } 3461.7}{\text{Co } 3417.2}$	$\frac{\text{Na } 3303.0}{\text{Co } 3443.6}$	$\frac{\text{V } 3184.0}{\text{Co } 3072.3}$

Table III. Analyses of Clay Catalysts

Sample No.	Presumed Concentrations[a], Weight %				Chemical Analyses, Weight %				Spectrographic Analyses, Weight %			
	Fe	Ni	Na	V	Fe	Ni	Na	V	Fe	Ni	Na	V
2300 Original	0.91	0.0025	0.14	0.0065	0.81	[b]	0.08	0.0057	0.79	0.0025	0.12	0.0064
					0.84		0.08	0.0077	0.90	0.0024	0.14	0.0061
					0.84		0.07	0.0068	0.93	0.0026	0.14	0.0062
					0.91		0.068	0.0060	0.93	0.0024	0.14	0.0063
					1.01		0.071		0.97	0.0026	0.14	0.0068
					1.04				0.91	0.0026	0.13	0.0072
Av.					0.91		0.074	0.0066	0.91	0.0025	0.14	0.0065
Standard deviation, % of sample					0.094			0.00086	0.062	0.0001	0.0089	0.00041
Standard deviation, % of element					10.3			13.0	6.5	4.0	6.7	6.3
2301-C												
Original	0.91	0.0025	0.14	0.0065	1.53	0.053	0.33	0.0145	1.34	0.056	0.50	0.0156
Added	0.47	0.0555	0.36	0.0090	1.52	0.051	0.33	0.0142	1.30	0.058	0.53	0.0150
Total	1.38	0.058	0.50	0.0155	1.50	0.060	0.32	0.0140	1.40	0.057	0.57	0.0168
					1.56	0.060	0.30	0.0142	1.42	0.060	0.51	0.0155
					1.52	0.058	0.30	0.0137	1.30	0.059	0.53	0.0160
									1.30	0.058	0.55	0.0148
									1.17	0.057	0.53	0.0141
Av.					1.53	0.056	0.32	0.0141	1.32	0.0578	0.53	0.0154
Standard deviation, % of sample					0.026	0.0042		0.0003	0.026	0.0014	0.023	0.0009
Standard deviation, % of element					1.7	7.5		2.1	1.9	2.3	4.4	5.7
2301-D												
Original	0.91	0.0025	0.14	0.0065	1.18	0.026	0.20	0.0107	1.06	0.030	0.32	0.0118
Added	0.23	0.0275	0.18	0.0045	1.23	0.026	0.20	0.0109	1.07	0.031	0.31	0.0110
Total	1.14	0.030	0.32	0.0110	1.23	0.030	0.20	0.0092	1.08	0.030	0.31	0.0114
					1.32	0.028	0.20	0.0092	1.23	0.031	0.28	0.0113
					1.26		0.18	0.0092	1.06	0.030	0.33	0.0110
									1.14	0.030	0.31	0.0112
									1.18	0.029	0.28	0.0111
Av.				....	1.24	0.028	0.20	0.0098	1.12	0.030	0.31	0.0113
Standard deviation, % of sample				....	0.051	0.0024		0.00088	0.068	0.0071	0.019	0.00028
Standard deviation, % of element				.....	4.1	8.6		9.0	6.0	2.4	6.3	2.5

[a] Nickel, sodium, and vanadium in 2300 were determined by addition spectrographic procedures, whereas iron was determined chemically. Known quantities were added to 2300 to give concentrations shown for 2301-C and 2301-D.

[b] Concentration below the accuracy of this method.

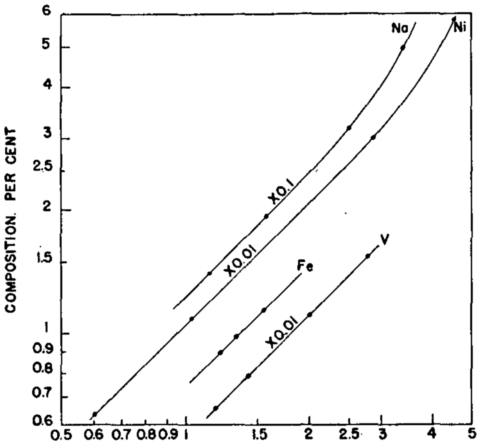


Figure 3. Working Curves for Natural Clay Catalyst

The chemical methods, or modifications thereof, which were used in these determinations are as follows:

1. Sodium was determined gravimetrically by weighing a precipitate of sodium-magnesium uranyl acetate (*12*).
2. Vanadium was determined colorimetrically by measuring the spectrophotometric absorption of a phosphotungstovanadate solution (*3*).
3. Nickel was determined colorimetrically, following the procedure reported by Wrightson (*13*), after the catalyst had been taken into solution by a procedure given by Sandell and Perlich (*11*).
4. Iron was determined colorimetrically by a modification of a dichromate-diphenylamine procedure (*1*).

An examination of Tables III and IV shows that, for most of the elements, there is fair agreement between the chemical and spectrographic methods. However, the latter is superior with respect to precision and reproducibility. The maximum standard deviation by this method was 10% in any one series of analyses, whereas the average was considerably better than this. Comparable results were not obtained by the chemical methods. The spectrographic method also provides an additional advantage—rapidity of analyses. The time required to complete a single analysis is approximately 3 hours and 15 minutes, distributed as follows: 30 minutes in grinding with a mechanical mortar and pestle, 2 hours in muffling, 10 minutes in preparing the pellet, 5 minutes in instrumentation, 20 minutes in developing and reading the film, and 10 minutes on the calculations. The grinding and heating require practically no attention, leaving the spectrographer free to perform other duties. The elapsed time may be substantially reduced by decreasing the grinding and muffling, without seriously affecting the results. The time required for each sample may be further reduced by analyzing groups of samples.

ACKNOWLEDGMENT

The authors wish to express their gratitude to R. J. Wilson, E. D. Alpert, R. G. Jewell, and Robert L. Dinsmore for the

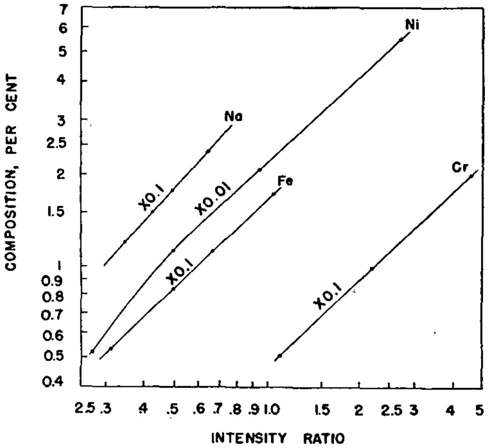


Figure 4. Working Curves for Synthetic Catalyst

Table IV. Analyses of Synthetic Catalysts

Sample No.	Presumed Concentrations, Weight %				Chemical Analyses, Weight %				Spectrographic Analyses, Weight %			
	Fe	Ni	Na	Cr	Fe	Ni	Na	Cr	Fe	Ni	Na	Cr
2561	0.053	0.005	0.12	Nil	0.050		0.092		0.051	0.0049	0.125	Nil
New clear synthetic					0.050		0.096		0.054	0.0052	0.112	Nil
					0.050		0.091		0.053	0.0053	0.124	Nil
					0.053		0.094		0.054	0.0047	0.112	Nil
Av.					0.051		0.091		0.053	0.0050	0.118	Nil
Standard deviation % of sample					0.0153		0.0030		0.0014	0.00028	0.0072	
Standard deviation % of element					3.0		3.3		2.6	5.6	5.6	
2562 (2561 fortified)												
Original	0.053	0.005	0.12	Nil	0.146	0.250	0.176	0.192	0.169	0.196	0.23	0.192
Added	0.119	0.198	0.109	0.198	0.146	0.215	0.194	0.196	0.168	0.208	0.26	0.205
Total	0.172	0.203	0.229	0.198	0.152	0.225	0.187	0.194	0.176	0.193	0.23	0.207
					0.150	0.220	0.218	0.192	0.156	0.202	0.24	0.192
Av.					0.149	0.228	0.194	0.194	0.167	0.200	0.24	0.199
Standard deviation % of sample					0.0034	0.014	0.018	0.0035	0.0083	0.0066	0.014	0.0081
Standard deviation % of element					2.3	6.1	9.3	1.8	4.7	3.3	5.9	4.1
2561-A[a]	...	...	...	...	0.082	[b]	0.109	0.054	0.099	0.0074	0.100	0.057
					0.085		0.140	0.052	0.097	0.0064	0.095	0.058
							0.137		0.097	0.0068	0.108	0.058
							0.077		0.098	0.0056	0.093	0.059
Av.				...	0.084		0.116	0.053	0.098	0.0066	0.099	0.058
Standard deviation % of sample				...	0.0021		0.0294	0.0014	0.001	0.00069	0.0067	0.0014
Standard deviation % of element				...	2.5		26.0	2.7	1.0	10.0	6.8	2.5

[a] Equilibrium catalyst from processing unit.
[b] Less than minimum detectable quantity.

chemical analyses, to Orland Eastin for the statistical analyses, and to Richfield Oil Corp. for permission to publish this report.

LITERATURE CITED

(1) Am. Soc. Testing Materials, "ASTM Methods of Chemical Analysis of Metals," pp. 242–3, 1950.
(2) Burdett, R. A., and Jones, L. C., ANAL. CHEM., **19**, 238–41 (1947).
(3) Cooper, M. D., and Winter, P. K., *Ibid.*, **21**, 605–9 (1949).
(4) Gunn, E. L., *Ibid.*, **21**, 599–605 (1949).
(5) *Ibid.*, **23**, 1354–8 (1951).
(6) Harvey, C. W., "Method of Semi-Quantitative Spectrographic Analyses," Glendale, Calif., Applied Research Laboratories, 1947.
(7) Harvey, C. E., "Spectrochemical Procedures," Chap. XIII, Glendale, Calif., Applied Research Laboratories, 1950.
(8) Hasler, M. F., and Harvey, C. E., private communication.
(9) Marling, J. B., ANAL. CHEM., **20**, 299–300 (1948).
(10) Nachtrieb, Norman, "Principles and Practices of Spectrochemical Analysis," pp. 193–212, New York, McGraw-Hill Book Co., 1950.
(11) Sandell, E. B., and Perlich, R. W., IND. ENG. CHEM., ANAL. ED., **11**, 309–11 (1939).
(12) "Scott's Standard Methods of Chemical Analysis," 5th ed., Vol. 1, pp. 879–81, New York, D. Van Nostrand Co., 1939.
(13) Wrightson, Frances, ANAL. CHEM., **21**, 1543–45 (1949).

RECEIVED for review June 17, 1952. Accepted September 18, 1952. Presented before the Division of Refining, American Petroleum Institute, San Francisco, Calif., 1952.

Polymer Degradation Studies by Absorption and Mass Spectrometry

B. G. ACHHAMMER

National Bureau of Standards, Washington 25, D. C.

THE analysis of the ingredients of unknown composition in a polymer and the detection of the changes in chemical structure resulting from degradative treatment at first thought appear to be intractable problems. It is true that the analytical problems involved in such a procedure would be insurmountable if the chemist had to rely on classical chemical methods. The various chemical methods established for identification and localization of functional groups in small organic molecules are not generally applicable in more complicated systems such as those found in polymers. For example, scission of the chains of a high molecular weight polymer could greatly decrease the molecular weight and considerably affect the physical properties of the material and yet be undetectable by standard analytical methods for functional groups. The formation of a few cross links between polymer chains could also change the physical properties of the polymer and present a similar analytical problem.

In some cases it is necessary to adapt chemical methods to polymer systems in spite of their limitations. For example, chemical methods are presently being used to detect hydroperoxide in polyvinyl chloride. Available methods, including the Karl Fischer method, are designed for study of a solution of the hydroperoxide-bearing material. Despite the insolubility of polyvinyl chloride in all common solvents, some measure of the hydroperoxide content can be obtained by adapting the Karl Fischer method so that the polymer is agitated in the presence of the reagent. Although the results obtained in this manner are of questionable quantitative accuracy, some agreement of the oxygen detected as hydroperoxide with the total oxygen content of the polymer as determined by the direct method of Walton, McCulloch, and Smith (*20*) is obtained (*4*).

In recent years spectroscopic methods have been emphasized for obtaining information on functional groups in macromolecules. A variety of physical-chemical techniques are now available for obtaining information on the structure of organic compounds. Without attempting a complete listing one can mention: mass spectrometry, infrared spectrophotometry, ultraviolet spectrophotometry, x-ray diffraction, electron microscopy, and dielectric measurements. All of the techniques available will not be

The use of absorption and mass spectrometry in a current study of polymer degradation mechanisms at the National Bureau of Standards is described. Infrared and ultraviolet spectrophotometry and mass spectrometry are discussed as tools for the determination of the chemical structure of polymers and for the detection of changes in the chemical structure of polymers which occur as a result of degradative treatment. Data obtained on specific polymers, including polystyrene, polyamides, and polyvinyl chloride, are presented to show how these techniques are applied to the determination of the composition of polymeric materials. Some limitations of absorption and mass spectrometric techniques for polymer studies are discussed.

applicable to all cases. Neither is it expected that one technique will give a complete answer.

A consideration of some of the analytical techniques used in the program on polymer degradation at the National Bureau of Standards will serve to indicate some of the problems which have been encountered and the type of progress which can be expected.

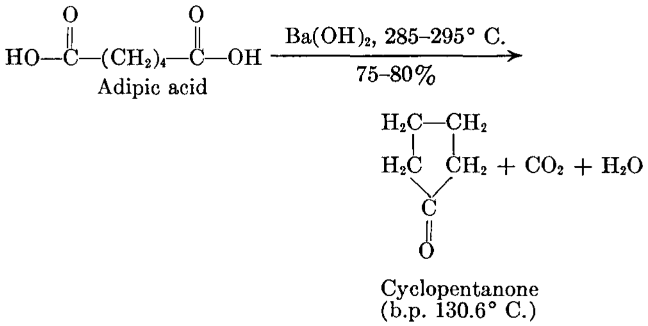
MASS SPECTROMETRY

The recent development of the mass spectrometer as an analytical tool (*8*, *9*, *18*, *19*) makes possible the identification of individual components in mixtures of gases when only a small quantity is available. This overcomes a major problem of the past which was to collect sufficient material to identify the products. A recent study of the gases evolved from polystyrene on exposures to heat and ultraviolet radiant energy showed that some twenty-five gases were identified by mass spectrometry (*2*). These gases for the most part could be attributed to residual contaminants, such as solvent and monomer, and to decomposition of oxygenated groups incorporated in the polymer during polymerization or storage. Benzaldehyde, acetophenone, formaldehyde, and formic acid were also identified on exposure of polystyrene to ultraviolet radiant energy in the presence of oxygen.

The evolution of styrene from the polymer will serve as an example of the technique (see Figure 1). These points are plotted as cumulative pressures which means that the pressure of the gas produced during the first 5 hours is added to the pressure obtained during the second 5 hours to give the point for the 10-hour exposure and so forth. It is interesting to observe that the amount of styrene obtained on heating polystyrene in vacuo at 120° C. (Δ, *V* curve) and in 20 mm. of oxygen at 115° C. (Δ, O_2 curve) levels off within a few hours indicating that the material produced was probably due to residual monomer. The *UV*, *V* curve represents the gas produced on exposure to ultraviolet radiant energy in vacuo at 120° C. and suggests that in this case sufficient energy is available to produce styrene by degradation of the polymer since the production of styrene continued throughout the treatment. The curve *UV*, *V*, O_2, *V* represents an exposure to ultraviolet at 120° C. in vacuo for 30 hours, followed by exposure at 118° C. in 20 mm. of oxygen for 40 hours and exposure at 120° C. in vacuo for 180 hours. During the period when oxygen was available (30 hours to 70 hours) the production of styrene was low. Subsequent treatment in vacuo caused an increase in the production of monomer, presumably as a result of decomposition of the polymer. The *UV*, O_2 curve representing exposure of polystyrene to ultraviolet radiant energy at 118° C. in 20 mm. of oxygen might then be interpreted as showing that any monomer evolved is oxidized to some other compound—perhaps benzaldehyde and formaldehyde or formic acid, which were also detected. A similar analysis of each of the other gases produced gives some insight into the method by which they were formed and the role they play in the degradation mechanism.

The mass spectrometer has made pyrolysis a valuable tool for studying decomposition mechanisms since the gases collected can be readily identified. Pyrolysis studies generally do not provide data which are directly applicable to mechanisms of degradation operative at service conditions but do provide information as to the mechanism of depolymerization. For example, it can be determined by pyrolysis whether polystyrene will decompose to give styrene monomer and/or other compounds. Wall and Madorsky (*8*, *9*, *19*) have contributed valuable information on the pyrolysis of polymers and copolymers. Pyrolysis can be utilized to give some idea of the structure of the polymer and the presence of weak linkages in the polymer chain.

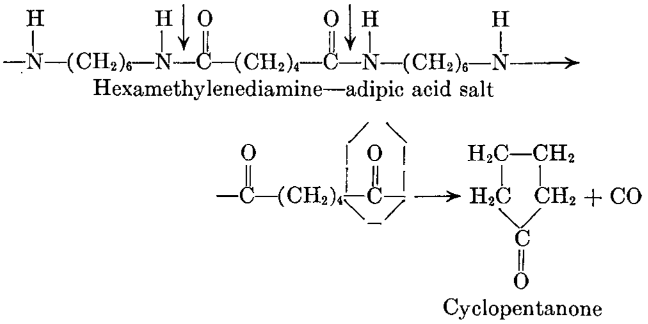
Analysis of the gases collected on pyrolysis of a polyamide will serve to show the value of this technique. Unidentified peaks of molecular weights were 84 and 56 found, and chemical reasoning suggested that cyclopentanone might be responsible for these mass values. A reference pattern run on cyclopentanone showed that it matched the mass spectral pattern of the unknown. Two mechanisms are available by which cyclopentanone could be a degradation product of a polyamide. It is known that pyrolysis of adipic acid, a component of the polyamides studied, will produce cyclopentanone, carbon dioxide, and water as shown in the following equation:



Adipic acid

Cyclopentanone
(b.p. 130.6° C.)

This could conceivably occur if the polymer contained excess adipic acid. Also, since the bond energy of the C—N (about 66 kcal.) is considerably less than that of the C—C bonds (about 82.5 kcal.) of the chain, it is possible to produce cyclopentanone from the polymer, as shown in the following equation, to give carbon monoxide in this case, or carbon dioxide if the adipic acid were at the end of a molecular chain.



Hexamethylenediamine—adipic acid salt

Cyclopentanone

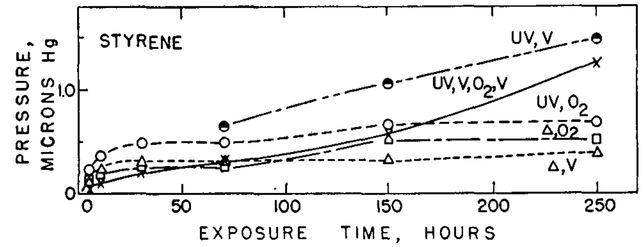


Figure 1. Mass Spectrometric Analysis for Styrene in Heat- and Ultraviolet-Treated Polystyrene

Both carbon dioxide and carbon monoxide were identified on degradation of the polyamide; however, other sources of these gases are also possible.

Although the mass spectrometer is a valuable tool for detecting and measuring degradation gases, it does have some rather definite limitations. High water yields, common in oxidation studies, decrease the quantitative accuracy of hydrocarbon analyses. Complete reference files of all suspected gases must be available or run as a check, as in the case of the cyclopentanone described previously. In this respect each analysis is potentially a research problem.

Another problem in using the mass spectrometer is the lack of background knowledge in analyzing certain gases and mixtures. Reference to some work in progress on the degradation of polyvinyl chloride will illustrate this situation. The degradation of this polymer is reported (*5*, *6*) to be due to loss of hydrogen chloride which produces conjugated double-bond systems which are in turn believed to be responsible for discoloration of the polymer, as well as providing reactive centers for subsequent oxidation of the polymer. An attempt to check this on a Consolidated mass spectrometer (Model 21–102) showed that no hydrogen chloride could be detected in the first 40 hours of heating in vacuo at 100° C. although discoloration occurred after the first hour of this treatment. Hydrogen chloride was detected after treatment for 200 hours (*7*).

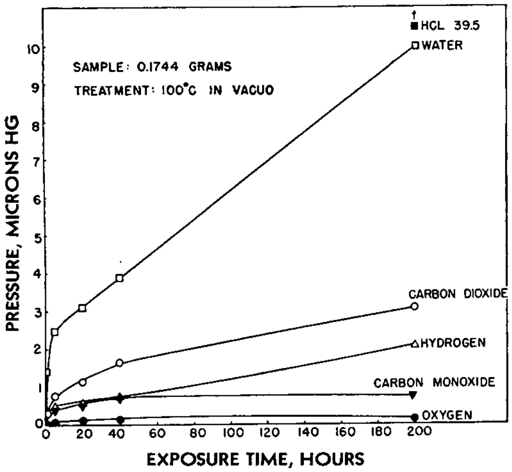


Figure 2. Degradation Products from Polyvinyl Chloride by Mass Spectrometry

The degradation products depicted in Figure 2 suggest that the water and possibly the carbon monoxide and carbon dioxide are associated with sorbed material and/or the breakdown of hydroperoxide in the polymer. The water could indicate that a hydroperoxide mechanism is the initiating step of the degradation. However, before one can conclude that a hydroperoxide mechanism is involved and that discoloration is not associated with loss of hydrogen chloride, the feasibility of detecting hydrogen chloride in the presence of water with the mass spectrometer must be investigated. Such a study was made with a Consolidated mass spectrometer (Model 21–102) and revealed that hydrogen chloride could not be determined in small quantities in the presence of water. In analyses involving the small amounts of materials encountered in degradation studies of this type, factors such as the metal inlet system and background water in the instrument must be considered.

INFRARED SPECTROPHOTOMETRY

When gases consisting of other than sorbed material are evolved from a polymer, structural changes must have occurred

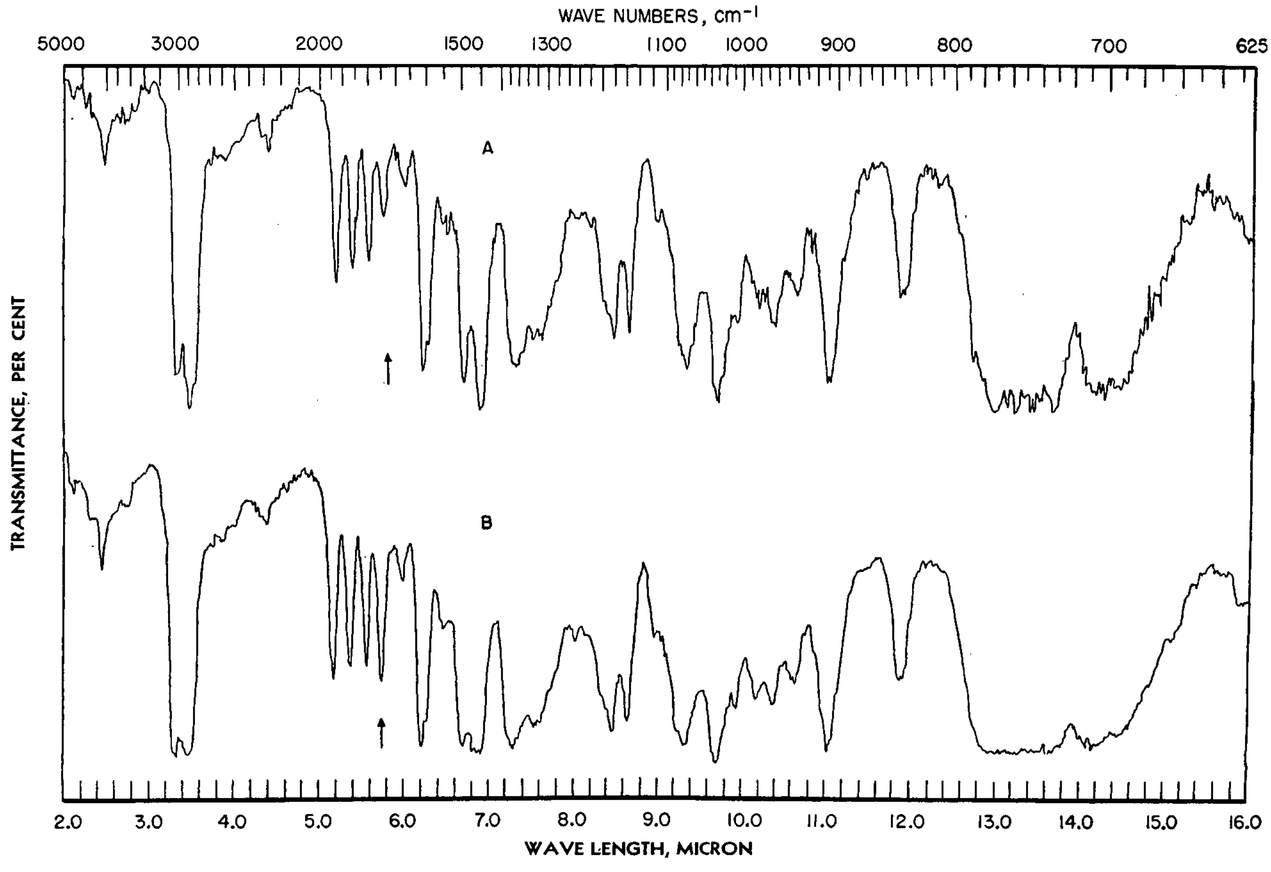


Figure 3. Infrared Spectra of Untreated Polystyrene Film

Thickness, 0.007 inch; solvent, benzene

in the residual material. Several spectroscopic techniques can be employed for determination of these changes.

In recent years infrared spectroscopic methods have been emphasized for obtaining information on functional groups in polymers, and considerable reference material is available. Traces of functional groups such as carbonyl, ether, and aliphatic double bonds can be detected by infrared. The presence of carbonyl groups, ether groups, and aliphatic double bonds as well as methyl groups has been established in commercial polyethylene polymers although this polymer is theoretically a simple chain of methylene groups (*10*). The methyl side groups are of interest in degradation studies since the presence of this group increases the rate of oxygen absorption which is used as a criterion of oxidizability (*12*). Carbonyl groups can be detected in commercial polystyrenes as shown by their infrared spectra. Carbonyl in polystyrene which was polymerized with benzoyl peroxide as the initiator, may indicate the presence of ester-type fragments of the catalyst attached to the ends of the polymer chains according to Pfann, Williams, and Mark (*13*).

Carbonyl absorption at approximately 5.8 microns in two untreated commercial polystyrene samples is shown in Figure 3. The material having the greater carbonyl absorption (curve *B*) can be shown to have a higher oxygen content by the direct method of Walton, McCulloch, and Smith (*20*).

Infrared spectrophotometry is also a valuable tool for describing chemical changes in this polymer as a result of degradative exposure (*1*). The effect of S-1 sunlamp radiation at 60° C. in air on the infrared spectrum of a purified polystyrene is shown in the *A* spectra of Figure 4. The solid line represents the untreated polymer, for little change is shown in the first 50 hours of treatment; the dotted line, the degraded specimen. Carbonyl formation as a result of the exposure is shown by the absorption in the region 5.7 to 6.0 microns; and hydroxyl formation is suggested by the absorption at 2.9 microns. A rather general increase in overall absorption between 7 and 16 microns results from the treatment. The *B* spectra of this figure show the effect of 4 months of outdoor exposure on the roof of the laboratory in Washington, D. C. The correlation with the accelerated test is considered to be very good.

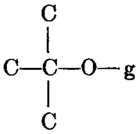
It is sometimes desirable to use infrared spectrophotometry for studies where little or no reference work has been done. Such a case exists in a current attempt to detect and measure hydroperoxide in polyvinyl chloride by a specific infrared absorption. It is hoped that this spectrophotometric method will be supported by the chemical method for detecting this group described previously. At least two attempts to detect hydroperoxide by infrared have been recorded (*14*, *17*). The work of Shreve, Heether, Knight, and Swern (*17*) indicates that this group should absorb in the region of 12 microns. Philpotts and Thain (*14*) attributed absorption at 12 microns to the  $\mathrm{C{-}\underset{C}{\overset{C}{C}}{-}O{-}}$ group which can be present in molecular forms other than hydroperoxides. These authors also showed that at higher molecular weights, it is difficult to distinguish between alcohol and peroxide. The infrared spectrum of untreated polyvinyl chloride (Figure 5) shows an absorption near 12 microns (*15*). This spectrum was obtained by suspending the insoluble polymer in a liquid of nearly equal refractive index in the visible.

Figure 6 shows the spectrum of a polyvinyl chloride sample which was prepared by mulling the polymer powder in Nujol. The absorption band in the region 5.75 to 6.5 microns suggests the presence of carbonyl, and the absorption between 2.75 and 3.0 microns may be associated with hydroxyl. This mulling technique resulted in degradation of the polymer powder. The absorption in the region of 10 microns is probably associated with oxidation of the polymer. It is interesting to note that the decomposition of hydroperoxide will usually produce hydroxyl and carbonyl groups (*11*). The detection and study of the decomposition mechanism of hydroperoxide are important because of this group's possible role in the initiation step of the oxidation and decomposition of various polymers.

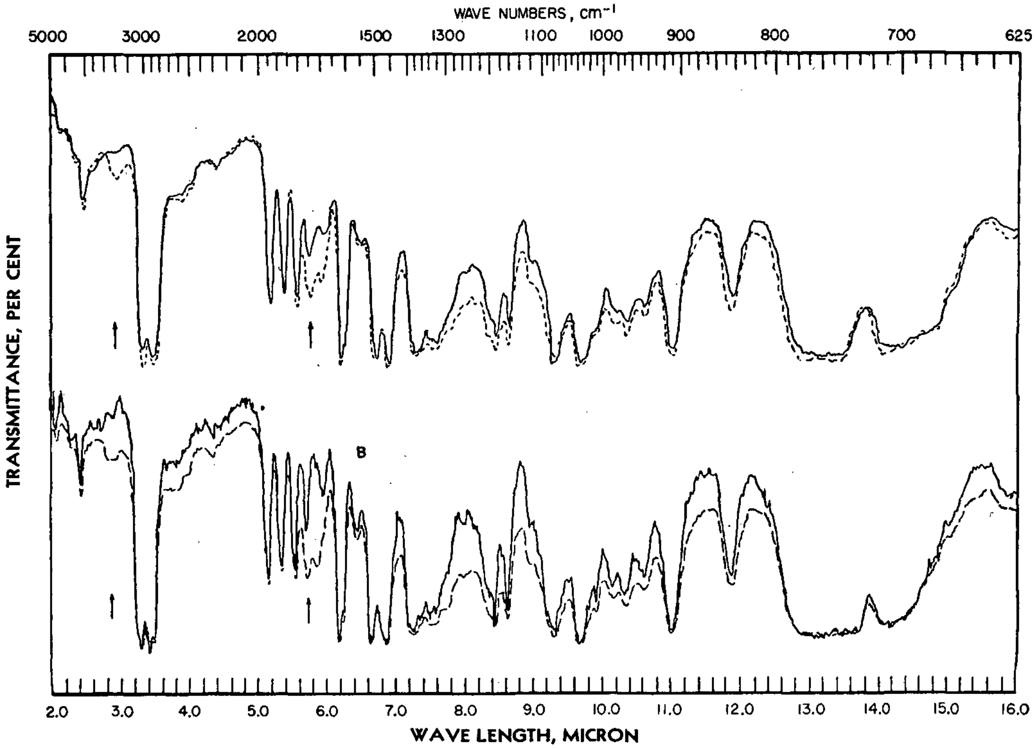


Figure 4. Infrared Spectra of Polystyrene Film

A. Thickness, 0.007 inch; solvent, benzene; treatment, —— 50 hours, S-1 sunlamp at 60° C. in air; - - - 200 hours, S-1 sunlamp at 60° C. in air

B. Thickness, 0.007 inch; solvent, toluene; treatment, —— untreated, - - - 4 months' outdoor exposure

As with any technique, infrared spectrophotometry has its limitations. One of the chief difficulties is the preparation of a sample suitable for both infrared measurement and degradation studies. A factor of major impact on degradation studies is the inability to detect molecular weight differences by infrared. However, changes in molecular weight can be detected for soluble polymers by viscometric methods. For example, the changes in molecular weight of a polyamide on exposure to degradative conditions is shown in Figure 7. The intrinsic viscosity decreases on both heating at 105° C. in air as shown in curve *A* and on ultraviolet exposure as shown in curve *B*. The effect of ultraviolet exposure is shown to be greater than the effect of heat for these particular treatments.

ULTRAVIOLET SPECTROPHOTOMETRY

Since the interpretation of the spectra in the ultraviolet is more involved than in the infrared, the analysis of the ultraviolet spectra of complicated molecules like high polymers is somewhat difficult. However, ultraviolet spectrophotometry can be used advantageously when the required chromophores are involved. Such a case is shown in Figure 8 by the detection of monomer styrene in polystyrene (*16*). Purified polystyrene is shown in curve *A*. Polystyrene containing monomer, absorbing at 292 mμ, is shown in curve *B*. The presence of monomer in a polymer to be used in degradation studies must be recognized before degradation studies are made in order to interpret properly the results of the exposure. The presence of monomer is known to affect the rate of degradation of polystyrene and has been associated with discoloration of the polymer (*5*).

When certain specific resonating groups are present in the polymer to produce absorption in the ultraviolet, ultraviolet spectrophotometry can also be used to follow the degradation of the polymer. The effect of heating and ultraviolet exposure of polyamide films on the ultraviolet transmission of the films (*3*) is shown in Figure 9. Curve *A* represents the spectrum of an untreated polyamide showing an absorption band with a maximum

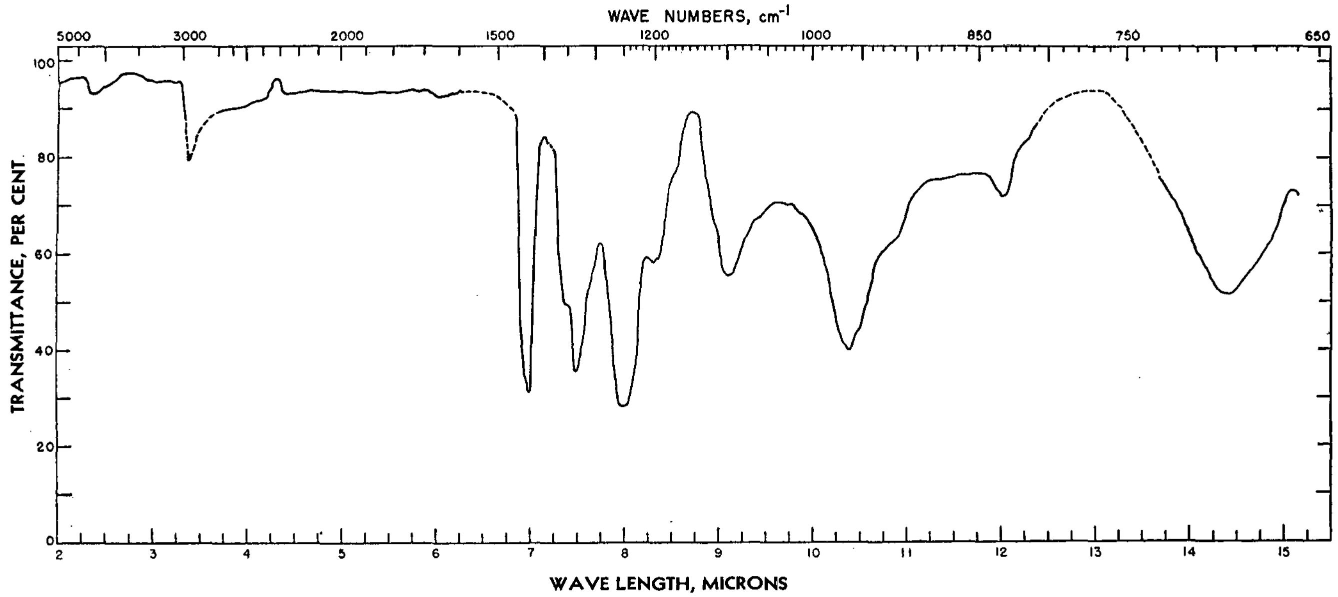


Figure 5. Infrared Spectrum of Polyvinyl Chloride

Thickness, 58 μ mixture compensated with 45 μ liquid of 1.56 n_D^{20}
Mixture, powder of particle size less than 44 μ mixed with a liquid of 1.56 n_D^{20}

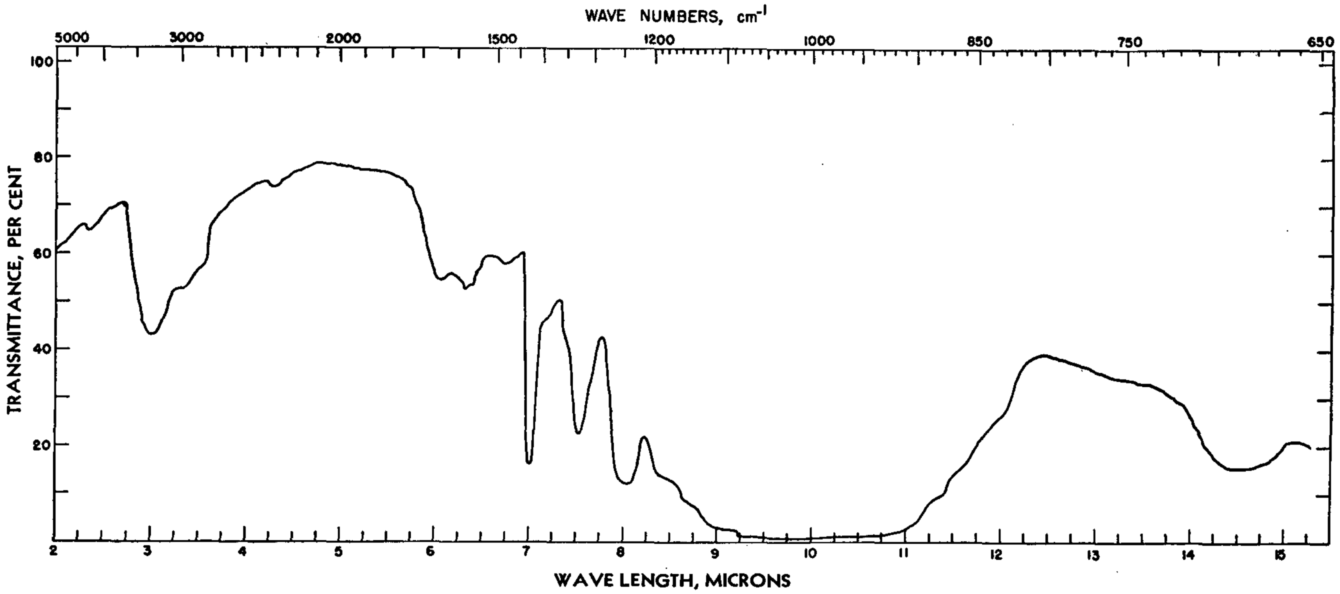


Figure 6. Infrared Spectrum of Polyvinyl Chloride

Thickness, 106.7 μ emulsion compensated with 96.5 μ Nujol
Emulsion, 0.15 gram of powder of particle size less than 44 μ mulled 0.375 ml. Nujol

at approximately 280 mμ. Exposure to S-1 sunlamp radiation results in increased transmission in this region of the spectrum as illustrated by curve *B*. The effect of heat on the ultraviolet transmission is shown in curve *C* where the absorption band is retained but an over-all decrease in transmission is recorded. As might be expected, a combination of heat and ultraviolet exposures results in elimination of the absorption band concurrent with a decrease in over-all transmission. This is shown by curve *D*. These changes are attributed to reactions involving the peptide linkage.

REMARKS

The techniques described represent the backbone of the present analytical attack on the problem of the degradation of polymers in this program. Infrared spectrophotometry is being utilized to detect and identify functional chemical groups in both untreated and degraded polymers. Mass spectrometry offers a means of identifying and measuring the gaseous products evolved from a polymer on degradative treatment, thereby making possible by chemical reasoning a reconstruction of the degradation reaction. Ultraviolet spectrophotometry provides valuable data on the degradation of polymeric materials when the necessary chromophoric groups are present in the polymer. Other techniques including electron diffraction, x-ray diffraction, and light microscopy have been used where a consideration of crystallinity was important to the mechanism of degradation. At times, however, a combination of two techniques, such as infrared absorption spectrophotometry, to detect functional groups, and x-ray diffraction methods, to establish spatial relationships, is sufficient.

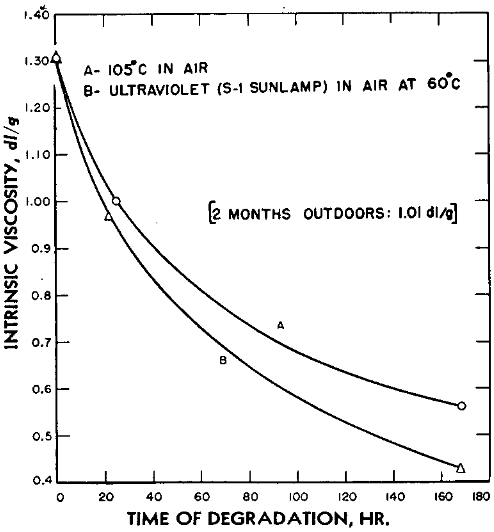


Figure 7. Effect of Degradation Conditions on Intrinsic Viscosity of a Polyamide

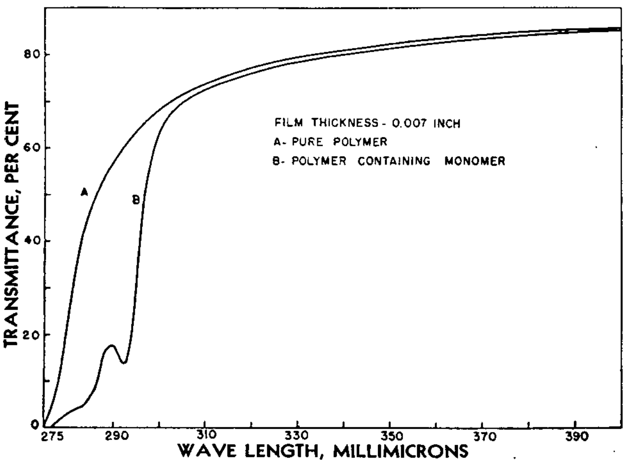


Figure 8. Ultraviolet Transmission of Untreated Polystyrene

The data from several techniques used to detect degradation are in any case then focused on the mechanism of the breakdown. Naturally, the analytical data have to be correlated and interpreted by the polymer chemist working in conjunction with the analytical chemist.

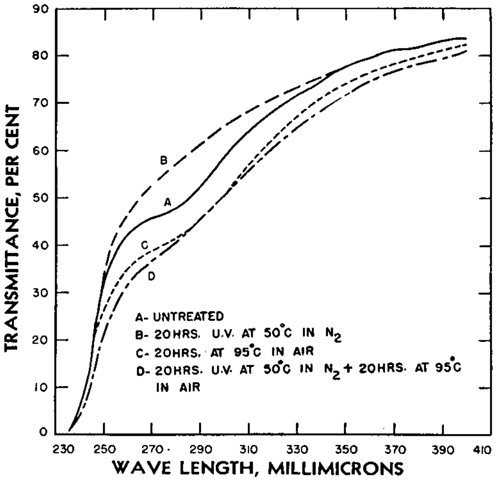


Figure 9. Ultraviolet Spectra of a Polyamide

Attacking the problem of the degradation of polymeric materials by studying changes in chemical structure will provide insight into the degradation mechanisms, make possible a more fundamental approach to the inhibitor problem, and provide data for designing more suitable accelerated aging tests. The importance of the analytical approach has been emphasized because progress in polymer chemistry is indeed dependent upon analytical methods.

LITERATURE CITED

(1) Achhammer, B. G., Reiney, M. J., and Reinhart, F. W., *J. Research Natl. Bur. Standards*, **47**, 116 (1951); RP 2235.
(2) Achhammer, B. G., Reiney, M. J., Wall, L. A., and Reinhart, F. W., *J. Polymer Sci.*, **8**, 555 (1952).
(3) Achhammer, B. G., Reinhart, F. W., and Kline, G. M., *J. Research Natl. Bur. Standards*, **46**, 391 (1951); RP2210.
(4) Bersch, C. F., unpublished data.
(5) Boyer, R. F., *J. Phys. & Colloid Chem.*, **51**, 80 (1947).
(6) Fox, V. W., Hendricks, J. G., and Ratti, H. J., *Ind. Eng. Chem.*, **41**, 1774 (1949).
(7) Ishihara, Shigeru, unpublished data.
(8) Madorsky, S. L., and Straus, Sidney, *Ind. Eng. Chem.*, **40**, 848 (1948).
(9) Madorsky, S. L., Straus, Sidney, Thompson, Dorothy, and Williamson, Laura, *J. Research Natl. Bur. Standards*, **42**, 499 (1949); RP 1989.
(10) Mark, H., and Tobolsky, A. V., "Physical Chemistry of High Polymeric Systems," 2nd ed., Vol. 2, p. 79, New York, Interscience Publishers, 1950.
(11) *Ibid.*, p. 479.
(12) Mesrobian, R. B., and Tobolsky, A. V., *J. Polymer Sci.*, **2**, 463 (1947).
(13) Pfann, H. F., Williams, V. Z., and Mark, H., *J. Polymer Research*, **1**, 14 (1946).
(14) Philpotts, A. R., and Thain, William, Anal. Chem., **24**, 638 (1952).
(15) Reiney, M. J., unpublished data.
(16) Reiney, M. J., Tryon, M., and Achhammer, B. G., manuscript in preparation.
(17) Shreve, O. D., Heether, M. R., Knight, H. B., and Swern, Daniel, Anal. Chem., **23**, 282 (1951).
(18) Taylor, R. C., Brown, R. A., Young, W. S., and Headington, C. E., *Ibid.*, **20**, 396 (1948).
(19) Wall, L. A., *J. Research Natl. Bur. Standards*, **41**, 315 (1948); RP 1928.
(20) Walton, W. W., McCulloch, F. W., and Smith, W. H., *Ibid.*, **40**, 443 (1948); RP 1889.

Received for review July 24, 1952. Accepted September 13, 1952. Presented before the Fifth Annual Summer Symposium, Ingredients of Unknown Consitution, East Lansing, Mich., June 20 and 21, 1952.

Constant Current Conductance

ROBERT P. TAYLOR AND N. HOWELL FURMAN
Frick Chemical Laboratory, Princeton University, Princeton, N. J.

This investigation was undertaken to study the possibility of using direct rather than alternating current for conductance measurements. Theoretical considerations indicated that if a constant direct current were passed across a solution through a pair of platinum primary electrodes, the voltage drop across a pair of tungsten secondary electrodes should be inversely proportional to the conductance of the solution. This conclusion was verified experimentally. The constant current was obtained from a 540-volt battery source in series with a high resistance. By plotting reciprocal voltage drop *vs.* volume of titrant, the usual conductance curves were obtained. Several different conductometric determinations were carried out. The cell constant was shown to remain constant over a hundredfold conductance range. Since the direct constant current method described uses a pH meter to measure conductance, the specialized or inconvenient apparatus of alternating current conductometry is avoided. Accuracy and precision compare favorably with usual conductance measurements.

DIRECT current conductance involves the difficulty of electrode polarization. This difficulty was overcome by Andrews and Martin (*1*), who measured the conductance of potassium chloride solutions by using calomel electrodes and low current density. Another method for measuring direct current conductance, introduced by Newbery (*7*), involved impressing a constant direct current voltage across a pair of nonpolarizable primary electrodes and measuring the voltage drop across the two nonpolarizable secondary electrodes. As the primary electrodes were not polarized, the current was constant and readily measured. Substitution of values for voltage across the secondary electrodes and for current into Ohm's law yielded the relative resistance of the solution across the secondary electrodes. The proportionality factor or cell constant was either deduced from the geometry of the cell (*7*) or determined from the conductance of the cell filled with mercury (*3*).

Investigators (*4, 5, 8*) have obtained direct current conductance values of various standard solutions which were within 0.03% or better of the accepted alternating current values, and consequently in direct current work the cell constant may be conveniently determined by measuring the conductance of a solution whose specific conductance is known from alternating current measurements.

Although direct current conductometry gives accurate results, it appears to have found little application. The neglect of the method seems to be due to the cumbersome experimental requirements. It is necessary to use small current to prevent electrode polarization, and the *IR* drop across the secondary electrodes has to be sufficiently high for convenient measurement. These two conflicting requirements were met by the use of long, narrow conductance cells. Such cells are not adapted to routine specific conductance measurements and the difficulty of stirring the solution practically precludes employing them in conductometric titrations.

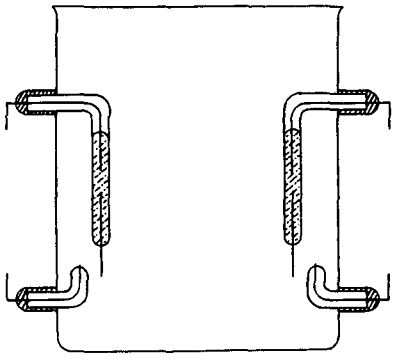


Figure 1. Conductance Cell

Another difficulty is the necessity of using nonpolarizable electrodes. Investigators have used calomel (*7*), mercurous sulfate (*3*), quinhydrone (*10*), and hydrogen electrodes (*8*). Not only are such electrodes less convenient than the platinum electrodes of alternating current conductometry, but they must be changed to conform to the solution being measured. The direct current conductance cell described below has neither of these disadvantages.

APPARATUS

The conductance cell, shown in Figure 1, consists of a 150-ml. beaker with two platinum and two tungsten electrodes. The platinum electrodes are placed close to the walls of the beaker and the tungsten electrodes are placed close to the platinum. This arrangement permits lowest current for given *IR* drop across the tungsten electrodes. The platinum primary electrodes through which constant current passes are made of 0.0126-inch platinum wire, cut flush with the glass. The electrodes are inverted to permit escape of the evolved gas. To aid further in the escape of the gas bubbles, the platinum electrodes occasionally were washed with a few drops of cleaning solution.

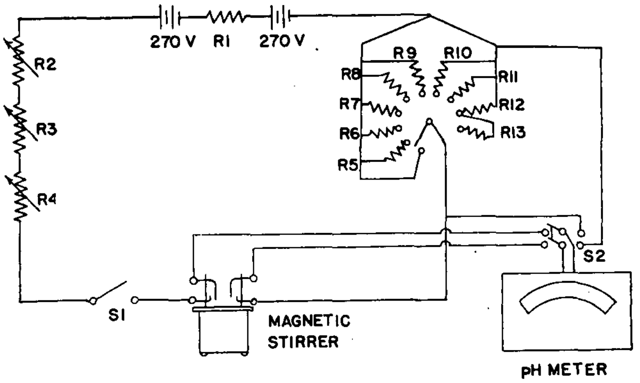


Figure 2. Direct Current Conductance Circuit Diagram

*R*1.	50,000 ohms	*R*6.	18 megohms	*R*11.	3961 ohms
*R*2.	0.1 megohm	*R*7.	80.3 ohms	*R*12.	6759 ohms
*R*3.	1 megohm	*R*8.	130.1 ohms	*R*13.	13160 ohms
*R*4.	5 megohms	*R*9.	405.9 ohms		
*R*5.	10 megohms	*R*10.	1298 ohms		

The secondary electrodes are 0.025-inch tungsten wire, approximately 0.5 inch long. This metal was chosen because it acts as a somewhat nonpolarizable electrode. Before the tungsten electrodes were fused into the cell, they were cleaned in molten sodium nitrite.

All the electrodes have short copper wire leads. At the platinum electrodes the contacts are soldered; at the tungsten electrodes mercury contacts are used. The external openings of the electrodes are sealed with wax to prevent breaking the platinum wire and to seal in the mercury.

The electrical circuit is shown in Figure 2.

Constant current is obtained by passing 540 volts through high variable resistors *R*2, *R*3, and *R*4. Where the solution resistance is large, either *R*5 or *R*6 can be thrown into the circuit. The voltage supply consists of twelve 45-volt radio B batteries in series. *R*1 is placed in the circuit both as a safety precaution and to prevent accidentally overloading the other resistances in the circuit. *R*7 through *R*13 are precision resistors which were calibrated after installation to 0.1% on a Wheatstone bridge. The current is measured by throwing one of these standard resistances into series and measuring the voltage drop across it. Of course, the current cannot be measured with the apparatus shown when *R*5 or *R*6 is in the circuit.

If the apparatus is not to be used for specific conductance measurements, it is not necessary to know the current, and the precision resistors may be omitted from the circuit. This would mean that instead of connecting the leads of the pH meter to the double-pole, double-throw switch, *S*2, they may be connected directly across the secondary electrodes.

The voltage drop across the secondary electrodes and across the precision resistors is measured with a Leeds & Northrup pH meter by throwing switch *S*2 into the required position.

A magnetic stirrer was used, the stirrer itself being made from a piece of iron nail fused into a glass tube about 10 mm. long. Two pieces of asbestos board were placed between the magnetic stirrer and the conductance cell to prevent the motor from heating the solution. When several titrations were performed in succession, the asbestos boards were changed.

PROCEDURE

The sample to be titrated was placed in the conductance cell and diluted to 100 ml. The solution was stirred at a moderate constant rate during the titration, the stirrer being left on as a matter of convenience when a conductance reading was made.

With the leads connected as shown in Figure 2 the pH meter was connected through switch *S*2, across the secondary electrodes. The pH meter was usually set on the 700-mv. scale, as this setting requires only half as much current as the 1400-mv. scale for given *IR* reading. To compensate for any polarization across the tungsten electrodes, the meter was zeroed with the zero switch up. Switch *S*1 was then closed and *R*2, *R*3, and *R*4, which may be thought of as a coarse, medium, and fine control, were adjusted to give a suitable *IR* reading.

For best accuracy the upper part of the millivolt scale should be used, as in that region a given change in conductance will result in the largest scale deflection. However, if the initial scale reading is set too high, there is the danger of having the *IR* drop at the end point off the millivolt scale. The voltage may be brought back on the scale by switching the pH meter to the 1400-mv. scale, but the change from one scale to the other is likely to introduce inaccuracies and this procedure is not recommended.

The correct setting for the initial *IR* drop may be estimated from a consideration of the type of conductance curve expected. For a titration in which the conductance remains approximately constant up to the end point, as in the titration of silver ion with chloride, the initial *IR* reading is set at approximately 600 mv., so that the *IR* drop after the end point occurs at the upper end of the scale. If the conductance rises throughout the titration, the initial voltage drop is set at 700 mv. For the titration of a strong acid with a strong base the initial *IR* drop is adjusted to about 250 mv. For other titrations in which the conductance decreases before the end point, a somewhat higher setting is used.

After the current has once been adjusted to give the required *IR* drop across the secondary electrodes, the variable resistances are left unchanged throughout the titration. The first *IR* reading is not recorded. The constant current is turned off for a minute and then turned on for a second *IR* reading, which is recorded. The titration is then performed in the usual manner by adding 1- or 2-ml. increments from a 10-ml. microburet. Each increment is stirred for about a minute. The pH meter is rezeroed if necessary, the constant current turned on, the *IR* drop read, and the current turned off. The constant current is left on as little as possible, both to save the batteries and to prevent battery polarization which would cause variation in the current. An average conductance reading requires about 5 seconds and an average titration about 15 minutes.

The reciprocals of the *IR* readings are obtained from a table and these values are corrected for volume and plotted against volume to give the usual conductance curves.

Unknown and titrant are made up using solvent at room temperature, but except for the use of asbestos squares under the conductance cell, there is no other temperature control during the titration.

EXPERIMENTAL

The following direct current conductance titrations were carried out: hydrochloric acid with sodium hydroxide, hydrochloric acid and acetic acid with sodium hydroxide, silver nitrate with sodium chloride, and perchloric acid with aniline in glacial acetic acid. Stock solutions of unknown and titrant were determined relative to each other by some nonconductometric procedure. These standard stock solutions were then diluted if necessary to give solutions of the normality desired for the conductometric titrations.

The conductometric titrations of hydrochloric acid with sodium hydroxide, hydrochloric acid and acetic acid with sodium hydroxide, and silver nitrate with sodium chloride have been explained by Britton (*2*) and are not elaborated on here. The stock solutions of 0.2 *N* hydrochloric acid and 0.2 *N* acetic acid were standardized potentiometrically against 0.2 *N* sodium hydroxide. The 0.2 *N* silver nitrate stock solution was standardized against dried sodium chloride using Mohr's method.

PERCHLORIC ACID WITH ANILINE

The 0.1 *N* perchloric acid was made by diluting 0.8 ml. of 70% perchloric acid to 100 ml. with glacial acetic acid, and a 0.1 *N* aniline solution was made by diluting 0.9 ml. of aniline to 100 ml. with glacial acetic acid. The relative standardization of these two solutions was obtained by titrating 8 ml. of the aniline solution with perchloric acid. Glacial acetic acid (75 ml.) was used as the supporting medium and 2 drops of a saturated chlorobenzene solution of methyl violet (*6*) was used as the indicator.

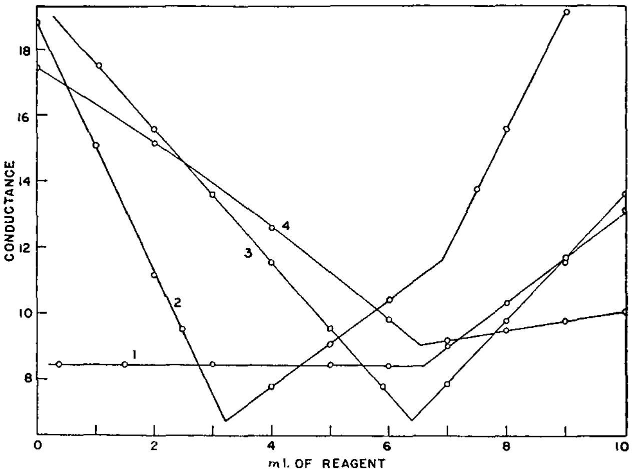


Figure 3. Typical Conductance Curves

1. 0.2668 mmole. $AgNO_3$ titrated with 0.04 *N* NaCl
2. 0.6461 mmole. HCl and 0.7402 mmole. HOAc with 0.2 *N* NaOH
3. 1.283 mmole. HCl with 0.2 *N* NaOH
4. 0.6547 mmole. $HClO_4$ with 0.1 *N* aniline in glacial HAc

Preliminary conductometric titrations of aniline with perchloric acid gave curves from which it appeared that the conductance change was caused largely by the water in the perchloric acid solution and no break was observed. This difficulty was overcome by reversing the procedure and titrating perchloric acid with aniline.

In order to exclude moisture partially the conductance cell was stoppered with a large cork through which a hole was bored for the microburet. After the stopper and buret had been put in place, the solution was stirred for several minutes before titrating to allow it to come to equilibrium with the atmosphere in the cell. The conductometric titration was then carried out in the usual manner. Because of the low conductance of glacial acetic acid solutions, an 18-megohm resistor, *R*6, was introduced into the constant current circuit.

Results of various titrations are given in Table I. Except for the silver nitrate solution, only the relative normalities of the stock solutions were known. For purposes of calculation therefore the 0.2 *N* sodium hydroxide solution was assigned a normality of 0.2000 and the 0.1 *N* aniline solution was assigned a normality of 0.1000.

Typical conductance curves obtained in this investigation are shown in Figure 3. The curves are the type that would be obtained in alternating current conductance titrations.

SPECIFIC CONDUCTANCE

As the conductance cell used does not have a defined volume, the cell constant may be expected to vary with volume of solution used. This effect was investigated by adding 0.02 *N* sodium chloride solutions from a buret into the conductance cell, and measuring the relative conductance as a function of total volume. The values obtained are plotted in Figure 4. The slope of the graph at a particular volume gives the accuracy with which the volume of solution must be known for a given accuracy in specific conductance. For example, at 100 ml., an inaccuracy of 0.5 ml. will cause a 1 part per 1000 inaccuracy in the measured conductance. This conductance change does not affect conductometric titrations, since over the range 100 to 110 ml., the change in conductance with change in volume is practically linear.

Table I. Analytical Results

Sample	Titrant	Mmoles Taken	Average Mmoles Found		No. of Detns.
0.04 *N* HCl	0.2 *N* NaOH	4.275	4.274	± 0.001	2
0.1 *N* HCl	0.2 *N* NaOH	1.283	1.285	± 0.002	3
0.001 *N* HCl	0.02 *N* NaOH	0.1283	0.1282	± 0.0002	3
0.006 *N* HCl	0.2 *N* NaOH	0.6461	0.6468	± 0.0030	3
0.007 *N* HAc		0.7402	0.7380	± 0.0066	3
0.015 *N* $AgNO_3$	0.2 *N* NaCl	1.442	1.444	± 0.003	3
0.0025 *N* $AgNO_3$	0.04 *N* NaCl	0.2668	0.2664	± 0.0002	4
0.0005 *N* $AgNO_3$	0.008 *N* NaCl	0.05352	0.05347	± 0.00004	3
0.007 *N* $HClO_4$	0.1 *N* aniline	0.6547	0.6529	± 0.0015	2

Table II. Effect of Concentration on Cell Constant

NaCl Molarity	Specific Conductance	*IR* (Soln.)	*IR* (Std.)	*R* (Std.)	Cell Constant
0.1	1.067 ± 10^{-2}	0.275	1.432	80.3	0.1645
0.05	5.555 ± 10^{-3}	0.528	1.426	80.3	0.1650
0.01	1.185 ± 10^{-3}	0.673	0.632	130.1	0.1642
0.005	6.032 ± 10^{-4}	1.028	0.489	130.1	0.1650
0.001	1.237 ± 10^{-4}	1.127	1.120	1298	0.1615
0.0005	6.225 ± 10^{-5}	1.218	0.625	1298	0.1575

The cell constant was measured with standard sodium chloride solutions over the range 0.1 to 0.0005 *N*. One-hundred-ml. samples of the sodium chloride solutions were brought to 25.0° C. in a constant temperature bath. The cell was rinsed with a sodium chloride solution of the molarity being measured and the 100-ml. sample transferred to the conductance cell. The *IR* drop across the secondary electrodes was measured within 8 seconds to minimize changes in temperature and the *IR* drop across a standard resistance was also determined. The cell constant was calculated from the formula

$$K = \frac{k\ (IR)\ \text{soln.}\ (R)\ \text{std.}}{(IR)\ \text{std.}}$$

where *k* is the specific alternating current conductance at 25° C. obtained by Shedlovsky (*9*).

The results are shown in Table II. Essentially the cell constant is unchanged over the concentration range 0.1 to 0.005 *M* sodium chloride.

ELECTROLYSIS

The inaccuracy introduced by electrolysis was investigated. Acid solutions will introduce the greatest error, because they have a high equivalent conductance and therefore require a large current for a given *IR* drop. With a 0.1 *N* hydrochloric acid solution the current amounts to 12 ma. for an *IR* drop of 0.5 volt. This current corresponds to the electrolysis of 1 part per 1000 in 1.4 minutes. Since during an average titration the current is on less than a minute, negligible decomposition of the solution occurs.

DISCUSSION

The direct current conductance method has certain advantages over the usual alternating current procedure. A pH meter is used to measure the resistance and the method would be useful when the specialized equipment required for alternating current conductometry is not available. The resistance measurements are made more conveniently, because the pH meter is a direct reading instrument. The electrolysis caused by the direct current has been shown to cause a negligible error.

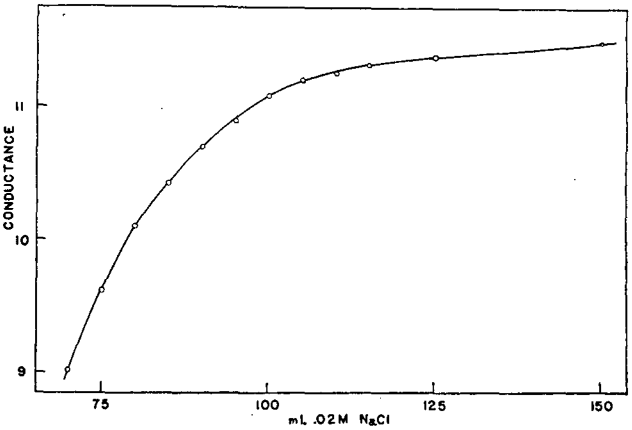


Figure 4. Volume of 0.02 *M* Sodium Chloride Solution *vs.* Conductance in Arbitrary Units

The cell described has the disadvantage of not being useful over the entire conductance range. At the upper limit are those solutions whose conductance corresponds to a 0.5 *N* sodium chloride solution. At this concentration the volume of gas evolved is sufficient to form a gas bubble at the primary electrodes, which results in fluctuating current. The upper limit may be extended by use of a low *IR* drop across the secondary electrodes, but this procedure would cause a decrease in accuracy. The limitation given above is not serious, as solutions titrated or measured conductometrically usually lie within the range over which the method is useful.

ACKNOWLEDGMENT

The work presented in this paper originated in a study of physical methods for following the course of extractions by observations on aqueous phases that were in contact with immiscible solvent phases. The work was supported by Contract AT(30-1)-937 Scope I of the U. S. Atomic Energy Commission to Princeton University.

LITERATURE CITED

(1) Andrews, L. V., and Martin, W. E., *J. Am. Chem. Soc.*, **60**, 871 (1938).
(2) Britton, H. T. S., "Conductometric Analysis," New York, D. Van Nostrand Co., 1934.
(3) Eastman, E. D., *J. Am. Chem. Soc.*, **42**, 1648 (1920).
(4) Gordon, A. R., *et al.*, *J. Chem. Phys.*, **11**, 18 (1943); **13**, 470, 473 (1945); **16**, 336 (1948); **19**, 752 (1951).
(5) Gunning, H. E., and Gordon, A. R., *Ibid*, **10**, 126 (1942).
(6) Keen, R. T., and Fritz, J. S., Anal. Chem., **24**, 564 (1952).
(7) Newbery, E., *J. Chem. Soc.*, **113**, 701 (1918).
(8) Palmer, R. F., and Scott, A. B., *J. Am. Chem. Soc.*, **72**, 482 (1950).
(9) Shedlovsky, T. S., Brown, A. S., and MacInnes, D. A., *Trans. Electrochem. Soc.*, **66**, 165 (1934).
(10) Siemens and Halske, A.-G., French Patent 819,625 (Oct. 22, 1937).

Received for review June 10, 1952. Accepted September 11, 1952.

Direct Determination of Aluminum Oxide in Portland Cement

A Chemical Method

C. L. FORD

Analytical Laboratories, Research and Development Division, Portland Cement Association, Chicago, Ill.

When an accurate determination of aluminum oxide in portland cement is required, present specification methods require four separate determinations, and then the estimation of aluminum oxide by difference. A direct and more rapid method giving comparable results is needed. A search of the literature and experimental work led to development of a procedure whereby aluminum was separated as the oxyquinolate from the usually interfering elements coprecipitated by ammonium hydroxide—iron, titanium, and phosphorus. Three methods were tested for determining as the oxide the aluminum content of the oxyquinoline precipitate. Two of these gave results comparable to and more rapid than the usual "by-difference" procedure. The new procedure provides producers and users with a method of directly determining with usual laboratory equipment the aluminum oxide content of portland cement with an accuracy comparable to that of the more laborious by-difference procedure.

IN THE usual analysis of portland cement, the aluminum oxide content is determined by substracting the separately determined amount of ferric oxide from the total amount of oxides precipitated by ammonium hydroxide, the difference being assumed to be entirely aluminum oxide. For most work this method is acceptable and provides a comparatively rapid way of determining the aluminum oxide.

However, phosphorus pentoxide and titanium dioxide are seldom absent from portland cement; thus the aluminum oxide values obtained are too high because these elements are precipitated with the ammonium hydroxide group (commonly referred to as R_2O_3). In the author's experience the positive error due to phosphorus pentoxide and titanium dioxide usually ranges from 0.25 to 0.40%, although at times it may be as high as 0.75%. Hence, when more accurate aluminum oxide values are needed for a cement sample to meet specification requirements or for other purposes, the interference caused by phosphorus pentoxide and titanium dioxide must be eliminated. This may be done by either determining these components separately and calculating the aluminum oxide content by difference, or determining the aluminum oxide content directly by procedures that eliminate the interfering components.

The present A.S.T.M. specification (*2*) for portland cement and those for air-entraining portland cement (*3*) contain a maximum limit for aluminum oxide and tricalcium aluminate for some types of cement. If a cement sample fails to meet these specifications when tested in accordance with the A.S.T.M. methods of test (*1*) phosphorus pentoxide must be determined separately and its value subtracted from the oxides precipitated by ammonium hydroxide. Any titanium dioxide that may be present is counted as aluminum oxide (*1*) Section 12. In some special cases, a similar correction may also be expressly specified for titanium dioxide. These separate procedures for phosphorus pentoxide and titanium dioxide are rather lengthy, and the final value thus obtained for aluminum oxide is subject to the summation of possible errors involved in all the determinations.

Therefore in those special cases where both phosphorus pentoxide and titanium dioxide are required merely as a means of obtaining a more accurate aluminum oxide value, a direct method for determining this value would be advantageous.

A polarographic method for the direct determination of aluminum oxide has been described by Ford and LeMar (*9*). Although the method shows considerable promise with respect to accuracy, it requires costly equipment and personnel with special training.

The purpose of the present paper is to describe an accurate and rapid direct chemical method which has been developed in these laboratories.

PRELIMINARY STUDIES

Because current A.S.T.M. methods already provide for the precipitation and separation of the ammonium hydroxide group, this study has been confined to a search for methods to separate aluminum oxide from the other constituents precipitated by ammonium hydroxide, which, in the case of portland cement, are those named above. Numerous procedures are described in the literature. Of these, at least two (*7, 12*) were developed specifically for cement and are described briefly below.

Chandler (*7*) proposed a method by which the cement is dissolved in dilute hydrochloric acid and the iron and titanium are separated by precipitation with cupferron. The aluminum in the filtrate is then precipitated with 8-quinolinol. The aluminum oxide content of the precipitate is determined either gravimetrically by weighing the dried aluminum oxyquinolate or by titration with a standard solution of potassium bromate and potassium bromide.

According to the second method developed by Kampf (*12*), silica is removed, then the aluminum and iron in the filtrate are precipitated together with 8-quinolinol. The precipitate, after being dissolved in acid, is titrated with a standard solution of potassium bromate-bromide, using methyl red as an internal indicator. The iron oxide content is determined on the same sample by titration with a standard sodium thiosulfate solution and the equivalent value is subtracted from the total potassium bromate-bromide titration. Comparative laboratory tests of Chandler's and Kampf's methods gave rather unsatisfactory results. They are discussed in more detail below.

Of all the other procedures studied, none was found that separated all the interfering elements in one operation; hence stepwise separations were studied. Methods for separating aluminum from iron have been based on (1) keeping the iron in solution while precipitating the aluminum, and (2) precipitating the iron and keeping the aluminum in solution.

Iron has been kept in solution by reduction to the ferrous state only (*5, 16, 21*) and by reduction to the ferrous state followed by the formation of soluble iron complexes (*13, 19, 20*). The author's results obtained by reducing iron to the ferrous state alone were unsatisfactory. Of the procedures utilizing the formation of iron complexes, only one, using thioglycolic acid as suggested by Welcher (*20*), was tried. The method was fairly successful, but the offensive odor of the reagent made a search for other methods desirable. Smith and Cagle's (*19*) procedure using 2,2'-bipyridine, and Kassner and Ozier's (*13*) procedure using potassium cyanide were not tested because of the high cost of 2,2'-bipyridine and the poisonous nature of potassium cyanide.

A number of methods for precipitating and separating iron (and in some cases titanium) from aluminum have been developed. Phenylhydrazine (*16*), urea and succinic acid (*5*, *21*), ammonium carbonate (*19*), cupferron (*7*, *16*), and sodium hydroxide (*6*, *11*, *15*) solutions have been used as precipitating agents. Cupferron and sodium hydroxide are more useful than the others because they remove titanium as well as iron. The procedure using cupferron has disadvantages in that the time-consuming process of destroying the excess of the cupferron reagent in the filtrate must be completed before the aluminum can be precipitated and the cupferron precipitate is often very bulky and may adsorb some of the aluminum, thus giving low results. The procedure using sodium hydroxide not only gives quantitative separations, but provides a solution which requires only neutralization before the separation of aluminum from phorphorus. Comparative studies on the two procedures in these laboratories indicated the latter method to be preferable.

The size of the sodium hydroxide precipitate depends on the amount of iron present in the sample analyzed. As all precipitates have a tendency to adsorb other substances, a study was made to determine the amount of aluminum retained in the sodium hydroxide precipitate. Bright and Fowler (*6*) in tests conducted on the separation of aluminum from iron and nickel by precipitation with sodium hydroxide, found that in a solution containing 0.0400 gram of aluminum, 0.040 gram of nickel, and 0.220 gram of iron, the loss of aluminum in the sodium hydroxide precipitate, after a single precipitation, was 0.0005 gram. In another solution containing 0.0400 gram of aluminum, 0.004 gram of nickel, 0.050 gram of chromium, and 0.240 gram of iron, the loss was 0.0004 gram. However, in each of their two solutions, the amount of iron present far exceeds the iron content of any cement sample used in this work, and furthermore, nickel is not present in cement. The authors findings, based on a study of the five cement samples used for testing, indicated the loss to be negligible.

According to the literature (*4*, *6*, *10*, *11*, *13–15*, *17*) quinolinol has been used extensively to precipitate aluminum quantitatively as the oxyquinolate after the separation of iron and titanium, leaving phosphorus in solution. The reaction takes place according to the following equation:

$$AlCl_3 + 3C_9H_6NOH \longrightarrow Al(C_9H_6NO)_3 + 3HCl \qquad (1)$$

Lundell and Knowles (*17*) have shown that after a single precipitation in a solution containing 0.05 gram of aluminum, none could be found in the filtrate. In another solution containing 0.05 gram of aluminum and 1.0 gram of phosphorus pentoxide, the precipitate contained less than 0.0002 gram of phosphorus pentoxide.

In establishing a suitable pH for the quantitative precipitation of aluminum, Knowles (*14*), basing his conclusion on the investigations of Fleck and Ward (*8*) and Goto (*10*), stated that the pH should never be less than 4.2 nor in excess of 7.0. In the author's work, described below, precipitation was conducted at a pH of approximately 5.0.

The following three procedures have been reported for determining the aluminum content of the oxyquinolate precipitate:

Method I. The precipitate is dried to constant weight at 120° to 140° C., cooled, and weighed, and the aluminum oxide content is calculated (*6*, *13*, *14*, *17*).

Method II. The precipitate is dissolved in hydrochloric acid and the solution thus obtained is titrated with an excess of a standard solution of potassium bromate–bromide (*6*, *14*, *17*). The aluminum oxyquinolate is quantitatively bromated to 5,7-dibromo-8-hydroxyquinoline according to the following equations:

$$Al(C_9H_6NO)_3 + 3HCl \longrightarrow AlCl_3 + 3C_9H_6NOH \qquad (2)$$

$$KBrO_3 + 5KBr + 6HCl \longrightarrow 3Br_2 + 6KCl + 3H_2O \qquad (3)$$

$$C_9H_6NOH + 2Br_2 \longrightarrow C_9H_4Br_2NOH + 2HBr \qquad (4)$$

Potassium iodide is added to the solution containing excess bromine liberated according to Equation 3 liberating free iodine:

$$2KI + Br_2 \longrightarrow 2KBr + I_2 \qquad (5)$$

The iodine liberated according to Equation 5, which is proportional to the excess potassium bromate-bromide solution, is then titrated with standard thiosulfate solution using starch as an indicator:

$$2Na_2S_2O_3 + I_2 \longrightarrow 2NaI + Na_2S_4O_6 \qquad (6)$$

Method III. The precipitate is decomposed with nitric and sulfuric acids, and the aluminum is precipitated as hydroxide, ignited to the oxide, and corrected for silica (*14*, *17*).

Knowles (*14*) has shown that if the amount of aluminum does not exceed 50 mg., good results may be obtained by weighing the dried aluminum oxyquinolate; that amounts greatly exceeding 25 mg. should not be titrated; and that amounts exceeding 50 mg. should be determined by weighing as the oxide.

The studies and preliminary tests discussed above led to the development of a general procedure for the separation of aluminum as the oxyquinolate and three possible methods of determining the aluminum content of the aluminum oxyquinolate. The detailed procedure is presented below.

PROCEDURE

PRELIMINARY SEPARATION

Section 1. Separate but do not weigh (unless desired) the silicon dioxide from a 0.500-gram sample of cement [*1*, Section 33(*a*) and (*b*)]. Volatilize the silicon dioxide thus obtained and recover the residue [*1*, Section 8(*d*)].

Precipitate the ammonium hydroxide group [*1*, Section 9(*a*) and (*b*)] and treat the precipitate according to [*1*, Section 9(*c*)]. Discard the filtrate (unless a determination of calcium oxide and magnesium oxide is desired).

SEPARATION OF ALUMINUM AS OXYQUINOLATE

Section 2. REAGENTS. Sodium Hydroxide Solution, 10%. Dissolve 100 grams of C.P. sodium hydroxide in distilled water and dilute to 1 liter.

Sodium Hydroxide Wash Solution, 5%. Dissolve 50 grams of C.P. sodium hydroxide and 5 grams of C.P. sodium sulfate in distilled water and dilute to 1 liter.

8-Quinolinol Solution, 2.5%. Treat 12.5 grams of 8-quinolinol with 25 ml. of C.P. glacial acetic acid and warm gently to effect solution. Pour the resulting solution into 450 ml. of distilled water at 60° C. Cool, filter if necessary, and dilute to 500 ml.

Ammonium Acetate Solution. Dissolve 100 grams of C.P. ammonium acetate in 100 ml. of distilled water.

PROCEDURE. Place the precipitate of the ammonium hydroxide group in a platinum crucible of approximately 40-ml. capacity. Dry and ignite the papers, first at a low heat until the carbon of the paper is completely consumed without inflaming, and finally at 1050° to 1100° C. for 10 minutes. Add 5 grams of fused C.P. potassium bisulfate to the crucible and heat below red heat until the residue is dissolved in the melt. (Start the heating slowly and with caution to prevent foaming and spattering.) Cool, and dissolve the fused mass in approximately 50 ml. of distilled water and 5 ml. of sulfuric acid (1 to 1) in a 250-ml. beaker. Nearly neutralize with the 10% sodium hydroxide solution (about 50 ml. is required), adjust the volume to about 100 ml., heat nearly to boiling, and pour slowly into 100 ml. of a hot sodium hydroxide solution (10%) as the latter is constantly stirred. Rinse out the beaker several times with small portions of hot water and add to the solution. Boil for 2 to 3 minutes, let settle on a steam bath for about 15 minutes, filter through an 11-cm. No. 41 Whatman paper (or equivalent) into a 600-ml. beaker, and wash the paper and precipitate several times with small portions of hot sodium hydroxide wash solution.

The combined filtrate and washings should have a volume of about 275 ml. Add hydrochloric acid (specific gravity 1.18) until the solution is just acid to methyl red. Heat the solution and add dilute ammonium hydroxide (1 to 1) until 1 drop just changes the color of the solution to yellow. At once add dilute hydrochloric acid (1 to 1), drop by drop, until the solution is again red and the precipitated aluminum hydroxide is just dissolved. (The presence of any aluminum hydroxide should be carefully checked at this point to be sure it is all in solution.) Cool the solution somewhat, then add 5 ml. of acetic acid (1 to 1) and 15 ml. of the 8-quinolinol solution. (One milliliter of the 8-quinolinol reagent suffices to precipitate 2.9 mg. of aluminum oxide. An excess of the reagent does no harm; in any case enough should be used to color the solution yellow.) Finally add, slowly and with stirring, 20 ml. of the ammonium acetate solution. Heat the solution to 60° to 70° C. and digest at this temperature for 5 minutes to facilitate crystallization and coagulation of the precipitate. Allow the precipitate to settle for 15 minutes, while cooling to room temperature. (The precipitate should be filtered within 1 hour. Prolonged standing may cause high results.)

Determine the aluminum oxide content of the precipitate by Section 4, 5, or 6.

DETERMINATION OF ALUMINUM OXIDE

Section 4. The precipitate is filtered, washed, dried, and weighed as anhydrous aluminum oxyquinolate, $Al(C_9H_6ON)_3$.

Procedure. Filter the precipitate, using moderate suction, through a weighed 30-ml. fritted-glass crucible of fine porosity. Wash the precipitate with warm ammonium hydroxide (1 to 40) until the washings are colorless, dry for 1.5 to 2 hours at 120° to 140° C., cool, and weigh as anhydrous aluminum oxyquinolate.

Blank. Make a blank determination following the same procedure and using the same amounts of reagents, and correct the results obtained in the analysis accordingly.

Calculation. Calculate the percentage of aluminum oxide to the nearest 0.01 as follows:

$$\%\ Al_2O_3 = W \times 22.198$$

where

W = weight of anhydrous $Al(C_9H_6ON)_3$

22.198 = molecular ratio of Al_2O_3 to $Al(C_9H_6ON)_3$ (0.11099) divided by sample weight (0.5) and multiplied by 100

Section 5. The precipitate is titrated, after solution in hydrochloric acid, with a standard solution of potassium bromate–potassium bromide.

Reagents. Standard Sodium Thiosulfate Solution, 0.35 *N*. Dissolve 88 grams of c.p. sodium thiosulfate ($Na_2S_2O_3.5H_2O$) in 300 ml. of recently distilled water, add 0.1 gram of c.p. sodium carbonate, and dilute to 1 liter. Standardize this solution against 0.1 *N* potassium dichromate prepared from National Bureau of Standards sample 136.

Standard Potassium Bromate–Potassium Bromide Solution, 0.35 *N*. Dissolve 9.743 grams of c.p. potassium bromate and approximately 34 grams of c.p. potassium bromide in 400 ml. of distilled water and dilute to 1 liter. Obtain the ratio of the strength of this solution to that of the standard sodium thiosulfate solution. Calculate the aluminum oxide value of the bromate-bromide solution and the bromate-bromide equivalent of the sodium thiosulfate solution. One milliliter of exactly 0.1 *N* bromate-bromide solution is equivalent to 0.0042485 gram of aluminum oxide.

Potassium Iodide Solution, 25%. Dissolve 25 grams of c.p. potassium iodide in 100 ml. of distilled water.

Starch Solution. To 500 ml. of boiling water add a cold suspension of 5 grams of soluble starch in 25 ml. of water. Cool, add a cool solution of 5 grams of c.p. sodium hydroxide in 50 ml. of water, then add 15 grams of c.p. potassium iodide and mix thoroughly.

Procedure. Filter the solution containing the oxyquinolate precipitate by suction through a 30-ml. fritted-glass crucible of fine porosity. Wash the beaker and precipitate with about 60 ml. of dilute ammonium hydroxide (1 to 99) heated to about 50° C. Place the crucible on a clean filter flask and pour 25 ml. of hot (75° C.) dilute hydrochloric acid (1 to 6) on the washed precipitate. Let the reaction proceed for a few minutes and stir occasionally with a small glass rod before applying suction. As soon as the precipitate has dissolved, apply suction and wash the crucible twice with 75-ml. portions of hot dilute hydrochloric acid (75° C.), then with 50 ml. of water. Dilute the acid solution to 250 ml., add 15 ml. of hydrochloric acid (specific gravity 1.18), and cool to room temperature (21° ± 4° C.). The hydrochloric acid content of the solution during the subsequent bromination should not be less than 8%. Add 25 ml. of the standard bromate-bromide solution from a buret or pipet. Stir the solution and allow to stand for 30 seconds to ensure complete bromination. Add 10 ml. of 25% potassium iodide solution. Stir the resulting solution well and then titrate very slowly with the standard sodium thiosulfate solution until the color of the iodine becomes faintly yellow. At this point add 2 ml. of the starch solution and titrate to the disappearance of the blue color.

Blank. Make a blank determination following the same procedure and using the same amounts of reagents and correct the results obtained in the analysis accordingly.

Calculation. Calculate the percentage of aluminum oxide to the nearest 0.01 as follows:

Correct the amount of bromate-bromide by subtracting from it the bromate-bromide equivalent of the sodium thiosulfate used.

$$\%\ Al_2O_3 = V \times F \times 200$$

where V = ml. of $KBrO_3$-KBr solution
F = grams of Al_2O_3 per ml. of KB_rO_3-KBr solution
200 = 100 divided by sample weight (0.5 gram)

Section 6. The precipitate is decomposed with sulfuric and nitric acids, and the aluminum is precipitated as hydroxide, ignited to the oxide, weighed, and corrected for silica.

Procedure. Filter through a Whatman No. 41, 11-cm. (or equivalent) filter paper into a 600-ml. beaker the solution containing the oxyquinolate precipitate and wash the precipitate with several small portions of hot ammonium hydroxide (1 to 40). Digest the paper and precipitate in an excess (about 30 ml.) of sulfuric acid (1 to 1), evaporate to fumes of that acid, and while fuming add successive 5-ml. portions of nitric acid (specific gravity 1.42) to destroy the bulk of the organic matter. Cool the solution slightly, add 10 ml. of nitric acid (specific gravity 1.42), and continue heating until the solution is colorless. Cool, dilute with water to about 200 ml., and boil until the anhydrous aluminum sulfate is completely dissolved. Cool the solution slightly, add a few drops of methyl red indicator, then treat slowly with freshly filtered ammonium hydroxide (1 to 1) until the color of the solution becomes yellow, and add 1 drop in excess. Bring the solution to boiling and boil for 10 to 15 seconds. Allow the precipitate to settle and filter. Wash four times with hot ammonium chloride solution (20 grams per liter). Place the paper and precipitate in a weighed platinum crucible. Dry and ignite the paper, first at low heat until the carbon of the paper is completely consumed without inflaming, and finally at 1050° to 1100° C. until the weight remains constant. The difference between this weight and the weight of the empty crucible represents the weight of aluminum oxide plus traces of silicon dioxide.

Add 5 grams of c.p. fused potassium bisulfate to the crucible and heat below red heat until the residue is dissolved in the melt. Cool, dissolve the fused mass in water containing 5 ml. of sulfuric acid (1 to 1), and evaporate the solution. Raise the temperature until copious fumes just begin to be evolved. Dissolve the mass in water, digest for 15 to 30 minutes at a temperature just below the boiling point, filter, and wash with hot water. Transfer the paper containing the residue to a platinum crucible, heat slowly until the paper is charred, and finally ignite to constant weight at 1050° to 1100° C. Treat the silicon dioxide thus obtained in the crucible with a drop of water, about 5 ml. of hydrofluoric acid (48%), and a drop of sulfuric acid (specific gravity 1.82), and evaporate cautiously to dryness. Finally, heat the crucible at 1050° to 1100° C. for 10 minutes, cool, and weigh. The difference between this weight and the first weight represents the amount of silicon dioxide. Subtract this weight from the weight of aluminum oxide obtained.

Blank. Make a blank determination following the same procedure and using the same amounts of reagents and correct the results obtained in the analysis accordingly.

Calculation. Calculate the percentage of aluminum oxide to the nearest 0.01 as follows:

$$\%\ Al_2O_3 = \frac{\text{weight of } Al_2O_3}{\text{weight of sample}} \times 100\ .$$

EXPERIMENTAL DATA AND DISCUSSION OF RESULTS

Determination of Aluminum Oxide in a Synthetic Solution. Preliminary tests were made on the reliability of the proposed method, using a synthetic solution containing a known content of aluminum. In order to simulate a solution of the oxides precipitated by ammonium hydroxide in the usual analysis of 0.5 gram of portland cement, known amounts of iron, phosphorus, and titanium were added to the solution.

Table I. Analysis of a Synthetic Solution

Methods used. R_2O_3, Fe_2O_3, P_2O_5, TiO_2, and Al_2O_3 by difference, current A.S.T.M. methods for portland cement.

Al_2O_3, direct methods.

- I. Weighed as aluminum oxyquinolate; Al_2O_3 calculated.
- II. Aluminum oxyquinolate titrated; Al_2O_3 calculated.
- III. Aluminum oxyquinolate dissolved; aluminum precipitated as $Al(OH)_3$; ignited and weighed as Al_2O_3.

All values are grams of oxides in 25 ml. of solution.

					Al_2O_3			
						Direct Methods		
Test No.	R_2O_3	Fe_2O_3	P_2O_5	TiO_2	By diff.	I	II	III
	Added							
	0.0550	0.0200	0.0030	0.0020	0.0300	0.0300	0.0300	0.0300
	Found							
1	0.0553	0.0200	0.0031	0.0020	0.0302	0.0300	0.0295	0.0298
2	0.0553	0.0201	0.0031	0.0020	0.0301	0.0297	0.0295	0.0301
3	0.0547	0.0200	0.0031	0.0020	0.0296	0.0295	0.0295	0.0297
4	0.0548	0.0200	0.0031	0.0020	0.0297	....	....	....
5	0.0553	0.0201	0.0031	0.0020	0.0301	....	....	....
Av.	0.0551	0.0200	0.0031	0.0020	0.0299	0.0297	0.0295	0.0299

Aluminum wire of 99.99% purity was used as a source of aluminum, iron wire of 99.89% purity for iron, C.P. potassium dihydrogen phosphate for phosphorus, and NBS sample 154 for titanium.

A 25-ml. aliquot contained 0.0300 gram of aluminum calculated as aluminum oxide, 0.0200 gram of iron as ferric oxide, 0.0030 gram of phosphorus as phosphorus pentoxide, and 0.0020 gram of titanium as titanium dioxide.

The solution was analyzed for "R_2O_3," ferric oxide, phosphorus pentoxide, and titanium dioxide. The value for aluminum oxide was calculated by difference. The analyses were made in accordance with the current A.S.T.M. procedures (*1*) for portland cement.

The results of the analysis of this solution are shown in Table I. The values for aluminum oxide calculated by difference show good agreement with the actual value. The greatest deviation from the actual value is 0.0004 gram, the smallest is 0.0001 gram.

The synthetic solution was then analyzed by the direct method, using all three ways for the determination of the aluminum oxide content of the oxyquinolate precipitate. These results also are given in Table I. A comparison of the values shows that (1) experimental results are in very good agreement with the calculated ones, (2) agreement between the three methods is good, and (3) when there is a difference between two results, the difference is within the limits permitted by the A.S.T.M. (*1*).

Determination of Aluminum Oxide in Portland Cements by A.S.T.M. Procedures. The value of the method for the analysis of a solution having been demonstrated, the practical applicability of the method was tested by determining the aluminum oxide contents of five different portland cements. The cements were selected for the following reasons:

Sample No.	Reason for Use in Tests
17596A	Laboratory reference sample, also identified as check sample 1
18169	High aluminum oxide content
LTS 23	High ferric oxide content
13895	High phosphorus pentoxide content
18277	High titanium dioxide content

The R_2O_3, ferric oxide, phosphorus pentoxide, titanium dioxide, and aluminum oxide were determined by the A.S.T.M. procedures (*1*). Silicon dioxide (present as an impurity in the R_2O_3) was determined and the amount present was subtracted from the total amount of R_2O_3. The analyses are shown in Table II. Inasmuch as the loss on ignition of a cement sample changes over a period of time—hence the apparent percentages of the various oxides—the values shown in this and succeeding tables have been calculated to the ignited (loss-free) basis. All these cements had been previously analyzed, four of them in these laboratories. The values shown are in good agreement with those previously obtained.

Determination of Aluminum Oxide in Portland Cements by Proposed Methods. Table III shows the aluminum oxide content of the five cements determined by the proposed procedure with its three different methods (described above) for determining the aluminum oxide content of the oxyquinolate precipitate. The differences from the by-difference values are also presented. As each method of estimating the percentage of the aluminum oxide, when used subsequent to the steps leading to the precipitation of aluminum oxyquinolate, constitutes an independent procedure, these methods are referred to in the discussion which follows as separate methods identified as I, II, and III.

Methods I and II were developed with the thought that they could be used as rapid or alternative methods. Accordingly, the experimental results shown in Table III were not corrected for blank determinations. Later work by other analysts showed that when corrected for blanks, Methods I and II were superior to Method III. In general, in spite of the lack of corrected values, good results were obtained by both Methods I and II. In the early stages of the work some high values were obtained—for example, cement 18169 gave values of 7.14 and 6.97% of aluminum oxide with differences of 0.62 and 0.45%, respectively, from the gravimetric value of 6.52%. As the experience of the analyst increased, better results were obtained. Such high results (all uncorrected for blanks) would be omitted from the calculation of averages, were it not that results, obtained by other analysts inexperienced in these methods, showed similar high results when not corrected for blanks. The average values obtained by Methods I and II, except the average including the high values noted above and one other, are all in good agreement with the by-difference values. Most of the individual values are within the A.S.T.M. (*1*) limits of 0.20% between duplicate gravimetric determinations.

Table II. Analysis of Portland Cements by Current A.S.T.M. Methods for Portland Cement

All values shown as percentages of ignited samples.
All values were corrected for blanks and are averages of 3 or more closely agreeing determinations.

Sample No.	R_2O_3	Fe_2O_3	P_2O_5	TiO_2	SiO_2	By Diff., Al_2O_3
17596A	9.16	2.81	0.08	0.30	0.12	5.85
18169	9.83	2.86	0.03	0.28	0.14	6.52
LTS 23	10.50	5.51	0.28	0.36	0.06	4.29
13895	8.49	2.69	0.89	0.37	0.10	4.44
18277	10.45	4.89	0.10	0.77	0.18	4.51

Most of the development work in this study was done with Method III using three of the five cements. Hence a considerable number of determinations are recorded for them. As it was thought at first that this was the most likely procedure for use as a "standard" or "referee" method, blank tests were made and the amounts of silica present as a contaminant were determined. For comparison purposes both corrected and uncorrected values are shown in Table III. Most of the values obtained by Method III, both uncorrected and corrected, are within the A.S.T.M. limit of 0.20%. Considering the average values, all except the uncorrected average value for cement 13895 are within the limit, and only one other varies by more than ±0.08% from the by-difference values. Although the results in this table show that the corrected values are not necessarily more accurate, tests by other analysts, discussed below, indicated that correction of the results obtained by Method III increased the accuracy.

Completeness of Separation of Aluminum from Iron, Phosphorus, and Titanium. A study was made to ascertain the amount of aluminum retained in the sodium hydroxide precipi-

Table III. Analysis of Portland Cement for Aluminum Oxide by Direct Methods by Analyst A

"Not Corr." values not corrected for blanks or SiO_2.
"Corr." values corrected for blanks by Methods I and II and for both blanks and SiO_2 by Method III.
"Diff." values are differences between direct method values and by-difference values.
All values calculated to "ignited" basis.
Values in parentheses not included in averages.

	Sample 17596A				Sample 18169				Sample LTS 23				Sample 13895				Sample 18277			
	Al_2O_3 Percentages by A.S.T.M. by-Difference Methods (Table II)																			
	5.85				6.52				4.29				4.44				4.51			
	Al_2O_3 Percentages by Direct Methods																			
Test No.	Not corr.	Diff.	Corr.	Diff.	Not corr.	Diff.	Corr.	Diff.	Not corr.	Diff.	Corr.	Diff.	Not corr.	Diff.	Corr.	Diff.	Not corr.	Diff.	Corr.	Diff.
	METHOD I																			
1	(6.35)	....	..		7.14	+0.62	..		4.22	−0.07	..		4.79	+0.35			(5.15)	..	..	
2	5.93	+0.08	..		6.97	+0.45	..		4.40	+0.11	..		4.67	+0.23			(5.11)	..	..	
3	5.93	+0.08	..		6.61	+0.09	..		4.31	+0.02	..		4.82	+0.38			4.50	−0.01	..	
4	5.92	+0.07	..		6.66	+0.14	..		4.21	−0.08	..		4.55	+0.11			4.59	+0.08	..	
5	6.04	+0.19	..		6.67	+0.15	..		4.36	+0.07	..		4.64	+0.20			4.72	+0.21	..	
6	5.93	+0.08	..		6.62	+0.10	..		4.31	+0.02	..		4.63	+0.19			4.69	+0.18	..	
7					6.57	+0.05	..		4.41	+0.12	..		4.49	+0.05			(5.97)	..	..	
8					6.60	+0.08	..		4.35	+0.06	..		4.45	+0.01			4.40	−0.11	..	
9													4.61	+0.17			4.50	−0.01	..	
10													4.46	+0.02						
Av.	5.95	+0.10			6.73	+0.21			4.33	+0.04			4.61	+0.17			4.57	+0.06		
	METHOD II																			
1	5.84	−0.01	..		6.40	−0.12	..		4.28	−0.01	..		4.60	+0.16			4.45	−0.06	..	
2	5.76	−0.09	..		6.28	−0.24	..		4.20	−0.09	..		4.43	−0.01			4.35	−0.16	..	
3	5.74	−0.11	..		6.38	−0.14	..		4.16	−0.13	..		4.32	−0.12			4.22	−0.29	..	
4	5.70	−0.15	..		6.35	−0.17	..		4.33	+0.04	..		4.44	0			4.44	−0.07	..	
5	5.85	..	..		6.55	+0.03	..		4.25	−0.04	..		4.37	−0.07			4.65	+0.14	..	
6	5.87	+0.02	..		6.44	−0.08	..		4.28	−0.01	..		4.54	+0.10			4.44	−0.07	..	
7					6.60	+0.08	..						4.45	−0.01						
8					6.60	+0.08	..						4.48	+0.04						
Av.	5.79	+0.06			6.45	−0.07			4.25	−0.04			4.45	+0.01			4.43	−0.08		
	METHOD III																			
1	6.04	+0.19	5.92	+0.07	6.71	+0.19	6.56	+0.04	(3.72)	..	(3.28)	..	4.75	+0.31	4.46	+0.02	4.37	−0.14	4.21	−0.30
2	5.90	+0.05	5.82	−0.03	6.75	+0.23	6.61	+0.09	4.53	+0.24	4.35	+0.06	4.75	+0.31	4.48	+0.04	4.31	−0.20	4.23	−0.28
3	6.02	+0.17	5.92	+0.07	6.75	+0.23	6.63	+0.11	4.07	−0.22	3.99	−0.30	4.63	+0.19	4.59	+0.15	(4.09)	..	(4.03)	..
4	5.90	+0.05	5.82	−0.03	6.75	+0.23	6.61	+0.09	4.43	+0.14	4.39	+0.10	(3.31)	..	..	..	4.39	−0.12	4.33	−0.18
5	5.92	+0.07	5.84	−0.01	6.79	+0.27	6.67	+0.15	4.39	+0.10	4.35	+0.06	(4.09)	..	(3.93)	..	4.19	−0.32	4.13	−0.38
6	6.04	+0.19	5.96	+0.11	6.79	+0.27	6.63	+0.11	4.01	−0.28	3.95	−0.34					4.29	−0.34	4.19	−0.32
7	6.00	+0.15	5.90	+0.10	6.85	+0.33	..										4.27	−0.24	..	
8	6.02	+0.17	5.88	+0.03	6.83	+0.31	..										4.43	−0.08	..	
9	5.98	+0.13	5.86	+0.01	6.61	+0.09	..										4.33	−0.18	..	
10	5.92	+0.07	5.80	−0.05	6.65	+0.13	..										4.45	−0.06	..	
11	5.92	+0.07	5.80	−0.05	6.40	−0.12	..										4.57	+0.06	..	
12	5.97	+0.12	5.64	−0.19	6.50	−0.02	..										4.57	+0.06	..	
13	(4.34)	..	(4.01)	..	6.30	−0.22	..										(3.85)	..	(3.39)	..
14	5.74	−0.11	5.56	−0.29	6.28	−0.24	(5.79)	..									(4.06)	..	..	
15	6.03	+0.18	5.84	−0.01	6.50	−0.02	6.44	−0.80									4.75	+0.24	4.71	+0.20
16	5.74	−0.11	5.54	−0.31	6.67	+0.12	6.61	+0.09									4.71	+0.20	..	
17					(6.12)	..	(6.08)										4.62	+0.11	4.52	+0.01
Av.	5.94	+0.09	5.80	−0.05	6.63	+0.09	6.60	+0.08	4.29	0	4.21	−0.08	4.71	+0.27	4.51	+0.07	4.44	−0.06	4.33	−0.18

Table IV. Impurities in Al_2O_3 Obtained by Decomposing Aluminum Oxyquinolate Precipitate, Precipitating Aluminum as $Al(OH)_3$, and Igniting to Al_2O_3 (Method III)

Methods used. SiO_2, P_2O_5, and TiO_2, current A.S.T.M. methods for portland cement.
Fe_2O_3, colorimetric method.
All values corrected for blanks.
All values percentages of ignited samples.

	Impurities in Al_2O_3				
Sample No.	SiO_2	Fe_2O_3	P_2O_5	TiO_2	Total
17596A	0.06	Faint	None	None	0.06
	0.06	Trace	None	None	0.06
	0.06	Trace	None	None	0.06
	Av. 0.06	Trace	None	None	0.06
18169	0.04	Faint	None	None	0.04
	0.04	Trace	None	None	0.04
	0.04	Trace	None	None	0.04
	Av. 0.04	Trace	None	None	0.04
LTS 23	0.06	Faint	None	None	0.06
	0.06	Trace	None	None	0.06
	0.06	Trace	None	None	0.06
	Av. 0.06	Trace	None	None	0.06
13895	0.14	Faint	None	None	0.14
	0.12	Trace	None	None	0.12
	0.08	Trace	None	None	0.08
	Av. 0.11	Trace	None	None	0.11
18277	0.14	Faint	None	Less than 0.01%	0.14
	0.14	Trace	None		0.14
	0.14	Trace	None		0.14
	Av. 0.14	Trace	None		0.14

tate. Confirming the findings of Bright and Fowler (*6*), the loss of aluminum in the precipitate was found to be insignificant. To estimate the aluminum, the precipitate was ignited in a platinum crucible until the paper was completely consumed, the residue was fused with a small amount of potassium bisulfate, the melt was dissolved in water, the iron and titanium were separated by precipitation with sodium hydroxide, and the filtrate was acidified, and, if necessary, concentrated to a smaller volume. Aluminum was determined on the filtrate by the colorimetric aurin tricarboxylic (aluminon) method (*18*). Although all five cements were tested in triplicate, none of the values for retained aluminum oxide was in excess of 0.1 mg.

Tests of the ignited aluminum oxide obtained by decomposing the aluminum oxyquinolate precipitate with acids, precipitating the aluminum as hydroxide, and igniting to the oxide, showed either the complete absence of iron, phosphorus, and titanium, or their presence in such minute quantities as to be wholly without effect on the determination. As shown in Table IV, of the impurities which may be present in the ignited aluminum oxide, only silicon dioxide was present in significant quantity. The fact that the sodium hydroxide method effected a good separation of iron and titanium from aluminum is evidenced by the presence of only a faint trace of iron and the complete absence of titanium in all except the sample which contains 0.77% titanium oxide. In this instance, the amount of titanium dioxide found in the ignited aluminum oxide is less than 0.01%. Phosphorus is absent, indicating the completeness of separation of aluminum from phosphorus by precipitation with 8-quinolinol.

Comparison of Results Obtained by Proposed and Other Direct Methods. Chandler's (*7*) and Kampf's (*12*) procedures for the direct determination of aluminum were referred to above as being unsatisfactory. This conclusion is based on data presented in Table V. The comparative tests were made on three of the five cements used in this study. Aluminum oxide values obtained by difference and by the proposed procedures are also shown in the table. Chandler's methods yielded low results. This confirms tests made some years ago by another analyst in these laboratories. The low results may be due to adsorption of a part of the aluminum by the bulky cupferron precipitate. Results by Kampf's method were much too high even after correction for ferric oxide as outlined in his procedure. The reasons for this are not entirely clear, although possible explanations are (1) the uncertainty of the end point due to gradual oxidation of the indicator, and (2) the presence of titanium, an interfering element, in the precipitate. It is also possible that some necessary details of the procedure that Kampf used in obtaining his excellent results were omitted from the published procedure.

Comparison of Results Obtained by Different Analysts Using Proposed Methods. To ascertain the reproducibility of results obtained by the direct method, the five cements were analyzed by three other analysts using the same three methods for determining the aluminum oxide content of the oxyquinolate precipitate. The comparative results, including average values obtained by the four analysts, are shown in Table VI. The individual values obtained by analyst A are the ones that appeared in Table III and are not repeated in Table VI. All values, however, were included in studying the data.

Table VI shows that in general the analysts, within tolerance limits, were able to reproduce their own results as shown by the individual values. Reproducibility was better with the corrected than with the uncorrected values. The accuracy of their corrected average results was good, as shown by the differences from the by-differences values. The uncorrected results were inaccurate in a number of instances.

Comparison of Proposed Methods. It appeared from a study of Table VI that the best estimate of the worth of the methods could be made by considering the results obtained by all analysts rather than only those of analyst A, who developed them. In the following discussion all the results for a given method and sample are considered as though obtained by one person.

Table V. Comparison of Aluminum Oxide Values Obtained by Proposed and Other Direct Methods

Methods used. Proposed Methods I, II, and III.
Chandler's gravimetric method (not corrected for blanks).
Chandler's volumetric method (not corrected for blanks).
Kampf's volumetric method (not corrected for blanks).
All values are percentages of Al_2O_3 calculated to ignited basis.

		Sample		
Method	Test	17596A	13895	18277
Grav. by difference	Av.	5.85	4.44	4.51
Proposed No. I (Table I)	Av. (not corr.)	5.95	4.61	4.57
Proposed No. II (Table I)	Av. (not corr.)	5.79	4.45	4.43
Proposed No. III (Table I)	Av. (corr.)	5.80	4.51	4.33
Chandler's gravimetric	1	5.18	4.02	4.04
	2	5.17	4.18	4.12
	3	5.27	4.16	4.10
	4	..	3.99	..
	Av.	5.21	4.09	4.09
Chandler's volumetric	1	5.08	4.04	4.10
	2	5.12	4.07	4.10
	3	5.12	4.10	..
	Av.	5.11	4.07	4.10
Kampf's volumetric	1	7.02	6.04	7.05
	2	7.02	6.04	7.10
	3	6.93		
	4	6.96		
	Av.	6.98	6.04	7.08

One estimate of the accuracy of the methods may be obtained from the differences from the by-difference aluminum oxide values. The number of determinations for each cement and the average differences are shown in Table VII. The by-difference values obtained by A.S.T.M. methods were subject to cumulative errors. However, because such values at present are considered as sufficiently accurate for acceptance or referee tests, the by-difference values are assumed to be correct in the following evaluations of the proposed methods. The weighted average differences appearing in Table VII show that the corrected results for Methods I and III are better than the corresponding uncorrected results, and that both the uncorrected and corrected average differences for Method II are low, therefore very good.

A better estimate of relative accuracy may be obtained by calculating standard errors with respect to the by-difference values. Such values, also shown in Table VII, indicate that the relative

Table VI. Analysis of Portland Cements for Aluminum Oxide by Direct Methods by Four Analysts

Average analyses by Analyst A (Table III) are included for reference. His individual values, although not shown in this table, were used in computing averages of all, differences and maximum and minimum values.
"Not Corr." values not corrected for blanks or SiO_2.
"Corr." values corrected for blanks by Methods I and II and for both blanks and SiO_2 by Method III.
"Diff." values difference between direct method values and by-difference values.
All values calculated to "ignited" basis.
Values in parentheses not included in averages.

Analyst	Test	Sample 17596A				Sample 18169				Sample LTS 23				Sample 13895				Sample 18277			
		Al_2O_3 Percentages by A.S.T.M. by-Difference Methods (Table II)																			
		5.85				6.52				4.29				4.44				4.51			
		Al_2O_3 Percentage by Direct Methods																			
		Not corr.	Diff.	Corr.	Diff.	Not corr.	Diff.	Corr.	Diff.	Not corr.	Diff.	Corr.	Diff.	Not corr.	Diff.	Corr.	Diff.	Not corr.	Diff.	Corr.	Diff.
METHOD I																					
A	Av.	5.95	+0.10	..	..	6.73	+0.21	..	..	4.33	+0.04	..	..	4.61	+0.17	..	..	4.57	+0.06	..	..
B	1	5.93	+0.08	5.80	−0.05	6.61	+0.09	6.47	−0.05	4.41	+0.12	4.27	−0.02	4.70	+0.26	4.56	+0.12	4.55	+0.04	4.42	−0.09
	2	5.86	+0.01	5.74	−0.11	6.56	+0.04	6.43	−0.09	4.47	+0.18	4.25	−0.04	4.55	+0.11	4.52	+0.08	4.62	+0.11	4.48	−0.03
	3	6.00	+0.15	5.86	+0.01	6.65	+0.13	6.51	−0.01	4.48	+0.19	4.35	+0.06	4.80	+0.36	4.67	+0.23	4.67	+0.16	4.54	+0.03
	Av.	5.93	+0.08	5.80	−0.05	6.61	+0.09	6.47	−0.05	4.46	+0.17	4.29	0.0	4.68	+0.24	4.58	+0.14	4.61	+0.10	4.48	−0.03
C	1	6.22	+0.37	5.90	+0.05	(6.54)	..	(6.24)	..	4.70	+0.41	4.40	+0.11	4.98	+0.54	4.67	+0.23	4.72	+0.21	4.41	−0.10
	2	6.28	+0.43	5.99	+0.14	6.91	+0.39	6.63	+0.11	4.80	+0.51	4.52	+0.23	5.02	+0.58	4.73	+0.29	4.80	+0.29	4.51	0.0
	3	6.11	+0.26	5.86	+0.01	6.79	+0.27	6.54	+0.02	4.56	+0.27	4.32	+0.03	4.88	+0.44	4.63	+0.19				
	4	6.16	+0.31	5.81	−0.04	7.02	+0.50	6.77	+0.25					4.96	+0.52	4.71	+0.27				
	5					6.87	+0.35	6.62	+0.10					17							
	Av.	6.19	+0.34	5.92	+0.07	6.90	+0.38	6.64	+0.12	4.69	+0.40	4.41	+0.12	4.96	+0.52	4.69	+0.27	4.76	+0.25	4.46	−0.05
	No. of detns.	12		7		15		7		14		6		17		7		11		5	
	Av. of all	6.03	+0.18	5.87	+0.02	6.75	+0.23	6.57	+0.05	4.44	+0.15	4.35	+0.06	4.70	+0.26	4.64	+0.20	4.61	+0.10	4.47	−0.04
	Maximum value	6.28	+0.43	5.99	+0.14	7.14	+0.62	6.77	+0.25	4.80	+0.51	4.52	+0.23	5.02	+0.58	4.73	+0.29	4.80	+0.29	4.54	+0.03
	Minimum value	5.86	+0.01	5.74	−0.11	6.56	+0.04	6.43	−0.09	4.21	−0.08	4.25	−0.04	4.45	+0.01	4.52	+0.08	4.40	−0.11	4.41	−0.10
	Standard error		0.22		0.07		0.29		0.12		0.21		0.11		0.32		0.21		0.15		0.06
METHOD II																					
A	Av.	5.79	−0.06	..	..	6.45	−0.07	..	..	4.25	−0.04	..	..	4.45	+0.01	..	..	4.43	−0.08	..	..
B	1	5.78	−0.07	5.61	−0.24	6.44	−0.08	6.26	−0.26	4.35	+0.06	4.17	−0.12								
	2	5.75	−0.10	5.57	−0.28	6.44	−0.08	6.26	−0.26	4.32	+0.03	4.15	−0.14	4.60	+0.16	4.42	−0.02	4.42	−0.09	4.25	−0.26
	3	5.88	+0.03	5.72	−0.13	6.49	−0.03	6.33	−0.19	4.40	+0.11	4.23	−0.06	4.58	+0.14	4.40	−0.04	4.58	+0.07	4.38	−0.13
	Av.	5.80	−0.05	5.63	−0.22	6.46	−0.06	6.28	−0.24	4.36	+0.07	4.18	−0.11	4.71	+0.27	4.53	+0.09	4.58	+0.07	4.42	−0.09
C	1	5.97	+0.12	5.74	−0.11	6.63	+0.09	6.40	−0.12	(4.38)	..	(4.10)	..	4.63	+0.19	4.45	+0.01	4.53	+0.02	4.35	−0.16
	2	(6.38)	..	(6.01)	..	6.77	+0.25	6.40	−0.12	4.58	+0.29	4.36	+0.07	4.88	+0.44	4.59	+0.15	4.87	+0.36	4.49	−0.02
	3	(5.70)	..	(5.40)	..	(6.32)	..	(6.03)	..	4.66	+0.37	4.40	+0.11	4.79	+0.35	4.57	+0.13	4.74	+0.23	4.45	−0.06
	4	6.01	+0.16	5.78	−0.07	6.69	+0.17	6.43	−0.09	4.62	+0.33	4.36	+0.07	4.86	+0.42	4.59	+0.15	4.64	+0.13	4.41	−0.10
	5	6.07	+0.22	5.80	−0.05																
	Av.	6.02	+0.17	5.77	−0.12	6.67	+0.15	6.42	−0.10	4.62	+0.33	4.37	+0.08	4.84	+0.40	4.58	+0.14	4.75	+0.24	4.45	−0.06
	No. of detns.	12		6		14		6		12		6		14		6		12		6	
	Av. of all	5.85	0.0	5.70	−0.15	6.50	−0.02	6.35	−0.17	4.37	+0.08	4.28	−0.01	4.57	+0.13	4.52	+0.08	4.54	+0.03	4.40	−0.11
	Maximum value	6.07	+0.22	5.80	−0.05	6.77	+0.25	6.43	−0.09	4.66	+0.37	4.40	+0.11	4.88	+0.44	4.59	+0.15	4.87	+0.36	4.49	−0.02
	Minimum value	5.70	−0.15	5.57	−0.28	6.28	−0.24	6.26	−0.26	4.16	−0.13	4.15	−0.14	4.32	−0.12	4.40	−0.04	4.22	−0.29	4.25	−0.26
	Standard error		0.11		0.17		0.13		0.19		0.18		0.10		0.22		0.11		0.17		0.13
METHOD III																					
A	Av.	5.94	+0.09	5.80	−0.05	6.63	+0.09	6.60	+0.08	4.29	0.0	4.21	−0.08	4.71	+0.27	4.51	+0.07	4.44	−0.07	4.33	−0.18
B	1	5.81	−0.04	5.67	−0.18	6.47	−0.05	6.29	−0.23	4.45	+0.16	4.25	−0.04	4.55	+0.11	4.41	−0.03	4.47	−0.04	4.33	−0.18
	2	6.10	+0.25	5.81	−0.04	(7.19)	..	6.23	−0.29	4.39	+0.10	4.19	−0.10	4.55	+0.11	4.39	−0.05	4.47	−0.04	4.35	−0.16
	3	6.12	+0.27	5.93	+0.08	6.53	+0.01	6.37	−0.15	4.65	+0.36	4.46	+0.17	4.66	+0.22	4.46	+0.02	4.51	0.0	4.37	−0.14
	Av.	6.01	+0.16	5.80	−0.05	6.50	−0.02	6.30	−0.22	4.50	+0.21	4.30	+0.01	4.59	+0.15	4.42	−0.02	4.48	−0.03	4.35	−0.16
C	1	6.30	+0.45	5.95	+0.10	7.07	+0.55	6.68	+0.16	4.78	+0.49	4.38	+0.09	5.00	+0.56	4.65	+0.21	5.14	+0.63	4.74	+0.23
	2	6.28	+0.43	5.89	+0.05	6.99	+0.47	6.60	+0.08	4.64	+0.35	4.28	−0.01	5.00	+0.56	4.61	+0.17	4.91	+0.40	4.53	+0.02
	3	6.08	+0.23	5.71	−0.14	6.91	+0.39	6.54	+0.02	4.72	+0.43	4.36	+0.07	5.23	+0.79	4.86	+0.42	4.93	+0.42	4.53	+0.02
	4	6.02	+0.17	5.67	−0.18	6.97	+0.45	6.62	+0.10	4.52	+0.23	4.15	−0.14	5.15	+0.71	4.80	+0.36				
	Av.	6.17	+0.32	5.81	−0.04	6.99	+0.47	6.61	+0.09	4.67	+0.38	4.29	0.0	5.10	+0.66	4.73	+0.29	4.99	+0.48	4.60	+0.09
D	1	6.13	+0.28	5.82	−0.03	(6.28)	..	(6.05)	..	4.65	+0.36	4.39	+0.10	4.79	+0.35	4.40	−0.04	4.98	+0.47	4.62	+0.11
	2	6.11	+0.26	5.84	−0.01	6.83	+0.31	6.61	+0.09	4.57	+0.28	4.43	+0.14	4.83	+0.39	4.61	+0.17	4.61	+0.10	4.22	−0.29
	3	6.01	+0.16	5.70	−0.15	6.63	+0.11	6.26	−0.26	(4.33)	..	(4.01)	..	5.10	+0.66	4.69	+0.25	4.73	+0.22	4.36	−0.15
	4					6.75	+0.23	6.40	−0.12					5.10	+0.66	4.81	+0.37	(4.40)	..	(4.02)	..
	Av.	6.08	+0.23	5.79	−0.06	6.74	+0.22	6.42	−0.10	4.61	+0.32	4.41	+0.12	4.96	+0.52	4.63	+0.19	4.77	+0.26	4.40	−0.11
	No. of detns.	25		25		25		18		14		14		14		14		23		16	
	Av. of all	6.00	+0.15	5.80	−0.05	6.69	+0.17	6.52	0.0	4.49	+0.19	4.28	−0.01	4.87	+0.43	4.59	+0.15	4.56	+0.05	4.40	−0.11
	Maximum value	6.30	+0.45	5.96	+0.11	7.07	+0.55	6.68	+0.16	4.78	+0.49	4.43	+0.14	5.23	+0.79	4.86	+0.42	5.14	+0.63	4.74	+0.23
	Minimum value	5.74	−0.11	5.54	−0.31	6.28	−0.24	6.23	−0.29	4.01	−0.28	3.95	−0.34	4.55	+0.11	4.39	−0.05	4.17	−0.34	4.13	−0.38
	Standard error		0.20		0.12		0.26		0.14		0.29		0.15		0.48		0.21		0.26		0.21

Standard error = $\sqrt{\frac{\Sigma d^2}{n}}$, where d = difference from by-difference values and n = No. of determinations.

Table VII. Summary of Mathematical Data

Cement	Method I Not corr.	Method I Corr.	Method II Not corr.	Method II Corr.	Method III Not corr.	Method III Corr.
	Number of Determinations					
17596A	12	7	12	6	25	25
18169	15	7	14	6	25	18
LTS 23	14	6	12	6	14	14
13895	17	7	14	6	14	14
18277	11	5	12	6	23	16
Total	69	32	64	30	101	87
	Differences, Averages for Four Analysts, %					
17596A	+0.18	+0.02	+0.0	−0.15	+0.15	−0.05
18169	+0.23	+0.05	+0.02	−0.17	+0.17	0.0
LTS 23	+0.15	+0.06	+0.08	−0.01	+0.19	−0.01
13895	+0.26	+0.20	+0.13	+0.08	+0.43	+0.15
18277	+0.10	−0.04	+0.03	−0.11	+0.05	−0.13
Weighted av.	+0.19	+0.06	+0.04	−0.07	+0.18	−0.02
	Standard[a] and Probable[b] Errors for Four Analysts with Respect to by-Difference Values, %					
17596A	0.22	0.07	0.11	0.17	0.20	0.12
18169	0.29	0.12	0.13	0.19	0.26	0.14
LTS 23	0.21	0.11	0.18	0.10	0.29	0.15
13895	0.32	0.21	0.22	0.11	0.48	0.21
18277	0.15	0.06	0.17	0.13	0.26	0.21
S.E. for all	0.26	0.13	0.17	0.16	0.29	0.17
P.E. for all	0.17	0.08	0.11	0.10	0.20	0.11
	Probable Errors[c] of Four Analysts with Respect to Average Direct Values, %					
17596A	0.09	0.05	0.08	0.06	0.09	0.08
18169	0.13	0.08	0.09	0.06	0.14	0.10
LTS 23	0.12	0.07	0.11	0.06	0.15	0.11
13895	0.12	0.05	0.11	0.06	0.16	0.11
18277	0.08	0.04	0.12	0.06	0.17	0.13
P.E. for all	0.11	0.06	0.10	0.06	0.14	0.10

[a] Standard error $= \sqrt{\frac{\Sigma d^2}{n}}$, where d = difference from by-difference values; n = no. of determinations.

[b] Probable error with respect to by-difference values = 0.6745 times standard error.

[c] Probable error with respect to average

$$= 0.6745 \sqrt{\frac{\Sigma x^2 - (n_1m_1^2 - n_5m_5^2)}{(n_1 - n_5) - 5}}$$

where x = individual values
n_1, etc. = number of determinations for each cement
m_1, etc. = average of n_1, etc., determinations.

accuracy of all methods, corrected, is good. It will be seen that for all methods the errors for the corrected values, with two exceptions, are less than for the corresponding uncorrected values. Considering the standard errors for all cements, Method II gave the lowest errors for uncorrected values.

The probable error values with respect to average direct method values, also shown in Table VII, show the precision of all the methods to be good. Results by Methods I and II for all determinations, corrected, show the lowest probable errors—namely, 0.06% in each case. The highest probable error for all determinations was 0.14% by Method III, uncorrected.

In considering the worth of the methods, the time requirements should be considered also. From a time standpoint, either Method I or II is preferable to Method III. Even Method III is faster than the A.S.T.M. by-difference method (*1*). Method II is the most rapid for one or two samples, provided all solutions have been prepared previously. For occasional tests, however, Method I is the most rapid. It is also the simplest for the analyst. On the other hand, Method II is the least affected by possible impurities in the oxyquinolate precipitate. This was shown in the mathematical studies discussed above, in which it was brought out that results by this procedure were good even when uncorrected for blanks.

SUMMARY

A chemical method for the direct determination of aluminum oxide in portland cement has been developed. By using the precipitate of the ammonium hydroxide group for the determination, the method can be incorporated as a part of the A.S.T.M. procedures for analysis of portland cement. After the separation of aluminum as oxyquinolate, the aluminum oxide content of the precipitate may be determined in any one of three ways.

Method I. The aluminum oxyquinolate precipitate is dried and weighed and the aluminum oxide content calculated.

Method II. The aluminum oxide content of the precipitate is determined volumetrically.

Method III. The precipitate is subjected to wet oxidation of the organic matter and aluminum is separated as the hydroxide and ignited and weighed as the oxide.

A synthetic solution containing aluminum oxide, ferric oxide, titanium dioxide, and phosphorus pentoxide was analyzed by each method with good results. Five commercial portland cements were analyzed by each method. Studies of various precipitates and filtrates showed that interference by other elements of the ammonium hydroxide group was eliminated.

Comparative tests showed the procedure to be superior to two other published methods. Reproducibility of results by other analysts was good, provided corrections were made for blank determinations.

A mathematical study of the data showed that accuracy and precision of all methods were good when corrections were made for blank determinations, but that Methods I and II were more accurate and precise than Method III. Method II is the least subject to errors due to possible contamination of the oxyquinolate precipitate.

On a time basis, Method I is the fastest for an occasional sample and Method II is the fastest as a routine procedure. In cases where it is required that the aluminum oxide values be corrected for phosphorus pentoxide and titanium dioxide, the direct methods are more rapid than the current A.S.T.M. (*1*) procedures.

ACKNOWLEDGMENT

W. S. Lui, formerly associate research chemist, Research and Development Division, Portland Cement Association, was responsible for a large share of the early work on the developments reported in this paper. Acknowledgment of his contributions is herewith extended.

LITERATURE CITED

(1) Am. Soc. Testing Materials, "Standards on Cement," Designation C 114-47.
(2) *Ibid.*, C 150-49.
(3) *Ibid.*, C 175-48T.
(4) Berg, R., *Z. anal. Chem.*, **70**, 341 (1927).
(5) Boyle, A. J., and Musser, D. F., IND. ENG. CHEM., ANAL. ED., **15**, 621 (1943).
(6) Bright, H. A., and Fowler, R. M., *J. Research Natl. Bur. Standards*, **10**, 330 (1933).
(7) Chandler, W. R., *Rock Products*, **39**, (8), 52 (1936).
(8) Fleck, H. R., and Ward, A. M., *Analyst*, **58**, 388 (1933).
(9) Ford, C. L., and LeMar, L., *ASTM Bull.*, No. **157**, 66 (March 1949).
(10) Goto, H., *J. Chem. Soc. Japan*, **54**, 725 (1933).
(11) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis," p. 76, New York, John Wiley & Sons, 1929.
(12) Kampf, Leo, IND. ENG. CHEM., ANAL. ED., **13**, 72 (1941).
(13) Kassner, J. L., and Ozier, M. A., *J. Am. Ceramic Soc.*, **33**, (8) 250-2 (1950).
(14) Knowles, H. B., *J. Research Natl. Bur. Standards*, **15**, 90 (1935).
(15) Kolthoff, I. M., Stenger, V. A., and Moskovitz, B., *J. Am. Chem. Soc.*, **56**, 812 (1934).
(16) Lundell, G. E. F., Hoffman, J. I., and Bright, H. A., "Chemical Analysis of Iron and Steel," 4th ed., p. 78, New York, John Wiley & Sons, 1946.
(17) Lundell, G. E. F., and Knowles, H. B., *J. Research Natl. Bur. Standards*, **3**, 92 (1929).
(18) Roller, P. S., *J. Am. Chem. Soc.*, **55**, 2437-8 (1933).
(19) Smith, G. F., and Cagle, F. W., IND. ENG. CHEM., ANAL. ED., **20**, 574 (1948).
(20) Welcher, F. J., "Organic Analytical Reagents," Vol. IV, p. 159, New York, D. Van Nostrand Co., 1948.
(21) Willard, H. H., and Tang, N. K., IND. ENG. CHEM., ANAL. ED., **9**, 357 (1937).

RECEIVED for review May 12, 1952. Accepted September 20, 1952.

Determination of the Carboxyl Content of Oxidized Starches

M. F. MATTISSON[1] AND K. A. LEGENDRE, *Clinton Foods Inc., Clinton, Iowa*

The determination of the carboxyl content of commercial oxidized starches has long been used to estimate the degree of oxidation. Existing methods are based on similar procedures for cellulose. The usual method requires the starch to be leached with hydrochloric acid to remove any existing cations, followed by exhaustive washing to remove the hydrochloric acid. The starch is then treated with calcium acetate solution, which unites calcium to carboxyl, freeing acetic acid which is determined by titration. A new method is proposed wherein the starch is pasted after the acid leaching and washing and the liberated carboxyl groups are titrated directly. This procedure eliminates the involved calcium acetate treatment of the older method, and is simple, shorter, and more precise.

COMMERCIAL oxidized cornstarches are widely used in papermaking, where their specific properties are used to close the pores of the paper to lay fuzz on the surface, to increase tensile fold and bursting strength, and to give a "feel" and rattle to the paper. The degree of oxidation is relatively small when compared to the periodate, chromic anhydride, and nitrogen dioxide starches frequently described in scientific research (*3*). Oxidized starches resemble the parent starch in that they retain essentially the original granule structure, preserve the typical polarization crosses, and show the typical blue staining with iodine. However, oxidized starches differ from the parent starch in that they require a shorter cooking time to produce a paste, and give a higher fluidity, increased adhesiveness, lower rate of congealing, and greater translucency of the pastes.

Commercial oxidized starches are usually produced by the treatment of an 18° Bé. starch slurry with a solution of sodium hypochlorite (7.5 to 9.0% chlorine). The degree of oxidation is, in general, controlled by fluidity determinations. After the desired degree of oxidation has been reached, the residual hypochlorite is eliminated by the addition of sodium bisulfite. The slurry is then acidified to a pH of 6.5, washed, filtered, and dried. The resulting products are less colored than the parent starch or the acid-modified starches.

Apparently, the free caustic of the sodium hypochlorite has neutralized most carboxyl groups formed during the oxidizing process; however, the subsequent acidification to pH 6.5 produces a small variable amount of acidity in the dry starch, and the washing introduces calcium cations, thereby transforming some sodium salts of carboxyl groups into the calcium salts. The acidity of the finished oxidized starches may result from one or more of the following causes:

1. Residual mineral acid or sulfur dioxide retained in the starch granules.
2. Lactic or amino acids retained by the starch from the steeping process.
3. Fatty acids contained in the extraneous fat adsorbed on the starch.
4. Carboxyl groups attached to anhydroglucose units of the starch molecule and freed from their cation content by the acidification of the starch slurry to pH 6.5.

The determination of this acidity is essential.

Since most of the carboxyl groups are not liberated from their salts by the acidification of the slurry to pH 6.5, all methods proposed for the determination of the carboxyl content of oxidized starch require a preliminary de-ashing treatment,—i.e., the starch is treated with diluted hydrochloric acid with subsequent removal of the excess hydrochloric acid with distilled water. The copious washing required removes not only cations, but also some of the sulfur dioxide, some of the organic acids and, undoubtedly, some of the water-soluble starch fractions of low degree of polymerization and therewith some carboxyl groups, for the solubility of oxidized starches increases with increasing degree of oxidation. The acidimetric value of the cation-freed starch as determined by various procedures has been used as a measure of the carboxyl content. This value should be corrected for the acidity of the original starch which has not been subjected to the de-ashing pretreatment but only washed with distilled water to remove the water-soluble acidic substances.

This correction is deemed advisable, especially in the case of the lower oxidized starches, for here the correction is of considerable magnitude and may influence the characterization of these starches.

These considerations have prompted an investigation of possible simplification and improvement of existing methods for carboxyl analysis, because a rapid and precise method for the determination of the carboxyl content of oxidized starches would aid starch chemists in identification and manufacturing control of these products.

SELECTION OF METHODS

The extensive literature on the determination of the carboxyl content of polysaccharides pertains almost exclusively to the carboxyl content of celluloses and oxidized celluloses. Of the various methods proposed, the calcium acetate method appeared to be the best for oxidized starches because it seemed the simplest and least time-consuming procedure. Lüdtke (*4*) was the originator of this method of cation exchange of carboxylic acid groups with calcium acetate and titration of the liberated acetic acid. He used 0.5% hydrochloric acid and carbon dioxide–free distilled water in the de-ashing pretreatment and 0.01 *N* sodium hydroxide for titrating at room temperature to the phenolphthalein end point.

Yackel and Kenyon (*9*) proposed as standard conditions for the method: 0.5 *N* calcium acetate, 2 hours' treatment time at 25 °C., and titration with 0.1 *N* sodium hydroxide. Other investigators found that concentrated solutions of calcium acetate had a strong buffering effect on the liberated acetic acid, resulting in unsatisfactory end points. For this reason, Heymann and Rabinov (*2*) critically reviewed previous research and investigated the normality of the calcium acetate and other salts for the liberation of the carboxylic acid. They found that 1.0 *N* calcium acetate solutions gave most unsatisfactory liberations and that 0.1 *N* calcium acetate was preferable to 0.5 *N* calcium acetate, as the liberation end point was sharper. Meesook and Purves (*5*) found that carboxyl values tend to be too low when the equilibrium pH of mixtures of oxidized polysaccharides and calcium acetate solutions falls below 6.3. Davidson and Nevell (*1*) specified an equilibrium pH of 6.5 to 6.7, the use of freshly prepared 0.1 *N* calcium acetate, slow agitation during a reaction time of 17 hours, and a mixed indicator to finish titration with 0.01 *N* sodium hydroxide at pH 8.4 to 8.6. They recommended the use of ammonia-free distilled water for the preparation of the solutions and for washing purposes. Kenyon and coworkers (*7*) proposed the use of electrometric titrations for determining the acetic acid liberated in the Yackel and Kenyon modification of the calcium acetate method.

---

[1] Present address, Commercial Solvents Corp., Terre Haute, Ind.

Finally, Wilson (*8*) investigated several methods and concluded that in the base exchange by the calcium ion for the hydrogen ion, the pH range must be within the limits of 7.0 to 8.6, for above pH 8.6 reactions other than acid-base equilibriums become significant. She showed that 0.1 *N* calcium acetate met the pH range specified, but that the slight slope of the neutralization curve resulted in a lack of precision.

Various suggestions advanced by the above authors have been investigated and incorporated into a new modification of the calcium acetate method, which is discussed below and is compared to another procedure which the authors developed and call the "paste titration method." It is based on a procedure which antedates the calcium acetate method and now serves frequently as a routine method for the analysis of starch samples or for manufacturing control—i.e., the starch sample is pasted and the paste is titrated hot with standard sodium hydroxide employing phenolphthalein as the indicator.

EXPERIMENTAL

Calcium acetate of 0.1 normality was chosen instead of 0.5 *N* calcium acetate because experiments confirmed the opinion of Heymann and Rabinov (*2*) and Wilson (*8*) that the calcium acetate–acetic acid system exerts less buffering capacity at the lower normality (see Figure 1, in which B refers to a sample of a low oxidized starch). All data and calculations are based on dry substance starch.

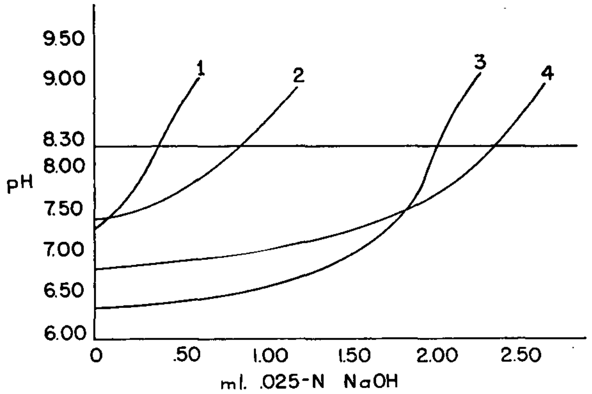


Figure 1. Titration of Calcium Acetate

1. 0.1 *N* calcium acetate
2. 0.5 *N* calcium acetate
3. 0.1 *N* calcium acetate plus 1 gram of starch B
4. 0.5 *N* calcium acetate plus 1 gram of starch B

Modified Calcium Acetate Method: Acidity of De-Ashed Starch. Water free of carbon dioxide and ammonia is used throughout for the preparation of solutions and de-ashing. Samples, 5.000 to 0.500 gram, of starch are used, the amount decreasing with the degree of oxidation, and are so chosen that the equilibrium pH of the calcium acetate solution at the end of this treatment falls within the range of 6.5 to 6.7. The sample is transferred to a 150-ml. beaker, slurried with 25 ml. of 0.1 *N* hydrochloric acid, and occasionally stirred. After a reaction time of 30 minutes, the slurry is filtered through a fritted crucible (medium porosity) and the starch is washed with ammonia-free distilled water until free from chlorides. The starch is transferred to a 100-ml. flask, 10 ml. of a 1 *N* calcium acetate solution or its equivalent are added, and the solution is made to volume with ammonia-free water, so that the final concentration of the calcium acetate is 0.1 *N*. The flasks are shaken occasionally during a reaction period of 30 minutes, the slurries are filtered into a dry suction flask, and a 50-ml. aliquot is titrated potentiometrically to pH 8.3 with 0.25 to 0.01 *N* sodium hydroxide. A blank titration is made on the calcium acetate solution used. The net de-ashed acidity is obtained by subtraction, *A*.

Blank, Acidity of Starch after Washing with Water. The procedure is the same as described above, but for the omission of the de-ashing pretreatment with 0.1 *N* hydrochloric acid. The samples are washed with the same volume of ammonia-free water which has been used in the above procedure and are then submitted to the calcium acetate treatment. The acidity is determined as described above, *B*. The corrected acidity is (*A*) – (*B*).

Calculations. $\frac{2(A-B)\times N}{\text{weight}} \times 100$ = milliequivalents of acidity/100 grams of starch. Milliequivalents of acidity/100 grams of starch × 0.045 = apparent % carboxyl.

Paste Titration Method: Acidity of De-Ashed Starch. Samples, 5.000 or 0.1500 gram, the latter for the more highly oxidized starches known as gums, are transferred to a 150-ml. beaker, 25 ml. of 0.1 *N* hydrochloric acid are added, and the mixture is allowed to remain for 0.5 hour with occasional stirring. The slurry is filtered through a fritted-glass crucible (medium porosity) and washed with distilled water until the wash water is free from chlorides. The de-ashed starch is transferred to a 600-ml. beaker, slurried in 300 ml. of distilled water, and heated to boiling over a gas burner or in a steam cooker. Sufficient time (about 5 to 7 minutes) should be allowed to ensure thorough gelatinization. The pasted starch is titrated hot with 0.1 *N* sodium hydroxide to the phenolphthalein end point, *C*.

Blank, Acidity of Original Starch. The weighed samples are transferred to a 600-ml. beaker, pasted, and titrated hot as described above, *B*. The de-ashing pretreatment is omitted as well as the washing with distilled water, since comparative tests with washed samples did not show any significant difference. The acidity is determined as described above, *D*. The corrected acidity is (*C*) – (*D*).

Calculations. $\frac{(C-D)}{\text{weight}} \times N \times 100$ = milliequivalents of acidity/100 grams of starch. Milliequivalents of acidity/100 grams of starch × 0.045 = apparent % carboxyl.

STARCH SAMPLES

A series of seven commercial oxidized starches was obtained, A to G, inclusive, representing increasing degrees of hypochlorite oxidation. A sample of common pearl starch was included for the first member of this series. The moisture was obtained, and all data are calculated to dry basis starch.

Table I. Modified Calcium Acetate Method

(Acidity as milliequivalents per 100 grams of starch)

Starch	*A* De-ashed	*B* Water-Washed	*A – B* Corrected
Pearl	1.6	1.6	0
Oxidized			
A	3.5	1.8	1.7 × 0.05
B	5.0	1.5	3.5 × 0.08
C	7.4	1.6	5.8 × 0.15
D	10.7	2.1	8.6 × 0.25
E	13.1	2.5	10.6 × 0.43
F	15.0	2.1	12.9 × 0.43
G	18.4	1.2	17.2 × 0.14

Table II. Paste Titration Method

(Acidity as milliequivalents per 100 grams of starch)

Starch	*C* De-ashed	*D* Original	*C – D* Corrected
Pearl	2.3	2.5	0
Oxidized			
A	4.4	2.6	1.8 ± 0.01
B	5.5	2.0	3.5 ± 0.05
C	8.7	2.0	6.7 ± 0.12
D	11.7	2.3	9.4 ± 0.03
E	13.9	2.2	11.7 ± 0.08
F	15.3	2.0	13.3 ± 0.11
G	19.7	1.2	18.5 ± 0.02

Methanol extraction was omitted because the results of tests made on samples B, D, and F, which were extracted with 85% methanol for 1 hour either once or five times according to Schoch (*6*), were not significant (Table IV).

EXPERIMENTS

The acidity determinations obtained by the modified calcium acetate method are shown in Table I, the corresponding data for the paste titration method in Table II. Duplicate determinations were run in each test. The deviations from the mean of the corrected acidities were calculated from the deviations from the mean of the uncorrected and the original acidities. Table III contains the results of the corrected acidity calculated as per cent carboxyl and moles of carboxyl per 100 anhydroglucose units (A.G.U.).

CONCLUSIONS

The data given in Tables I to IV indicate that the difference between the two methods is small and in most cases well within the limits of experimental error. The precision of the paste titration method is better because the buffering capacity of the pasted starch system is greater than that of the calcium acetate–acetic acid system. It is more rapid and is simpler than the corresponding calcium acetate method, and may even enable less experienced analysts to obtain reproducible data. The paste titration method is, therefore, recommended for routine control and analysis.

Both methods show that starches which were oxidized to a varying degree had essentially the same original acidity, which was approximately the same as the original acidity of the unoxidized starch. As the unoxidized starch contains only a small amount of carboxylic groups attached to anhydroglucose units—if any at all—it seems improbable that such carboxyl groups were the main cause for the original acidity of the oxidized starches. This acidity might be rather attributed to the presence of "acidic impurities," such as, for instance, fatty acids. Therefore, deducting the original acidity from the acidity of the de-ashed samples is recommended. This correction makes it also unnecessary to remove free fatty acids by solvent extraction before determining the acidity. The results of Table IV show that one methanol extraction which removes the greater part of the free fatty acids causes only a comparatively small decrease of the corrected acidity.

Base exchange methods of analysis or the paste titration method measure the corrected or uncorrected acidity of only that portion of a sample which is insoluble under the conditions of the de-ashing pretreatment. Therefore, the exact content of carboxylic groups attached to anhydroglucose units in the original starch samples has not been determined. Except in the case of very highly oxidized starches, the "apparent carboxyl content" of de-ashed oxidized starches based on the corrected acidity is believed to be a better approximation to the "real carboxyl content" than calculations based on the uncorrected acidity.

Table III. Corrected Acidity Expressed as Apparent Carboxyl

Oxidized Starch	% COOH Calcium acetate method	% COOH Paste titration method	Moles of COOH/ 100 A.G.U.
A	0.077	0.081	0.2 to 0.3
B	0.16	0.16	0.5 to 0.6
C	0.26	0.30	0.9 to 1.1
D	0.39	0.42	1.3 to 1.6
E	0.48	0.53	1.6 to 1.9
F	0.58	0.60	2.0 to 2.2
G	0.77	0.83	2.7 to 3.0

Table IV. Per Cent Decrease on Methanol Extraction

Oxidized Starch	No. of Extractions	Fat by Hydrolysis	De-Ashed Acidity[a]	Original Acidity[a]	Corrected Acidity[a]
B	1	48	13	35	0
	5	68	22	50	6
D	1	44	9	26	4
	5	73	16	37	10
F	1	46	9	32	5
	5	72	13	41	8

[a] Paste titration method for acidity.

LITERATURE CITED

(1) Davidson, G. F., and Nevell, T. P., *Shirley Inst. Mem.*, **21**, 85–100 (1947).
(2) Heymann, E., and Rabinov, G., *J. Phys. Chem.*, **45**, 1152–66 (1941).
(3) Kerr, R. W., "Chemistry & Industry of Starch," 2nd ed., Chap. III, XI, XXIV, New York, Academic Press, 1950.
(4) Lüdtke, M., *Angew. Chem.*, **48**, 650–1 (1935).
(5) Meesook, B., and Purves, C. B., *Paper Trade J.*, **123**, No. 18, 35–42 (1946); *Tech. Assoc. Papers Ser.*, **29**, 508–15 (1946).
(6) Schoch, T. H., *J. Am. Chem. Soc.*, **64**, 2954–6 (1942).
(7) Unruh, C. C., McGee, P. A., Fowler, W. F., Jr., and Kenyon, W. O., *J. Chem. Soc.*, **69**, 347–9 (1947).
(8) Wilson, K., *Svensk Paperstidn.*, **51**, 45–9 (1948).
(9) Yackel, E. C., and Kenyon, W. O., *J. Am. Chem. Soc.*, **64**, 121–7 (1942).

Received for review June 18, 1952. Accepted September 8, 1952.

Composition of a Typical Grape Brandy Fusel Oil

A. DINSMOOR WEBB, RICHARD E. KEPNER, AND ROBERT M. IKEDA

Department of Viticulture and Department of Chemistry, University of California, Davis, Calif.

A LONG-term program is under way in these laboratories, the primary objective of which is the isolation and identification of the substances contributing to the aromas and flavors of fresh grapes and grape fermentation products. The first phase of this project has been concerned with the investigation of the composition of a typical grape brandy fusel oil obtained from a large California distillery. Early work in the field has not resulted in a detailed knowledge of the composition of grape fusel oils (*2*, *6*, *8*, *10*, *15*) and in the more recent reports there have been errors in designating the isomers of amyl alcohol which are actually present. While the identity of the grapes used in the production of the fusel oil investigated was not recorded, it is very probable that they were all varieties of *Vitis vinifera L.*, as this genus predominates in California.

The composition of this sample of fusel oil is reported on a water- and ethyl alcohol–free basis, as the amount of these substances in a fusel oil depends upon the extent of the washing process used after the fusel oil has been removed from the rectifying column. Dehydration of the fusel oil sample prior to fractionation is necessary to avoid obtaining complex water-containing azeotropes. It was found, by titrations with the Karl Fischer reagent, that repeated dryings over magnesium sulfate, freshly dehydrated at 400° for 3 hours immediately before use, left about 0.2% water in the fusel oil. Complete dehydration was accomplished by adding a calculated excess of anhydrous ethyl alcohol and removing the water as the ethyl alcohol–water azeotrope at the beginning of the fractional distillation of the sample.

The dried fusel oil was found to contain about 0.02 meq. of acid and 0.15 meq. of ester per gram. The free acids were removed from a separate sample of the fusel oil by use of ion exchange resins. The acids present were identified by chromatographing the mixed *p*-phenylphenacyl derivatives according to the procedure of Kirchner, Prater, and Haagen-Smit (*7*). The principal free acids were found to be acetic and butyric, although there were traces of several other acids present in amounts too small to identify.

Reaction of an aliquot part of the fusel oil with 2,4-dinitrophenylhydrazine demonstrated that there were trace amounts of

The composition of grape brandy fusel oil, which is of considerable importance to the manufacturers of grape brandy and other fermented product distillates, has never been reported in the literature in detail. In the present investigation, the components of a *Vitis vinifera* L. grape brandy fusel oil have been determined to be 4.1% *n*-propyl alcohol, 1.9% *n*-butyl alcohol, 4.9% (−)-*sec*-butyl alcohol, 18.3% isobutyl alcohol, 9.6% (−)-2-methyl-1-butanol (act-amyl alcohol), 54.0% isoamyl alcohol, trace of *n*-amyl alcohol, 1.5% *n*-hexyl alcohol, 5.6% esters, and traces of acetic acid, butyric acid, and acetal. The esters are mainly ethyl, isobutyl, isoamyl, and act-amyl alcohol esters, which indicates their probable formation in the brandy rectifying column. The esterified acids are found to be mainly caproic, caprylic, capric, lauric, and palmitic. The observed optical activity, which has been generally assumed to result from the presence of active amyl alcohol in fusel oils, is in this sample one half contributed by the (−)-*sec*-butyl alcohol. Detailed knowledge of the composition of grape brandy fusel oil should be of value in the analyses for fusel oils in brandy, and possibly also in the design of brandy stills.

carbonyl compounds present, too small to permit identification. Elemental analyses on the dried fusel oil and on the several fractions obtained in a preliminary distillation showed the absence of compounds containing nitrogen, sulfur, or halogens.

The components of the fusel oil with boiling points not higher than that of isoamyl alcohol were identified by analysis of the fractions obtained by careful fractional distillation of approximately 83 grams of dried fusel oil at atmospheric pressure. The residue from this distillation did not contain sufficient material to permit appreciable separation of the higher boiling components by fractional distillation under reduced pressure. A large quantity of the higher boiling material was obtained from 289 grams of dried fusel oil by removing all of the lower boiling components up to and including isoamyl alcohol, using an efficient fractionating column operated at 75-mm. pressure. This high-boiling material, composed mainly of esters of high molecular weight fatty acids, was carefully fractionated under reduced pressure in order that individual esters might be identified in so far as possible.

Table I. Summary of Components of Fusel Oil

	Weight %	Substances Probably Present	Weight %
Acetic acid	Trace	Acetal	Trace
Butyric acid	Trace	Methyl salicylate	0.08
n-Propyl alcohol	4.1	Isoamyl caprate	1.10
(−)-*sec*-Butyl alcohol	4.9	Active amyl caproate Isoamyl caproate	0.08
Isobutyl alcohol	18.3		
n-Butyl alcohol	1.9	Active amyl caprylate	Trace
Active amyl alcohol	9.6	Isobutyl caprylate	0.08
Isoamyl alcohol	54.0	Active amyl caprate	0.11
n-Amyl alcohol	Trace	Active amyl laurate Isoamyl laurate	0.25
n-Hexyl alcohol	1.5		
Ethyl caproate	0.19		
Ethyl caprylate	0.60		
Isoamyl caprylate	0.52		
Ethyl caprate	1.32		
Isobutyl caprate	0.38		
Ethyl laurate	0.58		
Ethyl palmitate	0.25		
Butyrate ester	Trace		
Myristate ester	0.06		

The identity of the various alcohols obtained was established by their physical characteristics and by preparation of their 3,5-dinitrobenzoate derivatives. The esters in the fusel oil were identified by preparation of 3,5-dinitrobenzoate derivatives of the alcohols and *p*-phenylphenacyl derivatives of the acids resulting from saponification of the original ester. For fractions containing more than one pure compound, alcohol derivatives were separated chromatographically according to the method of White and Dryden (*16*) and the acid derivatives by the chromatographic technique developed by Kirchner, Prater, and Haagen-Smit (*7*).

Table I presents a list of the components of the fusel oil, with concentrations in weight per cent. Examination of the components listed reveals several points of interest. The optical activity of the sample of fusel oil, which customarily has been taken as a measure of the active amyl alcohol concentration, is in reality about one half contributed by the *sec*-butyl alcohol. The presence of levorotatory *sec*-butyl alcohol in grape fusel oils was inferred by Dupont and Dulou (*3*), who demonstrated its presence in technical propyl alcohol obtained from fusel oil.

The complete separation of isoamyl alcohol from active amyl alcohol was found to be impossible with the fractional distillation equipment available, nor was it possible to separate the 3,5-dinitrobenzoates of the two alcohols by chromatographic techniques. The composition of the fraction containing these two amyl alcohols was determined by using an average figure derived from the refractive index (which indicated 86% isoamyl), the optical rotation (which indicated 70% isoamyl), and a mass spectrometric analysis (which indicated 72% isoamyl). The fraction was considered to be 75% isoamyl alcohol. The composition calculated from the refractive index was based on the assumption of a linear relationship, which may in part explain the deviation from the other two methods of analysis.

On the basis of experimental evidence it has been proposed that the higher alcohols of fusel oil come from the naturally occurring amino acids of the grapes by means of the Ehrlich mechanism, or by a process which may involve the yeast proteins (*1, 4, 9, 13*). The presence of *n*-propyl, *n*-butyl, *n*-hexyl, and (−)-*sec*-butyl alcohols (alcohols which would require amino acids not known to occur naturally for their production by the Ehrlich mechanism) indicates that these alcohols were either present in the grapes or were formed during fermentation by some metabolic process other than that proposed by Ehrlich.

Although the presence of esters in grape brandy fusel oils has been demonstrated (*14*), there has always been some question as to the identity of the individual esters actually present. In the present work careful fractionation of the high boiling esters before saponification has made possible the identification of the actual esters present with a fair degree of certainty. Examination of the data listed in Table V will show that only one acid and one alcohol were obtained on saponification of certain of the distillation fractions. In such cases positive identification of the ester was accomplished. In other fractions two or three alcohols and two or three acids were identified, which indicated the presence of at least two or three esters in the original distillation fraction. It is logical to conclude that the boiling points of the esters in each distillation fraction are relatively close together, since the esters could not be separated by careful fractionation through an efficient column. Thus, the actual esters present in such fractions are most likely to be those combinations of the alcohols and acids identified which would give esters with boiling points of about the same magnitude. Esters identified only in this manner are listed in Table I as probably present. In certain of the other distillation fractions chromatographic analysis of the alcohol derivative gave an oily or low melting material from the lowest zone on the column (see Table V). The position on the column and an analysis in one case indicated that this material was a mixture of isoamyl and active amyl-3,5-dinitrobenzoates. Since, however, positive identification of active amyl alcohol as a product of the saponification of the esters was not possible, the esters from these distillation fractions are listed in Table I only as probably present.

In the group of esters listed, with the exception of methyl salicylate, each of the esters has as its alcohol portion ethyl, isobutyl, isoamyl, or what is probably active amyl alcohol. As ethyl, isobutyl, isoamyl, and active amyl alcohols are the principal alcohols present in the rectifying column during the distillation, and as the C_6 to C_{16} acids, being essentially water-insoluble but ethyl alcohol–soluble, would tend to remain in the lower part of the column, it seems reasonable to propose that the bulk of the esters withdrawn with the fusel oil are produced during the distillation of the brandy. Methyl salicylate probably comes from the grapes, as it seems unlikely that it would be produced in the rectifying column. The ester containing fractions obtained from the fusel oil had pleasant odors, a fact which suggests the desirability of returning an ester containing fraction separated from the crude fusel oil to the product brandy.

EXPERIMENTAL

Excess solid sodium chloride was added to the crude fusel oil and the saturated brine solution which separated as a second phase was removed. The fusel oil layer was dried for 4 weeks over freshly ignited magnesium sulfate which was replaced each week. Analysis by means of Karl Fischer reagent indicated that fusel oil dried in this manner still contained 0.19% by weight water. This remaining water was removed in subsequent fractionations of the fusel oil as the water–ethyl alcohol azeotrope by the addition of a slight excess over the calculated amount of absolute ethyl alcohol.

Seven and a half grams of absolute ethyl alcohol were added to an 82.7-gram sample of the dried fusel oil and the mixture was separated into 34 fractions by careful distillation through a Podbielniak Miniature Hyper-Cal column at atmospheric pressure. This distillation was run continuously over an 8-day period and was stopped when the pot temperature commenced climbing rapidly after removal of most of the isoamyl alcohol. These results are summarized in Table II.

Table II. Distillation of Fusel Oil

Distillation Fraction	Distillation Temp., ° C.	Fraction Wt., G.
1	77–78.4	12.20
2–8	78.4–107.3	10.19
9–12	107.3–107.5	9.96
13–14	107.5–128.3	4.00
15–29	128.3–131.3	28.08
30–33	131.3–131.5	13.09
34	Residue	11.50

Fraction 1 consisted mainly of ethyl alcohol with probably a trace of acetal present. Acetal and ethyl alcohol are reported (*5*) to form a binary azeotrope boiling at 77.95°. The sample was fractionated using a Piros-Glover microstill, atmospheric pressure model. The first 0.5 gram of distillate contained the odor of acetal, and gave a positive Schiffs test slowly, a positive test with 2,4-dinitrophenylhydrazine, and a negative test with Tollens reagent. Positive identification of the acetal was not possible because of the small amount of material present.

The material in fractions 2 to 8 was collected mainly over a boiling range of 90° to 107° with little evidence of a plateau to indicate the separation of any pure substances. These fractions were combined and refractionated using a Piros-Glover microstill, atmospheric pressure model.

Fractions 9 to 12 were constant boiling and had constant refractive index, n_D^{25} 1.3936.

Fractions 13 and 14 were combined and refractionated using a Piros-Glover microstill, atmospheric pressure model.

Fractions 15 to 29 were slightly levorotatory, indicating the presence of active amyl alcohol. The refractive index of these fractions decreased steadily from n_D^{25} 1.4060 to 1.4046. These fractions were combined and an unsuccessful attempt was made to separate the isoamyl and active amyl alcohols by distillation through the Piros-Glover microstill.

Fractions 30 to 33 did not rotate the plane of polarized light and had constant refractive index, n_D^{25} 1.4043.

Table III. Fractionation of Lower Boiling Constituents of Fusel Oil

Distilln. Fraction	Distilln. Temp., ° C.	Fraction Wt., G.	n_D^{25}	Acetyln. No.
A-1	<78.9	0.41	....	...
A-2	78.9–97.3	0.24	....	...
A-3	97.3–97.7	2.01	1.3839	64.4
A-4	97.7–98.9	0.94	1.3865	...
A-5	98.9–99.6	0.81	1.3912	...
A-6	99.6–99.8	2.84	1.3947	78.6
A-7	99.8–107.3	0.16	....	...
A-8	107.3–107.5	13.62	1.3936	75.0
A-9	107.5–118.1	0.23	....	...
A-10	118.1–118.2	1.27	1.3972	74.8
A-11	118.2–128.3	0.13	....	...
A-12	128.3–131.3	28.87	1.4051	88.4
A-13	131.3–131.5	13.09	1.4043	87.2
A-14	Residue	11.50	....	...

The data for the redistillation of fractions 2 to 8 and fractions 13 and 14 using the Piros-Glover microstill and the remaining data for the preliminary distillation using the Podbielniak column are summarized in Table III (fraction 1, Table II, is not included).

The acetylation numbers listed in Tables III and IV correspond to the grams of alcohol per hydroxyl group and were obtained by refluxing 30 to 50 mg. of the distillation fraction with an excess of standard acetyl chloride in toluene for an hour.

The apparatus used was vented to the atmosphere through a trap containing a measured amount of standard 0.2 *N* sodium hydroxide solution to prevent any loss of hydrogen chloride. At the end of the reflux period the contents of the trap were washed into the reaction flask with distilled water to hydrolyze the excess acetyl chloride and the remaining acid was titrated with additional 0.2 *N* sodium hydroxide. The decrease in equivalents of acid between a blank determination run with only the alcohol sample omitted and the determination run with the alcohol sample present is equal to the moles of alcohol in the sample.

In this work the 3,5-dinitrobenzoate derivatives of the alcohol fractions were prepared by allowing the alcohol and excess 3,5-dinitrobenzoyl chloride in anhydrous pyridine to stand for several hours or overnight at room temperature. The reaction mixture was poured into ether, and the ether layer was washed thoroughly successively with 3 *N* sulfuric acid, water, 10% sodium carbonate solution, and saturated sodium chloride solution. The ether layer was thoroughly dried over anhydrous magnesium sulfate and the ether was removed under reduced pressure to give the 3,5-dinitrobenzoate derivative.

IDENTIFICATION OF FRACTIONS

Fraction A-1. This fraction was essentially ethyl alcohol with no evidence for the presence of acetal.

Fraction A-3. This fraction was pure *n*-propyl alcohol and formed a 3,5-dinitrobenzoate ester, melting point 72–72.5°, mixed melting point with *n*-propyl-3,5-dinitrobenzoate, 72–72.5°. The physical constants and acetylation number agree closely with literature and theoretical values respectively for *n*-propyl alcohol.

Fraction A-4. This fraction was a mixture of *n*-propyl alcohol and (−)-*sec*-butyl alcohol. From the refractive index, assuming a linear relationship, the fraction was calculated to contain 27% (−)-*sec*-butyl alcohol. A 25.0-mg. sample of the 3,5-dinitrobenzoate ester of the fraction gave two sharp zones on a chromatographic column using the method of White and Dryden (*16*). The zones were eluted through the column and the lower zone gave 5.1 mg. of material, melting point 87–89°, and the upper zone gave 19.4 mg. of material, melting point 73–73.5°, mixed melting point with *n*-propyl-3,5-dinitrobenzoate 73–73.5°. From the chromatographic results the fraction was calculated to contain 21% (−)-*sec*-butyl alcohol. On the basis of these two methods of analysis the fraction was assumed to consist of 24% (−)-*sec*- butyl alcohol and 76% *n*-propyl alcohol.

Fraction A-5. This fraction was a mixture of *n*-propyl alcohol and (−)-*sec*-butyl alcohol. From the refractive index the fraction was calculated to contain 67% (−)-*sec*-butyl alcohol. Chromatographic separation of 24.6 mg. of the 3,5-dinitrobenzoate ester of the fraction gave two sharp zones. The lower zone contained 13.7 mg. of material, melting point 87–89°, and the upper zone contained 9.9 mg., melting point 73–74°, mixed melting point with *n*-propyl-3,5-dinitrobenzoate 73.0–73.5°. The chromatographic results indicated that the fraction contained 60% (—)-*sec*-butyl alcohol. From these two methods of analysis the composition of the fraction was calculated to be 64% (—)-*sec*-butyl alcohol and 36% *n*-propyl alcohol.

Fraction A-6. This fraction was essentially pure (—)-*sec*-butyl alcohol, $[\alpha]_D^{30}$ −10.0°. The physical constants and acetylation number agree closely with the literature and theoretical values, respectively, for *sec*-butyl alcohol. The 3,5-dinitrobenzoate derivative, melting point 89–90.5°, gave only one sharp band on the chromatographic column.

ANALYSIS. Calculated for $C_{11}H_{12}O_6N_2$: C, 49.25; H, 4.51; N, 10.45. Found: C, 49.38; H, 4.75; N, 10.42.

Fraction A-8. The physical constants and acetylation number agree closely with the literature and theoretical values, respectively, for isobutyl alcohol. The melting point of the 3,5-dinitrobenzoate was 84.5–85°, mixed melting point with isobutyl-3,5-dinitrobenzoate, 85–85.5°.

Fraction A-10. The physical constants and acetylation number agree closely with the literature and theoretical values, respectively, for *n*-butyl alcohol. The melting point of the 3,5-dinitrobenzoate was 62.8–63.0°, mixed melting point with *n*-butyl-3,5-dinitrobenzoate, 62.8–63.0°.

Fraction A-12. n_D^{20} 1.4071, d_4^{25} 0.8108, M_D, observed 26.77, calculated for amyl alcohol 26.82, acetylation number 88.4 α_D^{25} −2.3° (0.5-dm. microtube), $[\alpha]_D^{25}$ −1.95°. The molecular refraction and the acetylation number indicated that the sample was a mixture of amyl alcohols (calculated acetylation number 88.1), which were shown to be isoamyl alcohol and active amyl alcohol. Calculations from the index of refraction and specific rotation gave the composition of the fraction as containing 85 and 70% isoamyl alcohol, respectively. A determination of the composition by means of the mass spectrometer gave 72% isoamyl alcohol. Carefully purified synthetic samples of isoamyl alcohol from Sharples Chemicals, Inc., and 2-methyl-1-butanol from the Matheson Co. were used as reference standards in the mass spectrometric analysis. On the basis of these analyses the fraction was calculated to contain 75% isoamyl alcohol and 25% (—)-2-methyl-1-butanol (active amyl alcohol).

Fraction A-13. The physical constants and acetylation value agree closely with the literature and theoretical values, respectively, for isoamyl alcohol. The melting point of the 3,5-dinitrobenzoate was 59.5–60.5°, mixed melting point with isoamyl-3,5-dinitrobenzoate 59.5–61.0°.

Table IV. Fractionation of Higher Boiling Constituents of Fusel Oil

Distilln. Fraction	Distilln. Temp., ° C.	Press. Mm.	n_D^{25}	Fraction Wt., G.	Acetyln. No.	Neut. Eq.	Sapon. Eq.
	Up to 76	75		262.0			
B 1–7	67.4–68.2	49	1.4052 to 1.4042	17.71			
B-8	68.2–73.5	49	1.4070	0.42	105.5	77,100	786
B-9	73.5–83.8	49	1.4065	0.17	129.8		
B-10	83.8–88.6	49	1.4142	0.78	163.1	34,800	420
B-11	88.6–89.2	49	1.4140	1.32	118.6	23,900	1265
B-12	89.2–89.7	49	1.4147	0.84	107.9	>50,000	3608
B-13	68.1–68.5	15	1.4172	0.62	114.2	>50,000	2023
B-14	68.5–92.3	15	1.4242	0.53	197.3	3,200	627
B-15	92.3–99.7	15	1.4210	0.78	659	6,550	260
B-16	99.7–108.8	15	1.4322	0.35	542	15,940	248
B-17	76.4–91.0	2.5	1.4309	0.45	1016	36,530	250
B-18	87.3–91.1	2	1.4255	0.90	1298	43,770	243
B-19	91.1–92.9	2	1.4245	0.64	2344	36,320	239
B-20	92.9–106.1	2	1.4273	1.44	1854	10,810	268
B-21	106.1–117.4	2	1.4309	0.89	1358	10,320	272
B-22	117.4–120.7	2	1.4301	0.70	1331	20,820	254
B-23	120.7–122.6	2	1.4306	0.53	No. acet.	19,840	278
B-24	122.6	2	1.4304	1.19	7280	23,280	287
B-25	122.6–124.8	2	1.4305	0.54	5301	11,560	321
B-26	124.8–129.6	2	1.4372	0.27	6243	11,020	340
B-27	110–113	0.5	1.4394	0.66	2988	13,130	388
B-28	113–120	0.5	1.4490	1.07	1165	14,770	527
Residue (tars)				3.14			

Fractions A-2, A-7, A-9, and A-11. These break fractions from the distillation were mixtures of the alcohols boiling directly above and below them and were assumed to be equimolar mixtures in each case in calculating the composition listed in Table I.

Fraction A-14. This material was fractionally distilled through a Piros-Glover microstill, vacuum model, at 70-mm. pressure and contained 6.2 grams (53.5%) of isoamyl alcohol. The melting point of the 3,5-dinitrobenzoate was 61–61.5°, mixed melting point with isoamyl-3,5-dinitrobenzoate 60.8–61.5°. Fractionation of the small amount of higher boiling material at pressures ranging from 11 mm. on down to 0.5 mm. at the end of the distillation did not result in sufficient separation to allow identification of the components present.

Fractionation of Higher Boiling Constituents. A 289.0-gram sample of dried fusel oil to which was added 39.6 grams of absolute ethyl alcohol was distilled through the Podbielniak miniature column under 75 mm. pressure to remove the low boiling alcohols up to and including most of the isoamyl alcohol. The high boiling residue was fractionally distilled continuously over an 11-day period using the Piros-Glover microstill, vacuum model. The data for the distillation of this sample of fusel oil, and the acetylation numbers, neutral equivalents, and saponification equivalents for the various distillation fractions are summarized in Table IV. The method for determination of the acetylation numbers has been previously described. The neutral equivalents were determined on 4- to 6-mg. samples by titration with 0.1 *N* sodium hydroxide solution using a Gilmont ultramicroburet manufactured by the Emil Greiner Co. The saponification equivalents were determined on 7- to 10-mg. samples by the diethylene glycol method using approximately 0.1 *N* potassium hydroxide in diethylene glycol and titrating with 0.02 *N* hydrochloric acid. The diethylene glycol reagent was standardized under the same conditions as the determination.

Fractions B 1–7. The physical constants and the preparation of a 3,5-dinitrobenzoate derivative proved this material to be mainly isoamyl alcohol.

Fraction B-8. The analytical data indicated the presence of both free alcohols and esters in this fraction. The only alcohol which could be identified was isoamyl alcohol. Chromatographic analysis of the 3,5-dinitrobenzoate derivative gave only one major band, melting point 55–59°, mixed melting point with isoamyl-3,5-dinitrobenzoate 56–61°, 1-naphthylamine addition product of the 3,5-dinitrobenzoate derivative, melting point 101–103°, mixed melting point with the 1-naphthylamine addition product of isoamyl-3,5-dinitrobenzoate 101.5–103.5°. The identification of the ester portion of this fraction is discussed below and the results are summarized in Table V.

Fraction B-9. The amount of material in this fraction was too small to permit complete identification. Chromatographic analysis of the 3,5-dinitrobenzoate derivative of the alcohols present gave two faint bands near the top of the column which could not be identified and one major band which was eluted through the column. The major band could not be crystallized and was converted to a 1-naphthylamine addition product, melting point 80–81°, mixed melting point with the 1-naphthylamine addition product of *n*-amyl-3,5-dinitrobenzoate 81–83°.

Fraction B-10. The analytical data indicated the presence of free alcohols and esters in this fraction. Chromatographic analysis of the 3,5-dinitrobenzoate derivative of the alcohols present gave two faint bands near the top of the column which could not be identified and one major band which was eluted through the column. The low melting material from the major band was converted to the 1-naphthylamine addition

Table V. Analysis of Esters

	Acid Part					Alcohol Part				
Distilln. Fraction	M.P. of *p*-phenyl-phenacyl deriv.	Relative amount[a]	*p*-Phenyl-phenacyl ester of	M.P. of known deriv.[b]	Mixed m.p.	M.P. of 3,5-dinitro-benzoate deriv.	Relative amount[a]	3,5-Dinitro-benzoate ester of	M.P. of known deriv.[b]	Mixed m.p.
B-8	69.6–70.3		Caproic	69.5–70.0	69.0–69.8	92.0–92.5		Ethyl	93.0–93.2	92.0–93.0
B-10	69.5–70.0		Caproic		69.5–70.0	91.0–92.0		Ethyl		91.5–92.5
B-14	63–64	S	Caproic		63–65.5	89.5–91.5		Ethyl		90.5–92.5
	62–63	T	Caprylic	68.2–69.3	64–67					
	77.5–78.5	T	Butyric	82.0–82.2	77.5–80.5					
B-15	67.5–68.0		Caprylic		67.5–68.5	91–92		Ethyl		91.5–92.5
B-16	69.6–70.0	M	Caproic		69.2–70.0	91.5–92.5	M	Ethyl		92–93
	68.0–68.7	S	Caprylic		68–69	105.5–107.0	M	Methyl	106.5–106.8	106–107
	148–149	S	Salicylic	149.6–150.0	149–150	<35	M			
B-17	65–65.5	S	Caproic		68.5–70	89–91	M	Ethyl		90–92
	67–68	L	Caprylic		68.5–69.2	84–85.5	M	Isobutyl	85.5–86.0	85–86
	76.5–77.0	L	Capric	76.0–76.5	76.3–77.0	72–74.5[c]	M			
B-18	74–75.5		Capric		76–76.5	92.5–93.5		Ethyl		93.0–93.5
B-19	75–77		Capric		75.5–77	93–93.5		Ethyl		93.0–93.5
B-20	66.5–67.5[d]	M	Caprylic		67–68.5	86–89	S	Ethyl		91.5–93
	76–77	S	Capric		76–77	58–60	L	Isoamyl	60.0–61.0	58–60
B-21	74–75		Capric		75–76.5	89.5–91.5	S	Ethyl		91–93
						81–82.5	L	Isobutyl		82–85
B-22	65–67	T	Caprylic		65.5–67.5	91–93	L	Ethyl		92.5–93.0
	74.5–76	M	Capric		74.5–75.5	Oil	S			
	83.0–83.8	L	Lauric	84.4–84.8	83.5–84.5					
B-23	72.5–73[e,f]		Lauric[e]	74.0–74.5	73–74	90.5–92.0		Ethyl		92–93
B-24	74–75.5[g]		Capric		75.5–76.3	59.5–61	L	Isoamyl		60–61
						88–90	T	Ethyl		88–91.5
B-25	65–65.5[e,h]		Capric[e]	65.0–65.5	65–65.5	59.5–60.5		Isoamyl		60–61
B-26	75–76	L	Capric		75–76.5	Oil				
	80–82.5	T	Lauric		81.8–83.5					
B-27	72.5–73.5	T	Capric		73–76	105.5–107.0	T	Methyl		107–108
	79–80[i]	M	Lauric		79.5–83	91–92	S	Ethyl		92.5–93.5
						35.5–39[j]	L			
B-28	73.5–75	T	Capric		75–76	90.5–92	L	Ethyl		91.5–93.0
	82.5–83.7	L	Lauric		83–84	25–30	L			
	88.0–88.5	S	Myristic	90.3–91.0	87–88.5					
	93.8–94.2	L	Palmitic	93.0–94.0	93.5–94.6					
	84.0–84.5[e]		Palmitic[e]	84.5–85.0	84.0–84.7					

[a] L = large, M = medium, S = small, T = trace.
[b] The melting points of the known derivatives are listed only the first time each appears in the table.
[c] This band was between the ethyl and isobutyl bands on the chromatographic column but there was not sufficient material for positive identification.
[d] Anal. Calcd. for $C_{22}H_{26}O_3$: C, 78.07; H, 7.74. Found: C, 78.04; H, 7.98
[e] *p*-Br phenacyl derivatives.
[f] Anal. Calcd. for $C_{20}H_{29}O_3Br$: C, 60.45; H, 7.36. Found: C, 60.19; H, 7.61
[g] Anal. Calcd. for $C_{24}H_{30}O_3$: C, 78.65; H, 8.25. Found: C, 78.66; H, 8.20
[h] Anal. Calcd. for $C_{18}H_{25}O_3Br$: C, 58.54; H, 6.82. Found: C, 58.34; H, 6.83
[i] Anal. Calcd. for $C_{26}H_{34}O_3$: C, 79.15; H, 8.69. Found: C, 78.92; H, 8.59
[j] Anal. Calcd. for amyl-3,5-dinitrobenzoate, $C_{12}H_{14}O_6N_2$: C, 51.05; H, 4.99. Found: C, 51.42; H, 5.09

product, melting point 82–6°, mixed melting point with the 1-naphthylamine addition product of *n*-amyl-3,5-dinitrobenzoate 82–87°. The identification of the ester portion of this fraction is discussed below and summarized in Table V.

Fractions B-11 to B-13. The analytical data indicated that these fractions contained only alcohols. Chromatographic analysis of the 3,5-dinitrobenzoate derivative in each case gave only one major band (90% or greater), melting point 56–58°, mixed melting point with *n*-hexyl-3,5-dinitrobenzoate 56–58°.

Fraction B-14. The analytical data indicated the presence of free alcohols and esters in this fraction. Chromatographic analysis of the 3,5-dinitrobenzoate of the alcohols present gave only one major band, melting point 54–56.5°, mixed melting point with *n*-hexyl-3,5-dinitrobenzoate 54–57°. The identification of the ester portion of this fraction is discussed below and summarized in Table V.

Fractions B-15 to B-28. The analytical data indicated that these fractions consisted mainly of esters with at most only traces of free alcohols present. No attempt was made to identify free alcohols in these fractions. The esters in these fractions and in fractions B-8, B-10, and B-14 were saponified by the diethylene glycol method (*11*) using 0.2- to 0.4-gram samples.

The alcohols were distilled out of the basic solution using semimicro equipment and converted to the 3,5-dinitrobenzoate derivative by the method previously described. The entire sample of derivative was then placed on a chromatographic column (*16*) and the various zones were separated and identified by means of melting points and mixed melting points whenever possible. These results are summarized in Table V. In several fractions there was a trace zone high up in the column which could not be isolated for positive identification but from the position on the column was presumably a trace of the methyl derivative. In fractions B-8, and B-14 only ethyl-3,5-dinitrobenzoate was obtained in addition to the derivatives of the alcohols already shown to be present as free alcohols in the samples. In fractions B-16, B-22, B-26, B-27, and B-28 the lowest zone in the column gave an oily or low melting derivative which could not be separated into pure components by rechromatographing or by fractional crystallization techniques. A carbon-hydrogen analysis of the low melting derivative from fraction B-27 indicated that it was composed entirely of amyl-3,5-dinitrobenzoates (see Table V). The available data indicate that these low melting zones in each case are probably mixtures of active amyl and isoamyl-3,5-dinitrobenzoates.

The basic diethylene glycol solution remaining after removal of the alcohols wasc are fully neutralized with 3 *N* sulfuric acid and the acids were converted to the *p*-phenylphenacyl esters (*12*). After the hot solution was decolorized with carbon, the excess reagent and the derivatives were crystallized completely from the solution. All of the solid material obtained was then chromatographed using the technique of Kirchner, Prater, and Haagen-Smit (*7*). The excess reagent passed rapidly through the column first as a dark band, and the *p*-phenylphenacyl acid derivatives subsequently spread out as a broad, blue fluorescent band under ultraviolet light as the column was developed. This blue fluorescent band was developed through the column and the material collected as a large number of small samples. The solvent was removed from each sample under reduced pressure and the melting point of the resulting solid material determined.

Consideration of the melting points of consecutive samples indicated that certain of these small samples could be recombined in many cases and further purified by rechromatographing or by fractional crystallization techniques. The purified derivatives obtained by such methods were identified by melting points and mixed melting points with known derivatives and also by carbon-hydrogen analyses in certain cases. In the fractions which contained more than one ester the relative amounts of each acid derivative were estimated from the quantity of each derivative obtained. An exact determination of the relative quantity of each derivative could not be made, since the *p*-phenylphenacyl derivatives of the high molecular weight acids in many of the fractions could not be separated completely on the chromatographic column or by fractional crystallization techniques. These data are summarized in Table V.

Identification of Free Acids. The free acids of the dried fusel oil were removed by passing the fusel oil slowly through a column of Amberlite IRA 400 resin which was in the hydroxide form and which had been washed with 50% ethyl alcohol–water solution in order that the fusel oil would wet the resin. The higher alcohols were washed out of the column with successive portions of 50% ethyl alcohol–water solution and water, after which the anions were displaced by a wash with 10% sodium hydroxide solution. The excess sodium hydroxide and the sodium salts of the acids were then run through a column of Amberlite IR 120 which was in the hydrogen form. The effluent from the cation exchanger was neutralized with standardized sodium hydroxide solution, the excess water was evaporated on a steam bath, and the *p*-phenylphenacyl derivatives were prepared. The mixed derivatives were chromatographed by the method previously mentioned.

Five blue fluorescent zones were separated, two of which contained fairly large amounts of material. The material from the upper of these two zones recrystallized from ethyl alcohol as colorless plates, melting point 108.8–110.0°, mixed melting point 110.0°–111.0° with *p*-phenylphenacyl acetate. The lower large zone gave white plates on recrystallization from ethyl alcohol, melting point 79.7–80.8°, mixed melting point 81.0–82.0° with *p*-phenylphenacyl-*n*-butyrate.

ACKNOWLEDGMENT

The authors wish to acknowledge the financial assistance of the Wine Advisory Board in support of this research. They also wish to thank James F. Guymon for furnishing the fusel oil sample, and Amos Newton for making the mass spectrometric analyses.

LITERATURE CITED

(1) Castor, J. G. B., and Guymon, J. F., *Science*, **115**, 147 (1952).
(2) Claudon, E., and Morin, E.-Ch., *Compt. rend.*, **104**, 1187 (1887).
(3) Dupont, Georges, and Dulou, Raymond, *Ibid.*, **200**, 1860 (1935).
(4) Ehrlich, F., *Ber.*, **40**, 1027 (1907).
(5) Horsley, L. H., Anal. Chem., **19**, 531 (1947).
(6) Joslyn, M. A., and Amerine, M. A., Univ. Calif. Agr. Expt. Sta., *Bull.* **652** (1941).
(7) Kirchner, J. G., Prater, A. N., and Haagen-Smit, A. J., Ind. Eng. Chem., Anal. Ed., **18**, 31 (1946).
(8) Morin, E.-Ch., *Compt. rend.*, **105**, 1019 (1887).
(9) Neubauer, O., and Fromherz, K., *Z. physiol. Chem.*, **70**, 326 (1911).
(10) Ordonneau, Ch., *Compt. rend.*, **102**, 217 (1886).
(11) Shriner, R. L., and Fuson, R. C., "Identification of Organic Compounds," 3rd ed., p. 133, New York, John Wiley & Sons, 1948.
(12) *Ibid.*, p. 157.
(13) Thorne, R. S. W., *J. Inst. Brewing*, **55** (n.s. 46), 201 (1949).
(14) Ubeda, F. B., *Anales fis. quim.*, **37**, 356 (1941).
(15) Valaer, Peter, *Ind. Eng. Chem.*, **31**, 339 (1939).
(16) White, J. W., Jr., and Dryden, E. C., Anal. Chem., **20**, 853 (1948).

Received for review July 7, 1952. Accepted September 22, 1952.

Analysis of Pyrazine

Methods for Assay and Impurities

WILLIAM SEAMAN, J. T. WOODS, AND WLADIMIR LEIBMANN
Calco Chemical Division, American Cyanamid Co., Bound Brook, N. J.

As a part of a program of work on sulfapyrazine, a method was needed for determining pyrazine. A method is proposed whereby pyrazine is precipitated as a mercuric sulfate complex. The excess standard mercuric sulfate is titrated with thiocyanate. Piperazine, diethylenetriamine, ethylenediamine, and ethanolamine do not interfere. A small correction is applied for the solubility of the complex. The pyrazine values have a standard deviation of ±0.3% (absolute) and seem to suffer no systematic error. Some indication of the amount of the above-mentioned basic impurities, and of the presence of unidentified impurities, is obtained by a potentiometric titration with standard alkali after addition of an excess of standard acid.

IN CONNECTION with work on sulfapyrazine, a method of analysis was needed which would be able to differentiate pyrazine from basic impurities that were likely to be associated with it.

No such methods were found in the literature, but Stoehr (*1, 4*) had reported that pyrazine formed an addition compound with mercuric chloride which was insoluble in water and dilute acid. Utilization of this reaction as the basis of a convenient titrimetric method of assay of pyrazine would require titrating the excess mercuric chloride left after carrying out the reaction. Because chlorides interfere with the commonly used titration with thiocyanate (*2*), mercuric sulfate was used rather than mercuric chloride.

A potentiometric titration gives an indication of the type and amount of the basic impurities, mainly piperazine, ethylenediamine, diethylenetriamine, and ethanolamine, which may be present as a result of the particular synthesis which was used. It cannot be interpreted in terms of any specific impurity, but does, nevertheless, indicate to a small degree which of the expected bases may be present.

Water may be determined by the Karl Fischer reagent in the usual manner.

ASSAY METHOD

Reagents. Mercuric Sulfate Solution. A solution of 30 grams of reagent-grade mercuric sulfate in 850 ml. of water and 150 ml. of concentrated sulfuric acid is filtered through glass wool and standardized against 0.1 *N* ammonium thiocyanate, using ferric ammonium sulfate as the indicator.

Ammonium Thiocyanate Solution, 0.1 *N*. The ammonium thiocyanate is standardized against a standard silver nitrate solution.

Ferric Ammonium Sulfate. Ten milliliters of concentrated nitric acid are added to 125 grams of reagent-grade ammonium iron alum [$Fe_2(SO_4)_3 . (NH_4)_2SO_4 . 24H_2O$] dissolved in 100 ml. of water.

Procedure. The samples which were analyzed included both liquids and solids (pyrazine is a solid melting at about 53° C.). The solid samples were melted in an oven at 60° to 70° to ensure uniformity of sampling. A weighed 4- to 7-gram sample of the melt was dissolved in about 300 ml. of water and diluted to 1 liter in a volumetric flask. Fifty milliliters of standard mercuric sulfate solution was added to a 50-ml. aliquot dropwise from a buret, with mechanical stirring, over a period of 5 to 10 minutes. The reaction mixture was allowed to stand overnight at room temperature. (If somewhat poorer precision is acceptable, the overnight digestion is not necessary.) The reaction mixture was filtered with suction and the precipitate of the mercury complex was washed with about 100 ml. of water. The filtrate was titrated with 0.1 *N* ammonium thiocyanate, using 4 ml. of the ferric ammonium sulfate solution as the indicator.

The pyrazine content of the sample was calculated from the following equations:

$$C_4H_4N_2 + HgSO_4 \longrightarrow HgSO_4 . C_4H_4N_2 \qquad (1)$$

$$HgSO_4 + 2NH_4CNS \longrightarrow Hg(CNS)_2 + (NH_4)_2SO_4 \qquad (2)$$

A solubility correction of 0.50 ml. of 0.1 *N* thiocyanate was added to compensate for the solubility of the pyrazine-mercuric sulfate complex. This correction is discussed below.

Table I. Comparison of Mercury Contents of Precipitates Obtained under Various Conditions

Conditions of Precipitation	Mercury in Excess, Meq.	Pyrazine Found[a], %	Mercury in Precipitate[b], %
Dropwise addition of reagent	5.3	96.4	52.9
Dropwise addition of reagent	3.0	96.8	53.6
Dropwise addition of reagent	1.4	96.5	53.0
Dropwise addition of reagent	0.4	97.0	52.5
Rapid addition of reagent	4.0	100.8	52.6
Rapid addition of reagent	2.5	98.9	52.5
Rapid addition of reagent	0.5	98.1	52.6

[a] Pyrazine present, 96.7%.
[b] Theory, 53.2%.

Table II. Effect of Temperature of Precipitation

Conditions	Pyrazine Found, %
Recommended method	97.0, 96.5, 96.8, 96.4,
Av.	96.7
Precipitated at boiling point under reflux	96.2, 96.2
Precipitated hot in open beaker	86.9, 80.6

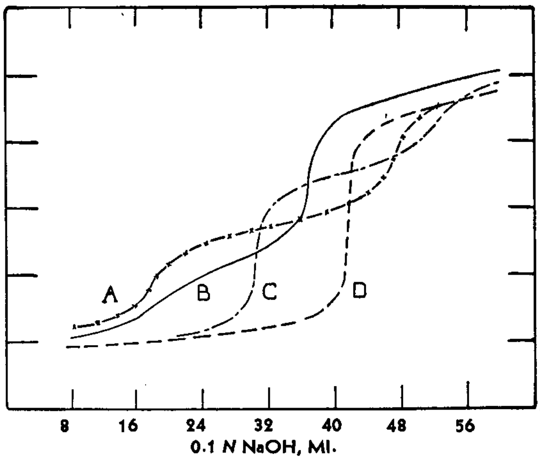


Figure 1. Potentiometric Titration of Impurities

Curve	Impurity	Weight, G.
A	Piperazine	0.2450
B	Diethylenetriamine	0.1946
C	Ethylenediamine	0.1402
D	Ethanolamine	0.2046

Method of Precipitation. The effect of the amount and manner of addition of mercuric sulfate is shown in Table I. For a dropwise addition of mercuric sulfate the values obtained do not depend on the excess of mercuric sulfate, over the range 0.4 to 5.3 meq. If the reagent is added more rapidly, such as in a steady stream from a pipet, higher values are obtained. To attempt to determine whether these high values are due to coprecipitation of impurities or to the formation of complexes containing greater amounts of mercury, the mercury was determined in some of the precipitates by dissolving about 0.2 gram of the precipitate (previously dried at 110° C.) in 20 ml. of concentrated nitric acid, diluting ten times with water in order to obtain a solution about 1.5 *N* in acidity, and titrating with ammonium thiocyanate, using ferric ammonium sulfate as the indicator. A comparison of the mercury values for the precipitates and the values obtained for the purity of the pyrazine is shown in column 4 of Table I. These data fail to disclose any evidence that the high values are caused by the precipitation of a complex containing a higher concentration of mercury. The higher values obtained by the rapid addition must therefore be due to the precipitation, because of local excesses of mercury, of mercury complexes of some of the impurities which are present in the sample. An attempt to eliminate this difficulty by precipitating hot in an open beaker, rather than at room temperature, led to low values. Under reflux there was some suspicion of lowered values. Losses of pyrazine on heating might occur because of the existence of an azeotrope with water which boils at 95.5° C. (uncorrected) (*3*). A comparison of the values obtained under these conditions is given in Table II.

Solubility Correction. The solubility correction was determined by suspending 50.0 mg. of the complex and varying amounts of mercuric sulfate in the same volume of solution and under the same conditions of acidity as those existing during the assay. After occasional shaking and then standing overnight, the remaining precipitate was filtered off and washed with 100 ml. of water and the mercuric sulfate in the filtrate was determined by means of ammonium thiocyanate in the usual manner. The values obtained are shown in Table III. From this it can

Table III. Solubility of Pyrazine–Mercuric Sulfate Complex

Temperature ° C.	Excess $HgSO_4$, as Ml. 0.1 *N* NH_4CNS	0.1 *N* NH_4CNS Consumed by $HgSO_4$ + Complex, Ml.	Solubility of Complex, as Ml. 0.1 *N* NH_4CNS	Av
Room	10.16	10.64, 10.64, 10.59	0.48, 0.48, 0.43	0.46
Room	39.56	40.10, 40.14, 40.14	0.54, 0.58, 0.58	0.57
Room	61.31	61.93, 61.98, 61.94	0.62, 0.67, 0.63	0.64
5	40.71	41.13, 41.22, 41.25	0.42, 0.51, 0.54	0.49
35.3	40.71	41.22, 41.23, 41.21	0.51, 0.52, 0.50	0.51
			Av.	0.53

Table IV. Effect of Aging of Precipitate

Digestion at Room Temperature, Hours	Pyrazine Found, %[a]	Av.
0	99.0, 98.2	98.6
1	98.9, 99.8, 98.5	99.1
2	98.8, 99.5	99.2
16–20	98.3, 98.5, 98.7, 98.5, 98.6, 98.6, 98.3, 98.5	98.5
48	99.2, 98.8	99.0

[a] Pyrazine present, 98.6%.

be seen that the solubility correction is reproducible and does not depend upon the temperature of precipitation. It may possibly depend to some extent upon the excess of mercuric sulfate but the data involved do not indicate this with certainty.

Precision and Accuracy. The precision of the method depends to some extent on the sampling and the time of digestion of the precipitate. For best conditions—that is, overnight digestion and using aliquots of the same volumetric solution—the standard deviation of a single value from its mean is ±0.3% (absolute). This value is calculated from data on four samples with four to eight replicates on each. If the solutions are filtered within a period of 2 hours, no systematic error is encountered, but the precision may be somewhat poorer. An indication of the effect of time of precipitation with aliquots of the same solution is shown in Table IV.

Table V. Effect of Impurities

Substance Added	Added, Gram	Pyrazine Found[a], Gram	$\frac{\text{Gram Pyrazine Found}}{\text{Gram Pyrazine Present}} \times 100$
Piperazine	0.060	0.3050	101.0
	0.120	0.3022	100.1
Ethylenediamine	0.015	0.3030	100.3
	0.030	0.3006	99.5
Diethylenetriamine	0.060	0.3018	99.9
	0.120	0.3030	100.3
Ethanolamine	0.060	0.3014	99.6
	0.120	0.3038	100.6

[a] 0.3020 gram of pyrazine present.

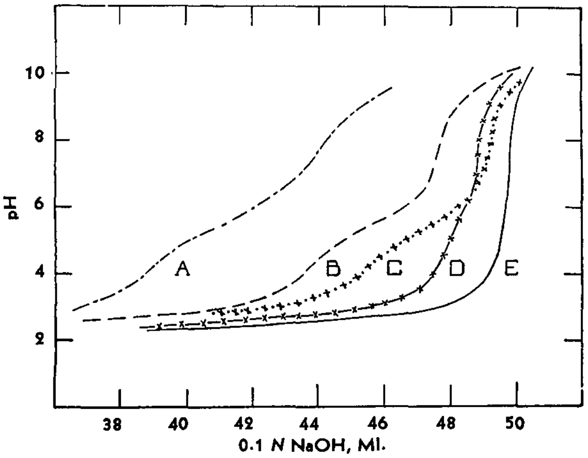


Figure 2. Titration of Impurities in Pyrazine Samples

Sample in 50 ml. of 0.1 N HCl titrated with 0.1 N NaOH

Curve	Sample	Weight, G.
A	8	1.76
B	1	2.18
C	5	2.53
D	2	2.35
E	Blank	

In regard to accuracy, there is no reason to believe that the method suffers from any systematic errors. A correction is applied for solubility of the complex, and the impurities most likely to be present, mainly diethylenetriamine, ethylenediamine, ethanolamine, and piperazine, do not interfere when amounts even up to 20 to 40% are present. The values obtained for the determination of pyrazine in the presence of these impurities are shown in Table V. A sample of pure pyrazine was not available, but a value of 99.1% pyrazine was obtained for one sample which contained 0.08% water and small amounts, probably tenths of 1%, of basic impurities.

METHOD FOR IMPURITIES

Basic substances that may be present in pyrazine because of the method of synthesis which was used may be determined by a potentiometric titration. The titration curves for the substances most likely to be present are shown in Figure 1. These curves have inflection points in the regions of pH 4 and 8. However, as there are at least four likely impurities and only two inflection points, it is impossible to give an unequivocal answer in terms of any one impurity or even any one type of impurity.

Procedure. A weighed sample of 2 to 5 grams is dissolved in 50.0 ml. of 0.1 N hydrochloric acid and titrated potentiometrically with 0.1 N sodium hydroxide, using a glass-calomel electrode system and a Beckman Model G pH meter. The shape of the curve obtained depends upon the amount and kind of the impurities present, but all of the samples exhibited two inflection points (Figure 2), one in the region of pH 4 and the other in the region of pH 8. Milliliters of 0.1 N sodium hydroxide to the inflection point at pH 4 may be expressed as C and to pH 8 as D. As several impurities may be present, the curve cannot be interpreted in terms of any one amine, so that it is necessary to report the results in terms of milliequivalents of alkali per gram of sample.

$$E = \text{milliequivalents of alkali consumed between pH 4 and 8 per gram of sample} = \frac{D - C}{10 \times \text{weight of sample in grams}} \quad (3)$$

$$F = \frac{50.0 - D}{10 \times \text{weight of sample in grams}} \quad (4)$$

$$G = \text{total milliequivalents of alkali consumed per gram of sample} = \frac{50.0 - C}{10 \times \text{weight of sample in grams}} \quad (5)$$

Discussion. G includes all the basic substances which may be present in the sample. E includes one equivalent of any di-acid base such as ethylenediamine and piperazine, and one equivalent of a tri-acid base such as diethylenetriamine. F includes strong mono-acid bases such as ethanolamine, one equivalent of piperazine and ethylenediamine, and two equivalents of diethylenetriamine.

From Table VI, a summary of the analysis of several samples, and from Figure 2 it may be seen that most of the samples contain relatively small amounts of these basic substances in view of the equivalent weight of the compounds concerned. Thus, samples 2 and 4 probably contain only a few tenths of 1% of these bases, while samples 1, 5, 6, and 8 contain considerably more, possibly 1 to 2%. Sample 7 contains considerably more im-

Table VI. Summary of Results

Sample	Pyrazine, %	*E*	*F*	% Water	Melting Range, ° C. (Cor.)
1	97.0, 96.5, 96.8, 96.4 Av. 96.7	0.174, 0.170 0.172	0.101, 0.117 0.109	0.23 0.19 0.21	49.5–52.2
2	98.6, 98.9 Av. 98.8	0.044	0.044	0.20, 0.20 0.20	51.6–53.1
3	98.3, 98.5, 98.7, 98.5, 98.3, 98.5, 98.6, 98.6 Av. 98.5	...	...	...	...
4	99.4, 99.8 98.9, 98.5 Av. 99.2	0.019	0.023	0.082 0.080 0.081	51.4–53.1
5	96.5, 96.0 Av. 96.3	0.137, 0.146 0.142	0.030, 0.038 0.034	...	...
6	91.1, 92.8 Av. 92.0	0.276	0.028	...	...
7	52.3, 52.6 Av. 52.5	3.03	1.89	...	(Liquid at room temp.)
8	94.5, 94.8, 94.8, 94.5 Av. 94.7	0.302	0.324	0.14, 0.15 0.15	45.9–50.4

purity than any of the other samples. It was a liquid while all the others were solids. In addition, it would appear that some of these samples contain an impurity which is not any of those considered, as all the latter compounds would give values for F which would be either equal to or greater than E. It is probable that these unknown impurities are weaker bases than those being considered.

Determination of Water. Water may be determined in the various pyrazine samples by the Karl Fischer reagent in the usual manner. A stable end point could be obtained.

LITERATURE CITED

(1) Beilstein, F., "Handbuch der organischen Chemie," 4te Auflage, Bd. XXIII, p. 91, Berlin, Julius Springer, 1936; Ann Arbor Mich., Edwards Bros., 1944.
(2) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis," p. 172, New York, John Wiley & Sons, 1929.
(3) Pfann, H. F., *J. Am. Chem. Soc.*, **66**, 155 (1944).
(4) Stoehr, *J. prakt. Chem.*, **51**, 459 (1895).

RECEIVED for review April 21, 1952. Accepted September 4, 1952. Presented before the Division of Analytical Chemistry at the 121st Meeting of the AMERICAN CHEMICAL SOCIETY, Buffalo, N. Y., and before the Meeting-in-Miniature of the North Jersey Section, AMERICAN CHEMICAL SOCIETY, January 9, 1950.

Paper Chromatographic Separation and Determination of Some Water-Soluble Vitamins

JAMES A. BROWN AND MAX M. MARSH
Eli Lilly and Co., Indianapolis, Ind.

With the large number of multiple vitamin preparations now being manufactured, the problem of adequate routine analytical control of these mixtures is becoming increasingly complex. As a potentially simpler general technique, paper chromatographic separation procedures for various vitamins were studied. It was found that four of the commonly encountered B complex vitamins—thiamine salts, riboflavin, nicotinamide, and pyridoxine hydrochloride—were readily separable on paper strips in a butanol–acetic acid–water system. By means of an automatic scanning device adapted for the Cary recording spectrophotometer, curves were plotted of total ultraviolet absorption *vs.* position on the strip. Quantitative results were obtained by measuring the areas of the discrete zones of absorption corresponding to each component and comparing them to those obtained from standard solutions. Preliminary studies indicate that the quantitative technique devised is sufficiently reproducible to be useful as a control method for certain types of pharmaceutical preparations. A single procedure may be utilized to analyze mixtures of many different and not necessarily related compounds.

EXAMINATION of the literature reveals the work of many on paper chromatography. It has become an accepted tool of the analytical chemist for the separation of closely related materials. With particular reference to vitamins, Hais and Pecáková (*7*) have used this procedure for separating riboflavin, Beran and Sicho (*1*) for thiamine, Kodicek and Leddi (*9*) and Huebner (*8*) for nicotinamide, and Wollish, Schmall, and Shafer (*13*) for nicotinic acid.

In this paper it is shown how four synthetic vitamins of the B complex—thiamine salts (hydrochloride or monitrate), riboflavin, nicotinamide, and pyridoxine hydrochloride—can be simultaneously separated, identified, and quantitatively estimated by means of the paper chromatographic technique, the apparatus for automatically scanning paper strips with the Cary recording spectrophotometer described by Parke and Davis (*10*), and the correlation of area measurements of the peaks on the resulting graphs.

Somewhat similar methods for the quantitative evaluation of components separated on a paper strip have been described (*2–6, 11, 12*). All of these make use of a relation between concentration and a measure of zone dimension—i.e., maximum absorbancy, total absorbing area, or length. In all cases, precision has been rather unsatisfactory and the discontinuous process of measurement very tedious.

The present method for plotting the absorbancy of the zones continuously in order to obtain accurate zone measurements is rapid and the precision obtainable is much greater than that outlined in other procedures.

EXPERIMENTAL

The paper chromatographic portion of the work involved the development of chromatograms on $^{17}/_{32}$ × 22.5 inch strips of Whatman No. 1 filter paper cut parallel to the grain of the paper. Ascending technique was used, the solvent being placed in the bottom of 12 × 24 inch cylindrical glass jars having flat, close-fitting glass lids. The jars were wrapped with opaque paper to prevent the destructive action of light. Jars made of nonactinic red glass have subsequently been obtained and are more convenient to use.

The solvent system finally chosen was the upper phase obtained by shaking together 40 parts of reagent grade *n*-butyl alcohol, 5 parts of reagent grade glacial acetic acid, and 55 parts of distilled water (by volume). This solvent is similar to that of Hais and Pecáková (*7*), but contains less acetic acid.

The vitamins (purest commercial grade available)—individually and in mixtures—were dissolved in 50% (v./v.) acetic acid in water (heating, if necessary) and finally diluted so the resulting solution was 10% (v./v.) acetic acid.

A 0.0175-ml. sample was applied 1.5 inches from the end of each strip. It was measured with a capillary pipet made from a common 0.09-ml. antibiotic pipet. The chamber of an antibiotic pipet was drawn out to a small volume and the outside dimension of the tip was reduced to about 1.5 mm. The chamber was filled by capillary action and discharged by the capillary action of the paper strip when the strip was touched to it. It was calibrated with distilled water, using a semimicrobalance to weigh the water delivered, and was found to discharge 0.0175 ± 0.0002 ml.

The strips were dried in a 50° C. oven after the sample was applied, 10 minutes' time in the oven being sufficient. They were then suspended in the jar with the end containing the sample dipping into the solvent in the bottom of the jar to a depth of approximately 0.25 inch. The lid was lubricated with stopcock grease and weighted to make a tight fit. The strips were developed overnight (15 hours) at room temperature and then dried in a 50° oven for 15 minutes.

The principal scanning to provide measurable areas was performed at predetermined wave lengths; absorbancy *vs.* a linear function of strip length was recorded as outlined by Parke and Davis (*10*). The instrument was set near its maximum sensitivity, the slit setting being about 2.75 mm. The slit width changed somewhat during the course of a run to compensate for voltage or light intensity changes.

Figure 1 shows the graphs of a typical strip both before and after development.

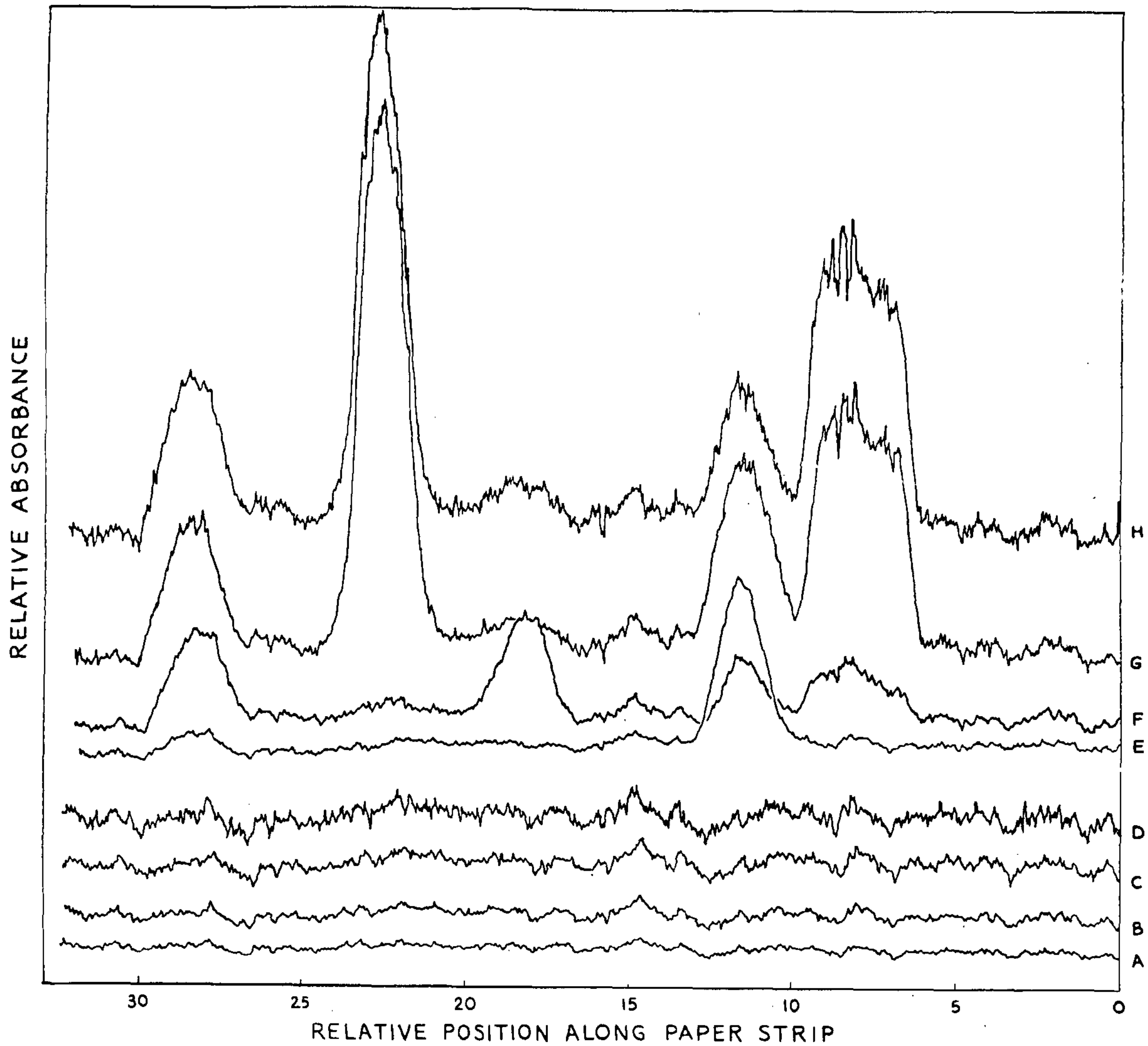


Figure 1. Scan of Filter Paper Strip before and after Chromatography

A. Before, at 370 mμ
B. Before, at 297 mμ
C. Before, at 264 mμ
D. Before, at 240 mμ
E. After, at 370 mμ (riboflavin)
F. After, at 297 mμ (pyridoxine hydrochloride)
G. After, at 264 mμ (nicotinamide)
H. After, at 240 mμ (thiamine mononitrate)

In this work, individual known solutions of a vitamin were compared to known solutions of a mixture of the four. In practice, unknown solutions of a vitamin preparation would be compared to known solutions of a mixture of the same vitamins. The area of each peak in the series was measured, after the base line had been inserted (see discussion), with a Keuffel and Esser polar planimeter readable to 0.01 sq. inch. Table III shows the resulting data.

DISCUSSION

The paper strips $^{17}/_{32}$ inch wide were used because the dimensions of the scanner are such that this width permits the maximum exposure of the zone to the light beam of the instrument. The beam width of the spectrophotometer was found to be about 0.5 inch at the phototube end of the cell compartment. It was further found that the paper must extend a minimum of $^{1}/_{64}$ inch beyond each side of the aperture to afford a guiding surface; the result was a $^{17}/_{32}$-inch strip which allowed 94% of the strip width to be scanned. When a strip wider than $^{17}/_{32}$ inch was used, a correspondingly lower percentage of the strip was scanned and the accuracy was decreased because of a variability in the lateral distribution of the material in the band. Subsequently it seemed desirable to redesign the scanner mechanism to incorporate an aperture $^{15}/_{32}$ inch wide in order to use a 0.5-inch strip, since 0.5-inch rolls of Whatman filter paper are a stock item. The reduction in sensitivity due to the slightly smaller aperture is not significant.

The cutting of 18 × 22.5 inch filter paper sheets parallel to the grain of the paper gave strips of a convenient length; the final results were as good as or superior to those obtained when strips cut perpendicular to the grain were used.

Whatman No. 1 paper was chosen because it has the most uniform structure of several types examined, thus giving the least variability in the background absorption. It was found that other types of paper sometimes contained impurities which seemed to destroy some of the vitamins. As is shown in Figure 1, there is considerable variation in background absorption of the paper strips. It is this variation which at present is one obstacle to the automatic integration of the curves and the resultant automatic evaluation of area. Improvements in this technique await the development of a more uniformly light-absorbing material possessing the capillary properties of paper.

Table I. Solvent Systems Tried for Vitamin Chromatography

System (Percentages Given by Volume)	Reason for Rejection
Ethyl acetate 48%–acetic acid 9%–water 43%	Too little travel of B_1 and B_2
Benzyl alcohol 40%–acetic acid 10%–water 50%	Too much travel of all vitamins
Butyl alcohol 48%–concd. HCl 2%–water 50%	Too little travel of all vitamins
Butyl alcohol 40%–acetic acid 20%–water 40%[a]	Too much travel of all vitamins
Butyl alcohol 40%–acetic acid 15%–water 45%[a]	Too much travel of all vitamins
Butyl alcohol 40%–acetic acid 13%–water 47%	Too much travel of all vitamins
Butyl alcohol 49%–acetic acid 1%–water 50%	Too little travel of B_1 and B_2
Butyl alcohol 50%–water 50%	B_1 and B_2 ran together.

[a] These systems were miscible and were used as such. The rest were not miscible and the water poor layer was used in all cases.

The solvent system chosen seemed to give the best band separation for the conditions of concentration and component limitations with which this paper is concerned. Table I gives a number of other systems tried and the reasons for their rejection.

The dissolving procedure and solvent for the vitamin samples were necessary because of the difficult solubility of riboflavin. Table II gives some other possible solvents, of which only the pyridine-water system seems feasible. It would be desirable to have a solvent volatile enough to dry quickly, if drying of the strips before development is desired. Drying is not absolutely necessary with the dilute acetic acid; however, when not dried, there is a pronounced tendency for the thiamine to appear as a double peak and there is some tendency for thiamine and riboflavin to run together.

The four vitamins were used in the chosen proportions because those ratios are commonly encountered in multivitamin preparations. The proportions vary among various products, but in nearly all cases where both nicotinamide and pyridoxine hydrochloride are present, the proportion of the former to the latter is of the order of 15 to 1.

Table II. Solvents for Vitamin Mixtures Containing Riboflavin

Solvent (Percentages Given by Volume)	Characteristics with Respect to Riboflavin
Water	Dissolves too slowly
10% acetic acid in water	Dissolves too slowly
50% acetic acid, then diluted to 10%	Dissolves in hot 50% acetic acid
Acetone	Maximum solubility too low
80% acetone in water	Solution O.K. when warm, supersaturated when cold
80% dioxane in water	Riboflavin destroyed
80% pyridine in water	Apparently satisfactory

The size of the samples used was near the maximum thought to be allowable. It seemed desirable to keep the area of the sample spot as small as possible. A length of about $^5/_8$ inch at the point of application of the sample to the strip appears to be about maximal; this corresponds to a sample size of about 0.02 ml. At the same time the volume of the sample must be kept as large as possible because of the limited solubility of riboflavin and the small amount of pyridoxine hydrochloride present. The highest concentrations shown in Table IV are maximal for the conditions described. If the absolute amount of nicotinamide on a strip were much higher than that given, the recorder pen would be driven off the chart. The area resulting from pyridoxine hydrochloride is low, but seems to be adequate. It has been possible to obtain higher concentrations of riboflavin in the dilute acetic acid, but such solutions are supersaturated. The drying of the strip after development is, of course, necessary. Because acetic acid absorbs in the ultraviolet region, even small amounts may affect the absorption pattern. However, trouble from solvent residues was never a problem if the strips were dried for 15 minutes at 50° C. It was found to be necessary to protect the strips from light at all times. The riboflavin band fades rapidly if exposed to daylight. When being scanned, the exposed length of strip was always covered.

The wave lengths used to obtain the graphs of a typical strip in Figure 1 are those of peak absorption of each substance and were determined originally on a similar strip by scanning at an approximate wave length to locate the bands and then positioning each band in the spectrophotometer beam and recording absorbancy *vs.* wave length in the conventional manner. The absorption spectra for the typical strip are shown in Figure 2. The peak wave lengths are constant for the solvent system used, but may change in other systems.

In all the spectrophotometry in this work it proved necessary to use a piece of untreated paper in the reference beam rather than to utilize the internal alternating current reference feature of the Cary instrument. The iris diaphragm attachment (*10*) was used for this purpose.

The characteristic peaks along the strip for each vitamin are very evident in Figure 1. The R_f value (location on the strip) of a band is constant in any given system and serves to help identify the substance. The peaks at the extreme left are located at the solvent front and are due to impurities in the system. These impurities are not detrimental and the peak serves the useful purpose of marking the front. The pattern resulting from the scanning is shown to be highly reproducible on the average except for the low amplitude "wiggle" of the line due to background "noise" in the instrument. This noise is characteristic of the Cary instrument when operating at the highest sensitivity

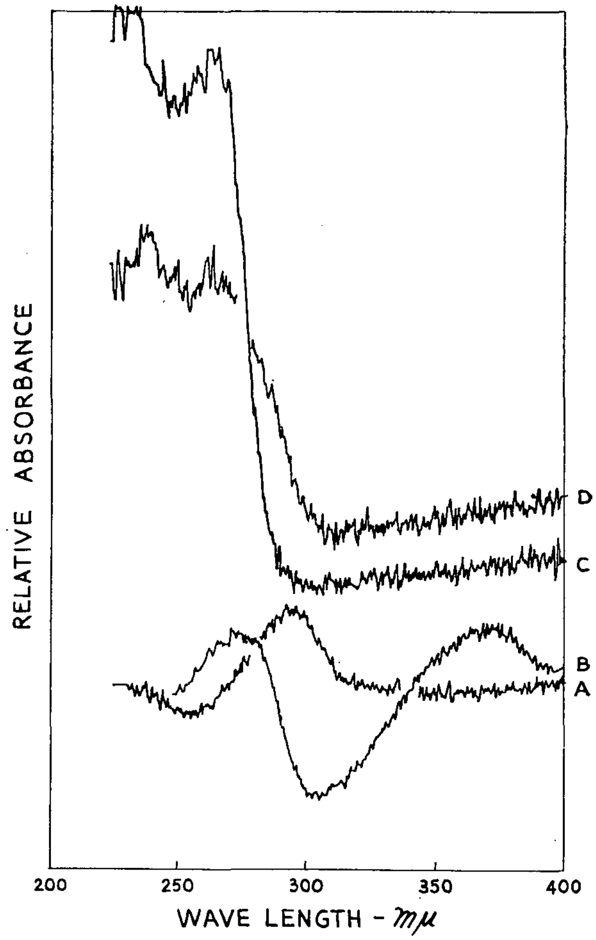


Figure 2. Ultraviolet Absorption Spectra of Zones on Paper Chromatogram

A. Pyridoxine hydrochloride
B. Riboflavin
C. Nicotinamide
D. Thiamine mononitrate

Table III. Concentration-Area Data for Four Vitamins Individually and Mixed Together

Solution A. 0.3331 g. nicotinamide per 100 ml.
0.0222 g. pyridoxine hydrochloride per 100 ml.
0.0530 g. riboflavin per 100 ml.
0.1112 g. thiamine mononitrate per 100 ml.
Solution B. 0.3331 g. nicotinamide per 100 ml.
Solution C. 0.0222 g. pyridoxine hydrochloride per 100 ml.
Solution D. 0.0530 g. riboflavin per 100 ml.
Solution E. 0.1112 g. thiamine mononitrate per 100 ml.

	Solutions of Individual Vitamins							
	Solution B λmax 264 mμ		Solution C λmax 297 mμ		Solution D λmax 370 mμ		Solution E λmax 240 mμ	
	1st day	2nd day	1st day	2nd day	1st day	2nd day	1st day	2nd day
Zonal areas, sq. inches	5.16	5.24	1.01	1.11	1.82	1.78	5.17	4.65
	5.47	5.19	1.06	1.11	1.85	1.73	5.07	4.72
	4.89	5.24	1.01	1.04	1.80	1.71	4.84	5.00
	5.69	5.57	1.03	1.09	1.82	1.73	5.06	4.96
Average	5.30	5.31	1.03	1.09	1.82	1.74	5.04	4.83
Dev. from av., %	+7.4	+4.9	+2.9	+1.8	+1.6	+2.3	+2.6	+3.5
	−7.7	−1.3	−1.9	−4.6	−1.1	−1.7	−4.0	−3.7
	Mixture of Four Vitamins (Solution A)							
	Nicotinamide λmax 264 mμ		Pyridoxine HCl λmax 297 mμ		Riboflavin λmax 370 mμ		Thiamine NO_3 λmax 240 mμ	
	1st day	2nd day	1st day	2nd day	1st day	2nd day	1st day	2nd day
Zonal areas, sq. inches	5.42	4.94	1.01	1.05	1.66	1.57	4.86	4.60
	5.41	4.93	1.10	0.98	1.67	1.63	5.01	4.68
	5.57	5.15	0.98	1.05	1.66	1.72	4.82	5.01
	5.37	5.36	0.96	1.05	1.78	1.75	4.90	4.94
Average	5.44	5.10	1.01	1.03	1.69	1.67	4.90	4.86
Dev. from av., %	+2.4	+5.1	+9.0	+1.9	+5.3	+4.8	+2.2	+4.2
	−1.3	−3.3	−5.0	−4.9	−1.8	−6.0	−1.6	−4.4

Four area values for each solution on each day represent replicate strips. Entirely new solutions were made for the second day's run, the concentration of both being identical.

(maximum dynode voltage), but has not so far been detrimental to the results of this work. The background pattern other than the noise makes the prescanning of the strips to be used absolutely necessary for quantitative work in order that a valid base line can be traced in for each peak. However, in practice it has not been found necessary to prescan at each wave length. Because the noise characteristics of the paper do vary somewhat with wave length, it was found desirable to run a base line at 370 mμ and one at 264 mμ, the latter being used for the 240 and 297 mμ traces as well. It was demonstrated experimentally that development of a "blank" strip in the solvent system did not alter the background absorption appreciably.

Table III shows among other things the reproducibility of the area measurements. It can be seen that the spread is on the average below 12%. For small areas the spread tends to be greater on a percentage basis than for large ones and occasionally the spread for areas of any size goes above the 12% value. Some trouble has been experienced with occasional apparent variations in the speed of the paper through the scanner which have affected the areas at any time a speed variation occurs while a band is passing the aperture. The occasional instances where one area is markedly different from the rest have been attributed partly to the speed variation and partly to variations in the lateral distribution of the vitamin in a band. The causes of the speed variations have not been fully determined, but at least part seems to be due to the soft character and the slightly varying thickness of the rubber on the rolls. A somewhat different design for the roller, which it is believed will eliminate the trouble from this source, is being developed. Often there is remarkably good agreement among replicates, which leads to the thought that ultimately as techniques and equipment improve and the causes of the variations are determined, the precision could increase considerably. As it requires very little additional effort to run a number of replicate strips per sample, adequate data can be obtained which will permit statistical evaluation of the results with almost any desired accuracy.

Table IV and Figure 3 show the results of a run at several concentrations of a given proportion of the four vitamins. The graphs of log concentration *vs.* log area show that the relationship gives a straight or nearly straight line when thus plotted. This corresponds to an equation of the type $A = kC^n$, where k and n are constants. Through further refinements it may ultimately be possible to establish a standard curve which would apply to all determinations under a given set of conditions.

The Cary recording spectrophotometer has proved to be an ideal instrument for this application. The sensitivity is high and the response of the recorder pen extremely rapid. With it, the ultraviolet spectrum of a given band can be rapidly determined. However, where such an instrument is not available, it may still be possible to apply the method. The authors have determined that a Beckman DU spectrophotometer with a Beckman photomultiplier attachment can be used to measure the absorbancies of bands on the paper strips with adequate sensitivity. A scanning mechanism similar to the present one has been designed for attachment to the Beckman DU monochromator and it is proposed to measure the per cent transmittance by using a

Table IV. Concentration-Area Data for Four Vitamins at Various Concentrations

Solution	1	2	3	4	5
Nicotinamide, g. per 100 ml.	0.0800	0.1600	0.2400	0.3200	0.4000
Pyridoxine HCl, g. per 100 ml.	0.0080	0.0160	0.0240	0.0320	0.0400
Riboflavin, g. per 100 ml.	0.0120	0.0240	0.0360	0.0480	0.0600
Thiamine NO_3, g. per 100 ml.	0.0280	0.0560	0.0840	0.1120	0.1400

	Zonal Areas, Square Inches				
	Nicotinamide, max. 264 mμ	Pyridoxine, max. 297 mμ	Riboflavin, max. 370 mμ	Riboflavin, max. 275 mμ	Thiamine, max. 240 mμ
Solution 1	2.12	0.48	0.52	0.97	1.37
	1.96	0.39	0.44	0.89	1.41
	2.00	0.41	0.49	0.89	1.46
	2.05	0.41	0.51	0.95	1.36
Av.	2.03	0.42	0.49	0.93	1.40
Solution 2	3.35	0.77	0.91	1.58	2.57
	3.36	0.75	0.95	1.45	2.49
	3.41	0.71	0.84	1.39	2.72
	3.55	0.78	0.91	1.68	2.99
Av.	3.42	0.75	0.90	1.53	2.69
Solution 3	4.35	1.12	1.29	1.93	3.40
	4.29	0.99	1.27	1.97	3.80
	4.39	0.98	1.25	1.94	3.82
	4.72	1.22	1.26	2.00	3.81
Av.	4.44	1.08	1.27	1.96	3.71
Solution 4	5.50	1.22	1.61	2.26	4.53
	6.05	1.28	1.57	2.30	4.80
	5.92	1.33	1.56	2.15	4.58
	5.80	1.40	1.51	2.20	4.85
Av.	5.82	1.31	1.56	2.23	4.69
Solution 5	6.18	1.75	1.89	2.30	5.47
	6.37	1.61	1.88	2.37	5.60
	6.75	1.61	1.97	2.37	5.03
	6.26	1.62	1.94	2.59	5.24
Av.	6.39	1.65	1.92	2.41	5.34

Each set of four values represents replicate strips.

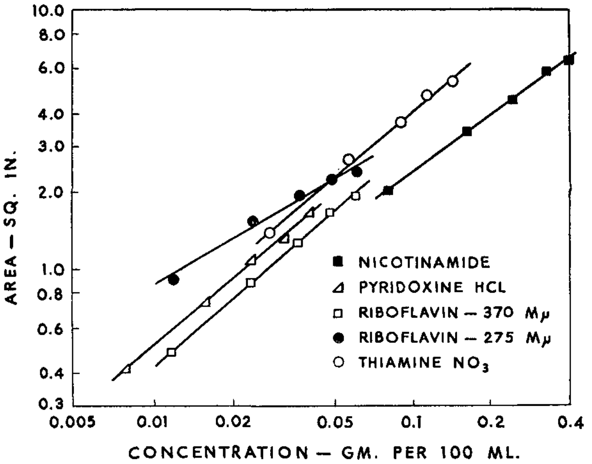


Figure 3. Log-Log Plot of Concentration *vs.* Area

photomultiplier tube and an amplifier to drive a fast-acting recorder. Thus, after the peaks for a given system have been determined (which can be done manually with the assembly described above), all the information needed can be obtained with a setup which is considerably less expensive than the Cary instrument. However, it is not possible at this time to report data obtained on such a setup, because of the slow delivery of suitable instrumental components. Plotting of per cent transmittance rather than density *vs.* time would seem to have some advantages, as the areas of the less intense bands would tend to be greater in relation to those of the more intense bands. The area-concentration relationships would no longer produce a substantially straight line on log-log paper but might do so on a semilog chart.

The development of this application of a new tool for the analytical chemist has involved only the four vitamins discussed for the sake of expediency. Work on several other vitamins as well as on other substances is in progress. It is believed that with special precautions to prevent deterioration, the method can be extended to include applications to many now difficult separation problems. The quantitative application of this technique may prove useful in evaluating natural vitamin sources.

ACKNOWLEDGMENT

Acknowledgment is made to W. W. Hilty of these laboratories for valuable suggestions and assistance and to Beckman Instruments, Inc., Chicago Office, for the loan of a photomultiplier attachment.

LITERATURE CITED

(1) Beran, M., and Sicho, V., *Chem. Listy*, **45**, 154–6 (1951).
(2) Block, R. J., *Science*, **108**, 608 (1948).
(3) Bull, H. B., Hahn, J. W., and Baptist, V. H., *J. Am. Chem. Soc.*, **71**, 550 (1949).
(4) Fisher, R. B., Parsons, D. S., and Morrison, G. A., *Nature*, **161**, 764–5 (1948).
(5) Fosdick, L. S., and Blackwell, R. Q., *Science*, **109**, 314–15 (1949).
(6) Fowler, H. D., *Nature*, **168**, 1123–4 (1951).
(7) Hais, I. M., and Pecáková, L., *Ibid.*, **163**, 768 (1949).
(8) Huebner, C. F., *Ibid.*, **167**, 119–20 (1951).
(9) Kodicek, E., and Leddi, K. K., *Ibid.*, **168**, 475–6 (1951).
(10) Parke, T. V., and Davis, W. W., ANAL. CHEM., **24**, 2019 (1952).
(11) Rockland, L. B., and Dunn, M. S., *J. Am. Chem. Soc.*, **71**, 4121 (1949).
(12) Tennent, D. M., Whitla, J. B., and Florey, K., ANAL. CHEM., **23**, 1748–9 (1951).
(13) Wollish, E. G., Schmall, M., and Shafer, E. G. E., *Ibid.*, **23**, 768–71 (1951).

RECEIVED for review July 7, 1952. Accepted September 8, 1952. Presented before the Division of Analytical Chemistry at the 122nd Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Determination of Free Acid in the Presence of Hydrolyzable Ions

LEONARD P. PEPKOWITZ, WILLIAM W. SABOL, AND DRAGOMIR DUTINA
Knolls Atomic Power Laboratory, General Electric Co., Schenectady, N. Y.

AN IMPORTANT and recurring problem is the determination of the free acid in the presence of hydrolyzable ions such as iron, chromium, aluminum, etc., or mixtures of such ions. Direct pH measurements are impossible in solutions of high acidity (greater than 1 *M*) with currently available electrodes (*3*); while in the interval between pH 0 and pH 1 the inherent error of measurement, ±0.1 to 0.3 pH unit in simple solutions, is prohibitive for the required determination, even without considering the effect of the high ionic strength of the test solutions. The dilution method, in which the pH is measured following dilution to an appropriate acid concentration range, is empirical and valid only if the solution contains one appreciable hydrolyzed ion and the concentration of this ion is accurately known, so that the measured pH can be corrected. A similar approach has recently been described for mixtures of ferric and chromic ions and chromic and aluminum ions by titrating the mixture and correcting for the hydrolyzed ions present (*6*). A method for the determination of nitric acid in aluminum nitrate solutions has been described by Blaedel and Panos (*2*).

The method is based on the conductometric titration of the free hydrogen ion by a strong base (sodium hydroxide) in the presence of a complexing agent. Previous to the development of this procedure, a large number of scouting experiments were performed to investigate the applicability of nonaqueous systems (*4*, *5*) for the determination. Such solvents as glacial acetic acid, formic acid, absolute methanol, absolute ethanol, and ethylene glycol–isopropyl alcohol were tried. However, none was completely satisfactory, although the methanol and formic acid systems showed some promise. A variety of bases, both organic and inorganic, were tried using electrometric and colorimetric methods for indicating the end points. All of these approaches were defective in that they did not prevent the interference by hydrogen ion released during hydrolysis, although some of the systems minimized this effect. As long as a complexing agent was required, it was simpler to utilize an aqueous system.

The actual mechanism of the hydrolysis of an ion such as iron is not as straightforward as usually indicated. Not only does the hydrolysis proceed through the formation of $FeOH^{++}$ and $Fe(OH)^{+}_2$, but the composition of the final precipitate varies according to the dilution. Evidence is available which indicates the presence of other acid complexes, such as ferrisulfuric acid, $(FeOH)(HSO_4)_2$. The various aspects of this complicated problem are thoroughly discussed by Arden (*1*). Analogous situations occur for aluminum and chromium.

The work was undertaken because a generalized method was needed for the determination of free acid in the presence of hydrolyzable ions in connection with rate and equilibrium studies. A conductometric titration procedure is described for the determination of free hydrogen in complex solutions containing such ions as iron, nickel, chromium, aluminum, and magnesium. The method has been applied to sulfuric acid, nitric acid, or mixtures of these acids in the presence of the ions listed. A standard deviation for precision of ±0.0016 was obtained, with a limit of sensitivity of 0.003 millimole of free acid. Because such small amounts of acid can be handled, the method is applicable to very small aliquots (100 microliters or less), which makes it especially useful for highly radioactive solutions. A variation of the procedure is described for the determination of acid deficiency in salt solutions.

In the course of the scouting experiments it was observed that the free acid–strong base equivalence point occurred at a pH of approximately 3, where the hydrolysis of iron and aluminum was starting with a release of hydrogen ion. This is also pointed out by Arden (*1*). The equivalence point could be detected conductometrically, but the titration curve was diffuse at the end point because the resulting $FeOH^{++}$ was a good conductor. The results were always high because of the released hydrogen ions. This behavior is similar to that indicated in Figure 4, *A*.

However, if the hydrolysis of the iron, etc., could be at least partially suppressed by an appropriate complexing agent, so that the acid-base end point could be attained without the hydrolysis complications, the free acid concentration could be determined quantitatively. This approach was borne out in practice. Typical titration curves are shown in Figure 4 (*B*, *C*).

Potentiometric detection of the end point was unsatisfactory, as the break was not sharp and was insensitive, because low acid concentrations gave no discernible breaks. Conductometric measurement, however, provided the required sharpness and sensitivity, even though the technique is not usually applied to solutions of such complexity.

APPARATUS

The conductivity measurements were made with a Leeds and Northrup Wheatstone bridge (No. 4760) utilizing an alternating current galvanometer (Leeds and Northrup No. 2370) to indicate the null point. The 60-cycle line current was used for energizing the bridge. This was taken off the dropping resistors in the galvanometer to ensure that the galvanometer and the bridge were in phase. This energizing current was limited by a simple potentiometer circuit to 2 volts to minimize polarization effects at this low frequency. The whole system was isolated by a 1 to 1 isolating transformer to eliminate capacitance effects with ground. In addition a 400-ohm resistor, R_1, Figure 1, was used across the moving coil of the galvanometer with solutions of initial low resistances (less than 100 ohms) in order to balance the galvanometer. At low initial resistances in the electrode circuit, the relatively high capacitance current caused a phase shift in the galvanometer armature. Resistance R_1 acted as a capacitance shunt, thus minimizing the effect. At high initial resistances the capacitance phase shift effect was not evident, so that the shunt could be removed with an attendant increase in sensitivity. The wiring diagram is shown in Figure 1.

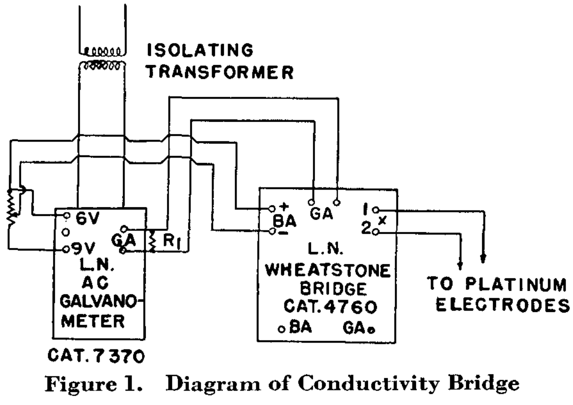


Figure 1. Diagram of Conductivity Bridge

The electrodes were of the conventional dipping-type construction and can be fabricated of either platinum foil or wire. Both types were used in the course of this investigation and gave equally good results. The foil electrodes were 0.64 sq. cm. in area and were rigidly held 1 cm. apart by glass rod fused to the corners. The wire type were of 60-mil wire, 1.5 cm. long, and similarly held 1 cm. apart by glass cane. Both types were platinized by the usual electrolytic procedure. A cell for remote operation utilizing wire electrodes is shown in Figure 2. Stirring was accomplished magnetically.

PROCEDURE

Add an appropriate sample, 100 microliters or less dependent on the activity of the sample, to 20 ml. of distilled water in the titration vessel. In the early stages of the work a 50-ml. beaker was used. Rinse the pipet quantitatively and add a calculated amount of 0.5 *M* sodium fluoride based on 3 mmoles of fluoride ion per millimole of cation. This will ensure sufficient fluoride to complex the iron and other ions in the sample. The influence of excess fluoride is discussed below.

Assemble the conductivity cell, including the stirring bar, and stir to mix the sample thoroughly. The stirrer remains in operation throughout the determination. Release the galvanometer needle clamp and lock the *GA* key on the bridge into position. With the ratio dial set at 0.1, balance the bridge by successively adjusting the bridge resistances and tapping the *BA* key until the galvanometer indicates a balance. This is the initial resistance of the test solution.

Titrate by adding 0.5 *N* sodium hydroxide in 0.1-ml. increments, balancing the bridge after each addition. The efficiency of the magnetic stirrer is such that no prolonged waiting period is required between additions to attain equilibrium. Four or five points should be determined on each side of the end point, if possible. The acid side is represented by increasing resistance and the basic side by decreasing resistance.

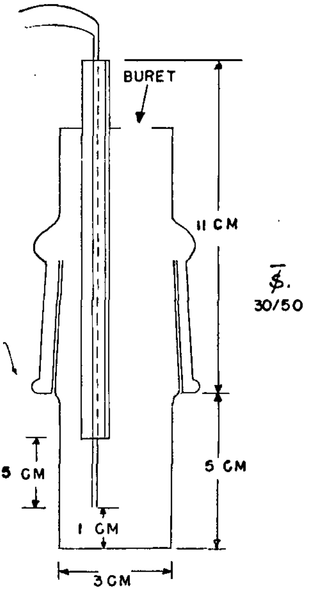


Figure 2. Complexing Action of Sodium Fluoride

Plot the experimental values against the milliliters of base added and draw the best straight lines through the two sets of points. The intersection denotes the end point of the determination.

EXPERIMENTAL

Following a few recovery experiments which indicated the feasibility of the method, a detailed study of the behavior of two hydrolyzable ions, aluminum and iron, was made.

An aluminum nitrate solution (1.45 *M*) was prepared by dissolving 13.4 grams of aluminum nitrate nonahydrate in 25 ml. of distilled water. This served as a stock solution for the subsequent experiments. Then 100-microliter samples of this solution were taken for the individual determinations and added to 20 ml. of distilled water in the titration vessel. This volume of water ensured complete coverage of the electrodes and provided a convenient initial resistance (150 to 200 ohms). A known amount of nitric acid was added and the aluminum was complexed with sodium fluoride as described in the procedure.

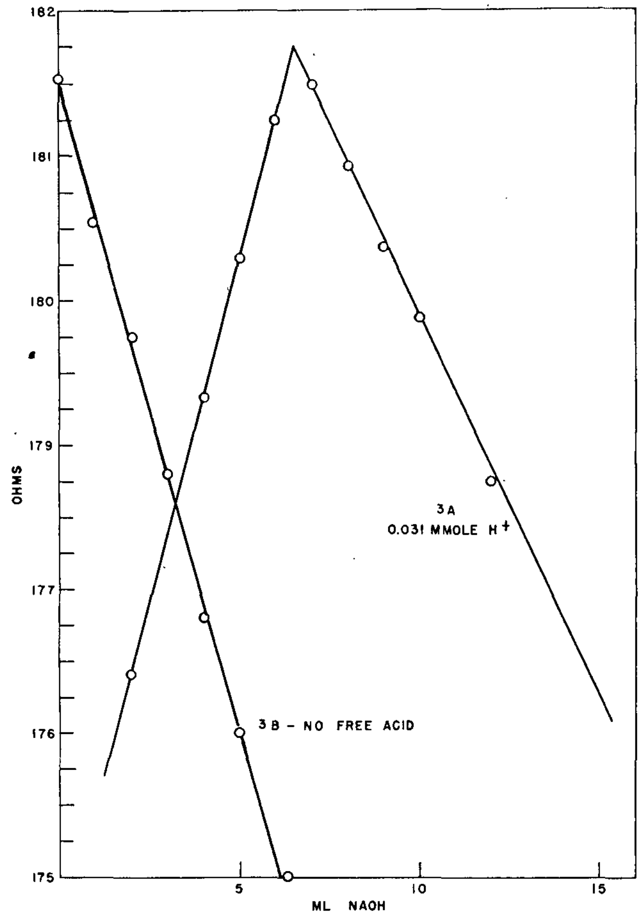


Figure 3. Titration of Synthetic Mixture

Table I. Recovery of Known Amounts of Acid in Presence of Aluminum

Acid Added, Mmole	Acid Found, Mmole		Difference, Mmole
0.243	0.248		+0.005
0.122	0.122		0.000
0.049	0.045		−0.004
		Av.	±0.003

The recoveries of the known amounts of acid in the presence of aluminum are given in Table I.

A similar study was made on the influence of iron on the determination of free acid, using fluoride as the complexing agent. Results comparable to those in Table I were obtained. The titration curves for both the aluminum and iron have the typical strong acid–strong base form.

Four test solutions were prepared, containing known amounts of free acid (nitric and sulfuric). These solutions contained in addition to aluminum (1.5 *M*) the following cations in 0.1 *M* concentration: iron, chromium, nickel, manganese, and magnesium. The solutions were submitted as unknowns, so as to eliminate any bias in construction of the titration curves on the part of the analyst; 100-microliter aliquots were used for the determination. The base was 0.047 *N* sodium hydroxide.

The results are given in Table II and two of the titration curves are reproduced in Figure 3.

The curves in Figure 3 are self-explanatory, but curve *B* points up an important attribute of the method. No blank is involved in the determination, which might be caused by a slight hydrolysis resulting in the liberation of hydrogen ion. This is indicated in *B*, Figure 3, by an immediate decrease in resistance with the first addition of base.

The mode of action of a complexing agent such as fluoride in a simple system containing a single hydrolyzable ion can be predicted. In the case of insufficient fluoride, the hydrolysis will produce a very diffuse end point, since the first hydrolysis products, such as $Fe(OH)^{++}$ are good conductors. With an excess of fluoride, slightly ionized hydrofluoric acid is formed by a metathesis with the strong acid present, so that the titration curve is typical of a very weak acid–strong base reaction.

The optimum conditions for the complex solutions were determined experimentally by adding various amounts of fluoride to 100-microliter aliquots of the test solution containing 0.039 mmole of hydrogen ion.

The curves in Figure 4 illustrate the results. *A* represents the effect of insufficient fluoride; *B* and *C* portray the effective concentrations; *D* shows the effect of excess fluoride ion. These results were as predicted above and indicate that the allowable excess fluoride ion cannot exceed 25% of the stoichiometrical amount required, assuming 3 mmoles of fluoride per millimole of aluminum.

During the earlier stages of this investigation, it was observed that ferricyanide was as effective as fluoride as a complexer for iron. In some of the early determinations a mixture of fluoride and ferricyanide was used, but subsequent results indicated that fluoride alone was sufficient.

The precision of the procedure was ascertained by repeated analyses of two levels of hydrogen ion concentration in synthetic solutions; 100-microliter aliquots were added to 20 ml. of distilled water and complexed with 1.0 ml. of 0.5 *M* sodium fluoride. The titrant was 0.047 *N* sodium hydroxide. The data obtained are given in Table III.

The limit of sensitivity of the procedure was found to be 0.0030 mmole of free acid in the complex mixtures. As an average of 5 determinations, the acid found was 0.0024 mmole as compared with 0.0030 mmole added. This corresponds to a standard deviation of ±0.0002. Extreme care must be taken when working at this level. The electrodes must be washed thoroughly to prevent carry-over of base from the preceding titration. The changes in cell resistance are small (0.5 to 1 ohm) with an associated difficulty in balancing the galvanometer. It was also necessary to dilute the titrant concentration to 0.018 *N*.

This series of experiments, indicates, however, that very small aliquots of highly radioactive solutions containing a sufficient concentration of hydrogen ion can be handled by the conducto-

Table II. Recovery of Added Acid from Test Solutions

Acid Added, Mmole	Acid Found, Mmole		Difference, Mmole
0.030	0.031		+0.001
0.020	0.021		+0.001
0.010	0.010		0.000
0.000	0.000		0.000
		Av.	±0.0005

Table III. Precision of Conductometric Method

Acid Added, Mmole	Average Acid Found, Mmole	No. of Detns.	Standard Deviation, Mmole
0.038	0.039	13	±0.0016
0.006	0.007	12	±0.0013

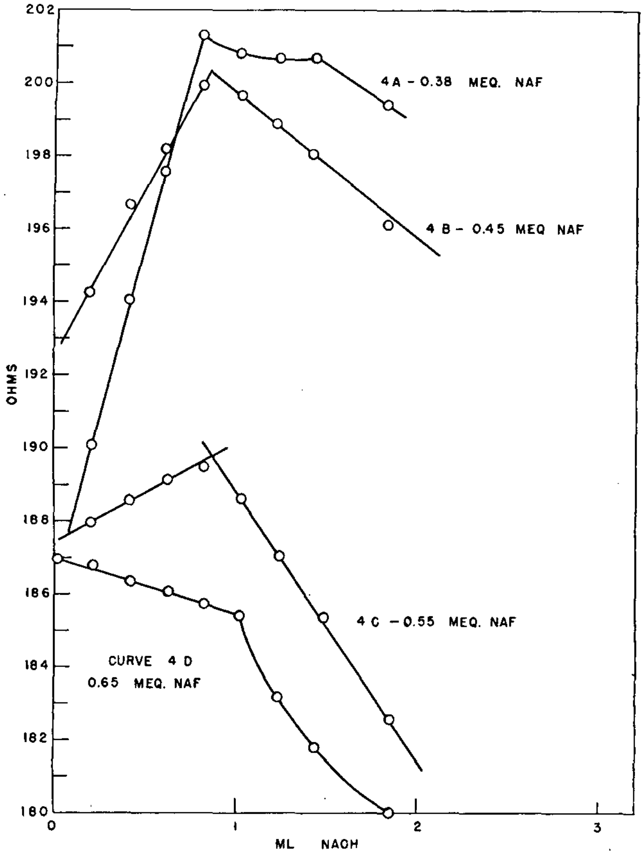


Figure 4. Conductometric Titration Cell

metric method—for instance, 10 microliters of 0.3 *M* hydrogen ion can be titrated. When possible, however, 0.01 mmole of acid should be the minimum amount used for an analysis.

The method is directly applicable to the determination of acid deficiency in salt solutions with the same precision as given above. This is accomplished by adding a known excess of acid to the acid-deficient solution and titrating as described.

CONCLUSION

A conductometric titration procedure is described for the determination of free hydrogen ion in complex solutions containing hydrolyzable ions. A relative precision of ±4% is obtained on aliquots containing 0.03 mmole of hydrogen ion. The limit of sensitivity is ~0.003 mmole. Accordingly, the method is applicable to very small aliquots (less than 100 microliters), which makes it especially useful for highly radioactive solutions. A variation of the procedure may be used for the determination of acid deficiencies in salt solutions. The generalized application of the procedure is dependent on finding an adequate complexing agent which will prevent hydrolysis at the acid-base end point.

ACKNOWLEDGMENT

The authors are indebted to C. A. Hansen of the Knolls Atomic Power Laboratory for the method of eliminating the capacitance phase shift effect which solved a very perplexing problem and to Mary Brennan of the Knolls Atomic Power Laboratory for the preparation of the unknown test solutions.

LITERATURE CITED

(1) Arden, T. V., *J. Chem. Soc.*, **1951**, 350.
(2) Blaedel, W. J., and Panos, J. J., ANAL. CHEM., **22**, 910 (1950).
(3) Dole, M., "The Glass Electrode," New York, John Wiley & Sons, 1941.
(4) Pifer, C. W., *et al.*, ANAL. CHEM., **24**, 300 (1952).
(5) Riddick, J. A., *Ibid.*, **24**, 41 (1952).
(6) Tanabe, H., and Hasegawa, F., *Ann. Rept. Takeda Research Lab.*, 9, 63 (1950).

RECEIVED for review August 7, 1952. Accepted October 4, 1952. The Knolls Atomic Power Laboratory is operated by the General Electric Co. for the Atomic Energy Commission. The work reported here was carried out under contract No. W-31-109 Eng-52.

Laboratory Evaluation of Fuel Oil Stability

A. R. RESCORLA, J. H. CROMWELL, AND DANIEL MILSOM
Cities Service Research and Development Co., New York, N. Y.

WITH the introduction of catalytic cracking of the heavier crude oil fractions, the furnace oil produced from these cracked stocks was found to be generally very unstable. The furnace oil produced from the catalytic cracking stocks has a tendency to become more rapidly oxidized in storage to form an insoluble precipitate and a dark colored oil which may have a high soluble residue. Although the fuel oils prepared from straight-run materials are more resistant to oxidation, when this material is mixed with the catalytic fuel oil the precipitation of the insoluble material is greatly accelerated. The consumer's first experience with the instability comes with the clogging of burner filters, and the dark oil removed from the system when clogging occurs.

There have been cases reported where fuel oil stored for a period as short as 2 months has caused filter clogging. The cause of this instability has not been definitely established, but various investigators have attributed it to sulfur compounds, particularly thiophenols and thiocresols, unstable nitrogen compounds, such as pyrroles, quinoline, and isoquinoline, colloidal carbenes and carboids, or unstable diolefinic hydrocarbons. Actually the instability may be due to any one or a combination of these compounds resulting from oxidation and polymerization reactions in the fuel oil. Regardless of the cause, it seemed logical first to devise a test procedure for evaluating fuel oil stability. To do this, it was necessary to define a stable fuel oil. It has been demonstrated in several laboratories (*10*) that any fuel oil containing as little as 2 mg. of insoluble gum per 100 ml. of oil can cause clogging in the average filter. Thus a stable fuel oil should contain less than 2 mg. of insoluble gum prior to actual consumption in the burner. It is a well recognized fact that fuel oils may be held in storage as long as 12 months from the time of actual production at the refinery until consumed in the customer's burner. In view of these two facts, it would appear that a stable fuel oil should be one that can be stored from 12 to 18 months with the formation of less than 2 mg. of insoluble gum.

DRUM STORAGE

With a stable fuel oil defined, the authors were assigned the problem of evaluating stability and of determining the period over which any fuel oil could be stored prior to the formation of 2 mg. of insoluble gum. To achieve the objectives it was decided to reproduce actual field storage conditions, and determine the insoluble gum formation during a definite storage period. This was accomplished by means of drum storage (*3*).

Several laboratory methods for determining fuel oil stability have been reported in the literature, but few have been correlated with actual storage. To develop an accelerated laboratory test that is directly correlated with storage, a series of oils was stored in steel drums for 18 months, and the insoluble gum was determined. Oven storage tests were completed on the same oils. An oil circulation and an air oxidation test were developed in the authors' laboratory. Both procedures are accelerated oxidation tests, providing for periodic removal of samples for determining insoluble gum content. The results of these tests were correlated with both oven and drum storage tests. Results of an investigation on a series of competitive fuel oils and also blends to which inhibitor had been added demonstrate the application of the laboratory test procedure. Actual field test results were obtained for comparison with the laboratory evaluation.

Oils were stored in 15-gallon steel drums in a nonsheltered area for 12 months. Arrangements were made to allow the drums to "breathe" normally, and the total contents of each drum were filtered to determine the insoluble gum contents.

The entire contents of the drum (10 gallons) were vacuum filtered by inserting a sintered, stainless steel beaker filter into the drum. The beaker filters employed were Micro-Metallic Corp. No. BF-150 beaker filters, $1^7/_8$ inch diameter, Grade F. Upon completion, the drum was rinsed with ASTM naphtha, and the washing was continued until the barrel and beaker filter with adherent insoluble matter were free of oil. The interior of the barrel was again rinsed with a 50–50 mixture of acetone and methanol for the removal of adherent insolubles. These washings were vacuum filtered through the beaker filter and retained in a clean filter flush. The recorded volume of the filtrate thus contained the dissolved insolubles from the barrel and sintered filter. An aliquot portion of the filtrate was evaporated in a tared beaker (*1*) and the results were reported as milligrams of insoluble gum per 100 ml. of oil. Sufficient drums were prepared on each sample to allow periodic sampling by selecting one of the drums, and filtering its entire contents to determine the amount of gum.

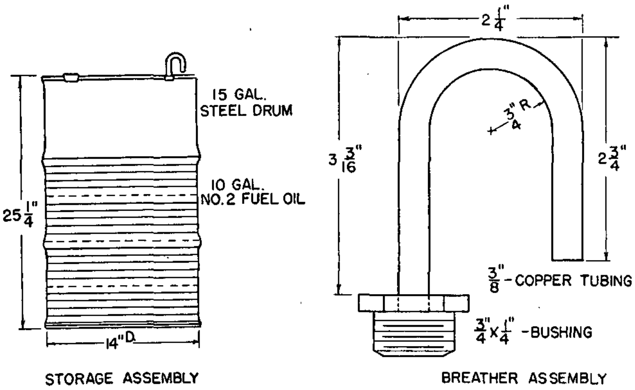


Figure 1. Drum Storage Assembly

Storage conditions, ambient temperatures

By means of these data it was possible to prepare a curve from which the insoluble gum content of any oil could be determined for any period during the 52 weeks' storage. Figure 1 shows a typical drum used in this investigation with the "breathing" arrangement.

OVEN STORAGE

The same series of fuel oils, which was subjected to drum storage, was stored in an oven at 110° F., using the Du Pont method of test (*4*). In this test, 60-ml. samples were stored, without catalyst, in 4-ounce dyestuff bottles, the plastic caps of which were drilled with a 0.25-inch hole to allow breathing. A diagram of the bottle is shown in Figure 2. The insoluble gum contents were determined on samples stored in the oven for various periods extending over 13 weeks.

In obtaining the amount of insoluble gum, the entire sample was filtered through a tared sintered-glass Gooch crucible (Pyrex No. 32960, fine porosity). The crucible and contents were washed with ASTM naphtha until free from oil and oven-dried to constant weight. The increase in the weight of the crucible was reported as milligrams of insoluble gum per 100 ml. of sample.

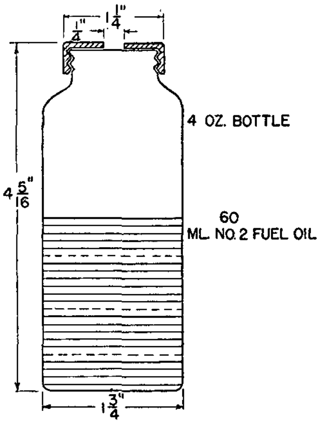


Figure 2. Oven Storage Bottle

Storage conditions, 110° F., circulating oven

From the data obtained by oven and drum storage the relationship shown in Figure 3 was developed. The weeks of drum storage are plotted against the weeks of oven storage on the basis of 2 mg. of insoluble gum per 100 ml. of sample. Assuming that 2 mg. of gum is the maximum that can be tolerated in the fuel oil, it is possible from the data contained in this graph to predict the actual weeks of drum storage from the oven storage data. In other words, the stability of an oil is expressed in weeks of storage.

The accelerated temperature in the oven storage decreases the actual drum storage time by a factor of 4.2. Thus it is possible in 13 weeks by means of oven storage to determine the stability of a fuel oil stored for a year under normal conditions, in drum or tank storage. Even with this accelerated oven storage oxidation, the time required to determine the stability of an oil is more than 3 months, a period not acceptable for routine analysis.

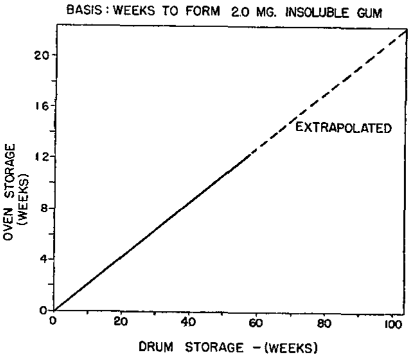


Figure 3. Correlation of Drum and Oven Storage

ACCELERATED LABORATORY TESTS

Once the correlation between drum and oven storage stability tests had been established in the laboratory, other accelerated laboratory procedures were investigated for the purpose of determining if such tests could be correlated with the storage test. The more important tests included in this investigation were the carbon arc (*8*), nitrogen bomb (*9*), steam jet gum test (*7*), acid flocculation (*5*), resin test (*2*), and oxygen bomb (*6*). Details of these tests can be found in the literature. After a considerable amount of investigation it was found that no simple correlation could be developed between these tests and the preceding storage procedures. It became necessary to develop two new accelerated laboratory procedures at the Cities Service Laboratory. The first of these accelerated tests was designated as the oil circulation test.

OIL CIRCULATION TEST

Purpose and Scope. This method has been developed for the laboratory evaluation of No. 2 fuel oils for resistance to oxidation by simulating the conditions encountered in domestic recirculatory oil burners.

Accelerative oxidizing conditions are provided by subjecting the oil to elevated temperatures while in contact with the copper, iron, and tin metallic surfaces of the equipment and a partial aeration of the oil.

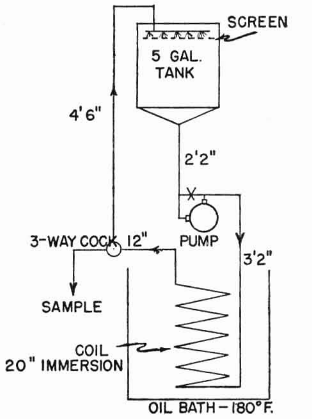


Figure 4. Oil Circulation System

3.5 gallons of oil circulated at 21 gallons per hour at 180° F. Coil, $^{3}/_{8}$-inch copper tubing, 15 feet 2 inches long

Lines, $^{5}/_{16}$-inch copper tubing, lengths as shown

Equipment. Oil Bath. Constant temperature 180° F., capacity for 20-inch. immersion.

Gear Pump. Viking, $^{3}/_{8}$-inch suction and discharge, motor driven. Capacity 21 gallons per hour.

Copper Tubing. $^{3}/_{8}$- and $^{3}/_{16}$-inch with essential Weatherhead fittings.

Filtering Equipment. Suitable for filtration at reduced pressures and accommodating a 7.0-cm. filter paper disk (Figure 5).

Filter Papers. No. 1 Whatman, 7.0 cm.

Procedure. In the 5.0-gallon reservoir are placed 3.5 gallons of the oil to be tested and the circulation of the oil through the system is started by means of the motor-driven pump.

The oil will thus be removed from the reservoir and pumped through the $^{3}/_{8}$-inch copper coil immersed in the oil bath maintained at 180° F. and returned to the reservoir. The discharge line into the reservoir is arranged in a horizontal position, the oil spraying into the tank through eight orifices, thus providing partial aeration.

When normal operating temperatures have been attained, the temperature of the oil entering the reservoir will be approximately 165° F. The temperature of the main volume of oil in the reservoir will be approximately 162° F.

At the end of each 24 hours of circulation 350 ml. of the oil are removed through the sample line and, after cooling to room temperature, are filtered through a 7.0-cm. No. 1 Whatman filter paper disk at the slightly reduced pressure of 740 mm. of mercury.

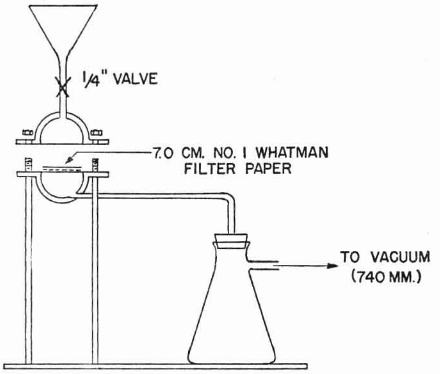


Figure 5. Filtering Assembly

Oil is removed from the filter paper disk by three consecutive washes with ASTM naphtha, after which the disk and adhering materials are removed from the filtering equipment and air dried.

A 350-ml. sample of the original oil is filtered and washed as previously described and the filter disk is retained as a control.

Sampling is continued at 24-hour intervals until the color intensity of the filter pad shows a definite increase as compared with the original pad, indicating a sludging formation and the maximum resistance of the oil to oxidation.

The density and quantity of the materials adhering to the filter paper are observed and photographically recorded.

The stability of the oil is recorded as the number of hours on test before sludge formation is indicated.

Cleaning Procedure. At the termination of the test, the remaining oil is removed from the circulatory system through the sampling line. The reservoir is spray-washed with a solvent blended in the following proportions:

Ethyl acetate	1600 ml.
Denatured alcohol	1200 ml.
Butyl alcohol	200 ml.
o-Dichlorobenzene	1000 ml.

One liter of the solvent is added to the reservoir and circulated through the system for 15 minutes. Consecutive washes are given until the system is essentially clean.

The solvent wash is followed by two 1-liter rinses with Stoddard solvent circulating for 5 minutes. The excess Stoddard solvent is removed from the system by disconnecting the lines and blowing with an air jet.

A final rinse is given the system by circulating 1 liter of the oil to be tested. After the system has been pumped as dry as possible, the assembly is charged with the 3.5-gallon test sample as indicated in the procedure.

In the oil circulation test 3.5 gallons of fuel oil are circulated by means of a pump through a $^{3}/_{8}$-inch copper coil immersed in an oil bath maintained at 180° F. The oil is then discharged into a 5-gallon reservoir through eight orifices which provide for partial aeration.

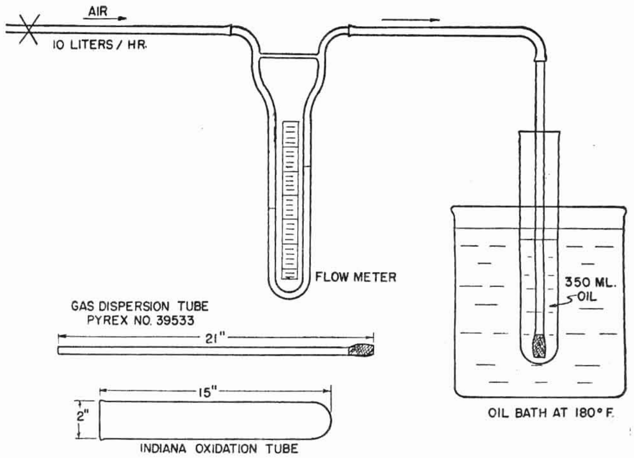


Figure 6. Air Oxidation System

Figure 4 is a sketch of the oil circulating system. The length of copper tubing is very critical because of its catalytic effect on the oxidation, and it is very important that the entire system be thoroughly clean at the start of a test. Every 24 hours 350-ml. samples of oil are withdrawn from the system, and the insolubles are determined by filtering this oil through a No. 1 Whatman filter paper at a pressure of 750 mm. of mercury. This sampling is continued at 24-hour intervals until the color intensity of the filter pad shows a definite increase. This is called the inflection point and the corresponding time in hours is reported as the expected stability. The density of the materials adherent to the filter pad can be observed and recorded photographically. Figure 5 is a sketch of the filtering assembly for preparing the filter pads.

The air oxidation procedure consists of oxidizing 350 ml. of fuel oil in a glass tube by passing 10 liters of air per hour through the oil while heating it to 180° F. A diagrammatic sketch of the apparatus is shown in Figure 6. To evaluate an oil, a series of tubes containing the oil to be tested is charged to the bath and the measured air flow admitted. These tubes are removed at definite time intervals and the contents are filtered through the assembly shown in Figure 5. The oxidation is continued until there is a distinct discoloring of the filter pads, and this point is designated as the inflection point, which is expressed in hours of oxidation. The inflection points, as determined by the air oxidation test, have been found reproducible within ±4 hours.

AIR OXIDATION TEST

Purpose and Scope. This method has been developed for the evaluation of No. 2 fuel oils for resistance to oxidation when subjected to a definite quantity of finely dispersed air at atmospheric pressure and at elevated temperatures.

Equipment. Oil Bath. Maintained at 180° ± 1.0° F.
Oxidation Tubes. 50 × 400 mm. Corning No. 9800.
Spargers. Fritted glass, Corning No. 39533–12 EC.
Flow Meters. Capacity 10 liters of air per hour.
Filter Mechanism. Capable of filtering a quantity of oil through a flat filter paper disk (No. 1 Whatman, 7.0 cm. in diameter) under slightly reduced pressure.

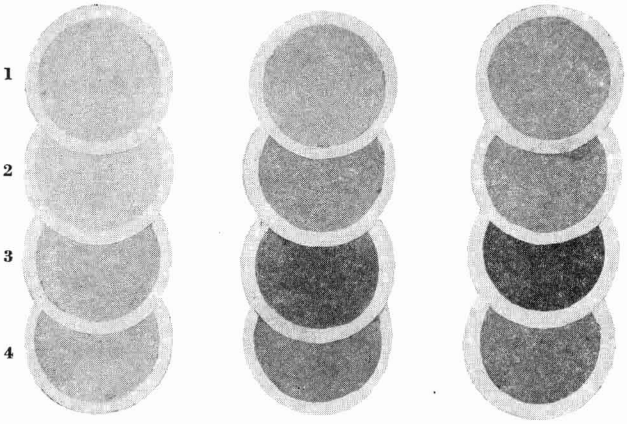


Figure 7. Comparison of Filter Pads from Air Oxidation and Oil Circulation

Original Oil No.	Air Oxidation, Hours	Oil Circulation, Hours
1	72	96
2	24	72
3	16	48
4	4	16

Procedure. The fuel oil under test (350 ml.) is placed in a 2 × 15 inch borosilicate glass tube. Tube and contents are immersed to the oil level in the tube, in a constant level oil both maintained at 180° F.

Air is bubbled through the oil sample at a rate of 10 liters an hour by means of a fritted-glass sparger immersed in the oil.

The tests are scheduled under these conditions for increments of 24 hours, separate samples being prepared for each increasing increment of 24 hours until the oil "breaks." In the event that the maximum resistance to oxidation is found to be less than 24 hours, tests are scheduled for the 4-, 8-, and 16-hour periods.

At the end of each time increment, the tube and contents are removed from the oil bath and reduced to room temperature.

The oil sample is filtered through a flat filter paper disk (No. 1 Whatman, 7.0 cm. in diameter) at slightly reduced pressure (740 mm. of mercury). Oil is removed from the filter paper by three consecutive 50-ml. washes of ASTM naphtha and air-dried after removal from the filter mechanism.

A 350-ml. sample of the oil is tested in the filter as above and preserved as a "control" filter pad.

The break point of the oil is indicated by a definite increase in the color intensity of the filter pads when compared with the unoxidized control sample.

The stability of the oil is recorded as the number of hours on test before sludge formation is indicated upon filtration of the oxidized oil sample.

The consecutive filter pads are arranged in sequence, including the control and break pad, and photographically recorded.

Comparison of Air Oxidation and Oil Circulation. It will be seen from the data contained in Figure 7 that the oxidation conditions by the oil circulation procedure are less severe than those by air oxidation. The times required to reach the inflection points are approximately three to four times greater in cases where the oxidation is 24 hours or less. Yet it will be noted that both methods rate the oils in the same manner, if the data in Figure 8 are observed. The relationship appears to be linear if the air oxidation is 24 hours or less. Since the oil circulation requires a considerably longer period of time to reach the inflection point, and the quantities of oil required for testing are much greater, it was decided to use this test procedure for special cases where an independent evaluation is required and a large oil sample is available.

Comparison of Air Oxidation and Oven Storage. It was considered advisable to develop the air oxidation procedure further. The first step in this direction was to correlate the air oxidation procedure in hours to that of oven storage in weeks. A series of fuel oil samples was subjected to both test procedures and the results were compared graphically in Figure 9. This graph shows that it is possible to predict the actual oven storage in weeks from the number of hours required by the air oxidation procedure to reach the inflection point. Once this was established, it was comparatively simple to convert the air oxidation to weeks of drum storage by means of a previous correlation. In the case of the air oxidation test no actual gum measurements were made, and only the discoloration of the pad was used as an indication of the inflection point of the oil. In the case of the oven storage tests the actual gum content of the oil was determined by weighing, and the weeks of oven storage reported represent the time required for 2 mg. of insoluble gum to form in the oil.

To confirm experimentally the relationship between air oxidation and oven storage, a series of 350-ml. samples was stored in the oven for various periods of time. This is the same amount of sample used in the air oxidation test. The entire quantity (350

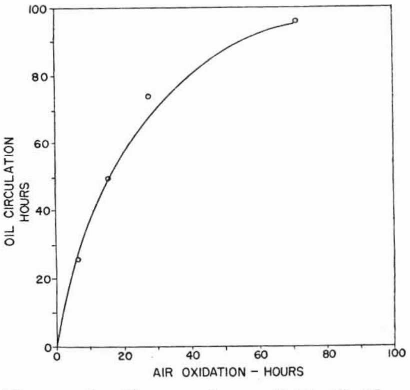


Figure 8. Comparison of Air Oxidation and Oil Circulation Inflection Points

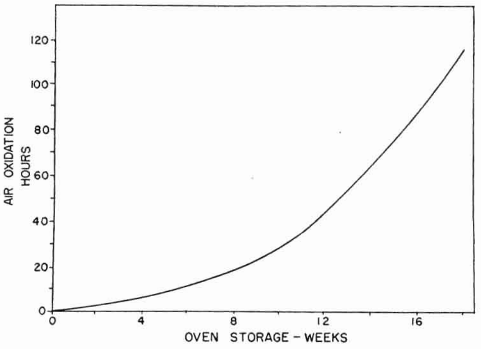


Figure 9. Air Oxidation to Inflection Point *vs.* Weeks of Oven Storage

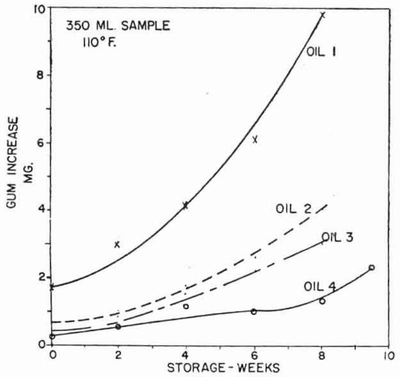


Figure 10. Increase in Insoluble Gum Content with Weeks of Oven Storage

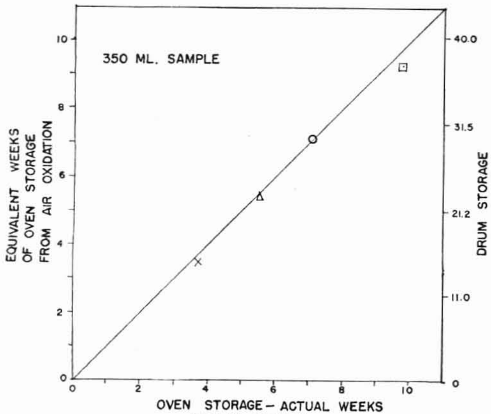


Figure 11. Weeks of Oven Storage *vs.* Converted Weeks of Oven Storage from Air Oxidation

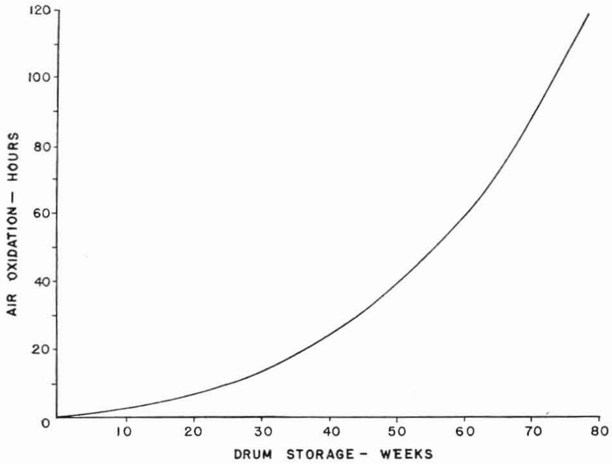


Figure 12. Direct Conversion

Air oxidation *vs.* drum storage

ml.) of fuel oil was filtered to determine the insoluble content. The curves of insoluble content versus weeks of oven storage are presented in Figure 10 for the four oils.

The same oils were subjected to the air oxidation test and the hours to reach the inflection point were determined for each oil. From the correlation contained in Figure 9 it was possible to convert the air oxidation results to the corresponding weeks of oven storage.

If the 2-mg. increase in insolubles is used as the criterion of an oil that will cause filter clogging, it is possible to predict from Figure 10 the actual weeks of oven storage that can be expected of each oil. The actual oven storage is compared with the predicted oven storage as determined with air oxidation in Figure 11. A study of this figure will indicate that the correlations of two results are linear, suggesting that the inflection point as determined by the air oxidation is indicative of an oil containing 2 mg. of insolubles per 100 ml. of fuel oil. Hence the inflection point as determined by the air oxidation test can be used with a reasonable assurance to predict the drum storage stability of fuel oils in accordance with the relation shown in Figure 12. Based on this test it is believed the air oxidation test is a reliable accelerated laboratory test procedure for measuring fuel oil stability and by means of this test it is possible in 60 hours to predict the equivalent of 60-week drum storage stability.

Air Oxidation Tests on Commercial Oils. At the Cities Service Laboratory a program for evaluating commercial oils has been in effect for the past 2 years. As these oils originate from totally different stocks, it was of interest to determine if the air oxidation procedure was applicable. In Figure 13 the storage stability as determined by air oxidation tests has been plotted for four oils. Each of these oils is marketed by a different company, and the stabilities for 1950 and 1951 are compared. In the case of Sample 1, the stability improved greatly in 1951, which suggests that the refining procedure was changed or the oil inhibited in some manner. The same condition exists with Sample II, but the results are less pronounced. Samples III and IV show very little change in stability levels and this would indicate no drastic refining methods were inagurated by these refiners, as in Samples I and II.

Numerous other evaluations on commercial oils have been completed in the authors' laboratories with some interesting results. In one case where the improvement was of much the same order of magnitude as in Sample I, it was found that the refiner was inhibiting the fuel oil. The date of improvement as indicated by air oxidation was in close agreement with the date the actual addition was made. Two similar cases brought to the authors' attention during the past year further confirm the practical application of this procedure to commercial fuel oil evaluation.

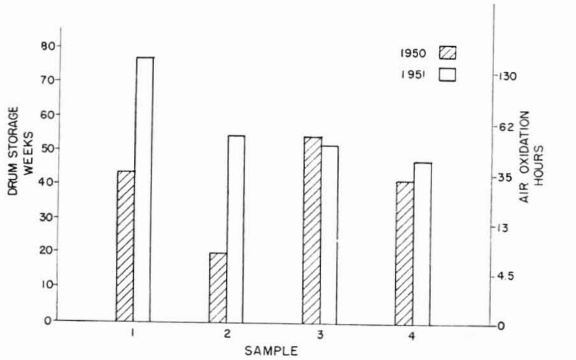


Figure 13. Drum Storage Stability of Commercial Oils

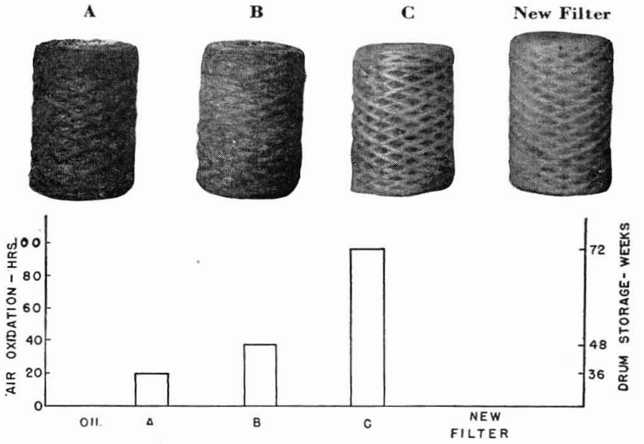


Figure 14. Laboratory Evaluations *vs.* Filter Discoloration of Commercial Oils

1800-gallon throughput

During the past heating season the authors have been able to secure filters from domestic and commercial installations using commercial oils with different air oxidation evaluations. In Figure 14 three of these filters have been photographed and the corresponding air oxidation results indicated. A study of this figure will indicate the same throughput of fuel oil in all cases and thus it represents an average consumption for a heating season in a domestic installation. The filter exhibiting the least discoloration and comparable to that of a new filter was rated with highest air oxidation evaluation and corresponding drum storage. These data would indicate that the air oxidation evaluates the fuel oils in much the same manner as the filter, regardless of stocks.

Air Oxidation and Inhibition Addition. The air oxidation test has been found very useful in the evaluation of inhibitors. In Figure 15, effectiveness curves for four different commercial inhibitors have been constructed by determining the inflection points, by the air oxidation test, of a base oil containing varying amounts of the respective inhibitors. Each inhibitor has its own characteristic curve, and the amount required to reach any stability level can be predicted once the graph has been established with any particular stock.

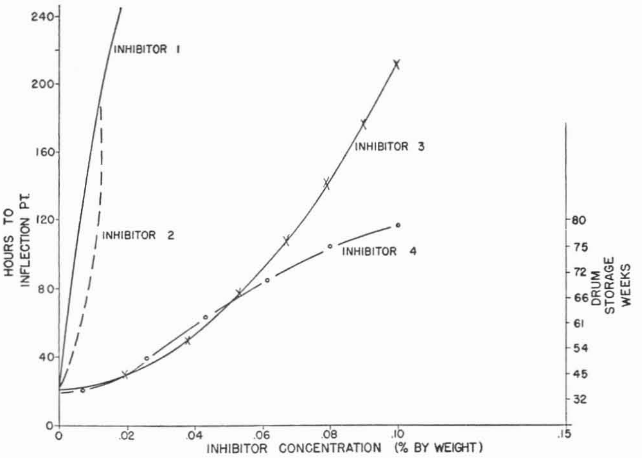


Figure 15. Evaluation of Fuel Oil Inhibitors

Similar curves have been prepared on some fourteen different inhibitors and the curves have been confirmed by repeating the test on the same stocks with different batches of inhibitors.

The results of a field test on an oil containing an inhibitor were made available to the authors. Air oxidation evaluations were completed on the inhibited and uninhibited oils. Actual filters were also obtained from domestic and commercial installations on both types of oil. Photographs of two typical filters from this investigation have been included in Figure 16, together with the air oxidation data. The oil having the highest air oxidation in hours also exhibited the least discolored filter. This tends to confirm that oils containing inhibitors can be rated by the air oxidation test for filter discoloration. Furthermore, inhibitor concentrations as low as 0.01% can be evaluated with a repeatability of ±2 weeks of drum storage.

CONCLUSIONS

It is possible to predict the stability of a fuel oil. A stable oil is defined as one that will not form more than 2 mg. of insoluble gum per 100 ml. of oil in a year of normal storage.

Drum storage can be directly correlated with laboratory oven storage tests. Accelerated laboratory stability tests have been developed—oil circulation and air oxidation. Correlative graphs have shown that the inflection point as measured by the air oxidation test can be used to predict drum storage.

Further work shows how the air oxidation test can be used to evaluate commercial oils. This means that the test procedure is applicable to all types of stocks.

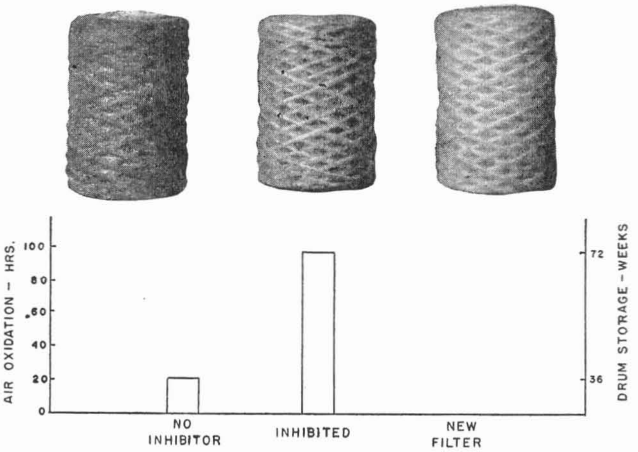


Figure 16. Laboratory Evaluations *vs.* Filter Discoloration of Inhibited Oils

1800-gallon throughput

The usefulness of the test in evaluating various inhibitors is also indicated. Other accelerated tests have been developed, but for the most part these tests are based on measurement of gum content and cannot be correlated directly to storage. It is for this reason, and this reason alone, that the accelerated laboratory tests described in this paper are recommended to the petroleum industry for predicting stability of fuel oil in storage.

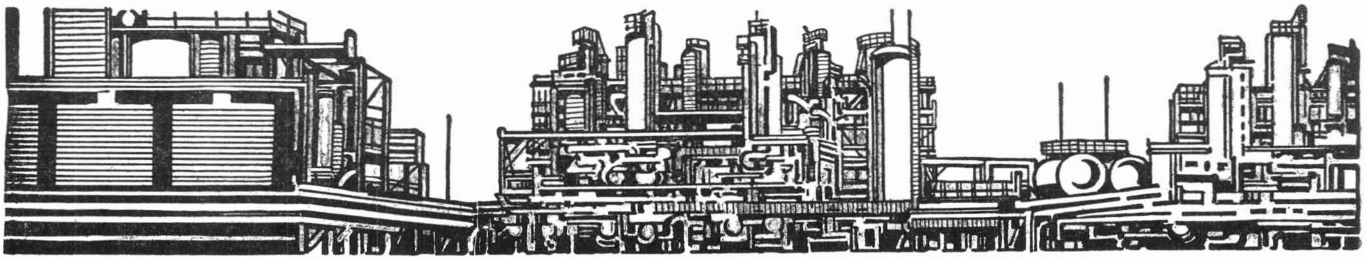
ACKNOWLEDGMENT

The authors wish to thank the Cities Service Research and Development Co., for permission to publish this article. The assistance of Lee Critides, Eugene Lang, and William Thaller in collecting the data is gratefully acknowledged.

LITERATURE CITED

(1) Am. Soc. Testing Materials, D 381–50.
(2) Cities Service Oil Co. (Delaware), Resin Formation in Distillate Oils, *Method* **PP 28.6–44.**
(3) E. I. du Pont de Nemours & Co., Fuel Oil Drum Storage Stability, Revised *PCL Method* **F1–D4.**
(4) E. I. du Pont de Nemours & Co., Method of Test for Storage Stability of Fuel Oils, Oven Storage at 110° F., Method **F1–B3.**
(5) Proell, W. A., and Bolt, J. A., *Oil Gas J.*, 44, No. 47, 234–8 (1946).
(6) Sabina, J. R., Korb, E. L., and Bender, R. O., "A Method for Evaluating the Oxidation Stability of Distillate Fuel Oils," paper presented before Division of Petroleum Chemistry, 105th Meeting, Am. Chem. Soc., Detroit, Mich., April 1943.
(7) Universal Oil Products Co., Laboratory Test Methods for Petroleum," *U. O. P. Method* **J-263-49 A,** Steam Jet Gum.
(8) *Ibid.*, **J-267-50 I,** Carbon Arc Test.
(9) *Ibid.*, **J-268-50 I,** Nitrogen Bomb Test.
(10) Universal Oil Products Co., private communication.

Received for review June 17, 1952. Accepted August 28, 1952. Presented before the Division of Refining, American Petroleum Institute, San Francisco, Calif., May 12, 1952.



Fluorometric Analysis

Report of round-table discussion held by Division of Analytical Chemistry at 121st Meeting, ACS, Buffalo, N. Y., March 1952

Moderator: CHARLES E. WHITE, *University of Maryland, College Park, Md.*
Panel: MARY H. FLETCHER, *U. S. Geological Survey, Washington, D. C.*
T. E. FRIEDEMANN, *U. S. Army Medical Nutrition Laboratory, Chicago, Ill.*
FREDERICK KAVANAGH, *Commercial Solvents Corp., Terre Haute, Ind.*
E. E. LEININGER, *Michigan State College, East Lansing, Mich.*
J. K. MURATA, *U. S. Geological Survey, Washington, D. C.*

FLUOROMETRIC procedures have been applied in many phases of chemical analysis. The object of this panel is to present some of the recent advances in fluorometric analysis in the fields of inorganic, mineralogical, biological, and organic chemistry in order to stimulate a discussion of these topics. The number of applications of fluorometric methods to biological analysis far exceeds those in any other branch of chemistry.

One of the most interesting developments in inorganic fluorometric analysis has been in the application of 8-quinolinol as a fluorometric reagent. It would seem that almost any element that can be precipitated with 8-quinolinol will lend itself to a fluorometric method. The general technique is to extract the metal-8-quinolinol with chloroform and to measure the fluorescence of the resultant solution. This method has proved useful in the determination of traces of aluminum, gallium, and indium. It permits the use of small original samples and avoids tedious separations. The decrease in the fluorescence of the aluminum 8-quinolinol can be used in the detection of fluorides. Methods for the determination of zinc and lithium using 8-quinolinol have also been developed.

DETERMINATION OF URANIUM

The melts obtained by the fusion of uranium salts with sodium fluoride fluoresce a brilliant yellow green when exposed to ultraviolet light. The intensity of the fluorescence is directly proportional to the weight of uranium in the melt and is used for the quantitative determination of uranium.

The reaction is specific for uranium if ultraviolet light of long wave length is used, but other ions may interfere by quenching the uranium fluorescence. The most serious quenchers are cerium, chromium, cobalt, gold, lanthanum, lead, manganese, nickel, neodymium, platinum, praseodymium, and silver. One to 10 micrograms of these elements quenches the uranium fluorescence of a 2-gram melt by 10% (the amount of quenching depends only upon the ratio of weight of quencher to weight of flux).

Interference from other ions may be overcome by several techniques. Price's dilution technique uses a sample so small that it contains less than the critical amount of quenchers. In other methods the uranium and quenchers are separated from each other either by precipitation of the interfering ions from the uranium solution, using a mixture of sodium and potassium carbonates, or by extraction of uranium nitrate from the other ions with an organic solvent using aluminum nitrate as the salting agent. Aluminum salts, which do not quench, are especially useful salting agents because aluminum complexes fluoride, sulfate, and phosphate ions, which if not complexed would prevent complete extraction of uranium.

The principal steps in the analysis of a sample for uranium are: decomposition of a weighed sample by acid attack or fusion; solution of the uranium in a known volume of acid; separation of uranium and quenchers if necessary; transference of sample aliquot to platinum container; evaporation and ignition of aliquot; addition of 2 grams of fusion mixture (9% sodium fluoride in equal parts by weight of sodium and potassium carbonates); fusion of the mixture; and measurement of the fluorescence of the cool melt. The fusion must be made carefully because the fluorescence depends upon the temperature and duration of fusion. If temperatures greater than 700° C. are used, platinum is dissolved from the container and quenches the uranium fluorescence.

Various types of fluorometers have been used for the measurement of the fluorescence. The transmission fluorometer developed at the U. S. Geological Survey is a simple rugged instrument that requires no elaborate optical system. In this fluorometer the exciting light and phototube are on opposite sides of the melt, and very close to it. As a result of this arrangement, both the instrument blank and the loss of light intensity due to the inverse-square law are greatly reduced. Consequently the instrumental sensitivity is high. As little as 0.0005 to 0.001 microgram of uranium in a 2-gram melt can be determined quantitatively. Variations in melt thickness that might occur are not critical and cause no significant errors. A completely battery-powered transmission fluorometer has been built for use where no electrical current is available. M.H.F.

GEOCHEMICAL APPLICATIONS

The manner in which geochemical information has been obtained from the study of fluorescent minerals was reviewed. Approximately 10% of the 1500 or so known species of minerals fluoresce. These are divisible into two classes: (1) those that are inherently fluorescent as a pure compound, such as $Ca(UO_2)_2(PO_4)_2\cdot 12H_2O$ (autunite), and (2) those that fluoresce only when they contain an impurity element as an activator of fluorescence, such as $Zn_2SiO_4{:}Mn$ (manganese-activated willemite). Advantage is taken of the invariable fluorescence of minerals of the first class by prospecting for them with portable ultraviolet lamps. Important deposits of calcium tungstate ($CaWO_4$, scheelite) have been discovered in this manner in the western states.

The more numerous minerals of the second class have been studied by a small group of geochemists in several countries, and a number of activator elements such as Mn^{++}, Eu^{++}, Cr^{+++}, and Sm^{+++} have been identified. Whenever feasible, the final proof of the correct identification of the activator has been obtained by synthesizing the fluorescent mineral. The fluorescence is often the only indication that unusual elements are present in the minerals. The intensity of fluorescence generally increases with activator concentration up to an optimum concentration and then decreases. The activators in most minerals of the second class are, as yet, unidentified.

The fluorescence properties of minerals may be altered by various physical and chemical changes that the minerals undergo subsequent to their formation. Some effects due to mechanical deformation, heat, and long exposure to feeble radioactivity have been recognized. Zoned fluorescence calls attention to fluctuations in the growth history of crystals and also to crystal-chemical forces that cause selective deposition of activator elements on certain faces of crystals.

The fluorescence of minerals, therefore, helps in identifying and localizing minerals, provides basic information on the distribution of a number of elements (activators) among minerals, and throws light on the natural history of these minerals in the earth's crust. J.K.M.

RELATION OF pH TO FLUORESCENCE

Many polycyclic and heterocyclic unsaturated organic compounds fluoresce when irradiated under proper conditions. Fluorescence as a function of pH may be characteristic of a compound. The pH-fluorescence curve is an indication of the influence of minor changes in structure upon the probability of internal conversion of absorbed energy into emitted energy. The fluorescent species can be a neutral molecule, a dipolar ion, a negative ion, or a positive ion. The ions may be univalent or polyvalent. A molecule may be fluorescent in more than one state of ionization. If it is, then the several fluorescent forms may be expected to fluoresce with different efficiencies and different colors.

The compounds considered here are those that give visible fluorescence in dilute aqueous solution when irradiated at room temperature by the 366 mμ lines of the mercury arc in the presence of dissolved oxygen and buffer salts. These conditions usually occur in most fluorometric methods of qualitative and quantitative analysis.

Factors which influence the shape of the pH-fluorescence curve are: pH, buffer composition, quenching by dissolved oxygen, efficiency of the fluorescent process, variation with pH of the absorption spectrum of the organic compound, color of the fluorescent light, and characteristics of the measuring instrument.

The three common sources of quenching are impurities accompanying the fluorescent substance, salts of the buffer, and dissolved oxygen. Quenching by oxygen is common and is well known to those who measure the fluorescence of carcinogenic hydrocarbons. Unless quenching by oxygen is very large, it is more convenient to obtain maximum quenching by saturating the solution with air than to remove all dissolved oxygen. Quenching by salts of the buffer may be appreciable and also cause a change in shape of the pH-fluorescence curve. An example is quinine sulfate measured in McIlwain's buffer and in nearly unbuffered sulfate solutions. The two curves coincide in the sulfuric acid range, but the fluorescence in McIlwain's buffer is less than that of the sulfate solution, showing that citric acid and phosphate quench fluorescence of quinine. Quenching by oxygen and buffer salts may be unimportant to the final results of qualitative and quantitative analysis because it will be the same for standard and sample. Quenching is important, however, when an absolute measure of fluorescence is required as in quantum yield experiments and in determining ionization constants from the shape of pH-fluorescence curves.

Four examples of compounds which fluoresce in these different states of ionization are quinine, anthranilic acid, riboflavin, and thiochrome.

Because there is no fluorescence without prior absorption of light, data for correlating absorption of energy with emission of energy were obtained by measuring the absorption spectra of the four compounds at several values of pH in the buffers used in obtaining the pH-fluorescence curves. The relative absorptions for the 366 mμ lines were computed by calling the maximum absorption 100. The shape of the anthranilic acid pH-fluorescence curve is that expected if a dipolar ion is the main fluorescing species, but the long tail of the curve suggests that an anion also fluoresces. The relative absorption of 366 mμ light also parallels the fluorescence curve. The quinine curve is about what would be expected if a constant fraction of the 366 mμ energy absorbed is emitted as fluorescent light. Both absorption and fluorescence follow fairly closely the relative concentration of the quinine doubly charged cation; the anion is very weakly fluorescent. The principal influence of pH in both quinine and anthranilic acid is on absorption of exciting energy. The pH-fluorescence curve for riboflavin is interesting because absorption of the 366 mμ lines is nearly independent of pH but fluorescence is not. There is a color change from greenish yellow at pH 10 to bluish at pH 11.3. Unlike the two previous examples, probably it is the conversion of absorbed light into fluorescent light, not the absorption itself, which is the function of pH. The shape of the curve suggests that the fluorescing structure is a neutral molecule or dipolar ion. The pH-fluorescence curve and the relative light absorption for thiochrome indicate that the main influence of change of pH on fluorescence is not upon the absorption of light.

The pH of the solution may affect markedly the color of the fluorescent light, which may vary from dark purple to deep red with blue the most common color. A change in color with change in pH indicates change in structure of the fluorescing substance. This color change is an important factor in determining the shape of the pH-fluorescent curve. If the photocell of the fluorometer is sensitive primarily to the blue, the pH-fluorescence curve will have a different shape from that determined with a photocell that is also sensitive to red light. Photocell spectral sensitivity and the characteristics of light filter over the photocell modify the shape of the pH-fluorescence curve and must be considered in comparing the curves obtained by different experimental arrangements.

Many fluorescing systems change under the influence of the light exciting fluorescence. The change can take the form of increase in fluorescence, decrease in fluorescence, or change in color of fluorescent light in various combinations.

An example of the utility of fluorometric methods may be cited in the identification of the blue-fluorescing substance in the 15-mm. portion of the oat root tip lying between 3 and 18 mm. from the tip. This part of the root had a wet weight of about 0.5 mg., yet the sensitivity of the method was such that a complete pH-fluorescence curve could be made on the extract from 30 root tips. The crude, unpurified chloroform extract of the root sections had a pH-fluorescence curve nearly identical with that of scopoletin, which was shown to be the blue fluorescing substance in that portion of the oat root. F.K.

ANALYSIS OF BIOLOGICAL MATERIALS

Fluorometry is an important analytical technique which has assumed prominence within the past 15 years. Its most dramatic and widest application occurred in the early years of the past decade, when it was used in vitamin assays, particularly of thiamine and riboflavin in foods and body fluids. Since then the interest in vitamin assays has waned, and instead, new interests have arisen in the application of fluorometry to inorganic analysis and the determination of hormones, metabolic intermediates, drugs, and decomposition products in prepared foods. The widest application of fluorometry still is to samples of biological origin, and it is in the analysis of such samples that the limitations of the techniques become most evident and often lead to serious error. Some of the difficulties are:

The ranges and the variations of concentration in biological materials are often very wide. Thus, it may be necessary to repeat the analysis until the photometric response is within the instrumental range. Obviously, an instrument with a wide range of sensitivity, which can be set readily to several narrow ranges, would be desirable.

The fluorescent compound of a fluorescent derivative may have strong light-quenching properties. Examples of such substances are the porphyrins, the colored adrenaline derivatives, and the colored pyridinium derivatives of nicotinic acid and other pyridine compounds. The fluorescence response is linear only at high dilution.

Many biological materials contain impurities and colored substances which may have strong light-quenching properties. The interference due to these substances may be minimized either by isolation procedures or, often more simply, by dilution. Obviously, the use of instruments of high sensitivity is indicated. Such an instrument has been used by the author since 1942 and is now available commercially from the Central Scientific Co., Chicago. This instrument is now known as the Cenco-Friedemann-Liebeck fluorometer and is a multirange type with two photocells, one of which is especially sensitive in the blue part of the spectrum and the other in the red.

The effect of dilution on the fluorescent properties of caramelized sugar and, generally, the interfering effect of colored materials in filtrates taken for analysis were discussed. At progressively smaller concentrations, but at constant hydrogen ion concentration, some substances like quinine exhibit decreased fluorescence; others, like riboflavin, do not. The hydrogen ion concentration is very important, especially at low concentrations. Multirange instruments are helpful in the study of the efficiency of adsorbing agents for use in chemical assay. Thus the recovery of thiamine after absorption on Decalso, a zeolite which is widely used in the analysis of thiamine, diminishes rapidly at progressively higher dilutions. The effect, although present, is not apparent in the single-range instruments in current use. On the other hand, the recovery of riboflavin from Supersorb, a magnesium silicate which is used in riboflavin assay, is quantitative over the range of 1.6 to 0.004 microgram per ml. T.E.F.

FLUOROMETRIC DETERMINATION OF CITRIC AND MALIC ACIDS

The fluorometric method for the determination of citric acid by the formation of ammonium citrazinate (*4*) has proved successful. When the method was devised, it was believed that the citric acid was changed to aconityl chloride with thionyl chloride. When the aconityl chloride is treated with ammonia gas, the expected product is aconitamide, which is transformed to citrazinic acid by ring closure upon heating with 76% sulfuric acid at 165° C.

Evidence has been found that the reactions do not follow this path completely. The aconityl chloride formed in the reaction with thionyl chloride was hydrolyzed to aconitic acid, which was then determined by polarographic reduction in hydrochloric acid solution (*6*). On the basis of the amount of aconitic acid found, it was impossible to account for the yield of citrazinate as determined by the fluorescence intensity. Therefore it is suggested that part of the citric acid is transformed to citrazinate by a different path, possibly involving citryl chloride and citramide.

The control of conditions at each step in the procedure is important. For example, it is essential that the moisture be removed from the sample completely, since it reacts with thionyl chloride and reduces the fluorescence intensity. After the reaction with thionyl chloride, the excess reagent must be removed completely without allowing the entrance of moisture. This is accomplished by alternate evacuation and flooding with dry air. The only drying agent which was completely satisfactory under the drastic conditions employed was Dehydrite. The completeness of ring closure was shown to be particularly dependent upon conditions. The ammonium citrazinate solution is not suitable for setting the fluorometer to a reading of 100, but a standard solution of sodium salicylate served fairly satisfactorily.

The method for the determination of malic acid (*5*) depends upon heating with 2-naphthol in 92% sulfuric acid. The blue fluorescence was believed to be due to the formation of 5,6-benzocoumarin. This has been corroborated by Goodwin and Kavanagh (*2*), who obtained identical pH *vs.* fluorescence curves for 5,6-benzocoumarin and a solution of malic acid treated with 2-naphthol and sulfuric acid as described. The fluorometric determination of malic acid by the methods of Barr (*1*) and Hummel (*3*) depends upon the formation of coumarin derivatives.

Fluorometric methods for a number of organic groups and compounds are possible and many applications of the methods already in existence might be made.

Methods depending upon the syntheses of fluorescent organic compounds might be expected to require rather close attention to detail; however, in spite of this, many useful methods are possible. E.E.L.

DISCUSSION

The determination of adrenaline provoked much discussion. Aluminum oxide does not seem to be the best agent for adsorbing protein to permit the determination of adrenaline. The use of a small sample gives the most satisfactory results and isobutyl alcohol has proved to be a good extracting agent. With the use of the dilution technique and an instrument of proper sensitivity, very satisfactory results can be obtained.

The browning of sugars and the discoloration resulting when foods are treated with sulfuric acid may cause a decrease or an increase in the intensity of the fluorescence of the products under determination. However, experiments in which dilute solutions were used with a multirange fluorometer seem to indicate that the fluorescence is always increased by this browning of food materials. The decrease in fluorescence in more concentrated solutions is probably due to the absorption of the ultraviolet radiation by the foreign material in the first few millimeters of the cell. This absorption can easily be observed by visual observation of the solution.

In reply to a question on the uranium method, it was stated that ordinary reagent grade aluminum nitrate is satisfactory for use when the uranium quantity is greater than 10^{-8} gram. With samples containing quantities smaller than this, it is necessary to purify most of the chemicals used.

A question concerning the effect of phosphate on the fluorometric determination of fluoride with aluminum-8-quinolinol provoked considerable discussion. Charles Horton made the following observations:

> In the fluorometric method for determining traces of fluoride with the 8-quinolinol extraction method, equilibrium is reached more quickly if an acetic acid solution of the reagent is added before extraction than if the reagent is placed in the extracting solvent. This method for the determination of fluorides depends on the relative stability of the metal-organic complex as compared to the metal-fluoride complex. Two or more of the fluoride complexes are generally present. Phosphate, sulfate, or other ions which interfere may form complexes with the fluoride, the metal, or the organic compound and affect the amount of the fluorescent compound present.
>
> In the titration of large amounts of fluoride, the photofluorometric method using quercetin or morin is superior to the use of Alizarin Red S in the visual method.
>
> Commercial morin is generally unsatisfactory as a reagent. It may be purified by ion exchange or sublimation. Morin of good quality can be obtained from Schuchardt in Germany.

Frank Grimaldi observed that in the determination of traces of aluminum in minerals with the 8-quinolinol, with the extraction fluorescence technique, it is very important to have a great excess of the reagent in order to prevent phosphate interference. Therefore, it is better to add the solution containing aluminum to the acetic acid solution of the 8-quinolinol than to employ the reverse procedure. In the analysis of phosphate rock a sample of about 0.1 mg. is used and the complex is extracted with chloroform at a pH of 4.6; 0.01% aluminum oxide can be determined in the 0.1-

mg. sample. This method is specific for aluminum and as much as 10% iron does not interfere.

A discussion concerning the source of ultraviolet radiation for the study of the fluorescence of minerals seemed to indicate that the high pressure and low pressure mercury vapor lamps with their essential radiation at 3650 and 2537A., respectively, are satisfactory for most purposes. The use of a monochromator to produce narrow bands for the excitation of the fluorescence of minerals does not seem warranted.

LITERATURE CITED

(1) Barr, C. G., *Plant Physiol.*, 23, 443 (1948).

(2) Goodwin, R. H., and Kavanagh, F., *Arch. Biochem.*, 27, 152 (1950).

(3) Hummel, J. P., *J. Biol. Chem.*, 180, 1225 (1949).

(4) Leininger, E., and Katz, S., ANAL. CHEM., 21, 810 (1949).

(5) *Ibid.*, p. 1375.

(6) Schwaer, L., *Collection Czechoslav. Chem. Commun.*, 7, 326 (1935).

THE report on the determination of uranium concerns work done on behalf of the U. S. Atomic Energy Commission and is published with the permission of the commission.

Spectrophotometric Determination of Small Quantities of 2,4-Dichlorophenoxyacetic Acid and 2,4,5-Trichlorophenoxyacetic Acid

Using Partition Chromatography

NATHAN GORDON[1], *Insecticide Division, Livestock Branch, Production and Marketing Administration, U. S. Department of Agriculture, Beltsville, Md., and* MORTON BEROZA, *Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Beltsville, Md.*

THE recent widespread use of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) as weed killers has unfortunately given rise to instances of contamination of liquid insecticide concentrates used on plants susceptible to these compounds. For example, large quantities of cotton were reported to be seriously damaged as a result of utilizing these contaminated liquid insecticide concentrates. A method which could detect small quantities of the contaminants as well as distinguish between them would be of use both in control and in regulatory enforcement work.

Previous methods have been published for the determination of small quantities of 2,4-D. A bioassay method involves the use of plants (*10*). A colorimetric qualitative test for 2,4-D has been described by Freed (*2*) and a modification of this test has been used to determine 2,4-D in milk (*6*). A method for determining 2,4-D in soil leachates by means of countercurrent distribution has also been described (*11*). These methods lack specificity, are time-consuming, and do not differentiate between 2,4-D and 2,4,5-T. After the present method was completed a method for the determination of 2,4-D by chromatography on kieselguhr containing a strong phosphate buffer was reported (*9*). The amount of 2,4-D is determined by titration.

The method described below involves the separation of the acids, after a suitable extraction procedure, by partition chromatography of the Martin and Synge type (*7*). After separation, the 2,4-D and 2,4,5-T are determined spectrophotometrically by measuring the ultraviolet absorbancy of the acids at 284 and 289 mμ, respectively.

In the chromatography of acids, Isherwood (*4*) recognized that narrower zones could be obtained by repressing the ionization of acids. He accomplished this by using 0.5 *N* sulfuric acid as the immobile phase. Lugg and Overell (*5*) used formic and acetic acids to repress the ionization of acids in paper chromatography and thus obtained narrower zones. This principle was utilized in the development of the method below; the method may be generally useful for the separation of acids which absorb in the ultraviolet.

A hydrolysis step is included to convert any ester, amide, or salt of 2,4-D or 2,4,5-T to the free acids. The hydrolysis of the esters, amides, and salts is complete after 1 hour's refluxing with 25% sodium hydroxide if wetting agent is present.

REAGENTS

Ether, USP. Carbon tetrachloride, C.P. Distill before use.

n-Hexane, commercial grade. Purify following the method of Graff, O'Connor, and Skau (*3*), passing the *n*-hexane through silica gel, and retaining only the portion sufficiently transparent to be read in the spectrophotometer at 230 mμ. Distill the effluent before use.

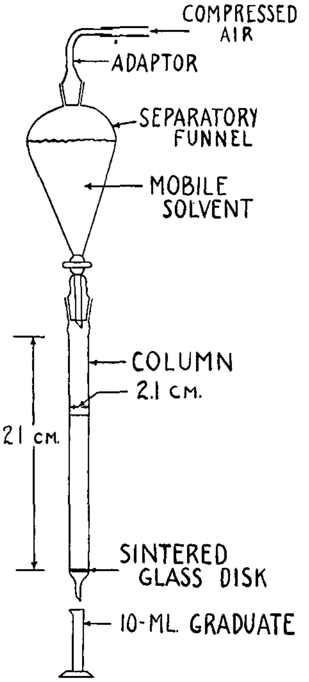


Figure 1. Chromatographic Column

Glacial acetic acid, C.P. Absolute methanol, C.P. Both used as received.

Formic acid (Eastman 139). Distill before using to prepare the 90% formic acid.

Immobile Solvent. Add 2 ml. of 1 to 5 90% formic acid–glacial acetic acid mixture to 18 ml. of 90% methanol and mix.

Mobile Solvent (*n*-hexane saturated with immobile solvent). Add approximately 400 ml. of purified *n*-hexane to 20 ml. of the immobile solvent contained in a 500-ml. separatory funnel and agitate vigorously for 5 minutes. Allow the layers to separate. Draw off the immobile solvent and reserve for use.

Silicic acid. Analytical reagent grade No. 2847 supplied by Mallinckrodt Chemical Works was used in the chromatographic separations.

Silica gel. Mesh size 28–200, used for the purification of *n*-hexane. Supplied by Davison Chemical Co., Baltimore, Md.

Pure 2,4-dichlorophenoxyacetic acid (2,4-D). Prepared by recrystallizing the

---

[1] Present address, Office of the Chief Chemical Officer, Washington 25, D. C.

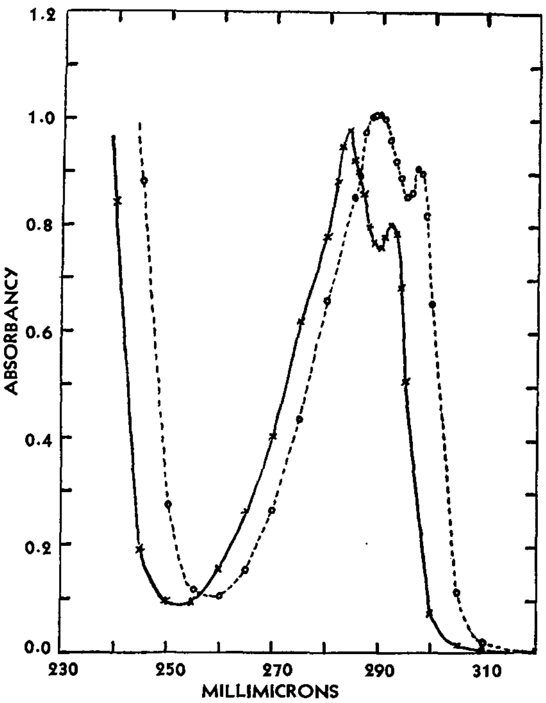


Figure 2. Ultraviolet Absorption Curves in Mobile Solvent

0.108 mg. per ml.
——— 2, 4-D
- - - - 2, 4, 5-T

commercial material from hot benzene to constant melting point, 140–141° (literature 140–141°).

Pure 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Prepared by recrystallizing the commercial material from hot benzene to constant melting point, 154° (literature 153°).

Sodium hydroxide, c.p., 15 and 25% aqueous solutions (freshly made).

Purified cotton. Extract in a Soxhlet apparatus for 24 hours with acetone, remove solvent, and finally dry at 100° overnight.

EQUIPMENT AND APPARATUS

All spectrophotometric measurements were made on a Beckman Model DU quartz spectrophotometer using 1.0-cm. square silica cells.

The chromatographic setup consists of a column, a reservoir, an adapter, and an air supply (Figure 1). The column is a glass tube, 2.1 cm. in inside diameter and 21 cm. long, with a sintered-glass disk sealed just above the constriction in the tube. A female 24/40 joint is sealed on the upper end of the column. The reservoir is a 250-ml. separatory funnel fitted with a male 24/40 joint at the lower end and a female 24/40 joint at the upper end. The adapter has a 24/40 male joint which fits into the upper end of the reservoir and delivers the air pressure to the apparatus. The air supply is regulated by an air pressure regulator and reducing valve which is attached to the laboratory compressed air line. The valve is provided with a pressure-indicating gage reading in pounds per square inch units and is connected to the adapter by means of pressure rubber tubing.

For solvent evaporation an all-glass apparatus is used.

DETECTION AND ESTIMATION

Both 2,4-D and 2,4,5-T absorb in the ultraviolet region and this property was used to detect and determine the quantity of these compounds. The absorption curves of 2,4-D and 2,4,5-T in the mobile phase are shown in Figure 2. The absorbancies of the maxima at 284 mμ for 2,4-D and 289 mμ for 2,4,5-T were found to obey Beer's law from 0 to 180 micrograms per milliliter for both compounds. The absorbancy index for 2,4-D at 284 mμ was found to be equal to 8.964 (molar absorbancy index = 1981) and the absorbancy index for 2,4,5-T at 289 mμ was found to be equal to 9.822 (molar absorbancy index = 2509).

PROCEDURE FOR ANALYSIS OF LIQUID INSECTICIDE CONCENTRATES

Extraction. Weigh out a 1-gram sample by means of a weighing buret and record the weight of the sample by difference. Transfer the sample into a Ŧ 125-ml. Erlenmeyer flask and add 10 ml. of freshly made 25% aqueous sodium hydroxide solution and one glass bead. Add about 3 to 4 drops of wetting agent if none is present. Attach the flask to a reflux condenser and reflux for 1 hour. At the end of this time, wash down the condenser with about 10 to 15 ml. of water and remove the flask. Cool the flask under tap water. Transfer the contents to a 125-ml. separatory funnel using a short-stemmed funnel and an irregular-shaped piece of glass to trap the glass bead and prevent clogging of the funnel. Wash flask and funnel with about 25 ml. of water.

Extract the alkaline solution twice with two 75-ml. portions of distilled ether, shaking for 1 minute each time. Allow the layers to separate completely. Backwash the ether extracts in reverse order—i.e., wash the second ether extract with 10 ml. of 15% sodium hydroxide solution, shaking for 2 minutes. Pass this alkaline wash into the first ether extract and shake again for 2 minutes. Add the alkaline wash to the main alkaline extract and discard the two ether extracts. Add 1 drop of phenolphthalein solution and carefully neutralize with 1 + 1 (by volume) sulfuric acid. Then add an excess of about 1 ml. While the acid is added, keep the separatory funnel cool under running tap water. Extract the acidified solution twice with two 25-ml. portions of distilled carbon tetrachloride, shaking for 3 minutes each time. Pass the two carbon tetrachloride extracts in succession through a small piece of purified cotton into a Ŧ long-necked round-bottomed 125-ml. flask containing two glass beads. Wash the funnel quantitatively with carbon tetrachloride. Distill off the carbon tetrachloride in a convenient all-glass assembly—e.g., a Friedrich's condenser—until about 5 to 10 ml. remains. Connect the flask by means of a ground-glass joint fitted with a stopcock to a vacuum source (about 100 mm.) and evaporate just to dryness while holding the flask in a 35° to 40° water bath. Gently swirl the flask during the evaporation to prevent bumping and ensure a smooth evaporation. The sample is now ready for placement on the column for analysis.

Preparation of the Column. Dry the silicic acid at 105° overnight and then store in a stoppered bottle until used. Weigh out 15 grams of the silicic acid into a mortar and add 10.5 ml. of immobile solvent. Grind vigorously for about 1 minute, then add mobile solvent to form a thick slurry and grind until smooth

The recent widespread use of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) as weed killers has unfortunately given rise to instances of contamination of liquid insecticide concentrates used on plants susceptible to these compounds. A method has been devised, which can detect small quantities of these contaminants as well as distinguish between them, for use in both control and regulatory enforcement work. The two acids are separated, after a suitable extraction procedure, by partition chromatography and determined spectrophotometrically by measuring the ultraviolet absorbancy of the acids at 284 and 289 mμ. The partitioning solvents are 90% methanol and *n*-hexane and the supporting medium is silicic acid. A 1 to 5 formic-acetic acid mixture is added to the 90% methanol (immobile phase) to repress ionization of the acids. A background correction is frequently necessary and the means of making such a correction is given. The method may be used for the determination of small quantities of 2,4-D in liquid insecticide concentrates, insecticide dusts, and alfalfa hay extracts. Other herbicidal compounds or intermediates used in the preparation of 2,4-D and 2,4,5-T do not interfere. Determinations are within 5% of added amounts of acid.

Add more mobile solvent until a free-flowing slurry is obtained (a total of 35 to 40 ml. of mobile solvent will accomplish this effect). Pour the slurry into the column (need not be quantitative). Wash down the sides of the column with mobile solvent until its level is about 1.5 inches from the top of the column. Attach the adapter directly to the top of column and apply air pressure (6 to 7 pounds per square inch) to force the mobile solvent through at the rate of 2 ml. per minute. Tap the sides of the column from time to time to remove air bubbles and to ensure an even surface to the top of the silicic acid column. Collect the effluent in a flask. The column height is generally about 12 cm. While the column is being readied, switch on the Beckman spectrophotometer with the hydrogen lamp in position for readings in the ultraviolet region.

Prepare the sample for the column by adding 3 ml. of mobile solvent to the residue in the 125-ml. round-bottomed flask and thoroughly wash down the sides of the flask with the aid of a dropping pipet. When the level of the mobile solvent on the column is just above the top surface of the silicic acid, release the air pressure and remove the adapter.

Operation of Column. Introduce the sample into the column with the aid of a dropping pipet by touching the pipet to the inside wall of the column and allowing the sample to flow slowly down the walls onto the surface of the silicic acid. After the sample has been introduced, apply the air pressure and place a 10-ml. graduate under the column to receive the effluent which should be collected at the rate of 2 ml. per minute. Release the air pressure when the level of the solvent is just above the top of the silicic acid. Do not ever allow the column to run dry. Rinse the round-bottomed flask with 1 ml. of mobile solvent and wash down the sides of the column. After this liquid has been forced into the gel, repeat this process once more.

Wash down the sides of the column with more mobile solvent and fill the column to within an inch of the joint. Fill the reservoir separatory funnel with mobile solvent and attach to the column. Open the stopcock (the stopcock must not contain stopcock grease, but is lubricated with mobile solvent instead) and attach the adapter to the top of the funnel. Apply air pressure (6 to 7 pounds per square inch) and collect 10-ml. fractions of effluent. Use two graduates. As one graduate fills up, replace it with the other. While a fraction is being eluted, read and record the absorbancy of the previous fraction at 284 and 289 mμ with the slit width knob set at 0.7.

Zone Boundaries. The 2,4,5-T is generally eluted between 120 and 180 ml. and the 2,4-D between 190 and 290 ml. on a 15-gram column. The exact boundary of each zone is readily judged by following the approximate absorbancy ratios of the individual fractions at 284 and 289 mμ (*1*). When the 2,4,5-T zone starts, a sudden rise in the 289 mμ absorbancy as compared with the 284 mμ absorbancy will be noticed. At the start of the 2,4-D zone, the 284 mμ absorbancy will rise more rapidly than the 289 mμ absorbancy. The fractions collected prior to the beginning of the 2,4,5-T zone,—i.e., the first 120 ml. of effluent—are held for solvent recovery. The portions of effluent used for reading the absorbancies and the volumes remaining in the 10-ml. graduate are collected and combined in a glass-stoppered 100-ml. graduate. Two 100-ml. graduates are required to collect the 2,4,5-T and 2,4-D zones. The total volume and absorbancy of the 2,4,5-T and the 2,4-D zones are read and recorded at 289 and 284 mμ, respectively.

Calculations. The amount of each acid is determined from the following formulas:

$$\text{Mg. of 2,4,5-T} = \frac{\text{ml.} \times \text{absorbancy at 289 m}\mu}{9.82}$$

$$\text{Mg. of 2,4-D} = \frac{\text{ml.} \times \text{absorbancy at 284 m}\mu}{8.96}$$

Corrections for Background. If the absorbancy readings just preceding and following a zone are significantly higher than the blank reading, a correction must be applied to the final absorbancy reading. When liquid insecticide concentrates of known composition were analyzed, the absorbancy readings just preceding and following each zone were a little higher than the blank reading. In such instances the arithmetic means of the absorbancy values preceding and following the zones were used to correct for this background error by assuming this background to be linear. In experiments with and without the addition of the acids, this assumption was shown to hold; and unless this correction was applied, the results were several per cent high. Thus a 60-ml. 2,4,5-T zone, starting at 0.030 and ending at 0.015, with an absorbancy reading of 0.320 would be corrected in the following manner:

$$\text{Mg. of 2,4,5-T} = \frac{\text{ml.} \times [A_{289} - 1/2\,(A_{289}\text{ at start} + A_{289}\text{ at end})]}{9.82}$$

$$= \frac{60 \times [0.320 - 1/2\,(0.030 + 0.015)]}{9.82}$$

where A_{289} = absorbancy at 289 mμ.

The same reasoning applies to 2,4-D, except that the absorbancy values (A_{284}) preceding and following the 2,4-D zone are used to correct for the background error as follows:

$$\text{Mg. of 2,4-D} = \frac{\text{ml.} \times [A_{284} - {}^1/_2\,(A_{284}\text{ at start} + A_{284}\text{ at end})]}{8.96}$$

where A_{284} = absorbancy at 284 mμ.

While this method of background correction has been found to give satisfactory results in the present analyses, it may be more accurate in some cases to apply other types of background corrections (*8*). For instance, if the background may be assumed to be linear between two wave lengths, such as 255 and 310 mμ, or experimental evidence indicates that such an assumption is justified, the background absorption at 284 and 289 mμ may be calculated and subtracted.

RESULTS

The data indicated that the method for the separation and determination of the pure compounds by partition chromatography was satisfactory. Recoveries of 2,4-D and 2,4,5-T from a column were within 1 to 5% of the added amounts (Table I). Combinations of from 0.5 to 4.0 mg. of each of the acids were separated completely.

Recoveries of 2,4-D and 2,4,5-T added to a 1-gram sample of a prepared liquid insecticide concentrate (40% toxaphene–20% DDT–24% xylene–5% petroleum distillates–11% Triton X-100) indicated that the acids may be determined with an accuracy of within 5% of the added amount (Table II).

The method was applied to three authentic contaminated liquid insecticide concentrates, consisting chiefly of toxaphene and DDT, which were responsible for considerable damage to cotton crops. They gave a positive qualitative test for a 2,4-D type compound by bioassay. The results showed that 2,4-D but no 2,4,5-T was present (Table III).

Table I. Recoveries of 2,4-D and 2,4,5-T from a Column

Added, Mg.		Recovered			
2,4,5-T	2,4-D	2,4,5-T, mg.	2,4-D, mg.	2,4,5-T, %	2,4-D, %
0.5	4.0	0.47	3.87	95	97
4.0	0.5	4.02	0.49	100	98
0.5	0.5	0.50	0.51	100	102
4.0	4.0	3.84	4.04	96	101

Table II. Recoveries of 2,4-D and 2,4,5-T Added to Prepared Liquid Insecticide Concentrate

Added, Mg.		Recovered			
2,4,5-T	2,4-D	2,4,5-T, mg.	2,4-D, mg.	2,4,5-T, %	2,4-D, %
0.5	0.5	0.48	0.51	96.0	102.0
0.5	0.5	0.48	0.52	96.0	104.0
1.0	1.0	0.95	1.04	95.0	104.0
1.0	1.0	0.97	1.05	97.0	105.0
2.0	2.0	1.98	2.02	99.0	101.0
2.0	2.0	1.94	1.99	97.0	99.0

Table III. Analysis of Three Authentic Contaminated Liquid Insecticide Concentrates

Sample No.	Wt. of Sample, G.	2,4-D Found, P.P.M.
1	0.8000	558
	1.0830	570
2	0.6990	684
	1.0350	675
3	0.8620	751
	0.8090	747

Recoveries of 2,4-D and 2,4,5-T added to a 1-gram sample of a prepared insecticide dust (18% BHC–5% DDT–40% sulfur–37% Attaclay) are shown in Table IV. These results were obtained by saponifying the entire sample, rather than an extract. It is probable that somewhat better results could have been obtained on an extract.

Recoveries of 2,4-D and 2,4,5-T added to an alfalfa hay extract are listed in Table V. In this study, 10 grams of alfalfa hay were extracted with ether for 8 hours in a Soxhlet apparatus. The 2,4-D and 2,4,5-T were added to the dried extract. The procedure for liquid insecticide concentrates was followed with one modification. The saponified mixture was filtered before extracting with ether.

Table IV. Recoveries of 2,4-D and 2,4,5-T Added to a Prepared Insecticide Dust

Added, Mg.		Recovered			
2,4,5-T	2,4-D	2,4,5-T, mg.	2,4-D, mg.	2,4,5-T, %	2,4-D, %
2.0	2.0	1.87	1.89	93.7	94.5
2.0	2.0	1.83	1.93	92.0	96.5

Table V. Recoveries of 2,4-D and 2,4,5-T Added to Alfalfa Hay Extracts

Added, Mg.		Recovered			
2,4,5-T	2,4-D	2,4,5-T, mg.	2,4-D, mg.	2,4,5-T, %	2,4-D, %
1.0	1.0	1.03	1.05	103	105
1.0	1.0	0.96	1.03	96	103
2.0	2.0	1.98	2.10	99	105
2.0	2.0	1.94	2.02	97	101

The combined results of all analyses indicated an average recovery of 96.1% for 2,4,5-T and 100.6% for 2,4-D. To obtain some idea of the precision of the method, the average deviations from the means for both 2,4-D and 2,4,5-T in Tables I, II, IV, and V were calculated and each was found to be 1.6%.

INTERFERING SUBSTANCES

Intermediates. 2,4-Dichlorophenol and 2,4,5-trichlorophenol are eluted prior to the acids on passage through a silicic acid column. Hence, these compounds do not interfere. Sodium chloroacetate does not absorb in the 285 mμ region and hence offers no source of interference.

Other Herbicidal Compounds. Twelve other herbicides were considered as possible interferers in this method. Two groups were prepared, each group containing about 2 mg. of each compound. The herbicidal compounds were commercial samples. The first group consisted of calcium cyanamide, ammonium sulfamate, sodium trichloroacetate, potassium cyanate, and maleic hydrazide. These five compounds were not expected to interfere and after this group had been subjected to the entire procedure, it was found, as predicted, that no interference occurred.

The second group consisted of sodium pentachlorophenate, pentachlorophenyl ethyl carbonate, sodium isopropyl xanthate, isopropyl *N*-phenyl carbamate, sodium dinitro-*o*-cresolate, ammonium dinitro-*o*-*sec*-butyl phenol, and phenylmercuric acetate. In the presence of all seven of these compounds, the "apparent" 2,4,5-T content was only 1.2% low while the "apparent" 2,4-D content was only 2.2% high, on a 2-mg. level of each acid with the background correction being applied. The spectra of the apparent 2,4,5-T and apparent 2,4-D zones were not at all similar to the spectra of 2,4,5-T and 2,4-D.

DISCUSSION

Of all the solvents used to extract the nonacidic material from the alkaline mixture of the hydrolyzed insecticide concentrates, only ether gave a clean separation of layers. Other solvents formed cloudy solutions or produced emulsions that were difficult to break. Extraction of the acidified solution with ether and a number of other solvents resulted in low recoveries of the acids, particularly the 2,4,5-T. However, recoveries were closer to theoretical when carbon tetrachloride was the extraction solvent.

A series of experiments, not reported herein, was conducted to determine the amount of acid to add to the immobile phase. Acetic acid repressed ionization, but somewhat better results were obtained when some formic acid was also added. Separations of 2,4-D and 2,4,5-T can be obtained without using either acetic or formic acid, or both, but the amounts employed were found to give the best results.

As greater proficiency was obtained in using the method, results could be duplicated better. It is believed that analyses should be within 2 to 3% of the true value once sufficient experience is gained in an analysis. With different lots of adsorbent it is possible that recoveries may vary owing to the adsorbent itself. With the pure acids the recoveries of 2,4,5-T averaged 97.8%, whereas corresponding recoveries of 2,4-D were 99.5% (Table I). In general, the recoveries of 2,4,5-T were slightly low and a correction of 2.2%, although not made, would have improved the results.

Lesser amounts of the acids can undoubtedly be determined if a narrower column or a larger sample is used.

Reagents of good quality were found to be necessary throughout the procedure. It was necessary to distill the ether, carbon tetrachloride, and formic acid. The sodium hydroxide solutions were prepared fresh each day. It was necessary to purify the cotton by acetone extraction to remove impurities that absorb in the ultraviolet. The hexane required the purification described under Reagents. Once purified, the hexane was re-used by washing the recovered solvent with alkali and water and then distilling to recover the hexane. As in all column work, it is necessary to ensure a smooth, even surface to the top of the silicic acid. This can be accomplished in a few trials and need offer no difficulties to the analyst.

The absorption spectra of both 2,4,5-T and 2,4-D shown in Figure 2 may be used to confirm the presence or absence of 2,4,5-T or 2,4-D.

LITERATURE CITED

(1) Beroza, M., Anal. Chem., 22, 1507 (1950).
(2) Freed, V. H., *Science*, 107, 98 (1948).
(3) Graff, M. M., O'Connor, R. T., and Skau, E. L., Ind. Eng. Chem., Anal. Ed., 16, 556 (1944).
(4) Isherwood, F. A., *Biochem. J.*, 40, 688 (1946).
(5) Lugg, J. W., and Overell, B. T., *Australian J. Sci. Research*, Series A, 1, 98 (1948).
(6) Marquardt, R. P., and Luce, E. N., Anal. Chem., 23, 1484 (1951).
(7) Martin, A. J. P., and Synge, R. L. M., *Biochem. J.*, 35, 1358 (1941).
(8) Mellon, M. G., "Analytical Absorption Spectroscopy," New York, John Wiley & Sons, 1950.
(9) Stroud, S. W., *Analyst*, 77, 63 (1952).
(10) Swanson, C. P., *Bot. Gaz.*, 106, 507 (1946).
(11) Warshowsky, B., and Schantz, E. T., Anal. Chem., 22, 460 (1950).

Received for review July 8, 1952. Accepted September 20, 1952. Presented in part before the Division of Analytical Chemistry at the 122nd Meeting of the American Chemical Society, Atlantic City, N. J. From the dissertation submitted by Nathan Gordon to the faculty of the Graduate School of Georgetown University, 1952, in partial fulfillment of the requirements for the degree of doctor of philosophy.

Determination of Small Amounts of Chlorides in Titanium Sponge

MAURICE CODELL AND JAMES J. MIKULA
Pitman-Dunn Laboratories Department, Frankford Arsenal, Philadelphia, Pa.

In titanium casting operations, even low concentrations of chloride cause spattering of molten metal and interfere with the production of satisfactory castings. It is therefore necessary to have a procedure by which the chloride content of titanium sponge can be accurately determined. An accurate and fairly rapid method for determining chloride in titanium sponge has been developed which is satisfactory for chloride concentrations in the range 0.001 to 0.20%. The production of titanium sponge is increasing rapidly and methods for quality control must be capable of handling large numbers of samples simultaneously. This procedure is well adapted for use in routine analysis and can be carried out with apparatus common to most laboratories.

A PHOTOMETRIC procedure for the determination of small amounts of chlorides in titanium sponge was chosen, because the absolute amounts of chloride present are so small. When an ammoniacal solution of silver chloride is treated with a soluble sulfide, a colloidal suspension of silver sulfide is formed. The resulting color is amber and the amount of silver can be quantitatively determined by photometric means (*7*).

Kuroda and Sandell (*4*) developed a satisfactory method for determining small amounts of chlorine in silicate rocks based on this procedure. Many other applications of this procedure have been made in biochemical analysis.

Titanium was originally dissolved by digesting with sulfuric acid, but this required several hours. It was found that solution could be effected rapidly through the use of hydrofluoric acid. The excess hydrofluoric acid was converted to the harmless fluoboric acid. This procedure proved entirely satisfactory.

When chlorides are precipitated from solution containing titanium, sufficient acidity must be maintained to prevent hydrolysis of titanium (*5*). The coagulation of silver chloride in the presence of titanium cannot be hastened by any of the usual procedures. Heating causes rapid hydrolysis of the titanium, and shaking by mechanical means appears to retard coagulation.

Considerable efforts were made to separate silver chloride by centrifuging, but in many cases results were low because of "creeping" of very fine silver chloride particles and their subsequent loss on decantation. It was found most practical to permit the precipitate to settle overnight in a dark place. The silver chloride could be readily filtered the following morning, redissolved with ammonium hydroxide, and treated with sodium sulfide to form a colloidal suspension of silver sulfide which is quantitatively determined by use of a spectrophotometer. Ions forming insoluble silver salts, such as bromides and iodides, must be absent.

It has been the authors' experience that at least 10 to 15 minutes are required after the addition of the sodium sulfide for full development of the color, which remains quite stable for about 1 hour, then gradually begins to deepen. The silver sulfide sol formed by the addition of sodium sulfide to the ammoniacal solution was found to be unstable on longer standing in the concentrations of chloride reported here. A cloudy solution resulted in most cases on standing overnight. Attempts were made to stabilize the silver sol by using gelatin as a protective colloid in accordance with the instructions of Snell and Snell (*7*) but no appreciable difference could be observed. Equally accurate results were obtained with or without the addition of gelatin solution. It was found advisable to take all transmittance readings at approximately the same time interval after the addition of the sodium sulfide, owing to the instability of the color. Low results were obtained by taking readings before full color development, while readings taken much later yielded high results. Repeated tests showed that readings taken between 15 to 30 minutes after the addition of the sulfide yielded the most accurate results. The calibration curves and all results reported here are, therefore, based on readings taken between 15 and 30 minutes after the sulfide addition.

SCOPE

This method is satisfactory for determining chlorides in titanium sponge when the chloride content lies within the range of 0.001 to 0.200%.

CONCENTRATION RANGE

The recommended concentration range is from 0.10 to 2.00 mg. of chloride in 100 ml. of solution and from 0.01 to 0.10 mg. of chloride in 10 ml. of solution using matched 13-mm. cells. In this procedure optically matched cells having a 13-mm. light path were used. Cells having other dimensions may be used, if suitable adjustments are made in the amounts of sample and the reagents used.

APPARATUS AND REAGENTS

Apparatus. Spectrophotometer, Universal Coleman Model 14 or equivalent apparatus having optically matched cuvettes.

Selas crucibles, No. 2001.

Plastic beakers of approximately 250-ml. capacity with covers (polyethylene or polystyrene).

A 500-ml. suction filtering flask with an adapter suitable for holding a No. 2001 Selas crucible and having a drawn-out stem which can fit into the neck of a 10-ml. volumetric flask.

Volumetric flasks, 100-ml. and 10-ml.

Reagents. Standard sodium chloride solution. Dissolve 0.1648 gram of dried C.P. sodium chloride in 1 liter of distilled water. 1 ml. = 0.10 mg. of chloride.

Dilute nitric acid, specific gravity approximately 1.20. Dilute 403 ml. of concentrated nitric acid (specific gravity 1.42) to 1 liter with distilled water.

Hydrofluoric acid, 48%, ACS reagent.

Boric acid, C.P. grade.

Silver nitrate solution, approximately 0.1 *N*. Dissolve 4.25 grams of C.P. silver nitrate in 250 ml. of distilled water.

Nitric acid wash solution, 1%. Dilute 5 ml. of concentrated nitric acid (specific gravity 1.42) to 500 ml., in a wash bottle with water.

Ammonium hydroxide solution, 1 to 1. Dilute 250 ml. of concentrated ammonium hydroxide (28%) with 250 ml. of water.

Sodium sulfide solution, approximately 0.1 *M*. Dissolve 2.5 grams of sodium sulfide nonahydrate in 100 ml. of distilled water.

PREPARATION OF CALIBRATION CURVES

A. Chloride Concentrations between 0.01 and 0.20%.

a. Place representative sized aliquots of standard sodium chloride solution, covering the desired range 0.10 to 2.00 mg. of chloride, in 200-ml. beakers, and carry through an additional beaker as a blank.

b. To each beaker add 60 ml. of distilled water, 5 ml. of dilute nitric acid, and 4 ml. of silver nitrate solution. Mix thoroughly and let stand in a dark place overnight.

c. Filter the precipitate on a Selas crucible. Wash beaker twice with small portions of nitric acid wash solution; finally, remove any remaining precipitate with a policeman, and wash twice with small portions of water.

d. Discard the filtrate and wash the flask thoroughly. Dissolve the precipitate completely by using 20 ml. of 1 to 1 ammonium hydroxide and wash several times with water.

e. Transfer the filtrate to a 100-ml. volumetric flask, add 1 ml. of sodium sulfide solution, dilute to volume with water, and mix.

f. After 15 minutes, transfer a suitable portion of the solution to an absorption cell and measure the transmittancy at 415 mμ, using as a reference cell the blank carried through from above containing all the reagents.

Plot the logarithm of the transmittancy values obtained against the concentration of chloride per 100 ml. of solution.

Table I. Accuracy of Method

Cl Added, %	Average Cl Found, %	Standard Deviation, %	No. of Determinations
0.0010	0.0010	....	1
0.0020	0.0023	0.0002	3
0.0040	0.0035	0.0004	3
0.0060	0.0060	0.0001	3
0.0080	0.0079	0.0004	3
0.0100	0.0102	0.0013	4
0.015	0.015	0.001	4
0.022	0.022	0.001	3
0.033	0.033	0.002	3
0.047	0.046	0.001	2
0.055	0.056	0.000	2
0.070	0.069	0.001	4
0.087	0.087	0.000	2
0.099	0.099	0.001	3
0.105	0.106	....	2
0.120	0.120	0.003	3
0.155	0.155	....	1
0.178	0.178	0.001	3
0.199	0.199	....	1

B. Chloride Concentrations between 0.001 and 0.01%.

a. From a buret measure exactly 50 ml. of standard sodium chloride solution into a 500-ml. volumetric flask. Dilute to the mark with distilled water and mix thoroughly. One milliliter will then contain 0.01 mg. of chloride.

b. Place representative sized aliquots of this standard sodium chloride solution, covering the desired range 0.01 to 0.10 mg. of chloride, in 200-ml. beakers, and carry through an additional beaker as a blank. Continue in accordance with *A*, *b* and *c*.

c. Break the suction and discard the filtrate. Place a 10-ml. volumetric flask inside the filtering flask and insert the drawn-out stem of the adapter into the neck of the volumetric flask. Add 2 ml. of 1 to 1 ammonium hydroxide to the crucible. Allow sufficient time for the precipitate to dissolve completely. Connect the suction and wash several times with 1-ml. portions of distilled water until the total volume is approximately 8 ml. Remove the volumetric flask and to it add 0.1 ml. of sodium sulfide solution. Dilute to volume, and shake. Continue in accordance with *A*, *f*.

d. Plot the logarithm of the transmittancy values obtained against the concentration of chloride per 10 ml. of solution.

PROCEDURE

To a 1-gram sample of titanium in a plastic beaker, add 10 ml. of distilled water and 4 ml. of hydrofluoric acid, and cover immediately with a plastic cover. Carry a blank through, adding everything but the sample. After the titanium is completely dissolved (15 to 20 minutes), add 1 gram of boric acid, mix thoroughly, and dilute to 20 ml.

Transfer to a 200-ml. beaker, add 5 ml. of dilute nitric acid, and heat carefully on a hot plate until the solution becomes colorless. Stir constantly and avoid excessive heat because of the danger of hydrolysis. Dilute to 60 ml., add 4 ml. of silver nitrate solution, and allow to stand overnight in a dark place. Continue in accordance with *A*, *c*, as in the preparation of the calibration curve.

For samples containing 0.01 to 0.20% chloride continue in accordance with *A*, *d*, *e*, and *f*. For samples containing 0.001 to 0.01% chloride, continue in accordance with *B*, *c*.

From the proper calibration curve determine directly the concentration of chloride present in the sample.

Run a blank with each set of determinations to eliminate any possible error due to varying amounts of chloride in the reagents from one determination to the other.

RESULTS

The results listed in Table I indicate the accuracy of the method. A 1-gram sample of chloride-free cast titanium drillings was used in each of these analyses. Chloride was added to the titanium in the form of sodium chloride solution of known concentration. The calculated concentration of chloride in the reagent blanks remained constant at 0.01% in 100 ml. and 0.001% in 10 ml. of solution, using distilled water as the reference cell.

DISCUSSION

Several colorimetric methods for the determination of chlorides exist. Clarke (*2*) determined chloride in water by adding acid, mercuric ion, and diphenylcarbazone to the chloride solution and measuring the excess of mercuric ion in terms of color intensity. Siggia (*6*) developed a colorimetric method for determining micro amounts of silver and silver halides based on a spot test described by Feigl (*3*). The reaction $AgX + K_2[Ni(CN)_4] \rightarrow K[Ag(CN)_2] + Ni(CN)_2 + KX$ is carried out in a pyridine-ammonia-water system containing dimethylglyoxime and the color intensity of the resulting solution is measured. Baker and Reedy (*1*) developed a sensitive test for silver and the halides based on the production of a bright orange color when a solution of potassium iodide that has been saturated with mercuric iodide is added to a silver chloride precipitate. They claim that this procedure is not quantitative, however, and the test must be made without diluting the reagent, as dilution causes the separation of a red precipitate of mercuric iodide.

Any of the preceding colorimetric methods or many others could possibly be adapted to yield satisfactory results in the determination of chloride in titanium sponge. However, no work along these lines has been attempted by the authors, as the procedure described above proved entirely satisfactory in precision, simplicity, and freedom from interferences.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Herbert Engelbert for his assistance in performing some of the analyses reported here.

LITERATURE CITED

(1) Baker, P. S., and Reedy, J. H., Ind. Eng. Chem., Anal. Ed., 17, 268–9 (1945).

(2) Clarke, F. E., Anal. Chem., 22, 553–5 (1950).

(3) Feigl, F., "Qualitative Analysis by Spot Tests," p. 254, New York, Nordeman Publishing Co., 1939.

(4) Kuroda, P. K., and Sandell, E. B., Anal. Chem., 22, 1144–5 (1950).

(5) McAlpine, R. K., and Soule, B. A., "Prescott and Johnson's Qualitative Chemical Analysis," p. 385, New York, D. Van Nostrand Co., 1933.

(6) Siggia, S., Anal. Chem., 19, 923–4 (1947).

(7) Snell, F. D., and Snell, C. T., "Colorimetric Methods of Analysis," Vol. 1, p. 533, New York, D. Van Nostrand Co., 1936.

Received for review July 24, 1952. Accepted September 12, 1952.

Microdetermination of Flash Point on Petroleum Products

PHILIP McCUTCHAN AND D. A. YOUNG
Research and Process Department, Union Oil Co. of California, Brea, Calif.

The need arose for the determination of the flash point on very small quantities of petroleum products, and suitable methods could not be found in the literature. The procedure and equipment, as developed, allow the observation of a visible flash from 0.3 ml. of sample heated in a flash chamber drilled into an aluminum block. The precision is excellent and the apparatus is simple to construct and operate. The values obtained are the same as from the Cleveland open cup procedure, ASTM D 92–46, which requires approximately 70 ml. of sample. This correlation enables one to obtain flash points on the small quantities of products frequently encountered in research investigations.

THE development of micromethods for the determination of physicochemical measurements of oils is made desirable by the limited amounts of sample often available during research on petroleum products. Some of the micromethods that have been reported in the literature are: the determination of viscosity of oils by Levin (*5*) and by Cannon (*3*), and the determination of pour point, titer, and vapor pressure by Levin, Morrison, and Reed (*6*).

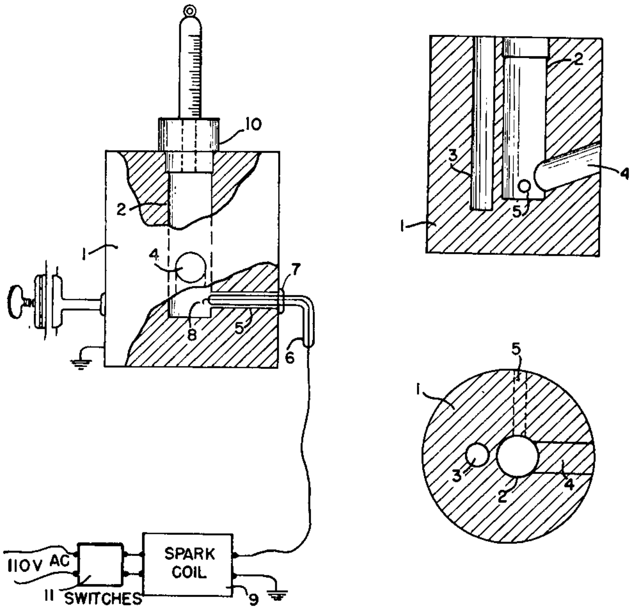


Figure 1. Micro Flash Block

One of the important properties of fuels and lubricants is the temperature at which the material flashes when it is exposed to a spark or flame. It is believed that a method for the determination of flash points on a micro scale would be of considerable value.

A number of methods have been proposed for flash point determination that use less material than required by ASTM methods.

Osmond and Abrams (*9*) devised a flash adapter that makes possible the use of 20-ml. samples in the Cleveland open cup and Able cup methods. Ettele (*4*) described an apparatus consisting of a grooved bar, heated at one end, in which the oil was placed and tested for flash point at various positions along the bar. Ormandy and Craven (*7*) determined flash points with 7 ml. of material in a test tube. An inverted bell was inserted in the test tube and an electric spark passed over the sample at frequent temperature intervals. These same workers (*8*) later devised another method for flash points in which 10 ml. of sample was tested at various pressures in a sealed chamber connected to a manometer. Ignition was by electric spark and the flash was detected by a surge in the manometer.

As no method was found in the literature for flash point determinations on a micro scale, the development of such a method was undertaken.

In considering the problem it was believed that the following conditions must be satisfied in order to obtain correct results:

1. The rate of temperature increase must be controlled
2. The accumulation of a flammable amount of vapor must be ensured by a controlled air circulation
3. To produce a distinct flash, the ignition must be made by an electric spark

Two different approaches to this problem have been followed in this laboratory.

The problem was first investigated by combining and modifying the procedures of Ormandy and Craven (*7, 8*). Although satisfactory results were obtained, this method was abandoned in favor of the simpler apparatus described below, which gave excellent results.

In the second method of investigation, the flash point was detected visually. The sample was placed in a chamber in an aluminum block that was heated at a controlled rate by a microburner. Heating by electrical means is no doubt possible, but was not tried because of the simplicity and ease of control afforded by the gas burner. The vapors were ignited by a spark directed against the side of the chamber. It was soon found that reproducible values could be obtained, but that the temperature at which a given flash point was observed was dependent on the size of the sample chamber and the circulation of air. These variables were balanced to give the Cleveland open cup (*1*) values on a micro scale.

Preliminary data indicated that modifications of the apparatus may allow correlation with Pensky-Martens closed tester (*2*) values. As yet, however, concordant results have not been obtained with all types of samples.

APPARATUS

The design of the apparatus is shown in Figure 1. It consists of a cylindrical aluminum block, 1, of 2-inch diameter and 2.5-inch length, containing a flash chamber, $^{31}/_{64}$ inch in diameter, 2, drilled to a depth of 1.875 inches with the bottom finished off flat. A 2-inch thermometer well, 3, is drilled close to the flash chamber. A slanted observation port, 4, of $^{11}/_{32}$-inch diameter, is drilled at a 70° angle, centered $1^{21}/_{64}$ inches from the top of the block, which will enter the flash chamber just above the surface of the sample. A horizontal $^{9}/_{64}$-inch hole, 5, centered $^{7}/_{32}$ inch from the bottom of the flash chamber, is drilled for insertion of the ignitor. The ignitor, 6, consists of a platinum wire sealed into a 3-mm. glass tubing. A proper position of the arcing point of the ignitor is assured by a flange, 7, on the tubing. The ignitor rests in the hole with the flange against the block as shown in Figure 1. Failure to insert the ignitor to the proper depth causes confusing reflections, and the incorrect position of the spark produces erroneous results. The ignitor is removed from the block after each determination to facilitate cleaning. The tip, 8, of the platinum wire should be bent downward so as to cause the spark to pass to the wall of the chamber on the lower side of the tubing. Current for the spark is conducted to the platinum wire from the secondary of a high voltage induction coil, 9, capable of furnishing a spark of at least 1-millejoule energy. The block is supported by a clamp on a vertical rod that gives facility in adjusting the height of the block. This provides a flexibility in heat control that is needed for samples of widely different flash points.

The flash chamber is closed by a cap, 10, bearing a center hole $^{11}/_{64}$ inch in diameter and with the cap fitting into the top of the chamber so as to extend down 0.25 inch. Total thickness of the cap is about $^{9}/_{16}$ inch.

The induction coil and aluminum block assembly are mounted in a three-sided case fashioned from sheet metal (see Figure 2 for details of assembly and wiring).

An ASTM open flash thermometer is used, graduated in Fahrenheit degrees, with the range from +20° to +760° F., and conforming to the requirements for thermometer 11 F, as described in the standard specifications for ASTM thermometers.

A high frequency coil of the Tesla type is required. A high frequency coil sold by the Central Scientific Co. is satisfactory for adaptation to the unit.

The danger of electrical shock is eliminated by connecting the primary of the induction coil through two push-button microswitches. The operator must use both hands to press these switches, and, therefore, cannot inadvertently contact the ignitor while there is high voltage. The flash block must be grounded at all times when in use. The sketch in Figure 2 shows the general arrangement.

PROCEDURE

Approximately 0.3 ml. of sample is introduced into the bottom of the flash chamber, and the cap is fitted into the top.

The sample may be conveniently added with a 1-ml. tuberculin syringe. The syringe is used without the needle.

The block is heated with a microburner at about 30° F. per minute until around 100° F. below the expected flash point and then at 10° F. per minute for at least the last 50° F. A spark should be made in the chamber at each 5° F. starting at about 30° F. below the flash point.

EXPERIMENTAL

In establishing the necessary balance of variables to attain the desired result, it was observed that the degree of circulation of the convection current of air above the sample was a very significant variable. This circulation was controlled by the diameter of the hole drilled through the aeration cap.

With no cap, flash was 20° F. high
With a $^{16}/_{64}$-inch hole, flash was 10° F. high
With an $^{11}/_{64}$-inch hole finally adopted, flash was correct
With cap closed, flash was 20° F. low

The ratio of the air space in the chamber to the sample was also important. Decreasing the volume of the flash chamber by about one third lowered the flash point about 15° F.

The proper dimensions for duplication of Cleveland open cup values over the range from 200° to 500° F. were found to be an ignition spark at about $^{1}/_{8}$ inch above the sample surface, a flash chamber of $^{31}/_{64}$-inch diameter and $1^{7}/_{8}$-inch depth, and an aeration cap bearing an $^{11}/_{64}$-inch hole with the cap fitted into and extending about 0.25 inch into the chamber.

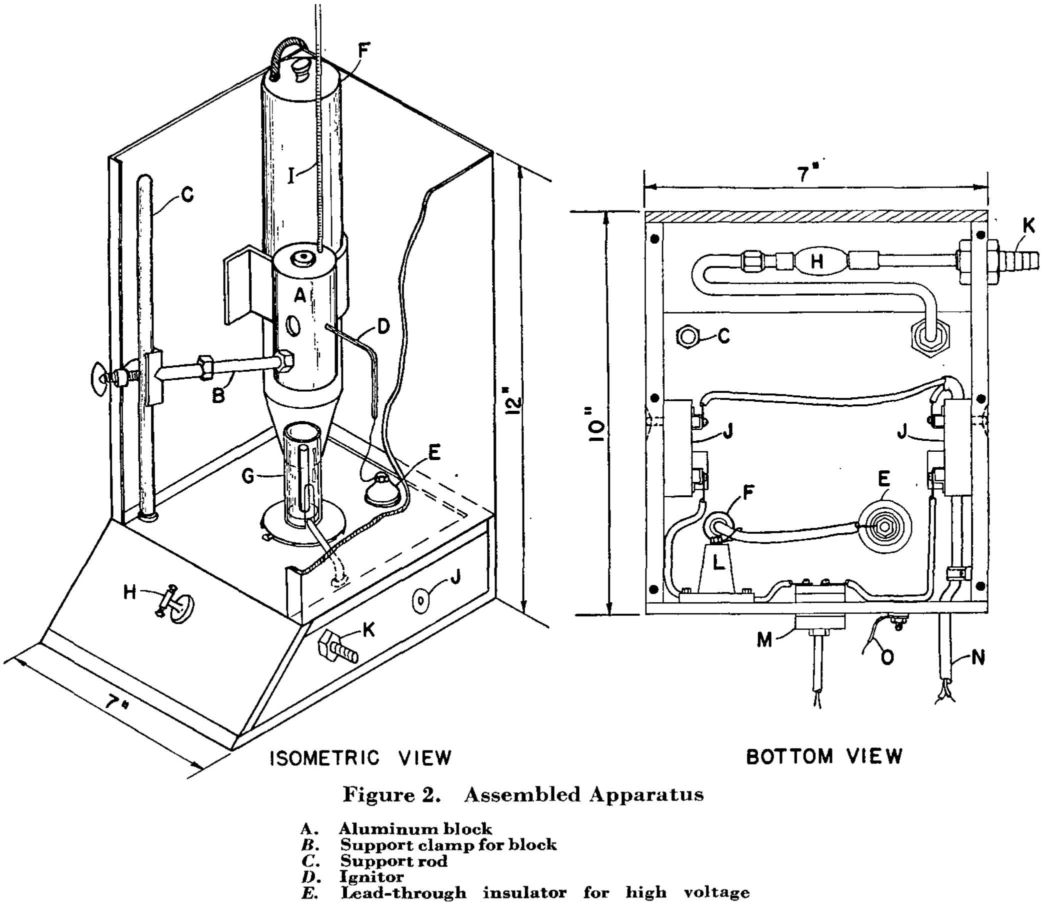


Figure 2. Assembled Apparatus

A. **Aluminum block**
B. **Support clamp for block**
C. **Support rod**
D. **Ignitor**
E. **Lead-through insulator for high voltage**
F. **High voltage induction coil**
G. **Microburner with flame shield**
H. **Valve for adjusting burner**
I. **Thermometer**
J. **Microswitches for operating sparker**
K. **Gas line hose connection**
L. **Standoff insulator**
M. **Plug and socket for induction coil**
N. **Line cord for induction coil**
O. **Ground connection**

Table I. Comparison of Cleveland Open Cup Flash Point with Micro Flash Point

Material	Cleveland Open Cup Flash Point (*1*), ° F.		Micro Flash Point, ° F.	
	Operator A	Operator B	Operator B	Operator C
Diesel receiver C	...	205 210	205 205	205 210
Spray oil A	305	300	305 305	...
Spray oil B	325	320	320 320 315	315
Spray oil C	340	345 340	345 345	340 340
Printing ink oil	360	360	360 360	... ...
SAE 20 lubricating oil	410	430	420 420	...
SAE 30 lubricating oil	435	435	440 445	445 450
SAE 30 compounded oil	450	465	455 460 460	...
Neutral oil	475	470	470 470	475 480
Mineral oil	500	490	490 495 480	...

RESULTS

Results have been obtained on samples covering a wide range of flash points with good duplicability throughout. For the range from 200° to 500° F., which is the range of normal interest for flash points by the Cleveland open cup, comparative results on 10 samples are shown in Table I. A large number of various types of oils have also been tested in the course of regular analytical work with good duplicability of results.

CONCLUSIONS

Apparatus has been designed and a method developed for obtaining reproducible flash points on a micro scale (0.3-ml. sample). In the range 200° to 500° F., the values are essentially the same as those obtained using the Cleveland open cup procedure described in ASTM method D 92–46. The micromethod, as compared to the macro, requires the same amount of time and gives equal precision, but uses only 0.3 ml. of sample instead of 50 to 70 ml.

The stated specifications must be closely followed, for the flash point values are very dependent on the dimensions of the apparatus. The design of the apparatus permits determinations in the open laboratory, because a dark hood is not required and the results are not affected by normal air currents.

ACKNOWLEDGMENT

The authors wish to express appreciation to the Union Oil Co. of California for permission to publish these data.

LITERATURE CITED

(1) Am. Soc. Testing Materials, "Standards on Petroleum Products and Lubricants," Designation D 92–46, September 1951.
(2) *Ibid.*, Designation D 93–46.
(3) Cannon, M. R., and Fenske, M. R., Ind. Eng. Chem., Anal. Ed., **10**, 297 (1938).
(4) Ettele, C., U. S. Patent 1,554,993 (Sept. 29, 1925).
(5) Levin, Harry, Ind. Eng. Chem., Anal. Ed., **9**, 147 (1937).
(6) Levin, Harry, Morrison, A. B., and Reed, C. R., Anal. Chem., **22**, 188–91 (1950).
(7) Ormandy, W. R., and Craven, E. C., *J. Inst. Petroleum Technol.*, **8**, 145–80 (1922).
(8) *Ibid.*, **9**, 39–45 (1923).
(9) Osmond, C. H., and Abrams, V. R., *Atlantic Lubricator*, **4**, No. 6 (February 1921).

Received for review June 17, 1952. Accepted August 18, 1952. Presented before the Division of Refining at the 17th Midyear Meeting of the American Petroleum Institute, San Francisco, Calif., May 12 to 15, 1952.

Colorimetric Estimation of Residual Benzene Hexachloride

WENDELL F. PHILLIPS
Beech-Nut Packing Co., Canajoharie, N. Y.

The lack of a sufficiently specific and sensitive chemical method for the detection of microgram quantities of benzene hexachloride prompted the investigation which led to the development of this colorimetric method. When benzene hexachloride is refluxed with an excess of aniline, a mixture believed to consist of diphenylamine and dichlorodiphenylamines is formed. This mixture forms a violet color with an absorption maxima at 510 mμ when oxidized with vanadium pentoxide in 50% sulfuric acid. Beer's law is obeyed over the range of 2 to 120 micrograms of the gamma isomer. The method described is readily adapted to routine quality control analyses and should be useful to the food industry.

SINCE Slade (*8*) announced the insecticidal activity of the gamma isomer of benzene hexachloride (1,2,3,4,5,6-hexachlorocyclohexane) it has become one of the major chemicals used in modern agriculture. The analytical methods (*1*) for determining this compound are not adequate for residue analysis, except for the recently described Schechter-Hornstein (*7*) method. The method proposed below is a modification of the procedure presented by Fairing (*3*) before the Division of Agricultural and Food Chemistry, at the 119th meeting of the American Chemical Society.

Among the published data on the chemistry of benzene hexachloride is a study reported in 1887 by Meunier (*6*), in which he noted a reaction between aniline and benzene hexachloride. Subsequent investigation revealed that, when a large quantity of gamma-benzene hexachloride is refluxed with aniline and the reaction products are separated, as indicated in the procedure, a mixture of compounds is obtained which forms a violet color (absorption maximum at 555 mμ) on oxidation in 50% sulfuric acid (v./v.) containing 0.05 mg. per ml. of vanadium pentoxide.

The mixture is believed to contain three components which form color when treated with vanadic acid. One of them is a dichlorodiphenylamine and the other two have not been satisfactorily identified, but they may be diphenylamine and another dichlorodiphenylamine. One of the unidentified compounds produces a red color in the vanadic acid solution which has an absorption maximum at 510 mμ. The dichlorodiphenylamine forms a purple color which has an absorption maximum at 555 mμ. With the same reagent, diphenylamine gives a blue color which has an absorption maximum at 595 mμ.

When microgram quantities of gamma-benzene hexachloride

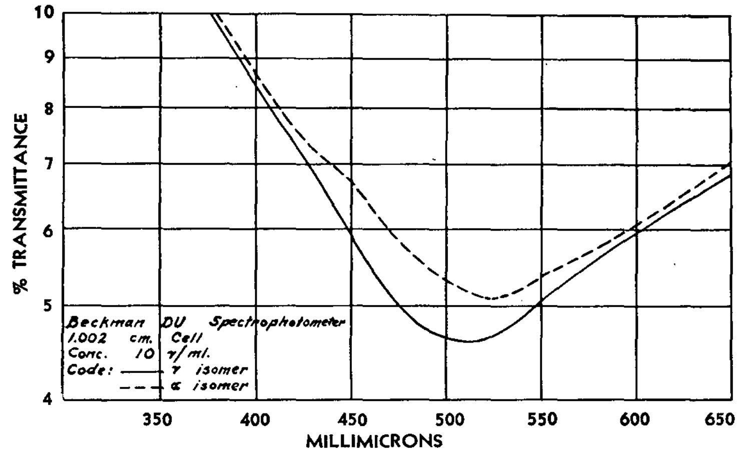


Figure 1. Absorption Spectra of Color Formed by Gamma and Alpha Isomers

are refluxed with aniline, a higher percentage of the red color-forming material is obtained and the color complex formed by these amounts of benzene hexachloride exhibits an absorption maxima at ca. 510 mμ.

The oxidation of diphenylamine to its blue quinoid imonium salt has been established (*5*, *9*) and it is believed that the colored body produced by the dichlorodiphenylamine is of similar structure.

The isomers of benzene hexachloride when treated under identical conditions do not yield the same intensity of color. The epsilon isomer produced 110%, the alpha 90%, the delta 40%, the beta approximately 5%, and technical about 80% of the color produced by an equal amount of the gamma isomer.

Table I. Insecticides Producing Color

Compound	Micrograms[a]
Dieldrin	5000
Aldrin	5000
DDT	4000
Aramite	950
Toxaphene	825
Heptachlor	700
Gamma-chlordan	675
Technical chlordan	450
Technical BHC	58
Gamma-BHC	50

[a] Micrograms of material necessary to form same intensity of color as produced by 50 micrograms of gamma-BHC.

Other chlorinated hydrocarbons that contain alkali-labile chlorine may produce colors when refluxed with aniline and treated as indicated in the procedure. Table I shows the amounts of some of the other insecticides necessary to produce the same amount of color formed from 50 micrograms of gamma-benzene hexachloride. In most cases the colors formed by other insecticides are characteristic of the blue color formed by diphenylamine and fade rapidly. Unless exceedingly large amounts of toxaphene or chlordan are present, no interference from them is encountered.

SPECIAL EQUIPMENT

Air Condensers, 150-mm. length of 10-mm. glass tubing, fitted with a ₸ 16/15 (or ₸ 19/38) male joint.

Büchner Funnels, fritted-glass, coarse porosity, 20-mm. and 60-mm. diameter.

Test Tubes, 16 × 150 mm., fitted with a ₸ 16/15 (or ₸ 19/38) female ground-glass joint and stopper.

Colorimeter. Glass beads are used as boiling aids throughout the procedure.

REAGENTS

Acid Celite. Adsorb on 100 grams of Celite 100 ml. of fuming sulfuric acid (15% SO_3) containing 0.75 gram per kg. of vanadium pentoxide.

Aniline. Redistill reagent grade aniline over a mixture of 10 grams of mossy zinc and 7 grams of magnesium turnings, for each 500-ml. charge of aniline. Store in a brown bottle with several lengths of 20-gage aluminum wire. Use an asbestos-wrapped, ground-glass jointed, Vigreux-type column for fractionation, and discard the first sixth and last fourth of distillate. Aniline purified in this manner will keep for several months.

Celite (Johns-Manville analytical grade or equivalent). Other inert filtering aids may be used, providing they do not adsorb any benzene hexachloride or contaminate the sample.

Ethyl Ether. ACS reagent grade; does not have to be anhydrous. It is advisable to check the ether before use. Mallinckrodt analytical reagent grade ether No. 0850 has been found satisfactory.

Hydrochloric Acid, dilute. One volume of hydrochloric acid, C.P. (36 to 37% HCl) plus 3 volumes of distilled water.

Pentane, Shell Co. In order to avoid the difficulty of interference from impurities present in petroleum solvents, they were purified by elution through a 1.5 × 120 cm. column. The lower third of the column was packed with a mixture of silica gel (28- to 100-mesh) and precipitated silicic acid (10 and 1 part, respectively) and the remainder was packed with 14- to 20-mesh silica

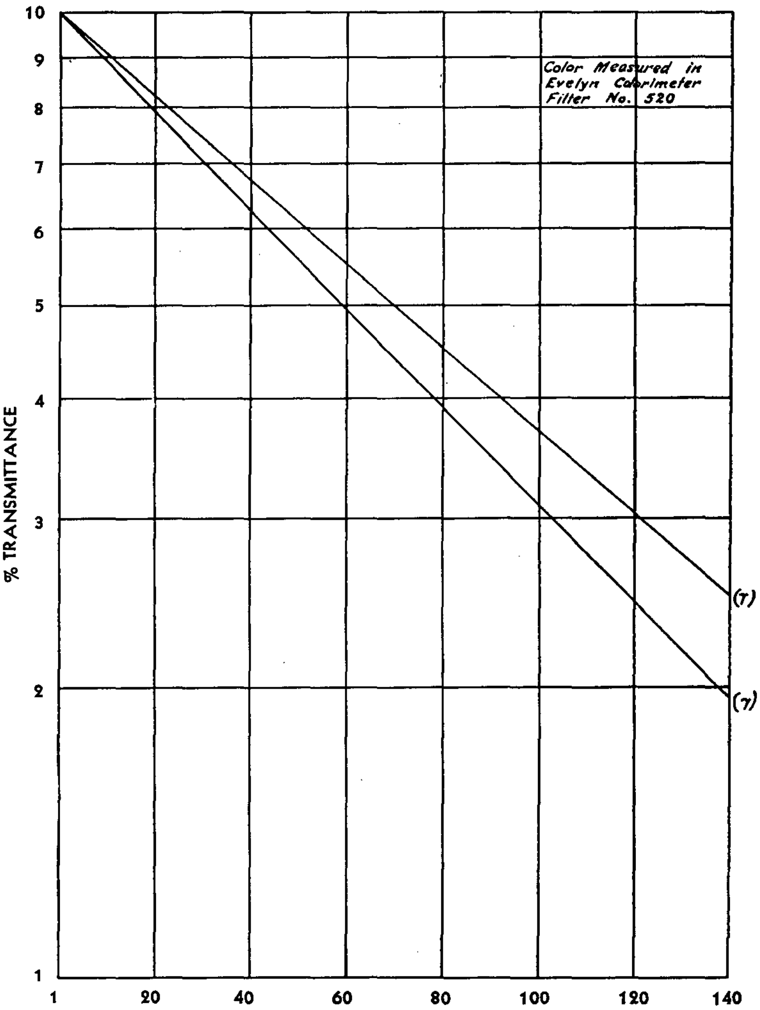


Figure 2. Typical Standard Curves

gel. The life of the column was prolonged when Shell pentane was used instead of Fisher petroleum ether. Mallinckrodt and Baker's petroleum ether were not found satisfactory.

Petroleum Ether, Fisher E-139, C.P., boiling range 30° to 60°.

Silica Gel, 28–200 and 14–20 mesh.

Sodium Sulfate, C.P. anhydrous, granular. The sodium sulfate was solvent-washed and dried before use to avoid possible contamination of the sample.

Sulfuric Acid Solution, 50%, 1 volume of sulfuric acid (specific gravity 1.84) to 1 volume of water.

Sulfuric Acid Solution, 5% by volume.

Sulfuric Acid–Vanadium Pentoxide, 0.05 mg. of vanadium pentoxide per ml. of 50% v./v. sulfuric acid solution.

Vanadium Pentoxide, C.P. anhydrous.

Table II. Recovery of Benzene Hexachloride Added to Strippings

Product	Aliquot, Ml.	BHC Added, γ[a]	Recovered, γ	Recovery, %	Average, %
Peanut butter	100	50 (L)	48	96	
	100	50 (T)	47	94	
	100	50 (T)	51	102	
	100	100 (L)	90	90	
	100	100 (L)	83	83	
	100	100 (L)	100	100	
	100	100 (T)	80	80	
	100	100 (T)	95	95	
	100	100 (T)	104	104	
	100	100 (T)	104	104	94.4
Sweet potatoes	75	100 (T)	102	102	
	50	50 (T)	48	96	
	50	50 (L)	49	98	
	50	100 (L)	104	104	
	50	100 (T)	103	103	101.5
Applesauce	75	100 (T)	103	103	
	50	50 (T)	52.5	105	
	50	50 (L)	49	98	
	50	50 (T)	48	96	
	50	50 (T)	43	86	
	50	100 (T)	103	103	
	50	100 (T)	103	103	
	50	100 (T)	86.5	86.5	
	50	100 (L)	102	102	98.5
Apricots and apples	50	50 (T)	46.5	93	
	50	50 (L)	47.5	95	
	50	100 (T)	95	95	
	50	100 (T)	78	78	89
Sweet potatoes	50	50 (T)	48	96	
	50	50 (T)	49	98	
	50	100 (T)	104	104	
	50	100 (T)	102	102	
	75	100 (T)	103	103	101.5
Peas	75	100 (L)	102	102	
Bananas	75	100 (L)	100.5	100.5	
Vegetables and beef	75	100 (L)	98	98	
	50	50 (T)	52	104	
	50	50 (T)	52	104	
	50	100 (T)	97.5	97.5	
	50	100 (T)	103	103	100.6

[a] L = Lindane.
T = Technical BHC.
Average deviation = 5.3%

Table III. Recovery of Technical Benzene Hydrochloride Added to Products Indicated

Product	Sample Weight, Grams	BHC Added, γ	Stripping Volume, Ml.	Aliquot, Ml.	BHC in Aliquot, γ	Recovery		Average, %
						γ	%	
Peanut butter	100	100	350	164	46.8	36	76.8	
	100	100	250	100	40.0	27.6	69	
	100	100	250	125	50	36	72	
	100	100	250	125	50	33.5	67	
	100	100	250	100	40	34	85	
	100	100	250	100	40	25.4	63.5	
	100	100	250	100	40	29.5	73.8	
	100	100	250	119	47.6	26	54.5	
	100	100	250	119	47.6	32	67	
	100	100	350	164	47.2	36	76.8	
	100	100	350	164	47.2	29	62	
	100	100	350	155	88.7	71	80.2	
	100	200	350	150	86.7	63	73.6	61.8
Sweet potatoes	100	100	250	93	37.2	27	72.5	
	100	100	250	93	37.2	26.5	71.2	
	100	100	250	97	38.8	29.5	76	
	100	100	250	97	38.8	34.5	88.8	
	200	100	200	77	38.5	26	67.5	
	200	100	200	77	38.5	31	80.5	
	200	100	250	100	40	37	92.5	
	200	100	250	68	27.25	24.5	90	79.7
Applesauce.	100	100	250	96	38.4	42	109	
	100	100	250	96	38.4	49	127	
	100	100	250	84	33.6	38.5	115	
	100	100	250	84	33.6	37	111	115.6
Apricots and apples	100	100	250	50	20	16.5	82.5	
	100	100	250	50	20	19	95	
	100	100	250	69	27.6	32	116	
	100	100	250	69	27.6	34	123	
	100	150	250	60	36	45	125	
	100	150	250	60	36	39	108	
	100	300	250	72	86.5	86.5	100	
	100	300	250	72	86.5	80.5	92.4	103.6
Vegetables and beef	100	100	250	57	22.8	23	100.8	
	100	100	250	56	22.4	23	102.8	
	100	100	250	56	22.4	23	102.8	
	100	100	250	56	22.4	23	102.8	102.2

PROCEDURE

The procedure consists primarily of six steps:

1. Extraction of benzene hexachloride from plant material
2. Separation of benzene hexachloride from other extractives
3. Reaction with aniline
4. Separation of reaction products
5. Development of color
6. Measurement of the intensity of color produced

The techniques described in the literature of solvent stripping for other organic insecticides such as DDT are, in general, applicable to benzene hexachloride. However, the solvent selected should be stable to sulfuric acid and should not react with aniline. Purified petroleum ether and pentane have been used successfully in this work, Shell pentane being the most desirable because of extremely low aromatic and unsaturate content.

Benzene hexachloride may be estimated in peanuts and peanut products in the following manner:

To a 100-gram sample in a Waring Blendor bowl is added 250 ml. of solvent and mixed for 4 to 5 minutes. Then 100 ml. of a 5% (by volume) sulfuric acid solution is added and blended for about 30 seconds. The mixture is transferred to stoppered bottles and centrifuged until a clear solvent layer is obtained (about 5 minutes), a 100-ml. aliquot of which is taken for analysis.

In a 400-ml. beaker 50 grams of the acid Celite is mixed with 20 grams of anhydrous sodium sulfate and the mixture is wetted with solvent. The stripping aliquot is added slowly and the mixture stirred with a glass rod [a modification of the Davidow (*2*) technique]. The slurry is thoroughly mixed until the solution is clear and then filtered with the aid of suction through a 60-mm. fritted-glass Büchner funnel. The solid material in the funnel is then washed with two 50-ml. portions of solvent. The filtrate and washings are combined and treated again with about 20 grams of the acid Celite and stirred mechanically for 5 minutes.

The solution is filtered with suction through a fritted-glass Büchner funnel and decanted into a 250-ml. separatory funnel, where it is washed with three 50-ml. portions of distilled water and is shaken 15 seconds each time. The washings are discarded and the solvent is passed through a 20-mm. fritted-glass Büchner funnel containing a 3-cm. layer of anhydrous sodium sulfate. (Interfering surface waxes may be removed by chromatographic separation, magnesium oxide–Celite mixture being satisfactory in most instances.) The funnel is then washed with 50 ml. of solvent and the filtrate plus washings is evaporated on a steam bath to a volume of 5 to 10 ml. The solution is transferred quantitatively to a 16 × 150 mm. test tube fitted with a ₮ 16/15 female joint and, while immersed in a bath of warm water (40° to 50° C.), is evaporated to dryness under a gentle current of air.

Approximately 2 ml. of purified aniline is introduced by means of an automatic pipet and the test tube is fitted with an air condenser equipped with a ₮ 16/15 male joint. The aniline is refluxed for 90 minutes and then the contents are allowed to cool to room temperature. It is essential that the aniline be refluxed vigorously and that condensation occur near the ground-glass joint.

The refluxed aniline is then transferred to a 250-ml. separatory funnel with ca. 50 ml. of ethyl ether, where it is washed with 50 ml. of dilute hydrochloric acid and 50 ml. of distilled water, respectively, being shaken with each for at least 10 seconds. The washed ethyl ether solution is filtered through a 20-mm. fritted-glass, coarse-porosity Büchner funnel containing a 2-cm. layer of anhydrous, ethyl ether–washed sodium sulfate and collected in a 100-ml. beaker containing two glass beads.

The funnel is washed with ca. 20 ml. of ethyl ether, and the combined filtrate and washings are evaporated on a steam bath to approximately 1 ml. and taken to dryness under a gentle current of air at room temperature. About 0.2 ml. of pentane is added to take up the residue, 10 ml. of sulfuric acid–vanadium pentoxide reagent is introduced, and the intensity of the color produced or optical density is read in a spectrophotometer at 510 mμ. For routine analyses a colorimeter using a filter with a transmittance peak between 550 and 570 mμ is satisfactory. The Evelyn colorimeter with filter No. 520M was used by this laboratory in obtaining the data shown in Tables I, II, and III.

When most fruits and vegetables are under examination, unwanted extractives may be readily removed by shaking or stirring the solution with 5 to 10 grams of the acid Celite for a few seconds and then filtering.

Essentially the same procedure may be employed for the analysis of other materials, except in cases where large amounts of surface waxes are encountered and special clean-up techniques are needed.

STANDARD CURVES

Because the reaction products of benzene hexachloride and aniline vary when large and small amounts of benzene hexachloride are used, standard curves for both the gamma isomer and technical benzene hexachloride must be prepared by carrying known amounts (0, 20, 40, 60, and 100 micrograms) of each through the entire procedure. The 0 sample or reagent blank is used as a reference standard when reading unknowns. The slopes of standard curves may vary slightly with changes of reagents and, therefore, should be checked frequently.

Recoveries may be checked by adding known amounts of benzene hexachloride to previously analyzed samples and carrying them through the procedure.

DISCUSSION

The dichlorodiphenylamine forming a purple color with a maximum absorption at 555 mμ on oxidation by the vanadic acid was obtained from the ether-soluble reaction products by vacuum distillation. Klein (*4*) obtained 22 grams of amber colored liquid, boiling at 145° C. at 2-mm. pressure, from 40 grams of material produced by refluxing 150 grams of gamma-benzene hexachloride with 350 ml. of redistilled aniline and separating the resulting mixture as indicated in the procedure. Total chlorine analysis indicated the amber colored liquid to be a dichlorodiphenylamine. Crystals obtained from this liquid after long storage (ca. 5 weeks) at −30° C. were recrystallized from hexane and melted at 63.0° to 63.2° C.

Orange crystals, which formed in the ether-soluble residue after the ether was completely removed, melted at 139° to 141° C., were insoluble in cold alcohol and hot water, and formed a red color with the acid reagent with a maximum absorption at 510 mμ.

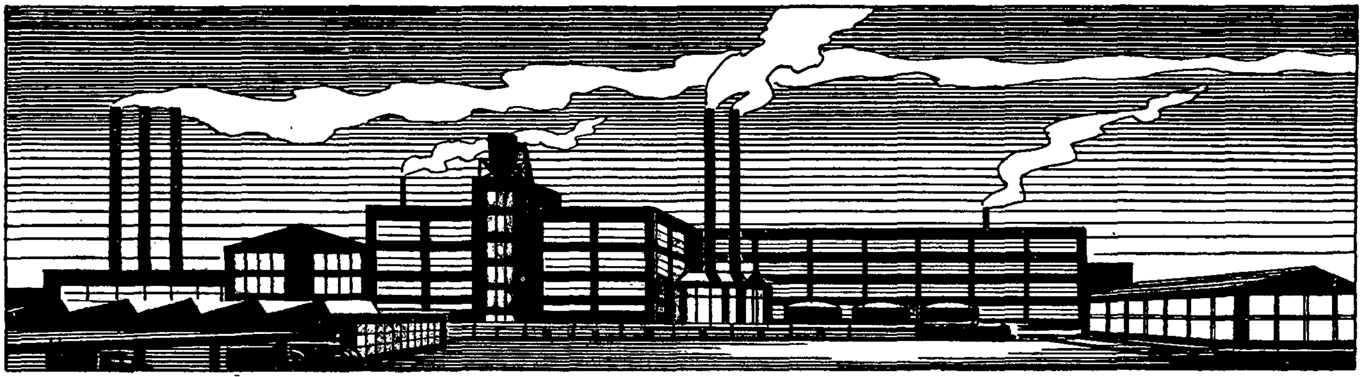
ACKNOWLEDGMENT

The author is indebted to the Dow Chemical Co., Hooker Electrochemical Co., and E. I. du Pont de Nemours & Co., for their generous contributions of chemicals necessary for this work and to A. K. Klein, Food and Drug Administration, Washington, D. C., for his many helpful suggestions and appreciation to Margaret French and Mary Wilson for their assistance in performing the large number of analyses required in carrying out this project.

LITERATURE CITED

(1) Bowen, C. V., *J. Assoc. Offic. Agr. Chemists*, **33**, 774–82 (1950).
(2) Davidow, B., *Ibid.*, **33**, 130 (1950).
(3) Fairing, J. D., and Phillips, W. F., Division of Agricultural and Food Chemistry, Symposium on Methods of Analysis for Micro Quantities of Pesticides, 119th Meeting, AM. CHEM. SOC., Boston, Mass.
(4) Klein, A. K., Food and Drug Administration, Washington, D. C., private communication.
(5) Kolthoff, I. M., and Noponen, G. E., *J. Am. Chem. Soc.*, **55**, 1448 (1933).
(6) Meunier, M. J., *Am. Chem.* (6), **10**, 223 (1887).
(7) Schechter, M. S., and Hornstein, Irwin, ANAL. CHEM., **24**, 544 (1952).
(8) Slade, R. E., *Chemistry and Industry*, **64**, 314 (1945).
(9) Wieland, H., *Ber.*, **46**, 3296 (1913); **52**, 886 (1919).

RECEIVED for review May 16, 1952. Accepted September 13, 1952.



Colorimetric Determination of Ruthenium with *p*-Nitrosodimethylaniline

J. E. CURRAH[1], ALICE FISCHEL, W. A. E. McBRYDE, AND F. E. BEAMISH

Department of Chemistry, University of Toronto, Toronto, Ontario, Canada

The growth in importance of the chemistry of ruthenium has brought with it demands for methods of determining very minute amounts of the metal. From several organic compounds tested for color formation with ruthenium salts, *p*-nitrosodimethylaniline has been selected for more detailed study. This reagent in aqueous solution will develop a bottle-green color when warmed with a solution of ruthenium chloride or bromide buffered to pH 4.1. Maximum absorption occurs at 610 mμ when this solution is compared with a reagent blank. Ruthenium may be separated from the other platinum metals by conventional distillation procedures and then determined in the distillate. The recommended procedure is simple, requires little of the operator's time, and is more sensitive than other methods in the literature.

THE colorimetric determination of ruthenium has been the subject of several investigations in recent years. Breckenridge and Singer (*4*) applied 5-hydroxyquinoline-8-carboxylic acid; Ayres and Young (*2*) and DeFord (*5*) independently studied thiourea as a colorimetric reagent; Ayres and Young (*3*) also examined rubeanic acid (dithio-oxamide) for this purpose. Sandell (*10*) discussed these and other methods. Marshall and Rickard (*7*) described a procedure in which the color of potassium ruthenate was made the basis of a colorimetric determination but this was applicable to higher concentrations than any of the foregoing methods.

In this paper *p*-nitrosodimethylaniline in aqueous solution is recommended as a colorimetric reagent of high sensitivity for ruthenium. This substance has already been successfully applied by Yoe and Overholser (*12*) to the colorimetric determination of palladium. Ogburn (*8*) reported that various nitroso compounds, including *p*-nitrosodimethylaniline, gave no visible reaction with ruthenium salts. However, the reaction described in this paper requires heating to develop the color, and Ogburn may have overlooked this.

APPARATUS AND SOLUTIONS

Optical Instruments. Absorption measurements were made mainly with a Klett-Summerson photoelectric colorimeter. The variation of absorbancy with wave length was examined with a Beckman Model DU spectrophotometer. In order to establish a relationship between the logarithmic scale of the Klett instrument and conventional absorbancy units, some comparisons were made with the same solution in 2-cm. rectangular cells in the Klett colorimeter, in 1-cm. rectangular cells in a Lumetron Model 402 EF colorimeter, and with the same filter in each instrument. The filter used was a Lumetron monochromatic No. M 610, having maximum transmittance at 610 mμ. It was found that, under these conditions, 519 Klett units equaled unit absorbancy.

Most of the measurements were made with the solutions contained in cylindrical Klett tubes, and these were carefully matched in the following manner. The absorption of samples of a large batch of green chromium(III) chloride solution was measured five times in each of a dozen Klett tubes. The entire sixty measurements were made in random order. The concentration of this solution was selected to give an absorption of about 200 Klett units when the 610 mμ filter was used. The results were examined by conventional statistical methods to compare the variance between tubes with that within tubes. On the basis of this examination it was possible to select sets of tubes for the following experiments for which the variance of measurements from tube to tube was not significantly greater (to a probability of 5%) than that of measurements within any one tube.

Standard Ruthenium Solution. A stock solution of "ruthenium chloride" was prepared by distillation of ruthenium tetroxide followed by refluxing in 6 *M* hydrochloric acid, according to the procedure of Rogers, Beamish, and Russell (*9*). The stock solution was standardized by the thionalide method described by these authors.

The thionalide precipitation continues to be successfully applied to the determination of ruthenium in this laboratory. Recent statements by Flagg (*6*) that results on semimicro quantities tend to be low by as much as 10% have never, so far as the authors are aware, been supported by experimental evidence. Such statements cannot be reconciled with the observations of Thiers, Graydon, and Beamish (*11*), made with the aid of radioactive ruthenium, that, within the prescribed acidity range, precipitation of 6 mg. of ruthenium was complete to less than 0.6 microgram.

Solutions of concentration suitable for colorimetric work were prepared by volumetric dilution from the stock solution. Two such solutions, which were used for many of the results in this paper, contained 44.6 and 4.46 micrograms of the metal per ml.

Buffer Solutions. Buffer solutions containing acetic acid and sodium acetate were made up in concentrated form, so that 1 ml., when diluted to 25 ml., gave the desired stabilized pH. The buffer in the recommended procedure was 4 *M* in acetic acid and 1 *M* in sodium acetate, and this gave a pH of 4.1 when diluted 1 to 25. Measurements of pH were all made with a Beckman Model G meter.

Color Reagent. *p*-Nitrosodimethylaniline (D.P.I. No. 188)

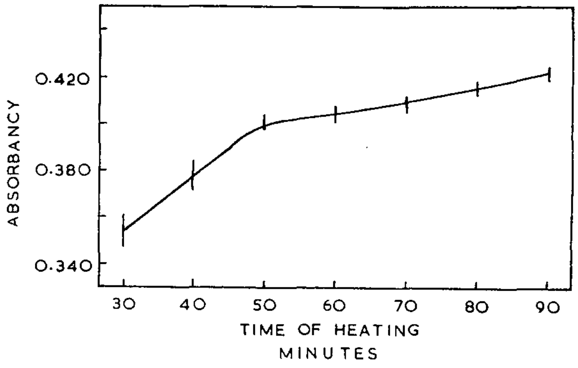


Figure 1. Effect of Time of Heating on Color Intensity

0.89 p.p.m. of ruthenium
Klett-Summerson colorimeter with tube cells. Vertical lines represent ranges of results.

---

[1] Present address, Canadian Industries, Ltd., McMasterville, Quebec, Canada.

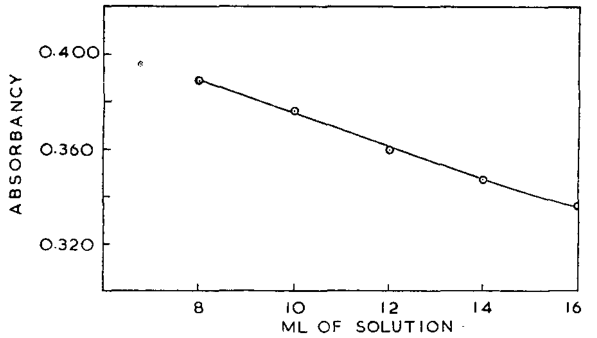


Figure 2. Variation of Color Intensity with Volume of Solution Heated

0.89 p.p.m. of ruthenium
Klett-Summerson colorimeter with tube cells

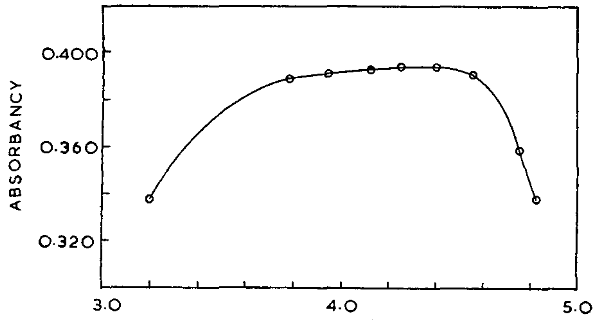


Figure 3. Variation of Color Intensity with pH

0.89 p.p.m. of ruthenium
Klett-Summerson colorimeter with tube cells

was prepared in aqueous solutions by dissolving 150 mg. per 100 ml. of solution. The solution was heated in a boiling water bath for about 25 minutes and then cooled under running water.

The dissolving of reagent is practically complete, and it appeared unnecessary to filter the solution. It is essential that this solution be cooled before using; otherwise the color development is incomplete. Solutions thus prepared have been found stable over a period of 2 weeks, and good agreement in color development was found between different solutions made up by the above procedure. The solution was not light-sensitive, and was stored at room temperature. Alcoholic solutions of the reagent, such as used for the determination of palladium, failed to produce a strong color with ruthenium; this was interpreted to mean that the ruthenium may have been reduced by the alcohol to an inactive oxidation state.

Solutions were also prepared from *p*-nitrosodiethylaniline (D.P.I. No. 522) and *p*-nitrosodiphenylamine (D.P.I. No. 1127).

Osmium Solution. An osmium solution containing very close to 200 mg. of the metal per liter of 1 *M* hydrochloric acid was prepared and standardized by hydrolytic precipitation in the usual manner. Fiftyfold dilution of this gave a solution containing 4 micrograms per ml.

COLOR REACTION

When an aqueous solution of *p*-nitrosodimethylaniline is heated with a solution of ruthenium chloride or bromide, a bottle-green colored solution is formed. The reagent itself has a yellow color in aqueous solution. The intensity of the green color depends on several factors, which must be controlled in order to obtain uniform results.

Time of Heating. At higher temperatures (90° to 100° C.) there is some tendency to get precipitation and hence less color. At lower temperatures color development is slower. The procedure adopted was to heat for 50 minutes at about 70° C. For shorter periods of heating the color development is not complete and results tend to be lower and not stable over 24-hour periods. For heating times longer than 50 minutes, increase in color is relatively small, as shown in Figure 1.

Volume of Solution Heated. The intensity of color for a fixed weight of ruthenium and fixed volumes of buffer and reagent, and for a fixed time of heating, was greater when the volume of liquid heated was kept small. This is evidently due to the fact that the approach to maximum color development is slow, and is hastened by having the concentrations of reactants as great as possible. The volume specified in the procedure given below is 8 ml., which allows for 5 ml. of ruthenium solution. In Figure 2 it is seen that less color develops when the same quantities of reactants contained in a larger volume are heated together.

Amount of Added Reagent. On a molar basis, a fair excess of reagent over ruthenium is added. A larger excess than that called for in this procedure would produce more intense coloration, but poorer precision, owing to precipitation in and on the walls of the heating tube.

pH of Solution. It was found that strong acid prevented the development of much color, and strong alkali brought on precipitation of a black material, possibly a hydrous oxide of the metal. For maximum color formation the solution should be buffered to a pH about 4 at the time it is heated. The variation in color intensity with pH is given in Figure 3.

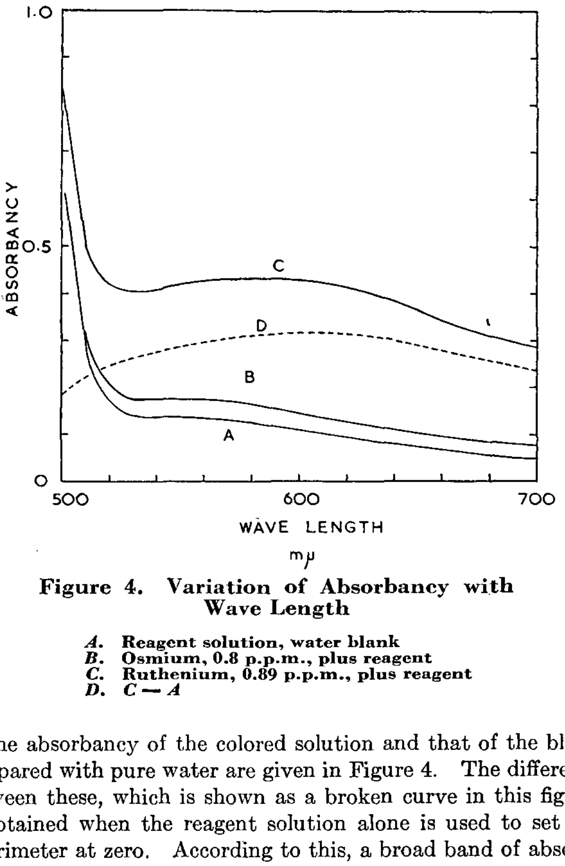


Figure 4. Variation of Absorbancy with Wave Length

A. Reagent solution, water blank
B. Osmium, 0.8 p.p.m., plus reagent
C. Ruthenium, 0.89 p.p.m., plus reagent
D. *C* — *A*

The absorbancy of the colored solution and that of the blank compared with pure water are given in Figure 4. The difference between these, which is shown as a broken curve in this figure, is obtained when the reagent solution alone is used to set the colorimeter at zero. According to this, a broad band of absorption, centered about 600 mμ, distinguishes the ruthenium from the reagent solution. In the photometric measurements a filter with transmittance centered at 610 mμ was used. Beer's law was found to hold throughout a wide range of absorbancy, as shown by the data in Table I. The solution in stoppered flasks was found to remain unchanged in absorption for periods of 24 hours.

The related compounds *p*-nitrosodiethylaniline and *p*-nitrosodiphenylamine were tested for activity toward ruthenium under these conditions. The former produced a somewhat less intense color, but the solution of the reagent denatured, so that progressively less color was obtained with each batch after the first day. No marked color was obtained in the second case. Both compounds are less soluble in water than the dimethylaniline compound, and it was necessary to prepare an acetone-water solution of these, from which there was considerable tendency to get precipitation.

The possibility of osmium occurring together with ruthenium in an analysis required examination of the reaction of the reagent with osmium solutions. A chloride solution of osmium, of concentration about equal to that of the ruthenium, was tested by the same procedure. The absorption compared to a blank was about 20% of that of a ruthenium solution of equal concentration. The absorption curve is included in Figure 4. As good separation of ruthenium from osmium is usually achieved by conventional distillation procedures, this interference is not a serious disadvantage. Also, as ruthenium is nearly always isolated by distillation before it is determined in an analysis, no extensive investigation of interfering elements was undertaken. It was established, in preparation for the distillation procedure to be used, that sodium chloride or bromide in concentrations up to 0.2 *M* and small amounts of magnesium chloride did not affect the color intensity. It was also established that sulfate and nitrate ions up to 0.5 *M* caused no significant change in color, but that perchlorate ion did result in a noticeable lessening of color intensity.

Table I. Conformity to Beer's Law

Concentration, P.P.M.	Absorbancy	Absorbancy, P.P.M.
0.17	0.075	0.44
0.34	0.152	0.45
0.51 (7)	0.233	0.451
0.68	0.310	0.456
0.86	0.393	0.457
1.03	0.468	0.454
1.38	0.624	0.452
1.55	0.707	0.456
1.72	0.782	0.455
2.07	0.942	0.455
2.58	1.19	0.461

Recommended Procedure. The sample of ruthenium, containing between 6 and 60 micrograms of the metal, is made approximately neutral and adjusted to a volume of 5 ml. It is treated with 1 ml. of a concentrated acetic acid–sodium acetate buffer solution (see above) and 2 ml. of the reagent solution. The resulting solution is then heated in a water bath at 70° ± 4° C. for 50 minutes. This heating can most conveniently be done in a 25 × 150 mm. borosilicate glass test tube with a pouring lip. The solution is then cooled under running water, transferred to a 25 ml. volumetric flask, and diluted to this volume with water. A blank solution is prepared containing only buffer and reagent, and this is used to set the zero on the colorimeter. One blank solution may be used for several determinations made over a period of several hours.

The size of the ruthenium sample recommended is based on the advisability of keeping the absorbancy of the colored solution between the limits 0.112 and 1.105. The somewhat arbitrary choice of these limits is based on the fact that, when Beer's law holds, the quantity $d\ln c/dT$ (*1*) exceeds 5 for solutions whose absorbancies lie outside these values. Since Beer's law holds in this case, the absorbancy is a linear function of concentration, and these error limits may be translated into concentration values. Where the final solution is diluted to a different volume, or different absorption cells or another instrument is used, these limits for the weight of ruthenium taken should be adjusted accordingly.

Sensitivity. The sensitivity when expressed by the notation of Sandell (*10*) appears to be the most concise way of expressing and comparing the results of different colorimetric procedures. The authors found that 2.8 micrograms per sq. cm. corresponded to unit absorbancy in the Beckman spectrophotometer at 610 mμ, and 2.9 micrograms per sq. cm. equaled unit absorbancy in the Klett-Summerson colorimeter where the sample was contained in rectangular cells. There was no practical decrease in sensitivity with the simpler instrument. This indicates that this reagent is more sensitive than either thiourea or rubeanic acid for the colorimetric determination of ruthenium. The authors were unable, perhaps through inexperience with the methods, to match the sensitivity claimed by Ayres and Young for either of these two reagents.

DISTILLATIONS

The colorimetric procedure was found suitable for determining ruthenium when isolated by distillation. The apparatus used was patterned after that of Thiers, Graydon, and Beamish (*11*) or that of DeFord (*5*). The former apparatus was employed for distillations in which ruthenium tetroxide was produced either by chlorine and sodium hydroxide, or sodium bromate and sulfuric acid, and received in 3% hydrogen peroxide. The procedure of DeFord is simpler and appeared to yield more satisfactory results for very small amounts of ruthenium. In this the tetroxide was formed by perchloric acid and a small amount of sodium bismuthate, and received in 6 *M* sodium hydroxide.

Hydrogen peroxide distillates were evaporated with hydrobromic acid in order to destroy excess peroxide. The ruthenium absorbed in sodium hydroxide was isolated as a hydrated oxide, which was then dissolved by warming with 6 *M* hydrochloric acid. In both cases some adjustment in pH was necessary before the reagent and buffer were added, owing to the high acidity of these solutions. This was accomplished by adding a saturated solution of sodium bicarbonate until the distillate was nearly neutral; the solution was then made up to known volume and aliquot portions were taken for colorimetric determination.

The distillation procedure of DeFord was applied to the distillation of as little as 200 micrograms of ruthenium, and this colorimetric procedure applied to one tenth of the distillate. When such small amounts as this were being handled, scrupulous attention must be paid to assure no contamination of reagents by nitrate. The following results are representative of distillations on this scale:

Ruthenium distilled	223 γ
Ruthenium sought (colorimetric)	22.3 γ
Ruthenium found (replicate distillations)	21.8, 22.2, 21.9, 21.4, 22.1 γ

Although these results are, on the average, about 2% low, the difficulty was shown to be connected with the distillation by analyzing other portions of each distillate by the thiourea procedure (*5*). Agreement between the colorimetric procedures was taken to indicate that the loss occurred during the distillation.

ACKNOWLEDGMENT

The authors wish to thank R. E. Thiers and Kay Fallis Balmer who, while students in this laboratory, performed various experiments and distillations which assisted in the development of this procedure.

LITERATURE CITED

(1) Ayres, G. H., Anal. Chem., **21**, 652 (1949).
(2) Ayres, G. H., and Young, F., *Ibid.*, **22**, 1277 (1950).
(3) *Ibid.*, p. 1281.
(4) Breckenridge, J. G., and Singer, S. A. G., *Can. J. Research*, **25B**, 49 (1947).
(5) DeFord, D. D., "Chemistry of Ruthenium," Oak Ridge, U. S. Atomic Energy Commission, *Document* **NP-1104** (December 1949).
(6) Flagg, J. F., "Organic Reagents," New York, Interscience Publishers, 1948.
(7) Marshall, E. D., and Rickard, R. R., Anal. Chem., **22**, 795 (1950).
(8) Ogburn, S. C., Jr., *J. Am. Chem. Soc.*, **48**, 2493 (1926).
(9) Rogers, W. J., Beamish, F. E., and Russell, D. S., Ind. Eng. Chem., Anal. Ed., **12**, 561 (1940).
(10) Sandell, E. B., "Colorimetric Determination of Traces of Metals," 2nd ed., New York, Interscience Publishers, 1950.
(11) Thiers, R. E., Graydon, W. F., and Beamish, F. E., Anal. Chem., **20**, 831 (1948).
(12) Yoe, J. H., and Overholser, L. G., *J. Am. Chem. Soc.*, **63**, 3224 (1941).

Received for review June 27, 1952. Accepted September 26, 1952.

Spectrophotometric Study of Dichromate-Chromic Salt Mixtures

Application to the Determination of Glycerol in Vinegar

D. T. ENGLIS AND LOUIS A. WOLLERMAN
University of Illinois, Urbana, Ill.

THE colors of chromium compounds in the various oxidation states are characteristic and lend themselves to the estimation of the element in the several states by spectrophotometric methods. Kasline and Mellon (*2*) have prepared and published absorption curves for chromate and dichromate solutions in the visual range. Sandell (*4*) gives an absorption curve for chromate through the ultraviolet range which was established by Rössler (*3*) using a spectrographic technique. The absorption maximum was at 370 mμ. Perhaps, because this is below the range of many early spectrophotometers, certain applications which it offers have not been realized. Sandell stresses the fact that the optical density values yielded by many filter instruments may not be proportional to concentration since the filter is not usually sufficiently selective and measurements are frequently made at the edge of an adsorption band. However, because of the fact that the sensitivity is so much greater in the shorter wave length region and good instruments for working in this range are now generally available, a study of possible applications of work in the ultraviolet region was undertaken. The determination of small amounts of glycerol and certain other products of microbiological action has been frequently accomplished by oxidation with an excess of dichromate followed by a volumetric titration of the excess oxidant with some suitable reducing agent. It was of interest to learn if this might be done more conveniently by a colorimetric procedure.

After the glycerol oxidation, both hexavalent and trivalent chromium are present in the final solution, which is commonly strongly acidic. The basicity necessary for conversion of the dichromate ion to chromate would cause the precipitation of the chromic ion and require removal of the insoluble chromic hydroxide or basic salt. Such a precipitation would require additional time and possible loss of some chromate in the gelatinous precipitate. Hence, the dichromate is the preferred form for estimation of the excess oxidant. Accordingly it was necessary to know not only the absorption characteristics of the dichromate in the ultraviolet region, but also that of the chromic salt to determine whether the latter would interfere with the estimation. Another factor to be established was the effect of the presence of sulfuric acid in the reaction mixture.

Equipment. A Cary Model 11 recording spectrophotometer was employed where complete absorption curves were to be established. When evaluations at selected single wave length positions were adequate, a Beckman Model DU spectrophotometer was used. For limited work in the visual range, some tests were made with a Lumetron filter-type photoelectric colorimeter, Model 400A.

ABSORPTION CHARACTERISTICS OF CHROMIUM COMPOUNDS

Aqueous solutions of chromate, dichromate, and chromic compounds were prepared so that each would contain the same concentration of chromium (32.95 p.p.m.). In a 1-cm. cell this solution gives an optical density of about 1 unit for the dichromate form. To simulate conditions which would be characteristic of the solutions after the glycerol oxidation, the acidity was adjusted so as to be 0.09 *N* in sulfuric acid for the dichromate and chromic compounds. The solutions were examined in the range 200 to 800 mμ using the Cary instrument. When the acid was added to the chromium solutions, it was added in identical amounts to the water which served as the reference solution.

The curves, Figure 1, show that the dichromate and chromate are similar in character in that each shows two maxima. These appear at 257.5 and 350 mμ for the dichromate and at 274 and 370 μ for the chromate. In the case of the dichromate these are of slightly lower optical density per unit weight of chromium than the corresponding maxima for the chromate. The chromic sulfate solution shows no significant absorption at this concentration (32.95 p.p.m. as chromium) between 250 and 800 mμ. Hence, under these conditions it will not interfere with the estimation of chromate or dichromate.

A much more concentrated solution of the chromic salt, containing the equivalent of 5.272 mg. per milliliter of chromium, shows (Figure 2) peaks at 416 and 587 mμ. The presence of the hexavalent forms would interfere to some degree in the 416 mμ range but would have no in-

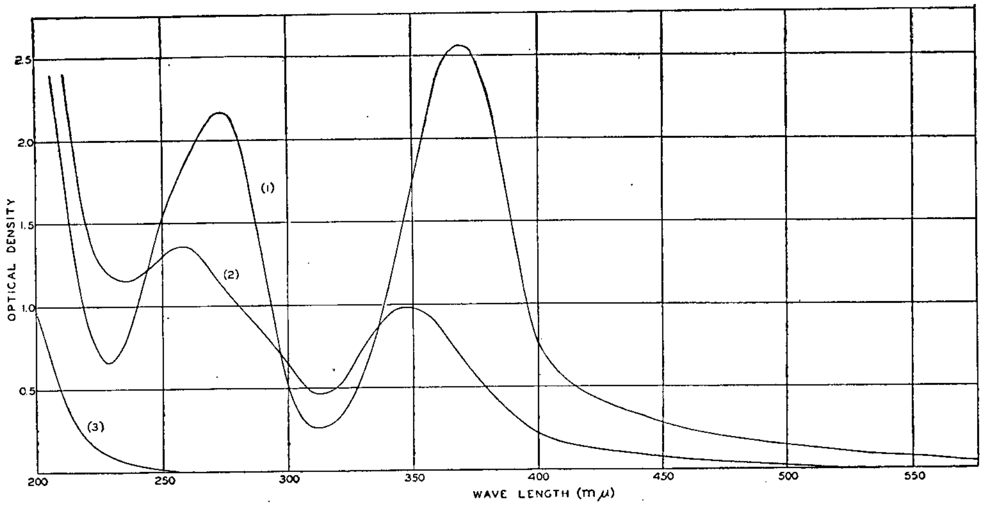


Figure 1. Absorption Curves of Chromium Compounds, Each Containing 32.95 P.P.M. of Chromium

1. Potassium chromate
2. Potassium dichromate
3. Chromic sulfate

fluence upon the determination of the chromic compound at 587 mμ since they do not absorb in this region.

In solutions of adequate concentration the measurement of the chromic form may be advantageous since dilution errors necessary for the estimation of the excess reagent as dichromate will be eliminated. Furthermore, the quantity of chromic salt is directly proportional to the material oxidized. In the determination of an excess oxidant, such as the dichromate forms, the desired value is secured by difference from the total quantity added. In this sense the method is indirect, but the increased sensitivity of the indirect procedure is imperative for low concentrations.

An application of the foregoing information and principles to the determination of glycerol was investigated.

DETERMINATION OF GLYCEROL BY DICHROMATE OXIDATION

The process of separation and purification of the glycerol in vinegar, wines, liquors, and flavoring extracts is a major problem in itself. The method of the Association of Official Agricultural Chemists for vinegar (*1*), which has been official for years, is tedious and time-consuming. It involves extractions and precipitations to eliminate sugars, proteins, organic acids, and other oxidizable substances. The method is generally conceded to be far from satisfactory. The authors have given attention to this phase of the determination with some success and efforts to improve the procedure are being continued. The work which follows has to do with the testing of the method based upon the absorption characteristics of the dichromate-chromic salt mixture and the comparison of the results with those obtained by the official titrimetric procedure. In order to eliminate all errors inherent in the purification process, the analyses were made upon solutions of pure glycerol in water.

Table I. Preparation of Potassium Dichromate–Chromic Sulfate Mixtures

No.	Standard Chromic Sulfate, Ml.	Standard Dichromate, Ml.	Concd. H_2SO_4, Ml.	Final[a] Volume of Diluted Sample, Ml.
1	0.00	30.00	25.00	250
2	5.00	25.00	25.00	250
3	10.00	20.00	25.00	250
4	15.00	15.00	25.00	250
5	20.00	10.00	25.00	250
6	25.00	5.00	25.00	250
7	30.00	0.00	25.00	250

[a] For photometry at 587 mμ.
For measurement at 350 mμ, 10 ml. of these solutions were diluted to 500 ml.

The glycerol content of vinegar varies from 0.0 to 0.5 gram per 100 ml. and only one half of the 100-ml. portion specified for treatment is finally subjected to oxidation. Therefore, the actual quantity determined is usually less than 0.25 gram. In the official procedure, this is oxidized with 30 ml. of potassium dichromate solution, 1 ml. of which is equivalent to 0.01 gram of glycerol and contains 26.36 mg. of chromium.

Oxidation Procedure. The oxidation procedure for the glycerol solution is essentially the same as is represented by the final steps in the official method and may be carried out as follows:

Measure into a 250-ml. volumetric flask a quantity of glycerol in water not to exceed 0.25 gram of glycerol. Add 30 ml. of the standard potassium dichromate solution and follow with the careful addition of 25 ml. of concentrated sulfuric acid. Heat in a boiling water bath for 20 minutes, cool, and make to volume with distilled water. This may be called solution *S*.

Estimation of Reagent Consumed. For the volumetric method the excess of dichromate is estimated as specified in the official method by titration of a portion of solution *S* with ferrous ammonium sulfate which has been standardized against the dichromate.

For the spectrophotometric methods, the amount of chromic salt is estimated from the color value of another portion of solution *S* at 587 mμ, and the residual dichromate is estimated by dilution of a 10-ml. portion of the solution *S* to 500 ml. with distilled water and evaluation of the optical density at 350 mμ. A sulfuric acid blank or reference solution must be employed in each case. For measurement at 587 mμ the acid should be 4.4 *N*, and for measurement at 350 mμ, it should be 0.1 *N*. Before the spectrophotometric evaluations may be made a standard curve must be constructed.

Preparation of Standards. In the present study a solution of chromic sulfate was prepared so as to contain the same amount of chromium (26.36 mg. per ml.) and sulfuric acid as the standard dichromate solution. Standards were then made up which contained varying amounts of the chromic sulfate and dichromate but always in quantities such that the total amount was equal to 30.00 ml. Thus each mixture contained the same amount of chromium, but the quantities of trivalent and hexavalent forms varied over the desired range. To each standard was added 25 ml. of concentrated sulfuric acid and the resulting solution was cooled and made up to 250 ml. in a volumetric flask. Such a series of solutions as has been described would be characteristic of the mixtures which result from reduction of dichromate with varying amounts of glycerol according to the official method.

Table I shows the quantities used in the preparation of the standards.

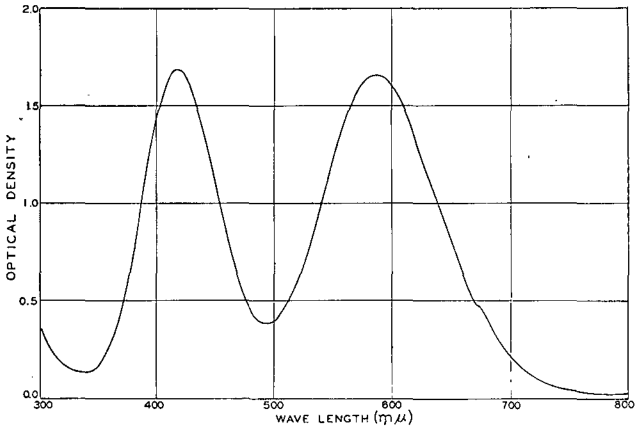


Figure 2. Absorption Curve of Chromic Sulfate Containing 5272 P.P.M. of Chromium

As was indicated in Figure 1, an evaluation of the optical density of the solution at 350 mμ gives a measure of the dichromate without interference by the chromic ion. Because of the high absorption at this wave length it is necessary to dilute 10 ml. of solution *S* to 500 ml. in order to secure optical densities in the working range of the Beckman instrument. These values, when plotted, show good conformance to Beer's law and establish the freedom from interference by chromic ion in the dichromate determination. The fact that chromium can be estimated as the dichromate at 350 mμ in a range of 0 to 50 p.p.m. indicates a sensitivity approaching that of the diphenyl carbazide method.

Table II. Determination of Glycerol by Dichromate Oxidation

(Evaluation of reagent consumed by different methods)

Sample No.	Glycerol, Grams: By titration of excess $K_2Cr_2O_7$ (*a*)	By Spectrophotometric Evaluation: At 350 mμ for excess $K_2Cr_2O_7$ (*b*)	At 587 mμ for chromic salt (*c*)
1	0.029	0.026	0.029
2	0.033	0.036	0.035
3	0.044	0.046	0.041
4	0.057	0.056	0.055
5	0.069	0.069	0.069
6	0.082	0.085	0.084
7	0.094	0.094	0.097
8	0.113	0.110	0.111
9	0.120	0.123	0.125
10	0.134	0.136	0.140
11	0.145	0.148	0.153
12	0.159	0.160	0.167

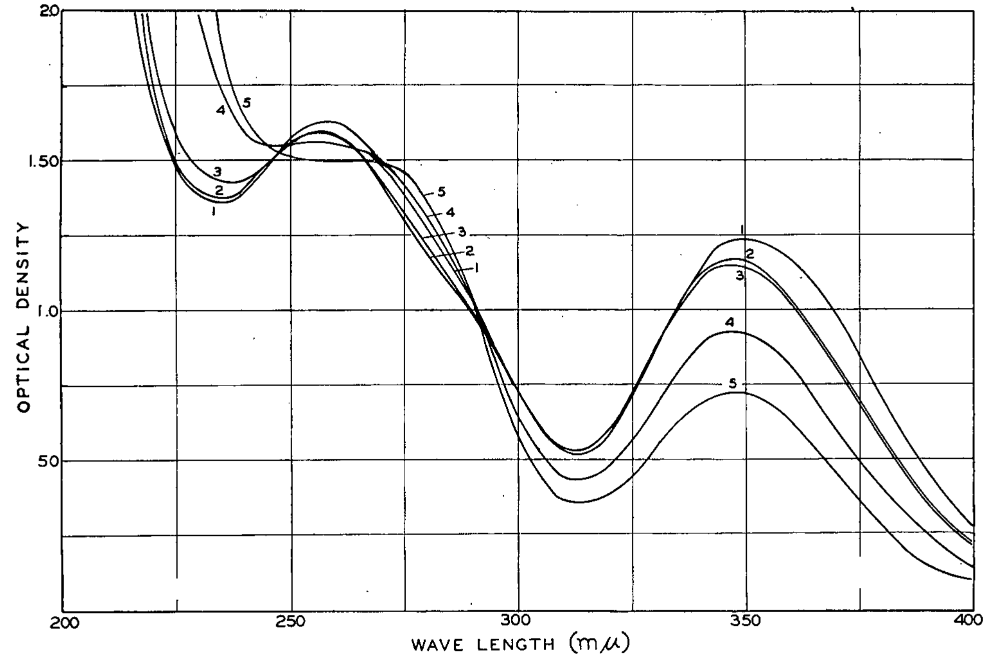


Figure 3. Curves Showing Effect of Addition of Sulfuric Acid upon Absorption of Potassium Dichromate Solution Containing 80 P.P.M. as Chromium

1. In water
2. In 0.01 N H_2SO_4
3. In 0.1 N H_2SO_4
4. In 1.0 N H_2SO_4
5. In 5.0 N H_2SO_4

In a similar way, the chromic salt concentrations were related to optical densities at 587 mμ but without the additional dilution. To test the performance with less refined equipment, a number of observations were made with the Lumetron colorimeter using the orange (580) filter. This gave satisfactory results also.

For the preparation of the standard curves and for the examination of samples to be referred to them, the concentration of the sulfuric acid should be the same in both standards and samples. It was observed that the adsorption at 587 mμ by chromic sulfate was decreased slightly as the concentration of sulfuric acid was increased. In the case of the dichromate an increase in concentration of the sulfuric acid from 0.01 to 4.4 N lowered the absorption peak at 350 mμ (Figure 3) about 40%. However a change in acidity, 0.01 to 0.1 N, had an almost insignificant effect.

Glycerol of the highest purity available was used to prepare the solutions subjected to analysis. However, it probably contained traces of moisture, so the results for the analyses cannot be expressed with reference to an absolute value, but as a comparison of values by the different methods. These values are given in Table II.

Since 1 ml. of potassium dichromate is equivalent to 0.01 gram of glycerol, the values in column (*a*) result from a subtraction of the excess dichromate from the total (30 ml.) and multiplying by 0.01.

The values indicated in column (*b*) were found by noting the value in micrograms per milliliter (or parts per million) of dichromate for the observed optical density at 350 mμ and inserting the value in the following expression:

$$\left[30 - \left(\frac{50 \times \text{micrograms per milliliter}}{4 \times 26.36}\right)\right] \times 0.01 = \text{grams of glycerol}$$

Similarly the values in column (*c*) were calculated by taking the milligrams per milliliter of chromium indicated by the optical density at 587 mμ and using the formula:

$$\frac{\text{Milligrams per milliliter} \times 250}{2636} = \text{grams of glycerol}$$

LITERATURE CITED

(1) Assoc. Official Agr. Chem., "Methods of Analysis," 7th ed., p. 489, Washington, D. C., 1950.
(2) Kasline, C. T., and Mellon, M. G., Ind. Eng. Chem., Anal. Ed., 8, 463 (1936).
(3) Rössler, G., *Ber.*, 59, 2605 (1926).
(4) Sandell, E. B., "Colorimetric Determination of Traces of Metals," p. 191, New York, Interscience Publishers, Inc., 1944.

Received for review June 23, 1952. Accepted August 23, 1952.

2-(*o*-Hydroxyphenyl)benzoxazole as a Volumetric Reagent for Cadmium

JOSEPH L. WALTER AND HENRY FREISER
Department of Chemistry, University of Pittsburgh, Pittsburgh 13, Pa.

SINCE the publication of the paper by Walter and Freiser involving the compound 2-(*o*-hydroxyphenyl)benzoxazole as a gravimetric reagent for the determination of cadmium (*2*), investigation has been carried out with this compound as a possible volumetric reagent for the determination of cadmium. The fact that the compound is a phenol and also that the compound and chelate are readily soluble in glacial acetic acid was utilized. These facts suggested the use of the bromate-bromide method for the determination of phenols. It was found that an accuracy of ±0.2 mg. could be expected when determining from 2 to 80 mg. of cadmium. The dead-stop indicator was used to determine the end point.

The rapid dissociation of the chelate in glacial acetic acid, and the fact that the compound is a phenol led to the use of the bromination technique as described by Siggia (*1*) substituting acetic acid for hydrochloric acid as the solvent. From the experimental results and also from a microanalysis performed on the purified bromo compound, it was found that two bromines substituted on the ring, most probably ortho and para on the phenol portion of the molecule.

The following equations best show the steps involved in the volumetric procedure:

First the cadmium is precipitated as the chelate,

$$Cd^{++} + 2C_{13}H_9O_2N \longrightarrow Cd(C_{13}H_8O_2N)_2 + 2H^+$$

Then the cadmium chelate is dissolved in glacial acetic acid,

$$Cd(C_{13}H_8O_2N)_2 + 2HOAc \longrightarrow Cd^{++} + 2C_{13}H_9O_2N + 2OAc^-$$

The dissociated chelate is brominated,

$$2C_{13}H_9O_2N + 4Br_2 \longrightarrow 2C_{13}H_7O_2NBr_2 + 4HBr$$

$$1 \text{ atom of Cd} \equiv 4\ Br_2$$

From this, then, one can readily calculate the per cent cadmium present in the sample.

REAGENTS AND APPARATUS

Bromate-Bromide Mixture. To make a 0.1 *N* bromate-bromide solution, 2.780 grams of c.p. potassium bromate and 10.00 grams of c.p. potassium bromide is dissolved in about 500 ml. of distilled water and diluted to 1 liter in a volumetric flask.

Table I. Volumetric Determination of Cadmium

Cadmium Taken, Gram	Vol. BrO_3-Br Used, Ml.	Cadmium Found, Gram	Error, Gram
0.0425	27.9	0.0427	+0.0002
0.0425	27.9	0.0427	+0.0002
0.0425	27.4	0.0419	−0.0006
0.0425	27.6	0.0421	−0.0004
0.0425	27.7	0.0423	−0.0002
0.0435	28.7	0.0439	+0.0004
0.0435	28.4	0.0435	0.0000
0.0435	28.4	0.0435	0.0000
0.0435	28.6	0.0437	+0.0002
0.0435	28.3	0.0433	−0.0002
0.0211	14.0	0.0213	+0.0002
0.0108	12.6	0.0111	+0.0003
0.0049	3.4	0.0051	+0.0002
0.0013	1.2	0.0017	+0.0004
		Average error	±0.0002

Standard Thiosulfate Solution. Exactly 24.820 grams of A.R. grade sodium thiosulfate is weighed into a 1-liter volumetric flask, and the volume is brought up to 1000 ml. with distilled water. This solution is standardized against the bromate-bromide solution.

Dead-Stop Indicator. A description of this apparatus is available (*3*, *4*).

PROCEDURE

The chelate, containing about 50 mg. of cadmium, precipitated at approximately pH 11 with the reagent, is filtered through a medium-porosity sintered-glass crucible with suction using the procedure previously described (*2*). It is washed thoroughly with 50% w./v. alcohol to which a trace of ammonia had been added. The chelate is then dissolved in about 50 ml. of hot glacial acetic acid, transferred to an iodine flask, and diluted with 20 ml. of distilled water. Thirty-five milliliters of a 0.1 *N* bromate-bromide mixture is pipetted into the flask. The flask is immediately stoppered, and 2 ml. of potassium iodide solution is added to the reservoir of the iodine flask to prevent any loss of bromine. The reaction is allowed to proceed for a period of 1 to 1.25 hours. A solution containing about 1.5 grams of potassium iodide is then added to the reaction. The liberated iodine is titrated with the standard 0.1 *N* sodium thiosulfate solution to the end point as detected with the dead-stop indicator.

This apparatus consists of two platinum electrodes, connected in series with a potential just sufficient to balance the back e.m.f. (about 20 mv.). During the titration, current will flow to give a full deflection of the galvanometer. When the end point is reached the needle returns to zero. Further addition of thiosulfate causes no deflection. This apparatus was used because of the difficulty encountered in detecting the end point visually with solid precipitate of the brominated compound present during the titration.

DISCUSSION

It was somewhat disappointing to find that less than the theoretical amount of bromine was taken up by the cadmium chelate. Despite the variation of the reaction temperature and the lengthening of the reaction time, only 90% of the theoretical bromine uptake was observed. However, while the reaction did not go to completion, the results were easily reproducible. Upon the application of an empirical factor, it was found that an accuracy of ± 0.2 mg. of cadmium could be obtained by this procedure in the presence of, or in the absence of, most metallic ions (*2*). Some of the results obtained can be seen in Table I. The number of grams of cadmium found in the samples (Column 3) was calculated by multiplying a quantity, the volume of thiosulfate used for a blank minus the volume used for the sample, by the normality of the thiosulfate, and finally by the empirical factor 0.0153.

ACKNOWLEDGMENT

The authors wish to express their appreciation to the United States Atomic Energy Commission for financial support.

LITERATURE CITED

(1) Siggia, S., "Quantitative Organic Analysis via Functional Groups," p. 111, New York, John Wiley & Sons, 1949.
(2) Walter, J. L., and Freiser, Henry, Anal. Chem., **24**, 984 (1952).
(3) *Ibid.*, in press.
(4) Willard, H. H., *et al.*, "Instrumental Methods of Analysis," pp. 212–13, New York, D. Van Nostrand Co., 1951.

Received for review July 7, 1952. Accepted September 26, 1952. Contribution No. 867 from the Department of Chemistry, University of Pittsburgh, Pittsburgh 13, Pa.

Extraction and Determination of Total Pectic Materials in Fruits

R. M. McCREADY AND E. A. McCOMB
Western Regional Research Laboratory, Albany, Calif.

ONE approach to the analysis of fruits for total pectic substances is to extract the tissue in turn with hot water, dilute mineral acid (pH 2), and cold alkali or ammonium oxalate (*3*). Three or four fractions of pectic substances are isolated, each presumed to have unique solubility characteristics. The high-methoxyl pectins are water-soluble; acid promotes solution of the protopectin and alkali or oxalate dissolves low-methoxyl pectins and pectates. Difficulties encountered in this procedure are due to overlapping solubilities of the various pectic substances. For example, sodium pectate is soluble in water, alkali, and ammonium oxalate, and partly soluble in hot dilute acid. Low-methoxyl pectins may be soluble in all extractants in some fruits and not in others, depending upon the cation composition. The quantitative multiextraction procedure is long and tedious and the final interpretation may be questionable.

A seemingly more reasonable approach to this problem in view of the present knowledge of the physical and chemical properties of high polymers assumes the existence in fruits of pectins of wide ranges of methoxyl contents and molecular weights and the possibility of extraction of all of the pectic substances in fruit tissue at pH 7 and below with very mild heating, provided that the tissue is sufficiently macerated and that the polyvalent cations are sequestered.

Pectin problems can be attacked by determining the total pectic substances as anhydrouronic acid (AUA) and by characterizing pectic substances extracted by mild means (*7*). Such information may permit deductions of the possible role of the pectic substances as they exist in the tissue under investigation. Some knowledge of the plant's cation composition is necessary because cations play significant roles in maintaining texture of fruits.

Pectic enzymes were used for the extraction of pectin from apples (*6*). A calcium sequestering agent and pectinase is used to extract total pectic substances from fruits without heating. The extraction procedure and method for determining anhydrouronic acid colorimetrically is presented.

REAGENTS

Versene Solution. Dissolve 5 grams of dry ethylenediaminetetraacetic acid tetrasodium salt (from Bersworth Chemical Co., Framingham, Mass., under the trade name Versene, regular) in 1 liter of solution.

Pectinase. A commercial Pectinol 100 D (from Rohm & Haas Co., Philadelphia, Pa.).

Acetic Acid. Reagent grade, glacial.

Sulfuric Acid. Reagent grade, concentrated.

Ethyl Alcohol. Reagent grade, 95%.

Ethyl Alcohol. Purified. Reflux 1 liter of 95% reagent grade ethyl alcohol with 4 grams of zinc dust and 4 ml. of 1 to 1 sulfuric acid for 24 hours. Distill, using all-glass apparatus. Add 4 grams of zinc dust and potassium hydroxide to the distilled alcohol and redistill.

Carbazole Reagent. Dissolve 0.150 gram of reagent grade carbazole in 100 ml. of purified ethyl alcohol. Solution is slow and stirring is required.

Galacturonic Acid Monohydrate. Reagent grade. Check the purity by titrating 0.5 gram with 0.1 *N* sodium hydroxide to pH 8.0. The theoretical formula weight of galacturonic acid hydrate is 212, and its final specific rotation in water $[\alpha]_d + 51, c = 1$.

VERSENE-PECTINASE EXTRACTION OF PECTIC SUBSTANCES

Dried Material. Weigh 1.0 gram of 40-mesh or finer sugar-free dried sample (extracted with 70% ethyl alcohol to remove sugars) containing 10 to 40% pectin into a 250-ml. beaker and moisten with 95% ethyl alcohol. Sequester the divalent cations with 200 ml. of 0.5% Versene solution. Adjust the pH to 11.5, if necessary, with 1 *N* sodium hydroxide and de-esterify the pectin and pectinates by holding at 25° C. for 30 minutes. Acidify the mixture to pH 5.0 to 5.5 with acetic acid. Add 0.1 gram of pectinase, stir for about 1 hour, dilute to 250 ml., and filter. Discard the first few milliliters of filtrate, dilute 2 ml. to 100 ml., and take 2-ml. aliquots for analysis.

Fresh Fruit. Blend 25.0 grams of fresh fruit in a Waring Blendor for 5 minutes with 125 ml. of 95% ethyl alcohol. Filter and discard the ethyl alcohol containing the sugars. Wash the pulp twice with 75% ethyl alcohol. Transfer the moist (not wet) pulp to a 250-ml. beaker. Sequester the cations and de-esterify the pectin with 200 ml. of a 0.5% Versene solution at pH 11.5 for 30 minutes. Proceed as described above.

Frozen Orange Concentrate, Total. Weigh 50.0 grams of thawed concentrate, acidify it to about pH 2.0, and add 2.5 volumes of 95% ethyl alcohol. Stir the mixture and centrifuge or filter. Discard the solution containing sugars and citric acid and wash the pulp twice with 75% ethyl alcohol. Add 200 ml. of Versene solution and proceed as described.

Frozen Orange Concentrate, Serum. Thaw and centrifuge about 100 grams of concentrate. Decant the turbid serum, add 2% filter aid, and filter. Take a 10-ml. aliquot of clear serum in a centrifuge tube and acidify with 5 drops of hydrochloric acid. Add 3 volumes of ethyl alcohol, stir, centrifuge, and discard the solution containing sugar and citric acid. Wash the precipitate once with 70% ethyl alcohol and dissolve it in 25 ml. of 0.05 *N* sodium hydroxide at pH 11.5 or above. After 30 minutes at 25° C., dilute to 500 ml. and take 2-ml. aliquots for analysis. Versene and pectinase are not required because the pectic substances are already in solution.

COLORIMETRIC DETERMINATION OF ANHYDROURONIC ACID

Measure 12.0 ml. of concentrated sulfuric acid into a 25 × 200 mm. culture tube. Cool the tube and contents to about 3° C. in an ice bath and add a 2-ml. aliquot of solution containing 5 to 80 micrograms of de-esterified galacturonide or polymer. Insert a 5-ml. beaker into the mouth of the test tube and mix the contents thoroughly. Replace the tube in an ice bath and cool to below 5° C. Heat the tube and contents for 10 minutes in a boiling water bath. Cool to about 20° C., add 1.0 ml. of 0.15% carbazole reagent, mix thoroughly, and allow to stand at room temperature for 25 ± 5 minutes. Determine the intensity of the color using light of wave length 520 $m\mu$. Analyze the samples in sequence, so that the time and temperature from the addition of the carbazole to the color determination are as reproducible as possible.

Use a standard curve to obtain the concentration of anhydrouronic acid in the samples, and to control daily variations, include a 40-microgram galacturonic acid hydrate (33.2 micrograms of anhydrouronic acid) standard with each series. The reproducibility falls within about 2%.

SPECIFICITY AND COMPARISON OF EXTRACTION PROCEDURES FOR TOTAL PECTIC SUBSTANCES

Materials Analyzed. Hot 70% alcohol was used to extract sugars and organic acids from grapefruit peel, cranberries, raspberries, and apricot halves. These were then dried and ground through 40-mesh screens. Raspberry seeds were removed from dried pulp by screening before grinding.

Pectate pulp (from the Sunkist Growers, Inc., Ontario, Calif.) was a dried commercial product made from citrus peel by enzymic de-esterification in situ, followed by pressing, drying, and grinding.

Lima bean pod and corncob hemicelluloses were separated from the corresponding holocellulose with 5% potassium hydroxide and purified as described by Carson and Maclay (*1*).

Anhydrouronic Acid. Dried material was analyzed for anhydrouronic acid by the yield of carbon dioxide resulting from heating the substance in 19% hydrochloric acid for 2 hours (*5*).

All of the diluted filtrates, obtained from the Versene-pectinase and other extractions, were analyzed for anhydrouronic acid by a colorimetric carbazole method (*4*). Agreement between the carbon dioxide and carbazole method was close, if the pectin was first de-esterified. Versene at pH 11.5 serves to sequester divalent cations and deesterify pectin and pectinates to pectates. Pectates are the substrates for the polygalacturonase present in the pectinase (*2*) and the resulting de-esterified degraded polymers were determined with the colorimetric method.

Table I. Comparison of Various Extractants for Pectic Substances

Dried Material	Anhydrouronic Acid, % 19% hydrochloric acid	Versene-pectinase	Hydrochloric acid, pH 2	Oxalate	Versene
Apricot pulp	15.3	14.6	14.0	...	13.8
Raspberry pulp	20.1	18.6	19.8	18.7	18.3
Grapefruit peel	38.1	36.4	33.0	...	35.8
Cranberry pulp	17.4	16.5	15.1	...	15.1
Pectate pulp	23.7	22.4	5.4	21.8	20.7
Corn cob hemicellulose	6.2	0.0	...	...	...
Lima bean pod hemicellulose	7.9	0.0	...	...	...

After 1 and 5 hours hydrolysis with pectinase, apricot pulp analyzed 14.6% anhydrouronic acid. Grapefruit pulp analyzed 36.4% and 37.2% anhydrouronic acid in 1 and 24 hours' hydrolysis, respectively. Pectate and raspberry pulp analyzed 22.4% and 18.6%, respectively, after 1 hour and remained unchanged for 24 hours' hydrolysis with pectinase. No significant increases in the anhydrouronic acid contents of the fruit tissue result in allowing pectinase extraction to proceed longer than 1 hour. The results of carbazole analysis for anhydrouronic acid on fruit tissue after 1 hour of hydrolysis are presented in Table I. The results, expressed on the dry basis in Table I, show that the anhydrouronic acid calculated from the carbon dioxide liberated by heating in 19% hydrochloric acid for 2 hours, is about 5% higher than the results obtained using the proposed Versene-pectinase extraction method. Corncob and lima bean pod hemicelluloses containing glucuronide units yield carbon dioxide when heated with 19% hydrochloric acid, amounting to 6.2 and 7.9%, respectively. Glucuronic acid gives color in the carbazole reaction. No interference was encountered because the pectinase is specific for pectic substances and no free glucuronic acid is liberated during the extraction. Neither hemicellulose, known to be free from pectic substances, yields anhydrouronic acid by the proposed pectinase-carbazole procedure. The latter procedure appears to be specific for pectic substances under these conditions and the difference between the results obtained by the 19%

hydrochloric acid method and those by the proposed method is believed to be due to glucuronide-containing hemicelluloses.

Pectic substances can be extracted from fruits with dilute hydrochloric acid, oxalate, Versene, or other extractants. Chemical agents require heating to be effective as pectin extractants. One-gram samples of 40-mesh dried fruit pulp were extracted with 200 ml. of boiling hydrochloric acid at pH 2 for 60 minutes; with 200 ml. of boiling 3% ammonium oxalate at pH 7 for 60 minutes; and with 200 ml. of boiling 0.5% Versene solution at pH 7 for 60 minutes. The extracted pectic substances were deesterified in solution by holding at pH 11.5 for 30 minutes, neutralized, diluted to 250 ml., and filtered. The first few milliliters of the filtrates were discarded, 2 ml. of each was diluted to 100 ml., and 2-ml. aliquots were taken for anhydrouronic acid analysis by the carbazole method. The results are summarized in Table I and compared with results of the carbon dioxide and Versene-pectinase extraction.

Hydrochloric acid extracts most of the pectic substances from fruit pulps under the conditions of the experiment. Pectate pulp contains pectates which are insoluble in the acid medium and only a small fraction is extracted. Boiling oxalate or Versene solution extracted pectic substances almost as completely as the pectinase method. Boiling Versene solution extracted 95% of the pectic substances from dried 40-mesh pectate, raspberry, cranberry, and apricot pulp in 30 minutes. No significant increases were obtained beyond 60 minutes' heating. Pectic substances were extracted from 150-mesh apricot and grapefruit pulp by boiling Versene solution for 30 minutes, but raspberry and cranberry pulp still required 60-minute extractions. Versene-pectinase is recommended, since heating is unnecessary and the method appears to be specific for extracting pectic substances from fruits.

Table II. Analysis of Undried Fruit Pulp and Frozen Orange Concentrate

For total pectic substances

Material Analyzed	Anhydrouronic Acid[a], % Versene	Anhydrouronic Acid[a], % Versene-pectinase
Apple pulp	0.51	0.54
Apricot halves	0.60	0.62
Orange concentrate, Calif., total	0.14	0.14
Orange concentrate, Calif., serum	0.05	0.05
Orange concentrate, Florida, total	0.26	0.26
Orange concentrate, Florida, serum	0.05	0.05

[a] On fresh-weight basis.

Fresh fruit and orange concentrate were analyzed for anhydrouronic acid by the carbazole method after extraction with Versene-pectinase and boiling 0.5% Versene. The results, presented in Table II, show that boiling 0.5% Versene solution at pH 7 for 60 minutes extracted the same amount of pectic substances from the undried pulps and frozen orange concentrates as the Versene-pectinase procedure and would serve as an alternative extraction procedure.

LITERATURE CITED

(1) Carson, J. F., and Maclay, W. D., *J. Am. Chem. Soc.*, 70, 293 (1948).

(2) Jansen, E. F., and MacDonnell, L. R., *Arch. Biochem.*, 8, 97 (1945).

(3) Kertesz, Z. I., "The Pectic Substances," pp. 99–104, New York, Interscience Publishers, Inc., 1951.

(4) McComb, E. A., and McCready, R. M., ANAL. CHEM., 24, 1630 (1952).

(5) McCready, R. M., Swenson, H. S., and Maclay, W. D., IND. ENG. CHEM., ANAL. ED., 18, 290 (1946).

(6) Meade, R. C., Fish, V. B., and Dustman, R. B., *Plant Physiol.*, 23, 98 (1948).

(7) Owens, H. S., McCready, R. M., Shepherd, A. D., Schultz, T. H., Pippen, E. L., Swenson, H. A., Miers, J. C., Erlandsen, R. F., and Maclay, W. D., Western Regional Research Lab., Albany, Calif., Bureau of Agr. Ind. Chem., U. S. Dept. of Agr., AIC-340 (mimeographed) 1952.

RECEIVED for review June 30, 1952. Accepted August 28, 1952. Mention of manufacturers and commercial products does not imply that they are endorsed or recommended by the department over others of a similar nature not mentioned.

Analytical Chemistry of Niobium and Tantalum

Atmospheric Chlorination of Hydrolyzed Oxide Precipitates

C. F. HISKEY, LEONARD NEWMAN[1], AND R. H. ATKINSON[2]

Polytechnic Institute of Brooklyn, Brooklyn, N. Y.

IN A recent group of papers (*1*), a method was presented for the atmospheric chlorination of some oxides associated with niobium and tantalum in their minerals. The method proposed employed octachloropropane as the chlorinating agent. A 3-hour reaction time and a temperature of 275° C. were required. Following chlorination, titanium and tin were separated quantitatively by means of a distillation procedure.

This separative process applied to the analysis of niobites and tantalites avoids many of the precipitations and reprecipitations of the Schoeller method (*2*). It is particularly advantageous because definite molecular entities are involved rather than colloidal oxides. Therefore, the separation is clear cut and requires only one operation. Another important feature is the simplification introduced in any analytical scheme by titanium removal. In most analytical reactions the properties of titanium compounds are intermediate to those of niobium and tantalum, making their separation from each other or their determination uncertain and difficult. These considerations indicate the desirability of introducing the chlorination-distillation step into separative schemes.

As previously indicated (*1*), the chlorination procedure was developed for synthetic oxide mixtures prepared from pure, freshly ignited oxides. It was thought that these mixtures would have a reactivity comparable with any oxide mixture regardless of method of preparation. The usual methods for gathering the earth oxides and titania include hydrolysis in ammoniacal solution or tannin precipitation from a weakly acid tartrate solution. The precipitates so formed are filtered and ignited at high heat in order to drive off water and burn off the filter paper. It was anticipated that these mixtures would chlorinate as readily as the synthetic ones. This did not prove to be the case, however. It was found that the chlorination failed in most cases when applied to oxide mixtures prepared from aqueous solution.

It became necessary to study this problem in an attempt to devise ways to circumvent this new difficulty. That investigation is the subject of this paper.

EXPERIMENTAL PROCEDURES

The oxide mixtures used in this study were prepared from the same analytical grade oxides referred to in the initial communication (*1*). Appropriate portions of each of the various oxides under test were separately ignited and weighed into a tared porcelain crucible (Coors No. 0). Roughly, fifteen times as much

---

[1] Present address, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Mass.

[2] Present address, Westinghouse Electric Co., Bloomfield, N. J.

potassium pyrosulfate was added and the mixture was gently heated to the melting point of the pyrosulfate to avoid any spattering. This was followed by a period of vigorous ignition until a clear melt resulted. The cooled melt was then extracted with 100 ml. of boiling 2 *N* hydrochloric acid, which dissolved the potassium salts and hydrolyzed the earth acid sulfates to their corresponding oxides. When tantalum and titanium are both present, considerable quantities of tantalum remain in solution. In the absence of titanium, the tantalum is nearly quantitatively precipitated at this point.

The acid slurries were next made strongly ammoniacal in order to precipitate the hydrous oxides completely. After filtration (Whatman Paper No. 41), the oxides are resuspended in hot 2 *N* hydrochloric acid solution and reprecipitated with ammonia in order to free them completely of potassium salts. After drying at 110° C. to volatilize the ammonium chloride, the precipitates were ignited in porcelain crucibles at 800° to 1000° C. for 1 hour. A fluffy, amorphous-appearing oxide mixture resulted except when tantalum was present in high concentrations. In such cases the apparent density was greater.

With exceptions noted here, the chlorination-distillation operation was performed as previously described (*1*). Still pot residues and the distillation column holdups were combined and analyzed for total titania present, for the quantity of R_2O_5 that had been chlorinated and sometimes for total R_2O_5. The determination of titania in the residues as well as in the distillate receiver has been described (*1*).

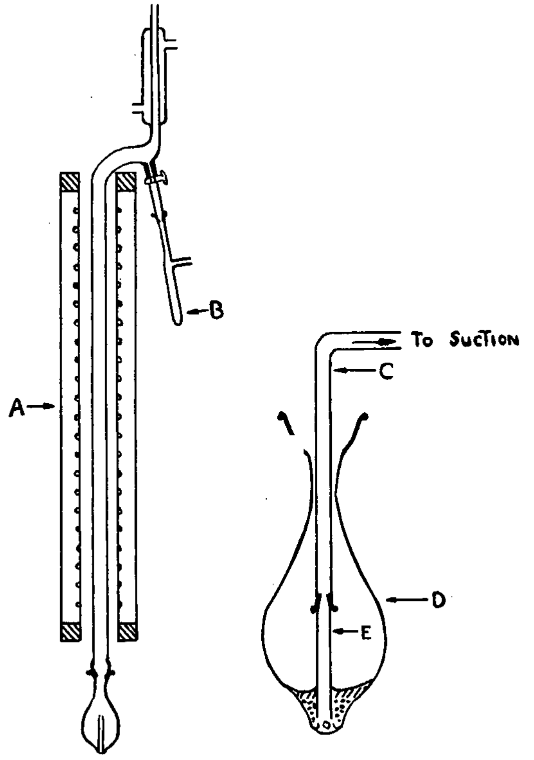


Figure 1. Diagram of Still Pot

Table I. Titanium Separation from Hydrolyzed and Ignited Oxide Mixtures

No.	Sample Composition, Grams of Each: Nb_2O_5	TiO_2	Ta_2O_5	ZrO_2	SnO_2	Sample Unreacted, %	Original TiO_2 in Residue, %	Notes
1	0.7501	0.0335	....	....	....	0.0	0.0	.....
2	0.6366	0.0684	....	....	....	0.8	0.1	.....
3	0.3956	0.0732	0.1037	....	....	..	9.8	.....
4	0.2953	0.0482	0.1697	....	....	..	10.0	.....
5	0.3028	0.0645	0.3111	....	....	..	20.3	.....
6	0.2014	0.0663	0.4025	....	....	..	31.5	.....
7	0.3149	0.0595	0.1900	0.0315	....	62.0	33.1	0.0315[a]
8	0.2971	0.0502	0.1512	0.0318	....	40.6	43.0	0.0318[a]
9	0.222	0.039	0.206	0.085	....	98.0	...	.....
10	0.2940	0.0501	0.1677	....	0.0267	12.2	1.4	0.0[b]
11	0.3016	0.0517	0.1492	....	0.0274	13.8	1.1	0.0[b]
12	0.3936	0.0710	0.1171	....	....	5.9	1.4	...[c]
13	0.3879	0.0676	0.2039	....	....	19.5	9.8	...[c]
14	0.5325	0.0704	0.2667	....	....	27.9	6.4	...[c]
15	0.3912	0.0688	0.2333	....	....	7.6	3.1	...[d]

[a] Gram of ZrO_2 in unreacted residue.

[b] Gram of SnO_2 in unreacted residue.

[c] Long-necked reaction flask substituted and heated to over 400° C. for 2.5 hours. Purpose of long-necked flask was to allow partial cooling of ball joint connecting it to distillation column. At high temperatures used here, there would have been vapor loss.

[d] Same as [c] except 30 grams of octachloropropane C_3Cl_8 used instead of usual 15 grams.

The procedure used for determining the amount of R_2O_5 that was chlorinated was as follows: After the distillation operation was complete, the still pot was cooled to room temperature while the column was kept hot. This served to condense the volatile chlorides in the still pot. The still pot was then disconnected from the column. Twenty-five milliliters of a mixture of 95% chloroform and 5% absolute ethyl alcohol were poured in and swirled about to dissolve the chlorides and excess octachloropropane. The resulting solution containing charred products and the unreacted oxides was filtered. The washing operation was repeated about three times more using smaller portions of the solvent. In addition, the column was washed down several times. The last traces of unreacted oxides were removed with solvent and a rubber policeman. All of the filtered washings were combined. The solution was concentrated by evaporation to about 15 ml. The organic matter was then destroyed with a boiling mixture of 25 ml. of concentrated sulfuric acid and 50 ml. of concentrated nitric acid. When a clear solution resulted, the nitric acid was removed by boiling down to fumes of sulfur trioxide followed by diluting with water and refuming. After dilution with water the solution was neutralized with an excess of ammonia and filtered. The precipitate was then ignited at 800° to 1000° C. and weighed.

The unreacted oxides were determined by igniting the filter paper containing them along with any charred products which may have been present, and then weighing. The total oxides represented the sum of the weights of the chlorinated earth oxides, the unreacted oxides, and the titania distilled as the tetrachloride.

Modification of Still Pot. A new modification of the still pot was required for the handling of oxide mixtures which were to be dried in it. This new still pot is illustrated in Figure 1 and differs from the previous one in the following way:

A male tapered ground-glass joint (10/30) is sealed to the bottom. At the periphery of the seal a number of small holes are provided to allow movement of liquid from the interior of the flask up through the male joint. This may be used as a filtering flask simply by attaching a female ground-glass joint to which suction is applied. The preparation of a mat for this filtration flask is as follows:

About 2 ml. of pure silica sand or pulverized borosilicate glass is first dropped into the bottom to provide a support for the asbestos mat. The procedure for forming the mat is similar to that for a Gooch crucible. The mat is washed thoroughly to free it of loose particles. Then, still maintaining suction, a hydrolyzed oxide suspension is poured in and filtered. It is washed with 1 *M* ammonium chloride solution to free it of adsorbed salts. Finally the ammonium chloride is washed out with pure distilled water. The precipitate is then sucked as dry as possible, after which the female member is removed. It may now be subjected to any drying schedule that the operator wishes. The advantage of this filtering flask is that it permits low temperature dehydrations without the difficulties of transferring the precipitate from a filtering crucible. Filter paper may not be used in this operation because it would react with the chlorinating agent resulting in excessive charring and oxychloride formation.

Chlorination of Hydrolyzed and Ignited Oxide Mixtures. In the first group of experiments various oxide mixtures prepared as described above and chlorinated in the usual way, except as noted below, were tested for completeness of chlorination and of titanium removal. Some typical results obtained are given in Table I. From an examination of these data a number of conclusions may be made and these are itemized:

Mixtures of niobia and titania react quantitatively (Nos. 1 and 2).

The addition of tantala to the niobia-titania mixture hinders titania chlorination (Nos. 3–6). Even with much more severe reaction conditions, this chlorination cannot be made quantitative, although substantial improvement results (Nos. 11–15).

The addition of even small percentages of zirconia to the oxide mixtures has a greater inhibiting effect than does the tantala upon the over-all chlorination. With 15% zirconia in the oxide mixture the chlorination is virtually prevented.

The presence of stannic oxide in the mixture has a beneficial effect on the over-all process, partially overcoming the inhibiting effect of the tantala.

In general, it is apparent that such a procedure would yield quantitative results for titanium only when the sample was extremely low in tantala and zirconia. This restriction is so great however, as to render the approach worthless for most of the minerals which it was expected to analyze. Consequently, it was necessary to overcome this defect or else to abandon the chlorination step in toto.

X-RAY EXAMINATION OF THE IGNITED PRECIPITATES. Since ignition of the precipitated oxide mixtures had produced such striking effects, the question arose as to the nature of possible structural changes which occurred during the ignition. Changes did not occur if mixtures of oxides ignited separately were combined and then reignited but appeared to require the intimate mixing which simultaneous precipitation afforded. To make a brief study of the changes that occurred on ignition, samples were prepared of pure tantala, niobia, and titania and then of the following mixtures (in 1 to 1 weight ratios): tantalum and niobium pentoxides, tantalum and titanium oxides, and niobium and titanium oxides. These ignited oxides and ignited mixtures were placed in the sample holder of a recording Geiger counter x-ray spectrometer and the detecting tube was swept through an angle from 60° to 18° in units of 2 θ. Analysis of the resulting curves showed that for the pure oxides markedly crystalline products resulted possessing characteristic diffraction patterns. The mixture of niobia and tantala retained the crystallinity of the two components. Its diffraction pattern was a simple combination of the two individual patterns for the pure oxides modified only by the dilution effect which each would have on the other. In the remaining two mixtures containing titania with each of the earth oxides, a diffraction pattern could barely be distinguished indicating a poorly crystalline almost amorphous material. A few weak diffraction peaks could be observed, but these bore no relation to the diffraction patterns of the pure oxides. Instead they suggested the formation of new phases extremely difficult to chlorinate.

To improve the chlorination of these ignited oxide mixtures, two general approaches were tried. One way to increase the chlorinating efficiency of the octachloropropane was to add a catalyst which would function more efficiently than niobium as a chlorine carrier. This approach was suggested by the beneficial effects produced by the stannic oxide. A second approach was to avoid the formation of these new chlorination-resistant phases altogether.

Addition of Chlorination Catalyst. The addition of a chlorine-carrying catalyst is suggested by the data in Table I. It will be recalled that the addition of a basic oxide like zirconia had a very deleterious effect on the chlorination. From previous work it was known that if such basic substances such as sodium oxalate, barium oxide, or barium carbonate were added to the ignited oxides, chlorination could be effectively prevented. On the other hand, the addition of a more acidic oxide, like stannic oxide, had a beneficial effect. The high volatility of stannic chloride compared to the chlorides of molybdenum and tungsten suggested that these latter elements might be better additives than tin. They would be superior for three reasons:

1. Their low volatility would concentrate them in the still pot where they were needed while the stannic chloride would be refluxing in the column.
2. They were elements not commonly found with earth oxide minerals and thus need not be determined.
3. Because of their ease of separation from the earth oxides, their initial presence would not greatly disturb the analytical program.

A series of samples containing niobia, tantala, titania, and zirconia was prepared according to the hydrolysis procedure given in the section on experimental procedures. Molybdenum(VI) oxide was added to these immediately prior to chlorination. In another experiment, tungsten(VI) oxide and stannic(IV) oxide were precipitated along with the other oxides to achieve more intimate mixing. The samples were then subjected to the usual chlorination-distillation procedure. The results obtained are summarized in Table II.

From an examination of these results it can be seen that improvement results in all cases over what might have been expected had the acidic oxide not been added. This supports the belief that the more acid chlorides function as chlorination catalysts. Nevertheless, the over-all prospective was one which gave little promise of quantitative chlorination for most oxide mixtures. Therefore, it seemed desirable to set aside this approach temporarily in favor of one which would avoid the formation of the unreactive oxide mixtures.

Low Temperature Dehydration. In this connection attempts were made to effect a dehydration of the oxide mixtures at temperatures lower than 800° C. It was hoped that a temperature might be found at which all of the water could be removed without the formation of the chlorination-resistant mixtures.

Table II. Effect of Acidic Oxides on Chlorination of Ignited Earth Oxide Mixtures

	1	2	3	4	5
Nb_2O_5, %	56.3	45.2	16.7	21.5	22.5
Ta_2O_5, %	29.3	40.7	68.0	60.5	60.3
TiO_2, %	9.6	9.6	8.2	10.6	11.1
ZrO_2, %	4.8	5.3	7.1	7.3	6.1
Wt. of sample, gram	0.5268	0.5166	0.5909	0.3409	0.3327
Added oxide, gram	MoO_3 0.5	MoO_3 0.5	MoO_3 0.5	WO_3 0.2	SnO_2 0.15
Original TiO_2 in unreacted residue, %	3.8	6.7	27.2	35.4	53.2
Wt. of unreacted residue, gram	0.0502	0.1178	0.3663	0.2789	0.2815

The new filtration flask described above was utilized in these experiments. In the preliminary studies a group of simple oxide mixtures of a wide variety of compositions was prepared, transferred into the filtration flasks, dehydrated under conditions of varying severity, and then chlorinated. From such studies as these it was soon found that dehydration overnight at 110° C. gave satisfactory results. With the aid of vacuum, the time of dehydration could be reduced to 4 or 5 hours. The time for oxide preparation could be reduced even further when the precipitates were given a final washing with volatile organic solvents like acetone or acetone followed by diethyl ether. From among

Table III. Chlorination of Oxide Mixtures Subjected to Low Temperature Dehydration

Samples weighed approx. 0.4 gram. 25 Grams of octachloropropane used. Chlorination conditions: 2 hours at 310° C. Alternate reflux distillation technique used. Temp. of still pot during distillation was 240° C., of column, 180° C. Reflux for 3 hours.

Nb_2O_5, %	Ta_2O_5, %	TiO_2, %	ZrO_2, %	Total Oxides Chlorinated, %	TiO_2 Distilled, %
...	...	100	...	88.8	...
5.8	...	94.2	...	97.5	...
10.5	...	89.5	...	99.7	...
19.4	...	80.6	...	99.9	...
...	100	...	...	95.0	...
...	100	...	...	95.1	...
...	100	...	...	95.8	...
2.9	97.1	...	...	99.1	...
12.3	87.7	...	...	99.7	...
21.4	78.6	...	...	100.0	...
...	...	...	100	5.5	...
10.3	...	...	89.7	56.1	...
61.7	...	...	38.3	84.5	...
45.0	45.0	10.0	...	99.5	...
37.5	38.5	6.5	17.5	99.0	...
44.3	42.5	8.7	4.5	...	99.3
46.0	41.0	7.5	5.5	...	99.5

the data obtained, a group of results has been taken to illustrate the results of this technique. These are given in Table III.

Pure titania and tantala are chlorinated to the extent of 89 and 95%, respectively. Only small amounts of niobia are required to produce quantitative chlorination in mixtures of these three oxides. Zirconia, which by all of the previous techniques was completely unreactive, is here shown in the pure state to be chlorinated to the extent of 5%. The percentage of zirconia which may be chlorinated is markedly improved by the addition of niobia. The distillation separation of titanium is, as might have been anticipated, quantitative even when sizable quantities of zirconia are present. A general conclusion therefore is that the reactivity of these oxides is enormously improved by a low temperature dehydration.

Samples which are not freed of potassium salts from the pyrosulfate melt are extremely difficult to chlorinate. When a precipitate is thoroughly washed it appears as a white, fluffy powder. Incomplete salt removal results in a gray color and a lumpy appearing precipitate. A number of such impure precipitates were treated with octachloropropane. It was found that pure titania and pure tantala so prepared chlorinated only to the extent of 10 and 60%, respectively, while niobia-titania oxide mixtures whose composition ratios were 6 to 94 and 10 to 90, respectively, were chlorinated only to the extent of about 10%. Thus, unless the oxide precipitates are washed clean, negative results may be anticipated.

DISCUSSION

Some conclusions may now be made with respect to the chlorination process in general and then more specifically with respect to the classes of minerals which may be treated successfully in this manner.

It has been noted in these studies that the more amorphous the pure oxide preparation, the more readily does it chlorinate. On the other hand, the more severe the ignition, the more crystalline, and therefore the less reactive it becomes. Such results are usually accounted for in terms of surface available for reaction with the chlorinating agent. Among the several oxides studied it is apparent that the chlorination rate varies in the order niobium pentoxide > tantalum pentoxide > titanium dioxide > zirconium dioxide—i.e., increasing as their acidity is increased. When very basic oxides are used, such as barium oxide, for example, chlorination fails completely. Thus it may be anticipated that chlorination of group III oxides will either fail completely or go only to a slight extent.

In oxide mixtures the more acid oxides assist the chlorination of those less easily chlorinated. This probably goes by way of a chlorine-oxygen interchange. In every case where a low temperature dehydration was used, increased chlorination of the less reactive species was observed on adding niobia.

Finally, the inhibiting effects observed in Table I when tantala and zirconia were incorporated in niobia-titania mixtures and ignited at high heat must be associated at least partially with a shrinkage of the surface or to the formation of new phases much more resistant to chlorination.

It is now possible to convert a wide variety of oxide mixtures and thus effect titanium removal from them. This means that such a class of minerals as the tantaloniobates can all be treated, since they are generally poor in titania and zirconia. Because they are rich in iron, a separation of this element must be made prior to chlorination.

With respect to the titanoniobates which may contain titania in amounts up to 35% (euxenite and aeschynite) and zirconia up to 5% (pyrochlore), some failures may be anticipated, particularly when the niobia becomes very low. From an inspection of older analyses summarized by Schoeller, it appears that when titania is high, the zirconia is low or absent, and vice versa. This situation eases the chlorination difficulties markedly, so that it may be concluded that most of this class will be chlorinated. With titanium rich minerals like the ilmenorutiles and dysanalyte (a calcium titanate containing niobia) chlorination may be expected. Minerals like naegite, hagalite, and zircon, or the silicozirconates, eudialyte, wöhlerite, läverite, etc., will almost invariably fail to chlorinate. On the other hand, the tin-containing minerals, ainalite and tantalocassiterite, will chlorinate with few exceptions.

ACKNOWLEDGMENT

The authors would like to acknowledge the assistance given to this investigation by Benjamin Post who took the diffraction patterns discussed above and to Joseph Steigman for a number of valuable suggestions as well as direct assistance during the course of the work. A part of this research was financed by the Chemical Division of the Office of Naval Research.

LITERATURE CITED

(1) Atkinson, R. H., Steigman, J., and Hiskey, C. F., Anal. Chem., 24, 477, 480, 484 (1952).
(2) Schoeller, W. R., "Analytical Chemistry of Tantalum and Niobium," London, Chapman and Hall, Ltd., 1937.

Received for review May 19, 1952. Accepted September 3, 1952. Presented in part at the Fifth Annual Symposium on Analytical Chemistry before the Pittsburgh Section of the American Chemical Society, March 1951. Taken from data submitted to the faculty of the Polytechnic Institute of Brooklyn by Russell H. Atkinson in partial fulfillment of the requirements for the Ph.D. degree in chemistry, June 1952, and the bachelor's thesis of Leonard Newman.

Refractive Indices of Some Cyclohexanol Solutions

CHARLES M. WHEELER, JR., AND FREEMAN S. JONES
University of New Hampshire, Durham, N. H.

CYCLOHEXANOL is an excellent solvent for a wide variety of organic compounds (*9*) and has an unusually high freezing point depression constant, 42.5° per gram molecular weight of solute per 1000 grams of cyclohexanol, calculated using the reported heat of fusion (*2*) and the freezing point obtained by the present authors. Cryoscopic measurements obtained in this laboratory for various binary systems containing cyclohexanol, indicate that solutions of cyclohexanol and a number of organic compounds deviate from Raoult's law over a wide concentration range. The occurrence of these abnormalities suggested the advisability of obtaining refractive index data for these solutions.

This paper presents analytical data which may be used for determining the compositions of binary solutions of cyclohexanol-benzene, cyclohexanol-toluene, cyclohexanol-chlorobenzene, cyclohexanol-aniline, and cyclohexanol–primary isoamyl alcohol. Graphic representations of the refractive indices of these binary systems have not been reported. However, refractive index values for the pure organic materials are found in the literature (*1, 4, 8*).

PURIFICATION OF REAGENTS

Cyclohexanol. Eastman Kodak white label cyclohexanol was most satisfactorily purified by freezing and decanting the supernatant liquid to remove most of the water which this extremely hygroscopic compound absorbs. The crystals of cyclohexanol were allowed to melt, and were stored over anhydrous calcium sulfate. The cyclohexanol was fractionated at a reflux ratio of

10 to 1 in a 35-theoretical plate column. The boiling temperature of the cyclohexanol was 90.5° C. at 50-mm. pressure. To protect this hygroscopic material, the distillation was carried out in a closed system and the distillate receivers were vented through drying tubes containing anhydrous calcium sulfate. The middle third of the constant boiling point distillate was collected in glass-stoppered bottles which were sealed with paraffin and stored in a desiccator over anhydrous calcium sulfate.

Purity of the cyclohexanol was checked by obtaining cooling curves using a Leeds and Northrup platinum resistance thermometer connected through a Leeds and Northrup mercury commutator to a Leeds and Northrup Mueller temperature bridge. A sealed cryoscope was used because of the hygroscopic nature of cyclohexanol. The cooling curves were analyzed by the method of Taylor and Rossini (*6*) and the freezing point of cyclohexanol was found to be 25.447° C. The highest reported freezing temperature for cyclohexanol is 25.5° to 25.6° C. (*5*). Many lower melting points have been reported (*3*, *7*), the low values apparently being due to the presence of water.

The other components of the binary solutions were fractionally distilled. Table I lists the refractive indices of the fractions used and literature values are shown for comparison.

Table I. Refractive Indices of Pure Components

	Experimental, n_D^{20}	Literature, n_D^{20}	Reference
Aniline	1.58620	1.58629	(*8*)
Benzene	1.50141	1.50144	(*8*)
Toluene	1.49602	1.49693	(*4*)
Chlorobenzene	1.52502	1.52479	(*8*)
	$n_D^{17.8}$	$n_D^{17.8}$	
Isoamyl alcohol	1.40853	1.4084	(*8*)

Table II. Refractive Indices of Cyclohexanol-Aniline Solutions

Experimental Data		Smoothed Values	
Aniline, weight %	Refractive index, n_D at 25.00° C.	Aniline, weight %	Refractive index, n_D at 25.00° C.
0	1.46472	0	1.46472
9.85	1.47468	10	1.47490
19.62	1.48463	20	1.48505
29.55	1.49479	30	1.49543
40.60	1.50649	40	1.50585
49.56	1.51612	50	1.51662
59.22	1.52685	60	1.52780
70.87	1.54092	70	1.53985
79.01	1.55169	80	1.55315
91.34	1.57011	90	1.56810
100.00	1.58311	100	1.58311

Table III. Refractive Indices of Cyclohexanol-Benzene Solutions

Experimental Data		Smoothed Values	
Benzene, weight %	Refractive index, n_D at 25.00° C.	Benzene, weight %	Refractive index, n_D at 25.00° C.
0	1.46472	0	1.46472
8.21	1.46659	10	1.46700
13.89	1.46790	20	1.46945
20.59	1.46957	30	1.47212
28.25	1.47165	40	1.47492
39.75	1.47481	50	1.47795
48.95	1.47763	60	1.48132
59.01	1.48096	70	1.48484
67.35	1.48389	80	1.48838
81.40	1.4887	90	1.49195
90.55	1.49215	100	1.49573
100.00	1.49573		

Table IV. Refractive Indices of Cyclohexanol-Chlorobenzene Solutions

Experimental Data		Smoothed Values	
Chlorobenzene, weight %	Refractive index, n_D at 25.00° C.	Chlorobenzene, weight %	Refractive index, n_D at 25.00° C.
0	1.46472	0	1.46472
8.75	1.46831	10	1.46885
16.25	1.47135	20	1.47305
24.59	1.47521	30	1.47795
31.00	1.47842	40	1.48325
39.71	1.48308	50	1.48878
49.03	1.48820	60	1.49435
60.01	1.49400	70	1.50000
69.25	1.49955	80	1.50642
79.76	1.50624	90	1.51382
86.59	1.51121	100	1.52195
93.45	1.51658		
100.00	1.52195		

Table V. Refractive Indices of Cyclohexanol Isoamyl Alcohol Solutions

Experimental Data		Smoothed Values	
Isoamyl alcohol, weight %	Refractive index, n_D at 25.00° C.	Isoamyl alcohol, weight %	Refractive index, n_D at 25.00° C.
0	1.46472	0	1.46472
10.26	1.45721	10	1.45740
19.52	1.45048	20	1.45015
30.45	1.44331	30	1.44355
39.63	1.43772	40	1.43752
49.14	1.43239	50	1.43195
61.02	1.42617	60	1.42670
68.15	1.42232	70	1.42132
79.80	1.41602	80	1.41588
89.51	1.41057	90	1.41032
100.00	1.40495	100	1.40495

Table VI. Refractive Indices of Cyclohexanol-Toluene Solutions

Experimental Data		Smoothed Values	
Toluene, weight %	Refractive index, n_D at 25.00° C.	Toulene, weight %	Refractive index, n_D at 25.00° C.
0	1.46472	0	1.46472
8.55	1.46641	10	1.46675
13.57	1.46753	20	1.46898
20.62	1.46992	30	1.47138
29.43	1.47124	40	1.47395
40.57	1.47401	50	1.47655
51.18	1.47680	60	1.47930
59.60	1.47918	70	1.48229
69.19	1.48203	80	1.48548
80.81	1.48570	90	1.48880
91.37	1.48921	100	1.49231
100.00	1.49231		

PREPARATION OF SOLUTIONS AND REFRACTIVE INDEX DETERMINATIONS

Solutions of varying cyclohexanol concentration were prepared by pipetting cyclohexanol into 25-ml. glass-stoppered weighing bottles containing a weighed amount of the other component of the binary solution. The actual amounts of each constituent added were determined by weighing to 0.1 mg. on an analytical balance.

Refractive indices were measured by means of a Pulfrich refractometer with a sodium vapor lamp light source. According to the manufacturer of the refractometer the readings of the refractive indices are exact within a unit of the fourth decimal. Using a constant temperature bath and circulating pump, the temperature was maintained at 25.00° ± 0.01° C. The samples were kept in a closed cup to prevent absorption of moisture from the air, while the refractive index measurements were being made. The sample was left in the covered cup for 30 minutes and the refractive index measured again. During this period no change in the refractive index was observed. Liquid cyclohexanol was supercooled to 25° C. and the refractive index measured.

Large-scale plots of the refractive index data were prepared and values obtained at even composition increments, from smooth curves through the points. Experimental data and smoothed values are presented in Tables II to VI.

LITERATURE CITED

(1) Auwers, K., Hinterseber, R., and Treppman, W., *Ann.*, **410**, 257 (1915).
(2) Cauquil, G., *Compt. rend.*, **180**, 1207 (1925).
(3) Crowe, R. W., and Smyth, C. P., *J. Am. Chem. Soc.*, **73**, 5406 (1951).
(4) Forziati, A. F., *J. Research Natl. Bur. Standards*, **44**, 373 (1950).
(5) Nitta, I., and Seki, S., *J. Chem. Soc. Japan, Pure Chem. Sect.*, **69**, 141 (1948).
(6) Taylor, W. J., and Rossini, F. D., *J. Research Natl. Bur. Standards*, **32**, 197 (1944).
(7) Timmermans, M. J., and Hennaut-Roland, Mme., *J. chim. phys.*, **34**, 693 (1937).
(8) Washburn, E. W., Ed., "International Critical Tables," Vol. VII, p. 38, New York, McGraw-Hill Book Co., 1929.
(9) Wilson, H. N., and Heron, A. E., *J. Soc. Chem. Ind.* (*London*), **60**, 168 (1941).

RECEIVED for review July 25, 1952. Accepted September 10, 1952. From a thesis submitted by F. S. Jones to the Graduate School of the University of New Hampshire in partial fulfillment of the requirements for the degree of master of science.

Simple Robot Drive for Craig Countercurrent Distribution Apparatus

FREDERICK C. HICKEY, O.P.
Medical Research Laboratory, Providence College, Providence, R. I.

THE great practicality of the Craig countercurrent distribution apparatus for the separation of small quantities of difficultly resolvable substances makes it a desirable addition to any synthetic, biochemical, or organic analytical laboratory. The original apparatus (*1*) was admittedly designed to contain the largest number of tubes which could be handled conveniently by one experienced operator. Obviously, smaller and more simplified versions are practical. They can now be purchased (H. O. Post Scientific Instrument Co., 6822 60th Road, Maspeth, N. Y.).

While completely manual operation is undoubtedly tedious, there is little advantage in partially automatic operation. If the operator must be on hand, for example, to add fresh solvent at each cycle, every 2 or 4 minutes, he might as well supply the other operations in the interval. Since entirely manual and completely automatic operation are the practical alternatives, it was thought desirable to construct a simple, inexpensive robot drive which, while lacking the elegance and flexibility of the very refined original or commercial model, could be constructed by anyone with ordinary shop facilities at hand. It contains 20 tubes identical with Craig's original design except that the small filling tube at the top of each cell has been lengthened, as suggested by Craig, to 10 cm. (*1*). With the longer tubes the solutions never reach the stoppers. Hence, corks may be used, in many cases, instead of specially ground glass stoppers.

The tubes are held in holes drilled in a wooden frame, *A*, as shown in Figure 1. The holes are sufficiently large in diameter to accommodate a rubber sleeve between the wood and the glass to prevent fracture with the swelling of the wood. Slits are sawed along the axis of the holes to provide visibility. To the ends of the wooden frame, flanges are secured by screws while the flanges are taper-pinned to the $^{3}/_{4}$-inch horizontal shaft. The latter revolves in pillow blocks bolted to a welded angle iron frame.

The essential motions of the apparatus are: (*a*) rocking of the tubes through angles of 35° either side of the horizontal for 15 complete oscillations (2 minutes) to establish equilibrium (by a change of timing wheels, of course, the number could be varied); (*b*) period of rest, the settling position, with the upper ends of the tubes elevated at the above angle to allow the phases to separate (1 minute); (*c*) elevation of the tubes to the vertical position, the decantation position, for a similar interval while the upper phases drain into the traps. While the tubes are in this position, the fraction collector advances one step. The first declination of the new shaking series is maintained sufficiently long to permit transfer of the upper phases to the next tubes. This, following Craig, is referred to as the transfer position. While the above motions are carried out automatically, provision is also made for the manual inversion of the tubes for draining and cleaning.

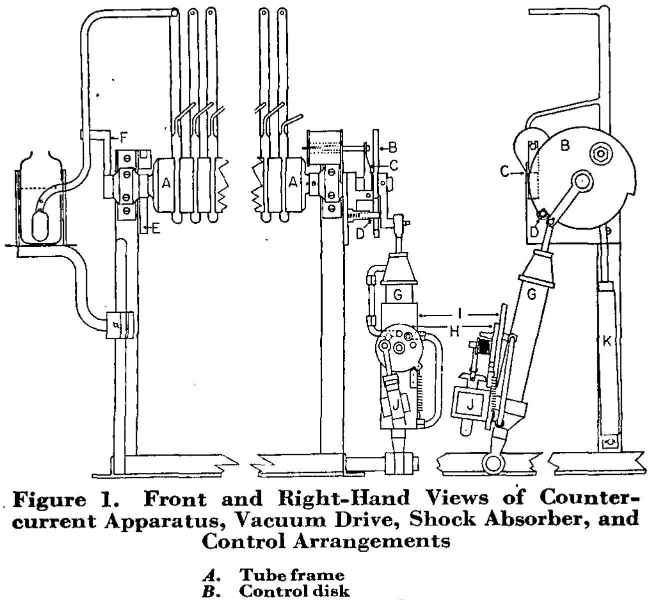


Figure 1. Front and Right-Hand Views of Countercurrent Apparatus, Vacuum Drive, Shock Absorber, and Control Arrangements

A. Tube frame
B. Control disk
C. Solenoid-operated stop
D. Depressible pin
E. Permanent stop
F. Filling tube brace
G. Cylinder
H. Brass valve plate
I. Stainless steel plate
J. Solenoid
K. Shock absorber

In order to provide positive limits to the various motions, a 6-inch circular disk of steel, *B*, from whose rim a 53° segment $^{1}/_{2}$-inch deep was removed, is fastened to the shaft by a taper pin. On the rising stroke, the edge at one end of this segment meets the solenoid-operated stop, *C*. This stop is operated by a direct current solenoid consisting of 1 pound of No. 24 enameled copper wire wound on a brass spool which is bolted to the frame. Its iron plunger is held in the out position by a light spring. When 60 volts direct current are applied, the plunger is drawn inward, withdrawing the stop from contact with the control disk. An alternating current solenoid could be employed and the need for direct current would be eliminated. On the lowering stroke, the opposite end of the segment meets the 0.25-inch depressible pin, *D*. The latter may be pushed in to allow the tubes to be inverted for draining and cleaning. The same stop limits the rising motion at the vertical, or decantation position, when the solenoid stop is withdrawn as shown in Figure 1. This disk is also fitted with the knuckle pin for the shock absorber.

At the opposite end of the shaft, the inner collar has a short length of 0.5-inch rod, *E*, brazed to its edge which, in contacting a projection from the frame, acts as a permanent stop to prevent the tubes from passing the vertical on their downward motion and eliminates the danger of breaking the filling tube against the frame. The outer collar has a similar projection, *F*, which acts as a brace for the filling tube and dipper. These collars are likewise held in position by taper pins.

DRIVING MECHANISM

To eliminate danger from fire and to provide the simplest mechanism, a vacuum drive is used. An Alemite grease gun [No. 6290-C (now obsolete, succeeded by Model No. 7510)], *G*, provides a substantial cylinder, a virtually leakproof piston, and very efficient packing around the piston rod. The unneeded interior parts are eliminated and the piston is secured directly to the piston rod. The latter is shortened, threaded, and provided with a crosshead which is connected to the shaft by means of a crank. A setscrew fastens the crank to the shaft and allows proper positioning. Upper and lower castings of the cylinder are drilled, threaded, and provided with $^{1}/_{8}$-inch elbows for the attachment of the copper tubing from the valve assembly described below. A 0.75-inch collar brazed to the lower casting and accepting a short shaft projecting from the frame, provides the pivot mounting of the cylinder as is shown from Figure 1. To the lower casting is brazed the base plate of the valve assembly.

VALVE ASSEMBLY

The valve assembly consists essentially of a circular brass disk, *H*, rotating about a central pin projecting from a stainless steel plate, *I*. The latter has two $^5/_{16}$-inch holes, $^1/_2$ inch apart, and at a common radius of $^7/_8$ inch from the center pin. These are connected by elbows and copper tubing, the left-hand hole to the top of the cylinder, the right-hand to the bottom. The close spacing required to reduce, as far as possible, the length of pull by the solenoid, necessitates silver soldering of the elbows to the base plate since there is insufficient space for rotation. The brass disk, which is ground to make a leakproof contact with the steel plate, contains three holes spaced so that in one position, the left and center holes coincide with those of the lower plate; in the second position, the center and right holes so coincide. The central hole of the upper plate is provided with a hose nipple for connection, by means of rubber tubing, to the vacuum pump. This plate is also provided with a knuckle pin for connection to the actuating solenoid and is held in firm contact with the base plate by a spring mounted on the central pin. When the return spring brings the disk to its stop, as is shown in Figure 1, vacuum is applied to the right-hand hole of the lower plate, thence to the lower end of the cylinder, while atmospheric pressure is admitted through the left-hand holes of upper and lower plate to the top of the cylinder. When the solenoid is actuated to its full extent, rotating the disk as far as possible in the opposite direction, the vacuum is applied to the left-hand hole of the lower plate and to the top of the cylinder while atmospheric pressure is admitted through the right-hand holes to the lower end of the cylinder. The solenoid used for the valve assembly, *J*, is a Philtrol No. 51C11560A, 110 alternating current model (Phillips Control Corp., Joliet, Ill.). A Pressovac pump is used to supply the vacuum.

SHOCK ABSORBER

To eliminate excessive mechanical shock in the various motions, an oil and spring shock absorber, *K*, is required. It consists of a brass pipe cylinder fitted, by lapping, with a steel piston and rod. Both piston and rod are drilled through their longitudinal axis to connect with a hole drilled transversely through the piston rod above the piston. The lower end of the piston is drilled and tapped to accept a $^1/_8$-inch brass plug through which a small hole (No. 57) is drilled to control the flow of oil. The cylinder is filled with No. 20 lubricating oil. On the downward stroke, the oil is forced through the channel in plug and piston upward. On the upward stroke, the oil is drawn through the same channel downward. On the piston rod, above the piston, is placed a spring 5 inches long, made of 0.040-inch wire, 4 turns per inch, which assists in absorbing the shock of the apparatus which would otherwise be violent on going from the settling to the decantation position, because the pump has had time to establish a high vacuum in the lower section. This spring also assists the return of the apparatus to the transfer position after decantation. The angles are so large that the driving mechanism is perilously close to dead center in the decantation position and some experimentation is necessary to find the proper position of the driving crank.

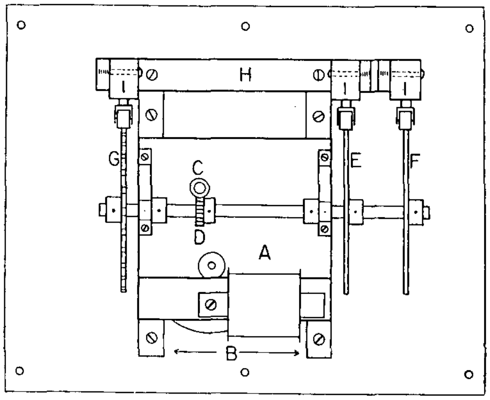


Figure 2. Top View of Timer

A. Timer motor
B. Mounting bars
C, *D*. Worm and gear
E, *F*, *G*. Timer wheels
H. Microswitch supporting bar
I. Microswitches

TIMER

The timer motor, *A*, Figure 2, was obtained from a No. 1001 automatic sign flasher (Automatic Electric Manufacturing Corp., Mankato, Minn.) with a shaft speed of 15 r.p.m.

A more powerful unit would provide a greater margin of safety against stalling but the present unit is satisfactory. After the removal of the relay panel and its switch, the motor is bolted to two 0.5-inch square steel bars, *B*, to provide space for the control wheels and to serve as a means of attachment to the base plate of a radio cabinet. A worm, *C*, is attached to the shaft by means of a short adapter and setscrews. A worm gear, *D*, having 30 teeth, is mounted on a $^1/_4$-inch shaft, 7 inches long, and the latter is attached to the motor frame by means of brass journals cut from $^1/_4$-inch plate. Collars with setscrews (radio shaft couplings cut in half) are used to hold the shaft in position. Brass blanks of $^3/_{32}$-inch plate are silver soldered to $^1/_4$-inch collars provided with set screws, and turned down to $3^3/_8$-inch diameter. Two of the wheels, *E* and *F*, are notched at one point on their periphery with a 25/64-inch router to a maximum depth of $^1/_{16}$ inch. The third wheel, *G*, is provided with 15 notches of the same size equally spaced around one half of its circumference. The first notch of this wheel is elongated approximately 0.25-inch to allow sufficient time for complete transfer of the upper phases from the traps to the equilibration tubes. The notch on the wheel, *E*, controlling the solenoid operated stop, is positioned 90° behind the last notch on the shaking control wheel, *G*. The notch on the fraction collector wheel, *F*, is set sufficiently farther away to prevent two switches from operating at the same time which would be an excessive load for the timer motor. Perpendicular to a 0.25 × 0.75 inch brass bar, *H*, 6.75 inches long, are silver-soldered three pieces of the same stock 2.375 inches long with tapped holes to secure the microswitches. The bar, *H*, is bolted to the steel bars, *B*. The microswitches, *I* (M.X.E.-1 Unimax switch, W. L. Marson Corp., 460 West 34th St., New York 1, N. Y.) are so positioned that the solid part of the timing wheels holds them in the off position—i.e., depressed—while the notches permit them to return to the on position. Final adjustment is made by enlarging the mounting holes of the switches to allow changing their position slightly and by hand filing the notches until all motions are smoothly carried out.

The whole timer is mounted in a steel radio cabinet, 10 × 8 × 7 inches in size, to protect it from dust. The front panel is provided with two plugs for the alternating and direct current input (differently polarized plugs are used to separate the two circuits) and three receptacles to provide one direct and two alternating current outlets. This panel also contains a double pole, single throw switch, one side of which is used to break the alternating current circuit and the other the direct current. Across the terminals of the direct current microswitch a 1-microfarad paper condenser in series with a 10-ohm resistor is connected, and across the direct current output terminals, a 40-microfarad electrolytic condenser is introduced to minimize arcing at the switch terminals. The timer, which contains the only source of electrical sparks, can be removed as far as necessary from the apparatus to eliminate danger of fire.

FRACTION COLLECTOR

While fraction collectors are described in the literature (*2*) and are available commercially, the unit required for this application could be simple. A 10.5-inch length of 0.5-inch steel shaft, *A*, Figure 3, is turned down to $^3/_8$-inch diameter for 3.625 inches

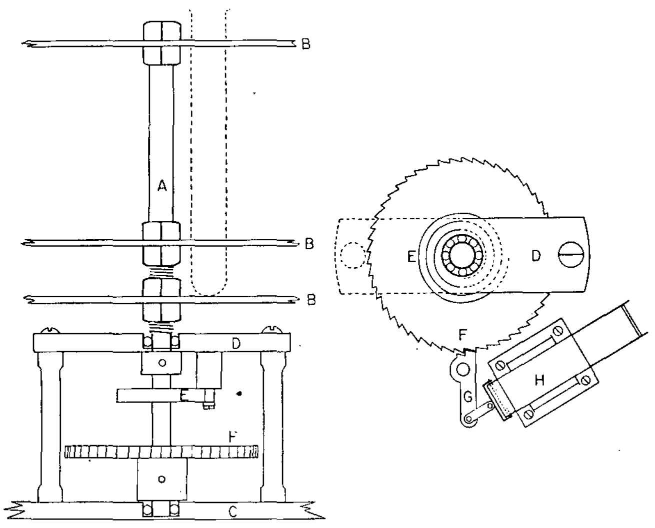


Figure 3. Front and Horizontal Sectional Views of Fraction Collector

A. **Main shaft**
B. **Test tube supporting disks**
C. **Base plate**
D. **Bearing support**
E. **Driving spring**
F. **Escapement wheel**
G. **Ratchet**
H. **Solenoid**

from one end. The 0.5-inch diameter section is threaded for a length of 1.25 inches at the end and for 2.25 inches above the shoulder. Beginning at a point 3.125 inches from the smaller end, two grooves $^1/_8$-inch apart, $^1/_8$-inch wide, and $^1/_{16}$-inch deep are cut. The remaining ridge between the grooves is filed away so as to leave only $^3/_{16}$ inch. This projection is so undercut, with a small file, as to grip securely the clock spring mentioned below.

Three circular disks, *B*, of 9.375 inches radius are cut from standard 19.25 × 19.125 × 0.125 inch aluminum radio panels. A 0.5-inch hole is drilled through the center of each disk. Around the edges of two of the plates are punched 50 holes 0.75 inch in diameter, on a radius of 8.75 inches center to center. These disks, the perforated ones on top, are held to the shaft between nuts.

The base plate, *C*, 8 inches square, with rounded corners, and the bearing support, *D*, are cut from $^3/_8$-inch steel. In the lathe, the cups to accept ball bearing sets for $^3/_8$-inch shaft are cut and 13/32-inch holes are drilled completely through the plates. The bearing support, *D*, is held rigidly above the base plate by pillars cut from $^1/_2$-inch shaft. Tapped holes in each end allow the pillars to be bolted directly between the two plates. The shaft is inserted through the bearings and secured by a collar and setscrew below the bearing support.

The driving force is obtained from an alarm clock spring, *E*, attached, as explained above, to the shaft and secured to a steel pedestal bolted to the bearing support. The stainless steel escapement wheel, *F*, is turned to 3.875-inch diameter and 0.25-inch thickness. Fifty teeth of the shape shown in the figure are milled in its edge. It is attached to the shaft by a taper pin through its hub. A ratchet, *G*, pivoted on a pedestal bolted to the base plate, allows one notch of the escapement wheel to pass each time the solenoid functions. The inertia of the moving assembly driven by the spring returns the solenoid, *H*, to its out position while the ratchet halts the motion as a fresh test tube is centered under the delivery funnel (not shown in Figure 1). Sufficient vertical length is allowed in the ratchet pivot and the link to the solenoid to permit the ratchet to be lifted above the escapement wheel for rewinding, which is accomplished by merely rotating the test tube platform in the clockwise direction with the ratchet in the elevated position. A spring normally holds the ratchet in its lower position.

The apparatus has been in use in this laboratory for several months and, within the limits imposed by its small number of tubes, has proved very satisfactory. With proper allowances the same driving and control arrangements could be used with a much larger apparatus.

LITERATURE CITED

(1) Craig, L. C., Hausmann, W., Ahrens, E. H., Jr., and Harfenist, E. J., Anal. Chem., 23, 1236 (1951).
(2) Stein, W. H., and Moore, S., *J. Biol. Chem.*, 176, 337 (1948).

Received for review June 23, 1952. Accepted September 2, 1952. Aided by grant from Damon Runyon Memorial Fund.

Photometric Determination of Microgram Quantities of Palladium with Beta-Furfuraldoxime

EUGENE W. RICE
Department of Biological Chemistry and Nutrition, Creighton University School of Medicine, Omaha, Neb.

IN CONNECTION with a research project involving the analysis of certain biochemical substances, numerous furane derivatives were investigated. Among the compounds examined was $\mathrm{Pd(OCH:CHCH:CCH:NOH)_2Cl_2}$, the addition compound formed by treating palladious chloride with β-furfuraldoxime. The formation of this precipitate has previously been adapted to both a gravimetric (*6*) and an oxidimetric method (*8*).

Preliminary study of this compound showed that it produces a pink color at room temperature in the dark with the *p*-bromoaniline acetate reagent of Roe and Rice (*7*). There was, however, a simultaneous reaction between palladium(II) and the thiourea contained in the original reagent (as an antioxidant). Hence, if the palladium-oxime compound is allowed to react with a *p*-bromoaniline acetate solution not containing thiourea, the pink color was found to form with more precision.

Spectral curves for several palladium(II) concentrations were determined over the wave length range from 450 to 700 mμ. The curves were identical to that previously reported of the color formed by interaction of *p*-bromoaniline reagent with furfural (*7*). The transmittancy minimum at 520 mμ was used for subsequent measurements.

It was the purpose of the present investigation to study the formation of the pink reaction product with a view to its application as a photometric method for the determination of palladium.

SOLUTIONS AND APPARATUS

Standard Palladium Solutions. Palladium chloride, 0.15 gram, was dissolved in 500 ml. of 3% (v./v.) hydrochloric acid. This solution, standardized gravimetrically with β-furfuraldoxime (*6*), contained 0.184 mg. of palladium per ml. Suitable concentrations were prepared by diluting this stock standard with 3% hydrochloric acid.

β-*syn*-Furfuraldoxime Reagent. The product (Eastman Kodak Co., No. 2907) was used after one crystallization from 70% ethyl alcohol. A 10.0% (w./v.) solution of the reagent is prepared shortly before use by dissolving a suitable quantity of the purified oxime in 95% ethyl alcohol.

Wash Solutions. Hydrochloric acid, 1% (v./v.); distilled water. Both of the solutions are kept in a refrigerator and used cold.

Tween-20. (Polyoxyalkylene ether of partial lauric acid ester.) Atlas Powder Co., Wilmington, Del.

Glacial Acetic Acid. Reagent grade chemical.

***p*-Bromoaniline Reagent.** Two grams of *p*-bromoaniline (Eastman Kodak Co. No. 473) are dissolved in enough 80.0% (v./v.) acetic acid to make 100.0 ml. of solution. The reagent, stored in a glass-stoppered brown bottle and kept in a refrigerator, should be prepared fresh every 3 or 4 days.

The spectral-transmittancy curves were determined with a Coleman Model 11 Universal spectrophotometer. Transmittancy measurements were made with an Evelyn photoelectric colorimeter, using filter 520.

EXPERIMENTAL

Based upon a study of the effect of variables, the following standardized procedure was developed:

Precipitation of Pd(II) with β-Furfuraldoxime. The procedure of Hayes and Chandlee (*6*) was adapted as follows:

Pipet a 1.00-ml. aliquot of the unknown palladium solution containing about 12 to 60 p.p.m. of palladium(II) into a 15-ml. conical graduated centrifuge tube. Adjust the acidity to 3 to 10% (v./v.) hydrochloric acid. Precipitate the palladium by adding 0.02 ml. of the alcoholic solution of the oxime from a serological micropipet. Mix the solutions thoroughly by careful rotation of the tube and allow to stand at room temperature for 30 minutes, or longer, to ensure complete precipitation. Add, with mixing, a small drop of Tween-20 to prevent creeping of the precipitate, and finally centrifuge at high speed for 10 minutes. Do not use an angle-head centrifuge.

Using a fine capillary and gentle suction, aspirate and discard the supernatant fluid, taking care not to disturb the precipitate. Do not attempt to decant the liquid. Pipet approximately 1 ml. of cold 1% hydrochloric acid down the sides of the tube, add a small drop of Tween-20, and mix the contents by twirling the tube carefully. Centrifuge at high speed for 5 minutes, remove the supernatant liquid, wash the precipitate again with Tween-20 and cold 1% hydrochloric acid, and discard the fluid. Finally, add another small drop of Tween-20 and wash once with cold distilled water in the above way. After the last centrifugation, carefully remove as much of the supernatant wash water as possible. The precipitate is now ready for colorimetric analysis. If necessary, the procedure may be interrupted at this point, by storing the unstoppered centrifuge tubes in a desiccator until the analysis is resumed.

Development of Color. Dissolve the palladium-oxime precipitate by adding to the centrifuge tube a definite volume (2.00 to 15.00 ml.) of glacial acetic acid. The volume of acid added is such that the final solution contains an expected concentration of 2.4 to 6.0 micrograms of palladium per ml. Shake the tube vigorously to ensure complete dissolution of the precipitate. A standard palladium solution (containing about 45 p.p.m.) is precipitated, the complex is dissolved in 10.00 ml. of glacial acetic acid, and a 1.00-ml. aliquot is analyzed concurrently with each set of unknowns.

Transfer a 1.00-ml. aliquot of each solution to a cuvet. Add exactly 5 ml. of the *p*-bromoaniline reagent and immediately mix by lateral shaking. Place the tubes at once in a water bath at 70° C. for exactly 5 minutes and then immerse in cold water for 2 or 3 minutes. After standing for 55 minutes at room temperature in the dark, determine the optical densities with the Evelyn colorimeter, using a 520 filter and setting the instrument to zero density with a blank tube containing similarly glacial acetic acid and reagent. Calculate the total amount of palladium present in the original sample by means of the customary formula of spectrophotometry.

EFFECT OF VARIABLES

Composition of Reagent. Optimum color intensity results with a reagent environment similar to that previously developed for the photometric analysis of aqueous furfural solutions (*7*). A final concentration of 83% acetic acid is obtained by adding 5 ml. of an 80% acetic acid reagent to 1 ml. of glacial acetic acid. As in the analysis of furfural (*7*) the reagent contains *p*-bromoaniline in a concentration of 2%.

Time of Color Development. The color reaches a maximum in approximately 50 minutes and remains constant for about 25 minutes, then fades slowly. If the reaction mixture is not heated, the color intensity increases slowly for about 135 minutes and then begins to fade approximately 30 minutes later.

Palladium Concentration. Attempts to precipitate less than 12 micrograms of palladium gave erratic results even when a 24-hour precipitation period was employed. On the other hand, quantities of palladium greater than about 60 micrograms produced an amount of precipitate that did not pack well upon centrifugation.

Figure 1 gives standard series data plotted as per cent absorptancy (100 − per cent transmittancy) against the logarithm of concentration (*1*). The system conforms to Beer's law for 2.4 to 6.0 p.p.m. of palladium. The range and accuracy of the method are also shown in Figure 1. The concentration range for optimum accuracy for the conditions and technique used, is 2.4 to 6.0 p.p.m. In this range the relative analysis error is about 2.8% per 1% absolute error in transmittancy. For a precision of 0.25% absolute transmittancy, the relative error is about 0.7% in the concentration range cited above.

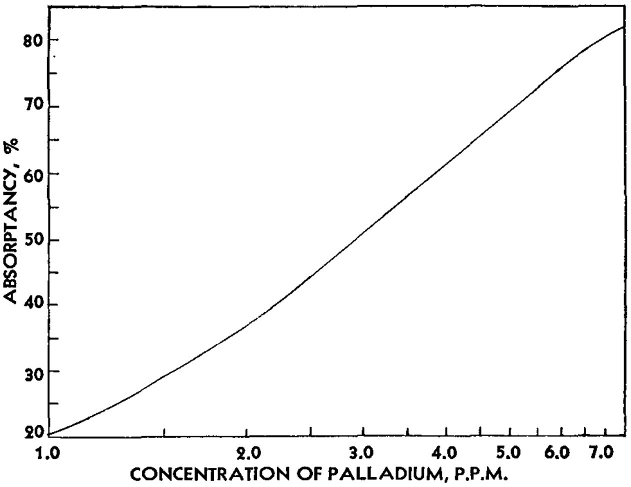


Figure 1. Range and Accuracy

Effect of Diverse Ions (*6*). Excess bromide and/or iodide interferes with the formation of the palladium-oxime compound. This is presumably due to the existence of palladium-bromide (*2*) or palladium-iodide (*5*) complexes. Cerium(IV) yields a color-producing oxime with the reagent and hence also interferes (*6*). Gold(III) is partially reduced to metallic gold by the reagent. However, the presence of gold(III) in the original solution, although interfering with the gravimetric determination (*6*), does not interfere with the proposed photometric method. However, before the solution containing the palladium-oxime is analyzed, it should be filtered to remove the fine suspension of gold particles. Moreover, in contrast to the gravimetric method (*6*), the present procedure is valid in the presence of silver(I), mercury(II), and lead(II), if the insoluble chlorides are removed by filtration.

DISCUSSION

A solution of β-furfuraldoxime alone was found to give the same pink color with the *p*-bromoaniline reagent as does the palladium-oxime compound. Hence, it is necessary to wash the precipitate entirely free of the initial precipitating medium.

Solutions of the palladium-oxime compound produce a constant color intensity for at least 24 hours; hence it appears that the compound is stable in acetic acid.

If palladious chloride solutions are treated with the geometrically isomeric α-*anti*-furfuraldoxime (*3*), the solution immediately turns a darker yellow, followed within 5 minutes by the formation of a typical light yellow precipitate. This precipitate produces the same intensity of color with the *p*-bromoaniline reagent as an equal quantity of the compound formed with the β-oxime.

This procedure for the photometric determination of palladium could be readily applied to the toxicological estimation of carbon monoxide in air (*4*) and in blood (*5*).

ACKNOWLEDGMENT

This work was supported by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

LITERATURE CITED

(1) Ayres, G. H., Anal. Chem., 21, 652 (1949).
(2) Ayres, G. H., and Tuffly, B. L., *Ibid.*, 24, 949 (1952).
(3) Brady, O. L., and Goldstein, R. F., *J. Chem. Soc. (London)*, **1927**, 1960.
(4) Christman, A. A., Block, W. D., and Schultz, J., Ind. Eng. Chem., Anal. Ed., **9**, 153 (1937).
(5) Christman, A. A., and Randall, E. L., *J. Biol. Chem.*, **102**, 595 (1933).
(6) Hayes, J. R., and Chandlee, G. C., Ind. Eng. Chem., Anal. Ed., **14**, 491 (1942).
(7) Roe, J. H., and Rice, E. W., *J. Biol. Chem.*, **173**, 507 (1948).
(8) Syrokomskiĭ, V. S., and Gubel'bank, S. M., *Zhur. Anal. Khim.*, **4**, 203 (1949).

Received for review June 30, 1952. Accepted July 28, 1952.

Estimation of Sodium Triphosphate

Improvement in Zinc Titration Method

RUSSELL N. BELL, A. R. WREATH, AND WILLIAM T. CURLESS
Research Laboratories, Victor Chemical Works, Chicago Heights, Ill.

SINCE the publication of the Bell method (*1*) the commercial production of sodium triphosphate has increased tremendously and with it the quality of the product. Formerly the commercial material often contained only 75 to 85% sodium triphosphate, the remainder consisting largely of tetrasodium pyrophosphate with occasional small percentages of ortho- and metaphosphates. Improvements in production methods have led to commercial products which now usually assay over 90% sodium triphosphate. Because of these recent advances, a more highly refined method for the estimation of sodium triphosphate became desirable.

The Bell method for the estimation of triphosphoric and pyrophosphoric acids in the presence of ortho- and metaphosphoric acids consists of the following steps:

1. Titration of the sulfuric acid liberated when a solution of zinc sulfate is added to a solution of the mixture adjusted to pH 3.8.
2. Gravimetric determination of the pyrophosphate precipitated as the zinc salt. Excess of liberated acid over that required by the pyrophosphate is calculated as triphosphate using an empirical factor.

$$H_2P_2O_7^{--} + 2Zn^{++} \longrightarrow Zn_2P_2O_7\downarrow + 2H^+$$

$$H_2P_3O_{10}^{---} + Zn^{++} \rightleftharpoons ZnP_3O_{10}^{---} + 2H^+$$

The present work was undertaken to determine the effect of certain variables on the method in an effort to increase the accuracy when applied to present-day commercial sodium triphosphate.

PREPARATION OF PHOSPHATES

Tetrasodium Pyrophosphate. The sodium pyrophosphate ($Na_4P_2O_7$) was recrystallized commercial material dried at 400° C.

Sodium Triphosphate Hexahydrate. The sodium triphosphate ($Na_5P_3O_{10}.6H_2O$) was prepared from commercial anhydrous sodium triphosphate by two methods.

1. A 14% aqueous solution of sodium triphosphate was prepared and filtered. Alcohol was added to the filtrate until the first permanent precipitate appeared. The slurry was allowed to stand until all of the triphosphate had precipitated. The crystalline product was filtered and washed first with 50% alcohol, then with 95% alcohol, after which it was air-dried.

Table I. Analysis of Phosphates

	$Na_4P_2O_7$		$Na_5P_3O_{10}$		
	Calcd., %	Found, %	Calcd., %	Found, % Method 1	Found, % Method 2
P_2O_5, %	53.4	53.4	44.7	44.2	44.7
Loss on ignition, %	0.0	0.0	22.7	23.2	23.0

2. A 25% solution of commercial sodium triphosphate, type II, was prepared and treated with a saturated sodium chloride solution. The amount of sodium chloride solution added was equivalent to 45% by weight of the sodium triphosphate solution. The sodium triphosphate hexahydrate which precipitated upon standing overnight was filtered and washed thoroughly with cold water. The product was air-dried.

Table I contains the results of the analysis of the tetrasodium pyrophosphate and the sodium triphosphate hexahydrate prepared by the methods described above.

DISCUSSION AND DATA

Variations in the pyrophosphate content of sodium triphosphate–sodium pyrophosphate mixtures have a marked effect on the results of the determination as shown in Table II. Between 15 and 20% sodium pyrophosphate must be present to assure complete precipitation of the pyrophosphate in the presence of sodium triphosphate. If a sample contains more than 25% sodium pyrophosphate, the amount of pyrophosphate apparently recovered is substantially greater than the actual amount present. This is possibly due to occluded sodium dizinc triphosphate.

The temperature was maintained at 25° ± 1° C. throughout each of the titrations. The temperature was found to affect both the titration value and the amount of zinc pyrophosphate precipitate. A temperature of 25° C. was found to give the most accurate results. Cale (*2*) also found that temperature affects the titration of triphosphate-pyrophosphate mixtures evidently to a much smaller degree than it does with this method.

The empirical factor for the calculation of sodium triphosphate as given by Bell (*1*) gives high values when applied to the conditions now used for the determination. Calculations of triphosphate and pyrophosphate content are interdependent; the per cent triphosphate found is high when the per cent of pyrophosphate found by precipitation is less than the amount actually present. A change in the triphosphate factor from 0.0258 to 0.0252 serves to correct the difficulty, as shown from the data presented in Table II.

The effect of raising the pH of the triphosphate-pyrophosphate solutions from pH 3.8 to pH 4.5 before addition of the zinc solution was also investigated and was found to aid in the precipitation of the pyrophosphate. However, zinc hydroxide tends to precipitate excessively during the titration at the higher pH and is difficult to redissolve. Sodium dizinc triphosphate also tends to precipitate prematurely. Adjustment of the solution to be analyzed to pH 3.8 is therefore recommended.

METHOD

Apparatus. Glass electrode pH meter with outside titration assembly and mechanical agitator.

Table II.[a] **Effect of Variations in Pyrophosphate Content**

Composition		Extra $Na_4P_2O_7$ added from Standard Solution, Gram	Found		
			$Na_5P_3O_{10}$, %		
$Na_5P_3O_{10}$, %	$Na_4P_2O_7$, %		Old factor (0.0258)	New factor (0.0252)	$Na_4P_2O_7$, %
70.0	30	None	68.4	66.8	31.4
75.0	25	None	76.0	74.3	25.0
80.0	20	None	82.4	80.6	19.2
85.0	15	None	87.4	85.3	13.9
90.0	10	None	97.4	96.1	4.9
80.0	20	0.0750	74.5	73.3	24.2[b]
85.0	15	0.0750	81.3	79.5	17.6[b]
90.0	10	0.0750	90.3	88.3	10.8[b]
95.0	5	0.0750	96.2	94.5	5.4[b]
97.0	3	0.0750	98.5	96.4	3.0[b]
100.0	0	0.0750	..	..	−2.6[c]

[a] All percentages and calculations based on anhydrous $Na_5P_3O_{10}$ and anhydrous $Na_4P_2O_7$. Each determination is average of two or three determinations.

[b] Corrected for added $Na_4P_2O_7$.

[c] Less $Na_4P_2O_7$ was recovered than originally added.

Reagents. Sodium hydroxide, 0.1 *N*. Hydrochloric acid, 0.2 *N*. Zinc sulfate, 12.5% solution of zinc sulfate heptahydrate adjusted to pH 3.8.

Bromophenol blue indicator, 0.04% solution.

Standard tetrasodium pyrophosphate solution, 1.33 grams of sodium pyrophosphate per 100 ml. of solution.

Procedure. Weigh accurately 0.5 gram of sample and dissolve in 50 ml. of water. Place the sample on the glass electrode assembly, previously rinsed with ca. 1 *N* hydrochloric acid and adjust to ca. pH 3.8 with 0.2 *N* hydrochloric acid. Four drops of the bromophenol blue reagent may be used if desired. Dilute the sample to 100 ± 2 ml. with water and make a final adjustment to exactly pH 3.8 with 0.2 *N* hydrochloric acid or 0.1 *N* sodium hydroxide. The temperature of the solution should be maintained at 25° ± 1° C. throughout the entire titration. Add 70 ml. of zinc sulfate solution at 25° ± 1° C. and allow the solution to agitate 1 to 2 minutes. Titrate to a pH of 3.8 with 0.1 *N* sodium hydroxide.

The entire titration should be carried out slowly. When a pH of 3.7 is reached, stop the addition of sodium hydroxide and stir the solution for 2 minutes to allow equilibrium to be established. Continue the titration to pH 3.8 by adding small increments of sodium hydroxide. At least 30 seconds should elapse between additions. When it is believed that the end point has been reached, continue agitation for another 2 minutes.

If the value of the titration equals 23 ml. ± 1 ml. of 0.1 *N* sodium hydroxide, the determination may be continued. (A small amount—e.g., 1 to 2%—of either a meta or an orthophosphate may be present without interfering appreciably with the results. However, larger amounts will result in a lower titration value due to dilution of the sample with these nontitrating substances.) If the titration is less than 22 ml. of 0.1 *N* sodium hydroxide, it is to be expected that all of the pyrophosphate has not been titrated and therefore has not precipitated. It is then necessary to repeat the titration, adding a known amount of standard pyrophosphate solution such that the total titration value will lie between 24 and 28 ml. of sodium hydroxide (0.0133 gram of sodium pyrophosphate equals 1 ml. of standard base). If the amount of liberated acid requires more than 28 ml. of 0.1 *N* sodium hydroxide the pyrophosphate, when determined gravimetrically, will be high and the triphosphate result correspondingly low.

After the titration as described in the paragraph directly above has been completed, wash the titration assembly off into the beaker containing the precipitate and allow the precipitate to settle for 30 to 60 minutes. Filter, using a rapid paper such as S. and S. 604, and wash the precipitate three times with water at room temperature. Then reprecipitate the zinc pyrophosphate as follows:

Puncture the filter paper and wash the precipitate into a clean 400-ml. beaker. Any adhering precipitate may be dissolved by washing with 10 ml. of 0.2 *N* hydrochloric acid. Dilute to approximately 250-ml. volume and add sufficient 0.2 *N* hydrochloric acid to dissolve all the precipitate. Add 25 ml. of the zinc sulfate reagent and adjust the pH to exactly 3.8 with 0.1 *N* sodium hydroxide. The pH should be brought back to 3.8 as quickly as possible after dissolving the precipitate to prevent hydrolysis. Allow the precipitate to settle and filter on S. and S. 604 or similar, fast ashless paper. Wash well with water at room temperature and ignite the precipitate at 500° to 600° C. until the residue is gray; then at 900° C. until it is light gray or white. Weigh as $Zn_2P_2O_7$. A blank should be run containing no triphosphate and only the amount of pyrophosphate added to the sample.

Most of the steps in the above procedure are critical. It is therefore necessary to follow each step exactly if accuracy is to be achieved.

Calculation.

Corrected weight of ppt. = total weight of ppt. − weight of blank ppt.

Corrected titration = total titration − blank titration

$$\frac{\text{Corrected weight of ppt.} \times 0.872 \times 100}{\text{Weight of sample}} = \%\ Na_4P_2O_7$$

$$\frac{[\text{Corrected titration} - (\text{corrected weight of ppt.} \times 65.5)]\ 0.0252 \times 100}{\text{Weight of sample}} = \%\ Na_5P_3O_{10}$$

ACKNOWLEDGMENT

The authors wish to thank L. F. Audrieth for his helpful suggestions and criticism.

LITERATURE CITED

(1) Bell, R. N., Anal. Chem., 19, 97 (1947).
(2) Cale, W. R., *Can. Chem. Process Inds.*, 32, 741 (1948).

Received for review June 23, 1952. Accepted September 5, 1952.

Identification of Molybdenum and Tungsten Oxides

By X-Ray Powder Patterns

ARNE MAGNÉLI, GEORG ANDERSSON, BIRGITTA BLOMBERG, AND LARS KIHLBORG
Institute of Chemistry, University of Uppsala, Uppsala, Sweden

In a previous investigation carried out at this institute (*7*) the (approximate) compositions and other data were obtained for the molybdenum and tungsten oxides formed by reducing the trioxides with the corresponding metal at about 700° and 1000° C., respectively. The phase analyses were partly performed by means of powder photographs, which, however, in several cases could not be interpreted in detail owing to the very complicated x-ray patterns of these substances. Similar investigations have been made by Glemser and Lutz (*4*) on molybdenum oxides and by Glemser and Sauer (*5*) on tungsten oxides. The results of these authors are essentially in agreement with those obtained here as regards the number and approximate compositions of the existing phases. Further studies carried out at this institute by means of single-crystal methods have revealed the crystal structures of these compounds and in this way also made it possible to determine the accurate compositions of the complicated intermediate oxides (*8*, *9*, *11–13*). [For a survey of the results of these investigations see (*14*).] The existence of the following phases was thus established.

Molybdenum oxides: MoO_2, Mo_4O_{11}, Mo_8O_{23}, Mo_9O_{26}, and MoO_3
Tungsten oxides: WO_2, $W_{18}O_{49}$, $W_{20}O_{58}$, and WO_3

The considerable technical importance of the molybdenum and tungsten oxides has caused the authors to investigate whether the x-ray powder method might offer a convenient way to identify these substances.

PREPARATION OF OXIDES

The oxides were generally prepared by heating weighed mixtures of trioxide and metal in evacuated, sealed-off silica tubes for a few days. The starting materials were of purissimum or reagent grade. The control of the heating temperature had to be fairly accurate for the samples of intermediate molybdenum oxides. Molybdenum dioxide was also prepared by cathodic reduction of a melt of potassium polymolybdate.

X-RAY INVESTIGATION

The practical experience gained when carrying out the phase analyses suggested that the successful application of the powder method for identification purposes must presuppose the use of an x-ray camera allowing low angle reflections to be registered and giving very good resolution of the lines and also a low background. These conditions are fulfilled by the camera used in these experiments—viz., a Guinier camera built at this institute by Hägg and provided with a cut and bent quartz monochromator, which focuses the reflections transmitted through a thin sample of the crystal powder on the film of a cylindrical film holder of 80-mm. diameter. Reflections within the diffraction range $3.0° < \Theta < 45.3°$ could be registered, the lower limit corresponding to a "cut-off" of 14.7 A. for Cu-K α radiation ($\lambda = 1.5418$ A.), which was used throughout this investigation.

Table I. X-Ray Diffraction Data on Molybdenum Oxides

MoO_2

d, A.	I/I_1	h k l
4.78	0.20	001
3.41	1.00	110,$11\bar{1}$
2.804	0.30	$20\bar{1}$
2.433	0.50	200
2.420	0.85	020,111
2.405	0.40	$20\bar{2}$
2.398	0.50	002
2.176	0.30	210
2.171	0.10	$12\bar{1}$
2.147	0.30	012
1.833	0.35	201,$22\bar{1}$
1.718	0.55	$31\bar{1}$,220
1.704	0.80	$22\bar{2}$,112,022
1.692	0.50	$11\bar{3}$

Mo_4O_{11}

d, A.	I/I_1	h k l
12.3	0.30	200
5.19	0.10	301
4.53	0.25	401
4.24	0.45	011
4.00	1.00	211
3.95	0.65	501
3.75	0.95	311
3.48	0.85	411,601
3.33	0.10	102
3.26	0.20	610
3.11	0.15	302
2.787	0.50	212
2.771	0.15	502
2.722	0.60	020
2.700	0.45	312
2.663	0.70	810,220
2.593	0.30	602
2.473	0.10	221,512
2.423	0.10	702
2.241	0.20	521
2.225	0.30	103,1010
2.207	0.15	203
2.146	0.30	621

MoO_3

d, A.	I/I_1	h k l
7.0	0.15	020
3.82	0.85	110
3.47	0.20	040
3.44	0.40	120
3.27	1.00	021
3.01	0.10	130
2.707	0.40	101
2.657	0.70	111
2.613	0.05	140
2.528	0.15	041
2.377	0.30	131
2.312	0.20	060
2.277	0.25	150
2.135	0.25	141
1.982	0.40	200
1.965	0.50	210,061
1.907	0.05	220
1.852	0.80	002
1.822	0.35	230
1.788	0.20	022
1.754	0.05	161
1.735	0.65	080,211
1.720	0.05	240
1.694	0.40	221
1.666	0.70	112
1.630	0.40	042,122

Mo_8O_{23}

d, A.	I/I_1	h k l
8.1	0.10	200
6.15	0.30	201
4.47	0.60	202
4.22	0.20	$40\bar{1}$
4.05	1.00	400,010
3.96	0.60	$40\bar{2}$
3.47	0.70	$40\bar{3}$,$11\bar{2}$
3.42	0.90	203
3.32	0.10	$20\bar{4}$,211
3.26	0.25	$31\bar{1}$,310
3.11	0.15	$31\bar{2}$
3.04	0.10	311
3.01	0.40	$11\bar{3}$,212
2.836	0.35	$41\bar{2}$
2.806	0.10	113
2.704	0.60	312,600
2.674	0.40	$20\bar{5}$
2.638	0.25	$41\bar{3}$
2.611	0.45	213

Mo_9O_{26}

d, A.	I/I_1	h k l
8.4	0.10	200
7.5	0.15	$20\bar{1}$
7.0	0.20	201
5.75	0.35	$20\bar{2}$
4.37	0.60	$20\bar{3}$
4.18	0.20	400
4.11	0.10	$40\bar{1}$
4.03	0.90	010
3.90	0.70	401
3.79	0.55	111,$40\bar{2}$
3.56	0.10	$21\bar{1}$
3.47	0.65	211,402,$11\bar{2}$
3.44	1.00	$20\bar{4}$
3.41	0.35	112
3.26	0.70	310
3.22	0.30	$31\bar{1}$
3.14	0.10	311
3.04	0.10	$31\bar{2}$
3.01	0.35	403,113
2.961	0.20	$21\bar{3}$
2.809	0.40	411
2.689	0.20	114
2.677	0.65	$60\bar{2}$
2.657	0.50	205
2.630	0.30	412
2.614	0.50	$21\bar{4}$

The photographs were evaluated according to the method given by Hägg (*6*). A scale, divided in 0.1 mm. was thus printed on each film before the development and the positions of the diffraction lines on this scale were measured in a projection comparator. In this way, the proper correction for the shrinkage of the film was automatically introduced. The fine primary beam line was used as a reference line. The camera had been calibrated with sodium chloride.

By comparisons with the single-crystal photographs it was possible to interpret the powder patterns in detail and to determine accurate unit cell dimensions of all the investigated oxides. Photographs of samples with compositions corresponding to the two-phase regions between the pure phases showed the lines of the latter at unaltered positions, which indicates that the homogeneity ranges of the oxides are very narrow. This fact highly facilitates the identification of the various phases when appearing in mixtures.

FURTHER OXIDE PHASES

This investigation has been concerned only with molybdenum and tungsten oxides prepared at about 700° and 1000° C., respectively. However, further molybdenum oxides have been found to form at other temperatures, when mixtures of trioxide and metal were heated, and also by thermal decomposition of molybdenum trioxide at a reduced pressure of oxygen (unpublished experiments, further studies in progress). No tungsten oxides except those listed above have been found with certainty. The coexistence of molybdenum and tungsten in a sample may confuse the analyses by the formation of double oxides [(*10*), also unpublished experiments].

OXIDES INVESTIGATED

In the following, a survey is given of the preparation and properties of the oxides investigated. Powder patterns of the molybdenum oxides are listed in Table I and those of the tungsten oxides in Table II.

MoO_2 (*13*). A mixture of molybdenum trioxide and molybdenum (of composition $MoO_{2.00}$) was heated at 700° C. for 40 hours. Brown crystal powder. Dimensions of the monoclinic unit cell:

$a = 5.608 \pm 0.005$ A. $b = 4.842 \pm 0.005$ A.
$c = 5.517 \pm 0.005$ A. $\beta = 119.75° \pm 0.07°$

Mo_4O_{11} (*9*). $MoO_{2.75}$ heated at 700° C. for several days. Reddish-violet crystal powder. Dimensions of the orthorhombic unit cell:

$a = 24.40 \pm 0.02$ A. $b = 5.450 \pm 0.005$ A.
$c = 6.723 \pm 0.005$ A.

Mo_8O_{23} (*8*). $MoO_{2.90}$ heated at 700° C. for 50 hours. [The preparation temperature given in (*7*) is somewhat too low.] Blue-violet crystal aggregates. Dimensions of the monoclinic unit cell:

$a = 16.90 \pm 0.02$ A. $b = 4.055 \pm 0.005$ A.
$c = 13.38 \pm 0.01$ A. $\beta = 73.73° \pm 0.07°$

Mo_9O_{26} (*8*). $MoO_{2.90}$ heated at 750° C. for 50 hours. [The temperature given in (*7*) is somewhat too low.] Blue-violet crystal aggregates. Dimensions of the monoclinic unit cell:

$a = 16.74 \pm 0.02$ A. $b = 4.019 \pm 0.005$ A.
$c = 14.53 \pm 0.02$ A. $\beta = 95.45° \pm 0.05°$

MoO_3 (*2, 3, 15*). Recrystallized commercial preparation. Dimensions of the orthorhombic unit cell:

$a = 3.966 \pm 0.005$ A. $b = 13.88 \pm 0.01$ A.
$c = 3.703 \pm 0.005$ A.

WO_2 (*13*). $WO_{2.00}$ heated at 950° C. for 40 hours. Brown crystal powder. Dimensions of the monoclinic unit cell:

Table II. X-Ray Diffraction Data on Tungsten Oxides

d, A.	I/I_1	*h k l*
	WO_2	
4.78	0.15	001
3.45	1.00	$110,11\bar{1}$
2.828	0.20	$20\bar{1}$
2.446	0.45	020
2.436	0.55	200
2.428	0.20	$20\bar{2}$
2.418	0.80	111
2.393	0.50	002
2.181	0.15	$12\bar{1},210$
2.150	0.10	012
1.847	0.20	$22\bar{1}$
1.827	0.20	201
1.731	0.40	$31\bar{1}$
1.724	0.65	220
1.709	0.45	022
1.698	0.50	$11\bar{3}$
	$W_{18}O_{49}$	
12.9	0.35	001
8.3	0.15	101,200
6.55	0.05	$20\bar{2}$
6.1	0.10	$30\bar{1}$
5.22	0.10	102
4.59	0.15	$10\bar{3}$
4.54	0.10	$40\bar{1}$
4.43	0.20	$40\bar{2},301$
4.37	0.10	$30\bar{3}$
3.78	1.00	010
3.73	0.50	103
3.63	0.55	$50\bar{2},011$
3.48	0.05	302
3.44	0.55	111,210
3.40	0.40	$50\bar{3}$
3.385	0.50	$10\bar{4}$
3.31	0.05	500
3.25	0.15	203
3.21	0.05	$31\bar{1}$
3.17	0.30	004
3.05	0.10	$60\bar{2}$
3.02	0.15	$50\bar{4}$
2.958	0.15	$60\bar{3}$
2.937	0.15	402
2.910	0.40	$501,11\bar{3},41$
2.871	0.05	$41\bar{2},311$
2.800	0.15	$30\bar{5}$
2.759	0.15	600
2.743	0.35	$40\bar{5}$
2.654	0.55	113
2.620	0.50	$51\bar{2}$
2.522	0.50	$51\bar{3},11\bar{4}$
	$W_{20}O_{58}$	
4.28	0.20	105
3.89	0.55	$30\bar{2}$
3.77	1.00	010
3.70	0.55	$30\bar{3}$
3.64	0.60	106
2.821	0.15	115
2.729	0.80	$20\bar{8},403$
2.707	0.45	$31\bar{2}$
2.640	0.50	$31\bar{3}$
2.620	0.60	116
2.211	0.70	$21\bar{8},413$
	WO_3	
3.835	1.00	001
3.76	0.95	020
3.64	1.00	200
3.41	0.05	011
3.34	0.50	120
3.11	0.50	$11\bar{1}$
3.075	0.50	111
2.684	0.75	021
2.661	0.60	$20\bar{1}$
2.617	0.90	201,220
2.528	0.35	$12\bar{1}$
2.509	0.40	121
2.172	0.50	$22\bar{1}$
2.149	0.60	221

$$a = 5.650 \pm 0.005 \text{ A.} \quad b = 4.892 \pm 0.005 \text{ A.}$$
$$c = 5.550 \pm 0.005 \text{ A.} \quad \beta = 120.42° \pm 0.07°$$

$W_{18}O_{49}$ (*11*). $WO_{2.72}$ heated at 1000° C. for 40 hours. Small, reddish-violet crystal needles. Dimensions of the monoclinic unit cell:

$$a = 18.28 \pm 0.02 \text{ A.} \quad b = 3.775 \pm 0.005 \text{ A.}$$
$$c = 13.98 \pm 0.02 \text{ A.} \quad \beta = 115.14° \pm 0.07°$$

$W_{20}O_{58}$ (*12*). [The formula $W_{20}O_{58}$ is preferred to $W_{10}O_{29}$ for structural reasons. Cf. (*12*).] $WO_{2.90}$ heated at 1050° C. for several days. Dark blue, very thin crystal needles. Dimensions of the monoclinic unit cell:

$$a = 12.05 \pm 0.01 \text{ A.} \quad b = 3.767 \pm 0.005 \text{ A.}$$
$$c = 23.59 \pm 0.02 \text{ A.} \quad \beta = 94.72 \pm 0.05°$$

WO_3. According to Braekken (*3*) the symmetry of tungsten trioxide is triclinic but very nearly orthorhombic. Goniometric measurements by Wyart and Foëx (*16*) also showed triclinic symmetry. Recent x-ray investigations on single crystals, carried out at this institute by Andersson (*1*), have confirmed the pseudo-orthorhombic arrangement of the tungsten atoms. Powder photographs of preparations of various origin differed in the sharpness of the reflections. However, they never showed the multiplet structure of the lines required by triclinic symmetry, but were in full agreement with the quadratic form of a monoclinic structure (*1, 10*) with the unit cell dimensions:

$$a = 7.285 \pm 0.003 \text{ A.} \quad b = 7.517 \pm 0.003 \text{ A.}$$
$$c = 3.835 \pm 0.002 \text{ A.} \quad \beta = 90.90° \pm 0.03°$$

ACKNOWLEDGMENT

The authors wish to thank G. Hägg for his kind interest in the investigation and for the facilities put at their disposal. This study forms a part of a research program on oxides and oxide systems supported by the Swedish Natural Science Research Council.

LITERATURE CITED

(1) Andersson, G., *Acta Chem. Scand.* (to be published).
(2) Andersson, G., and Magnéli, A., *Ibid.*, **4**, 793 (1950).
(3) Braekken, H., *Z. Krist.*, **78**, 484 (1931).
(4) Glemser, O., and Lutz, G., *Z. anorg. Chem.*, **263**, 2 (1950).
(5) Glemser, O., and Sauer, H., *Ibid.*, **252**, 144 (1943).
(6) Hägg, G., *Rev. Sci. Instruments*, **18**, 371 (1947).
(7) Hägg, G., and Magnéli, A., *Arkiv Kemi, Mineral. Geol.*, **A19**, No. 2 (1944).
(8) Magnéli, A., *Acta Chem. Scand.*, **2**, 501 (1948).
(9) *Ibid.*, p. 861.
(10) *Ibid.*, **3**, 88 (1949).
(11) Magnéli, A., *Arkiv Kemi*, **1**, 223 (1949).
(12) *Ibid.*, **1**, 513 (1950).
(13) Magnéli, A., *Arkiv Kemi, Mineral. Geol.*, **A24**, No. 2 (1946).
(14) Magnéli, A., *Nova Acta Regiae Soc. Sci. Upsaliensis*, [4] **14**, No. 8 (1950).
(15) Wooster, N., *Z. Krist.*, **80**, 504 (1931).
(16) Wyart, J., and Foëx, M., *Compt. rend.*, **232**, 2459 (1951).

RECEIVED for review May 29, 1952. Accepted September 6, 1952.

Ethylenediamine

A Carbonate-Free Alkali for Carbon Dioxide Absorption

ROBERT W. SWICK, DONALD L. BUCHANAN, AND AKIRA NAKAO
Division of Biological and Medical Research, Argonne National Laboratory, Chicago, Ill.

COMPLETE recovery of purified carbon dioxide from "wet" oxidations, acidified carbonates, and other reactions has been obtained in a vacuum apparatus by the use of a proper sequence of cold traps and heated reaction tubes (*1*). However, in many reactions which yield carbon dioxide, gaseous impurities are evolved which, like carbon dioxide, do not condense in a dry ice trap but only at the temperature of liquid nitrogen. Most of these impurities are best removed by trapping the gas mixture in alkali and subjecting the alkaline carbonate to a vacuum before acidifying to release the carbon dioxide. Despite careful preparation and handling, solutions of alkali usually contain measurable quantities of carbonate.

A simple and effective method of eliminating the undesired blank is to select an alkali that can be separated from its carbonate by distillation. Some amines such as ethanolamine and diethanolamine, which are used industrially for carbon dioxide scrubbing (*3*), are not sufficiently alkaline to prevent the release of some carbon dioxide during distillation. Furthermore, these compounds have relatively low volatility. Ethylenediamine, a more alkaline and more volatile compound, can be readily separated from its carbonate by vacuum distillation.

Figure 1 shows the partial pressure of carbon dioxide (32° C.) which equilibrates with a 20% solution of the base at various stages of neutralization. The least carbonate contamination will result when small quantities of ethylenediamine are distilled from a sizable volume of the commercially available compound.

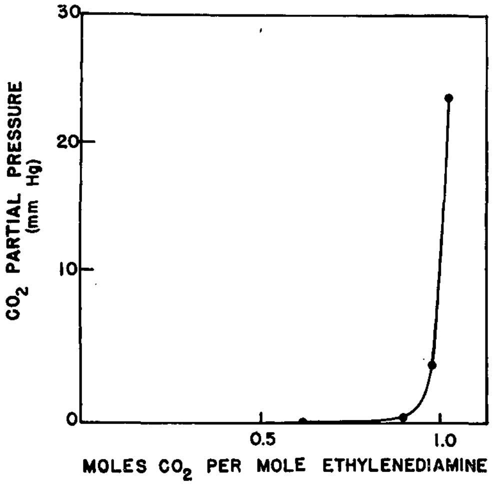


Figure 1. Equilibrium Partial Pressure of Carbon Dioxide in 20% Ethylenediamine in Water at 32° C.

Only one of the two equivalent amino groups can be relied on to bind carbonate firmly, as would be anticipated from the pH-neutralization curve (*2*).

Ethylenediamine (95 to 100%) and water are distilled from reservoirs on the vacuum manifold and frozen into a vessel fitted with a stopcock. After the impure carbon dioxide is also frozen into this vessel (with liquid nitrogen), the stopcock is closed and the mixture warmed to room temperature. Evacuation then removes neutral and alkaline gases. Subsequent acidification with sulfuric acid, followed by heating, releases carbon dioxide quantitatively. With an excess of water, hydrogen chloride, present as a contaminant, is not evolved in measurable quantities, but sulfur dioxide, a weaker acid, is not retained upon acidification. Other acid gases probably behave as these do—retention depending on their acid strength.

The measured blank of vacuum distilled ethylenediamine was 0.001% of its carbon dioxide binding capacity. To test completeness of recovery, 5 purified carbon dioxide samples were measured manometrically and then absorbed in a tenfold excess of a 1 to 4 ethylenediamine-water mixture. After evacuation the samples were acidified with a twofold excess of 50% sulfuric acid, heated to about 80° C., and the evolved carbon dioxide collected and again measured. The mean recovery was 99.8 ± 0.2% (standard error). When the solutions were unheated or first heated and allowed to cool before collecting the carbon dioxide, the recovery was low, usually by 2 to 4%.

It is suggested that ethylenediamine, which is so easily freed of carbonate, might find wide usefulness as an absorbing agent in chemical and radioactive analyses requiring the collection of carbon dioxide.

LITERATURE CITED

(1) Buchanan, D. L., and Nakao, A., *J. Am. Chem. Soc.*, 74, 2389 (1952).
(2) Carbide and Carbon Chemicals Corp., New York, N. Y., "Organic Nitrogen Compounds," July 31, 1946.
(3) Gregory, L. B., and Scharmann, W. G., *Ind. Eng. Chem.*, 29, 514 (1937).

RECEIVED for review July 3, 1952. Accepted September 15, 1952.

Quantitative Determination of Methylol Phenols by Paper Chromatography

J. H. FREEMAN, *Westinghouse Research Laboratories, East Pittsburgh, Pa.*

THE use of paper chromatography to achieve a successful separation and identification of each of the several possible mono- and polymethylol phenols present in a phenol-formaldehyde reaction mixture was described in a preceding paper (*5*). Further application of this method as a quantitative tool is highly desirable. Past attempts to use paper chromatography for quantitative determinations have employed measurement of total or maximum density of the spot (*7, 9*), radioactivity (*8, 10*); or elution of individual spots followed by colorimetric, spectrometric, or chemical determination of the eluate.

Optical methods were considered unsatisfactory for use in determination of the methylol phenols because of the uneven background color produced by the diazonium indicator used and because of further changes in both spot and background color with time. Elution methods were not attempted because the indicator reaction is irreversible and, in some cases, individual compounds were not sufficiently separated to permit blind sectioning of uncolored areas of the strip. For use as a routine analytical tool it was also desirable that a technique be developed which would be rapid and would not require any special apparatus or training.

Fisher and his colleagues (*4*) have shown that there is a linear relationship between the size (or density) of the spot of a substance found on a paper chromatogram and the logarithm of the amount initially applied to the strip. This relationship has been shown to hold for amino acids (*1, 4*) and for sugars (*9*) and is presumably true for any compound-indicator combination yielding a clearly discernible spot. Brimley (*2*) advanced a theoretical explanation for this based on diffusion analogies. The originators of the method have pointed out the further need for consideration of the concepts of partition distribution theory (*3*).

Determination of spot areas by means of a planimeter as originally described by Fisher *et al.* (*4*), was found to work satisfactorily with chromatograms of the methylol phenols if all conditions are adequately controlled. However, the repeated tracing of spot outlines with the instrument is tedious. Equal or perhaps slightly better accuracy may be achieved and the process considerably expedited by simply cutting out the spots with scissors and weighing them individually on the analytical balance. The weight of the paper comprising the spot may serve as a measure of spot area but it is also more directly related to concentration than is the corresponding area itself. When spot weight is plotted against the logarithm of the concentration of component which produced it, a straight line results. Unknown concentrations may then be directly obtained from the determined weight of their corresponding spots.

A comparison of areas and weights of several pairs of spots representing duplicate concentrations of substance run on the same paper strip is given in Table I. The per cent variation in weight is seldom found to be greater, and frequently is less than the variation in areas of spots representing equal concentrations of a substance. This is to be expected since determination of spot weight instead of area will tend to compensate for errors due to variation in density or thickness of the paper. Both factors must be presumed constant in methods involving direct measurement of surface area as an indication of concentration.

When paper chromatography is used for quantitative determinations by such procedures, it is essential that standard samples of known concentration be run in parallel, on the same sheet of paper as the unknown, for each compound to be analyzed. The curve for logarithm concentration versus spot weight for each compound must be established anew for each chromato-

Table I. Comparison of Spot Weight and Spot Area for Duplicate Concentrations of Substance on Paper Chromatogram

Substance	Approx. Amount, γ	Spot Area, Square Inch		Variation, %	Spot Weight, Mg.		Variation, %
		Sample 1	Sample 2		Sample 1	Sample 2	
Saligenin	8	0.39	0.39	0.0	23.7	23.1	2.5
	16	0.57	0.50	12.3	31.8	28.1	11.6
	25	0.62	0.63	1.6	36.3	35.2	2.2
p-Hydroxybenzyl alcohol	10	0.33	0.31	6.1	18.8	17.8	5.3
	15	0.41	0.43	4.7	24.5	24.3	0.8
	25	0.59	0.54	8.5	34.7	32.1	7.5
2,6-Dimethylol phenol	4	0.25	0.22	12.0	14.1	12.5	11.3
	6	0.33	0.30	9.1	19.1	17.5	8.4
	16	0.46	0.51	9.8	26.8	27.8	3.6
2,4-Dimethylol phenol	7	0.29	0.29	0.0	16.5	16.2	1.8
	23	0.52	0.54	3.7	30.0	30.3	1.0
	31	0.54	0.57	5.3	31.1	32.4	4.0
2,4,6-Trimethylol phenol	10	0.31	0.30	3.2	17.4	17.8	2.2
	20	0.36	0.37	2.7	20.9	20.7	1.0
	31	0.43	0.46	6.5	25.3	25.8	1.9
	41	0.48	0.48	0.0	27.5	27.4	0.4

graph in order to avoid errors due to differences in time, temperature, actual distance traversed by spot, composition of paper, etc., between runs.

In the apparatus described in the previous paper (*5*), it is convenient to run ten samples simultaneously on a single sheet of paper. Six of these are allocated to standard samples from which the least squares curve is established, and four are used for analysis of unknown compositions. Since variations are observed in weights (or areas) of spots representing equal concentrations of a substance, it has been found most advantageous to run duplicate pairs representing three concentrations of a given substance and to use as data for plotting, the average weight of each pair of duplicates. The curve obtained from the six points (three average values each representing two determinations) is not much improved by employing eight spaces for known samples.

The four spaces devoted to unknowns are utilized to provide simultaneous analyses of two different samples. However instead of using two duplicate samples of each unknown mixture, the unknown sample is applied in different amounts of known ratio. Usually applications of 1 and 2, or 2 and 3 microliters of unknown solution are made to the paper but 1-microliter samples of solutions of different, but accurately known, dilution may also be used. After the value for each aliquot has been determined from the graph and the dilution ratio taken into account, the values are averaged.

This method has the advantage of making the unknown determination at two different places along the curve rather than duplicating a determination at only one point on the curve. If greater certainty is desired at the expense of speed, all four spaces can, of course, be used for quadruplicate analysis of one unknown. However, using the method as outlined, an accuracy of better than ±5% on samples containing 10 to 35 micrograms of each substance may be expected after a little experience with the method. The primary source of error lies in the accurate delineation by eye of the edge of each respective spot.

Table II shows results of typical analyses made using prepared solutions of accurately known concentration as unknowns.

EXPERIMENTAL

Apparatus. The apparatus, solvent, and indicator are described in (*5*). Preparation of the compounds studied is described in (*6*).

Procedure. A sheet of Whatman No. 1 filter paper, approximately 9 by 22 inches, with the grain in the longer direction was used. Ten samples were spotted at 20-mm. intervals along a line 6 cm. from the top of the sheet. Samples were applied in 1-microliter amounts from a self-adjusting micropipet. The transfer of solution from pipet to paper was interrupted several times for each sample in order to permit solvent to evaporate and to keep the initial spot size as small and uniform as possible (less than 3 mm. in diameter). Repeated pipettings were made to build up concentrations or create mixtures of components on a single spot. The same micropipet was used for all samples on one strip. The pipet was rinsed with methanol between transfers of each different solution, and was rinsed three times with each solution to be used prior to transfer of solution to the paper. For each transfer, the outer edge of the pipet tip was touched to a vertical ground glass surface (mouth of volumetric flask) to remove adhering solution before discharging it to the paper.

The strip was developed in the usual manner (*5*). Most runs were made at 25° C. The specific temperature is not critical but it should remain uniform throughout a run.

After development of the strip and application of the indicator, each spot was outlined with a sharp pencil as soon as the strip was dry. This ensures that no small spots containing only trace amounts of substance will be missed. However, it is not possible to make the final marking for quantitative determination immediately. When the sheet contains a large number of spots, fading which occurs during the time required for marking introduces a significant error. About 20 minutes after the strip is dry the rate of fading has decreased considerably and it was found that the strip could be most successfully marked in the interval from 20 to 40 minutes after it became dry. Generally the strip was turned over and the final outline of each spot was marked on the back. The spot borders recede and the final spots are appreciably smaller than the initial outlines. The strip was allowed several hours to equilibrate with the atmosphere before weighing. Spots were then excised carefully by trimming with scissors along the inner markings and weighed individually. The entire strip need not be completed in a day, but all spots corresponding to a given single compound should be weighed at one sitting.

Table II. Errors in Quantitative Determination of Methylol Phenols by Paper Chromatography

Compound	Taken[a], γ	Found[a, b], γ	Error, γ	Error, %
Saligenin	13.99	13.33	−0.66	−4.7
	13.99	14.75	+0.76	+5.4
	13.99	13.03	−0.96	−6.8
p-Hydroxybenzyl alcohol	14.10	13.68	−0.42	−3.0
	14.10	14.80	+0.70	+5.0
	14.10	13.33	−0.77	−5.4
2,6-Dimethylol phenol	15.03	14.50	−0.53	−3.5
	15.03	15.85	+0.82	+5.4
2,4-Dimethylol phenol	14.97	14.48	−0.49	−3.3
	14.97	15.00	+0.03	+0.3
2,4,6-Trimethylol phenol	16.83	16.88	+0.05	+0.3
3,3′,5,5′-Tetra(hydroxymethyl) 4,4′-dihydroxydiphenylmethane	13.98	13.15	−0.73	−5.2

[a] Amounts given as micrograms/1-microliter spot.
[b] Average of two determinations.

LITERATURE CITED

(1) Block, R. J., ANAL. CHEM., **22**, 1327 (1950).
(2) Brimley, R., *Nature*, **163**, 215 (1949).
(3) Fisher, R., Parsons, D., and Holmes, R., *Ibid.*, **164**, 183 (1949).
(4) Fisher, R., Parsons, D., and Morrison, G. A., *Ibid.*, **161**, 764 (1948).
(5) Freeman, J. H., ANAL. CHEM., **24**, 955 (1952).
(6) Freeman, J. H., *J. Am. Chem. Soc.*, in press.
(7) Gustafsson, C., Sundman, J., and Lindh, T., *Paper and Timber (Finland)*, **33**, 1 (1951).
(8) Lindberg, O., and Hummel, J. P., *Arkiv. Kemi*, **1**, 17 (1949).
(9) McFarren, E. F., Brand, K., and Rutkowski, H. R., ANAL. CHEM., **23**, 1146 (1951).
(10) Page, J. E., Biochemical Society Symposia, No. 3, p. 32, R. F. Williams, ed., London, Cambridge University Press, 1950.

RECEIVED for review August 22, 1952. Accepted September 8, 1952.

Use of Magnesium Oxide in Determination of Carbon and Hydrogen

In Fluoro-organic Compounds

W. H. THROCKMORTON AND GLENNA H. HUTTON

Research Laboratories, Tennessee Eastman Co., Kingsport, Tenn.

IN THE determination of carbon and hydrogen in fluoro-organic compounds, difficulties are encountered because the fluorine-containing combustion products react with the silica of combustion tubes to form silicon tetrafluoride (*2*). A preliminary report of a method which appears to overcome these difficulties is presented here.

Although metallic silver reacts with any hydrogen fluoride present to remove its fluorine, it has no effect on the silicon tetrafluoride. Belcher and Goulden (*2*) successfully used sodium fluoride at 270° C. to remove the latter compound. Carbon analyses on fluoro-organic compounds containing no hydrogen were 1 to 2% low, however, unless hydrogen was supplied by the addition of a known weight of a pure substance, such as benzoic acid. It also appeared that correct carbon values could be obtained when water vapor was introduced into the system.

Most of the procedures described in the literature do not permit the simultaneous determination of carbon and hydrogen. Teston and McKenna (*11*) determined carbon, fluorine, and chlorine in completely halogenated hydrocarbons. Other procedures deal with the determination of carbon and fluorine (*10*), hydrogen only (*5, 8*), or carbon only (*6*).

For the determination of carbon in uranium tetrafluoride, Warf (*9, 12*) mixed the sample with an equal weight of ignited magnesium oxide and covered the sample with additional oxide. The results, corrected by a blank determination, were stated to be from 2 to 5% high, as determined from added amounts of uranium carbide.

Methods for the determination of fluorine in biological materials make use of magnesium acetate or peroxide to prevent loss of fluorine during the ashing procedure (*3, 14*).

The procedure described here makes use of pellets of magnesium oxide as part of the combustion tube filling. The results obtained with a limited number and type of compounds ranging in fluorine content from 15 to 30% indicate that this filling reacts effectively with all the fluorine-containing combustion products, including the silicon tetrafluoride.

EXPERIMENTAL

Apparatus. The apparatus used was essentially that described by Niederl and Niederl (*7*). The "combination" filling for the combustion tube was modified only by replacing 3.5 to 4.0 cm. of the copper oxide in the front end of the quartz tube with magnesium oxide pellets. The Sargent micro combustion apparatus was used with the temperature of the long furnace set at 775° C. (*13*) and that of the sample furnace at 800° C. Temperatures were checked in an empty tube with a thermocouple at the mid-points of the furnaces.

Materials. COMPOUNDS ANALYZED. The sample of 1,1,2-trichloro-3,3,3-trifluoropropene was obtained from the Columbia Organic Chemicals Co. The other samples were obtained from the Hooker Electrochemical Co. All materials were redistilled before use. The boiling points and refractive indices of the fractions analyzed and the types of distilling columns used are shown in Table I.

MAGNESIUM OXIDE PELLETS. Reagent grade magnesium oxide powder was mixed with water to form a creamy paste. This was poured into a flat-bottomed dish and dried at 110° C. The dry cake was cut into 8- to 20-mesh pellets which were then ignited for 1 hour at 800° C. in a muffle furnace to convert any carbonate present to oxide.

Procedure. The customary techniques of sample weighing, combustion, and handling of absorption tubes were followed (*7*). The sample capillaries contained potassium chlorate. The sample furnace was set for automatic drive at a rate of 4 mm. per minute and with a 5-minute burning time directly over the sample boat. The combustion was repeated at a rate of 12 mm. per minute but without stopping the furnace over the sample boat.

Fluorine was determined by Parr bomb combustion using potassium chlorate as the accelerator. The combustion was followed by the volumetric lead chlorofluoride method essentially as described by Hillebrand and Lundell (*4*).

Experiments. The first trials with magnesium oxide consisted of covering the sample, contained in a sample capillary in a long platinum boat, with magnesium oxide powder and using the "combination" filling described by Niederl and Niederl (*7*). The results were rather poor, although somewhat better results were obtained on a solid sample contained in a boat and covered with the oxide.

The oxide powder was next placed in a platinum boat 45 mm. long which was then positioned so that it was about half covered by the long furnace. An improvement in results was noted, especially after the magnesium oxide became scattered about in the tube because of the flashing of a rather volatile sample. This indicated that better contact between the gases and the oxide was needed.

It followed, then, that the use of pellets as a part of the tube filling might accomplish this result. The results obtained with the pellets in analyzing five fluoro-organic compounds are presented in Table II. Fluorine analyses of these compounds are also included in the table.

DISCUSSION OF RESULTS

The data of Table II indicate that the carbon and hydrogen values obtained are within the generally acceptable limits, although there does appear to be a tendency for both to be slightly high.

The indication of some hydrogen for the completely halogenated hydrocarbons is possibly due to the dehydration of the lead peroxide used in the filling. This appears to be confirmed by the correct results obtained with the compounds not containing nitrogen when the lead peroxide was replaced by silver wool. (The results obtained with this filling will be discussed in a subsequent article.) It was necessary in this case to deduct a small blank from the weight of the water absorption tube. The blank was determined by following all the steps of the procedure without using any sample. It was of the order of 0.09 mg. of which approximately 0.06 mg. was found to be due to the opening of the combustion tube for the introduction of the sample boat. [While the manuscript was in preparation, Backeberg and Israelstam

Table I. Compounds Analyzed

Compound	Boiling Point: Distillation[a], ° C. (uncor.)/mm.	Boiling Point: Micro, ° C. (cor.)/mm.	Refractive Index, n_D^{20}
m-Nitrobenzotrifluoride	199–200/730[b]	201.0/731.2	1.4726
m-Chlorobenzotrifluoride	135.6–136.1/737–9[c]	136.6/732.8	1.4467
1,1,2,2,3-Pentachloro-3,3-difluoropropane	165.5–166.0/730.5[b]	166.6/731.2	1.4618
1,1,2,2-Tetrachloro-1,2-difluoroethane	89.0/731[c]	92.0/734	1.4458 (25° C.)
1,1,2-Trichloro-3,3,3-trifluoropropene	86.8–86.9/725.8[d]	87.3/732.8	1.4095

[a] Although distillation data indicate boiling ranges of the fractions used, temperatures are uncorrected. Therefore, micro boiling points, which are corrected, are included.

[b] 22-mm. × 18-inch Podbielniak Heligrid distilling column.

[c] 8-mm. × 72-inch Podbielniak Nichrome wire-spiral distilling column.

[d] 7-mm. × 36-inch Podbielniak Nichrome wire-spiral distilling column.

Table II. Results of Analyses

Compound		Analyses Carbon	 Hydrogen	 Fluorine[a]
m-Nitrobenzotrifluoride	Calcd.	43.99	2.11	29.83
	Found (14)	44.22 ± 0.078[b, c]	2.23 ± 0.061[b, c]	29.74
m-Chlorobenzotrifluoride	Calcd.	46.56	2.23	31.57
	Found (8)	46.69 ± 0.119[b, d]	2.37 ± 0.084[b, d]	31.59
1,1,2,2,3-Pentachloro-chloro-3,3-difluoropropane	Calcd.	14.28	0.40	15.06
	Found	14.43	0.46	14.96
		14.41	0.50	
		14.35	0.46	
		14.44	0.43	
1,1,2,2-Tetrachloro-1,2-difluoroethane	Calcd.	11.78	None	18.64
	Found	11.84	0.22	...[e]
		11.85	0.12	
1,1,2-Trichloro-3,3,3-trifluoropropene	Calcd.	18.07	None	28.59
	Found	17.86	0.08	28.75
		18.05	0.20	
Acetanilide (NBS)	Calcd.	71.09	6.70	...
	Found (8)	71.16 ± 0.094[b, f]	6.72 ± 0.040[b, f]	

[a] Average found by Parr bomb combustion followed by volumetric lead chlorofluoride method (*4*).

[b] Averages with ± limits at 95% confidence level for number of determinations indicated in parentheses.

[c] Standard deviation $\left(\sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}}\right)$ for carbon = 0.135% C and for hydrogen = 0.106% H.

[d] Standard deviation for carbon = 0.142% C and for hydrogen = 0.101% H.

[e] Obtained only 88 to 90% of calculated value.

[f] Standard deviation for carbon = 0.112% C and for hydrogen = 0.048% H.

(*1*) reported their findings concerning this water blank. The reader is referred to their article for a more complete discussion.] With a tube containing lead peroxide it is obviously impossible to determine a blank that will apply correctly to compounds which contain hydrogen as well as to those which do not. No blank was used with the analyses given in Table II. The results obtained with acetanilide indicate that none was required with this particular apparatus to obtain correct hydrogen values with compounds containing considerable hydrogen.

SUMMARY AND CONCLUSIONS

The addition of magnesium oxide to the combustion tube filling appears to offer promise as a simple means of overcoming the difficulties encountered in the determination of carbon and hydrogen in fluoro-organic compounds. There are indications that dehydration of the lead peroxide leads to high hydrogen values with compounds which contain little or no hydrogen.

A more detailed study is in progress. The study will include the determination of the optimum temperature for the magnesium oxide, its position in the combustion tube, and its physical form; the use of an external absorbent for the oxides of nitrogen; and the application of the procedure to a wider variety of fluoroorganic compounds.

This preliminary report is offered at this time in the hope that others may find the suggested procedure helpful and make improvements upon it.

LITERATURE CITED

(1) Backeberg, O. G., and Israelstam, S. S., Anal. Chem., **24**, 1209–14 (1952).

(2) Belcher, R., and Goulden, R., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 679–89 (1951).

(3) Crutchfield, W. E., Jr., Ind. Eng. Chem., Anal. Ed., **14**, 57–8 (1942).

(4) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis," pp. 604–5, New York, John Wiley & Sons, 1929.

(5) Miller, J. F., Hunt, H., Hass, H. B., and McBee, E. T., Anal. Chem., **19**, 146–47 (1947).

(6) Miller, J. F., and McBee, E. T., "Quantitative Determination of Carbon in Polyfluorocompounds," "Monthly Technical Report on Research at Purdue University for October 1944," by McBee, E. T., *Rept.* **A-1515**, pp. 25–9.

(7) Niederl, J. B., and Niederl, J., "Micromethods of Quantitative Organic Analysis," 2nd ed., pp. 101–23, New York, John Wiley & Sons, 1942.

(8) Pearlson, W. H., Brice, T. J., and Simons, J. H., Ind. Eng. Chem., Anal. Ed., **18**, 330–1 (1946).

(9) Rodden, C. J., *et al.*, "Analytical Chemistry of the Manhattan Project," p. 279, New York, McGraw-Hill Book Co., 1950.

(10) Simons, J. H., and Block, L. P., *J. Am. Chem. Soc.*, **61**, 2964 (1939).

(11) Teston, R. O., and McKenna, F. E., Anal. Chem., **19**, 193–6 (1947).

(12) Warf, J. C., U. S. Atomic Energy Comm., *Rept.* **CC-433** (Jan. 26, 1943).

(13) Willits, C. O., and Ogg, C. L., *J. Assoc. Offic. Agr. Chemists*, **34**, 609 (1951).

(14) Winter, O. B., *Ibid.*, **19**, 359–65 (1936).

Received for review July 10, 1952. Accepted September 6, 1952.

Quantitative Determination of Pentoses with Anthrone

ROBERT ROY BRIDGES

Masonite Corp., Laurel, Miss.

In 1946 Dreywood reported that anthrone in concentrated sulfuric acid gives a permanent green coloration with carbohydrate material (*1*). Morris later showed that the green color intensity obeyed Beer's law at 620 mμ for such carbohydrates as glucose, lactose, glycogen, and maltose (*2*). Other investigators have successfully used the reagent for the quantitative determination of minute amounts of sucrose, cellulose, and starch with a sensitivity exceeding that of previously used tests for these materials (*3*, *6*).

Sattler and Zerban, as a result of a study of the reaction, concluded that the color was due to the formation of furfural compounds in strong sulfuric acid (*4*). This was confirmed when Shriver, Webb, and Swanson revealed that the spectral curves for glucose and xylose with the reagent were almost identical with those given by hydroxymethyl furfural and furfural, respectively, with the reagent (*5*). They further confirmed a minimum at 620 mμ and the validity of Beer's law for hexoses and methyl pentoses (fucose and rhamnose) at this wave length. With pentoses such as arabinose and xylose, however, the blue-green color responsible for the minimum at 620 mμ appeared too transient to permit transmittance readings. The blue-green color was observed to form, but to change rapidly to an amber color having a spectral curve with no distinct minimum at 620 mμ and for which Beer's law is not obeyed at any wave length.

It was found that the blue-green color with pentoses remains stable long enough to make accurate, reproducible transmittance readings, provided the reaction mixture is cooled in an ice bath immediately after mixing the sample with the reagent.

EXPERIMENTAL PROCEDURE

A 2.00-ml. sample of sugar solution in water from 0.001 to 0.01%, to be analyzed in a 16 × 144 mm. test tube has added to it carefully as a lower layer, 4.00 ml. of a 0.05% (w./w.)-solution of anthrone in concentrated sulfuric acid. The acid is added with a pipet so as not to mix with the aqueous layer. The two layers are then quickly mixed using an oscillating motion of

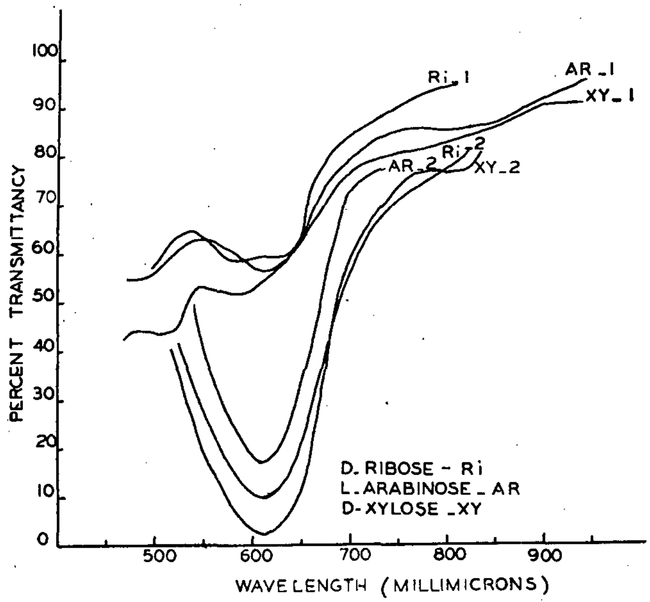


Figure 1. Spectral Curves of Pentoses Reacted with Anthrone Reagent

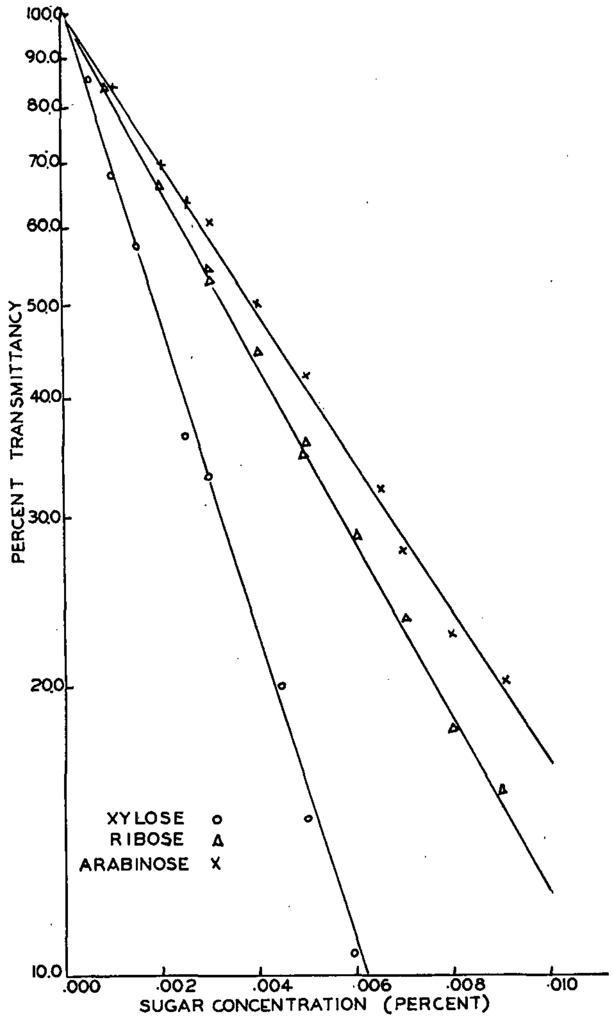


Figure 2. Transmittance Versus Pentose Concentration

Cooling technique used

the tube for about 10 seconds. Heat generated by the mixing raises the temperature to around 95° C. The test tube is then immediately immersed in an ice bath (0° C.) for 5 minutes. Before transmittance measurements are taken the tube is brought to room temperature. A blank for the spectral measurements is provided for with 2.00 ml. of distilled water in place of the sample. Spectral measurements are made with a Beckman Model DU spectrophotometer.

The solution of anthrone in sulfuric acid darkens noticeably after a day's standing, so a fresh solution is prepared each day and allowed to stand about 4 hours to ensure color stability.

DISCUSSION OF RESULTS

Solutions of the three pentoses at 0.010% concentration, when reacted with 0.05% anthrone in sulfuric acid and allowed to cool at room temperature for 5 minutes, give spectral curves with no pronounced minimum at 620 mμ characteristic of the blue-green color. These solutions after standing hot have gone over to the amber color (Figure 1). If, however, the tubes are cooled immediately after mixing, the minimum at 620 mμ is observed.

Using the cooling technique, when the per cent transmittance (on a logarithmic scale) is plotted against the concentration (0.001 to 0.010%) at 620 mμ, straight lines are obtained for all three pentoses, thus showing that Beer's law is obeyed, and making the technique suitable for quantitative analysis (Figure 2).

The transmittances for the various concentrations used have been shown to be constant—45 minutes for D-xylose, 75 minutes for L-arabinose, and 90 minutes for D-ribose. If measurements are made within these limits no color instability will be noticeable.

CONCLUSIONS

The use of the anthrone reagent, previously shown to be suitable for the quantitative determination of hexoses, has been extended to the quantitative determination of pentoses by employing a simple modification of operating technique.

Hexoses will interfere if they are present, so the operating conditions as they now stand apply only to pentose sugars.

ACKNOWLEDGMENT

The author wishes to thank the Masonite Corp., and especially Robert M. Boehm for permission to publish this material.

LITERATURE CITED

(1) Dreywood, R., Ind. Eng. Chem., Anal. Ed., **18**, 499 (1946).
(2) Morris, D. L., *Science*, **107**, 254–5 (1948).
(3) Morse, E. E., Anal. Chem., **19**, 1012–13 (1947).
(4) Sattler, L., and Zerban, F. W., *Science*, **108**, 207 (1948).
(5) Shriver, E. H., Webb, M. B., and Swanson, J. W., *Tech. Assoc. Pulp Paper Ind.*, **33**, 578–86 (1950).
(6) Viles, F. J., and Silverman, L., Anal. Chem., **21**, 950–3 (1949).

Received for review May 8, 1952. Accepted August 29, 1952.

Correction

In the summary of one of my papers, reviewed by S. K. Love [Anal. Chem., **24**, 299 (1952)], the correct text is as follows:

"To 1 ml. of sample add 10 ml. of sulfuric acid and heat just until it fumes. Cool below 100° C., add 2 ml. of azo dye (0.125 gram of chromotrop 2B in 500 ml. of sulfuric acid), dilute with sulfuric acid to 15 ml., and keep for 30 minutes at 100° C. Cool, and add 0.04 ml. of sodium cobaltinitrite solution. Shake well and keep for 8 hours in darkness. Read color in photometer. Range of application is 0.5 to 25 micrograms of boron per 15 ml."

Hegedus Andras

Pazsit-u. 13,
Budapest II,
Hungary

A Spectrophotometric Method for the Determination of Hydrazine

GEORGE W. WATT AND JOSEPH D. CHRISP
The University of Texas, Austin, Tex.

THE yellow color developed upon addition of *p*-dimethylaminobenzaldehyde to solutions of hydrazine in dilute hydrochloric acid solutions has been used as the basis for the development of a spectrophotometric method for the determination of hydrazine. These systems are characterized by a transmittancy minimum at 458 mμ. Good agreement with Beer's law is displayed at hydrazine concentrations up to 0.77 p.p.m.; the optimum concentration range is 0.06 to 0.47 p.p.m., over which the relative error does not exceed 1%. Data are given for the per cent relative error introduced by the presence of urea and semicarbazide; ammonium ion does not interfere with the determination.

Methods for the determination of hydrazine depend upon its basic character or reducing properties (*5–9, 11*) and are, of course, subject to interferences owing to the presence of other substances having similar properties. In connection with a research program in progress there arose the need for an analytical method relatively free of interferences resulting from the presence of appreciable concentrations of urea and semicarbazide. The procedure described in this paper is based upon the observation by Pesez and Petit (*10*) to the effect that a characteristic color results upon addition of a solution of *p*-dimethylaminobenzaldehyde in ethyl alcohol and hydrochloric acid to hydrazine in dilute hydrochloric acid solution.

EXPERIMENTAL

Apparatus. Transmittancy measurements were made with a Beckman Model DU spectrophotometer using Corex cells of 1.003-cm. light path. The instrument was operated at constant sensitivity using slit widths of the order of 0.02 to 0.10 mm., corresponding to nominal band widths of about 0.3 to 1.6 mμ.

Materials. The purity of hydrazine dihydrochloride (Eastman No. 1117) and semicarbazide hydrochloride (Eastman No. 226) was established as 99.8 and 99.4%, respectively, by means of the Jamieson method (*6*). *p*-Dimethylaminobenzaldehyde (Eastman No. 95) was used as received; urea was purified by recrystallization from methanol. All other materials employed in this work were reagent grade chemicals that were used without further purification.

Preparation of Standard Hydrazine Solutions. Solutions of 0.5 to 1.0 gram of hydrazine dihydrochloride in 1 liter of distilled water were standardized by titration with standard potassium iodate solution (*6*) as follows: A mixture consisting of 10.0 ml. of the hydrazine solution, 20 ml. of distilled water, 25 ml. of concentrated hydrochloric acid, and 15 ml. of chloroform was prepared in an iodine bottle and cooled to about 10° C. in an ice bath. After addition of approximately one half of the quantity of potassium iodate solution required in the complete titration, additional concentrated hydrochloric acid solution was added to give a total acid concentration within the range 3 to 6 *M*, and the titration was then carried to completion. If all of the required acid is added initially, the pink color of the iodine-chloroform layer does not appear at the beginning of the titration. Appropriate aliquots of these stock solutions were diluted to the desired concentration, made 1 *M* with respect to hydrochloric acid, and used in the color development.

Color Development. The color reagent employed had the following composition: *p*-dimethylaminobenzaldehyde, 0.4 gram; ethanol, 20.0 ml.; concentrated hydrochloric acid, 2.0 ml. Ten milliliters of this reagent was added to aliquots of the standardized hydrazine solution selected so that the final hydrazine concentration would be within the range 0.02 to 0.8 p.p.m., and the resulting mixtures were diluted to a total volume of 25.0 ml. with 1 *M* hydrochloric acid solution. Blanks consisted of 10.0 ml. of the color reagent in 25.0 ml. of solution 1 *M* with respect to hydrochloric acid.

At room temperature the yellow color develops immediately and is stable after a period of 10 minutes. For a given concentration of hydrazine the per cent transmittancy is unchanged if the hydrochloric acid concentration is less than 1 *M* but increases at acid concentrations greater than 1 *M*. Thus, for a hydrazine concentration of 0.13 p.p.m., the relative transmittancy is increased 1.6% if the acid concentration is 2 *M*, and 4.5% if the acid concentration is 3 *M*. For hydrazine concentrations of the order of 0.26 p.p.m. in the presence of 1 *M* hydrochloric acid, use of 15.0 ml. rather than 10.0 ml. of the color reagent results in a decrease in transmittancy amounting to only 2.5%.

Spectral Characteristics. The transmittancy of hydrazine solutions of different concentrations was measured at frequent intervals over the range 400 to 700 mμ; per cent transmittancy is plotted against wave length in Figure 1, which shows one transmittancy minimum at 458 mμ. A plot of log transmittancy (458 mμ) against concentration showed good agreement with Beer's law over the concentration range investigated—i.e., up to a hydrazine concentration of 0.77 p.p.m.

Stability of Color. Colored solutions containing different quantities of hydrazine showed no measurable change in transmittancy in 12 hours, an increase of 0.6% (absolute) in 34 hours, and an increase of 1.6% in 5 days.

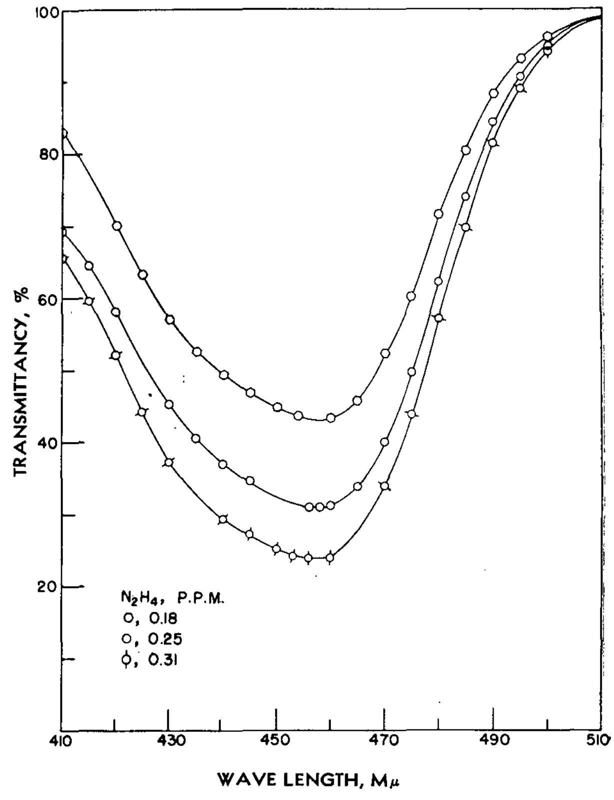


Figure 1. Spectral Curves for Hydrazine with *p*-Dimethylaminobenzaldehyde

Effect of Temperature. For a color-developed sample containing hydrazine at a concentration of 0.2 p.p.m., the effect of temperature over the range 20° to 40° C. was found to amount to +0.14% absolute transmittancy per 1° C.; this effect was completely reversible.

Reproducibility. Over the hydrazine concentration range 0.1 to 0.3 p.p.m., samples having the same hydrazine concentration gave an average deviation of 0.1% absolute transmittancy. Solutions color-developed with color reagent that had stood for 1 week gave the same per cent transmittancy as those color-

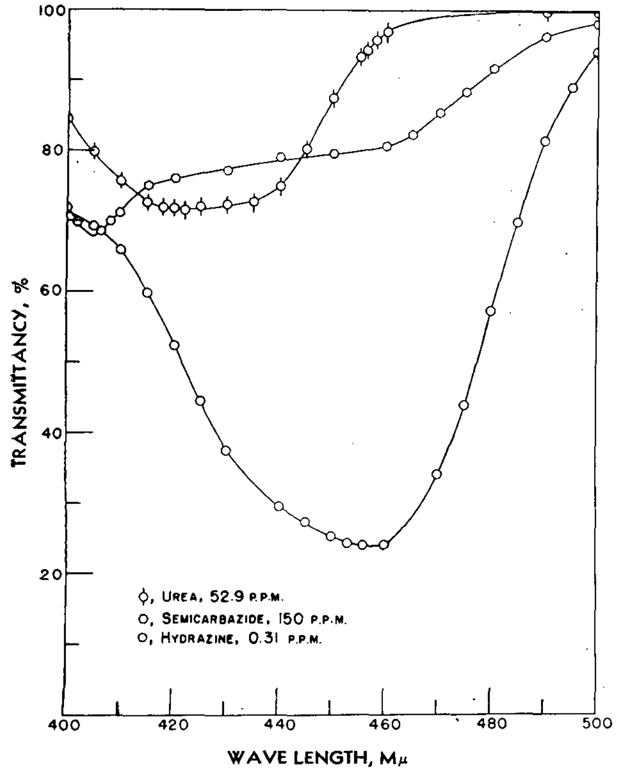


Figure 2. Spectral Curves for Hydrazine, Semicarbazide, and Urea with *p*-Dimethylaminobenzaldehyde

developed with freshly prepared reagent. As a check on the stability of hydrazine in hydrochloric acid solution, standard solutions of hydrazine dihydrochloride (0.65 p.p.m. hydrazine in 1 *M* hydrochloric acid) were stored for 6 days in a glass-stoppered flask, color-developed in the usual manner, and used in transmittancy measurements. The resulting values corresponded to from 4 to 5% relative decrease in hydrazine concentration. However, no change in hydrazine could be detected during the first 12 hours of this storage period.

Six standard samples containing hydrazine at concentrations unknown to the analyst were analyzed over the working range of concentration. The relative error was found to vary from 0.6 to 1.1%.

Interferences. The extent of interference owing to the presence of semicarbazide, urea, and ammonium salts was evaluated by measuring the transmittancies of color-developed solutions containing fixed quantities of hydrazine (0.26 or 0.13 p.p.m.) and varying quantities of the potentially interfering additive. Transmittancy measurements were made over a considerable interval on either side of 458 mμ. The mole ratio of semicarbazide and/or urea to hydrazine and the corresponding per cent relative errors are listed in Table I. Both of these substances lead to a decrease in per cent transmittancy; a shift in the minimum was observed using solutions containing 110 moles of urea per mole of hydrazine. Colored solutions containing up to 5000 moles of ammonium chloride or nitrate per mole of hydrazine showed no change in per cent transmittancy and no shift in the minimum.

Table I. Per Cent Relative Error Resulting from Presence of Semicarbazide and/or Urea

Mole Ratios		Hydrazine, P.P.M.	
		0.13	0.26
Semicarbazide to hydrazine	Urea to hydrazine	% Error relative to hydrazine	
2.3			0.0
20		1.4	
25			1.3
30		2.1	
46			3.2
50		4.6	
69			4.3
96			6.0
138		12.5	
	29		0.8
	55	1.6	
	110	4.3	
	220	7.6	
5	5	1.6	0.6
15	15	2.7	1.0
25	25	2.7	1.6

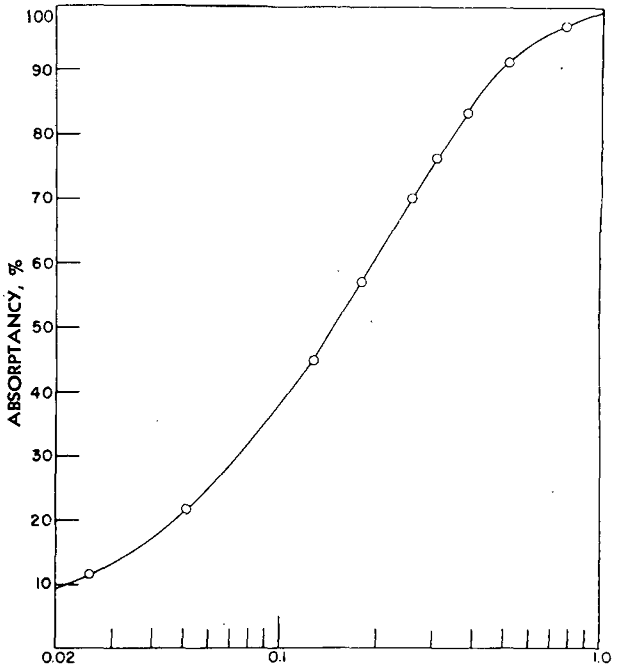


Figure 3. Calibration Curve for Hydrazine with *p*-Dimethylaminobenzaldehyde at 458 Mμ

The spectral curves for semicarbazide and urea (Figure 2) indicate that high concentrations of these substances may be tolerated without serious interference. The colored solutions of urea were stable, but those containing semicarbazide were unstable. A spectral curve was plotted for a colored solution of semicarbazide every 24 hours for 3 days. The curve characteristic of semicarbazide was progressively replaced by that characteristic of hydrazine with a minimum at 458 mμ.

DISCUSSION

The calibration curve for this method for the determination of hydrazine is shown in Figure 3, in which per cent absorptancy (100 − % transmittancy) at 458 mμ is plotted against log concentration of hydrazine in parts per million; each experimental point was established by many replicate measurements. The utility of this method of plotting for evaluation of the most appropriate working range and maximum accuracy has been discussed by Ayres (*2*). The curve exhibits maximum slope at about 63% absorptancy, in agreement with Beer's law, hence a maximum accuracy corresponding to 2.7% relative analysis error per 1% absolute photometric error, or about 0.6% relative error for a precision of 0.2% in making the measurements. To attain this precision it was found necessary to exercise every precaution to avoid cross contamination of solutions via transfer pipets. Maximum accuracy is obtained at hydrazine concentrations of the order of 0.2 p.p.m., but in view of the observed temperature coefficient of transmittancy, it is necessary to hold the temperature constant in order to achieve this accuracy.

In order to keep the relative analysis error within 1.0%, the hydrazine concentration must be within the limits 0.06 to 0.47 p.p.m.; these limits were determined exactly as described by Ayres and Young (*3*), assuming 0.2% absolute photometric error.

The susceptibility of hydrazine to catalytic oxidation and/or decomposition (*1*, *4*) was confirmed; results show that hydrazine solutions should be analyzed as soon as possible after preparation. In the present method, errors become appreciable after 12 hours. For this reason, freshly prepared standard hydrazine solutions were used in all measurements involved in the calibration data.

With reference to interferences, it is clearly evident that the progressive change in the spectral curve for semicarbazide results from the slow hydrolysis of this component to produce hydrazine. Although the rate of hydrolysis is slow, the color reagent is more sensitive to hydrazine than to semicarbazide, and this causes a shift in the minimum transmittancy to that characteristic of hydrazine. In the study of interferences, therefore, freshly prepared solutions of semicarbazide were employed. As shown by the data of Table I, at a hydrazine concentration of 0.26 p.p.m. (29.7% transmittancy at 25° C.), a molar concentration of semicarbazide 25 times as great as that of hydrazine introduces a relative error of only 1.3%, but the magnitude of the error increases with decrease in hydrazine concentration. A relative error of 1.6% results when the molar concentration of urea is 55 times as great as that of hydrazine. If both urea and semicarbazide are present, the relative error is increased over that attributable to either component in the absence of the other, but the increase is not great. Thus, if the hydrazine concentration is 0.26 p.p.m., the per cent relative error is 1.6 if the solution contains 25 moles of urea and 25 moles of semicarbazide per mole of hydrazine.

ACKNOWLEDGMENT

The work described in this paper was supported by the U. S. Navy Bureau of Ordnance under Contract N123s-67363, Task Order 2.

LITERATURE CITED

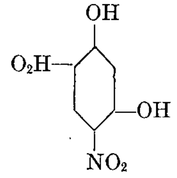
(1) Audrieth, L. F., and Mohr, P. H., *Ind. Eng. Chem.*, **43**, 1774 (1951).
(2) Ayres, G. H., Anal. Chem., **21**, 652 (1949).
(3) Ayres, G. H., and Young, Frederick, *Ibid.*, **22**, 1280 (1950).
(4) Bray, W. C., and Cuy, E. J., *J. Am. Chem. Soc.*, **46**, 858 (1924).
(5) Gilbert, E. C., *J. Am. Chem. Soc.*, **46**, 2648 (1924).
(6) Jamieson, G. S., *Am. J. Sci.*, **33**, 352 (1912).
(7) Kolthoff, I. M., *J. Am. Chem. Soc.*, **46**, 2009 (1924).
(8) Kurtenacker, A., and Kubina, H., *Z. anal. Chem.*, **64**, 388 (1924).
(9) Penneman, R. A., and Audrieth, L. F., Anal. Chem., **20**, 1058 (1948).
(10) Pesez, M., and Petit, A., *Bull. soc. chim. France*, 1947, 122–3.
(11) Smith, G. F., and Wilcox, C. S., Ind. Eng. Chem., Anal. Ed., **14**, 49 (1942).

Received for review June 10, 1952. Accepted September 25, 1952.

CRYSTALLOGRAPHIC DATA

63. 4,6-Dinitroresorcinol

Contributed by **WALTER C. MCCRONE** and **IRENE CORVIN**, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.



Structural Formula for 4,6-Dinitroresorcinol

EXCELLENT crystals of 4,6-dinitroresorcinol can be obtained either by sublimation or by recrystallization from ethyl alcohol. Both techniques give massive crystals and tablets showing the forms: prism, {110}, orthopinacoid {100}, and basal pinacoid {001}. Good crystals can also be obtained from thymol on a microscope slide.

Crystal Morphology
Crystal System. Monoclinic.
Axial Ratio. $a:b:c = 3.394:1:2.344$.
Interfacial Angles (Polar). $110 \wedge 1\bar{1}0 = 136° 40'$.
Beta Angle. 48°.

Optical Properties
Refractive Indices (5893 A.; 25° C.). $\alpha = 1.598 \pm 0.002$. $\beta = 1.673 \pm 0.002$. $\gamma = 2.01$ (calculated from α, β, and $2V$).
Optic Axial Angles (5893 A.; 25° C.). $2V = 50°$ (+) (measured). $2E = 91°$.
Dispersion. $r > v$.
Optic Axial Plane. 010.
Acute Bisectrix. γ.
Extinction. $\gamma \wedge a = 3°$ in acute β.
Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.752$. R (calcd.) = 40.2; R (obsd.) = 45.7.

X-Ray Diffraction Data
Cell Dimensions. $a = 17.07$ A.; $b = 5.03$ A.; $c = 11.79$ A.
Formula Weights per Cell. 4 (4.01 calculated from x-ray data).
Formula Weight. 200.11.
Density. 1.786 (flotation in aqueous zinc chloride); 1.781 (x-ray).

Principal Lines

d	I/I_1	d	I/I_1
12.07	0.04	2.45	0.07
6.28	0.04	2.40	0.06
5.91	0.04	2.34	0.04
4.68	0.37	2.27	0.04
4.35	0.42	2.18	0.04
4.23	0.31	2.14	0.04
3.92	0.13	2.09	0.07
3.68	0.11	2.01	0.05
3.29	1.00	1.951	0.04
3.06	0.04	1.850	0.04
2.93	0.03	1.821	0.02
2.85	0.26	1.778	0.02
2.74	0.05	1.729	0.02
2.67	0.17	1.640	0.04
2.60	0.04	1.571	0.02
2.52	0.12	1.533	0.02

Fusion Data. 4,6-Dinitroresorcinol sublimes readily to give large well-formed crystals (Figure 1). Some of the crystals show an off-center optic axis interference figure with $2V = 50°(+)$ and strong inclined dispersion, $r > v$. On further heating melting occurs with slight decomposition at 215° C. The melt solidifies spontaneously in large areas of uniform orientation separated by large gas bubbles (Figure 3). The shrinkage cracks are very characteristic. Crystals showing the "hour-glass" type of crack give a Bx_o figure; crystals showing the straight cracks show an off-center optic axis figure.

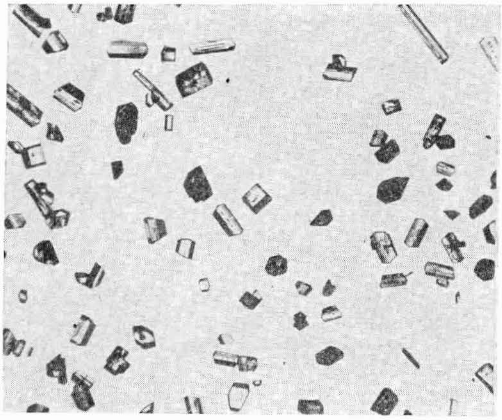


Figure 1. Crystals of 4,6-Dinitroresorcinol by Sublimation

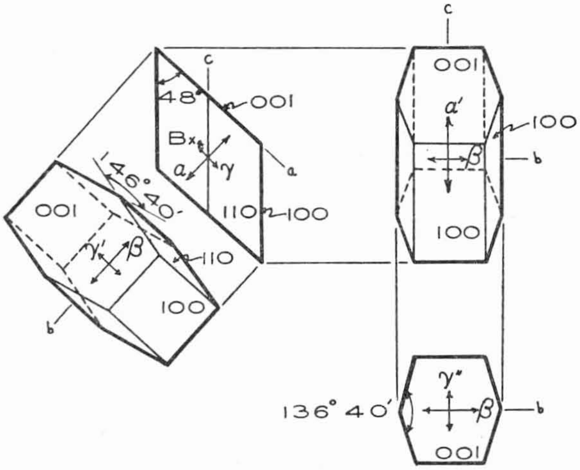


Figure 2. Orthographic Projection of Typical Crystal of 4,6-Dinitroresorcinol

Crystals of 4,6-dinitroresorcinol grow into thymol elongated parallel to *b*. The terminal angles are all about 147°. Interference figures between Bx_o and the optic axis are obtained.

ACKNOWLEDGMENT

Much of the work described above was performed under a contract between Cornell University and the Office of Scientific Research and Development during World War II. Alfred T. Blomquist was technical representative of OSRD Section B-2-A supervising progress of this work. Sien-Moo Tsang and John H. Andreen were also associated with this project and contributed to the above description.

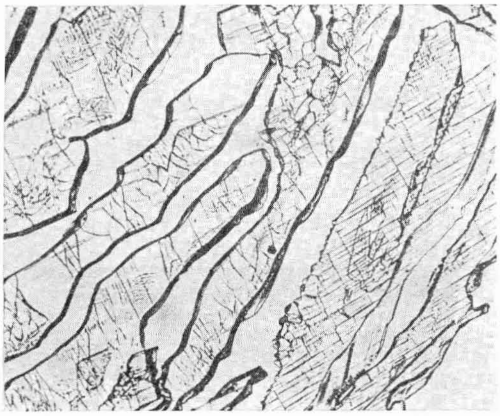


Figure 3. 4,6-Dinitroresorcinol from Fusion

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Section, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

Correspondence

Reliability of Photoelectric Photometry

SIR: In the article "Reliability of Photoelectric Photometry" [Gridgeman, N. T., ANAL. CHEM., 24, 445 (1952)] reference is made to an equation presented by Stearns (Stearns, E. I., "Analytical Absorption Spectroscopy," M. G. Mellon, ed., page 338, equation 7.11, New York, John Wiley & Sons, 1950) with the implication that the equation involves the statistically abhorrent simple addition of errors. This implication is incorrect.

In Gridgeman's article, the Martens photometer is not treated at all; the article deals only with the situation in which the error in reading the transmittance is considered to be constant over the entire transmittance scale. The familiar form of the error equation is

$$\frac{\sigma D/D}{\sigma T} = \frac{1}{T \ln T} \tag{1}$$

Gridgeman points out that if the error in setting the 100% line, considered to be equal in magnitude to the error in the test reading, is taken into consideration, the equation becomes

$$\frac{\sigma D/D}{\sigma T} = \frac{\sqrt{T^2 + 1}}{T \ln T} \tag{2}$$

He also points out that if the errors of test reading and 100% line reading are not added under the radical, the following abhorrent equation results,

$$\frac{\sigma D/D}{\sigma T} = \frac{T + 1}{T \ln T} \tag{3}$$

By the purest coincidence, this equation is practically identical in form with equation 7.11, which is

$$\frac{C\Delta S}{\Delta C} = \frac{T_s \log T_s}{1 + T_s} \tag{4}$$

since concentration is proportional to absorbance. Gridgeman correctly implies that log should be written ln in Equation 4. However, the two equations deal with two entirely different optical arrangements. Equation 4 corresponds to Equation 1, but deals with the specific case of the Martens photometer which is governed by the laws of polarizing prisms. The $(1 + T_s)$ factor in Equation 4 arises from a series of cancellations of trigonometric functions and not from any statistical reasoning, faulty or otherwise.

Incidentally, while the precision of setting the 100% line is of importance in the type of instrument discussed by Gridgeman, it is of no importance in the method of interpreting spectrophotometric curves recommended by Shurcliff and Stearns [*J. Opt.*

Soc. Amer., **39,** 72 (1949)]. The reference point is obtained by the use of a calibrated gray filter having a transmittance approximately equal to that of the sample.

Calco Chemical Division,
American Cyanamid Co.,
Bound Brook, N. J.

EUGENE ALLEN
E. I. STEARNS

SIR: The odd coincidence of the two equations, one statistically weak, the other optically correct, confused me and I see that my censure was misplaced. The assumed misprint was that "log" meant "$\log_{10}$" and should have been printed "$\log_e$" or "ln."

At the time I was encountering wrong equations, in particular the $(T + 1)$ additive-error one, often in the literature. I saw that this equation specifically referred to the Martens-Rochon setup (with which I am not familiar), but could not follow the derivation. I now understand that equation 7.11 is the counterpart of the Twyman-Lothian equation in the special circumstance of rotating-prism instruments.

I think I should have better understood 7.11 if 7.9 had been written

$$\Delta L = \sin^2 A - (T_s + \Delta T_s) \cos^2 A$$

for that is the real but hidden key to the derivation.

I agree with the last paragraph of the letter from Allen and Stearns; it ties up with the general advantageousness of comparative work with transmittancy differences minimized.

Division of Applied Biology,
National Research Council,
Ottawa 2, Canada

N. T. GRIDGEMAN

Dead-Stop End Point

SIR: The recent article of Stone and Scholten (*11*) concerning the "dead-stop" end point advances a very logical and tenable explanation of the phenomena occurring at the indicator electrodes. However, they failed to cite a number of articles dealing with the same types of electrode systems and offering similar explanations of the phenomena observed.

Attention is called to the article of Myers and Swift (*7*) in which the end point of a coulometric titration was observed by use of a pair of platinum indicator electrodes inserted in the titration mixture. Their experimental procedure involved a refinement of the dead-stop technique in that the end point was determined by extrapolation of straight lines rather than by a change in the magnitude of the indicator current. Myers and Swift were able to relate the magnitude of the indicator current to the concentrations of substances present through Fick's laws of diffusion, as had been done previously by others (*4, 5*) for somewhat similar situations. The fact that the reagent was produced by electrolytic generation with a second pair of electrodes rather than added from a buret as in Stone and Scholten's work does not alter the mechanism of the indicator electrode system. Furthermore, the latter's plot of the current *vs.* volume for a complete titration is similar to that of Wooster, Farrington, and Swift (*12*), which should have been noted.

A number of examples have appeared in which the end point of a titration is determined by a pair of identical electrodes, one of which becomes unpolarized at the end point. Among the redox couples that have been used successfully in this manner are: the iodine-iodide system (*8*), the bromine-bromide system (*1, 2, 10*), the chlorine-chloride system (*3*), and the cupric-cuprous system in chloride media (*6*). Reilley, Cooke, and Furman (*9*) have explained the dead-stop end point in terms of a three-dimensional model relating polarography, amperometry, potentiometry, and polarized end-point phenomena. The dead-stop method is considered to be a special case of the more general treatment offered.

LITERATURE CITED

(1) Brown, R. A., and Swift, E. H., *J. Am. Chem. Soc.*, **71,** 2717 (1949).
(2) Carson, W. N., Jr., ANAL. CHEM., **22,** 1565 (1950).
(3) Farrington, P. S., and Swift, E. H., *Ibid.*, **22,** 889 (1950).
(4) Kolthoff, I. M., and Lingane, J. J., "Polarography," p. 435, Interscience Publishers, New York, 1946.
(5) Laitinen, H. A., and Kolthoff, I. M., *J. Phys. Chem.*, **45,** 1079 (1941).
(6) Meier, D. J., Myers, R. A., and Swift, E. H., *J. Am. Chem. Soc.*, **71,** 2340 (1949).
(7) Myers, R. A., and Swift, E. H., *Ibid.*, **70,** 1047 (1948).
(8) Ramsey, W. J., Farrington, P. S., and Swift, E. H., ANAL. CHEM., **22,** 332 (1950).
(9) Reilley, C. N., Cooke, W. D., and Furman, N. H., *Ibid.*, **23,** 1226 (1951).
(10) Sease, J. W., Niemann, C., and Swift, E. H., *Ibid.*, **19,** 197 (1947).
(11) Stone, K. G., and Scholten, H. G., *Ibid.*, **24,** 671 (1952).
(12) Wooster, W. S., Farrington, P. S., and Swift, E. H., *Ibid.*, **21,** 1457 (1949).

ROBERT L. PECSOK

University of California,
Los Angeles, Calif.

ASTM Committee D-2 on Petroleum Products and Lubricants

THREE important publications of the American Society for Testing Materials Committee D-2 are nearing completion: "ASTM Standards on Petroleum Products and Lubricants"; a revised and considerably extended edition of "Manual of Engine Test Methods for Rating Fuels," and a new book—"ASTM-IP Petroleum Measurement Tables."

Technical Committee A on Gasoline (H. M. Smith, chairman) has revised ASTM D 439, specifications for gasoline (tentative). Research method octane number (ASTM D 908) has been adopted to replace the motor method octane number (ASTM D 357). On the basis of the winter 1951–52 Bureau of Mines survey research octane numbers of 78 minimum for regular and 85 minimum for premium-price gasolines have been selected.

The committee has been reorganized to include:

Section I. Specifications, J. M. Campbell, chairman
Section II. Volatility, R. C. Alden, chairman
Section III. Gum and varnish, S. S. Kurtz, Jr., chairman
Section IV. Sulfur and corrosion, P. C. White, chairman
Section V. Antiknock value, F. C. Burk, chairman
Section VI. Tetraethyllead, C. M. Gambrill, chairman
Section VII. Storage stability, W. R. Power, chairman

Section VII has prepared a report showing correlation of data on storage stability of gasolines with data on gasolines tested by ASTM D 525.

Technical Committee B on Lubricating Oils (W. S. James, chairman) has active work under way in Section U-III on industrial gear oils (C. L. Pope, chairman). Section U-V on instrument oils (E. H. Erck, chairman) is investigating performance, spreading, corrosion, and oxidation characteristics of instrument oils.

A Special Subcommittee on Railway Car Journal Lubrication was formed (J. J. Laudig, chairman).

Section I on Oiling Systems (F. E. Rosenstiehl, chairman) of **Technical Committee C on Turbine Oils** (F. C. Linn, chairman) plans to study rusting of turbine oil systems in service. The section also acts as a joint ASME–ASTM Committee on Turbine Lubrication, and is continuing studies preparatory to issuing "Recommended Practices on Design of Turbine Lubricating Systems" and "Preparation of Turbine Lubricating Systems for Layup."

ASTM D 665, test for rust-preventing characteristics of steam-turbine oil in the presence of water (tentative), was revised.

Further revisions to D 665 and to ASTM D 943, test for oxidation characteristics of inhibited steam-turbine oils, are planned.

Work is continuing on rotary-bomb oxidation tests to determine oil life. Other projects include correlation of neutralization values with peroxide content, development of emulsion tests, and preparation of a report on the compatibility of new and used turbine oils.

Technical Committee F on Diesel Fuels (W. K. Simpson, chairman) is continuing to study ASTM D 975, classification of Diesel fuel oils (tentative), to keep the classification in line with requirements of modern-day Diesel engine. A method for estimating cetane numbers has been appended. The committee will attempt to develop a test to differentiate wax cloud point of Diesel fuels from water or moisture cloud point.

Technical Committee G on Lubricating Grease (R. C. Adams, chairman to June 1952; J. M. Bryant, present chairman) completed long-term projects on thixotropic properties of lubricating greases, new methods for determination of lead in greases, and effect of grease on copper. Data were collected for the establishment of a high-temperature performance tester and a wheel bearing grease tester as standard equipment.

ASTM D 217, test for cone penetration of lubricating grease, was revised to include new specifications for the penetrometer and a method for testing greases worked more than 60 strokes. The method was reverted to tentative.

A study will be made to develop improved methods for the chemical analysis of constituents of grease not covered by ASTM D 128, methods of analysis of grease, and of low-temperature measurements of grease and oil separation of grease.

The committee sponsored an ASTM Symposium on Fretting Corrosion during the 50th anniversary meeting of the society.

Technical Committee H on Light Hydrocarbons (W. G. Lovell, chairman) co-sponsored a Symposium on Analytical Methods in the Manufacture and Utilization of Butadiene in cooperation with the Synthetic Rubber Division of the RFC, in Washington February 6, 1952, in conjunction with the ASTM Committee D-2 meeting.

The committee recommended that tentative ASTM methods D 1020, acetylene in polymerization-grade butadiene (silver nitrate method); D 1023, separation of residue from butadiene; and D 1025, nonvolatile residue of polymerization-grade butadiene, be advanced to standard. Specific gravity, copper strip corrosion, and water methods for liquefied petroleum gases were considered.

ASTM D 1090, factors and tables for volume correction and specific gravity conversion of liquefied petroleum gases, was withdrawn from "ASTM Standards on Petroleum Products and Lubricants." Such tables are included in "ASTM-IP Petroleum Measurement Tables."

The Natural Gasoline Association of America will be asked for recommendations relative to the commercial propane residue test (mercury freeze test) and the LPG weathering test. Liaison with the Natural Gasoline Association and with ASTM Committee D-3 on Gaseous Fuels will be continued.

Technical Committee J on Aviation Fuels (J. T. Hendren, chairman) is continuing study of the performance of aviation fuels. A new grade, 108/135, is under active consideration.

Increasing emphasis on jet propulsion fuels is showing Section VII on Jet Fuels (A. B. Crampton, chairman) has laboratory tests for the filterability of jet fuels under consideration, and plans to study smoke point tests for jet fuels.

Section II on Detonation (E. A. Droegemueller, chairman) is continuing cooperative work with the Armed Services-Industry Cooperative Group. Work on fuel rating shows progress.

Technical Committee K on Cutting Fluids (E. M. Kipp, chairman) has an active program under way in Sections I on Laboratory Evaluation of Cutting Fluids (L. H. Sudholz, chairman); II on Plant Evaluation of Cutting Fluids (M. E. McKinney, chairman); and III on Nomenclature (O. W. Boston, chairman).

A report will give the results of a testing program involving Federal Stock Specification VV-O-283, determination of active sulfur in cutting oils.

The committee is continuing study of flank wear and finish properties tests, film-strength testers, and methods for the stability of water emulsions of soluble oils and rust-preventing characteristics of water emulsions of soluble oils.

Two special sections are being considered: possible methods for surveying industrial practices in the selection and application of cutting fluids, and consulting services to Technical Committee K groups on statistical planning of laboratory test programs and evaluating cooperative test results.

Technical Committee L on Tractor Fuels (E. M. Hughes, chairman) recommended the publication of the proposed definition and specifications for tractor fuels, which appeared as Appendix VIII to the 1950 Report of Committee D-2. The definition and specifications were adopted by the ASTM this year as tentative.

Technical Committee M on Petroleum Wax (A. M. Heald, chairman) functions as a joint TAPPI-ASTM committee. A test method for tensile strength of paraffin wax has been developed.

The committee has other test methods for wax under study: bending, penetration, accelerated oxidation and direct oxygen absorption, odor, oil content, blocking, sealing strength, and scuff and gloss of wax surfaces.

ASTM D 721–47, test for oil content of paraffin wax, was withdrawn from "ASTM Standards on Petroleum Products and Lubricants" in favor of ASTM D 721–51T.

Research Division I on Combustion Characteristics (J. B. Macauley, Jr., chairman) completed a test program directed at improving reproducibility of knock ratings by elimination of the effects of barometric pressure variations.

An article in the *ASTM Bulletin* gave information on the development of micromethods for knock rating small samples. Three micromethods were published in the 1951 "ASTM Standards on Petroleum Products and Lubricants." A micromethod has been applied to the detection of impurities in iso-octane and *n*-heptane; 0.1 octane number impurity in iso-octane and 0.2 octane number impurity in *n*-heptane, when blended 50/50 with iso-octane, can be detected.

Ignition delay measuring instrumentation for use in the cetane (ASTM D 613) method was investigated. Institute of Petroleum (United Kingdom) laboratories joined in part of this work. Efforts to improve the supercharge (ASTM D 909) method included recommendation of hard-faced (Eatonite) exhaust valves, as well as investigation of valve rotators, electrical ignition system, experimental knockmeter, and chromium-plated cylinders and spark plugs. Efforts to improve D 613 and D 909 in equipment and instrumentation are continuing.

Publication of a new edition of the "ASTM Manual of Engine Test Methods for Heating Fuels" is expected.

A "check out" fuel was established for the aviation (ASTM D 614) and supercharge (ASTM D 909) methods. A more accurate calibration of leaded primary reference fuels, used as engine standardization fuels on the motor (ASTM D 357) and research (ASTM D 908) methods was obtained.

Development of new check fuels, comprised of pure hydrocarbons, for motor and aviation knock test methods was initiated.

The division is attempting to develop micrometer guide curves for the motor and research methods for rating fuels from 100 octane number up to iso-octane plus 3 ml. of TEL per gallon.

A report is planned outlining the precision of fuel rating for 1947 to 1951.

Division II on Measurement and Sampling (L. C. Burroughs, chairman) completed large projects—"ASTM-IP Petroleum Measurement Tables" and "Method of Calibrating Liquid Containers—Section A, Upright Tanks." Both were adopted as tentative.

Close contact is being maintained with the Institute of Petroleum for coordination of methods of measurement and sampling.

Methods contained in the "ASTM Manual on Measurement and Sampling of Petroleum and Petroleum Products" underwent revision. A method for sampling liquefied petroleum gases has been included.

Two new sections were formed: Section B on Gravity (J. G. Detwiler, chairman) and Section H on Water and Sediment (C. H. Lynam, chairman).

Research Division III on Elemental Analysis (C. M. Gambrill, chairman) reported that a method for the determination of mercaptan sulfur in jet fuels (color-indicator method) was adopted as tentative.

Section A on Determination of Sulfur (B. J. Heinrich, chairman) is continuing a study of methods for determining sulfur in liquefied petroleum gases and butadiene.

Section B on Determination of Chlorine (R. C. Mallatt, chairman) is continuing a study of quartz-tube combustion and sodium peroxide fusion methods for chlorine in oils and greases.

Section D on the Determination of Metals (W. C. Woelfel, chairman) is studying volumetric and colorimetric procedures for the determination of barium, calcium, and zinc in new oils.

It is planned to revise ASTM D 482, test for ash content of petroleum oils.

Section E on Trace Elements (R. O. Clark, chairman) is studying flame photometric and spectrographic methods for the determination of small amounts of metals in residual fuels.

Section F on Determination of Tetraethyl Lead (J. B. Rather, Jr., chairman) has developed a polarographic procedure for tetraethyllead in gasoline, which may eventually supplement ASTM D 526, test for tetraethyllead in gasoline (tentative). More rapid methods will be considered.

Section G on Determination of Carbon, Hydrogen, Nitrogen, and Oxygen (R. Matteson, chairman) applied ASTM D 1018, test for hydrogen in petroleum fractions by the lamp method (tentative), to the determination of hydrogen in petroleum waxes. The error introduced in applying D 1018 to testing stocks containing chlorine and sulfur will be studied.

ASTM D 809 was withdrawn from "ASTM Standards on Petroleum Products and Lubricants." It was made obsolete by the more accurate ASTM D 1091, test for phosphorus in lubricating oils, lubricating oil additives, and their concentrates.

Research Division IV on Hydrocarbon Analysis (S. S. Kurtz, Jr., chairman) conducted an investigation in cooperation with ASTM Committee D-16 on Industrial Aromatic Hydrocarbons, with respect to bromine index of petroleum source aromatics. Benzene and other aromatics for use as chemical raw materials should contain little olefin; therefore, the bromine index should be low.

A method of test for hydrocarbon types in jet propulsion fuels, fluorescent indicator adsorption method, was approved. It will be studied for its applicability to the determination of aromatics in the 400° F. plus fraction of jet fuels.

A cooperative test program is under way to evaluate a silver mercuric nitrate method for olefins in gas samples.

Tentative methods for the precise determination of density and refractive index of knock test reference fuels—iso-octane, *n*-heptane—were approved. Work is in progress on the measurement of refractive index of hydrocarbons at 80° to 100° C.

A method for determination of cracked C_4 hydrocarbons by infrared spectrophotometry will be studied.

Revisions to ASTM D 1017, test for benzene and toluene by ultraviolet spectrophotometry, are under consideration.

Research Division V on Analysis of Fuels (C. A. Neusbaum, chairman) completed studies to support the expansion of ASTM D 381, test for existent gum in gasoline (air-jet evaporation method), to cover the testing of aircraft, turbine, and jet engine fuels. Further development of the method to cover the testing of Diesel fuels is planned.

Studies to improve ASTM D 95, test for water in petroleum products and other bituminous materials, are being continued.

Research Division VI on Analysis of Lubricants (H. P. Ferguson, chairman) conducted cooperative tests on ASTM D 94, test for saponification number of petroleum products by color-indicator titration, to check the merits of *p*-xylenol blue as an indicator and the effect of saponification time.

A thin-film technique for dark-colored oils using ASTM D 974, test for neutralization value (acid and base numbers), was studied.

Cooperative tests were run on new lubricating oils to compare mixtures of cyclohexane and heptane with ASTM precipitation naphtha. The work will determine whether more uniform precipitation can be obtained from the naphtha used.

Progress was made on revisions to the methods for carbon residue of petroleum products—ASTM D 189 (Conradson carbon residue), and ASTM D 524 (Ramsbottom coking method). Further work will be done on correlation between the methods on stocks of 6% and higher carbon residue values.

Work is planned to improve the precision of the precipitation test (ASTM D 91) on all new oils, including, if necessary, a filter technique.

A special group in Section A, Newtonian Liquids, of **Research Division VII on Flow Properties** (J. C. Geniesse, chairman), is preparing a technical paper outlining the steps necessary to bring ASTM tables, charts, and viscosity methods in line with a new value for the absolute viscosity of water, which is to be adopted by the National Bureau of Standards July 1, 1953. The paper will serve as a basis for a recommendation from Research Division VII to Committee D-2.

ASTM D 445, test for kinematic viscosity, was completely revised. The revision will appear in the 1952 "ASTM Standards on Petroleum Products and Lubricants."

Research Division VIII on Volatility (G. G. Lamb, chairman) proposed revisions to ASTM distillation methods D 86, D 158, D 216, D 1160, and ASTM flash point methods D 56, D 92, and D 93.

Studies are under way on distillation procedures to give more satisfactory recoveries for products of high vapor pressure, or distillations at high altitudes. Work is continuing on the consolidation of distillation methods D 86, D 158, and D 216.

Efforts to develop a method using null-point head and manometer as a substitute for Bourdon-type gages prescribed in ASTM D 323, test for vapor pressure of petroleum products (Reid method), are continuing.

Additional reduced pressure distillation data have been supplied for the American Petroleum Institute Project on Vapor Pressure–Temperature Relationships, at Northwestern Technological Institute.

A study has been initiated to determine whether increased precision can be achieved by substituting a vacuum-jacketed column and mantle-type heater for equipment now specified in ASTM D 1160, test for reduced pressure distillation of petroleum products.

Research Division IX on Color (H. M. Hancock, chairman) is considering refinements and improvements in ASTM D 156, test for color of refined petroleum oil by Saybolt chromometer.

New glass color standards are being developed for the Union colorimeter by Research Division IX, Hellige, Inc., and the National Bureau of Standards. The new color scale features uniform differences between color standards, and spectrometric units for specifying color. The color difference between standards is smaller.

Research Division X on Corrosion Tests (F. D. Tuemmler, chairman) is studying a bomb for making corrosion tests of aviation gasolines and other products.

Section B on Humidity Cabinets (H. L. Leland, chairman) is at work developing a humidity cabinet test.

Research Division XI on Calorimetry (A. J. Kraemer, chairman), has been organized for "the promotion of knowledge of calorimetry in its application to the technology of petroleum, petroleum products, and fuels, and the development, standardization, promulgation, and improvement of apparatus and methods for calorimetric measurements."

A questionnaire was sent to laboratories engaged in oxygen-bomb determinations of the calorific value of liquid and solid fuels, requesting descriptions of equipment and procedures used.

A study panel was appointed to make recommendations regarding the formation of a section on indirect methods for estimating calorific values of liquid fuels.

The precision of the current oxygen bomb methods will be evaluated by a cooperative test program.

Research Division XII on Graphite Tests (Gus Kaufman, chairman) continued its activities on nomenclature, analysis, abrasion testing, particle size determination, and sampling. As a result of cooperative programs, considerable progress has been made on analysis, abrasion testing, and particle size. Extensive discussions have been held on definitions, sampling techniques.

The design of an abrasion tester will be completed and cooperative work done. A proposed method on particle size is to be revised for cooperative testing. A sampling procedure will be written. A proposed method for chemical analysis of graphite will be prepared as a basis for further activity.

Subcommittee I on Pharmaceutical Tests (C. F. W. Gebelein, chairman), is developing a standard suitable for calibrating the grease penetrometer used in ASTM D 937, test for penetration of petrolatum. The life of the standard (amber petrolatum) is being studied. Color standards for USP white and yellow grade petrolatums are planned.

Subcommittee XVII on Plant Spray Oil Tests (L. Mittelman, chairman) proposed revisions to ASTM D 483, test for unsulfonated residue of plant spray oils, and ASTM D 447, test for distillation of plant spray oils.

Subcommittee XXIV on Petroleum Sulfonates (C. F. W. Gebelein, chairman) developed a method for analyzing calcium and barium petroleum sulfonates which was adopted by the ASTM as tentative. ASTM D 855, methods of analysis of petroleum sulfonates (tentative), was revised. Work on both methods is planned.

Special Subcommittee on Extreme Pressure Properties Measurement (Harry Levin, chairman) has been evaluating the Timken test machine for determining the extreme pressure properties of lubricating oils and greases.

Observations based on the results of a cooperative testing program, involving the testing of twelve lubricating oils of varying extreme pressure levels in the Timken machine, were published in the April 1952 *ASTM Bulletin*. The committtee has investigated the possibility of standardizing the Timken machine by use of liquid standards of mineral oils containing the extreme pressure additive agents methyl dichlorostearate and hexachloroethane. Results obtained in a cooperative test program with greases of low, medium, and high extreme pressure ratings, indicate that the Timken machine rates the extreme pressure quality of a grease with precision equivalent to that for an oil.

Coordinating Division on Nomenclature (S. S. Kurtz, Jr., chairman) proposed revisions to ASTM D 288, definitions of terms relating to petroleum. Editorial revisions to the definitions for petroleum naphtha and Stoddard solvent will be considered.

Coordinating Division on Test Methods (F. D. Tuemmler, chairman) is studying recommended practices for applying precision data given in ASTM methods of test for petroleum products and lubricants. The division hopes to recommend the practices for adoption as tentative next year.

Coordinating Division on Research (H. P. Ferguson, chairman) developed three research projects which have been submitted to ASTM Headquarters: precipitation of insoluble sediment from furnace oils containing catalytically cracked gas oils, effect of gasoline composition on weathering losses, and storage stability of oils containing pour point depressants under fluctuating temperature conditions.

Close cooperation has been established between Committee D-2 and the Standardization Committee of the Institute of Petroleum (United Kingdom) with the object of promoting international standards and test methods for petroleum and its products.

Book Reviews

Semimicro Qualitative Analysis. *Paul Arthur* and *Otto M. Smith.* Third ed. xi + 285 pages. McGraw-Hill Book Co., Inc., 330 West 42nd St., New York 36, N. Y., 1952. Price, $4.00.

The authors have somewhat revised and rearranged the material in the second edition of their text in preparing this edition. The first half of the book is devoted to a capable treatment of chemical theories and background material. Chapters on review of elementary principles, heterogeneous equilibrium and solubility product, colloids and coprecipitation, hydrogen sulfide and metal sulfides, ionization of water, pH and hydrolysis, complexion and amphoteric substances, and oxidation-reduction reactions are included. Many examples of typical numerical problems are worked out in detail. The summary at the end of each chapter is followed by a set of review questions and problems, many with answers given.

The experimental section of the book is introduced by a short section on semimicrotechnique. The cation analysis procedure follows the conventional hydrogen sulfide system. The anion analysis divides the anions into four groups and occupies about 20% of the section. A listing of chemical properties and a set of preliminary experiments precede the outline of each group analysis. Numerous notes and questions on the procedure and a complete set of net reactions conclude the presentation.

The text gives good directions for the manipulations required and in addition presents a clear picture of the theory behind the analysis schemes. The principal objection to the text lies in its format. Illustrative problems, review questions and problems, chemical characteristics, group analysis schemes, and notes on the analysis are all set in small print. This format consolidates a large amount of information into a relatively small book which is useful for reference material but is not convenient for an everyday text. Nevertheless the authors have managed to include a wealth of material in a book which should be carefully considered by anyone selecting a text in this field.

W. WAYNE MEINKE

Polarized Light in Metallography. *G. K. T. Conn* and *F. J. Bradshaw,* Editors. xi + 130 pages. Academic Press, Inc., 125 East 23rd St., New York 10, N. Y., 1952. Price, $3.80.

This book is the first in the neglected field of polarized light microscopy of metals. The basic theory, procedures, and application of this tool are carefully discussed in seven chapters prepared by a group of experts in this field. The book covers the various optical phenomena such as reflection, refraction, and depolarization that occur at the surfaces of either metals or the nonmetallic inclusions in metals. The minor modifications required to adapt the usual metallograph or metallographic microscope are covered in detail. Finally many applications of these methods to the identification of inclusions are illustrated.

The methods are generally useful for the identification of alloy phases and inclusions as well as orientation studies on these phases. The book is written to serve as a concise handbook of techniques and applications. It is well illustrated and should be available in every metallography laboratory.

W. C. McCRONE

Ammonium Dichromate Safety Data Sheet. Important physical and chemical properties of ammonium dichromate are given in this safety data sheet. Instructions for the safe handling and use of the compound are included. A section on health hazards and their control outlines first aid measures, personal protective equipment, and precautions to be observed by workers who come in contact with the chemical. Manufacturing Chemists' Association, Inc., 15th and H Sts., Washington 5, D. C. Price, 25 cents.

Sodium, Sodium Metal, Metallic Sodium Safety Data Sheet. Sodium can be used safely if a number of hazards and dangers involved in its handling, in both its solid and molten forms, are known to the user. This safety data sheet outlines these dangers and gives specific directions for coping with sodium in its various forms. Manufacturing Chemists' Association, Inc., 15th and H Sts., Washington 5, D. C. Price, 25 cents.

NEW BOOKS

Acid-Base Titrations in Nonaqueous Solvents. *James S. Fritz.* 47 pages. The G. Frederick Smith Chemical Co., 867 McKinley Ave., Columbus, Ohio. Paper-backed copy gratis. Board-bound copies $1.00.

Theoretical Basis of the Bouguer-Beer Law of Radiation Absorption—Correction

In the article on "Theoretical Basis of the Bouguer-Beer Law of Radiation Absorption" [ANAL. CHEM., **24,** 338 (1952)] several errors were made in the equations. The correct forms of Equations 9, 10, 12, 22, 25, 27, and 28 are given here.

$$A_m = \frac{a_1n_1}{S} + \frac{a_2n_2}{S} + \ldots\ldots \tag{9}$$

$$T_m = 10^{-\frac{a_1n_1}{S}-\frac{a_2n_2}{S}-\ldots\ldots} \tag{10}$$

$$= 0.4343a'_i \tag{12}$$

$$\underline{T_m =} \left(\frac{dP}{dP_0}\right)_{b,c_1,c_2,\ldots} = \frac{\partial P}{\partial P_0} = \frac{\partial P/\partial \lambda}{\partial P_o/\partial \lambda} = \frac{P_\lambda}{P_{\lambda,o}} \tag{22}$$

$$P = \int_{\lambda_1}^{\lambda_2} P_{\lambda,o}e^{-a_1'bc_1-a_2'bc_2-\ldots}d\lambda \tag{25}$$

$$dP_B = P_{\lambda,o}e^{-a_2'bc_2-a'_3bc_3-\ldots}d\lambda \tag{27}$$

$$T_{r,m} = \frac{\partial P}{\partial P_B} = e^{-a_1'bc_1} \tag{28}$$

The equations on page 340, second column, should be:

$$P_o\left(=\int_{\lambda_1}^{\lambda_2} P_{\lambda,o}d\lambda\right)$$

and

$$P\left(=\int_{\lambda_1}^{\lambda_2} P_\lambda d\lambda\right)$$

F. C. STRONG

Third International Congress of Phytopharmacy

THE Third International Congress of Phytopharmacy met in Paris September 15 to 21, 1952. Abstracts of papers presented before Sections I and II on chemical, physical, and physicochemical studies are given here.

Polarographic Study of DDT. J. Prat and D. Bodin, Vert-le-Petit, France.

The influence of different factors on the form of the wave, its height, and the half-wave potential has been determined. These factors are: isomerism, hydrolysis, solvent, concentration in water, electrolyte, supports, etc.

Partition Chromatographic Method of Assaying the Gamma Isomer of Hexachlorocyclohexane. N. B. Van Buren, United States.

To determine with sufficient accuracy the gamma content of a product containing a high percentage of gamma isomer of hexachlorocyclohexane, a relatively simple partition chromatographic method has been established by the Association of Official Agricultural Chemists in the United States. The method does not require elaborate and expensive apparatus and most normally equipped chemical laboratories can produce satisfactory and reliable results. The equipment used is a borosilicate glass partition column, a solvent evaporator, a blender, and some Erlenmeyer and volumetric flasks as well as the usual laboratory equipment. Reagents are *n*-hexane, nitromethane, silicic acid, and a dye solution (1-hydroxy-4-*p*-toluinoanthraquinone).

Contribution to the Chromatographic Determination of Hexachlorocyclohexane. Granger, Salindres, France.

A practical process was described, using 50 grams of silica by diverse operative techniques, chromatography in the presence of nitromethane on 0.05 gram of γ-hexachlorocyclohexane, and chromatography in the presence of water on 0.2 gram of γ-hexachlorocyclohexane. Applications to the analysis of various insecticidal products, in both powder and liquid form, were given.

Promising Spectrochemical Method for Determining Chlorine in Insecticides and Other Materials. Lais Helena de Paiva Azevedo, São Paulo, Brazil, and Alston W. Specht, Beltsville, Md.

An emission spectrographic method has been developed for producing the calcium chloride band head at 5934 A., using an insecticide such as technical DDT, BHC, toxaphene, and aldrin as a source of chlorine and monocalcium phosphate as a source of calcium. This band head is produced by a special type of carbon electrode which provides for the simultaneous delivery of calcium and chlorine in the gaseous state to a direct current arc burning at 150 volts and 24 amperes. The lower limit of colored chlorine detection by this method is approximately 48 micrograms. An analytical curve derived from spectrograms produced by standard homogeneous mixtures of pure DDT and monocalcium phosphate indicates that it is possible to measure chlorine quantitatively in insecticides when the electrode charge contains between 48 and 120 micrograms of the element.

Determination of Small Quantities of Nicotine in Biological Materials. Application to the Study of the Absorption and Elimination of the Alkaloid in Subjects Exposed. R. Fabre, R. Truhaut, and Cl. Boudene, Paris, France.

Following Corcoran and his collaborators and Mme. Garreau-Perdreau (1946) the authors have applied to the determination of nicotine the color reaction which gives, with pyridine and certain of its derivatives, cyanogen bromide in the presence of an aromatic amine, such as benzidine (red-orange coloration). Several improvements have been brought about by operating at pH 5.3 in the presence of a suitable quantity of acetone. Not only is the sensitivity much increased, but because clear colored liquids are obtained, the photometric reading is greatly facilitated. For application to biological materials and especially to urine, which constitutes the way of eliminating by far the most important part of the alkaloid, distillation by steam in an alkaline medium is used and the distillate is received in a weak hydrochloric medium. After extraction by ether to eliminate certain impurities, the photometric determination is carried on. This method permits observation of subjects exposed to the absorption of nicotine. It is applicable to vegetable materials and may serve also for studying the retention of the alkaloid by plants treated with insecticides.

Determination of Aldrin in Agricultural Materials. S. Z. Perry, Louis Lykken, F. R. Brooks, A. E. O'Donnell, and E. J. Agazzi, Emeryville, Calif.

The merits of several different methods for determining the insecticide aldrin at the level of 0.1 p.p.m. were discussed. A procedure was described for a nonspecific method based on a sensitive means for determining total halogen in microgram amounts.

Results obtained with control crop samples containing known amounts of added aldrin were given. It was concluded that this method gives satisfactory results at the desired level, provided the history of crop treatments is known and interferences can be overcome.

Some Applications of Phosphorus-32 to the Study of Systemic Insecticides. D. F. Heath, Great Britain.

The use of phosphorus-32 as a radioactive indicator in the study of organophosphorus systemic insecticides has made practicable many researches which would have been very difficult or impossible to perform with only ordinary chemical methods of analysis. It is not only more sensitive and swift, but, as naturally occurring phosphorus does not interfere, much more specific. Methods involving the use of partition between immiscible solvents in analyzing plant macerates for systemic insecticides and their decomposition products were described. The general principles were illustrated by summaries of experiments performed to determine the rates of decomposition in plants of octamethylpyrophosphoramide and other systemic insecticides and the toxicities of their decomposition products, and to find a suitable method for the routine analysis for toxic residues of bis-dimethylaminofluorophosphine oxide.

Preparation of Samples of Hexachlorocyclohexane in the Light of the Determination of Their Active Product Content. J. Prat and A. Colas, Vert-le-Petit, France.

Examination of the drying curves of different hexachlorocyclohexanes under varying conditions was discussed, and conclusions were drawn regarding the preparation of samples in the light of their analysis.

New Analytical Methods for Plant Protective Materials. M. May, Berlin, Germany.

Ever since inorganic insecticides and fungicides have been utilized in phytopharmacy in the form of products for pulverization or powdering, standard methods of examination based on the study of physical or chemical properties, which have been the object of numerous publications, have been employed. But with the discovery or invention of active organic materials, and their utilization in the form of sprays or mist, the needs have increased especially for the definition of physical qualities. New methods of assay have become indispensable.

New methods were presented, with examples of (1) esters as insecticides, (2) wettable colloidal sulfurs as fungicides, and (3) hexachlorocyclohexane as a product for the treatment of sols.

1. Examination of the initial action and persistence by the *Drosophilus* test. Properties of emulsions in relation to the chemical stability of active matter (hydrolysis) and wetting power (measure of superficial tension using a torsion balance).
2. Microscopic measurement of the size of particles. Method of studying sedimentation. Assay of adherence (persistence tested by the effect of artificial rain). Distribution on plants, advantages and disadvantages.
3. Influence of the density of gas on the method of application. Different forms of application according to whether or not the gas is dense (hexachlorocyclohexane lighter than air may be buried in the soil, ester heavier than air may be utilized in sprinkling).

Preservation of Insecticides during Long Periods of Storage. P. Lapérouse, Lyon, France.

More frequent publication of the results of tests of preservation of antiparasitic products during storage would be desirable. The case of rotenone was studied as an example. The results do not show a particularly rapid destruction of the active principle. A complete review bearing on these products and a clear statement of data already obtained should be prepared.

New Process for Obtaining Insecticide Aerosols. A.-J. COURTIER AND J. BOUSCHARAIN, Paris, France.

The authors described a new process for obtaining insecticidal aerosols. The process is based on the carrying over of organic products as vapor by azeotropic distillation. Indications of the physical structure of the particles were given. The activity of the aerosols on different insects has been studied. Measurements have been made in confined space on flies, domestic crickets, weevils, and caterpillars. The practical efficacy of aerosols has been studied on the *Tineides* and in the forest on oak and pine caterpillars.

New Type of Concentrated Pesticides. J.-R. DE JONG AND N. VAN TIEL, Amsterdam, Holland.

As the existing forms of pesticides have certain drawbacks, a new form of product has been developed. The essential feature of this type of product is that, in addition to mineral oil and water, it contains a high percentage of the toxicant (40 to 50% by weight) in the form of very small solid particles; it has the consistency of a creamy paste and is easy to pour and to dilute with water. Many solid toxicants can be brought into this form.

Extensive biological investigations have shown that the most important advantages of these products are: The toxicant, in the diluted spray, has a low rate of sedimentation. There is no loss of activity due to penetration into porous substrate, which is the case with dissolved toxicants. The toxicant adheres tenaciously to the substratum. The deposited toxicant has a great resistance to rain. The residue of the spray has a high initial toxicity. The product does not contain phytotoxic or inflammable solvents or other noxious auxiliary material.

Products of this type have been employed in actual practice or in field trials with success in several cases, by both the high-volume and the low-volume technique: with DDT for the control of cotton jassids (Sudan), Colorado beetles and larvae (Netherlands), and tsetse flies and malaria mosquitoes (Uganda); with benzene hexachloride against apple saw fly, cabbage weevils, cabbage pollen beetle, and cabbage gallmidge in the Netherlands; with both DDT and benzene hexachloride as sheep dip against Psoroptes scab; with TMTD against apple scab; and with the ammonium salt of DNOC as aphicide on apple trees in early spring, and as a weed killer.

Applications of Ultrasounds to Certain Problems of the Phytopharmaceutical Industry. R.-G. BUSNEL, Jouy-en-Josas, France.

The laboratory results of the application of ultrasounds to certain problems of the chemistry of emulsions and colloids indicate that the technique of ultrasonic vibrations may eventually be extended to industry and especially to phytopharmacy.

The well known dispersive property of ultrasounds may be applied to the production of nonmiscible liquids, to the dispersion of organic or metallic compounds, to the precipitation of colloids, to depolymerization, and to the formation of aerosols. The mechanisms of action are beginning to be well known, but the physical factors (wave length, wave system, intensity) must be studied in each case.

Certain recent applications of ultrasounds are: formation of aqueous suspensions of DDT and hexachlorocyclohexane; emulsion of oils, mercury, and lead in water; production and dispersion of organic or metallic ions by ultrasonics in the course of electrolysis (copper); aerosols of aqueous solutions or oil; precipitation of colloids (sulfur).

Eighth Southwest Regional Meeting

THE Eighth Southwest Regional Meeting of the AMERICAN CHEMICAL SOCIETY was held in Little Rock, Ark., December 4 to 6, 1952, with the Central Arkansas, South Arkansas, and University of Arkansas Sections as hosts. Abstracts of papers of particular interest to analytical chemists are given here. Program chairman is Edgar Wertheim, Department of Chemistry, University of Arkansas, Fayetteville, Ark.

Spectrophotometric Determination of Aliphatic Sulfides. S. H. HASTINGS, Humble Oil & Refining Co., Baytown, Tex.

A real need exists for rapid and sensitive analytical procedures for the sulfur compound types present in petroleum and petroleum products. In response to this need, development work has been carried out on a new procedure for the determination of aliphatic sulfides based on the intense absorption shown by the iodine complexes of these compounds at 308 mμ. It has been found that iodine forms complexes with aliphatic sulfides (both acyclic and cyclic) having absorptivities at 308 mμ in the league of 50 liters per gram cm. (based on the sulfide sulfur content) for a constant iodine concentration of 0.1 gram per liter. This coefficient is relatively constant for nearly all the sulfides examined. A light-dark equilibrium phenomenon which was observed was discussed and a procedure was given which is sensitive to 1 p.p.m. of sulfide sulfur under ideal conditions.

Use of Ethylenediaminetetraacetic Acid in Polarography. CECIL H. HALE AND MARGIE N. HALE, Southeastern Analytical Chemists, Austin, Tex.

The ability of ethylenediaminetetraacetic acid to form stable complexes with a large number of metal ions makes it useful for preventing the interference of many of these ions in polarography. In a solution alkaline with tetrabutylammonium hydroxide, ethylenediaminetetraacetic acid prevents the polarographic reduction of calcium, magnesium, barium, aluminum, cobalt(II), nickel, cadmium, manganese(III), chromium(III), and zinc ions. The reduction potentials of sodium and potassium are unaffected, whereas those of lead, copper(II), and iron(III) are shifted to more negative values. Ethylenediaminetetraacetic acid itself is not reducible in tetrabutylammonium hydroxide solution. As an illustration of the application of this complexing agent, alkali metal impurities can be determined polarographically in calcium, magnesium, and barium salts. In the absence of ethylenediaminetetraacetic acid, even small amounts of calcium, magnesium, or barium ions interfere with the polarographic determination of sodium or potassium.

Polarographic Characterization of *p*-Nitroacetophenone and Related Compounds. T. S. BURKHALTER AND BOBBY G. MESSICK, Texas A. & M. College, College Station, Tex.

The polarographic properties of *p*-nitroacetophenone, *p*-bromoacetophenone, *p*-aminoacetophenone, and *p*-nitrophenacylbromide were reported. The order of reduction of the various groups, effects of pH upon the half-wave potential and diffusion currents, the number of electrons involved in the reduction, and the probable reversibility of the reactions have been determined. All reductions are apparently thermodynamically irreversible. With the exception of nitro reduction below a pH of 4, all half-wave potentials are independent of pH. The keto reduction exhibits two waves above a pH of 4 when the nitro group is para but only one wave for the bromo or amino substituent.

Identification of Polymers by Infrared Analysis of Their Pyrolysis Products. PAUL F. KRUSE, JR., AND WILLIAM B. WALLACE, Sandia Corp., Albuquerque, N. M.

Analysis of pyrolysis products of rubber and plastics materials by infrared absorption spectroscopy was presented as a method for more rapid, reliable, qualitative identification of these materials than color tests, spot tests, and chemical analytical procedures. A variety of natural and synthetic rubber and thermoplastic and thermoset plastics were pyrolyzed for 2 minutes at 830° to 8700° F. into carbon tetrachloride. The infrared spectra of the pyrolyzates were obtained, compensating for the solvent. The spectra of the pyrolyzates from several samples of each of the materials were found to contain unique features which were reproduced closely. Examples of bands which were useful in identifying the parent materials included: GR-S, 1600, 1490, 907, and 695 cm.$^{-1}$; natural rubber, 889 cm.$^{-1}$; Butyl, 1212 cm.$^{-1}$, and a doublet at 1372 and 1360 cm.$^{-1}$; Buna-N, 1740 and 1250–1000 cm.$^{-1}$; Kel-F, 1785 cm.$^{-1}$ Spectra-structure correlations were discussed, and the absence of bands was shown to be often useful. Slides of the spectra of pyrolyzates of GR-S, natural, natural and GR-S mixture, Butyl, neoprene, and Buna-N rubber and Kel-F, vinylite, and diallyl phthalate plastics were shown and discussed to illustrate the method.

Improved Results in Flame Photometry.

John T. Wiley and Thomas B. Smitherman, Texas Co., Port Arthur, Tex.

The scope of flame analysis has been extended in recent years; flame photometric analysis has become a great time and money saver in a number of laboratories, and in some cases analyses have been made which hardly could have been made by other methods. Numerous difficulties are encountered in flame photometry which, if not recognized and overcome by flame photometer operators, cause inaccurate analytical results. Conditions such as flame instability, flame contamination, contamination of solutions from various sources, effects of various solutes and extraneous cations on the flame analysis of metals, and improperly prepared standard solutions may cause serious errors, especially when determining weak concentrations of ions. Recognition of and steps to correct conditions such as these make this type of analysis a very practical analytical tool in industrial laboratories.

Study of pH Variations in the Catalytic Decomposition of Hypochlorite.

Max H. Booth and Gilbert H. Ayres, University of Texas, Austin, Tex.

A study has been made on the system in which sodium hypochlorite solutions are catalytically decomposed by compounds and/or complexes of iridium. Experimental evidence indicates that the pH of this system varies with time between wide limits, depending upon the conditions employed. The type and amount of variation have been found to be dependent upon the initial pH value of the system. Analysis of the decomposition products also indicates that the initial pH of the system determines the course of the reaction.

The catalyst is added to the hypochlorite solutions in the form of an acid solution of sodium chloroiridate, which forms a colloidal system under the experimental conditions. The stability of the resulting colloidal system is dependent upon the concentration of the hypochlorite as well as upon the initial pH of the solution. Reproducibility of the catalyst has proved difficult, presumably because of its colloidal nature.

The observed rapid changes in pH are accompanied by extreme color transformations, which may be interpreted as being due to variation of the colloidal particle size. The charge on the colloidal particles is negative in all cases. A possible explanation of the general shape of the pH-time curves was presented by postulating a series of oxidation-reduction reactions, which could account for the experimental observations.

Phosphates in Sea Water Determined by a Butanol Extraction Procedure.

Charles M. Proctor and Donald W. Hood, Texas A. & M. College, College Station, Tex.

The need for more sensitive methods for determination of phosphates in sea water is indicated by the large number of zero values obtained by present methods. The significance of phosphate in chemical and biological processes in the oceans makes its accurate determination of primary interest.

The present procedure is a modification of the butanol extraction of Berenbloom and Chain [*Biochem. J.*, **32**, 295(1938)]. To eliminate interference by silicate and arsenate, the phosphomolybdate is extracted from an acid reaction mixture into isobutyl alcohol and the alcoholic extract is washed with acid. The isobutyl alcohol extract is then shaken with stannous chloride solution to develop the phosphomolybdenum blue color in the alcohol layer. This is again washed with acid, a little isopropyl alcohol or ethanol is added to bring water droplets into solution, and the absorbance is determined at $\lambda = 730$ mμ. The color is stable for about an hour, reproducibility compares favorably with existing methods, and sensitivity is about one order of magnitude greater.

Electronic Titrimeter for Salinity Determinations.

K. E. Harwell, Celanese Corp. of America, College Station, Tex.

Research in oceanography requires the analysis of great members of samples of sea water to determine their salinity or salt concentration. A high accuracy is necessary in these analyses because minute changes in salinity reflect changes in the density of the water which cause profound effects in the circulation of the oceans. The salinity determinations are usually made by titrating the halide ions with standard silver nitrate. The errors involved in locating the exact position of the end point have given difficulty when the color-change type indicators have been used. In theory the end-point error could be essentially eliminated by the use of the electrometric method, provided that a sufficiently sensitive voltage-indicating instrument is used.

The titrator described was designed for use with silver–silver chloride electrodes but may be used equally well with other types of electrodes. It is essentially a bridge-type vacuum tube voltmeter. It uses a dual triode having a common cathode. The electrode potential is applied to one grid, a reference potential is applied to the other grid, and equality of the two potentials is indicated by a microammeter connected across the two triode plates. The reference potential is obtained from a helical potentiometer which may be read to better than 1 part per thousand. The instrument also contains a voltage-regulated power supply and a source of reference potential. It has a range of 0 to 1.5 volts and a sensitivity 1.5 mv. per division. Its simplicity and economy surpass those of commercially available instruments.

Chemical Analysis by Nuclear Magnetic Resonance Measurements.

John P. O'Meara, Southwest Research Institute, San Antonio, Tex.

The recent techniques of nuclear magnetic resonance absorption and induction have been used extensively in studies of chemical structure as revealed by chemical shifts, relaxation times, and line shape transitions in crystals. The possible use of nuclear magnetic resonances methods for qualitative and quantitative chemical analysis has received little attention as yet, although many interesting applications appear to be feasible. The various methods for detecting nuclear magnetic resonance transitions were described, and some of their advantages and disadvantages were cited. Qualitative analysis by these methods can be relatively straightforward. Quantitative analysis, on the other hand, must take into consideration several parameters such as inhomogeneity of the steady magnetic field, amplitude of the applied radiofrequency field, frequency and amplitude of the magnetic field modulation, presence of paramagnetic impurities in the sample, and physical state of the sample. The requirements of high stability and minimum noise in the associated electronic equipment are severe. Studies now in progress at Southwest Research Institute were described briefly.

Determination of Lead by Centrifugation of Homogeneously Precipitated Lead Sulfate.

Richard C. Jarnagin and Charles T. Kenner, Southern Methodist University, Dallas, Tex.

A rapid method for the determination of lead by centrifugation of homogeneously precipitated lead sulfate was proposed. The lead was precipitated by sulfate ion which was produced homogeneously throughout the solution by the hydrolysis of sulfamic acid catalyzed by the presence of potassium chlorate. The precipitate was centrifuged in special type Goetz tubes and the volume read with the aid of a cathetometer. The effects of variations in factors which would affect the particle size and the uniformity of the precipitate were studied and the precipitates were found to be more uniform than those produced by normal precipitation. The method is accurate to better than 1% of the amount present.

Reaction between Salicylaldoxime and Zinc(II).

S. H. Simonson and P. Christopher, University of Texas, Austin, Tex.

Previous studies have shown that the reaction between salicylaldoxime and zinc(II) is not suitable for the determination of zinc because the pH range for complete precipitation is very limited and the precipitate does not have a definite composition. This investigation has been made in an attempt to learn more about the composition of the precipitate. Precipitates were prepared under varying conditions of pH, digestion, and neutral salts present. The precipitates were analyzed for zinc and nitrogen and examined by x-ray diffraction methods. Amperometric titrations were made under varying conditions of precipitation.

A precipitate corresponding to zinc(II) monosalicylaldoximate was obtained by adjusting the pH of a solution containing zinc(II) and salicylaldoxime to 8.5 by the slow addition of dilute ammonium hydroxide. If the precipitation was made from solutions of lower pH, the precipitate was of indefinite composition.

Determination of Ash in Petroleum.

Philip W. West, Gerald Perkins, and Hans Suter, Louisiana State University, Baton Rouge, La.

The authors demonstrated experimentally that an error is incurred in the ash determination of crude oils if "fly ash" is not taken into account. Several experimental setups were described by which the loss of ash constituents during the combustion of the oil can be shown. With some refinements, the apparatus employed could be used for routine ash determinations. Evidence was presented that fly ash may be caused by volatilization of organometallic compounds (especially chelate compounds) during the combustion of the oil sample prior to ashing.

Isolation and Identification of Biphenyls from West Edmond Crude Oil. NORMAN G. ADAMS AND DOROTHY RICHARDSON, Ethyl Corp., Baton Rouge, La.

The possible deleterious effect of aromatic hydrocarbons on the combustion characteristics of Diesel fuels has stimulated interest in their detection in such fuels. The authors, in an earlier investigation, found strong evidence in ultraviolet absorption spectra of the presence of biphenyl and a methyl derivative of biphenyl in close-out fractions of a West Edmond crude oil. The presence of biphenyl and 3-methylbiphenyl, concentrated by silica gel absorption from a straight-run fraction of West Edmond crude oil and isolated and purified by derivative formation, has been confirmed by infrared spectra. The 2- and 4-methyl derivatives, as well as higher alkylated biphenyls, are also thought to be present in West Edmond crude oil fractions, but this has not been confirmed.

System Acetic Acid–Water-Dimethylaniline. Liquid Phase Behavior and Analysis. LEO GARWIN AND PHILIP O. HADDAD, Kerr-McGee Oil Industries, Inc., Oklahoma City, Okla.

Analysis, by simple means, of the ternary system acetic acid–water-dimethylaniline became of interest during a study of methods to improve the separation of acetic acid and water. Two properties are needed to fix the composition of a ternary system. Those selected for study here were the refractive index of the ternary mixture and direct titration of the acetic acid by standard alkali. The results obtained show this procedure to represent a convenient and accurate method, and one particularly suited for routine determinations.

The solubility envelope for the ternary system and tie line compositions were determined as an incidental part of the program. The data indicate that dimethylaniline possesses only slight selectivity for acetic acid relative to water in a liquid-liquid extraction process at 25° C.

Determination of Serum and Tissue Polysaccharides by the Anthrone Reaction. M. R. SHETLAR AND VIRGINIA RICHMOND, University of Oklahoma, Oklahoma City, Okla.

Blood serum polysaccharides have been shown to be polymers of galactose, mannose, and glucosamine. These polymers are conjugated with serum protein. Tissue also contains, in addition to polysaccharides of this type, glycogen and polysaccharides containing uronic acids and glucosamine.

The use of anthrone as a quantitative reagent for serum and tissue polysaccharides is under investigation. The reaction of anthrone with serum protein results in an absorption curve with maxima at 520 to 530 mμ and at 620 mμ, while only the maximum at 620 mμ is present in pure hexose-anthrone curves. The maximum found in the serum-anthrone curves at 520 to 530 mμ was shown to be due to an interaction between anthrone, tryptophan, and carbohydrate.

In the presence of excess tryptophan, the absorption maximum at 620 disappeared, and that at 520 increased. Under these conditions the absorption at 520 is quantitatively related to the carbohydrate concentration. The use of this interaction was studied as a basis for a new colorimetric procedure for the estimation of polysaccharides in the presence of protein.

Methods for the Microdetermination of Mixed Halogen and Amide Group. L. I. DIUGUID AND N. L. CRUMP, Du-Good Chemical Laboratory, St. Louis, Mo.

Determination of mixed halogens. A micromethod for the determination of chlorine in the presence of bromine or iodine in organic compounds has been developed by the modification of the procedure described in a previous paper ("Modifications in the Micro Carbon, Hydrogen, Alkoxyl, and Halogen Determinations," presented at 115th Meeting AMERICAN CHEMICAL SOCIETY, San Francisco, Calif., April 1949). The micromethod consists of: (1) bombing the sample (micro-Parr), precipitation of the halogen ions with excess silver nitrate, and filtering and weighing the precipitated silver halide (AgCl, AgBr, and AgI). (2) The weighed silver halide (mixed) is subjected to chlorination at elevated temperature (120°) for a short time and thus the mixed halide is converted quantitatively into silver chloride. From the weight of the mixed silver halide and the weight of the silver chloride after chlorination, the percentage of chlorine, bromine, or iodine can be computed. The method is applicable to a wide range of compounds containing mixed halogens.

Determination of Primary Amide Group. A rapid micromethod for the determination of primary amide group of the type $RCONH_2$ (R = alkyl or aryl) has been developed by a modification of the procedure for the micro-Kjeldahl nitrogen determination. The primary amide group can be selectively determined in the presence of other nitrogen-containing groups such as ring nitrogen, primary, secondary, and tertiary amines with no interference. Hydrolysis is accomplished by refluxing the amide with dilute sulfuric acid for 30 minutes or longer, if required. A blank titration is required for the presence of ammonium salts should their presence be suspected. The method has been successfully employed on a wide range of primary amides. Solubility and unreactive type compounds present limitations to the method and require further modification of conditions stated.

Punch Card System for Chemical Classification Studies of Surface and Underground Waters. MARYANN DUGGAN AND A. V. METLER, Magnolia Petroleum Co., Dallas, Tex.

Results of mineral analysis of water samples may be applied to a variety of problems in the petroleum industry. Some of these applications are concerned with identification of unknown geological structures, calculation of electrical resistance values in well-logging procedures, location of subsurface leaks in cased wells, or establishing treating programs for complex brines. Samples of many different types of water from a number of mechanical, geological, or geographical sources were analyzed for the major dissolved components. The results of the mineral analysis may be expressed in ionic concentrations, hypothetical combinations, or other methods indicative of chemical proportionation of the dissolved ions. Graphical presentation of the dissolved components in natural waters permits a rapid identification of specific waters.

The large number and the variability of types of waters encountered necessitated the development of a Keysort punch card system for effective filing purposes as well as for chemical classification studies of mineral waters. An 8 × 10.5 inch card was selected, so that all information and chemical data regarding the sample could be entered on one side of the card. Waters may be sorted on the basis of geological or geographical location as well as by chemical composition or behavior. Space is provided on the card for the graphical presentation of the proportionation of components in each sample. The selection of sorting codes for each individual property of the water was described.

The simplicity and flexibility of the system should prove of value in establishing principles of geological, geographical, and chemical interrelationships of natural waters.

Serum Protein-Bound Iodine Determination. CARLETON W. SHAW, Shaw Laboratories, Birmingham, Ala.

The European 19th century discovery of iodine in the thyroid gland established a relationship between the ancient seaweed treatment for goiter and iodine. The need for a reliable, economical method became apparent. Investigations have continued for a chemical method or methods whose results would reflect the clinical condition of the patient. Approximately 90% of the blood serum iodine behaves like thyroxine in its solubility properties and the concentration of this iodine depends almost directly upon the degree of thyroid activity.

The technique of the Chaney procedure modified by Conner consists of four main steps: (1) precipitation of the serum protein by $ZnSO_4$ and NaOH, (2) digestion and oxidation of the precipitate by a chronic-sulfuric acid mixture, (3) reduction and distillation of the iodine in the presence of phosphorous acid, and (4) estimation of the amount of iodine by its catalytic effect upon ceric sulfate–arsenious acid mixture under carefully controlled conditions. The reaction results in a blanching effect on the ceric sulfate in proportion to the amount of iodine present.

The procedure, being ultramicro in character, requires the utmost care throughout. One of the main problems is the variable activity of different lots of phosphorous acid. This remains a problem to be investigated in greater detail. Recovery of added iodine to beef serum in the range in human protein-bound iodine is 100 ± 10% and is usually not more than ±5%. The procedure has been found reliable and clinically acceptable. Normal values with this procedure range from 3.5 to 6.0 gamma %.

Determination of Free and Combined Amino Acids. ROBERT L. POLLACK AND CHARLES H. EADES, JR., University of Tennessee, Memphis, Tenn.

Difficulty in obtaining analyses of "free" amino acids in urine is overcome by destroying the urea without hydrolyzing the "bound" forms of the amino acids. The urease treatment and the microbiological methods for determining the free and bound amino acids were described in detail. Several organisms are used in parallel assays to ascertain some of the microbiologically available amino acids that are in the combined form. Analyses of hydrolyzed samples of urine yield total amino acid content both free and bound.

Statistical Analysis and Determination of the Thallous Ion. John G. Surak and David A. H. Roethal, Marquette University, Milwaukee, Wis.

During the past few years thallium has come to assume a much more important position among the elements. A prime cause of this phenomenon was the second world war and its unforeseen demands for materials possessing special properties which could be used in furthering the war effort. Recourse was made to thallium probably because of its similarity to lead and much was added to the limited knowledge of the element. New ways for determining thallium were devised and these, along with older methods of analysis, afforded a variety of ways in which to compute the amount of thallium present in a particular substance. In such a situation one procedure must be superior to the others with regard to accuracy, precision, and ease of manipulation. The problem is to find which one this might be. Statistics affords the chemist an accurate and sure means for making this decision. A statistical study of several procedures were presented in an attempt to decide which method for quantitatively determining the thallous ion is best qualified to do the job.

Further Investigation of Molecular Association by the High Frequency Oscillator Method. Joseph C. Trantham and Roby B. Bevan, Baylor University, Waco, Tex.

An investigation on utilization of a high frequency oscillator for measurements of molecular association was extended to solutions of several substituted phenols and corresponding anisoles in benzene or chloroform. The object of this work was to establish a firm basis for the method, so that the measurements could be made on various other types of compounds with confidence in the significance of the results. In these measurements, as in the case of phenol and anisole, the compounds which were known to undergo molecular association produced curves when the degree of oscillator loading (in terms of microamperes change of plate current) was plotted as a function of concentration. Substances which did not associate gave straight-line graphs. During the measurements improvements in oscillator design were accomplished, resulting in an instrument possessing greater stability, higher sensitivity, and temperature control.

Determination of the Molecular Weight of Gum Arabic by Light Scattering. H. B. Williams and Eugene W. Berg, Louisiana State University, Baton Rouge, La.

The molecular weight of a thrice recrystallized commercial preparation of gum arabic was found to be approximately 3×10^5 by light scattering techniques. The depolarization values indicated that the anisotropy of the molecule could be ascribed almost completely to intrinsic anisotropy of structure and not to size or shape. These results were established for the acid gum in water solution at pH 4.0. The theory and techniques of the method were reviewed. The probable size and shape of the gum arabic particle under the stated experimental conditions were discussed.

The Analyst's Calendar

Symposium on Modern Methods of Analytical Chemistry

THE fifth annual Symposium on Modern Methods of Analytical Chemistry will be held February 2 to 5, 1953, at the Louisiana State University, Baton Rouge, La. In addition to the formal program, time will be provided for informal discussions. Representatives of scientific supply houses will be present, and exhibits of instruments are planned.

Chemistry of Specific, Selective, and Sensitive Reactions and Analytical Research. Fritz Feigl, Rio de Janeiro.

Chromatography. Harold H. Strain, Argonne National Laboratory.

Electroanalysis. Paul Delahay, Louisiana State University.

Microscopy in Research and Analysis. Claude Arceneaux, Ethyl Corp.

Analytical Development in the Petroleum Industry. Louis Lykken, Julius Hyman & Co.

Infrared Spectroscopy. Van Zandt Williams, Perkin-Elmer Corp.

Promoting Analytical Chemistry. C. E. Starr, Jr., Standard Oil Development Co.

Quantitative Organic Microanalysis. E. W. D. Huffman, Huffman Microanalytical Laboratories.

Aquametry. John Mitchell, Jr., E. I. du Pont de Nemours & Co., Inc.

Analytical Uses of Complex Ions and Molecules. Philip W. West, Louisiana State University.

Preregistration is requested where possible. Correspondence should be addressed to Philip W. West, Coates Chemical Laboratories, Louisiana State University, Baton Rouge, La.

Society for Applied Spectroscopy

The Society for Applied Spectroscopy will meet on January 6 at the Socony-Vacuum Training Center, 63 Park Row, New York, N. Y., preceded by dinner at 6 P.M. at Tosca's, 118 Fulton St. Frank Hochgesang, Socony-Vacuum Laboratories, will speak on "Computation of Analytical Results." C. A. Jedlicka, Lucius Pitkin, Inc., 47 Fulton St., New York 7, N. Y., is secretary of the society.

Philadelphia Meeting-in-Miniature

The fifth Meeting-in-Miniature of the Philadelphia Section, American Chemical Society, will be held on January 29, 1953. The program will include an Analytical Section headed by J. N. Bartlett, St. Joseph's College, Philadelphia 31, Pa. Abstracts of the papers will be available at the meeting.

Priestley Lectures

The 27th Annual Priestley Lectures, sponsored by the Mu Chapter of the Phi Lambda Upsilon honorary chemistry fraternity, will be given by James J. Lingane, Harvard University, April 20 to 24, 1953, at the Pennsylvania State College, State College, Pa. The subject of the lectures will be "Innovations in Electroanalytical Chemistry."

Sixth Annual Symposium on Modern Methods of Analytical Chemistry. Louisiana State University, Baton Rouge, La., February 2 to 6, 1953

Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. William Penn Hotel, Pittsburgh, Pa., March 2 to 6, 1953

American Chemical Society. 123rd National Meeting, Los Angeles, Calif., March 15 to 19, 1953

Sixth Annual Summer Symposium. Rensselaer Polytechnic Institute, Troy, N. Y., June 1953

AIDS FOR THE ANALYST.....

Circular Slide Rule for Calculating Spectral Intensities.

Leo D. Frederickson, Jr., Aluminum Research Laboratories, New Kensington, Pa.

CALCULATIONS in quantitative emission spectrography are usually accomplished through the use of graphical-type calculating boards which employ rectangular coordinate scales to convert per cent transmittance or density readings to the required logarithmic intensity values. Many variations of this type of calculator have been described in the literature and are available commercially, some of which are designed to utilize directly charts made on a particular recording densitometer. Because cost and limited utility of certain of these commercial types are important, especially where nonrecording densitometers are used, the present slide-rule calculator (Figure 1) was designed and constructed. It provides the necessary scales for making the essential spectrochemical calculations in a portable, compact unit at a minimum expense.

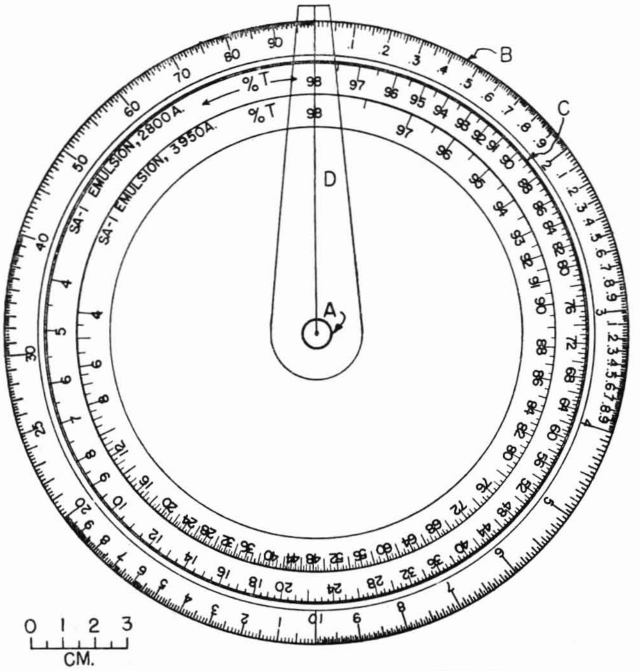


Figure 1. Spectral Intensity Calculator

The circular slide rule consists of two disks placed together and moving independently on a central pivot, *A*. The larger, or outer disk, *B*, contains a 2-cycle logarithmic scale around its circumference. The smaller disk, *C*, has similarly located per cent transmittance scales, which are in effect the emulsion calibration curves for the indicated emulsion type and wave-length region. A movable vernier arm, *D*, allows for setting the scale index and determining the desired relative intensity for any given per cent transmittance value.

Construction of the slide rule has made use of heavy white-backed cardboard, which is convenient for lettering the scales with India ink. The vernier arm is of transparent plastic with an engraved hairline for accurate positioning. New disks are readily prepared when emulsion calibration changes make this necessary, although a single disk may contain several calibration scales on its surface. This is illustrated in Figure 1, where disk *C* contains calibration scales for an Eastman S.A.-1 emulsion at both 2800 and 3950 A. A variety of scales may be easily prepared by using any suitable duplicating process to provide paper impressions which are subsequently affixed to a durable disk material such as wood, plastic, or metal.

The log scale was drawn on the circumference of disk *B* with the aid of a drafting divider; suitable short distances were measured with the instrument on any desired length of a straight-line log scale, and this increment was transferred to the disk circumference. The smallest increments taken will produce the most accurate disk scale. It is not essential that the log scale extend completely around the circumference of the disk, although in the interests of good appearance and maximum distance between scale divisions this has been done. The calibration scales are prepared with the assistance of the emulsion calibration curves drawn on semilog paper. The highest practicable per cent transmittance value from the paper plot is drawn on the smaller disk scale opposite the index on scale *B* (98% *T* opposite 1 in the figure). While the two disks are maintained fixed with respect to each other, the vernier arm is moved to the relative intensity value on disk *B* corresponding to a transmittance of 96% on the graph paper plot. A scale mark is drawn on disk *C* representing 96% *T*. Successive repetitions of this procedure every 1 to 2% result in the complete calibration curve being transferred to the disk scale.

The accuracy with which such a calculator can be used is governed chiefly by the length of the scales employed. This rule may be expected to provide reading accuracy comparable to a large calculating board, since an equal length of scale is contained in a smaller working area.

The unit is also adaptable for use in place of the Dunn-Lowry type of calculator when it is desired to convert per cent transmittance figures for an analytical line directly into per cent concentration. In this application a separate disk may be used to support two three, or more analytical line slopes.

Automatic Spectrophotometry of Paper Strip Chromatograms.

T. V. Parke and W. W. Davis, Lilly Research Laboratories, Indianapolis, Ind.

COMPONENT spots on a paper strip chromatogram have been detected by microbiological activity, fluorescence, reactivity with a reagent, or ultraviolet or visible light absorption. Several devices have been described for spot detection using light

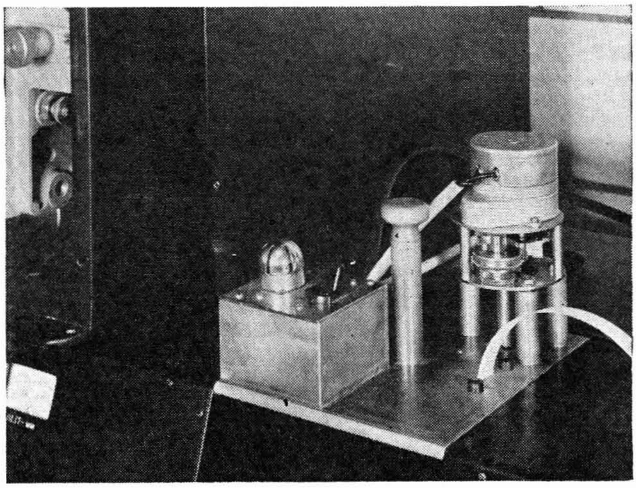


Figure 1. Paper Strip Chromatogram Scanning Device

In position for use in Cary recording spectrophotometer absorption (*2–4*). The present procedure utilizes the high sensitivity and flexibility of the Cary recording spectrophotometer to scan the paper strip at predetermined wave lengths and to obtain an absorption spectrum of individual separated spots.

The device shown in Figures 1 and 2 has been constructed to replace the sample cell-well cover of the Cary spectrophotometer and to provide a means of transporting a 0.75-inch paper strip vertically across the beam in synchronism with the movement of the recorder chart. A second track unit utilizing paper strips down to $^{17}/_{32}$ inch in width can be readily interchanged for the 0.75-inch track unit. When $^{17}/_{32}$-inch paper strips are used, the beam scans about 93% of the width of the strip. The paper strip is held in a channel close to the phototube port to utilize as large a solid angle of scattered radiation as possible. In this way most of the light passing through the paper is collected by the photomultiplier, in spite of the fact that essentially all of the light has been scattered. A simple adjustable slit variable from 0 to 0.5 inch permits variation in "resolution" of spots on the chromatogram. The strip is transported by rubber-covered rollers driven by a synchronous clock motor at 3 r.p.m. The rollers bear against small free-turning brass rollers. A 15-inch paper strip can be scanned in approximately 2 minutes.

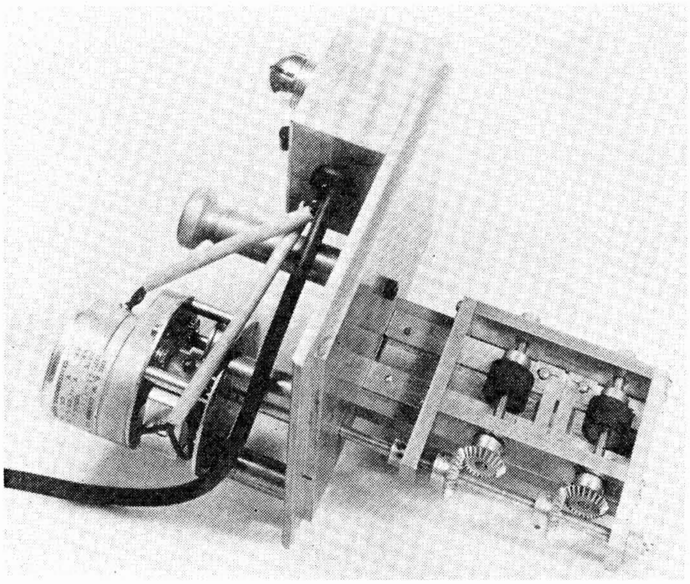


Figure 2. Paper Strip Transporting Mechanism of Scanning Device

The only needed alteration of the spectrophotometer is the milling of a slot $^{3}/_{16} \times {}^{7}/_{8}$ inch in the plate between sample and reference cell compartments to permit the scanned strip to pass into the lower reference compartment. Measurements can be made with the selector switch in A.C. position and with the reference cell compartment door open, in which case the reference beam is not used. More quantitative data are obtained by using a similar piece of filter paper in the reference beam and operating the spectrophotometer in the conventional manner. When the reference beam is used in this way, it is necessary to keep the emerging paper strip from interfering with the reference beam. It has been found convenient to use a small sheet metal tube carrying an iris diaphragm and the reference filter paper in order to shield the reference beam from the emerging filter paper strip, to hold the filter paper reference, and to permit adjustment of the reference beam intensity. The reference paper must be placed close to the phototube port, so that the solid angle of scattered radiation intercepted by the phototube is essentially the same as in the sample beam.

Absorption spectra of spots of the initial mixture or resolved components may be obtained directly from a stationary paper strip. For this purpose it is necessary to use a comparable piece of paper in the reference beam as described above. A spectrum of the initial mixture made either from a solution or from a spot of the mixture on filter paper indicates the wave lengths at which to scan the strip. Absorption spectra on the separated spots serve to identify the separated components.

The record obtained by scanning the paper strip across the beam at a fixed wave length shows changes in absorption with distance along the strip. The absorption of spots must be significantly higher than random variations in absorption and scattering of the paper to permit ready recognition of the spots. This involves both the amount and absorptivity of the component. For quantitative work, it has been found desirable to run a "baseline" scanning curve on each individual strip. The strips for such quantitative chromatographic analyses are numbered and scanned in advance at each wave length to be used in scanning the developed chromatograms.

These techniques have been applied to the identification of antibiotics, the separation and identification of alkaloids, and the simultaneous quantitative analysis of four water-soluble vitamins (*1*).

ACKNOWLEDGMENT

The authors gratefully acknowledge contributions to this project by F. R. Van Abeele, J. A. Brown, and Elmer Lee.

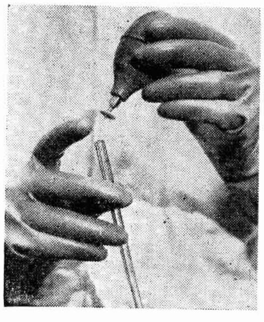
LITERATURE CITED

(1) Brown, J. A., and Marsh, M. M., Anal. Chem., **24**, 1952 (1952).
(2) Haines, W. J., and Drake, N. A., *Federation Proc.*, **9**, 180 (1950).
(3) Sober, H. A., and Kegeles, G., private communication, 1951.
(4) Tennent, D. M., Whitla, J. B., and Florey, K., Anal. Chem., **23**, 1748 (1951).

Presented before the Division of Analytical Chemistry at the 122nd Meeting of the American Chemical Society, Atlantic City, N. J.

Simple Pipetter. S. L. Hood, University of Tennessee Atomic Energy Commission Agricultural Research Program, Oak Ridge, Tenn.

Hand pipetters are necessary in handling radioactive, toxic, or fuming liquids and are useful generally. A simple hand pipetter has been developed and used for over 2 years by workers in this laboratory. It is preferred over several other such devices available. The flexibility and ease of control of the old method of mouth pipetting are retained. The pipetter is inexpensive, made quickly from common materials, requires no experience to use, and can be used with any macropipet. It is practical to have a large number available so that one can be set aside or discarded if contaminated.



The pipetter, shown in the illustration, is assembled from a 1-cm. rubber disk cut from Gooch tubing, a rubber bulb of 10- to 30-ml. capacity, and a No. 18 to 23 hypodermic needle. The smaller sized needles are best used with the 10-ml. bulbs. The needle is broken off to $^{3}/_{16}$ inch in length, and the tip filed smooth. The needle shank is inserted into the bulb, and the pierced disk slipped on the tip.

Flexible 1- to 4-ounce plastic bottles can be substituted for the rubber bulb, although they are stiffer than the bulbs. The needle is inserted through the cap from the inside out and the cap screwed on tightly. The rubber disk must be used, as the cap is not flexible enough to make a seal with the pipet.

In use the pipet is manipulated as in ordinary mouth pipetting. The pipetter is held in one hand, and the pipet in the other. The needle is inserted as a guide into the bore of the pipet, the rubber disk making a seal. Suction to fill or pressure to blow out is applied as desired. When the pipet is filled the pipetter is removed, and the meniscus level controlled by finger as usual. Pipets larger than 15 ml. require more than one draw with the pipetter to fill.

ANALYTICAL CHEMISTRY

Volume 24—1952

Published by the
AMERICAN CHEMICAL SOCIETY
1155 Sixteenth Street, N.W., Washington 6, D. C.

Sheets of errata for 1952, arranged so that they may be clipped out and pasted over the incorrect material, are available without charge from the reprint department, American Chemical Society, 1155 Sixteenth St. N.W., Washington 6, D. C.

AUTHOR INDEX

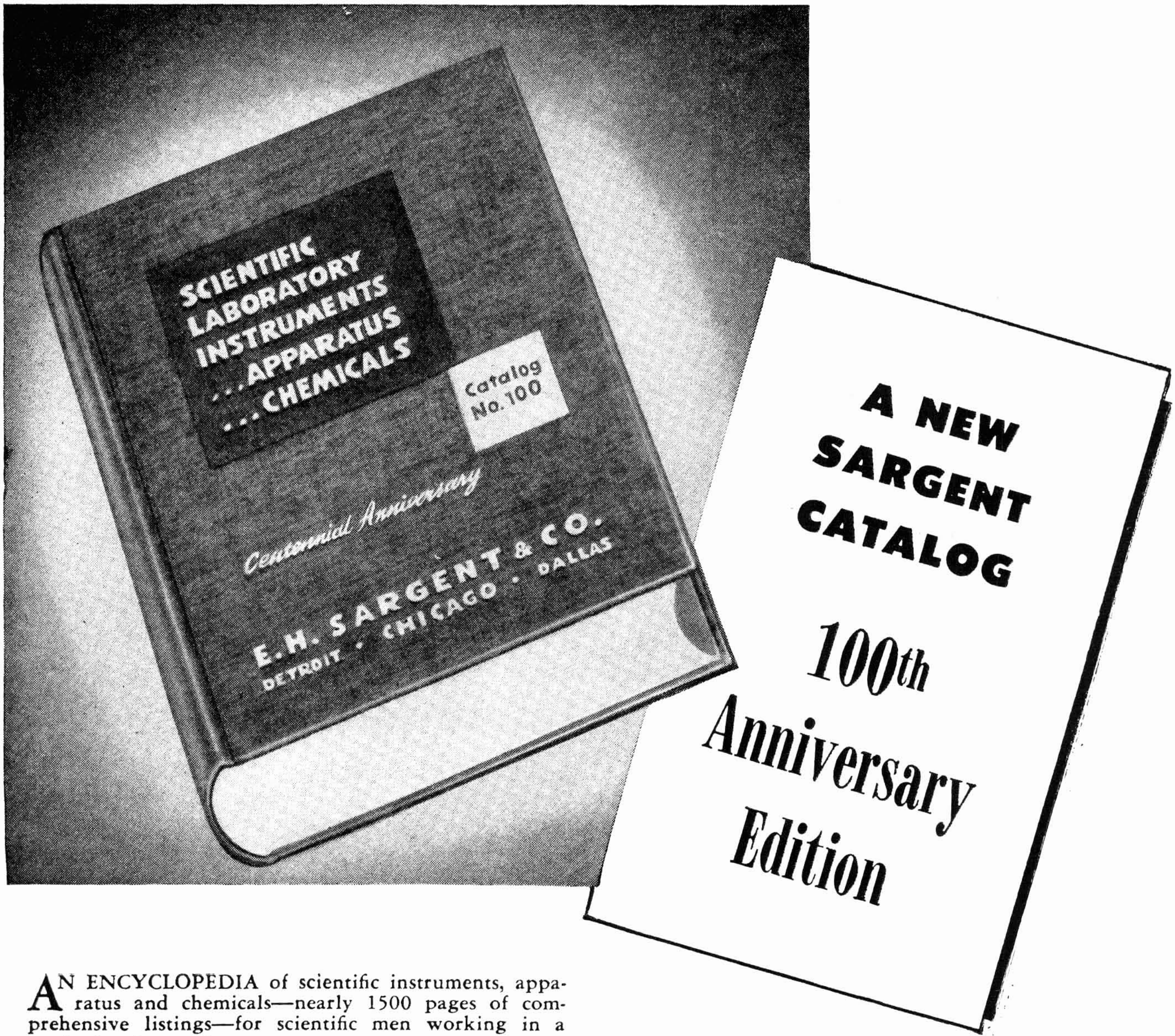
VOLUME 24—1952

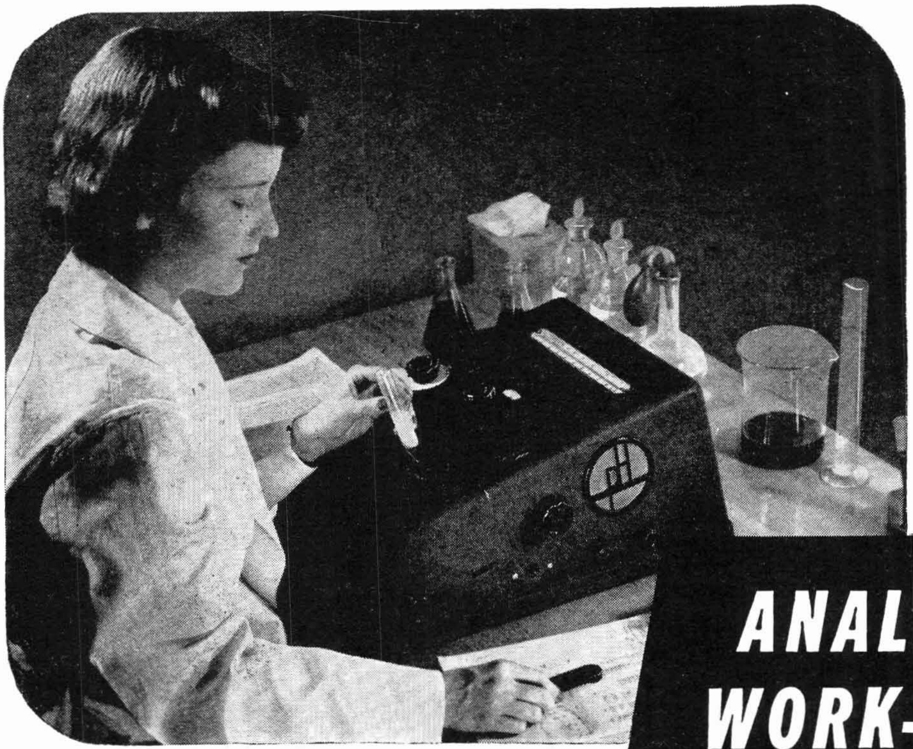
Numbers followed by letter "A" refer to pages in the advertising section

SUBJECT INDEX

VOLUME 24—1952

Numbers followed by letter "A" refer to pages in the advertising section


SCIENTIFIC LABORATORY INSTRUMENTS ...APPARATUS ...CHEMICALS
Catalog No. 100
Centennial Anniversary
E. H. SARGENT & CO.
DETROIT · CHICAGO · DALLAS
A NEW SARGENT CATALOG
100th Anniversary Edition



"It's the 'Work-Horse' of our laboratory. We have a number of analytical instruments, but it is the Model 14 that really carries our load. It's working all day, every day and it turns out more and better work than any other instrument we have."*

ANALYTICAL WORK-HORSE

The Coleman Universal has always been known as a "Working Instrument" for it performs routine spectrophotometric measurements faster and easier than any other instrument made. Yet it has the flexibility to meet the special problems that arise in every laboratory; long-path cells for trace determinations; fluorescence attachments for vitamins or other fluorophors; micro accessories for small sample quantities; an adapter for nephelometry; and measuring circuits of a precision not exceeded by *any* instrument of its kind.

Here's why the COLEMAN is so convenient

Use of a diffraction grating	The diffraction grating creates a true undistorted spectrum, unlike prisms whose spectrums appear condensed in the short wavelengths, much wider in the longer ones.
Allows using a fixed bandpass	The true spectrum used in the Universal, makes possible a fixed slit. The bandpass is 35 mu and it is constant at *all* wavelengths. Only Coleman Spectrophotometers fully select color with a *single* dial, and without secondary adjustment of the slit.
For easier operation and full reproducibility	The Coleman's fixed Band-pass allows *valid* use of calibration curves, eliminates need for continual preparation and use of concurrent standards; insures reproducibility, simplifies and speeds instrument operations. No vacuum tubes and no mechanical linkages means lower maintenance, greater reliability.

*Name on request.

For complete details write to:
Coleman Instruments, Dept. A,
318 Madison St., Maywood, Ill. ----Ask for Bulletin B-212

COLEMAN *UNIVERSAL* SPECTROPHOTOMETER

INSTRUMENTATION

Newly developed high-precision resistance thermometer recorder with automatic range extension will have many important applications in analytical chemistry

by Ralph H. Müller

On numerous occasions we have referred to the constant improvement of recording instruments. One outstanding development is the new Leeds and Northrup Speedomax high-precision resistance thermometer recorder with automatic range extension. This instrument combines the precision of a laboratory bridge with an automatic recorder and covers the entire platinum resistance thermometer range from −260° to +500° C. within ±0.01° C. We are indebted to J. C. Melcher of the Leeds and Northrup Co. for advance information on this instrument and to the company's associate director of research, A. J. Williams, Jr., for a reprint of his recent publication concerning further details (*Trans. Am. Inst. Elec. Engrs.*, September 1952).

The instrument is based on an earlier development by D. R. Stull of the Physical Research Laboratory, Dow Chemical Co., Midland, Mich. [*Rev. Sci. Inst.*, **16**, 318 (1945)]. This study showed the advantage of automatic range extension of a recorder. As embodied in the Leeds and Northrup instrument, it combines the precision of the manually balanced Mueller bridge with the reliability of the Speedomax recorder. Basically the instrument is a d.c. Wheatstone bridge, single-point, curve-drawing recorder with a chart reading 0 to 1 ohm with 100 divisions plus 1 division overtravel at both ends of the chart. For deflections above and below the nominal chart span (0 to 1 ohm) decade switches are actuated automatically and as required, and the integral values so selected are printed to the nearest ohm in the left margin of the strip chart. In effect, then, whenever a resistance is presented to the input terminals of the recorder within its range of 0 to 100 ohms (absolute), the decade selector will print the resistance value to the nearest ohm and the pen chart system will interpolate to the nearest 0.001 ohm. The accuracy is 0.01% of the reading or 0.003 ohm, whichever is greater. As L. & N. has so effectively pointed out, this provides an effective chart width of 75 feet, or more than the combined width of three 24-sheet outdoor billboards! Although we hope our readers will consult the above references, it may be of interest to note here how some of the difficulties in attaining this high precision have been met.

The design of this instrument is justified by the fact that the platinum resistance thermometer is the interpolating device for the 1948 International temperature scale from −182.97° to +630.5° C. When properly made and aged, the reproducibility of its resistance justifies its calibration and use for measurements to 0.01° C. or 1/80,000th of its temperature range. Heretofore, the requisite precision in measurement could be attained by the Mueller bridge, but with increasing use of the platinum resistance thermometer in measuring freezing points, boiling points, cooling curves, etc., the labor of following changes manually is enormous, and one may well miss important deflections.

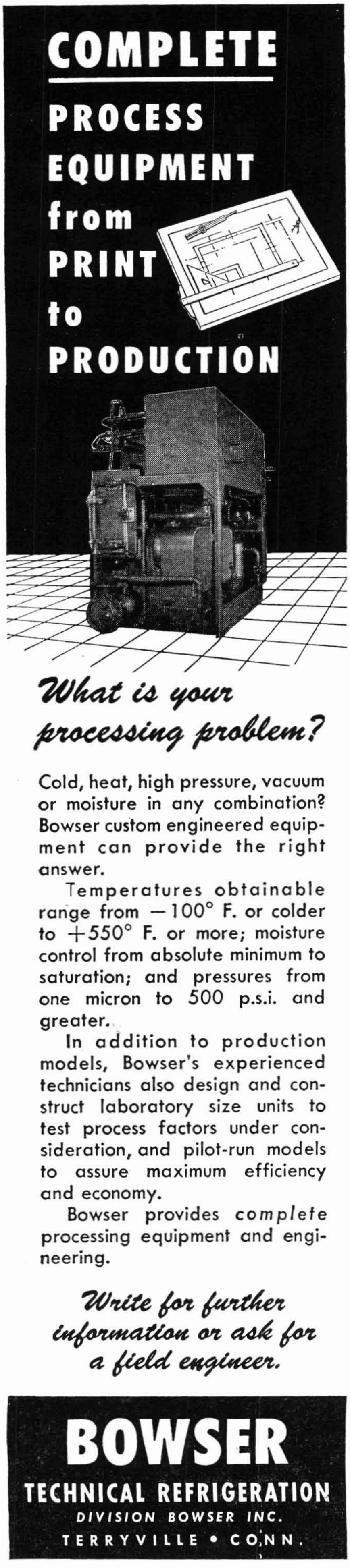
Bridge Circuit. Among the elegant features of this instrument is the bridge circuit proper. The 1-ohm and 10-ohm decades are in the same arm as the resistance thermometer; hence a substitution type of measurement is made. The effect of contact resistance in the 10-ohm decade is diminished by a factor of 5 by placing it in a relatively high-valued ratio arm, and in the 1-ohm decade, contact resistance errors are diminished by a factor of 6 or more by the use of a shunted decade similar to the Mueller bridge. The substitution principle provides constant sensitivity; the use of a simple Wagner ground avoids the need for checking and adjusting the ratio arms specifically. All critical resistors in the bridge network are enclosed in a box, the temperature of which is controlled to within 1° C. As the instrument requires a constancy in these resistors to 1 part in 100,000, this is accomplished by temperature control and choice of resistor material. The control point is chosen as 55° C. because the temperature inside the recorder may rise 15° above a possible ambient temperature of 40° C. All resistors were made to have a minimum temperature coefficient at 55° C. by making them partly of copper and partly of Manganin, in series combination. The slide-wire is continuous (not wound on a mandrel) and thus places no limit on the sensitivity of the recorder. It is housed in a magnetic shield. For factory checking and in intercomparison of the various decade units, a microscope attachment permits reading slide-wire displacements to 0.001 inch or 1/15,000th of total travel. This permits checking to within 0.00001 ohm for calibration purposes, whereas in actual use a sensitivity of 0.001 ohm is adequate.

A reversible capacitor-type motor drives the print wheels. A Geneva movement drives the decades. Trip switches start the motor whenever the pen approaches the upper or lower limits of its travel. The trip switches are set to allow the pen to travel a little more than 1.00 ohm without tripping, in order to avoid frequent switching of the decades when the thermometer resistance is hovering around some integral value.

The balancing amplifier in the recorder embodies many of the advanced design features previously described [Williams, A. J., Tarpley, R. E., and Clark, W. R., *Trans. Am. Inst. Elec. Engrs.*, **67**, 47 (1948)]. These are primarily in high zero and gain stabilization. An extra stage of amplification permits the reduction of thermometer current to 2 ma., the value used by the National Bureau of Standards for calibration of platinum resistance thermometers. Some of the additional gain can be used to make the balancing motor responsive to random noise. This minimizes striction effects and the extreme sensitivity is useful for self-checking purposes.

The attainment of 0.01% accuracy in this instrument would be impossible if dimensional changes in the chart, as a result of humidity changes, were neglected. Although automatic correction for this effect was suggested many years ago, the present instrument utilizes a new method in which a spider is positioned axially by the chart. Through a groove, engaging pin, bell crank, and long lever, the pen is moved in space to compensate for chart expansion. Performance tests with this corrector indicate that a fixed pen will show an error of as high as 0.5% with humidity increase, whereas the trace made by the compensated pen shows no change.

Many readers of ANALYTICAL CHEMISTRY will recall the stimulating and pioneering paper in which the potentialities of this type of instrument were outlined [Stull, D. R., IND. ENG. CHEM., ANAL. ED., **18**, 234 (1946)]. The recorder is of particular value in the examination of many organic compounds in the 98 to 100% purity range. Upon cooling, the



recorder draws the time-temperature, showing supercooling and the freezing point. The freezing point of the pure material and the mole fraction of impurity are readily calculated from the curve. Applications to ebulliometry are equally important. If the boiling point apparatus with accurate pressure control is used, the boiling point vapor pressure data are easily obtained with convenience and high precision. The instrument also affords an almost automatic means of purity determinations by continuously recording boiling points as a function of quantity of distillate. Other applications include the general field of calorimetry—i.e., specific heats, heat of reaction, etc.

Although the analyst has numerous criteria of purity and uses many instruments to detect, identify, and estimate impurities, he will continue to rely upon increasingly accurate physicochemical constants. To the extent that these involve thermal quantities and a precise temperature measurement, the new Leeds and Northrup instrument marks a new departure in the field. It measures nothing more precisely than heretofore, but by retaining equivalent precision and being automatic, it affords almost infinite detail of minute thermal changes, something which would be prohibitively time-consuming and exhausting if not impossible by the older methods.

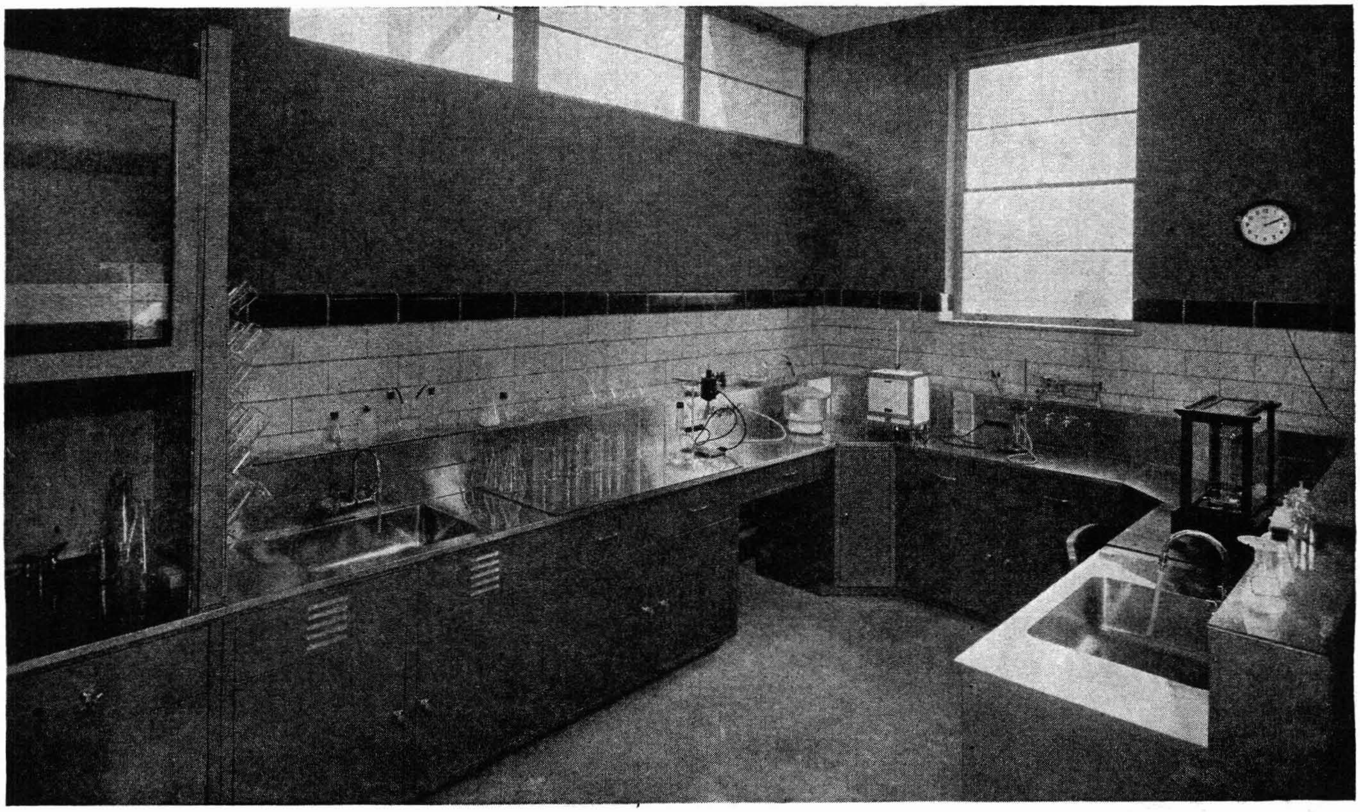
Transistors

The November *Proceedings of the Institute of Radio Engineers* is devoted almost exclusively to contributed papers on transistors. There are 48 original contributions, an editorial, and three general commentaries on the present status of transistor theory, devices, and applications. This is practically a monograph on the subject. It would be impossible to do justice to all these papers; indeed, few of them are of direct or present interest to the analyst. The startling effect of the collection is the fact that transistor theory and practice are in a very advanced state and an understanding of the basis of their operation is much greater than that of the electron tube at the time when it was first used in communications. Many entire transistor circuits draw power from the operating source, which is one thousandth part of that required merely to heat the cathode of miniature vacuum tube. If we may requote a prominent authority in the field, "It is not proper to speak of flea-power in connection with transistors—rather, their power consumption corresponds to a very tired flea jumping once every 6 seconds."

Two of the papers may be quoted briefly, to indicate some of the very radical developments in this field. "New Photoelectric Devices Utilizing Carrier Injection" are described by K. Lehovec, [*Proc. Inst. Radio Engrs.*, **40**, 1407 (1952)]. The detection of injected carriers by their absorption and by emission due to their recombination with majority carriers is discussed. Two new components are proposed by the author, (1) the photomodulator, which permits modulation of a light beam by the change in absorption due to injected carriers; (2) the "graded seal junction" which permits the light to be transmitted to and from a P-N junction without substantial absorption in the bulk semiconductor. The author has suggested that the photomodulator combined with a phototransistor might be useful as an amplifier.

Of more immediate interest to the analyst is the description of "properties of M-1740 P-N junction photocell [Shive, J. N., *Proc. Inst. Radio Engrs.*, **40**, 1410 (1952)]. A year or two ago, a contact transistor having photoelectric response was announced, but this new type employing a special *p-n* junction has fantastic properties. It has a sensitivity of 30 ma. per lumen for light of 2400° K. color temperature. This corresponds to a quantum yield (electrons per incident photon) of approximately unity in the spectral range from the visible to the long wave cutoff at 1.8 microns. To put it in less elegant terms, this is a perfect photocell! By comparison, the best photocathodes have an efficiency of about 0.1%, or about 20 to 60 μa. per lumen. The photomultiplier, despite its enormous sensitivity, still employs an inefficient photocathode. The high gain is merely a matter of internal amplification by secondary emission at the successive dynodes. The new M-1740 P-N junction photocell exhibits a dark current of a few microamperes at room temperature, with temperature coefficient of about +10% per degree Centigrade. Both dark and photocurrents exhibit saturation in the range of 1 to 90 volts applied. The frequency response is flat into the 100-Kc. region. The photocell element is encapsulated in a $^1/_4 \times {}^3/_{16} \times {}^3/_8$ inch plastic housing, from which it might be inferred that a dozen of these photocells could easily be lost in the back recesses of a small drawer.

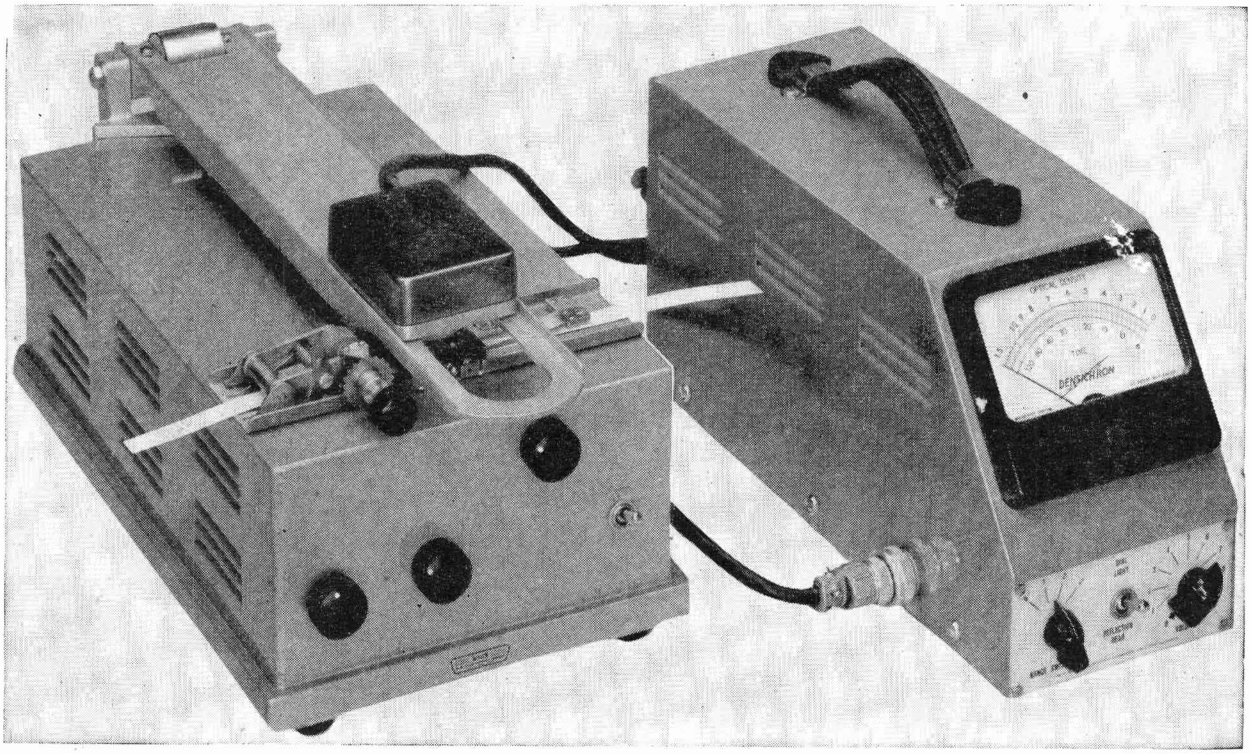
Transistor devices of all classes will not be readily available until the first half of 1953. The problems of mass production are prodigious because germanium and silicon of fantastically high initial purity are required and the subsequent introduction of minute traces of intentional impurities has involved exhaustive research and development. Several major companies promise to be in production shortly. It is to be hoped that the military will not absorb the major part of this production for stockpiling, although their interest is understandable. Many chemists have wondered if they could ever catch up with the rapid advances in electron tube developments. The transistor era may solve the problem; they will either give up the chase in despair, or what is more likely, they will have unprecedented, simple, and minute devices for the solution of their problems.



The Welch DENSICHRON® + Transmission Light Source

makes a complete

TRANSMISSION DENSITOMETER *for* QUANTITATIVE PAPER CHROMATOGRAPHY



No. 3835B

The special Welch Transmission light source with a filter paper guide for holding and advancing the papergram at 3 mm increments, when used with the Densichron becomes a complete transmission densitometer for quantitative paper chromatography. The quantitative analysis of complex compounds such as PROTEINS, SUGARS, VITAMINS, etc., has been simplified by reading maximum densities of papergrams with the DENSICHRON.

This new apparatus and procedure will speed up your work and permit the analysis of very small samples. Works equally well for quantitative paper electrophoresis.

This instrument was demonstrated in September at the Instrument Show in Cleveland and the Chemical Exposition in Chicago.

Write for literature describing the production of papergrams and the use of the Densichron for quantitative determination by the maximum density method.

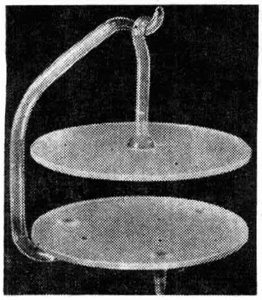
$425.00

W. M. WELCH SCIENTIFIC COMPANY
DIVISION OF W. M. WELCH MANUFACTURING COMPANY
ESTABLISHED 1880
1515 SEDGWICK STREET, DEPT. A CHICAGO 10, ILLINOIS, U.S.A.

NEW PRODUCTS FOR ANALYSTS

Equipment, Apparatus, Instruments, Reagents, Materials

Weighing Glass



Metro Industries has available a weighing glass which permits rapid determination of total volatiles in emulsions and similar viscous products, residues, and oxidations.

This apparatus, called the Heidbrink weighing glass, consists of two equal ground glass plates, 6 cm. in diameter, held about 15 mm. apart by means of a frame of glass tubing. A small sample, 11 to 200 mg., is placed on the lower precision ground surface (which is attached to the glass frame), and immediately covered by the upper, movable plate, to prevent loss or take-up of moisture. After weighing, the top plate is pulled off and hung on an attached glass hook. In this position, the apparatus and sample are placed in a desiccator or drying oven, until constant weight is obtained. **1**

Vacuum Fusion Apparatus

Vacuum fusion gas analysis apparatus is being manufactured by National Research Corp.

A wide variety of ferrous and nonferrous pure metals and alloys including titanium, molybdenum, stainless steels, high temperature alloys, and electronic alloys can be quantitatively analyzed for total oxygen, nitrogen, and hydrogen content. Sensitivity as high as one part in 10,000,000 is possible.

The system is furnished with mechanical and diffusion pumps, mercury, furnace assembly, and built-in control panel. A National Research technician assembles, tests, calibrates the instrument, and trains the customer's operators. **2**

Spectrophotometer

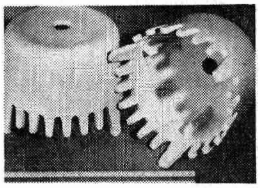
A kinetic spectrophotometer for the instantaneous display of spectrophotographic curves of various materials through the entire visible range of the spectrum has been announced by American Optical Co.

It is a combination of optical spectrophotometer and electronic indicating device which traces curves at 60 per second on the face of a cathode ray tube against a linear wave length–percentage grid. Transparent materials up to 100 mm. in thickness can be accommodated in the instrument's sample chamber. Spectrophotometric reflectance curves may be obtained from opaque materials of approximately 1 square inch dimensions by means of a reflection attachment.

Because of its speed, the instrument is adaptable to the study of rapid color changes and offers possibilities for monitoring flowing liquid in which spectral absorption can be used as a criterion for control. For more detailed study or for permanent records, photographs of the tube face can be made with a standard 5-inch oscilloscope camera. **3**

Bubble Caps

For general use in fractionating and distillation towers where severe corrosion limits the useful service life of metal caps, Coors Porcelain Co. is offering high strength ceramic bubble caps. These caps can be made in a variety of designs and in several types of ceramic materials.



The alumina ceramic caps shown have a tensile strength of 18,000 to 20,000 pounds per square inch. They have a $^3/_8$-inch wall and an outer diameter of $8^3/_4$ inches, and are 7 inches high. Only hydrofluoric acid and strong caustic solutions will affect the caps which are also resistant to abrasive conditions. **4**

Chemicals

Rohm & Haas Co. has announced the availability in development and pilot plant quantities of *tert*-butylamine, glacial methacrylic acid, glycolonitrile, and Acryloid B-82 resin.

In any reaction where primary or secondary amines of similar molecular weight have been used, *tert*-butylamine will merit examination. It forms stable, distillable, monomeric aldimines by reaction with aromatic or aliphatic aldehydes.

Glacial methacrylic acid is especially useful in nonaqueous

Circle desired items for further information on items listed in this section

Inquiry Card Is Valid Six Months from Publication Date

TECHNICAL DATA DEPARTMENT
ANALYTICAL CHEMISTRY

DECEMBER 1952

I am interested in the following circled items:

New Products 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Manufacturers' Literature 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45

Name________ Position________

Company________

Street________

City________ Zone________ State________

(PLEASE PRINT OR TYPE)

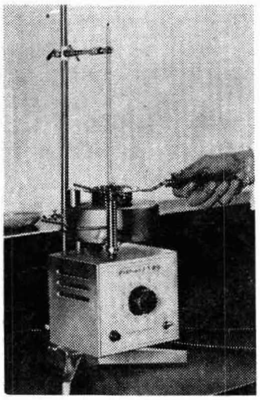
polymerizations or in reactions such as esterification and acid chloride formation where water cannot be tolerated.

Glycolonitrile undergoes esterification, ether formation, reductive methylation, hydrolysis, simultaneous dehydration and hydrolysis and offers a convenient method of cyanomethylation with a single reagent.

Acryloid B-82 softens in the range 70° to 80° C. and flows at 140° to 155° C. It is soluble in toluene, xylene, and other aromatics. **5**

Flash Point Testers

Three newly designed open cup flash and fire point testers, one gas and two electric, are available from Fisher Scientific Co.



Gas and electric heaters are integral parts of the stand assembly, with gas inlet on both models mounted in the rear at the bottom of the stand, making the whole a portable assembly. A smaller grid has been substituted in the burner so that the flame will not backfire at the slow heating rate required for testing.

The handle of the sample cup is made of stainless steel tubing to reduce amount of heat conducted from hot cup to the grip. Electrical models operate on 50- to 60-cycle a.c. and come with ASTM thermometers. The gas-heated model uses either artificial or natural gas. **6**

Cross Section Paper

Ogilvie Press, Inc., has developed a new type of cross-section paper, the cross-section ruling of which will not reproduce. It is available in 8 × 8 and 10 × 10 to the inch in rolls 20 yards and 50 yards by 30 inches wide, as well as sheets up to 24 × 36 inches. **7**

Microscope

William J. Hacker & Co., Inc., is marketing a research microscope developed by the Optische Werke C. Reichert A.-G., Vienna, Austria.

The microscope incorporates several completely new features. There is a monocular-binocular tube which permits instant change-over from binocular observation to microphotography. The binoculars revolve at an angle of 120° permitting persons other than the operator to witness the image without interference.

Examinations in reflected as well as transmitted light can be made. Phase contrast accessories are supplied for transmitted light. The stage is 130 × 120 mm. **8**

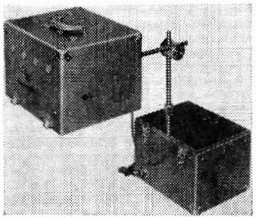
Optical Pyrometer

Ferro Corp. is distributing a pocket-sized telescopelike pyrometer weighing only $5^{1}/_{3}$ ounces. It is used for direct reading of temperatures up to 3300° F. produced by heat radiating from a hollow space.

The pyrometer uses a small bimetal spiral spring as radiation tracer, whose free end is provided with a point subject to the influence of temperature. The measuring spiral is artificially aged by temperature changes before being installed in the pyrometer. The bimetal indicator-thermometer is arranged in a cylindrical housing of approximately $2^{1}/_{3}$ inches outside diameter and $6^{1}/_{2}$ inches total length in the focus of a convergent lens. There is a built-in eyepiece on the opposite side. Through this, the observer can view the object as well as the deflection of the needle on a calibrated scale. **9**

Ultraviolet Unit

Cooper Hewitt Electric Co. is supplying an all-purpose ultraviolet laboratory unit built into a storage and carrying case.



Ultraviolet energy is furnished by a 400-watt high pressure quartz mercury arc lamp with an envelope of clear fused quartz. A mounting assembly allows adjustment of the lamp housing to any position in the vertical or horizontal plane. A double-tiered filter holder is supplied with each unit to accommodate selective filters for fluorescence investigation or other special requirements.

It is suggested that the "researcher" will find many uses in bacteriology, fluorescence analysis, photochemistry, spectroscopy, and weathering. **10**

Meter

An extremely sensitive but none the less rugged meter has been achieved by means of new meter movement incorporated in the instrument being offered by Columbus Products Corp.

The instrument contains a removable and replaceable cartridge. Its movable coil, suspended on bifilar or twin wires, being free of frictional losses, is extremely sensitive to current variations.

This meter can be had in sensitivities from 1 microampere

PLACE
2¢ STAMP
HERE

TECHNICAL DATA DEPT.

ANALYTICAL CHEMISTRY

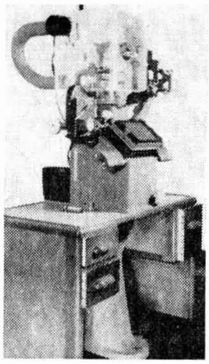
330 W. 42nd St.

New York 36, N. Y.

Use this handy return card to save yourself time. It will bring information of use to chemists and engineers in laboratory, pilot plant, and production. The items listed in this special section have been selected by the editors of ANALYTICAL CHEMISTRY for their value and timeliness in helping you to keep abreast of the latest developments in the field.

full scale, and up. It is usually of the d.c. type but can be supplied to a.c. measurements in conjunction with suitable rectifying means. It can be used directly for checking and measuring transistor circuit values, as well as in vacuum tube circuits, where at present only a vacuum tube voltmeter can serve. **11**

Plenary Microscope



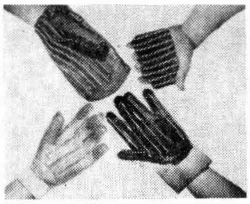
Lovins Engineering Co.'s plenary microscope uses as its base the Vickers projection microscope. It is offered either with or without an incubator surrounding the stage and substage, a motion picture camera, and controls for time-lapse photography. The microscope has a beam-splitter and mirror which enable the observer at all times to see, in the eye tube, a portion of the light passing either to the projection system for still plate photography or to the motion picture camera. Change-over from still to motion picture photography is accomplished by operating a push button. External screen projection is possible.

The incubator temperature is controlled by a sensitive thermoregulator to within 0.5° F.

Illumination may be phase contrast, polarized light, dark field, incident light, oblique light, or bright field. Special accessories available include multiple-beam interferometry, crystallographic, microhardness, and macrophotography. **12**

Work Gloves

The Surety Rubber Co. has designed work gloves made with flexible metal zippers permanently bonded to fingers and palms in synthetic rubber which provide positive gripping action with maximum pliability.



These work gloves are available in all sizes and styles for jobs requiring protection for palms and fingers. Gloves, mittens, and palm guards are shown in the picture. **13**

Protective Coating

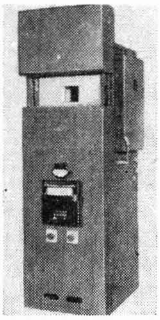
The Flexrock Co. has developed a coating which gives protection against corrosion caused by atmospheric conditions, vapors, fumes, spillage, and splash of chemicals. This coatting, Flexcoat, can be brushed, sprayed, or dipped, and applied over old paints. It has good adhesion to metal, wood, or concrete and is resistant to acids and alkalies. It does not contain oxidizing oils or resins; it dries by solvent evaporation alone. **14**

Card System

A timesaving system of records control for following the progress of routine analytical work on large numbers of samples has been developed by the analytical section of Sam Tour & Co., Inc.

The system is based on a set of 4 × 6 inch cards. Each set has a master card which contains information about the origin of the sample, the series of analyses required, and the results of each analysis as they are reported. Each of the remaining cards in the set corresponds to an individual analysis and these cards are distributed to the analysts. They identify the sample and give the expected range of results. All data needed for subsequent calculations are written in a space where they are used directly in the calculations. As each phase of the analysis is completed, the results are recorded and these data cards are then attached to the master card in a file. **15**

Muffle Furnace



Burrell Corp. is manufacturing a dual purpose muffle furnace for both low and high temperature use. Routine operations such as ashing, drawing, igniting, and sintering can be performed at temperatures from 600° to 2000° F. Above 2000° F., and up into the high white, the same furnaces are adaptable for such operations as sintering, melting, clinkering, fusing, and high speed hardening. Working temperatures can be quickly changed, up or down.

The furnace can be mounted on the floor and has an easily accessible panel which opens from the front for inspection and servicing. Heating chambers are offered in various sizes. Rod-shaped silicon carbide elements are used. The furnace operates by connection to the existing power supply, either 115 volts, 50- to 60-cycle, single-phase. **16**

Drawer Loading Oven

Lydon Brothers, Inc., is manufacturing a cabinet oven for use in sintering fluorocarbons at temperatures to 750° F. It features an internal air circulation system.

The drawers have insulated fronts, ball bearing drawn rollers that run in internal guides, so that no tray-supporting extensions are required in front of the oven. An excess temperature cutout is provided. There is a vent hood over the front of the oven, vent slots at the sides, and an oven exhaust with separate direct connected exhauster. There are ten drawers 20 inches in width, 14 inches in depth, and 3 inches in height. **17**

Bath Clamp

Labline has designed a bath clamp that is quickly attached to the edge of glass jars that are generally used in visibility water baths. It holds thermometers, pipets, and thermostats at any desired distance from 0.25 to 5 inches from bath wall. It holds thermometers either clamped in a V or suspended on a hook through a thermometer ring. The clamp is easily adjusted at any angle through 360° or extended by thumb screw adjustment without removing the clamp from bath wall or object from clamp. **18**

Temperature Humidity Cabinet

A new development in control makes it possible to hold humidity within 1° wet bulb in the Food Technology, Inc., temperature and humidity cabinets. Temperature is controlled by thermostats which turn heaters or open valves admitting a coolant. Humidity is controlled by a wet bulb device which atomizes water into a separate chamber through which the air is circulated. This method avoids overloading the air during the lag waiting for the wet bulb thermostat to respond. During this period atomization is controlled by a relay which opens the spray valve intermittently for a few seconds at a time. These cabinets provide a range from 40° to 150° F. and controlled degree wet bulb. **19**

Humidity Controller

The Abbeon Supply Co. has designed an automatic "plug-in" humidity controller to operate electrically powered humidifiers and dehumidifiers.

The control operates in much the same way as a room-type thermostat. It is hung on the wall and all that is necessary is to turn the dial to the percentage of relative humidity desired. When used as a dehumidifier control, if the humidity rises above the setting, the control will turn on the dehumidifier. When the humidity falls below the set percentage of relative humidity, the dehumidifier will automatically shut off. When used with humidifiers the reverse is true.

To use the control in conjunction with existing humidifiers or dehumidifiers, the unit is plugged into the control and the control is plugged into the nearest 110-volt wall outlet. The control is powerful enough to handle most types of electric humidifiers and dehumidifiers. **20**

Battery Caps

An entirely new storage battery cap for all types of batteries has been developed by Industrial Research Inc. The new cap keeps water in the battery at a safe level 8 times longer than has been hitherto possible. It prevents corrosion by capturing the corrosive sulfuric acid fumes which commonly escape from batteries and washing them back into the battery. In addition, Hydrocaps give warning of overcharge; they normally operate warm to the touch but when they turn hot to the touch, they indicate that the voltage regulator needs adjusting.

Hydrocap contains a palladium catalyst which converts a battery's escaping hydrogen and oxygen gases back into water. These caps are available in sets of three and are designed to replace the battery's conventional caps. They are available in all sizes to fit any make of battery. **21**

MANUFACTURERS' LITERATURE

Chemical Information. A 20-page booklet, "Physical Properties of Synthetic Organic Chemicals," tabulates physical properties of over 320 products, including 56 new research chemicals. Union Carbide and Carbon Corp. (*Booklet F-6136*) **22**

Water Stills. A 12-page illustrated and detailed bulletin gives specifications for steam, gas, and electrically heated units. Precision Scientific Co. (*Bull. 950*). **23**

Nephelometry. Illustration of equipment for and discussion of nephelometry are included in 4-page publication called "Waco Nephelos Notes." Wilkins-Anderson Co. **24**

Chemicals. Eleven-page catalog lists over 1000 special, rare, and reagent chemicals and includes a description of standard solutions and newly designed equipment. Jasonols Chemical Corp. **25**

Liquid Handling Equipment. Colorful, illustrated 15-page catalog discusses many types of liquid handling equipment including filters, filter sheets, mixers, cappers, vacuum bottle fillers, and pumps. Ertel Engineering Corp. (*Cat. 52*). **26**

Translations. Translations of German and Russian literature on manufacturing processes, patent applications, and research records pertaining to sulfur and sulfur compounds, chlorine and chlorine compounds, miscellaneous manufacturing processes, and articles from Russian scientific journals. Research Information Service (*Bull. 71*). **27**

Organic Silicofluorides. Complete product data sheets for each of six new silicofluorides now available commercially. The Davison Chemical Corp. **28**

Surface Active Agents. Catalog lists anionic, cationic, and nonionic agents and supplies data on physical and chemical properties, concentration, general applications, and specific suggested uses. Onyx Oil & Chemical Co. **29**

Methylenebisacrylamide. An 11-page new product bulletin includes physical and chemical properties, reactions, potential applications, and toxicity of *N,N'*-methylenebisacrylamide which is now available in trial-lot quantities. American Cyanamid Co. (*No. 24*). **30**

Kjeldahl Apparatus. This 14-page booklet illustrates and discusses the Kjeldahl nitrogen apparatus, the Goldfisch fat extraction apparatus, and crude fiber condensers. Laboratory Construction Co. **31**

Laboratory Supplies. Four-page folder illustrating and describing over 15 necessary laboratory items. E. Machlett & Son. **32**

Organic Chemicals. Catalog of over 100 reactive organic chemicals including ethanedithiol, tetranitromethane, the sodium salt of benzenesulfinic acid, and acetylenedicarboxylic acid. Aldrich Chemical Co., Inc. **33**

Monochromators. Bulletin illustrates and gives detailed information about 250- and 500-mm. focal length monochromators for irradiation, ultraviolet microscopy, and photochemistry. Bausch & Lomb. **34**

Reflux Condenser. Three-page, illustrated bulletin tabulates specifications for vertical thermosyphon reboiler and reflux condenser. Doyle & Roth Manufacturing Co., Inc. (*Bull. VT83-52*). **35**

Converter. An 11-page illustrated bulletin describes in detail a new analog-to-digital converter. Consolidated Engineering Corp. (*Bull. CEC-3002*). **36**

Analog Computer. A 10-page illustrated catalog describing applications, methods, and features of analog computer which functions as an equation solver, simulator, or tester. Beckman Instruments (*Bull. 309*). **37**

Products for Processing Industries. Catalog covers chemical properties, temperature limitations, sizes, and recommended working pressures of products for use in the chemical processing industries. U. S. Rubber Co. **38**

Submerged Combustion. Description of submerged combustion installation and list of suggested applications. Submerged Combustion Co. of America, Inc. **39**

Electroplated Rhodium. A 1-page technical data sheet completely characterizing electroplated rhodium and announcing availability of heavy rhodium plated deposits for first time. Technic Inc. **40**

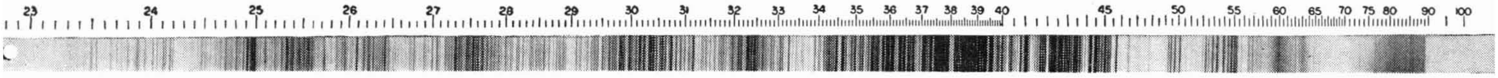
Laboratory Equipment. Sixteenth edition of a publication called, "What's New for the Laboratory." Scientific Glass Apparatus Co., Inc. **41**

Optics and Photography. A 15-page bulletin lists research records, manufacturing processes, and patent applications from the files of German manufacturers in the field of optics and photography. An abstract of each in English is included. Research Information Service (*Bull. No. 68*). **42**

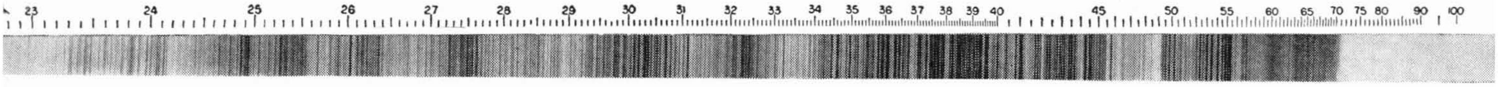
Industrial Chemicals. Thirty-three-page, 1953 catalog contains specifications, properties, and typical uses of 54 of the company's industrial chemicals and lists the various fields in which each compound finds its greatest use. Tennessee Eastman Co. (Sixth Edition). **43**

Volatile Corrosion Inhibitors. Two illustrated bulletins, one technical and one general, on the use of volatile corrosion inhibitors for protecting ferrous metals and aluminum from rust and corrosion without using oil or grease. Berlin and Jones Co., Inc. (*Bulls. VT-1, GB-1*). **44**

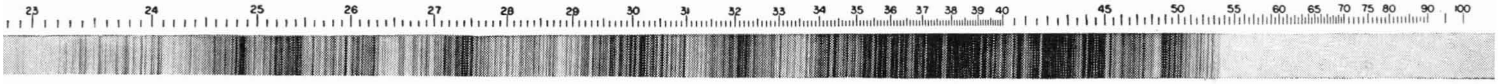
Nuclear Instruments. A complete catalog of nuclear instruments, accessories, miscellaneous products, and services available. Nucleonic Co. of America. **45**



Class L sensitization provides reasonably uniform sensitivity throughout the entire range from 240 to 900 mμ. It has found wide use for preliminary surveys in spectrographic analysis.



Class F sensitization is valuable for its high sensitivity in the red up to 680 mμ and for its uniform response over the whole visible spectrum.



Class O represents the unmodified color sensitivity of silver halide, which has a sharp cutoff just above 550 mμ. It is available in all emulsion types, including one that can resolve 1,000 lines per millimeter.

To capture the light you want . . .

This is the iron arc spectrum as seen on three different Kodak Spectroscopic Plates, each with a spectral sensitivity suited to a particular purpose.

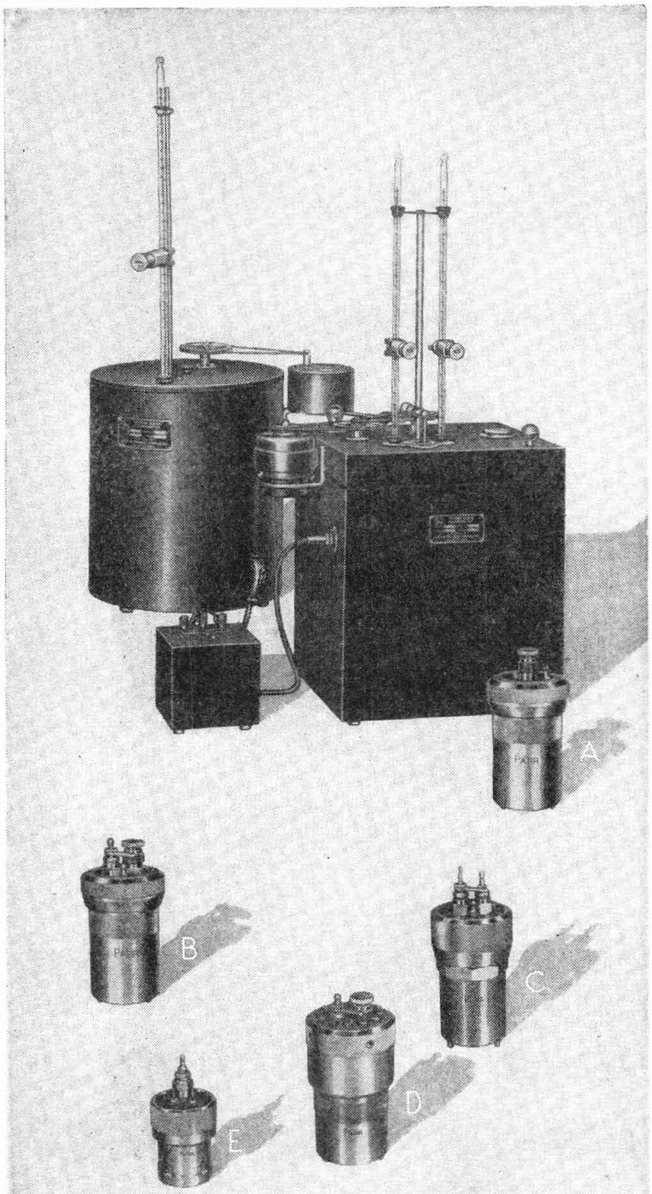
Kodak Spectroscopic Plates and Films come in 18 different kinds of spectral sensitization. There are 10 different emulsion types to take these sensitizations, varying in speed, contrast, and granularity. There are even two emulsion types for the very low light levels where the reciprocity between illumination and exposure time otherwise falls off.

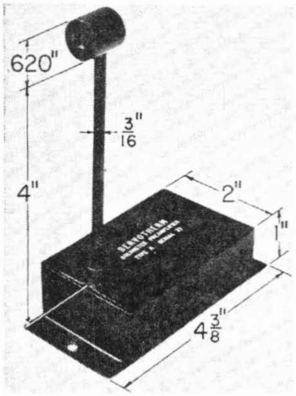
Whether your interest lies in spectroscopy, astronomy, or nuclear physics; in studies of luminescence, emission, absorption—you'll find it helpful to have the booklet which serves as a guide to the plates, films, and pellicles we make for you. It's called "Kodak Sensitized Materials for the Scientific and Industrial Laboratory," and we shall be happy to send you a copy without charge. Eastman Kodak Company, Industrial Photographic Division, Rochester 4, N. Y.

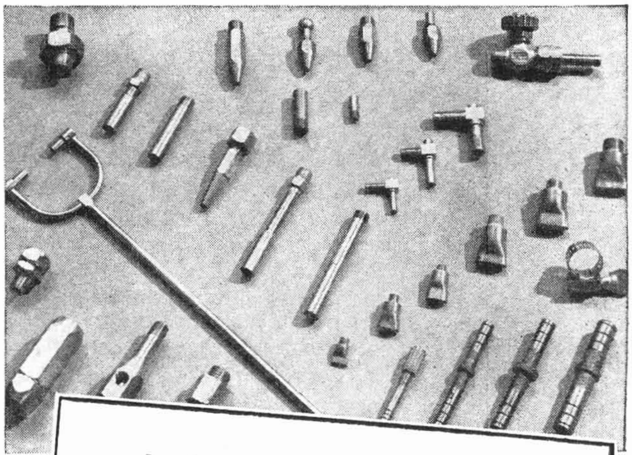
SPECTROGRAPHY

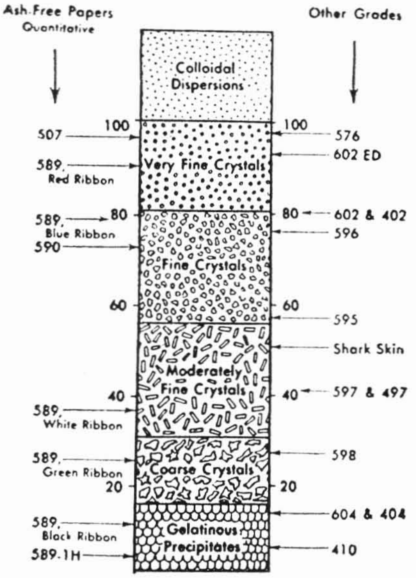
. . . an important function of photography

Kodak
TRADE-MARK

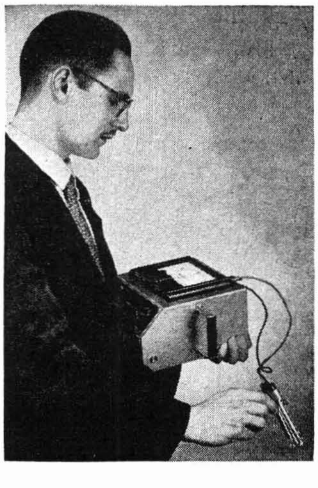

A
B
C
D
E

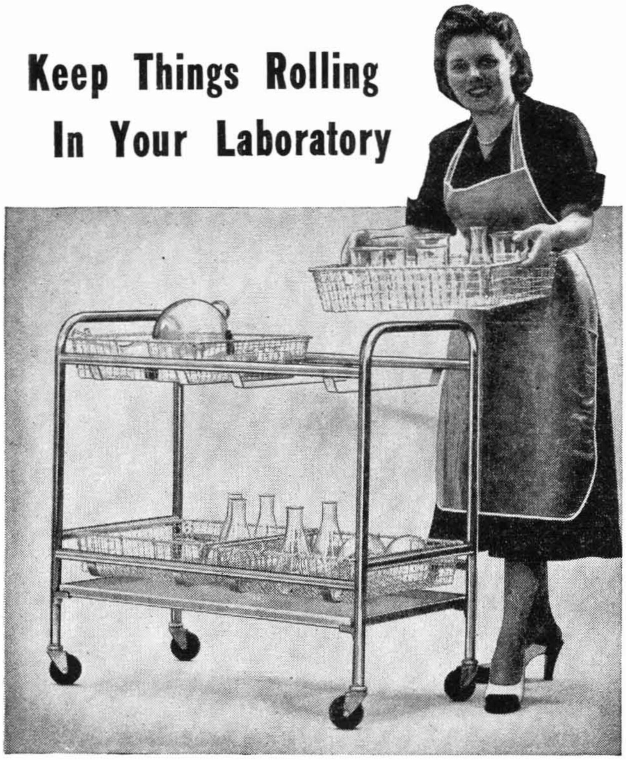

.620"
3/16"
4"
2"
1"
4 3/8"

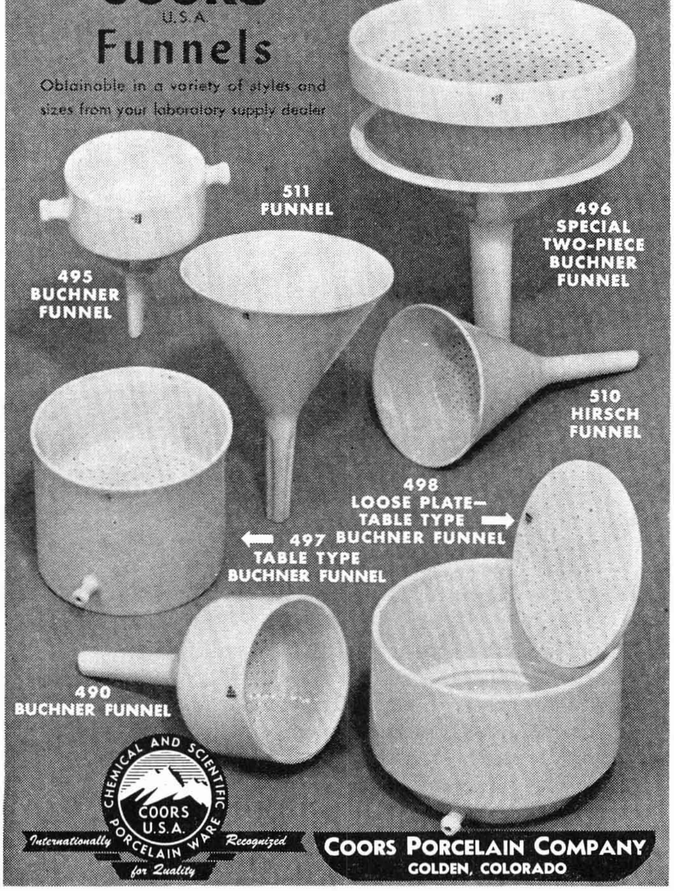


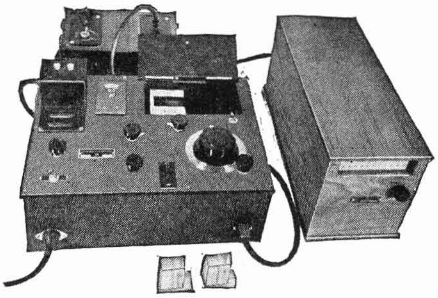

Ash-Free Papers
Quantitative
Other Grades
Colloidal Dispersions
100
507
576
602 ED
589, Red Ribbon
Very Fine Crystals
589, Blue Ribbon
80
602 & 402
596
590
Fine Crystals
60
595
Shark Skin
Moderately Fine Crystals
40
597 & 497
589, White Ribbon
589, Green Ribbon
Coarse Crystals
598
20
604 & 404
589, Black Ribbon
Gelatinous Precipitates
589-1H
410

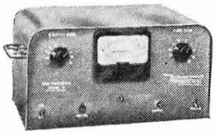

PHOTOVOLT
ELECTRONIC pH METER
MODEL 125
PHOTOVOLT CORP.
NEW YORK CITY
OPERATION

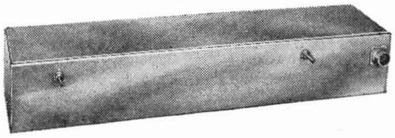




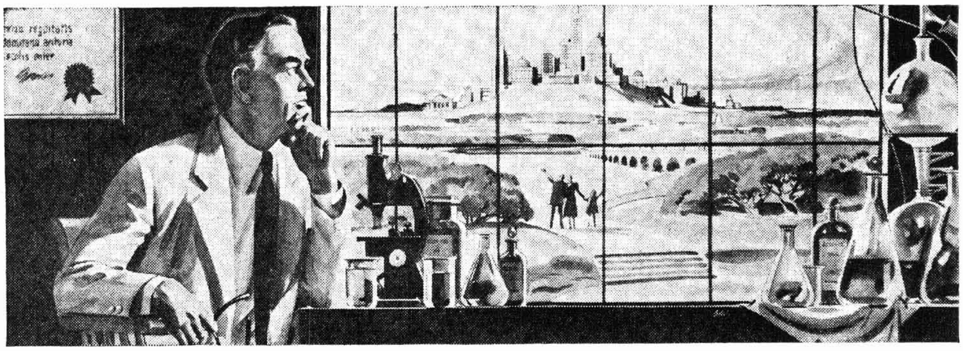

511 FUNNEL
496 SPECIAL TWO-PIECE BUCHNER FUNNEL
495 BUCHNER FUNNEL
510 HIRSCH FUNNEL
498 LOOSE PLATE—TABLE TYPE BUCHNER FUNNEL
497 TABLE TYPE BUCHNER FUNNEL
490 BUCHNER FUNNEL
CHEMICAL AND SCIENTIFIC PORCELAIN WARE
COORS U.S.A.
Internationally Recognized for Quality





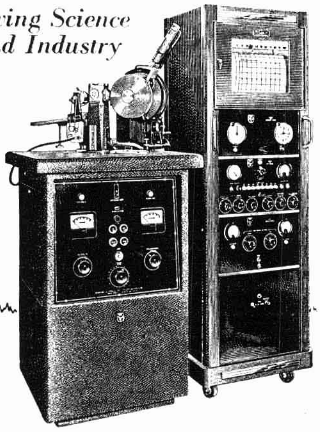


Will X-Ray Diffraction Reveal the Hidden Secrets



of Cancer . . . Heart Diseases . . . Hardening of Arteries?

Serving Science and Industry



DOCTORS at New York's Mount Sinai Hospital are using NORELCO X-ray Diffraction equipment designed by Philips—world leader in X-ray development — to observe molecular behavior in the calcium salts of bone cancer, calcium salt deposits in artery walls, and the composition and formation of kidney stones. New observations have been made concerning heart structure and behavior. In X-ray Diffraction, there is indeed new hope.

Applied to industry, NORELCO X-ray Diffraction equipment has already revolutionized metallurgical and chemical methods — improved controls for production, processing and end-product analysis.

In the scientific laboratory, it has permitted non-destructive analysis impossible for traditional chemistry.

You may find NORELCO X-ray Diffraction equipment just as valuable for your company or research organization. Write for free consultation. Also free booklet—"What is X-ray Diffraction?"

A *Norelco*

PRODUCT OF NORTH AMERICAN PHILIPS COMPANY, INC.

Dept.IE-12 • 100 East 42nd St., New York 17, N. Y.

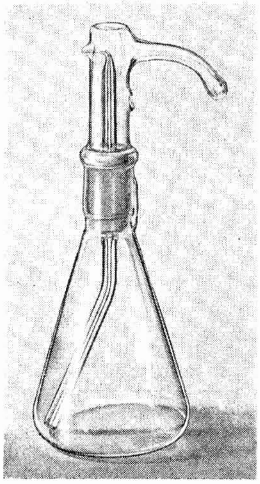
In Canada: Rogers Majestic Electronics Ltd., 11-19 Brentcliffe Road, Leaside, Toronto 17, Ontario

Paper Partition CHROMATOGRAPHY Equipment

A new and complete line of paper chromatographic equipment carefully engineered for use in this new field of analysis.

- CHROMATOCABS
- STEEL OR GLASS RACKS
- DENSITOMETERS
- ULTRAVIOLET LAMPS
- INDICATOR SPRAYS
- DRYING OVENS
- DISC CHAMBERS
- SOLVENT TROUGHS
- MICRO PIPETS
- CLIPS

Prompt Delivery



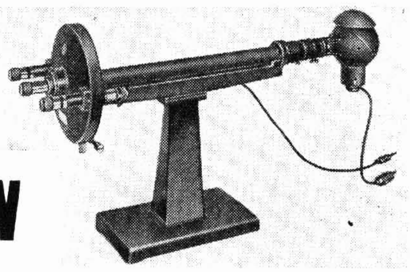
PYREX INDICATOR SPRAYS

Write for Catalog

Research Equipment CORPORATION

1135 THIRD STREET, OAKLAND 20, CALIFORNIA DEPT. A

NEW



Lippich Type Polarimeter

(Schmidt & Haensch)

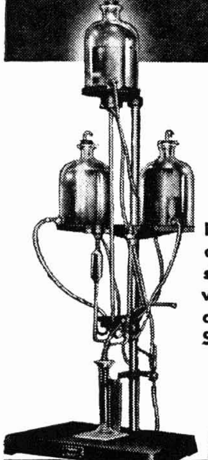
A precision instrument for measuring the rotation of the plane of polarization of all optically active substances. Big advantage is its glass circle instead of a metal circle. The scale on glass, in conjunction with a micrometer plate, permits direct reading without vernier, resulting in greater speed and precision of reading. Glass circle is rigidly connected with the analyzer and carries two scales, an angular scale in degrees arc, and a scale in international sugar degrees. Direct connection of the light source with the instrument reduces adjustments to a minimum.

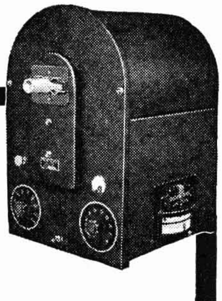
Model A with receiving trough for tubes up to a length of 220 mm.
Model B with receiving trough for tubes up to a length of 400 mm.

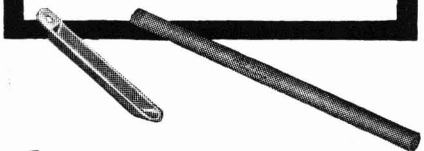
Bulletin SH-344 on request

FISH-SCHURMAN CORPORATION
72 Portman Road New Rochelle, N. Y.

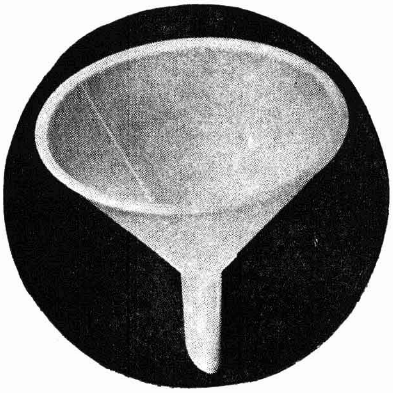
Fish-Schurman

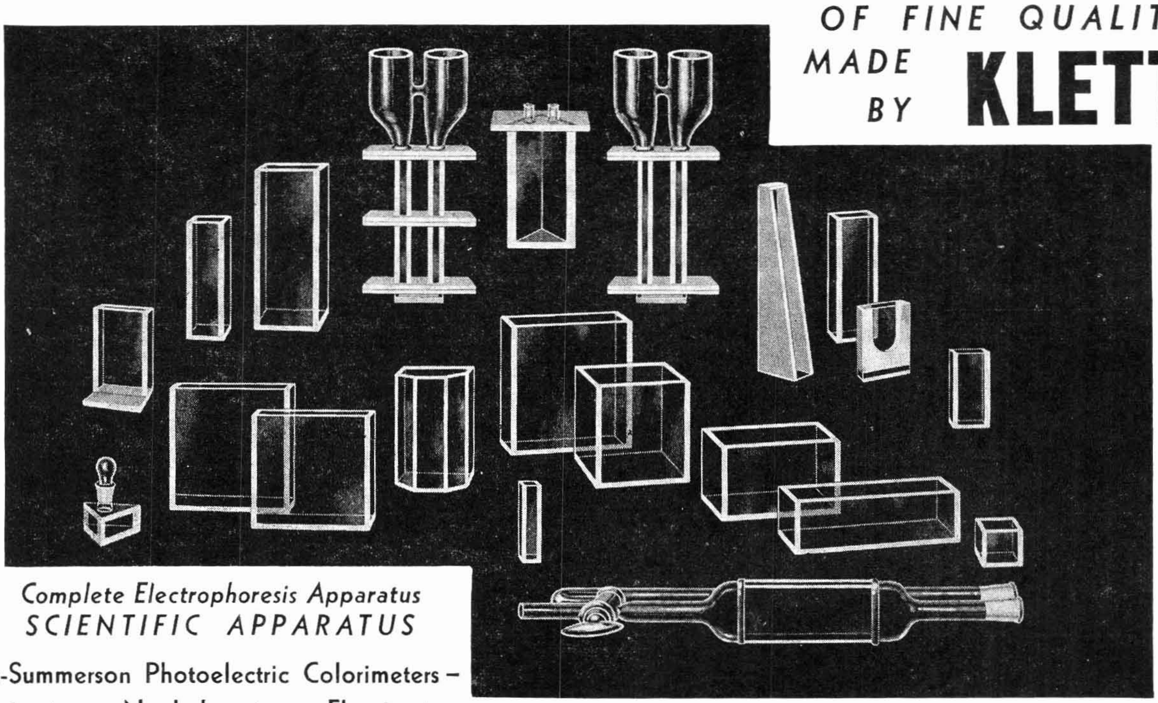


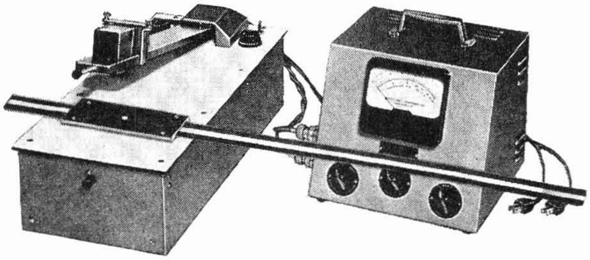


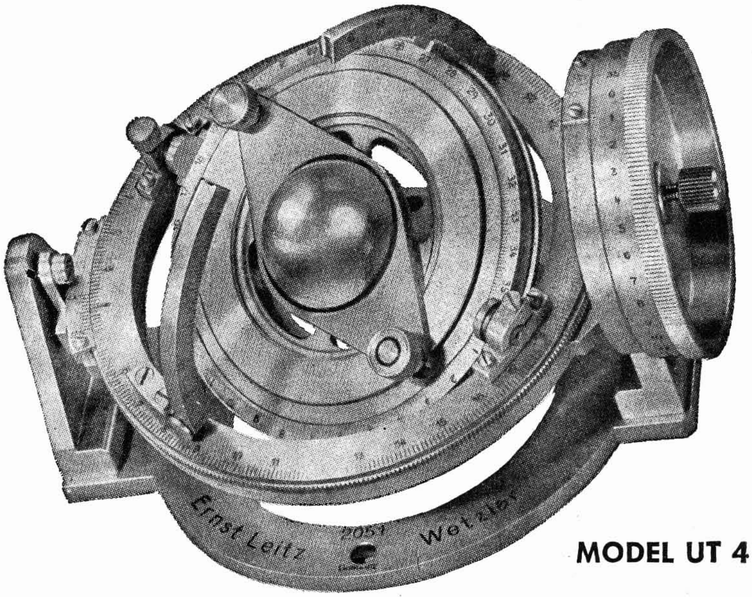



COSTLY BREAKAGE
LAB.








Ernst Leitz
2051
Wetzlar

DURACHRON PRECISION STOP-CLOCK for LABORATORY and INDUSTRIAL TIMING

—the accuracy of a fine stopwatch—but much easier to read—built to take hard knocks.

Durachron is a precise spring-wound stopclock with the same action as a stopwatch. Just as easy to use, far easier to read, and infinitely more durable. Durachron frees both hands for whatever manipulation must be done during the timing operation.

USEFUL wherever timing observations to a split-second are required.

EASY TO READ. Legible 4 inch dial, visible across room. Glass front keeps dial clear.

ACCURATE. Superb timing movement, with fine stopwatch precision.

DEPENDABLE. Freedom from the delicacy of small stopwatches.

RUGGED. Heavy metal case. Constructed for years of rigorous use in laboratory atmosphere.

ECONOMICAL. Costs less than a stopwatch, and will outlast a stopwatch five to one.

COSTS LESS THAN AN ORDINARY STOP-WATCH

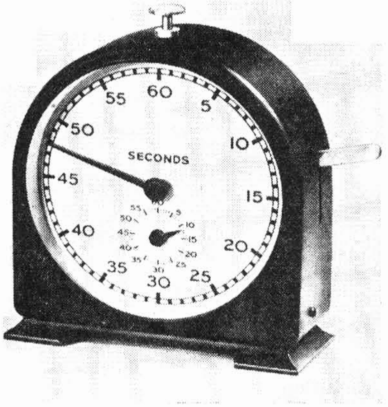
Meticulous craftsmanship and superb quality distinguishes Durachron from inferior makes of timing devices. Yet its cost is less than an ordinary stopwatch, and it will perform dependably for years, while saving you money on stopwatch repair bills. A truly fine value!

only $17.50 each

STANDARD SCIENTIFIC SUPPLY CORP.

LABORATORY APPARATUS • CHEMICALS • REAGENTS

34 West 4th Street
New York 12, New York



3 WAY TIMING CONTROL

Control lever at side stops and starts the sweep hand and recording hand instantaneously. Pressing the stem at top returns both hands to zero.

1. **TIME-OUT.** Stop the action, read the elapsed time, then resume timing without returning to zero.
2. **STRAIGHT-TIMING.** Stop the hands, read elapsed time, then return to zero and start over.
3. **SNAP-BACK TIMING.** Read elapsed time "on the fly", then snap the hands back to zero by pressing the stem. On releasing the stem, the sweep hand starts around again instantaneously to begin another reading.

SELAS MICRO-POROUS PORCELAIN LABORATORY FILTER WARE

Complete Line FOR ALL PHASES OF LABORATORY FILTRATIONS

Selas Micro-Porous Porcelain laboratory filter ware can be used for all types of laboratory filtrations, from the clarification of chemicals to the cold sterilization of pharmaceuticals.

Rapid, microscopic filtrations are possible with Selas Micro-Porous Porcelain filter media . . . a fine porous membrane with precisely controlled pore sizes. All Selas filter ware is chemically inert, highly resistant to heat, and mechanically strong.

The complete line of Selas filter ware comprises:

- Filter Crucibles
- Filters for Micro and Semi-Micro Analyses
- Filter Funnels
- Filter Candles

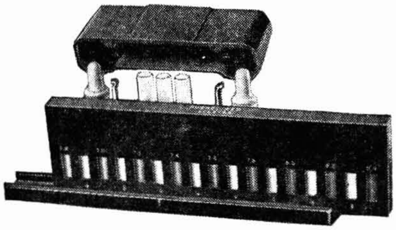
Special items, incorporating Selas Micro-Porous Porcelain Filter Media are available upon request.

WRITE FOR CATALOG

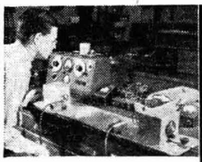
SELAS CORPORATION OF AMERICA

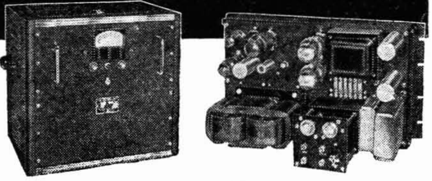
Scientific Equipment Division

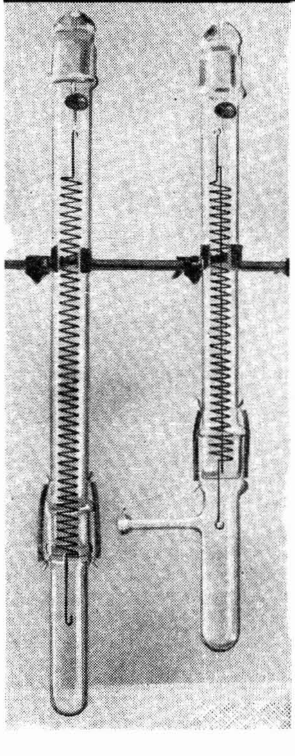
PHILADELPHIA 34, PENNSYLVANIA



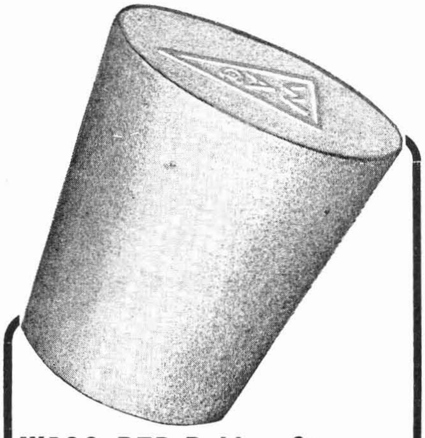

MODERN
pH AND
CHLORINE
CONTROL







THE LABORATORY SUPPLY CENTER



WACO RED Rubber Stoppers

Cost less—In The Long Run

WACO RED rubber stoppers are dense, soft and extremely TOUGH. Do not harden . . . even on your Kjeldahl. Savings appear through infrequent replacement.

No. 7347A—One Hole
No. 7348A—Two Hole
$2.00 Per Pound

Try a five pound box today!

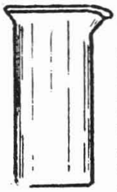
LABORATORY SUPPLIES AND CHEMICALS
WILKENS-ANDERSON CO.
4525 W. DIVISION CHICAGO 51, ILL.

WISCONSIN ALUMNI RESEARCH FOUNDATION
LABORATORY SERVICES

Vitamin and amino acid assays
Proximate analyses and mineral determinations
Food chemistry and microbiology
Screening of compounds for insecticidal, fungicidal, and bactericidal properties
Warm-blooded toxicity studies
Other biological, chemical, and microbiological services

Write for price schedule

WISCONSIN ALUMNI RESEARCH FOUNDATION
P. O BOX 2059, MADISON 1, WISCONSIN



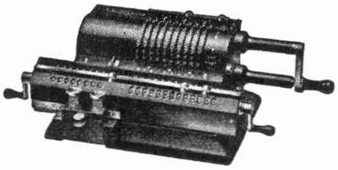
QUARTZ WORKING FACILITIES NOW AVAILABLE

In addition to design and manufacture of micro and ultramicro laboratory apparatus, we are now able to manufacture many laboratory items of clear fused quartz . . . taper joints, pipets, graded seals, breakers, crucibles, flasks, dishes, test tubes, large diameter tubing. Also other items to your specifications or drawings.

Inquiries invited.

MICROCHEMICAL SPECIALTIES CO.
1834 University Ave. • Berkeley 3, Calif.

SINCE 1874
ORIGINAL-ODHNER
HANDY CALCULATORS



For The Chemist and Engineer

Sturdy: Low Priced: Weight 12 lbs.
Easy to Learn: New Back Transfer
Device Speeds up Calculations

Ask For Bulletin BQ-123

SOLE DISTRIBUTORS FOR U. S. A.
IVAN SORVALL, INC.
210 FIFTH AVE., NEW YORK 10, N. Y.

REAGENT TABLETS . . . TO YOUR ORDER

If your procedures require routine weighing of small quantities of reagents, perhaps these can be dispensed more conveniently in tablet form—with assured uniformity and great saving of time . . . also an economical way to dispense very expensive reagents.

We specialize in small-lot tablets to order. Write us about any problem along this line.

R. P. CARGILLE LABORATORIES, INC.
117 Liberty St. Dept. AC-RT N. Y. 6, N. Y.

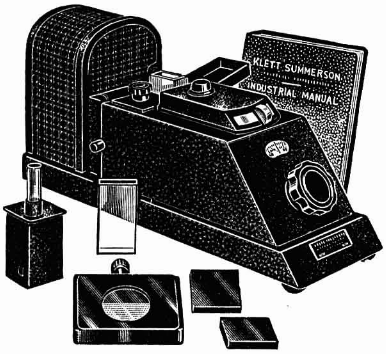
The latest book of
ACS SPECIFICATIONS

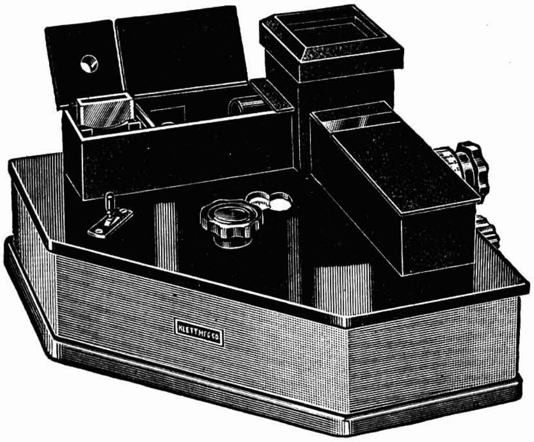
"Reagent Chemicals"

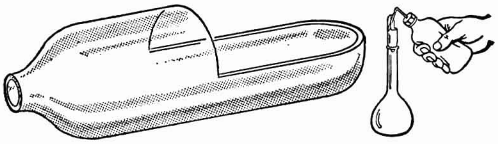
376 pages of data on 177 reagents, their important properties, acceptable limits of usual impurities, and approved test method for each . . . plus 30 pages of related text.

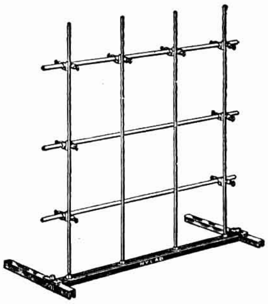
Price, Postpaid . . (Cloth Bound) . . $5.00

Order from AMERICAN CHEMICAL SOCIETY
1155 Sixteenth St., N.W., Washington 6, D. C.


KLETT SUMMERSON
INDUSTRIAL MANUAL


KLETT MFG CO






NYLAB
Catalog 7
Scientific Instruments and Apparatus

FOR EXACTING TECHNICAL APPLICATIONS

VITREOSIL* (vitreous silica) tubing possesses many characteristics you seek. A few of its properties are: Chemical and catalytic inertness. Usefulness up to 1000°C and under extreme thermal shock. Homogeneity and freedom from metallic impurities. Unusual electrical resistivity. Best ultra-violet transmission (in transmission quality).

VITREOSIL tubing available promptly from stock in four qualities.

TRANSPARENT · SAND SURFACE
GLAZED · SATIN SURFACE

Stock sizes transparent up to 32 mm. bore, opaque to 4½" bore. Available for prompt shipment. Larger diameters can be supplied on special order.

Can be had in all normal lengths.

Send for Bulletin No. 9 for specifications and prices.

VITREOUS VITREOSIL REG'D TRADE MARK SILICA

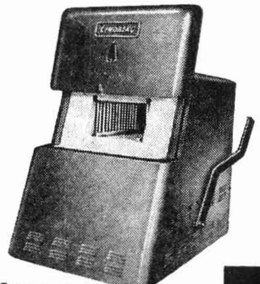
*®

THE THERMAL SYNDICATE, LTD.

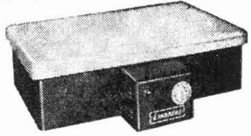
14 BIXLEY HEATH LYNBROOK, N. Y.

---

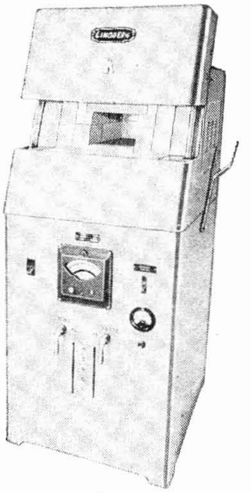
rugged... accurate... dependable...



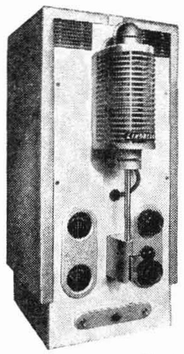
Box Furnaces



Hot Plates



G-10



High Frequency Carbon Determinator

LINDBERG LABORATORY EQUIPMENT

Here's a complete line of laboratory heating equipment . . . designed and built to fit the exacting standards of accuracy needed in precise laboratory work. Accuracy, rugged construction and dependability are built into all Lindberg equipment — from giant industrial furnaces to the small hot plates for lab use.

Write for complete details on specifications, sizes and applications of individual units in the Lindberg Laboratory Line.

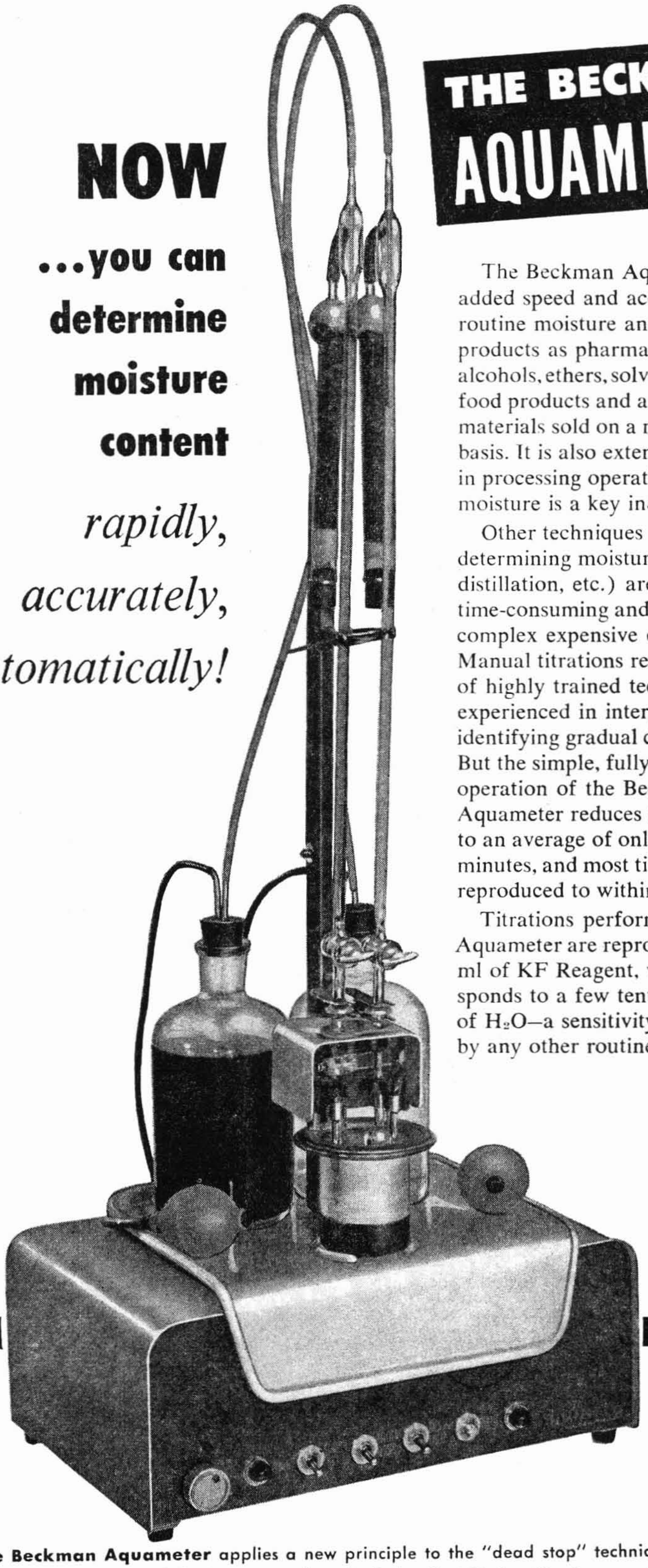
LINDBERG LABORATORY DIVISION

2440 West Hubbard Street
Chicago 12, Illinois

INDEX TO ADVERTISERS

DIRECTORY

REINHOLD PUBLISHING CORPORATION
Advertising Management for the American Chemical Society Publications
330 W. 42nd Street, New York 36, N. Y.
ADVERTISING SALES REPRESENTATIVES
Merald Lue, Advertising Sales Manager
New York 36—BRyant 9-4430; Eastern Advertising Sales Manager, B. Franklyn LaRue; District Managers—James J. Sullivan, Thomas F. Casey, Brayton C. Nichols
Cleveland 13—630 Terminal Tower, PRospect 1-5583; District Manager—Rodney D. Long
Chicago 2—111 West Washington Street, RAndolph 6-8497; Western Advertising Sales Manager, G. E. Cochran; District Managers—Edward J. Fregan, Frederick C. Dorr
San Francisco 5—McDonald-Thompson, 625 Market Street, YUkon 6-0647; Roy M. McDonald
Los Angeles 5—McDonald-Thompson, 3727 West Sixth Street, Dunkirk 7-5391; E. T. Thompson
Seattle 1—McDonald-Thompson, Terminal Sales Bldg., Main 3860; Harry Abney
Dallas 5—McDonald-Thompson, 6617 Snider Plaza. Logan 6283; Dick Worthington
Denver 2—McDonald-Thompson, Colorado National Bank Building, Keystone 4669; Robert H. Heidersbach



THE BECKMAN AQUAMETER

NOW ...you can determine moisture content *rapidly, accurately, automatically!*

The Beckman Aquameter brings added speed and accuracy to routine moisture analyses of such products as pharmaceuticals, alcohols, ethers, solvents, antibiotics, food products and a host of other materials sold on a moisture-free basis. It is also extensively used in processing operations where moisture is a key indication.

Other techniques now used for determining moisture (oven-drying, distillation, etc.) are tediously time-consuming and necessitate complex expensive equipment. Manual titrations require the skill of highly trained technicians experienced in interpreting and identifying gradual color variations. But the simple, fully automatic operation of the Beckman Aquameter reduces titration time to an average of only one or two minutes, and most titrations can be reproduced to within ±0.05 ml.

Titrations performed on the Aquameter are reproducible to 0.05 ml of KF Reagent, which corresponds to a few tenths milligram of H_2O—a sensitivity unsurpassed by any other routine method.

The Beckman Aquameter applies a new principle to the "dead stop" technique, recognized as the most sensitive of the instrumental methods for Karl Fischer titrations, and recommended by the U. S. Pharmacopoeia. Initial adjustment is simple and easily made, and any number of sequential titrations can be carried out with no further checking or setting, simply by adding the sample and pushing a button. The titration is completed automatically and without attention.

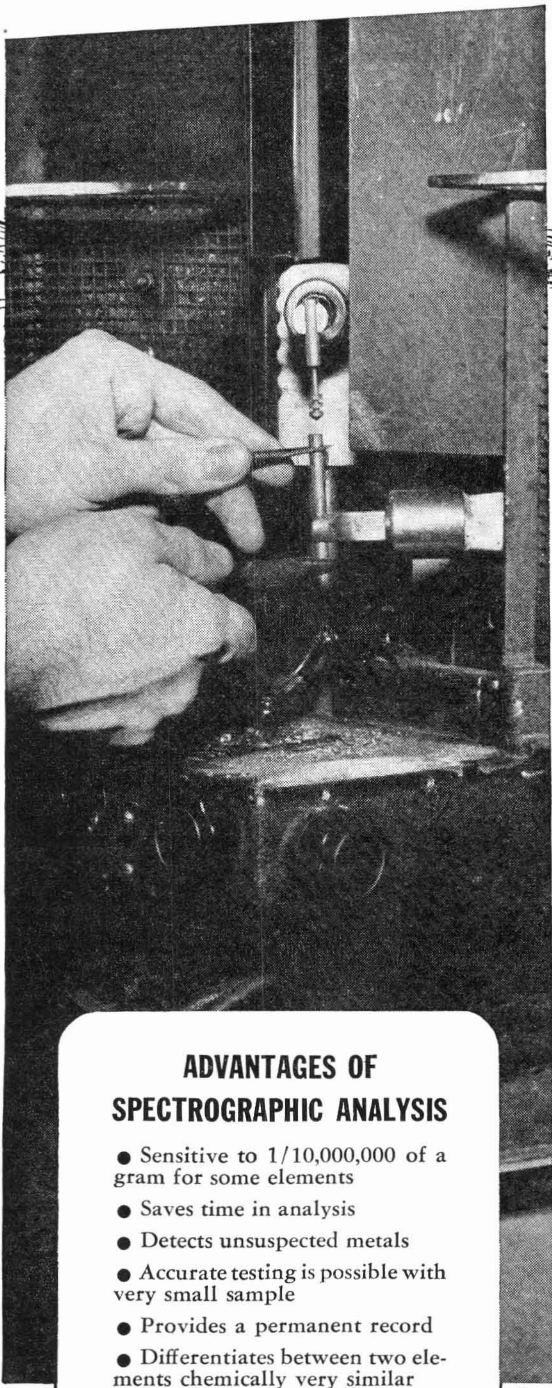
See your Beckman Dealer or write for Data File #41-15

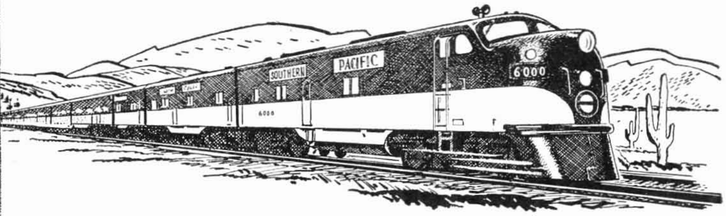
BECKMAN INSTRUMENTS
control modern industries

BECKMAN INSTRUMENTS, INC.
18 SOUTH PASADENA, CALIFORNIA
Factory Service Branches: New York—Chicago—Los Angeles

BECKMAN INSTRUMENTS include: pH Meters and Electrodes . Spectrophotometers . Radioactivity Meters . Special Instruments

UNUSUAL APPLICATIONS OF SPECTROGRAPHIC ANALYSIS...





MATERIAL IDENTIFICATION and QUANTITATIVE ANALYSIS BY S-P

Uses of the spectrograph by Southern Pacific Company offer striking evidence of the method's speed, accuracy and versatility for both qualitative and quantitative determinations.

In their Sacramento General Laboratory, S-P uses spectrography for a quick double-check of the specifications on nearly a score of such purchased items as welding rods; chrome and moly alloys and tool steels; solders and other non-ferrous metal ingots.

Qualitatively, the spectroscope identifies the material in diesel parts requiring replacement or repair and unknown steels from Tool Room Treating.

Special analyses have yielded valuable information on a diversity of S-P items, including band saws to be welded; tank car sediment; residues from ice machines and alcohol heaters; additive oils, greases, paints and pigments.

●

HAVE YOU A JOB FOR THE SPECTROGRAPH?

Spectrography will identify and quantify any element within the range of emission-type spectra *much faster than any other method of comparable accuracy.*

BE SURE OF ELECTRODE PURITY: specify "National" electrodes — the purest obtainable — — accompanied by a statement of purity which enables your analyst to allow fully for trace-element spectra.

●

ADVANTAGES OF SPECTROGRAPHIC ANALYSIS

- Sensitive to 1/10,000,000 of a gram for some elements
- Saves time in analysis
- Detects unsuspected metals
- Accurate testing is possible with very small sample
- Provides a permanent record
- Differentiates between two elements chemically very similar
- Analysis can be made in some cases without destroying sample

*The term "National"
is a registered trade-mark of
Union Carbide and Carbon Corporation*

NATIONAL CARBON COMPANY
**A Division of Union Carbide and
Carbon Corporation**
30 East 42nd Street, New York 17, N. Y.

District Sales Offices:
Atlanta, Chicago, Dallas, Kansas City, New York, Pittsburgh, San Francisco

In Canada: National Carbon Limited,
Montreal, Toronto, Winnipeg

I WISH TO SUBSCRIBE TO

ANALYTICAL CHEMISTRY

STARTING WITH THE JANUARY 1953 ISSUE FOR THE PERIOD OF
☐ 3 YEARS AT $10. ☐ 2 YEARS AT $7. ☐ 1 YEAR AT $4.
☐ REMITTANCE ENCLOSED ☐ BILL ME 45387

NAME
STREET
CITY ZONE STATE
COMPANY TITLE
PRODUCTS

☐ I AM A MEMBER OF THE ACS AND SHOULD BE BILLED AT RATE OF $3.50, $6. OR $9. 12/52

I WISH TO SUBSCRIBE TO

ANALYTICAL CHEMISTRY

STARTING WITH THE JANUARY 1953 ISSUE FOR THE PERIOD OF
☐ 3 YEARS AT $10. ☐ 2 YEARS AT $7. ☐ 1 YEAR AT $4.
☐ REMITTANCE ENCLOSED ☐ BILL ME 45387

NAME
STREET
CITY ZONE STATE
COMPANY TITLE
PRODUCTS

☐ I AM A MEMBER OF THE ACS AND SHOULD BE BILLED AT RATE OF $3.50, $6. OR $9. 12/52

No
Postage Stamp
Necessary
If Mailed in the
United States

Postage
Will be Paid
by
Addressee

BUSINESS REPLY CARD
First Class Permit No. 14-11R (Sec. 34.9 P.L.&R.) Washington, D. C.

AMERICAN CHEMICAL SOCIETY
1155 16th St., N. W.
Washington 6, D. C.

Attn: George G. Taylor

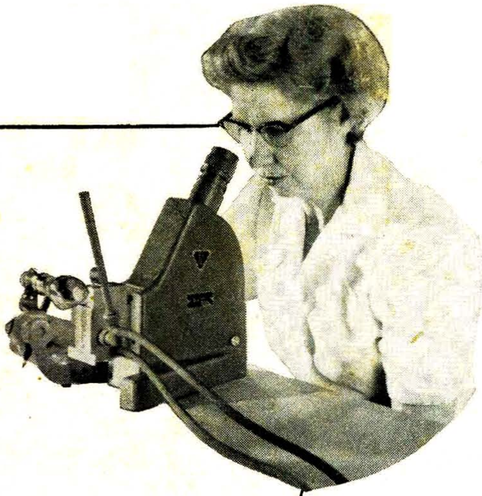
No
Postage Stamp
Necessary
If Mailed in the
United States

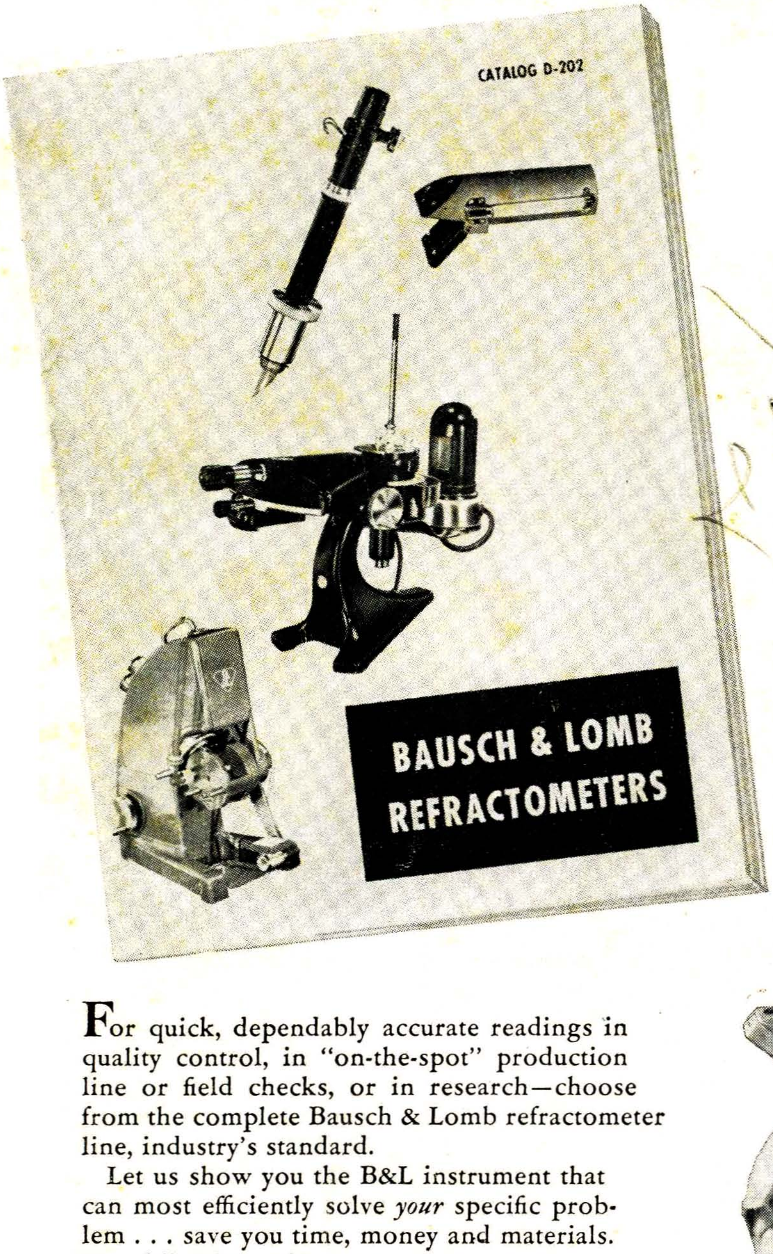
Postage
Will be Paid
by
Addressee

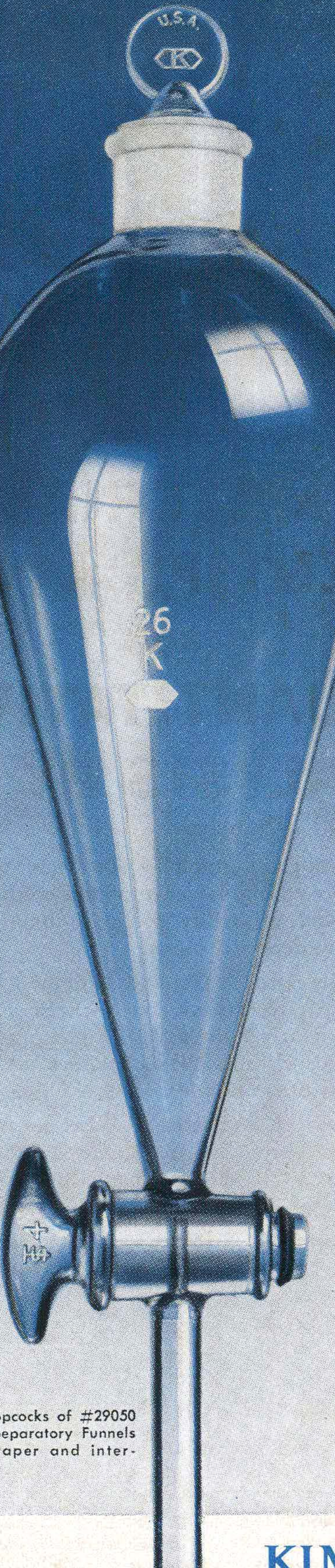
BUSINESS REPLY CARD
First Class Permit No. 14-11R (Sec. 34.9 P.L.&R.) Washington, D. C.

AMERICAN CHEMICAL SOCIETY
1155 16th St., N. W.
Washington 6, D. C.

Attn: George G. Taylor




CATALOG D-202
BAUSCH & LOMB REFRACTOMETERS


U.S.A.
K
26
K