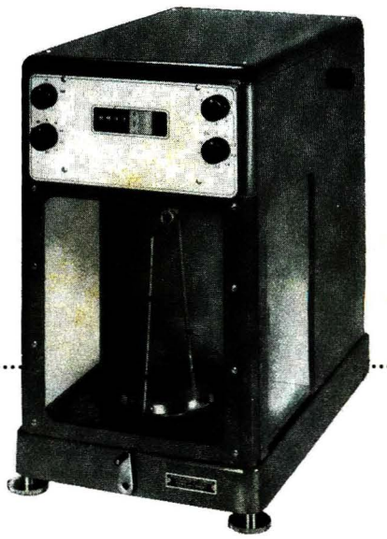
ANALYTICAL CHEMISTRY

JUNE 1951

FOR AMERICA'S LABORATORIES...

A New, Completely Direct-Reading Analytical Balance...

THE *Gram-atic* BALANCE



. . . weighs in one-third the time required by the usual balance.

Its control knobs are manipulated to select built-in weights. Corresponding figures appear on the direct reading scale.

Weights under 100 milligrams are indicated automatically.

The Gram-atic Balance weighs samples up to 200 grams and has constant sensitivity throughout this entire range.

For 115 and 230 volt 50-60 cycle A. C. . . . . $895.00 each.

Fast (20 second weighing)

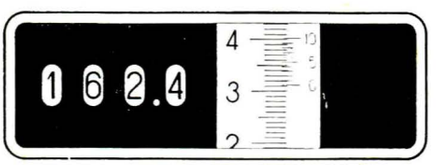
Eliminates all weight handling

Beam under constant load

One-scale reading

Constant sensitivity

Weighs below 100 milligrams automatically



Entire weight of sample is read on direct-reading indicator.

Write for Folder No. 4B

Headquarters for Laboratory Supplies

FISHER SCIENTIFIC COMPANY

Stocks at:

717 Forbes, **Pittsburgh** (19), Penna.
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635 Greenwich, **New York** (14), New York
7722 Woodbury, **Silver Spring,** Md.

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

ANALYTICAL CHEMISTRY

Volume 23, No. 6

Issued June 15, 1951

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What a Reagent Survey told us about you

In a recent widespread survey, you chemists told us a good deal about your likes and dislikes.

You *liked* 'Baker Analyzed' C. P. Chemicals and you ranked them as the No. 1 line of Laboratory Chemicals in *dependability*, in *known quality*, and in *availability*. That was fine praise, and *we liked it.*

Then, some of you told us some things you *didn't* like:

You asked us not to call our chemicals 'C. P.'—but to use the term 'Reagent.'

You felt that 'Baker Analyzed' Labeling could be improved. You wanted the actual analysis on the label, but you wanted it *in type* that could be more easily read. You also wanted the chemical name to appear in *bolder* display.

We welcome your suggestions and we're planning to follow them. Presently, you will begin to see the new 'Baker Analyzed' Reagent label, similar to specimen below.

Of course, the transition will have to be gradual. It will take a long time before all 'Baker Analyzed' Reagents reach you with the new label—for Baker distributors will continue to receive laboratory chemicals labeled both ways until this transition can be completed.

This new label is another contribution to the needs of the laboratory chemist by the makers of Baker Reagents, already first in dependability, first in known quality and first in availability. J. T. Baker Chemical Co., *Executive Offices and Plant:* Phillipsburg, New Jersey.

Baker CHEMICAL

Baker Chemicals

REAGENT • FINE • INDUSTRIAL

WARNING!

Causes Severe Burns to Skin and Eyes

Avoid contact with skin, eyes, and clothing.
Do not take internally.
When handling, wear goggles or face shield.
While making solutions, add Potassium Hydroxide slowly to surface of solution to avoid violent spattering.
In case of contact, immediately flush skin with plenty of water and wash with vinegar; for eyes, flush with plenty of water for at least 15 minutes and get medical attention.

POISON

Antidote - EXTERNAL: Flood with water, then wash with vinegar. If in eyes, wash with saturated boric acid solution. INTERNAL: Do not use emetics. Give water with large amount of diluted vinegar, lemon or orange juice. Follow with milk or white of eggs beaten with water. CALL A PHYSICIAN.

1-3140 1 LB.(453.6g.)

Potassium Hydroxide

KOH PELLETS F. W. 56.104

'Baker Analyzed' *REAGENT*

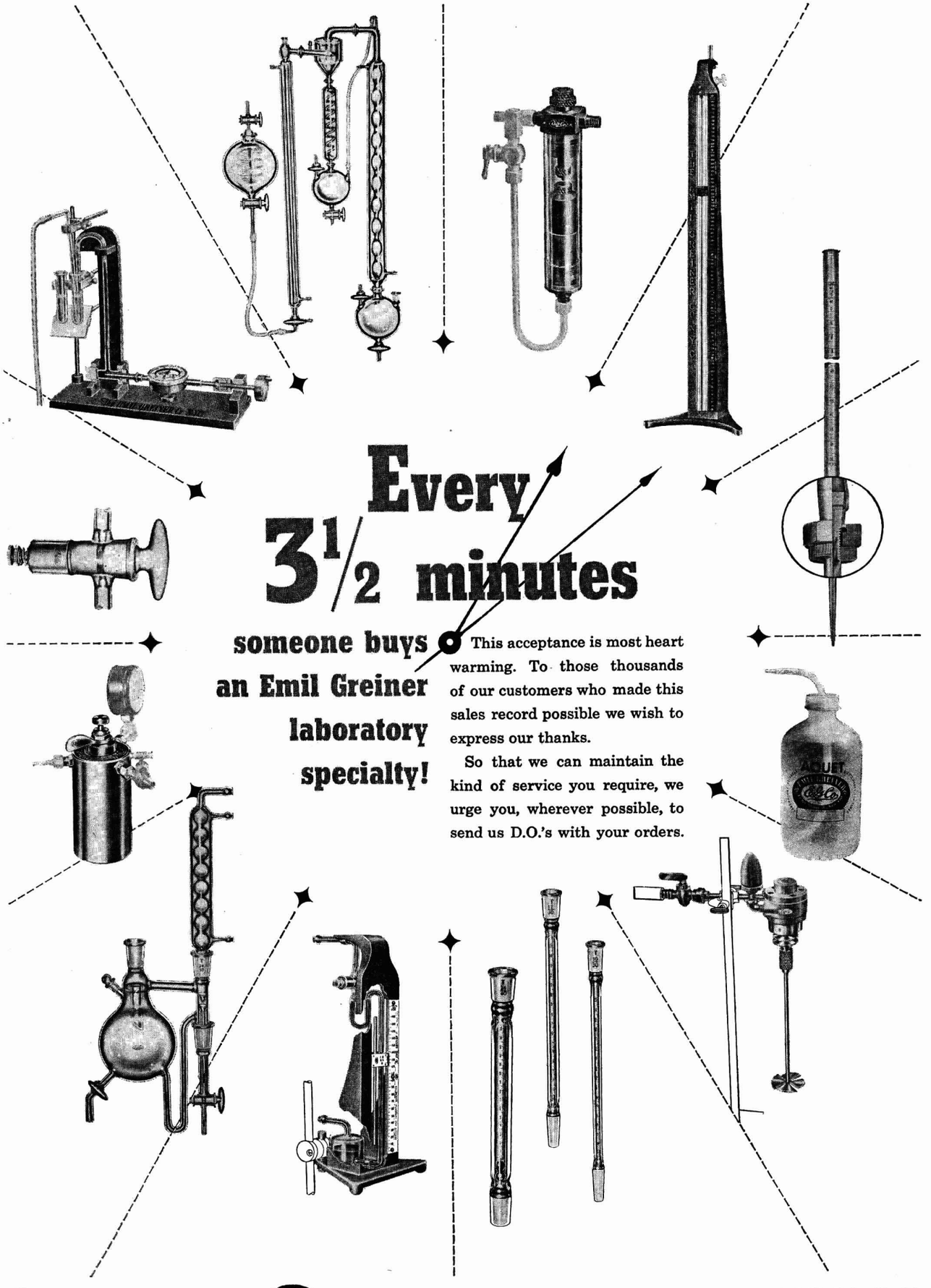
ANALYSIS OF LOT NO. 5150 MEETS A. C. S. SPECIFICATIONS

Assay (KOH)	88.8	%
Potassium Carbonate (K_2CO_3)	1.8	%
Chloride (Cl)	0.008	%
Nitrogen Compounds (as N)	0.0004	%
Phosphate (PO_4)	0.0004	%
Sulfate (SO_4)	0.001	%
Ammonium Hydroxide Precipitate	0.008	%
Heavy Metals (as Ag)	0.0005	%
Iron (Fe)	0.0004	%
Sodium (Na)	0.002	%

MADE IN U.S.A.

Baker CHEMICAL

J.T. BAKER CHEMICAL CO., PHILLIPSBURG, N.J.



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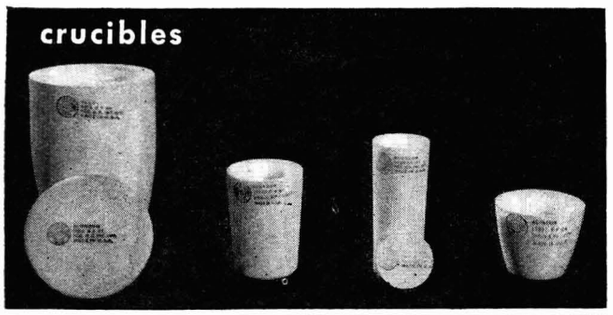
someone buys an Emil Greiner laboratory specialty!

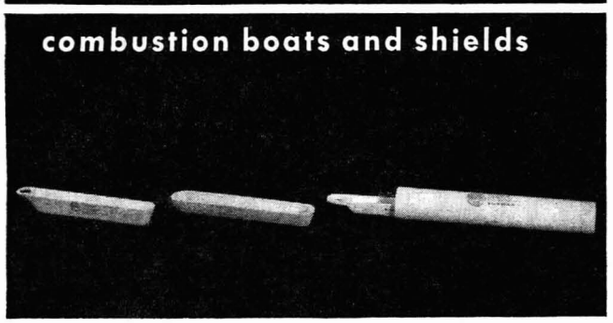
This acceptance is most heart warming. To those thousands of our customers who made this sales record possible we wish to express our thanks.

So that we can maintain the kind of service you require, we urge you, wherever possible, to send us D.O.'s with your orders.

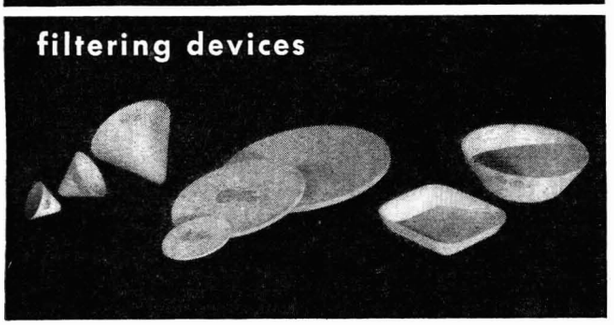
The EMIL GREINER Co. E&Co 20-26 NORTH MOORE STREET • NEW YORK 13, N. Y.

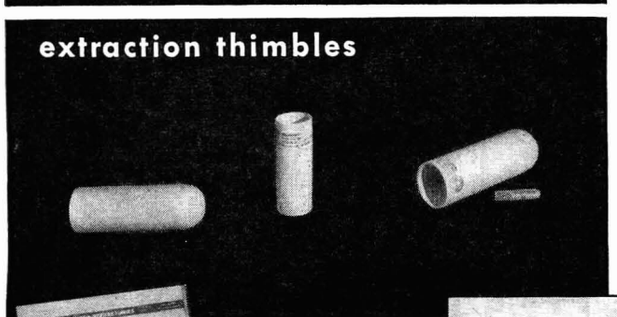
How does your REFRACTORY LABORATORY WARE *compare with this?*





MADE OF FUSED ALUMINA, containing at least 85% alumina (up to 99% in special mixtures), this highly refractory Norton Alundum* laboratory ware is not attacked by any organic solvent.





Your laboratory development, experimental or analytical work is important enough to justify the best materials money can buy. That's why you owe it to yourself and your work to compare Norton Alundum ware with whatever you are now using.

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Chemical Stability — Norton Alundum ware is not attacked by any organic solvent. Only hydrofluoric acid, concentrated phosphoric acid or concentrated alkalies can affect it.

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Norton Alundum laboratory ware includes general purpose crucibles, ignition capsules, incinerating dishes, melting crucibles, filter crucibles, filter cones, filter dishes, filter discs, extraction thimbles, and combustion boats. . . all in many sizes and shapes.

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Get Bulletin 793

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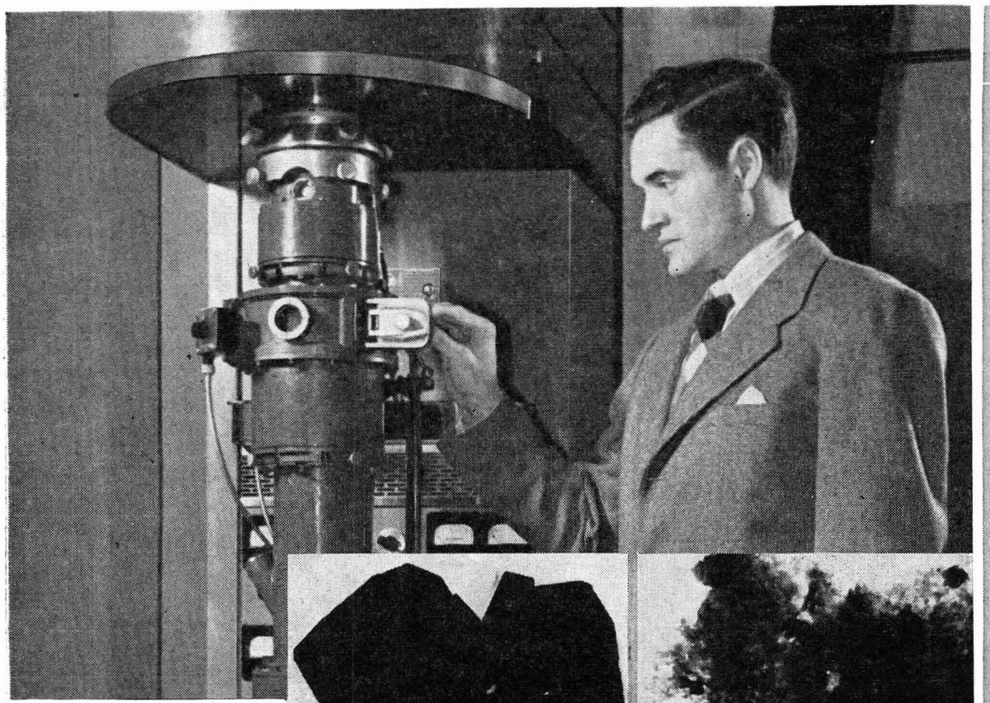
CARRIED IN STOCK BY MOST LABORATORY SUPPLY HOUSES



Edwin B. Bradford of DOW placing a specimen in the RCA Electron Microscope. The Dow Chemical Company uses three of these powerful instruments.

HOW LIME BREAKS DOWN

Fresh lime (Magnified 7,000 x)

Inactive lime, broken down from use (Magnified 8,000 x)

How the RCA *electron microscope* helps

DOW CHEMICAL

with processing problems

TAKE THE TROUBLE with lime, for example —an important ingredient used in one of DOW's processing operations.

All signs pointed to the possibility that the activity of lime dropped off with use. Yet careful chemical tests failed to show that any significant change took place.

Then the RCA Electron Microscope gave the answer!

Electron micrographs of lime—magnified more than 24,000 times—*clearly revealed that lime particles did break down extensively into finely divided, gelatinous masses. And when the action was completed, all chemical activity ceased!*

Just one example of how the RCA Electron Microscope is helping DOW research solve many problems in chemistry, metallurgy, physics, and biology.

Consider the possibilities of the RCA Electron Microscope in your own research and production program. This powerful instrument can produce sharp images at direct magnification from 500 to 23,000 diameters—with photographic enlargements to 100,000 diameters. Resolving power is 20 to 100 times greater than the best light microscope. Depth of field is 75 to 150 times greater than the best light microscope.

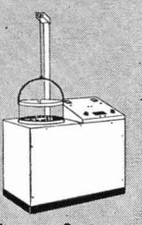
For more information about the RCA Electron Microscope, simply write Dept. R-98, RCA Engineering Products, Camden, New Jersey. No obligation, of course.

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

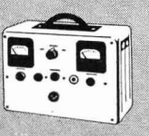
In Canada: RCA VICTOR Company Limited, Montreal

RCA Scientific Instruments for Industry

Micro-second Timer



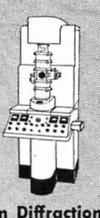
Vacuum System



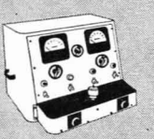
Vacuum Gage

Count-Rate Meter

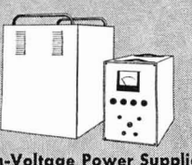
New Low-Cost Electron Microscope



Electron Diffraction Unit

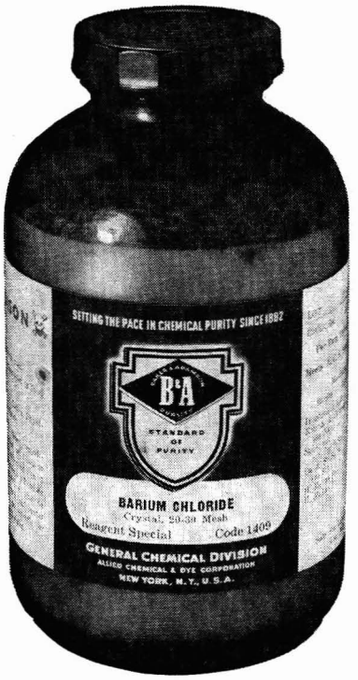


Vacuum Leak Detector



High-Voltage Power Supplies

3 reasons why you should specify B&A reagents!



1 WIDE SELECTION...
B&A has over 1,000 laboratory chemicals for you to choose from.

2 HIGHEST PURITY...
always meet or exceed A.C.S. specifications.

3 READILY AVAILABLE...
from B&A's own well-stocked distributing stations situated in key centers everywhere.

B&A SAVES YOU *time, money, effort in your laboratory chemical buying.* When you specify B&A, you can choose from more than 1,000 purity products of Reagent, A.C.S., C.P., U.S.P., N.F., and Technical grades.

THE BAKER & ADAMSON LABEL *is your assurance of the highest purity.* Reagent chemicals that bear the B&A "Shield of Quality" *always meet or exceed A.C.S. specifications* . . . the exacting purity standards set by the chemical profession itself!

YOU GET *prompt and efficient service.* Extensive stocks of "B&A Quality" products are supplied through B&A's own chain of distributing stations located from coast to coast.

Contact the B&A office nearest you and tell them your needs. Your B&A salesman will work with you in having our local stocks built to meet your requirements.

REAGENTS
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FINE CHEMICALS

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ALLIED CHEMICAL & DYE CORPORATION
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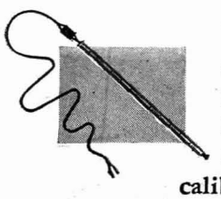
SETTING THE PACE IN CHEMICAL PURITY SINCE 1882

* Complete stocks are carried here.

For accurate temperature measurement, use meters calibrated for Chromel-Alumel thermocouples

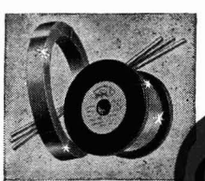


Wherever accurate temperature measurement is important to the finished quality of work . . . in laboratory analysis or on heat treat operations . . . you'll find that nearly always the pyrometers in use are calibrated for Hoskins CHROMEL-ALUMEL thermocouple alloys.

And for good reasons, too. Because CHROMEL-ALUMEL thermocouple wire carries a positive accuracy guarantee . . . ±5° F. at temperatures up to 660° F., and ±¾% from 660° F. and over. It's highly responsive to temperature fluctuations . . . so resistant to oxidation that you need not pack the protection tube . . . and it maintains its fine accuracy over longer periods of time than any other base metal thermocouple material. So if you're responsible for accurate temperature measurement, better check your pyrometers to make sure they're properly calibrated, then specify couples by name . . . CHROMEL-ALUMEL thermocouples. Available through your instrument manufacturer or pyrometer service company.

PS—And while you're at it . . . complete your chain of accuracy with CHROMEL-ALUMEL "extension" leads. For, by using wires of identical compositions for both couples and leads, you eliminate all possibility of "cold-end" errors.

Complete technical information is contained in our Catalog 59-R . . . want a copy?



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Chromel®

**the original nickel-chromium resistance alloy that first made electrical heating practical*

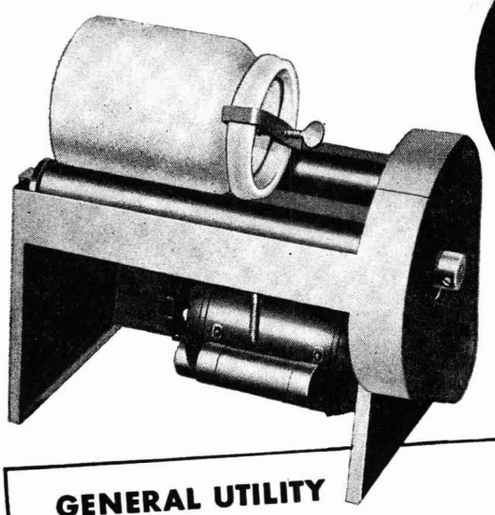
NEW ADJUSTABLE ROLLER TYPE LABORATORY JAR MILLS

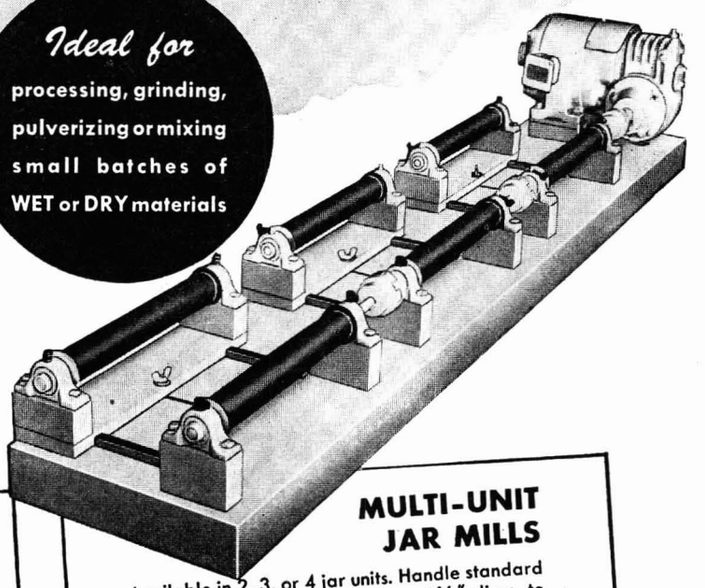
These new "Roller-Type" mills are adjustable through 3 positions enabling units to handle standard jar mills or round containers of 2½" diam. to 15" diam. (roughly from ½ pint to 6 gal. capacity). "Roller-type" Jar Mills are sturdily built with welded steel frames. The rollers are made of a special rubber formulation molded on a ⅝" diam. steel core; they are 12½" long and average approximately 2" in diam. One roller is convex, one concave, and they revolve in heavy duty ball bearings. The entire construction is designed to provide long, trouble-free life, low power costs and minimum upkeep expense.

Outstanding Features

- Will roll any round container—mill jar, bottle, can, etc.
- No clamping in frames or fastening required. Merely place container on the rollers.
- Special patented design of the rollers automatically centers the jar or container—prevents creepage, minimizes breakage.

Ideal for processing, grinding, pulverizing or mixing small batches of WET or DRY materials





GENERAL UTILITY LAB MILL

Powered by a ¼ h.p., 110 volt, 60 cycle open-type motor. Rollers adjustable through 3 positions; handles standard mill jars or round containers of 2½" diam. to 12" diam. (Roller length 12½"). Overall dimensions: 16" long x 14" wide x 16" high. Roller speed approximately 175 R.P.M. Net weight 75 lbs.

CAT. NO. N1930 . . . . $172.50

MULTI-UNIT JAR MILLS

Available in 2, 3, or 4 jar units. Handle standard mill jars or round containers from 2½" diam. to 15" diam. Roller speed, 216 R.P.M.

Cat. No.	No. of Jars	Overall Dimensions L	W	H	Motor H.P.	Price AC	DC
N1931	2	54¾"	15"	12⅞"	½	$453.50	$480.50
N1932	3	76¼"	15"	12⅞"	½	551.50	628.50
N1933	4	96"	15"	12⅞"	¾	673.50	706.50

SPECIFY VOLTAGE.
Available with Explosion Proof Motor. Prices on Request.

ROALAX MILL JARS

Made of white chemical porcelain. Lid securely tightened by a novel locking cross-bar controlled by an extra large non-slip hand wheel. Gasket material is of rubber but chemically resistant tygon gaskets or cork can be supplied.

Size	Capacity	N1937 Chemical Stoneware	N1938 White Porcelain
00	1 qt.		$17.50
0	.46 gal.	$16.50	20.50
1	1.17 gal.	32.50	40.00
2	2.7 gal.	50.50	62.50
4	4.4 gal.	63.00	76.50
6	6.8 gal.	74.00	100.00

GRINDING MEDIA

No. **N-2131** "Borundum" Tubular Grinding Media. Cylindrical in shape, approximately 13/16" in diam. by 13/16" long. Weigh 75-80% more, and up to 50% harder, than conventional media. Afford greater contact area for faster, better quality grinding. Reduced "wear-off" assures correspondingly less contamination of the mill charge.

Average No. per Pound	18
Price per Pound	Less than 100 lbs.—$.88 per lb. 100 to 500 lbs.—$.74 per lb.

Cat. No. **N-2132** Flint pebbles. Specially suited for grinding jobs requiring less speed and moderate quality. Exceedingly hard, uniform in size and free from holes.

Size	1" x 1½"	1½" x 1¾"	1⅝" x 2"
Aver. No. per Pound	8	4.2	2.5
Price per Pound	.20	.20	.20

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SCHAAR & COMPANY 754 W. LEXINGTON ST. CHICAGO 7, ILLINOIS

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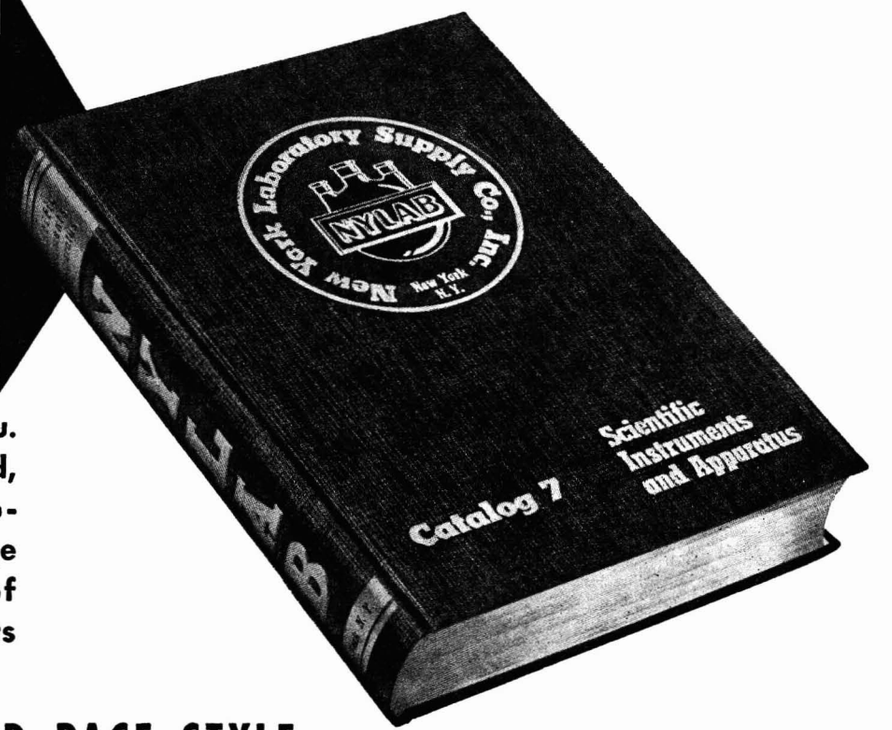

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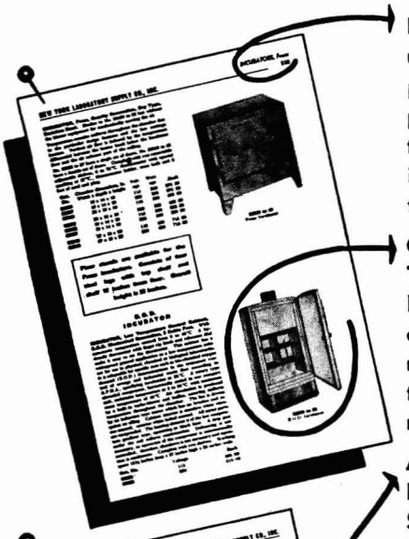
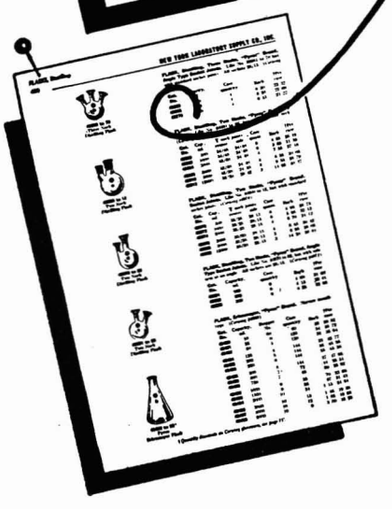


EACH PAGE INDEXED. The upper outside corner of each page carries the individual page listing directly above the page number to assist in identifying the proper section of the catalog.

CLEAR FACTUAL ILLUSTRATIONS. Over 3,500 large illustrations arranged alongside the descriptive material, with each illustration bearing the catalog number and description.



AN INDIVIDUAL NUMBER FOR EACH STYLE AND SIZE. For fast, easy ordering, each size and style of an item carries its own BOLD FACE catalog number. Your assurance of receiving the item you desire by merely writing one number instead of the customary lengthy description.

FEATURES OF THE NEW NYLAB CATALOG 7

- Alphabetic listings with each item and size of item carrying its own catalog number in bold face type.
- Fully illustrated with 3,547 individual illustrations.
- Large page size, 8 x 10¾ inches, printed on a heavy stock, bound in durable Fabrikoid and embossed in green and gold.
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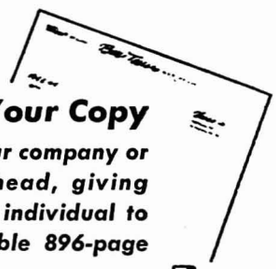

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the analyst's column

IN JANUARY we outlined the scope and general methods of geochemical prospecting. A paper by Ray E. Gilbert given at the 1950 Metal Mining Convention presents some details from an actual survey:

Since June 1949, the New Park Mining Co. has been conducting experiments in geochemical prospecting for ore on its property in the southeastern part of the Park City district, Utah. Ore in the area studied is found principally in veins localized along faults and fractures cutting Paleozoic sediments and quartz diorite intrusives. The veins of quartz, with varying quantities of sulfides of lead, zinc, and some copper, are concealed under residual soil, 5 to 30 feet thick.

Sampling is done on a prearranged geometrical pattern. The humus layer is scraped away, and 10 to 20 grams of the first soil encountered, usually at a depth of 3 to 6 inches, with twigs and pebbles removed, are taken as the sample. Samples are taken at this depth because of the convenience, but, more important because in general it has been found that the shallow sample contains more metal and tends to emphasize the mineralized areas more than samples taken at greater depths. This metal enrichment of topsoil is explained as being caused by the action of plants in bringing up mineral matter from the subsoil.

While variations of 75 to 150 p.p.m. on resampling are common, these variations are not great enough to conceal anomalies indicative of mineralization where values of above 600 p.p.m. of total metal are obtained compared to 200 to 400 p.p.m. in barren areas.

The analytical field method involves taking a 0.5-gram sample and fusing over a gasoline stove with a bisulfate flux until a bright orange melt results. The melt is dissolved in water, and acetate buffer and dithizone test solution are added. This reagent gives a pink color with heavy metals, and the concentration is measured in a simple photometer previously standardized. If the total metal is greater than 300 p.p.m., sodium thiosulfate is added to the dithizone layer, and the resulting mixed color is again measured in the photometer and recorded as zinc.

"Total metal" values are used in preference to zinc because they tend to emphasize mineralization more than the zinc values alone, and because they are more apt to give a clue to the structural pattern in areas of weak mineralization. Whenever the "total metal" content is high, a zinc determination, made as a check on the "total metal" determination, makes it possible to contour the zinc pattern also, in areas of consistently high values.

All data are plotted on 28- by 36-inch sheets of tracing cloth, on a scale of 1 inch equals 100 feet. A base map is made, showing the location and identification numbers of all survey stations and sample points, and separate overlays are made of the following: one of mining claims and property boundaries, one of any underground workings and geology, one of topography and surface geology, one of "total metal" values, and one of zinc values.

The results of this study show that a vein 2 to 5 feet wide has a heavy metal halo in the overlying soil 50 to 100 feet wide but with the values above 600 p.p.m. in a belt less than 50 feet across. The background topsoil showed less than 400 p.p.m. The study also showed that mineralization 20 feet in depth can be detected by topsoil analysis.

A three-man crew was found to be the most convenient unit to handle all the field, laboratory, and office work involved in soil sampling. Although men with less training could be used on most of this work, college graduates with more or less education in geology, engineering, and chemistry are preferred. It is desirable that each man be capable of doing both field and laboratory work to provide maximum flexibility of the crew, and to give each an occasional change from his usual routine.

A trained crew is doing well to collect and analyze an average of 45 samples per day, or 15 samples per man-shift. This figure may be higher in reconnaissance sampling or in brush-free areas, and will be somewhat lower in areas of thick brush and steep slopes.

Sampling on a 200-foot checkerboard for reconnaissance work would require roughly one sample per acre. Based on the 45 samples per day average, preliminary sampling could be conducted at the rate of 15 acres per man-shift. Sampling in more detail along profile lines 100 feet apart, with samples taken every 50 feet on the lines, slows the rate down to less than 2 acres per man-shift.

THERE are excellent sessions planned for analytical chemists at the World Chemical Conclave. So keep the date—September 3 to 15—open for these events which will be held in Washington, D. C., and in New York City.

Associate Editor

ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

Academic Trends in Analytical Chemistry

DOUGLAS G. Nicholson of the Fisher Scientific Co. is a very much traveled individual, spending a considerable portion of each year interviewing teaching staffs in our schools of chemistry and chemical engineering. He is the next best thing to a Gallup Poll when one wishes to find out the latest trends in teaching, particularly in the field of analytical chemistry. We are indebted to Nicholson for his paper "Trends in Academic Analytical Chemistry" given before the 1951 Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. In his paper he lists ten important trends that we would like to summarize briefly for our readers.

Growth of Semimicro Techniques. Semimicroprocedures in elementary qualitative analysis are being employed in from 90 to 95% of the schools contacted and several other institutions plan to institute such programs in the fall of 1951. The use of "semi" procedures is spreading into general chemistry laboratories as well as into many elementary organic courses. Approximately 30 to 40% of the schools visited by Nicholson in the past 12 months now use semi procedures in their elementary general chemical laboratories, and from 10 to 20% use the semi approach in elementary organic courses.

Analytical Procedures Not Using Hydrogen Sulfide or Sulfides as Such. Nicholson reports a definite reduction or complete elimination of the use of hydrogen sulfide in qualitative analytical schemes. The use of aqueous solutions of organic compounds which hydrolyze to produce a sulfide ion in contact with the metal ion solution has eliminated the long-familiar hydrogen sulfide Kipp generator. Any procedure that helps to eliminate the use of hydrogen sulfide in qualitative analysis is welcome to teacher and student alike and indicates basic progress in the science of analytical separations.

Streamlined Kjeldahl Techniques. Analytical chemists welcome a method of Kjeldahl nitrogen determination which dispenses with the distillation procedure. Nicholson reports as No. 4 in his list of trends Kjeldahl digestion aspiration trains devised for laboratories not adequately equipped with ventilation or fume hoods. Both modifications are particularly welcomed by teachers of analytical chemistry in schools where large numbers of students must utilize limited laboratory facilities.

Applied Electronics Instruction for Chemists. Current analytical instrumentation work involves wide application of physical methods in chemical analyses. It is reassuring to hear that emphasis is being placed in our colleges and universities on the use of electronics in instrumentation. It is important that the student learn the fundamentals of instrumentation rather than the mere operation of equipment. To do this he should be steeped in the theory of electronics and should be provided with the opportunity to repair an instrument, to modify it, or even to devise a new circuit to accommodate specific immediate needs.

Enumeration of the remaining special trends reported by Nicholson will emphasize their growing importance in the minds of analytical chemists.

Paper chromatography is used as an analytical chemical tool not only in undergraduate courses but in our organic and biological research laboratories.

Similarly, the use of ion exchange resins in analytical chemical procedures is widely recognized not only in the training of analytical chemists, but as a very important day-by-day tool in quantitative separations in research and in industrial laboratories.

The extent of the interest and possibilities of tracer techniques in chemical research is reflected in the large number of manuscripts which have appeared and are appearing in scientific and technical journals. The use of these "tagged" atoms in research will lead us into heretofore entirely unexplored fields and add greatly to our scientific knowledge in widely diversified fields.

One observation of Nicholson frankly does not meet with our approval. He reports that in many instances qualitative analysis is being eliminated from chemical engineering curricula. We appreciate the time element that probably is responsible for such deletion, but we feel that our chemical engineers of the future will lose a great deal of solid basic training as the result of the elimination of courses in qualitative analysis.

Finally, we are very happy to note an increase in the popularity in the courses of analytical instrumentation, as reported by Nicholson. Instrumentation is not a passing fad. There are sound basic reasons why it should continue to develop and serve the needs of the chemical analyst. It is well for us to remember, however, that the instrument must always be the servant, not the master. Accordingly, our academic institutions must be primarily concerned with the development of scientists rather than technicians—men and women who are thoroughly familiar with the fundamentals involved, who are capable of fundamental research, not mere knob-twisters following printed instructions, for instrumentation is based in one way or another upon very definite advances in fundamental knowledge. When we fail to place proper emphasis on fundamental research in instrumentation in our academic institutions, then we can expect to lose the present pace that has characterized its growth in the past decade.

Analysis by Electromigration plus Chromatography

HAROLD H. STRAIN AND JAMES C. SULLIVAN
Argonne National Laboratory, Chicago, Ill.

This investigation was undertaken to provide widely applicable analytical methods for continuous operation and of potential industrial importance. To this end, electromigration was combined with various chromatographic techniques. With flow of electrical current at right angles to the flow of solvent in a special cell, mixtures of various ions were separated completely and continuously. With this method one ion could be substituted continuously for another, and in the presence of complexing agents that affect the adsorbability and the ionic mobility of various solutes, many different kinds of substances could be isolated and detected. Modifications provide sensitive and rapid discontinuous procedures for the separation of mixtures of ionic substances, as in qualitative analysis.

ELECTROMIGRATION (*2, 4, 10, 12*) and chromatography (*3, 15, 17, 19, 20, 24*) have long been employed for the resolution of mixtures of solutes. In 1939, Strain utilized electromigration, without flow of solvent, for the separation of dyes in a column of moist porous adsorbent (*21*). Under the name of electrochromatography, this method has been widely applied to the resolution of mixtures in columns and in strips or stacks of filter paper (*3, 8, 9, 13–16, 23, 25*). It has proved effective with charged particles in dispersed systems as well as with the inorganic ions (*6, 8, 23, 25*).

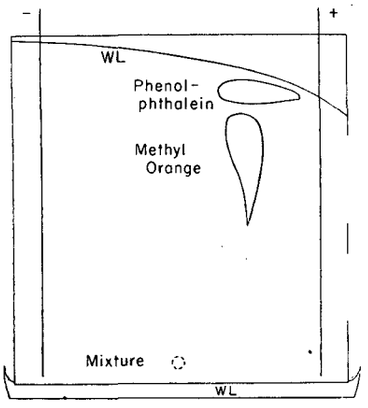


Figure 1. Separation of 0.02 *M* Methyl Orange and 0.02 *M* Phenolphthalein

Separated by upward flow of wash liquid, *WL* (4 *M* ammonium hydroxide), and by horizontal flow of electric current. Paper (Grade 301 or 625, 0.030 inch thick, Eaton-Dikeman), clamped between plastic plates to prevent evaporation of ammonia. 300 volts, 25 to 35 ma., 1 hour

In 1948, Haugaard and Kroner employed electrical migration plus simultaneous flow of solvent for the discontinuous separation of ionized substances in a sheet of paper, the current flowing at right angles to the flow of solvent (*11*). A modification of this method, which requires only about an hour for resolution of a mixture, is shown in Figure 1.

In 1949, Svensson and Brattsten utilized electrical migration plus simultaneous flow of solvent for the continuous separation of dyes in a cell filled with powdered glass on which there was no adsorption of the ionized solutes (*22*), and Strain designed an analogous analytical system for the continuous separation of solutes by electrical migration plus chromatographic adsorption (*18*). An elaboration of this latter method forms the basis for this report.

APPARATUS

Most experiments summarized here were performed in electrographic cells constructed of adsorptive paper held between glass plates. Cells designed for continuous operation were constructed of paper with a paraffined central compartment as indicated by Figure 2. Cells designed for discontinuous operation were constructed without this compartment, compartment *D,D* extending continuously from side to side.

In both types of cells, the adsorptive material was commercial filter paper (0.1 inch or 2.5 mm. thick). Two papers (Filpaco, No. 046, The Filter Paper Co., Chicago, Ill.; and Grade 320, Eaton-Dikeman, Mt. Holly Springs, Pa.) gave reproducible results and exhibited exceptionally high filtration rates. Both papers occasionally contained iron and copper in small spots (probably in the form of oxides).

For preparation of the cells, the sides of the paper and the central compartment were brushed with a saturated solution of paraffin in carbon tetrachloride or in petroleum ether (boiling range 65° to 110° C.), and the saturated areas were allowed to dry. With paper designed for the continuous procedure, the dried, central paraffined area was cut with a sharp razor blade, and a narrow strip of untreated filter paper (Eaton-Dikeman, Grade 625, thickness 0.030 inch) was inserted as indicated by the figure. The inserted paper served as a wick for the continuous addition of a narrow zone or stream of the mixture to be resolved. For this purpose, the common analytical papers also proved useful.

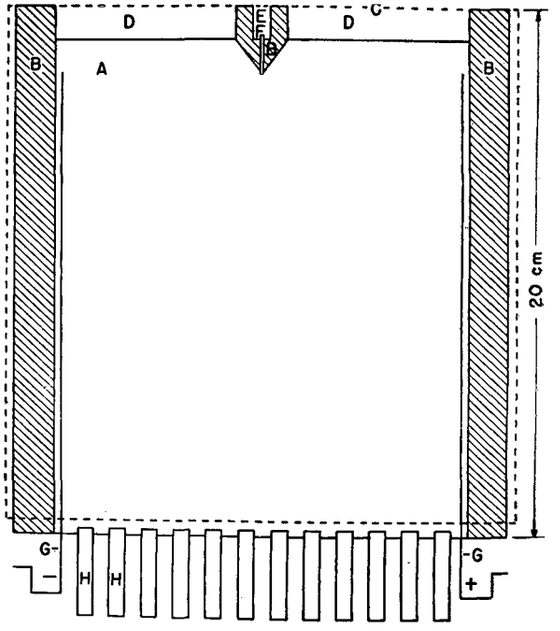


Figure 2. Electrographic Cell for Continuous Operation

A. Filter paper
B. Regions of paper impregnated with paraffin
C. Outline of glass plates
D. Compartments for addition of wash liquid
E. Compartment for addition of solute mixture
F. Paper wick through paraffined region
G. Platinum electrodes in grooves in glass plate
H. Paper strips to promote uniform flow of wash liquid

This prepared paper was placed between two glass plates as indicated by Figure 2. Platinum electrodes were inserted in grooves ground in one of the plates, and the plates were pressed to the paper with screw clamps. This electrographic cell was clamped in an upright (vertical) position between two ring stands. A pan for the collection of the effluent was placed under the cell, and separatory funnels for the continuous addition of wash liquid were supported on the ring stands.

CONTINUOUS OPERATION OF THE CELLS

For continuous operation of the electrographic cells, wash liquid was added continuously to compartments *D,D*, indicated in Figure 2. When this liquid flowed through the cell, small strips of filter paper were placed in contact with the moist paper protruding below the glass plates. These paper strips, which were held in place by capillary forces, promoted uniform dripping of the wash liquid. They could also be moved in order to channel the effluent into particularly narrow regions. The cells themselves retained slightly over 60 ml. of the wash liquid and approximately 20 minutes were required for the flow of this quantity of liquid through the cell.

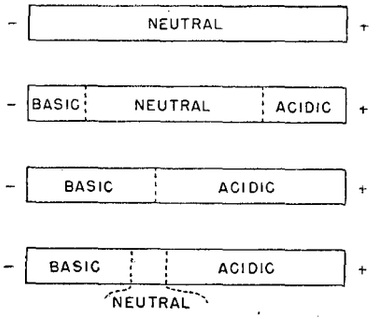


Figure 3. Migration of Acidic and Basic Zones Formed by Electrode Reactions with Ionized Salts in Paper Strips

With continuous flow of wash liquid through the cell, the solution of the mixture of ions was added to the central compartment, *E*, whence it too flowed continuously into the cell. Potential was then applied across the cell. Under these conditions, the several ions followed separate, narrow paths through the cell, so that they could be collected separately and continuously in the respective portions of the effluent. To this end, small beakers or test tubes were placed in suitable positions below the paper strips.

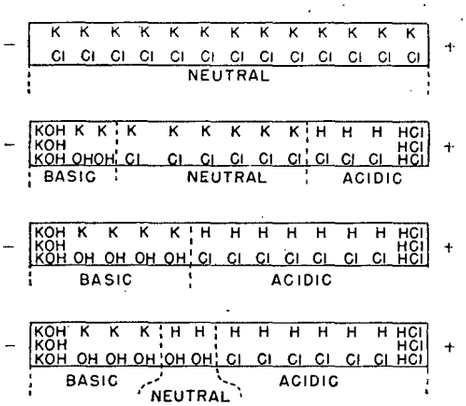


Figure 4. Simplified Mechanism for Migration of Acidic and Basic Zones Shown in Figure 3

The paths followed by the ions did not vary, provided the electrical current and solvent flow remained constant. Paths followed by dyes and by colored ions as copper and ferric iron were readily visible. Many ions, such as nickel and cobalt, could be located in the effluent by addition of reagents to the paper strips or to portions of the percolate collected from the paper strips. With many solutes it was possible to discontinue the operation of the cell, to remove the glass plates, and to add reagents to the paper so that the ion paths were rendered visible. Reagents were usually added as gases or as solutions which were brushed or sprayed onto the paper. As some colored products were rather unstable, the paths were outlined with indelible pencil, thereby yielding a permanent record.

DISCONTINUOUS OPERATION OF CELLS

For discontinous separations, the electrographic cells without the central paraffined compartment were saturated with the wash liquid. A small quantity of the mixture (0.01 to 0.1 ml.) was then delivered from a fine-tipped pipet to the upper edge of the paper. Potential was applied and enough wash liquid (about 60 ml.) was filtered through the cell so that nonadsorbed solutes were carried nearly to the bottom of the paper. The electrical current was interrupted, the cell was opened, and various reagents were applied to the paper.

This discontinuous procedure revealed the several ions as a series of zones or spots. Resolution of the mixtures in this way required about 20 minutes from the time of their addition or a total operational time of about 40 minutes.

PROPERTIES OF ELECTROGRAPHIC SYSTEMS

Numerous variable conditions affect the separations in the electrographic cells: the dimensions and the construction of the cells, the voltage and the electrical current, the properties of the solvent or wash liquid including its reactions at the electrodes, the concentration and the electrical properties of the ions, the adsorbability of the ions, and the nature of the complexes formed by the ionic mixtures with the wash liquids.

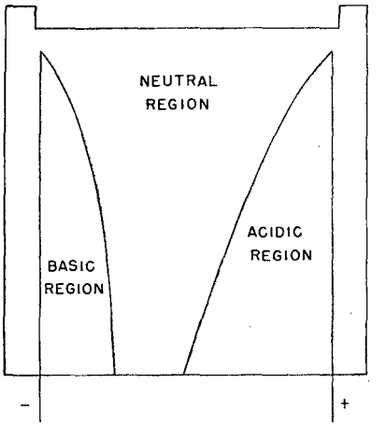


Figure 5. Acidic and Basic Regions Formed in Electrographic Cell with 0.1 *M* Ammonium Acetate

300 volts, ca. 50 ma.

The construction of the cells was varied in many ways. Plates of plastic (6 mm. thick) were employed as well as plates of glass, and papers of various filtration rates were also tested. For the discontinuous procedure, holes were drilled through one of the plastic covers so that the mixture of ions could be added to the paper through this opening, which was then plugged while the cell was washed with the fresh solvent. This procedure offered little advantage over the direct addition of the mixture to the upper edge of the paper. With ions that migrated principally in one direction, the mixture could be added near the side of the cell, thereby increasing the effective area.

The dimensions of the cells were also varied. Thick cells were prepared by placing several sheets of paper between the glass plates. Large cells were prepared from sheets of paper about 60 cm. (24 inches) square. With both modifications of the cells, similar separations of ions were obtained, provided the same

current density was maintained per unit cross section of the cell. A few experiments carried out in cells constructed of plastic plates and filled with inorganic adsorbents such as heat-treated siliceous earth (Celite) yielded results analogous to those obtained with paper (*21*).

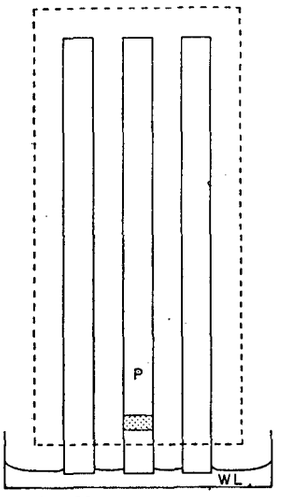


Figure 6. Chromatographic Separation of Mixtures of Ions in Paper Strip, *P*, between Glass Plates

The electrical current was adjusted in relation to the flow and the electrical conductivity of the wash liquid. With the coarse paper described above, an electrical potential ranging from 160 to 400 volts and providing a current of about 50 to 100 ma. was sufficient for separation of many ionic mixtures. With the highest voltages and currents, the cells became rather warm. As cool wash liquid flowed into the cells at the top, the regions of highest temperature were near the bottom. This effect caused the ions to move slightly faster in the lower regions of the cells than in the upper cooler regions, so that the paths followed by the migrating ions were often slightly curved.

The electrical properties of the wash liquid determined the conductivity of the cells and the separability of many ions. Owing to their low conductivity, weak acids and bases could be employed as wash liquids at much higher concentration than neutral salts. With these acids and bases, considerable variation of pH was possible without enormous variation of the electrical conductivity of the cells. Moreover, some of these acids and bases formed complex or chelated ions which facilitated the separation of many mixtures.

Electrolysis of the wash liquid often had a great effect upon the pH in different regions of the electromigration systems. With salt solutions in paper strips (Figures 3 and 4) or in the electrographic cells (Figure 5), basic regions were formed at the cathode and acidic regions at the anode. These zones, which were readily detectable with a strip of test paper, migrated toward each other. Once they met, the resistance of the cell increased rapidly. The mechanism for this effect is shown by Figure 4, a simplified drawing that does not portray the differential migration rates of the ions or the evolution of gas at the electrodes. When the ions being separated were affected by acid or alkali, these secondary reactions became very troublesome. They were avoided by use of wide cells and, as in most of the experiments reported here, by the use of weak acids or weak bases as the wash liquids. In the electrographic cells, gases liberated at the electrodes escaped through the grooves in the glass plate.

The wash liquid itself had a pronounced effect upon the adsorbability of ions. From acid solutions or from salt solutions, most cations were scarcely adsorbed on paper. From weakly alkaline solutions many cations were strongly adsorbed on paper. Through variation of the wash liquid, both the electromigration and the adsorption of ionic substances could be varied in order to improve the separations.

ELECTROMIGRATION AND CHROMATOGRAPHIC COMPONENTS OF ELECTROGRAPHIC CELLS

In the electrographic cells there are two principal migration forces—the flow of electrical current and the flow of solvent. These two components were studied separately in sheets or strips of paper, as illustrated by Figures 6 and 7. For investigation of the chromatographic factor, the solution of ions was added as a band near the end of a paper strip, or as spots near one edge of a wide paper sheet. This paper was then placed between glass plates, and the protruding paper was dipped into the wash liquid as shown in Figure 6. Adjoining paper strips reduced evaporation of the wash liquid. With porous filter paper (Eaton-Dikeman, Grade 301 or 625, 0.030 inch thick), development of the chromatogram could often be accomplished in 10 to 20 minutes. The relative migration rates of colorless ions in these chromatographic systems were revealed by the addition of reagents to the paper.

For determination of the relative migration rates of ions in the electrical field, paper sheets or strips were supersaturated with the wash liquid. The excess liquid was removed with blotting paper, and the saturated paper was placed on a glass plate between two parallel paper strips also saturated with the wash liquid. Solute mixtures were added as narrow zones or as spots. Platinum wires were inserted through holes in the paper strips, or they were pressed across the ends of the wider sheets. With applied potentials of 160 to 400 volts, the relative migration rates of the ions could be determined in 20 to 40 minutes.

From theoretical considerations, the relative adsorbability of ions in heterogeneous systems plays an important role in separations by electromigration as well as by flow of solvent. With flow of electrical current, the migration of ions depends upon the constituent mobility in the solvent and upon the proportion of the ions remaining unadsorbed. With flow of solvent, the migration of the ions depends solely upon the proportion remaining unadsorbed (*20*).

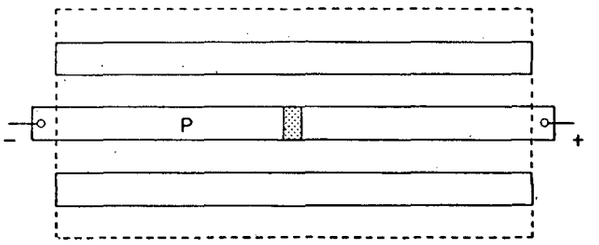
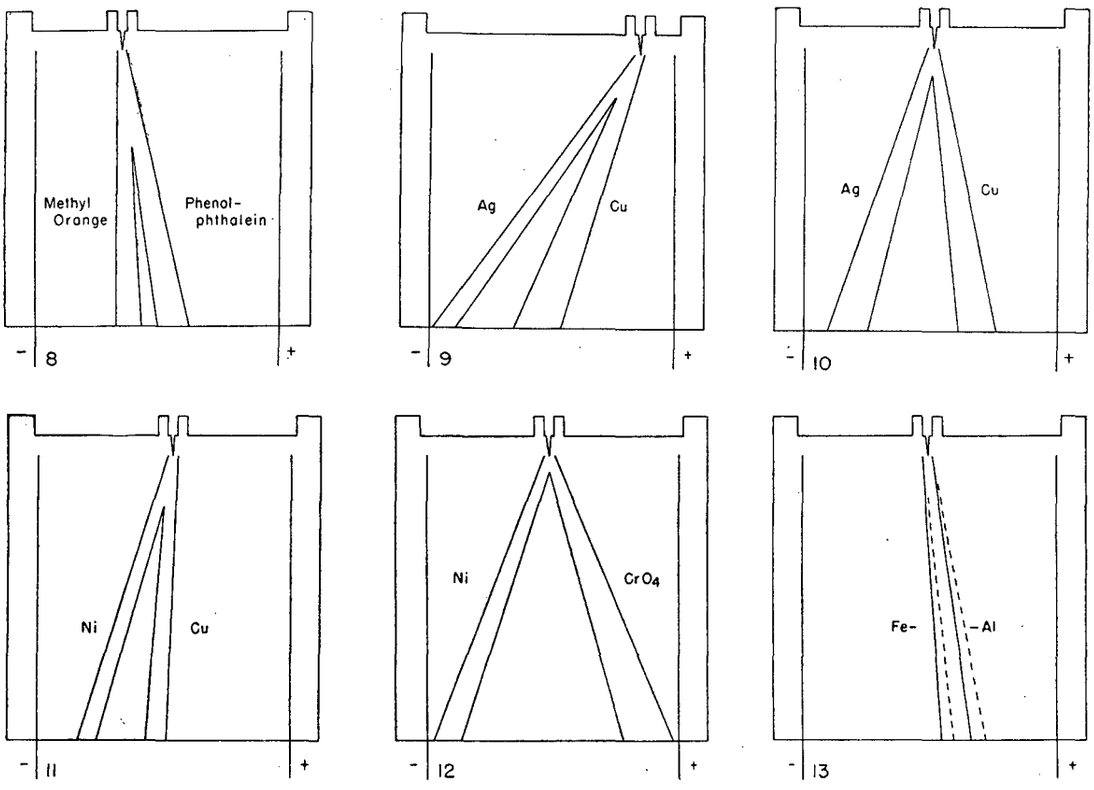


Figure 7. Electrochromatography of Ions in Paper Strip, *P*, between Glass Plates

In the combined electromigration and chromatographic methods, the separability of mixtures should depend upon the relative adsorbability and the relative constituent mobility in the two-phase system. As this mobility also depends upon the adsorbability, variation of the degree of adsorbability without simultaneous variation of the relative adsorbability should not affect the separability of the ionic mixtures. The greater the adsorbability, the lower the migration rates.

In the electrographic cells, the path described by a given ion should be approximately a straight line whose angle with the vertical is determined by the direction cosine of the resultant obtained upon vector addition of the chromatographic and electromigration factors. If the direction cosine of the resultant is the same for two different ions (irrespective of the absolute magnitude of the components), the ions will follow the same path (Figure 21).



Figures 8 to 13. Continuous Separations

8. Methyl orange and phenolphthalein, each 0.005 *M* in *WL*, 4 *M* NH_4OH. 230 volts, ca. 90 ma.
9. Silver and copper nitrates, each 0.005 *M* in *WL*, 0.1 *M* lactic acid. Reagents (*R*), dithio-oxamide and H_2S. 200 volts, 80 ma.
10. Silver and copper nitrates, each 0.005 *M* in 4 *M* NH_4OH. *WL*, 4 *M* NH_4OH plus 0.01 *M* Versene. *R*, diphenylthiocarbazone. 160 volts, over 100 ma.
11. Nickel and copper nitrates, each 0.005 *M* in 4 *M* NH_4OH. *WL*, 0.01 *M* disodium tartrate in 4 *M* NH_4OH. *R*, dithio-oxamide. 160 volts, over 100 ma.
12. Nickel nitrate and potassium chromate, each 0.01 *M* in 4 *M* NH_4OH. *WL*, 0.01 *M* disodium tartrate in 4 *M* NH_4OH. *R*, dithio-oxamide and lead salts. 160 volts, 100 ma.
13. Ferric and aluminum nitrates, each 0.005 *M* in 0.01 *M* tartaric acid and 4 *M* NH_4OH. *WL*, 0.005 *M* tartaric acid in 4 *M* NH_4OH. *R*, aluminon in 50% acetic acid. 200 volts, 100 ma.

When two ionic species of opposite sign or mobility are added to the electrographic cell, the separation of the ions should be absolute (100%), whereas in the usual chromatographic separations the trailing solutes are always contaminated by traces of the leading solutes (Figures 8, 10, 12, and 20). For continuous operation of the cells, the separation of ions will be effective if the chromatographic factor is eliminated but not if the electromigration factor is eliminated. For discontinuous operation, separations may be effective if either the electromigration factor or the chromatographic factor is eliminated.

SPECIFICITY OF THE ELECTROGRAPHIC CELL

The separability of various mixtures in chromatographic columns is the resultant of a complex equilibrium in the two-phase chromatographic system. This equilibrium has so many variable factors that it is difficult to ascribe chromatographic selectivity solely to the solvent or to the adsorbent (*17*). Similar considerations apply to the chromatographic factor in the electrographic cell.

Owing to the importance of electrical mobility upon the migration of ions in the electrographic cell, the separability of ionic mixtures may often hinge upon the possibilities for variation of this property. The most promising methods for variation of the mobility are oxidation, reduction, and the formation of complex or chelated compounds (*1*, *5*, *7*). Even nonionized solutes that form complexes with ionized reagents should be amenable to examination in the electrographic cell.

RESULTS WITH CONTINUOUS SEPARATIONS

The continuous separation of various binary, ternary, and quaternary mixtures and the conditions utilized for the separations are illustrated by Figures 8 to 20. These examples demonstrate the separation of mixtures of anions, of cations plus anions, and of cations. They also illustrate the separation of mixtures of inorganic substances, of organic substances, and of inorganic and organic substances. Figures 9 and 10, 13 and 14, and 18 to 20 illustrate the effect of different conditions upon the separability of similar ionic mixtures.

In most of the examples with ammoniacal solutions (Figures 8, 10, 11, 13, and 14), the chromatographic factor was somewhat smaller than the electromigration factor. The addition of salts, such as potassium nitrate or ammonium acetate, to the ammoniacal solutions usually reduced the adsorbability of cations. With the acidic solutions, the chromatographic factor was very small relative to the electromigration factor. In most separations of cations or of anions, the chromatographic sequence and the electromigration sequence were identical.

The effect of complex-forming solutes on the separation of silver and nickel ions is illustrated by Figures 18 to 20. Inseparable in the presence of ammonia, these two species were readily separable in the presence of oxalate or of ethylenediamine tetraacetic acid (Versene).

As indicated by the sensitive dithio-oxamide test, silver and copper ions were separated completely in the presence of Versene, which converts copper ions into an anionic complex (Figure 10).

Similarly, nickel cations were removed completely from chromate anions, the nickel being confined to the narrow zone (Figure 12).

In the ammoniacal solution (Figure 18), the relative electromigration rates of silver and nickel ions were equal to the relative chromatographic movement, as shown by measurements summarized in Figure 21. This latter figure also illustrates the relative effects of the flow rate of the wash liquid and of the electrical current on the migration of the ions in the cell. These results indicate that silver ions move through the electrographic cell faster than the nickel ions, even though both ions follow the same pathway. During the initial stages of the separation, the advancing front of the zone should contain only silver ions, as indicated by the distances Ag to Ni and (Ag) to (Ni). This conclusion was confirmed by examination of the electrographic cell before the silver had been carried into the percolate as in Figure 22. During the continuous operation of the cell (Figure 18), nickel ions were, therefore,, migrating at a much slower rate than the silver ions. As this rate was less than that predicted from electromigration or from relative adsorbability, chromatographic and electromigration factors must have been effective.

Figure 21 provides additional evidence for the simultaneous action of electromigration and differential adsorption. Electromigration alone and chromatography alone separate silver from nickel. In the electrographic cell, these two factors (vectors) for each ion have identical cosine resultants. Under these conditions, chromatography and electrical migration play equally significant roles in determining the path followed by an ion.

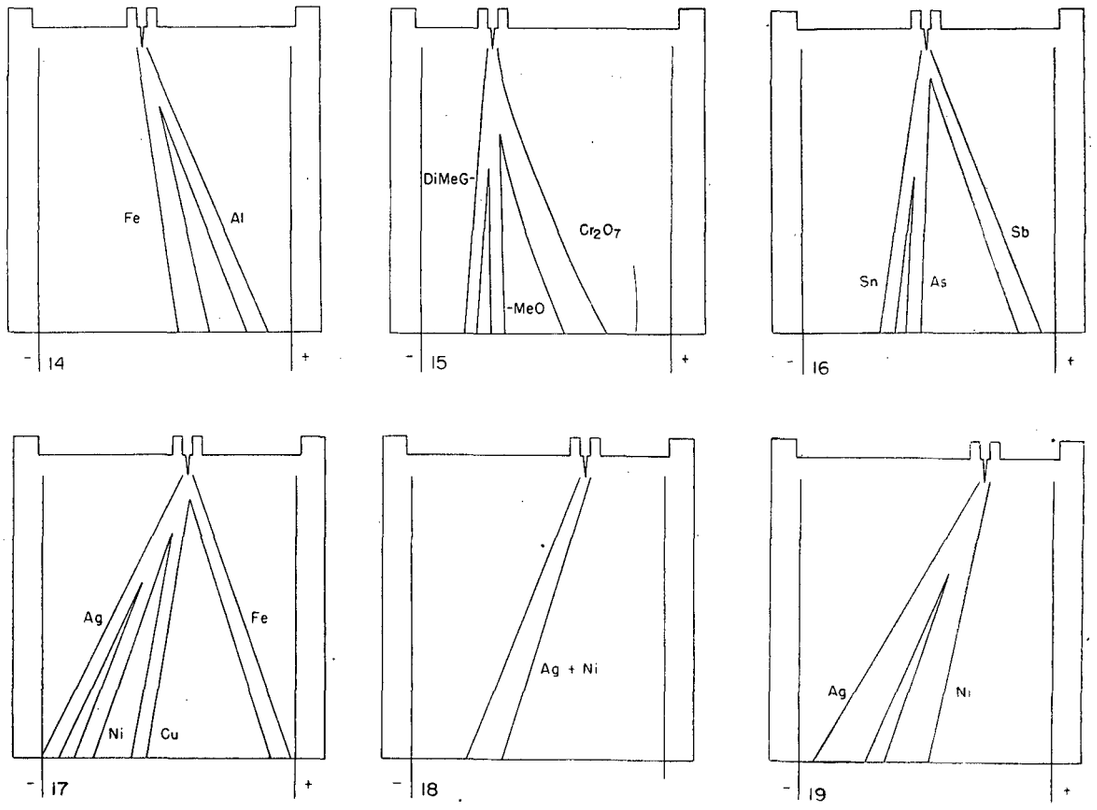
For ions of opposite sign or mobility as in Figures 10, 12, and 20, both electromigration and differential adsorption determine the paths in the electrographic cell (Figure 23). Under these conditions, the ions will always follow separate courses, even though electromigration and chromatographic migration should play equivalent roles for both ions.

Determination of the relative electromigration and chromatographic rates of silver and of copper ions under the conditions represented by Figure 10 yielded the results summarized in Figure 23. In this figure, the chromatographic migration of the copper ions in the paper strip was much less than the migration found in the electrographic cell, an effect attributable to a slowly reversible reaction between the copper ions and the cellulose.

In some of the continuous separations, the concentration of the ions was increased in order to determine the effectiveness of the electrographic cells. With ferric and nickel ions in the presence of tartrate and ammonia, the separations were effective with solutions 0.2 *M* with respect to each cation. With increasing concentration, the courses followed by the ions became wider and their separation decreased.

RESULTS WITH DISCONTINUOUS SEPARATIONS

Many examples of the separation of similar elements by the discontinuous procedure are illustrated in Figures 24 to 31. In some of these separations, the ions reacted with the wash liquid to form insoluble products that remained fixed in the paper. One example is the precipitation of metallic mercury by treatment of mercurous mercury with aqueous ammonia (Figure 27).



Figures 14 to 19. Continuous Separations

14. Ferric and aluminum nitrates, each 0.005 *M* in *WL*, ca. 0.005 *M* dimethylglyoxime and 0.01 *M* tartaric acid in 4 *M* NH_4OH. *R*, aluminon in ca. 50% acetic acid. 250 volts, ca. 90 ma.
15. 0.005 *M* dimethylglyoxime, 0.0001 *M* methyl orange, and 0.005 *M* dichromate. *WL*, 0.1 *M* acetic acid. *R*, nickel plus ammonia. 250 volts, 30-32 ma.
16. Stannous, arsenious and antimonous chlorides, each 0.005 *M* in *WL*, 0.02 *M* lactic acid, 0.02 *M* tartaric acid, and 0.04 *M* *dl*-alanine. *R*, H_2S. 300 volts, 95 ma.
17. Silver, nickel, copper, and ferric nitrates, each 0.005 *M* in *WL*, 0.01 *M* disodium tartrate and 0.01 *M* ammonium oxalate in 4 *M* NH_4OH. *R*, dithio-oxamide and H_2S. 160 volts, over 100 ma.
18. Silver and nickel nitrates, each 0.005 *M* in *WL*, 4 *M* NH_4OH. *R*, dithio-oxamide and diphenylthiocarbazone. 160 volts, 60 ma.
19. Silver and nickel nitrates, each 0.005 *M* in *WL*, 0.01 *M* ammonium oxalate in 4 *M* NH_4OH. *R*, dithio-oxamide and diphenylthiocarbazone. 160 volts, over 100 ma.

Another example is the precipitation of nickel by dimethylglyoxime contained in the ammoniacal wash liquid (Figures 28 and 30). Under these conditions, the principles of chemical precipitation, electromigration, and chromatographic separation were utilized simultaneously.

Ions such as silver and nickel, which were inseparable under the conditions of continuous flow (Figure 18), were separated quickly by the discontinuous procedure (Figure 24). Ions of copper and nickel, likewise inseparable by the continuous procedure when ammonium acetate in concentrated ammonium hydroxide was the wash liquid, were quickly separated by the discontinuous procedure (Figure 25).

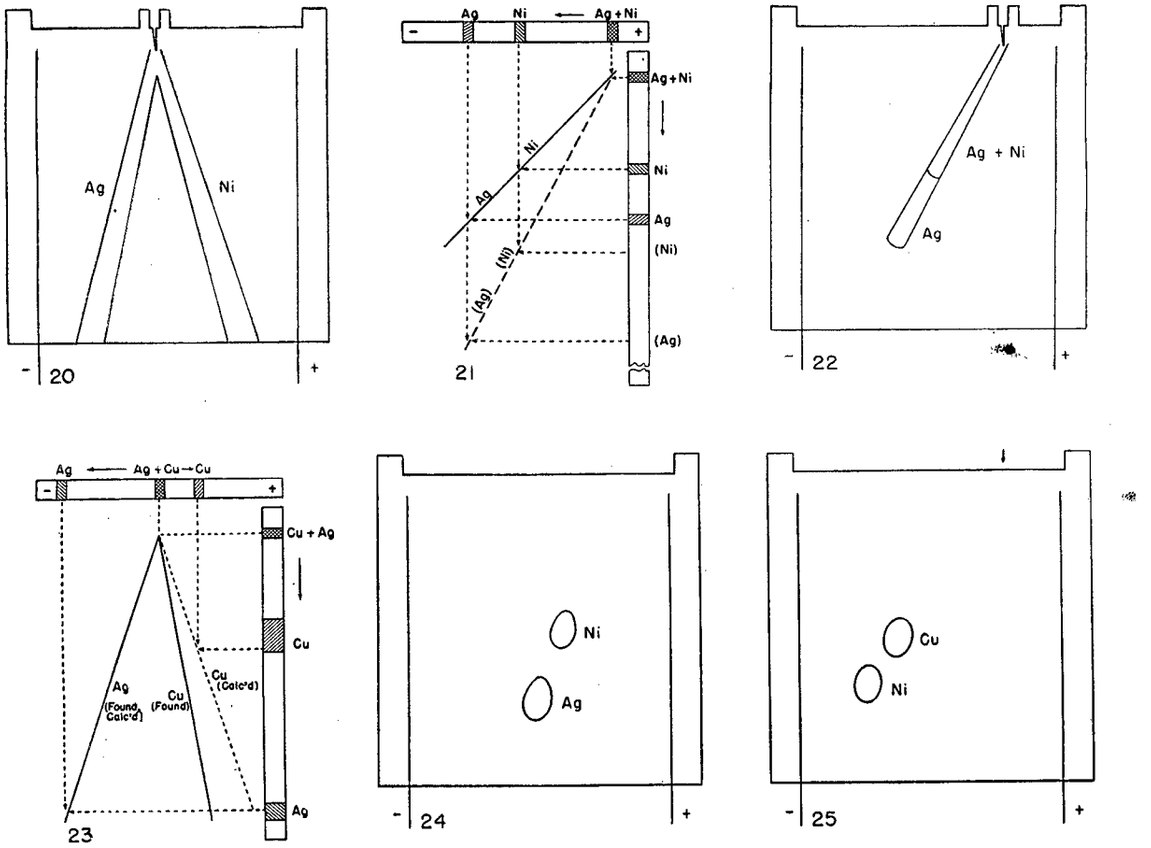
In the presence of complex-forming reagents, most ions migrated as a single zone in the electromigration and chromatographic systems. An exception to this relationship was observed with cobaltous ions that were added to a solution of tartaric acid in aqueous ammonia and allowed to stand with air for several hours. With these oxidized solutions, both anionic and cationic forms of cobalt were separated by electromigration.

In acidic solutions, cobalt and nickel ions were slowly separable by electromigration and by chromatography. In freshly prepared, aerated, ammoniacal solutions, the oxidized cobalt ions were more adsorbed than nickel ions and migrated at a much slower rate. Separate tests showed the oxidized cobalt to migrate slower than nickel in chromatographic systems as well as in electrochromatographic systems.

As indicated by Figure 31, arsenious and antimonous ions were readily separable by the discontinuous procedure. Under the same conditions, stannous ions could not be located in the electrographic cell with sulfide, although these ions were readily detectable in the cells operated continuously, as shown by Figure 16. Tests in paper strips indicated that the stannous ions were more adsorbed than arsenious and antimonous ions and that they formed a large trailing zone. As a consequence, their concentration in the discontinuous procedure was reduced until they were not readily detectable.

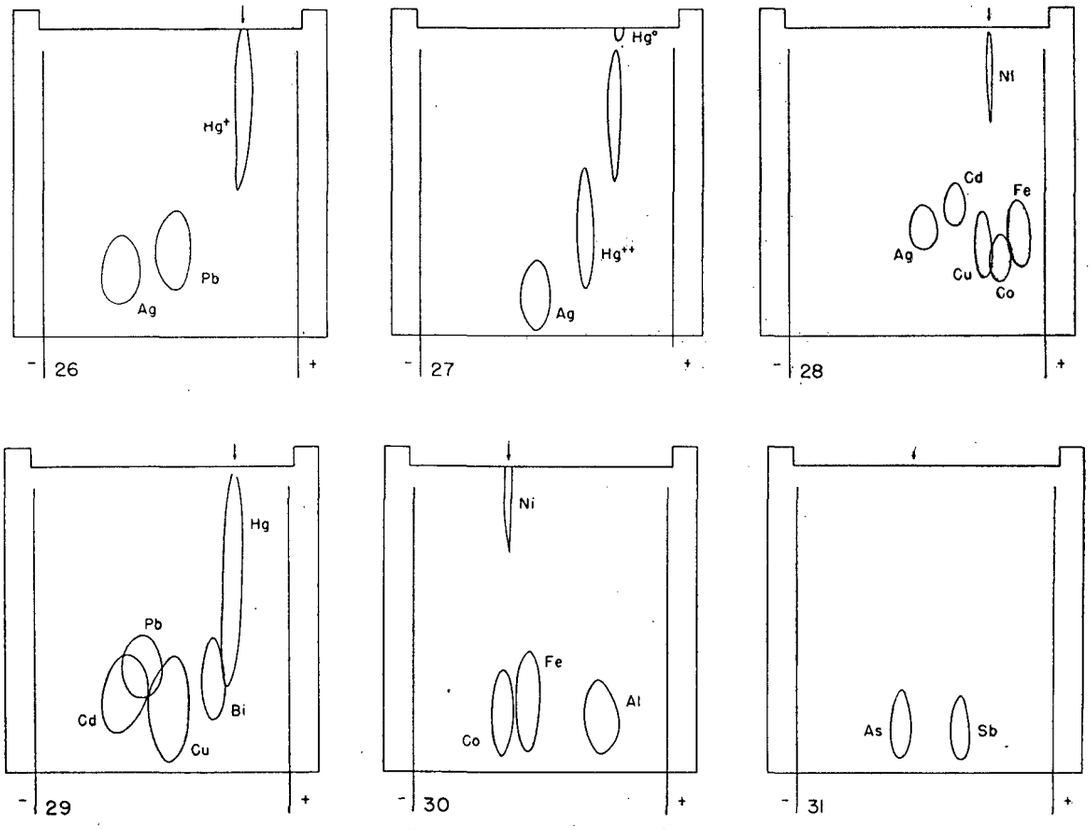
DISCUSSION

Separation of mixtures in the electrographic cells presents a number of advantages over the usual chromatographic systems. This method not only provides a means for the continuous resolution of mixtures, but it also provides a basis for the complete separation of many groups of ions from one another. With ethylenediamine tetraacetic acid as a complex-forming solute, this electrographic procedure makes possible the complete and continuous separation of monovalent cations from divalent and polyvalent cations. It also provides a continuous method for the



Figures 20 to 25. Continuous and Discontinuous Separations

20. Silver and nickel nitrates, each 0.005 *M* in *WL*, 0.005 *M* Versene in 4 *M* NH_4OH. *R*, diphenylthiocarbazone and dithio-oxalic acid plus HCl. 160 volts, 95 ma.
21. Silver and nickel separated by electrochromatography, upper paper strip, and by chromatography, paper strip at right. Each ion 0.005 *M* in electrolyte and *WL*, 4 *M* NH_4OH. *R*, dithio-oxamide and diphenylthiocarbazone. Solid vector, calculated path of silver and nickel in electrographic cell. Dashed vector, calculated path of silver and nickel with low electrical current and rapid flow of *WL*
22. Relative migration rates of silver and nickel nitrates, each 0.005 *M* in *WL*, 4 *M* NH_4OH. 160 volts, ca. 60 ma., ca. 18 minutes
23. Silver and copper separated by electromigration, upper paper strip, and by chromatography, paper strip at right. Each ion 0.005 *M* in *WL*, 0.01 *M* Versene in 4 *M* NH_4OH. *R*, dithio-oxamide and diphenylthiocarbazone. Solid lines, observed paths in electrographic cell
24. Silver and nickel nitrates, each 0.005 *M* (0.01 ml.). *WL*, 4 *M* NH_4OH (60 ml.). Arrow, point of addition. *R*, dithio-oxamide and diphenylthiocarbazone. 160 volts, ca. 40 ma., 20 minutes
25. Copper and nickel nitrates, each 0.005 *M* (0.01 ml.). *WL*, 0.01 *M* ammonium acetate in 15 *M* NH_4OH (60 ml.). *R*, dithio-oxam de. 200 volts, ca. 35 ma., 20 minutes



Figures 26 to 31. Discontinuous Separations

26. Mercurous, lead and silver nitrates, each 0.05 *M* in 1 *M* HNO_3 (0.01 ml.). *WL*, 0.1 *M* lactic acid (60 ml.). *R*, H_2S. 250 volts, 100 ma., 20 minutes
27. Mercurous, lead and silver nitrates, each 0.05 *M* in 1 *M* HNO_3 (0.01 ml.). *WL*, 0.008 *M* citric acid in 4 *M* NH_4OH (60 ml.). *R*, H_2S. 160 to 170 volts, 100 ma., 20 minutes
28. Nickel, ferric, cobalt, copper, cadmium, and silver nitrates, each 0.05 *M* in 0.1 *M* tartaric acid (0.01 ml.). *WL*, 0.01 *M* ammonium tartrate, ca. 0.005 *M* dimethylglyoxime in 4 *M* NH_4OH (60 ml.). *R*, H_2S. 160 volts, 95 to 100 ma., 20 minutes
29. Mercuric, bismuth, copper, lead, and cadmium nitrates, each 0.05 *M* in 1 *M* HNO_3 (0.01 ml.). *WL*, 0.1 *M* lactic acid (60 ml.). *R*, diphenylcarbazide (Ag), diphenylthiocarbazone (Cd), dithio-oxamide (Cu), dithio-oxamide plus NH_4OH (Pb), Na_2S (Bi). 250 volts, 100 ma., 20 minutes
30. Nickel, cobalt, ferric, and aluminum nitrates, each metal 0.005 *M* in 0.01 *M* tartaric acid and 0.005 *M* dimethylglyoxime (0.025 ml.). *WL*, 0.01 *M* tartaric acid and 0.005 *M* dimethylglyoxime in 4 *M* NH_4OH (60 ml.). *R*, dithio-oxamide, aluminon in 50% acetic acid. 150 volts, 100 ma., 20 minutes
31. Arsenious and antimonous chlorides, each 0.01 *M* (0.05 ml.). Solution and *WL*, 0.04 *M* *dl*-alanine in 0.1 *M* lactic acid. *R*, H_2S. 300 volts, 100 ma., 20 minutes

removal of particular cations or anions from a solution, and it makes possible the continuous substitution of one cation or anion for another. In these latter respects, it provides a promising approach to various decontamination and purification procedures.

The discontinuous electrographic method provides a convenient means for the rapid resolution of mixtures and for the identification of the components. As in conventional chromatography, resolved ionic substances may be identified by their location relative to other ionic species and by radiographic tracer techniques. By analogy with separations obtained in columns, the separation of ions at tracer levels should also be possible.

In the continuous procedure, separation of two ions is impossible if their relative electromigration and chromatographic rates are the same. In the discontinuous method, two ions will be inseparable only if both the electromigration and the chromatographic rates are identical. For this reason, the discontinuous method is more selective than the continuous method.

In the experiments reported here, the use of organic solutes has facilitated the separation of various mixtures of cations. Conversely, various cations may now be utilized to aid in the separation of neutral organic compounds that will form complex ionic species. Similarly, the chromatographic and the electrochromatographic behavior of ions in various solvents should give indication of the formation and the nature of complex ions. Through variation of the solvent and the complexing agents and through use of various adsorptive agents, the electrographic method should prove fully as adaptable and as useful as the familiar chromatographic procedures.

LITERATURE CITED

(1) Bailar, J. C., Jr., *Chem. Revs.*, **21**, 1 (1937).
(2) Butler, J. A. V., and Stephen, J. M. L., *Nature*, **160**, 469 (1947).
(3) Clegg, D. L., ANAL. CHEM., **22**, 48 (1950).
(4) Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **40**, 33 (1946).
(5) Copley, M. J., Foster, L. S., and Bailar, J. C., Jr., *Chem. Revs.*, **30**, 227 (1942).
(6) Cremer, H.-D., and Tiselius, A., *Biochem. Z.*, **220**, 273 (1950).
(7) Diehl, H., *Chem. Revs.*, **21**, 39 (1937).
(8) Durrum, E. L., *Chem. Eng. News*, **27**, 601 (1949); *J. Am. Chem. Soc.*, **72**, 2943 (1950).
(9) Garrison, W. M., Haymond, H. R., and Maxwell, R. D., *J. Chem. Phys.*, **17**, 665 (1949).
(10) Gordon, A. H., Keil, B., and Sebesta, K., *Nature*, **164**, 498 (1949).
(11) Haugaard, G., and Kroner, T. D., *J. Am. Chem. Soc.*, **70**, 2135 (1948).
(12) Kendall, J., *Science*, **67**, 163 (1928).
(13) Kraus, K., and Smith, G. W., *J. Am. Chem. Soc.*, **72**, 4329 (1950).
(14) Lecoq, H., *Bull. soc. roy. sci. Liége*, **13**, 20 (1944).
(15) Lederer, E., "Progrès Récents de la Chromatographie," Part 1, Paris, Hermann & Cie., 1949.
(16) Schoofs, F., and Lecoq, H., *Bull. acad. roy. méd. Belg.*, **9**, 122 (1944).

(17) Strain, H. H., ANAL. CHEM., **22**, 41 (1950).
(18) Strain, H. H., *Carnegie Inst. Wash., Year Book*, **48**, 87 (1949).
(19) Strain, H. H., "Chromatographic Adsorption Analysis," New York, Interscience Publishers, 1942.
(20) Strain, H. H., in "Frontiers in Colloid Chemistry," Vol. VIII, pp. 29–63, New York, Interscience Publishers, 1950.
(21) Strain, H. H., *J. Am. Chem. Soc.*, **61**, 1292 (1939).
(22) Svensson, H., and Brattsten, I., *Arkiv Kemi Mineral. Geol.*, **1**, 401 (1949).
(23) Turba, F., and Enenkel, H. J., *Naturwissenschaften*, **37**, 93 (1950).
(24) West, P. W., ANAL. CHEM., **22**, 79 (1950).
(25) Wieland, T., and Fischer, E., *Naturwissenschaften*, **35**, 29 (1948); *Angew. Chem.*, **60**, 313 (1946); *Ann.*, **564**, 152 (1949).

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Techniques and Reagents for Paper Chromatography

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This report deals with the choice of paper for chromatography, the use of hydrochloric acid as a vehicle for the application of amino acids, and the use of a comb for application of solutions in horizontal strips. It describes an inexpensive, transparent tank with accessories and means of color development on chromatographed sheets. All reagents are applied by dipping, instead of spraying; ninhydrin, in anhydrous acetone. Also described are nitroprusside and cyanide dipping reagents of low water content, which produce relatively permanent colors with —SH and —S—S—, as well as a platinum and a palladium reagent for detection of reducing compounds in general. An arrangement is shown for production of inexpensive chromatographic records by three-filter photography in transmitted light.

APPLICATION of the methods of amino acid paper chromatography (*1*) to specific problems has led to several improvements in general technique.

THE PAPER

Chiefly as a result of difficulties in the recovery of small amounts of methionine, Schleicher and Schuell paper No. 589 Green Ribbon was selected. With certain other papers there is either a marked loss of methionine—e.g., Schleicher and Schuell No. 589—or a much slower movement of diffusing liquid—e.g., Whatman No. 1. Three different lots of the 589 paper differed slightly in their properties, although 3 micrograms of methionine were detectable in all cases by ninhydrin after a phenol-ammonia run. The rate of flow is practically independent of the direction with reference to the water marks.

APPLICATION OF SOLUTIONS

Application of amino acids or protein hydrolyzates in 50 or 100% formic acid solutions results in small initial spots and compact spots after chromatography. In order to apply a uniform strip of substance across the width of the paper for certain types of one-dimensional work, the authors use a hard rubber comb, the teeth of which are ground down to a length of about 0.5 mm. The comb is filled from a small aluminum trough coated with paraffin. Combs of 82-mm. length (100 teeth, 2.5 mm. wide) deliver 50 to 60 μl. of an aqueous 12% amino acid solution, with an error of ± 1 μl. Calibration was made by coloring the solution with phenol red and determining its amount spectrophotometrically after extraction from the paper by 0.1 *N* hydrochloric acid in 85% alcohol.

TANK AND ACCESSORIES

The authors use a fish aquarium available in pet stores, approximately 20 × 12 × 10 inches, with a combination of ascending and descending flow. The tank and some accessories are shown in Figure 1. The lid consists of a glass plate fitted with a sponge rubber gasket, the outside pores of which are sealed with neoprene paint. The upper rim of the tank is covered with Parafilm. The tank is equipped with removable glass rods as shown, to accommodate two sheets of paper. The rods, flattened at the ends to prevent rolling, are supported by plates of Bakelite or hard rubber cemented to roughened areas on the walls with Dekadhese (Technical Specialties Co., Malden, Mass.). A pair of aluminum or glass hooks is used to hold the lowest pair of rods apart. The papers, 40 × 46 cm. in size, are marked off in centimeters on the long edges to aid in adjustment and observation of flow rate, and are held in place by glass "clothespins." The starting line is 6 cm. from the end of the sheet and should be located above the lowest rod. The solvent is contained in a semicircular glass trough (51 mm. in outside diameter, 48 cm. long, Yonkers Laboratory Supply Co., Yonkers 2, N. Y.). Each sheet passes from the bottom of the trough, on the inside of one of the lowest pair of rods, and turns back over the upper rod and along the inside of the middle rod. A long filling funnel (Figure 1) with a ground inner valve near the bottom is used to add the solvent without disturbance after the sheets have been placed.

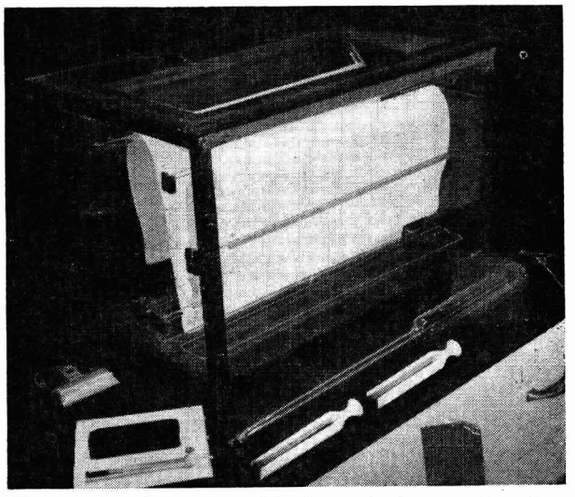

Figure 1. Aquarium Tank and Accessories for Chromatography

Parafilm-sheathed binders clip, comb, aluminum trough, filling funnel, and plastic tongs shown outside the tank

A typical phenol run at 20° to 25° C. requires approximately 20 hours. A time plot shows that there is no break in the rate of flow at the turning point of the paper. Contact between paper and glass rods also has no effect, but contact between the two sheets above the liquid in the tray must be prevented.

In addition to simplicity and visibility this equipment has the advantage over the ordinary descending technique of providing for automatic stoppage of flow because of the difference in height between the terminal edge of the paper and the liquid in the trough. Thus, termination of a run during the night hours causes no trouble. However, when papers are left in the tank

for 3 days after the end of the run there is evidence of some spreading of spots by lateral diffusion. For uniform flow across the width of the paper and for reproducible results, control of room temperature (±1°) has been found important, but not sufficient. It is further necessary to allow several hours for "conditioning" the paper in the atmosphere of the solvent, before beginning the run. This effect of conditioning is demonstrated by the example shown in Figure 2.

DRYING

The wet sheets are suspended under the hood from their terminal edge with the aid of binders clips (sheathed with Parafilm when acid is present on the paper) and dried in the air stream

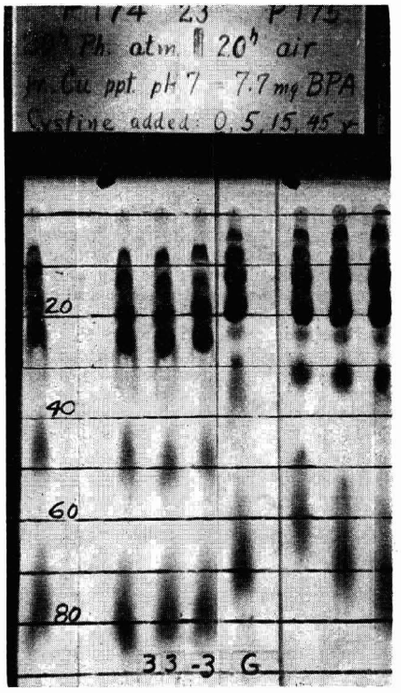

Figure 2. Effect of Conditioning Sheets in Atmosphere of Medium. Example of Photograph with Identification

Chromatograms 174 and 175, film 33, exposure 3, green filter. Each spot contains 400γ of copper-free substance obtained by cupric precipitation at pH 7 from a hydrolyzate of 7.7 mg. of bovine plasma albumin, without and with added cystine (0, 5, 15, 45γ, respectively, for the first four and for the second four spots). Before the run the sheets were left in the tank for 20 hours in the presence (left four spots) or absence (right four spots) of a phenol atmosphere. In each group the smaller strip represents one end and the larger strip the other end, reversed, of a sheet carrying 12 spots (as seen, the original order of spots is 1, 12, 11, 10; 1, 12, 11, 10)

of a fan for a few hours. Small residues of phenol perceptible by odor do not interfere with the action of the ninhydrin, nitroprusside, or palladium reagents (see below), but may interfere with the visibility of the platinum reaction. Adequate removal of hydrochloric acid vapors, in the case of chromatograms run in their presence, is shown by the absence of darkening of the paper when a clipping is heated at 90°.

REAGENTS AND DEVELOPMENT

Ninhydrin. The troublesome spraying with the ninhydrin-butanol reagent has been replaced by the procedure of dipping the sheets in an acetone solution of ninhydrin.

In one-dimensional work it is convenient to cut the sheet into halves. The half-sheet, held on each end by means of a pair of plastic tongs (Figure 1), is passed slowly through the solution in a photographic tray. The acetone evaporates in a few minutes and color development may be observed at room temperature or be brought to completion in a few minutes by heating in a 90° oven. Comparison of sprayed acetone and butanol reagents shows more rapid and more intense color as well as greater persistence when acetone is used. The dipping reagent is a 0.25% (w./v.) solution of ninhydrin in anhydrous (>99%) acetone. Color development with this reagent is uniform, intense, rapid, and undistorted. The presence of 10% water in the reagent results in spreading of spots and delayed color development.

Nitroprusside. The following dipping reagents have been found useful in the study of sulfur compounds.

I. Dissolve 1.5 grams of sodium nitroprusside in 5 ml. of 2 *N* sulfuric acid, add 95 ml. of methanol and 10 ml. of ammonia water (28%), and pour through a filter to remove precipitated salts. This alkaline reagent in the alcoholic medium is stable for many days in the refrigerator, in contrast to its well-known lability in an aqueous medium.

II. Dissolve 2 grams of sodium cyanide in 5 ml. of water and add 95 ml. of methanol.

III. Combine equal volumes of Reagents I and II made up in double concentrations.

The presence of —SH on the paper is revealed by the immediate appearance of a brilliant red color after dipping in Reagent I. To detect —S—S—, the paper, while still damp from Reagent I after having been briefly hung in the air, is dipped (under the hood) in Reagent II, and resuspended. The color resulting from the cyanide cleavage of —S—S— groups appears within a few seconds, and reaches its maximum in about 10 minutes, thus giving time for photography. On further drying in air, the background color, which at first is pale yellow, turns into a strong green while the red reaction color, slightly diminished, persists. The red nitroprusside color produced under these conditions, as well as the green background, then remains intact for weeks. If the water content of Reagent II is increased to 10% the background is yellow and the red color does not last for more than 15 minutes. Arginine was found to give an orange color with the nitroprusside reagent when the background was yellow, or a gray-blue when the background was green.

If detection of —SH is not desired, it is more convenient to use Reagent III in one operation. The lower limit of response lies between 0.6 and 0.3 microgram of cystine in a spot 18 mm. in diameter.

Platinum Reagent. The platinum reagent previously described (*2*) has been modified as follows for use as a dipping reagent: 4 ml. of 0.002 *M* platinochloric acid plus 0.25 ml. of 1 *M* potassium iodide plus 0.4 ml. of 2 *N* hydochloric acid plus 76 ml. of acetone. The components must be combined just before use. After the paper has been dipped and dried at room temperature,

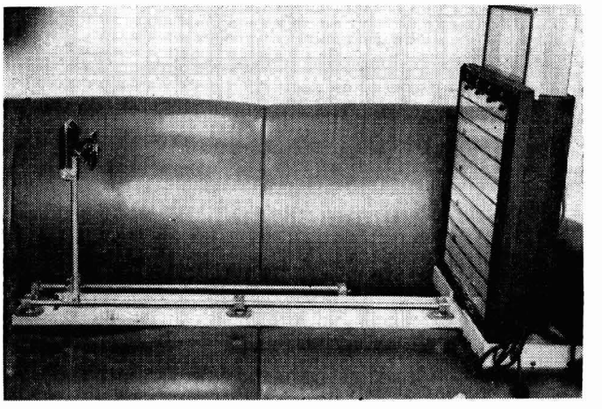

Figure 3. Setup for Chromatogram Photography

This arrangement is also convenient for photography of charts, etc., in reflected light at distances from 10 to 32 inches, with the aid of standard portrait attachment lenses

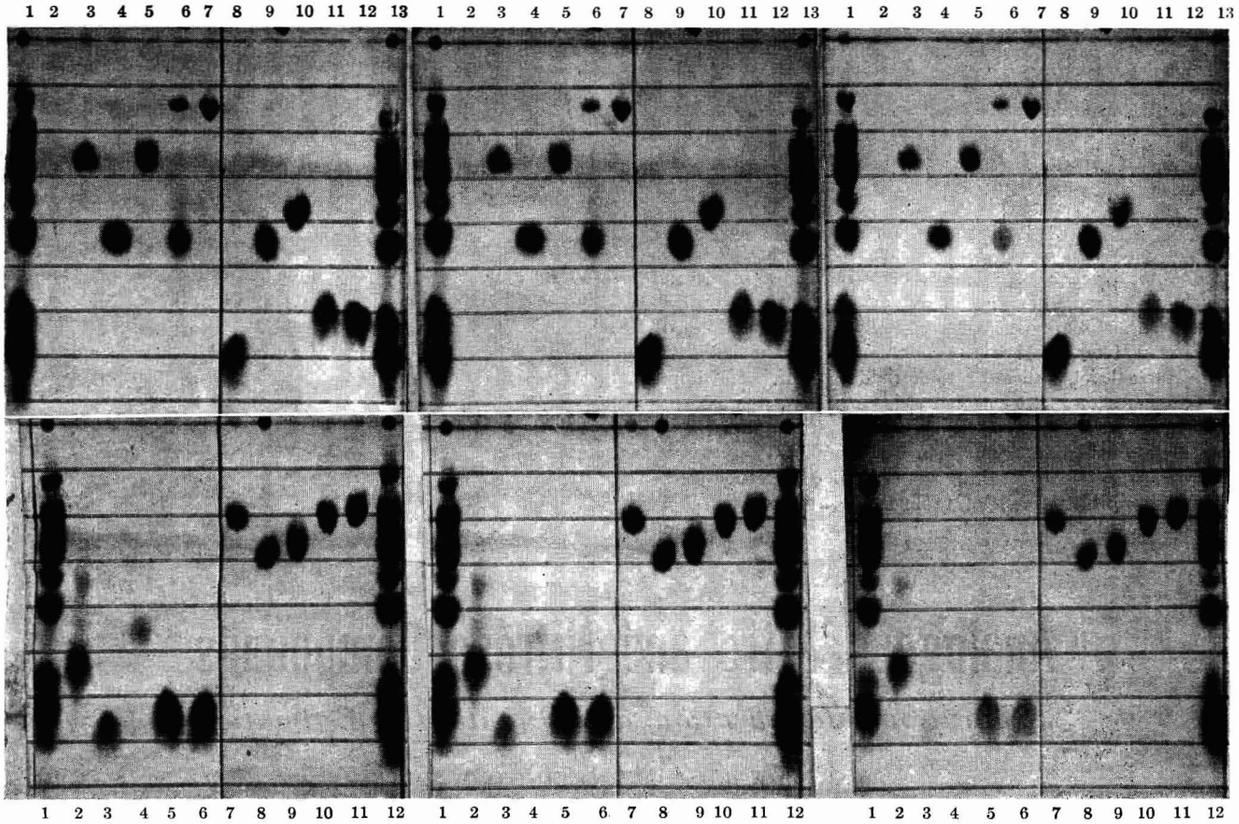

Figure 4. One-Dimensional Run of Amino Acids, etc., at 37° C.

Medium. Phenol–isopropyl alcohol–water (70–5–25 by weight). A hydrolyzate of 1080γ of bovine plasma albumin in 10 μl. of 3 *N* HCl in 50% HCOOH. Amino acids and ammonium chloride, 0.5 μ*M* each in same solvent

Upper three pictures.

1, 13.	Protein hydrolyzate	5.	Serine	9.	Tyrosine
2.	Ammonium chloride	6.	Cysteine	10.	Threonine
3.	Glycine	7.	Cystine	11.	Methionine
4.	Alanine	8.	Phenylalanine	12.	Valine

Lower three pictures.

1, 12.	Protein hydrolyzate	5.	Leucine	9.	Arginine
2.	Tryptophan	6.	Isoleucine	10.	Histidine
3.	Proline	7.	Aspartic acid	11.	Lysine
4.	Hydroxyproline	8.	Glutamic acid		

white to pale yellow areas, indicative of reducing sulfur compounds—e.g., cystine, methionine—appear on a pink background. The background color may be intensified by exposure to hydrochloric acid vapors. For best results the paper should be completely freed of phenol odor (by drying at 90°). Methionine sulfoxide has a weak bleaching action, while methionine sulfone, erroneously stated to respond positively (*2*), does not bleach.

Palladium Reagent. This reagent is analogous to the platinum reagent but produces much greater contrast. Its composition is 4 ml. of 0.002 *M* palladous chloride in 0.1 *N* hydrochloric acid plus 0.25 ml. of 1 *M* potassium iodide plus 0.4 ml. of 2 *N* hydrochloric plus 76 ml. of acetone. Cystine gives immediately a clear bleached spot against a deep tan-brown background. However, the bleaching effect reaches its maximum only after several days. The exact specificities of this reagent require further investigation.

Superimposability. It is sometimes desirable in the characterization of spots or areas to apply different tests to the same chromatogram. It is possible to apply the platinum reagent first, mark the perimeter of the bleached areas by perforations for subsequent photography, permit the background color to fade, by exposure to the air for several days, and then to apply either nitroprusside-cyanide or ninhydrin reagent. The nitroprusside reagents, because of their basic reactions, cannot be followed by the other reagents, nor can a ninhydrin-developed sheet be used for subsequent tests, primarily because of the great persistence of the ninhydrin colors developed by the acetone reagent.

PHOTOGRAPHY

Figure 3 shows the equipment which has been found convenient for the routine preparation of photographic records of all chromatograms.

The stand is constructed from plywood, Flexaframe units, and a ruler. With the x-ray film viewer lying on its back, the chromatogram to be photographed is placed upon the screen and covered by a glass plate which carries permanent horizontal black lacquer lines for the estimation of R_f values (all chromatograms are exactly 400 mm. long).

In the case of papers treated with nitroprusside the outer glass plate is omitted, because pictures must be taken a few minutes after application of the reagent while the paper is still slightly wet (avoid exposure to hydrogen cyanide vapors!), in order to obtain maximum intensities.

Individual photographs are identified by the use of pieces of Plexiglas marked with black lacquer, which are inserted at the bottom of the plate. Legends may be written on the paper with black crayon or on an inexpensive sign box (3 × 6.25 × 12.25 inches, as commercially available for EXIT signs) which is placed on top of the viewer (Figures 2 and 3). The sign box carries an ordinary 60-watt bulb, which should be shielded by paper over its center on the inside of the glass. The camera is a Kodak Flash Bantam (K.A. Special *f*/4.5 lumenized lens No. 26); it is set for *f*/16 and 2.5 feet and carries Kodak Panatomic-X (FX828) film. Pictures are taken in a dark room in the transmitted light of the two 15-watt fluorescent bulbs of the x-ray viewer, either without filter or with the following Wratten filters attached to the lens of the camera by Kodak adapter rings: blue No. 49-C4, green No. 61-N, and red No. 29-F.

Sheets treated with the nitroprusside, platinum, or palladium reagents are photographed through the green filter, while three

pictures are generally taken of ninhydrin-treated sheets: blue filter, no filter, red filter. The green filter may be used in place of the no-filter exposure if maximum contrast is desired. The following exposure times have been established: 2 seconds without filter, 60 seconds with the 49 filter, 25 seconds with the 61 or 29 filter. Films are developed and printed to 2.75 × 4 inch size (2.5 times enlarged) by a commercial photographer, using an automatic printing machine. A file of these prints provides an objective, permanent, compact, and inexpensive record of all chromatographic runs.

Figure 4 shows the photographic records of one-dimensional runs of twenty amino acids, ammonium chloride, and a protein hydrolyzate, developed with ninhydrin. Both rows of pictures show how the red filter aids in differentiation within the hydrolyzate chromatograms: The area at R_f60 is greatly "thinned" by the red filter, as are the methionine and valine spots on the same level, indicating their reddish color. The upper series further shows that cysteine yields a primary reddish spot at R_f43 and a smaller, gray-blue spot due to cystine. In the lower series it is evident that tryptophan is accompanied by two secondary spots, probably resulting from acid decomposition. The yellow or brownish colors of proline and hydroxyproline are recorded by enhanced blackness in the blue-filter picture, and near-disappearance in the red-filter picture. The relative intensities of the blue-filter and no-filter prints also suggest that the weak color shown by the hydrolyzate at R_f45 is not due to hydroxyproline.

ACKNOWLEDGMENT

The authors are indebted to Richard J. Block for recommending the use of an aquarium tank with ascending-descending flow.

They are also indebted to the MacCallum Stores of Ardmore, Pa., for their friendly cooperation in the development of the photographic procedure.

LITERATURE CITED

(1) Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38**, 224 (1944).
(2) Winegard, H. M., Toennies, G., and Block, R. J., *Science*, **108**, 506 (1948).

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Analogous Nitro and Nitroso Compounds

Separation, Identification, and Quantitative Estimation

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MANY reactions of nitrogenous organic substances, particularly oxidations, result in the formation of mixtures containing, among other things, analogous nitro and nitroso compounds. Difficulties are encountered when the separation of such coexisting products is attempted by the usual methods. Similarities in solubilities make the use of selective solvents unsatisfactory, and such circumstances as unsuitable vapor pressures and insufficient stabilities may interfere with distillation procedures. These difficulties may reach prohibitive proportions when the desired products exist in small total quantity, in dilute solution, and in the presence of other substances.

The present work describes a method for the separation of such compounds from solution and from each other by chromatography; for the partial identification of each by observation of the position and color (natural or developed) of its adsorption zone; and for the further identification and quantitative estimation of each by spectrophotometry. The method would serve equally well for analysis of a solution containing only one such compound. The five pairs of *C*-nitro and *C*-nitroso compounds studied responded well to both chromatographic and spectrophotometric treatments; it seems probable that many similar pairs would do the same. Study of one *N*-nitroso compound indicated a possible extension of the method to mixtures of such related substances as nitramines and nitrosamines. The fact that extremely small quantities of solutes in very dilute solutions were separated and estimated with at least approximate accuracy under mild conditions gave renewed evidence of the usefulness of such methods in work with compounds which are relatively unstable when heated or isolated, and particularly in the field of high explosives research.

Trueblood and his coworkers (*14*) have employed similar techniques to advantage in this specialized field. The present work was also approached at one point by the papers of Gullstrom *et al.* (*5*), describing the separation and estimation of small amounts of *p*-benzoquinone monoxime (tautomerically equivalent to *p*-nitrosophenol) from mixtures containing also the dioxime and the by-products formed during nitrosation and oximation of phenol. Other uses of chromatography in conjunction with spectrophotometry have been described by various authors (*1, 2, 6, 12*).

APPARATUS

A No. 1 chromatographic column (9 × 130 mm.) was employed for most of the separations. It was filled with adsorbent to a height of 80 mm., using the technique described by Strain (*13*).

Oxidations and other reactions of nitrogenous organic compounds frequently produce mixtures containing analogous nitro and nitroso compounds, whose separation and analysis by other methods are in some instances relatively difficult. The chromatographic characteristics of five *C*-nitro compounds, their *C*-nitroso analogs, and one *N*-nitroso compound were determined. All were recovered effectively from very dilute solutions by this method. Each nitroso compound was much more strongly adsorbed than its nitro analog, a circumstance favoring accurate separation. Standard spectrophotometric absorption curves were constructed, suitable for their identification and estimation. The combined chromatographic and spectrophotometric procedures offer a means of analyzing a solution containing minute quantities of a nitro compound and its nitroso analog, or either one. Specifically, the method has been applied to five such pairs; it appears probable that it could be extended to others. It should have special value in work with explosives.

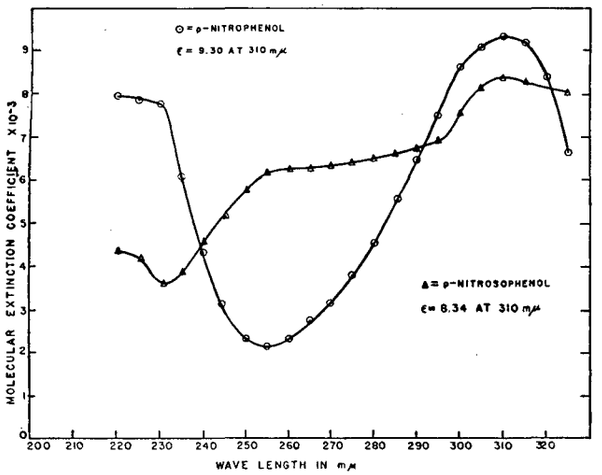

Figure 1. Absorption of *p*-Nitrophenol and *p*-Nitrosophenol in Absolute Ethyl Alcohol

In two specified instances, a No. 2 column (20 × 220 mm.) was used. In the preliminary purifications of the nitro and nitroso compounds, a No. 4 column (48 × 300 mm.) was employed.

Extinction coefficients were determined by use of a Beckman Model DU spectrophotometer.

MATERIALS

The solutes obtained or prepared as indicated in Table I were further purified chromatographically immediately before use; about 0.4 gram of material in each run was treated with solvents, adsorbents (prewashed), and developers identical to those shown in Table III. The appropriate zones were eluted with absolute alcohol, and the solvent was removed by evaporation at room temperature.

When Celite was used as a filter aid, the proportions were: 2 parts by weight of silicic acid to 1 part of Celite. The adsorbents were standardized (Table II) by determination of the R values, with respect to them, of 0.6-ml. portions of a 0.01 M solution of *o*-nitroaniline in benzene (method of LeRosen, *9*). Such standardization permits conversion of data obtained with one adsorbent system to other systems.

Adsorbents were not prewashed, except when this is mentioned specifically. In such instances it was accomplished by employing, in succession, 1 volume of acetone, 1 volume of ether, and 2 volumes of petroleum ether; "1 volume" is defined as the quantity which would barely wet the entire column, so that its top became dry just as the first liquid reached the bottom.

The petroleum ether was purified as follows: Commercial petroleum ether *B* was shaken with several successive portions of fuming sulfuric acid, standing overnight in contact with the first portion. It was then washed three times with 10% aqueous sodium carbonate and three times with water, dried over anhydrous sodium sulfate, and distilled over sodium metal, the fraction boiling at 65° to 67° being retained for use.

EXPERIMENTAL PROCEDURES

The chromatographic characteristics of the individual nitro and nitroso compounds (Table III) were determined as follows:

Table I. Materials

Name	Source	Reference	Melting Point, ° C.
Solutes			
p-Nitrophenol	Eastman Kodak Co., Research Laboratory		113
p-Nitrosophenol	Synthetic, nitrosation of phenol	(*7*)	123–125
2,4-Dinitroresorcinol	Synthetic, two-step nitration of resorcinol[a]	(*8, 15*)	146.5
2,4-Dinitrosoresorcinol	Eastman Kodak Co., Research Laboratory		167[b]
1-Nitro-2-naphthol	Synthetic, oxidation of 1-nitroso-2-naphthol with nitric acid	(*4*)	102.5
1-Nitroso-2-naphthol	Eastman Kodak Co., Research Laboratory		109.5
2-Nitro-1-naphthol	Synthetic, oxidation of 2-nitroso-1-naphthol with alkaline hydrogen peroxide	(*4*)	127–128
2-Nitroso-1-naphthol	Eastman Kodak Co., Research Laboratory		162–163
2,2-Dinitropropane	Synthetic, oxidation of 2-nitroso-2-nitropropane with chromic acid[c]	(*10*)	53
2-Nitroso-2-nitropropane	Synthetic, nitrosation of 2-nitropropane	(*11*)	75
Diethylnitrosamine	Synthetic, nitrosation of diethylamine	(*3*)	176[d]
Solvents			
Benzene	Merck, reagent grade, thiophene-free		
Ethyl alcohol (absolute)	U. S. Industrial Chemical Co., U.S.P.		
Acetone	Merck, reagent grade, redistilled and dried		
Ether (anhydrous)	Mallinckrodt Chemical Works, reagent grade		
Petroleum ether	Skellysolve Petroleum Co., Skellysolve B[e]		
Adsorbents			
Silicic acid	Merck, reagent grade		
Celite	Johns-Manville		

[a] 2-Nitroresorcinol formed as intermediate.
[b] Decomposed.
[c] Much improved yield obtained by conducting oxidation at 15–16° for 24 hours, instead of using elevated temperature described in reference.
[d] Boiling point.
[e] Purified; see text.

Table II. Standardization of Adsorbents

Adsorbent	Solution	Developer	R Value
Silicic acid	0.01 M *o*-nitroaniline in benzene	Benzene	0.374
Silicic acid–Celite	0.01 M *o*-nitroaniline in benzene	Benzene	0.534
Silicic acid–Celite (prewashed)	0.01 M *o*-nitroaniline in benzene	Benzene	0.512

Table III. Chromatographic Characteristics of Nitro and Nitroso Compounds

Compound	Solvent	Adsorbent	Developer	R Value	Color of Zone: Before streaking	Color of Zone: After streaking[a]	Sensitivity (after Streaking), M
p-Nitrophenol	Benzene	Silicic acid and Celite	Benzene (96%) Acetone (4%)	0.378	Colorless	Yellow	0.00004
p-Nitrosophenol	Benzene	Silicic acid and Celite	Benzene (96%) Acetone (4%)	0.222	Colorless	Yellow	0.00008
2,4-Dinitroresorcinol	Benzene	Silicic acid and Celite	Benzene (96%) Acetone (4%)	0.382	Yellow	Yellow	0.00008
2,4-Dinitrosoresorcinol	Benzene	Silicic acid and Celite	Benzene (96%) Acetone (4%)	0.100[b]	Colorless	Green-blue[c]	0.0005
1-Nitro-2-naphthol	Benzene	Silicic acid	Benzene	0.894	Yellow	Yellow	0.00004
1-Nitroso-2-naphthol	Benzene	Silicic acid	Benzene	0.284	Red-brown	Red-yellow	0.0002
2-Nitro-1-naphthol	Benzene	Silicic acid and Celite	Benzene	0.960	Yellow	Yellow	0.00004
2-Nitroso-1-naphthol	Benzene	Silicic acid and Celite	Benzene	0.215	Yellow	Yellow	0.00004
2,2-Dinitropropane	Petroleum ether	Silicic acid	Benzene (50%) Petroleum ether (50%)	0.850[d]	Colorless	Blue[e]	....
2-Nitroso-2-nitropropane	Petroleum ether	Silicic acid	Benzene (50%) Petroleum ether (50%)	0.416	Blue[f]	Blue[f]	0.0004
Diethylnitrosamine	Petroleum ether	Silicic acid	Petroleum ether	0.060[d]	Colorless	.....	.....

[a] Streaked with 6 N NaOH unless otherwise indicated. Except where noted, colors existing before streaking were intensified by streaking.
[b] Approximate value, boundaries of zone relatively indefinite.
[c] Streaked with solution containing 0.005 M $KMnO_4$ and 0.125 M NaOH.
[d] Approximate values, positions of zones determined spectrophotometrically.
[e] Streaked with diphenylamine in H_2SO_4. Boundaries indefinite and unreliable.
[f] Colorless when dry, color restored by streaking with benzene.

Preliminary experiments were made to ascertain a combination of solvent, adsorbent, and developer which would serve efficiently for the isolation of each compound. Where necessary, the rate of movement of an adsorbed zone was increased by dilution of the adsorbing silicic acid with Celite, or by dilution of the benzene usually used as the developer with acetone, or both. All initial solutions were 0.01 *M* except three containing solutes of relatively low solubility: *p*-nitrosophenol (0.0004 *M*), 2,4-dinitrosoresorcinol (0.0025 *M*), and 2-nitroso-2-nitropropane (0.0025 *M*).

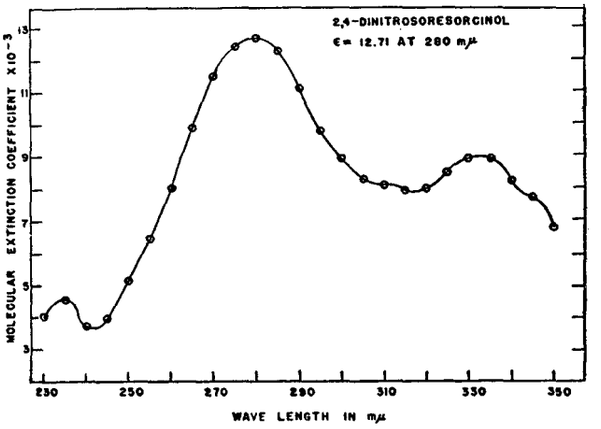

Figure 2. Absorption of 2,4-Dinitrosoresorcinol in Absolute Ethyl Alcohol

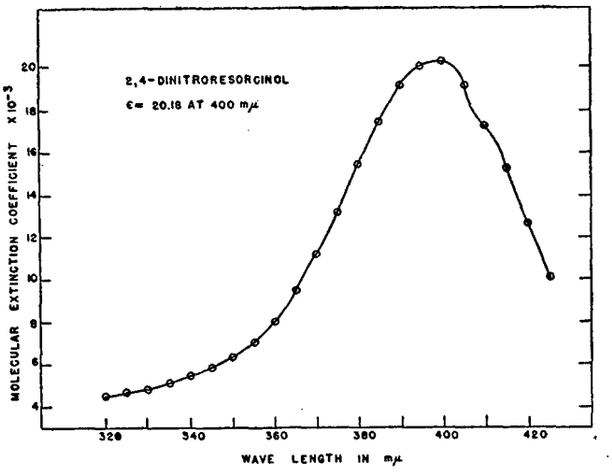

Figure 3. Absorption of 2,4-Dinitroresorcinol in Absolute Ethyl Alcohol

One milliliter of a solution of the compound undergoing examination was delivered to the top of the column. As its upper edge disappeared into the adsorbent, addition of one volume of the developer was begun. The *R* value of the solute under the experimental conditions was determined. Streaking agents were employed to produce or to intensify zone color. Similar runs were then made with increasingly dilute solutions to determine the sensitivity of the method for each compound—that is, the lowest concentration which would give a dependably visible zone. The zones of 2,2-dinitropropane and diethylnitrosamine were colorless, and no streaking agents were discovered which would indicate their boundaries with sufficient precision, though a solution of diphenylamine in sulfuric acid produced a recognizable but ill-defined blue shade with the former. The positions of these zones, therefore, were determined by systematic spectrophotometric examination of successive portions of the columns.

Spectrophotometric data for the nitro and nitroso compounds were obtained from standard solutions in absolute ethyl alcohol, with readings at 5 mμ intervals. The elapsed time between final purification of each solute and its spectrophotometric measurement was made as short as possible to minimize errors due to

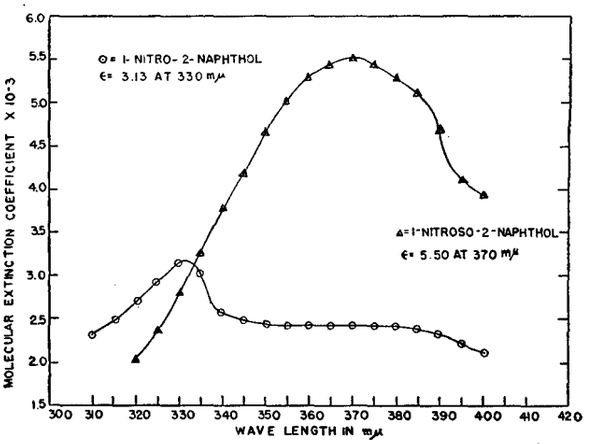

Figure 4. Absorption of 1-Nitro-2-naphthol and 1-Nitroso-2-naphthol in Absolute Ethyl Alcohol

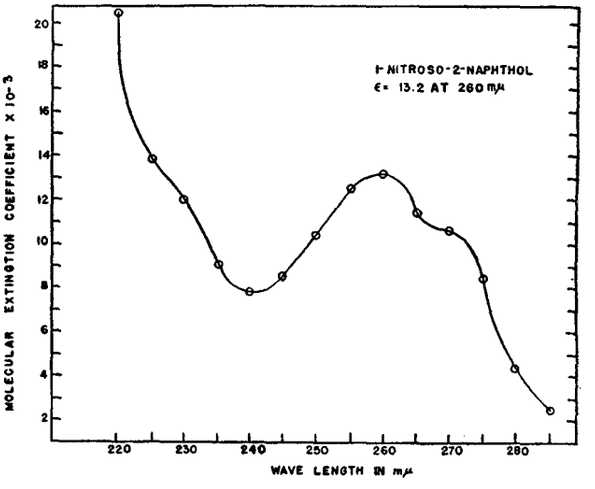

Figure 5. Absorption of 1-Nitroso-2-naphthol in Absolute Ethyl Alcohol

Table IV. Recoveries of Nitro and Nitroso Compounds from Solutions

Compound	Concentration, *M*	Quantity Chromatographed, Ml.	Size of Column	% Recovery[a]
p-Nitrophenol	0.01	1	No. 1	97
p-Nitrophenol	0.01	5	No. 2	98
p-Nitrosophenol	0.00041	1	No. 1	92
p-Nitrosophenol	0.00041	5	No. 2	98
2,4-Dinitroresorcinol	0.01	1	No. 1	94
2,4-Dinitrosoresorcinol	0.0025	1	No. 1	88
1-Nitro-2-naphthol	0.01	1	No. 1	94
1-Nitroso-2-naphthol	0.01	1	No. 1	91
2-Nitro-1-naphthol	0.01	1	No. 1	95
2-Nitroso-1-naphthol	0.01	1	No. 1	93
2,2-Dinitropropane	0.005	1	No. 1	88
2-Nitroso-2-nitropropane	0.0025	1	No. 1	79
Diethylnitrosamine	0.01	1	No. 1	85

[a] Averages of 3 determinations. Extreme variation, ±3%.

chemical change or evaporation; some nitroso compounds, in particular, gave altered results after long standing. Normally, the absorption range between 220 and 310 mμ was covered; if a satisfactory absorption maximum was not found within this range, measurements were extended to other wave lengths. Figures 1 to 9, inclusive, contain curves constructed from these data by plotting molecular extinction coefficients (ε) against wave lengths. Measurements of standard solutions of different concentrations showed that these compounds obeyed Beer's law

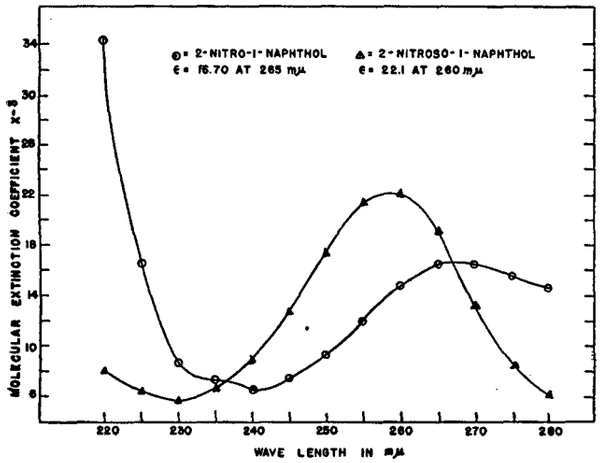

Figure 6. Absorption of 2-Nitro-1-naphthol and 2-Nitroso-1-naphthol in Absolute Ethyl Alcohol

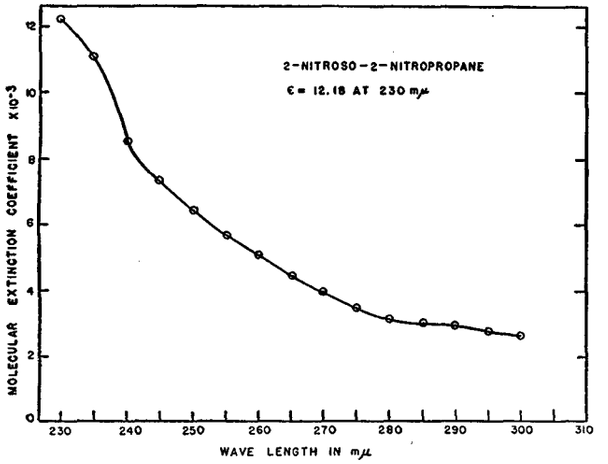

Figure 7. Absorption of 2-Nitroso-2-nitropropane in Absolute Ethyl Alcohol

for dilute solutions; the data could be used, therefore, for quantitative estimations. For some types of more precise work, spectrophotometric measurements at shorter intervals should be made.

The procedure followed for the identification and estimation of related nitro and nitroso compounds in unknown solutions containing one or both (and possibly other solutes) was as follows:

One milliliter of the solution was chromatographed in the manner previously described for the determination of chromatographic characteristics. After developing, and without allowing any part of it to become dry, the column was washed with a little more than one volume of petroleum ether to remove any adsorbed developer. The column was extruded, and the zone containing the compound sought was cut out. If the compound was one which gave a colorless zone, two columns were run under identical conditions. One was streaked to develop a color, and the position of the zone was thus determined; the second was cut "blind" at the same position, and the material in this second (unstreaked) column was used for the subsequent spectrophotometric examination. When analogous nitro and nitroso compounds were both present, the latter was always much more strongly adsorbed, so that no difficulty was experienced in separating the zones.

Each zone thus isolated was powdered, dried at room temperature, put back on the column, and eluted with sufficient absolute ethyl alcohol to dissolve all adsorbed material. The eluent was diluted suitably, and a significant portion of its spectrophotometric absorption curve was determined. Concentration of the solute was calculated from its extinction coefficient at or near a peak; for this purpose the appropriate wave length specified in Figures 1 to 9 was selected, representing an actual point of measurement of the standard solution. Identification of the solute was accomplished in the same process by comparing the position and color of the chromatographic zone, and the shape and dimensions of the spectrophotometric curve, with the corresponding characteristics shown by the standard solution of the same compound.

Table V. Recoveries of Solutes from Solutions Containing Mixtures of Analogous Nitro and Nitroso Compounds[a]

Composition of Solution	Recovery[b], %
p-Nitrophenol, 0.002 *M*	96.7
p-Nitrosophenol, 0.005 *M*	91
2,4-Dinitroresorcinol, 0.005 *M*	96
2,4-Dinitrosoresorcinol, 0.00125 *M*	85
1-Nitro-2-naphthol, 0.005 *M*	96
1-Nitroso-2-naphthol, 0.005 *M*	94
2-Nitro-1-naphthol, 0.005 *M*	96
2-Nitroso-1-naphthol, 0.005 *M*	90

[a] Data obtained by chromatographing 1 ml. of each solution on No. 1 column.
[b] Averages of two or more determinations.

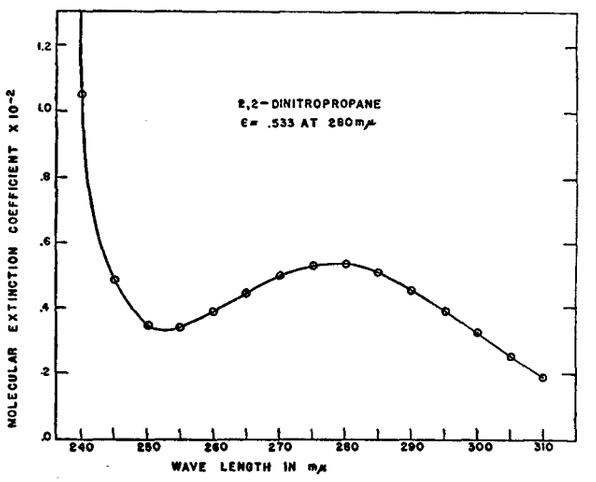

Figure 8. Absorption of 2,2-Dinitropropane in Absolute Ethyl Alcohol

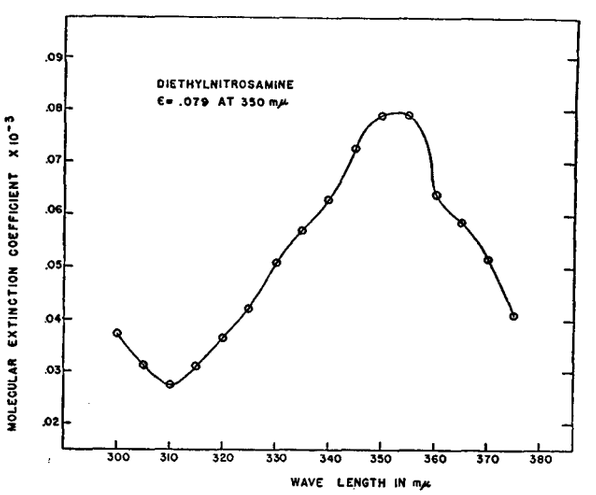

Figure 9. Absorption of Diethylnitrosamine in Absolute Ethyl Alcohol

Because of the relative weakness with which they were adsorbed, 2-nitro-1-naphthol and 2,2-dinitropropane formed zones so near the bottom of the chromatographic column that washing with petroleum ether as described above might have caused appreciable loss. For these compounds, the chromatographic procedure was modified.

After developing, the column was extruded without washing. The zone was cut out, powdered, dried, put back on the column, and eluted with absolute ether. The ethereal solution was collected in a 25-ml. volumetric flask, and the ether evaporated. The residue was dissolved in absolute ethyl alcohol, without removal from the flask, and subjected to spectrophotometric examination.

Tables IV and V show the efficiencies of the method; the former lists recoveries from solutions each of which contained a single solute, and the latter describes the results obtained with solutions containing pairs of analogous nitro and nitroso compounds. Recovery was only semiquantitative; however, with the possible exception of 2,2-dinitropropane, it was much superior to parallel recoveries of the same materials from similar dilute solutions by other methods. Probably a large part of the loss was incurred in transferring the small samples required by use of a No. 1 column. Preliminary work with a No. 2 column, using correspondingly larger samples, substantiates this contention; losses were reduced materially, as shown in the first four lines of Table IV. Circumstances permitting, it seems probable that the accuracy of the method could be increased further by the use of still larger columns and samples, perhaps merely by use of a larger sample with a No. 1 column. Where the nitro or nitroso compound is volatile or unstable (notably in the cases of 2-nitroso-2-nitropropane and diethylnitrosamine) some further loss is inevitable, but it should be smaller than such loss when other methods are employed.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Brown, R. A., Emmett, A. D., Ewing, D. T., and Kingsley, G. V., IND. ENG. CHEM., ANAL. ED., **15**, 301 (1943).
(2) Dewitt, J. B., and Sullivan, M. X., *Ibid.*, **18**, 117 (1946).
(3) Geuther, A., *Ann.*, **128**, 151 (1863).
(4) Grandmougin, E., and Michel, O., *Ber.*, **25**, 972 (1892).
(5) Gullstrom, D. N., Burchfield, H. P., and Judy, J. N., IND. ENG. CHEM., ANAL. ED., **18**, 613 (1946).
(6) Halpern, G. R., *Ibid.*, **18**, 621 (1946).
(7) Houben, J., "Die Methoden der Organischen Chemie," 3rd Auflage, Vol. 4, p. 109, Leipzig, Germany, George Thieme, 1941.
(8) Kauffmann, H., and DePay, E., *Ber.*, **37**, 725 (1904).
(9) LeRosen, A. L., *J. Am. Chem. Soc.*, **64**, 1905 (1942); **67**, 1683 (1945).
(10) Meyer, V., and Locher, J., *Ann.*, **180**, 133 (1876).
(11) Nygaard, E. M., U. S. Patent 2,401,268 (May 26, 1946).
(12) Smith, F. H., IND. ENG. CHEM., ANAL. ED., **18**, 41 (1946).
(13) Strain, H. H., *Ibid.*, **14**, 245 (1942).
(14) Trueblood, K. N., Schroeder, W., and coworkers, Office of Scientific Research and Development, *OSRD Rept.* 5952 (1945).
(15) Vermeulen, M. H., *Rec. trav. chim.*, **38**, 106 (1919).

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Mass Spectrometry of Heavy Hydrocarbons

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IN RECENT years the chemical constitution of the high boiling portions of petroleum has become of considerable interest to the petroleum industry. Numerous analytical problems have arisen with these materials since many of the methods and techniques ordinarily applicable to lighter hydrocarbons (gasolines) are either not applicable at all or give misleading or noninterpretable data. The ability to measure composition in any detail becomes extremely difficult for petroleum products boiling above the gasoline range.

In mass spectrometry the components of a mixture are dispersed into groups of ionized molecules (and molecular fragments) of identical mass. This type of information seemed to offer some possibilities as an aid to the analysis of heavy hydrocarbons. Analytical mass spectrometry has been successfully applied to the analysis of many light hydrocarbons (*2, 3, 13, 17, 18*) and other low boiling organic compounds (*5, 7, 8, 11, 16*). However, its use has been entirely limited to materials exhibiting at least a small vapor pressure (~0.05 mm.) at room temperature. The low resolving power (about 1/200) of most commercially available instruments, the lack of an adequate heated inlet system, and the anticipated complexity of spectral interpretation have been discouraging factors to the extension of the method to hydrocarbons above 200 molecular weight. Various instruments have been described (*6, 9, 10*) having high resolving power, but these instruments have been designed for the single purpose of mass measurement (accurate measurement of the mass scale rather than intensities).

In the application of mass spectrometry to organic materials of low volatility, the instrument should be highly stable and reproducible as are analytical instruments that have gained wide acceptance in recent years. In order to include the heavy hydrocarbons of interest to the petroleum industry, the resolution should be such that masses up to about 600 could be recorded satisfactorily. Such a molecular weight range would be sufficient to include hydrocarbons to above C_{40}. In order to vaporize these materials at a suitable pressure, the entire sample introduction system should be capable of operating at about 300° to 400°C. The instrument stability should be such that relative intensities in the spectra of pure compounds could be reproduced under a given set of operating conditions.

The modification of a commercially available instrument appeared to be the simplest method of meeting the above requirements. The use of a basic instrument design was considered important because the data obtained would be comparable to a great deal of published pure compound spectra (*1*). In addition, the spectra of heavy pure compounds would be usable by similarly modified instruments since the instrument-to-instrument variations probably would not be sufficient to negate their use from a qualitative standpoint.

EXPERIMENTAL

A standard Consolidated Model 21-102 analytical mass spectrometer (*4*) was modified in order to scan satisfactorily and resolve the mass scale up to about m/e 600. The instrument resolution was increased by decreasing the ion slit widths from 0.008 inch normally employed to 0.004 inch and the exit slit from 0.025 to 0.006 inch. The resultant loss of sensitivity (to about one

The purpose of this work was to provide a means for examining the mass spectra of heavy hydrocarbons, and to determine and correlate structure and spectral characteristics of a limited variety of pure compounds. A Consolidated Engineering Corp. analytical mass spectrometer was modified to provide complete, resolved spectra of compounds having molecular weights up to about 600. A heated sample introduction system was so built that the sample could be measured as a liquid volume and there would be complete vaporization of hydrocarbons up to C_{40}. Mass spectra are given for several types of hydrocarbons between C_{14} and C_{32} with some preliminary relations between the spectra and molecular structure. Results indicate that by extension of this type of data, the technique may prove useful in the analysis of petroleum fractions boiling above the gasoline range. As an example, the analysis of a petroleum wax is given as determined by the mass spectrometer. The detection of impurities in pure compounds is shown for several heavy hydrocarbons.

third of that normally obtained) was compensated for by increasing the amplifier sensitivity to give an over-all sensitivity slightly less than that normally obtained. The magnet coils were heavily insulated to withstand the higher temperatures accompanying continuous operation at high magnet currents. The critical components of the magnet power supply (transformer, chokes, etc.) were increased in size to supply the higher currents for the magnet. A selector circuit was added to the magnet control system so that any one of the five preset magnet currents (from 0.2 to 1.6 amperes) could be employed by a simple selector switch. Two additional filter stages were added to increase the stability of the ion accelerating voltage. A multiple scanning circuit was added to the ion acceleration voltage control so that the normal 4000- to 500-volt range could be scanned in either 6, 14, or 44 minutes.

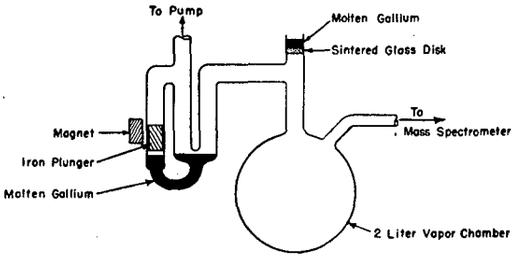


Figure 1. Diagram of Inlet System

The inlet system design is shown in Figure 1. The apparatus consists of a glass vapor reservoir with a molten gallium Y-tube cutoff isolating the pumping system from the inlet system. The gallium cutoff is similar to that previously described (*15*) for use with mercury at room temperature. The boiling point of gallium (2060° C.) is so high that the system can be operated at 300° to 400° C. with no vaporization of the molten metal. A gallium-covered sintered disk allows for sample introduction in the same manner that mercury-covered sintered disks have been used (*14*). The entire sample introduction line is heated, including that portion inside the envelope. The higher temperature necessitated a water-cooled coverplate in order to preserve the wax seal.

The resolution as obtained from the modified instrument is illustrated by a partial mass spectrum in the m/e 600 region of a heavy oil as shown in Figure 2.

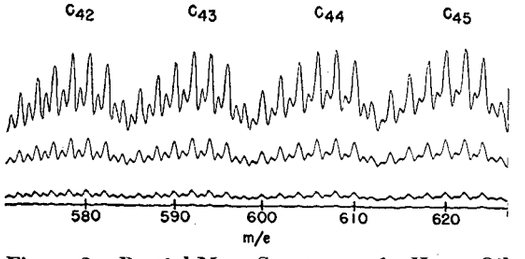


Figure 2. Partial Mass Spectrum of a Heavy Oil

Table I. Mass Spectrum of *n*-Decane

	Relative Intensities	
m/e	Literature[a]	This work[b]
26	1.27	1.21
27	28.0	26.9
29	38.0	36.2
30	0.79	0.80
37	0.04	0.08
38	0.47	0.33
39	12.7	11.3
40	2.35	2.11
41	43.4	41.2
42	16.8	15.4
43	100.0	100.0
44	3.30	3.37
45	0.06	0.07
50	0.13	0.17
51	0.50	0.46
52	0.28	0.26
53	2.17	1.97
54	1.45	1.27
55	14.3	13.4
56	17.1	16.4
57	81.8	81.7
58	3.52	3.58
59	0.04	0.07
63	0.08	0.07
64	0.02	0.02
65	0.23	0.20
66	0.12	0.09
67	0.62	0.56
68	0.46	0.39
69	3.66	3.42
70	12.2	11.2
71	30.0	30.6
72	1.59	1.68
73	0.03	0.04
77	0.12	0.12
78	0.04	0.04
79	0.11	0.09
80	0.02	0.02
81	0.07	0.07
82	0.16	0.15
83	0.91	0.79
84	7.35	6.40
85	21.3	21.0
86	1.32	1.40
87	0.04	0.04
97	0.14	0.17
98	3.59	3.09
99	4.56	3.84
100	0.34	0.31
111	0.04	0.04
112	1.47	1.19
113	2.76	2.36
114	0.23	0.24
126	0.01	0.03
127	0.02	0.04
142	6.74	3.86
143	0.68	0.44
144	0.04	0.03

[a] Mass spectral data, No. 109 (*1*), m/e 43/58 n-C_4 = 8.10.
[b] Hign resolution spectrometer operated at 340° C. (same conditions as for heavy hydrocarbons), m/e 43/58 n-C_4 = 9.60.

The operating characteristics of the instrument are not unlike the standard Consolidated instruments. Table I shows the relative intensities obtained for n-decane with the high resolution instrument compared to literature values obtained for this compound with a standard low resolution instrument (*1*). Close agreement is obtained at all masses except the parent peak (m/e 142). The lower parent mass (and to a less extent the lower values for the other heavy masses) is characteristic of data obtained at the elevated temperatures—i.e., greater cracking, as evidenced by the 43 to 58 ratios for n-butane.

The method of sample introduction was tested by measurement of the linearity of the peak at m/e = 57 (singly charged butyl ion, $C_4H_9^+$, the most prominent peak) with incremental volume additions of n-hexadecane and n-tetracosane. The peak height–sample volume relationship was found to be linear (deviation from mean about 1%) in both cases. In the case of n-tetracosane

Table II. Mass Spectra of Hydrocarbons

m/e	Natl. Bur. Standards, n-C16	A.P.I. No. 537	A.P.I. No. 541	Eastman Kodak, n-C32	A.P.I. No. 109	22	67	8	76	74	69	122	80	517	173	179
12	0.06	0.41	0.12	0.54	0.41	0.67	0.33	0.09	0.78	0.59	0.05	0.15	0.84	0.83	...	0.55
13	0.06	0.26	0.12	0.54	0.48	0.93	0.51	0.02	1.01	0.75	...	0.11	1.44	1.28	...	0.66
15	0.70	1.61	0.91	3.31	3.23	4.33	3.01	0.30	5.57	3.99	0.33	0.68	6.95	6.78	0.37	3.00
16	0.28	1.12	0.66	2.76	2.75	3.37	2.42	0.07	4.61	3.71	0.10	0.34	6.18	4.82	0.12	1.48
17	...	...	...	...	...	...	...	...	...	3.02	...	...	...	...	...	...
19	...	...	...	...	...	0.26	...	...	0.18	0.22	...	...	...	0.36	...	0.35
19½	...	...	...	...	...	0.17	...	...	...	0.17	...	...	...	0.23	...	0.14
20	...	...	...	...	...	0.20	...	...	0.18	0.31	...	...	...	0.26	...	0.31
20½	...	...	...	...	...	...	...	...	...	...	...	...	...	0.11	...	...
24	0.05	0.18	0.07	0.31	0.31	0.67	0.26	...	0.60	0.53	0.03	...	0.94	0.96	0.04	0.45
25	0.14	0.59	0.29	1.09	0.89	2.31	0.96	0.05	2.27	1.65	0.03	0.15	3.25	2.36	0.10	1.55
25½	...	...	...	...	...	...	...	...	...	...	...	...	...	0.26	...	...
26	0.99	3.38	1.73	6.42	5.57	13.3	5.60	0.40	13.1	9.66	0.41	1.09	18.9	13.8	0.54	9.04
27	12.6	16.3	8.93	17.0	16.8	29.3	14.7	4.81	33.3	26.8	6.07	7.25	34.8	36.5	5.16	23.5
29	29.0	31.1	21.8	22.1	25.9	30.7	16.0	16.8	37.3	33.3	21.2	16.7	20.5	8.95	14.4	17.3
30	0.76	0.84	0.70	1.25	1.13	1.81	0.72	0.40	1.85	1.48	0.51	0.45	1.64	0.67	0.35	1.35
31	...	...	...	...	...	0.13	...	...	0.12	0.17	...	...	0.10	0.26	...	0.17
33	...	...	...	...	...	...	...	...	...	...	...	...	...	0.11	...	...
36	...	0.08	...	...	0.14	0.24	0.15	0.02	0.30	0.31	0.03	...	0.25	0.52	...	0.24
37	0.11	0.39	0.19	0.82	0.65	1.48	0.98	0.05	1.62	1.34	0.05	0.15	1.79	3.66	0.10	1.48
37½	...	...	...	...	...	...	...	...	...	...	...	...	...	0.18	...	...
38	0.19	0.69	0.36	1.36	1.17	2.74	1.62	0.04	2.69	2.35	0.15	0.26	2.73	6.13	0.13	2.55
38½	...	...	...	...	...	...	...	...	...	...	...	...	...	0.11	...	...
39	4.94	7.72	5.17	10.4	10.1	16.3	11.8	1.86	22.0	18.4	2.94	4.68	4.81	38.5	1.87	13.8
39½	...	...	...	...	...	...	...	...	...	...	...	...	...	0.11	...	...
40	1.14	1.89	1.09	2.84	2.23	4.44	2.84	0.40	5.38	5.00	0.72	1.09	4.07	7.14	0.42	3.87
41	35.9	44.4	32.7	42.8	46.7	55.1	36.3	23.7	87.6	82.0	43.3	44.5	41.7	43.6	16.6	28.9
42	11.5	15.0	10.5	17.2	16.2	21.3	10.9	5.82	27.8	23.4	8.09	4.23	18.1	13.7	2.46	16.5
43	86.6	89.8	85.7	88.7	100.0	84.8	32.9	79.6	96.9	98.4	85.5	28.1	29.4	2.67	25.7	11.1
44	2.90	3.05	2.85	4.12	2.71	3.19	0.87	2.68	3.53	3.55	2.53	0.79	1.14	0.34	0.90	0.72
45	...	...	...	...	...	...	...	...	...	...	...	...	...	0.11	0.60	0.07
46	...	...	...	...	...	...	...	...	...	...	...	...	...	0.08	...	...
48	...	...	...	...	...	...	...	...	0.24	...	...	...	...	0.18	...	...
49	...	...	...	...	0.14	0.24	0.23	0.02	0.30	0.31	...	0.04	0.40	1.52	...	0.17
49½	...	...	...	...	...	...	...	...	...	...	...	...	...	0.21	...	...
50	0.05	0.24	0.10	0.51	0.58	0.89	0.83	0.05	1.55	1.06	0.08	0.19	2.90	10.8	0.12	1.03
50½	...	...	...	...	...	...	...	...	...	...	...	...	...	0.11	...	...
51	0.16	0.35	0.21	0.74	0.65	1.09	0.88	0.09	2.03	1.48	0.13	0.57	6.28	23.2	0.25	1.45
51½	...	...	...	...	...	...	...	...	...	...	...	...	0.20	0.62	...	...
52	0.13	0.20	0.10	0.39	0.34	0.56	0.36	0.05	1.20	0.89	0.10	0.34	2.23	8.89	0.19	0.41
52½	...	...	...	...	...	...	...	...	...	...	...	...	...	0.26	...	...
53	1.41	1.93	1.07	2.49	2.75	3.81	2.39	0.79	6.88	5.78	1.77	4.53	3.28	10.3	1.08	1.48
54	2.01	2.99	3.02	5.83	4.74	5.13	3.07	2.81	16.2	10.5	8.14	8.72	3.20	14.2	0.67	0.83
55	18.6	25.3	26.6	39.9	43.1	15.0	20.7	28.2	100.0	77.2	73.0	50.7	27.9	28.6	13.6	10.0
56	15.0	18.2	16.4	25.1	20.0	9.50	54.9	13.4	35.6	30.7	18.4	4.83	13.7	3.83	2.25	8.07
57	100.0	100.0	100.0	100.0	79.7	100.0	100.0	100.0	89.8	100.0	98.3	16.8	13.8	0.52	9.78	2.35
57½	...	...	...	...	...	...	...	...	...	...	...	...	0.32	0.88	...	...
58	4.42	4.34	4.46	4.47	3.51	4.61	4.41	4.44	4.07	4.58	4.12	0.79	0.99	0.50	0.50	0.21
58½	...	...	...	...	...	...	...	...	...	...	...	...	...	0.06	...	...
59	...	0.08	...	...	...	0.09	...	0.09	0.12	...	0.08	...	...	0.12	...	...
60	...	...	...	...	...	...	...	...	...	...	...	...	...	0.20	...	...
61	...	...	...	...	...	0.07	...	...	...	...	...	...	0.13	0.55	...	0.05
62	...	...	...	...	...	0.17	...	...	...	...	...	0.02	0.25	1.60	...	0.40
63	...	...	...	...	0.04	0.09	...	...	0.16	0.14	...	0.15	0.73	4.62	0.13	1.07
63½	...	...	...	...	...	...	...	...	...	...	...	...	...	0.17	...	...
64	...	...	...	...	...	...	...	...	0.06	...	...	0.02	0.18	1.43	0.10	0.18
64½	...	...	...	...	...	...	...	...	...	...	...	...	...	0.25	...	...
65	0.29	0.28	0.20	0.28	0.31	0.43	0.18	0.19	1.01	0.87	0.47	1.65	1.22	4.98	0.27	0.43
66	0.19	0.23	0.20	0.39	0.28	0.33	0.15	0.26	1.27	3.58	0.82	2.16	0.22	1.25	0.12	0.13
67	2.02	2.58	2.86	4.61	0.16	5.28	2.17	4.15	28.7	25.8	22.1	57.6	1.80	10.3	1.94	0.99
68	1.87	2.51	3.07	4.96	3.56	4.04	1.53	3.56	9.20	15.3	7.90	10.7	0.98	1.25	0.50	0.24
69	9.78	13.3	16.2	22.8	22.3	24.6	10.0	21.5	49.1	68.4	44.5	45.6	6.43	1.16	6.35	1.81
69½	...	...	...	...	...	...	...	...	...	...	...	...	...	0.13	...	...
70	12.0	12.6	11.7	14.2	43.0	10.8	5.61	9.60	16.9	14.4	14.1	3.58	3.08	0.55	1.23	1.41
70½	...	...	...	...	...	...	...	...	...	...	...	...	...	0.30	...	...
71	57.0	58.0	57.8	54.8	65.2	56.5	15.1	58.7	46.6	50.3	55.2	6.11	4.95	0.54	3.18	0.65
71½	...	...	...	...	...	...	...	...	...	...	...	...	...	0.08	...	...
72	3.32	3.10	3.17	3.02	3.56	3.11	0.82	3.24	2.57	2.82	3.07	0.36	0.28	0.10	0.25	0.05
73	...	0.08	...	...	0.07	0.09	...	0.07	0.09	0.08	0.08	...	0.06	0.32	0.02	0.04
74	...	...	...	...	...	...	...	...	...	...	...	0.05	0.36	1.90	0.03	0.58
75	...	...	...	...	...	...	...	...	...	...	...	0.07	0.29	1.72	0.03	0.81
76	...	...	...	...	...	...	...	...	...	...	...	...	0.34	1.81	0.03	1.12
77	...	0.28	...	...	0.21	0.43	0.16	0.16	1.24	0.81	0.55	3.45	2.42	18.0	0.32	0.92
78	...	0.13	...	...	0.07	0.15	0.04	0.05	0.44	0.28	0.18	1.42	3.09	12.5	0.14	0.31
79	0.25	0.41	0.29	0.57	0.42	0.85	0.31	0.47	3.01	2.26	1.92	3.88	1.30	11.1	0.41	0.27
80	0.07	0.15	0.12	0.35	0.24	0.30	0.12	0.24	1.52	1.28	1.13	7.24	0.18	1.38	0.08	0.04
81	0.48	0.95	1.09	2.76	2.00	2.81	1.21	2.42	13.6	10.7	12.0	97.1	0.87	3.05	0.91	0.24
82	1.17	1.81	2.56	5.13	2.88	2.50	1.25	3.15	92.0	23.1	86.7	11.5	0.65	5.96	0.22	0.22
82½	...	...	...	...	...	...	...	...	...	...	...	...	...	0.07	...	...
83	5.19	7.79	11.5	20.5	15.3	13.9	6.70	16.8	98.4	73.7	100.0	28.4	3.58	17.7	2.44	0.31
84	7.40	7.64	7.16	9.09	9.65	5.54	2.78	5.36	14.4	9.61	12.7	2.30	1.44	1.33	0.31	0.56
85	37.8	39.7	40.1	39.5	30.1	41.3	9.51	42.4	30.2	33.9	39.0	3.48	2.92	0.16	0.94	0.40
86	2.53	2.51	2.65	2.54	2.00	2.74	0.60	2.72	1.97	2.21	2.48	0.21	0.20	0.28	0.07	0.29
87	...	0.08	...	...	0.05	0.09	...	0.09	0.09	0.14	0.03	0.02	0.09	0.50	...	0.61
88	...	...	...	...	...	...	...	...	...	...	...	0.02	...	0.21	...	1.14
89	...	...	...	...	...	...	...	...	...	...	...	0.03	0.39	1.77	0.06	1.07
90	...	...	...	...	...	...	0.04	...	...	...	...	0.02	0.35	0.51	0.05	0.09
91	...	0.18	...	...	0.18	0.30	0.10	0.12	0.98	0.26	0.53	4.56	100.0	30.4	2.29	1.27
92	...	0.07	...	...	...	0.07	0.03	0.03	0.25	0.20	0.10	1.22	10.0	2.79	0.22	0.18
93	...	0.07	...	...	0.09	0.22	0.09	0.13	0.89	0.78	0.72	11.0	0.58	0.32	0.14	0.09
93½	...	...	...	...	0.07	...	...	...	...	...	...	...	...	...	...	...
94	...	0.05	...	...	0.07	0.11	0.05	0.08	0.57	0.84	0.43	10.2	0.09	0.22	0.03	0.29
95	0.12	0.33	0.51	1.58	1.02	1.48	0.65	1.18	6.82	10.6	6.75	100.0	0.42	0.41	0.48	0.51
96	0.44	0.80	1.10	3.00	1.67	1.91	0.60	1.73	11.6	6.42	12.3	12.3	0.29	0.19	0.10	0.22
97	2.98	4.75	7.46	14.7	10.4	10.4	3.68	11.3	45.6	52.2	46.5	17.8	3.29	0.14	1.29	0.09
98	5.35	5.27	5.11	6.07	3.12	3.22	1.26	3.12	7.17	6.34	7.07	1.58	0.75	0.27	0.17	0.27
99	8.50	8.73	10.0	10.1	7.47	11.6	2.63	11.5	7.39	8.24	10.3	0.79	0.63	0.12	0.11	0.34
100	0.64	0.67	0.77	0.81	0.58	0.93	0.21	0.89	0.57	0.64	0.80	0.05	0.05	0.07	...	1.14
101	...	...	...	...	...	0.07	...	0.04	...	...	0.03	0.03	0.12	0.50	...	3.27
102	...	...	...	...	...	...	...	...	...	...	...	0.03	0.64	2.61	0.29	0.78
103	...	...	...	...	...	...	...	...	...	...	0.08	0.17	3.60	12.8	...	0.25
104	...	...	...	...	...	...	...	...	0.06	0.14	0.05	0.24	14.0	32.2	0.70	0.31
105	...	...	...	...	0.07	0.07	...	0.06	0.25	0.22	0.15	1.63	13.1	100.0	2.01	0.33
106	...	...	...	...	...	0.04	...	0.06	0.06	0.08	0.08	0.69	1.39	86.2	0.21	0.31

Table II. Mass Spectra of Hydrocarbons (*Continued*)

m/e	Natl. Bur. Standards, n-C_{16}	A.P.I. No. 537	A.P.I. No. 541	Eastman Kodak, n-C_{32}	A.P.I. No. 109	22	67	8	76	74	69	122	80	517	173	179
107	...	...	...	...	...	0.07	...	0.04	0.38	0.19	0.25	6.12	0.11	7.57	0.06	0.76
108	...	...	...	...	...	0.04	...	0.04	0.41	0.36	0.30	12.7	0.06	0.40	0.04	1.50
109	...	0.11	0.19	0.70	0.42	0.50	0.21	0.53	5.62	3.83	4.85	24.1	0.13	0.14	0.13	0.31
110	0.20	0.36	0.58	1.28	0.70	0.69	0.24	0.93	3.30	2.43	2.57	2.93	0.11	0.30	0.51	1.50
111	1.21	2.06	3.37	6.67	4.44	4.41	1.65	5.46	17.0	25.1	20.9	8.06	0.78	0.23	0.52	1.56
112	3.99	3.90	3.68	5.00	1.60	6.00	0.66	2.23	3.81	3.71	3.48	0.82	0.37	0.04	0.98	2.95
113	6.04	6.16	6.85	7.02	4.98	8.07	1.85	8.04	4.66	4.86	6.78	0.58	0.40	0.18	0.51	2.62
114	0.52	0.52	0.58	0.65	0.44	0.67	0.16	0.78	0.38	0.42	0.38	0.14	0.05	0.18	1.17	5.01
115	...	...	...	...	...	...	...	0.03	0.06	0.08	0.02	...	1.89	4.58	1.62	3.13
116	...	...	...	...	...	...	...	0.38	...	...	0.58	0.17	0.88	1.80	0.91	0.94
117	...	...	...	...	...	...	...	0.02	...	0.11	0.03	0.53	7.13	3.25	2.47	0.29
118	...	...	...	...	...	...	...	0.01	...	0.11	0.02	0.15	7.32	1.16	0.63	0.18
119	...	...	...	...	0.05	...	...	0.09	...	0.08	0.03	0.77	3.57	0.41	0.20	0.52
120	...	...	...	...	...	...	...	...	...	0.05	...	0.58	0.51	1.04	0.03	1.14
121	...	...	...	...	...	...	...	0.01	0.16	0.19	0.13	8.40	0.05	0.12	0.08	1.39
122	...	...	...	...	...	...	...	0.01	0.19	0.42	0.15	3.84	0.03	0.03	0.01	0.51
123	...	...	...	...	0.14	0.19	0.08	0.20	1.14	2.18	1.20	30.1	0.05	...	0.05	0.25
124	0.07	0.16	0.31	0.67	0.37	0.31	0.16	0.63	2.35	1.92	1.65	3.33	0.05	...	0.03	0.18
125	0.43	0.85	1.48	2.96	1.84	1.96	0.59	2.61	6.57	11.9	10.1	4.22	0.29	...	0.10	0.60
126	3.04	3.09	3.00	3.35	0.93	1.69	0.70	1.66	2.44	2.62	2.80	0.48	0.22	0.32	0.05	1.66
127	5.02	5.03	5.29	5.26	3.70	3.85	1.45	6.02	3.33	3.41	4.90	0.43	0.41	1.53	0.65	0.92
128	0.47	0.49	0.52	0.54	0.37	0.41	0.14	0.60	0.32	0.36	0.48	0.39	0.57	5.16	2.88	0.92
129	...	...	...	...	...	...	...	0.04	...	...	...	0.69	0.88	7.02	5.63	0.60
130	...	...	...	...	...	...	...	...	...	...	...	0.43	0.55	2.46	3.90	0.24
131	...	...	...	...	...	...	...	0.03	...	...	...	0.63	2.48	1.13	4.75	0.29
132	...	...	...	...	...	...	...	...	...	...	...	0.21	0.58	0.34	1.59	0.22
133	...	...	...	...	...	...	...	...	...	...	...	0.72	2.17	0.06	0.50	0.42
134	...	...	...	...	...	...	...	...	...	...	...	2.40	0.28	0.34	0.09	0.18
135	...	...	...	...	...	...	...	...	...	0.11	0.12	42.9	0.02	0.07	0.10	0.14
136	...	...	...	...	...	...	...	...	...	0.28	0.05	94.5	0.06	...	0.15	0.07
137	...	...	...	...	0.07	0.11	0.03	0.10	0.47	1.56	0.58	89.7	0.03	...	0.20	0.18
138	...	0.08	0.18	0.50	0.30	0.50	0.11	0.53	1.43	1.23	1.00	9.91	0.03	...	0.04	0.29
139	0.11	0.26	0.59	1.28	0.77	1.07	0.28	1.31	3.81	6.00	4.85	2.52	0.09	0.41	0.08	0.63
140	2.38	2.50	2.48	2.72	0.58	1.13	0.45	1.64	1.62	1.87	1.90	0.27	0.14	0.12	0.07	0.16
141	4.34	4.29	4.40	4.19	2.84	3.06	1.15	4.61	2.47	3.02	3.57	0.51	0.37	2.13	6.43	0.61
142	0.46	0.46	0.46	0.48	0.28	0.33	0.11	0.45	0.25	0.36	0.38	0.15	0.14	2.04	1.69	0.16
143	...	...	...	...	...	...	...	0.03	...	...	0.02	0.69	0.33	2.28	12.2	0.16
144	...	...	...	...	...	...	...	...	...	...	...	0.38	0.16	2.24	3.20	0.09
145	...	...	...	...	...	...	...	0.03	...	...	...	5.99	0.96	0.48	100.0	0.13
146	...	...	...	...	...	...	...	...	...	...	...	1.01	2.20	0.12	12.9	0.09
147	...	...	...	...	...	...	...	...	...	0.36	...	0.39	45.1	0.04	1.02	0.05
148	...	...	...	...	...	...	...	...	...	0.08	...	0.51	7.25	...	0.07	0.04
149	...	...	...	...	...	...	...	...	...	0.08	0.02	3.50	0.54	...	0.06	0.16
150	...	...	...	...	...	...	...	...	0.06	0.08	0.02	4.10	...	...	0.04	0.58
151	...	...	...	...	0.03	0.11	...	0.06	0.70	0.39	0.28	7.43	...	0.07	0.06	0.76
152	...	0.07	...	...	0.19	0.43	0.04	0.39	19.5	0.59	0.83	1.30	0.03	0.23	0.25	0.99
153	0.02	0.11	0.30	0.76	0.47	1.02	0.11	0.84	30.8	4.11	3.25	1.36	0.08	0.22	0.61	0.38
154	1.68	1.96	2.11	2.26	0.42	25.0	0.36	0.90	4.54	1.54	1.60	0.24	0.14	0.19	0.59	0.11
155	3.83	3.79	3.78	3.50	2.28	11.1	0.97	3.61	2.08	2.73	2.82	0.24	0.27	0.29	1.27	0.14
156	0.46	0.46	0.46	0.46	0.26	1.13	0.10	0.44	0.22	0.33	0.33	0.05	0.06	0.28	0.69	0.04
157	...	...	...	...	...	0.07	...	0.03	...	...	0.02	0.15	0.11	0.34	2.04	0.11
158	...	...	...	...	...	...	...	...	...	...	...	0.24	0.12	0.32	1.86	0.04
159	...	...	...	...	...	...	...	...	...	...	...	0.22	0.19	0.12	4.84	0.05
160	...	...	...	...	...	...	...	...	...	...	...	0.07	0.41	0.04	0.76	0.02
161	...	...	...	...	...	...	...	...	...	...	...	0.39	1.51	...	0.16	0.09
162	...	...	...	...	...	...	...	...	...	...	...	0.22	0.25	...	0.03	0.11
163	...	...	...	...	...	...	...	...	...	...	...	1.37	...	0.04	0.03	0.74
164	...	...	...	...	...	...	...	...	...	...	...	0.45	...	0.04	0.02	0.31
165	...	...	...	...	...	0.07	...	0.04	0.35	0.25	0.13	2.57	0.05	0.25	0.31	2.03
166	...	...	...	...	0.18	0.28	0.89	0.44	1.36	0.67	0.60	0.75	...	0.12	0.15	0.42
167	...	0.07	0.20	0.54	0.35	1.00	1.72	0.98	2.32	3.41	2.55	0.98	0.02	0.21	0.71	0.16
168	1.15	1.55	1.86	2.00	0.33	3.02	2.08	0.81	1.14	1.59	1.37	0.17	0.09	0.14	0.26	0.04
169	3.15	3.43	3.33	3.05	1.91	2.92	1.15	3.10	1.59	1.62	2.52	0.21	0.11	0.11	0.96	0.11
170	0.40	0.46	0.41	0.41	0.23	0.37	0.13	0.40	0.19	0.25	0.33	0.08	0.03	0.26	0.39	0.14
171	...	...	...	...	...	...	...	0.02	...	...	...	0.24	0.05	0.76	2.63	0.11
172	...	...	...	...	...	...	...	...	...	...	...	0.05	0.06	0.91	0.71	0.29
173	...	...	...	...	...	...	...	...	...	...	...	0.07	0.14	0.28	0.63	0.04
174	...	...	...	...	...	...	...	...	...	...	...	0.05	0.12	0.06	0.13	0.29
175	...	...	...	...	...	...	...	...	...	...	...	0.38	1.09	...	0.06	0.38
176	...	...	...	...	...	...	...	...	...	...	...	0.19	0.17	...	0.03	1.08
177	...	...	...	...	...	...	...	...	...	...	...	0.48	...	...	0.03	0.51
178	...	...	...	...	...	...	...	...	...	...	...	0.24	...	0.12	0.07	2.22
179	...	...	...	...	...	0.06	...	0.01	0.09	0.42	0.07	1.34	...	0.11	0.13	0.74
180	...	...	...	...	0.14	0.07	0.06	0.30	0.57	0.70	0.60	0.26	...	0.18	0.06	0.16
181	...	...	0.16	0.37	0.25	0.19	0.09	0.52	1.36	1.98	2.12	1.01	...	0.10	0.27	0.05
182	0.59	1.18	1.55	1.74	0.25	1.02	1.76	1.06	0.89	1.42	1.08	0.17	0.06	0.11	0.09	0.04
183	1.95	3.09	3.01	2.65	1.63	1.96	1.16	2.74	1.30	1.48	2.18	0.10	0.06	0.04	0.37	0.04
184	0.28	0.44	0.41	0.39	0.22	0.26	0.14	0.39	0.16	0.25	0.32	0.05	...	0.54	0.15	...
185	...	...	...	...	...	...	...	0.03	...	...	...	0.14	...	0.08	0.95	0.04
186	...	...	...	...	...	...	...	...	...	...	...	0.03	...	1.00	0.25	0.05
187	...	...	...	...	...	...	...	...	...	...	...	0.03	0.06	0.21	0.26	0.61
188	...	...	...	...	...	...	...	...	...	...	...	0.02	0.05	19.6	0.06	0.33
189	...	...	...	...	...	...	...	...	...	...	...	0.15	0.87	2.83	0.05	2.95
190	...	...	...	...	...	...	...	...	...	...	...	0.14	0.14	0.22	0.02	0.98
191	...	...	...	...	...	...	...	...	...	...	...	0.57	...	...	0.05	2.80
192	...	...	...	...	...	...	...	...	...	...	...	0.19	...	...	0.03	0.65
193	...	...	...	...	...	...	...	0.03	0.06	0.19	0.07	1.17	...	...	0.04	0.24
194	...	...	...	...	0.11	0.06	0.03	0.24	0.47	0.58	0.53	2.32	...	...	0.03	0.05
195	...	...	0.09	0.24	0.19	0.09	0.04	0.40	0.96	0.92	1.57	3.31	...	...	0.10	0.02
196	0.30	0.85	1.33	1.50	0.21	0.28	0.09	0.47	0.70	0.42	0.93	0.50	0.02	...	0.04	...
197	1.08	2.58	2.71	2.41	1.42	1.57	0.42	2.27	1.11	1.01	1.48	0.10	0.17	...	0.11	0.04
198	0.17	0.38	0.41	0.37	0.21	0.26	0.06	0.35	0.16	0.17	0.20	0.02	0.05	...	0.06	0.14
199	...	...	...	...	...	0.04	...	0.02	...	...	0.10	0.03	...	...	0.25	0.22
200	...	...	...	...	...	...	...	0.01	...	...	0.13	0.02	...	...	0.12	1.34
201	...	...	...	...	...	...	...	0.01	...	...	0.08	0.02	0.02	...	0.13	1.07
202	...	...	...	...	...	...	...	0.02	...	...	0.17	0.03	0.06	...	0.07	8.39
203	...	...	...	...	...	...	...	0.01	...	...	...	0.03	0.65	...	0.03	4.34
204	...	...	...	...	...	...	...	0.01	...	...	0.03	0.03	0.09	...	0.02	3.78
205	...	...	...	...	...	...	...	0.01	...	...	...	0.25	...	...	0.03	1.32
206	...	...	...	...	...	...	...	...	...	...	...	0.14	...	...	0.02	0.52
207	...	...	...	...	...	...	...	0.09	0.06	0.17	0.07	1.06	...	...	0.04	0.18

(*Continued on next page*)

Table II. Mass Spectra of Hydrocarbons (*Continued*)

m/e	Natl. Bur. Standards, n-C_{16}	A.P.I. No. 537	A.P.I. No. 541	Eastman Kodak, n-C_{32}	A.P.I. No. 109	22	67	8	76	74	69	122	80	517	173	179
208	...	...	...	...	0.09	0.04	0.03	0.32	0.38	1.31	0.50	0.33	...	...	0.03	0.04
209	...	...	...	...	0.16	0.09	0.17	0.40	0.79	0.78	1.18	3.48	...	...	0.04	...
210	...	0.47	1.13	1.33	0.18	1.85	0.12	0.43	0.63	0.86	0.75	0.58	0.08	...	0.03	0.02
211	0.03	1.67	2.52	2.19	1.24	6.98	0.38	2.11	0.98	0.86	1.20	0.09	...	...	0.07	0.27
212	...	0.28	0.39	0.35	0.18	1.15	0.05	0.35	0.16	0.17	0.18	...	...	...	0.05	0.13
213	...	...	...	...	...	0.11	...	0.02	...	...	0.03	0.02	...	...	0.10	1.70
214	...	...	...	...	...	...	...	...	...	...	...	0.02	...	...	0.11	0.63
215	...	...	...	...	...	...	...	...	...	...	...	0.03	0.02	...	0.11	4.07
216	...	...	...	...	...	...	...	...	...	...	...	0.02	0.07	...	0.03	4.59
217	...	...	...	...	...	...	...	...	...	...	...	0.03	0.50	...	0.03	6.58
218	...	...	...	...	...	...	...	...	...	...	...	0.03	0.09	...	0.01	1.57
219	...	...	...	...	...	...	...	...	...	...	...	0.09	...	...	0.01	0.67
220	...	...	...	...	...	...	...	...	...	...	...	0.07	...	...	0.01	0.20
221	...	...	...	...	...	...	...	0.01	...	0.11	0.07	0.84	...	...	0.03	0.11
222	...	...	...	...	0.09	0.41	...	0.18	0.32	1.23	0.53	0.26	...	...	0.01	0.09
223	...	...	0.04	0.15	0.12	1.74	0.04	0.32	0.70	1.73	0.85	0.31	...	...	0.02	0.13
224	0.08	0.28	0.94	1.17	0.18	0.74	0.78	0.39	0.38	0.56	0.53	0.05	...	...	0.03	0.99
225	0.05	0.93	2.33	2.00	1.09	1.24	1.68	1.69	0.73	0.75	1.03	...	...	...	0.04	0.61
226	10.6	0.18	0.40	0.46	0.18	0.20	0.28	0.03	0.16	0.14	0.15	...	...	...	0.03	5.37
227	1.85	...	0.03	0.07	...	...	...	0.02	...	...	0.02	...	...	...	0.06	2.71
228	0.15	...	...	...	...	...	...	...	...	...	...	...	...	...	0.11	27.4
229	...	...	...	...	...	...	...	...	...	...	...	0.02	...	...	0.15	27.0
230	...	...	...	...	...	...	...	...	...	...	...	0.02	0.05	...	0.05	8.68
231	...	...	...	...	...	...	...	...	...	...	...	0.02	0.31	...	0.03	3.98
232	...	...	...	...	...	...	...	...	...	...	...	0.02	0.07	...	0.02	9.67
233	...	...	...	...	...	...	...	...	...	0.08	...	0.10	...	...	0.02	6.35
234	...	...	...	...	...	...	...	...	...	0.11	...	0.12	...	...	...	1.48
235	...	...	...	...	...	...	...	...	0.03	0.84	0.03	1.25	...	...	0.02	0.27
236	...	...	...	...	0.07	0.06	...	0.09	0.25	38.1	1.97	0.22	...	...	...	0.07
237	...	...	0.02	0.13	0.11	0.09	...	0.19	0.47	36.4	2.70	0.03	...	...	...	0.83
238	...	...	0.81	1.04	0.14	2.54	0.09	0.34	0.29	6.28	0.78	...	...	...	...	0.33
239	...	0.03	2.17	1.89	0.98	1.39	0.25	1.23	0.38	1.06	0.90	...	...	...	0.05	6.91
240	...	...	0.38	0.39	0.18	0.22	0.03	0.23	0.06	0.14	0.15	...	...	...	0.03	2.41
241	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.10	33.9
242	...	...	...	...	...	...	...	0.02	...	...	...	...	...	...	0.10	12.6
243	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.33	25.4
244	...	...	...	...	...	...	...	...	...	...	...	...	0.06	...	0.09	7.58
245	...	...	...	...	...	...	...	...	...	...	...	0.03	0.22	...	0.06	100.0
246	...	...	...	...	...	...	...	...	...	...	...	0.03	0.05	...	0.03	24.1
247	...	...	...	...	...	...	...	...	...	...	...	0.15	...	...	0.01	5.42
248	...	...	...	...	...	...	...	...	...	...	...	0.46	...	...	...	2.55
249	...	...	...	...	...	...	...	...	...	...	0.03	0.72	...	...	...	0.58
250	...	...	...	...	0.05	...	...	0.04	0.19	1.06	0.82	0.14	...	...	...	0.78
251	...	...	...	...	0.09	0.04	...	0.10	0.38	1.03	1.23	0.02	...	...	...	0.27
252	...	0.23	0.68	0.93	0.14	0.37	0.04	0.33	0.95	0.61	0.42	...	...	...	0.02	3.11
253	...	0.08	2.01	1.74	0.88	0.50	0.17	0.80	0.22	0.47	0.72	...	...	...	0.04	3.04
254	...	9.89	0.38	0.35	0.16	0.11	0.03	0.16	...	0.08	0.17	...	...	...	0.03	2.60
255	...	1.94	...	...	...	...	...	0.02	...	...	...	...	...	...	0.05	4.10
256	...	0.20	...	...	...	...	...	...	...	...	...	...	...	...	0.07	2.98
257	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.16	2.88
258	...	...	...	...	...	...	...	...	...	...	...	...	0.06	...	0.11	4.12
259	...	...	...	...	...	...	...	...	...	...	0.05	0.02	0.14	...	0.03	3.58
260	...	...	...	...	...	...	...	...	...	...	...	0.02	...	...	...	1.93
261	...	...	...	...	...	...	...	...	...	...	0.03	0.27	...	...	...	1.18
262	...	...	...	...	...	...	...	...	...	...	0.02	0.22	...	...	...	4.72
263	...	...	...	...	...	...	...	...	...	...	0.18	0.10	...	...	...	1.63
264	...	...	...	...	0.05	...	...	0.02	0.16	0.08	14.1	0.07	...	...	...	0.45
265	...	...	...	...	0.07	...	...	0.05	0.16	0.39	22.8	0.03	...	...	0.02	2.24
266	...	...	0.56	0.83	0.21	0.13	0.05	0.98	0.16	0.55	4.48	...	0.02	...	0.02	1.05
267	...	...	1.84	1.65	0.70	0.26	0.12	0.48	0.09	0.25	1.00	...	...	...	0.03	1.37
268	...	...	0.37	0.35	0.14	0.07	...	0.08	...	0.05	0.15	...	...	...	0.02	0.72
269	...	...	...	...	...	...	...	0.01	...	...	0.02	...	...	...	0.05	1.84
270	...	...	...	...	...	...	...	...	...	...	...	0.02	...	...	0.04	0.94
271	...	...	...	...	...	...	...	...	...	...	...	0.07	...	...	0.11	1.65
272	...	...	...	...	...	...	...	...	...	...	...	0.03	0.05	...	0.07	1.08
273	...	...	...	...	...	...	...	...	...	...	...	0.09	0.03	...	0.07	1.25
274	...	...	...	...	...	...	...	...	...	...	...	0.09	0.02	...	0.03	0.51
275	...	...	...	...	...	...	...	...	...	...	...	0.60	...	...	...	0.27
276	...	...	...	...	...	...	...	...	...	...	...	0.33	...	...	...	0.42
277	...	...	...	...	...	...	...	...	...	...	0.07	0.34	...	...	0.03	0.36
278	...	...	...	...	0.05	...	...	...	0.35	0.17	0.67	0.07	...	...	...	0.24
279	...	...	...	...	0.07	...	...	...	0.25	0.17	0.82	0.05	...	...	0.06	0.40
280	...	...	0.48	0.78	0.18	0.19	0.11	0.59	29.9	0.42	0.32	0.03	...	...	0.06	1.27
281	...	...	1.61	1.59	0.53	0.72	0.09	0.70	16.4	0.45	0.50	0.15	...	...	1.06	0.54
282	...	...	0.34	0.35	0.11	0.13	...	0.13	2.92	0.08	0.10	0.15	...	...	0.39	0.25
283	...	...	...	...	...	...	...	0.01	0.32	...	...	0.22	...	...	1.65	0.49
284	...	...	...	...	...	...	...	...	...	...	...	0.57	...	...	1.41	1.08
285	...	...	...	...	...	...	...	...	...	...	...	1.54	...	...	29.3	0.52
286	...	...	...	...	...	...	...	...	...	...	...	1.77	0.02	...	8.57	0.24
287	...	...	...	...	...	...	...	...	...	...	...	1.82	0.01	...	1.15	0.38
288	...	...	...	...	...	...	...	...	...	...	...	1.30	...	...	0.11	0.16
289	...	...	...	...	...	...	...	...	...	...	...	3.21	...	...	0.03	0.27
290	...	...	...	...	...	...	...	...	...	...	...	34.7	...	...	0.03	0.14
291	...	...	...	...	...	...	...	0.02	...	...	...	36.9	...	...	0.03	0.22
292	...	...	...	...	0.04	...	...	0.03	0.32	0.22	0.23	7.60	...	...	0.02	0.16
293	...	...	0.04	0.04	0.05	...	...	0.07	0.63	0.19	0.32	0.86	...	...	0.06	1.50
294	...	...	0.25	0.74	0.58	9.74	0.33	18.7	0.16	14.0	0.28	0.17	...	...	0.09	0.43
295	...	...	1.12	1.54	0.72	29.6	0.66	24.2	0.06	16.4	0.37	0.03	0.02	...	0.57	0.22
296	...	...	0.22	0.35	0.14	6.37	0.13	4.94	...	3.38	0.08	...	0.02	...	0.22	0.16
297	...	...	...	...	...	0.70	...	0.54	...	0.36	...	0.02	...	...	0.32	0.89
298	...	...	...	...	...	0.07	...	0.03	...	...	...	0.02	...	...	0.24	0.34
299	...	...	...	...	...	...	...	...	...	...	...	0.02	0.12	...	7.88	0.20
300	...	...	...	...	...	...	...	...	...	0.08	...	0.05	0.58	...	1.90	0.24
301	...	...	...	...	...	...	...	...	...	0.11	...	0.12	11.2	...	0.39	0.60
302	...	...	...	...	...	...	...	...	...	0.08	...	0.05	3.04	...	0.07	0.45
303	...	...	...	...	...	...	...	...	...	...	...	0.10	0.41	...	0.02	0.16
304	...	...	...	...	...	...	...	...	...	...	...	0.07	0.03	...	...	0.11
305	...	...	...	...	...	...	...	...	0.16	...	...	0.07	...	...	0.02	0.24
306	...	...	...	...	0.04	0.06	...	0.01	7.30	0.08	0.10	0.05	...	...	0.01	0.09
307	...	...	...	...	0.05	0.06	...	0.01	17.3	0.08	0.17	0.03	...	...	0.07	0.07
308	...	...	0.16	0.65	0.35	0.37	5.13	0.57	4.13	0.78	0.10	0.02	...	...	0.06	0.05
309	...	...	0.61	1.46	0.26	0.56	6.60	0.75	0.57	0.98	0.18	0.02	...	...	0.25	0.05
310	...	...	0.13	0.37	0.05	0.13	1.41	0.16	0.06	0.22	0.03	0.02	...	...	0.07	0.05
311	...	...	...	...	...	...	0.16	0.01	...	...	...	...	...	...	0.39	0.11

Table II. Mass Spectra of Hydrocarbons (*Continued*)

m/e	Natl. Bur. Standards, n-C_{16}	A.P.I. No. 537	A.P.I. No. 541	Eastman Kodak, n-C_{32}	A.P.I. No. 109	22	67	8	76	74	69	122	80	517	173	179
312	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.10	0.05
313	...	...	...	...	...	...	...	...	...	...	...	0.02	...	...	0.30	0.16
314	...	...	...	...	...	...	...	...	...	...	...	0.02	0.16	...	0.10	0.14
315	...	...	...	...	...	...	...	...	...	...	...	0.05	0.06	...	0.16	0.31
316	...	...	...	...	...	...	...	...	...	...	...	0.02	0.05	...	0.04	0.13
317	...	...	...	...	...	...	...	...	...	...	...	0.03	...	...	...	0.18
318	...	...	...	...	...	...	...	...	...	...	...	0.02	...	...	...	0.09
319	...	...	...	...	...	...	...	...	...	0.03	...	0.02	...	...	...	0.11
320	...	...	...	...	...	...	...	...	0.32	0.05	0.08	0.03	...	...	...	0.04
321	...	...	...	...	...	...	...	...	0.44	0.05	0.12	0.03	...	...	0.01	0.04
322	...	...	...	0.61	0.35	0.07	...	0.19	0.13	...	3.37	0.02	...	...	0.01	0.04
323	...	...	0.03	1.46	0.72	0.15	...	0.29	...	...	2.28	0.02	...	...	0.04	0.03
324	...	...	...	0.37	0.18	0.06	...	0.06	...	...	0.48	...	...	...	0.03	0.04
325	...	...	...	...	...	...	...	...	...	...	0.05	...	...	...	0.05	0.04
326	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.03	0.09
327	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.22	0.07
328	...	...	...	...	...	...	...	...	...	...	...	0.03	0.02	...	0.07	0.07
329	...	...	...	...	...	...	...	...	...	...	...	0.03	0.02	...	0.02	0.09
330	...	...	...	...	...	...	...	...	...	...	...	0.07	0.03	...	...	0.20
331	...	...	...	...	...	...	...	...	...	...	...	0.02	...	...	...	0.09
332	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.09
333	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.04
334	...	...	...	...	...	...	...	...	0.06	...	...	...	...	...	...	0.07
335	...	...	...	...	...	...	...	...	0.13	...	...	...	...	...	...	0.14
336	...	...	0.19	0.54	14.0	...	...	0.09	...	...	0.03	...	...	...	...	0.29
337	...	...	0.10	1.39	31.3	0.06	...	0.17	...	...	0.10	0.10	...	...	...	0.11
338	...	...	7.36	0.35	7.65	...	...	0.04	...	...	0.12	0.03	...	...	0.02	1.45
339	...	...	1.95	0.07	0.96	...	...	0.01	...	...	0.02	...	...	...	0.02	1.46
340	...	...	0.24	...	0.07	...	...	...	...	...	...	...	...	...	0.04	20.0
341	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.05	6.62
342	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.05	18.4
343	...	...	...	...	...	...	...	...	...	...	...	0.07	...	...	0.02	5.57
344	...	...	...	...	...	...	...	...	...	...	...	0.02	...	...	0.02	68.9
345	...	...	...	...	...	...	...	...	...	...	...	0.03	...	...	...	19.0
346	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2.78
347	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.29
348	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.14
349	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.05
350	...	...	...	0.48	...	...	...	0.06	...	...	0.07	0.03	...	...	...	0.72
351	...	...	...	1.35	0.05	...	1.65	0.11	...	...	0.05	...	...	...	...	0.22
352	...	...	...	0.37	...	...	0.45	0.03	...	...	14.0	...	0.19	...	...	0.20
353	...	...	...	...	...	...	0.05	...	...	...	11.5	...	...	...	...	0.20
354	...	...	...	...	...	...	...	...	...	...	2.55	...	...	...	0.02	1.10
355	...	...	...	...	...	...	...	...	...	...	0.35	...	...	...	0.03	0.47
356	...	...	...	...	...	...	...	...	...	...	...	...	0.23	...	0.02	0.65
357	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.42
358	...	...	...	...	...	...	...	...	...	0.11	...	...	7.39	...	...	1.36
359	...	...	...	...	...	...	...	...	...	0.08	...	...	2.01	...	...	0.47
360	...	...	...	...	...	...	...	...	...	...	...	...	0.28	...	...	0.27
361	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.07
362	...	...	...	...	...	...	...	...	0.35	..	...	...	...	...	...	0.05
363	...	...	...	...	...	...	...	...	0.16	...	...	...	...	...	...	0.02
364	...	...	...	0.39	0.21	...	...	0.03	1.53	...	...	...	...	...	...	0.02
365	...	...	...	1.31	0.11	0.17	0.03	0.08	0.41	...	...	...	...	...	...	0.04
366	...	...	...	0.35	0.12	0.11	0.02	0.03	0.06	...	...	...	...	...	...	0.02
367	...	...	...	...	...	0.06	...	...	...	...	...	...	...	...	...	0.16
368	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.11
369	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.01	1.19
370	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.01	0.42
371	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.01	0.52
372	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.14
373	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.96
374	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.27
375	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.13
376	...	...	...	...	...	...	...	...	...	0.19	...	...	...	...	...	0.04
377	...	...	...	...	...	...	...	...	...	0.14	...	...	...	...	...	0.07
378	...	...	...	0.39	...	...	...	0.02	...	0.78	...	...	...	...	...	0.02
379	...	...	...	1.26	...	...	...	0.05	...	0.22	...	...	...	...	...	0.02
380	...	...	...	0.35	...	...	...	...	...	...	...	...	...	...	...	...
381	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.02
382	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.14
383	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.04
384	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.01	0.07
385	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.02
386	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.09
392	...	...	...	0.35	...	...	...	...	...	...	...	...	...	...	...	...
393	...	...	...	1.15	...	...	...	0.02	...	...	...	...	...	...	...	...
394	...	...	...	0.35	...	...	...	...	...	...	...	...	...	...	...	...
396	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.02	...
397	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.03	...
398	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.02	...
399	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.02	...
406	...	...	...	0.22	...	...	...	...	...	...	...	...	...	...	...	...
407	...	...	...	0.80	...	...	...	0.01	...	...	...	...	...	...	...	...
408	...	...	...	0.24	...	...	...	...	...	...	...	...	...	...	...	...
409	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.01	...
410	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.03	...
411	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.02	...
412	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.05	...
413	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.02	...
414	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.02	...
416	...	...	...	...	...	...	...	...	...	...	...	0.17	...	...	0.02	...
417	...	...	...	...	...	...	...	...	...	...	...	0.03	...	...	0.01	...
418	...	...	...	...	...	...	...	...	...	...	...	0.07	...	...	0.05	...
419	...	...	...	...	...	...	...	...	...	...	...	0.02	...	...	0.02	...
420	...	...	...	...	...	...	...	...	...	...	...	0.17	...	...	0.32	...
421	...	...	...	0.59	...	...	...	...	...	...	...	0.05	...	...	0.11	...
422	...	...	...	0.44	...	...	...	...	...	...	...	0.21	...	...	1.55	...
423	...	...	...	...	...	...	...	...	...	...	...	0.05	...	...	0.50	...
424	...	...	...	...	...	...	...	...	...	...	...	0.03	...	...	3.56	...
425	...	...	...	...	...	...	...	...	...	...	...	0.02	...	...	1.14	...
426	...	...	...	...	...	...	...	...	...	...	...	0.19	...	...	5.02	...

(*Continued on next page*)

Table II. Mass Spectra of Hydrocarbons (*Concluded*)

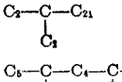
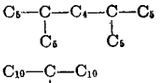
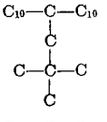
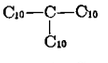
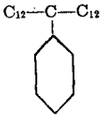
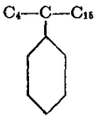
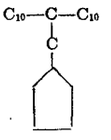
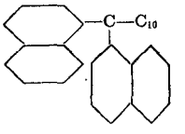
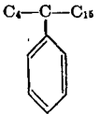
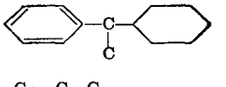
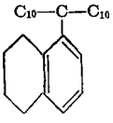
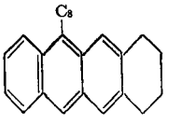
m/e	Natl. Bur. Standards, n-C_{16}	A.P.I. No. 537	A.P.I. No. 541	Eastman Kodak, n-C_{32}	A.P.I. No. 109	22	67	8	76	74	69	122	80	517	173	179
427	...	...	...	...	...	...	...	...	...	...	...	0.07	...	...	1.61	...
428	...	...	...	...	...	...	...	...	...	...	...	0.14	...	...	0.25	...
429	...	...	...	...	...	...	...	0.07	...	...	...	0.03	...	...	0.04	...
430	...	...	...	...	...	...	...	...	...	...	...	0.02	...	...	0.02	...
431	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.02	...
432	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.04	...
433	...	...	...	...	...	...	...	0.02	...	...	...	...	...	...	0.02	...
434	...	...	...	...	...	...	...	0.01	...	...	0.08	...	...	...	0.18	...
435	...	...	...	0.11	...	...	...	0.04	...	...	0.03	...	...	...	0.07	...
436	...	...	...	0.04	...	...	...	0.03	...	...	0.03	...	...	...	0.12	...
437	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.05	...
438	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.22	...
439	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.10	...
440	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1.19	...
441	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.40	...
442	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.08	...
443	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.03	...
448	...	...	...	0.67	...	...	...	...	...	...	...	...	...	...	...	...
449	...	...	...	0.28	...	...	...	...	...	...	...	...	...	...	...	...
450	...	...	...	4.36	...	...	...	...	...	...	...	...	...	...	...	...
451	...	...	...	1.46	...	...	...	...	...	...	...	...	...	...	...	...
452	...	...	...	0.24	...	...	...	...	...	...	...	...	...	...	...	...
464	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.07
465	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.02
466	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.40
467	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.13
468	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.18
469	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.04
470	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.40
471	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.13
472	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.02
478	...	...	...	0.39	...	...	...	...	...	...	...	...	...	...	...	...
479	...	...	...	0.13	...	...	...	...	...	...	...	...	...	...	...	...
Base peak, div/μ	26.2	24.6	25.0	33.0	31.8	28.2	60.7	149.8	15.8	18.8	90.0	77.8	41.3	16.8	212.5	54.2
n-butane 43/58	7.05	7.05	7.05	7.05	7.05	7.05	7.05	9.87	7.05	7.05	9.87	9.87	7.05	7.05	9.70	9.60
58 div/μ	5	5	5	5	5	5	5	19	5	5	19	19	5	5	19	19

it was necessary to maintain the pipet at an elevated temperature (100° C.) because the melting point is above room temperature.

The rate of pump-out was measured with *n*-hexadecane and *n*-dotriacontane. After 5 minutes of pumping (with an initial pressure of ~100μ) the *m/e* 57, most sensitive peak, was reduced to less than 0.1% of the initial value for the C_{16}. With the C_{32} about 0.5% of the initial peak remained after 5 minutes of pumping. These pumping rates compare favorably to those obtained with light hydrocarbons in a nonheated inlet system.

MASS SPECTRA OF PURE COMPOUNDS

The mass spectra of fourteen compounds prepared at the Pennsylvania State College by Research Project 42 of the American Petroleum Institute (*12*) are given in Table II and reproduced in Figures 3 to 18. The materials include normal and isoalkanes, cycloalkanes, and aromatics, and represent a spread of molecular weight with between 14 and 31 carbon atoms per molecule. The materials are as follows:

A.P.I. No.	Name	Structure
PSC 537	*n*-Octadecane	*n*-C_{18}
PSC 541	*n*-Tetracosane	*n*-C_{24}
PSC 109	3-Ethyltetracosane	
PSC 22	6,11-di-*n*-amylhexadecane	
PSC 67	11-Neopentylheneicosane	
PSC 8	11-*n*-decylheneicosane	
PSC 69	13-Cyclohexylpentacosane	
PSC 76	5-Cyclohexyleicosane	
PSC 74	11-Cyclopentylmethylheneicosane	
PSC 122	1,1-Di(α-decalyl)hendecane	
PSC 80	5-Phenyleicosane	
PSC 517	1-Phenyl-1-cyclohexylethane	
PSC 173	11-α-*ar*-tetralylheneicosane	
PSC 179	9-*n*-Octyl(1,2,3,4-tetrahydro)naphthacene	

Eastman Kodak dicetyl (n-C_{32}) and National Bureau of Standards hexadecane (n-C_{16}) are included for extension of the n-alkane data.

Table III shows the instrument operating conditions under which the spectra were obtained.

These materials represent a variety of hydrocarbon types as well as a wide range in boiling point. The mass spectra of these compounds should thus encompass roughly the range of spectral variations to be expected of the hydrocarbon types present in petroleum fractions.

RELATION OF MASS SPECTRA TO MOLECULAR STRUCTURE

n-Alkanes. The mass spectra of the n-alkanes examined (Figures 3 to 6) are very similar one to another in regard to the intensity of the C_nH_{2n+1} cracked fragments. Figure 19 shows a comparative plot of all C_nH_{2n+1} fragments and the parent peaks for each of three n-alkanes: n-C_{16}, n-C_{24}, and n-C_{32}. The C_nH_{2n+1} relative intensities of these compounds are identical up to fragments within three carbon numbers of the parent compound. Thus it becomes a simple matter to predict unknown spectra by constructing a family of curves relating relative intensities to carbon number for the various fragmentation peaks.

Table III. Mass Spectrometer Operating Conditions

Instrument model: Consolidated No. 21-102 (modified)
Magnet currents: 0.6 amp. (m/e 12 to ~60)
1.3 amp. (m/e ~60 to 600)
Scan rate: 4000 to 500 volts accelerating potential/44 min.
Ion accelerating voltages (magnet current, 1.3 amp.):

m/e	Volts
100	2130
200	1030
300	720
400	530

Ionizing voltage: 70 volts
Electron current (catcher): 13.5 microamp.
Ionization chamber temperature: ~250° C.
Vapor temperature: 340° C.
Basis of pressure measurement: Calculated (PVT)

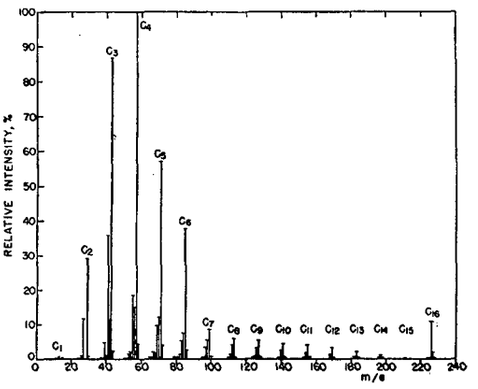


Figure 3. Mass Spectrum of n-Hexadecane, n-C_{16}

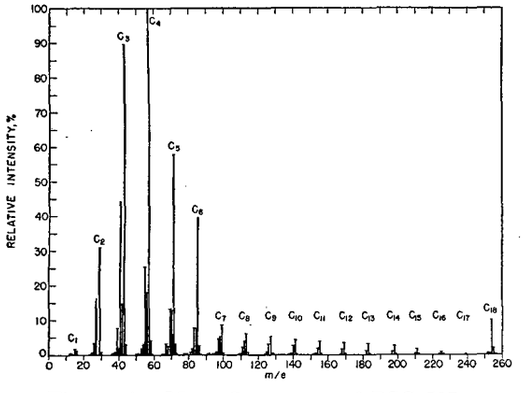


Figure 4. Mass Spectrum of PSC 537, n-Octadecane

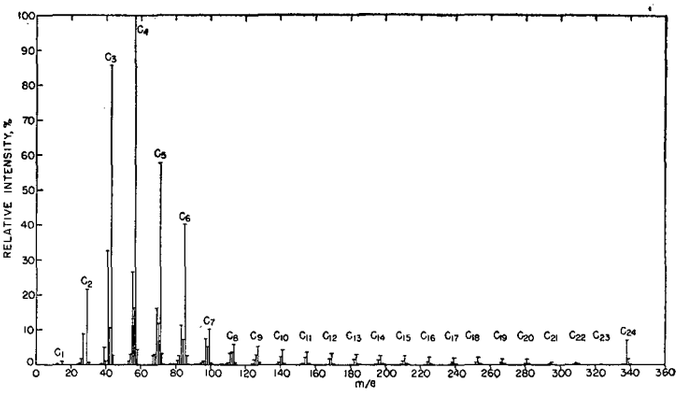


Figure 5. Mass Spectrum of PSC 541, n-Tetracosane

Table IV. Comparison of Calculated and Determined Mass Spectral Intensities of n-Octadecane

m/e	Carbon No.	Calcd. Relative Intensity[a]	Detd. Relative Intensity[a]
254	C_{18}	9.9	9.90
239	C_{17}	0.03	0.04
225	C_{16}	0.9	0.93
211	C_{15}	1.7	1.67
197	C_{14}	2.5	2.58
183	C_{13}	2.9	3.09
169	C_{12}	3.3	3.43
155	C_{11}	3.7	3.79
141	C_{10}	4.2	4.29
127	C_9	5.1	5.03
113	C_8	6.8	6.16
99	C_7	9.5	8.74
85	C_6	39.0	39.7
71	C_5	52.0	58.0
57	C_4	100.0	100.0
43	C_3	87.0	89.8
29	C_2	25.0	31.1
15	C_1	1.5	1.61

[a] Basis: m/e 57 = 100.

These relationships are shown in Figure 20 for the peaks corresponding to C_nH_{2n+2}, $C_{n-1}H_{2n-1}$, $C_{n-2}H_{2n-3}$, $C_{n-3}H_{2n-5}$, and $C_{n-4}H_{2n-7}$. The $C_{n-4}H_{2n-7}$ is the limiting curve, and all fragments smaller than four carbon atoms less than the parent compound fall on this curve. The relative intensities for n-octadecane and its fragments were read from these curves and compared with the experimentally determined values. This comparison is shown in Table IV. Excellent agreement was obtained between the calculated and determined values above m/e 100. The lower masses show large values and somewhat greater error is found. However, the worst case (about 10%) is not believed to be serious disagreement and may be within the accumulative errors of the data and subsequent correlations.

The lower intensity peaks (C_nH_{2n}, C_nH_{2n-1}, C_nH_{2n-2}, etc.) in the n-alkane spectra appear to increase in relative magnitude with the molecular weight of the compound. For example, the C_nH_{2n}, C_nH_{2n-1}, etc., peaks at most carbon numbers appear to increase with increasing molecular weight, whereas the C_nH_{2n+1} peaks remain constant and the C_nH_{2n-2} peaks decrease.

Isoalkanes. Examination of the isoalkane spectra (Figures 7 to 10) indicates a general spectral appearance similar to that of n-alkanes with large peaks due to fragmentation of the prominent branches superimposed thereon. The parent peak is very small compared to that of the n-alkanes. Several interesting features in regard to fragmentation are apparent. The simplest molecule

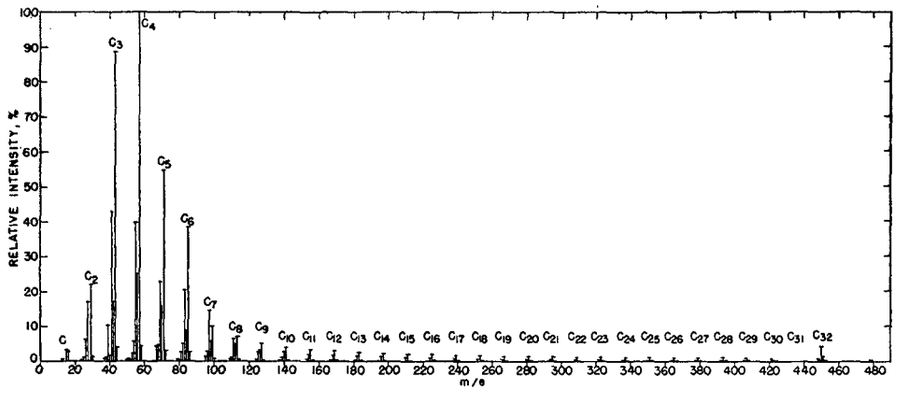


Figure 6. Mass Spectrum of *n*-Dotriacontane, *n*-C_{32}

of this group, $C_2H_5—\underset{\displaystyle C_2H_5}{\underset{|}{CH}}—C_{21}H_{43}$, in addition to the *n*-alkane pattern shows the following: a large $C_4H_9^+$ peak, equivalent to the loss of an ethyl radical; a larger $C_5H_{11}^+$ peak than in *n*-alkanes (relative to the $C_4H_9^+$), equivalent to the loss of the C_{21} radical; and a larger $C_3H_7^+$ peak than in *n*-alkanes, equivalent to the loss of both *n*-$C_{21}H_{43}$ and a C_2H_5 group (plus a rearrangement to give *m/e* 43 rather than 42). It is of interest to note the very small $C_{21}H_{43}^+$ peak, which indicates that although the $C_{21}H_{43}$ was dissociated from the parent molecule to leave a C_5H_{11} ion, the $C_{21}H_{43}$ did not ionize to any appreciable extent. The outstanding modes of dissociation and ionization that are apparent can be summarized as follows, using the terminology

R^+ = positively charged ion

R = negatively or zero charged ion (nonpositive ion)

(*r*) = rearrangement

$$C_2H_5—\underset{\displaystyle C_2H_5}{\underset{|}{CH}}—C_{21}H_{43} \longrightarrow C_2H_5—CH—C_{21}H_{43}^+ + C_2H_5$$

$$\longrightarrow C_2H_5—CH—C_2H_5^+ + C_{21}H_{43}$$

$$\longrightarrow C_2H_5—CH_2^+\ (r) + C_{21}H_{43}^+ + C_2H_5$$

Similar reasoning indicates the following modes of dissociation for the other isoalkanes (the reactions of each compound are not necessarily arranged according to magnitude):

$$C_5H_{11}—\underset{\displaystyle C_5H_{11}}{\underset{|}{CH}}—C_4H_8—\underset{\displaystyle C_5H_{11}}{\underset{|}{CH}}—C_5H_{11} \longrightarrow C_5H_{11}—\underset{\displaystyle C_5H_{11}}{\underset{|}{CH}}—C_4H_8—CH—C_5H_{11}^+ + C_5H_{11}$$

$$C_5H_{11}—\underset{\displaystyle C_5H_{11}}{\underset{|}{CH}}—C_4H_8^+ + C_5H_{11}—CH—C_5H_{11}^+$$

$$C_{10}H_{21}—\underset{\displaystyle CH_2}{\underset{|}{CH}}—C_{10}H_{21} \longrightarrow C_{10}H_{21}—\underset{\displaystyle CH_2}{\underset{|}{CH}}—C_{10}H_{21}^+ + CH_3—\underset{\displaystyle CH_3}{\underset{|}{C}}—CH_3^+$$

$$CH_3—\underset{\displaystyle CH_3}{\underset{|}{C}}—CH_3 \longrightarrow C_{10}H_{21}—CH_2—CH_2^+\ (r) + CH_3—\underset{\displaystyle CH_3}{\underset{|}{C}}—CH_3^+ + C_{10}H_{21}$$

$$\longrightarrow C_{10}H_{21}—CH_2—CH_2—CH_2^+\ (r) + CH_3 + C_{10}H_{21}$$

$$\longrightarrow C_{10}H_{21}—\underset{\displaystyle CH_3}{\underset{|}{\overset{\displaystyle CH_3}{\overset{|}{C}}}}—CH_2—CH_3^+ + C_{10}H_{21}$$

$$\longrightarrow C_{10}H_{21}—\underset{\displaystyle C_{10}H_{21}}{\underset{|}{CH}}—CH_2—\underset{\displaystyle CH_3}{\underset{|}{C}}—CH_3^+ + CH_3$$

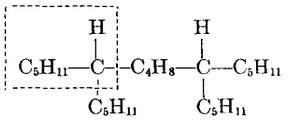
$$C_{10}H_{21}—\underset{\displaystyle C_{10}H_{21}}{\underset{|}{CH}}—C_{10}H_{21} \longrightarrow C_{10}H_{21}—CH—C_{10}H_{21}^+ + C_{10}H_{21}$$

(Dissociations such as R, $R_1^+ + R_2$, and $R_1 + R_2^+$ are summarized as $R \rightarrow R_1^+ + W_2^+$.)

The intensities of peaks corresponding to the dissociation fragments are not clearly defined as a function of the molecular structure. However, a branched chain molecule, $R_1—\underset{\displaystyle R_2}{\underset{|}{C}}—R_3$, where $R_3 > R_2 > R_1$, appears generally to dissociate to yield primary fragments whose intensities are in the following order: $R_1—C—R_2 > R_1—C—R_3 > R_3—C—R_2$. This may be due, at least in part, to the probable higher sensitivity of the lighter ions. Apparently there is no ion formation of dissociated branches unless the branch contains a tertiary or quaternary carbon.

The presence of a C_nH_{2n} peak at the same carbon number as the primary fragment, C_nH_{2n+1}, can be noted in all cases. The explanation of this peculiarity is obscure unless a hydrogen atom is lost (possibly the hydrogen attached to the tertiary carbon) leaving two unattached bonds on the tertiary carbon or the loss from an adjacent carbon resulting in the formation of a double bond. In the light of rearrangements that occur rather readily (discussed below) it would seem that the latter might be the better possibility.

A number of peaks in the isoalkane spectra are due to structural rearrangements since they cannot be explained by simple dissociation. Referring to Figure 8, it will be seen that the relative intensity at *m/e* 85 ($C_6H_{13}^+$) is about as large for this molecule $\left(C_5H_{11}—\underset{\displaystyle C_5H_{11}}{\underset{|}{CH}}—C_4H_8—\underset{\displaystyle C_5H_{11}}{\underset{|}{CH}}—C_5H_{11}\right)$ as for *n*-alkanes. This peak is due to the ion C_6H_{13} which can result from the parent molecule only by a hydrogen shift or rearrangement and not by direct dissociation since the ion C_6H_{12} would thereby be formed:



Similarly all ions of the class C_nH_{2n+1} between C_6H_{13} and $C_{10}H_{21}$, $C_{16}H_{33}$ and $C_{20}H_{41}$, in this compound are due to rearrangements. Similar rearrangement mechanisms are apparent in the other isoalkane spectra in that the rearrangement peaks occur in approximately the same magnitude as those that are not necessarily due to a rearrangement. Such data lend support to a theory that in such a dissociation process a molecule can be visualized as having some degree of atom mobility.

Cycloalkanes. The spectra of three monocycloalkanes are shown in Figures 11, 12, and 13. As in the case of the normal and isoalkanes, the largest peaks occur in the C_3 to C_4 range. As

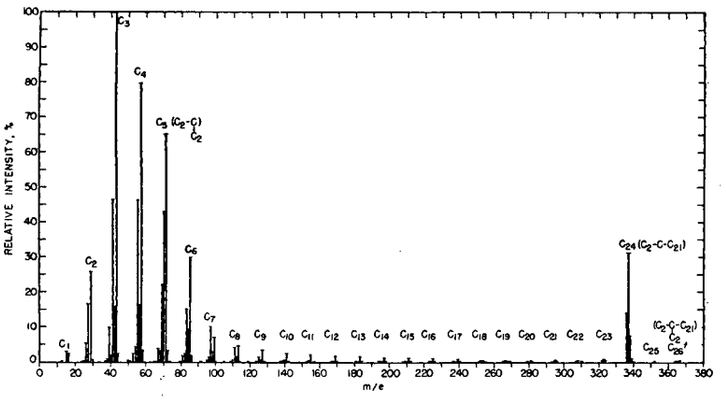


Figure 7. Mass Spectrum of PSC 109, 3-Ethyltetracosane

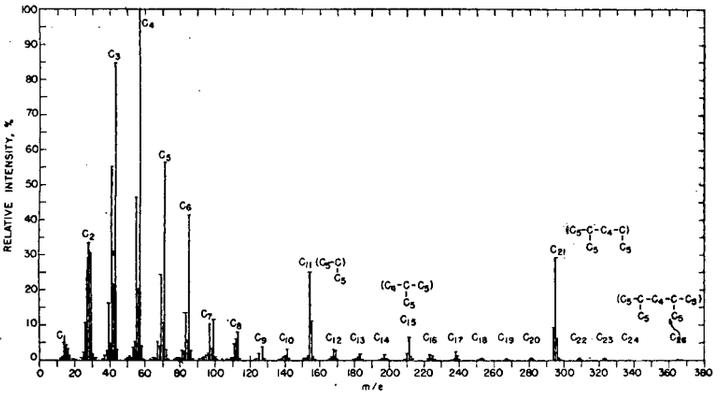


Figure 8. Mass Spectrum of PSC 22[6] 6,11-di-*n*-amylhexadecane

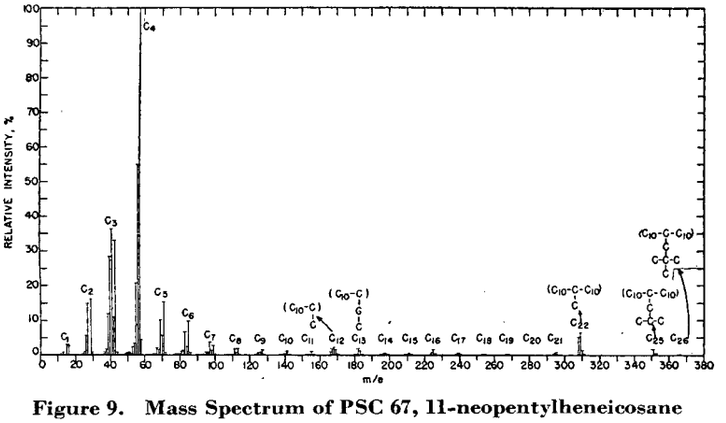
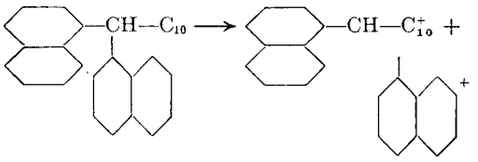


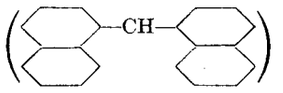
Figure 9. Mass Spectrum of PSC 67, 11-neopentylheneicosane

in the case of isoalkanes, molecular dissociation occurs at tertiary carbon atoms irrespective of the cyclic linkage. The double peak (at each fragmentation group) caused by hydrogen dissociation or double bond formation at the tertiary carbon is perhaps more pronounced than in the isoalkanes. Rearrangement peaks are also apparent in the spectra of the cycloalkanes. The cyclic C_7 peak occurs at m/e 97 ($C_6H_5CH_2$) rather than at m/e 96 (C_6H_5CH), which would result from the simple dissociation of two alkyl groups. The alkyl fragments (C_nH_{2n+1}) also show rearrangement in the same manner as did the isoalkanes.

The polycycloalkane structure (Figure 14) shows a shift to higher masses for the most abundant ions with the peaks corresponding to the ring structures predominating. Primary dissociation occurs as follows:

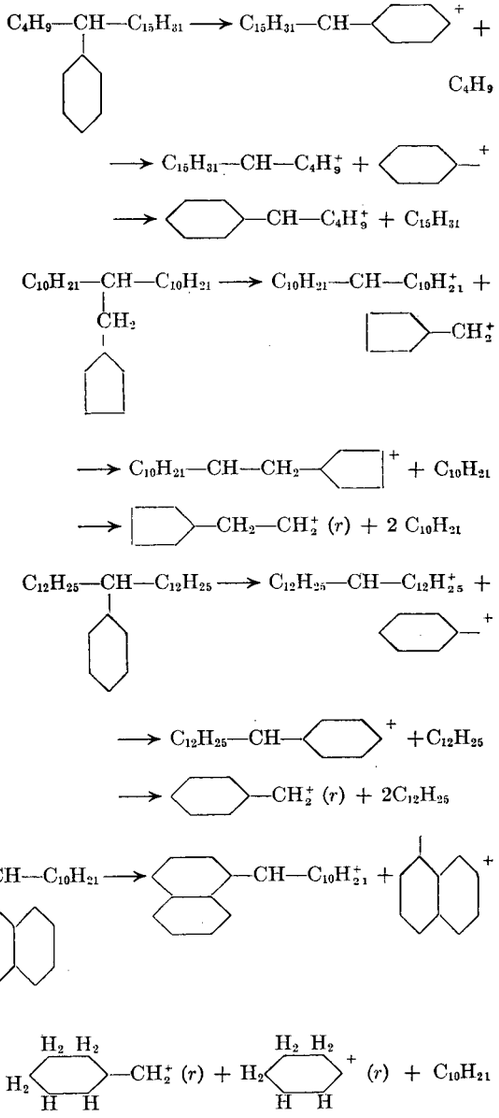


The absence of a prominent didecalylmethyl ion



is noteworthy, but the reason is obscure except that such an ion probably is unstable and tends to dissociate at the CH group.

The outstanding modes of dissociation can be summarized for the cycloalkanes as follows:



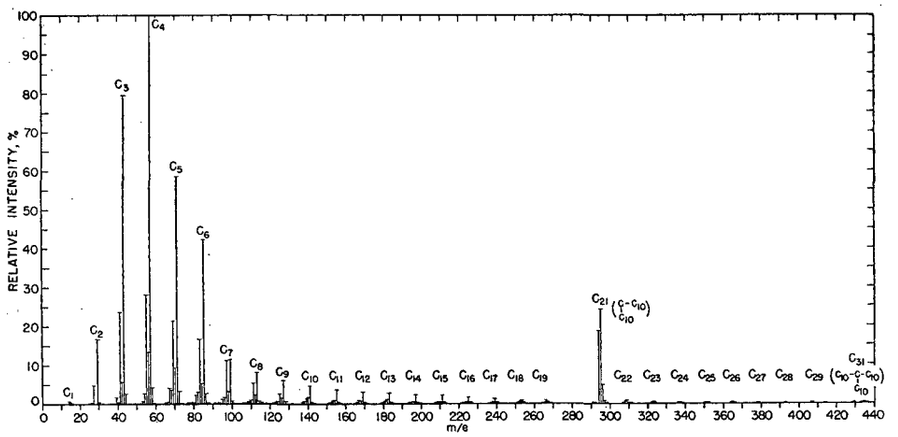
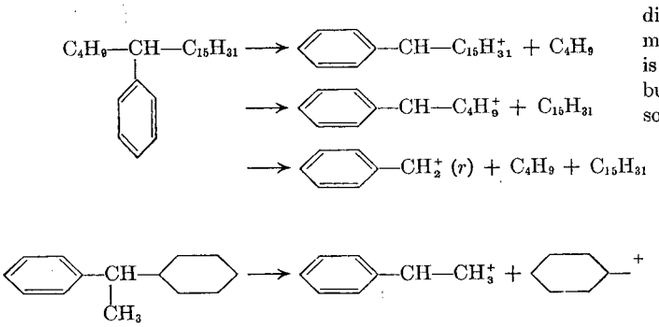


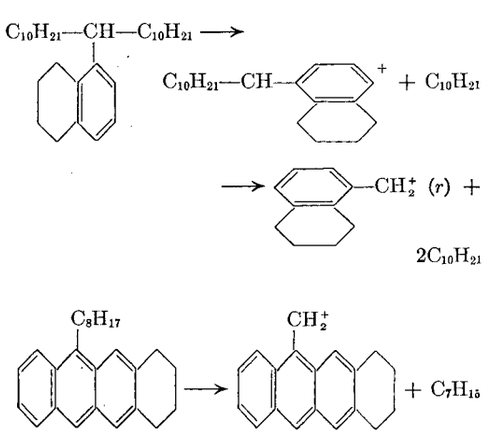
Figure 10. Mass Spectrum of PSC 8, 11-*n*-decylheneicosane

The parent mass intensity of the branched monocycloalkanes, although a factor of about 10 greater than the isoalkanes, is about one fifth that of *n*-alkanes of corresponding molecular weight. It is presumed that straight chain or monoalkyl cycloalkanes may have greater parent mass intensity than the branched members of the family.

Aromatics. The spectra of four aromatic compounds are shown in Figures 15 through 18. In general, the aromatics show a greater distribution of ions above the C_5 group than do the aliphatic compounds. A relatively large parent peak is generally obtained compared to the similar aliphatic structures with the base—i.e., most prominent—peak generally due to a substituted aromatic radical.

The major dissociation modes for the aromatic compounds can be summarized as follows:





The spectrum of 5-phenyleicosane is to be compared with that of 5-cyclohexyleicosane since the structures differ only by the type of ring. The dissociation peaks are similar in the two molecules with the notable exception that the phenyl ring does not dissociate as a group, but remains attached to the tertiary carbon atom. The cyclohexyl ring, however, dissociates from the tertiary carbon in the same manner as do the alkyl groups. An interesting feature of the 5-phenyleicosane spectrum is that the most prominent peak is due to a rearrangement

$$\left(m/e\ 91 = \text{C}_6\text{H}_5\text{—CH}_2\right).$$

Similarly, 1-phenyl-1-cyclohexylethane shows the most prominent peak at

$$m/e\ 105\ \left(\text{C}_6\text{H}_5\text{—CH—CH}_3\right)$$

and the second largest peak at

$$m/e\ 106\ \left(\text{C}_6\text{H}_5\text{—CH}_2\text{—CH}_3\right)$$

the latter produced by rearrangement.

The intensity ratio of

$$m/e\ 91\ \left(\text{C}_6\text{H}_5\text{—C}\right)\ \text{to}\ m/e\ 105\ \left(\text{C}_6\text{H}_5\text{—C}_2\right)$$

is rather large for the phenyleicosane, whereas it is rather small for the phenylethane. This is apparently due to the fact that both the alkyl groups (C_4 and C_{15}) are relatively heavy and are dissociated from the tertiary carbon atom (but not from the aromatic ring). In the case of the phenylethane, the methyl group is relatively light and does not dissociate appreciably. An *n*-butyl group replacing the methyl group would be expected to dissociate and result in the largest peak at the aromatic C_7 group.

The other two aromatic compounds dissociate in a similar manner—i.e., loss of the alkyl groups with the carbon atom adjacent to the aromatic ring not showing appreciable dissociation.

DETERMINATION OF IMPURITIES

In three of the spectra given above obvious impurities are detectable. The *n*-C_{32} shows a significant amount (~15%) of C_{30} and C_{34} *n*-alkanes. The 11-α-*ar*-tetralylheneicosane shows a significant amount of material of mass 440. This

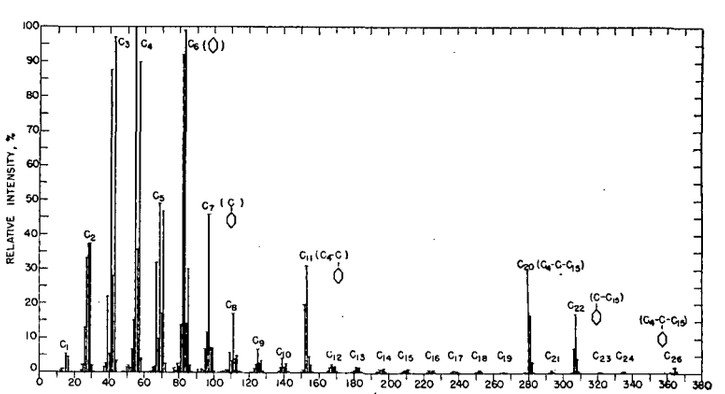


Figure 11. Mass Spectrum of PSC 76, 5-Cyclohexyleicosane

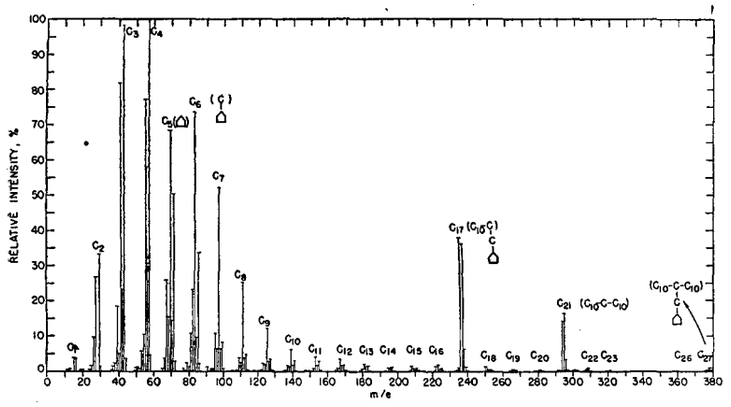


Figure 12. Mass Spectrum of PSC 74, 11-Cyclopentylmethylheneicosane

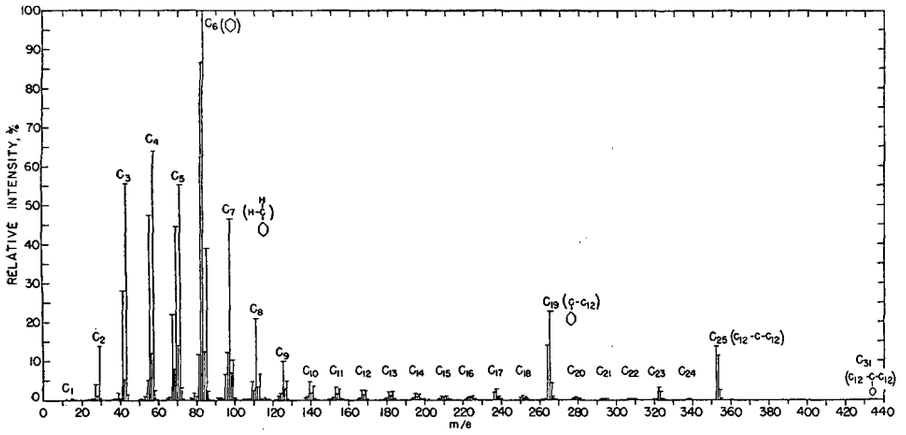


Figure 13. Mass Spectrum of PSC 69, 13-Cyclohexylpentacosane

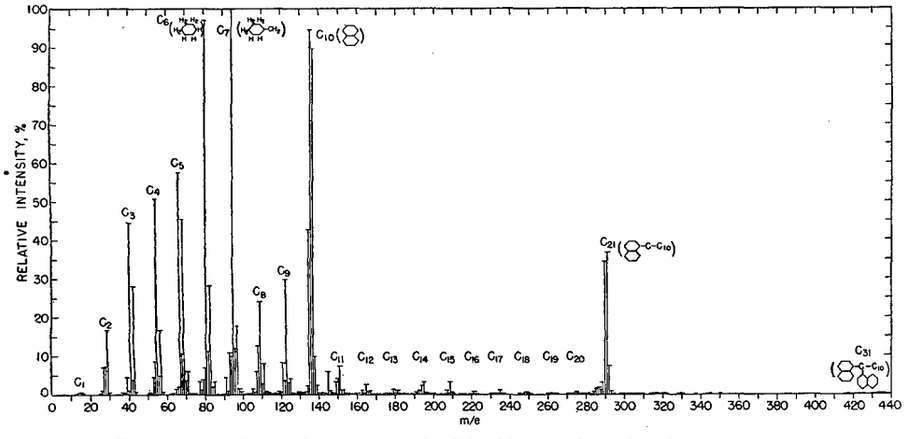
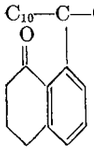


Figure 14. Mass Spectrum of PSC 122, 1,1-di(α-decalyl)hendecane

peak could be caused by a compound of the same structure as the major constituent with an additional CH_2 group. In the same manner the impurity could be an oxidized structure such as $C_{10}—C(=O)—C_{10}$, this perhaps being the more likely as a result of per-



oxidation. As confirmation of this oxidation, Schiessler, director of A.P.I. Project 42, found that the viscosity of the sample had increased by about 50% since the original determination of the physical properties at the time of preparation of the sample. The spectrum of 9-*n*-octyl(1,2,3,4-tetrahydro)naphthacene shows a group of small peaks heavier than the parent mass. The peaks are present between mass 350 and 385 and at about 470. The materials here cannot be adequately interpreted at this time, but the data illustrate that even such apparently small amounts of impurity can be detected by the mass spectrometric technique. The spectra of several other compounds show small peaks at masses lower than the parent peak that may be due to small amounts of impurities.

From the above discussion it is apparent that the mass spectrometer may be used to detect certain impurities in the synthesis and isolation of pure compounds that are difficult to observe by other means. As more data become available on pure compounds it may become possible to identify individual compounds that have not been prepared previously.

PETROLEUM WAX ANALYSIS

As an illustration of the application of the mass spectrometer method to heavy petroleum fractions the composition of a petroleum wax was computed from its mass spectrum as follows:

All peaks were corrected to a monoisotopic basis.

The *n*-alkanes were determined from the C_nH_{2n+2} peaks by using the calibration correlations described above for materials between C_{16} and C_{32}. (Isoalkanes do not contribute appreciably to the C_nH_{2n+2} peaks.)

The residual peak heights at C_nH_{2n+1} and C_nH_{2n} were computed by subtracting the *n*-alkane fragment contribution to these peak heights based on the calibration data and the C_nH_{2n+2} peaks one or more carbon atoms higher.

The C_nH_{2n+1} peaks below C_{21} were found to be entirely accounted for by the mentioned *n*-alkane contributions, but a residual peak remained for each C_nH_{2n+1} between C_{23} and C_{31}. These residuals are attributable to cracked fragment of isoalkanes. The fact that no residual C_nH_{2n+1} peaks were obtained below C_{21} indicates that the isoalkanes present in the wax essentially undergo primary dissociation (as in the case of the pure compound isoalkane spectra) with no secondary dissociation products. In addition, it is reasonable to assume that the isoalkanes of each carbon number have very nearly the same average structure. Thus the residual C_nH_{2n+1} peak height is an approximate measure of an isoalkane mixture of some single higher carbon number (since at least one alkyl group has been dissociated from the parent molecule). Thus, the residual C_nH_{2n+1} peak height

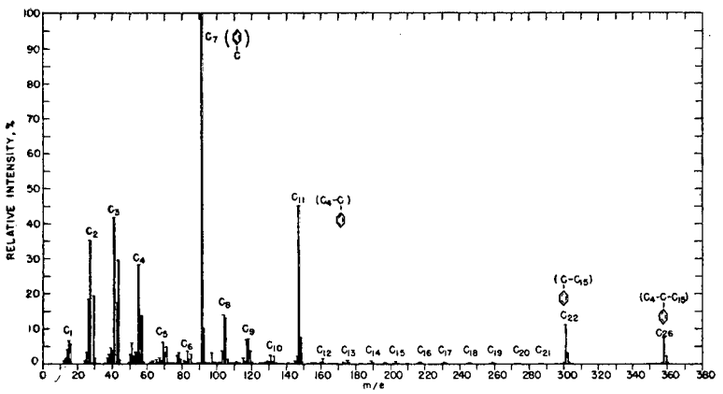


Figure 15. Mass Spectrum of PSC 80, 5-Phenyleicosane

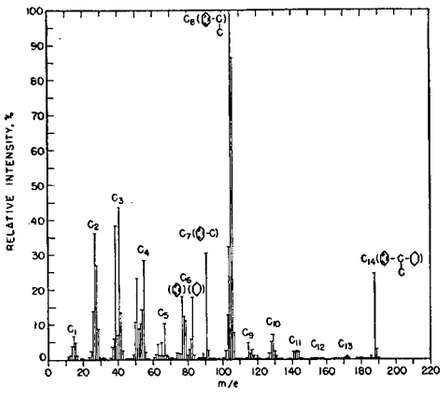


Figure 16. Mass Spectrum of PSC 517, 1-Phenyl-1-cyclohexylethane

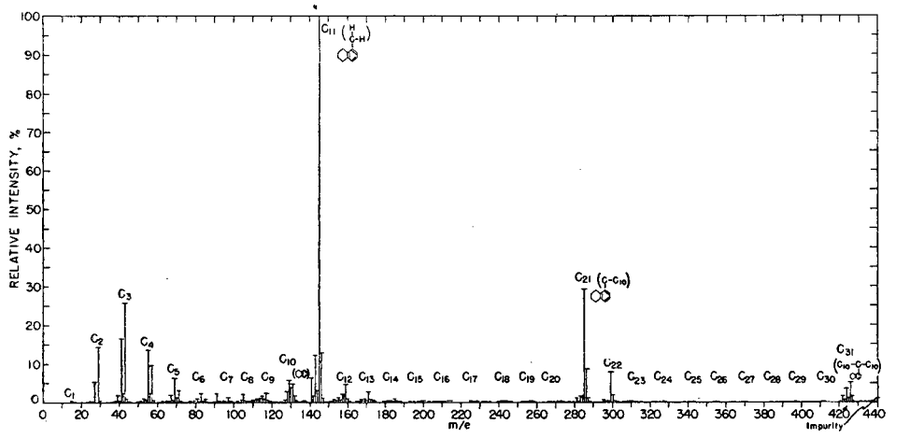


Figure 17. Mass Spectrum of PSC 173, 11-α-*ar*-tetralylheneicosane

(after correction for *n*-alkane contribution) at each carbon number must be corrected by addition of a constant number of carbon atoms to obtain the parent C_nH_{2n+2} structure. This correction must be the equivalent of at least one carbon atom (since the C_nH_{2n+1} is a cracked fragment) but probably not greater than three carbon atoms (assuming simple branching of the carbon chain). This correction was arbitrarily taken as equivalent to two carbon atoms. Therefore, the pure compound 3-ethyltetracosane was used as a calibration material since its spectrum is similar to that expected under the above-stated assumptions. A sensitivity ratio of 1 to 6 for the normal to isoalkanes (at C_nH_{2n+2} and C_nH_{2n+1}, respectively) was thus obtained. This assumption is hardly more than a guess at present, and can be fully established only by careful separation techniques to simplify such mixtures. The relative carbon number distribution of the isoalkanes should be more nearly accurate than the total isoalkane content or the absolute carbon number distribution.

The C_nH_{2n} peaks were corrected for the hydrogen dissociation or double bond formation by isoalkanes (discussed above). A contribution to a C_nH_{2n} peak of about 50% of the corresponding C_nH_{2n+1} isoalkane peak has been found to be about average for the isoalkanes in the wax range. Use of this factor gave a small residual C_nH_{2n} peak in the wax spectrum lying in the range C_{31} to C_{23}. Below C_{23} the peak was completely accounted for. It appears that the correction was fairly accurate since the C_nH_{2n} peak was found to be not overly corrected and a net peak height of zero was obtained from C_{23} to about C_{17}. Inaccuracies involved became apparent below C_{17}. Thus, the residual peak at C_nH_{2n} was used as a measure of monocycloalkanes. The same sensitivity factor was used for the cycloalkanes as for isoalkanes as a crude value. Since the cycloalkane content was small the sensitivity values were not critical.

The results of the above computations are shown in Figure 21. As might be expected the peak of the three distribution curves is highest in carbon number for cycloalkane and lowest for *n*-alkanes. The average molecular weight as computed from these data is 351 as compared to 349 ± 5 determined by the ebullioscopic method. Although the molecular weight check is a rough indication that the *n*-alkane distribution is accurate, it does not serve as an accuracy check on the total non-*n*-alkane data.

CONCLUSIONS

Experimental difficulties of obtaining mass spectra of high molecular weight hydrocarbons appear to have been largely overcome. The spectra of several heavy hydrocarbons indicate some interesting and useful relationships between the mass spectra and molecular structure. These correlations help to reduce the complexity of possible future analytical mass spectrometric techniques as applied to heavy hydrocarbon mixtures. The spectra and correlations should be useful to other laboratories since the ordinary variation from instrument to instrument in the relative behavior of a series of materials is not sufficient to negate the usefulness of the technique. The spectra of more compounds should provide greater quantitative accuracy.

ACKNOWLEDGMENT

The authors wish to acknowledge the help and advice of members of the staff of Consolidated Engineering Corp., especially H. F. Wiley, C. E. Berry, and H. W. Washburn, in the design and construction of the special spectrometer tube and magnet in this work. The authors also desire to express their appreciation to the Advisory Committee of the American Petroleum Institute Research Project 42, and especially to R. H. Schiessler, for the loan of most of the pure hydrocarbons employed in this work.

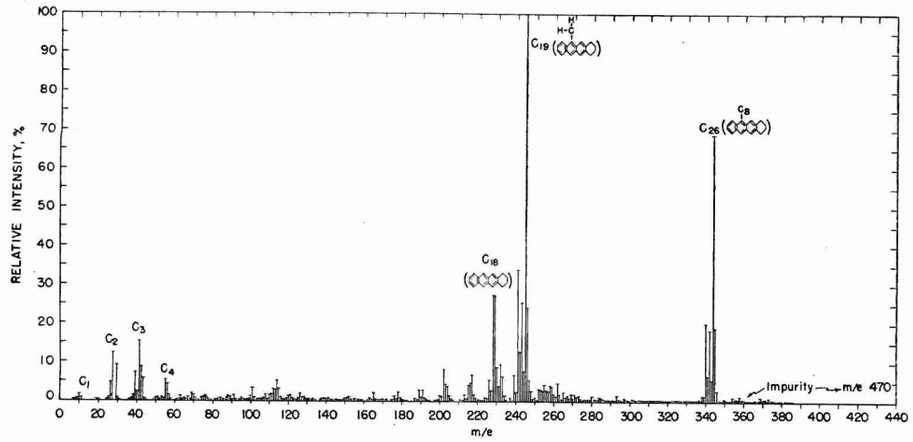


Figure 18. Mass Spectrum of PSC 179, 9-*n*-Octyl(1,2,3,4-tetrahydro)naphthacene

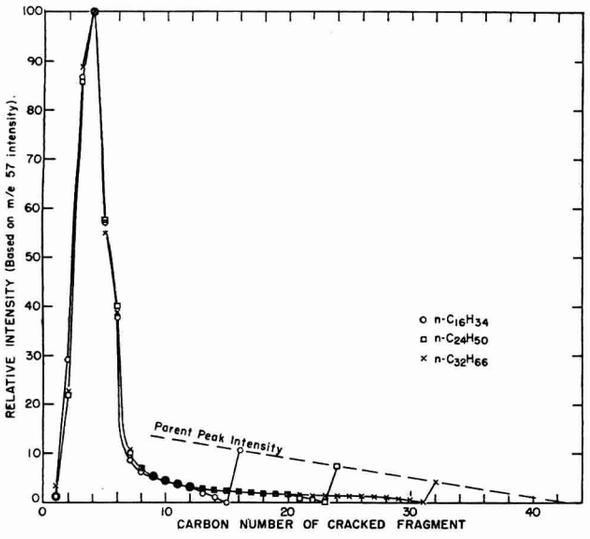


Figure 19. Relative Intensity of C_nH_{2n+1} Cracked Fragments from *n*-Alkanes

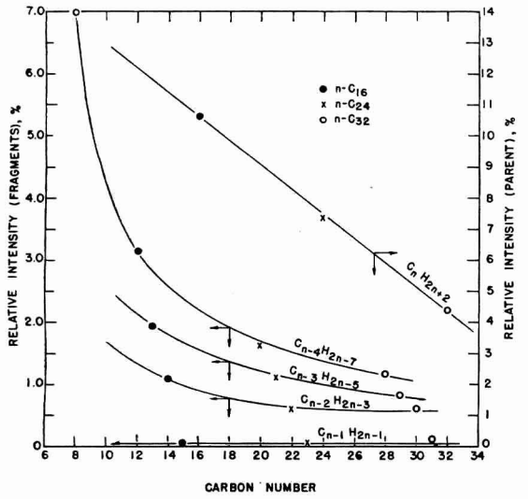


Figure 20. Relation of Carbon Number to Fragment Intensity for *n*-Alkanes

Basis: *m/e* 57($C_4H_9^+$) = 100

LITERATURE CITED

(1) Am. Petroleum Inst., Research Project 44, "Catalog of Mass Spectra Data," National Bureau of Standards, Washington, D. C.

(2) Brown, R. A., Consolidated Engineering Corp., *Mass Spectrometer Group Rept.* **71** (November 1949).

(3) Brown, R. A., Taylor, R. C., Melpolder, F. W., and Young, W. S., ANAL. CHEM., **20**, 5 (1948).

(4) Consolidated Engineering Corp., Pasadena, Calif., *Bull.* **C.E.C. 21-102.**

(5) Gifford, A. P., Rock, S. M., and Comaford, D. J., ANAL. CHEM., **21**, 1026 (1949).

(6) Hipple, J. A., Sommer, H., and Thomas, H. A., *Phys. Rev.*, **76**, 1877 (1949).

(7) Kistemaker, J., *Anal. Chem. Acta*, **2**, 522 (1948).

(8) Langer, A., and Fox, R. E., ANAL. CHEM., **21**, 1302 (1949).

(9) Mariner, T., and Bleakney, W., *Rev. Sci. Instruments*, **20**, 297 (1949).

(10) Richards, P. I., Hays, E. E., and Goudsmit, S. A., *Phys. Rev.*, **76**, 180 (1949).

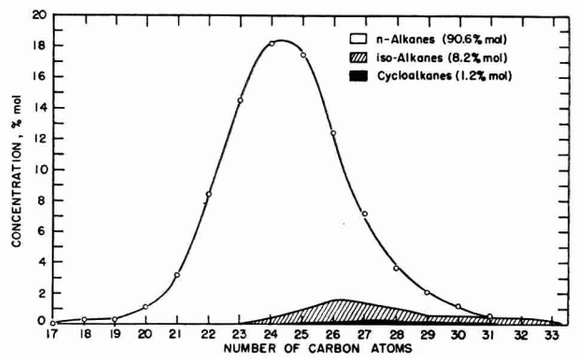


Figure 21. Composition of a Petroleum Wax from Mass Spectrometer Analysis

(11) Roberto, R. H., and Johnsen, S. E. F., ANAL. CHEM., **20**, 1225 (1948).

(12) Schiessler, R. W., Herr, C. H., Rytine, A. W., Weisel, C. A., Fischl, F., McLaughlin, R. L., and Kuehner, H. H., *Proc. Am. Petroleum Inst.*, **26, III**, 254 (1946).

(13) Shephard, M., *J. Research Natl. Bur. Standards*, **38**, 491 (1947).

(14) Taylor, R. C., and Young, W. S., Atlantic Refining Co., *Consolidated Group Rept.* **13** (February 1945).

(15) *Ibid.*, **31** (February 1946).

(16) Thomas, B. W., and Seyfried, W. D., ANAL. CHEM., **21**, 1022 (1949).

(17) Washburn, H. W., Wiley, H. F., and Rock, S. M., IND. ENG. CHEM., ANAL. ED., **15**, 541 (1943).

(18) Washburn, H. W., Wiley, H. F., Rock, S. M., and Berry, C. E., *Ibid.*, **17**, 74 (1945).

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CORRECTION. In the paper on "Spectrophotometric Analysis of Amithiozone Preparations" [Levy, G. B., and Fergus, David, ANAL. CHEM., **23**, 384 (1951)], Figure 2 was unfortunately printed upside down.

Particle Size Determination by Centrifugal Pipet Sedimentation

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Particle size analyses of dusts and industrial powders are frequently required in size ranges below the limits of gravity sedimentation methods. An apparatus was developed which permits particle size analyses by centrifugal pipet sedimentation in the range of 0.1 to 2 microns. This method is analogous in principle to the well-known gravity pipet sedimentation method and gives results comparable with it. A theory of this centrifugal method was worked out and equations were derived for calculating particle size distributions from the experimental data. The centrifugal and gravity sedimentation methods jointly permit complete particle size distributions to be obtained for many fine materials.

SEDIMENTATION methods are used for measuring the particle-size distributions of powders in the subsieve range—i.e., below 44-micron particle diameter. In these methods, the particles of the powder are dispersed in a fluid through which they settle under gravity or centrifugal force, and their diameters are measured in terms of their terminal free settling velocities by applying Stokes's law. Gravity sedimentation methods, which were the first developed, are applicable down to about 1- or 2-micron particle diameter. In recent years, centrifugal sedimentation methods have been developed to extend this range down to about 0.1 micron and in some cases even lower.

Both gravity and centrifugal sedimentation methods admit of many variations, with respect to basic principles and technique (*4*, *5*). With respect to principles (which involve also variations in technique) the main variations are the following: The sedimenting particles may originally be dispersed uniformly throughout the fluid, or they may be introduced at the surface; the fluid may be stagnant or maintained in a state of turbulence; the concentration of solids in suspension or the amount of sediment at the bottom of the suspension may be measured; and either of these quantities may be measured as a function either of settling time or of settling distance (or mathematically equivalent variables). With respect to technique only, equally many variations are known—for example, the pipet, hydrometer, manometer, sedimentation balance, turbidimetric, and other methods.

For gravity liquid sedimentation, the pipet method—in which the concentration of a quietly settling, originally uniform suspension of a powder in a liquid is measured as a function of time by intermittently withdrawing small samples of suspension from a known depth below the surface and determining their content of suspended solids—has come to be regarded as one of the most accurate and dependable variations of sedimentation methods of particle size analysis. Consequently, when it is necessary to determine particle sizes smaller than this method will permit, using only gravity as the settling force, an exactly analogous centrifugal method is a natural choice. The method described in this article is analogous, in so far as possible, to the gravity pipet sedimentation method. It applies over a size range from about 2 to 0.1 micron, depending to some extent on the particle density. The main features which distinguish it from centrifugal methods previously described are the type of flask used to centrifuge the suspension and the mathematical method used to calculate the particle size distribution.

The method which was developed requires a special type of centrifuge flask, which is described below. The flask is sector-shaped, because particles in a centrifugal field follow radial paths, rather than substantially parallel ones as in a gravity field; and it is equipped with means for removing a sample of suspension while the centrifuge is in motion, thereby avoiding the disturbance to the suspension which occurs when the centrifuge is stopped.

In pipet sedimentation under the force of gravity, calculation of the particle size distribution of the powder is a straightforward matter. Using Stokes' law, the diameter of a sphere (of the same density as the material being analyzed) is calculated which would just settle the distance from the surface of the suspension to the sampling point during the settling time which elapsed prior to withdrawing each sample. The fraction by weight of the powder consisting of particles smaller than the diameter so calculated is obtained by dividing the weight of powder in the sample by the weight that would have been contained in an equal volume of the original uniformly dispersed suspension. In centrifugal pipet sedimentation, the calculation method is more complicated, because the centrifugal force acting

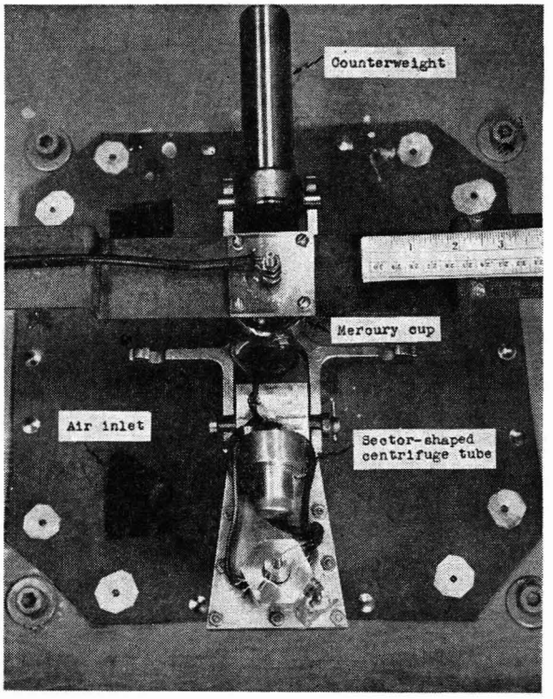

Figure 1. Centrifuge for Particle-Size Analysis by Centrifugal Sedimentation

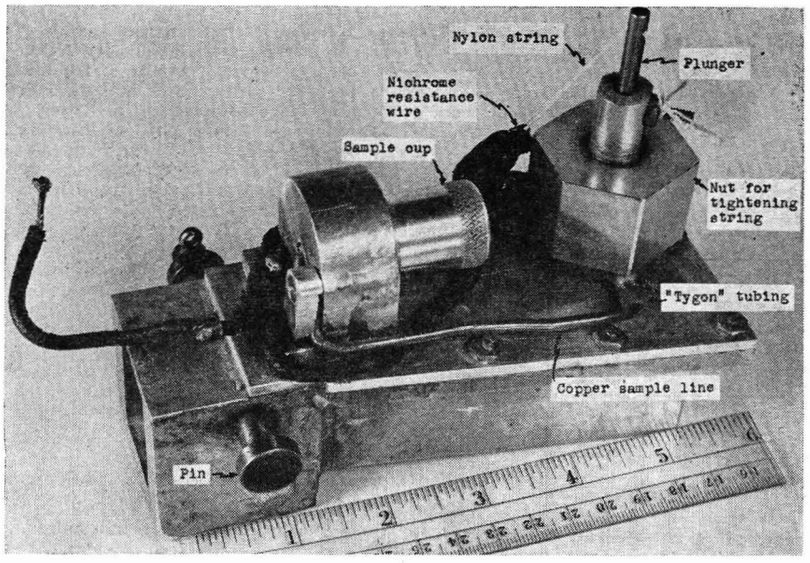

Figure 2. Sector-Shaped Centrifuge Tube

on a particle, unlike the force due to gravity, increases as the particle settles, because the radius of rotation is increasing. Thus, the particles continue to accelerate as they settle, whereas in gravity sedimentation the particles settle at constant velocity (except for an extremely brief initial acceleration which can be ignored for particles of the sizes to which the method is applied). Moreover, in a centrifugal field, the particles move apart as they settle, because their motion is radial, rather than parallel as it is (for practical purposes) in a gravitational field.

Because of these two effects which arise in the centrifugal sedimentation method, the particle size distribution is found to be related to the concentration of the suspension through an integral equation (Equation 20) for which no usable exact mathematical solution is known. This difficulty could have been avoided by arranging the centrifuge so that the variation in centrifugal force over the distance of settling of the particles would be negligible, but this would entail making the centrifuge awkwardly large. It was decided instead to use a centrifuge of comparatively small diameter and to solve the integral equation in question by an approximate method which is described below.

The centrifugal pipet sedimentation method of particle size analysis gives results which are consistent with gravity pipet sedimentation analyses—i.e., when the two methods are applied to the same powder (the coarse part of the distribution being obtained by the gravity method, the fine part by the centrifugal method) the two distribution curves fall in line when plotted on logarithmic-normal (also called logarithmic-probability) paper.

APPARATUS

The centrifuge consisted of a vertically mounted 1-hp. direct current motor which could be operated at any desired speed up to 1800 r.p.m. by means of a variable-voltage supply from a Westinghouse Mo-To-Trol alternating-direct current converter. On the free shaft above the motor was mounted a standard centrifuge head of the kind used in International Equipment Co. laboratory centrifuges. The centrifuge flask was hung in this head by a steel pin, and a counterweight was hung in the opposite position of the head. The flask rotated in a horizontal plane.

The flask, mounted in the centrifuge, is shown in Figure 1, and a close-up of the flask alone in Figure 2. Figure 3 is a diagrammatic cross section showing the essential details of its construction.

The flask was sector-shaped—i.e., its vertical sides were inclined at an angle of about 16°, with the vertex at the center of the shaft, while the horizontal sides were parallel. The flask was made of aluminum, had a capacity of 150 ml., and weighed 1.9 pounds (860 grams) when filled with water.

The upper horizontal side of the flask was a separate plate, bolted to the flask, which carried the sampling equipment. This equipment consisted of a copper sampling line $1/16$ inch in inside diameter, a valve, and a cup to receive the sample. The sampling line entered the sedimentation flask at the "bottom"—i.e., the side furthest removed from the center of rotation—and turned up at the center of the bottom, extending radially inward for 1 inch (2.5 cm.). The end of the line (inside the flask) was brazed to a copper rod as shown in Figure 3. This rod provided mechanical support and prevented solids from depositing on the end of the sample line. Two holes $1/32$ inch in diameter were drilled through the sample line near its end, 1 inch above the bottom of the flask. The sample line ran through the cover plate and up the outside of it, making a loop just below the level of the surface of the suspension, and terminated with a $1/32$-inch orifice above the sample cup, which had a capacity of 12 ml.

The "valve" consisted of a short section of Tygon tubing inserted in the sampling line outside the flask. The tubing was normally held closed by a brass plunger fastened down by a nylon string, one end of which was anchored to a Nichrome resistance wire. When current was passed through this wire, the string melted, releasing the plunger, and opening the valve. Current was supplied to the Nichrome wire from a 6-volt automobile storage battery and was carried to the rotating flask through a slip ring. The sample line acted as a siphon; when the valve

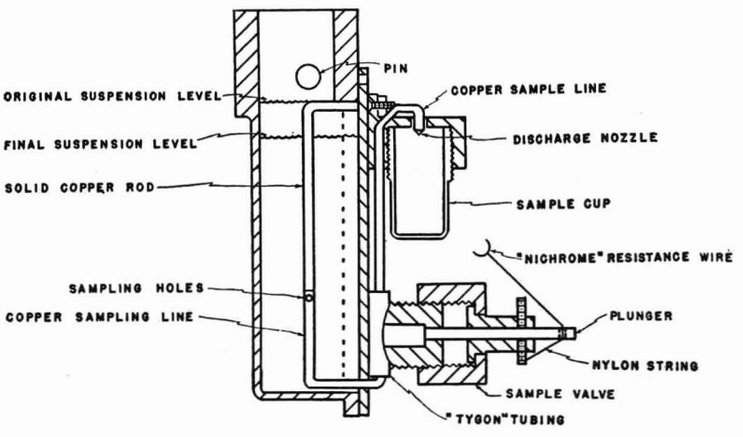

Figure 3. Sampling System of Sector-Shaped Centrifuge Tube

opened, suspension flowed through it until the liquid surface inside the flask reached the level of the orifice on the end of the sample line outside the flask. This was so located that the volume of sample withdrawn was about 10 ml.

The initial suspension surface was at a radius of rotation of 3.5 inches, and the sampling point (the two $1/32$-inch holes near the inner end of the sampling line) at a radius of 6.5 inches.

None of the dimensions given above are critical. The particular values used were based on the following considerations

The suspension used for analysis must be sufficiently dilute to allow the particles to disperse and settle without interfering with one another, yet sufficiently concentrated to allow accurate gravimetric determination of the solids content. A suspension of 1% concentration by weight was employed to meet these conditions. The size of sample withdrawn was then dictated by the precision desired in the results. With a 10-ml. sample and an ordinary analytical balance for weighing the dried solids, the solids concentration can be determined with an accuracy of 0.2% of the original concentration. The sample volume in turn determined in a general way the minimum dimensions of the suspension flask, which had to be large enough to prevent the walls and the surface of the suspension from interfering with the sample. The ratio of the distance of the sampling point from the center of rotation to the distance of the suspension surface from the center of rotation was selected so that, over the range of particle sizes to which the method was applied, the duration of centrifugation fell in a convenient range.

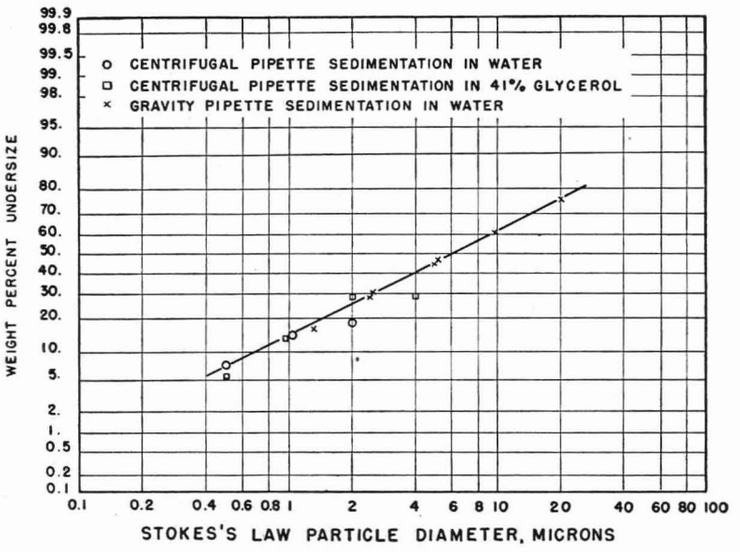

Figure 4. Particle-Size Distribution of Barytes Sample

The pipet flask described above had one disadvantage as compared with the Andreasen flask used for gravity pipet sedimentation—viz., only one pipet sample can be withdrawn during a centrifugation. An improved model of the apparatus is now being made, in which four flasks will be centrifuged simultaneously. This seems to be a simpler approach than to design a single flask from which multiple samples might be taken during a centrifugation. There are, of course, many other methods besides the one described above by which sampling of the suspension might be accomplished during centrifugation.

PROCEDURE

The procedure for making a particle size analysis with this apparatus was as follows:

An adequate supply (about 1 liter) of a 1% by weight suspension of the powder to be analyzed was prepared, using wetting and dispersing agents and mechanical agitation as required to assure essentially complete dispersion of the particles. Of this suspension, 150 ml. were poured into the flask with the valve closed. The flask was mounted in the centrifuge and revolved at a suitable speed and for a suitable length of time, as determined by the particle size of interest and the density of the particles (see discussion of theory, below). At the end of this predetermined interval, a pulse of current was sent through the Nichrome wire, causing the valve to open and an approximately 10-ml. sample to flow into the sample cup, as described above. Because of the orifice in the end of the sampling line, the withdrawal of the sample required about 8 to 16 seconds (depending on the speed of rotation), so that very little disturbance was wrought in the suspension around the sampling point.

After sufficient time had been allowed for withdrawal of sample, the centrifuge was stopped. As the flask was free to swing vertically, it assumed its natural position of balance between gravity and centrifugal force as it decelerated, and no liquid spilled from the flask or sample cup during stopping. The sample in the cup was weighed and transferred to a crucible, from which the water was evaporated, and the residual solids were weighed.

The procedure was repeated with additional batches of suspension, at other speeds and centrifuging times selected so that the particle diameters spanned the size range of interest, within the limitations of the apparatus.

To obtain the particle size distribution it was necessary to calculate for each withdrawn sample the limiting particle diameter (see Theory) and the sample concentration (weight of solids per weight of sample). The sample concentration was then expressed as a percentage of the concentration of the original uniform suspension, and this ratio was plotted against the corresponding limiting particle diameter on logarithmic-normal paper (*2*). A smooth curve was drawn through the points and the fractional concentrations were read from this curve for a set of particle diameters chosen in a $\sqrt{2}$ sequence. These concentrations were substituted in Equation 29 to give the required values of the cumulative weight percentage of the powder smaller than each particle diameter. This is discussed at more length under Theory.

RESULTS

The particle size distribution of a sample of pulverized barytes is shown in Figure 4. This distribution was determined by gravity and centrifugal pipet sedimentation, in the latter case

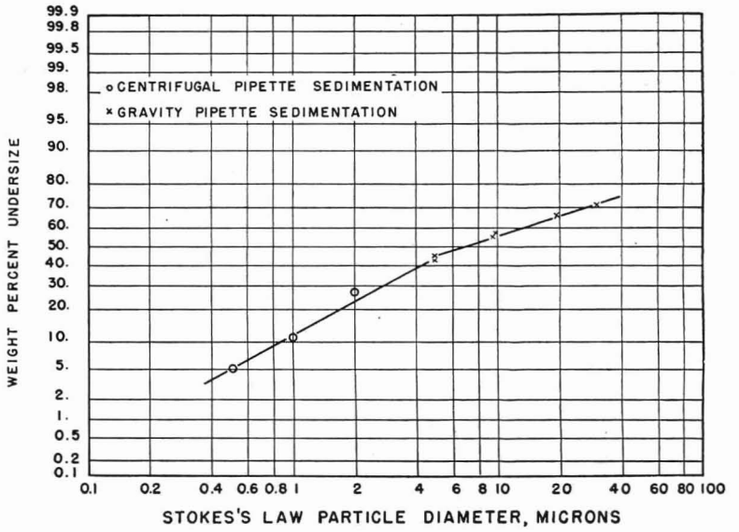

Figure 5. Size Distribution of Crude Dye Sample

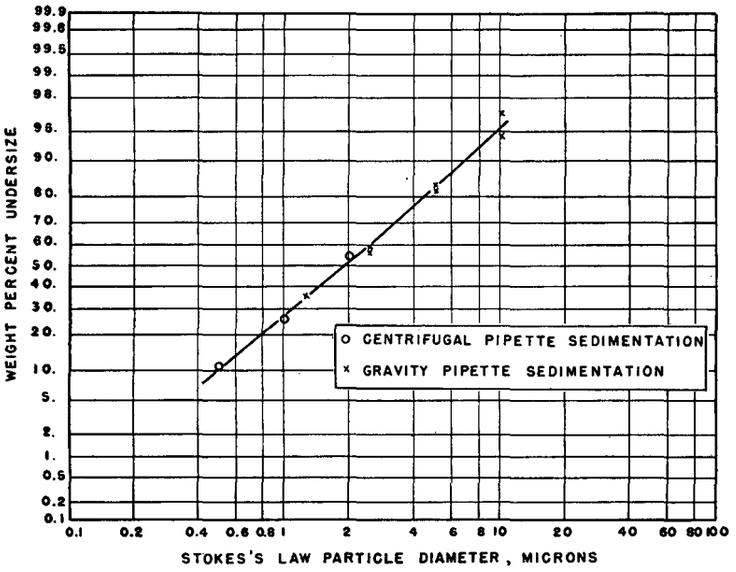

Figure 6. Particle-Size Distribution of Talc Sample

both in water and in 41% aqueous glycerol (which has a viscosity four times that of water). The cumulative distribution is a straight line on logarithmic-normal paper, and the points found by centrifugal sedimentation fall on the line determined by gravity sedimentation. An exception is that the point for the largest size determined centrifugally falls below the line, both for settling in water and for settling in 41% glycerol. The cause of these deviations is not yet known. They may be due to the fact that for the largest particle size the centrifuging time and speed are both very low, to the nature of the approximate method used for calculating the size distribution, or to some other cause.

Other size distributions by gravity and centrifugal pipet sedimentation analysis are shown in Figures 5 and 6. In general, the two methods give consistent results, and, because independent checks have shown the gravity sedimentation method to be accurate, this agreement attests to the validity of the centrifugal sedimentation results. These results do not, of course, prove the correctness of the method. This would require measurements of particle-size distributions over the size range of the centrifugal method, by some independent method.

The powder whose size distribution is shown in Figure 5 is a crystalline organic compound whose particles are acicular. The agreement between the gravity and centrifugal sedimentation analyses in this case, in spite of the marked deviation from particle sphericity, is possible because in both methods the particle diameter is defined in terms of the same property of the particles—i.e., their settling velocity—so that particle shape plays the same role in both methods. Such good agreement could not be expected if a comparison were made, say, between a sedimentation analysis and a microscopic count. Similarly, in Figure 6, good agreement is obtained between gravity and centrifugal sedimentation analyses of talc, whose particles are thin flakes.

THEORY

The general principle on which the method is based is that, when an initially well-mixed dilute suspension of a powder in a liquid is allowed to settle, the particles move with velocities determined by their sizes in accordance with Stokes' law, and that, consequently, the concentration of solids at any point within the suspension at any time thereafter is a function of the particle size distribution.

As is usual in the theory of particle size analysis, equations are derived based on the assumption that the particles are spheres, and the diameter of a nonspherical particle then is defined as the diameter of a sphere (of the same density) which behaves in the same way, specifically, in the present case, as the diameter of a sphere which settles at the same velocity as the particle in question, when in the same environment.

Consider first a single spherical particle of diameter y which is immersed in a liquid that is rotating as a rigid body at constant angular velocity about a fixed axis which is external to the liquid. The particle will be assumed to be small enough so that Stokes' law holds for its motion. The condition for this is that

$$yv'\rho_f/\mu < 1 \tag{1}$$

It will be assumed that the "centrifugal force" is great enough to make the effect of gravity on the sphere negligible:

$$\omega^2 r/g \gg 1 \tag{2}$$

Then the sphere can be considered as being acted on by only two forces, the liquid drag and the liquid buoyancy, and it will move in a plane perpendicular to the axis of rotation. The liquid drag is given by Stokes' law:

$$\mathbf{R} = -3\pi y\mathbf{v}'\mu \tag{3}$$

and the buoyancy is taken care of by using the "effective mass" of the sphere, as follows

$$m = \pi y^3(\rho_s - \rho_f)/6 \tag{4}$$

The acceleration of the particle is, therefore:

$$\frac{d^2\mathbf{r}}{dt^2} = \mathbf{R}/m = -2z\mathbf{v}' \tag{5}$$

where

$$z = 9\mu/y^2(\rho_s - \rho_f) \tag{6}$$

Equation 5 represents the vector differential equation of motion of the particle, from which two simultaneous scalar equations can be obtained by resolving the vectors into their radial and tangential components. Thus, for example, if $\mathbf{r}_1$ is a unit vector directed along the radius vector of the particle, and $\boldsymbol{\theta}_1$ a unit vector orthogonal to $\mathbf{r}_1$ (in the plane of motion) and in the direction of rotation of the liquid, then

$$\mathbf{r} = r\mathbf{r}_1$$

so

$$\mathbf{v} = \frac{d\mathbf{r}}{dt} = \frac{dr}{dt}\mathbf{r}_1 + r\frac{d\mathbf{r}_1}{dt} = \frac{dr}{dt}\mathbf{r}_1 + r\omega\boldsymbol{\theta}_1$$

and

$$\mathbf{a} = \frac{d\mathbf{v}}{dt} = \frac{d^2r}{dt^2}\mathbf{r}_1 + 2\frac{dr}{dt}\omega\boldsymbol{\theta}_1 + r\frac{d\omega}{dt}\boldsymbol{\theta}_1 - r\omega^2\mathbf{r}_1 \tag{7}$$

also

$$\mathbf{v}_f = r\omega_f\boldsymbol{\theta}_1$$

so:

$$\mathbf{v}' = \mathbf{v} - \mathbf{v}_f = \frac{dr}{dt}\mathbf{r}_1 + r(\omega - \omega_f)\boldsymbol{\theta}_1 \tag{8}$$

So, substituting Equations 7 and 8 in Equation 5 there results:

$$\frac{d^2r}{dt^2}\mathbf{r}_1 + 2\frac{dr}{dt}\omega\boldsymbol{\theta}_1 + r\frac{d\omega}{dt}\boldsymbol{\theta}_1 - r\omega^2\mathbf{r}_1 + 2z\frac{dr}{dt}\mathbf{r}_1 + 2zr(\omega - \omega_f)\boldsymbol{\theta}_1 = 0$$

And, because $\mathbf{r}_1$ and $\boldsymbol{\theta}_1$ are independent vectors:

$$\begin{cases} \dfrac{d^2r}{dt^2} + 2z\dfrac{dr}{dt} - \omega^2 r = 0 & (9) \\ r\dfrac{d\omega}{dt} + 2\omega\dfrac{dr}{dt} + 2z\omega r - 2z\omega_f r = 0 & (10) \end{cases}$$

Because of the assumed inequalities 1 and 2, the solution to Equation 9 is given to an extremely close approximation by the equation

$$2z\frac{dr}{dt} - \omega_f^2 r = 0 \qquad (11)$$

for the radial motion of the sphere. Integration of this equation gives

$$\frac{r}{r_i} = e^{y^2(\rho_s - \rho_f)\omega_f^2 t/18\mu} \qquad (12)$$

where r_i is the radius of rotation of the particle at zero time. It can also be shown, on the same grounds, that the tangential drift of the sphere relative to the liquid, the so-called Coriolis motion, is negligible, so that

$$\omega = \omega_f \qquad (13)$$

Consider next a uniformly dispersed suspension of spheres of diameter y and assume that the suspension is sufficiently dilute so that the particles move independently of one another, in accordance with Equation 12. Let r_o be the radius of rotation of the free surface of the liquid. Then it is clear from Equation 12 that after a centrifugation time t there will be a suspension-liquid interface at a radius of rotation

$$r_s = r_o e^{y^2(\rho_s - \rho_f)\omega_f^2 t/18\mu} \qquad (14)$$

There will, of course, be some diffusion of particles across this interface, due to their Brownian motion, but this is a negligible effect for the conditions of centrifugation employed.

To determine the concentration of particles in suspension below the level $r = r_s$ a procedure is used similar to that employed by Brown (*1*). Referring to Figure 7, consider a thin cylindrical shell of suspension of thickness Δr at a radius of rotation, r. Evidently the particles in this region were initially contained in a cylindrical shell at some radius of rotation r_i, of thickness Δr_i, where, according to Equation 12

$$r_i = re^{-y^2(\rho_s - \rho_f)\omega_f^2 t/18\mu}$$

$$r_i + \Delta r_i = (r + \Delta r)e^{-y^2(\rho_s - \rho_f)\omega_f^2 t/18\mu}$$

Consequently, the ratio of the concentration of particles at the radius of rotation r to the initial uniform concentration is equal to the ratio of the volume of the cylindrical shell at r_i of thickness Δr_i to the volume of the cylindrical shell at r of thickness Δr:

$$c = \frac{(r_i + \Delta r_i)^2 - r_i^2}{(r + \Delta r)^2 - r^2} = e^{-2y^2(\rho_s - \rho_f)\omega_f^2 t/18\mu};\ r \geq r_s$$

$$c = 0 \qquad r < r_s \qquad (15)$$

where r_s is given by Equation 14.

Consider now a polydisperse suspension of particles such that the weight fraction of particles smaller than diameter y is $F(y)$. Then, from Equation 15, and in view of the assumed independent settling of individual particles, there results for the fractional concentration of particles at a radius of rotation r:

$$c = \int_o^D e^{-2y^2(\rho_s - \rho_f)\omega_f^2 t/18\mu} f(y)dy \qquad (16)$$

where

$$f(y) = dF/dy \qquad (17)$$

and

$$D = \left[\frac{18\mu \ln (r/r_o)}{(\rho_s - \rho_f)\omega_f^2 t}\right]^{1/2} \qquad (18)$$

Equation 16 gives the fractional concentration of suspended solids at any radius of rotation r, and at any time of centrifugation t. D, defined by Equation 18, is the diameter of a sphere which settles from the surface of the suspension (radius r_o) to the radius of rotation r, during the centrifugation time, t. It therefore is the largest particle diameter which is present in the suspension at the radius of rotation, r, and is called the "limiting particle diameter."

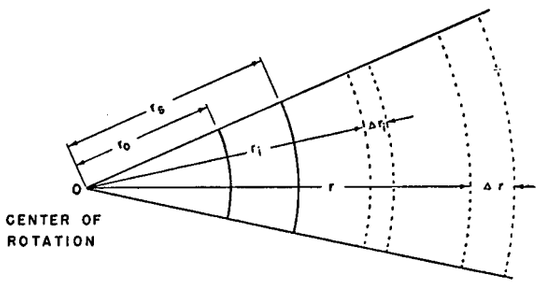

Figure 7. Diagram for Calculating Concentration of Particles in Centrifuge Flask

Equation 16 can be regarded as an integral equation expressing the concentration of suspension, which is measurable, in terms of the particle size distribution, which it is desired to find. There are two cases of interest. In the first, the concentration is measured as a function of the ratio, r/r_o, with all other variables (ρ_f, μ, ω_f; t) held constant. In this case, Equation 16 may be written in the form:

$$c = \int_o^D e^{-ky^2} f(y)dy \qquad (19)$$

where

$$k = 2(\rho_s - \rho_f)\omega_f^2 t/18\mu$$

is constant. In the second case, concentration is measured as a function of $\omega_f^2 t$, and r/r_o is held constant. In this case, Equation 16 may be written in the form:

$$c = \int_o^D e^{\frac{y^2}{D^2}\ln (r_o/r)^2} f(y)dy \qquad (20)$$

It is this case which has been employed in the centrifugal pipet sedimentation method described above. This case is called the "variable time" method of centrifugal sedimentation, while the first case is called the "variable height" method. In the variable time method, the variables μ, ρ_f, and ω_f which

enter into the definition of D could also be used, in principle. In practice, only ω_f and t are varied, for obvious reasons.

The use of the variable time method of centrifugal pipet sedimentation requires a solution to Equation 20. A formal mathematical solution is possible (*3*), but has no practical utility. A practical approximate solution may be derived, however, which depends on the solution to the variable height method, formulated in Equation 19. Equation 19, fortunately, has a simple exact solution, which will now be derived. Differentiating Equation 19 with respect to D, and in view of Equations 17 and 18:

$$\frac{dc}{dD} = e^{-kD^2} f(D) = \frac{r_o^2}{r^2}\frac{dF}{dD}$$

This equation is subject to the boundary conditions:

$$\left.\begin{aligned} c &= 1 \text{ when } t = 0 \text{ for all } r \\ c &= 0 \text{ when } r = r_o \text{ for } t > 0 \end{aligned}\right\} \qquad (21)$$

and the condition

$$F = 0 \text{ when } D = 0 \qquad (22)$$

Consequently,

$$F(D) = \int_0^c \left(\frac{r}{r_o}\right)^2 dc \qquad (23)$$

is the solution to Equation 19. With this solution it is now possible to derive an approximate solution to Equation 20. Referring to Figure 8, if c is plotted as a function of $s = r^2/r_o^2$ with $t' = \omega_f^2 t$ as parameter, a family of curves is obtained whose shapes depend on the particle size distribution function, f. In view of conditions 21, however, all the curves except that for $t' = 0$ will pass through the point $c = 0$, $s = 1$, and they will all be asymptotic to the curve for $t' = 0$, which has the equation $c = 1$. Furthermore, by Equation 23, the area under any curve is equal to $F(D)$.

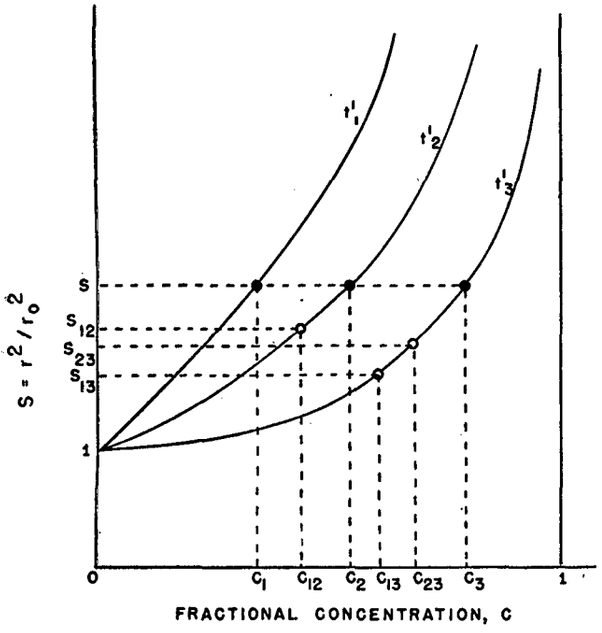

Figure 8. Typical Sedimentation Curves

Suppose, then, that at a fixed known value of s a set of measurements of c have been made, say $c_1, c_2, \ldots c_n$, for each of a set of known values of $\omega_f^2 t = t'$, say $t_1', t_2', \ldots t_n'$, and let $t_1' > t_2' > t_3' \ldots$. Then one point is known on each curve in Figure 8, in addition to the common point $s = 1$, $c = 0$. Such a set of points is illustrated by the black circles in Figure 8. To each such point corresponds a known value D_i, obtained by substituting the constant value of $\sqrt{s} = r/r_o$ and the value of $t_i' = \omega_f^2 t$ corresponding to the point into Equation 18. Furthermore, the area included between each curve, the concentration axis, and the ordinates $c = 0$ and $c = c_i$, is equal to the value of $F(D_i)$ or, for short, F_i. Thus $F(D)$ can be approximated by the trapezoidal rule.

For, first of all, approximately:

$$F_1 = \frac{1}{2}(1 + s)c_1$$

by integrating under the curve for t_1'. Now, considering the curve for t_2', a point can be found on it corresponding to D_1—i.e., a point such that the area under the curve up to this point is F_1, which is now known. For if the ordinate of this point is called s_{12}, and the abscissa c_{12}, then by Equation 18

$$D_1 = \left[\frac{9\mu \ln s_{12}}{(\rho_s - \rho_f)t_2'}\right]^{1/2}$$

and since

$$D_2 = \left[\frac{9\mu \ln s}{(\rho_s - \rho_f)t_2'}\right]^{1/2}$$

we have

$$s_{12} = s^{(D_1/D_2)}$$

Then

$$c_{12} = \frac{2F_1}{1 + s_{12}} = \frac{1 + s}{1 + s_{12}}c_1$$

Now having found the coordinates s_{12} and c_{12} of the point on the curve for t_2' corresponding to D_1, there results:

$$F_2 - F_1 = \frac{1}{2}(s + s_{12})(c_2 - c_{12})$$

Proceeding in this manner, thus in effect approximating the first curve by one chord, the second by two, and so on, a general formula for F_n can now be written down as follows:

$$F_n - F_{n-1} = \frac{1}{2}(s + s_{n-1,\,n})(c_n - c_{n-1,\,n}) \qquad (24)$$

where

$$s_{ij} = s^{(D_i/D_j)^2} \qquad (25)$$

$$c_{n-1,\,n} = c_{n-2,\,n} + \frac{2(F_{n-1} - F_{n-2})}{s_{n-2,\,n} + s_{n-1,\,n}} \qquad (26)$$

$$D_n = \sqrt{9\mu \ln s/(\rho_s - \rho_f)t_n'} \qquad (27)$$

By using Equations 26 to eliminate the c_{ij} from Equations 24, there results:

$$F_i = \frac{1}{2}(s + s_{i-1,\,i})c_i + \sum_{j=1}^{i-1}\left[\frac{s + s_{i-1,\,i}}{s_{j+1,\,i} + s_{j,\,i}} - \frac{s + s_{i-1,\,i}}{s_{j,\,i} + s_{j-1,\,i}}\right]F_j \qquad (28)$$

$$i = 1, 2, \ldots n$$

where $D_o = 0$. Equation 28 is a general approximate solution to Equation 20, in recursive form.

Equations 28 are a set of linear equations which express the desired values of F_i explicitly in terms of the measured values of c_i. The coefficients in the equations depend on the values of D_i (corresponding to the values of t_i') at which the concentrations c_i are measured; more exactly, the coefficients depend on the ratios of the values of D_i, as shown by Equation 25. Consequently, if the values of D_i are chosen in a geometric sequence, the coefficients of Equations 28 are considerably easier to calculate and the equations themselves are also simplified. It is usual and desirable, for other reasons also, to choose particle sizes in a geometric sequence when making particle size analyses or reporting particle size distributions. Usually a sequence with common ratio $\sqrt{2}$ is used.

The coefficients in Equations 28 depend also on the value of $s = r^2/r_o^2$—that is, on the dimensions of the centrifuge flask employed. For the flask described above, $s = 3.44$ and Equations 28 assume the form (for sizes in a $\sqrt{2}$ sequence):

$$F_i = 2.65c_i - 1.71c_{i-1} - 0.08c_{i-2} + 0.12c_{i-3} + 0.03c_{i-4} \quad (29)$$
$$i \geq 5$$

the subsequent terms being negligible. For the first four values of i, the equations are slightly altered, as follows:

$$\left.\begin{aligned} F_1 &= 2.22c_1 \\ F_2 &= 2.65c_2 - 1.90c_1 \\ F_3 &= 2.65c_3 - 1.71c_2 - 0.10c_1 \\ F_4 &= 2.65c_4 - 1.71c_3 - 0.08c_2 + 0.14c_1 \end{aligned}\right\} \quad (29a)$$

The method of using these equations to compute size distributions is as follows:

The experimental data consist of a series of fractional concentrations, c, measured at known values of ω_f and t, from which a value of D for each value of c is calculated from Equation 18. The values of c *vs.* D are plotted (usually on logarithmic-normal paper) and a smooth curve is drawn through the points. Values of c are read from this curve at any convenient set of values of D in a $\sqrt{2}$ sequence, D_1, $D_2 = \sqrt{2}D_1$, $D_3 = 2D_1$, etc. The corresponding values of c are called c_1, c_2, . . . c_n. These values are substituted in Equation 29 to give the values of F_1, F_2, . . . F_n. The values of F_1, F_2, F_3, and F_4 can be found either by using Equations 29a or by extrapolating the curve of c *vs.* D to get additional points to use in Equation 29.

NOMENCLATURE

Any consistent units may be employed.

- $\mathbf{a}$ = acceleration of a particle in a centrifugal field, (cm./second)/second.
- c = concentration of suspended solids, as a fraction of the concentration of uniform suspension prior to settling, dimensionless
- D = Stokes's law particle diameter of a particle which settles from a radius r_o to a radius r in time t, cm.
- $F = F(D)$ = fraction by weight of particles smaller than diameter D, in a powder, dimensionless
- f = dF/dD, 1/cm.
- g = acceleration due to gravity, (cm./second)/second
- m = effective mass of a sphere immersed in a liquid, grams
- $\mathbf{R}$ = Stokes's law drag force, dynes
- r = distance of particle from axis of rotation; also, specifically, distance of sampling point from axis of rotation, cm.
- r_o = distance of surface of suspension from axis of rotation, cm.
- r_i = initial distance of particle from axis of rotation (when t = 0), cm.
- $\mathbf{r}_1$ = unit vector in direction of radius vector of a particle, cm.
- s = $(r/r_o)^2$, dimensionless
- t = time, seconds
- t' = $\omega_f^2 t$, 1/second
- $\mathbf{v}$ = particle velocity, cm. per second
- $\mathbf{v}'$ = particle velocity relative to liquid medium, cm. per second
- $\mathbf{v}_f$ = velocity of liquid in neighborhood of particle, cm. per second
- y = Stokes's law particle diameter, cm.
- z = $9\mu/y^2(\rho_s - \rho_f)$, 1/second
- μ = liquid viscosity, poises
- ρ_f = liquid density, grams per cc.
- ρ_s = particle density, grams per cc.
- θ_1 = unit vector orthogonal to $\mathbf{r}_1$, cm.
- ω_f = angular velocity of rotation of liquid, radians per second
- ω = angular velocity of rotation of particle, radians per second

Letters in bold face represent vector quantities.

LITERATURE CITED

(1) Brown, C., *J. Phys. Chem.*, 48, 246–58 (1944).
(2) Codex Book Co., Norwood, Mass., Coordinate Paper No. 32376, "Logarithmic-normal."
(3) Lovitt, W. V., "Linear Integral Equations," New York, Dover Publications, 1950.
(4) Martin, S. W., Symposium on New Methods for Particle Size Determination in the Subsieve Range, pp. 66–89, Am. Soc. Testing Materials, March 4, 1941.
(5) Robison, H. E., and Martin, S. W., *J. Phys. Chem.*, 52, 854–81 (1948).

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Particle-Size Determination in Radioactive Aerosols by Radioautograph

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A RADIOAUTOGRAPH technique has been used to study particle-size distributions in aerosols of an alpha-emitting compound. The active material was collected on filter paper and placed in contact with nuclear track plates for various exposure times. By counting the number of tracks in the emulsion for a given exposure time, the size of each emitting particle was calculated from the formula:

$$d = \left(\frac{KC}{t}\right)^{1/3}$$

where C = number of tracks in emulsion from particle of diameter d microns, t = autograph exposure time, and K = constant.

Particles as small as 0.2 micron have been accurately determined in aerosols containing as little as 0.8 $\mu\mu$g. of active material per liter of air.

DERIVATION OF EQUATION

For a sphere of diameter d microns containing N_o atoms of an alpha-emitter whose decay constant is λ per minute, collected as

One of the problems encountered in the study of radioactive aerosols is the low abundance of these particles relative to atmospheric dust in the sample. Most methods of particle size analysis do not make any distinction between the radioactive and the inert particle. Therefore, a new method of discriminating and measuring alpha-emitting radioactive particles was developed. A filter paper sample of an alpha-ray-emitting aerosol was placed in contact with nuclear track plates for various exposure times. By counting the number of tracks in the emulsion for a given exposure time, the size of each emitting particle was calculated from the derived equation

$$d = \left(\frac{KC}{t}\right)^{1/3}$$

where d = particle size in microns, C = the number of tracks in the emulsion emitted from the particle, t = exposure time, and K is a constant for a given radioactive material. Although the method is not limited to aerosols, it is particularly useful in health-physics studies where the permissible air concentration of alpha-emitting particles in the size range from 0.1 to 10 microns is extremely low.

a compound having density ρ and molecular weight M, the equation is:

$$(dN) = \lambda N_o(dt)$$

$$= \lambda \frac{WfA}{M}(dt)$$

$$= \lambda \frac{(V\rho)fA}{M}(dt)$$

$$= \lambda \frac{\pi d^3 \rho f A}{6M}(dt) \times 10^{-12}$$

where dN = number of atoms disintegrating in time dt, W = weight of particle, grams, A = Avogadro's number, V = volume of particle, cc., and f = number of radioactive atoms per molecule of compound.

$$(dN) = kd^3(dt)$$

where k is a constant for a given compound

$$k = \frac{\lambda f A \rho \pi 10^{-12}}{6M}$$

Assuming no significant change in the value of N_o during exposure, and a 50% geometry for intimate contact between emulsion and sample, the number of tracks, C, will be:

$$\frac{\Delta N}{2} = C = \frac{kd^3}{2}t$$

or

$$d = \left(\frac{KC}{t}\right)^{1/3} \quad (1)$$

where t = exposure time in minutes and

$$K = 6.32 \times 10^{-12}\left(\frac{M}{\lambda \rho f}\right)$$

As the diameter is a cube-root function of both exposure time and number of tracks, the method is precise.

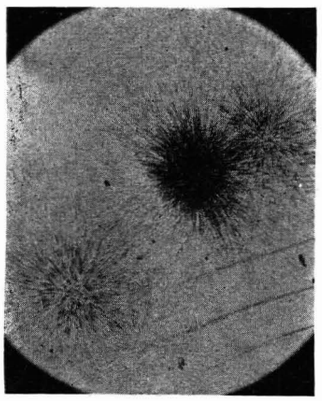


Figure 1. Typical Group (100×)
24-hour exposure

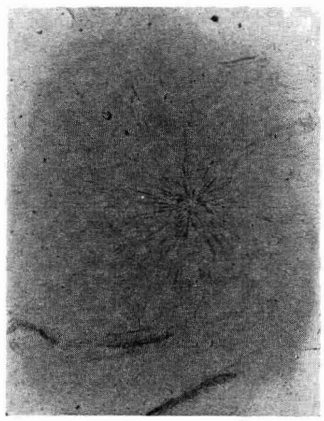


Figure 2. 0.9-Micron Particle (125×)
24-hour exposure

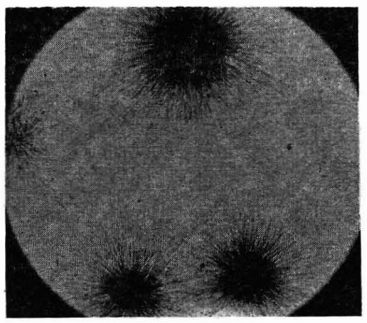


Figure 3. Orientation Group (110×)
24-hour exposure

GENERAL METHOD

The slide or filter paper sample on which the material has been collected is placed in intimate contact with a nuclear track plate (Kodak, Type NTA, 25 microns) and exposed for the desired time in a simple "camera." After development for 2.5 minutes in Kodak developer D-19, the emulsion is fixed in acid hypo, washed, and dried. The plate is then scanned microscopically at a magnification of about 200.

Figure 1 shows a typical group of clusters which can be resolved by decreasing the autograph exposure time. If a cluster remains too dark to count on the second exposure, a third shorter exposure is made, etc. By this method of varying exposures, each cluster may be reduced to a countable number of tracks (approximately 10 to 100). A cluster containing the maximum number of tracks for counting is shown in Figure 2. For such large clusters, only one quadrant need actually be counted.

If the concentration of particles on the sample is high enough, it is necessary to count only a small representative area. However, a minimum of 200 particles should be counted per sample. In going from one exposure to another for a given sample, the same area can be located by the use of an orientation group such as that shown in Figures 3 and 4. The coordinates of this group are determined on the microscope stage and used as the center of the area to be scanned. In using a contaminated filter paper as the autographer, the group shown was not disturbed, although it was in intimate contact with each emulsion for six exposures.

For very short exposures, serious error will result if any active particles are transferred to the emulsion. However, there was no evidence of this in any autographs of filter paper deposits.

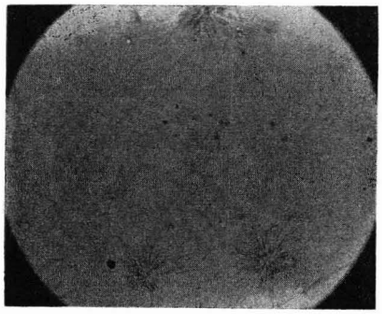


Figure 4. Orientation Group of Figure 3 (100×)
1-hour exposure

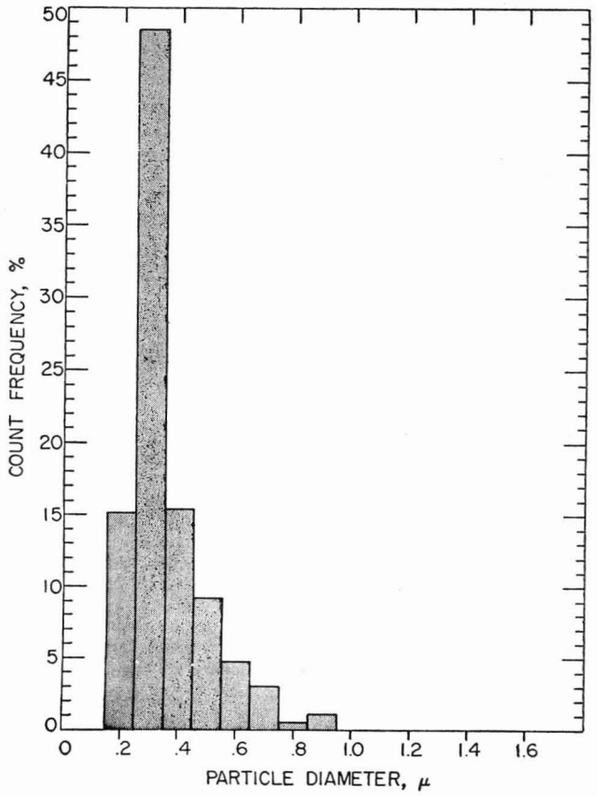


Figure 5. Particle Size Distribution on Surface of Filter Paper

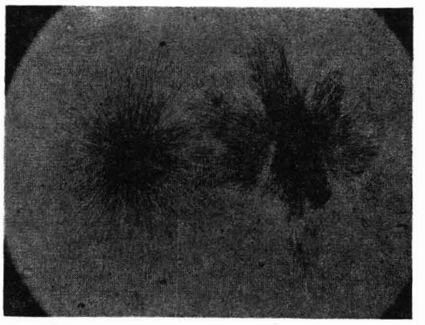
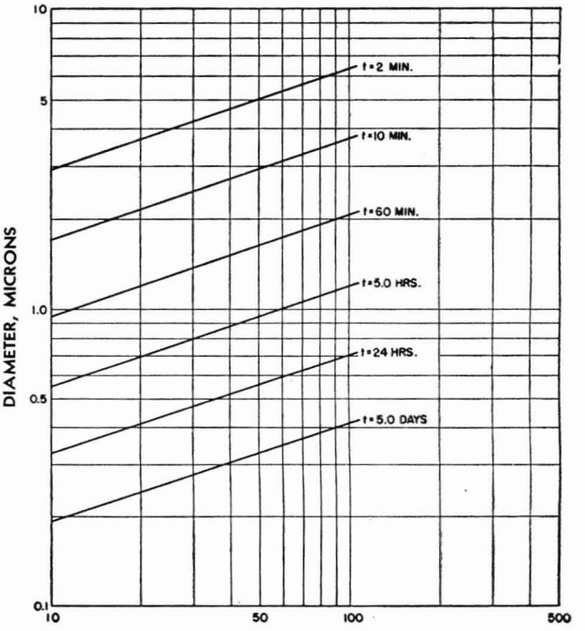


Figure 6. Large Particle and Agglomerate (100×)
24-hour exposure

From the number of particles in each size group in the area scanned, the number of particles on the total filter paper or slide sample can be computed from the ratio of total sample area to area scanned. This total abundance may then be converted to a concentration in the air stream from which the sample was taken.

DETERMINATION OF SIZE-FREQUENCY DISTRIBUTION ENTERING AND LEAVING PILOT PLANT

This method has been used to determine the size-frequency distribution of particles in the feed to and discharge from an air-decontamination pilot plant. Standard methods of determining particle size were inadequate, because the discharge concentration of radioactive material was about 0.8 μμg. per liter of air. The filter paper samples which had been used to determine removal efficiency were used as radioautograph sources. Figure 5 is a typical distribution of the effluent from the plant obtained by this technique.



C

Figure 7. Calibration Curve

As the alpha-particle will not penetrate any appreciable thickness of filter paper, the distributions reported are for the surface of each sample of filter paper. In Figure 5 there appears to be a decrease in the count frequency in going from 0.4 to 0.2 micron. This is probably due to penetration of the paper (Hollingsworth-Vose H-70) to a greater depth by the smaller particles.

The presence of agglomerates may also be detected by this method. Figure 6 shows a track cluster of an agglomerate consisting of radioactive and nonradioactive material. The latter stops the alpha-particles from reaching the emulsion, which results in a very irregular cluster pattern. The particle shown was greater than 5 microns, and is probably duct scale. Only two such particles were present in a total of about 1000 particles observed.

For the material used in these particular tests, the value of K in Equation 1 was 5.04. A calibration curve for the formula:

$$d = \left(\frac{5.04C}{t}\right)^{1/3}$$

was constructed to give the particle size as a function of the number of tracks for a given exposure time (Figure 7).

Cascade impactor samples of the feed to the system taken at a later date verified the results obtained by radioautograph. Analysis by the method of Hatch and Choate (*1*) indicated that 50% of the mass of sample was composed of particles less than 0.9 micron, while 98.6% of the mass was made up of particles less than 5 microns.

LIMITATIONS OF RADIOAUTOGRAPH METHOD

Obviously, the aerosol being studied must consist of radioactive particulates, as inert material is not detected. However, if inert material is also present, the method gives an analysis for this hazardous material only.

If there is a very great abundance of large particles, the finer particles will be obscured in the radioautograph unless the large particles can be removed and studied separately.

The isotope being studied (as well as interfering decay products) must be identified. In addition to standard electronic detection devices, the range and track appearance in the emulsion will help determine the radiation characteristics of the particle.

The method is feasible as a research tool on only a few samples, as counting tracks in hundreds of clusters is a tedious and expensive procedure. Projection of the field on a screen is helpful in counting individual tracks.

A simpler modification of this method would be to observe the entire cluster for each particle rather than individual tracks. By establishing a standard minimum cluster of about 300 tracks and making several exposures, the particles can be grouped by size range. A photodensitometer could be used to count such large clusters. However, this method is not as precise as counting individual tracks.

As the actual particles are never observed, this method does not reveal the true particle shape. An effective diameter for the particle is measured, which is based on the mass of each particle.

Gamma-emitters must be considered as a separate problem, because they do not produce tracks. By a previous exposure-density calibration, the size of the particle might be determined, but this possibility has not been investigated.

LITERATURE CITED

(1) Dalla Valle, J. M., "Micromeritics," New York, Pitman Publishing Co., 1943.

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Infrared Spectra of Phosphorus Compounds

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Application of infrared spectroscopy to analytical and structural problems in phosphorus chemistry has been limited by the lack of spectral data on reference compounds, and by the inadequacy of information concerning characteristic frequencies of molecular groups containing phosphorus. This information was sought through study of a large number of phosphorus compounds containing a variety of molecular groups of interest. Empirical correlation of sixty reference spectra yielded characteristic frequency ranges for a number of groups: P—H, 2350 to 2440 cm.$^{-1}$; P—F, 850 to 980 cm.$^{-1}$; P—Cl, 430 to 585 cm.$^{-1}$; P$^+$→O$^-$, 1170 to 1310 cm.$^{-1}$; P$^+$→S$^-$, 700 to 770 cm.$^{-1}$; P—C (aliphatic), 650 to 750 cm.$^{-1}$; phenyl-phosphorus group, near 1000 cm.$^{-1}$ and 1440 cm.$^{-1}$; P—O—H, 2550 to 2700 cm.$^{-1}$; P—O—C, 1030 to 1090 cm.$^{-1}$; phosphinic acids, near 1665 cm.$^{-1}$ The usefulness of these group frequencies for qualitative interpretation of spectra in terms of molecular structure is discussed and illustrated.

CONSIDERABLE work has been published on the Raman spectra of phosphorus compounds, but infrared data on only a few of the simpler compounds are found in the literature (*11*, *17*). A study more extensive than heretofore reported has therefore been made of the infrared spectral properties of phosphorus compounds. It has dealt mainly, but not entirely, with organophosphorus compounds. For this type of compound, in particular, very few data have been published.

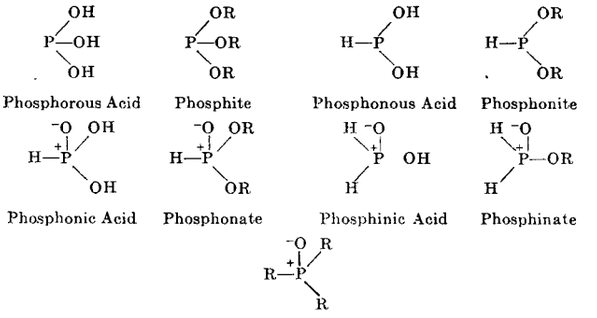
EXPERIMENTAL

Nomenclature and Materials. The nomenclature of phosphorus compounds is somewhat complex and at present in a state of flux. Accordingly, a few definitions will simplify discussion of materials and data. *Chemical Abstracts* has been followed wherever possible in the scheme of nomenclature presented in Table I.

The sources of materials are given in the legends of the sixty spectra (Figures 1 to 60). The samples were the best available, but in many cases some evidence of impurity was found in the spectra. Samples prepared at the Naval Research Laboratory were constant-boiling liquids or recrystallized solids of constant melting point.

Equipment and Measurements. A research-type, recording prism spectrometer (*13*) was used for all spectral measurements. Liquid samples were placed in amalgam-sealed cells; the thickness of the cell used is given on the spectral curve in millimeters. In those cases where solutions were used, the solvent is also noted. Solid samples were mortar-ground to a fine powder and mulled with a small amount of petrolatum to form a smooth paste, which was then placed between potassium bromide plates separated by a spacer and mounted in a suitable holder. The effect of petrolatum absorption was canceled out roughly by measuring the mulls relative to a petrolatum blank.

Table I. Nomenclature of Phosphorus Compounds



GROUP FREQUENCIES IN PHOSPHORUS COMPOUNDS

P—H and P—D bonds. In phosphine, the fundamental stretching vibrations of the P—H bonds appear at 2327 and 2421 cm.$^{-1}$, while those at 991 and 1121 cm.$^{-1}$ may be described approximately as bending motions of P—H bonds (*10*).

Seven compounds believed to contain a P—H bond have been studied, and, as summarized in Table II, all have an absorption band in the region 2350 to 2440 cm.$^{-1}$ Because other phosphorus compounds exhibit little or no absorption in this region, these bands may confidently be assigned to stretching vibrations of the P—H bond. Little can be said concerning the dependence of the P—H stretching vibrations within this 100 cm.$^{-1}$ range. All of the compounds studied have one P—H bond only, and none contained trivalent phosphorus.

Replacement of hydrogen by deuterium should produce an isotopic frequency shift of about 650 cm.$^{-1}$ for the stretching vibrations. Upon deuteration of benzenephosphinic acid (with heavy water) it was observed that the P—H absorption at 2381 cm.$^{-1}$ decreased in intensity while a new band, undoubtedly due to the P—D vibration, appeared at 1750 cm.$^{-1}$

Absorption due to the P—H bending vibration has not been identified in these compounds. It is evidently weak and spread over a greater spectral interval than the stretching vibration, facts which make it difficult to identify. It probably occurs in the region from 950 to 1150 cm.$^{-1}$, where it is masked by other intense bands not related to P—H bonds.

Table II. P—H Absorption Frequencies

Compound	Figure	Position, Cm.$^{-1}$	Position, μ
Diethyl phosphonate	7	2433	4.11
Di-*n*-butyl phosphonate	17	2410	4.15
Ethyl benzenephosphinate	5	2350	4.25
Benzenephosphinic acid	4	2381	4.20
p-Toluenephosphinic acid	9	2440	4.10
Naphthalenephosphinic acid	10	2391	4.18
p-Ethylbenzenephosphinic acid	11	2391	4.18

P—F Bond. The P—F stretching frequencies may be expected in the region from about 840 to 980 cm.$^{-1}$ by analogy with the positions of these frequencies in phosphorus trifluoride (*9*) and phosphoryl trifluoride (*6*), as listed in Table III. Three additional compounds of higher-valent phosphorus, all containing a single P—F bond, have strong bands between 850 and 900 cm.$^{-1}$ which are undoubtedly due to this vibration. Compounds of this

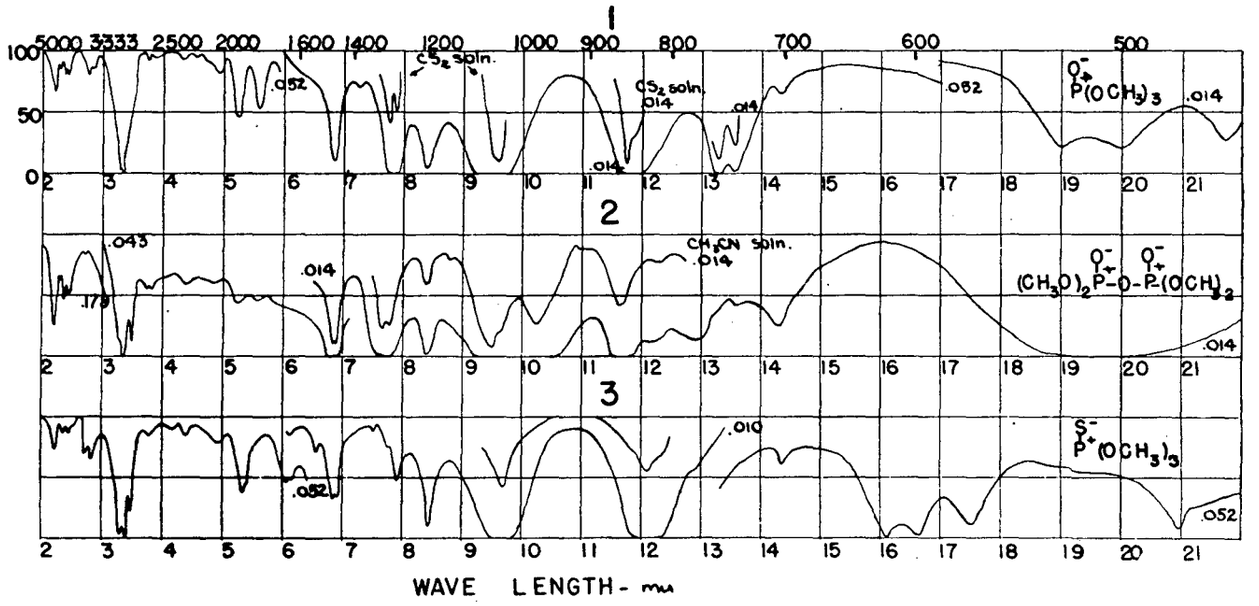


Figure 1. Trimethyl Phosphate (Naval Research Laboratory)
Figure 2. Tetramethyl Pyrophosphate (Victor Chemical Works)
Figure 3. Trimethyl Thionophosphate (University of Chicago Toxicological Laboratory)

Table III. P—F Stretching Frequencies

Compound	Formula	Position Cm.$^{-1}$	Position μ	Figure
Phosphorus trifluoride	PF_3	840, 890	11.9, 11.2	
Phosphoryl trifluoride	PCF_3	865, 980	11.7, 10.2	
Thiophosphoryl dichloride fluoride	$PSCl_2F$	900	11.1	40
Dimethyl fluorophosphate	$(CH_3O)_2POF$	860	11.4	59
Diethyl fluorophosphate	$(C_2H_5O)_2POF$	880	11.4	60
N,N-diethylaminophosphorus difluoride	$(C_2H_5)_2N—PF_2$	740, 800	13.5, 12.5	58

type, but with two P—F bonds, have not been studied, and may not always absorb within this region.

The spectrum of only one fluoride of trivalent phosphorus has been obtained (Table III), so that for this type of compound it is known only that P—F frequencies as low as 740 cm.$^{-1}$ may occur. Until additional fluorides of trivalent phosphorus and higher-valent phosphorus compounds containing the PF_2 groups are studied, our knowledge of P—F frequencies will remain inadequate.

P—Cl Bond. According to previous results (*6*, *10*) each of the inorganic compounds phosphorus trichloride, phosphoryl trichloride, and thiophosphoryl chloride has two frequencies around 500 cm.$^{-1}$ which correspond roughly to stretching motions (in phase and out of phase) of P—Cl bonds. These frequencies are listed in Table IV, together with the observed positions of bands believed to arise from P—Cl stretching in seven additional compounds. In each case one band is observed between 475 and 540 cm.$^{-1}$ For chlorides of trivalent phosphorus the bands fall in the region from 485 to 525 cm.$^{-1}$, while for the compounds of higher-valent phosphorus they occur between 430 and 585 cm.$^{-1}$ Those compounds containing two or more P—Cl bonds usually exhibit two bands within these regions.

Table IV. P—Cl Stretching Frequencies

Compound	Formula	Position Cm.$^{-1}$	Position μ	Figure
Phosphorus trichloride	PCl_3	488, 511	20.5, 19.6	
Phenyldichlorophosphine	$C_6H_5PCl_2$	500	20.0	28
Ethyldichlorophosphine	$C_2H_5PCl_2$	488, 502	20.5, 19.2	38
Phosphonitrilic chloride (trimer)	$(PNCl_2)_3$	488, 521	20.5, 19.2	47
Phosphoryl chloride	$POCl_3$	485, 581	20.6, 17.2	56
Phenyldichlorophosphine oxide	$C_6H_5POCl_2$	488, 572	20.5, 17.5	25
Diphenylchlorophosphine oxide	$(C_6H_5)_2POCl$	521	19.2	26
Thiophosphoryl chloride	$PSCl_3$	433, 538	23.1, 18.6	
Phenyldichlorophosphine sulfide	$C_6H_5PSCl_2$	500, 524	20.0, 19.1	27
Thiophosphoryl dichloride fluoride	$PSCl_2F$	476, 565	21.0, 17.7	40

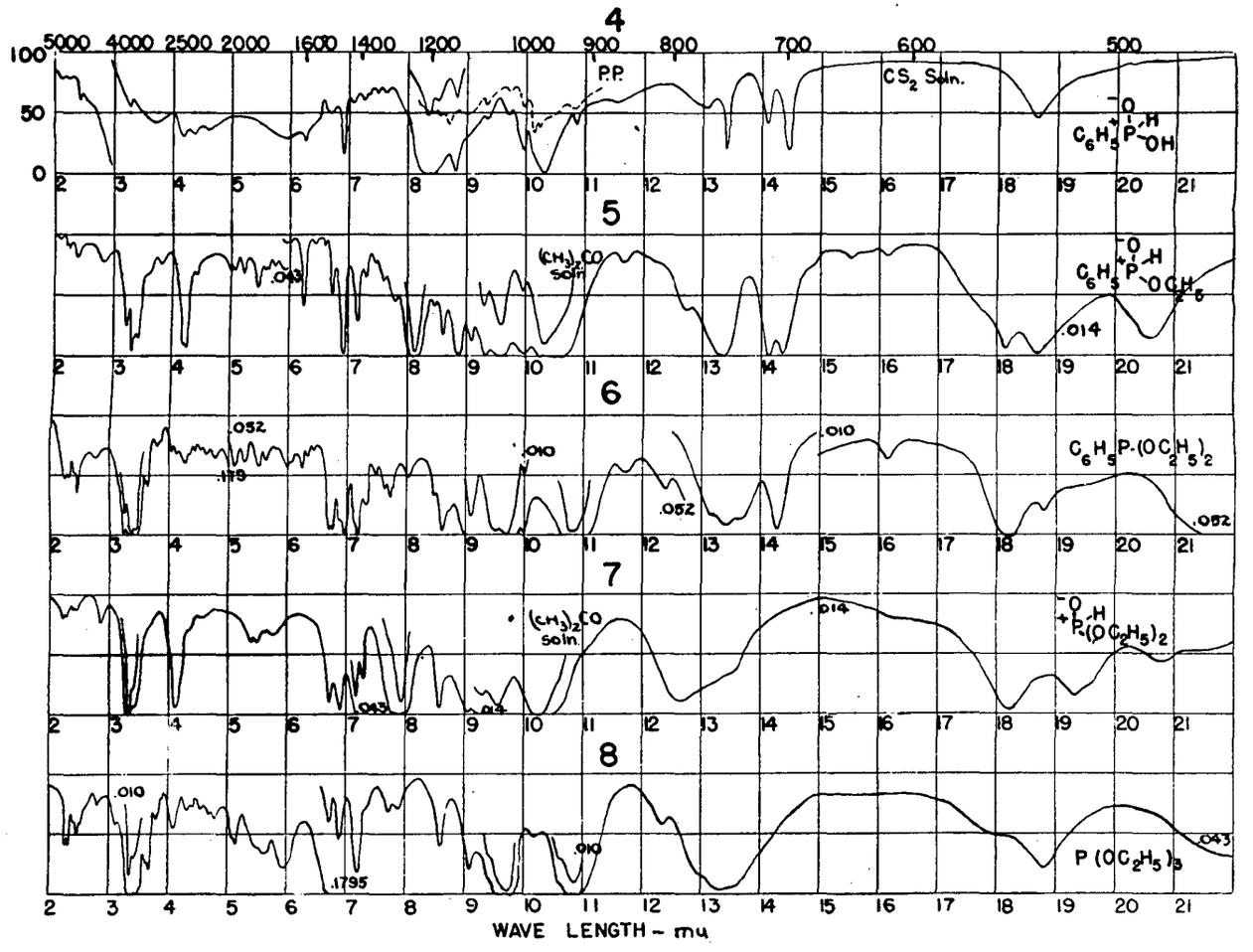


Figure 4. Benzenephosphinic Acid (Naval Research Laboratory)
Figure 5. Ethyl Benzenephosphinate (Naval Research Laboratory)
Figure 6. Diethyl Benzenephosphonite (Naval Research Laboratory)
Figure 7. Diethyl Phosphonate (Naval Research Laboratory)
Figure 8. Triethyl Phosphite (Monsanto Chemical Co.)

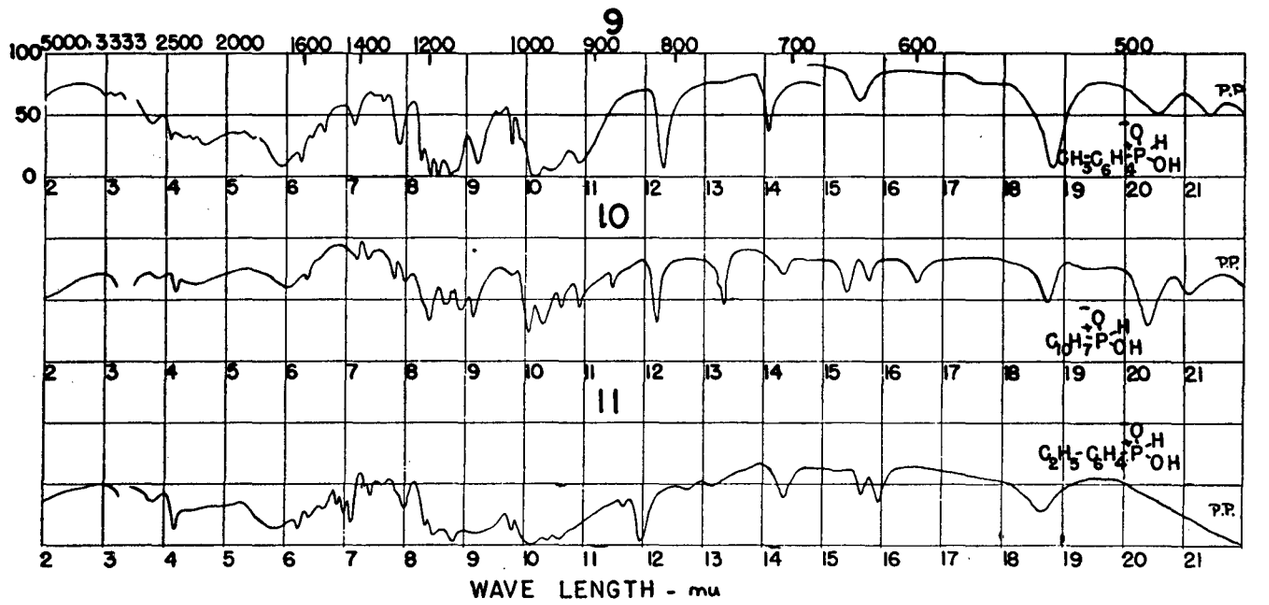


Figure 9. *p*-Toluenephosphinic Acid
Figure 10. Naphthalenephosphinic Acid
Figure 11. *p*-Ethylbenzenephosphinic Acid

Naval Research Laboratory

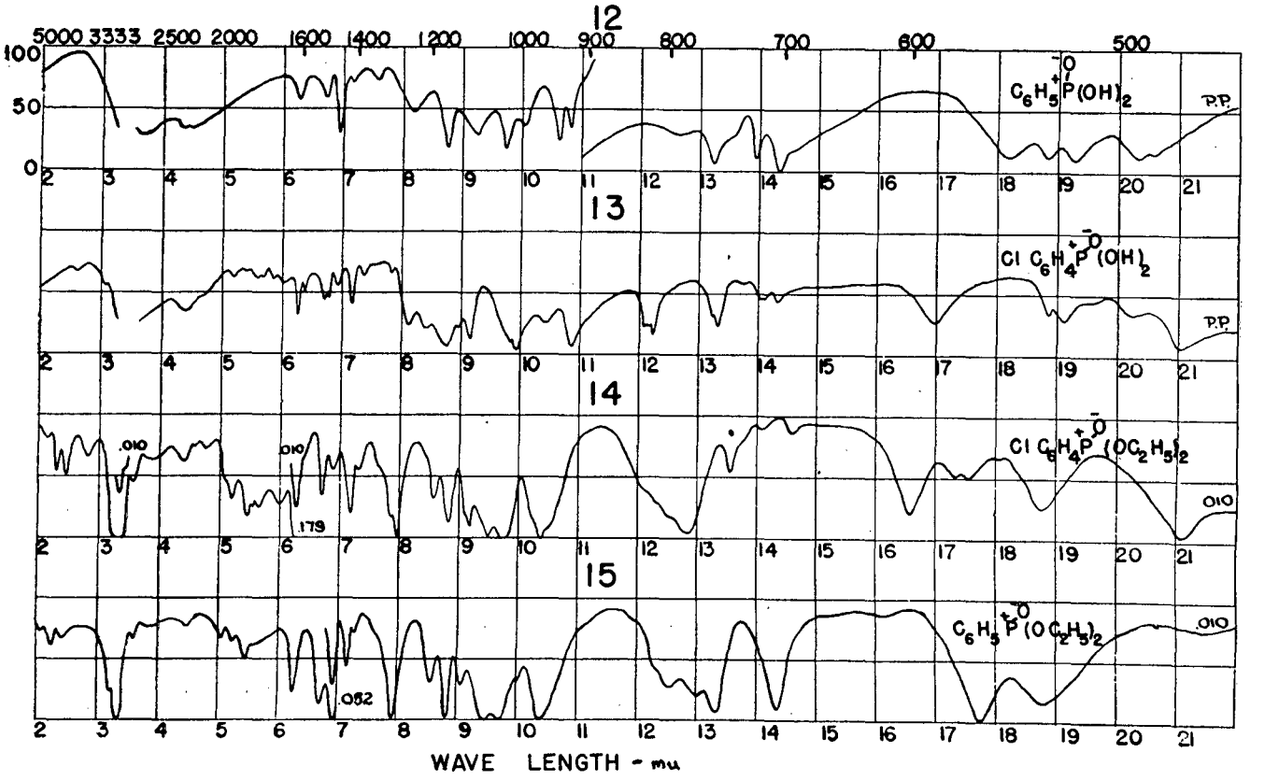


Figure 12. Benzenephosphonic Acid (Naval Research Laboratory)
Figure 13. *p*-Chlorobenzenephosphonic Acid (Monsanto Chemical Co.)
Figure 14. Diethyl *p*-Chlorobenzenephosphonic Acid (Monsanto Chemical Co.)
Figure 15. Diethyl Benzenephosphonate (Naval Research Laboratory)

Phosphoryl Group. When an oxygen atom is bonded only to a phosphorus atom, the resulting group, $\equiv(\overset{+}{P}—\overset{-}{O})$, will be called the phosphoryl group. All phosphoryl halides (OPX_3) exhibit a strong band near 1280 cm.$^{-1}$ due to the stretching vibration of this group (*6, 10*). An intense band in this same region, and having the same origin, has also been observed for other compounds containing the phosphoryl group, as listed in Table V. Compounds of trivalent phosphorus usually do not absorb strongly in this region.

Table V. Dependence of Phosphoryl Frequency upon Electronegativity of Substituents

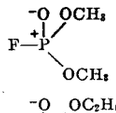
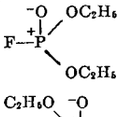
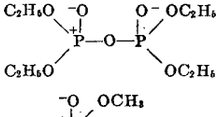
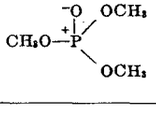
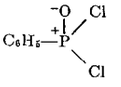
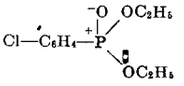
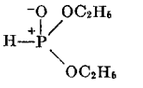
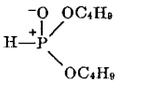
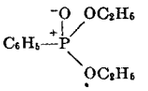
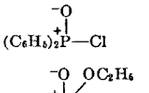
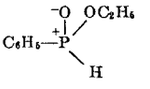
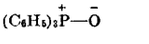
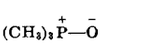
Compound	Figure	No. of Electronegative Substituents	Position Cm.$^{-1}$	Position μ
	59	3	1305	7.61
	60	3	1309	7.58
	30	3	1290	7.75
	1	3	1275	7.85

Table V (*Continued*)

	25	2	1275	7.85
	14	2	1265	7.90
	7	2	1265	7.90
	17	2	1265	7.90
	15	2	1257	7.95
	26	1	1236	8.10
	5	1	1236	8.10
	23	0	1190	8.40
	24	0	1176	8.50

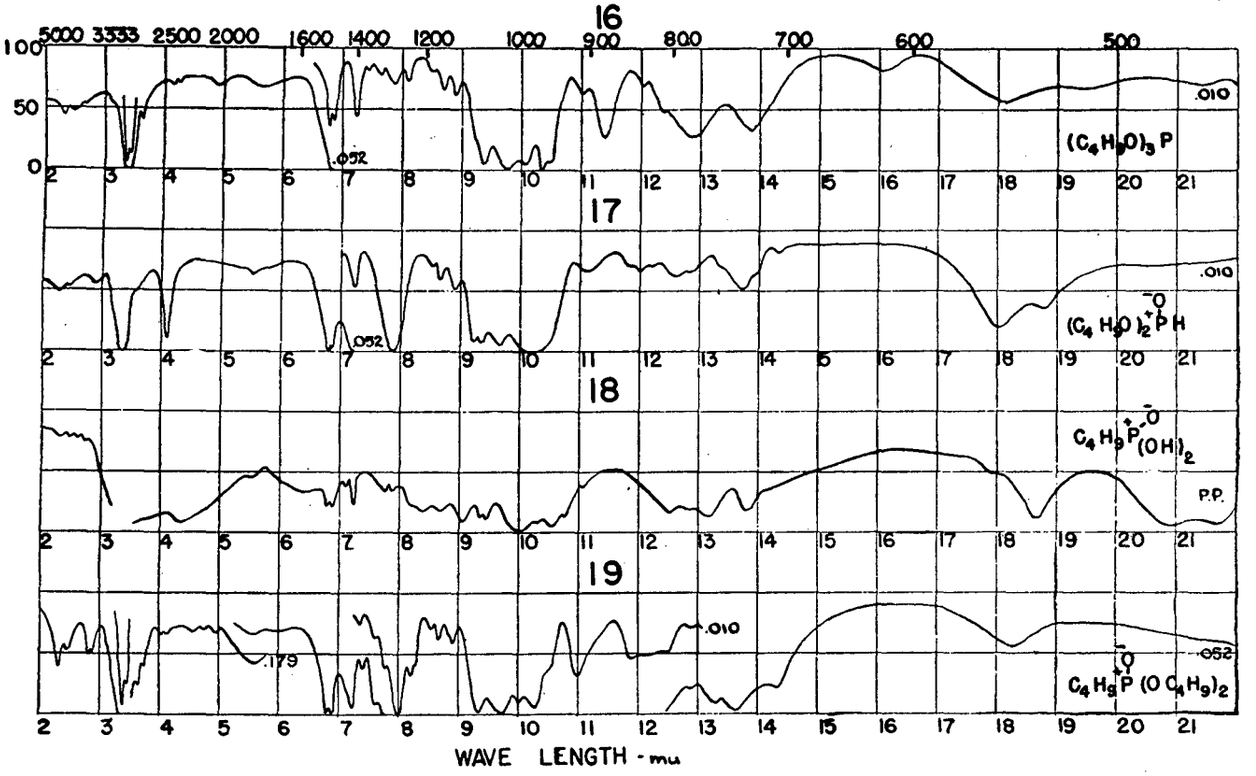


Figure 16. Tri-*n*-butyl Phosphite
Figure 17. Di-*n*-butyl Phosphonate
Figure 18. *n*-Butanephosphonic Acid
Figure 19. Di-*n*-butyl *n*-Butanephosphonate

The phosphoryl frequency occurs in a rather wide range extending from 1170 to 1310 cm.$^{-1}$ The position of the band within this range appears to depend neither upon the type of compound (as shown by the intermixing of phosphonates, phosphinates, and phosphine oxides in Table V) nor upon the size of the substituents (as shown by the intermixing of compounds of different molecular weight). There is, however, a definite correspondence between the phosphoryl frequency and the electronegativity of the other substituents on the phosphorus atom (as shown in Table V), the high frequencies always being associated with high electronegativities. This relationship is of considerable help in identifying the substituent groups, as illustrated by the examples cited below.

Acids have not been included in this correlation. In the case of phosphonic acids (Figures 12, 13, and 18) and phosphinic acids (Figures 4, 9, 10, and 11), both the phosphoryl band and the hydroxyl band are displaced toward lower frequencies and so broadened that they are difficult to identify, particularly in the spectra of the solid samples. The effects are clearly due to hydrogen bonding between the acid hydrogen and the phosphoryl oxygen. In dilute solutions the effects are greatly diminished, as shown by the spectra of benzenephosphinic acid (Figure 4) in the 1200 cm.$^{-1}$ region.

Thiophosphoryl Group. The valence vibration of the thiophosphoryl linkage appears as a medium to strong band at 753 cm.$^{-1}$ in thiophosphoryl chloride and at 718 cm.$^{-1}$ in thiophosphoryl bromide (*6*). Thiophosphoryl dichloride fluoride (Figure 40) and phenyldichlorophosphine sulfide (Figure 27), each has a strong band in the 745 to 750 cm.$^{-1}$ region, although in the latter case the absorption would be expected from the presence of the phenyl group. Similarly, five compounds of the type $SP(C_6H_5)(NR_2)_2$ (Figures 42 to 46) have one band at approximately 720 cm.$^{-1}$, and another between 745 and 765 cm.$^{-1}$ One of these (probably the former) is undoubtedly associated with the phenyl group, and the other may be related to the thiophosphoryl group. The spectrum of the compound known as trimethyl thionophosphate (Figure 3) is peculiar in that the thiophosphoryl absorption is displaced, weak, or absent, whereas in other compounds it appears as a medium to strong band in the region 715 to 770 cm.$^{-1}$

P—C (Aliphatic) Bond. In trimethylphosphine the two vibrations which involve mainly a stretching of P—C bonds appear at 653 and 708 cm.$^{-1}$ (*14*). In trimethylphosphine oxide the corresponding vibrations appear at 671 and 756 cm.$^{-1}$ (*5*). If characteristic frequencies for P—C stretching vibration exist, they should appear in the approximate range 650 to 750 cm.$^{-1}$, although the size and structure of the alkyl groups and the identity of the

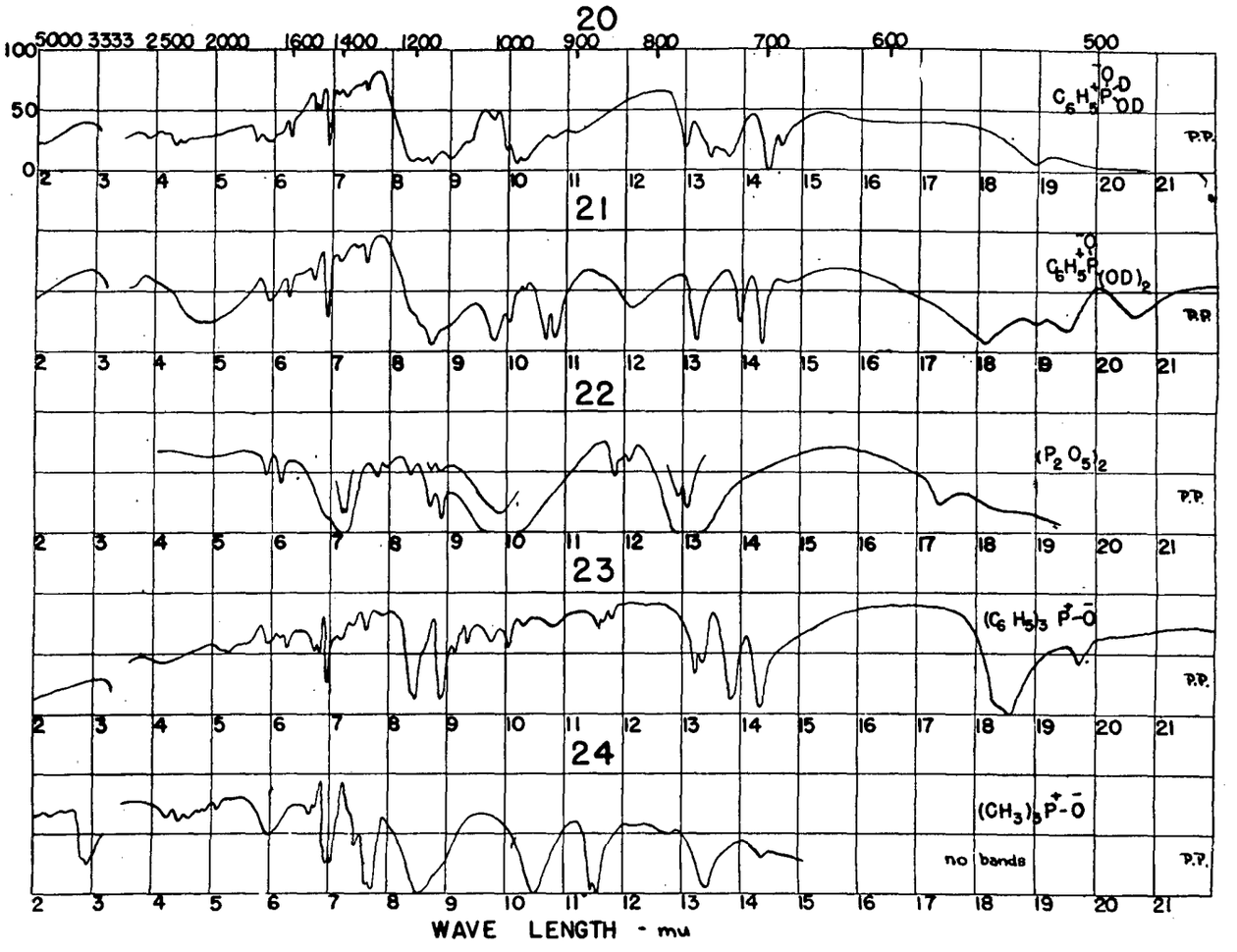


Figure 20. Benzenephosphinic Acid-d_2 (Naval Research Laboratory)
Figure 21. Benzenephosphonic Acid-d_2 (Naval Research Laboratory)
Figure 22. Phosphorus Pentoxide (J. T. Baker Chemical Co.)
Figure 23. Triphenylphosphine Oxide (Naval Research Laboratory)
Figure 24. Trimethylphosphine Oxide (Naval Research Laboratory)

other substituents on the phosphorus atom may be expected to have some effect.

In addition to the two compounds already mentioned, four other compounds with P—C (aliphatic) bonds have been studied (Table VI). All have bands in the region of 750 cm.$^{-1}$, but because absorption appears in this region for practically all phosphorus compounds, the origin of the bands is uncertain. In the case of methyl group substituents, further investigation may establish some correlations of value, but it does not appear likely that organophosphorus molecules in general can be recognized by characteristic infrared absorption involving vibrations of the P—C (aliphatic) bond.

Phenyl-Phosphorus Group. It is known from previous work at this laboratory and elsewhere (*3, 4, 15*) that the aromatic ring in hydrocarbons produces a number of characteristic absorption bands which not only confirm the presence of the ring but also indicate the number and positions of the substituents. It is clear that if the aromatic ring in a phosphorus compound is bonded only to carbon, these same correlations will apply. In the present work it has been found that they also apply when the aromatic ring is bonded directly to phosphorus. Thus, all the compounds containing a phenyl group (Table VII) or a *p*-substituted benzene ring (Figures 9, 11, 13, and 14) exhibit the characteristic aromatic frequencies corresponding to the respective structures, even though the ring is attached to phosphorus in each case. Presumably other polysubstituted aromatic groups having one phosphorus substituent will also exhibit the characteristic frequencies by which they may usually be identified.

There are two additional frequencies near 1000 and 1440 cm.$^{-1}$ in the spectra of all compounds containing a phenyl group attached directly to phosphorus (Table VIII). The band at 1000 cm.$^{-1}$ is usually stronger than observed for hydrocarbons and the other always occurs below 1450 cm.$^{-1}$ in phosphorus compounds but above 1450 cm.$^{-1}$ in hydrocarbons. Although one or both of these bands may arise from ring vibrations, they

Table VI. Phosphorus-Carbon (Aliphatic) Frequency

Compound	Formula	Position Cm.$^{-1}$	Position μ	Figure
Trimethylphosphine	$(CH_3)_3P$	653, 708	15.3, 14.1	..
Trimethylphosphine oxide	$(CH_3)_3PO$	671, 756	14.9, 13.4	24
Di-*n*-butyl *n*-butanephosphonate	$C_4H_9PO(OC_4H_9)_2$	735, 752	13.6, 13.3	19
Diethylphenylphosphine	$C_6H_5P(C_2H_5)_2$	740	13.5	39
Ethyldichlorophosphine	$C_2H_5PCl_2$	725, 758	13.8, 13.2	38
Diethyl trichloromethaue-phosphonate	$Cl_3CPO(OC_2H_5)_2$	740, 766	13.5, 13.0	35

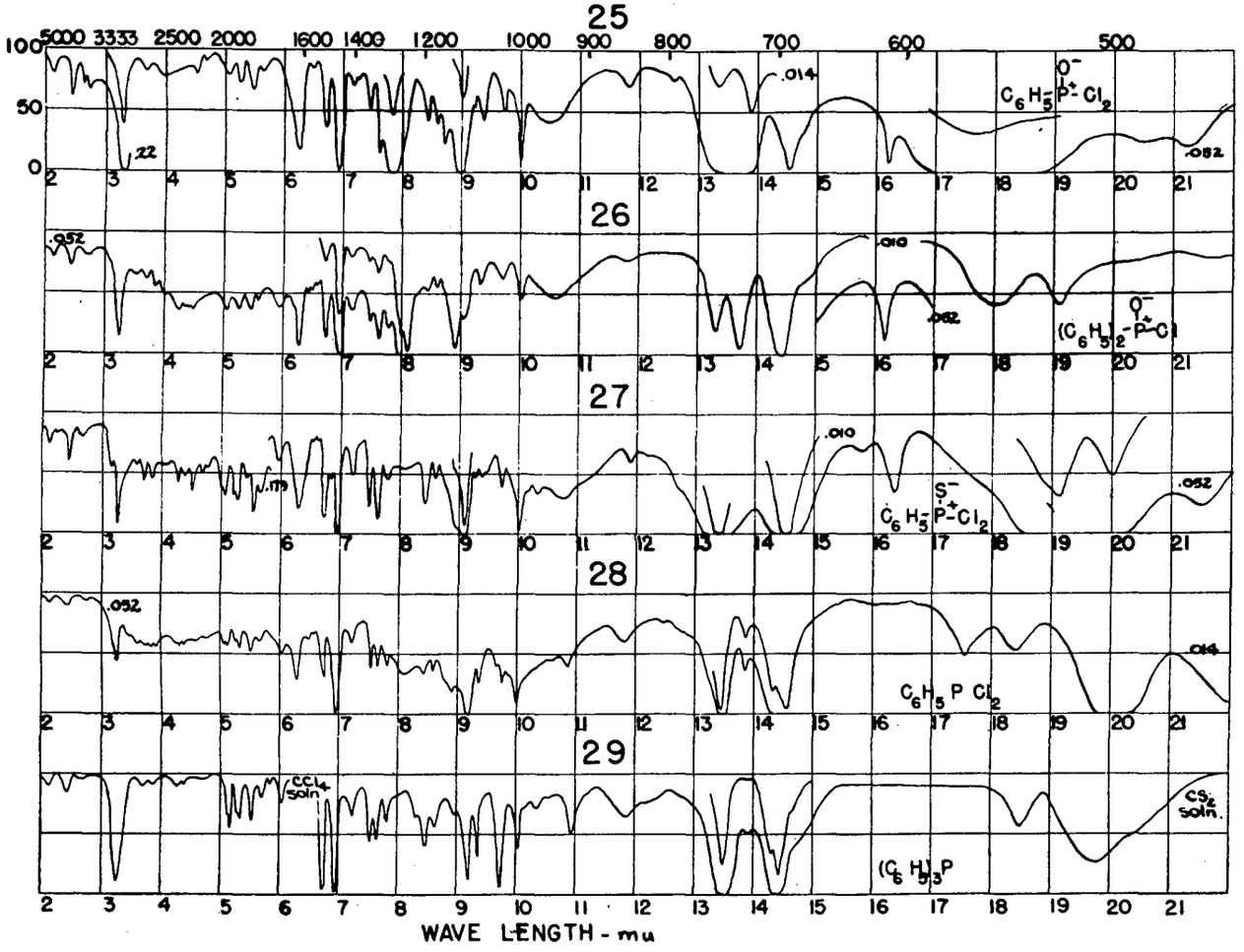


Figure 25. Phenyldichlorophosphine Oxide (Naval Research Laboratory)
Figure 26. Diphenylchlorophosphine Oxide (Naval Research Laboratory)
Figure 27. Phenyldichlorophosphine Sulfide (Victor Chemical Works)
Figure 28. Phenyldichlorophosphine (Naval Research Laboratory)
Figure 29. Triphenylphosphine (Naval Research Laboratory)

appear to be useful for the identification of the phenyl-phosphorus group. In previous work at this laboratory, characteristic bands at approximately these same positions have been observed for compounds containing a phenyl ring bonded to a silicon atom. The origin of these bands, believed to be the same for the two groupings, may be related to the fact that the phosphorus and silicon single bonds have about the same force constants, at least for bonds to halogen (*18*) or to aliphatic carbon atoms (*7*).

Hydroxyl Group. In the present work no evidence of a free hydroxyl group in phosphorus compounds has been found. Such groups should have characteristic absorption due to the stretching vibration of the OH bond at about 3620 cm.$^{-1}$ (*3*, *4*, *15*). In every case where it was sought to identify free hydroxyl absorption in an acid supposedly containing trivalent phosphorus—that is, containing no polar phosphoryl groups and consequently having the possibility of free hydroxyl groups—there was observed, instead, the characteristic absorption of the phosphoryl group, an indication that the acid contained a phosphorus atom in its higher valent state. In the presence of these highly polar groups, any hydroxyl groups may be expected to show hydrogen-bonding effects.

The stretching frequency could be expected in the 3000 cm.$^{-1}$ region, which is partially masked by the absorption of petrolatum used in the preparation of the solid samples. However, one compound, benzenephosphinic acid, which was soluble enough to be studied in carbon disulfide solution, showed (Figure 4) a strong, broad band at approximately 2680 cm.$^{-1}$ Absorption at about this same position was also observed for several other compounds studied as solids. It is believed to be due to bonded hydroxyl groups, especially as other compounds which cannot contain hydroxyl groups are considerably more transparent in this region. The data are summarized in Table VIII. Although the frequency range is somewhat lower than that usually observed for hydrogen-bonded hydroxyl groups in alcohols, aliphatic acids, phenols, etc., it is not much lower than that reported for benzoic acid (*16*).

Table VII. Phenyl-Phosphorus Group Frequency

Compound	Frequencies, Cm.$^{-1}$		Figure
Benzenephosphinic acid	1442	1002	4
Ethyl benzenephosphinate	1443	1000	5
Diethyl benzenephosphonite	1441	1000	6
Benzenephosphonic acid	1440	998	12
Diethyl benzenephosphonate	1450	1000[a]	15
Benzenephosphinic acid-d_2	1443	1003	20
Benzenephosphonic acid-d_2	1440	998	21
Triphenylphosphine oxide	1440	996	23
Phenyldichlorophosphine oxide	1444	998	25
Diphenylchlorophosphine oxide	1443	997	26
Phenyldichlorophosphine sulfide	1440	999	27
Phenyldichlorophosphine	1436	999	28
Triphenylphosphine	1435	999	29
Diphenyl benzenephosphonate	1450	1005	34
Benzenephosphonic dianilide	...	1000	36
Benzenephosphonic diphenylhydrazide	1440[a]	1000[a]	37
Diethylphenylphosphine	1440	1000	39
N,N-Dimethyl benzenethiophosphonic diamide	1440	995[a]	42
N,N-Diethyl benzenethiophosphonic diamide	1440	1002[a]	43
N,N-Di-*n*-butyl benzenethiophosphonic diamide	1440	1000[a]	44
N,N-Diisobutyl benzenethiophosphonic diamide	1435	995[a]	45
N,N-Didecyl benzenethiophosphonic diamide	1435	990[a]	46

[a] Weak band.

P—O—R Linkage. Organic esters have a characteristic band at about 1110 cm.$^{-1}$ which has been ascribed to the C—O—C linkage (*3*, *4*, *15*). Substitution of phosphorus for carbon in an

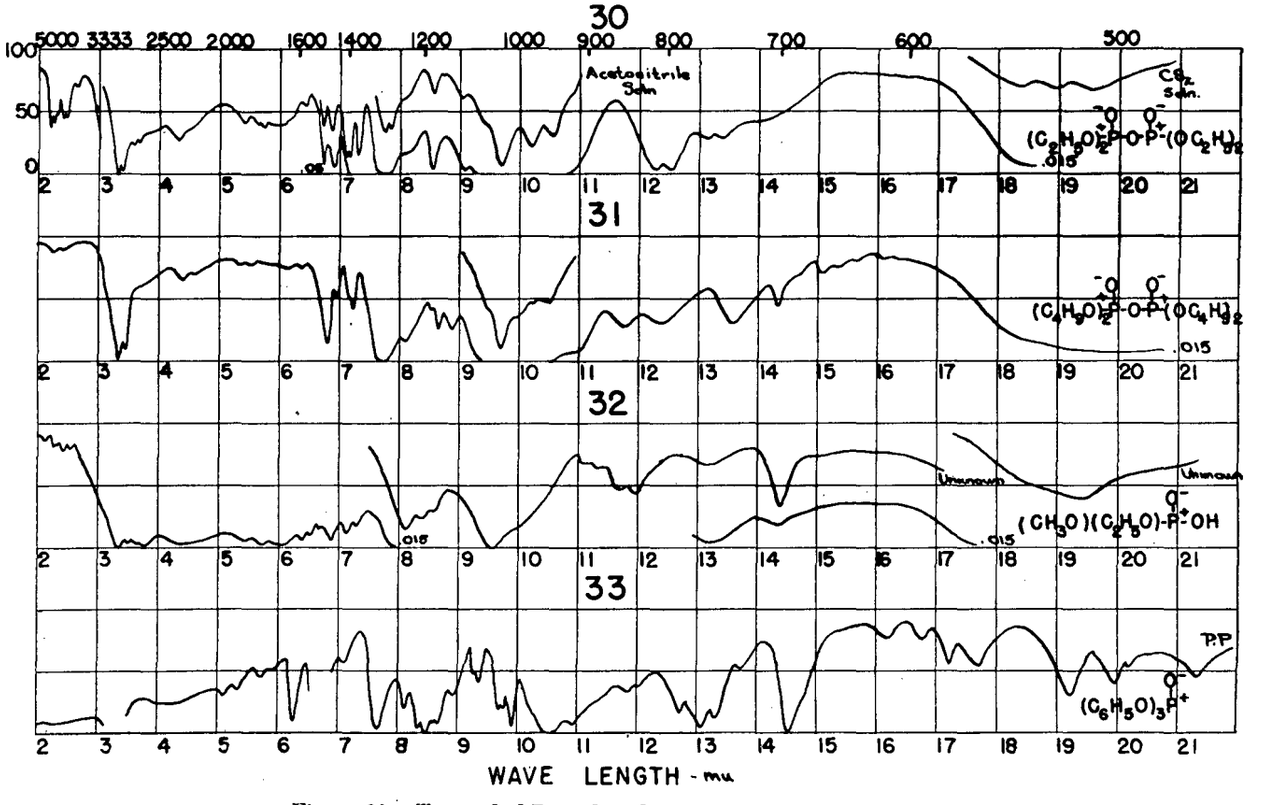


Figure 30. Tetraethyl Pyrophosphate (Victor Chemical Works)
Figure 31. Tetra-*n*-butyl Pyrophosphate (Victor Chemical Works)
Figure 32. Methyl Ethyl Phosphate (Victor Chemical Works)
Figure 33. Triphenyl Phosphate (Eastman Kodak Co.)

Table VIII. Possible Bonded Hydroxyl Group Frequencies

Compound	Formula	Position Cm.$^{-1}$	Position μ	Figure
Benzenephosphinic acid	$C_6H_5PH(O)OH$	2680	3.73	4
p-Toluenephosphinic acid	$CH_3—C_6H_4PH(O)OH$	2640	3.79	9
Naphthalenephosphinic acid	$C_{10}H_7PH(O)OH$	2550	3.92	10
p-Ethylbenzenephosphinic acid	$C_2H_5—C_6H_4PH(O)OH$	2640	3.79	11
Benzenephosphonic acid	$C_6H_5POH(O)OH$	2690	3.72	12
p-Chlorobenzenephosphonic acid	$Cl—C_6H_4POH(O)OH$	...	...	13
n-Butanephosphonic acid	$C_4H_9POH(O)OH$	2550–2800	3.92–3.57	18
Methyl ethyl phosphate	$CH_3O(C_2H_5O)P(O)OH$	2650	3.77	32

ester group will normally shift the absorption band toward lower frequencies. Indeed, in the twenty phosphorus esters listed in Table IX there was found near 1050 cm.$^{-1}$ a strong, moderately broad band envelope which was absent in each of four parent acids studied and which is believed to be characteristic of the P—O—R linkage in alkyl esters. The frequencies given in the table are the estimated centers of the band envelopes which sometimes have rather sharp absorption peaks superimposed on them.

Table IX. Characteristic Absorption Bands of the P—O—C Linkage

Compound	Formula	Approximate Position of Center of Band Envelope Cm.$^{-1}$	Approximate Position of Center of Band Envelope μ	Figure Compound	Figure Parent acid
Di-*n*-butyl *n*-butanephosphonate	$C_4H_9P(O)(OC_4H_9)_2$	1035	9.65	19	18
Diethyl benzenephosphonate	$C_6H_5P(O)(OC_2H_5)_2$	1052	9.5	15	12
Diethyl *p*-chlorobenzenephosphonate	$ClC_6H_4P(O)(OC_2H_5)_2$	1042	9.6	14	13
Ethyl benzenephosphinate	$C_6H_5PH(O)OC_2H_5$	1052	9.5	5	4
Diethyl benzenephosphonite	$C_6H_5P(OC_2H_5)_2$	1042	9.6	6	
Dimethyl fluorophosphate	$FP(O)(OCH_3)_2$	1052	9.5	59	
Diethyl fluorophosphate	$FP(O)(OC_2H_5)_2$	1052	9.5	60	
Trimethyl phosphate	$P(O)(OCH_3)_3$	1042	9.6	1	
Tetramethyl pyrophosphate	$(CH_3O)_2P(O)OP(O)(OCH_3)_2$	1064	9.4	2	
Trimethyl thionophosphate	$P(S)(OCH_3)_3$	1030	9.7	3	
Diethyl phosphonate	$HP(O)(OC_2H_5)_2$	1052	9.5	7	
Triethyl phosphite	$P(OC_2H_5)_3$	1030	9.7	8	
Tri-*n*-butyl phosphite	$P(OC_4H_9)_3$	1030	9.7	16	
Tetra-*n*-butyl pyrophosphate	$(C_4H_9O)_2P(O)OP(O)(OC_4H_9)_2$	1035	9.65	31	
Diethyl trichloromethanephosphonate	$Cl_3CP(O)(OC_2H_5)_2$	1030	9.7	35	
Methyl phosphonitrilate (trimer)	$(PN(OCH_3)_2)_3$	1042	9.6	49	
n-Propyl phosphonitrilate (trimer)	$(PN(OC_3H_8)_2)_3$	1035	9.65	51	
Isopropyl phosphonitrilate (trimer)	$(PN(OC_3H_8)_2)_3$	1030	9.7	52	
Octyl phosphonitrilate (trimer)	$(PN(OC_8H_{17})_2)_3$	1042	9.6	53	
Ethyl phosphonitrilate (polymer)$_x$	$(PN(OC_2H_5)_2)_x$	1030	9.7	55	

When the R group is methyl (Figures 1, 2, 3, 32, and 49) the P—O—R absorption appears as a single, well-defined, strong band, and there is observed, in addition, a weak but sharp band at about 1190 cm.$^{-1}$ Furthermore, the methyl group absorption (which appears at 1379 cm.$^{-1}$ in hydrocarbons) is not observed at its usual position. When the R group becomes ethyl (Figures 5, 6, 7, 8, 14, 30, 32, 35, 50, and 55) the P—O—R band becomes somewhat broadened and frequently shows subsidiary maxima, the methyl group (in C_2H_5) absorbs near its usual 1379 cm.$^{-1}$ position (but as a sharp band at

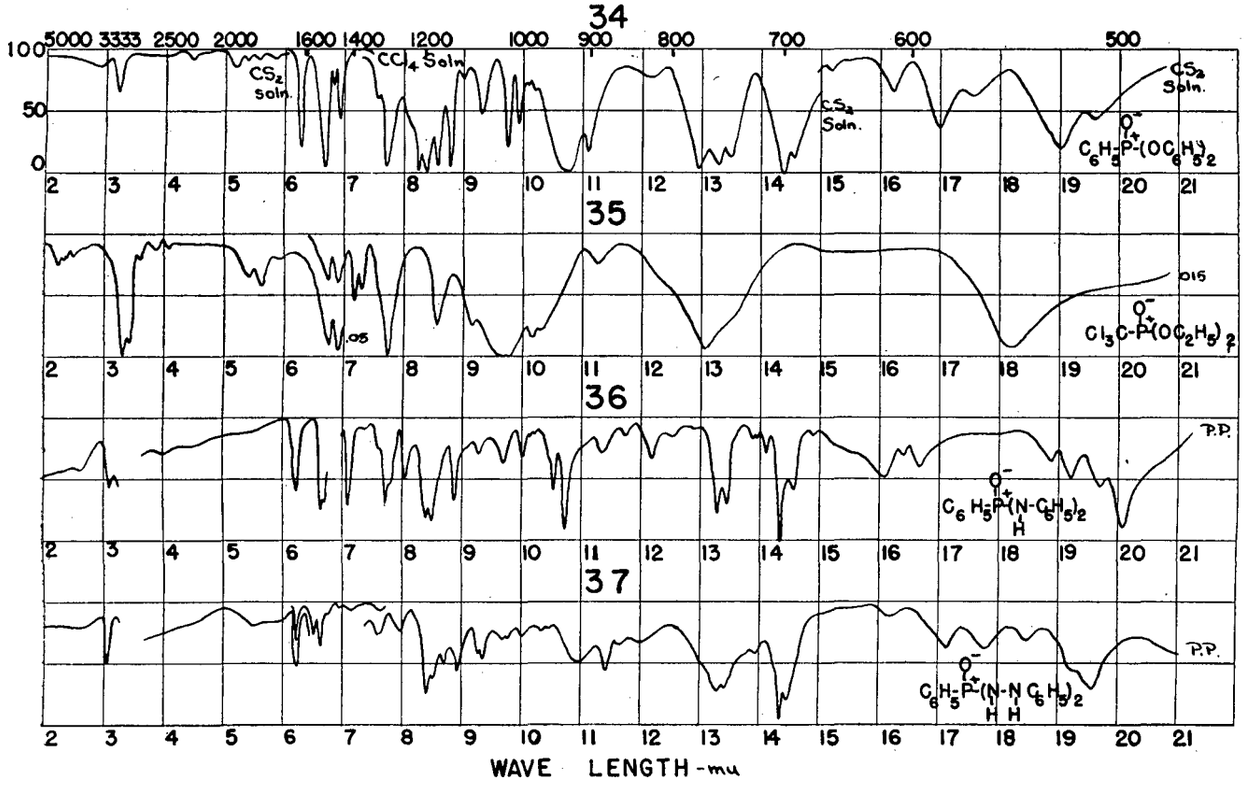


Figure 34. Diphenyl Benzenephosphonate (Naval Research Laboratory)
Figure 35. Diethyl Trichloromethylphosphonate (Monsanto Chemical Co.)
Figure 36. Benzenephosphonic Dianalide (Naval Research Laboratory)
Figure 37. Benzenephosphonic Diphenylhydrazide (Naval Research Laboratory)

1388 with a weaker branch at 1370 cm.$^{-1}$), and in addition there appears a sharp band of medium intensity at 1165 cm.$^{-1}$

Only three compounds (triphenyl phosphite, triphenyl phosphate, and diphenyl benzenephosphonate) have been studied in which R is aromatic. The characteristic P—O—R absorption does not appear in the spectra of these aromatic esters (Figures 33, 34, and 41) at the same position as for the alkyl esters. Additional data are required to determine whether the band has shifted toward lower (875 to 950 cm.$^{-1}$) or higher (1200 to 1250 cm.$^{-1}$) frequencies.

Phosphites and Phosphonates. A report (*17*) that phosphites and phosphonates could be quantitatively analyzed according to type by two characteristic bands at 870 and 940 cm.$^{-1}$ has recently been withdrawn (*11*). Absorption data in the 825 to 1000 cm.$^{-1}$ for typical compounds of several types here investigated are shown in Figure 61. No basis for the proposed analytical procedure is evident. However, all four phosphites of higher molecular weight (above ethyl) have a band between 870 and 880 cm.$^{-1}$, in which region other classes of phosphorus compounds are usually transparent and which, therefore, may be of some use for qualitative analysis. Phosphonates have a band between 940 and 990 cm.$^{-1}$ rather than at exactly 940 cm.$^{-1}$, but this knowledge is of little use, even for qualitative analysis, because many compounds of other types (including phosphites) also absorb in this region.

Phosphinic Acids. All five acids of the type RPO(H)OH showed definite spectral similarities (Figures 4, 9, 10, 11, and 20), some of which are due to the characteristic absorption of P—H, OH, and P—O, and of the aromatic ring which was present in each compound. In addition, each has a broad band of medium or low intensity at 1665 cm.$^{-1}$ which is not observed in other classes of compounds and which has been useful for identification of phosphinic acids.

DISCUSSION

In Figure 62 are summarized in graphical form probable characteristic frequency ranges for several molecular groups, as derived from the data presented. Because these correlations are based upon a limited number of compounds, they must be applied with due reserve, especially in cases where other than organophosphorus compounds are considered. Those of a particularly tentative nature are indicated by broken-line boxes. The ranges given for P—H and phosphoryl group frequencies are in agreement with the correlations of Colthup (*4*) and data cited by Thompson (*11*). Thompson has also suggested that the P—O link in P—O—C produces a band at 795 cm.$^{-1}$, but this is not particularly evident in the authors' curves and does not appear to be as useful as the 1050 cm.$^{-1}$ absorption. The latter probably arises from the O—C part of the P—O—C group.

Application of these correlations to determination of molecular structure is most fruitful in the case of pure materials, for in this case the minimum number of different groups is present in the sample. To illustrate, Figures 15 and 25 are chosen, assuming for present purposes that these are the spectra of unidentified phosphorus compounds.

	For Figure 15	
Cm.$^{-1}$	Observation	Interpretation
3000	Strong band	C—H present
1480	Strong band	C—H present

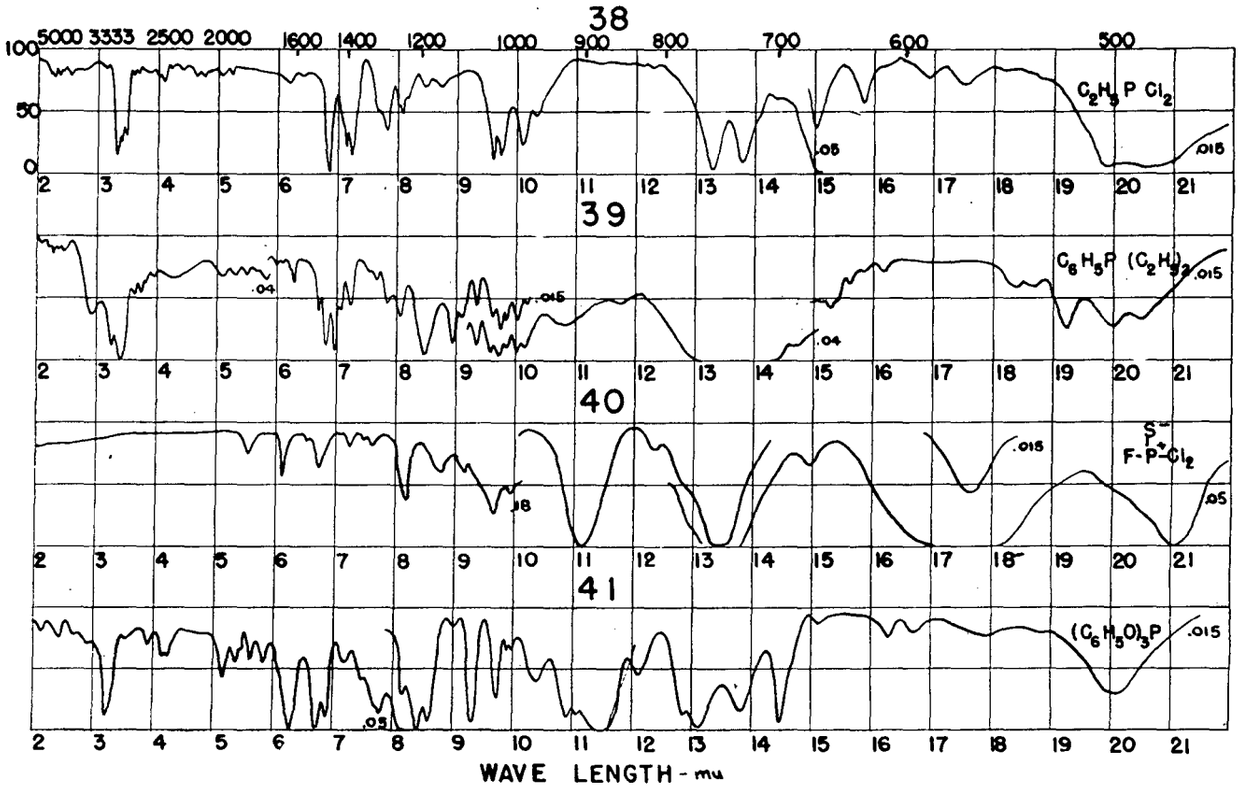


Figure 38. Ethyl dichlorophosphine (Naval Research Laboratory)
Figure 39. Diethylphenylphosphine (University of Chicago Toxicological Laboratory)
Figure 40. Thiophosphoryl Dichloride Fluoride (University of Chicago Toxicological Laboratory)
Figure 41. Triphenyl Phosphite (Eastman Kodak Co.)

For Figure 15 (*Continued*)

Cm.$^{-1}$	Observation	Interpretation
2665	Absorption weak	No O—H; not an acid
2350–2450	No sharp band	No P—H
1260	Intense band	Phosphoryl group present, probably with two or three electronegative substituents
1050	Region of intense absorption	P—O—R present, an ester (alkyl)
1200	No band	Not a methyl ester; probably an ethyl ester
1165	Sharp band	
700	Strong sharp band	Phenyl group present
750	Strong sharp band	
1030	Strong sharp band	
1605	Strong sharp band	
3050	Sharp band	
1450	Strong sharp band	Phenyl group attached to phosphorus
1000	Weak sharp band	
980	Intense absorption	Many phosphorus compounds absorb here

Conclusion. The compound is probably a phosphonate of structure $C_6H_5P(O)(OR)_2$ where R is greater than methyl, probably ethyl; it could also be a phosphinate of structure $C_6H_5-\overset{-O}{\underset{|}{\overset{|}{P^{+}}}}-X$ where R is greater than methyl (probably ethyl) and X is an electronegative group.

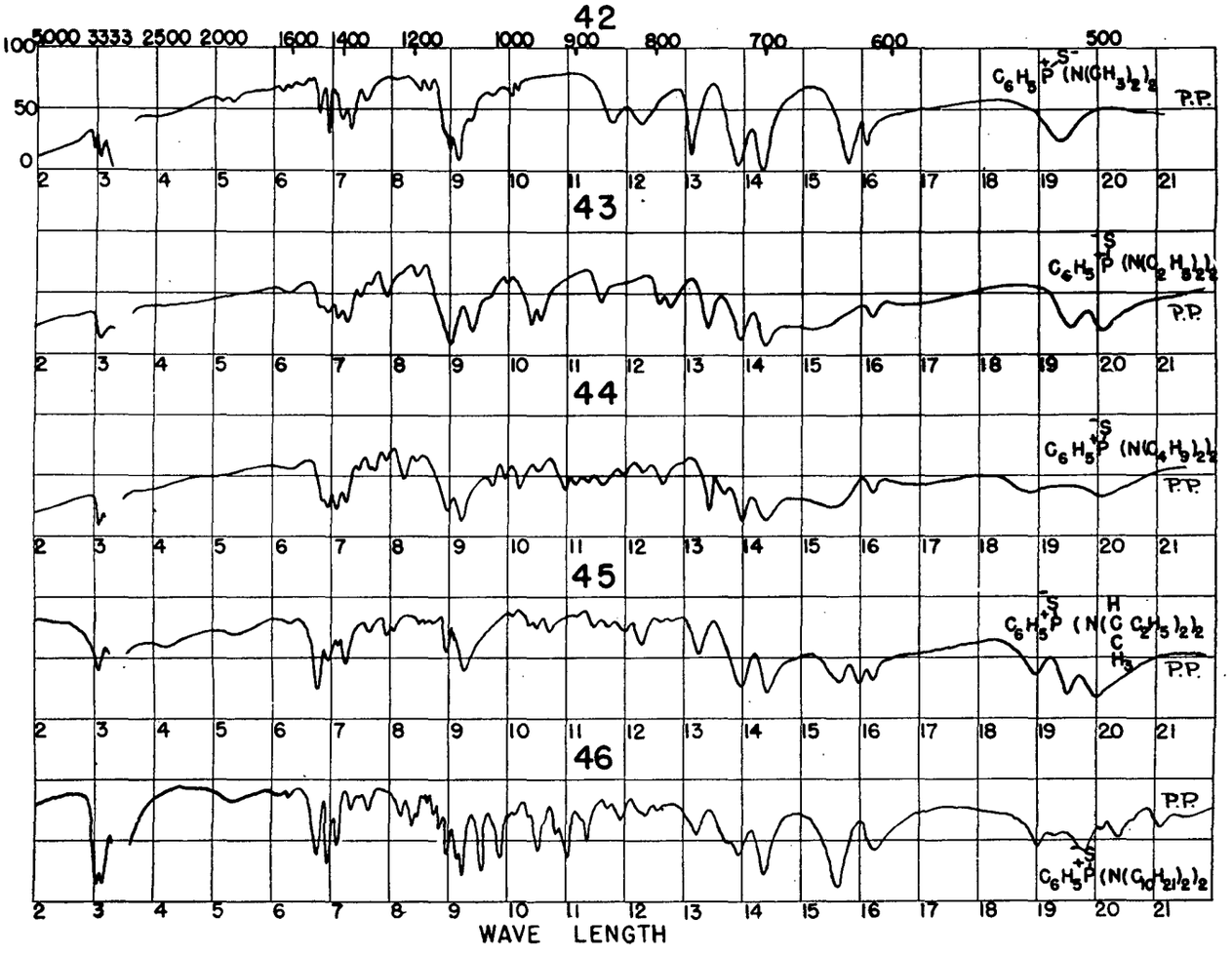
OR

Actual compound. Diethyl benzenephosphonate, $C_6H_5-\overset{-O}{\underset{OC_2H_5}{\overset{OC_2H_5}{P^{+}}}}$

For Figure 25

Cm.$^{-1}$	Observation	Interpretation
3000	No band	No paraffinic C—H
2665	Absorption weak	No O—H, not an acid
2350–2450	No sharp band	No P—H
690	Strong band	
750	Strong band	
1030	Sharp band	Phenyl group present
1605	Strong band	
3050	Sharp band	
1450	Strong sharp band	Phenyl group attached to phosphorus
1000	Sharp band	
1275	Intense band	Phosphoryl group present, with two or three electronegative substituents (since one substituent is phenyl, remaining two must be electronegative)

(*Continued on next page*)



Figures 42 to 46. *N,N*-Dimethyl-, *N,N*-Diethyl-, *N,N*-di-*n*-butyl-, *N,N*-Diisopropyl-, and *N,N*-Didecylbenzenethiophosphonic Diamides

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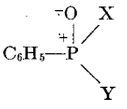
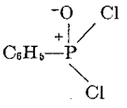
For Figure 25 (*Continued*)

Cm.$^{-1}$	Observation	Interpretation
1050	No intense band	Not an ester
1110	Intense band	?
970	Broad band	Many phosphorus compounds absorb here
750	Intense absorption	Possibly P—F
570	Broad band	Possibly P—Cl

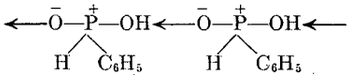
Conclusion. Compound must be a phosphine oxide with structure



where both X and Y are electronegative, possibly fluorine or chlorine. Actual compound. Phenyldichlorophosphine oxide,



When other information—for example, the method of preparation or the physical and chemical properties of a material—is available, those data should also be taken into consideration. As a case in point, the spectrum of benzenephosphinic acid is readily interpreted in terms of the simple structure shown on Figure 4, except that here (and in all acids studied) hydrogen bonding is observed. It has been found independently by molecular weight determinations in benzene solution (*8*) that the acid exists in trimeric or higher polymeric form. Thus, the actual structure probably consists of cyclic (or possibly linear) polymers in which the monomers are joined by hydrogen bonds, as in the structure



Spectral methods, as illustrated, have proved very useful in the course of work for the identification of other types of phosphorus compounds, including most of those listed in Table I. Many

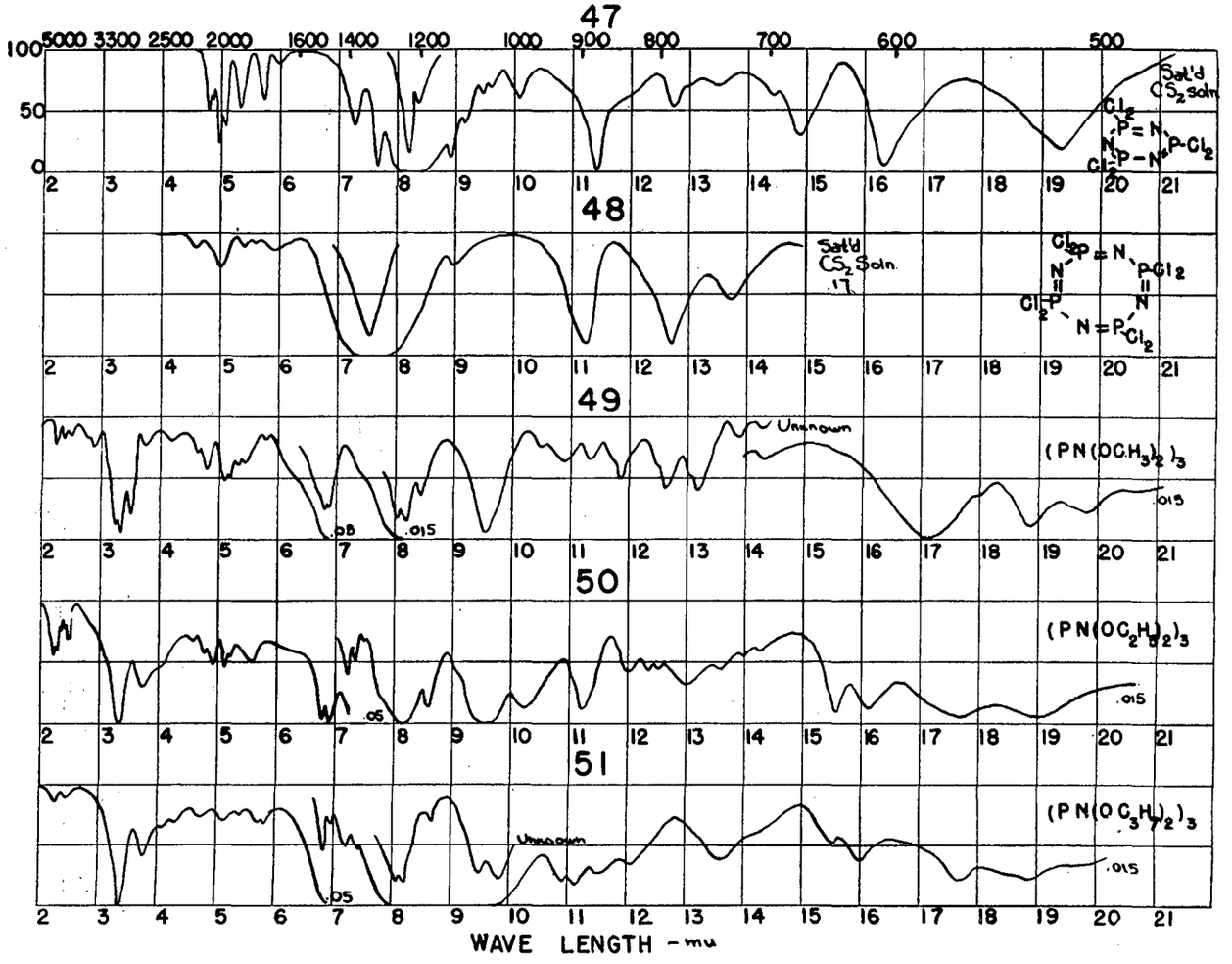
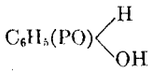


Figure 47. Phosphonitrilic Chloride Trimer
Figure 48. Phosphonitrilic Chloride Tetramer
Figure 49. Methyl Phosphonitrilate Trimer
Figure 50. Ethyl Phosphonitrilate Trimer
Figure 51. *n*-Propyl Phosphonitrilate Trimer

Naval Research Laboratory

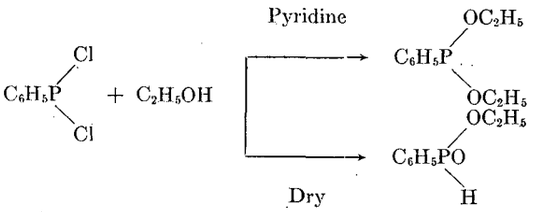
cases have been encountered in which a reaction product does not have the structure expected on the basis of chemical considerations alone, or in which two or more structures were possible. In such cases identification by chemical methods can undoubtedly be made, but spectral methods appear to be much simpler, faster, and sometimes more reliable, particularly in connection with the preparation of new compounds.

For example, the acid obtained by hydrolysis of phenyldichlorophosphine ($C_6H_5PCl_2$) is not the expected benzenephosphonous acid, $C_6H_5P(OH)_2$, but rather the monobasic benzenephosphinic acid,



as shown by the presence of characteristic P—O (phosphoryl), OH, PH, and C_6H_5P frequencies in its spectrum (Figure 4). In fact, in this investigation no acids of trivalent phosphorus have been found! In every case where it was sought to identify a phosphorous or phosphonous acid there was found, instead, the corresponding phosphonic or phosphinic acid.

Again, two different esters can be formed by the reaction of ethyl alcohol with phenyldichlorophosphine, depending upon how the reaction is carried out:



In the presence of pyridine the phosphonite is obtained (Figure 6), whereas reaction of the pure materials yields the phosphinate, as shown clearly by the presence of P—H and phosphoryl group frequencies in the spectrum (Figure 3).

Similarly, three different esters may be obtained from the reaction of phosphorus trichloride and alcohol in the presence of pyridine (*9*), as indicated by the following equations:

$PCl_3 + 3ROH + 3$ pyridine $\longrightarrow P(OR)_3 + 3$ pyridine hydrochloride (I)

$PCl_3 + 3ROH + 2$ pyridine $\longrightarrow HPO(OR)_2 + 2$ pyridine hydrochloride $+$ RCl (II)

$PCl_3 + 3ROH + 1$ pyridine $\longrightarrow H_2O_2P—OR + 1$ pyridine hydrochloride $+ 2RCl$ (III)

The spectra of the *n*-butyl esters prepared by Reactions I and II are shown in Figures 16 and 17, respectively. While I yielded tributyl phosphite, as expected, II yielded dibutyl phosphonate, as shown by the spectral differences in the regions of

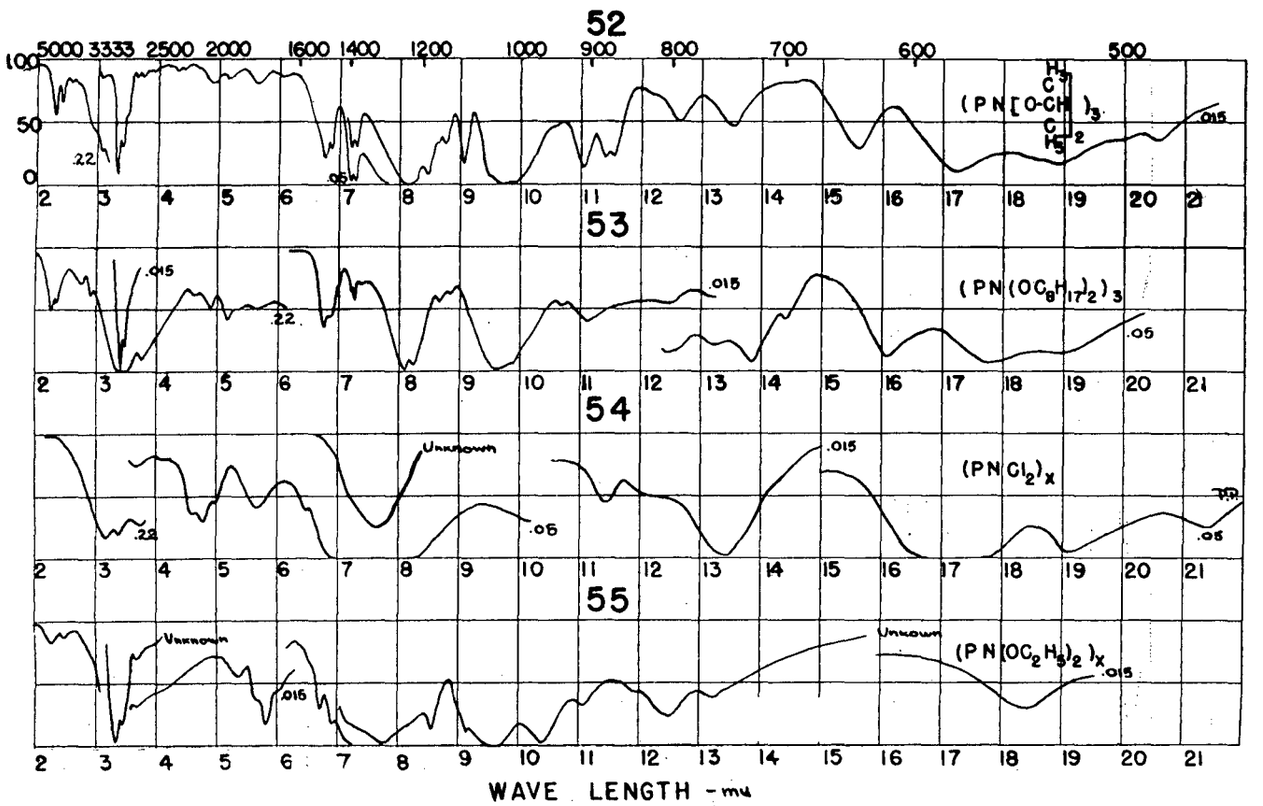


Figure 52. Isopropyl Phosphonitrilate Trimer
Figure 53. Octyl Phosphonitrilate Trimer
Figure 54. Phosphonitrilic Chloride Polymer
Figure 55. Ethyl Phosphonitrilate Polymer

Naval Research Laboratory

P—H and phosphoryl absorptions near 2400 and 1275 cm.$^{-1}$, respectively. These differences are again illustrated in the ethyl esters in Figures 7 and 8. Reaction III has not yet been studied, but the spectrum of the ester should readily show whether the alkyldihydrogen phosphite, RO—P—$(OH)_2$, or the more likely alkyl hydrogen phosphonate, RO—(PO)$\langle^{OH}_{H}$, is obtained.

Uncertainty as to the structure of esters also arises because of the possibility of rearrangement. For example, in the Arbusov transformation (*1*, *2*) trialkyl phosphites are converted into dialkyl alkane phosphonates by alkyl halide according to the reaction:

$$(RO)_3P + R_1X \longrightarrow R_1\text{—}\overset{-O}{\underset{+}{P}}\text{—}(OR)_2 + RX \quad (R_1 \text{ can be equal to R})$$

Moreover, this conversion is reported to occur in certain instances without alkyl halide (*12*) and to have the properties of a catalytic reaction (*1*, *2*). It is frequently desirable, therefore, to check the structure of an ester to determine whether rearrangement has occurred, and this is easily done by means of the phosphoryl frequency. In the present work tri-butyl phosphite and di-*n*-butyl *n*-butanephosphonate (Figures 16 and 17) have shown no tendency to rearrange at room temperature.

Finally, the characteristic group absorptions have been found useful in determining the purity of esters which tend to hydrolyze. For example, samples of pure triethyl phosphite, $P(OC_2H_5)_3$, and diethyl benzenephosphonite, $C_6H_5P(OC_2H_5)_2$ do not exhibit strong characteristic absorption of phosphoryl, P—H, or OH (bonded), but most preparations of these compounds do have some absorption in these regions (Figures 6 and 8). Although these weak bands may be due to combination frequencies, it is more likely that they are caused by a small amount of hydrolytic impurity. When the samples were purposely exposed to the atmosphere for short periods of time, the intensity of these bands rapidly increased.

ACKNOWLEDGMENT

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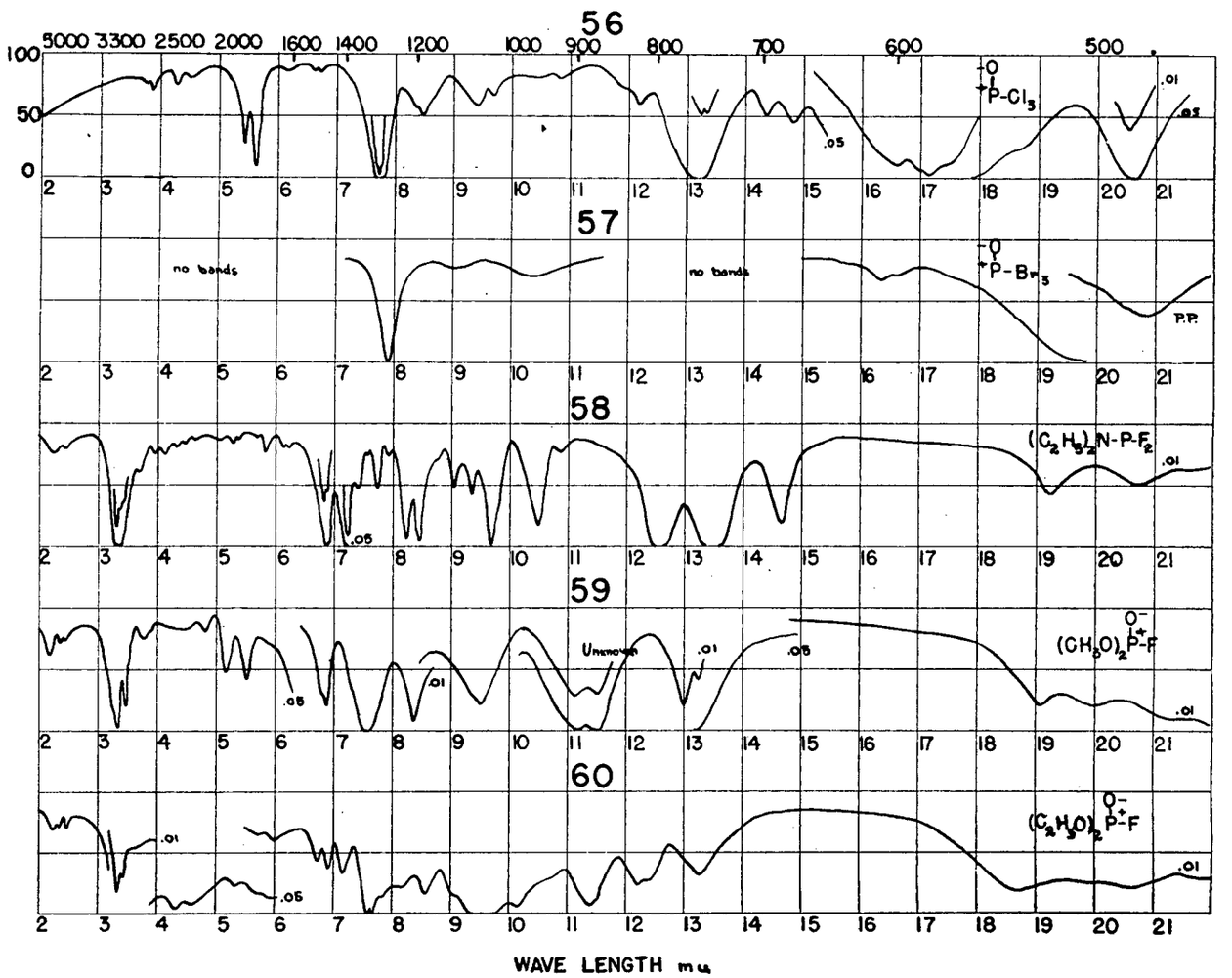

Figure 56. Phosphoryl Trichloride (Naval Research Laboratory)
Figure 57. Phosphoryl Tribromide (Naval Research Laboratory)
Figure 58. *N,N*-Diethylaminophosphorus Dichloride (University of Chicago Toxicological Laboratory)
Figure 59. Dimethyl Fluorophosphate (University of Chicago Toxicological Laboratory)
Figure 60. Diethyl Fluorophosphate (University of Chicago Toxicological Laboratory)

Bernard Buchner, who synthesized many of the compounds studied. Thanks are also extended to those organizations who contributed samples and to Mrs. E. J. Butler for her assistance in obtaining some of the spectra.

LITERATURE CITED

(1) Arbusov, A., "Catalytic Phenomena in the Field of Phosphorus Chemistry," dissertation, Kazan, 1914.
(2) Arbusov, A., *J. Russ. Phys. Chem. Soc.*, **38** (ii), 687 (1906).
(3) Barnes, R., Gore, R., Stafford, R., and Williams, V., ANAL. CHEM., **20**, 402 (1948).
(4) Colthup, N., *J. Optical Soc. Am.*, **40**, 397 (1950).
(5) Daasch, L., and Smith, D. C., *J. Chem. Phys.*, **19**, 22 (1951).
(6) Delwaulle, M., and François, F., *Compt. rend.*, **220**, 817 (1945); **222**, 1391 (1946); **224**, 1422 (1947).
(7) Duchesne, J., *J. Chem. Phys.*, **7**, 87 (1939).
(8) Dye, W. T., Naval Research Laboratory, unpublished data.

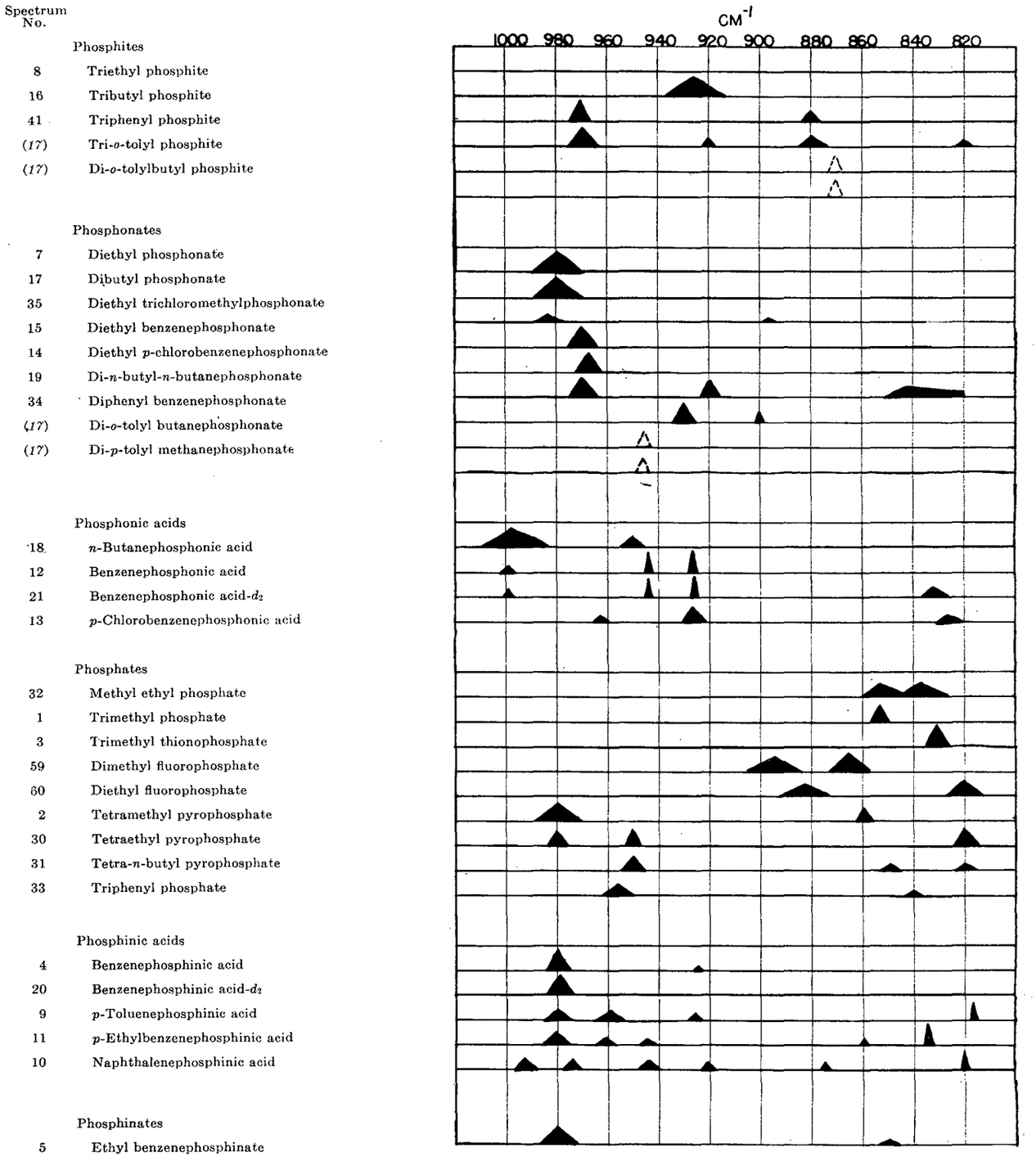

Figure 61. Absorption in 800–1000 Cm.$^{-1}$ Region for Phosphorus Compounds

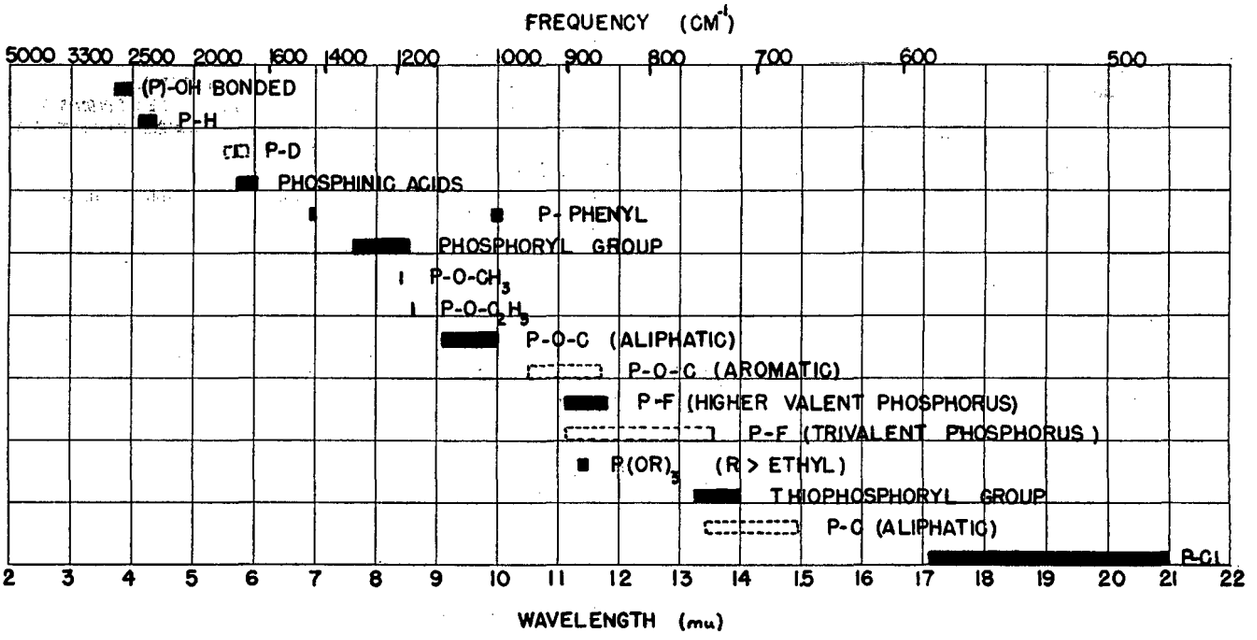

Figure 62. Group Frequency Correlations for Phosphorus Compounds

(9) Gerrard, W., *J. Chem. Soc.*, **1940**, 1464.
(10) Herzberg, G., "Infrared and Raman Spectra," p. 164, New York, D. Van Nostrand Co., 1945.
(11) Meyrick, C., and Thompson, H., *J. Chem. Soc.*, **1950**, 225.
(12) Milobedzki, T., and Szulgin, K., *Chem. Polski*, **15**, 66 (1917).
(13) Nielsen, J. R., Crawford, F., and Smith, D. C., *J. Optical Soc. Am.*, **37**, 296 (1947).
(14) Rosenbaum, E., Rubin, D., and Sandberg, C., *J. Chem. Phys.*, **8**, 366 (1940).
(15) Thompson, H., *J. Chem. Soc.*, **1948**, 328.
(16) Wall, F., *J. Chem. Phys.*, **7**, 87 (1939).
(17) Whiffen, F., Torkington, P., and Thompson, H., *Trans. Faraday Soc.*, **41**, 204 (1945).
(18) Wu, T., "Vibrational Spectra and Structure of Polyatomic Molecules," pp. 202–33, KuMing, China, National University of Peking, 1939.

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Titration of Dissolved Oxygen Using Acid-Chromous Reagent

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AMONG the methods for the determination of dissolved molecular oxygen which have been used during the past 60 years, the Winkler (*12–14*) method and its modifications appear most frequently in the literature. There are, however, important disadvantages to Winkler's method, such as troublesome manipulations, the need for relatively large volumes for each determination, and the considerable length of time required for each analysis. For these reasons, the possibility of a new method based on the reaction of molecular oxygen with the acid-chromous reagent seemed worthy of investigation. The success of this reagent in the determination of oxygen in gaseous mixtures (*8*) suggested that it might work equally well for dissolved molecular oxygen in water.

The reaction, which for practical purposes is instantaneous, may be represented by the following equation:

$$4H^+ + 4Cr^{++} + O_2 = 4Cr^{+++} + 2H_2O \qquad (1)$$

In the method presented here, the sample of water was added to a quantity of standard chromous chloride–hydrochloric acid solution in excess of that required to react with the oxygen in the sample. The excess chromous ion was then oxidized by adding standard potassium iodate solution, which reacted according to Equation 2. The iodate remaining was determined by adding a

$$6H^+ + 6Cr^{++} + IO_3^- = 6Cr^{+++} + I^- + 3H_2O \qquad (2)$$

crystal of potassium iodide and then titrating the iodine formed with the standard chromous chloride. The whole procedure was carried out under an atmosphere of carbon dioxide.

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PREPARATION OF REAGENTS

Acid-Chromium Chloride Solution. An approximately 0.008 *M* solution was prepared by mixing 1.05 grams of C.P. chromic chloride hexahydrate with 8.5 ml. of 6 *M* hydrochloric acid and then diluting to 500 ml. with distilled water. The chromium (III) was reduced to the chromium (II) state by passing this solution through a reductor, containing 1% mercury on zinc, into the storage titration apparatus depicted in Figure 1. The method used to accomplish this is described in detail by Stone (*7*).

Oxygen-Free Potassium Iodate Reagent. A 0.6-gram sample of C.P. potassium iodate was dissolved in distilled water and diluted to 500 ml. After this solution was forced into a storage titration apparatus, shown in Figure 1, by the method used for the chromous solution, the apparatus was inverted so that the open cock of the buret was well above the water level. The solu-

The need was felt for a method of determining molecular oxygen dissolved in water which would eliminate some of the disadvantages of the commonly used Winkler procedure. This work was undertaken to ascertain whether or not the reaction between divalent chromium and molecular oxygen could be made the basis for such a determination. The investigation developed a practical method for determining dissolved oxygen, which is simple, fast, and reproducible. Results given for the oxygen content of air-saturated water agree with those of previous investigators. Applications of this method should be of interest to all analytical chemists concerned with problems of sanitation involving water supplies and waste waters. The method is adaptable for use in the field as well as in the laboratory.

tion in the storage flask was then boiled vigorously until the 500-ml. volume had been reduced to approximately 300 ml. This required about 20 minutes. The stopcock was then closed and the flame removed at once. After the apparatus and contents had cooled to room temperature, the pressure in the storage flask was brought to approximately 1 atmosphere above the room pressure with oxygen-free nitrogen as described by Stone (*7*).

Potassium Iodate Standards Reagent. A standard solution of pure potassium iodate, approximately 0.002 *M*, was prepared by the usual quantitative procedures. Measured volumes of this solution were boiled to remove the dissolved oxygen and used to determine the concentration of the chromous solution, which in turn was used to standardize the oxygen-free potassium iodate solution. The method of standardization is described in detail below.

Water of Standard Oxygen Content. This reagent was prepared in the apparatus diagramed in Figure 2. A thermostat, not shown, provided water at constant temperature that was circulated by a pump through the water jacket, *A*, surrounding the buret reservoir, *B*. Corrosion was inhibited in the thermostatic bath by making the water 0.0005% in sodium nitrite and adding just enough sodium hydroxide to keep the bath faintly alkaline to phenolphthalein. This method of inhibiting corrosion was suggested by Wachter (*9*). Compressed air, filtered through glass wool, was bubbled through the thermostated water sample for 4 hours. Then the air supply was turned off and an interval of 1 hour was allowed to elapse before aliquot portions were taken for analyses.

PROCEDURES

Standardization of Chromium (II) Reagent. A 25 × 80 mm. heat-resistant test tube was fitted with a rubber stopper in which three suitable holes had been made. Two of the holes were for the inlet and outlet of the carbon dioxide stream used to exclude the air. The third hole was the opening through which the delivery tips of the burets could be inserted. The fit of the buret tip was loose, so that carbon dioxide also escaped past the tip when it was in place. A glass stopper closed this third hole

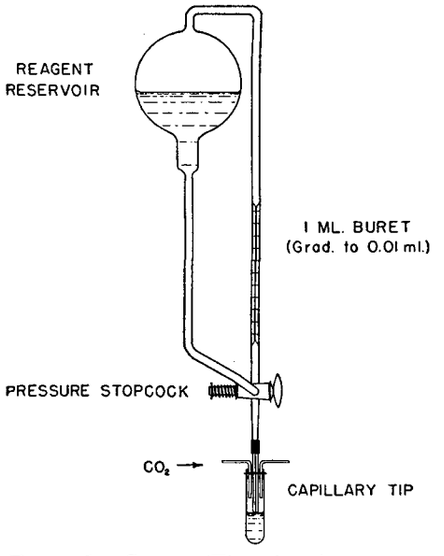

Figure 1. Storage Titration Apparatus

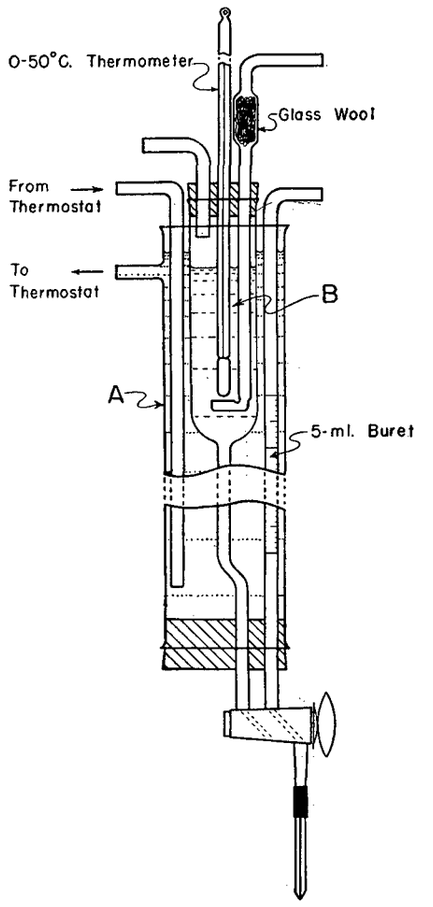

Figure 2. Apparatus for Preparing Water of Standard Oxygen Content

when it was not occupied by a buret tip. A cylinder equipped with a pressure-reducing valve provided carbon dioxide, free from molecular oxygen, for flushing the titration tube and excluding the air during the titrations. The rubber tube from the cylinder to the titration tube was made as short as was convenient. When this was not over 2 feet long, a 2-minute flushing, at a rate of 400 ml. of carbon dioxide per minute, was found sufficient for this work. The flow of gas was regulated by the reducing valve and measured on a calibrated U-tube orifice meter.

After the air had been purged from the titration flask, a measured volume of standard potassium iodate solution was introduced through a hole in the stopper to the titration tube. The 400 ml. per minute flow of carbon dioxide was maintained throughout the purging and titration process. A very low flame from the laboratory burner, with the air ports completely closed, was used to cause the solution to boil quietly. The gentle boiling was continued for about 5 minutes, by which time a fifth of the solution had boiled away. Experiments with varying times showed that further boiling did not change the titration value

Table I. Titrations of Molecular Oxygen in Distilled Water

	Temperature, ° C.						
	15.0 ± 0.2	25.3 ± 0.2	27.2 ± 0.2	27.2 ± 0.2	31.2 ± 0.2	31.8 ± 0.2	38.1 ± 0.2
0.008237 *N*	0.900	0.766	0.739	0.740	0.700	0.690	0.643
$CrCl_2$ required to titrate O_2 in 5.00 ml. of water, ml.	0.905	0.764	0.733	0.738	0.700	0.687	0.643
	0.905	0.765	0.737	0.736	0.700	0.687	0.645
	0.895	0.764	0.738	0.738	0.703	0.688	0.644
	0.897	0.765	0.737		0.702		0.642
	0.893	0.766					
Av.	0.8992	0.7648	0.7380	0.7380	0.7010	0.6880	0.6434
Av. deviation	0.0042	0.0009	0.0015	0.0010	0.0010	0.0010	0.0009
Probable error	0.0016 (0.2%)	0.0003 (0.05%)	0.0007 (0.1%)	0.0005 (0.1%)	0.0005 (0.1%)	0.0005 (0.1%)	0.0005 (0.1%)
Oxygen found, p.p.m.	10.04	8.26	7.90	7.91	7.42	7.26	6.69

In each titration 0.200 ml. of O_2-free KIO_3 (equivalent to 0.1374 ml. of $CrCl_2$) was added.

of the standard. This is in line with the observations of White (*11*) and his coworkers, who found that in their special hydrogen purged and evacuated distilling apparatus, the oxygen was completely removed from aqueous solution when one tenth of the solution had been distilled off. With the volumes used in the standardization, this amount was easily boiled away in 2 minutes. Great care was used during the boiling process to prevent spattering of the solution high up on the walls of the titration tube where the drops could not be easily included in the titration to follow.

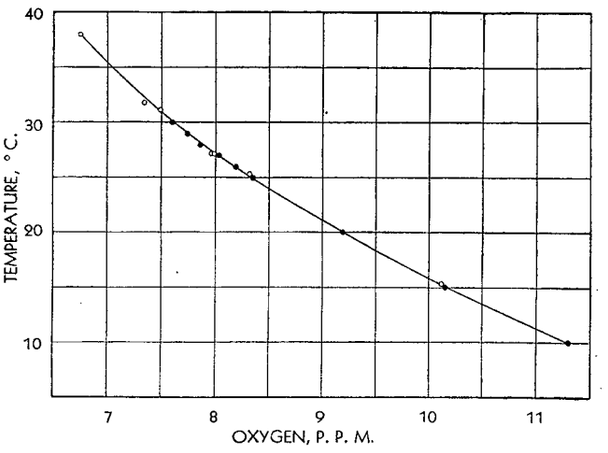

Figure 3. Solubility of Oxygen in Air-Saturated Water at 760 Mm.

● Values accepted by American Public Health Association
○ Values calculated from chromous titration

After the solution and tube had cooled to room temperature, the capillary tip of the buret containing chromous chloride was brought in contact with the surface of the solution and a volume of chromous reagent nearly equivalent to the standard iodate was delivered. Mixing was accomplished by means of a magnetic stirrer, the stirring element consisting of a 10-mm. length of iron wire sealed in glass. Next, a crystal of potassium iodide and a drop of starch indicator were added and the titration was completed by the addition of more chromous chloride solution. As an aid in seeing the end point change under the varying conditions of laboratory daylight, a blue daylight bulb was installed. This resulted in a more nearly uniform lighting of the end point at all times. The end point color change was from the pale blue of the starch-iodide-iodine to the light sparkling green of the dilute chromium (III).

Standardization of Oxygen-Free Potassium Iodate Reagent. This reagent was titrated against the now standardized chromous chloride by following same procedure used for the standardization of the chromous reagent itself, save that no boiling was required. This had already been done in the pressure-storage apparatus prior to the standardization.

Determination of Molecular Oxygen in Water Saturated with Air at Various Temperatures. The method, almost identical with that used for the standardizations previously cited, is here summarized to include points peculiar to the analysis.

The apparatus was purged of air with carbon dioxide flowing at a rate of 400 ml. per minute for 2 minutes. A volume of chromous chloride–hydrochloride acid reagent, which was more than equivalent to the expected oxygen content of the sample, was delivered to the titration tube. Next, the water sample was added from the buret with the capillary tip touching the chromous solution. If the solution being analyzed was at a lower temperature than the reagent, the test tube containing the chromous solution was cooled before the water sample was added. This was done to minimize the possible loss of oxygen due to a rise in temperature before it reached the chromous reagent.

If the water sample being titrated was at elevated temperatures, the titration tube and contents were always cooled to room temperatures before the final step of the titration. This was done because other investigators (*2*, *3*) have shown that starch indicator loses sensitivity rapidly as the temperature rises above 20° C.

After the solutions had been thoroughly mixed with the aid of the magnetic stirrer, a slight excess of oxygen-free standard potassium iodate solution was added and the solution was again stirred. As soon as a single crystal of potassium iodide and a drop of starch indicator had been added, the iodine formed was titrated by the addition of more chromous reagent. Because the excess of iodate was kept small, based on the results of a preliminary titration, the possibility of loss of iodine by volatilization was at a minimum. The end point color change was from a pale blue to the sparkling green.

DISCUSSION

A complete titration can be carried out in 4 or 5 minutes, and a series of four, from which a very accurate value for the oxygen content may be found, can be made in 20 minutes.

This indirect titration procedure was chosen, in preference to the direct titration of the excess acid-chromous reagent with the standard potassium iodate-iodide, because it was found by experiment that the end point was better by the indirect method.

Table I shows the results of the titrations of the molecular oxygen in distilled water saturated with air at various temperatures. These indicate that the reliability of oxygen content values determined by this method is better than 0.2%. Figure 3 graphically compares the results obtained by use of the acid-chromous reagent with those chosen by the American Public Health Association for oxygen in air-saturated water over the same temperature range. These values (*1*) are based on the work of Fox (*5*) as calculated by Whipple and Whipple (*10*).

Fox employed a purely physical method of analysis based on Estreicher's (*4*) adaptation of the method described by Ostwald (*6*). This depended on precise measurements of volume, temperature, and pressure before and after the water was saturated by air. While other sources give somewhat different values, a thorough search of the literature convinced the authors that these values, accepted by the American Public Health Association, were the most reliable.

EFFECT OF NITRITE

Other methods of determining the oxygen dissolved in water have failed in the presence of interfering ions, particularly nitrite.

Table II. Effect of Nitrite

	Approximate Nitrite Concentration							
	2 P.P.M.		10 P.P.M.		20 P.P.M.		20 P.P.M.	
	Before boiling	After boiling	Before boiling	After boiling	Before boiling	After boiling	Before boiling	After boiling
0.008237 N $CrCl_2$ required to titrate O_2 in 5.00 ml. of water, ml.	0.823	0.192	1.000	0.382	1.275	0.588	1.251	0.630
	0.821	0.190	1.000	0.385	1.271	0.592	1.254	0.628
	0.822	0.191	1.000	0.380	1.280	0.590	1.252	0.624
	0.822				1.273			0.629
Av.	0.822	0.191	1.000	0.3817	1.275	0.590	1.2523	0.6283
Difference before and after boiling, ml.	0.631		0.6183		0.685		0.624	
Oxygen found, p.p.m.	8.32		8.15		9.02		8.22	
Oxygen in control, p.p.m.	8.30		8.09		8.94		8.09	
% above control	0.3		0.7		1.0		1.6	

In each case 0.200 ml. of O_2-free KIO_3 (equivalent to 0.1374 ml. of $CrCl_2$) was added.

To determine the applicability of the chromous method to solutions containing nitrite, solutions of sodium nitrite were made up and titrated. A series of titrations on the same solution showed that a constant value was obtained, but the result was significantly higher than that of pure water at the same temperature. It appeared reasonable to believe that the actual oxygen content of the nitrite solution could be found by running one sample by the ordinary procedure and another after boiling off the dissolved gas. The difference between the two titrations would then be equivalent to the oxygen in the original sample. The results of determinations made in this way are shown in Table II. The solutions used were made by adding various volumes of a known sodium nitrate solution to flasks containing distilled water. The flasks were stoppered, shaken well, and allowed to stand for over an hour. Samples were taken in a pipet. Control values were obtained by using a similar procedure on a flask of water containing no nitrite.

It is significant that all the determinations of nitrite-containing water show a higher apparent oxygen content than the control. Perhaps sufficient carbon dioxide dissolves in the solution to raise the hydrogen ion concentration to a point at which an appreciable amount of the nitrite is in the form of nitrous acid when boiled, and is thus lost. It was thought that the addition of a small amount of base before boiling might eliminate this, if the solution were acidified before titrating. When this was tried, basic solutions had a greater tendency to bump, spattering solution on the sides of the test tube. The attempts made with this procedure produced inconsistent results.

LITERATURE CITED

(1) Am. Public Health Assoc., New York, "Standard Methods for the Examination of Water and Sewage," 9th ed., p. 137, 1946.
(2) Bairstow, F., and Wyatt, G. H., *Analyst*, **72**, 340 (1947).
(3) Bond, G. W., *J. S. African Chem. Inst.*, **27**, 25 (1944).
(4) Estreicher, Tadeusz, *Z. physik. Chem.*, **31**, 176 (1899).
(5) Fox, C. J. J., *Trans. Faraday Soc.*, **5**, 68 (1909).
(6) Ostwald-Luther, "Physikochemische Messungen," 2nd Aufgabe, p. 274, Leipzig, W. Engelmann, 1902.
(7) Stone, H. W., ANAL. CHEM., **20**, 747 (1948).
(8) Stone, H. W., and Skavinski, E. R., IND. ENG. CHEM., ANAL. ED., **17**, 495 (1945).
(9) Wachter, A., IND. ENG. CHEM., **37**, 749 (1945).
(10) Whipple, G. C., and Whipple, M. C., *J. Am. Chem. Soc.*, **33**, 362 (1911).
(11) White, A. H., *et al.*, *Proc. Am. Soc. Testing Materials*, **36** (II), 697 (1936).
(12) Winkler, L. W., *Ber.*, **22**, 1773 (1889).
(13) *Ibid.*, **34**, 1410 (1901).
(14) Winkler, L. W., *Z. anal. Chem.*, **28**, 366 (1889).

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Colorimetric Estimation of Various Metal Derivatives of Sodium Diethyldithiocarbamate

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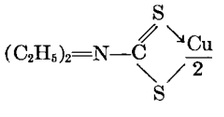
The organic reagent, sodium diethyldithiocarbamate, has been widely used in the colorimetric determination of copper. This investigation was undertaken to show that trace quantities of many other elements can also be accurately determined colorimetrically using this reagent. Bismuth, cobalt, chromium, iron, nickel, and uranium as well as copper can be determined colorimetrically using this reagent and subsequent extraction into a chloroform solution. The extraction is quantitative over a wide range of acidity. Application of this method to the determination of bismuth should be particularly valuable. This work shows that all the elements mentioned will interfere in the determination of copper unless provision is made for their removal; by controlling the acidity of the solution from which the extraction is made, copper can be separated successfully from uranium and chromium.

MOST of the investigations dealing with sodium diethyldithiocarbamate as an analytical reagent have dealt with its application to the analysis of copper; little work has been reported showing that the colored solutions obtained with other metals obey Beer's law. The object of this paper is to investigate the use of sodium diethyldithiocarbamate as a color-forming complex, and to study its application to the colorimetric determination of various elements.

In 1908 Delepine (*4*) published a paper describing the properties of methyldithiocarbamates, including the copper compounds, and in the same year (*3*) he published a paper on the detection of copper and iron with a solution of dialkyl dithiocarbamate.

Sodium diethyldithiocarbamate reacts with solutions of copper salts to give a brown color or precipitate. The compound formed in this reaction has been assigned the following formula (*6*).



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In very dilute copper solutions, a colloidal suspension is formed which is suitable for a colorimetric comparison (*1*). Gum tragacanth, gelatin (*8*), and gum arabic (*7*) may be used as protective colloids to prevent coagulation of the colloidal precipitate. The inherent difficulties of such a method may be eliminated, however, by application of the fact that the copper diethyldithiocarbamate, though insoluble in water, is soluble in a number of organic liquids, such as amyl alcohol, amyl acetate, bromobenzene, carbon tetrachloride, and chloroform. Hence the colored copper complex can be extracted with a solvent which is immiscible with water and subsequently measured in a photometer or color-matching apparatus. Further, by using the extraction method, the sensitivity of the reaction is increased, and the interference by certain colored ions can more readily be prevented (*11*).

The last reference cited lists 69 references dealing with applications of sodium diethyldithiocarbamate to analytical methods. Practically all of these methods are concerned with the determination of copper in such materials as sea water, milk and dairy products, blood and biological materials, fabrics, dyes, rubber, and both ferrous and nonferrous alloys. Callan and Henderson (*1*) studied the colorimetric determination of copper, and in addition found that sodium diethyldithiocarbamate forms precipitates with aluminum, antimony, barium, bismuth, cadmium, calcium, chromium, cobalt, iron, lead, magnesium, manganese, mercury, nickel, silver, tin, titanium, uranium, and zinc. Consequently the use of the reagent was extended to include the determination of some of these metals. However, not all of these precipitates are colored, and the methods developed were not colorimetric. In fact, even though the cobalt diethyldithiocarbamate is colored, Scacciati (*9*) extracted it with chloroform and proceeded to determine that metal gravimetrically.

In more recent work performed by Chernikhov and Dobkina (*2*) the qualitative reactions of sodium diethyldithiocarbamate with various metals were reaffirmed. The list of Callan and Henderson was expanded to include molybdenum, selenium, tellurium, vanadium, indium, gallium, thallium, rhenium, and tungsten. However, no indications were made concerning application of the reactions to colorimetric determinations.

QUALITATIVE STUDY

Reagents. 1. Buffer Solutions. pH 3.7, 120 ml. of glacial acetic acid and 27 grams of ammonium acetate were dissolved in a total volume of 1 liter. pH 7.2, the pH 3.7 buffer was adjusted to a pH of 7.2 by the addition of ammonium hydroxide. pH 9.5, 200 grams of ammonium acetate and 70 ml. of concentrated ammonium hydroxide were dissolved in a total volume of 1 liter.

2. Metal Ion Solutions. The theoretical amount of reagent grade salt required to give a concentration of 5 mg. of metal ion per ml. was dissolved in a total volume of 250 ml.

3. Reagent. Sodium diethyldithiocarbamate (2 grams, Eastman Kodak) was dissolved in 100 ml. of distilled water.

4. Organic Solvents. Reagent grade chloroform and carbon tetrachloride and C.P. grade amyl acetate were used.

Experimental Procedure. One milliliter of a solution containing 5 mg. of metal ion per ml., and 1 ml. of a 2% solution of sodium diethyldithiocarbamate were added to each of three 125-ml. separatory funnels. Twenty milliliters of each of the buffers with pH values of 3.7, 7.2, and 9.5 were poured into the first, second, and third funnels, respectively. The mixtures were shaken and observed for any colored precipitates. Chloroform (10 ml.) was then added to each funnel and the mixtures were shaken. The aqueous and chloroform layers were then observed to see whether the chloroform had dissolved any precipitate.

This procedure was repeated using carbon tetrachloride and then amyl acetate as solvents for extraction. The whole procedure was also repeated without adding a solution of metal ion to be sure that any colored solutions formed with the solvents when the metal ion was added were not due to the presence of the reagent alone.

Results. The following ions did not yield a precipitate or a color in any of the organic solvents at the three pH's mentioned: As^{+++}, As^{+++++}, Ce^{+++}, Cr^{+++}, Pt^{++++}, or Ru^{++++}.

Table I. Color of Metal Complexes in Chloroform

Ion	Color	Ion	Color
Bi^{+++}	Yellow	Mo^{++++++}	Red
Co^{++}	Green	Ni^{++}	Yellow-green
Cu^{++}	Brown	Sn^{++}	Orange
Cr^{++++++}	Green	Sn^{++++}	Orange
Fe^{++}	Brown	U^{++++++}	Reddish brown
Fe^{+++}	Brown		

The following ions yielded white or cream-colored precipitates but no color in any of the organic solvents at the three pH's mentioned: Sb^{+++}, Cd^{++}, Ir^{++++}, Hg^{+}, and Hg^{++}.

The ions listed in Table I yielded the same color in all three of the organic solvents used with the exception of the uranium and molybdenum complexes which were insoluble in carbon tetrachloride.

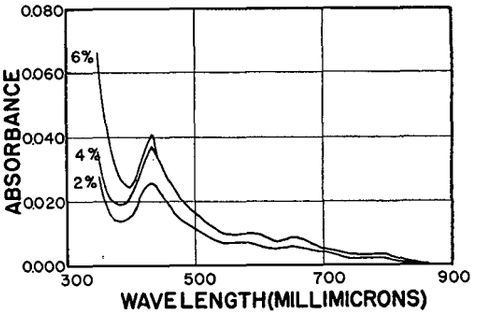

Figure 1. Absorption Spectra of Various Concentrations of Sodium Diethyldithiocarbamate in Chloroform

The amyl acetate extractions would involve some inconvenience in an analytical method because this solvent forms the upper layer when in contact with water. Therefore, chloroform was chosen as the solvent for all subsequent work because of its more universal solvent action for the diethyldithiocarbamates investigated.

QUANTITATIVE STUDY

The same reagents were used with the exception of the buffer solution which was a solution of ammonium acetate and acetic acid of pH 6.3.

The standard metal ion solutions were made to contain 1.00 mg. of metal ion per ml. of solution using an accurately weighed quantity of an appropriate salt according to Welcher (*10*). These solutions were further checked using an appropriate gravimetric or volumetric procedure.

The spectrophotometric measurements were made in 1-cm. cells using a Beckman spectrophotometer, Model DU, and the pH measurements were made using a Beckman pH meter, Laboratory Model G.

Absorption Spectrum of Sodium Diethyldithiocarbamate in Chloroform. Before measuring the absorption spectra of the various metal complexes in chloroform, it was necessary to determine the absorption spectrum of the reagent.

One milliliter of a 2% solution of sodium diethyldithiocarbamate was added to a 125-ml. separatory funnel; 2 and 3 ml. of the same solution were added to a second and a third funnel, respectively, making solutions which were equivalent to 4 and 6% solutions of sodium diethyldithiocarbamate. A buffer of pH 6.3 (10 ml.) was added to each funnel, and the aqueous solutions were extracted three times with 15-ml. portions of chloroform. The chloroform layers were withdrawn through 9-cm., No. 41 Whatman filter papers into 50-ml. volumetric flasks. The solutions in the flasks were diluted to the mark, and the absorption spectra were measured from 350 to 1000 mμ.

Figure 1 indicates that a maximum occurs at 425 mμ, but that even at a concentration of 6% sodium diethyldithiocarbamate, the absorbancy at this maximum is very low. It was decided to use a concentration of 4% (2 ml. of 2%) of the reagent in all subsequent work because, at this concentration, the blank is

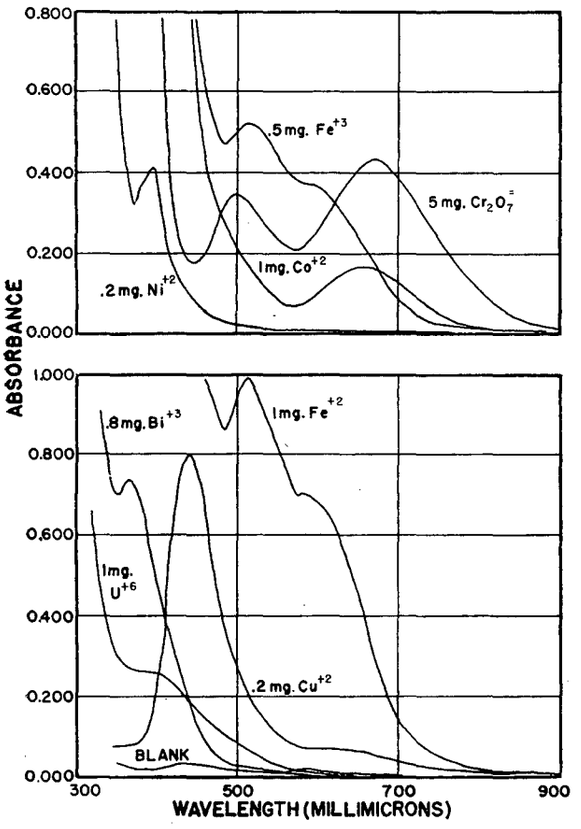

Figure 2. Absorption Spectra of Metal Diethyldithiocarbamates

sufficiently low. Theoretical calculations, based on the formula assigned the copper diethyldithiocarbamate by Dubsky (*6*), indicate that this concentration is 30 times the amount necessary to react with the maximum amount of copper used. Even assuming a difference in the nature of the other complexes, this was thought to be a sufficient excess of reagent. Such proved to be the case except in the extraction of the dichromate ion, when it became necessary to use 4 ml. of 2% sodium diethyldithiocarbamate solution for complete precipitation of the complex.

ABSORPTION SPECTRA OF VARIOUS METAL DIETHYLDITHIOCARBAMATES IN CHLOROFORM

Previous work with sodium diethyldithiocarbamate has not produced complete spectral data for solutions of the metal complexes other than that of copper. Drabkin (*5*) obtained some spectral data for nickel, cobalt, and bismuth while studying the interference of these ions with the copper determination. His data, however, were obtained over the wave-length range of 420 to 660 mμ and were not complete enough for application to determinations of these metals. In the present investigation, the absorption spectra of the diethyldithiocarbamates of the ions listed in Table I were measured over a wave-length range of 350 to 1000 mμ.

Experimental Procedure. The proper concentration of metal ion to be used was determined experimentally. This was done by first extracting a solution containing 1 mg. of metal ion and measuring the absorbancy. The concentration was then adjusted to keep the intensity of color at such value that the maximum absorbancy would not be above 0.800 on the spectrophotometer scale. This value was chosen as the upper limit because it represents the approximate limit of the best sensitivity range of the Beckman instrument.

A 2% solution of sodium diethyldithiocarbamate (2 ml.), and 10 ml. of a buffer of pH 6.3 were added to the appropriate amount of metal ion solution in a 125-ml. separatory funnel. Two extractions with 20-ml. portions of chloroform were used in all cases, inasmuch as two extractions gave as complete extraction as three. The chloroform layer was withdrawn into a 50-ml. volumetric flask through a 9-cm., No. 41 Whatman filter paper, and diluted to the mark. The absorbancy of the solution was measured from 350 to 1000 mμ.

The absorption spectra are shown in Figure 2. The wave lengths of maximum absorbancy are shown in Table II.

Table II. Wave Lengths of Maximum Absorbancy

Ion	Wave Length, mμ	Ion	Wave Length, mμ
Bismuth	370	Ferric	515
Cobaltous	650	Ferrous	515
Cupric	440	Nickelous	395
Dichromate	500 and 670[a]	Uranyl	390

[a] This wave length was used in all subsequent work.

During this part of the investigation, it was observed that the colored chloroform solutions of the tin and molybdenum diethyldithiocarbamates faded rapidly. Consequently, the absorption spectra for these complexes could not be accurately measured.

The chloroform solutions of the other complexes were measured at the wave length of maximum absorbancy over a period of 3 hours and no deviations from the original absorbancy readings were observed. The nickel, bismuth, and uranium complexes were stable over a 24-hour period. The absorbancies of the other complexes were not measured after 3 hours.

Although the absorption spectra for the nickel and cobalt complexes seem to indicate the possibility of determining cobalt in the presence of nickel, a subsequent investigation, using a quantity of nickel salt solution equivalent to 5 mg. of nickel, showed that the nickel complex also absorbs slightly at a wave length of 650 mμ.

The chloroform solution of the uranium complex does not actually have an absorption maximum at 390 mμ. However, there is a sufficiently broad absorption plateau in the region of this wave length to permit reproducible absorption measurements.

EFFECT OF pH ON EXTRACTIONS OF SODIUM DIETHYLDITHIOCARBAMATE COMPLEXES

The effect of the pH on the extractions of various metal complexes of sodium diethyldithiocarbamate was studied for the metals listed in Table II.

Experimental Procedure. Buffer solutions with pH values from 0.1 to 9.5 were prepared. This was done by adjusting buffers of a pH 3.7, 7.2, and 9.5 with either ammonium hydroxide or acetic acid to obtain the desired pH value. A buffer solution of hydrochloric acid and potassium chloride was used for extractions at a pH of 0.1 to 1.0.

A sufficient quantity of metal ion solution was added to a 125-ml. separatory funnel. The quantity was determined, as previously explained, to keep the absorbancy below 0.800. Two milliliters of a 2% sodium diethyldithiocarbamate solution were added, except in the dichromate investigation which required 4 ml. Ten milliliters of the desired buffer solution were added, and the complex was extracted twice with 20-ml. portions of chloroform. The chloroform was withdrawn through a 9-cm., No. 41

Whatman filter into a 50-ml. volumetric flask and diluted to the mark.

The absorbancy of the chloroform solution was measured at the wave length of maximum absorbancy indicated in Table II. The results are shown in Figure 3.

The curves which show the effect of the pH on the extractions of the complexes indicate that only the uranium complex is not extracted in acid solutions and, therefore, would not interfere with those complexes which extract at low pH values. The complex formed with the dichromate ion extracts well up to a pH of 6.3 and decreases rapidly at higher pH values. The other complexes are extracted almost quantitatively over the entire pH range which was investigated; only the copper complex shows a slight decrease at about pH 4.0. It might be possible, however, to effect a separation of the complexes by studying the extractions at higher acidities.

Beer's Law Relationships. The purpose of this part of the investigation was to determine if the chloroform solutions of the colored diethyldithiocarbamate complexes adhered to Beer's law of linear proportionality between absorbancy and concentration.

Experimental Procedure. Three different quantities of metal ion solution were added to three 125-ml. separatory funnels. These quantities were so chosen that the absorbancy reading would be approximately equally spaced over the absorbancy range from 0.000 to 0.800. Two milliliters of 2% sodium diethyldithiocarbamate were added, except in the dichromate investigation which required 4 ml. A 10-ml. portion of the optimum pH buffer (as noted in Figure 3) was added, and the complexes were extracted twice with 20-ml. portions of chloroform. The chloroform was withdrawn through a 9-cm., No. 41 Whatman filter into a 50-ml. volumetric flask and diluted to the mark. The absorbancy of each solution was measured at the wave length of maximum absorbancy.

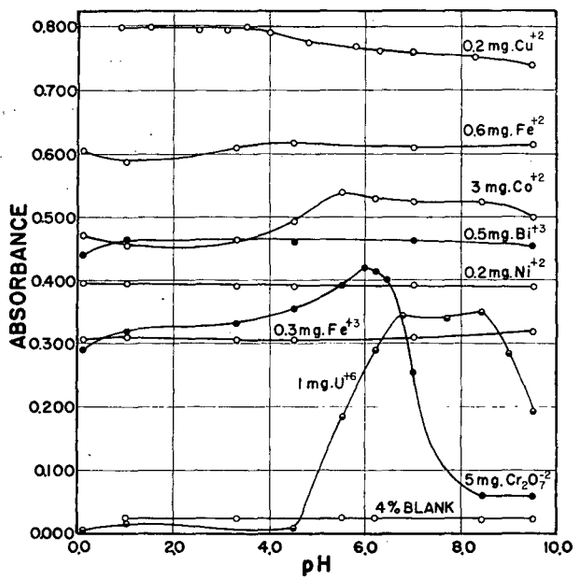

Figure 3. Effect of pH of Aqueous Phase on Extractions of Diethyldithiocarbamate Complexes

The results are shown in Figure 4. These plots have not been corrected for the blank (Figure 1).

The linear relationships of the absorbancy *vs.* concentration curves indicate that the bismuth, cobaltous, cupric, dichromate, ferric, ferrous, nickelous, and uranyl ions can be colorimetrically determined by using sodium diethyldithiocarbamate as a reagent and chloroform as the extracting solvent.

Both the ferrous and ferric complexes were measured but the points were so similar that only one straight line is shown in Figure 4 for both Fe^{++} and Fe^{+++}.

The data in Figure 4 show that the reagent is most sensitive for copper, whereas it is least sensitive for the dichromate ion. The sensitivity of the reagent for the various ions arranged in the order of decreasing sensitivity is as follows: cupric, nickelous, ferric and ferrous, bismuth, uranyl, cobaltous, and dichromate.

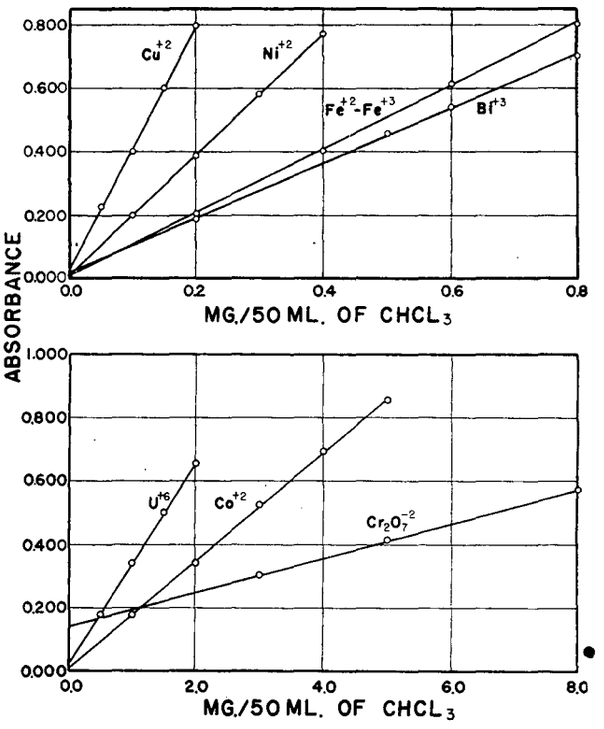

Figure 4. Beer's Law Relationships for Various Metal Diethyldithiocarbamate Complexes

The data presented show that trace quantities of these elements can be quantitatively determined using sodium diethyldithiocarbamate as a color-forming reagent—for example, the determination of bismuth by this method should be equal or superior to existing methods. Furthermore, in using this reagent for copper with present procedures, serious interferences from these same elements can be expected unless provision is made for their removal.

LITERATURE CITED

(1) Callan, T., and Henderson, J., *Analyst*, 54, 650–3 (1929).
(2) Chernikhov, Y., and Dobkina, B., *Zavodskaya Lab.*, 15, 1143-9 (1949).
(3) Delepine, M., *Bull. soc. chim.*, [3] 4, 652–4 (1908).
(4) Delepine, M., *Compt. rend.*, 146, 981–4 (1908).
(5) Drabkin, D. L., *J. Assoc. Offic. Agr. Chemists*, 22, 320 (1939).
(6) Dubsky, J. V., *Mikrochemie ver. Mikrochim. Acta*, 28, 145-72 (1940).
(7) Hoar, T. P., *Analyst*, 62, 657–61 (1937).
(8) Moseley, H. W., Rohwer, A. G., and Moore, M. C., *Science*, 79, 507–8 (1934).
(9) Scacciati, G., *Chimica e industria (Milan)*, 17, 592–4 (1935).
(10) Welcher, F. J., "Chemical Solutions," New York, D. Van Nostrand Co., 1942.
(11) Welcher, F. J., "Organic Analytical Reagents," Vol. 4, p. 84, New York, D. Van Nostrand Co., 1948.

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Spectrophotometric Determination of Nickel in Steel

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This investigation was conducted for two purposes: to develop a method that would provide consistently good color stability, and to establish the nature and degree of interference of copper and manganese. This paper defines the range of ratios of manganese and copper to nickel which can be tolerated in the sample solution, and it presents a simple method of correcting for the interference. The description of the retarding effect of manganese on color development, which may lead to serious error in the determination of nickel at trace levels in steel, should constitute a warning to those who may be inclined to ignore its presence. Comparative data show that this spectrophotometric method is superior to the familiar gravimetric and titrimetric methods from the standpoint of precision as well as speed.

THE spectrophotometric method, presented here, is a modification of one published by Haywood and Wood (*7*). Although the reagents used are essentially the same, the order of addition has been changed, with resultant improvement in stability. Specifically, this method is characterized by the oxidation of nickel in the presence of dimethylglyoxime, the iodine solution being added to the ammoniacal solution of the sample. The absorbancy of the nickel compound, when it is formed in the absence of other metallic elements, reaches a maximum within 2 or 3 minutes and remains constant for at least 25 minutes.

In further contrast to work that has been described previously (*8–10*), this investigation comprises an advance in the study of interferences. Hence, it is shown that the degree of stability (constancy of the absorbancy with time) characteristic of solutions containing only nickel is not necessarily a measure of the stability when nickel is associated with certain other elements. Because in the analysis of steel it is generally impractical, and in some cases impossible, to isolate the nickel quantitatively, it must be determined in the presence of the other constituents of the alloy. Among these are invariably manganese and almost inevitably copper. These elements may seriously affect both the constancy and the magnitude of the absorbancy.

REAGENTS

Sulfuric-phosphoric acid mixture. Add 150 ml. of sulfuric acid (specific gravity 1.84) and 150 ml. of phosphoric acid (85%) to 500 ml. of water, cool, and dilute to 1 liter.

Ammonium citrate solution. Dissolve 540 grams of ammonium citrate in water and dilute to 1 liter.

Ammoniacal dimethylglyoxime solution. Dissolve 1 gram of dimethylglyoxime in 500 ml. of ammonium hydroxide (specific gravity 0.90) and dilute to 1 liter.

Ammonium hydroxide (1 to 1). Dilute one volume of ammonium hydroxide (specific gravity 0.90) with one volume of water.

Iodine solution (0.02 *N*). Dissolve 8 grams of potassium iodide in a minimum of water, add 2.6 grams of iodine, dissolve, and then dilute to 1 liter.

Standard nickel solution, 1.000 mg. of nickel per ml. Dissolve 1.000 gram of pure (Hilger) nickel in 15 ml. of nitric acid (specific gravity 1.42) (supplied by the Adam Hilger Co., Ltd., London, England), add 10 ml. of sulfuric acid (specific gravity 1.84), and evaporate to fumes. Cool, add 50 ml. of water, and digest until salts are dissolved, and then cool and dilute to 1 liter.

Standard nickel solution, 0.0100 mg. of nickel per ml. To 10.00 ml. of the standard nickel solution containing 1.000 mg. of nickel per ml. add approximately 10 ml. of sulfuric acid (1 to 1) and dilute to 1 liter. Use this solution for calibration involving the 1-cm. cells.

Standard nickel solution, 0.0020 mg. of nickel per ml. To 100.0 ml. of the standard nickel solution containing 0.0100 mg. of nickel ml. add approximately 5 ml. of sulfuric acid (1 to 1) and dilute to 500 ml. Use this solution for calibration involving the 5-cm. cells.

Standard copper solution, 0.100 mg. of copper per ml. Prepare according to the method described for the standard nickel solution (1.000 mg. per ml.) using 0.1000 gram of pure (Hilger) copper.

Standard manganese solution, 0.30 mg. of manganese/ml. Dissolve 0.3000 gram of pure (Hilger) manganese in 15 ml. of nitric acid (specific gravity 1.42), add 50 ml. of water, boil 5 minutes, cool, and dilute to 1 liter.

PROCEDURE

Transfer the sample to a borosilicate glass volumetric flask, add sulfuric-phosphoric acid mixture, and heat on the steam plate until action ceases. The sample size, capacity of the volumetric flask, and volume of the acid mixture should be correlated according to the following considerations:

The aliquot of the solution of the sample should not exceed 25 ml.; a 20-ml. aliquot is preferable.

The aliquot should represent not more than 50 mg. of sample; if the percentage of manganese is greater than 0.9, the maximum sample may be calculated as 45/% manganese.

The apparent percentage of nickel, uncorrected for manganese interference, must be at least 0.0277 × % manganese.

The aliquot should contain approximately 0.5 ml. of the sulfuric-phosphoric acid mixture.

The sample weight should be great enough to preclude a significant error in weighing.

Add nitric acid (specific gravity 1.42) dropwise to the hot solution, while swirling the flask, until the iron is oxidized. Complete oxidation of the iron is indicated by a sharp increase in the rate of evolution of oxides of nitrogen. Add a few drops of nitric acid in excess and heat the solution to boiling. Remove the flask, add about 50 ml. of water, and boil the solution for a few minutes to remove oxides of nitrogen. Cool, dilute to volume, mix, and transfer identical aliquots to each of two 50-ml. volumetric flasks.

To one of the aliquots add the following reagents:

1. 5 ml. of ammonium citrate solution. Mix well to assure complete complexing of the iron and other elements which precipitate in the presence of excess ammonium hydroxide.
2. 10 ml. of ammonium hydroxide (1 to 1). Mix.
3. 5 ml. of iodine solution (0.02 *N*). Agitate the solution rather vigorously during the addition and for about 5 seconds. Quickly rinse down the neck of the flask with water, then continue the agitation for an additional 10 seconds in order to effect complete removal of nitrogen which is formed in the reaction between iodine and ammonium hydroxide. This solution constitutes a reference solution.

To the other aliquot add the following reagents:

1. 5 ml. of ammonium citrate solution. Mix well.
2. 10 ml. of dimethylglyoxime solution. Mix mildly (excessive mixing may cause precipitation of unoxidized nickel dimethylglyoxime).
3. 5 ml. of iodine solution (0.02 *N*). Introduce the iodine as soon as possible after the addition of dimethylglyoxime in order to avoid precipitation of unoxidized nickel dimethylglyoxime. Agitate the solution and rinse the neck of the flask as described above for the preparation of the reference solution.

Dilute the solutions to volume and mix thoroughly.

Measure the transmittancy of the test solution, 10 minutes after the addition of iodine, against the reference solution at the wave length of 540 mμ and slit setting of approximately 0.02 mm. for the

Beckman spectrophotometer. For other instruments, maintain conditions established in calibrating.

Calculate the absorbancy, A (log $1/T$), apply the factor (mg. Ni/A) found in calibrating the spectrophotometer, and then calculate the percentage of nickel on the basis of the number of milligrams of sample represented by the aliquot of the sample solution. Correct the percentage nickel value for manganese by subtracting F (Mn) × % Mn, and for copper by subtracting F (Cu) × % Cu. The latter is not significant, as a rule. [The values of the factors, F (Mn) and F (Cu), must be determined according to methods described below. This may be especially important if instruments other than the Beckman are used.]

CALIBRATION OF SPECTROPHOTOMETER

The standard nickel solution containing 0.0100 mg. of nickel per ml. is appropriate for the calibration involving the 1-cm. cells. Using 5- to 25-ml. pipets, transfer identical aliquots to each of two 50-ml. volumetric flasks. Add 0.5 ml. of sulfuric-phosphoric acid mixture to each solution, and at each of the five levels of nickel concentration (Table I) prepare one solution as a reference and the other as a test solution as previously described. Measure the transmittancy at the wave length of 540 mμ for the Beckman spectrophotometer, and calculate the absorbancy (log $1/T$).

Follow the same procedure for the calibration involving the 5-cm. cells, using the standard nickel solution containing 0.0020 mg. of nickel per ml. (see Table II). Determine the reagent blank by treating in a similar manner approximately 20 ml. of distilled water in each of two 50-ml. volumetric flasks. Subtract the reagent blank, calculated in terms of its absorbancy value, from the absorbancy value found at each level of nickel concentration.

For each set of data calculate the ratio, mg. Ni/A, at each nickel level. Calculate the average value of the ratio, thus obtaining the factor to use to convert absorbancy to milligrams of nickel. Weighting the values according to the magnitude of the absorbancy is recommended, because it tends to reduce the significance of the less reliable measurements characteristic of the low absorbancy values.

Typical calibration data are shown in Tables I and II.

COPPER INTERFERENCE IN DETERMINATION OF NICKEL IN STEEL

The behavior of copper alone when treated according to the method for developing the soluble nickel dimethylglyoxime is different from its behavior in the presence of nickel. Solutions containing copper alone exhibit a rather high absorbancy initially, diminishing with time as shown in Figure 1. Each curve is based upon the measurement of the absorbancy of a solution of copper in the presence of dimethylglyoxime against a reference solution containing an identical amount of copper. The sample and reference solutions were treated according to the procedure previously described; each solution contained 0.5 ml. of the sulfuric-phosphoric acid mixture prior to the addition of the reagents. Time measurement was started when the sample solution was agitated.

In Figure 2 is shown a family of curves resulting from plotting absorbancy (1-cm. cells) against time. These curves indicate a relatively stable condition during the first 10 minutes. As time

Table I. Calibration Data for 1.000-Cm. Cells

Mg. Ni/50 Ml.	Absorbancy	Mg. Ni/A	Weighted Av., Mg. Ni/A
0.0600	0.125	0.4800	
0.1197	0.251	0.4770	
0.1797	0.376	0.4780	
0.2397	0.499	0.4805	
0.2990	0.622	0.4810	0.4795

Table II. Calibration Data for 5.000-Cm. Cells

Mg. Ni/50 Ml.	Absorbancy	Mg. Ni/A	Weighted Av., Mg. Ni/A
0.01200	0.123	0.0975	
0.02393	0.249	0.0961	
0.03595	0.377	0.0954	
0.04795	0.499	0.0961	
0.05980	0.623	0.0960	0.0960

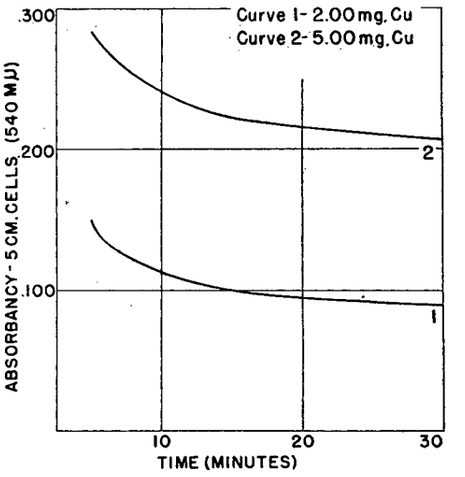

Figure 1. Effect of Copper and Dimethylglyoxime

progresses, however, fading is definitely indicated. Solutions containing only copper fade excessively during the first 10 minutes, and then become stable. This, perhaps, indicates that copper reacts with dimethylglyoxime to form decomposition products, which, although having little effect on the absorbancy due to copper, cause instability of the nickel compound. Further investigation in this direction would prove to be largely of academic interest.

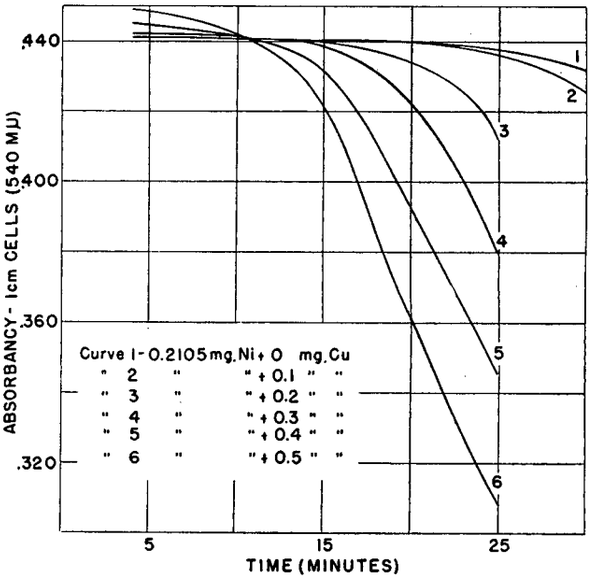

Figure 2. Effect of Copper

From the practical standpoint, according to Figure 2, it may be concluded that a copper to nickel ratio of approximately 2.5 to 1 may be tolerated without significant error, provided the absorbancy is measured 10 minutes after the sample solution is agitated. Measurements made earlier may lead to slightly high results, while those made after 10 minutes may be seriously low.

The curves shown in Figure 3 indicate that, by reducing the concentration of nickel to a level which permits the use of the 5-cm. cells, the ratio of copper to nickel may be extended to 12.5 to 1. Within these limits the added copper effects an orderly in-

crease in the observed absorbancy. The data shown inset in Figure 3 support this statement, and constitute calibration data on which the magnitude of the copper interference was established in this investigation. For convenience the copper correction was evaluated in terms of percentage nickel according to the following equation: % nickel = 0.0038 × % copper. In other words, if the presence of 1% copper in the steel sample were ignored, the nickel value would be high by 0.0038. Because a steel sample containing a high percentage of copper is seldom encountered, the copper correction is usually small.

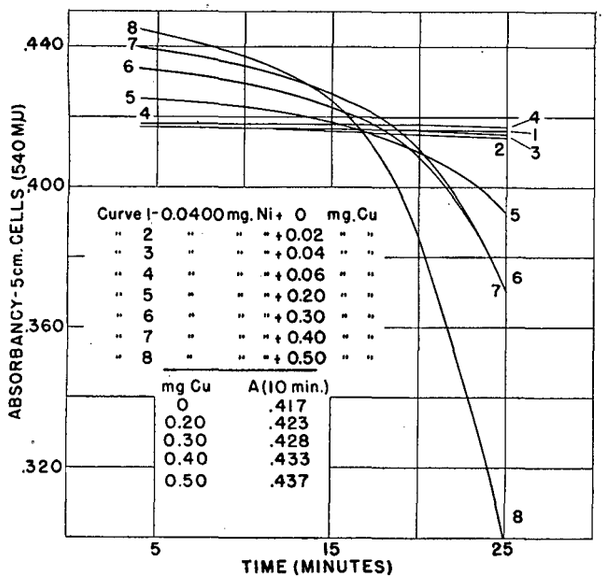

Figure 3. Effect of Copper

However, erratic values for nickel may be obtained, due to induced fading, if the ratio of copper to nickel exceeds the limits defined previously.

EVALUATION OF COPPER INTERFERENCE

Although the method of evaluating the copper interference has been implied in the preceding discussion, it is considered advisable to include the following outline.

Transfer 20.00 ml. of the standard nickel solution (0.04 mg. of nickel) containing 0.0020 mg. of nickel per ml. to each of two 50-ml. volumetric flasks. Add 5.00 ml. of standard copper solution (0.1000 mg. of copper per ml.) to each, followed by 0.5 ml. of sulfuric-phosphoric acid mixture. Prepare one as a reference solution and the other as a test solution as previously described. Measure the transmittancy in 5-cm. cells 10 minutes after the solution is agitated. Calculate the absorbancy (log $1/T$), subtract the absorbancy found for 0.0400 mg. of nickel in calibrating, and record the difference as the increase in absorbancy, A (Cu), due to 0.50 mg. of copper.

Calculations. Mg. Ni/A $_{(5\text{ cm.})}$ × $\frac{A\ (\text{Cu})}{0.50}$ = copper correction in terms of per cent nickel, F (Cu).

INTERFERENCE OF MANGANESE

The interference of manganese in the determination of nickel by spectrophotographic methods involving dimethylglyoxime is mentioned by the American Society for Testing Materials (*1*). In a footnote it is suggested that the effect of manganese may be compensated for by using a standard of similar manganese content. With the purpose of overcoming this objectionable feature, the following study of the effect of manganese was made.

Using the approach described in the study of the copper interference, aliquots of a standard nitric acid solution of pure (Hilger) manganese were added to aliquots of standard nickel solution. Duplicates were prepared, one as a reference, and the other as a test solution in each case. A time study of the effect of varied amounts of manganese on the constancy of the absorbancy and its magnitude was made at eight levels of nickel, four of which involved the 5-cm. cells and four the 1-cm. cells. An example is shown in Figure 4.

The interference of copper dictates that readings must be taken 10 minutes after preparing the sample solution. Therefore, in order to correlate the increase in absorbancy due to manganese, with manganese concentration, the range, in this case, is established to be between 0 and 0.9 mg.

SAMPLE SIZE LIMITATIONS

In regard to the behavior of manganese at the nickel levels for which the 1-cm. cells are appropriate, it was found that above a certain critical ratio of manganese to nickel a precipitate forms and precludes the possibility of reliable absorbancy measurement.

Precipitation may occur when nickel is determined in the presence of more than 50 mg. of iron. This limitation is not serious, because percentages of nickel, as low as 0.01%, may be determined satisfactorily by utilizing the 5-cm. cells.

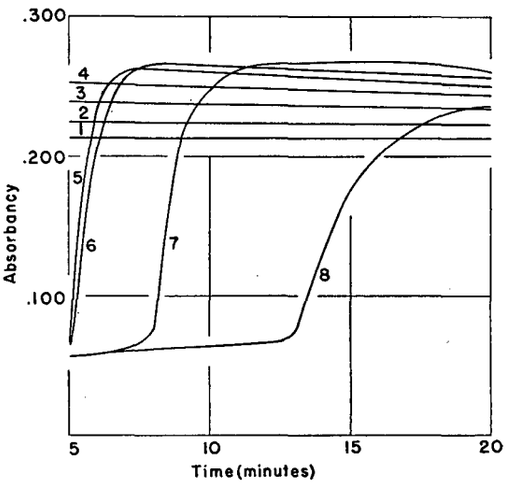

Figure 4. Effect of Manganese

Curve	0.02 mg. of nickel plus: Mn, Mg
1	0.0
2	0.2
3	0.4
4	0.6
5	0.8
6	0.9
7	1.0
8	1.1

Figure 5 summarizes the study of the manganese interference. The maximum amount of manganese which may be present with a given amount of nickel determines the position of each of the eight points shown. The tabular data of Figure 5 express the relationship in terms of percentage nickel and manganese based on a 50-mg. sample.

In planning the method for recommendation to the routine laboratory, the practical approach has been adopted. Thus it was considered advisable to fix the maximum sample size at 50 mg., although there are indications that larger samples might be permitted in certain isolated cases, so far as the effect of iron is concerned. The conclusions which are reached in the following summary of sample size limitations imposed by manganese were influenced by the same practical considerations. At the two ex-

tremes of the nickel range investigated, 0.02 to 0.25 mg. (Figure 5), the maximum amount of manganese that may be tolerated is 0.45 mg. Between these extremes less serious restrictions are shown to exist. Because reliable advance information in regard to the approximate percentage of nickel in a given steel is not always available, the aim of the following guide is to avoid false starts and the resultant loss of efficiency.

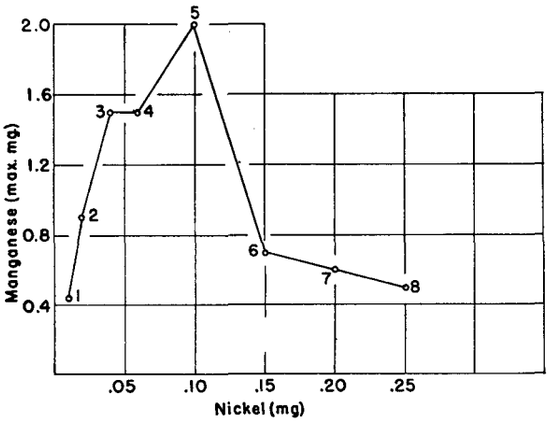

Figure 5. Limits of Per Cent Manganese

Point	50-Mg. Samples Ni, %	Mn (Max.), %
1	0.02	0.9
2	0.04	1.8
3	0.08	3.0
4	0.12	3.0
5	0.20	4.0
6	0.30	1.4
7	0.40	1.2
8	0.50	1.0

Assuming that the sample solution must contain not more than 0.45 mg. of manganese, the maximum permissible sample size may be calculated as 45/% manganese in the steel sample. If, however, the ratio of manganese to nickel is less than 45 to 25, or if % nickel/% manganese is greater than 0.55, the sample size is governed solely by the nickel content of the alloy and may be calculated as 25/% nickel. The maximum sample size is 50 mg. in any case.

The possible interference of manganese in the development of the oxidized nickel compound, where the ratio of manganese to nickel is greater than 45 to 1, dictates that a minimum amount of nickel must be provided. If the absorbancy value indicates that the percentage of nickel in the alloy, when corrected for manganese, is equal to 0.0222 × % manganese, the nickel value may be considered to be reliable. (The apparent percentage nickel, uncorrected, must be at least 0.0277 × % manganese.) If the nickel value falls below this level, the ratio must be adjusted by adding a known amount of nickel to the test solution. In case it is necessary to make the standard addition, it must be considered in the final calculation of percentage nickel in the alloy.

EVALUATION OF MANGANESE INTERFERENCE

Transfer 20.00 ml. of the standard nickel solution (0.0400 mg. of nickel) containing 0.0020 mg. of nickel per ml. to each of two 50-ml. volumetric flasks. Add 5.00 ml. of the standard manganese solution (0.30 mg. of manganese per ml.) to each, followed by 0.5 ml. of sulfuric-phosphoric acid mixture. Prepare one as a reference solution and the other as a test solution as previously described. Measure the transmittancy in 5-cm. cells 10 minutes after the solution is agitated. Calculate the absorbancy (log $1/T$), subtract the absorbancy found for 0.0400 mg. of nickel in calibrating, and record the difference as the increase in absorbancy, A (Mn), due to 1.5 mg. of manganese.

Calculations. Mg. $\mathrm{Ni}/A_{(5\ \mathrm{cm.})} \times \frac{A\ (\mathrm{Mn})}{1.5}$ = manganese correction in terms of per cent nickel, F (Mn).

One per cent manganese has been found to be equivalent to 0.0057% nickel.

Table III. Composition of Steel Samples

Sample No.	Mn	Cu	Ni	Si	Cr	Mo	Sn	V
2-1	1.58	0.04	...	0.262	0.044	0.0078	0.005	0.0015
1-1	1.42	0.083	...	0.102	0.212	0.0525	0.0114	....
1-4	0.91	0.176	...	0.362	0.515	0.199	0.0362	....
3-7	0.968	...	...	0.392	1.18	0.079	....	0.112
NBS 32C	0.752	0.099	1.20	0.281	0.654	0.063	....	0.003
NBS 111	0.662	0.122	1.75	0.292	0.272	0.215	....	0.003
NBS 100	1.38	0.124	0.151	0.191	0.042	0.006	....	
NBS 11e	0.451	0.105	0.045	0.316	0.036	0.007	....	
NBS 12e	0.71	0.14	0.058	0.28	0.050	0.015	....	
NBS 13d	0.924	0.022	0.010	0.265	0.023	0.002	....	

COBALT INTERFERENCE

Although it is of little significance in the analysis of low-alloy steel, cobalt interferes seriously. A modified version of this method, which, however, provides for the determination of nickel in cobalt-base materials, such as certain high-temperature alloys, will be published later.

SELECTION OF WAVE LENGTH

In Figure 6 are shown absorbancy–wave length curves which were plotted according to data based upon measurements made with the Beckman spectrophotometer with a band width of less than 1 mμ using 1-cm. cells. Curve 1 indicates an increasing absorbancy by the nickel compound toward wave lengths shorter than 500 mμ, but in order to avoid the interference of ferric citrate (curve 4) 540 mμ was chosen as the wave length to use in the nickel determination. Curve 2 is for copper as it appears in the reference solution and 3 is for chromium.

ANALYTICAL RESULTS

Analytical results and precision data according to the spectrophotometric method are shown in Table IV. The composition of the steel samples is given in Table III. The first four samples listed are those for which results found by other methods are shown (see Tables V and VI).

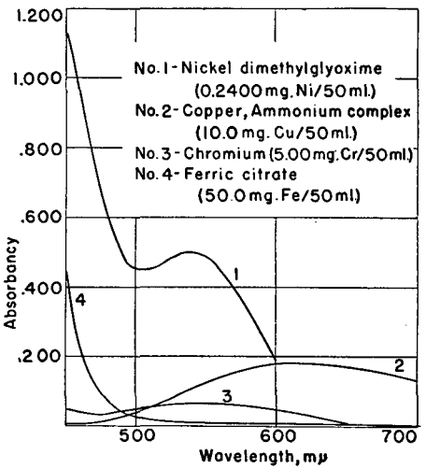

Figure 6. Absorbancy–Wave Length Curves

On the basis of ten determinations, in each case the precision of the spectrophotometric method, expressed as the standard deviation, was found to range from 0.25 to 0.5%. Attention is called to the magnitude of the manganese correction; the copper correction is hardly significant. For sample 2-1 if the manganese correction were neglected, the value for nickel would be high by

Table IV. Analytical Results by Spectrophotometric Method

Sample No.	No. of Detns.	Av. % Ni (Uncorr.)	Mn Corr., %	Cu Corr., %	Ni Corr., %	Max. Dev., %	Std. Dev., %
2-1	10	0.0474	−0.0090	−0.0002	0.0382	0.004	0.4
1-1	10	0.238	−0.008	.....	0.230	0.002	0.5
1-4	10	0.525	−0.005	.....	0.520	0.006	0.5
3-7	10	2.115	−0.005	.....	2.110	0.010	0.25
NBS 32C 1.20% Ni	3	1.205	−0.004	.....	1.200	0.004	.....
NBS 111 1.75% Ni	3	1.750	−0.004	.....	1.745	0.005	.....
NBS 100 0.151% Ni	3	0.1605	−0.0079	−0.0006	0.152	0.001	
NBS 11e 0.045% Ni	3	0.0488	−0.0026	−0.0004	0.0458	0	.....
NBS 12e 0.058% Ni	3	0.0632	−0.0040	−0.0005	0.0586	0.0002	.....
NBS 13d 0.01% Ni	3	0.0179	−0.0053	−0.0001	0.0125	0.0006	.....

Table V. Analytical Results and Precision Data for Gravimetric Method

Sample No.	Sample Wt., G.	No. of Detns.	% Nickel	Max. Dev., % Ni	Std. Dev., %
2-1	7.5	10	0.0385	0.0015	1.9
1-1	5.0	10	0.227	0.004	0.9
1-4	2.5	10	0.518	0.003	0.4
3-7	0.75	10	2.120	0.015	0.3

25% of the amount present; for No. 1-1, the error would amount to nearly 4%, and for No. 1-4, less than 1%.

As a further check of the method National Bureau of Standards samples were analyzed. Two of these, Nos. 32C and 111, represent the percentage range in which the 1-cm. cells are used. In neither case was the manganese correction of great significance.

The remaining samples provide a more rigorous test of the validity of the method with respect to the manganese and copper corrections.

For sample 100 the manganese correction is equivalent to 5% of the nickel value.

The copper correction for samples 11e and 12e amounts to nearly 1% of the nickel value.

Sample 13d is the most significant, because the manganese correction amounts to 40% of the nickel value. In this sample the ratio of manganese to nickel is too high to permit the direct determination of nickel, according to limitations described above. The analysis was conducted successfully, however, by adding enough standard nickel solution to adjust the ratio sufficiently. The absorbancy due to nickel in the steel sample, plus that due to copper and manganese, was taken by difference and the nickel content of the sample was then calculated. The difference between the value obtained and the certified value is not disturbing as the latter is apparently not accurately known. The values shown on the certificate range from 0.006 to 0.016% nickel.

GRAVIMETRIC AND TITRIMETRIC METHODS

The gravimetric dimethylglyoxime method, which has been known for more than 30 years, is considered extremely accurate for the determination of nickel. The cyanide titration method, when certain details are carefully observed, belongs in the same category. However, there has always been some doubt of the reliability of both these methods in the analysis of steel containing less than 0.06% nickel. Any appraisal of this investigation, which is concerned primarily with refining the spectrophotometric method for nickel, must be based upon a comparison of the results obtained by the improved method with those found by methods that have been accepted previously. Analyzing the same sample of steel by each of the methods provides the necessary comparative data.

A routine titrimetric method, which, according to a private communication, is commonly used, is included in this study. It is characterized by neglect of certain critical factors in the interest of performing more rapid analyses. By virtue of the purpose for which it has evolved, it is referred to as the "rapid" cyanide titration method in this comparative study.

Although space does not permit a complete description of the gravimetric and titrimetric methods as applied, the most significant details are presented in condensed form.

Procedures. In the separation of nickel prior to the determination by the gravimetric or the titrimetric method, the following considerations were borne in mind:

Because copper tends to coprecipitate, and it is difficult to wash the precipitate entirely free of salts, a double precipitation should be made (*11*). (In the rapid cyanide titration method a single precipitation was performed.)

The separation of nickel from copper (*3*), and from large amounts of manganese (*2*), is facilitated by the use of acetic acid.

Nitrates prevent precipitation of nickel, which may be serious if the amount of nickel is small (*4*).

More complete precipitation occurs if the solutions are allowed to stand overnight (*5*). (Twelve hours' standing time was used except in the case of the rapid cyanide titration method, in which the precipitation is performed by digesting the solutions for 1 hour.)

Filtering the solutions while hot, and washing the precipitate with hot water, lead to low results (*6*). (These practices are recommended by analysts who resort to the rapid cyanide titration method.)

The solubility of the precipitate is increased in solutions containing alcohol (*6*). (An aqueous solution of sodium dimethylglyoxime was used except in the rapid cyanide titration method, in which an alcoholic solution of dimethylglyoxime was employed.)

As a rule faintly ammoniacal media are recommended for the precipitation; therefore pH value of 7.1 to 7.2 were considered to be appropriate. A pH meter was used to facilitate the pH adjustment.

Following the second precipitation each gravimetric result was obtained by filtering the solution through a glass filtering crucible of medium porosity (Corning No. 32940), washing with cold water, drying for 1 hour at 110° to 120° C., and weighing.

In the titrimetric methods the cyanide solution was standardized against a standard solution of pure (Hilger) nickel.

The rapid cyanide titration method has come into use in some laboratories where emphasis is placed on speed. The results obtained by this method are included because they demonstrate the error that may be introduced by disregarding certain precautions which have been cited.

The analytical results and precision data related to the gravimetric, titrimetric, and the rapid cyanide titration methods are shown in Tables V, VI, VII. The composition of each sample is shown in Table III.

SUMMARY

Figure 7 graphically presents the comparative data obtained by the methods studied. The shaded area of each vertical block

Table VI. Cyanide Titration Method (Double Precipitation)

(Analytical results and precision data)

Sample No.	Sample Wt., G.	No. of Detns.	% Nickel	Max. Dev., % Ni	Std. Dev., %
2-1	7.5	10	0.0375	0.001	1.3
1-1	5.0	10	0.225	0.003	0.8
1-4	2.5	10	0.516	0.004	0.4
3-7	0.75	10	2.110	0.010	0.3

Table VII. Rapid Cyanide Titration Method (Single Precipitation)

(Analytical results and precision data)

Sample No.	Sample Wt., G.	No. of Detns.	Theoretical % Nickel	% Nickel Found	Range of Values, %	Max. Dev., % Ni	Std. Dev., %
1-1	5.0	10	0.230	0.208	0.196-0.212	0.012	1.7
1-4	2.5	10	0.520	0.506	0.503-0.541	0.008	0.65
3-7	0.75	10	2.110	2.090	2.085-2.110	0.020	0.45

represents the range of values found in each case; a horizontal line has been drawn through the area at the level of the average value obtained. The scale for the sets of blocks is adjusted in such a manner that a given vertical distance represents a given percentage of the amount present.

CONCLUSIONS

This investigation serves to demonstrate the superiority of the spectrophotometric method from the standpoint of precision and speed over the familiar gravimetric method and the cyanide titration method for nickel in steel. The greater reliability of the spectrophotometric method is evident over the entire range of nickel concentration which was studied; it is especially pronounced at the lower nickel levels.

A cyanide titration method, which has been shown to yield erroneous results, evolved as the result of the quest for increased speed in the routine analysis of steel. The time required per determination when a group of 20 samples is analyzed by this method is 30 minutes; by the spectrophotometric method, less than 20 minutes. A single determination by the rapid cyanide titration method requires 2 hours, whereas the spectrophotometric method requires an elapsed time of only 1 hour.

LITERATURE CITED

(1) Am. Soc. Testing Materials, Philadelphia, Pa. "A.S.T.M. Methods for Chemical Analysis of Metals," p. 332, 1946.
(2) Diehl, Harvey, "Application of the Dioximes to Analytical Chemistry," p. 25, Columbus, Ohio, G. Frederick Smith Chemical Co., 1940.
(3) *Ibid.*, p. 26.
(4) *Ibid.*, p. 27.
(5) *Ibid.*, p. 28.

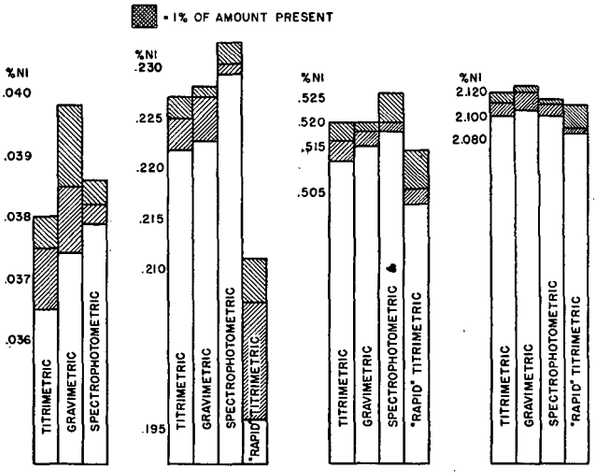

Figure 7. Comparison of Analytical Results

(6) *Ibid.*, p. 29.
(7) Haywood, F. W., and Wood, A. A. R., "Metallurgical Analysis," p. 62, London, Adam Hilger, Ltd., 1944.
(8) Makepeace, G. R., and Craft, C. H., IND. ENG. CHEM., ANAL. ED., **16**, 375–7 (1944).
(9) Mitchell, A. M., with Mellon, M. G., *Ibid.*, **17**, 380–2 (1945).
(10) Murray, W. H., Jr., and Ashley, S. E. Q., *Ibid.*, **10**, 1–5 (1938).
(11) U. S. Steel Corp. Chemists, "Sampling and Analysis of Carbon and Alloy Steels," p. 135, New York, Reinhold Publishing Corp., 1938.

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Spectrophotometric Determination of Nickel in Aluminum Alloys

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This work was undertaken for the purpose of ascertaining the applicability of the method described for the determination of nickel in aluminum alloys. Because the ratio of copper to nickel in these alloys is usually more unfavorable than in steel, preliminary removal of copper is in order where a high degree of accuracy is required. Two methods, which differ with respect to the method of removing copper, and a third method with satisfactory precision for routine analysis, are described. Because nickel is determined in the presence of copper, the time requirements are reduced one half.

THE procedure covering the decomposition of the aluminum sample and the preparation of the sample solution is similar to that of the American Society for Testing Materials (*1*). A detailed interpretation of the ASTM procedure and directions for finishing the determination spectrophotometrically are given here.

REAGENTS

Sodium hydroxide solution, 200 grams of sodium hydroxide dissolved in water and diluted to 1 liter.

Hydrogen sulfide.

Hydrogen sulfide wash solution, one volume of sulfuric acid (specific gravity 1.84) added to 99 volumes of water and saturated with hydrogen sulfide at room temperature.

Hydrochloric acid (1 to 1), one volume of hydrochloric acid (specific gravity 1.19) diluted with one volume of water.

Nitric acid (specific gravity 1.42).

Sulfuric acid (1 to 1), one volume of sulfuric acid (specific gravity 1.84) diluted with one volume of water.

Copper solution (1 mg. per ml.). Dissolve 1.000 gram of pure (Hilger) copper in 15 ml. of nitric acid (specific gravity 1.42) in a 300-ml. Erlenmeyer flask, add 20 ml. of sulfuric acid (specific gravity 1.84), and heat to fumes. Cool, add 50 ml. of water digest until the salts are dissolved, cool, and dilute to 1 liter.

Additional reagents are used as listed in the preceding paper (*2*).

PROCEDURE

Transfer a sample of suitable size to a 300-ml. Erlenmeyer flask, add 15 ml. of 20% sodium hydroxide solution, and when the reaction is complete, dilute to 150 ml. with hot water and mix. Using a glass rod to facilitate the quantitative handling of the sample, filter the hot solution through a Whatman No. 40 paper or its equivalent. Rinse the flask twice, and wash the filter three or four times with hot water. Wipe the lip of the flask with a small piece of wet filter paper, and place the paper on the filter. Discard the filtrate.

Place the original flask under the filter. Pour through the paper about 10 ml. of hot hydrochloric acid (1 to 1) to which approximately 1 ml. of nitric acid (specific gravity 1.42) has been added. Wash the filter three or four times with hot water, pour through 10 ml. of the acid mixture, wash with hot water, then repeat the cycle, and finally wash the filter thoroughly with hot water. Discard the paper with any undissolved residue. Remove the flask, add 10 ml. of sulfuric acid (1 to 1), and evaporate to fumes.

After the sample has been fumed for approximately 10 minutes, cool, and if much silica is present, add approximately 1 ml. of hydrofluoric acid (48%). Swirl the flask a few times and evaporate to fumes again in order to volatilize the silica and remove excess hydrofluoric acid. Cool, add 50 ml. of water, and digest until the salts are dissolved.

The procedure as described thus far is followed in all cases. Variations in the succeeding steps, which lead finally to the evaluation of the nickel content of the alloy, depend upon the method used in circumventing the copper interference.

REMOVAL OF COPPER AS SULFIDE

Dilute the solution to 100 ml. and fit into the flask a two-hole stopper fitted with one length of glass tubing as a delivery tube extending through the stopper nearly to the bottom of the flask and another length of glass tubing extending approximately 1 cm. below the stopper. The latter may be connected by means of rubber tubing to the delivery tube of another flask. By connecting a series of flasks in this manner, as many as six or seven samples may be treated simultaneously with a single stream of the gas. Connect the delivery tube to the source of hydrogen sulfide, and maintain a steady flow of the gas until the solution is saturated.

Set the sample aside for approximately 2 hours, and then filter through a Whatman No. 42 paper or its equivalent. Rinse the delivery tube and flask, and wash the filter thoroughly with hydrogen sulfide wash solution. Discard the paper and precipitate.

Evaporate the filtrate to a volume of approximately 20 ml., and then add nitric acid (specific gravity 1.42) dropwise to the hot solution to oxidize the iron, and add a few drops in excess. Add 50 ml. of water, boil the solution 2 or 3 minutes to remove oxides of nitrogen, cool, transfer to a volumetric flask, dilute to volume, and mix.

Transfer identical aliquots to each of two 50-ml. volumetric flasks. The sample size, dilution of the sample solution, and aliquot taken should be correlated in such a manner that an optimum amount of nickel will be provided and not more than the equivalent of 0.5 ml. of sulfuric acid (specific gravity 1.84). The volume of reagents to be added dictates that the aliquot should not exceed 20 ml.

To one of the aliquots add the following reagents:

1. 5 ml. of ammonium citrate solution. Mix well in order to assure complete complexing of the iron and other elements which precipitate in the presence of excess ammonium hydroxide.
2. 10 ml. of ammonium hydroxide (1 to 1). Mix.
3. 5 ml. of iodine solution (0.02 N). Agitate the solution rather vigorously during the addition and for about 5 seconds. Quickly rinse down the neck of the flask with water; then continue the agitation for an additional 10 seconds in order to effect complete removal of nitrogen which is formed in the reaction between iodine and ammonium hydroxide. This solution constitutes a reference solution.

To the other aliquot add the following reagents:

1. 5 ml. of ammonium citrate solution. Mix well.
2. 10 ml. of dimethylglyoxime solution. Mix mildly. (Excessive mixing may cause precipitation of unoxidized nickel dimethylglyoxime.)
3. 5 ml. of iodine solution (0.02 N). Introduce the iodine as soon as possible after the addition of dimethylglyoxime, in order to avoid precipitation of unoxidized nickel dimethylglyoxime. Agitate the solution and rinse the neck of the flask as described above for the preparation of the reference solution.

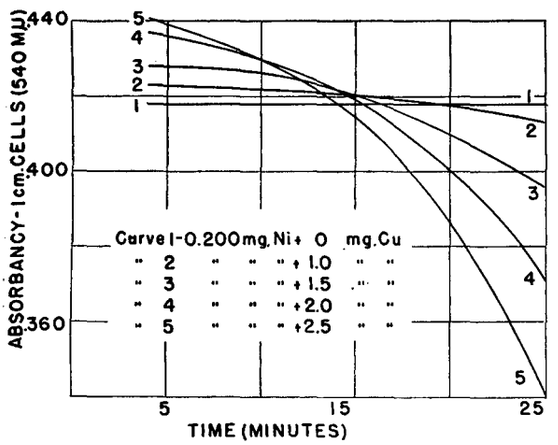

Figure 1. Effect of Copper on Determination of Nickel in Aluminum

Dilute the solutions to volume and mix thoroughly.

Measure the transmittancy of the test solution, 10 minutes after the addition of iodine, against the reference solution at the wave length of 540 mμ and slit setting of approximately 0.02 mm. for the Beckman spectrophotometer. For other instruments conditions established in calibrating should be maintained.

Calculate the absorbancy, A (log $1/T$), apply the factor (mg. Ni/A) found in calibrating the spectrophotometer, and then calculate the percentage of nickel on the basis of the number of milligrams of sample represented by the aliquot of the sample solution.

REMOVAL OF COPPER BY ELECTROLYSIS

Transfer the sulfuric acid solution of the sample to a 200-ml. electrolysis beaker, dilute to approximately 150 ml., and add 2 or 3 ml. of nitric acid (specific gravity 1.42). Electrolyze to remove copper. (The work reported was based upon removal of copper using the Sargent-Slomin electrolytic analyzer equipped with platinum gauze electrodes, and the anode was rotated during electrolysis. The current was limited to 1 ampere for not more than 5 minutes and then increased to 2.5 amperes for 20 minutes. The sample solutions which were electrolyzed contained approximately 28 mg. of copper, which was found to be removed practically quantitatively by this procedure.)

Rinse the electrodes thoroughly while removing them from the solution, and then transfer the solution to a volumetric flask, dilute to volume, and mix.

Transfer identical aliquots to each of two 50-ml. volumetric flasks, and treat them according to the method for solutions from which copper was removed as the sulfide.

NICKEL IN PRESENCE OF COPPER

Transfer the sulfuric acid solution of the sample directly to a volumetric flask, dilute to volume, and mix. Transfer identical aliquots to each of two 50-ml. volumetric flasks. To each aliquot add 5.0 ml. of ammonium citrate solution and 2.5 ml. of ammonium hydroxide (specific gravity 0.90). Cool the solutions to room temperature, then prepare one as a reference solution by adding ammonium hydroxide (1 to 1) and iodine solution. To the test solution add ammoniacal dimethylglyoxime solution and iodine, observing the directions regarding agitation of the solutions and other details described previously.

Careful timing is essential in this procedure, especially in case of a high ratio of copper to nickel. Start the timing so that the transmittancy measurement may be made 10 minutes after the sample solution is agitated. Dilute the test and reference solution to volume, and measure the transmittancy at the end of the 10-minute time interval at 540 mμ.

Measure the transmittancy of the reference solution against water at 615 mμ. This value constitutes datum on which the evaluation of the copper correction at 540 mμ is based.

Convert the transmittancy values to their corresponding absorbancy values (log $1/T$), and subtract values obtained for a blank determined by performing all of the steps of the procedure with sample omitted. Multiply the corrected absorbancy at 615 mμ by the factor obtained in calibrating the spectrophotometer for known copper in the presence of nickel. Subtract this value from the absorbancy at 540 mμ.

Convert the corrected absorbancy to milligrams of nickel, and calculate percentage of nickel in the alloy on the basis of the sample weight represented by the aliquot of the sample solution. (The percentage of copper in the alloy may be roughly evaluated on the basis of the absorbancy of the reference solution.)

CALIBRATION OF SPECTROPHOTOMETER

Follow the procedure described (*2*). In order to provide for the determination of nickel in the presence of copper, the copper interference must be evaluated.

COPPER INTERFERENCE IN DETERMINATION OF NICKEL IN ALUMINUM ALLOYS

The nature of the interference of copper has been described in connection with the determination of nickel in steel (*2*). In the determination of nickel in aluminum alloys, some rather

Table I. Copper Interference

Mg. Cu	A_{540}	ΔA_{540}/Mg. Cu	A_{615} (Observed)	Mg. Cu/A_{615} (Av.)	$\Delta A_{540}/A_{615}$ (Av.)
		1-Cm. Cells, 0.2000 Mg. of Nickel			
0	0.418	...	0.001		
1.00	0.423	0.005	0.198a		
1.50	0.426	0.006	0.028b		
2.00	0.430	0.006	0.037c		
2.50	0.429	0.004	0.046d	55.6[a]	0.28[b]
		5-Cm. Cells, 0.0400 Mg. of Nickel			
0	0.418	...	0.001		
0.05	0.420	0.040	0.011		
0.10	0.421	0.030	0.018		
0.20	0.425	0.035	0.031e		
0.40	0.439	0.053	0.044f		
0.50	0.445	0.054	0.053g	11.3[c]	0.63[d]

[a] Based on values b, c, and d.
[b] Based on values a, b, and c.
[c] Weighted average of values e, f, and g.
[d] Based on values f and g.

troublesome ratios of copper to nickel are frequently encountered. Consequently, spectrophotometric methods of the past have provided for a preliminary removal of copper.

In this investigation a method has been developed which permits the determination of nickel in the presence of copper where the ratio of copper to nickel is as high as 12.5 to 1. The copper interference in the steel method imposes the limitation of a ratio of 2.5 to 1, at least at the nickel levels for which the 1-cm. cells are recommended. In the steel method the ammoniacal solution of dimethylglyoxime is added to the acid solution of the sample. By complexing the copper, by adding an excess of ammonium hydroxide prior to the addition of the reagent solution, the deleterious effect of the element on stability is minimized.

Figures 1 and 2 show the effect of copper at two nickel levels. A procedure, in which the copper correction is based on an additional absorbancy measurement, has been developed. This procedure precludes the need for prior information in regard to the copper content of the alloy.

Evaluation of Copper Interference. To each of two 50-ml. volumetric flasks transfer 20.00 ml. of the standard nickel solution (0.0100 mg. of nickel per ml.) and 2.00 ml. of the standard copper solution (1.000 mg. of copper per ml.). Add to each solution 2.0 ml. of dilute sulfuric acid (1 to 3) and 5.0 ml. of ammonium citrate solution, followed by 2.5 ml. of ammonium hydroxide (specific gravity 0.90). Cool the solutions to room temperature. Add 10 ml. of ammonium hydroxide (1 to 1), and 5.0 ml. of iodine solution to one of the solutions; to the other add 10 ml. of ammoniacal dimethylglyoxime solution and 5.0 ml. of iodine solution, in the order and manner previously described. Dilute the solutions to volume and mix. Measure the absorbancy of the test solution against the reference solution, 10 minutes after the test solution is agitated, at a wave length of 540 mμ, using the 1-cm. cells. Measure the absorbancy of the reference solution against water at 615 mμ. The latter value must be corrected for the absorbancy due to constituents other than copper, which may be called "background absorbancy."

Determine the background absorbancy by measuring the absorbancy of a solution containing the sulfuric acid, ammonium citrate, ammonium hydroxide, and iodine, against a water reference at 615 mμ.

The method of utilizing the data may be summarized briefly as follows:

$$A_{540}(\text{Ni}) = A_{540}(\text{Ni} + \text{Cu}) - f \cdot A_{615}(\text{Cu})$$

$$f = \frac{\Delta A_{540}(\text{Cu})}{A_{615}(\text{Cu})} \text{ or } \frac{A_{540}(\text{Ni} + \text{Cu}) - A_{540}(\text{Ni})}{A_{615}(\text{observed}) - A_{615}(\text{background absorbancy})}$$

Where $\Delta A(\text{Cu})$ = change in absorbancy of the nickel dimethylglyoxime due to copper.

A similar procedure may be used to obtain calibration data for the 5-cm. cells.

Calibration data used for evaluating the copper interference are shown in Table I for the 1- and 5-cm. cells.

Table II. Composition of Alloys

No.[a]	Si	Fe	Cu	Ni	Cr	Mn	Ti	Bi	Mg
1	10.1	0.93	3.50	0.304	0.07	0.25	0.09	..	0.11
2	0.32	0.31	0.30	0.607	0.15	0.16	0.07	0.10	0.29
S-1	9.10	0.84	3.0	0.347	0.08	0.24	0.07	0.01	0.14
S-2	6.90	0.64	2.0	0.444	0.11	0.21	0.07	0.05	0.19
S-3	4.70	0.45	1.0	0.540	0.13	0.18	0.07	0.08	0.25

[a] S-1, 2, and 3 prepared by combining alloys 1 and 2.

SELECTION OF WAVE LENGTH

The absorbancy–wave length curves shown in Figure 6 of (*2*) provide the information on which the choice of wave lengths for the determination of nickel in aluminum alloys is based.

ANALYTICAL RESULTS

A measure of the precision of the methods was obtained by analyzing two alloys furnished by Committee E-3 of ASTM. The composition of the alloys is shown in Table II. In order to check the method for determining nickel in the presence of copper at other levels of copper concentration, three "synthetic" alloys were analyzed. The amount of each of the first two alloys required to furnish copper equivalent to 1, 2, and 3% of the total weight, while maintaining a constant amount of nickel, was calculated. The calculated amount of each alloy was transferred to the Erlenmeyer flask, and the mixture was treated as a single sample in each case.

Analytical results and precision data are shown in Table III. Alloys 1 and 2 were first analyzed by the procedure which in-

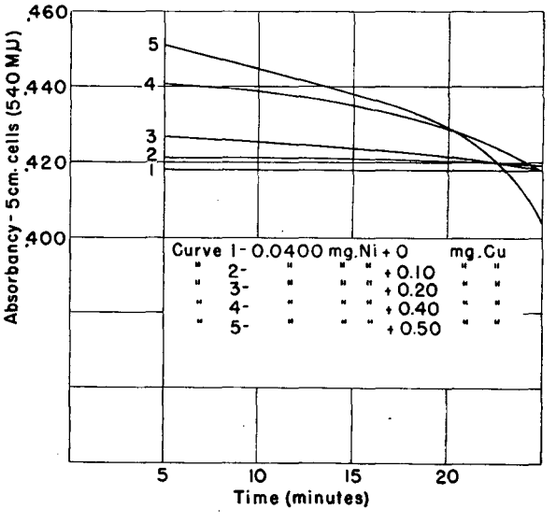

Figure 2. Effect of Copper on Determination of Nickel in Aluminum

Table III. Analytical Results for Nickel in Aluminum Alloys

Alloy No.	No. of Detns.	Method[a] No.	% Cu	% Ni (by Method 1)	% Ni Found	Max. Dev., %	Std. Dev., %
1	11	1	3.5	...	0.304	0.001	0.28
2	11	1	0.3	...	0.607	0.007	0.65
1	10	2	3.5	0.304	0.303	0.001	0.22
1	11	3	3.5	0.304	0.300	0.003	0.6
2	11	3	0.3	0.607	0.607	0.006	0.5
S-1	3	3	3.0	0.347	0.346		
S-2	3	3	2.0	0.444	0.440		
S-3	3	3	1.0	0.540	0.539		

[a] Method 1. Copper removed as sulfide.
Method 2. Copper removed by electrolysis.
Method 3. Nickel determined in the presence of copper.

volves the removal of copper as the sulfide, described previously. In the ensuing study of proposed procedures, the values found by this method were accepted as true values.

RELATIVE ECONOMY OF METHODS FOR NICKEL IN ALUMINUM ALLOYS

Aside from the obvious evils of hydrogen sulfide, the method in which copper is removed by electrolysis has the advantage, from the standpoint of the time required for a determination, of 45 minutes compared with 55 to 60 minutes required in the sulfide method.

The method by which nickel is determined in the presence of copper is recommended for the routine analysis of aluminum alloys. By this method 31 samples were analyzed in 15 hours. This indicates a saving of time which should be valuable to laboratories where large numbers of nickel determinations are required routinely.

LITERATURE CITED

(1) Am. Soc. Testing Materials, Philadelphia, Pa., "Methods of Chemical Analysis of Metals," pp. 160–1, 1946.
(2) Cooper, M. D., ANAL. CHEM., 23, 875 (1951).

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Simple Methylol Determination

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During a study of the cure of phenol alcohols and one-stage phenolic resins it became very important to be able to determine their methylol content. By reaction of phenol alcohols and similar materials containing methylol groups with an excess of phenol, it was found that a mole of water is formed for each methylol group. When benzene is added to the phenol containing the sample and refluxed, the water formed by reaction of the methylol groups with the phenol distills as a benzene-water azeotrope. The water is collected as a separate phase in a calibrated Bidwell and Sterling trap, where it is measured to give an estimate of the methylol content of the sample. The method not only provides a simple procedure for determination of the methylol content of a variety of compounds, but makes possible a more systematic study of condensation polymers based on formaldehyde and phenol, urea, etc.

UNTIL recently a simple method has not been available for determining the methylol content of phenol alcohols or phenolic resins containing methylol groups. This has been due largely to the fact that a reagent which will react quantitatively with a methylol group and yet not attack the phenolic hydroxyl group or the benzene ring has been difficult to find.

Lilley and Osmond report that iodine in alkaline solution will oxidize a methylol group without attacking the phenolic group (*8*). However, the iodine reacts with the ring hydrogens of the phenol, so that corrections for this side reaction become necessary. Runk (*10*) has reported his inability to obtain reliable results with this method and the author has experienced similar difficulties. Sprung (*11*) has shown that *o*- and *p*-hydroxybenzyl alcohol react with bromate-bromide solution to add 3 atoms of bromine—that is, these phenol alcohols react with bromine as if the methylol group were not present. However, the formation of a diphenylol methane by condensation of a phenol alcohol reduces the number of positions reactive toward bromine by one per phenol nucleus for each methylene bridge established. Thus, if the quantity of formaldehyde added to a phenol-formaldehyde reaction mixture is known, determination of the amount of formaldehyde reacted and the reduction in bromine absorption caused by condensation will give an indirect measure of the methylol content. However, the method is limited, as Ruderman (*9*) has shown that the methylol groups of most phenol alcohols, with the exception of the two mentioned above, are not quantitatively displaced by bromine.

Even in determining the methylol content of phenol-formaldehyde reaction products containing these phenol alcohols, some error may be introduced because the isomeric diphenylol methanes which result from their condensation absorb different quantities of bromine, depending on the position at which the methylene linkage has been established. Furthermore, the method is not applicable to dehydrated resins where part or essentially all unreacted phenol and formaldehyde are removed, inasmuch as all points of reference for the necessary calculations are lost.

The author has now found a simple means for determining methylol content, based on the well-established reaction of phenol alcohols with phenols to give diphenylol methanes and water (*7*). This reaction is catalyzed by acids and is favored by an excess of phenol.

$$RCH_2OH + C_6H_5OH \longrightarrow RCH_2C_6H_4OH + H_2O$$

R represents a group, such as *o*- or *p*-hydroxyphenyl, which will activate the methylol group sufficiently so that reaction will occur with phenol under the experimental conditions used in this procedure. Because a mole of water is eliminated for each methylol group reacted, measurement of the water liberated provides a means for estimating methylol content. This paper presents the results of a study of the use of this reaction as applied to pure phenol alcohols, benzyl alcohols, phenolic resins, and methylol derivatives of urea and nitromethane. The results obtained upon treating some related compounds under the conditions of the test are also included.

REAGENTS

Phenol, redistilled, commercial grade.

Benzene, commercial grade, used as received.

p-Toluenesulfonic acid, monohydrate. Eastman Kodak Co. used as received.

2,6-Bis-(hydroxymethyl)-4-chlorophenol, prepared from *p*-chlorophenol according to Weiler and Berres (*14*). Recrystallized twice from alcohol; melting point 164° C. Melting point reported, 165° C. Calculated for $C_8H_9O_3Cl$: Cl, 18.83%. Found: Cl, 18.69, 18.85%.

2,6-Bis-(hydroxymethyl)-4-methylphenol, prepared from *p*-cresol and formaldehyde according to Ullmann and Brittner (*13*); melting point 129.5–130.5°. Melting point reported, 130°.

Saligenin, Eastman Kodak Co., recrystallized from benzene, melting point 85–86°.

Benzyl alcohol, Eastman Kodak Co., redistilled once, boiling point 199–202° at 732 mm.

Benzhydrol, Eastman Kodak Co. Used as received, melting point 68–69°.

p-Chlorobenzyl alcohol, Heyden Chemical Co., used as received, melting point 71–72°.

Dibenzyl ether, Advance Solvents and Chemical Corp., redistilled once.

Tris-(hydroxymethyl)nitromethane, Commercial Solvents Corp., recrystallized once, melting point 155–156°.

o-Allyloxy benzyl alcohol, prepared from saligenin and allyl bromide according to Claisen and Eisleb (*1*), boiling point 110–120° at 1 to 5 mm.

Bis-(hydroxymethyl)urea, prepared from formaldehyde and urea according to D'Alelio (*2*). Recrystallized twice from alcohol, melting point 125–126°. Melting point reported, 126° (*3*).

2,2'-Dihydroxy dibenzyl ether, prepared according to Gladstone (*6*) by heating saligenin at 100° for 8 hours. The product was washed with water and recrystallized three times from benzene, melting point 120–121°. Melting point reported, 119–120° (*12*).

EXPERIMENTAL

A solution containing 500 grams of phenol, 250 ml. of benzene, and 15 grams of *p*-toluenesulfonic acid monohydrate was placed in a 1-liter flask equipped with a Bidwell and Sterling take-off trap provided with a stopcock and reflux condenser. The take-off trap was of 5-ml. capacity, graduated in units of 0.1 ml. The phenol solution was dried by refluxing vigorously, any water being removed by distilling into the take-off trap where it was withdrawn. The trap was calibrated by adding known quantities of water from a pipet to the phenol solution and distilling until no more water collected in the trap. In calibrating the trap it was necessary to add the water to the phenol solution and distill it into the trap rather than add the water directly to the trap, as a small quantity of phenol codistills with the water. The correction was mainly due to the large contact angle between the water and the glass and changed from about 0.12 ml. for 1 ml. to 0.15 ml. for 4 ml. By treating the inside of the trap and condenser with GE Dri-Film ® water repellent (applied in the form of a toluene solution and cured by baking 1 hour at 150° C.), the tendency for water droplets to adhere to the glass and not sink to the bottom of the trap was essentially eliminated.

After the trap was calibrated, a sample of known weight and sufficient size to yield 2 to 4 ml. of water was introduced into the phenol solution. The solution was refluxed until no more water separated. The volume of aqueous phase collected in the trap was measured and corrected, by use of the calibration data, to give the quantity of water formed by reaction of the sample with the phenol. From this figure the methylol content can be calculated. For routine tests on resins it was found practical to run a number of samples without changing the phenol solution; care was taken to see that reaction was complete for one sample before a second sample was added. Where it seems advisable to use fresh phenol for each test, 100 to 150 ml. of phenol solution are adequate.

Table I shows typical results and the percentage error found when the methylol content of a number of compounds was determined according to the above procedure. Table II gives data on the methylol content of three dehydrated phenolic resins prepared by reacting different ratios of phenol and formaldehyde using an alkaline catalyst. The water evolved by some related compounds when treated with phenol under the conditions described for the methylol determination is shown in Table III.

For small samples, where direct measurement of the water evolved is impractical, titration of the water with Karl Fischer (*5*) reagent after separation from the phenol solution by distillation has been suggested. This procedure is now under study.

DISCUSSION OF RESULTS

The method gave results with pure phenol and benzyl alcohols which were in all cases within 3% of the calculated values, and in general the agreement was much better than this figure. The results with phenolic resins, although they cannot be checked because of lack of an independent method of analysis, appear reasonable, based on knowledge of the mode of preparation. The fact that dibenzyl ethers react with phenol to give a mole of water per mole of ether shows that this group, where present, would interfere. However, Sprung and Gladstone (*12*) have shown, based on their study with saligenin, that ether formation is not an important reaction in the base-catalyzed condensation of phenol alcohols. In one-stage resins, where basic catalysts are almost always employed, the quantity of ether groups present would not appear sufficient in most cases seriously to affect the accuracy of an analysis.

Table I. Determination of Methylol Content

Compound	Methylol Content, % Calcd.	Found	Error
Saligenin	25.0	24.6	−1.6
2,6-Bis-(hydroxymethyl)-4-methylphenol	36.9	36.8 36.7	−0.3 −0.5
2,6-Bis-(hydroxymethyl)-4-chlorophenol	32.9	32.6 32.6	−0.9 −0.9
Benzyl alcohol	28.7	28.5 29.4	−0.7 +2.4
p-Chlorobenzyl alcohol	21.6	21.8	+0.9
o-Allyloxy benzyl alcohol	18.8	18.6	−1.0
Tris-(hydroxymethyl)-nitromethane	60.4	61.8	+2.3
Bis-(hydroxymethyl)-urea	51.7	50.3	−2.7

Table II. Methylol Content of Dehydrated Phenolic Resins

Resin	Phenol-Formaldehyde Ratio	Methylol Content, %
1	1:0.9	4.25
2	1:1.05	7.1
3	1:1.3	11.4

Table III. Water Evolved

Compound	Water Calculated, %	Water Found, %
Benzhydrol	9.78	9.75
Dibenzyl ether	9.09	9.07
2,2'-Dihydroxy dibenzyl ether	7.83	7.80

Although water interferes with the determination, the method can be applied to wet resins by first measuring the water content of the resin with Karl Fischer reagent (*5*) or by the method of Feith (*4*); the former is more reliable. It is obvious, however, that for water-soluble resins, where the water content may be very high, the accuracy of the method will be impaired. For resins of very low methylol content, large samples will be required. Care must also be exercised to see that the water content of the resin is accurately determined, if reliable results are to be obtained.

Formaldehyde reacts with phenol to liberate a mole of water for each mole of aldehyde reacted. While dry phenolic resins contain very little unreacted formaldehyde, resins before dehydration often contain free formaldehyde. In such cases a correction must be made for the unreacted formaldehyde in the reaction mixture.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Claisen, L., and Eisleb, O., *Ann.*, **401**, 30–1 (1913).
(2) D'Alelio, G. F., "Laboratory Manual of Plastics and Synthetic Resins," p. 28, New York, John Wiley & Sons, 1943.
(3) Einhorn, A., and Hamburg, A., *Ber.*, **41**, 24 (1908).
(4) Feith, F., *Kunststoffe ver Kunststoff-Tech. u. Anwend.*, 34, 71–6 (1944).
(5) Fischer, K., *Angew. Chem.*, **48**, 394–6 (1935).
(6) Gladstone, M. T., private communication.
(7) Koebner, M., *Chem.-Ztg.*, **54**, 619 (1930).
(8) Lilley, H. S., and Osmond, D. W. J., *J. Soc. Chem. Ind.* (*London*), **66**, 425–7 (1947).
(9) Ruderman, I. W., IND. ENG. CHEM., ANAL. ED., **18**, 753–9 (1946).
(10) Runk, R. H., *Ind. Eng. Chem.*, **42**, 2110–14 (1950).
(11) Sprung, M. M., IND. ENG. CHEM., ANAL. ED., **13**, 35–8 (1941).
(12) Sprung, M. M., and Gladstone, M. T., *J. Am. Chem. Soc.*, **71**, 2907–13 (1949).
(13) Ullmann, F., and Brittner, K., *Ber.*, **42**, 2539–48 (1909).
(14) Weiler, M., and Berres, K., Ger. Patent 510,447 (1929).

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Colorimetric Determination of Rosin and Rosin Esters

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This work was undertaken to develop a satisfactory qualitative test for rosin in paints and varnishes, for quality control and acceptance, and to develop quantitative methods for either free rosin or rosin esters in paint products. Certain types of rosin modification are desirable in some paints used for Army Ordnance materiel, but in the absence of means of determining the type of rosin modification, it has been necessary to prohibit use of rosin in most paints. Procedures for distinguishing between free rosin and rosin esters provide a means of determining either type of product quantitatively under certain conditions. Analysts will be able not only to determine the nature of the rosin products present, but in most cases to determine it quantitatively, thus providing a way to control the formulation of products containing desirable rosin modification and prohibit undesirable forms.

ROSIN is the most widely used natural resin in both the varnish and plastic fields; rosin is cheap and easily available and by reason of its acid character it reacts to form salts and esters.

There has been no completely suitable method of determining rosin and rosin derivatives in paint products, either qualitatively or quantitatively although many methods have been recommended for detecting rosin in other resins, in varnishes, and in a wide variety of materials. None of these qualitative tests seems reliable, and many of the tests have been subjected to numerous modifications (*3*). The Liebermann test (*2*) has been the most widely used, although many interfering substances have been named. One investigator (*7*) has concluded that the Liebermann test is significant only if a negative result is obtained. In developing a systematic procedure for identifying synthetic resins and plastics, Shaw (*4*) employed the Liebermann test along with some twenty or more other confirmatory tests to identify most resins of commercial importance. Many of the synthetic resins containing no rosin or rosin esters gave color reactions similar to that of rosin products. The reaction with butyl phenol formaldehyde resin is identical with the reaction of some rosin products. There is a definite need for a satisfactory qualitative test for rosin and its products.

In a recent study of the varied methods of detecting rosin, it was noted that under certain conditions it was possible to distinguish between the colors formed by free rosin and rosin esters, employing the color reactions characteristic of the Liebermann test. As a result of further investigation, two quantitative colorimetric procedures, one for rosin and the other for rosin esters, were developed. Free rosin can be determined quantitatively in any type of vehicle, with the exception of lacquers containing cellulose derivatives. None of the esterified or modified rosin products interferes with the determination of free rosin; the method can be applied to incompletely esterified rosin products such as mono- and diglycerides, and the results agree reasonably well with the acid number of such esters. Esterified and modified rosin products can be determined quantitatively by a separate colorimetric procedure, but the method is limited to nonalkyds and samples in which the rosin has been completely esterified. Simple qualitative tests will indicate whether or not the procedure is applicable to the sample under analysis.

Both methods are based on the violet color formed by the reaction of abietic acid (the principal ingredient of rosin), sulfuric acid, and acetic anhydride. When rosin products in benzene are shaken with 50% sulfuric acid and acetic anhydride in certain proportions, the violet color formed by free rosin or rosin soaps goes into the sulfuric acid layer, while the color formed by rosin esters remains in the benzene layer. In the procedure for free rosin and rosin soaps, the color of the reaction is extracted with 50% sulfuric acid from benzene and compared with a standard by means of an electrophotometer. In the procedure for rosin esters, the color is developed in the benzene medium and is sufficiently stable to permit comparison with standard potassium permanganate colors.

It is not possible to distinguish by these procedures between different types of rosin esters. Cumar and dammar, natural resins which interfere in the usual qualitative Liebermann spot test, do not interfere with either of the newly developed procedures. Some rosin products, such as hydrogenated rosin, previously reported as giving a color different than rosin by the usual qualitative spot test, have been found actually to produce the same color as any other form; only the intensity is different. No actual color variations between different rosin products have been discovered in these investigations. However, certain oils and other paint constituents char readily in the presence of sulfuric acid as used in the original spot test, and form colors which make the test uncertain. The diluted conditions used in the new procedures eliminate this type of error.

ANALYTICAL PROCEDURE

Qualitative Method for Detecting Rosin and Rosin Esters. The following instructions apply to resin solutions such as paint vehicles. If dry resins are to be tested qualitatively, 5 to 10 mg. are dissolved in the specified volume of benzene.

ROSIN ESTERS. Two drops of the resin solution to be tested are added to 50 ml. of benzene in a 100-ml. glass-stoppered graduate, and 0.5 ml. of acetic anhydride is added and mixed with the sample. One large drop of concentrated sulfuric acid is added and the graduate is shaken until color develops. If no color develops in 15 seconds of vigorous shaking, one more drop of acid is added and the agitation is continued 10 to 15 seconds longer. If no red to violet color forms throughout the benzene, rosin esters are absent.

FREE ROSIN OR RESINATE DRIERS. Two drops of the resin solution to be tested are added to 75 ml. of benzene in a 100-ml. glass-stoppered graduate. Five milliliters of 18 *N* sulfuric acid are added and mixed with the sample by shaking. Acetic anhydride is added in 1-ml. portions, followed by vigorous shaking and cooling in cold water for approximately 30 seconds between additions of the anhydride. A red to violet color in the lower layer of sulfuric acid, after the addition of 5 to 10 ml. of the anhydride, indicates the presence of free rosin or resinate driers. However, a faint coloration in this test, when applied to a sample which shows rosin esters from the previous test, does not indicate substantial amounts of free rosin, and the test should be confirmed by the quantitative method for free rosin, as outlined.

Preparation of Sample for Quantitative Analysis. The following instructions apply to resin solutions such as paint vehicles. Dry resins may be weighed directly or dissolved in benzene so that aliquots may be taken. In either case, the recommended sample size is indicated.

The degree of accuracy obtained by either method of analysis is determined by two factors: the accuracy with which the small

sample is weighed, and the number of aliquots of varying size withdrawn for confirmatory runs.

The sample used is necessarily small and should be weighed to the nearest 0.0001 gram by difference from a special dropping vial. The recommended sample bottles have molded screw cap and pipet of clear glass, capacity 15 or 30 ml. (Fisher Scientific Co. catalog No. 3-337). Before pigmented materials are analyzed, the vehicle must be isolated by supercentrifuging until clear.

A sample of the resin solution, weighing not more than 0.5 gram (0.1 to 0.2 gram of nonvolatile solid), is weighed by difference from the special sample bottle into a 100-ml. volumetric flask containing benzene, ACS reagent grade. It is then diluted to volume with benzene and aliquots are withdrawn for analysis as indicated in the detailed procedure.

COLORIMETRIC PROCEDURE FOR FREE ROSIN AND ROSIN SOAPS. A sample aliquot, estimated to contain 4 mg. or less of rosin, is transferred to a dry 250-ml. separatory funnel (Squibb, pear-shaped) containing benzene, ACS reagent grade. The volume is then made up to 100 ml. with benzene. After mixing, 5 ml. of 18 *N* sulfuric acid are added from a buret. This mixture is thoroughly shaken and 1 ml. of acetic anhydride (C.P., 98% minimum) is added from a 10-ml. buret. The separatory funnel is immediately and vigorously shaken for approximately 5 seconds and immediately immersed in a reservoir of water at room temperature. The level of water in the bath should be adjusted so that it equals or slightly exceeds the level of benzene in the separatory funnel. When the funnel has been in the bath 45 seconds, it is withdrawn, 1 ml. of acetic anhydride is added quickly, and the funnel is immediately shaken for 5 seconds and returned to the water bath for 45 seconds. This process is continued until 10 ml. of acetic anhydride have been added.

Upon removal from the bath after the tenth addition of acetic anhydride, excess water is quickly blotted from the outside of the funnel. Eighty milliliters of 18 *N* sulfuric acid are added from a graduate or dispensing buret and the funnel is tumbled (not shaken) five times to mix the contents. As soon as the liquid phases have separated, which takes only a few seconds, the lower or sulfuric acid layer is withdrawn directly into a dry, fritted-glass filter crucible, medium porosity, of 30-ml. capacity, prepared with an additional thin mat of medium-fiber-length filtering asbestos. The use of a Fisher Filtrator is recommended and the sample is collected in a dry 250-ml. beaker. Porosity of the crucible should be such that the 85 ml. of sulfuric acid will be filtered in 3 or 4 minutes.

The sample is transferred to a 100-ml. volumetric flask, diluted to volume with 18 *N* sulfuric acid, and compared at once on the Fisher electrophotometer with a green light filter (wave length 525 mμ). The rosin content of the sample aliquot is determined by consulting a graph previously prepared in like manner, using 1-, 2-, 3-, and 4-mg. samples of purified rosin. If the color intensity of a sample aliquot equals or exceeds the color equivalent to 4 mg. of rosin, the test should be repeated using a smaller aliquot sample. If rosin esters are present in the sample, as indicated either by previous qualitative test or by coloring of the benzene layer in the above procedure, the test must be repeated, using a proportionately smaller aliquot until analysis of the aliquot shows approximately 1 ± 0.5 mg. of rosin.

Details of preparing the rosin standard are discussed in a later paragraph. Total time for conducting the extraction procedure, from drawing of aliquot to comparison of color on electrophotometer, should not exceed 20 minutes.

COLORIMETRIC PROCEDURE FOR ROSIN ESTERS. A sample aliquot, estimated to contain the equivalent of 2 to 15 mg. of abietic acid, is withdrawn from a predried buret into a dry 100-ml. glass-stoppered graduated cylinder and diluted to the 50-ml. mark with benzene, ACS reagent grade, and 0.500 ml. of acetic anhydride (C.P., 98% minimum) is added from a dry 10-ml. buret. The contents of the graduate are thoroughly mixed and placed in a reservoir of cold water maintained at a temperature of 15° to 18° C. by means of ice. After 3 minutes the graduate is withdrawn and quickly wiped dry, and one drop of concentrated sulfuric acid (95.5% minimum) is added. The graduate is immediately stoppered and shaken with maximum speed and vigor until color develops and for 3 seconds longer. It is immediately placed in an upright position before a light source and matched at once with potassium permanganate standards previously prepared as outlined below. Fading usually starts in 10 to 15 seconds and the test should be repeated with varied aliquot sizes until one or more of the permanganate standards has been perfectly matched. The abietic acid equivalent content of the sample aliquot is determined from Table I, or, if desired and if the type of rosin ester is known, it may be calculated in terms of the ester present.

PREPARATION OF POTASSIUM PERMANGANATE COLOR STANDARDS. C.P. potassium permanganate (0.100 gram) is dissolved in 1 liter of distilled water. If the solution is not free of suspended matter, it is filtered through a fritted-glass filter funnel. Ten aliquots, ranging in volume from 1 to 10 ml., are withdrawn from a buret into separate 100-ml. glass-stoppered, graduated cylinders of equal size, shape, and height of graduations. These graduates match those used to develop color in the sample aliquots. The permanganate aliquots are diluted to 50 ml. with distilled water. Both the diluted and undiluted permanganate standard solutions are unstable and must be prepared fresh the same day they are used. The usual methods of stabilizing permanganate standard solutions are unsuitable for these dilutions.

Calculation of Rosin Esters. Matching of a large number of commercial samples of high quality and low acid number has established the relations shown in Table I. The equivalent weights of abietic acid are calculated from the theoretical content of completely reacted ester gum and maleic rosin ester.

ACCURACY

Accuracy of the methods is hard to establish, because of the difficulty of obtaining suitable standards. The amount of abietic acid in rosin has never been definitely established and undoubtedly varies with grades of commercial rosin. The color of reaction, assumed to be due to abietic acid, may be partially due to other rosin acids present. Rosin is not completely stable to heat, and heat is involved in the manufacture of many rosin products. Various samples of lead resinate, on analysis, show varying composition in lead and in rosin content. The analysis of resins containing free rosin shows a decided decrease in abietic acid with time of storage. The acid number of such samples likewise decreases. Powdered rosin has shown a decrease of 5 to 9 acid number units in 6 weeks on exposure to air (*3*). As nearly as can be estimated, however, the methods appear to have a maximum deviation of 1.0%, although reproducibility in some cases appears much higher.

Some analytical results are shown in Table II. These results are not conclusive, as the estimated ester content of the samples is based on bulk laboratory weighings which are known to cause variations of 1 to 2% from intended composition. Table III shows the free rosin content of some dry, incompletely esterified ester gums. The rosin content of these gums is calculated from their acid numbers as compared to the acid number of the rosin from which they were made—N-wood rosin having an acid number of 162.

Table I. Abietic Acid Equivalents

0.01% $KMnO_4$ Diluted to 50-Ml. Volume with H_2O, Ml.	Equivalent Ester Gum, Mg.	Equivalent Maleic Rosin Ester, Mg.	Calculated Equivalent Abietic Acid, Mg.
2	3.33	4	3.2
4	6.67	8	6.4
6	10.0	12	9.6
8	13.33	16	12.8
10	16.7	20	16.0

DISCUSSION

Attempts to substitute benzoyl chloride for acetic anhydride, as recommended by LaLande (*1*) in his survey of qualitative rosin tests, were unsuccessful. Acetyl chloride was also unsatisfactory.

The color developed in both procedures is affected by heat. The color forms faster at elevated temperatures but fades rapidly. At excessively low temperatures, color does not form at all. The extraction procedure for determining free rosin must be conducted on a timed schedule, and the operator must be able to carry the determination through without interruption. There is apparently no fading of the color developed prior to dilution with sulfuric acid. However, the fading proceeds very slowly from this point, and results of good reproducibility have been obtained by different operators. This accounts for the desirability of a filter crucible of pretested filtering rate.

The graph for determining free rosin is a straight line, but be-

cause of the many opportunities for variation, it will be necessary to develop color on each known aliquot sample twice and plot the majority of points. An attempt was made to extend the sample size limit beyond the equivalent of 4 mg. To do so required withdrawing the sulfuric acid layer and repeating the process so as to continue the color extraction. Although this technique is possible, it was found to be less accurate and unnecessary. When the sample size is 4 mg. of abietic acid or less, all the color is extracted by the procedure as outlined.

Table II. Typical Analyses of Rosin Esters

Type of Resin Solution	Estimated Content as Rosin Ester (Solids Basis), %	Composition by Analysis (Solids Basis), %
Maleic rosin ester (varnish)	20	19.1
Maleic rosin ester (varnish)	40	41.1
Ester gum varnish	25	26.0
Ester gum lacquer	25	24.4

The use of wood or gum rosin as a standard for preparing the color graph is unsatisfactory. It is practically impossible to obtain reproducible results from different samples of high grade rosin. Large lumps of rosin give more color than an equal weight of small lumps taken from the same source, and powdered rosin gives even less color. This is apparently due to the rapid oxidation of free rosin. This same effect is not apparent when dealing with rosin esters. It has been noted, however, that a recheck of a sample that has been standing 24 hours, dissolved in benzene, gives slightly less color than the original test. It is therefore recommended that all tests be completed the same day the samples are prepared. It is necessary to use a purified form of abietic acid as standard for the free rosin procedure. Abietic acid for use as a standard was prepared from two different sources. Although the two samples were not identical in appearance, they yielded identical color intensities. One source, which gave pure white crystals, was a sample of tall oil that had been on hand for approximately one year. In this time, a considerable quantity of crystals had separated from solution. The oil was filtered through paper of coarse porosity and the crystals were transferred and washed thoroughly with 75% ethyl alcohol (by volume). After air-drying, the crystals were placed in a vacuum at room temperature for 1 hour. The resulting product was practically odorless. A suitable standard can also be prepared from wood rosin by a method proposed by Steele (*5*). Seventy grams of wood rosin are refluxed with 50 ml. of 98% acetic acid for 2 hours and filtered while hot through paper. After standing 12 hours or longer in a closed container, agitation will cause crystals of abietic acid to separate from solution. These are filtered through paper in a Büchner funnel and washed with 75% ethyl alcohol (by volume) until the filtrate is practically colorless.

The color developed in the procedure for rosin esters matches that of potassium permanganate perfectly. A reddish appearance, compared to permanganate of equal intensity, indicates a charred or brownish coloration existing with the characteristic violet. This condition can be corrected by using smaller sample aliquots and matching to standards of less intensity. The color standards could be extended as high as 20 ml. of permanganate in 50-ml. volume and would match 40 mg. of maleic rosin ester, but the distinctions between concentrations are harder to make. The comparison of color with potassium permanganate was suggested by Stock (*6*) in 1926. A daylight fluorescent tube placed horizontally 2 inches above a table top was found to be an excellent light source for color comparison. The amount of concentrated sulfuric acid added in the procedure for rosin esters should be fairly constant. It has been found that a "medicine dropper" with a large opening will not deliver more than 0.025 ml. of concentrated sulfuric acid. This is a suitable quantity; a larger volume is slow in settling and does not permit prompt comparison of color.

The fact that rosin salts are determined by the same procedure as free rosin would seem to limit the usefulness of this procedure. That such is not the case is due to the great difference in the amount of rosin anticipated in a sample which contains metallic resinates only, as compared to a sample to which free rosin has been added as an adulterant. The behavior of rosin salts, reacting as free rosin, is probably due to reaction with sulfuric acid which liberates abietic acid. Rosin esters, being more stable to the acid, behave differently. Incompletely esterified ester gums, having acid numbers above 5.0, produce a highly intensified color when tested by the procedure for rosin esters. The intensity increases rapidly with increasing acid number. Similarly, the color formed with these gums fades rapidly when developed; the speed of fading increases with acid number. Such gums are probably mixtures of mono-, di-, and triresinates, and will show a positive qualitative test for free rosin and cannot be analyzed for ester content. Attempts to complete the esterification so that analysis could be made, and efforts to separate rosin from rosin esters by saponification, were unsuccessful. High temperature saponification destroys the color-producing properties of rosin esters. When hydrogenated rosin is tested qualitatively, it gives positive tests for free rosin and for rosin ester; when tested quantitatively, the combined composition does not exceed 100%. Methyl abietate behaves like free rosin and rosin salts.

Table III. Free Rosin Content of Dry Ester Gums (Incompletely Esterified)

Acid No. of Ester	Free Rosin Content, %	
	Calculated	Analyzed
5.6	3.5	3.1
10.5	6.5	5.9
19.0	11.7	13.3
36.0	22.2	20.3

When rosin-modified glyceryl phthalate resins are tested by the procedure for rosin esters, an intense, rapidly fading color forms which is from four to six times higher than anticipated from a known composition. The method for determining rosin esters is not recommended for phthalic anhydride alkyd resins, although an approximate composition can be obtained by running the test as outlined and dividing the result by 5. It is interesting to note, however, that a mixture of a known amount of ester gum with an alkyd which contains no rosin can be analyzed accurately. It is the combination of rosin, glycerol, and phthalic anhydride which produces the intensified color. Free rosin and resinate driers can be determined on resin of any type, including the alkyds. There are several types of finishes in which rosin modification would be desirable, but in the absence of a suitable method of determining either the nature or amount of rosin, it has been necessary to prohibit its use entirely in many paint specifications.

Unsaturated oils such as tung or linseed do not affect either determination. Certain fish oils produce slight brown coloring in the rosin ester test, but the use of small aliquots prevents interference.

When lacquers containing nitrocellulose are added to benzene and the insoluble nitrocellulose separates from solution, much of the rosin ester present is coprecipitated. Attempts to redissolve and reprecipitate to recover these esters have not been completely successful. Further investigations will be conducted along these lines because rosin esters are used extensively in lacquers. If no separation occurs when a lacquer is added to benzene, the tests may be applied. No solvent, other than benzene, has been found suitable for use in the two procedures.

Additional work will be done to broaden the applicability of the procedures. It is hoped that the investigations, as reported,

will stimulate the interest of other workers in the field. Although the investigation was conducted primarily with paints, the analytical procedures should prove useful in the wide variety of industries in which rosin products are used.

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LITERATURE CITED

(1) LaLande, W. A., Jr., *J. Am. Chem. Soc.*, **55**, 1536 (1933).
(2) Liebermann, *Ber.*, **17**, 1884 (1884).
(3) Mattiello, J. J., "Protective and Decorative Coatings," Vol. I, "Raw Materials for Varnishes and Vehicles," Chap. 7 on "Rosin," by P. O. Powers, New York, John Wiley & Sons, 1941.
(4) Shaw, T. P. G., IND. ENG. CHEM., ANAL. ED., **16**, 541 (1944).
(5) Steele, L. L., *J. Am. Chem. Soc.*, **44**, 1333 (1922).
(6) Stock, E., *Farben Ztg.*, **31**, 2777 (1926).
(7) Wolff, H., *Ibid.*, **26**, 648 (1920).

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Determination of Alpha- and Beta-Lactose in Dry Products of Milk from Rates of Crystallization

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The lactose in dry products of milk manufactured by most commercial processes is known to exist in an amorphous state with the alpha and beta forms present in approximately the same equilibrium ratio as in fluid milk. However, with certain manufacturing processes and in products of high moisture content the ratio of α- to β-lactose is altered by the crystallization of part of the lactose as the alpha hydrate. A convenient method for estimating these two forms of lactose in dry milk products is, therefore, desirable. It was found that β-lactose can be determined from its rate of crystallization in a saturated lactose solution abundantly seeded with crystalline alpha hydrate, and α-lactose can be obtained as the difference between the total lactose and β-lactose. This method does not require special equipment and yields results in satisfactory agreement with those obtained by other methods.

ACCORDING to Hudson (*4*) the rates of solution and crystallization of lactose are controlled entirely by the rate of conversion of one form of the sugar into the other. Hudson (*4*) found that when a large excess of finely powdered lactose hydrate is shaken continuously with water at constant temperature, a definite amount dissolves initially and then more continues to go into solution slowly until a final solubility is attained. In the initial stage the process is one of ordinary solution and is very rapid because of the large area of solid-liquid interface. In the second stage the rate of solution is controlled entirely by the rate of isomerization of the alpha to the more soluble beta modification and cannot be further increased by increasing the contact between the solid and liquid phases. This limiting rate has been referred to by Hudson (*4*) as "the maximum rate" of solution, although it is actually much slower than that of the initial stage of solution. The initial solubility represents the equilibrium concentration of the alpha form of lactose, while the difference between the final and initial solubilities gives the equilibrium concentration of the beta modification. On this basis both equilibrium and rate equations have been derived by Hudson (*4*). The solubility method (*1*) for α- and β-lactose, previously published by the authors, was based upon the equation for the maximum rate of solution.

In a solution in which the concentrations of both the α- and β-lactose are in excess of their equilibrium concentrations Hudson (*4*) found that addition of a large amount of fine crystals of α-lactose hydrate and application of vigorous agitation cause the immediate crystallization of the excess alpha modification, followed by a slow conversion of the beta to the alpha form, which then crystallizes as soon as it is formed. The rate of crystallization in this latter stage is controlled entirely by the rate of transformation of the beta to the alpha modification. This limiting rate, referred to by Hudson (*4*) as "the maximum rate of crystallization," is described by the equation,

$$k_2 t = \log \frac{C_0 - S_\infty}{C_t - S_\infty}$$

where k_2 is the rate constant, C_0 is the total initial lactose concentration after seeding with lactose hydrate crystal, S_∞ is the final solubility, and C_t is the concentration of lactose at any given time t. From this relationship an additional method has been developed for the determination of α- and β-lactose in dry products of milk.

The lactose in commercial dry products of milk exists either predominantly as crystalline alpha hydrate or as a glass with the two modifications in equilibrium proportion. Of the two forms of lactose the beta, whether in the glass or crystalline state, dissolves rapidly in a saturated solution of lactose; the crystalline alpha hydrate does not dissolve; and the alpha glass dissolves but immediately crystallizes when agitated with enough crystalline alpha hydrate. As the final solubility of lactose is not affected by the constituents of milk (*5*), the increase in concentration of lactose that results from the addition of dry milk to a continuously agitated saturated solution of lactose containing crystalline alpha hydrate is, therefore, from the β-lactose of the sample. This β-lactose, once dissolved, slowly disappears as a result of its conversion to the alpha hydrate. If the quantity of lactose in solution is determined at several known time intervals and the logarithm of $(C_t - S_\infty)$ is plotted against t in accordance with the equation for the maximum rate of crystallization, a straight line should result, whose intercept at zero time gives the β-lactose in the original sample. The difference between the total lactose and the β-lactose thus obtained is the alpha component.

APPARATUS

The apparatus consists of a borosilicate glass cylinder, 5.5 cm. in diameter and 15 cm. long, fitted with a two-hole rubber stopper. Through one hole an electrically driven stirrer is inserted. The other hole is used as a sampling outlet and stoppered when not in use. The cylinder is placed in a constant temperature water bath maintained at 25° C.

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PROCEDURE

Prepare a saturated solution of lactose by dissolving exactly 22.8 parts of C.P. α-lactose hydrate in 100 parts by weight of distilled water. Solution may be hastened by slight warming of the mixture. Cover the final solution with a layer of toluene to prevent mold growth and store the solution at 25° C. for at least a day before use.

Weigh 30.0 grams of dry milk of less than 5% moisture and mix thoroughly with 35 grams of α-lactose hydrate powder. If the moisture content is above 5%, the sample should be dried at about 65° C. to reduce it below this level before analyzing. Quickly add the mixture to the borosilicate glass cylinder containing 122.8 grams of saturated lactose solution at 25° C. and note the time. Before transferring the cylinder and contents to the water bath it is necessary to dislodge any clump of powder that may adhere to the wall of the cylinder, so that rapid and complete dispersion is facilitated. Apply stirring at a rate sufficient to keep the solids in suspension. Withdraw 20 to 25 ml. of the suspension at several known time intervals. Centrifuge immediately in a 50-ml. centrifuge tube at approximately 1000 r.p.m. for 3 minutes. Carefully decant the supernatant liquid into a clean, dry test tube. Weigh accurately 6 to 7 grams of the centrifugate and determine lactose by the Hinton-Macara method (*3*). Reconstitute 10.00 grams of the dry milk sample to a final volume of 100 ml. and determine total lactose by the same method.

On a separate portion of the centrifugate determine the water content by means of the following procedure: Weigh accurately 2 to 3 grams of the centrifugate in an aluminum dish of the type used in the determination of total solids of milk. Evaporate on a steam bath for 15 to 20 minutes and then dry in a vacuum oven at 100° C. under a pressure of 2 to 3 mm. of mercury for 2 hours. The loss in weight is calculated as water.

Express the lactose result obtained by the Hinton-Macara method (*3*) as grams of lactose hydrate per 100 grams of water, using the water content determined by the above procedure. From this value subtract 22.80, the lactose content of the saturated solution, and express the difference as per cent of the total lactose found in the 30.0 grams of dry milk used for the determination. When the logarithm of this quantity is plotted against the corresponding time, a straight line should result, whose intercept at zero time should give the β-lactose content expressed as per cent of the total lactose in the sample of dry milk. The difference between this and 100% is the amount of α-lactose.

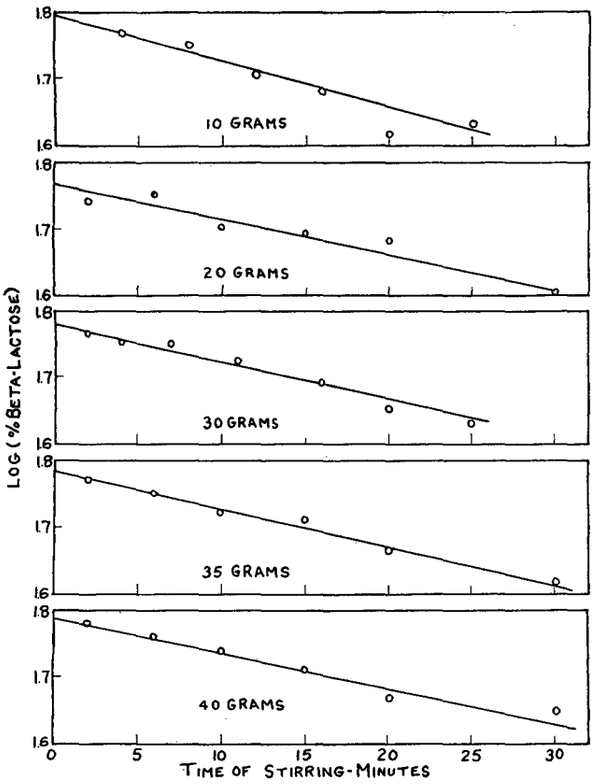

Figure 1. Effect of Variation of Sample Size

Grams of spray process nonfat dry milk solids per 100 grams of water

RESULTS AND DISCUSSION

To determine the range of sample size within which this method is applicable, varying quantities of a sample of spray process nonfat dry milk solids were used. Data are plotted in Figure 1 with the least squares line drawn through each set of points. Values for α- and β-lactose calculated from the vertical intercepts ar presented in Table I.

The results show that sample size from 10 to 40 grams, inclusive, yielded essentially the same results. With increasing quantity of sample used the relative accuracy of lactose analysis may be improved because of the larger amount of lactose present, but the viscosity of the suspension also increases. A sample size of 30 grams was found to be a convenient quantity to use.

Table I. α- and β-Lactose Content of Nonfat Dry Milk Solids

(As per cent of total lactose)

Weight of Sample, Grams	Lactose Distribution	
	% beta	% alpha
10.0	61.8	38.2
20.0	59.0	41.0
30.0	60.3	39.7
35.0	61.0	39.0
40.0	61.4	38.6

In Figure 1 the slope of the lines, which is a measure of the rate constant, k_2, is approximately 0.006 for all concentrations of milk solids used. This value was also found when other samples of spray and roller process nonfat dry milk solids were analyzed. While Herrington (*2*) was not able to measure the mutarotation velocity, $k_1 + k_2$, of lactose in milk by the polaroscopic method because of interference from the turbidity of milk, both the solubility method (*1*) and the present crystallization method offer means of obtaining such data. The equilibrium constant, K, which may be expressed as the ratio of the two velocity constants, k_1/k_2, is known at several temperatures (*6*). The rate constant, k_2, can be obtained by either the solubility method or the present crystallization method. From these two quantities the mutaro-

Table II. α- and β-Lactose Content of Some Dry Products of Milk

(As per cent total lactose)

Sample	Crystallization Method		Solubility Method	
	% beta	% alpha	% beta	% alpha
Spray nonfat dry milk solids				
1	62.5	37.5	...	...
2	63.0	37.0	...	...
3	60.4	39.6	...	...
Roller nonfat dry milk solids				
1	61.7	38.3	...	...
2	61.0	39.0	...	...
3	62.1	37.9	...	...
Dry whey solids				
1	60.9	39.1	62.6	37.4
2	62.2	37.8	59.6	40.4
3	62.0	38.0	62.0	38.0
4	9.1	90.9	11.0	89.0
5	28.5	71.5	30.1	69.9
6	28.2	71.8	28.5	71.5
7	20.0	80.0	19.4	80.6
8	20.0	80.0	20.2	79.8
9	13.0	87.0	15.9	84.1
10	11.1	88.9	13.2	86.8

tation velocity, $k_1 + k_2$, can be calculated. In the equations derived by Hudson for the maximum rates of solution and crystallization, common logarithm is employed. If the rate constants are to be applied to kinetics problems, they must be multiplied by the logarithmic conversion factor 2.3026.

In Table II results on the α- and β-lactose distribution in different dry products of milk as determined from rates of crystallization are presented along with some data obtained by the solubility method (*1*). The values for nonfat dry milk solids are in agreement with those reported by Sharp and Doob (*7*) and by the present authors using the solubility method (*1*). The ratio of β- to α-lactose is very close to that found in fresh fluid milk at ordinary temperature, a fact which indicates that the drying operations cause very little disturbance in the equilibrium of the two forms of lactose. This is also true of dry whey solids samples 1, 2, and 3. In the remaining samples of dry whey solids, in which crystallization of the lactose had been induced in the processing, the alpha modification predominates.

In general, results obtained by the solubility method (*1*) and by the crystallization method are in good agreement. The techniques employed and the length of time required per analysis are very similar in both methods.

LITERATURE CITED

(1) Choi, R. P., Tatter, C. W., O'Malley, C. M., and Fairbanks, B. W., *J. Dairy Sci.*, **32**, 391 (1949).
(2) Herrington, B. L., *Ibid.*, **17**, 659 (1934).
(3) Hinton, C. L., and Macara, T., *Analyst*, **52**, 668 (1927).
(4) Hudson, C. S., *J. Am. Chem. Soc.*, **26**, 1065 (1904).
(5) Hunziker, O. F., and Nissen, B. H., *J. Dairy Sci.*, **9**, 517 (1926).
(6) Kendrew, J. C., and Moelwyn-Hughes, E. A., *Proc. Roy. Soc. (London)*, **A176**, 352 (1940).
(7) Sharp, P. F., and Doob, H., Jr., *J. Dairy Sci.*, **24**, 589 (1941).

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Potentiometric Analytical Methods for Hydrazino Compounds

Hydrazine Sulfate

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This potentiometric study of the oxidation of hydrazine to nitrogen was made in order to develop general procedures for the quantitative determination of hydrazine nitrogen in organic compounds. Experimental conditions are systematically varied, so that the reduction of iodate ion proceeds to the three distinct equivalence points: the iodine monochloride, the iodine, and the iodide ion. The relative effect of hydrogen ion and chloride ion concentrations on the iodate reduction is studied in both the presence and absence of chloroform. Recommended potentiometric procedures for the quantitative determination of hydrazine sulfate are described. Because potentiometric procedures may be varied to a greater degree than most generally accepted methods allow, it is probable that the hydrazine nitrogen in other compounds may be determined by a proper choice of one of the procedures described.

THE quantitative oxidation of hydrazine to nitrogen with various oxidants has led to numerous analytical methods for the determination of hydrazine. A comprehensive review of quantitative methods is given by Penneman and Audrieth (*2, 8*).

Jamieson (*5*) applied the Andrews method (*1*), essentially titration with potassium iodate in a strong hydrochloric acid solution with formation of iodine monochloride, to several hydrazine salts. The potentiometric study of this reaction in at least 4 *F* hydrochloric acid was found by Singh (*10*) to give accurate and reproducible results with a sharp inflection point when using a platinum foil electrode versus a calomel cell. Stelling (*11*) reported that the potentiometric titration of hydrazine with iodic acid gave iodine as an end product in sulfuric acid solutions and iodine monochloride in hydrochloric acid solutions.

Bray and Cuy (*3*) found that the oxidation of hydrazine to nitrogen in 0.5 to 2.0 *N* sulfuric acid was complete in 3 minutes when treated with a known excess volume of iodic acid. The excess iodic acid was determined by reduction to iodine with potassium iodide and titration with potassium thiosulfate. The oxidation of hydrazine by iodine (*3*) was found to be rapid in alkaline solution and slow in acid solution. In the presence of air in alkaline solution hydrazine undergoes slow decomposition due to a reaction with oxygen. By proper addition of reagents this decomposition was minimized to give agreement within 0.2% of theory.

Kolthoff (*6*) observed that the concentration of hydrochloric acid solution had considerable effect upon the quantitative aspects of the iodate-hydrazine reaction when carbon tetrachloride was used as a solvent indicator. A decrease of the final hydrochloric acid concentration from 2.9, 2.3, 1.7, to 0.9 volume *F* increased the results from 0.0, 0.4, 1.0, to 4.0%, respectively, above the theoretical value. All these observers in performing the Jamieson method or modifications stress the importance of maintaining a final concentration in the hydrochloric acid solution greater than 3.0 *F*.

Swift (*12*), however, in a series of experiments determined the relative effect of the concentration of hydrochloric acid solutions upon the titration of iodide ion with iodate ion by the Andrews method, and concluded that the high concentration of acid is necessary for a rapid rate of reaction and not for prevention of hydrolysis of the iodine monochloride. His results were in good agreement when the final concentration of hydrochloric acid was 1.0 *F*. Because of the small potential change at low acid concentrations, the electrometric method of analysis (*13*) may be in error in 2 *F* hydrochloric acid. Philbrick (*9*) in examining the hydrolysis of iodine monochloride reiterates Swift's opinion that the high acid concentration used is not necessary to reduce hydrolysis, but is needed to increase the reaction rate. The intensity or position of the absorption band for iodine monochloride in aqueous solution does not change with hydrochloric acid or alkali

chloride concentration in going from 0.2 to 10 F (*4*). This effect is added evidence for the essentially complete prevention of hydrolysis in solutions of hydrochloric acid or alkali chloride which are as low as 0.2 F. Lang (*7*) has shown that the hydrochloric acid in the Andrews method, provided its concentration does not fall below 10% by volume (1.2 F), may be replaced almost completely by potassium chloride. It is reasonable to assume that this same effect may also be produced by other alkali chlorides.

Audrieth (*2*) expresses the opinion, based upon some preliminary work by Bray and Cuy (*3*), that the oxidation of hydrazine by iodate ion proceeds slowly in neutral or alkaline solutions at room temperature. The basis for this opinion, not discussed, is apparently either the lack of formation of iodine in the solution or the slow liberation of nitrogen. The iodide end point lefinitely is of theoretical interest as a stage in the reduction of iodate ion, especially if the reaction is performed under an inert atmosphere of nitrogen in an alkaline medium.

This potentiometric investigation of the titrimetric analysis of hydrazine sulfate with potassium iodide is primarily concerned with stages of the iodate ion reduction, determination of the optimum conditions for developing analytical procedures for hydrazino-containing compounds, evaluation of the recommended analytical procedures for hydrazine sulfate, and the theoretical aspects of the reaction mechanism.

EXPERIMENTAL

Apparatus and Materials. The electrical apparatus consisted of a Model G Beckman pH meter arranged to measure electromotive force for oxidation-reduction reactions. The platinum electrode, attached to the upper pin-jack of the pH meter in all titrations, served as the indicating electrode and the saturated calomel electrode as the reference half-cell. An Ivan Sorvall magnetic stirrer with a glass-covered bar served as an agitator for the titration performed in a 200-ml., 3-necked, round-bottomed flask. The flask was fitted with rubber stoppers, in which were placed the two electrodes and the tip of the 50-ml. buret. The system was rearranged slightly for the alkaline titrations performed under nitrogen.

The standard solution of potassium iodate, Merck reagent grade, was 0.02500 volume F (5.3505 grams per liter). When the solution of potassium iodate was to be used for titrations in alkaline systems, it was prepared from boiled, distilled water and stored under nitrogen. The hydrazine sulfate was prepared from distilled hydrazine hydrate and sulfuric acid; it was recrystallized several times from distilled water and dried at 140° C. The analysis of the hydrazine sulfate for purity (99.6 to 99.8%) gave consistent and reproducible results either potentiometrically (*10*) or by the Jamieson method (*5*). The slight stoichiometric discrepancy existing between potassium iodate, as the primary standard, and the hydrazine sulfate is inconsequential for the purpose of this investigation. All other reagents used were chemically pure.

General Analytical Procedure. The customary procedure was to dissolve the hydrazine sulfate in a small volume of hot water to facilitate solution. The required amount of acid or salts then was added to the remaining portion of water, and after cooling, this solution was added to the hydrazine solution. The initial volume of all the solutions was 60 ml.; the initial temperature was 25° to 30° C. No apparent difference existed between rapid and slow addition of potassium iodate solution, provided that nearly equilibrium conditions were attained near the end point.

DISCUSSION OF IODATE ION REDUCTION STAGES

Iodine Monochloride End Point. The potentiometric titration of hydrazine sulfate with potassium iodate to the iodine monochloride end point is quantitative under the different experimental conditions employed. The net reaction may be formulated by the equation

$$N_2H_5^+ + IO_3^- + H_3O^+ + Cl^- \longrightarrow ICl + N_2 + 4H_2O \quad (1)$$

The intermediate reduction of iodate ion to iodine with the subsequent oxidation to iodine monochloride is the characteristic reaction of the Andrews method (*1*) and is not formulated in Equation 1.

Table I illustrates the effect of varying the initial concentration of hydrochloric acid solution from 0.5 to 9.0 F in the absence of chloroform. The potential change at the end point progressively increased from 100 to 5000 mv. per ml. The experimental evidence indicates that acid concentrations greater than 2.0 F are convenient though not absolutely necessary for the quantitative determination of hydrazine. The titrations were reproducible within 0.2% in the 2.0 to 9.0 F range; the 0.5 and 1.0 F solutions gave slightly high results.

Table I. Effect of Hydrochloric Acid and Chloride Ion Concentration on Titration of Hydrazine Sulfate to Iodine Monochloride End Point

Initial Formality[a]		$N_2H_4 \cdot H_2SO_4$,	0.02500 F KIO_3,	% of Theory,	Potential Change,
HCl	Cl^-	Gram	Ml.	Ml.obsd./Ml.calcd.	Mv./Ml.
0.5	0.5	0.1045	31.70-33.60	98.7-104.6	Erratic 30-70
0.5	0.5	0.1095	34.00	101.0	40
0.5	1.0	0.1118	34.60	100.7	151
0.5	1.0	0.1058	32.71	100.7	220
0.5	3.0	0.1065	32.67	99.8	300
0.5	3.0	0.1059	32.40	99.5	350
0.5	6.0	0.1091	33.49	99.9	510
0.5	6.0	0.1061	32.49	99.6	450
0.5	9.0	0.1098	33.65	99.7	2800
0.5	9.0	0.1090	33.44	99.8	2200
1.0	1.0	0.1075	33.04	100.0	190
1.0	1.0	0.1040	31.92	99.8	260
2.0	2.0	0.1056	32.30	99.5	500
3.0	3.0	0.1031	31.60	99.7	570
3.0	3.0	0.1031	31.60	99.7	670
6.0	6.0	0.1213	37.18	99.7	2900
6.0	6.0	0.1020	31.19	99.5	2100
6.0	6.0	0.1142	34.93	99.5	2300
6.0	12.0	0.1010	30.98	99.8	3500
6.0	12.0	0.1148	35.18	99.7	4000
9.0	9.0	0.1006	30.86	99.8	5500
9.0	9.0	0.1010	30.98	99.8	8800
9.0	9.0	0.1039	31.87	99.8	3300

[a] Original volume, 60 ml. in all titrations in these tables. Volume formal concentrations, formula weights per liter of solution, used to express all concentrations. Chloride ion concentration increased by addition of either lithium or sodium chloride.

The high results cannot be explained by the hydrolysis of iodine monochloride because the final concentration of the solutions in respect to hydrochloric acid was still above 0.2 F. Perhaps the high results are best attributed to the slowness of the oxidation of iodine to iodine monochloride by iodate ion and the relatively small potential change at the end point, less than 100 mv. per ml. As Kolthoff obtained much higher deviations from theory at higher acid concentrations (*6*), it was decided to use 15 ml. of chloroform in an experiment identical to the 1.0 F trial (Table I). No definite end point was determined by the time 109.5% of the theoretical volume of potassium iodate solution had been added. The color of iodine was still observed in the chloroform layer and required 1 hour of stirring to disappear. The essential difference between the two solutions is the concentration of iodine in the aqueous phase. Thus it would seem that the relative concentration of iodine and iodate ion definitely influences the rate of reaction in solutions of low acid concentration to give an apparent high stoichiometric relationship.

The Jamieson method (*5*) requires maintaining the final hydrochloric acid concentration from 3 to 6 F. When the acid concentration becomes too low, the reaction rate decreases rapidly and results are high. The presence of a solvent layer for the iodine causes the reaction to proceed at a still slower rate, presumably owing to the reduced concentration of iodine in the aqueous phase. A high acid concentration favors the direct formation of the iodine monochloride, with only a small amount of iodine yielding a faint and disappearing end point. Both conditions tend to inhibit the attainment of equilibrium near the end point.

The effect of alkali chlorides on the reaction was investigated by maintaining an initial acid concentration of 0.5 F and increasing the chloride ion concentration from 0.5 to 9.0 F by the addition of either sodium or lithium chloride within their respec-

Table II. Effect of Sulfuric Acid Concentration on Titration of Hydrazine Sulfate with Potassium Iodate to Iodine End Point

Initial Formality, H_2SO_4	$N_2H_4.H_2SO_4$, Gram	0.02500 *F* KIO_3, Ml.	% of Theory, $Ml._{obsd.}/Ml._{calcd.}$	Potential Change, Mv./Ml.
0.0	0.1013	24.90	99.9	110
0.0	0.1156	28.00	98.5	100
0.0	0.1094	26.49	98.5	60
0.0	0.1038	25.21	98.8	160
0.5	0.1054	25.85	99.7	1600
0.5	0.1048	25.72	99.8	5800
1.5	0.1073	26.25	99.5	2000
1.5	0.1212	29.67	99.5	3000
1.5	0.1044	25.58	99.7	4900
1.5	0.1031	25.29	99.8	5300
3.0	0.1092	26.28	99.7	5600
3.0	0.1024	25.05	99.5	5600
4.5	0.1118	27.43	99.8	5400
7.5	0.1048	25.70	99.7	7500

tive solubility limits (Table I). Quantitative results within 0.2% were obtained with initial chloride ion concentrations of 3.0, 6.0, and 9.0 *F* with satisfactory potential changes at the end points. High results were obtained at 0.5 and 1.0 *F* chloride ion concentrations with smaller potential changes. These results obtained by a potentiometric procedure are in complete agreement with Lang's work (*7*) performed by a modified Jamieson method.

Iodine End Point. The potentiometric titration of hydrazine sulfate with potassium iodate to the iodine end point gave reproducible results within 0.2% in solutions of initial sulfuric acid concentration from 0.5 to 7.5 *F* (Table II). The potential change at the end point progressively increased from 1600 to 7500 mv. per ml. with the increase in sulfuric acid concentration. The net reaction may be formulated as

$$5N_2H_5^+ + 4IO_3^- \longrightarrow 5N_2 + 2I_2 + 11H_2O + H_3O^+ \quad (2)$$

This study was an extension of Bray and Cuy's evidence (*3*) that hydrazine is oxidized quantitatively to nitrogen in 0.5 to 2.0 *N* sulfuric acid.

The titrations performed in a sulfuric acid medium without a solvent layer all produced solid iodine which supposedly existed in equilibrium with its saturated solution. Two tendencies were noted in the titrations: Apparent low results were obtained when the hydrazine sulfate was titrated in distilled water; and increasing length of time was required for the potential to become stable with increased sulfuric acid concentrations. Identical experiments in the presence and absence of chloroform were carried out with the hydrazine sulfate in distilled water and in 1.5 *F* sulfuric acid. In the titrations performed in distilled water the addition of chloroform gave nearly theoretical values with respect to the volume of potassium iodate solution required. The results of the titrations performed in distilled water without the addition of sulfuric acid have a limited significance, because the decreased potential change at low acid concentrations results in a corresponding decrease in the sensitivity of the method. In a 1.5 *F* sulfuric acid solution the length of time for the titration could be shortened from nearly 1 hour to about 15 minutes by the addition of chloroform. The results obtained by titrating rapidly in the absence of chloroform were likely to be slightly high. This effect might be due conceivably to the rather slow formation of iodine at low concentrations of iodate ion and hydrazine in solutions of relatively high concentrations of sulfuric acid and iodine. Again the low results found by the titration of hydrazine sulfate in distilled water may be explained by similar reasoning—namely, that the iodine formed is partially reduced by the hydrazine to iodide ion, which in turn is rather slowly oxidized to iodine by iodate ion in the presence of relatively large concentrations of iodine. This hypothesis is supported by the observation that, in the titration, the iodine color formed in the aqueous phase, after the initial addition of a few milliliters of iodate solution, completely disappears, thus indicating a further reduction to iodide ion.

Both the iodine and the iodine monochloride end points are observed in hydrochloric acid solutions 6 *F* or less. When the acid concentrations were 1 or 3 *F*, both end points were quantitative; the same was true of a solution which was 0.5 *F* with respect to hydrogen ion and 3.0 *F* with respect to chloride ion (Table III, Figure 1). Although a potential change is noted at the iodine equivalence point in a 6 *F* hydrochloric acid solution, there is no potential change noted in the solution 6 *F* with respect to hydrogen ion and 12 *F* with respect to chloride ion (Figure 1). The effect of the chloride ion is evident on comparing curve *A*, which is a potential-volume curve of the iodine monochloride end point in a solution 6 *F* with respect to hydrogen ion and 12 *F* with respect to chloride ion, and curve *B*, which is a similar curve of both the iodine monochloride and the iodine end points in 3 *F* hydrochloric acid.

Iodide End Point. The potentiometric titrations of hydrazine sulfate with potassium iodate in alkaline solutions, which are 0.2

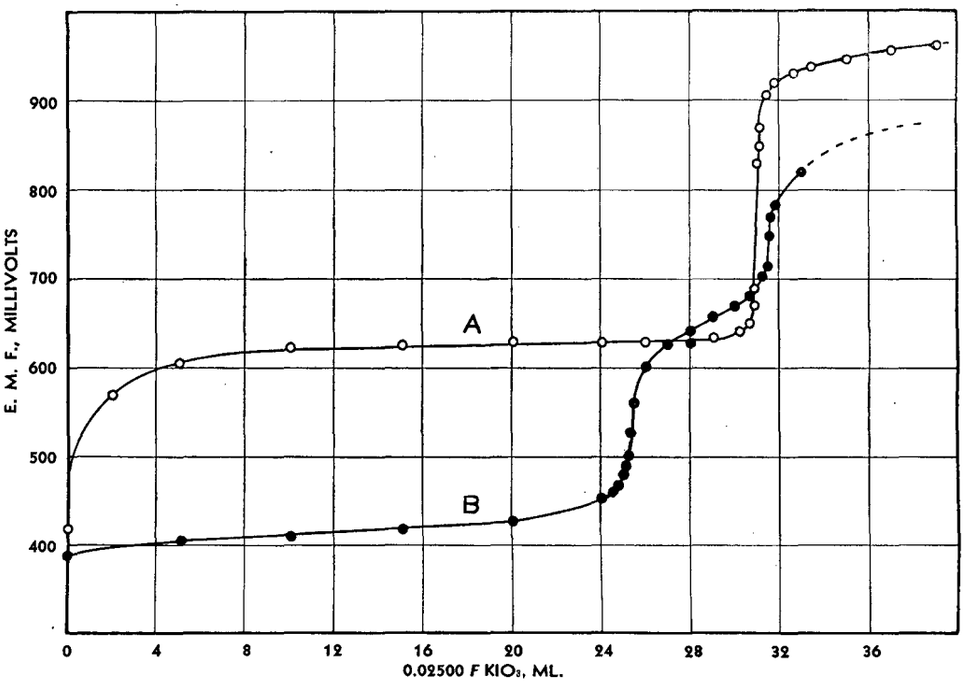

Figure 1. Iodine Monochloride End Points

A. In solution 6 *F* with respect to hydrogen ion and 12 *F* with respect to chloride ion.
B. In 3 *F* hydrochloric acid solution.

to 2.5 *F* with respect to hydroxyl ion, proceed rapidly to the iodide end point with the evolution of nitrogen. Low results, 3 to 4%, due to the concurrent oxidation of hydrazine by air were noted when the hydrazine sulfate was titrated in solutions of potassium hydroxide (Table IV). When the titration was performed in an alkaline medium under nitrogen, the volume of iodate solution required approached within 0.2 to 1.0% of the calculated theoretical volume (Table IV). Because the oxidation of hydrazine with iodate ion is quantitative to the iodine stage and the oxidation of hydrazine with iodine is quantitative to the iodide stage in the presence of excess iodine (*3*), the quantitative reduction of iodate ion to iodide ion logically appears to follow. The quantitative reaction in alkaline solution could be formulated by the net reaction

$$3N_2H_4 + 2IO_3^- \longrightarrow 3N_2 + 6H_2O + 2I^- \qquad (3)$$

Table III. Comparison of Iodine and Iodine Monochloride End Points in Same Solution

Conditions	% of Theory, $Ml._{obsd.}/Ml._{calcd.}$		Potential Change, Mv./Ml.	
	I_2	ICl	I_2	ICl
1 *F* HCl	100.0	99.8	330	260
3 *F* HCl	99.8	99.7	260	670
6 *F* HCl	100[a]	99.5	70	2100
0.5 *F* H^+, 3.0 *F* Cl^-	99.6	99.5	280	350

[a] Establishment of equilibrium too slow to be useful at such a high hydrochloric acid concentration.

The sharp inflection point and the agreement with theory of several of the titrations, especially in relation to similar titrations performed in air, indicate that the difference might be due to an incomplete elimination of oxygen from the system. A more complete investigation of this reduction stage is in progress at the present time in order to ascertain the reason for this discrepancy.

RECOMMENDED ANALYTICAL PROCEDURES

Iodine Monochloride End Point. The hydrazino compound is dissolved in 15 ml. of water, and 45 ml. of hydrochloric acid (specific gravity 1.18 to 1.19) are added. The solution is cooled before the initially rapid addition of iodate solution. The end point may be determined with a reproducibility of better than 0.2% in either of two ways: by allowing equilibrium to be attained during the dropwise addition, in which case the end point is taken as the maximum potential change per volume; or by slow dropwise addition until the potential continues to increase to between 700 and 800 mv., in which case the end point is taken as the volume where the continued increase is noted. The essential difference between the two methods is the time involved in the titration. The amount of acid required may be varied according to the individual circumstance.

Table IV. Comparison of Iodide End Point under Air and Nitrogen in Alkaline Solution

Initial Conditions	$N_2H_4.H_2SO_4$, Gram	0.02500 *F* KIO_3, Ml.	% of Theory, $Ml._{obsd.}/Ml._{calcd.}$
0.2 *F* KOH[a]	0.1004	20.00	97.2
0.3 *F* KOH[a]	0.1005	19.80	96.2
0.9 *F* KOH	0.1042	21.15	99.0
0.9 *F* KOH	0.1038	21.10	99.2
0.8 *F* NaOH	0.1185	24.00	98.9
0.8 *F* NaOH	0.1055	21.50	99.4
1.3 *F* NaOH	0.1067	21.75	99.5
1.3 *F* NaOH	0.1020	20.75	99.3
2.5 *F* NaOH	0.1023	20.70	98.7

[a] Performed under air.

Iodine End Point. The hydrazino compound is dissolved in 45 ml. of water to which 15 ml. of sulfuric acid (specific gravity 1.84) are added. The end point is determined in the same manner as the iodine monochloride end point with the same 0.2% reproducibility. In the second method 15 ml. of chloroform are added initially. This procedure may again be varied as the occasion demands.

Iodide End Point. After the hydrazino compound is dissolved in 60 ml. of boiling water, the solution is maintained continuously under nitrogen while it is cooled; after the addition of 3 grams of solid sodium hydroxide, the solution is again cooled to room temperature. The standard solution of potassium iodate is added initially as rapidly as possible and near the end point is added dropwise in order to permit equilibrium to be established.

A comparison of the three recommended procedures and the Jamieson method (*5*) is summarized in Table V. In the iodine monochloride, the iodine, and the iodide methods, the potential at the end point changes, respectively, from 600 to 900, 500 to 800, and −900 to −400 mv. with the recommended analytical procedures.

SUMMARY

Quantitative procedures for the determination of hydrazine sulfate with potassium iodate have been developed for the iodine monochloride and the iodine equivalence points which are reproducible within 0.2%. The method for the iodide equivalence point gives results varying from 0.2 to 1.0% low. While the iodide method is not recommended for most compounds, it may be the only one available for certain organic compounds.

Table V. Comparison of Recommended Analytical Procedures

Method	$N_2H_4.H_2SO_4$, Gram	0.02500 *F* KIO_3, Ml.	% Hydrazine N_2 Found (Theory 21.53)
Iodide	0.1067	21.75	21.42
	0.1020	20.75	21.37
Iodine	0.1044	25.58	21.46
	0.1031	25.29	21.48
Iodine monochloride	0.1010	30.98	21.49
	0.1006	30.86	21.49
Jamieson	0.1025	31.41	21.47
	0.1062	32.52	21.45

Because these procedures may be varied to a greater degree than most generally accepted methods allow, it is probable that the hydrazine nitrogen in organic compounds may be determined by a proper choice of one of the procedures described. For this reason the application of the potentiometric procedures to a series of organic hydrazino compounds will be a subject of later investigation.

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LITERATURE CITED

(1) Andrews, L. W., *J. Am. Chem. Soc.*, **25**, 756 (1903).
(2) Audrieth, L. F., *et al.*, "The Chemistry of Hydrazine," University of Illinois (Contract N6 ori-71, Task No. 20), Chap. 7, April 1949.
(3) Bray, W. C., and Cuy, E. J., *J. Am. Chem. Soc.*, **46**, 858 (1924).
(4) Gillam, A. E., and Morton, R. A., *Proc. Roy. Soc.*, **132**, 152 (1931).
(5) Jamieson, G. S., *Am. J. Sci.*, **33**, 352 (1912).
(6) Kolthoff, I. M., *Ibid.*, **46**, 2013 (1924).
(7) Lang, R., *Z. anal. Chem.*, **102**, 12 (1935).
(8) Penneman, R. A., and Audrieth, L. F., ANAL. CHEM., **20**, 1058 (1948).
(9) Philbrick, F. A., *J. Am. Chem. Soc.*, **56**, 1257 (1934).
(10) Singh, B., and Ilahi, I., *J. Indian Chem. Soc.*, **14**, 376 (1937).
(11) Stelling, O., *Svensk Kem. Tid.*, **45**, 3 (1933).
(12) Swift, E. H., *J. Am. Chem. Soc.*, **52**, 894 (1930).
(13) Swift, E. H., and Gregory, C. H., *Ibid.*, **52**, 901 (1930).

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Separating Asphalt into Its Chemical Constituents

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A knowledge of the composition of asphalt on the basis of molecular size and chemical type, comparable to the analysis of the overhead cuts of petroleum, was desired. The method developed involved molecular distillation to yield a size separation; silica gel chromatography to separate saturates, aromatics, and resins; solvent dewaxing of the saturates to determine wax content; urea-complex formation to separate long-chain paraffins; alumina chromatography to separate monocyclic aromatics; peroxide oxidation followed by chromatography to remove thiophene analogs; and thermal diffusion in the liquid state to segregate naphthenes on the basis of ring number. This method of analysis has proved satisfactory for establishing the concentration of paraffins, naphthenes, aromatics, resins, and asphaltenes in an asphalt, as well as their distribution on the basis of molecular size.

IN COMPARISON with overhead distillate products from petroleum, the chemical composition of asphalt is practically unknown. Although a great deal of excellent work has been done on the fractionation of asphalt, most of it has been directed toward either comparing a series of asphalts or ultimately meeting specifications. Consequently, such analyses involve considerable empiricism. The method described here illustrates a separation of asphalt comparable to the analysis of overhead cuts from petroleum. The objective was to separate the wide spectrum of chemical individuals in asphalts into a limited number of groups of components, each embracing definite chemical classes and molecular sizes. A California coastal asphalt was selected to illustrate most of the methods used.

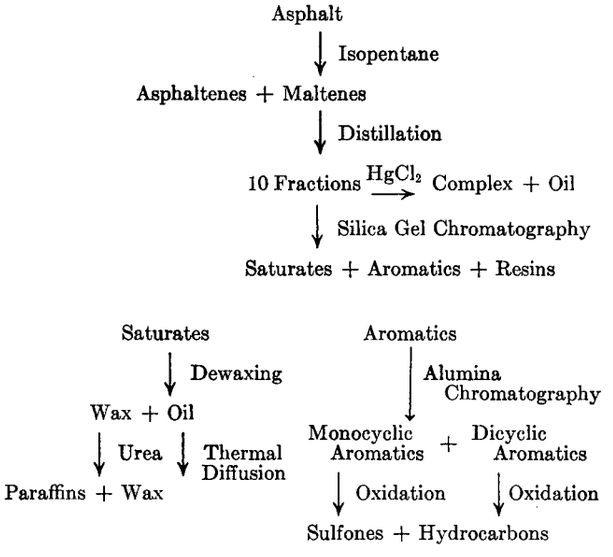

Figure 1. Schematic Diagram of Method of Separation

Most physical methods of separation into chemical types are partially vitiated when there is a broad distribution of molecular sizes. For example, solvent separation of aromatics and paraffins is not completely satisfactory, inasmuch as an aromatic of low molecular weight may be more soluble than a paraffin of high molecular weight in a solvent selected to remove paraffinic constituents. Chromatographic separation of lubricating oils leads to considerable overlapping of bands, unless the oil has previously been fractionated into narrow molecular weight ranges. The procedure described here is based on the premise that sharpness of separation can be greatly enhanced by first separating the asphalt on the basis of molecular size. Unfortunately, as the molecular weights of the individual fractions vary from about 300 to 3000, a compromise has to be made between narrowness of cuts and a convenient number of samples.

After the asphalt has been separated by distillation into fractions of fairly narrow molecular size, the individual fractions may be analyzed in a manner similar to the analysis of lubricating oil. Physical separation by silica gel chromatography into saturates, aromatics, and resins, dewaxing of the saturates to yield a wax and an oil, and urea treatment of the wax to remove paraffins have been found satisfactory. The dewaxed saturates and the urea raffinate can be further separated by means of thermal diffusion. The aromatic fractions from the majority of asphalts can then be separated into mono- and dicyclic aromatics by further chromatography. The benzothiophene homologs can be removed from the aromatics by oxidation to the sulfones and chromatographing. On a second sample of the original distilled fractions the most polar nitrogen and sulfur compounds are separated by treatment with mercuric chloride. A schematic diagram of the method generally used is given in Figure 1.

FRACTIONAL DISTILLATION

The asphalt under investigation may be distilled as such, or it may be separated into its maltene and asphaltene fractions and the maltenes distilled separately. The former method has the advantage of fractionating the asphalt in its original state, but the much higher viscosity limits the distillation. The latter method permits deeper distillation, but does not yield as representative samples as does distillation of the entire product. For example, distillation of a California coastal asphalt yielded overhead fractions considerably more waxy than did distillation of the maltenes. Moreover, fractional separation of the asphaltenes gave some fractions of lower molecular weight than the heavier maltene fractions. However, roughly 10% more of the original asphalt can be distilled if the asphaltenes are removed first.

The distillation was conducted on a conventional 14-inch cyclic molecular still obtained from Distillation Products, Inc. This apparatus was selected because the thermal lability of asphalt necessitates minimizing the time of heating and the temperature to which it is heated. With this still the feed is passed over a heated rotor in a fraction of a second, and the reservoirs are maintained at the minimum temperature required to preserve fluidity. At no time was the temperature of the material permitted to exceed 250° C. Fractions of 1000 molecular weight can be distilled overhead at this temperature.

Inasmuch as this still was designed for oil having a much lower viscosity than asphalt, a few modifications were made on the original design. All sections of the still where solidification of the asphalt could occur were equipped with electrical heaters and the degassing unit was by-passed, because it proved a major source of plugging. It was found that distillation could take place at a much lower rotor temperature if the film from which distillation occurred was maintained at a minimum thickness. This was accomplished by substituting a variable-speed motor for the original pump motor, and slowing down the feed rate

as the viscosity increased. Pressures were maintained at 4 to 10 microns during the distillation.

Figure 2 illustrates the molecular weight distribution of two typical California asphalts of the same penetration. It is apparent that there is considerable difference in component distribution just on the basis of molecular weights. It was also found that carbon-hydrogen ratio, nitrogen and sulfur content, specific gravity, and viscosity increased as the molecular weight increased.

PREPARATION OF MALTENES

The procedure employed in the preparation of the maltenes is the conventional method for obtaining separation of the insoluble asphaltenes and soluble maltenes. Isopentane (2-methylbutane) was selected as solvent because it is readily obtainable, is liquid at atmospheric pressure, and is sufficiently volatile to be removed from the maltenes without resorting to high temperatures. The asphalt sample was dissolved in an equal volume of benzene and poured with stirring into 40 volumes of isopentane. After 24 hours' standing the mixture was filtered and the insoluble asphaltenes were washed repeatedly with isopentane. The asphaltenes were stored still wet with solvent under an atmosphere of carbon dioxide until required for further experiments. The solvent was removed from the maltenes under vacuum.

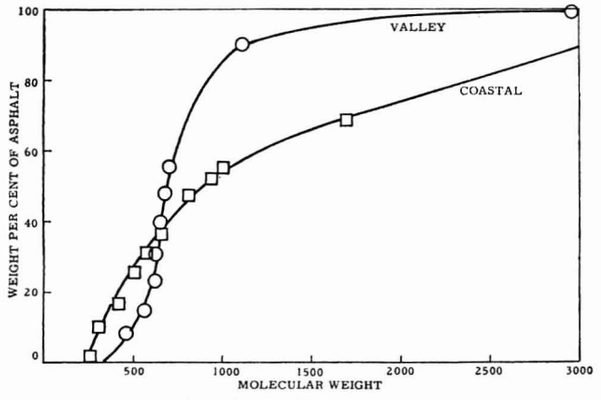

Figure 2. Molecular Weight Distribution of Two California 40/50 Penetration Asphalts

Essentially the same values are obtained whether benzene is used, or the asphalt is cooled to −75° C. and pulverized by grinding, or the asphalt is liquefied with small amounts of isopentane before being added to the bulk of the isopentane. The amount of asphalt used is immaterial, as the same percentage of asphaltenes was obtained with 15-gallon samples as with 10-gram samples.

MERCURIC CHLORIDE COMPLEX FORMATION

Inasmuch as no direct correlation could be made between the sulfur content of the asphalts investigated and their physical properties, various methods were tried for the separation of sulfur compounds. It was found that mercuric chloride was the most satisfactory reagent for this purpose. Mercuric chloride is known to form salts or complexes with a number of sulfur and nitrogen compounds. Treatment of a number of asphalts with mercuric chloride showed that the amount of sulfur compounds complexing was not proportional to the sulfur content. In some cases 80% of the sulfur was removed, whereas in others almost none of the sulfur compounds reacted. Indications are that disubstituted benzothiophenes and substituted dibenzothiophenes are the predominant sulfur compounds where complex formation did not occur. With asphalts of high nitrogen content, the complexed material is frequently higher in nitrogen content than in sulfur. The mercuric chloride–treated oils were of lighter color and had a much lower viscosity than the original material. Figure 3 illustrates the results of mercuric chloride treatment of a California coastal asphalt as a function of molecular weight, as well as the distribution of sulfur in both the complexed and uncomplexed material. Examples of the results of this separation are given in Table I.

Table I. Results of Mercuric Chloride Treatment

	Molecular Weight	% C	% H	% N	% S
Regenerated complex	583	75.7	9.7	0.7	12.2
$HgCl_2$ raffinate	583	83.4	11.3	0.4	4.3
Regenerated complex	935	76.9	9.7	0.7	10.0
$HgCl_2$ raffinate	935	83.0	11.0	0.5	4.6

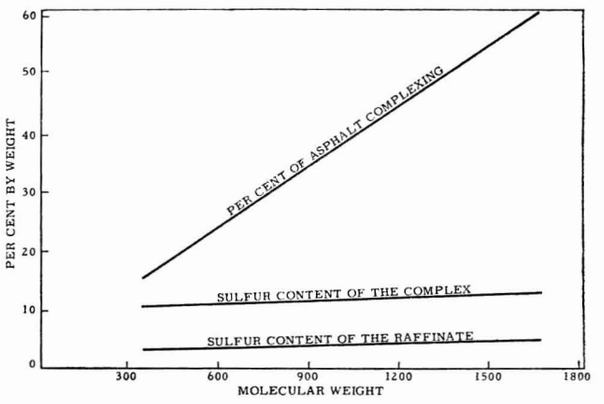

Figure 3. Mercuric Chloride Treatment of California Coastal Asphalt

Experimentally, a 10% solution of distilled fraction in isopentane was vigorously mixed with an equal volume of saturated aqueous mercuric chloride solution. Most of the reactive material complexes immediately, but the mixture was allowed to stand 24 hours with occasional shaking to ensure complete reaction. The solid complex was filtered, and washed thoroughly with isopentane to remove occluded oil and with water to remove excess mercuric chloride. The filtrate was washed with water until a test for mercuric ion was negative, then dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. The complex was decomposed by suspending in dilute hydrochloric acid, adding benzene, and after warming, passing hydrogen sulfide through the mixture. The mercuric sulfide formed was filtered from the benzene solution and the solution was washed repeatedly with saturated aqueous sodium sulfide solution to remove colloidal mercuric sulfide, and then with water. The benzene was removed from the residue under vacuum. Molecular weight determination of the regenerated complexed material showed that no polymerization had occurred.

CHROMATOGRAPHIC SEPARATION

The use of chromatography in asphalt analysis is not new. Grader (*3*) and others have used this method of separation. However, in the molecular weight range of asphalt many high molecular weight paraffins are sufficiently insoluble in paraffinic elutrients that low molecular weight aromatics are frequently eluted first. By fractionally distilling the original material into narrower molecular weight fractions, this difficulty is avoided. Furthermore, a large molecule having only one aromatic ring has almost the same solubility as a saturated hydrocarbon. Consequently, ultraviolet absorption must be determined frequently on the effluent solution during the chromatographing to determine when aromatics are being eluted. The criterion used for the first change of elutrients was the attainment by the effluent solution of an extinction coefficient of 0.1 liter per gram cm., a value corresponding to $\leq 0.2\%$ by weight aromatics in the saturates, ac-

cording to the ultraviolet definition of aromatics shown in Table III. This analytical method is more sensitive than that provided by refractive index measurements and does not lose sensitivity with increasing molecular weight.

Although chromatographic separation in the laboratory restricts the amount of material which may be handled, as much as 1 kg. of distilled fraction has been separated in a single column. Experimentally the saturates were eluted with isopentane, samples being removed periodically for ultraviolet examination for the presence of aromatics. When the absorption spectrum showed aromatics to be present in the effluent, the receiver was replaced, the solvent was changed to benzene, and the remainder of the aromatics were eluted. The benzene elution of aromatics yielded a highly colored solution. When the benzene came through the columns colorless, the eluted aromatics were removed and alcohol was substituted to remove the resins. Resins are defined here as that black, resinous fraction, containing the most strongly adsorbed molecules, which is not eluted from silica gel by benzene. The alcohol elution was continued until the adsorbent was essentially colorless. Ultimate recoveries amounted to 98 to 99% by weight of the charge. Figure 4 illustrates the distribution of saturates, aromatics, and resins for a California coastal asphalt.

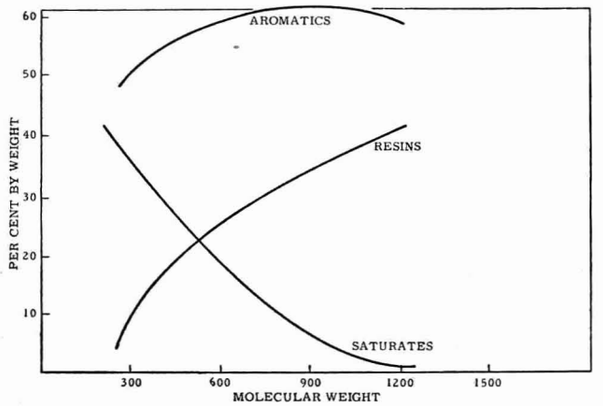

Figure 4. Distribution of Asphalt Fractions

With most asphalts the saturates are 99.1% or more carbon and hydrogen and some asphalts are 50% or more saturates with as little as 10% resins. The material removed by mercuric chloride is concentrated in the resin fraction, although only about half of the resins react with mercuric chloride. Nitrogen- and oxygen-containing compounds, as well as nonaromatic sulfur compounds, are principally in the resin fraction. Substituted benzothiophenes are eluted in the aromatic fraction, and may contain as much as 65% of the sulfur compounds present. Some typical analyses are given in Table II, illustrating the type of separation attained.

The ultraviolet absorption of these fractions is given in Table III for a few characteristic wave lengths.

The chromatograph columns consist of two sections of glass tubing, 5 feet long by 3 inches in diameter, which are water-jacketed for cooling, because the initial adsorption is accompanied by considerable evolution of heat. Sintered-glass plates are fused in the bottom of the columns to hold the adsorbent. The lower end is equipped with a stopcock for removing samples during the course of the elution, and with a distilling flask and heater for flashing off the solvent. The upper end of the column is furnished with a condenser and nitrogen inlet tube. The distilling flask is connected to the condenser by an insulated bypass tube which conveys the vaporized solvent back to the top of the column, so that elution is continuous. The apparatus thus acts very much like a large Soxhlet extractor. The nitrogen inlet tube permits the separation to be conducted in the absence of air and, hence, minimizes oxidation.

Table II. Chromatographic Separation
(Molecular weight = 867)

	% by Wt.	% C	% H	% N	% S	Empirical Formulas
Original	100	86.0	11.3	0.35	2.00	$C_{62}H_{97}N_{0.2}S_{0.5}$
Saturates	32	85.8	13.7	...	0.18	$C_{62}H_{118}$
Aromatics	53	86.2	10.7	0.20	2.6	$C_{62}H_{92}N_{0.1}S_{0.7}$
Resins	15	84.0	9.7	1.2	3.1	$C_{61}H_{83}N_{0.7}S_{0.8}$

Table III. Ultraviolet Extinction Coefficients of Chromatographed Fractions[a]

Wave Length, A.	Saturates	Aromatics	Resins
		Liters/Gram-Cm	
3000	0.018	19.3	17.7
2600	0.030	39.4	29.0
2400	0.035	47.2	38.6
2200	0.080	52.1	43.9

[a] Values obtained by Spectroscopic Department.

Packing of the columns is carried out in a very simple manner. Inasmuch as isopentane is the first solvent used, the columns are partially filled with isopentane and the silica gel is introduced. The heat evolved causes the isopentane to boil and this agitation results in very compact packing of the gel. A mixture of Davison 28/200 and passing 200-mesh gel is used throughout, all efforts being made to minimize the time of exposure to air. After packing, nitrogen pressure is applied and a few liters of isopentane are circulated to ensure maximum consolidation of the gel and thereby minimize channeling. An isopentane solution of the oil to be analyzed is then poured on top of the adsorbent.

With low molecular weight fractions of asphalt a gel to oil ratio of 6 to 1 was satisfactory. However, with fractions of very high molecular weight as much as 50 to 1 must be used. Addi-

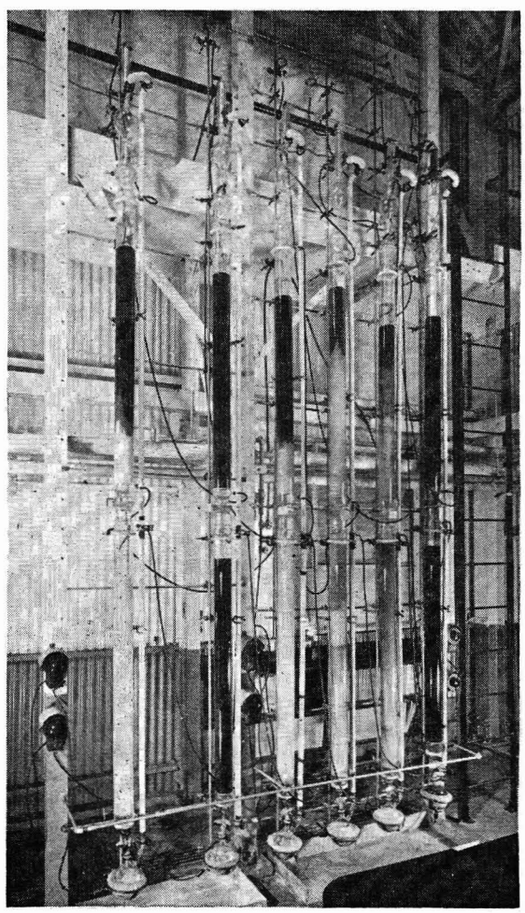

Figure 5. Chromatograph Columns

tion of 1 liter of alumina gel to the top of the columns decreases the amount of silica gel required because of its much greater absorptive capacity for resins, but alters the separation of aromatics and resins.

Figure 5 is an illustration of the columns used.

SEPARATION OF SATURATES

The saturated hydrocarbons obtained by chromatographing the asphalt fractions were separated into wax and oil by more or less conventional solvent dewaxing. This is done primarily because an absolute value of wax content is important to the asphalt technologist, since wax is regarded as deleterious. Furthermore, the *n*-paraffins are concentrated in the wax fraction and hence are more readily complexed with urea. The use of urea for the removal of *n*-paraffins has been reported (*1*). The greater part of the paraffins are removed by complex formation with urea.

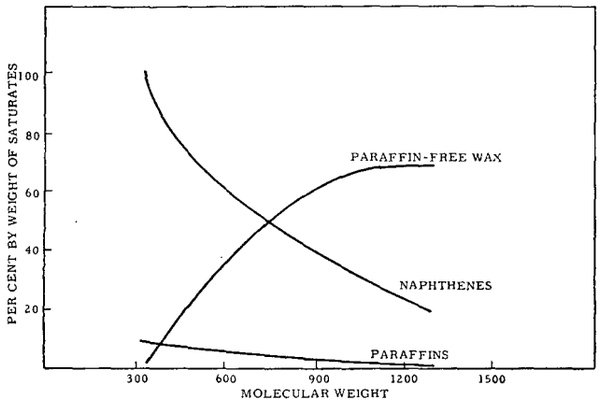

Figure 6. Distribution of Saturates

The saturated fraction of most asphalts is principally a naphthenic oil. Next in abundance is a naphthenic wax, and least of all is the quantity of paraffins. No attempts have been made to separate *n*-paraffins from isoparaffins because of the spread in molecular weights even in distilled fractions. Furthermore, the amount of isoparaffins present would undoubtedly be slight.

Inasmuch as these molecules are free of aromatics, judging by their transparency in the ultraviolet region, a knowledge of the degree of condensation may be obtained from their hydrogen deficiency compared with C_nH_{2n} for a simple naphthene. Figure 6 illustrates the characteristic distribution obtained for a California coastal asphalt. Table IV contains analyses of a typical, separated saturate fraction of 867 molecular weight for another typical asphalt. Despite the hydrogen deficiency calculated for the paraffins, the melting point and refractive index corresponded with those of a *n*-paraffin.

Table IV. Analysis of a Saturate Fraction

(Molecular weight = 867)

	% by Wt.	% C	% H	% S	Hydrogen Deficiency
Paraffins	5.6	85.6	14.3	0.2	0.6
Paraffin-free wax	75.4	86.1	13.7	0.2	6
Naphthenic oil	19	86.4	13.4	0.2	10

The dewaxing operation was carried out by cooling to −50° C. a methyl isobutyl ketone solution containing 10% of saturate fraction. After the wax cake had been filtered in a precooled, jacketed Büchner funnel, the wax cake was slurried with fresh methyl isobutyl ketone at −50° C. and filtered again. The occluded solvent was pressed from the wax with a cooled, glass stopper. Distillation of the methyl isobutyl ketone solution under vacuum yielded a water-white oil.

The wax cake, containing methyl isobutyl ketone, was dissolved in a minimum of additional methyl isobutyl ketone and stirred vigorously with excess, saturated aqueous urea solution. With the fractions of higher molecular weight, elevated temperatures were required to effect solution. When this was necessary, the wax solution was reacted with urea solution saturated at the same temperature. The mixtures were stirred several hours to ensure completion of the reaction, and filtered. The precipitate was washed thoroughly with fresh methyl isobutyl ketone to remove occluded oil and then with ether to remove methyl isobutyl ketone. The filtrate was washed several times with water to remove all urea present. Distilling off the solvent under reduced pressure gave a soft, waxy residue from the filtrate. Boiling the filter cake with water decomposed the urea complex and liberated the paraffins. Repeated treatments with water removed all urea, and the solid paraffins were dried in a vacuum desiccator.

Table V. Thermal Diffusion of Paraffin-Free Wax

(Molecular weight = 579)

	% C	% H	H Deficiency
Cut 1 (top)	85.6	14.2	1.3
2	85.8	14.1	2.1
3	86.0	14.0	2.9
4	86.6	13.7	5.1
5 (bottom)	86.3	13.1	8.2

SEPARATION OF NAPHTHENES

Although fractional precipitation of the naphthenic waxes is satisfactory, this method cannot be applied to the naphthenic oils. For this separation, thermal diffusion has yielded some interesting results, and has the advantage that no solvent is required, high temperatures are not used, so that thermal hazards do not exist, and a low expenditure of labor is required. This method can also be applied to the separation of the naphthenic wax, if the cool wall is maintained at a temperature slightly above the melting point of the wax. The method is essentially that of Clusius and Dickel (*2*). The type of separations which can be achieved by this method has been very clearly demonstrated by Korsching and Wirtz (*5*) and Kramers and Broeder (*6*). Although the theory of the process is complicated (*7*), it may be stated that the most viscous material always remains at the cold wall and hence concentrates at the bottom, while the least viscous material tends to go to the hot wall and is thereby concentrated at the top of the column. Table V contains the analyses of a typical naphthenic wax. The hydrogen deficiency listed indicates the degree of unsaturation compared with C_nH_{2n}, thus giving a measure of the number of rings present.

The column used for obtaining the above separation consists of three concentric glass tubes. Steam is passed through the center tube and cooling water through the outer tube. The annulus formed by the carefully ground and centered inner tubes contains the sample. In order to increase the effective capacity of the column, five reservoirs are spaced equally along the tube, which merely extends the time required to reach equilibrium and does not affect the degree of separation. To facilitate sample removal without any mixing, each reservoir is equipped with a small capillary stopcock. Samples are removed periodically for refractive index determination to ascertain when equilibrium is reached. The length of time required for each separation is a function of the viscosity of the material, the width of the annulus, and the size of the reservoirs. Anything from 2 to 20 days may be necessary for equilibrium, depending upon the sample.

Figure 7 gives a schematic diagram of the column used.

SEPARATION OF AROMATICS

The aromatic fractions obtained from the silica gel chromatograms are too broadly defined to be entirely satisfactory, as aromatic fractions of the same molecular weight from two different asphalts may have entirely different physical properties. To

help resolve this difference, aromatic fractions were rechromatographed over alumina gel into monocyclic and dicyclic aromatics. In the asphalts investigated, the ultraviolet absorption spectra were incompatible with the presence of catacondensed tricyclic aromatic molecules in amounts greater than 1%. The relative amount of benzenoid and naphthenoid molecules varies from predominantly benzenoid, in the lower molecular weight range, to a predominance of naphthenoid compounds in the fractions of higher molecular weight. Indications are that substituted Tetralins comprise a large part of the benzenoid molecules.

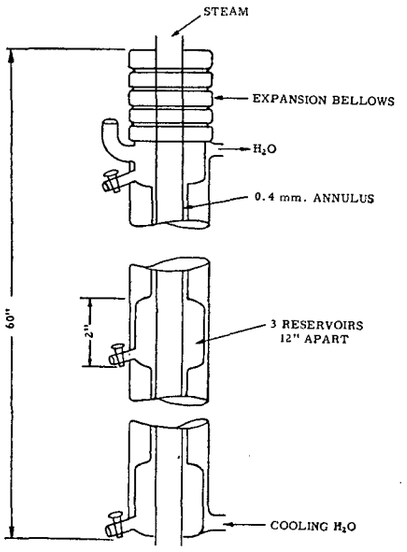

Figure 7. Thermal Diffusion Column

Table VI illustrates a typical separation of monocyclic and dicyclic aromatic compounds. This does not imply that higher condensed rings, where some of the rings are hydrogenated, are not present—for example, the monocyclic aromatics undoubtedly contain a high percentage of Tetralins. It is, therefore, not inconceivable that a similar situation obtains with the dicyclic aromatic fraction. Furthermore, two or three benzenoid fragments linked by alkyl chains would probably be desorbed along with substituted naphthalenes. These would be classified with the dicyclics by the method used, although their absorption spectra would be that of the monocyclic aromatics. However, no method has been found for further separation, with the possible exception of thermal diffusion.

Experimentally, the samples were dissolved in isopentane and poured into a column filled with Alcoa F-20 activated alumina. During the elution with isopentane, samples were removed periodically and ultraviolet absorption spectra obtained. Substituted naphthalene spectra were evident while still eluting with isopentane. As soon as substituted naphthalenes appeared, the receiver was changed and the remainder eluted with benzene or alcohol. Carefully eluting with a graded series of solvents yielded fractions of increasing optical density in the ultraviolet region, but the character of the absorption remained unchanged.

REMOVAL OF AROMATIC SULFUR COMPOUNDS

It is obvious from an inspection of the empirical formulas that many aromatic fractions contain a predominance of sulfur compounds. In order to examine the aromatic fractions without the possibility of confusion from the sulfur compounds, it was necessary to remove them. This was accomplished by oxidizing the sulfur compounds with hydrogen peroxide and chromatographing. Because indications are that the sulfur compounds in the aromatic fractions are principally substituted benzothiophenes, the oxidation was conducted in a manner suitable for the conversion of the sulfur atoms to the dioxides for ease of removal. Table VII illustrates the differences obtained with two typical aromatic fractions. Even in those oils where there was more than one sulfur atom per molecule, according to the empirical formula, it was possible to obtain some hydrocarbons.

Table VI. Separation of Aromatics

(Molecular weight = 460)

	% C	% H	% N	% S	Empirical Formulas
Monocyclics	85.2	12.0	0.1	2.1	$C_{33}H_{55}N_{0.03}S_{0.30}$
Dicyclics	86.2	10.5	0.3	2.6	$C_{33}H_{48}N_{0.1}S_{0.37}$

Table VII. Removal of Aromatic Sulfur Compounds

	Mol. Wt.	% C	% H	% N	% S	Empirical Formulas
Original	650	82.3	10.4	0.37	6.4	$C_{44}H_{67}N_{0.17}S_{1.3}$
Treated	650	86.6	12.6	0.1	0.1	$C_{47}H_{81}N_{0.05}S_{0.02}$
Original	460	85.2	12.0	0.1	2.1	$C_{33}H_{55}N_{0.03}S_{0.30}$
Treated	460	87.6	12.3	0.07	0.1	$C_{34}H_{56}N_{0.02}S_{0.01}$

The standard procedure for oxidizing benzothiophenes to sulfones by means of hydrogen peroxide was used (*4*). Three times the theoretical amount of hydrogen peroxide, based on the sulfur content, was added to an acetic acid solution of the oil. This was heated to 70° C. and stirred 8 hours. After working up by conventional procedures, the oxidized material was separated by chromatography. The oxidized sulfur compounds remain on the adsorbent until the very end of the elution, and are generally removed by eluting with alcohol.

ANALYSIS OF ASPHALTENES

The asphaltene content, obtained by precipitation with isopentane, varies from 5 to 35% by weight, depending on the source of the asphalt. Analysis of these asphaltenes is more difficult than the analysis of the maltenes because of their high molecular weight and the ease with which they change on standing.

The presence of wax in the asphaltenes has been established by pouring a benzene solution of asphaltenes on a large volume of silica gel. Extraction of the gel with copious quantities of isopentane yielded 5 to 10% of an essentially colorless wax of high melting range.

Fractional precipitation of asphaltenes from a benzene solution by gradual addition of isopentane yielded fractions of atomic carbon-hydrogen ratios varying from 0.82 to 0.71, and with trends in both sulfur and nitrogen content. However, no chemical interpretation could be assigned to the results, other than that the most aromatic molecules have the highest sulfur and nitrogen content and are least soluble.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Bengen, F., Ger. Patent Appl. O.Z. 12,438 (1940); Technical Oil Mission, Reel 6, p. 263.
(2) Clusius, K., and Dickel, G., *Z. physik. Chem.*, (B) 44, 397 (1939).
(3) Grader, R., *Oel u. Kohle*, 38, 867 (1942).
(4) Hinsberg, O., *Ber.*, 43, 289 (1910).
(5) Korching, H., and Wirtz, K., *Ibid.*, 73, 249 (1940).
(6) Kramers, H. A., and Broeder, J. J., *Anal. Chim. Acta*, 2, 687 (1948).
(7) Ritchie, M., *Proc. Roy. Soc. Edinburgh*, 62, 308 (1946).

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Analysis of Mercuric Ion-Anion Mixtures

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Mercuric ion and acetone were observed to react, with the production of hydrogen ion and a complex mercury compound. As a result of the complex formation, mercuric ion may be determined alkalimetrically and chloride, bromide, thiocyanate, and iodide ions may be determined argentimetrically. The method described is a more simple procedure than those commonly employed for the analysis of mercuric ion-anion mixtures.

THE analytical procedure herein described was developed when it was attempted to perform a thiocyanate determination of mercuric ion remaining in a mercuric chloride–acetone reaction mixture. The thiocyanate method requires the complete absence of halide ion and, for this reason, analyses of mercuric ion–halide ion mixtures are normally preceded by isolation of the mercury as mercuric oxide via alkali precipitation. Application of this procedure failed to effect any precipitation in the solution under consideration; the only reaction observed was a neutralization of the alkali and the appearance of a slight yellow turbidity which disappeared on standing. Investigation of the literature disclosed that this behavior had been observed as early as 1871 (*12*) and that several mercuric ion–acetone complexes have been investigated (*1–3, 9, 10, 13, 15*). However, no information is available regarding the stoichiometry of alkali, acetone, and mercuric ion. Accordingly, preliminary investigations were undertaken to determine whether any quantitative relationship could be established between mercuric ion in acetone solution and the quantity of added alkali.

A standard alkali solution was added to an aqueous-acetone solution of mercuric chloride until the solution was definitely alkaline and clear. Standard acid was added to neutralize the excess base, and the amount of acid necessary to effect various lower pH values was noted. Back-titration to the colorless phenolphthalein end point involved an over-all proportion of 2 moles of hydroxide ion per mole of mercuric ion. On the basis of this stoichiometry and in view of the gradual increase in acidity of a mercuric chloride–acetone solution on standing, the following equation is suggested as a reasonable expression of the reaction.

$$Hg^{++} + 2CH_3{-}\overset{\overset{O}{\|}}{C}{-}CH_3 \rightleftarrows Hg(CH_3{-}\overset{\overset{O}{|}}{C}{=}CH_2)_2 + 2H^+$$

These observations were applied to the analysis of a 0.02468 *M* solution of mercuric chloride. A mean of 123.8 ± 0.29 mg. was found on analyses of seven aliquot samples containing 123.9 mg. of mercuric ion. The average deviation from the mean was ±2.4 parts per thousand, the standard deviation was ±2.9 parts per thousand, and the probable deviation was ±2.0 parts per thousand.

The effect of excess amounts of chloride ion was next investigated. As indicated by the data of Table I, relatively large concentrations of chloride ion—as much as 10 moles of chloride ion per mole of mercuric ion—had no adverse effect upon the accuracy of the method.

Table I. Determination of Mercuric Ion in Presence of Chloride and Bromide Ion

Equiv. Halide/ Equiv. Hg^{++} in Sample	Hg^{++} Present *Mg.*	Hg^{++} Found *Mg.*
1.0 (Cl^-)	250.8	249.7
1.8 (Cl^-)	250.8	251.2
2.6 (Cl^-)	250.8	251.0
3.4 (Cl^-)	250.8	250.9
4.2 (Cl^-)	250.8	251.0
5.0 (Cl^-)	250.8	250.9
1.2 (Cl^-)[a]	250.8	250.8
1.0 (Br^-)[b]	250.8	249.9
2.0 (Br^-)[b]	250.8	250.7

Unless otherwise indicated, mercuric chloride was source of mercuric ion.
[a] Solution prepared by addition of 1.25 millimoles of mercuric oxide to nitric acid followed by addition of indicated amount of potassium chloride.
[b] Analysis performed by prior precipitation of bromide as described in experimental section.

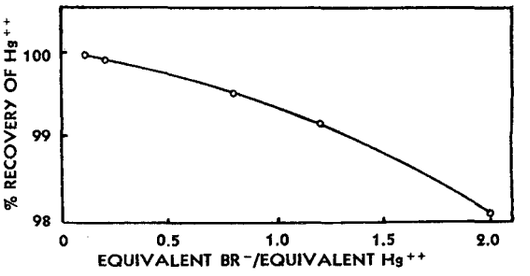

Figure 1. Per Cent Recovery of Mercuric Ion
From a 0.05 *M* solution of $HgCl_2$, plotted against equivalent Br/equivalent Hg^{++}

Two other complications were next considered. Obviously, an acidified solution of mercuric chloride would require a modification of the method and certain anions because of the stability of their mercury complexes would be expected to preclude the use of this method. The complication of added acid presents no difficulty, provided that the anion of the acid does not interfere. If interfering anions are absent, the solution is adjusted to the bromocresol green end point (the pH of a mercuric chloride solution) prior to the titration.

The anion complication, however, imposes a limitation on this method. With the exception of chloride and bromide ions, all complex-forming anions introduced erroneous results. In the case of bromide ion, quantitative results are possible if certain modifications are made. Such modifications are required because the stability of the mercuric ion–bromide complex produces low results, as indicated by Figure 1. The bromide ion complication may be solved in either of two ways: This ion may be removed nearly quantitatively prior to the analysis, or a correction from Figure 1 may be applied to the results obtained from the unmodified method. The former method is preferred and is described in detail in the experimental section.

Although cyanide, bromide, iodide, and thiocyanate ions limit the applicability of the mercuric ion determination, the converse is not true—i.e., all of the ions listed, with the exception of the cyanide ion, may be determined quantitatively in a mercuric ion mixture after the mercuric ion–acetone complex has been formed. On formation of the complex and adjustment of pH, the chloride,

Table II. Determination of Anion in Presence of Mercuric Ion

Equiv. Hg^{++} / Equiv. Anion	Anion Present *Mg.*	Anion Found *Mg.*
1.0	88.65 Cl^-	88.75
2.0	126.9 Cl^-	126.9
4.0	126.9 Cl^-	127.0
1.0	199.8 Br^-	199.8
2.0	199.8 Br^-	199.8
3.0	199.8 Br^-	199.8
1.0	317.3 I^-[a]	317.8
1.0	158.3 SCN^-	158.1
2.0	158.3 SCN^-	158.3

[a] Determined gravimetrically. All other analyses by Mohr method.

bromide, and thiocyanate ions may be determined by the Mohr method and the iodide ion may be determined gravimetrically as silver iodide. Typical results of such anion analyses are shown in Table II. In the opinion of the authors, the method herein described permits a much more convenient means for the determination of these anions than the previously published procedures (*4–8, 14, 16*). The latter methods require the removal of mercuric ion from the reaction mixture prior to analysis, and are accordingly more tedious and difficult.

The determination of chloride and bromide ions in mercuric ion solutions by the Mohr method is as readily accomplished as their determination in the presence of the alkali metal ions. This similarity permits a new and interesting standardization of silver nitrate. Under the conditions described, mercuric chloride may be used as a primary standard. It presents an advantage over sodium chloride because of its higher equivalent weight, 135.76 as compared with 58.45 for sodium chloride. Application of this method to the standardization of silver nitrate yielded results which differed no more than 2 parts per thousand from the values obtained by the Mohr standardization with sodium chloride as described by Pierce and Haenisch (*11*).

EXPERIMENTAL

Determination of Mercuric Ion in Presence of Chloride Ion. A 25-ml. sample, 0.02 to 0.2 *M* in mercuric ion (as the chloride or nitrate), was adjusted with 1 *M* potassium chloride if necessary to assure that chloride ion was present in amount at least equivalent to mercuric ion. (The chloride ion is required to prevent precipitation of mercuric oxide if the solution requires adjustment to the bromocresol green end point.) If the solution was not already at the bromocresol green end point—i.e., if it contained added acid—it was adjusted to this end point with carbonate-free sodium hydroxide or sulfuric acid. In the event that the solution was highly acidic, the adjustment was made with 1 *N* sodium hydroxide until the end point was nearly attained. The final adjustment was then made with 0.1 *N* sodium hydroxide or sulfuric acid as required.

A 25-ml. portion of acetone was then added and standard 0.1 *N* sodium hydroxide was added to attain the red phenolphthalein end point. The yellow precipitate of mercuric oxide which first formed dissolved in a few minutes and the completely clear solution was back-titrated with 0.1 *N* sulfuric acid to the colorless phenolphthalein end point. The volume of the added alkali after deduction of the base equivalent of the added acid and the acetone blank represents the amount of mercuric ion present.

The acetone blank is determined by mixing 25 ml. of acetone with about 25 ml. of water, followed by titration to the red phenolphthalein end point. Commercial acetone (Carbide and Carbon) was found to be entirely satisfactory for analytical purposes and consistently provided a blank of about 0.1 ml. of 0.1 *N* sodium hydroxide.

The sodium hydroxide solution used in all determinations was carbonate-free, and was standardized against primary standard grade potassium acid phthalate using phenolphthalein as an indicator. The sulfuric acid solution was standardized against the sodium hydroxide solution.

Two observations should be noted at this point: The proportion of acetone is not critical, except in so far as the lower limit should be in the proportion of 0.45 ml. of acetone per ml. of water at the end point, and care should be exercised to assure that the colorless phenolphthalein end point has been attained. While definite and unambiguous, the phenolphthalein end point is not as definite as that observed in an ordinary alkalimetric determination and acid should be added until the solution is completely colorless. As a precaution, the authors found it advisable to add one more drop of indicator at the apparent end point. On occasion the additional indicator produced an evident red color in a previously colorless solution.

Determination of Mercuric Ion in Presence of Bromide Ion. A 25-ml. sample containing 250.8 mg. of mercuric ion and 1 to 2 equivalents of bromide ion was mixed with excess 1.0 *M* silver nitrate solution to assure precipitation of silver bromide. An excess of 1 *M* potassium chloride was then added to assure complete precipitation of silver ion and to provide that chloride ion was present in amount at least equivalent to mercuric ion. The solution was filtered through a sintered-glass crucible and the filtrate treated as described above.

Determination of Chloride, Bromide, or Thiocyanate Ions in Presence of Mercuric Ion. A 25-ml. sample containing various amounts of mercuric ion and approximately 0.1 *N* in halide or thiocyanate ions was mixed with 10 ml. of acetone in the case of halides and with 20 ml. of acetone in the case of thiocyanate. The solution was taken slightly beyond the red phenolphthalein end point with approximately 1 *N* sodium hydroxide. A 1-ml. portion of chromate indicator was added and the halide or thiocyanate determined by the Mohr method. The indicator solution was 1 *M* in potassium chromate and 1 *M* in sodium bicarbonate.

Determination of Iodide Ion in Presence of Mercuric Ion. A solution containing iodide ion and varying amounts of mercuric ion from mercuric nitrate was mixed with 25 ml. of acetone and heated if necessary to dissolve any precipitate of mercuric iodide. The solution was made alkaline with 0.1 *N* sodium hydroxide and 4 ml. of 6 *M* ammonium hydroxide was added. Excess 0.1 *N* silver nitrate was then added to assure completeness of precipitation. After digestion for 2 hours, the precipitate was removed by filtration on a weighed sintered-glass crucible and washed successively with an acetone–ammonium hydroxide solution, water, 0.01 *N* nitric acid, and finally with water. The acetone–ammonium hydroxide solution was prepared by mixing 5 ml. of acetone, 100 ml. of water, and 5 drops of 6 *M* ammonium hydroxide. The washed precipitate was dried at 100° for 4 hours, and cooled in a desiccator for 1.5 hours prior to weighing.

CONCLUSIONS

Mercuric ion may be determined in the presence of chloride ion and acetone by alkalimetric procedures. Bromide ion interferes, but mercuric ion may be determined by the method of this paper by near-quantitative removal of bromide ion prior to the analysis or by application of a correction based upon the amount of bromide ion present. The method is of no value in the determination of mercuric ion in the presence of iodide, cyanide, or thiocyanate ions.

Chloride, bromide, and thiocyanate ions may be determined in mercuric ion mixtures by the Mohr method after the mercuric ion–acetone complex has been formed. Iodide ion may be determined similarly by gravimetric procedure.

LITERATURE CITED

(1) Auld, S. M., and Hantzsch, A., *Ber.*, 38, 2683 (1905).
(2) Biilmann, E., *Ibid.*, 35, 2584 (1902).
(3) Hofmann, K. A., *Ibid.*, 31, 2783 (1898).
(4) Kohn, M., *Z. anorg. Chem.*, 59, 108 (1908).
(5) *Ibid.*, p. 271.
(6) Kohn, M., and Ostersetzer, A., *Ibid.*, 80, 218 (1913).
(7) Koszegi, D., and Tomori, N., *Z. anal. Chem.*, 100, 257 (1935).
(8) Lang, R., *Z. anorg. allgem. Chem.*, 142, 229 (1925).
(9) Lasserre, A., *J. pharm. chim.*, (6) 22, 264 (1905).
(10) Oppenheimer, C., *Ber.*, 32, 986 (1896).
(11) Pierce, W. C., and Haenisch, E. L., "Quantitative Analysis, 3rd ed., pp. 303–4, New York, John Wiley & Sons, 1948.
(12) Reynolds, J. E., *Ber.*, 4, 483 (1871).
(13) Reynolds, J. E., *Chem. News*, 23, 217 (1871).
(14) Rupp, E., and Lehmann, F., *Pharm. Ztg.*, 52, 1020 (1907).
(15) Sand, J., and Genssler, O., *Ber.*, 36, 3703 (1903).
(16) Uzumasa, Y., and Osaki, M., *J. Chem. Soc. Japan*, 62, 489 (1941).

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Ultraviolet Spectrophotometric Determination of Vanadium

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Ultraviolet adsorption spectra of inorganic complex compounds are being studied with the purpose of developing new or improved spectrophotometric methods of analysis. It was desired to ascertain the wave length of maximum absorbancy, the optimum concentration range for vanadium, the effect of the concentration of reagents, and the effect of diverse substances for the peroxyvanadic acid system. Although pervanadyl ions exhibit strong general absorption in the ultraviolet region, peroxyvanadic acid had a characteristic absorbancy maximum at 290 mμ in addition to that at 460 mμ. Absorptiometric measurement gives somewhat greater sensitivity in the ultraviolet than in the visible region. The tolerances for dichromate and titanic ions are larger when the ultraviolet spectrophotometric method is used. Ferric and nitrate ions are the main interfering ions whose effect cannot be circumvented by using a compensating blank. The general procedure which was developed is suitable for the determination of 0 to 125 p.p.m. of vanadium and should be applicable to a variety of samples. The existence of an ultraviolet absorbancy maximum for peroxyvanadic acid is especially significant.

ONE of the widely utilized colorimetric methods for vanadium is based upon the formation of a reddish-brown color by a reaction between quinquevalent vanadium and hydrogen peroxide in an acidic solution (*5*). The color is probably due to peroxyvanadic acid, HVO_4 (*1*), although a vanadium peroxide sulfate, $(VO_2)_2(SO_4)_3$ (*3*), containing quinquevalent vanadium in the cation, has also been suggested as being the compound formed. The method was first proposed by Slowik (*4*), and was further studied by McCabe (*2*). Several modified procedures have been developed (*5*). Wright and Mellon made a spectrophotometric study of the peroxyvanadic acid complex, in the visible region (*8*). Weissler utilized the peroxyvanadic acid complex in the visible region for the simultaneous spectrophotometric determination of vanadium, titanium, and molybdenum (*7*).

In an investigation of the acidic peroxymolybdate complex (*6*), it was found that peroxyvanadic acid also exhibited maximum absorbancy in the ultraviolet region. Therefore, this study was carried out in order to ascertain the suitability of the peroxyvanadic acid complex for an ultraviolet spectrophotometric determination of small amounts of vanadium.

GENERAL EXPERIMENTAL WORK

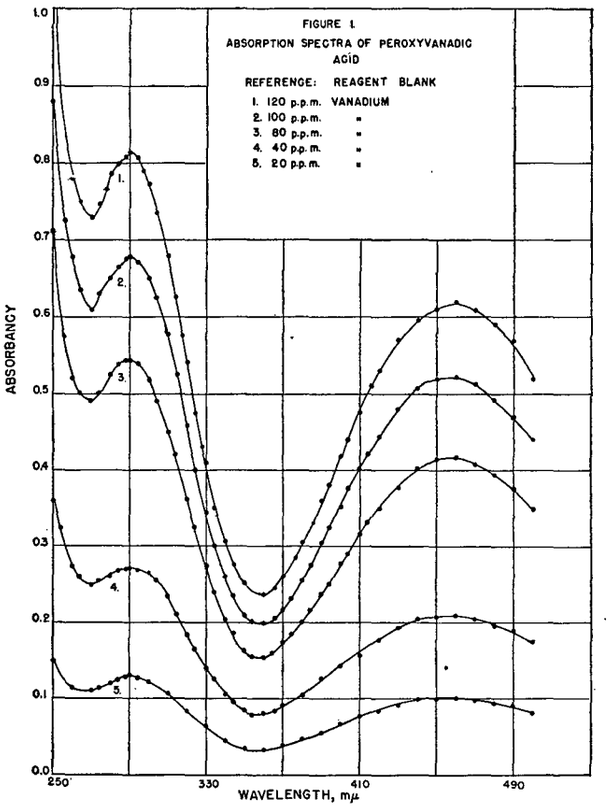
Apparatus. The absorbancy measurements were made with a Beckman Model DU spectrophotometer equipped with an ultraviolet accessory set, and 1.000-cm. silica cells. The spectrophotometer was equipped with an ultraviolet-sensitive phototube for high sensitivity in the 200 to 625 mμ region of the spectrum. The reference cell contained a reagent blank solution containing the reagents used, unless otherwise stated.

Solutions. A standard vanadate solution was prepared by dissolving 1.1475 grams of pure ammonium metavanadate in 50 ml. of concentrated sulfuric acid and diluting to 500 ml. with redistilled water. One milliliter of this solution contained 1.00 mg. of vanadium. The perchloric and phosphoric acids used were, respectively, 72 and 85% reagent grade. The hydrogen peroxide used was 3% analytical reagent grade. Other acids used were C.P. reagent grade.

Color Reaction. The reaction of an acidic solution of vanadate ions with hydrogen peroxide results in the formation of the peroxyvanadic acid complex, HVO_4. This complex has a reddish-brown color with characteristic absorption in the ultraviolet and visible regions.

In order to study the effect of certain variables on the maximum absorbancy in the ultraviolet region, the following procedure was used:

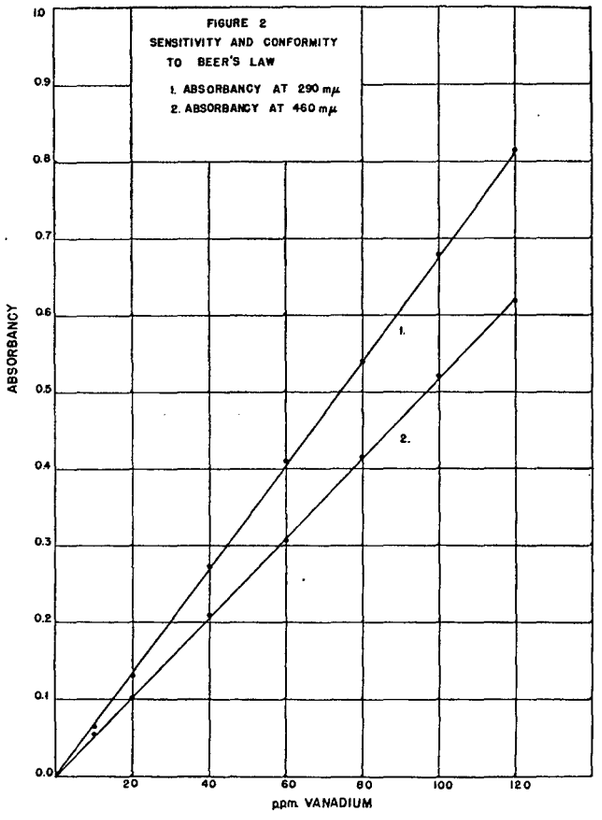
A definite amount of the standard vanadate solution was transferred to a 50-ml. volumetric flask. The desired amount of acid was added and the volume was adjusted to 50 ml. with redistilled water. After addition of 1 ml. of 3% hydrogen peroxide, the contents of the flask were thoroughly mixed and absorbancy measurements were taken from 250 to 500 mμ at 2 mμ intervals. The color formation is immediate and the system is stable for at least 12 hours. In the study of diverse ions, a definite amount of solution containing each ion was added, followed by dilution and addition of hydrogen peroxide.



EFFECT OF VARIABLES

Vanadium Concentration. The ultraviolet absorption spectra for various concentrations of vanadium were determined and conformity to Beer's law was found at 290 mμ for concentrations from 0 to 125 p.p.m. of vanadium. A good absorbancy maximum occurs at 290 mμ with a reagent blank solution in the reference cell, as shown in Figure 1. Figure 2 shows a comparison of sensitivity at 290 mμ as compared to the usual visible measurements taken at 460 mμ and conformity to Beer's law.

Reference Cell. The significance of the proper selection of the reference solution was studied. Figure 1 shows the absorption spectra obtained using a solution containing the reagents as the reference solution. Figure 3 shows the absorption spectra obtained using redistilled water as the reference solution. A marked difference in the shape of the resulting curves in the ultraviolet region was noted. Therefore, it seems desirable to utilize a reagent blank solution in the reference cell, so that a true ultraviolet absorption spectrum of the peroxyvanadic acid can be obtained.



Acid Concentration. The effect of various concentrations of perchloric acid was determined using 100 p.p.m. of vanadium and 1 ml. of hydrogen peroxide in a final volume of 50 ml. It was found that a minimum of 1 ml. and varying amounts up to 15 ml. of perchloric acid have no effect upon the maximum absorbancy measured at 290 mμ. From this study, 5 to 10 ml. of perchloric acid per 50 ml. of solution were deemed to be sufficient for attainment of maximum absorbancy. The study of the effect of various acids indicated that 10 ml. of either a 1 to 1 mixture of perchloric acid and sulfuric acid, or a 1 to 1 mixture of perchloric acid and hydrochloric acid, have little effect. Small concentrations of phosphoric acid were found to intensify the coloration and also increase the absorbancy as measured at 290 mμ. Very reproducible readings were obtained using 10 ml. of a 1 to 1 mixture of perchloric and phosphoric acids, but a gradual decrease in absorbancy was noted. Unless otherwise stated, 10 ml. of perchloric acid were used.

Hydrogen Peroxide. The effect of various concentrations of hydrogen peroxide was studied using 100 p.p.m. of vanadium and 10 ml. of perchloric acid in a final volume of 50 ml. It was found that 0.10 to 1.5 ml. of hydrogen peroxide were sufficient to attain the maximum absorbancy. A large excess of hydrogen peroxide is known to diminish the color intensity (*8*). A decrease in absorbancy was also found for measurement at 290 mμ when a large excess of hydrogen peroxide was added. Therefore, 1 ml. of hydrogen peroxide in a final volume of 50 ml. is sufficient for attainment of maximum absorbancy.

Diverse Ions. The effect of various diverse ions was studied using 100 p.p.m. of vanadium. Absorbancy readings were taken at 290 mμ in order to determine any changes in the maximum absorbancy. A negligible error was obtained with 1000 p.p.m. of acetate, aluminum, ammonium, borate, bismuth, cadmium, calcium, cobaltous, cupric, chlorate, chloride, lithium, magnesium, malonate, manganous, mercuric, nickelous, oxalate, plumbous, stannate, sulfate, silicate, sodium, zinc, and zirconyl ions; and with 500 p.p.m. of fluoride and thiocyanate ions. Table I lists the interfering ions and their effect. The permissible amount of titanium and dichromate is much larger for absorptiometric measurement in the ultraviolet region than in the visible region (*8*).

Compensating Blank. In the study of the effects of such ions as nitrate, ferric, molybdate, titanic, and tungstate it seemed advisable to utilize a compensating blank containing these ions. The compensating blank solution contained the same concentration of acid, hydrogen peroxide, and diverse ion as the solution placed in the unknown cell. This technique of external compen-

Table I. Interfering Diverse Ions

Ion	Added as	Amt. Added, P.P.M.	Error, % of Desired Constituent	Permissible Amt., P.P.M.
$CbO(C_2O_4)_3^{---}$	$K_3CbO(C_2O_4)_3$	100	+12.5	5
$C_4H_4O_6^{--}$	$(NH_4)_2C_4H_4O_6$	500	−9.0	150
$C_6H_5O_7^{---}$	$Na_3C_6H_5O_7$	360	−11.0	100
$Cr_2O_7^{--}$	$K_2Cr_2O_7$	115	−3.0	100
Fe^{+++}	$Fe_2(SO_4)_3$	13	+35.0	0
MoO_4^{--}	Na_2MoO_4	4	+2.0	4
Ti^{++++}	$Ti(SO_4)_2$	100	+18.0	20
WO_4^{--}	Na_2WO_4	20	+10.0	10
NO_3^-	NH_4NO_3	300	+7.0	100

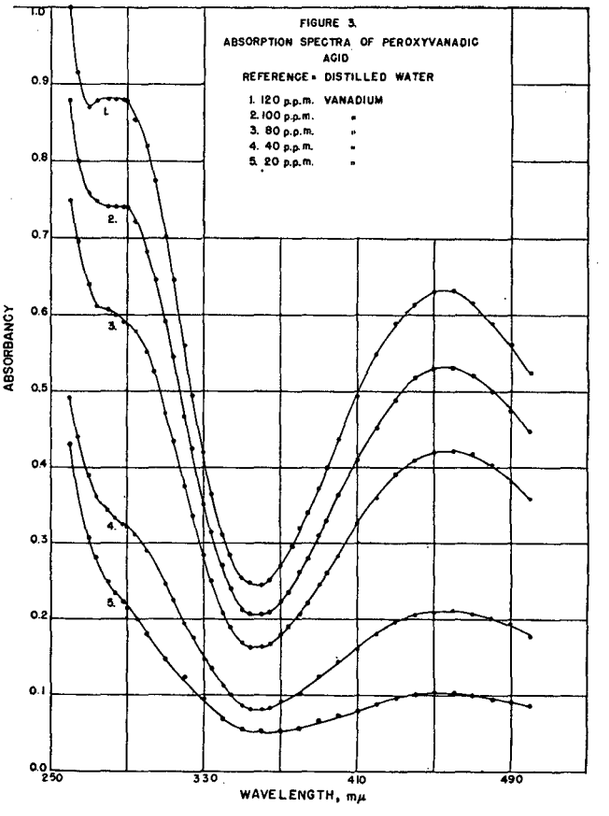


Table II. Effect of Compensating Blank

(Absorbancy of 100 p.p.m. vanadium as peroxyvanadic acid[a] at 290 mμ is 0.680 and at 460 mμ is 0.522)

Ion	Added as	Amt. Added, P.P.M.	Absorbancy At 290 mμ	Absorbancy At 460 mμ
MoO_4^{--}	Na_2MoO_4	100	0.683	0.526
Ti^{++++}	$Ti(SO_4)_2$	100	0.681	0.485
WO_4^{--}	Na_2WO_4	100	0.682	0.520
NO_3^-	NH_4NO_3	200	0.700	0.524
Fe^{+++}	$Fe_2(SO_4)_3$	13	0.838	0.522

[a] Data have been thoroughly checked. The abnormal effect of nitrate ions may be due to the formation of nitrous acid or nitrogen dioxide. The anomalous effect of ferric ions may be due to catalytical decomposition of peroxyvanadic acid to pervanadyl ions, which exhibit slightly greater absorption than peroxyvanadic complex.

sation would cancel any absorption effects due to these diverse ions. Table II lists the effect of using a "compensating" blank solution.

The data in Table II show that the measurement of absorbancy at 290 mμ using a compensating blank is very much superior to measurement at 460 mμ when titanium is present. Furthermore, the interference due to molybdate, titanium, and tungstate ions, which was observed when the absorbancy was measured at 290 mμ, can be minimized by using a compensating blank. The elimination of the interferences due to ferric, molybdate, and tungstate ions by use of a compensating blank solution when absorbancy is measured at 460 mμ is also significant.

DISCUSSION

This study has presented the ultraviolet absorption spectra of the peroxyvanadic acid complex. A characteristic absorbancy maximum was found at 290 mμ, heretofore unmentioned in the literature. In the study of the diverse ions it was found that a greater concentration of titanium and dichromate ions can be tolerated in the ultraviolet than in the visible region. Furthermore, the utilization of a compensating blank solution minimizes interferences in amounts of 100 p.p.m. of the tungstate, titanic, and molybdate ions.

RECOMMENDED GENERAL PROCEDURE

Sample. Procure a representative sample of the material and subject it to the necessary preparative treatment. Weigh, or measure by volume, a sample containing an amount of vanadium such that the final solution contains not more than 1.00 mg. of vanadium per ml. of solution. Make this solution just acidic to litmus with perchloric acid and dilute to a definite volume in a volumetric flask.

Desired Constituent. Transfer a suitable aliquot of this prepared solution to a 50-ml. volumetric flask and add 10 ml. of perchloric acid and sufficient redistilled water to bring the meniscus to the mark. Add 1 ml. of 3% hydrogen peroxide and mix thoroughly. Use a compensating solution, depending upon the material being analyzed, in the reference cell. Measure the absorbancy at 290 mμ in 1.000-cm. silica cells.

LITERATURE CITED

(1) Latimer, W. M., and Hildebrand, J. H., "Reference Book of Inorganic Chemistry," p. 342, New York, Macmillan Co., 1940.
(2) McCabe, C. R., *Chem. Eng.*, **13**, 243–7 (1911); *J. Ind. Eng. Chem.*, **5**, 736 (1913).
(3) Meyer, J., and Pawletta, A., *Z. physik. Chem.*, **125**, 49 (1927).
(4) Slowik, P., *Chem. Ztg.*, 34, 648 (1910).
(5) Snell, F. D., and Snell, C. T., "Colorimetric Methods of Analysis," p. 453, New York, D. Van Nostrand Co., 1949.
(6) Telep, G., and Boltz, D. F., ANAL. CHEM., **22**, 1030 (1950).
(7) Weissler, A., IND. ENG. CHEM., ANAL. ED., **17**, 696 (1945).
(8) Wright, E. R., and Mellon, M. G., *Ibid.*, **9**, 251 (1937).

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Determination of Pentoses

Effect of Varying Proportions of Components of Bial's Color Reagent

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Bial's color reagent has been applied to the determination of pentose nucleic acids in tissues. Although many compositions of the reagent have been proposed, no investigator has demonstrated what differences, if any, may result under the different conditions. To obtain the most favorable results with the test, a study of the variables involved was important. When the concentrations of orcinol, iron, or hydrochloric acid in the reagent are increased, the color intensities increase to maxima, then decrease. The color develops more rapidly at high than at low levels of orcinol or hydrochloric acid, but changes only slightly when ferric alum is varied. Increases in the levels of each component also produce increases in the blanks. On the basis of the results obtained, the different proportions of the components used by previous investigators could be evaluated and the most favorable ones chosen.

FOR the qualitative detection of pentoses Bial (*2, 3*) described a reagent composed of orcinol, ferric ion, and hydrochloric acid. When heated with this reagent, pentoses give a greenish-blue color. For quantitative applications of the test, more recent investigators (*1, 4–8*) have used various modifications of Bial's reagent, which are outlined in Table I. No data are readily available, however, to show the effects of changes in the composition of the reagent. The present study was, therefore, carried out to provide a basis on which to choose the best conditions for the test. Ribose nucleic acid was selected as the most suitable pentose-containing test material because of interest in its determination in different tissues.

METHOD

Pure orcinol was used throughout the tests, because impure material produced low color intensities and turbidity. It was obtained by recrystallizing 1 part of commercial Coleman and Bell or Mackay orcinol from 20 parts of benzene. Merck reagent ferric alum was used as the source of ferric ion (*5*). The hydrochloric acid used was Baker's analyzed and contained approximately 44 grams of hydrochloric acid per 100 ml. The ribose nucleic acid used as the test substance was obtained from Schwarz Laboratories.

Reagents containing different proportions of the components were made up from suitable stocks of ferric alum in hydrochloric acid, to which weighed samples of solid orcinol were added just before the tests were carried out. The stock ribose nucleic acid

Table I. Compositions of Bial's Reagent Used by Different Investigators

Reagent	Concentrations of Reagents[a], Grams per 100 Ml.				
	Mejbaum	McRary and Slattery, and Brown	Militzer	Albaum and Umbreit	Drury
Orcinol	0.50	0.15	0.147	0.476	0.476
Ferric alum[b]	0.15	0.10	0.44	0.142	0.047
Hydrochloric acid[c]	22	26	26	21	21

[a] Based on final mixtures of sample plus reagent.
[b] Or ferric chloride calculated as ferric alum.
[c] Calculated on basis of 44 grams of hydrochloric acid per 100 ml. in concentrated hydrochloric acid.

solution was made up to contain 1 mg. per ml. in water. The amount of ribose nucleic acid solution used in the tests was kept at 0.1 ml., and the final volumes of reagent plus test substance were brought to 2 ml. The concentrations of the orcinol in the final mixtures were varied to give nine different levels, up to 3 grams per 100 ml.; the ferric alum, at eight levels, up to 3 grams per 100 ml.; the hydrochloric acid, at four levels, from 17.6 to 33.0 grams per 100 ml. For measurements of the rates of color development, tests were made at six different periods of heating, up to 60 minutes.

The reactions were carried out in 10 × 100 mm. tubes, selected for optical uniformity. The mixtures were heated in a constant-level water bath (*9*) kept boiling by means of a heating mantle. They were then cooled in another water bath at room temperature, and their color intensities were measured in a Klett-Summerson photoelectric colorimeter with the aid of a No. 66 filter. Blanks were also run and zero adjustments were made with unheated blanks. An adapter was constructed to accommodate the tubes in the colorimeter.

The colorimeter responded more sluggishly with the small 10 × 100 mm. tubes than with the standard colorimeter tubes. Furthermore, it showed a linear response only for amounts of ribose nucleic acid up to 0.04 mg., whereas the response of a Beckman Model DU spectrophotometer, used for comparative purposes, was linear up to 0.16 mg. or higher. These limitations in application of the Klett-Summerson instrument did not, however, affect the interpretation of the data of this study, although they should be given consideration when more precise analyses are required. The varying response of different photoelectric colorimeters in the orcinol test has also been noted by McRary and Slattery (*6*).

To present most effectively the considerable amount of data thus obtained, they were plotted in the form of contour maps. First, two-dimensional graphs, relating each variable alone to color intensity, were drawn, and data obtained from cross sections of these were then used to locate the contours.

RESULTS

The effects of changes in concentrations of orcinol, iron, and hydrochloric acid on the amount of color developed with ribose nucleic acid are shown by the contour maps of Figure 1. Each map represents a surface which relates concentrations of orcinol and ferric alum to color intensity. The different maps show the surfaces obtained at different levels of hydrochloric acid. All data of this set were obtained with a constant period of heating of 30 minutes. Horizontal cross sections show that with increasing concentrations of orcinol, the color intensities rise, then fall; vertical cross sections show that increases in ferric alum produce a similar effect; and data taken from each map at constant levels of orcinol and iron reveal that increases in hydrochloric acid also lead to a rise, then a fall, in color intensity.

The plateaus on the surfaces signify the conditions where the color intensities are maximal and where they are least affected by changes in the concentrations of orcinol and iron. Optimum proportions of the components may, therefore, be judged on the basis of the maximum colorimeter readings, indicated by crosses on the graphs. They may be seen to be different at different levels of hydrochloric acid. The maximal color intensities increase with

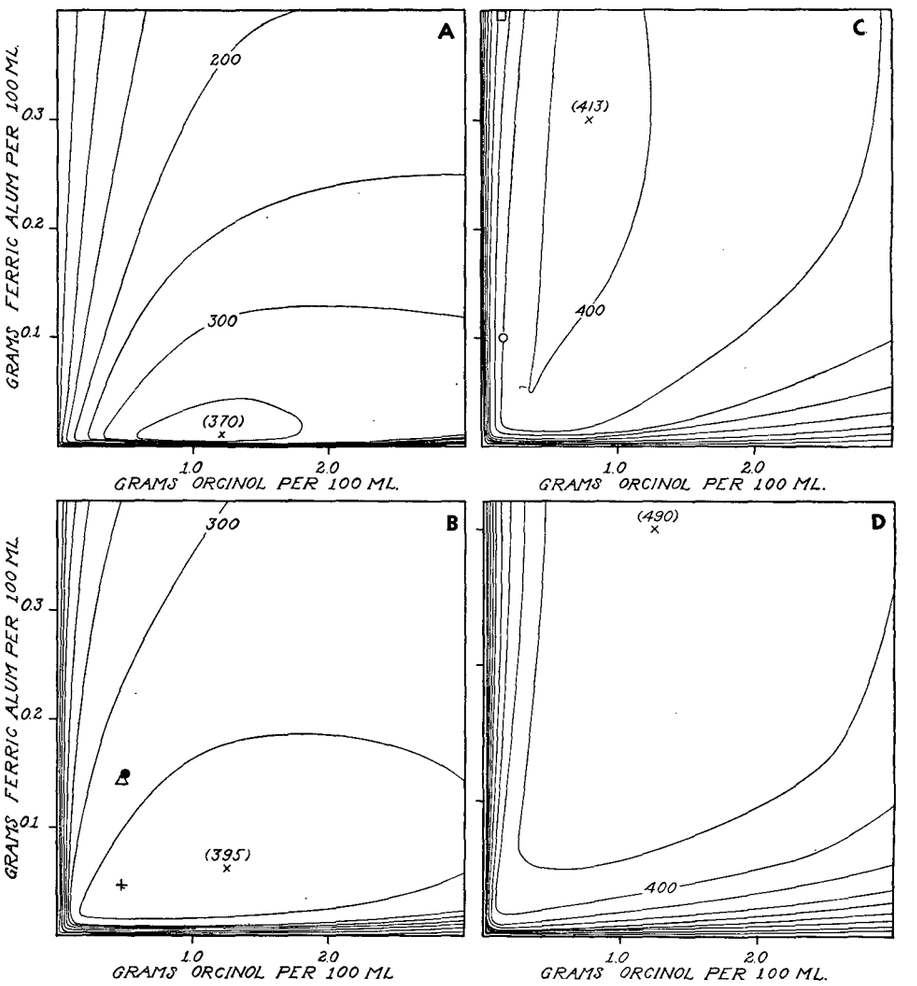

Figure 1. Contour Maps of Surfaces Relating Concentrations of Orcinol and Ferric Alum to Color Intensity at Different Levels of Hydrochloric Acid

Ordinate levels for *D* are 3.0, 2.0, 1.0; for *C*, 0.3, 0.2, 0.1 gram of ferric alum per 100 ml.
Grams of hydrochloric acid per 100 ml.
A. 17.6
B. 22.0
C. 26.4
D. 33.0

Levels of color intensity are indicated at intervals of 50 units of colorimeter reading. Numbers inserted in contour lines are guides to magnitudes of readings. Numbers in parentheses are maximum readings, positions of which are shown by crosses. Points corresponding to conditions of other investigators are: ● Mejbaum. ○ McRary and Slattery, and Brown. □ Militzer. △ Albaum and Umbreit. + Drury.

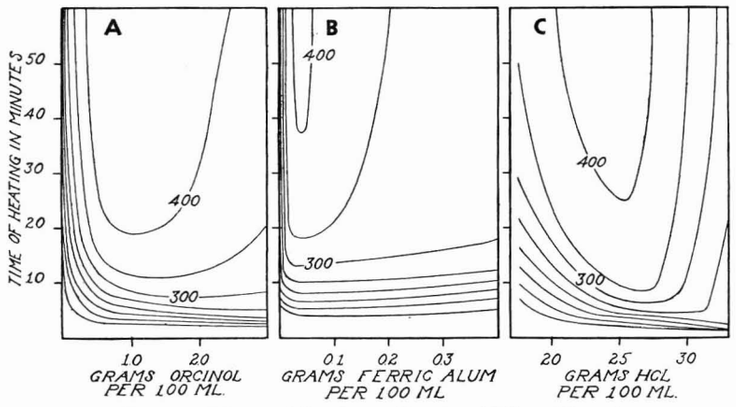

Figure 2. Contour Maps of Surfaces Obtained When Two Components Are Held at Optimal Concentrations and Third Component and Time of Heating Are Varied

A. 0.047 gram of ferric alum and 21 grams of hydrochloric acid per 100 ml.
B. 0.476 gram of orcinol and 21 grams of hydrochloric acid
C. 0.047 gram of ferric alum and 0.476 gram of orcinol
Levels of color intensity indicated at intervals of 50 units of colorimeter reading. Numbers inserted in contour lines are guides to magnitudes of readings.

an increase in hydrochloric acid concentration, provided the level of ferric alum is also increased.

The effects of changes in orcinol, iron, and hydrochloric acid on the rate of color development are exemplified by the contour maps of Figure 2. Only a limited study of these effects was carried out, because a complete study would have required the redetermination at several different heating periods of every point on which the maps of Figure 1 were based. In obtaining the data of Figure 2 it was assumed, for reasons discussed below, that Drury's conditions (Table I) were optimal; on this basis it was simple to test the effects of varying each component while the remaining components were held constant at their respective assumed optimum levels. Vertical cross sections of the contour maps show that the color develops much more rapidly at high than at low levels of either the orcinol or the hydrochloric acid, but that it changes only slightly when the ferric alum is varied. Horizontal cross sections show that, except at the shortest periods of heating, an increase in the concentration of each constituent caused a rise, then a fall, in the amount of color developed. This confirmed the previous findings of Figure 1, which were based on only a 30-minute period of heating.

The effects of changes in orcinol, iron, and hydrochloric acid on the color intensities of the blanks were also found to be significant. Thus, increases in the levels of each of the constituents cause increases in the color intensities of the blanks, and the effects are roughly additive. At the lowest levels of the constituents, the colorimeter readings of the blanks are about 4; at the highest, about 60.

DISCUSSION

Ideal conditions for the pentose test would be those which bring about the most color, the least sensitivity of the color to small changes in the conditions of the test, the most rapid development of color, and the lowest blank reading. It is clear from the data given that not all of these criteria can be met at the same time, and that a compromise must be made. The relative color intensities which are obtained with the reagents of the different previous investigators are indicated, as closely as possible within the limits of the data, by appropriate points on the contour maps of Figure 1. Those obtained with Drury's reagent are the highest, although most of the others are comparable, except for Militzer's, which are definitely low. The proportions of the orcinol and iron which were used do not, however, in any instance produce the maximum possible color intensities.

The conditions used by Drury appear better than those of Mejbaum or Albaum and Umbreit because they not only produce more color but also require less iron. The use of less iron was based on a recommendation of Ogur as quoted by Drury (*5*). Drury's conditions may even be favored over those which give the maximum color, as indicated by the cross on Figure 1, *B*. Drury's conditions require less iron and much less orcinol, and have been found to produce a lower blank reading—namely, about 8, compared with a reading of 20 for the conditions giving the maximum color. The slightly lower amount of color and slightly lower rate of color development under Drury's conditions are, for practical purposes, insignificant.

Drury's conditions also appear better than those of McRary and Slattery because the latter require a smaller volume of sample than of reagent, owing to the use of higher hydrochloric acid levels, whereas the former provide for equal volumes of sample and reagent. The disadvantage of having to use a smaller volume of sample, which applies to all the conditions of Figure 1, *C* and *D*, as well as to those of McRary and Slattery, nullifies the advantages of higher color intensities and more rapid color development which the high concentrations of hydrochloric acid otherwise make possible. This is not serious, if the test sample contains sufficient pentose. The use of larger proportions of the sample than of the reagent, as is possible at the low hydrochloric acid level represented by the contour map of Figure 1, *A*, is of no further advantage, however, because of the decreased color intensities which are obtained under these conditions.

Doubling of the heat period from 30 to 60 minutes, under Drury's conditions, causes an increase in color intensity of only 4%. The shorter period should, therefore, suffice for routine measurements of ribose nucleic acid, provided the unknown and standard are treated identically. In Drury's procedure, as previously in Albaum and Umbreit's, the orcinol is conveniently made up in alcohol and is added to the hydrochloric acid and ferric alum in this form. In special experiments the presence of alcohol was found to have no effect on the results other than dilution and the slight reduction in the effective concentrations of the components of the reagent.

It may finally be concluded that the levels of each of the components of Bial's reagent for pentoses have, in general, marked effects both on the intensity of color and on the rate of color development obtained in the test. Furthermore, the present data provide a firm basis for choosing Drury's conditions as the most favorable for carrying out the test.

LITERATURE CITED

(1) Albaum, H. G., and Umbreit, W. W., *J. Biol. Chem.*, **167**, 369 (1947).
(2) Bial, M., *Deut. med. Wochschr.*, **29**, 253 (1902); *Chem. Zentr.*, **1902**, II, 295.
(3) Bial, M., *Deut. med. Wochschr.*, **29**, 477 (1903); *Chem. Zentr.*, **1903**, II, 1021.
(4) Brown, A. H., *Arch. Biochem.*, **11**, 269 (1946).
(5) Drury, H. F., *Ibid.*, **19**, 455 (1948).
(6) McRary, W. L., and Slattery, M. C., *Ibid.*, **6**, 151 (1945).
(7) Mejbaum, W., *Z. physiol. Chem.*, **258**, 117 (1939).
(8) Militzer, W. E., *Arch. Biochem.*, **9**, 85 (1946).
(9) Miller, G. L., *J. Lab. Clin. Med.*, **36**, 325 (1950).
(10) Miller, G. L., Golder, R. H., and Miller, E. E., *Federation Proc.*, **9**, 206 (1950).

RECEIVED January 2, 1951. Preliminary reports were presented at the meetings of the Federation of American Societies for Experimental Biology, 1950 (*10*), and at the Fourth Meeting-in-Miniature of the Philadelphia Section, AMERICAN CHEMICAL SOCIETY, January 1951. Investigation supported in part by a research grant from the National Cancer Institute of the National Institutes of Health, Public Health Service, and in part by a grant from the American Cancer Society.

Microscopic Analysis of Benzene Hexachloride

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A microscopic study of the crystal habits of five isomers of benzene hexachloride was made in order to develop simple qualitative methods for identification of each isomer as well as a rapid quantitative method of determination for high γ-benzene hexachloride. A melting point depression method (% impurity = 1.77 × melting point depression in ° C.) designed for use with a micro hot stage was developed. For concentrations of 90 to 100% γ-benzene hexachloride, single determinations may be duplicated with a precision of ±1% impurity. Supplementing this method with qualitative fusion pattern studies and comparative melting point observations has improved its accuracy. A system of application of simple fusion techniques has been demonstrated and used for purity determinations of the tetrachlorobenzenes as well as for α-, δ-, and γ-benzene hexachloride. These techniques may find useful application in purity determinations of other organic-crystalline material.

THE insecticidal activity of benzene hexachloride (1,2,3,4,5,6-hexachlorocyclohexane) has been attributed to the gamma isomer. Commercial "technical benzene hexachloride," however, is composed principally of five stereoisomers (alpha, beta, gamma, delta, epsilon); hence, quantitative methods of analysis for all of these isomers have been of interest. The commercial production of lindane (99% gamma) further emphasizes these analytical needs, because additional methods are required for use in evaluating and setting specifications for this insecticide. These requirements are being met by contributions in the fields of infrared spectroscopy (*3*), partition chromatography (*1*, *5*, *12*), polarography (*4*), and chemical analysis (*8*).

The purpose of the present investigation was to supplement the available methods of benzene hexachloride analysis by the application of chemical microscopy and fusion techniques to develop qualitative methods of analysis for each isomer, and to provide a rapid and accurate means of ascertaining the purity of materials used for standards in infrared analysis. The development of a rapid and simplified method of determining the purity of high γ-benzene hexachloride for use as a control analysis for lindane was an additional aim of this investigation. The initial step was a study of published crystallographic data, undertaken for the purpose of obtaining possible practical applications of this knowledge.

APPARATUS

The polarizing microscope used throughout this work was the Bausch & Lomb Model MA instrument, and all photomicrographs were taken with a Bausch & Lomb Model L photomicrographic camera.

For micromelting point determinations as used in this study, a Kofler hot stage (Arthur H. Thomas and Co.) is recommended. This instrument has a variety of uses, but is designed primarily for micromelting point determinations (*7*). The thermometers used for this instrument are calibrated in terms of melting points of sharply melting pure chemicals. This eliminates the need for a calibration curve. Furthermore, the use of a hot stage in determining melting points enables the operator to observe other changes, such as a tendency toward sublimation, etc., which occur in the crystal fragment as the temperature is elevated toward the melting point.

CRYSTAL HABITS OF BENZENE HEXACHLORIDE ISOMERS RECRYSTALLIZED FROM SOLVENTS DIRECTLY ON MICROSCOPE SLIDES

Examination of the crystallographic data obtained by Daasch (*3*), shown in Table I, reveals interesting diagnostic characteristics of the various benzene hexachloride isomers. The isotropic character of the beta isomer distinguishes it from all others and provides a possible semiquantitative as well as qualitative means of determination. It is also obvious that the alpha isomer may be determined qualitatively by observing apparent optic angle ($2V$). This, however, necessitates the proper orientation of the crystals for obtaining interference figures. The similarity of the crystal structure of the gamma and delta isomers is rather marked, but the very high birefringence of delta may provide a qualitative means of distinguishing between the two.

Table I. Crystallographic Data

Isomer	Type	Sign	Apparent Optic Angle	Birefringence	Refractive Index Range
Beta	Isoaxial	...	...	None	1.630
Alpha	Biaxial	+	30°	Medium	1.60 –1.626
Gamma	Biaxial	+	65°	Medium	1.60 –1.635
Delta	Biaxial	+	...	Very high	1.576–1.674
Epsilon	Biaxial	+	75°	Medium	1.60 –1.635

Crystals of the isomers may be obtained simultaneously from a concentrated benzene solution of crude benzene hexachloride directly on a microscope slide by recrystallization at slightly below room temperature (Figure 1). When examined between crossed nicols of the polarizing microscope, the larger crystals show various degrees of interference colors, but the smaller triangularly shaped crystals black out completely. These are obviously crystals of beta. A rough estimate of the beta content of a benzene hexachloride sample may thus be obtained by microscopic inspection.

The pure beta isomer recrystallizes very easily from concentrated benzene solution into well-shaped isotropic crystals (Figure 2), which often show triangular symmetry, and appear colorless in plane polarized light.

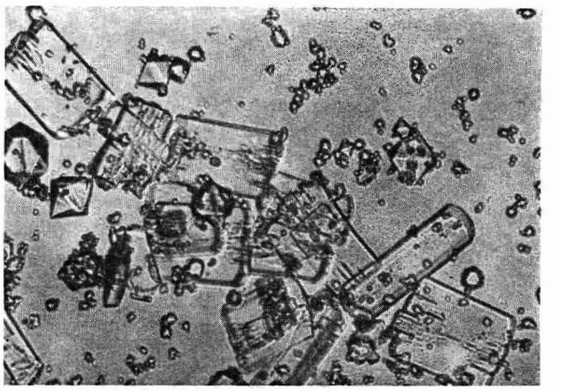

Figure 1. Photomicrograph of Crude Benzene Hexachloride

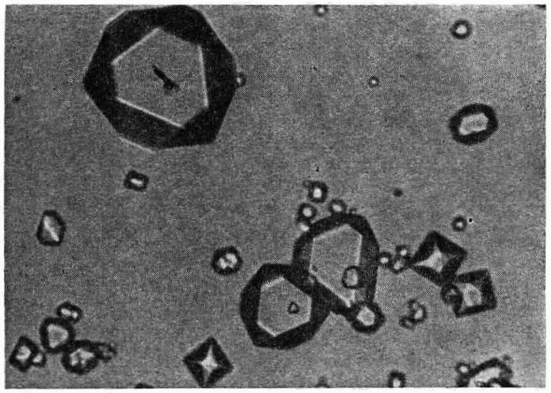

Figure 2. Beta Crystals Obtained by Evaporation of Concentrated Benzene Solution Directly on Microscope Slide

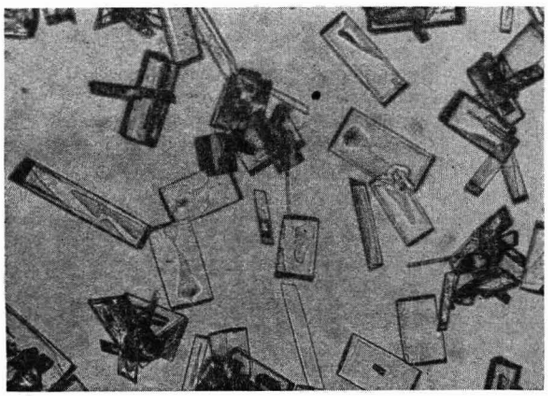

Figure 3. α-Benzene Hexachloride Recrystallized from Benzene Solution Directly on Microscope Slide

Well-defined crystals of the alpha isomer (Figure 3) grow very easily on a microscope slide from a concentrated benzene solution of the pure isomer. The individual crystals are mainly prismatic, appear colorless in plane polarized light, and show a medium order of interference colors when viewed between crossed nicols.

The epsilon isomer crystallizes fairly easily from a concentrated benzene solution of the pure isomer, and appears in two crystal habits (Figure 4). One form shows prismatic crystals very similar to alpha crystals. The other form shows a six-sided symmetry. Both types of crystals may appear together, and both appear colorless in plane polarized light. The birefringence of these crystals is very similar to that of the alpha isomer. These observations regarding the epsilon isomer are in agreement with that made by Kauer (*6*).

The extremely high birefringence of the delta isomer is difficult to demonstrate with on-the-slide preparations. However, well-shaped crystals of delta (Figure 5) may be obtained from a benzene solution of the pure isomer on a microscope slide by slow evaporation of the solvent. As predicted from the crystallographic data, these crystals show a very high order of interference colors when viewed between crossed nicols. Rapid evaporation of a solution of this isomer from most solvents will yield a typical pattern of spherulites (Figure 6). This characteristic habit provides a quick way of identifying the delta cuts when this isomer is prepared chromatographically (*12*). This phenomenon may often be distinguished with the naked eye.

As in the case of delta, it is equally difficult to obtain well-shaped crystals of gamma on a microscope slide. However, when the solvent is allowed to evaporate slowly, this isomer crystallizes from concentrated isopropyl alcohol solution of the pure isomer in the form of plates, tablets, and sometimes rods (Figure 7). By rapid evaporation from its most common solvents, gamma crystallizes in typical treelike patterns or dendrites (Figure 8). As in the case of delta, this habit provides a quick way of indentifying the gamma cuts when preparing this isomer by partition chromatography (*12*). This habit may also quite often be seen with the naked eye.

FUSION STUDIES OF γ-, δ-, AND α-BENZENE HEXACHLORID

The beta and epsilon isomers are not included in these fusion studies because their crystals sublime very readily. Under the usual conditions of fusion between a cover glass and slide, the samples disappear completely without transformation to the liquid phase.

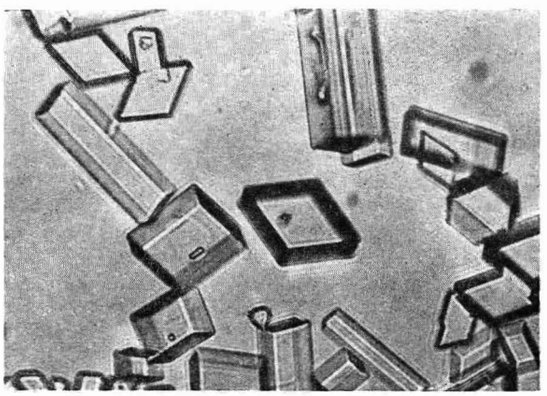

Figure 4. ε-Benzene Hexachloride Recrystallized from Benzene Solution Directly on Microscope Slide

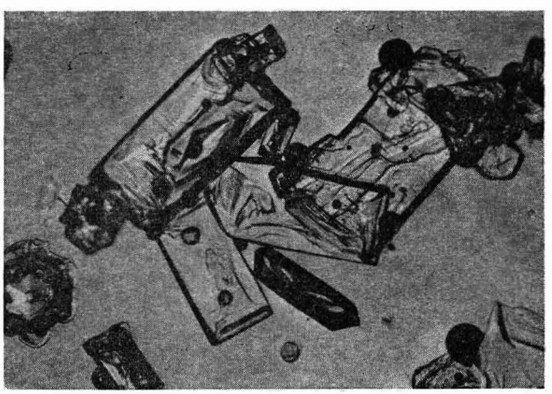

Figure 5. Delta Crystals Obtained by Slow Evaporation of Benzene Solution Directly on Microscope Slide

The works of Kofler (*7*) and his school in Europe and the efforts of McCrone *et al.* (*9–11*) in this country have resulted in a revival of interest in the use of fusion methods in identification of organic crystalline material. These methods permit rapid analysis with small quantities of material and require little specialized training to perform the operations after the initial fusion data have been determined.

The technique employed in this study for preparing samples for obtaining fusion patterns is rather simple.

A few milligrams of the material placed between a slide and cover glass are gradually heated with a microflame until the

material melts. The cover glass is pressed down to obtain a thin film of the melt under the cover glass, and held until the material begins to crystallize. Observations are made between crossed Nicols at low magnification (30× to 90×). To ensure uniformity of sampling for both micromelting point determinations and fusion preparations, a few grams of the sample are melted by gradual heating on a hot plate in a porcelain crucible. The molten material is mixed by stirring and a few drops transferred to a microscope slide. After this material solidifies, it is scraped from the slide and crushed in a micro mortar for use in the determinations.

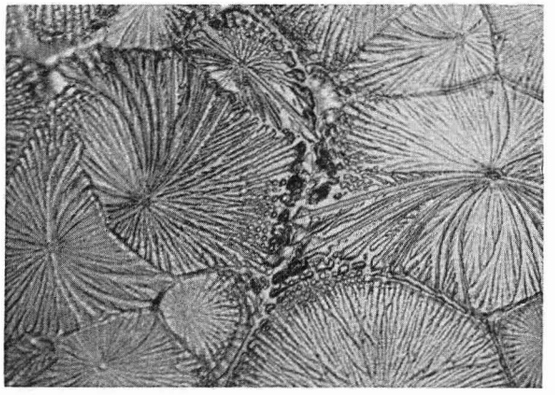

Figure 6. δ-Benzene Hexachloride Obtained by Rapid Evaporation of Solvent
Illustrating typical spherulitic habit

The fusion data for gamma, delta, and alpha (shown in Table II) were intended for use in identifying these pure isomers by simple procedures and not intended as a table of complete fusion data. McCrone (*11*) reports fusion data of the gamma isomer in agreement with those given for gamma in Table II.

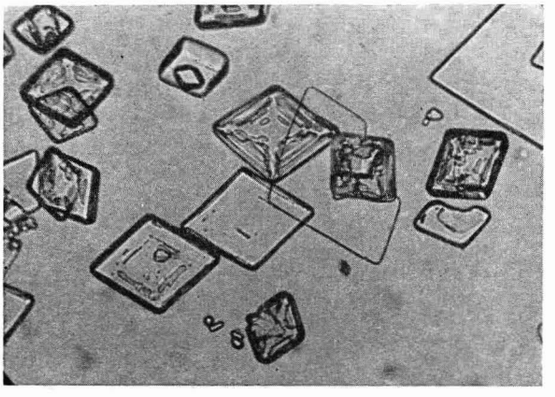

Figure 7. γ-Benzene Hexachloride Recrystallized from Isopropyl Alcohol Solution on Microscope Slide

Since the gamma isomer recrystallizes in three different polymorphic forms (Table II), it is difficult to apply conventional fusion methods of purity determinations. However, the general appearance of the gamma pattern after recrystallization from the melt is fairly reproducible when the fusion operation is performed in the manner described above.

The final fusion pattern of the pure isomer shows the herringbone effect (Figure 9) due to polymorphic form II. This phenomenon is observed only in preparations containing over 99% gamma. As the purity decreases the pattern loses its regularity and assumes a more fibrous appearance (Figures 10 through 12). A study of synthetic samples in this manner enables the operator

Table II. Fusion Data

Isomer	Micro-melting Point, °C.	Remarks
Gamma	114	Melts with slight tendency toward sublimation; three polymorphic forms crystallize from fusions; recrystallizes on spontaneous cooling; form III crystallizes in rods of low birefringence; form II grows slowly with herringbone pattern; form I grows from melt by seeding (highly birefringent rods)
Delta	138	Melts with slight tendency toward sublimation; definite softening of crystal occurs at 134° C.; loses birefringence at 138° C.; recrystallizes from melt in thin rods of very low birefringence
Alpha	160	Melts with strong tendency toward sublimation; transition to well-shaped rods occurs before melting; recrystallizes from melt in spherulites of medium birefringence (yellow, brown, and dull red)

to evaluate qualitatively the purity of a preparation by inspection of the fusion pattern. The principal value of fusion patterns in quantitative analysis is in determining whether a sample contains 99% or more gamma, thus providing a rapid control method for lindane.

Bowen and Pogorelskin (*2*) have published a method for determining the gamma content of crude benzene hexachloride, based on the effect of impurity on freezing point depression. In this investigation the quantitative effect of impurity on melting point depression was used to develop a method of purity determination for lindane.

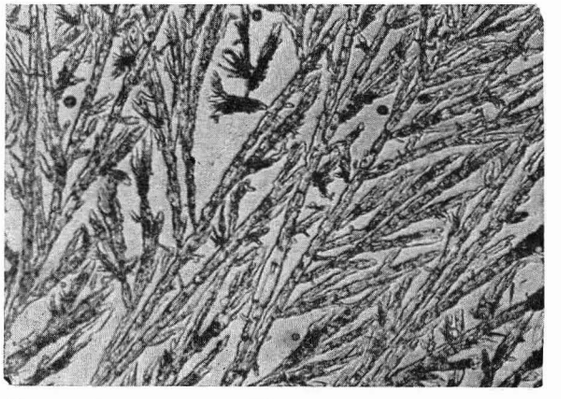

Figure 8. γ-Benzene Hexachloride Obtained by Rapid Evaporation of Solvent
Illustrating typical dendritic habit

Figure 9. Pure Gamma Fusion Showing the Herringbone Effect Due to Polymorphic Form II

Figure 10. 99.5% Gamma Fusion Still Showing the Herringbone Effect with Less Regularity of the Pattern

Micromelting point studies were made with synthetic samples of high γ-benzene hexachloride containing pure alpha as the impurity since that isomer is most likely to be the predominant impurity in lindane. The effects of delta, epsilon, and beta on micromelting point depressions were also investigated. Delta and epsilon produce essentially the same effect as alpha, but the presence of beta yields erratic results. This effect is not too serious since the beta isomer will normally be removed early in the process of concentrating gamma from crude benzene hexachloride by most procedures, owing to the relative insolubility of this isomer in most organic solvents. The presence of beta in a sample is usually detected during the fusion operation by the milky appearance of the melt.

Figure 11. 98.5% Gamma Fusion

Figure 12. 95% Gamma Fusion

The working curve (Figure 13) shows the quantitative effect of impurity on melting point depression of high γ-benzene hexachloride [least squares lines: % impurity = 1.77 × (114 − apparent melting point in ° C.)]. Single determinations may be duplicated with a precision of ±1% impurity. This provides a rapid method of estimating the purity of high γ-benzene hexachloride based on micromelting point depression, which may be checked simultaneously with a qualitative fusion pattern study. In this manner gamma concentrations from 90 to 100% may be satisfactorily estimated at 1% intervals, and these results compare favorably with those obtained by infrared analysis (Table III). A complete determination including a fusion preparation and micromelting point determination requires less than 20 minutes' time.

The above method has been designed primarily as a control method of analysis; however, the accuracy may be improved by taking an average of several melting point determinations. Furthermore, the purity of a sample may be more accurately

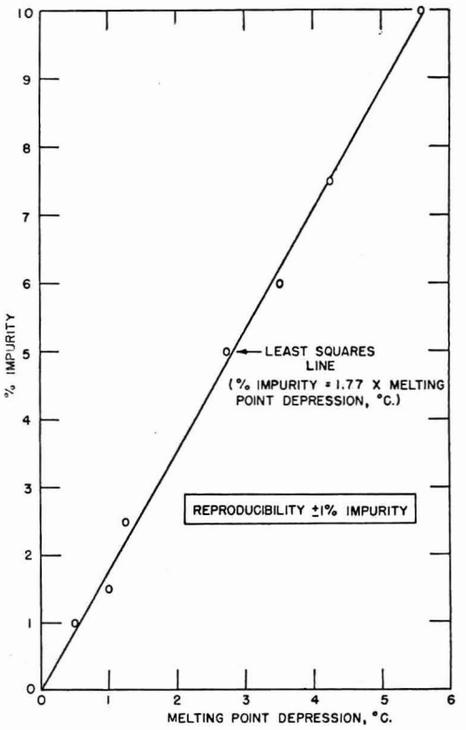

Figure 13. Working Curve

Each point represents the average of several determinations

Table III. Comparative Analyses

Sample	Wt. % Gamma: Micro-melting point fusion method	Wt. % Gamma: Infrared method
1	96	97
2	98	98
3	99+	98
4	96	95.5
5	95	95
6	96	95
7	99+	100
8	99+	100
9	99+	100
10	99+	100

estimated by direct comparison with synthetic samples of known composition on the same slide (Figure 14). For this purpose a slide is divided in quadrants by a glass marking pencil, and as many as three controls may be observed with one unknown.

The fusion patterns obtained with pure α- and pure δ-benzene hexachloride (Table II, Figures 15, 16) are typical and reproducible. In the presence of impurities (in the form of other isomers) smaller and more irregularly shaped spherulites, which usually show brighter interference colors, appear in the alpha fusion pattern (Figure 17). The fusion pattern of impure delta also shows a marked increase in birefringence together with wider and more irregular plates (Figure 18).

From the above data, it appears that the method of micromelting point depression and fusion pattern study may also be used for determining the purity of α- and δ-benzene hexachloride, as has been demonstrated for gamma.

CONCLUSIONS

This investigation has provided an independent approach to the study of benzene hexachloride analysis. It has incorporated a study of the microscopic characteristics of all the isomers in one report, and has provided qualitative means of detection for each of the benzene hexachloride isomers. It has demonstrated a system of application of simple fusion techniques for purity determinations of high γ-benzene hexachloride, thus providing a control method of analysis for lindane. Adaptations of this method have been successfully used in this laboratory for purity determinations of the tetrachlorobenzenes as well as α- and δ-benzene hexachloride. These techniques may also find application in purity determinations for numerous other organic crystalline material.

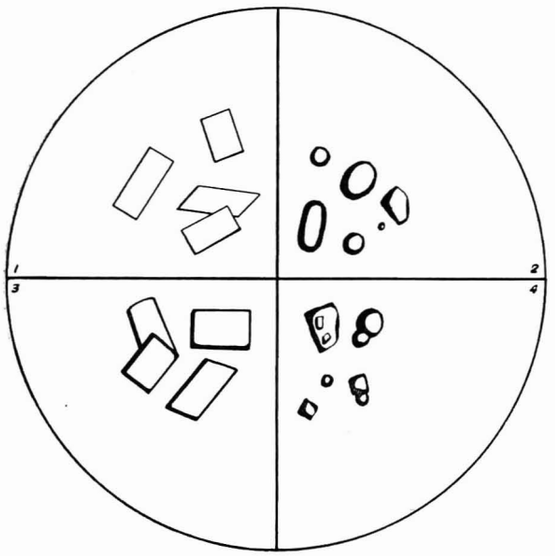

Figure 14. Simultaneous Hot Stage Observation of Controls and Unknowns on Same Slide

Four samples of crystalline material at same temperature. 1, not yet melted; 2, completely melted; 3, beginning to soften around the edges; 4, at its melting point. Liquid and solid phases are in equilibrium

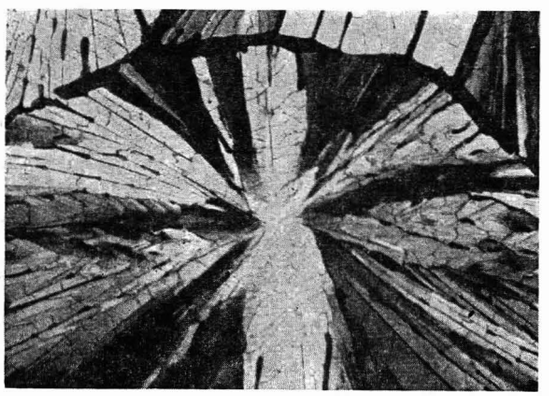

Figure 15. Pure α-Benzene Hexachloride Fusion (Crossed Nicols)

Figure 16. Pure Delta Fusion (Crossed Nicols)

Figure 17. 95% Alpha Fusion (Crossed Nicols) Showing Smaller and More Irregularly Shaped Spherulites

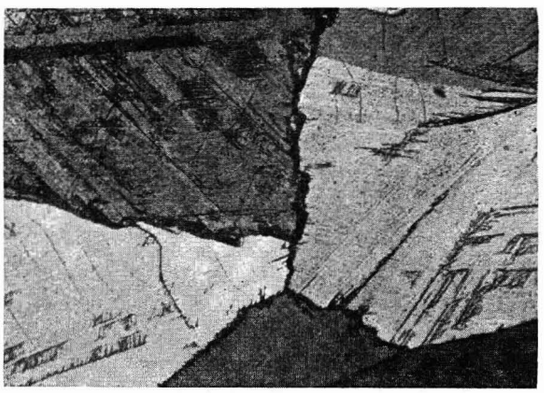

Figure 18. 95% Delta Fusion (Crossed Nicols)

ACKNOWLEDGMENT

The author wishes to thank Robert Herzog for his helpful suggestions and his assistance in evaluating the data, George Calingaert for his suggestion in the use of controls in micromelting point observations, and J. V. Tokos for conducting the infrared analyses.

LITERATURE CITED

(1) Aepli, O. T., *et al.*, ANAL. CHEM., 20, 610 (1948).
(2) Bowen, C. V., and Pogorelskin, M. A., *Ibid.*, 20, 346 (1948).
(3) Daasch, L. W., *Ibid.*, 19, 779 (1947).
(4) Dragt, C., *Ibid.*, 20, 737 (1948).
(5) Harris, T. H., *J. Assoc. Offic. Agr. Chemists*, 32, 684 (1949).
(6) Kauer, K. C., *et al.*, *Ind. Eng. Chem.*, 39, 1335 (1948).
(7) Kofler, L., and Kofler, A., "Mikromethoden zur Kennzeichnung Organisher Stoffe und Stoffgemische," Innsbruck, Universitätsverlag Wagner, 1948.
(8) LaClair, J. B., ANAL. CHEM., 20, 241 (1948).
(9) McCrone, W. C., *et al.*, *Ibid.*, 18, 578 (1946).
(10) *Ibid.*, 21, 436 (1949).
(11) *Ibid.*, p. 882.
(12) Ramsay, L. L., and Patterson, W. I., *J. Assoc. Offic. Agr. Chemists*, 29, 337 (1946).

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Microdetermination of Carbon and Hydrogen

A Statistical Study of Factors

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A collaborative study of the microdetermination of carbon and hydrogen was conducted to find an accurate method suitable for adoption by the Association of Official Agricultural Chemists. The results were analyzed statistically to determine the effects of different variables on the accuracy and precision of the method. Of the twelve factors considered, those which affected the accuracy of the carbon values most were the size of the sample and the treatment the absorption tubes received before being weighed. None of the factors, however, had much influence on the precision of the carbon values and on the accuracy and precision of the hydrogen values. This statistical analysis indicated that certain details of the carbon and hydrogen method should be eliminated, that others were not necessary, and that a more simplified procedure than is often used should produce more accurate and equally precise results.

THE micromethods for carbon and hydrogen in general use today and their semimicro modifications are all based on the catalytic combustion of the organic material to form carbon dioxide and water, followed by the absorption and weighing of these two products. Although the principle of the methods is the same, the details of the procedures vary from laboratory to laboratory.

A collaborative study was undertaken to determine which of these variations should be included in a standard method for the Association of Official Agricultural Chemists. Twenty laboratories participated in this study and performed 115 carbon and hydrogen analyses on nicotinic acid (sample 1) and 95 on benzylisothiourea hydrochloride (sample 2). Each analyst was asked to perform the analyses by his own method under his normal working conditions and to report all values obtained. Details of the procedures used were obtained from each laboratory by a questionnaire designed to give uniform and fairly complete information.

Analysis of the questionnaires showed that for a number of steps in the procedure two different techniques were used. This made possible a simple division of the data for statistical treatment. Thus, all the data could be classified as micro or semimicro, depending on the sample size, or be grouped according to whether or not some treatment or particular modification of the apparatus was used—for example, were the absorption tubes wiped; was the choking plug omitted?

Table I. Data from Collaborative Study of Carbon and Hydrogen Analysis

Collab. No.	Micro Samples (2–10 Mg.)	Absorption Tubes Wiped	O_2 Replaced with Air	Choking Plug	O_2 Aspirated with Mariotte Bottle	Silver Alone	CuO-Pt Catalysts	Electric Sample Burner	Mechanically Operated Burner	Quartz or Vycor Tubes	Lab Air-Conditioned	Balance Adjacent to Furnace
0	—	—	—	—	—	×	—	×	×	×	×	×
2	—	×	—	—	—	—	×	—	—	—	×	—
10	×	×	?	×	×	×	—	—	—	×	—	×
12	×	×	×	×	×	—	—	—	—	×	×	×
13	×	×	×	—	×	×	×	—	—	—	—	—
17	×	×	×	—	—	—	—	×	×	—	—	—
23	×	×	—	×	—	×	—	—	—	×	—	—
24	×	—	—	—	×	×	×	×	×	×	—	×
27	×	—	×	×	×	×	×	—	—	—	—	×
28	×	×	—	×	—	×	—	—	—	—	—	×
31	×	×	—	×	×	×	×	—	—	×	×	×
35	×	—	—	—	—	×	×	—	—	×	×	×
39	×	—	—	×	—	×	×	×	×	×	×	×
40	×	—	×	—	—	×	×	—	—	×	—	—
41	×	×	×	×	—	×	—	×	—	×	×	—
44	×	—	×	×	×	×	×	×	×	×	—	—
45	×	×	×	×	—	×	×	—	—	×	—	—
46	—	—	—	—	×	×	×	×	×	×	×	—
49	×	×	×	×	×	—	×	×	×	—	×	—
50	×	×	×	×	×	×	×	×	×	×	—	—

× Analyst employed procedure listed at head of column.
— Analyst employed alternate procedure.

Table II. Data from Collaborative Study of Carbon and Hydrogen Analysis

Collab. No.	Carbon Data						Hydrogen Data					
	Sample 1			Sample 2			Sample 1			Sample 2		
	n	$\bar{X}$	log s^2	n	$\bar{X}$	log s^2	n	$\bar{X}$	log s^2	n	$\bar{X}$	log s^2
0	8	58.47	2.6201	8	47.38	1.2788	8	4.10	2.3636	8	5.44	2.4150
2	4	58.44	2.4771	3	47.32	1.8751	4	4.21	1.9823	3	5.57	2.0607
10	8	58.75	2.6739	8	47.43	2.7803	8	4.68	2.1818	8	5.90	2.2900
12	2	58.75	1.9294	2	47.72	0.6990	2	4.10	0.0000	2	5.48	1.1139
13	3	58.99	1.4914	3	47.68	0.8451	3	3.98	1.4314	3	5.35	2.4786
17	8	58.64	2.3201	2	47.44	1.5051	8	4.27	1.9031	2	5.68	2.7619
23	3	58.53	2.0682	4	47.42	2.5079	3	3.97	2.0828	4	5.41	2.5092
24	3	58.64	1.7160	3	47.51	2.1790	3	4.16	1.7853	3	5.56	1.3010
27	3	58.57	1.7076	3	47.48	1.4914	3	4.09	1.4314	3	5.44	1.2304
28	3	58.61	2.7202	3	47.39	2.6702	3	4.04	2.1644	3	5.46	2.4065
31	2	58.40	0.6021	3	47.55	2.5966	2	4.06	1.9912	3	5.37	2.3856
35	8	58.52	3.8790	5	47.51	3.8899	8	4.22	2.3118	5	5.51	2.2672
39	4	58.70	2.7084	4	47.42	2.5276	4	3.95	1.9085	4	5.35	1.9685
40	4	58.62	1.5563	4	47.58	1.4472	4	4.09	1.8633	4	5.48	2.0569
41	5	58.92	1.7853	4	47.66	2.0253	5	4.37	1.9912	4	5.77	1.8573
44	4	58.73	1.9912	3	47.14	1.5682	4	4.20	1.4314	3	5.53	1.4314
45	8	58.64	2.5551	10	47.59	2.4456	8	4.17	1.6232	10	5.56	1.9294
46	6	58.66	2.1847	3	47.45	2.3032	6	4.32	1.7559	3	5.68	2.1239
49	21	58.73	3.0249	17	47.55	2.3201	21	4.12	2.5391	17	5.42	2.6253
50	8	58.67	2.1430	3	47.53	2.5786	8	4.11	2.0934	3	5.61	1.3424
Theory		58.53			47.40			4.09			5.47	

Tables I and II show those variables whose effects were compared and a summary of each collaborator's data. In these tables, n is the number of analyses reported by each analyst, $\bar{X}$ is the mean of his values, and log s^2 is the log of the variance of his data. Each collaborator was asked to report all the values he obtained, after his method had been proved satisfactory by analysis of a standard compound of his choice. All data received were used in calculating the $\bar{X}$ and log s^2 values.

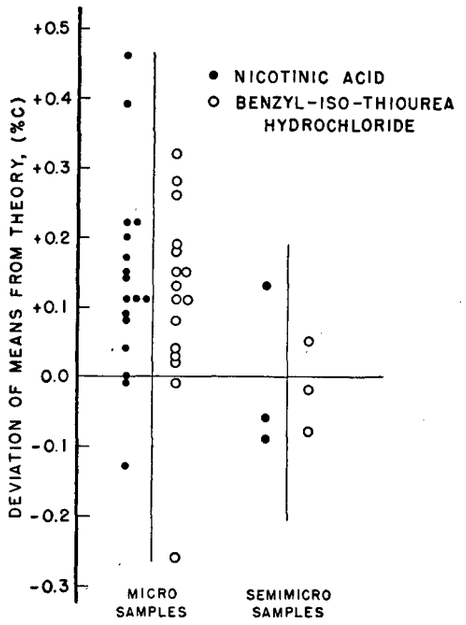

Figure 1. Effect of Sample Size on Carbon Results

In the 12 columns showing the different variables studied, × means that the analyst employed the procedure listed at the head of the column, whereas — marks the alternate procedure.

Following are the procedures listed in Table I plus the alternates with which they were compared:

Micro samples (2 to 10 mg.) *vs.* semimicro samples (10 to 20 mg.)

Absorption tubes wiped before being weighed *vs.* not wiped

Oxygen in absorption tubes replaced with air before weighing *vs.* oxygen not replaced

Use of a choking plug in exit end of combustion tube *vs.* omitting this plug

Oxygen aspirated with Mariotte bottle *vs.* pressure only to force oxygen through system

Use of silver alone to remove sulfur oxides *vs.* silver plus lead chromate

Copper oxide plus platinum catalyst *vs.* all other combinations used

Electric sample burner *vs.* gas burner

Mechanically operated sample burner *vs.* manually operated burner

Quarts or Vycor combustion tubes *vs.* borosilicate glass and other heat-resistant tubes

Analyses conducted in air-conditioned laboratory *vs.* analyses from non-air-conditioned laboratory

Balance located adjacent to furnace irrespective of air-conditioning *vs.* balance in air-conditioned balance room

To determine whether or not there was any marked difference in accuracy, the carbon data obtained with micro- and semimicroprocedures were plotted (Figure 1) as suggested by Tukey (*2*). Inspection of this simple plot of the deviation of the means from the theoretical values readily shows that the micro values were in general higher than the semimicro. Student's t test (*1*) was applied to these data to determine if this apparent difference was really significant.

$$t = \bar{x}\sqrt{\frac{n_a n_b\,(n_a + n_b - 2)}{(n_a + n_b)\,(S_a x^2 + S_b x^2)}}$$

where $\bar{x} = \bar{X}_a - \bar{X}_b$ (difference between means of two groups)

n_a = No. of values in group a, micro

n_b = No. of values in group b, semimicro

$$S_a x^2 + S_b x^2 = \Sigma(X_a - \bar{X}_a)^2 + \Sigma(X_b - \bar{X}_b)^2$$

The t value calculated from these data was 2.24 and the critical $t_{0.05}$ obtained from the table of t values was 2.03. The difference between the two means, therefore, was critical at the 95% level. This indicates that semimicromethods will produce more accurate results than microprocedures, if the data were representative and not biased by other variables.

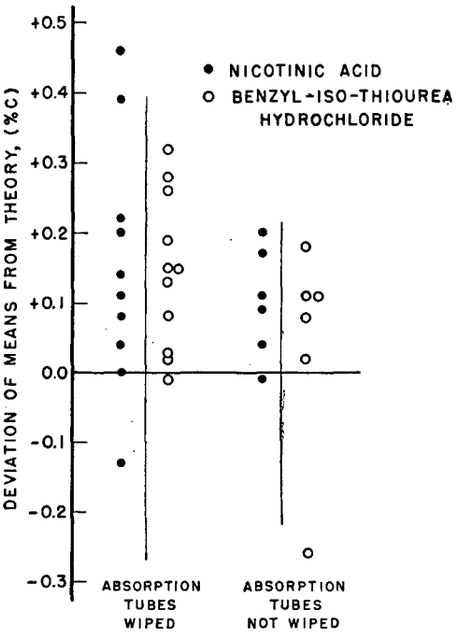

Figure 2. Effect of Wiping and Not Wiping Absorption Tubes

Because there was a significant difference between the micro and semimicro values, it seemed desirable to eliminate the effect of this variable from subsequent comparisons. Therefore, the values obtained using micro samples were treated separately, so that the effect of the other variables on the accuracy of the carbon values could be determined without bias due to the sample size.

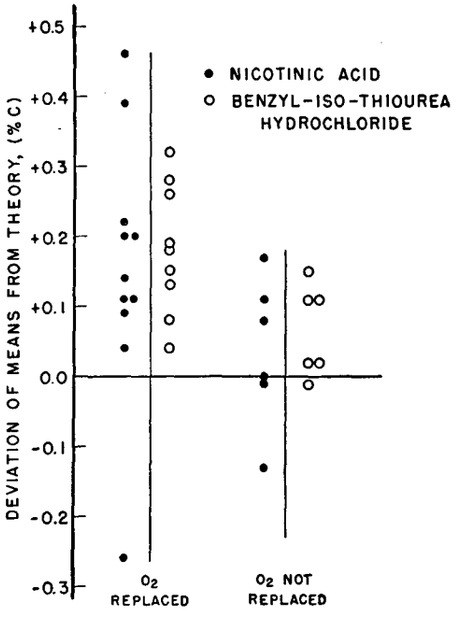

Figure 3. Effect of Replacing Oxygen in Absorption Tubes with Air

Using only micro values, the effect of wiping or not wiping the absorption tubes before weighing was analyzed by this same procedure. Figure 2 shows a plot of the data, and on inspection it appears that wiping has a tendency to cause high results. When the t test was used to determine the significance of the difference, the calculated value was 1.67. Thus the difference was significant at the 90% but not at the 95% level.

Figure 3 shows the values obtained when the oxygen in the absorption tubes was replaced before weighing and when it was not replaced. The difference that can be seen here is significant, as the t value for the difference between means was greater than $t_{0.95}$. No significant differences in the accuracy of the carbon values were found between the alternate procedures for the remaining nine variables listed in Table I.

The question arose as to whether or not the two apparently critical variables biased the micro data for the other variables, so that no critical differences appeared when in reality some actually existed. To determine this, the effect of the two variables which appeared to be important (wiping the absorption tubes and replacing the oxygen) had to be at least partially eliminated by adjusting the data. Figure 4 shows that there was considerable overlapping of the data for these two variables; seven analysts both wiped the tubes and replaced the oxygen and only three each used the other three combinations of these two variables. Adjusting the data to eliminate the effect of either of these variables also made the other variable not critical. Therefore, rather than attempting to select the more important variable, the data were adjusted to eliminate the effect of both. The median deviation of the mean from the theoretical value for the 14 carbon results obtained by those who both wiped the tubes and replaced the oxygen was +0.20%. The median for those who did neither was +0.11%, and for those who performed either one or the other operation it was +0.06. This latter value should logically fall between 0.20 and 0.11%, and the fact that it did not may mean that there was an interrelationship between the two variables or that the value was low by accident. Regardless of this, it can be concluded with reasonable certainty that the effect of both wiping the tubes and replacing the oxygen was to cause the results to be high by approximately 0.10%. To adjust for this effect 0.10% was subtracted from the values obtained by those who performed both operations. These adjusted data were then re-examined to determine if any of the previously noncritical differences would become significant. However, the only effect of any importance

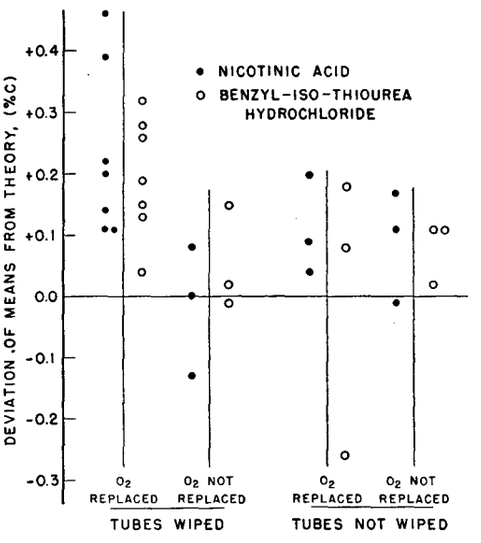

Figure 4. Effect of Wiping Tubes and Replacing Oxygen

Table III. Statistical Data for Variables Causing Critical Differences in Carbon and Hydrogen Results

	Comparison	n	$\bar{X}$	Sx^2	$t_{calcd.}$	$t_{0.05}$
Carbon	Micro samples (2–10 mg.)	34	+0.12	0.6149	2.24	2.03
	Semimicro samples (10–20 mg.)	6	−0.01	0.0384		
	Absorption tubes wiped[a]	22	+0.15	0.4073	1.67	2.04
	Absorption tubes not wiped[a]	12	+0.07	0.1630		
	Oxygen replaced with air[a]	20	+0.17	0.4233	2.53	2.04
	Oxygen not replaced with air[a]	12	+0.05	0.0800		
	Tube wiped and O_2 replaced	14	+0.21	0.1695	2.38	2.10
	Tubes not wiped and O_2 not replaced	6	+0.09	0.2325		
	Micro samples[b]	32	+0.09	0.4846	1.94	2.03
	Semimicro samples	6	−0.01	0.0371		
			log s^2			
	Oxygen replaced with air	20	1.87	7.148	2.16	2.03
	Oxygen not replaced with air	18	2.33	8.227		
Hydrogen	Oxygen replaced with air	20	1.76	7.648	2.31	2.03
	Oxygen not replaced with air	18	2.15	2.050		
	Oxygen aspirated (Mariotte bottle)	20	1.76	7.708	2.58	2.03
	Pressure alone	20	2.15	1.935		

[a] Micro data only used in calculations.
[b] Micro data corrected by subtracting 0.10 from values obtained when tubes were wiped and oxygen replaced with air.

was on the micro-semimicro comparison and this was to reduce the difference, as only micro values were lowered by adjustment of the data. The t value using the adjusted data was 1.94, which is still critical at the 90% but not at the 95% level.

The t values of importance for the whole study and the data necessary to calculate these values are shown in Table III.

In addition to the effect on accuracy, it was desired to know the effect of the variables on the precision of the results. A method for doing this, suggested by Tukey (*2*), consisted of determining the variance of each analyst's carbon values, taking the logarithm of these, and applying Student's t test as if they were means. When this test was applied to the carbon values, only one significant difference (95% level) in precision appeared. As 11 separate tests were made, one would be expected to appear critical about half $[1-(0.95)^{11}]$ of the time. This critical difference was for oxygen in the absorption tubes replaced with air before weighing as compared with not replacing the oxygen. The calculated t value was 2.16 as against a critical value of 2.03 at the 95% level. This particular variable apparently had the greatest effect on the accuracy and on the precision of any of the variables evaluated. While replacing the oxygen caused significantly better precision, it also caused significantly poorer accuracy, showing that the two criteria by which data are judged are not the same or related, even when working with a large number of values.

Information concerning the effect of the variables on the accuracy and precision of the hydrogen data was also desired. The same tests were applied to the hydrogen data as to carbon. None of the variables appeared to have a significant effect on the accuracy of the hydrogen determination, although two variables affected the precision. One, as in the carbon study, was whether or not the oxygen in the absorption tubes was replaced with air before weighing, and the other was whether pressure plus aspiration with a Mariotte bottle or pressure alone was used to drive oxygen through the combustion train. Table III shows the calculated and critical t values for these variables.

CONCLUSIONS

The purpose of the study was to determine which combination of techniques should produce the best carbon and hydrogen results. To do this, it was necessary to arrange the desired objectives of a good method in the order of their importance. The following order was used:

Accuracy of carbon results
Accuracy of hydrogen results
Precision of carbon values
Precision of hydrogen values

In addition to these objectives, such items as the complexity of apparatus and technique, the number of determinations possible per apparatus per day, amount of the analyst's time spent per analysis, and the possibility of simultaneous operation of two apparatus were also considered. With these objectives and consideration in mind, a study of the result led the authors to conclude that the carbon and hydrogen procedure should include the following: electric furnaces with mechanical operation, quartz or Vycor combustion tubes with a filling of copper oxide plus platinum catalysts and silver wire or ribbon, no choking plug, pressure only for oxygen flow; no treatment of the absorption tubes other than to allow them to equilibrate before weighing; and use of samples weighing 10 to 15 mg. if possible.

Directions for a method including these features were written and submitted to a number of collaborators, so that the conclusions drawn from this study could be tested. The results will be published in a referee report to the Association of Official Agricultural Chemists.

ACKNOWLEDGMENT

The authors express their thanks to John W. Tukey, Princeton University, who in reviewing this paper made many helpful suggestions.

LITERATURE CITED

(1) Snedecor, G. W., "Statistical Methods," 4th ed., p. 82, Ames, Iowa, Iowa State College Press, 1946.
(2) Tukey, J. W., private communication.

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Microdetermination of Arsenic and Its Application to Biological Material

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THE most satisfactory method for the determination of arsenic in biological materials appears to be a combination of wet ashing with sulfuric, nitric, and perchloric acids, oxidation to pentavalent arsenic with various oxidizing agents, and the development of molybdenum heteropoly blue arsenic complex with ammonium molybdate and hydrazine sulfate (*1–14*).

An investigation to develop a more accurate and sensitive method for the determination of micro quantities of arsenic in samples containing less than 0.1 microgram of arsenic with a sensitivity of 0.01 microgram was undertaken when it was evident that the literature contained little information for normal arsenic content of body tissues such as serum, kidney, liver, and muscle. Arsenic levels in normal urine have been reported from 2.8 to 7.8 micrograms % (*6, 11, 14*). Claims have been made that arsenic is not present in whole blood of humans (*6, 14*) and horses (*12*) but others found 6 to 25.5 micrograms % in normal whole blood of humans (*9, 13*).

METHOD

Reagents. Stock Standard Arsenic Solution. Dissolve 0.1320 gram of arsenic trioxide in 50 ml. of distilled water and 0.50 ml. of 70% sodium hydroxide. Neutralize with 0.50 ml. of 70% sulfuric acid and dilute to 100 ml. in a volumetric flask. One milliliter of this solution equals 1.0 mg. of arsenic.

Working Standard Arsenic Solution. Dilute 1 ml. of stock solution to 100 ml. in a volumetric flask with distilled water for photometer (Coleman Model No. 14) standardization (1 ml. = 0.010 mg. of arsenic). Dilute 1 ml. of stock solution to 500 ml. in a volumetric flask with distilled water for photometer (Beckman Model DU) standardization (1 ml. = 2.0 micrograms of arsenic).

Stock Iodine 0.02 *N* Solution. Dissolve 2.54 grams of iodine and 8 grams of potassium iodide in 25 ml. of distilled water. When solution is complete, dilute to 1 liter in a volumetric flask. Store in a dark bottle.

Working Iodine Solutions, 0.002 *N* and 0.001 *N*. Dilute 10 ml. and 5 ml. of stock solution to 100 ml. in a volumetric flask with distilled water.

Ammonium Molybdate (1% in 5 *N* sulfuric acid). Add 70 ml. of concentrated sulfuric acid to about 300 ml. of distilled water. Dissolve 5 grams of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, in the warm acid mixture. When solution is complete, cool and dilute to 500 ml. in a volumetric flask with distilled water.

Hydrazine Sulfate (0.15%). Dissolve 0.3 gram of C.P. hydrazine sulfate ($N_2H_4.H_2SO_4$) in about 150 ml. of distilled water and then dilute to 200 ml.

An accurate and sensitive method for the determination of micro quantities of arsenic in samples containing less than 0.1 microgram of arsenic with a sensitivity of 0.01 microgram was developed for the measurement of normal arsenic content of body tissues for study of arsenic exposure. A normal range of 3.5 to 7.2 micrograms % of arsenic in serum and 4.6 to 20.6 micrograms in 24-hour urinary excretion of arsenic was found in normal human adults. This investigation indicates that an accurate, sensitive determination of arsenic in biological material can be made simply with the apparatus described without preliminary oxidative digestion and that arsenic is probably not firmly bound in biological material. A constant normal range of arsenic content of biological material was established for interpretation of results obtained on individuals or animals exposed to arsenic.

Potassium Iodide. Prepare a 15% solution in distilled water. Prepare fresh before use.

Stannous Chloride. Prepare a 40% solution in distilled water. Prepare fresh before use.

Hydrochloric Acid. Concentrated C.P.

Zinc, Mossy. C.P. Use pieces weighing 0.7 to 1 gram.

Lead Acetate. Prepare fresh saturated solution.

Standardization. Add to 2.5 ml. of 0.002 *N* iodine solution in a cuvette, 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.7, 0.8, and 1.0 ml. of appropriate working arsenic standard solutions for Beckman Model DU or Coleman Model No. 14 photometer standardization. Dilute to 5 ml. with distilled water. Add 0.5 ml. of ammonium molybdate, mix, and then add 0.2 ml. of hydrazine sulfate and mix. Place in boiling water bath for 10 minutes. Cool. Read at 830 mμ with the Coleman or 865 mμ with the Beckman spectrophotometer with water blank set at 100% transmission. Color is stable for 24 hours.

PROCEDURE

Procedure for Specimens Containing Less Than 15 Micrograms of Arsenic. (Specimens of normal body fluids and tissues containing less than 7 micrograms of arsenic may be measured more accurately with the Beckman Model DU photometer if the arsenic is distilled into 2.5 ml. of 0.001 *N* iodine and the color developed with 0.25 ml. of molybdate solution and 0.1 ml. of hydrazine sulfate reagent.) To 5 ml. of serum and 20 ml. of distilled water (or 25 ml. of urine) in a 125-ml. Erlenmeyer flask (Figure 1), add 10 ml. of concentrated hydrochloric acid and 2 glass beads. Mark total volume on flask. Digest slowly (to prevent excessive foaming) for 15 minutes on a 350-watt Cenco cone heater. Cool to room temperature. Add 10 ml. of concentrated hydrochloric acid and dilute to volume of first digestion with distillation water. Add 2 ml. of 15% potassium iodide and 0.5 ml. of 40% stannous chloride. Allow to stand for 15 minutes at room temperature. Stopper into a ground-glass standard-taper (lubricate with distilled water) flask a 15-mm. coarse fritted-disk funnel (No. 36060 funnel, borosilicate glass) containing a small piece of cotton saturated with lead acetate solution (three drops) to absorb the hydrogen sulfide evolved. Stopper a bent, fritted gas-dispersion cylinder (12-mm. coarse, No. 39533 tube, borosilicate glass) into the ground-glass standard taper of the fritted-disk funnel and lubricate with distilled water. Quickly add 2 to 3 pieces of mossy zinc and allow gas to bubble for 1 hour through the fritted gas-dispersion cylinder into 5 ml. of 0.001 *N* iodine in a photometer cuvette immersed in cracked ice. Add 0.5 ml. of ammonium molybdate solution and 0.2 ml. of hydrazine sulfate (mixing after each addition) to the iodine solution. Place in a boiling water bath for 10 minutes. Cool and read against blank set at 100% transmission. Use 865 mμ light transmission if Beckman Model DU is used, or use P-5 filter and 830 mμ light transmission for Coleman Model No. 14. Blank is prepared by running 25 ml. of distilled water through the above procedure.

Procedure for Specimens Containing 15 to 40 Micrograms of Arsenic. Continue as directed in previous procedure but bubble gas into 10 ml. of 0.001 *N* iodine for 1 hour and then add 1.0 ml. of ammonium molybdate and mix Add 0.4 ml. of hydrazine sulfate and mix. Place in a boiling water bath for 10 minutes. Cool, and make photometric measurements as directed.

Calculations. General formula, concentration in micrograms per 100 ml. equals K (2 − log % transmission).

EXPERIMENTAL

Digestion of Specimens. Wet digestion with sulfuric, nitric, and perchloric acids for the complete oxidation of the sample was not necessary for full recovery of arsenic from various animal tissues. A simple preliminary digestion of 5 grams of sample with 20 ml. of distilled water and 10 ml. of concentrated hydrochloric acid in a 125-ml. Erlenmeyer flask for 15 minutes was established as the optimum condition for the arsenic distillation. Solid tissues such as muscle, heart, kidney, and liver were emulsified by mixing one part of tissue with 4 parts of distilled water in a Waring Blendor for 10 minutes. Liquid tissues such as serum and whole blood were digested directly.

If the digestion of the specimen with the dilute hydrochloric acid was carried out under a reflux condenser instead of an open flask, less than 50% of arsenic added to or present in the specimen could be distilled from such a preparation. Hydrogen sulfide and other compounds containing mercapto (—SH) groups are retained by the reflux condenser to combine with the arsenic and prevent its distillation. The same loss of arsenic occurs if hydrogen sulfide is bubbled into the open flask during digestion.

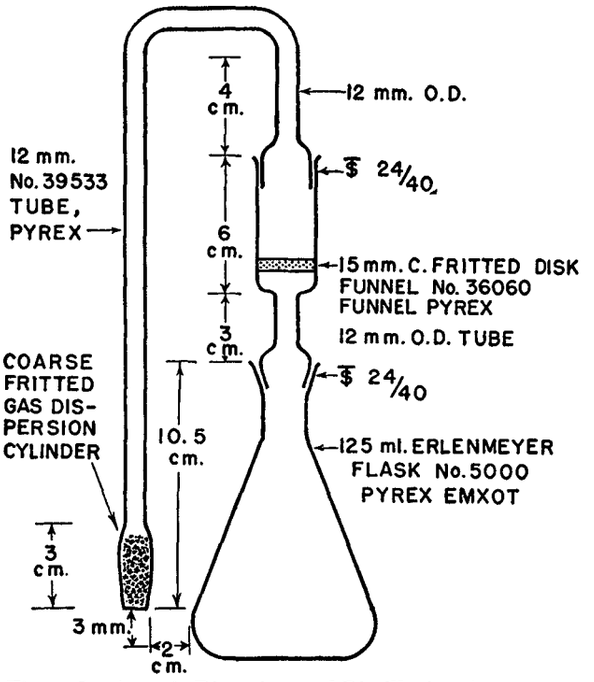

Figure 1. Arsenic Digestion and Distillation Apparatus

After the hydrochloric acid digest was cooled to room temperature, the addition of 10 ml. of concentrated hydrochloric acid and water to the original volume of the digest before the start of digestion gave the best reaction when 2 ml. of 15% potassium iodide and 0.5 ml. of 40% stannous chloride were added and allowed to stand 15 minutes before the start of the distillation of arsenic as arsine. Apparently the addition of potassium iodide is not necessary for the complete recovery of naturally occurring arsenic from animal fluids and tissues as complete recoveries were obtained in its absence. Complete recovery of trivalent arsenic added as (3-amino-4-hydroxyphenyl) dichloroarsine hydrochloride to serum was also obtained when potassium iodide was omitted. However, only about 50% of pentavalent arsenic

Table I. Recovery of Different Amounts of Arsenic Added to 5 Ml. of Serum

Arsenic in Serum, γ	Arsenic Added, γ	Total Arsenic Found γ	Total Arsenic Found γ %
	Beckman Model DU Spectrophotometer		
	(2.5 ml. of 0.001 *N* iodine absorbant)		
0.25	1.0	1.25	100.0[a]
0.25	2.0	2.25	100.0[a]
0.25	5.0	5.05	96.1[a]
0.25	7.0	6.95	95.7[a]
0.25	10.0	8.75	85.5[a]
	(5 ml. of 0.002 *N* iodine absorbant)		
0.26	0.1	0.37	102.8[b]
0.26	0.2	0.47	102.2[b]
0.24	0.5	0.76	104.0[b]
0.24	0.7	0.93	98.5[b]
0.24	1.0	1.24	100.0[b]
0.26	2.0	2.11	93.4[b]
0.26	3.0	2.93	89.5[b]
0.26	4.0	3.70	86.5[b]
	Coleman Model No. 14 Spectrophotometer		
	(5 ml. of 0.001 *N* iodine absorbant)		
0.25	0.25	0.51	104.0[b]
0.25	0.50	0.76	102.0[b]
0.25	0.75	0.99	98.6[b]
0.25	1.00	1.21	96.0[b]
0.25	2.00	2.10	92.5[b]
0.25	3.00	2.95	90.0[b]
0.25	4.00	3.75	87.5[b]
0.25	5.00	5.25	100.0[a]
0.25	6.00	5.50	87.5[b]
0.25	10.00	10.05	98.0[a]
0.25	15.00	14.85	97.3[a]
0.25	20.00	19.05	93.5[a]
0.25	25.00	21.95	87.3[a]
	(10 ml. of 0.001 *N* iodine absorbant)		
0.25	20.00	19.85	98.0[a]
0.25	40.00	38.65	96.2[a]
0.25	50.00	45.25	90.2[a]

[a] 0° to 4° C.
[b] 20° to 25° C.

added as the sodium salt of *N*-(phenylglycine amide) *p*-arsonic acid or as arsenic acid was recovered in the absence of potassium iodide.

The addition of 1 mg. of phosphorus as orthophosphoric acid or 1 mg. of silicon as sodium silicate to the digest produced no increase in arsenic blanks.

Distillation of Arsenic. A simple combination digestion and distillation, all-borosilicate glass apparatus (Corning Glass Co.) with a hydrogen sulfide trap was designed for arsenic determinations by the new method (Figure 1). Three pieces or less of coarse mossy zinc weighing together 2 to 3 grams gave the desired rate of evolution of hydrogen gas. If the mossy zinc is too finely divided, objectionable foaming occurs. Various distillation times at room temperature were tried from 15 minutes to 2 hours; 1 hour was satisfactory inasmuch as no differences were observed between 1- and 2-hour distillations.

DEVELOPMENT, PHOTOMETRIC MEASUREMENT, AND STANDARDIZATION OF MOLYBDENUM BLUE COLOR

Light transmission ranging from 610 to 725 mμ (*6*, *11*) was used for photometric measurement of arsenic heteropoly blue complex until Sultzaberger (*14*) obtained maximum absorption of the colored arsenic complex at 840 mμ light transmission. However, an investigation of Boltz and Mellon (*1*) showed that maximum density of the arsenic complex could be changed by variation in the concentration of the chemical reagents in the development of the color and the kind of reductant used. The absorption curve may also be influenced by the ratio of arsenic to excess molybdate as a larger excess of molybdate forms pentavalent molybdenum ions which change the absorption curve at the blue end of the spectrum.

Under the conditions for developing molybdenum blue color with arsenic distilled from biological material as described herein, it was discovered that the maximum light absorption of this colored complex occurred at 865 mμ (Figure 2), as measured with the Beckman Model DU spectrophotometer at different arsenic concentrations. Blank determinations on reagent-grade reagents gave 96 to 97% transmission with the Beckman and 98% transmission with the Coleman spectrophotometer. According to the manufacturer, the reagents—mossy zinc, hydrochloric acid, and sulfuric acid—which were used contained 0.000001% arsenic. Stannous chloride contained 0.0002% arsenic; analytical data for the arsenic content of the other reagents could not be obtained from the manufacturer.

STANDARDIZATION OF BECKMAN AND COLEMAN SPECTROPHOTOMETERS

Standardization curves for the Beckman and Coleman spectrophotometers are given in Figure 3. A fairly linear curve is given by the Coleman up to 30% transmission using 830 mμ light transmission and 5 ml. of 0.001 *N* iodine absorbant. The standardization curves for the Beckman are linear to 35% transmission using 865μ light transmission. The Beckman spectrophotometer is 2.5 times as sensitive at 35 to 100% transmission as the Coleman instrument when an equal volume of 0.001 *N* iodine absorbant is used; this sensitivity may be doubled again by halving the volume of the absorbant (Figure 3). The sensitivity of the Beckman instrument was further increased to 0.005 microgram of arsenic at 0.01-mm. slit by substituting Duodial, Model R, No. R10L for the sensitivity knob (Helipot Corp., South Pasadena, Calif.). The absence of linearity below 35% transmission with the Beckman instrument may indicate that arsenic at higher concentrations tends to form a more colloidal molybdenum blue complex through which linear transmissions of 865 mμ light is more critical than at 830 mμ (Figure 3).

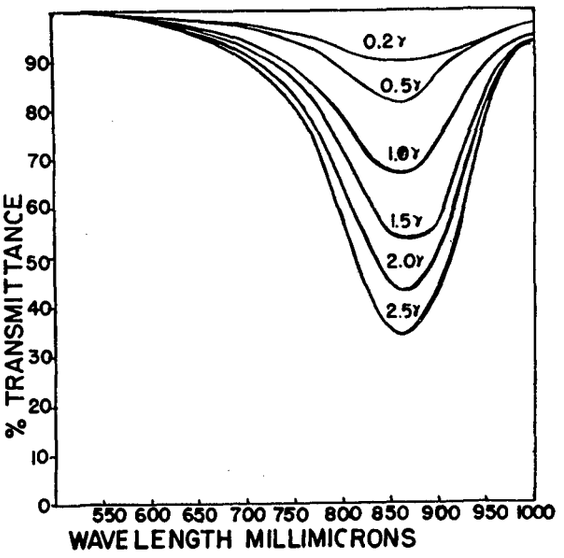

Figure 2. Spectral Transmittance of 0.2 to 2.5 Micrograms of Arsenic

Reaction mixture of 5 ml. of 0.001 *N* iodine, 0.5 ml. of 1% ammonium molybdate in 5 *N* sulfuric acid, and 0.2 ml. of 0.15% hydrazine sulfate in 1-cm. cell of Beckman Model DU spectrophotometer, 0.02-mm. slit

The ammonium molybdate reagent was made up in 1 to 10 *N* sulfuric acid to test the effect of pH on the development of the molybdenum blue color. Increasingly higher blanks were given at normalities below 5 *N*. Optimum color development with a low blank occurred at 5 *N*. Although blanks remained constant at higher normalities, less molybdenum blue color was developed. The proportion of ammonium molybdate and hydrazine sulfate used was similar to that reported by Case (*2*).

RECOVERY EXPERIMENTS

Table I indicates the limits of the amounts of arsenic added as arsenious acid to serum which can be measured by the Beckman

Table II. Comparison of Spectrophotometers for Serum Arsenic Determinations

(5 ml. of 0.001 *N* iodine absorbant)

Sample No.	Serum Used, Ml.	Arsenic, γ %: Beckman[a]	Arsenic, γ %: Coleman[b]
1	2	5.2	5.1
2	2	6.2	6.2
3	2	4.2	3.9
4	2	6.1	6.2
5	2	5.2	5.1
6	2	5.2	5.3
7	2	5.9	6.2
8	5	7.6	7.9
9	5	4.2	4.5
10	5	6.9	6.7

[a] 865 mμ light transmission.
[b] 830 mμ light transmission.

Table III. Comparison of Methods for Arsenic Determination in Blood Serum and Urine of Adult Patients

(Photometric measurements made on Coleman spectrophotometer)

Sample No.	Arsenic in Urine[a], γ/24 Hours: Chaney-Magnuson	Arsenic in Urine[a], γ/24 Hours: Kingsley-Schaffert	Sample No.	Arsenic in Serum[b], γ %: Chaney-Magnuson	Arsenic in Serum[b], γ %: Kingsley-Schaffert
1	80	70	1	6.7	7.0
2	110	105	2	5.6	5.1
3	37	39	3	6.7	6.2
4	43	37	4	3.9	3.7
5	90	84	5	4.5	4.2
6	24	22	6	5.1	5.1
7	64	60	7	4.5	4.2
8	11	11	8	3.6	3.4
9	15	18	9	3.1	3.4
10	9	9	10	4.2	4.2
11	36	38	11	5.3	5.1
12	14	14	12	2.3	2.3
13	42	38	13	4.2	3.7
14	10	9	...	...	...
15	22	27	...	...	...
Av.	40.5	38.8		4.6	4.4

[a] 25-ml. urine specimen used.
[b] 5-ml. serum specimen used.

Table IV. Comparison of Methods for Determination of Arsenic in Fresh Beef Tissues

Tissue	Kingsley-Schaffert, γ %: Spec. I	Kingsley-Schaffert, γ %: Spec. II	Kingsley-Schaffert, γ %: Av.	Chaney-Magnuson, γ %: Spec. I	Chaney-Magnuson, γ %: Spec. II	Chaney-Magnuson, γ %: Av.
Muscle	14.0	14.7	14.4	15.7	13.5	14.1
Liver	3.0	3.0	3.0	3.0	3.0	3.0
Kidney	5.7	5.2	5.5	6.0	5.0	5.5

and Coleman spectrophotometers when arsenic is distilled into different amounts of 0.001 *N* iodine absorbant at room and ice-bath temperatures. Recoveries of 95% or more were obtained with the Beckman spectrophotometer for 1.5 micrograms at room temperature with 5 ml. of 0.001 *N* iodine absorbant and 7 micrograms at 0 to 4° C. with 2.5 ml. of 0.001 *N* iodine absorbant. Good recoveries on larger amounts of added arsenic up to 15 micrograms were obtained at 0° to 4° C. with 5 ml. of 0.001 *N* iodine absorbant with the Coleman spectrophotometer; recoveries were further increased to 40 micrograms when 10 ml. of 0.001 *N* iodine absorbant were used. The higher arsenic concentrations gave molybdenum blue solutions too dense to be read directly with the photometer but gave quantitative proportionate values when diluted with various amounts of distilled water up to 1 part in 100 parts of water. No change was noted for several hours in the light absorption of these dilutions.

Although light transmissions of 865 mμ could not be used in making readings with the Coleman spectrophotometer of the arsenic molybdenum complex, arsenic analyses of serum compared favorably with those obtained with the Beckman spectrophotometer (Table II).

COMPARISON OF CHANEY-MAGNUSON AND KINGSLEY-SCHAFFERT METHODS

The Chaney and Magnuson method was used in this investigation as a method of comparison for the determination of arsenic in serum, urine, and body tissues. The arsenic distillation method of Chaney and Magnuson (*3*) which has been widely used has had two suggested modifications—the use of potassium bromide at the start of distillation to ensure distillation of arsenic in pentavalent form (*9*), and the addition of 3 ml. instead of 2 ml. of 1 *N* hydrochloric acid to the molybdate solution for color development to prevent the reduction of ammonium molybdate which was believed to be the cause of high blanks (*10*) with this method. The following two suggested modifications (*4*) were used:

(a) The digestion reagents were combined into a single mixture of 125 ml. of C.P. concentrated sulfuric acid, 85 ml. of 70% perchloric acid, and 290 ml. of C.P. concentrated nitric acid. This mixture (20 ml.) was used for the digestion of each 5-gram sample.

(b) Potassium chloride (15.75 grams), 6.00 grams of potassium bromide, and 5.00 grams of hydrazine sulfate were used in the reducing reagent for the digest.

The sensitivity of the Chaney-Magnuson method was also increased by diluting the distillate to only 10 ml. A 5-ml. aliquot of the distillate was treated in the same manner as described previously by adding 0.5 ml. of ammonium molybdate, 0.2 ml. of hydrazine sulfate, and heating on a water bath for color development.

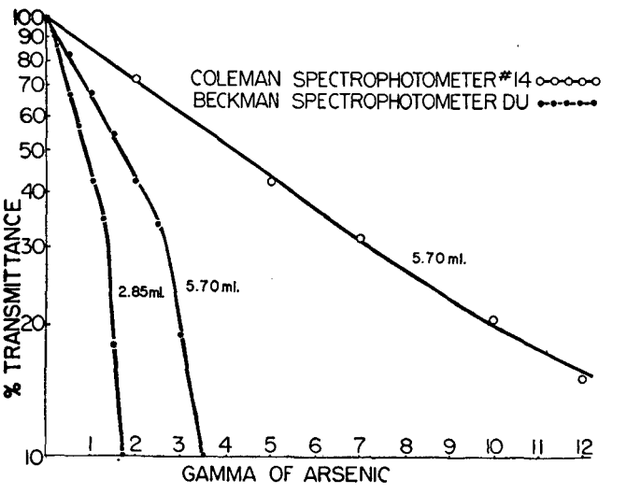

Figure 3. Relation between Arsenic Concentration in Sample and Per Cent Transmittance

Comparison of the Kingsley-Schaffert and Chaney-Magnuson methods for the determination of arsenic in urine and serum are shown in Table III which indicates good agreement. As simple digestion with hydrochloric acid was used in the new method, some difficulty in releasing arsenic from solid tissues was expected. However, preliminary emulsification of tissues in water with a Waring Blendor was sufficient for hydrochloric acid to free all arsenic as shown by the good agreement of the new method with the Chaney-Magnuson method in the determination of arsenic in beef tissues (Table IV).

NORMAL RANGE OF ARSENIC IN FLUIDS, TISSUES, AND FOODS

Twenty-four hour excretion of arsenic in the urine of normal adult males averaged 10.6 micrograms % (4.6 to 19.8 micrograms % as shown in Table V); for females the average was 12.8 micrograms % (5.2 to 20.6 micrograms % as shown in Table VI). The normal fasting serum levels averaged 5.1 micrograms % (3.5 to 7.0 micrograms %, Table V), in males; in females the average was 5.4 micrograms % (3.5 to 7.2 micrograms %, Table VI). The partition of arsenic in whole blood and serum is given in Table VII with an additional comparison with the Chaney-Magnuson method. Apparently the arsenic content of the red cell is two to three times that of serum which roughly approximates the difference in their protein content. The arsenic content of various human tissues was consistently lower than that of serum or red cells as shown in Table VIII.

Table V. Blood Serum and 24-Hour Urine Arsenic Levels of Normal Adult Males

(Photometric measurements made with Coleman Model No. 14 spectrophotometer)

Sample No.	24-Hour Urine, γ			Blood Serum, γ %		
	Spec. I	Spec. II	Av.	Spec. I (5 ml.)	Spec. II (2 ml.)	Av.
1	9.7	10.0	9.9	4.2	4.2	4.2
2	14.2	13.8	14.0	3.4	3.5	3.5
3	8.4	8.6	8.5	5.6	5.6	5.6
4	10.9	11.1	11.0	5.6	5.5	5.6
5	4.6	4.6	4.6	5.6	5.8	5.7
6	14.0	14.4	14.2	4.8	4.8	4.8
7	19.8	19.8	19.8	3.9	4.0	4.0
8	5.6	5.3	5.5	5.6	5.5	5.6
9	4.6	4.8	4.7	5.6	5.6	5.6
10	18.5	16.0	17.3	6.1	6.2	6.2
11	13.6	14.1	13.9	3.9	4.2	4.1
12	8.3	8.9	8.6	4.8	4.6	4.7
13	5.0	5.3	5.2	4.4	4.6	4.5
14	...	...	...	3.5	...	3.5
15	...	...	...	4.9	...	4.9
16	...	...	...	4.0	...	4.0
17	...	...	...	5.9	...	5.9
18	...	...	...	6.3	...	6.3
19	...	...	...	7.0	...	7.0
20	...	...	...	5.0	...	5.0
21	...	...	...	5.6	...	5.6
22	...	...	...	4.2	...	4.2
23	...	...	...	4.5	...	4.5
24	...	...	...	5.2	...	5.2
25	...	...	...	5.6	...	5.6
26	...	...	...	7.0	...	7.0
27	...	...	...	5.6	...	5.6
28	...	...	...	5.2	...	5.2
Over-all av.			10.6			5.1

Table VI. Blood Serum and 24-Hour Urine Arsenic Levels of Normal Adult Females

(Photometric measurements made with Coleman Model No. 14 spectrophotometer)

Sample No.	24-Hour Urine, γ			Blood Serum, γ %		
	Spec. I	Spec. II	Av.	Spec. I (5 ml.)	Spec. II (2 ml.)	Av.
1	11.2	11.4	11.3	4.2	4.0	4.1
2	8.4	9.0	8.7	3.7	3.8	3.7
3	6.3	6.7	6.5	5.6	5.5	5.6
4	5.2	5.2	5.2	3.4	3.5	3.5
5	11.1	11.6	11.4	7.0	7.3	7.2
6	20.8	20.4	20.6	5.6	5.8	5.7
7	20.0	20.0	20.0	4.5	4.7	4.6
8	18.5	18.5	18.5	3.9	4.0	4.0
9	14.2	13.2	13.7	6.2	5.9	6.1
10	8.4	9.3	8.9	4.5	4.5	4.5
11	20.4	19.6	20.0	7.0	6.7	6.9
12	8.3	8.9	8.6	4.8	4.6	4.7
13	...	...	...	4.5	4.2	4.4
14	...	...	...	6.7	6.9	6.8
15	...	...	...	7.0	...	7.0
16	...	...	...	5.6	...	5.6
17	...	...	...	5.6	...	5.6
18	...	...	...	6.3	...	6.3
19	...	...	...	5.6	...	5.6
20	...	...	...	7.0	...	7.0
21	...	...	...	4.2	...	4.2
22	...	...	...	6.3	...	6.3
23	...	...	...	4.2	...	4.2
24	...	...	...	6.3	...	6.3
25	...	...	...	6.0	...	6.0
Over-all av.			12.8			5.4

The arsenic content of rabbit serum, although varying over a wide range, averaged about the same as that of the guinea pig, but both were lower than that of man (Table IX). The arsenic content of some food materials is shown in Table X.

A study of arsenic in sea animals indicated that 90% of the arsenic was associated with proteins (*5*). The present investigation indicated that 88% of the naturally occurring arsenic in serum is precipitated with the proteins by trichloroacetic acid, and 100% with zinc or cadmium hydroxide. Only 5 to 10% of 0.1 to 1.0 microgram of arsenic added to 5 ml. of serum is precipitated with the proteins by trichloroacetic acid but 90 to 100% is precipitated by zinc or cadmium hydroxide. These data indicate that the arsenic present in serum is bound for the most part to proteins. This finding has been reported by others (*7*).

EFFECT OF SMALL DOSES OF ARSENIC ON BLOOD AND URINE ARSENIC LEVELS

The effect of small doses of arsenic (Fowler's solution), from 1 to 12 mg. per 24 hours, upon serum and urine levels was studied

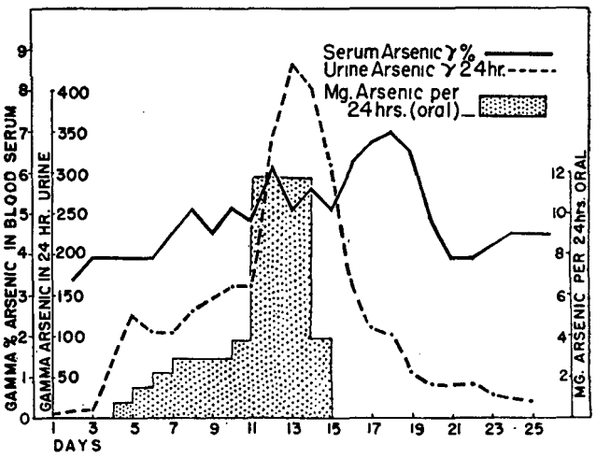

Figure 4. Serum and Urinary Arsenic of Adult Male Patient Receiving Arsenic

Table VII. Partition of Arsenic in Human Serum and Whole Blood

Specimen	Kingsley-Schaffert Method, γ %		Chaney-Magnuson Method, γ %	
	Serum	Whole blood	Serum	Whole blood
Polycythemia male	7.2	12.3	7.2	11.9
Normal female	3.7	10.0	3.6	10.3
Normal male	4.2	5.5	4.1	5.6
Normal male	5.1	9.8	5.0	...

Table VIII. Arsenic Content of Fresh Tissues Obtained at Autopsy of Human Males

Sample No.	Diagnosis	Age	Tissue, γ %					
			Kidney	Liver	Heart muscle	Thigh	Brain	Lung
1	Hypertension	40	3.4	3.2	...	...	...	1.8
2	Hypertension	50	3.1	3.4	...	...	3.1	...
3	Carcinoma of lung	60	3.7	3.9	3.7	4.3	3.7	...
4	General arteriosclerosis myocardial infarction	41	2.6	3.1	2.4	3.7	2.6	2.9
5	Pulmonary tuberculosis	60	2.6	3.1	2.9	3.1	2.6	2.6
6	Arteriosclerosis	51	2.6	3.0	2.6	5.8	2.4	2.9
7	Bronchopneumonia	50	2.9	3.1	2.8	4.0	2.5	2.9

Table IX. Blood Serum Arsenic Levels of Normal Adult Rabbits and Guinea Pigs

Sample No.	Arsenic in Rabbit Serum, γ %		Sample No.	Arsenic in Guinea Pig Serum, γ %
	2 ml. of serum[a]	5 ml. of serum[b]		5 ml. of serum[a]
1	2.8	2.8	1	2.8
2	1.1	1.4	2	2.5
3	2.8	2.8	3	3.1
4	0.6	0.6	4	3.6
5	5.0	4.5	5	2.5
6	3.9	3.5	6	2.0

[a] Photometric measurements made with Beckman Model DU spectrophotometer.
[b] Photometric measurements made with Coleman Model No. 14 spectrophotometer.

Table X. Arsenic Content of Some Food Material

Item	γ %
White bread	1.9
Whole wheat bread	1.8
Milk	1.3[a]
Canned asparagus	1.0
Tomato	1.0
Orange	1.1
Egg	1.3
Peach	1.2
Cabbage	1.2
Potato (white)	1.0
Squash	1.1
Beef meat (fresh)	5.6
Beef liver (fresh)	2.6
Tap water	2.5[a]

[a] Volume per cent.

in a patient with myelogenous leukemia before, during, and after administration over a period of 25 days (Figure 4). During this whole period the serum arsenic levels, although showing a tendency to increase after arsenic administration, ranged from 3.5 to 7.0 micrograms % which is within the normal range. The 24-hour urinary arsenic increased from 5.6 micrograms before medication to 500 during medication, and was still elevated (30 micrograms) 10 days after the last administration of arsenic.

LITERATURE CITED

(1) Boltz, D. F., and Mellon, M. G., ANAL. CHEM., 19, 873 (1947).
(2) Case, O. P., *Ibid.*, 20, 902 (1948).
(3) Chaney, A. L., and Magnuson, H. J., IND. ENG. CHEM., ANAL. ED., 12, 691 (1940).
(4) Chaney, A. L., and Magnuson, H. J., private communication.
(5) Gorgy, S., Rakestraw, N. W., and Fox, D. L., *J. Marine Research* (Sears Foundation), 7, 22 (1948).
(6) Hubbard, D. M., IND. ENG. CHEM., ANAL. ED., 13, 915 (1941).
(7) Hunter, F. T., Kip, A. F., and Irvine, J. W., Jr., *J. Pharmacol. Exptl. Therap.*, 76, 207, 221 (1942).
(8) Maechling, E. H., and Flinn, F. B., *J. Lab. Clin. Med.*, 15, 779 (1930).
(9) Magnuson, H. J., and Watson, E. B., IND. ENG. CHEM., ANAL. ED., 16, 339 (1944).
(10) Maren, T. H., *Ibid.*, 18, 521 (1946).
(11) Morris, H. J., and Calvery, H. O., *Ibid.*, 9, 447 (1937).
(12) Reyes, E. A., *Rev. quim. farm. (Santiago, Chile)*, 4, 2 (1947).
(13) Satterlee, H. S., and Blodgett, G., IND. ENG. CHEM., ANAL. ED., 16, 400 (1944).
(14) Sultzaberger, J. A., *Ibid.*, 15, 408 (1943).

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Microdetermination of Fluorine in Solid Halocarbons

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During investigation of the structures of some laboratory-synthesized halocarbons, the need arose for rapid microanalytical determination of fluorine in solid compounds which contained fluorine, carbon, chlorine, and nitrogen, and, particularly, for a method that could be used in the presence of nitrogen. A method for the microdetermination of fluorine in solid organic compounds containing fluorine, chlorine, bromine, and nitrogen in addition to carbon has been developed. By decomposing the sample at 1100° C. in a stream of moist oxygen and determining the fluorine colorimetrically with a standard ferric ion–salicylic acid solution, 200 to 1500 micrograms of fluorine can be determined with an average deviation of from 1 to 2% a precision of 3.9% of the fluorine present on a 95% confidence interval. A determination of fluorine can be made in 30 minutes in most instances. The use of quartz, platinized quartz, or platinum combustion tubes in the pyrohydrolysis of halocarbons caused no significant differences in the amounts of fluorine that were recovered.

THE synthesis of high boiling halocarbons required procedures for ultimate determination of carbon, chlorine, fluorine, nitrogen, sulfur, and hydrogen. Of the available procedures, those for fluorine were the least satisfactory with respect to accuracy, precision, and time required for a single determination. This paper describes a rapid method for the determination of fluorine in solid halocarbons.

There are two aspects of the problem of fluorine determination in halocarbons—decomposition of the sample, and collection and determination of the liberated fluorine.

PREVIOUS WORK

Sample Decomposition. The methods of fluorocarbon decomposition have been reviewed by Elving and Ligett (*4*).

Halocarbons containing fluorine can be quantitatively decomposed by prolonged digestion with sodium and liquid ammonia at room temperature in sealed glass reaction tubes (*13*) or at 400° C. in an evacuated tube in much shorter periods of time (*4*). Kimball and Tufts (*11*) found the sodium and liquid ammonia treatment insufficient for many halocarbons and recommended a bomb fusion with potassium at 550° C.

Teston and McKenna (*20*) used a direct combustion technique in which the sample was burned in a stream of oxygen at 1000° C. in a quartz combustion tube packed with platinum and crushed quartz. Fluorine reacted with the quartz to form silicon tetrafluoride which was sorbed on activated alumina at 175° C. The exact conditions necessary for quantitative sorption of silicon tetrafluoride on alumina have not been definitely established. Miller and McBee (*14*) obtained quantitative sorption at 650° C. Horton and Kirslis (*9*) studied the reaction of silicon tetrafluoride on alumina at various temperatures and found that quantitative sorption requires careful preparation of the alumina.

Schumb and Radimer (*18*) described a combustion technique, applicable to volatile organic compounds, depending upon pyrohydrolysis of the sample in a stream of moist oxygen in a platinum combustion tube at 1100° C. Milner (*15*) modified this procedure to permit analyses of volatile and nonvolatile compounds. Gaseous halocarbons have been quantitatively decomposed using steam by Cline and Westbrook (*3*).

Fluorine Determination. Methods for determining fluoride have been reviewed recently by Rinck (*16*).

Fluorine may be determined gravimetrically as lead chlorofluoride (*13*), volumetrically using thorium nitrate (*21*), conductometrically (*8*), amperometrically (*12*), or colorimetrically using the bleaching effect of fluoride ion on metallo-organic complexes such as ferric ion–salicylic acid (*5–7, 19*).

The moderately high solubility of lead chlorofluoride makes the use of this compound unsatisfactory for microdeterminations. The thorium nitrate titration using alizarin red S as an indicator is too insensitive for microgram amounts of fluoride. The fluorescent titration of Horton (*10*) can be used in the low microgram region, but requires pure morin which was not available at the time of this study. Langer (*12*) has titrated fluoride amperometrically using thorium nitrate in concentrations as low as 5 micrograms per ml.

Colorimetric procedures employing various iron complexes are extremely sensitive to fluoride when a spectrophotometer is used to measure color density—for example, the method described by Greenspan and Stein (*7, 17*) is usable in the fluoride concentration range of 2 to 50 micrograms per ml. Fluoride bleaches the wine-colored ferric ion–salicylic acid solution. This bleaching action is caused by the preferential formation of soluble fluoride complexes with ferric ions which no longer show the color reactions of ferric ions alone.

EXPERIMENTAL

The experience in this laboratory with sodium or potassium fusions in bombs has been that not all fluorocarbons can be com-

pletely decomposed without damaging the bombs and necessitating frequent replacement. Fusion with sodium peroxide has resulted in explosions. Of the procedures reviewed above, the method of decomposition used by Cline and Westbrook (*3*) in combination with the Greenspan and Stein (*7*) method of determination appeared most applicable to the compounds encountered.

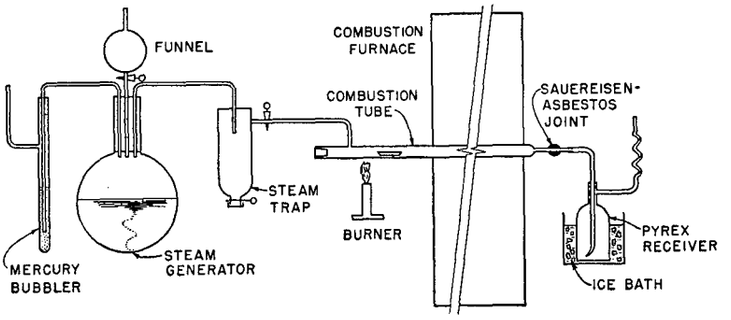

Figure 1. Pyrohydrolysis Assembly

Reagents. Sodium fluoride solution, 100 micrograms per ml. Ferric ammonium sulfate, 0.007 *M* solution. Salicylic acid, 0.01 *M* solution. Hydrochloric acid, pH 3.1.

Apparatus. A pyrohydrolysis train was assembled as shown in Figure 1. The mercury bubbler assembly and a filling funnel were connected to the steam generator, which consisted of a round-bottomed 1-liter flask. A 500-ml. steam trap was placed between the steam generator and the combustion tube. The combustion tube was approximately 60 cm. long and 10 mm. in inside diameter and was made of quartz, platinized quartz, or platinum. The portion of the tube which was within the combustion furnace was packed with platinum stars. The combustion furnace consisted of a steel shell lined with firebrick and asbestos, through which four 44 × 1 cm. globar rods were placed. A 220-volt power source gave an efficient operating temperature of 1100° C.

The fluoride, which was absorbed in a borosilicate glass receiver joined to the combustion tube by a borosilicate glass delivery tip, was determined with a Beckman Model DU spectrophotometer using 1-cm. Corex cells. All weighings were made with an Ainsworth keyboard microbalance.

Colorimetric Procedure. Fluorine was determined by measuring the decrease in absorbancy of a standard ferric ion–salicylic acid solution at 530 mμ. This solution was made by adding 80 ml. of the ferric ammonium sulfate solution to 90 ml. of the salicylic acid solution and diluting to 1 liter with the hydrochloric acid solution. The final pH was 3.1 ± 0.2 and this stock solution was allowed to stand in a dark bottle for 24 hours before use. Greenspan and Stein (*7*) have shown that the absorbancy of the ferric ion–salicylic acid solution is a maximum at pH 2.9 to 3.2 and there is a very rapid decrease in absorbancy on either side of this range. In practice it has been found that maintaining the pH of the reference solution and of the sample within this range is the most critical aspect of the determination.

The absorbancy of the stock solution compared with that of distilled water at 530 mμ was between 0.820 and 0.880 and remained constant for at least a week. Figure 2 shows characteristic absorption curves of a ferric ion–salicylic acid solution for various fluoride concentrations measured against distilled water.

Each new stock solution requires a calibration curve, as it was found difficult to prepare different solutions with the same absorbancy. The slopes of the curves are the same, however. This may be due to variations in the purity of ferric ammonium sulfate lots.

In contrast to most spectrophotometric methods, the sample solution had a lower absorbancy than did the reference solution. Therefore, the fluoride solution was set to zero absorbancy on the spectrophotometer and the absorbancy of the reference solution was measured against it.

The system does not follow a linear relationship between absorbancy and fluoride concentration.

Analytical Procedure. Organic samples weighing from 1 to 10 mg. were weighed into a platinum micro combustion boat, which was then placed in the combustion tube approximately 7.5 cm. (3 inches) from the furnace entrance. The receiver containing 25 ml. of stock solution was then mounted on the system, an ice bath was placed around the receiver, and then steam was permitted to flow through the combustion tube. The rate of steam flow was maintained at approximately 1 gram per minute by the mercury bubbler, but could be adjusted by varying the amount of mercury in the bubbler. Heat from a burner was then cautiously applied 5 cm. (2 inches) in front of the sample and as the sample hydrolyzed the burner was moved toward the furnace entrance. The burning cycle was repeated to ensure complete sample decomposition. The receiver was removed from the system and the solution cooled to room temperature. It was then transferred to a 100-ml. volumetric flask and diluted to volume with the pH 3.1 hydrochloric acid solution.

The absorbancy of this solution was considered to be zero and the absorbancy of the reagent blank (15 ml. of stock solution in a 25-ml. volumetric flask, made to volume with the pH 3.1 hydrochloric acid) was measured against the sample solution at 530 mμ. Fluoride ion concentration was obtained from a calibration curve.

The ferric ion concentrations in the reagent blank and the sample solution differ. The ratios chosen need not be identical, so long as a calibration curve is used, and it was found that the ratio used here made the colorimetric determination more sensitive.

The time for one determination was about 20 minutes, but varied depending upon the ease of sample decomposition.

Presentation of Data. Data for the fluorine determination in seven solid halocarbons, prepared by the Fluorocarbon Section of this laboratory, are presented in Table I.

Table I. Determination of Fluorine in Solid Samples

Compound	Combustion Tube	No. of Detns.	Fluorine, % Theo.	Fluorine, % Found	Av. Dev., %
$C_{10}H_{16}NO_2F_2$	Platinum	10	17.3	17.1	±0.3
	Platinized quartz	12	17.3	17.3	±0.3
	Quartz	10	17.3	17.2	±0.3
$(CF_2CFCl)_n$	Platinum	9	49.1	49.1	±0.5
	Platinized quartz	9	49.1	49.6	±0.5
	Quartz	9	49.1	48.9	±0.5
$C_7H_{11}NOClF$	Platinized quartz	2	10.6	10.4	±0.4
$C_{16}H_{27}NO_2F_2$	Platinized quartz	3	12.5	12.3	±0.2
$C_7N_2NO_2F_2$	Platinized quartz	3	21.5	21.3	±0.3
$C_8H_{10}O_6F_4$	Platinized quartz	3	27.3	28.3	±0.1
$C_8H_{10}O_6F_4$			27.3	27.8[a]	
C_8H_7NOClF	Platinum	3	10.1	10.0	±0.2
	Platinized quartz	3	10.1	10.3	±0.4

[a] Clark Microanalytical Laboratory, Urbana, Ill.

To test the effect of different combustion tube materials, determinations were made on two different compounds using a quartz tube, a platinized quartz tube, and a platinum tube.

Statistical analysis of variances and covariances (*1*) of the data presented in Table I showed no significant difference between any of the combustion tube materials in the fluoride analysis. Accordingly, the data for each compound were considered to have been obtained from the same combustion tube and calculations to establish the precision of the method were based upon all the determinations for each compound taken as one set. The data obtained are presented in Table II.

DISCUSSION

Pyrohydrolysis of Sample. The data in Table I show that quartz, platinized quartz, or platinum combustion tubes may be used without causing significant difference in fluoride recovery However, it is desirable to use a quartz tube partially platinized

Table II. Precision of Replicate Determinations

Compound	No. of Detns.	Precision (95% Confidence Interval), %	
		Single detns.	Group mean[1]
$C_{10}H_{16}NO_2F_2$	32	0.68	0.12
$(CF_2CFCl)_n$	28	1.61	0.31

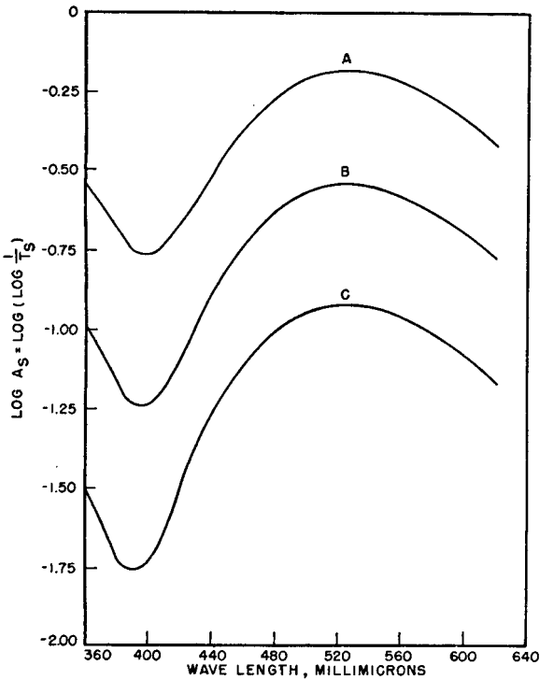

Figure 2. Characteristic Spectral Absorption Curves of Ferric Ion–Salicylic Acid Solution

Various fluoride concentrations measured against distilled water
A. 1×10^{-4} mole of fluoride per liter
B. 3×10^{-4} mole of fluoride per liter
C. 6×10^{-4} mole of fluoride per liter

so that the preburn may be observed as samples must be vaporized slowly to ensure complete decomposition during passage through the combustion tube. Some samples may hydrolyze more readily than others; hence each must be burned slowly A carbonaceous residue in the receiver is considered evidence. of incomplete combustion.

To ensure complete sample decomposition, any residue remaining in the platinum boat after the first burn was heated to a red heat again, but the ferric ion–salicylic acid solution was kept below boiling temperature to prevent its decomposition.

Liquid halocarbons are difficult to control during the vaporization step in the heating cycle, because they distill through the system too rapidly.

Interferences. Elements which act as reducing agents after passing through the system may reduce ferric ions to ferrous and act as interferences. Sulfur acts probably as a reducing agent, causing an interference. Phosphorus compounds were not investigated, but phosphate is known to complex the ferric ion. When chlorine, bromine, and nitrogen were present in the organic compounds no interference was observed.

Alkali and alkaline earth fluorides formed by hydrolysis of metallo-organic compounds are not completely decomposed by pyrohydrolysis (*2*, *17*, p. 239) and, therefore, will interfere with this method without modification. The modification consists in bedding the sample in the combustion boat with uranouranic oxide (U_3O_8) (*3*). Other colored complexes that are bleached by fluoride, such as ferric-feeron (*5*), ferric-thiocyanate (*6*), and titanic acid (*19*) may, in some cases, be substituted for ferric ion–salicylic acid where specific interferences are present.

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LITERATURE CITED

(1) Brownlee, K. A., "Industrial Experimentation," 2nd rev. American ed., Brooklyn, Chemical Publishing Co., 1948.
(2) Cline, W. D., and Warf, J. C., Iowa State College, *Rept.* **CC-2723**, 1945.
(3) Cline, W. D., and Westbrook, J. A., Carbide and Carbon Chemicals Corp., K-25 Plant, Oak Ridge, Tenn., *Rept.* **K-262**, 1948.
(4) Elving, P. J., and Ligett, W. B., IND. ENG. CHEM., ANAL. ED., **14**, 449–53 (1942).
(5) Fahey, J. J., *Ibid.*, **11**, 362–3 (1939).
(6) Foster, M. D., *J. Am. Chem. Soc.*, **54**, 4464–5 (1932).
(7) Greenspan, J., and Stein, S. J., Kellex Corp., *Rept.* **KZ-891**, 1944.
(8) Harms, J., and Jander, G., *Z. Elektrochem.*, **42**, 315–19 (1936).
(9) Horton, A. D., and Kirslis, S. S., Carbide and Carbon Chemicals Corp., K-25 Plant, Oak Ridge, Tenn., *Rept.* **K-372**, 1949.
(10) Horton, C. A., Ph.D. thesis, University of Michigan, 1949.
(11) Kimball, R. H., and Tufts, L. E., ANAL. CHEM., **19**, 150–3 (1947).
(12) Langer, A., IND. ENG. CHEM., ANAL. ED., **12**, 511–14 (1940).
(13) Miller, J. F., Hunt, H., and McBee, E. T., ANAL. CHEM., **19**, 148–9 (1947).
(14) Miller, J. F., and McBee, E. T., Purdue University, *Rept.* **A-1515**, 25–9, 1944.
(15) Milner, O. I., ANAL. CHEM., **22**, 315–17 (1950).
(16) Rinck, E., *Bull. soc. chim., France*, **1948**, 305–24.
(17) Rodden, C. J., "Analytical Chemistry of the Manhattan Project," p. 241, New York, McGraw-Hill Book Co., 1950.
(18) Schumb, W. C., and Radimer, K. J., ANAL. CHEM., **20**, 871–3 (1948).
(19) Steiger, G., *J. Am. Chem. Soc.*, **30**, 219–25 (1908).
(20) Teston, R. O'D., and McKenna, F. E., ANAL. CHEM., **19**, 192–6 (1947).
(21) Willard, H. H., and Winter, O. B., IND. ENG. CHEM., ANAL. ED., **5**, 7–10 (1933).

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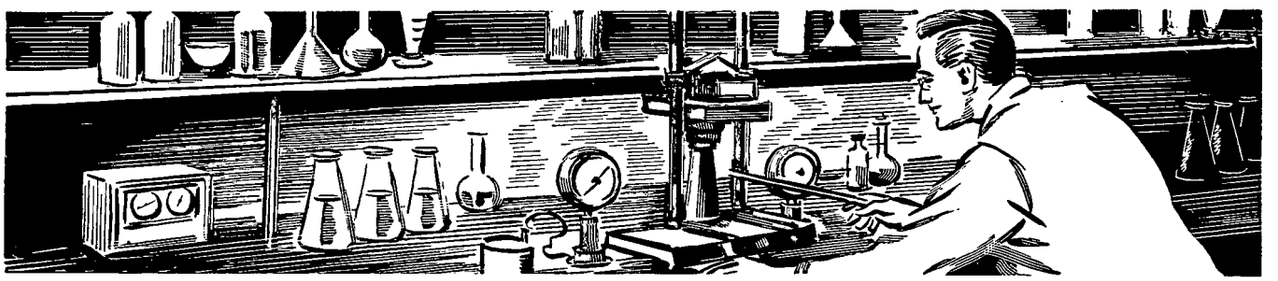

NOTES ON ANALYTICAL PROCEDURES . . .

Determinations of Small Amounts of Ammonia in the Presence of Hydrazine

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IN CONNECTION with studies on anhydrous hydrazine, it was desirable to be able to determine small amounts of ammonia in the presence of hydrazine. None of the methods described in the literature was found to be entirely satisfactory.

Penneman and Audrieth (*6*) have described a method for determining both hydrazine and ammonia in mixtures by means of the difference between the titration of the total basic constituents with standard acid and the titration of the hydrazine with potassium iodate.

A few preliminary experiments carried out in this laboratory before publication of Penneman and Audrieth's article were unsatisfactory, apparently because 0.1 *N* hydrochloric acid was used rather than at least 0.4 *N* as recommended by them. This method, although rapid, has the disadvantage that the ammonia content is determined by difference.

Fuchs and Niszel (*4*) and Milligan (*5*) removed the hydrazine from mixtures containing ammonia by oxidation with alkaline cupric salts (Fehling's solution), and then distilled the ammonia into a measured quantity of standard acid. This method depends upon the removal of the hydrazine by oxidation to nitrogen. Unfortunately, unless experimental conditions are more carefully controlled than is practicable in routine analysis, small amounts of ammonia are formed from the decomposition of the hydrazine (*1, 2*).

Experiments to determine whether a blank could be used to correct for the traces of ammonia formed under routine operating conditions showed that the blanks, although small, were not consistent enough for accurate results. However, if most of the hydrazine is removed by precipitation, the small amount remaining produces a negligible quantity of ammonia upon oxidation with Fehling's solution. Consequently, satisfactory blanks are obtained. The ammonia can then be distilled into standard acid and titrated in the usual manner.

The hydrazine can be precipitated in an acidic solution by formation of the sparingly soluble salt, $CuSO_4.(N_2H_5)_2SO_4$. Curtius and Schrader (*3*) report a solubility of 0.871 gram per liter at 10° C. for this salt. The solubility is repressed by sulfuric acid.

This is especially convenient, because a slight excess of copper(II) sulfate is used as the precipitant for the hydrazine. Thus one needs only to add Rochelle salt and potassium hydroxide to form the Fehling's solution needed for oxidation of the unprecipitated hydrazine and at the same time produce the necessary condition for the distillation of ammonia.

EXPERIMENTAL

Because anhydrous hydrazine–ammonia mixtures and alkaline solutions of the mixtures are rapidly decomposed by air, the samples must be handled by means of an appropriate technique until acidified.

Some of the synthetic samples used for checking the method of analysis were made from commercial anhydrous hydrazine (98.2% N_2H_4). A portion was transferred to a small, weighed bottle in a nitrogen-filled dry box. After weighing, the bottle was opened under the surface of a dilute solution of sulfuric acid, to which was added a definite amount of a standard ammonia solution. Individual samples or aliquots of the acidified solution of larger samples may be used.

If aliquots are used, it is advisable to calculate carefully the amount of sulfuric acid necessary to neutralize the sample and to add only a slight excess, as sulfuric acid represses the solubility of hydrazine sulfate to a large degree. The presence of sulfuric acid will not interfere with the determination of the hydrazine by titration with potassium iodate, although it may be necessary to reduce the quantity of hydrochloric acid added in that determination (*5*).

Other synthetic samples were made from weighed portions of recrystallized hydrazine sulfate and portions of a standard ammonia solution.

Reagents. C.P. chemicals selected for low ammonia content were used for all solutions.

The solution of copper sulfate contained 140 grams of cupric sulfate pentahydrate and 100 ml. of concentrated sulfuric acid per liter of solution. The Rochelle salt solution contained 242 grams of sodium potassium tartrate tetrahydrate and 500 grams of potassium hydroxide per liter of solution. Equivalent amounts of potassium or sodium tartrate may be substituted for the Rochelle salt if desired.

Other necessary reagents are 10 *N* potassium hydroxide, and standard solutions of 0.1 *N*, carbonate-free, sodium hydroxide and 0.1 *N* hydrochloric acid. Methyl purple (commercial modified methyl red) was used as the indicator because of its easily detectable color change.

Standard Procedure. A sample, of such size that upon final dilution a 25-ml. aliquot will contain 0.25 gram of anhydrous hydrazine, is weighed and dissolved in distilled water containing sufficient sulfuric acid just to neutralize the hydrazine. Care must be taken to protect the original sample from coming into contact with air or oxygen.

One milliliter of concentrated sulfuric acid is added to the 25-ml. aliquot of the sample. Some hydrazine sulfate will precipitate, and it is redissolved by boiling the sample gently for a few minutes. As soon as the hydrazine sulfate redissolves, 15 ml. of the solution of copper sulfate are added together with 4 ml. of concentrated sulfuric acid (cautiously). The mixture is placed in a refrigerator (temperature approximately 5° C.) and allowed to remain at least 4 hours. The sample is filtered while cold by means of a medium fritted funnel and suction. About 50 to 75 ml. of a cold 5% solution of sulfuric acid are used to transfer and wash the precipitate.

The filtrate and washings are transferred to a 250-ml. Kjeldahl

Table I. Determination of Known Amounts of Ammonia in Ammonia-Hydrazine Mixtures

N_2H_4 Added, Gram	NH_3 Added, Gram	NH_3 Found, Gram	NH_3[b] Calcd., %	NH_3[b] Found, %
0.2463[a]	0.0171_7	0.0172_1	6.52	6.53
0.2463	0.0170_7	0.0170_5	6.48	6.47
0.2463	0.0173_8	0.0173_9	6.58	6.60
0.2463	0.0106_6	0.0107_9	4.16	4.16
0.2463	0.0062_9	0.0062_0	2.47	2.49
0.2463	0.0019_4	0.0019_8	0.79	0.77
0.2463	0.0018_7	0.0018_2	0.75	0.76
0.2463	0.0018_6	0.0017_9	0.73	0.72
0.2463	0.0010_4^1	0.0010_6^9	0.42	0.43
0.4801[c]	0.0235_9	0.0233_7	5.46	5.41
0.3731	0.0092_6^9	0.0091_2^7	2.42	2.39
0.3458	0.0051_9^6	0.0051_3^0	1.48	1.46
0.3573	0.0009_9^9	0.0009_5^5	0.28	0.27
0.4137	0.0010_6^9	0.0011_3^3	0.26	0.27

[a] 1.0000 gram $N_2H_4.H_2SO_4$ added contains 0.2463 gram N_2H_4.

[b] % NH_3 calculated as $\frac{\text{grams } NH_3}{\text{grams } NH_3 \text{ added} + \text{grams } N_2H_4} \times 100\%$.

[c] Commercial anhydrous hydrazine samples.

flask and diluted to approximately 150 ml. with distilled water. Sixty milliliters of 10 *N* potassium hydroxide and 20 ml. of the Rochelle salt solution are mixed together and added as rapidly as possible to the flask, which is immediately connected to a trap and condenser. The condenser adapter extends below the surface of a measured amount (about 25 ml.) of standard 0.1 *N* hydrochloric acid in the receiver.

After distillation of about 150 ml. of the flask contents, the condenser is rinsed, the receiver is removed, and the excess hydrochloric acid is titrated with standard base, using methyl purple indicator.

When the Rochelle salt solution and potassium hydroxide are added, the formation of the blue copper tartrate complex serves to indicate that the contents are alkaline. Shortly thereafter, some copper(I) oxide will be formed from the reaction with the residual hydrazine. If a large excess of potassium hydroxide is not present, the contents of the flask will foam seriously during the distillation. Blanks should be run using the reagents and ammonia-free hydrazine.

A determination requires about 2 hours to run exclusive of cooling time. It was usually found convenient to precipitate the samples and allow them to remain in the ice box overnight.

RESULTS

Table I shows the results of the determination of known amounts of ammonia in synthetic samples. The first series of samples was made from weighed amounts of hydrazine sulfate with ammonia added in the form of a standard solution. The second series was made from weighed amounts of commercial anhydrous hydrazine and a standard solution of ammonia. From Table I, the least significant difference for a mean of two determinations is calculated to be ±0.03%.

Results of the blank determinations for several lots of reagents are shown in Table II. If the blanks are higher than 0.5 mg. of ammonia, it is well to select new reagents.

Table II. Blank Determinations

Series A[a] Reagents		Series B Reagents		Series C Reagents		Series D Reagents	
$N_2H_4.H_2SO_4$	NH_3	$N_2H_4.H_2SO_4$	NH_3	N_2H_4	NH_3	N_2H_4	NH_3
Gram	*Mg.*	*Gram*	*Mg.*	*Gram*	*Mg.*	*Gram*	*Mg.*
1.0000	0.22	1.0000	0.37	0.4422	0.27	0.3443	0.16
1.0000	0.24	1.0000	0.37	0.3919	0.26	0.3895	0.24
1.0000	0.19	1.0000	0.26	0.4167	0.27	0.3229	0.08
1.0000	0.26	1.0000	0.30	0.3154	0.32	0.4115	0.28
Av.	0.23	Av.	0.33	Av.	0.28	Av.	0.19

[a] Series refer to different lots of reagents.

ACKNOWLEDGMENT

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This paper is published with the permission of L. T. E. Thompson, technical director of the U. S. Naval Ordnance Test Station.

LITERATURE CITED

(1) Bray, W. C., and Cuy, E. J., *J. Am. Chem. Soc.*, **46**, 858 (1924).
(2) Browne, A. W., and Shetterly, F. F., *Ibid.*, **31**, 783 (1909).
(3) Curtius, T., and Schrader, F., *J. prakt. Chem.*, **50**, 311 (1894).
(4) Fuchs, W., and Niszel, F., *Ber.*, **60B**, 209 (1927).
(5) Milligan, L. H., *J. Phys. Chem.*, **28**, 544 (1924).
(6) Penneman, R. A., and Audrieth, L. F., ANAL. CHEM., **20**, 1058 (1948).

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Spectrophotometric Determination of Dihydrostreptomycin

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STREPTOMYCIN may be determined spectrophotometrically by measuring the maltol (*4*) formed by alkaline hydrolysis in the visual (*1*, *2*) or ultraviolet (*5*) range of the spectrum. In this laboratory it is estimated by measuring the absorbance at 275 and 290 mμ of a solution of streptomycin, 1 *N* in sodium hydroxide, which has been heated for 3 minutes at 100° C. and immediately neutralized (unpublished). Dihydrostreptomycin does not give the maltol reaction (*3*). In the ultraviolet region it shows only end absorption even after prolonged treatment with alkali.

When streptomycin is heated in acid solution, its ultraviolet spectrogram, as determined using the Beckman DU quartz spectrophotometer, shows absorption maxima at 245 and 315 mμ with a minimum at 285 mμ. When dihydrostreptomycin is treated similarly, a single absorption maximum appears at 265 mμ. The extent of these maxima depends upon the normality of the acid and the heating time employed. On refluxing solutions of streptomycin sulfate or the calcium chloride complex which are normal in sulfuric acid, the maxima show their greatest absorbance after 30 minutes' treatment. On more prolonged heating the maximum at 315 mμ decreases rapidly, while that at 245 mμ broadens but does not decrease significantly. When a similar solution of dihydrostreptomycin is refluxed, the maximum at 265 mμ requires 1.5 to 2 hours to reach its greatest value. After 30 minutes' refluxing another absorption peak begins to appear at 220 mμ. Its position shifts to longer wave lengths and its absorbance increases as the refluxing time is lengthened. After 2 hours its position is at 227.5 mμ and it absorbance is greater than that of the 265 mμ maximum.

To determine if use could be made of the maximum at 265 mμ for the quantitative estimation of dihydrostreptomycin with the mode of heating changed from refluxing on a sand bath to heating in boiling water, solutions in which the concentration of sulfuric acid varied from 0.25 to 5 *N* were heated from 30 minutes to 2 hours. When a heating time of 2 hours was used for a solution of dihydrostreptomycin in 0.25 *N* sulfuric acid, the absorbance was as great as in a 1 *N* solution refluxed for 2 hours. The secondary absorption peak did not appear under these conditions. Figure 1 shows the absorption spectra of solutions of streptomycin sulfate and dihydrostreptomycin in 0.25 *N* sulfuric acid which have been heated in boiling water for 2 hours. It was necessary to dilute the solutions before taking readings in the Beckman spectrophotometer. The concentration of acid in the final solutions was 0.06 *N*. It is evident that the quantitative estimation of dihydrostreptomycin is possible.

For the determination of dihydrostreptomycin the following procedure is used in this laboratory:

In a 25 × 200 mm. test tube place an aqueous aliquot containing 1 to 3 mg. of dihydrostreptomycin. Make to a volume of 3 ml. with water. Add 3 ml. of 0.5 *N* sulfuric acid. Insert a foil-wrapped rubber stopper through which a 12-inch (30-cm.) piece of glass tubing has been passed to serve as an air condenser. Heat the tube in boiling water for 2 hours. Cool. Transfer the contents to a 25-ml. volumetric flask and make to volume with water. Measure the absorbance of the solution at 265 and 380 mμ. The difference in absorbance is proportional to the dihydrostreptomycin present.

With seventy-five aliquots containing 0.25 to 4 mg. of dihydrostreptomycin a regression line was established using this procedure. The equation of the line was $Y = 104.011847\ X - 0.4236$ where X is the difference in absorbance and Y the micrograms of dihydrostreptomycin in 1 ml. of the final solution. The correlation coefficient was +0.9998 and the standard error of estimation ±0.97.

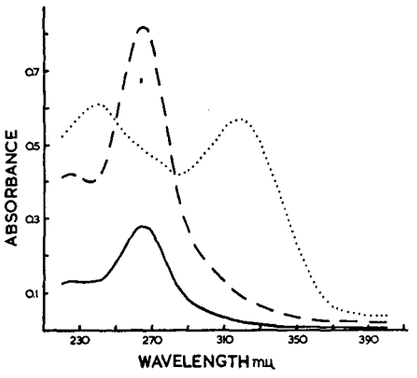

Figure 1. Spectrograms of Streptomycin Sulfate and Dihydrostreptomycin

Heated in 0.25 N H_2SO_4 for 2 hours in boiling water
Dihydrostreptomycin. 30 micrograms/ml. 0.06 N H_2SO_4
Dihydrostreptomycin. 90 micrograms/ml. 0.06 N H_2SO_4
Streptomycin sulfate. 90 micrograms/ml. 0.06 N H_2SO_4

Although one reading must be made at 265 mμ, there is a choice of wave lengths for the second reading; 380 mμ was chosen as being well within the horizontal part of the curve and yet not too close to the upper limit of the ultraviolet range. Readings may be made at 235 and 265 mμ for the quantitative determination of dihydrostreptomycin, but the difference in absorbance is not as great as with the two wave lengths chosen and the sensitivity of the method is less. A reading at 235 mμ is useful as a check on the identity of the compound.

Table I. Comparison of Methods for Determination of Dihydrostreptomycin

Sample	Potency, Micrograms/Mg. Bioassay	Potency, Micrograms/Mg. Ultraviolet	Ultraviolet as % of Bioassay
54550	702	733	104.4
53827	701	685	97.7
54281	736	697	94.7
55278	690	751	108.8
50207	696	700	100.6
50323	742	741	99.9
50419	676	668	98.8
50381	775	760	98.1
55626	739	746	100.9
5038	765	772	100.9
5040	807	798	98.9
5072	825	793	96.1
50317	798	811	101.6
50318	792	770	97.2
50327	793	741	93.4
55245	736	752	102.2
55477	731	755	103.3
51051	733	712	97.1
51825	737	696	94.4
50830	834	764	91.6
50256	696	742	106.6
5020	791	770	97.3
55936	790	756	95.7
50513	697	710	101.9
		Mean	99.5

In Table I results by the ultraviolet assay are compared with those by the bioassay on twenty-four commercial samples of dihydrostreptomycin. There is no significant difference between the potencies obtained by the two methods, as the t value is 1.050. The fourth column of the table lists the ultraviolet determinations as per cent of the bioassay. The bioassay figures were determined by Miss K. Fitzpatrick.

LITERATURE CITED

(1) Boxer, G. E., Jelinek, V. C., and Leghorn, P. M., *J. Biol. Chem.*, **169**, 153 (1947).
(2) Eisenman, W., and Bricker, C. E., ANAL. CHEM., **21**, 1507 (1949).
(3) Peck, R. L., Hoffhine, C. E., and Folkers, K., *J. Am. Chem. Soc.*, **68**, 1390 (1946).
(4) Schenk, J. R., and Spielman, M. A., *Ibid.*, 67, 2277 (1945).
(5) Titus, E., and Fried, J., *J. Biol. Chem.*, **174**, 57 (1948).

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Quantitative Test for Nornicotine

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A NEW quantitative test has been found and developed for the *Nicotiana* alkaloid, nornicotine. Nicotine fails to give any similar color even when taken in amounts of 80 times that of nornicotine. Anabasine develops a color only 80% as intense as nornicotine when taken in an amount 80 times that of the nornicotine. Synthetic and natural nornicotines react quantitatively like each other.

It was observed that nornicotine produced a very intense violet color when added to an acetone solution of 1,3-diketohydrindene if diisopropyl ketone was also present (*1*). The violet color, however, failed to appear with the use of a new batch of diisopropyl ketone. The old bottle of ketone had a cork stopper and an experiment was set up to extract a new cork with the new ketone. This cork extract caused the violet color to appear with nornicotine and the acetone solution of 1,3-diketohydrindene. Other experiments showed that tannic acid, gallic acid, or *p*-hydroxybenzoic acid could be used to make the new ketone reactive.

The present method employs as reagents, acetone, diisopropyl ketone, *p*-hydroxybenzoic acid, and 1,3-diketohydrindene. Us-

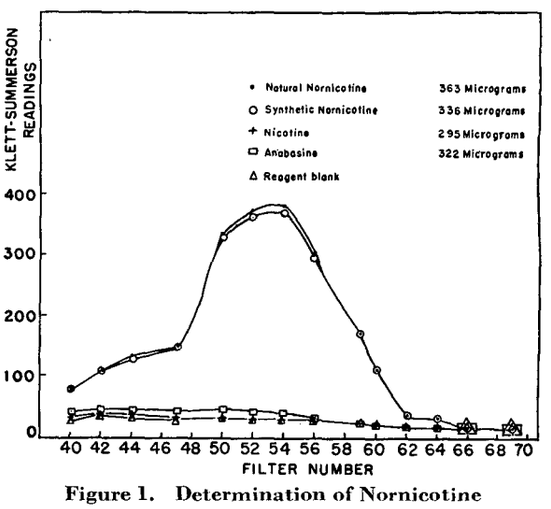

Figure 1. Determination of Nornicotine

ing *p*-hydroxybenzoic acid and omitting the diisopropyl ketone allowed the violet color due to nornicotine to appear, but color development continued even over 20 hours. Further experiments showed that diisopropyl ketone (new) added to the reagents acted as a reaction stabilizer and the violet color 1 hour after starting the reaction was stable for over a 1-hour period.

ANALYTICAL PROCEDURE

Reagents. Acetone, A.C.S.

Diisopropyl ketone, Eastman's P3244.

p-Hydroxybenzoic acid, Eastman's 1520, 2% by weight/volume in diisopropyl ketone.

1,3-Diketohydrindene, Eastman's P3565, 0.3% by weight/volume in diisopropyl ketone.

Nornicotine.

Preparation of Standard Curve. Dissolve 0.0400 gram of nornicotine in 500 ml. of acetone. Place 2, 3, 4, and 5 ml. of the resulting solution in glass-stoppered flasks and add acetone to bring to 5 ml. where necessary. Add to each flask 15 ml. of diisopropyl ketone followed by 2 ml. each of the *p*-hydroxybenzoic acid and 1,3-diketohydrindene reagent solutions. Stopper the flasks, shake to mix, and place the flasks in the dark for 60 minutes. Determine color density of the solution with the colorimeter, using a No. 54 filter, and plot the readings against the concentration.

Figure 1 shows that natural and synthetic nornicotine react alike. The same quantity of nicotine and anabasine gives very little more color than the reagent blank.

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LITERATURE CITED

(1) Feinstein, L., and McCabe, E. T., *Science*, **112**, 534 (1950).

RECEIVED July 29, 1950. Contribution from the United States Department of Agriculture, Agricultural Research Administration, Bureau of Entomology and Plant Quarantine, Beltsville, Md. Report of a study under the Research and Marketing Act of 1946.

Determination of Formal Oxidation Potentials of Ferric-Ferrous and Dichromate-Chromic Systems

Selection of Substituted 1,10-Phenanthroline Indicators for Determination of Iron at Low Acidity

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THE determination of iron in hydrochloric acid solution by dichromate oxidation, according to existing procedures, is accompanied by irksome inhibitions. Diphenylamine-type indicators, which are ordinarily employed, leave much room for improvement. Because the oxidation of iron by dichromate gives a green solution due to the chromic ion, 1,10-phenanthroline–type indicators would be preferable. The formal potentials involved have not been previously studied systematically, particularly in the lower range. The problem is complicated by the low oxidation potential of the dichromate-chromic ion system, particularly at low acid concentrations.

The present work has for its objective determination of the requisite formal oxidation potentials and selection of suitable 1,10-phenanthroline–type ferrous complex ions to serve as indicators in the determination of iron by dichromate oxidation. The procedures described involve the use of 0.1 to 1.5 *F* hydrochloric acid.

Previous Investigations. The study of polysubstituted 1,10-phenanthrolines for use as oxidation-reduction indicators in the form of their ferrous complex ions together with the determination of various pertinent physical constants has been made by Brandt and Smith (*1*). The determination of iron by dichromate oxidation in 2 *F* sulfuric or hydrochloric acid employing the 5,6-dimethyl-1,10-phenanthroline ferrous ion as indicator has been described by Smith and Brandt (*3*). Phenanthroline-type ferrous complex ions for use as oxidation-reduction indicators are now known which have color transition potentials covering the range 0.84 to 1.27 volts. The lower potentials, 0.84 to 1.10 volts, are represented by the various methyl substituted types (*1*), and the higher values, 1.10 to 1.28 volts, by the chloro-, bromo-, and nitro-substituted types described by Smith and Richter (*5*). Applications in volumetric microdeterminations of iron, arsenic, calcium, and the oxalate ion have been described by Smith and Fritz (*4*) and by Salomon, Gabrio, and Smith (*2*). Ferroin-type indicators have not previously been known and available for use in the titration of ferrous iron by dichromate in 0.1 to 0.25 *F* hydrochloric acid. The dichromate titration of iron using a series of familiar indicators has been studied by Stockdale (*6*).

DETERMINATION OF OXIDATION POTENTIALS

Solutions of known or determined concentration of ferric chloride and potassium dichromate of 0.1 *N* strength are required. In addition, hydrochloric acid solutions of graded formalities of 0.1 to 4.0 with small increments of increase in strength between 0.1 and 1.5 *F* were to be prepared. Solutions of the indicators, 4,7-dimethyl- and 3,4,7,8-tetramethyl-1,10-phenanthroline ferrous complex ions, which were 0.01 *N* are prepared by reaction of weighed portions of the organic base with hydrated ferrous sulfate, addition of water to promote solution and complex formation, and dilution to calculated final volume. The ferric chloride solutions were prepared as follows:

One-tenth molecular weight of ferric chloric hexahydrate was dissolved in 8.245 ml. of reagent hydrochloric acid (specific gravity 1.19, 37.5% hydrochloric acid) and diluted with water with stirring. The solution thus obtained was transferred to a 1000-ml. graduated flask, diluted to the mark, and thoroughly mixed. The procedure was repeated with appropriate increase in added hydrochloric acid to prepare approximately 0.1 *N* ferric iron in 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, and 4.0 *F* hydrochloric acid in addition to the 0.1 *F* hydrochloric acid solution described.

Solutions of the same hydrochloric acid formality were prepared to serve for volume dilutions.

Solutions of potassium dichromate in 0.1 to 4.0 *F* hydrochloric acid were prepared as follows:

Samples of pure potassium dichromate weighing 4.9035 grams (0.1 equivalent weight) were accurately weighed and transferred to 1000-ml. beakers, dissolved in water, and diluted to 800 to 900 ml. with water. With constant stirring the proper volume of reagent hydrochloric acid was then added (8.245 ml. for 0.1 *F* acidity, 82.45 ml. for 1 *F* acidity, etc.). Finally, the beaker contents were transferred quantitatively to a 1000-ml. graduated flask, diluted to the mark, and thoroughly mixed. Solutions of potassium dichromate above 1 *F* in hydrochloric acid form some free chlorine upon storage and should be made up just before use. The presence of a very low chlorine content will become evident through the attainment of high results for the oxidation potential of the chromate-chromic ion couple. For this reason the solutions are prepared precisely as described to eliminate the presence of free chlorine, and solutions more than a few hours old are discarded.

The potential and potentiometric titration studies were evaluated using an assembly of a Leeds & Northrup student potentiometer and decade resistance box, Weston standard cell, and lamp and scale galvanometer. The electrode system was a saturated calomel reference electrode and a platinum wire indicator elec-

Table I. Determination of Formal Single Electrode Potentials of Ferric-Ferrous and Dichromate-Chromic Systems in 1 *F* Hydrochloric Acid

(24.40 ml. of 0.1 *N* $K_2Cr_2O_7$ are equivalent to 25.00 ml. of ferrous chloride sample)

Vol. of Cr_2O_7 Ml.	Starting Potential Ref. H_2 Electrode, Volt		Iron 50% Oxidized Fe^{+++}-Fe^{++} e.m.f., Volt		Equivalent Point e.m.f., Volt		100% Excess Cr_2O_7 e.m.f., Volt	
	Soln. 1	Soln. 2	Soln. 1	Soln. 2	Soln. 1	Soln. 2	Soln. 1	Soln. 2
0.00	0.5811	0.5851	...	...	...	...	...	...
0.20	0.5956	0.6043	...	...	...	...	...	...
12.20	...	...	0.7013	0.7023	...	...	...	...
24.30	...	...	...	...	0.8288	0.8656	...	...
24.40	...	...	...	...	0.8854	0.8966	...	...
24.50	...	...	...	...	0.8933	0.9053	...	...
48.80	...	...	...	...	...	...	1.0015	1.0034

Table II. Formal Oxidation-Reduction Potentials of Dichromate-Chromic and Ferrous-Ferric Systems in Various Strengths of Hydrochloric Acid

(Values in parentheses are for sulfuric acid solutions)

Acid Formality	0.10	0.25	0.50	0.75	1.00	1.50	2.00	3.00	4.00
Fe^{+++}-Fe^{++} system, volt	0.73 (0.68)	0.73 ...	0.72 (0.68)	0.71 ...	0.70 ...	0.70 ...	0.69 ...	0.68 ...	0.66 ...
Cr^{++++++}-Cr^{+++} system, volt	0.93 (0.92)	0.96 ...	0.97 (1.08)	0.99 ...	1.00 ...	1.02 ...	1.05 ...	1.08 ...	1.10 ...

trode. The single electrode potentials reported in this work are believed to be accurate to within 0.01 volt. Magnetic stirring was provided.

Determination of Formal Electrode Potentials. The solutions of ferric chloride were reduced to ferrous chloride using the Walden silver reductor. The initial 75 to 100 ml. of solution to pass through the reductor were discarded and the required volume for a given determination was collected in a clean dry beaker. Pipetted 25.00-ml. portions were transferred to titration beakers and standardized after dilution to 150 ml. with water. The solutions were fortified by addition of 50 ml. of cold sulfuric acid (1 volume of reagent sulfuric acid plus 1 volume of water), and titrated by 0.1 *N* dichromate as oxidant with ferroin as indicator. By this process the dichromate value of 25.00 ml. of the approximately 0.1 *N* ferrous chloride solutions was determined for each solution at the various formalities of hydrochloric acid present.

In a typical procedure, exactly 25.00 ml. of approximately 0.1 *N* ferrous chloride in 0.1 *F* hydrochloric acid were transferred to a 400-ml. beaker and the sample was diluted to 150 ml. volume by the addition of 0.1 *F* hydrochloric acid. Magnetic stirring was provided and the ferrous iron oxidized by the addition of 0.1 *N* dichromate which was 0.1 *F* in hydrochloric acid. The oxidation procedure was carried out in four stages with potential determinations at each separate stage. The initial potential was observed, that at the point representing 50% oxidation of iron, that at the equivalence point, and finally that at the point of 100% excess of dichromate. The determinations were all made in duplicate.

Typical data are given in Table I for the oxidation of iron in 1 *F* hydrochloric acid.

Data duplicating those given in Table I were taken for nine different hydrochloric acid formalities (Table II).

Graphical representation of the potentiometric titrations of ferrous iron by dichromate in 0.1 and 0.5 *F* hydrochloric and 0.5 and 0.1 *F* sulfuric acid is given in Figure 1.

SELECTION OF FERROIN-TYPE INDICATORS FOR LOW ACID FORMALITIES IN OXIDATION OF IRON

An examination of Figure 1 shows that the vertical "break" in potential for the oxidation of iron by dichromate at 0.5 *F* hydrochloric acid, curve I, extends over the range 0.86 to 0.93 volt. The corresponding values for 0.5 *F* sulfuric acid, curve III, are 0.85 to 0.95 volt. The indicator selected for these titrations was the ferrous sulfate complex of 4,7-dimethyl-1,10-phenanthroline; the potentiometric transition potential in 0.5 *F* acid is known to be 0.88 volt. It was found to apply admirably and gave a sharp indication of the equivalence point. The value of the e.m.f. at the point on the titration curves indicated by arrows is in both cases higher than 0.88 volt because the reduced color form (red) predominates in intensity over the oxidized form, which is faint blue. This necessitates a slightly higher potential to bring about the maximum color change (Figure 1).

The vertical break in potential for the corresponding oxidation of iron by dichromate in 0.1 *F* hydrochloric acid, curve II is 0.85 to 0.91 volt. For 0.1 *F* sulfuric acid the vertical break is 0.81 to 0.87 volt. These values call for the use of an indicator of lower oxidation potential than that found serviceable in 0.5 *F* acid concentrations, and the ferrous sulfate complex of 3,4,7,8-tetramethyl-1,10-phenanthroline was applied, having an oxidation potential of 0.85 volt. This indicator gave excellent color transitions at points indicated by the arrows in Figure 1. Here again the color change was observed at a somewhat higher e.m.f. These ferroins not only have the proper visual oxidation potentials but have the extremely favorable molecular extinction coefficients of 13,800 and 14,000, respectively. These values indicate a superior color intensity at a minute indicator concentration with accompanying negligible indicator correction. The reversibility of the indicator color change is superior, as is the stability of its reduced and oxidized forms. In actual test titrations its performance was found to be as good as or better than that described by Smith and Brandt (*3*) for the case of ferroin made from 5,6-dimethyl-1,10-phenanthroline when employed for the same oxidation-reduction procedure at higher acid formalities.

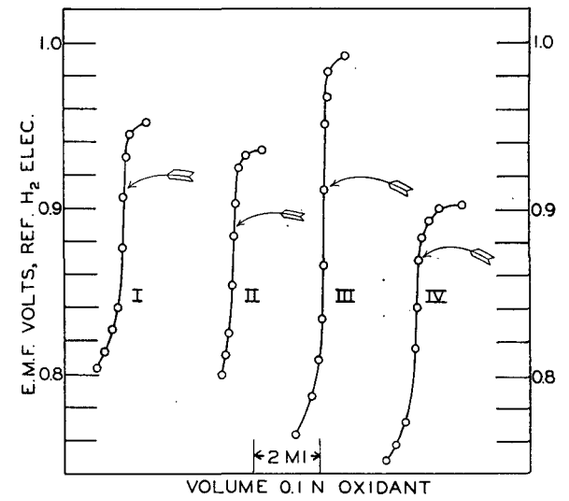

Figure 1. Equivalence Point Potential

Oxidation of Fe^{++} by $Cr_2O_7^{--}$
I. 0.5 *F* HCl
II. 0.1 *F* HCl
III. 0.5 *F* H_2SO_4
IV. 0.1 *F* H_2SO_4
Arrows indicate visual indicator transition points

For the oxidation of ferrous to ferric ion employing dichromate as oxidant in 0.1 *F* hydrochloric and sulfuric acid solutions, in the region of the equivalence point, fully 0.5 minute must be allowed between dropwise addition of oxidant. If this precaution is observed, the visual color change of the indicator is sharp and reproducible as well as precise and stoichiometric. The indicator color transition is better defined in 0.1 *F* sulfuric acid solutions than in the presence of hydrochloric acid, where the yellow color of ferric chloride affects the color transition phenomenon.

CONCLUSIONS

The selection of a ferroin-type indicator suitable for the oxidimetric determination of iron in 0.1 to 4 or 6 *F* sulfuric or hydrochloric acid is now possible. The proper ferroin dye base can be selected to give a ferrous sulfate complex indicator with any oxidation potential from 0.82 to 1.10 volts in increments of 0.02 to 0.04 volt (*1*). In every case the molecular extinction coefficient is between 11,100 and 14,500 and therefore an intensely colored indicator is assured. All selections give rise to complex indicators of favorable instability constants. A potentiometric titration of the reaction in question is the only necessary prerequisite to the selection of a suitable indicator. For every reaction condition, because of the marked change in oxidation potential of the two half-cell reactions, $Fe^{+++} + e^- = Fe^{++}$, and $Cr^{+6} + 3e^- = Cr^{+3}$, a potential is defined at which the change in e.m.f. with added increment of oxidant is the maximum. The oxidation potential of the indicator selected should correspond precisely with this value to provide maximum proficiency. The visual indication of indicator transition is somewhat higher than the potentiometrically defined oxidation potential (0.05 to 0.06 volt). This value is not of sufficient magnitude to require a correction blank in titrational macroprocedures.

Potentiometric study of the oxidation of ferrous iron in 0.1 *F* perchloric acid has shown that the two half-cells involved give the following formal potentials:

$$Fe^{+++} + e^- = Fe^{++} \text{ (e.m.f. = 0.735 volt)}$$
$$Cr^{++++++} 3e^- = Ce^{+++} \text{ (e.m.f. = 0.84 volt)}$$

At these values the equivalence point break is practically negligible (0.01 to 0.02 volts), and the determination of iron under these conditions is impossible.

LITERATURE CITED

(1) Brandt and Smith, ANAL. CHEM., **21**, 1313 (1949).
(2) Salomon, Gabrio, and Smith, *Arch. Biochem.*, **11**, 433 (1946).
(3) Smith and Brandt, ANAL. CHEM., **21**, 948 (1949).
(4) Smith and Fritz, *Ibid.*, **20**, 874 (1948).
(5) Smith and Richter, "Phenanthroline and Substituted Phenanthroline Indicators," Columbus, Ohio, G. Frederick Smith Chemical Co., 1944.
(6) Stockdale, D., *Analyst*, **75**, 150 (1950).

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Ultramicronitrometer for Use in Determination of Nitrogen in Mineral Oil

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AND

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IN THE course of the analysis of mineral oils, high values of nitrogen were frequently obtained with the common Dumas apparatus due to incomplete combustion. These difficulties were readily overcome by using the modified Dumas method described by Kirsten (*1*, *2*). However, the quantity of nitrogen in the analyzed oils was so small that it was impossible to get good readings with the common micronitrometer. As it was difficult to burn larger samples than about 60 to 70 mg. of motor oil because of the formation of tar and hard-burnt carbon in the capsule, a new nitrometer was constructed which allows the measurement of smaller quantities of nitrogen than the type commonly used. The nitrometer is shown in Figure 1.

The graded part is a capillary with a length of ca. 140 mm. and a capacity of 0.2 ml.; s_3 is a three-way stopcock with one connection to the leveling bulb, a_1, which is filled with mercury. Stopcock s_4 leads from the bottom of the nitrometer. The leveling bulb, a_2, is filled with potassium hydroxide.

PROCEDURE

The capsule is filled with copper oxide grains up to the ground joint. The grains are covered with a layer of copper oxide powder. A 50-mg. sample of oil is weighed into a platinum or nickel boat and the boat is filled with copper oxide powder. The capsule is kept in a horizontal position and the boat is put inside the ground joint of the capsule by means of a pair of tweezers. The capsule is then turned upright and a mixture of copper oxide grains and powder is poured upon and around the boat until the ground joint is filled. Then the rest of the capsule is filled with copper oxide grains. Apparatus and nitrometer are now swept with carbon dioxide. Residual nitrogen is taken out from the nitrometer by raising a_2 and turning s_3 in such a manner that the nitrogen passes out into a funnel, u. After closing s_3, a_2 is lowered, a_1 is raised, and s_3 is turned so that mercury from a_1 passes down the graded capillary and some drops fall down to the bottom of the nitrometer. Then s_3 is closed and a_1 is placed upon the table.

The capsule with sample is put into the apparatus and the sweeping and combustion are carried out (*2*). The nitrogen of the sample is accumulated under the mercury. After sweeping, s_3 is opened cautiously, so that the mercury slowly passes up and partly into a_1. The nitrogen follows the mercury up into the graded part of the capillary. Then s_3 is closed and the quantity of nitrogen is read after 20 minutes. When reading, the length between the two menisci is taken for the calculation. Possible errors from the manner of reading are compensated, as the blanks are read in the same manner.

As the quantities of nitrogen are very small, special attention must be paid to possible errors. The dry ice in the thermos flask should be of good quality and a blank without copper oxide should be run after every filling of the flask in order to check it. With the present apparatus the value of the blank lies at about 0.002 to 0.003 ml. of nitrogen, when the dry ice is of good quality. The copper oxide in the capsule should be used only once. Higher and somewhat irregular values were obtained when the copper oxide had been regenerated for further analyses, probably because of changes in the surface structure caused by the reduction and reoxidation. With good reagents the total value of the blank lies between 0.005 and 0.007 ml. of nitrogen.

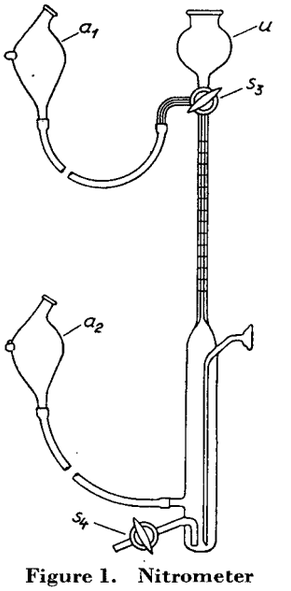

Figure 1. Nitrometer

The filling of the combustion tube has to be reoxidized after about ten analyses by leading a stream of oxygen through the hot apparatus for about 2 hours. After the tube is kept at working temperature (1000° C.) for 1 hour in the carbon dioxide stream it can immediately be used again for the analysis of compounds containing nitrogen not bound to oxygen. After one analysis of mineral oil so much nickel oxide has been reduced that the tube can be used also for analysis of samples that give off nitrogen oxides during combustion.

When the quantity of mercury on the bottom of the nitrometer has increased after

Table I. Analyses of Mineral Oil

Oil	Weight of Sample, Mg.	N_2, Normal Pressure and Temp., Ml.	N Found, %
A	42.7	0.0022	0.006
	45.9	0.0023	0.006
B	40.3	0.0020	0.006
	41.7	0.0020	0.006
C	40.2	0.0023	0.007
	44.5	0.0027	0.008
D	44.0	0.0027	0.008
	42.4	0.0026	0.008
	47.3	0.0027	0.007
E	45.5	0.0036	0.010
	40.7	0.0036	0.011
F	39.3	0.0033	0.011
	41.2	0.0032	0.010
G	47.6	0.0040	0.011
	41.2	0.0040	0.012
H	42.3	0.0065	0.019
	41.8	0.0074	0.022
I	42.6	0.0081	0.024
	41.6	0.0083	0.025
K	40.5	0.0089	0.028
	43.5	0.0098	0.028
L	38.5	0.1624	0.527
	38.0	0.1647	0.542

All analyses were carried out by one of the authors (B. W.).

several analyses, a_2 is raised and the excess of the mercury is allowed to flow out by opening s_4.

When the amount of barium hydroxide specified by Pregl (*3*) is added to the solution of the potassium hydroxide available to the authors, an excess of barium hydroxide goes into solution, and when the liquid is used in the nitrometers this excess is precipitated as barium carbonate. This can be avoided by using less barium hydroxide, but in this case the liquid seems to retain a tendency to foam. Satisfactory nonfoaming potassium hydroxide was obtained by dissolving the quantity of barium hydroxide specified by Pregl (*3*) in the solution and precipitating the excess by adding some dry ice. After the precipitate has settled, the liquid is decanted and filtered through a sintered-glass funnel, or still better centrifuged. When the gas bubbles in the nitrometer tend to stick to the mercury, a trace of carbon disulfide is added to the potassium hydroxide in the leveling bulb and the latter is shaken. After a few minutes the carbon disulfide has dissolved and is washed down into the nitrometer. A thin layer of a black precipitate is formed on the mercury, which effectively prevents the sticking of the bubbles. The stopcocks or stoppers of the nitrometers should be lubricated only with vaseline and never with silicone grease, as the latter seems to cause foaming.

ACKNOWLEDGMENT

The authors are indebted to Einar Stenhagen for reviewing the paper and to Lars Finn for drawing the figure. The apparatus may be obtained from Norstedt & Söner, Stockholm.

LITERATURE CITED

(1) Kirsten, W., ANAL. CHEM., 19, 925 (1947).
(2) *Ibid.*, 22, 358 (1950).
(3) Pregl-Grant, "Quantitative Organic Microanalysis," p. 72, London, J. & A. Churchill, 1945.

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Modified Vacuum Fusion Apparatus for Determination of Oxygen, Hydrogen, and Nitrogen in Certain Metals

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THE vacuum fusion method for the determination of hydrogen, oxygen, and nitrogen in metals has been modified to increase its rate of output. This should enable this valuable method of analysis to receive more widespread use. The changes suggested are in the construction of the apparatus rather than in the theoretical principles of the method. With these revisions the vacuum fusion apparatus becomes a more easily assembled piece of laboratory equipment, less expensive to construct and to maintain than the basic apparatus described by Alexander, Murray, and Ashley (*1*).

Three major structural changes were made:

A melting chamber similar to that introduced by Guldner and Beach (*2*) was substituted.

Ground-glass joints were located at strategic points throughout the apparatus (note Figure 1) to facilitate assembling, repairing, and cleaning of the equipment.

Two measuring systems were attached to the single melting furnace and the pumping system. Each is a complete unit, consisting of a mercury diffusion pump, oxidizing furnace, trap, and calibrated volume. This enables one operator to analyze twice as many samples per day as with the ordinary single-unit system. This important cost- and time-saving feature makes the vacuum fusion method a more practical tool for many laboratories.

To facilitate the most advantageous use of laboratory space and equipment, the apparatus has been installed on a small table (3.5 × 2 × 2.5 feet) on casters which enable the equipment to be rolled to the less easily moved 5-kv.-amp. oscillator. In many laboratories, several other kinds of vacuum equipment must be

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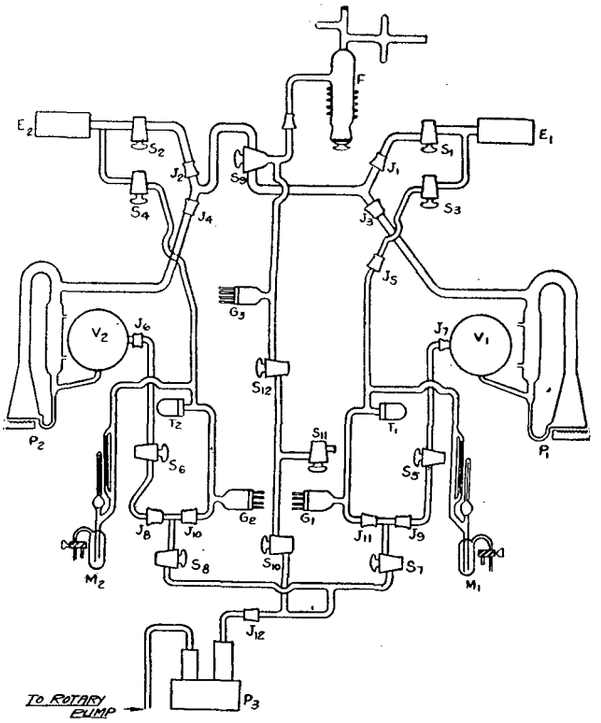

Figure 1. Schematic Diagram

run for varying periods of time, depending on the demand for a particular type of analysis with only a single oscillator.

The melting furnace described by Guldner and Beach (*2*) was modified, although the important features of this chamber—ease of maintenance, visibility of interior, and simple cooling—have been retained. In place of the borosilicate glass outer chamber, a clear quartz envelope was installed to give complete assurance that this part of the assembly will withstand the hot zone surrounding the crucibles in the inner quartz thimble. This quartz envelope was fitted with joints to connect it to the measuring systems and to the sample loading arm. Rather than use costly graded seals at these points, Vycor joints, which can be sealed directly to quartz, were utilized and these were waxed to the joints of the rest of the system.

The large three-way stopcock, S_9 (Figure 1), connects the furnace area to either of the two measuring units. The most time-consuming part of the analysis is the cycling, freezing, expanding, and measuring of constituent gases. Doubling this part of the equipment virtually doubles the output per day by the same personnel. The actual melting of the sample is a short procedure; consequently only one furnace is needed. In addition, only one set of vacuum pumps is used by which both measuring units are evacuated. With such a setup, a complete analysis of about ten samples for hydrogen, oxygen, and nitrogen can be made in an 8-hour day.

From the schematic diagram (Figure 1) it can be seen that each measuring unit consists of a high speed pump, P_1 and P_2, a 5-liter volume, V_1 and V_2, a cold trap, T_1 and T_2, a copper oxide furnace, E_1 and E_2, and connecting stopcocks. Opening stopcock S_{10}, which leads to the oil diffusion pump, P_3, serves to maintain a vacuum on the furnace tube, F, when S_9 is in the "off" position. Stopcock S_{11} serves to let air either into the furnace area alone or into the whole system. Thermocouple gage glass envelopes, G_1, G_2, and G_3, are located so that a continual pressure reading can be obtained in the furnace area and the measuring systems. With a little experience, the analyst can tell by the gage reading when gases are fully oxidized after cycling, when gases are at equal pressures after coming to room temperature, etc. Final pressure readings are taken on the McLeod gages, M_1 and M_2.

Ground-glass joints, J_1, J_2, ... J_{15}, were located to enable one to assemble the apparatus with the greatest ease and the least amount of glassblowing. Another advantage of these locations is in the repair and cleaning of sections, which are easily removed from the system with no glassblowing necessary. The number and locations of these joints can be varied to suit individual needs. High vacuum stopcock grease such as Apiezon L was used on the stopcocks. Apiezon wax W was used on the ground-glass joints.

With these suggestions, the authors believe that any laboratory can utilize this versatile vacuum fusion apparatus with the least outlay of space, time, and cost. The double measuring system alone cuts the cost of operation in half, while the use of ground-glass joints and the recommended combustion chamber further reduce the cost of assembly and maintenance.

LITERATURE CITED

(1) Alexander, L. E., Murray, W. M., Jr., and Ashley, S. E. Q., ANAL. CHEM., 19, 417–22 (1947).
(2) Guldner, W. G., and Beach, A. L., *Ibid.*, 22, 366 (1950).

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Improved Procedure for Extraction of DDT in Milk

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THE determination of DDT in milk by the colorimetric method described by Schechter *et al.* (*4*) is a lengthy process; it is advantageous to introduce every time-saving device possible. The procedure heretofore used in this laboratory in carrying out the method has included as the first step four successive treatments of the milk with ethyl alcohol, ethyl ether, and petroleum ether (Skellysolve B), to extract the butterfat as described by Carter (*1*). It is then necessary to remove the solvent from the extract by evaporation, after which the residue is taken up in chloroform for further treatment with sulfuric acid. Recently the observation was made that the addition of glacial acetic acid to milk curdled it and caused the butterfat to rise to the top, and that the subsequent addition of a buffer salt prevented the formation of emulsions during extraction with chloroform. As a result, the procedure described herein has been developed, which eliminates the time-consuming extraction of the milk with ethyl alcohol, ethyl ether, and petroleum ether.

REAGENTS

Acetic acid, glacial, C.P.

Potassium acetate, C.P.

Sodium Sulfate–Sulfuric Acid. Dissolve 100 grams of C.P. anhydrous sodium sulfate in 1 liter of C.P. concentrated sulfuric acid (specific gravity 1.84) with the aid of heat, and cool to room temperature.

Fuming Sulfuric Acid–Concentrated Sulfuric Acid. A mixture of equal volumes of fuming sulfuric acid (20 to 30% sulfur trioxide) and concentrated sulfuric acid (specific gravity 1.84).

Sodium bicarbonate, 5%.

Technical chloroform, redistilled.

PROCEDURE

Shake 50 grams of milk, which has been thoroughly mixed before sampling, in a 500-ml. separatory funnel with 35 ml. of glacial acetic acid until the butterfat rises to the top. Add 45 grams of potassium acetate (a 50-ml. beaker full) and shake well. Now extract the solution with 150 ml. of redistilled chloroform. (A mechanical extraction apparatus is a time and labor saver in both this extraction and the sulfuric acid extractions to follow, *3*.) After the layers have separated, filter the chloroform solution through a plug of cotton held in a large glass Gooch crucible holder resting in the neck of another 500-ml. separatory funnel. Extract the sample with another 150-ml. portion of chloroform and filter into the same funnel as before. The chloroform in this funnel contains almost all the butterfat and the DDT, and it is analogous to the first funnel in the Schechter sulfuric acid procedure. Filter a third extraction with 150 ml. of chloroform through the same plug of cotton but into another 500-ml. separatory funnel. This funnel is similar to the second or lower funnel in the Schechter sulfuric acid method.

To make the method more sensitive a slight modification has been made in the Schechter sulfuric acid procedure. Extract the chloroform solutions successively with (1) 75 ml. of sodium sulfate–sulfuric acid, (2) 75 ml. of sodium sulfate–sulfuric acid, (3) 75 ml. of fuming sulfuric acid–concentrated sulfuric acid, (4) 75 ml. of fuming sulfuric acid–concentrated sulfuric acid, and (5) 75 ml. of sodium sulfate–sulfuric acid. Drain each acid wash (lower layer) from the first funnel into the second funnel and finally into a 1-liter Erlenmeyer flask to be discarded. After the extractions have been completed, combine the chloroform solutions in the upper funnel. Drain off any acid that settles out before the chloroform solution is filtered through a plug of cotton held in a large glass Gooch crucible holder resting in the neck of the cleaned lower funnel. (If the stopper is kept in the neck of the upper funnel during this filtration, the level of the liquid is regulated so that it requires no attention.) In the lower funnel wash the chloroform solution with a sodium bicarbonate solution and again filter through a plug of cotton into a 500-ml. Erlenmeyer flask with a standard-taper 24/40 joint. Wash the bicarbonate solution remaining in the funnel with two successive 30-ml. portions of chloroform, which are also run through the plug of cotton into the Erlenmeyer flask. Complete the analysis as described by Schechter *et al.*, but use Clifford's (*2*) suggestion of heating the sample in a drying oven at 100° C. for 1 hour before developing the color.

Table I. DDT Recovered

DDT Added, P.P.M.	Uncorrected, P.P.M.		Corrected, P.P.M.		% Recovery	
	Acetic acid procedure	Extraction procedure	Acetic acid procedure	Extraction procedure	Acetic acid procedure	Extraction procedure
0 (blank)	0.07	0.08	..	..	..	..
0 (blank)	0.05	0.10	..	..	..	..
0.40	0.42	0.40	0.36	0.31	90	78
0.40	0.43	0.37	0.37	0.28	93	70
1.00	1.00	1.01	0.93	0.92	93	92
1.00	1.05	1.00	0.99	0.91	99	91
2.00	1.93	1.90	1.87	1.81	94	91
2.00	1.89	1.88	1.83	1.79	92	90

DISCUSSION

The method of extraction of the milk differs from the procedures described by Schechter and Carter in that the solvent employed (chloroform) is later used during the sulfuric acid extraction, thereby eliminating the ethyl ether–petroleum ether–alcohol mixture entirely and reducing by one third the over-all time consumed.

With the amounts of reagents described in the acetic acid–chloroform procedure, emulsions which do not break in 2 minutes will be formed only rarely. If an emulsion is formed, the addition of 1 or 2 ml. of acetic acid will separate the chloroform quickly.

The chloroform solution is highly buffered, but the buffer is removed along with some fat during the first sodium sulfate–sulfuric acid extraction.

The complete procedure, starting with the extraction of the milk sample, was tested by adding 0.0, 20.0, 50.0, and 100.0 micrograms of pure 75–25 *p,p'–o,p'* DDT in duplicate to 50-gram samples of milk. A comparison with the ethyl ether–petroleum ether–ethyl alcohol extraction method was made by extracting with ethyl ether–Skellysolve B as described by Carter (*1*), 50 grams of milk to which had been added the same amounts of DDT as above. The acetic acid procedure, when applied to milk containing as low as 0.40 p.p.m., gave an easily discernible characteristic blue color. The blue color is obtainable in either procedure at 0.20 p.p.m., but the accuracy is questionable. The results of these analyses are given in Table I.

Blank milk from the same sample will vary from 0.04 to 0.12 p.p.m., the results depending upon the amount of interfering substances left unremoved. Correcting for the higher blanks in the ethyl ether–petroleum ether–alcohol procedure resulted in low percentage recovery for the 0.40–p.p.m. sample.

The results indicate that the acetic acid–potassium acetate treatment gives fully as good recovery as the longer ethyl ether–petroleum ether–ethyl alcohol extraction procedure.

LITERATURE CITED

(1) Carter, R. H., Ind. Eng. Chem., Anal. Ed., **19**, 54 (1947).
(2) Clifford, P. A., *J. Assoc. Offic. Agr. Chemists*, **30**, 337–49 (1947).
(3) Mann, H. D., U. S. Bur. Entomol. Plant Quarantine, *Bull.* ET-268 (June 1949).
(4) Schechter, M. S., Pogorelskin, M. A., and Haller, H. L., Ind. Eng. Chem., Anal. Ed., **19**, 51 (1947).

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Separation of Iron(III) from Aluminum

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SMALL quantities of iron are not easily separated from aluminum prior to the gravimetric determination of aluminum.

Employment of a cation exchanger for the simultaneous separation of iron(III) and aluminum, followed by selective removal of the aluminum with sodium hydroxide, has been reported by Lur'e and Filippova (*6*), although the data of Samuelson (*8*) indicate that this method results in some loss in iron.

Separation by precipitation with cupferron (*4*) leaves small amounts of iron in solution. The ether extraction (*1*) of less than 1 mg. of iron is not easily accomplished. There are not sufficient quantitative data to appraise the chloroform extraction of either ferric 8-hydroxyquinolate (*3*) or ferric cupferronate (*2*) in the presence of aluminum. The use of the mercury cathode often results in incomplete removal of iron (*7*).

By the method described in this paper, iron(III) is removed as a negatively charged ferric thiocyanate complex ion on the anion exchanger, Amberlite IRA-400A. It is necessary that this strongly basic ion exchanger be converted to the chloride prior to its use. This method serves to separate 1 to 2 mg. of iron from up to 80 mg. of aluminum, so that the gravimetric determination of aluminum by precipitation as aluminum hydroxide is easily accomplished. The removal of iron in order to employ colorimetric methods for trace quantities of aluminum is being investigated.

MATERIALS USED

Ion Exchangers. Amberlite IRA-400 and Amberlite IRA-400A were treated with 3 *N* hydrochloric acid and used to prepare columns of ion exchanger approximately 25 cm. in height and 1.3 cm. in diameter.

Pure Aluminum Solution. Aluminum metal, containing 0.5% iron, was dissolved in hydrochloric acid. After removal of silica by filtration, ammonium thiocyanate was added in sufficient quantity to complex iron(III), the pH adjusted to 1.0, and the iron then removed by passage through a long column of the ion exchanger. The eluate was evaporated to dryness with aqua regia. This purified aluminum salt was used to prepare solutions which were gravimetrically standardized by precipitation of aluminum hydroxide. The precipitates of aluminum oxide thus obtained were pure white, although analysis indicated 0.02% ferric oxide.

Other Materials. Ferric chloride (low phosphorus), analytical reagent grade, was used to prepare solutions containing approximately 1 mg. of iron(III) per milliliter.

Ammonium thiocyanate, reagent grade, was prepared as a 3 *M* solution. The potassium salt can be used with equal success.

EXPERIMENTAL

General Procedure. Except in a very few cases the ion exchanger, previously treated with 3 to 4 *N* hydrochloric acid to convert it to the chloride, was rinsed with 50 ml. of 0.3 *M* ammonium thiocyanate, adjusted to pH 1.0 with hydrochloric acid, prior to the introduction of solutions containing iron(III) and aluminum. The solutions passed through the ion exchanger were 0.0004 to 0.0008 *M* in iron(III) and 1.5 *M* in ammonium thiocyanate, contained varying amounts of aluminum, and were at pH 1. The presence of the ferric thiocyanate complex is indicated by its reddish color on the yellow ion exchanger. After passage of the solution, the column was washed with several portions of 0.3 *M* ammonium thiocyanate. The aluminum in the eluate was then gravimetrically determined by a standard pro-

cedure (*5*). The column was easily regenerated with 3 to 4 *N* hydrochloric acid, so that it could be used again.

Effect of Thiocyanate Concentration. For solutions up to 0.0008 *M* in iron(III), the concentration of ammonium thiocyanate should be 1.5 *M*, although this may vary between 0.5 *M* and 2.0 *M*. Below 0.5 *M*, some iron may pass through the column. The effect of ammonium thiocyanate concentration above 2.0 *M* was not studied.

Table I. Effect of Flow Rate on Residual Iron

Aluminum taken = 0.0782 gram
Iron taken = 1.0 mg.

Flow Rate	Fe Found	Final Iron Present in Al as Impurity
Ml./Min.	*Mg.*	%
8–10	0.013	0.02
4–5	0.0035	0.005
1	0.0014	0.002

Effect of pH. At a pH of 1.0, satisfactory removal of iron is effected. While this value may vary slightly in either direction, it should not be much higher, in order to prevent precipitation of ferric hydroxide and not much lower, else the ferric thiocyanate complex will be removed from the column. A 1 *N* hydrochloric acid solution will elute the ferric thiocyanate complex.

The column was washed free of aluminum salts with 0.3 *M* ammonium thiocyanate adjusted to pH 1.0. At pH 2.0, some loss of aluminum was noted. At lower pH values than 1.0 it is probable that there will be removal of the ferric thiocyanate complex.

Table II. Determination of Aluminum in Absence of Iron

No.	Al Taken	Al Found	Difference
	Gram	*Gram*	*Mg.*
1[a]	0.0176	0.0177	+0.1
2[a]	0.0176	0.0175	−0.1
3[b]	0.0782	0.0784	+0.2
4[b]	0.0782	0.0783	+0.1

[a] Column washed 15 times with 10-ml. portions of 0.3 *M* NH_4CNS (pH 1.0).
[b] Column washed 20 times with 10-ml. portions of 0.3 *M* NH_4CNS (pH 1.0).

Effect of Flow Rate. The effect of flow rate on residual iron is shown in Table I. In these experiments the initial solutions which were passed through the column contained 1 mg. of iron (III) and 78.2 mg. of aluminum in 50 ml., were 1.5 *M* in ammonium thiocyanate, and were at pH 1.0. Ten washings with 10-ml. portions of 0.3 *M* ammonium thiocyanate at pH 1.0 were made in each case. Residual iron was determined spectrophotometrically.

DETERMINATION OF ALUMINUM IN ABSENCE OF IRON

Results obtained by passage of aluminum solutions 1.5 *M* in ammonium thiocyanate and at pH 1.0 followed by the gravimetric determination of the aluminum are shown in Table II. The flow rate was 8 to 10 ml. per minute.

DETERMINATION OF ALUMINUM AFTER REMOVAL OF IRON BY AMBERLITE IRA-400A

Results obtained in the presence of iron are shown in Table III. The flow rate was 8 to 10 ml. per minute. Twenty portions of wash solution were necessary for the quantitative removal of the larger quantities of aluminum from the column. It is probable that larger quantities of iron could be removed on columns of increased dimensions, but it might be difficult to wash out all aluminum salts.

PROCEDURE FOR SEPARATION OF IRON

The solution from which iron is to be removed may contain up to 2 mg. of iron(III) and up to 80 mg. of aluminum in a volume of about 25 ml. and it should be at approximately pH 1. It is feasible to separate larger quantities of iron with correspondingly larger columns of ion exchanger, but difficulty in quantitative removal of aluminum may be encountered.

Table III. Determination of Aluminum after Removal of Iron on Amberlite IRA-400A

No.	Fe Taken	Al Taken	Al Found	Difference
	Mg.	*Gram*	*Gram*	*Mg.*
1[a]	2	0.0176	0.0175	−0.1
2[a]	2	0.0176	0.0176	±0.0
3[b]	2	0.0176	0.0177	+0.1
4[b]	2	0.0176	0.0173	−0.3
5[a]	1	0.0414	0.0412	−0.2
6[a]	1	0.0414	0.0411	−0.3
7[c]	1	0.0414	0.0414	±0.0
8[d]	1	0.0782	0.0780	−0.2
9[d]	1	0.0782	0.0781	−0.1
10[d]	2	0.0782	0.0784	+0.2
11[d]	2	0.0782	0.0782	±0.0

[a] Column washed 8 times with 10 ml. of 0.3 *M* KCNS (pH 1.0).
[b] Column washed 4 times with 10 ml. of 0.3 *M* KCNS (pH 1.0).
[c] Column washed 15 times with 10 ml. of 0.3 *M* NH_4CNS (pH 1.0).
[d] Column washed 20 times with 10 ml. of 0.3 *M* NH_4CNS (pH 1.0).

Amberlite IRA-400A was treated with 3 to 4 *N* hydrochloric acid to convert it to the chloride, and washed several times with distilled water. A column of suitable dimensions was prepared, with the water slurry of ion exchanger. The usual precautionary measure was followed to ensure a column in which passage of solution was not accompanied by channeling. Liquid level was never allowed to fall below the top of the column. The column was further treated by passing 50 ml. of 0.3 *M* ammonium thiocyanate, adjusted to pH 1 with hydrochloric acid, through it at a flow rate of 8 to 10 ml. per minute.

An equal volume of 3.0 *M* ammonium thiocyanate was added to the solution containing iron(III) and aluminum, then adjusted to pH 1.0. This solution was passed at a flow rate of 8 to 10 ml. per minute through the previously prepared ion exchange column, and washed twenty times with 10-ml. portions of 0.3 *M* ammonium thiocyanate. Aluminum was determined directly in the combined eluates by precipitation as aluminum hydroxide.

ACKNOWLEDGMENT

The authors wish to thank Howard Bewick of the Solvay Process Division, Allied Chemical and Dye Corp., for his assistance in checking the iron content of certain of the eluates. Thanks are also due the Rohm and Haas Co. for making available the Amberlite IRA-400A ion exchanger used in this investigation.

LITERATURE CITED

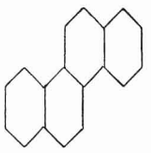
(1) Dodson, R. W., Forney, G. J., and Swift, E. H., *J. Am. Chem. Soc.*, **58**, 2575 (1936).
(2) Furman, N. H., Mason, W. B., and Pekola, J. S., ANAL. CHEM., **21**, 1325 (1949).
(3) Gentry, C. H. R., and Sherrington, L. G., *Analyst*, **75**, 17 (1950).
(4) Hoffman, J. I., and Lundell, G. E. F., *J. Research Natl. Bur. Standards*, **18**, 1 (1937).
(5) Kolthoff, I. M., and Sandell, E. B., "Textbook of Quantitative Inorganic Analysis," rev. ed., pp. 326–7, New York, Macmillan Co., 1947.
(6) Lur'e, Yu. Yu., and Filippova, N. A., *Zavodskaya Lab.*, **13**, 539 (1947).
(7) Parks, T. D., Johnson, H. O., and Lykken, L., ANAL. CHEM., **20**, 149 (1948).
(8) Samuelson, O., *Svensk Kem. Tid.*, **57**, 158–68 (1945).

RECEIVED March 10, 1950. From a dissertation submitted by Harry Teicher to the Graduate School of Syracuse University in partial fulfillment of the requirements for the degree of master of science in chemistry.

CRYSTALLOGRAPHIC DATA

45. Chrysene

Contributed by JOHN KRC, JR., Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.



Structural Formula for Chrysene

EXCELLENT crystals of chrysene can be obtained from ethyl alcohol solutions. Rapid crystallization favors the plate habit, while slow crystallization favors the tablet or massive form. Figure 1 shows plates of chrysene from ethyl alcohol. Figure 2 is an orthographic projection of a typical tablet from ethyl alcohol.

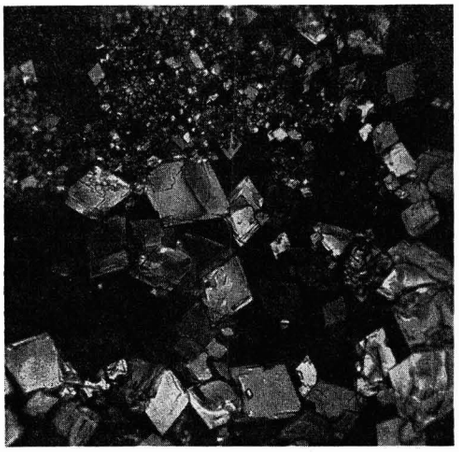


Figure 1. Crystals of Chrysene from Ethyl Alcohol

Crossed Nicols

CRYSTAL MORPHOLOGY

Crystal System. Monoclinic.

Form and Habit. Massive or plates lying on orthopinacoid, 100, showing clinodome, {011}, and occasionally basal pinacoid, {001}.

Axial Ratio. $a:b:c = 4.04:1:1.35$.

Interfacial Angle (not a polar angle). $011 \Lambda 0\bar{1}1$ projection on 100 = 73°.

Beta Angle. 115.8° (*1*).

Twinning Plane. 100.

OPTICAL PROPERTIES

Refractive Indexes (5893 A.; 25° C.). $\alpha' = 1.616 \pm 0.001$ (in 100 plane); $\alpha = 1.578 \pm 0.001$; $\beta = 1.775 \pm 0.005$; $\gamma = 2.01 \pm 0.02$ (calcd. from α, β, and $2V$).

Optic Axial Angles (5893 A.; 25° C.). $2H = 104°$; $2V = 84.5°$ (calcd. from β and $2H$).

Dispersion. $r > v$.

Optic Axial Plane. 010.

Sign of Double Refraction. Negative.

Acute Bisectrix. α.

Extinction. $a \Lambda \gamma = 6°$ in obtuse β; $a \Lambda \gamma = 10°$ in obtuse β (*1*).

Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.78 \pm 0.01$; R (calcd.) = 73.0; R (obsd.) = 73.6.

X-RAY DIFFRACTION DATA

Space Group. C_{2h}^6 (*1*).

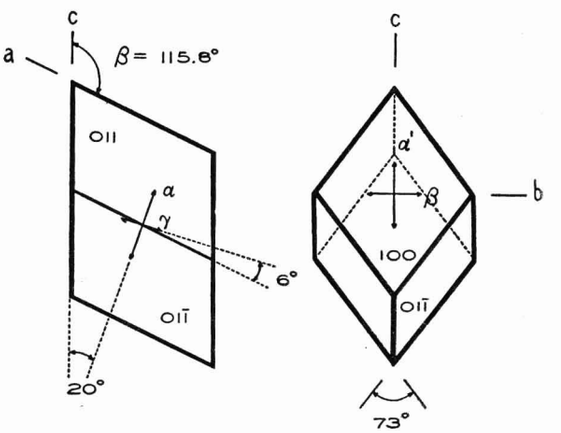


Figure 2. Orthographic Projection of Typical Tablet of Chrysene from Ethyl Alcohol

Cell Dimensions. $a = 25.0$ A.; $b = 6.18$ A.; $c = 8.34$ A. (*1*).

Formula Weights per Cell. 4.

Principal Lines

d	I/I_1	d	I/I_1
11.29	1.00	2.714	Very weak
5.99	Very weak	2.627	0.06
5.68	0.15	2.552	Very weak
4.94	0.34	2.481	0.20
4.77	1.00	2.351	0.05
4.30	0.08	2.258	0.09
4.15	0.53	2.153	0.03
3.76	0.21	2.090	0.07
3.48	0.19	2.072	0.06
3.36	0.79	2.011	0.06
3.24	Very weak	1.944	0.07
3.19	Very weak	1.883	0.08
3.02	Very weak	1.857	Very weak
2.903	Very weak	1.741	Very weak
2.838	Very weak	1.731	Very weak

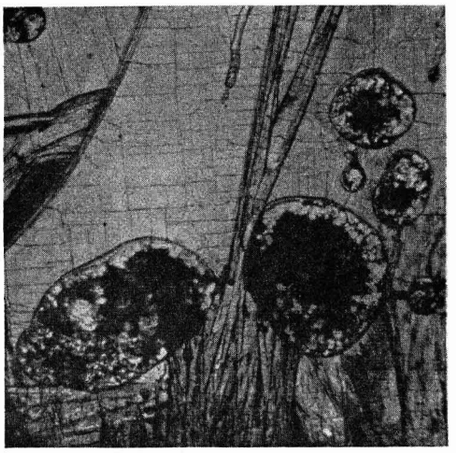


Figure 3. Crystals of Chrysene from Fusion and Chrysene Sublimate

Crossed Nicols

Formula Weight. 228.27.
Density. 1.298 (flotation); 1.307 (x-ray).

FUSION DATA. Chrysene melts at 260° C. with slight decomposition and with considerable sublimation. If cooled slowly just below the melting point or rapidly at room temperature, it crystallizes as highly birefringent crystals oriented preferentially to give an off-centered Bx_0 interference figure similar to that obtained on the 100 face of chrysene crystals from ethyl alcohol. No polymorphs were observed during this study. Figure 3 shows chrysene crystals from the melt and chrysene sublimate.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the assistance of Irene Corvin and Anne Humphreys, who determined the powder x-ray spacings and intensities.

LITERATURE CITED

(1) Bernal, J. D., Iball, J., and Robertson, J. M., *Nature*, 132, 751 (1933).

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, supervisor, Analytical Section, Armour Research Foundation of the Illinois Institute of Technology, Chicago, Ill.

Book Reviews

Physical Methods in Chemical Analysis. *Walter G. Berl*, editor. Volume II. xii + 640 pages. Academic Press, Inc., 125 East 23rd St., New York 10, N. Y., 1950. Price, $13.50.

The editor and the authors of the twelve chapters in Volume II of this work have produced a worthy complement to Volume I. Whereas the first volume dealt for the most part with methods based on the interaction of matter and radiant energy, the present volume is concerned with a wider variety of subjects: chromatography, radioactive tracer techniques, gas analysis by thermal conductivity, vacuum methods, measurement of surface tension and area of surfaces, magnetic methods, and four types of electrical methods.

The aims and general organization of the material are the same as before. The authors, carefully selected authorities in their respective fields, have been eminently successful in presenting their material in such a way that the nonexpert in a given field, with a good background of chemistry and physics, may read with understanding and acquire some degree of familiarity with the principles and practice of the various physical methods of chemical analysis. Each author discusses the fundamental principles and theoretical basis of his topic, describes apparatus and manipulative technique, indicates known or probable practical applications, gives consideration to the inherent accuracy, the limitations, and the sources of error, and cites pertinent literature references.

The volume is well bound and printed on good quality paper, with good type, and the illustrations seem to be exceptionally well done.

PAUL K. WINTER

Spectrochemical Analysis. *L. H. Ahrens.* xxiv + 269 pages. Addison-Wesley Press, Inc., Cambridge 42, Mass., 1950. Price, $10.

This authoritative book is devoted entirely to the analysis of rocks, soils, minerals, and related materials. The emphasis is laid almost exclusively upon direct current arc methods of sample excitation. It provides a comprehensive discussion of the theoretical principles and practical applications of this phase of emission spectrochemical analysis.

In Part I the physical characteristics of the direct current arc are thoroughly discussed. The many factors which affect the intensities of spectrum lines excited in this source are described. Especially well handled is the phenomenon of selective volatilization; listing of the methods recommended for minimizing fractional distillation should be very helpful. The mode of operation of spectroscopic buffers and the recommendations given for their selection are well formulated.

In Part II the author draws upon his wide experience in geochemical analysis for a discussion of methods of determining traces of particular elements in rocks and minerals. One especially useful chapter is devoted to methods for the determination of major constituents.

On unnumbered pages following the text are given tables of wave lengths of the most sensitive lines of the elements, together with the wave lengths of possible interfering elements. Values of the ionization potential of the element and of the excitation potentials of the most sensitive lines enhance the usefulness of the tables.

An adequate bibliography is given, although this reviewer would have preferred to see the references in footnote form near the point of reference rather than collected alphabetically by author name at the end of the text. Many of the tables lack captions and thereby lose some of their value out of context.

NORMAN H. NACHTRIEB

X-Ray Studies on Polymorphism. *Tei-Ichi Ito.* 231 pages. Maruzen Co., Ltd., P. O. Box Tokyo Central 605, Tokyo, 1950. Price, $10.

One of the most promising signs of the rehabilitation of the highest type of scientific research in Japan after defeat and several years of isolation from intellectual contact with the rest of the world is this book by the distinguished professor of mineralogy of the University of Tokyo. Trained as a student in the laboratories of Niggli in Switzerland and of Sir Lawrence Bragg at Cambridge, to both of whom Ito gives warmest appreciation for guiding him into "our beloved science in the making," he brings to this contribution an expert knowledge of crystal structure analysis.

The book is not a text, but a collection of research papers on experimental studies of mineral structures, with a central unifying theme of polymorphism based on the thesis of submicroscopic twinning in crystals. In 1938 Ito proposed the theory of twinned space groups, obtained by superimposing a group of operations called a twinning group onto one of the conventional 230 space groups of Schoenflies. Thus adjacent unit cells may be brought into twinned relationships by rotation, reflection, and gliding (echelon, alternate, and complex) while maintaining the homogeneity of the lattice as a whole. A twinned space group may be identical with one of the 230 Schoenflies groups, but generally it differs in the introduction of enhanced symmetry, experimentally indicated by extra regularities of x-ray diffraction spectra which are not accounted for by usual space group criteria. However, symmetry may also be degraded in some cases of twinning by gliding. The polysymmetric synthesis may take place on a smaller scale than the unit cell (internal twinning) or a larger one (twinning en bloc).

Ito proceeds to attempt to show these types of twinning in the polymorphism of pairs or groups of minerals subjected to detailed single crystal analyses by accepted x-ray diffraction techniques, including two-dimensional Fourier syntheses of electron density contour maps. Examples are eudidymite and epididymite ($HNaBeSi_3O_8$), essentially inner twins; the hexagonal feldspar, α-celsian ($BaAl_2Si_2O_8$) from which the structures of monoclinic and triclinic feldspars may be derived; the orthorhombic pyroxene, enstatite, as a twin of monoclinic diopside instead of an independent species; anthophyllite as a twinned tremolite; zoisite [$HCa_2(Al,Fe)Al_2Si_3O_{13}$] (space group Pn_ma) derived strictly as a twinned form of epidote (space group $P2_1/m$);

boleite ($26PbCl_2.24CuO.9AgCl.27H_2O$) with a complex space group obtained by operating a twinning group on a cubic space group; monoclinic wollastonite as a twinned lattice of a triclinic form; two monoclinic forms of TNT representing polysynthetic structures composed of rhombic cells; and moonstone, illustrating twinning en bloc, with an intricate structure of three kinds of monoclinic and one or more triclinic felspars existing side by side. The evidence from these examples for submicroscopic twinning as a potential crystallographic phenomenon seems convincing.

In an appendix the author reports individual structures for tourmaline, kotoite ($Mg_3B_2O_6$), lieurite, antigorite, ludlamite (a rare iron phosphate), and orpiment, together with a method of indexing a powder pattern of a crystal regardless of its symmetry, a generalization of a mathematical procedure proposed in 1917 by Runge.

Ito writes in English with facility and he has illustrated his structures with a considerable number of good diagrams. The book is fairly well printed, though a leaflet of two pages is required for 33 errata. Crystallographers will find in this rather highly specialized research compilation of the work of one man and his associates a fearless experimental attack upon some very complex mineral structures and a stimulating and not unreasonable theory for interpretation and prediction. In most of the very limited number of examples there is admittedly not an ideal correspondence between twinning theory and experimental results, because configurations will not coincide on reverse twinning operations. But at least here is an idealized guiding principle which may greatly simplify future work on the sometimes great complexities of nature's building plan.

G. L. CLARK

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Microchemical Nitrogen Apparatus

Since publication of the paper "Recommended Specifications for Microchemical Apparatus. Micro-Kjeldahl Nitrogen" [ANAL. CHEM., **23**, 523–8 (1951)] by the Committee for the Standardization of Microchemical Apparatus, private communications have disclosed the origin of the one-piece model. It was designed by E. C. Noonan, U. S. Naval Ordnance Laboratory, Silver Spring, Md., while he was working as a graduate student in the laboratory of H. A. Iddles at the University of New Hampshire in 1935. The work is unpublished. AL STEYERMARK

The Analyst's Calendar

American Society for Testing Materials. Atlantic City, June 18 to 22
American Association for the Advancement of Science. Gordon Research Conference, Colby Junior College, New London, N. H., and New Hampton School, New Hampton, N. H., July 23 to August 10
International Union of Crystallography. Second General Assembly, Stockholm, Sweden, June 27 to July 3
International Congress on Analytical Chemistry. United Kingdom, August 8 to 13

AIDS FOR THE ANALYST . . . .

Apparatus for Liquid-Liquid Extraction without Formation of Emulsions. Frederic E. Holmes, Clinical Laboratory, Christ Hospital, Cincinnati, Ohio.

THE apparatus shown in Figure 1 prevents formation of emulsions in liquid-liquid extractions, maintains an adequate area of interface between the two solvents, and is simple in construction and operation. It has been used for extraction by ethyl ether of fats and fatty acids, of hippuric acid and barbiturates from urine and other body fluids and from feces and ground tissues suspended in an aqueous medium, and for extraction by benzene of machine oil held in boiler feed water by emulsifying agents. The process is similar to that in the conventional extractor, except that the refluxed solvent, instead of passing through the aqueous layer, flows across its surface.

Some specimens form relatively stable emulsions with the solvents. Even large drops of solvent passing through an interface, or slight turbulence where aqueous and nonaqueous layers flow around the shaft of a stirrer, may produce stubbornly persistent coarse emulsions, or suspensions of finely divided solids, which are carried over into the final extract. Washing or settling chambers have been used to promote separation of such entrained matter (*1, 4, 6, 8*). However, use of the horizontal interface alone, and of the completely submerged, magnetically operated stirrer, prevents formation of emulsions.

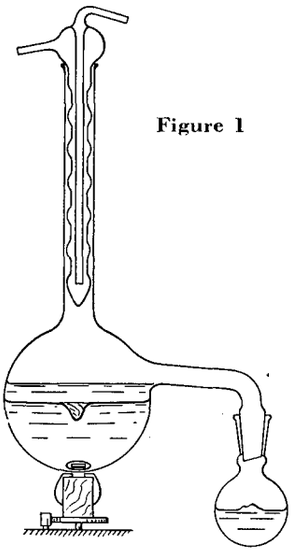

Figure 1

Several devices provide more area of contact than the horizontal interface, but may be otherwise unsatisfactory. The vertically disposed interface formed by a heavier immiscible solvent flowing down the walls of the extraction chamber (*7*) is inherently less stable than the horizontal interface. Dispersal of solvent in minute droplets by passage through many fine orifices, or by vigorous stirring or shaking, makes possible practically unlimited increase of interfacial area, but aggravates the difficulty of separating the extract. On the other hand, the area of interface between the solvent and the sample provided in the middle of the spherical extraction chamber of the present apparatus is considerably greater than the area of the corresponding plane of contact plus that of the surface of drops of solvent rising through the sample in a conventional extractor of the same capacity, even when drops are forced to follow a spiral (*5*) or devious (*3*) path.

In an alternative form the round extraction chamber consists of a standard short-necked round-bottomed flask with standard-taper joint for attachment of a standard condenser. An extractor with capacity for 500 ml. of sample requires a 1-liter flask. In both forms, on extractors of 500-ml. capacity or less, the side tube connecting with the boiler receiver is fused to the chamber at a point such that solvent will overflow into it when the level reaches 15 to 20 mm. above the mid-line. For extractors of 3-liter capacity, the distance above the mid-line is doubled.

Depth of the supernatant solvent is initially controlled by addition of water to the chamber. It may be adjusted by tipping the extractor to raise or lower the side tube. When the aqueous phase increases in volume through absorption of solvent, or wavelike surges occur due to asymmetry in the stirring motion, the side tube may be raised slightly. When circulation is smooth, the side tube may be lowered to decrease the depth and volume of the solvent pool and effect a more rapid turnover (*2*).

The apparatus may be tipped to decant off the last portion of extract into the boiler receiver. Extracts too large to be handled in a small evaporating dish may be concentrated without exposure to oxidation, danger of fire, or risk of spattering, by raising the side tube and distilling the solvent back into the chamber.

Batch extraction may be preferred when continuous extraction results in accumulation in the extract of unwanted substances which are slightly soluble in the solvent; substances such as hippuric acid may interfere in the gravimetric determination of fats. During prolonged batch extractions, the condenser prevents loss of solvent. At the end of the extraction, the extract is decanted into the boiler receiver. Solvent may be distilled back, decanted over to wash the last traces of extract into that previously collected, and then concentrated in the apparatus without danger of oxidation, fire, or loss.

The stirrers are made by sealing short pieces of soft iron rod into glass tubes which are tapered to form stubby or slender spindles 15 to 35 mm. long for use with small or large volumes of aqueous sample, different viscosities of solutions, and different curvatures of the round extraction chambers. The magnet for actuating the stirrers is mounted in a wooden block on the idling wheel of a phonograph turntable. The speed is controlled by the wall thickness of the rubber tubing placed over the shaft of the motor to drive the idling wheel.

The performance of the extractor was compared with that of a common type of laboratory extractor (Table I).

The conventional extractor consists of a cylindrical body with a side tube connecting with the boiler receiver, the space below the side tube serving as the extraction chamber. A condenser of the type shown in Figure 1 hangs in the part above the side tube, and a 7-mm. glass tube with funnel top conducts the solvent to the bottom of the chamber, where it escapes through notches in the flared end. The method used to measure rate of extraction was suggested by that of Bewick, Currah, and Beamish (*1*). Time of extraction of approximately 40% of the substance sought at equal rates of reflux was taken as a measure of rate of extraction, and was controlled by identical conditions of heating. Turnover of ether was estimated by counting drops. Hippuric acid was introduced in a solution of known concentration. The fatty specimen was prepared by grinding together skim milk, casamino acid (Difco), acacia, starch, soluble starch, olive oil fatty acids, olive oil neutral fat, Turgatol 7, Permutit, and finally gradually increasing amounts of water until a smooth paste and then a uniform thick emulsion suspension were obtained. On direct titration, 10 ml.

Table I. Performances of Extractors

Substance extracted	Hippuric Acid		Artificial Fatty Specimen	
Apparatus	Conventional	Present	Conventional	Present
Solute or sample, g.	0.087	0.087	10 ml.	10 ml.
NaOH in receiver, ml.	2 (0.1 *N*)	2 (0.1 *N*)	1 (1 *N*)	1 (1 *N*)
$CaCl_2$ in receiver, ml.	...	...	5 (10%)	5 (10%)
Aqueous layer, ml.	600	650	625	675
Ether layer, ml.	100	100	75	75
Estimated turnover, of ether, ml./hour	100–150	100–150	250–350	250–350
Aqueous layer, depth, mm.	205	60	215	63
Interfacial areas, sq. cm.				
Sum of drops	8	None	Estd. 20	None
Horizontal plane	28	120	28	120
Total	36	120	Estd. 48	120
Extraction of "40%," min.	378	347	992	497

were equivalent to 2.5 ml. of 1 *N* sodium hydroxide. Extraction was continued until the phenolphthalein in the standard aqueous alkaline solution in the boiler receiver became decolorized. (Calcium chloride was used with the sodium hydroxide to form the calcium soaps, which are not alkaline to phenolphthalein in aqueous solution.)

LITERATURE CITED

(1) Bewick, H. A., Currah, J. E., and Beamish, F. E., ANAL. CHEM., 20, 740 (1948).
(2) Dubbs, C. A., private communication.
(3) Francis, A. W., IND. ENG. CHEM., ANAL. ED., 1, 15 (1929).
(4) Knox, W. T., Weeks, R. L., Hibshman, H. J., and McAtear, J. H., *Ind. Eng. Chem.*, 39, 1579 (1947).
(5) Mathews, A. P., "Physiological Chemistry," 4th ed., p. 1006, New York, Wm. Wood and Co., 1925.
(6) Pearl, I. A., IND. ENG. CHEM., ANAL. ED., 16, 62 (1944).
(7) Stracy, L. C., Hunter, T. G., and Nash, A. W., *Ind. Eng. Chem.*, 29, 278 (1937).
(8) Wylie, A. B., and Schmulovitz, M. J., *Science*, 83, 398 (1936).

Use of pH Meters in Conjunction with Chromatographic Columns. R. N. Jeffrey, Division of Tobacco, Medicinal, and Special Crops, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md.

ONE of the drawbacks to the use of various chromatographic procedures in the isolation of colorless substances, whether the procedures are based on adsorption, partition, or ion exchange, has been the difficulty of determining which portion or portions of the separated material contain the interesting compounds. This is particularly true when a flowing column is used, from which the solution is collected continuously. However, this type of procedure has certain advantages in analytical methods for the determination of compounds which can be measured readily when relatively pure, but cannot be determined accurately in the presence of some of the other constituents of the original mixture. Quantities of material can be used which permit the accurate determination of the compounds separated, and this is not always true when a single sheet of paper is used instead of a column. The flowing column also has advantages from a manipulative standpoint over the older procedure of extrusion and separation of various portions of the solid material in the column.

The best method of selecting a fraction from the flowing column which contains all of the desired compound without contamination with interfering substances naturally depends on the compounds being studied. When weak acids or bases are determined, the use of a pH meter with electrodes in the effluent from the column assists in this selection.

In this laboratory the composition of cured tobacco leaves grown using different cultural practices is being studied. An effort is being made to determine separately each of the alkaloids and the organic acids present in these samples. The quantities of various other substances in these complicated mixtures affect the volume in which a given substance is eluted. Some evidence has been obtained that a continuous indication of the pH of the effluent assists materially in selecting a fraction that will contain the maximum amount of the ingredient for which the particular analysis is being conducted, with the minimum amount of interfering substances. The use of pH values, as such, applies only to water solutions, such as compose the effluents from ion exchange columns and partition columns in which water is the solvent for the moving phase. In the more common type of partition column, in which the solvent of the moving phase is an organic liquid, variations sometimes occur in the potential read on the pH meter as the effluent moves through the cell, but these should not be referred to the pH scale.

The cell in use in this laboratory was made from a 50-ml. distilling flask. The neck was cut off 2 cm. below and 1 cm. above the side arm and the side arm was cut to a length of 5 cm. The bottom of this piece was flared slightly and the top made oval in cross section, to allow the two standard 2.5-inch Beckman electrodes to enter it while they are mounted in the usual way on the door of the Model G meter. A small-diameter glass tube was bent into U-shape, connected to the bottom of the ion exchange column, and passed up through the hole in the bottom of the Bakelite beaker holder, where it was inserted into a short rubber stopper which fits the bottom end of the cell. The side arm was bent downward about 2 cm. from its end, which allows a 100-ml. graduate to be placed under it, so that records of the volume of effluent can be kept along with records of pH value, and the graduate can be replaced when desired. The cell thus formed has a net volume of 1.3 ml. when the electrodes are in place.

One can also obtain a commercial flow-type electrode assembly or can make a flowing microcell modified from the one described by Dietz [*Science*, **108**, 338–9 (1948)].

Micro Still Pot Suitable for Column Calibration. T. J. Walsh and E. H. Phelps, Case Institute of Technology, Cleveland, Ohio.

THE most satisfactory method of measuring the efficiency of a distillation column is to take spot samples of the column overhead and pot liquids while the column is operating at equilibrium conditions on a test mixture of known properties. Securing a pot sample from a microstill without disturbing the thermal equilibrium of the still is possible, using a hypodermic syringe with a 6-inch needle attached permanently to the still pot.

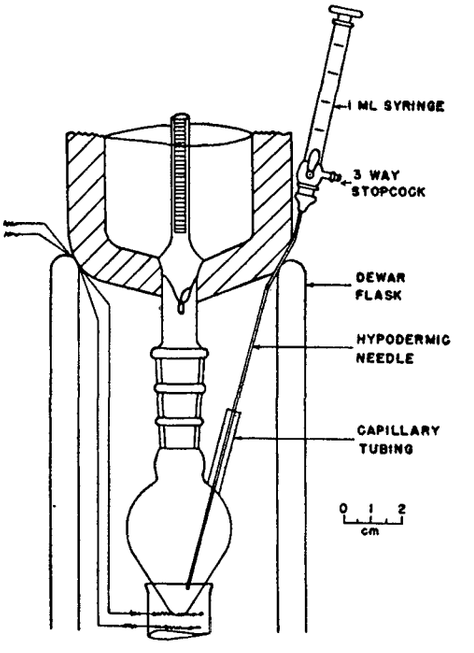

Figure 1. Syringe Attachment for Obtaining Still Pot Samples

The hypodermic needle is inserted through a piece of capillary borosilicate glass tubing before the tubing is sealed to the shoulder of the still pot. In sealing, the glass flows around the needle forming a tight seal. The seal may be ensured by flowing de Khotinsky cement into the capillary above the glass constriction.

Outside the still pot, the needle should be long enough to extend beyond the insulating flask. The outer end is connected to a 1-ml. hypodermic syringe through a capillary three-way stopcock. Samples as small as 2 drops may be taken by drawing a few tenths of a milliliter of liquid into the syringe and discharging through the third port of the stopcock. Excess material may be returned to the still pot.

This arrangement also permits changing the still pot composition without dismantling the still.

Sargent Constant Temperature Bath

The 0.01° C. Sargent Constant Temperature Water Bath, which is employed in many laboratories throughout the world where a precise, reliable thermostat is required, is now being supplied with an improved relay unit and heating system. The central heating and circulating unit of the bath is now equipped with three cylindrical heating elements rated at 200, 300 and 400 watts respectively. The 200 watt heater is controlled by the No. 81835 mercurial thermoregulator through a thyratron tube and saturable core reactor in the relay unit. (The use of a saturable core reactor obviates the difficulties commonly encountered with mechanical relaying systems such as pitted contacts, broken moving parts and freezing.) By means of a control mounted on the panel of the relay the output of this heater can be varied from the full 200 watts to approximately 60 watts, thus permitting such adjustment of the heater output that positive overshooting of the regulatory temperature is minimized. With the improved relay system this bath can be adjusted to a precision of ±.005° C. when operating in the vicinity of 25° C.

An autotransformer, adjusted by means of a knob on the relay unit panel, permits the setting of the 300 watt heater so that heat loss from the bath is almost compensated and negative overshoot is reduced to a negligible quantity.

The 400 watt heater may be used to quickly raise the bath temperature to the desired operating level or, in the case where sacrifice of some precision of regulation is of no consequence, to permit operation of the bath at temperatures above 60° C.

In addition, the relay unit is equipped with a master switch, a switch for each heater and a pilot light to indicate that the circuit to the 200 watt heater is closed. Maximum power consumption 1100 watts.

S-84805 WATER BATH—Constant Temperature, 0.01° C., Sargent. Complete with Pyrex jar, 16 inches in diameter and 10 inches in height; central heating and circulating unit; constant level device; cooling coil; No. 81835 thermoregulator and relay unit with cord and plug for connection to standard outlets. For operation from 115 volt 50/60 cycle circuits **$250.00**

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INSTRUMENTATION

Analog computers, already used in mass spectrometry and infared spectrometry, can assume the dynamic role of process simulation, saving time and expense

by R. H. Müller

AT LEAST two important methods of instrumental analysis can use analog computers to advantage. These are mass spectrometry and infrared spectrometry. It may be expected that as more of our instrumental methods continue to turn out vast amounts of data, particularly in the analysis of polycomponent mixtures, the need for rapid data assimilation will increase.

The automatic, or semiautomatic, accumulation of analytical data has already been extended to process control. Whereas plant process control has heretofore largely implied the control of temperature, pressure, flow, pH, and a few other variables, there has always been the tacit assumption that such control will lead to an acceptable product. This has been justified in the majority of cases and it has been successful to the extent that laboratory research, followed by pilot plant experience, established the correct conditions which the plant instruments were then supposed to maintain. The newer approach analyzes the final product or desired condition automatically, and controls the pertinent variables in terms of actual rather than predicted performance. This seems very logical and sensible, but its achievement is by no means simple, especially in a complex multistage process. Control engineers have long contended with the difficulties inherent in transfer lags, capacity factors, and the influence of process upsets. There are limitations to what can be calculated in advance and, indeed, one of the reasons for pilot plant experimentation is largely that of confirming small scale research and revealing unsuspected sources of deviation, not revealed either by experience or calculation.

For a long time, it has been found profitable to set up models or analogs of a real physical system, usually in terms of an equivalent electrical network. Then, to the extent that the electrical components can be made to simulate a variable, faithfully, the electrical network can be subjected to an infinite variety of fluctuations or forcing functions, individually or in combination, and the over-all effect can be observed continuously. This approach has been very useful in heating and ventilating problems, in aerodynamics, ballistics, and navigation. Application to plant process control is equally feasible, and in this connection, the instrumental analyst supplies part of the essential information.

An analog computer is by no means restricted to the role of accepting data and turning out an "autopsy" of process failure. It can assume the dynamic role of process simulation and the designer can then manipulate the "process" in infinite variety with a continuous indication of performance and at an enormous saving of time and expense. Giant aircraft are now being "built, flown, crashed, and immediately restored to their original form" —all in terms of simulators of this nature.

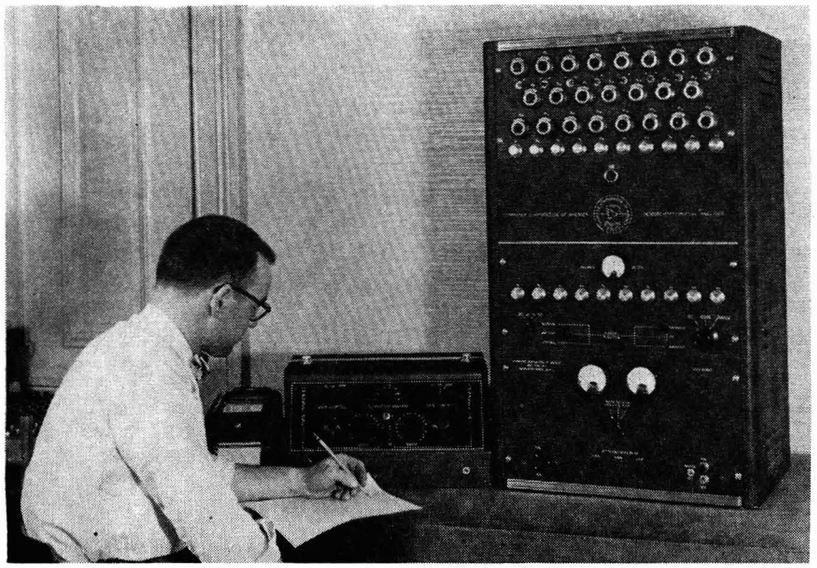


Figure 1. Electronic Analog Computer

Electronic Analog Computer

A recent example of this kind of computer is shown in Figure 1. It is the electronic analog computer often referred to as the integro-

(Continued on page 26 A)

INSTRUMENTATION

differential analyzer of IDA and is manufactured by the Computer Corp. of America, 149 Church St., New York 7, N. Y. IDA may be used for qualitative and quantitative dynamic studies in many fields. It may be used to solve linear differential equations with constant coefficients up to the eighth order. Eight initial conditions other than zero may be set into the problem and varied by means of potentiometers. By means of auxiliary equipment, the computer can be used to solve differential equations of higher order than eight, differential equations with nonconstant coefficients, and nonlinear differential equations.

The problem's analog is set up by simple interconnections or a setup board, one of which is shown in Figure 1 behind the operator. Connections are made with jumper wires and scale-factor resistors on push-type binding posts. The setup board is then slid into place in the computer and automatically makes all electrical contacts necessary for the solution of the problem. Twenty high-gain, stable d.c. amplifiers are provided which use both positive and negative feedback. These are extremely compact, identical, interchangeable, completely closed and quickly replaceable plug-in units. One of these is illustrated in Figure 2. Each amplifier can be used as an integrator, summer, or sign changer, and the inputs are easily set to any convenient multiplying factor. There are eight precision integrating capacitors of 1% tolerance and 23 precision, 10-turn helical potentiometers, eight of which may be used as initial condition potentiometers.

The control panel has two knobs for applying the forcing function and for starting or stopping the computing process. Two other controls select internal or external forcing function and adjust its level. The recorder and its control unit are shown in Figure 1 (left and center, respectively). The recorder is a 2-channel, direct-inking magnetic oscillograph. All computed quantities and the forcing function are automatically available at the control unit and any inputs or outputs can be recorded by the oscillograph two at a time. Any quantity can be viewed on an oscilloscope, with or without simultaneous pen recording.

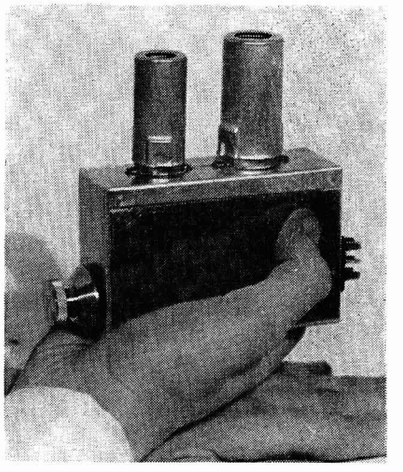


Figure 2. Amplifier

This computer is very compact and can handle many problems which previously required elaborate and prohibitively costly installations.

Basic Instrumentation Report

The third quarterly report from the Office of Basic Instrumentation of the National Bureau of Standards has been released. Each of these reports is more interesting than its predecessor and indicates a well-thought out and important program. We referred, some time ago, to the stimulating article in *Science* by the director of the Bureau, wherein he defined the science of instrumentation. Very shortly thereafter his conclusions were implemented by the establishment of the Office of Basic Instrumentation. Under the able direction of W. A. Wildhack this group is studying fundamental, long range aspects of instrumentation.

NEW PRODUCTS FOR ANALYSTS

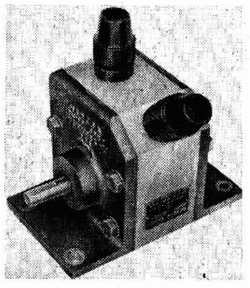
Equipment, Apparatus, Instruments, Reagents, Materials

Humidity Indicator

The new humidity indicator offered by Andrew Technical Service consists of a card measuring 6.75 × 1.88 inches. Its vertical column contains seven color spots which denote relative humidities from 10 to 70%. Usable at temperatures from 50° to 200° F., the Humigraph is said to be superior in accuracy to inexpensive hygrometers at low and normal humidities. For small changes in humidity, the card responds in about 10 minutes. For a change of 40%, the response time is about 30 minutes. Changes in humidity cause the spots to turn color. The reading is made at the top blue spot. The indicator may be used as an insert for long-term packaging of materials affected by humidity changes or in checking humidity without recourse to instruments or charts. **1**

Polyethylene and Vinyl Pump

The Vanton Pump Corp. has reported the availability of its new P series of noncorrosive and noncontaminating "flex-i-liner" pumps in which the fluid transferred comes in contact only with a polyethylene body block and a vinyl flex-i-liner. The pump is able to withstand the extreme corrosive action of acids such as sulfuric, hydrochloric, nitric, and hydrofluoric, as well as caustics and other chemicals to which polyethylene and vinyl are resistant. The design of this unit has eliminated such pumping trouble sources as stuffing boxes, packing glands, shaft seals, check valves, and gaskets.

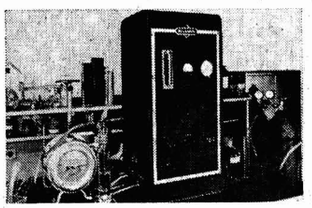


An eccentric rotor mounted on a sealed-in ball bearing rides inside and activates the molded vinyl flex-i-liner, creating a squeegee action between the outside of the liner and the inside of the polyethylene body block. Molded flanges on the liner seal off the fluid passageway from contact with any of the moving parts of the pump. As a result of this particular type of pumping action, high polymer slurries will not be coagulated, nor will solids be removed from suspension. The pumping action can be so closely controlled that even fresh blood can be passed through the pump without the destruction of blood cells or coagulation.

Pump body blocks may be obtained in Bakelite or Bakelite with graphite filler. Flex-i-liners made of natural rubber, pure gum rubber, Hycar, Perbunan, or neoprene are available. At present, P series pumps are obtainable in capacities up to 5 gallons per minute. Terminals are either drilled and tapped with 0.50- or 0.75-inch standard pipe thread, or supplied with standard saran tubing fittings or hose connections. The pump requires a 0.25-hp. motor and can be furnished either alone or completely mounted and connected to a constant- or variable-speed drive. **2**

Laboratory Ozonator

A new laboratory ozonator, Model T-23, has been introduced by the Welsbach Corp. The instrument is intended for use in testing the potential value of ozone as an oxidizing agent in various chemical processes, in phenol and cyanide destruction in industrial wastes, and in the reduction of tastes, odors, and color in water purification. The company produces an entire line of ozonators which range in size from the new laboratory model to much larger units which make possible the low-cost production of tonnage ozone. **3**



Photomultiplier Microphotometer

A new photomultiplier microphotometer for the precise measurement and comparison of light intensities from 20 micromicrolumens to 20 lumens at selected wave lengths has been devised by the American Instrument Co. It is used in film densitometry, colorimetry, turbidimetry, fluorometry, light scattering, and other studies.

The instrument provides readings of densities from 0 to 9 and phototube currents from 10^{-5} to 10^{-11} ampere. This range can be extended with neutral filters. Full-scale de-

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flections of the meter are given with photomultiplier (phototube) currents of 0.01, 0.1, 1, and 10 microamperes. Provision is made for an additional position on the range switch of 0.001 microampere full scale where this amplification is usable. The meter indicates per cent transmission, density, and photocurrent microamperes directly without correction factors. Any filter 2 inches square may be positioned in the filter holder, either singly or in combinations up to 0.625 inch thick.

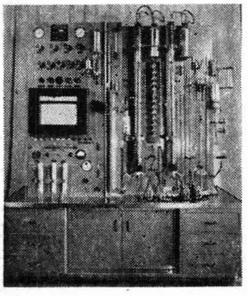
An outlet on the panel provides 10 mv. at 100 microamperes for the operation of a commercial recorder. The instrument may be operated from any 100- to 140-volt, 60-cycle source. No batteries are needed. **4**

Water Purification

The mixed-bed deionizing equipment offered by the Illinois Water Treatment Co. will produce water containing less than 0.1 p.p.m. of dissolved solids and less than 0.02 p.p.m. of silica. A series of six packaged mixed-bed units is available in sizes ranging from 12 to 1000 gallons per hour. Larger units are engineered to meet specific requirements. **5**

Low-Temperature Distillation Analyzer

A new model automatic analyzer for low-temperature distillations offers increased accuracy as well as decreased time per distillation. Manufactured by Podbielniak, Inc., the instrument has a temperature range of −200° to +100° C., a pressure range of atmospheric pressure to 1 mm. of mercury absolute, a capacity of 0.5 to 100 ml., and an accuracy of 0.1 to 0.5%.



The Brown Electronik strip-chart-type potentiometer incorporated in the assembly is available with a second extrasensitive range of 0 to 2 mv. As a result, the sensitivity of temperature measurements is increased fivefold. A high-capacity, high-efficiency, Super-Cool column permits the analysis of samples at much faster rates and with greater accuracy than previously possible. The total distillation time is reduced one half or more, even though larger samples are taken to minimize holdup and other errors. In this respect, the greatest time saving is realized on "dry" gas samples because of the very high permissible "venting" rates. Even though high rates may be obtained, liquid holdup per plate is not increased and therefore small samples may be effectively distilled.

In this column, the distilling tube packing and kettle are now separable and interchangeable with various sizes and types of kettles. This interchangeability is made possible by a new type of glass wetting solder which remains vacuum-tight indefinitely at liquid nitrogen temperatures. A tubular thermocouple assembly having increased sensitivity is supplied for distillate temperature measurement. This thermocouple assembly appreciably reduces liquid holdup in the reflux section of the column, thereby improving the sharpness of separation.

A special rotameter is built into the gas entering line to permit the measurement and control of high gas rates. All stopcock manifold and manifold-connecting lines are made of stainless steel. Rubber tubing connections to glassware are eliminated through the use of a special vacuum-tight seal. All relays are electronic or of the hermetically sealed plug-in type. Solenoids and other electrical assemblies are provided with special connections facilitating easy replacement. **6**

Ketosteroid Determinations

Dajac Laboratories announces the availability of 2-hydroxy-3-naphthoic acid hydrazide, which is useful in histochemical demonstration of active carbonyl groups, such as those found in the adrenal cortex, ovary, placenta, and testis. One application involves the reaction of the naphthol hydrazone precipitated in the tissues or cells with a diazonium salt to form a brightly colored dye. Tissues stained in this manner may be permanently mounted. The quantitative measurement of ketosteroids from body fluids by colorimetric methods has also been developed. **7**

Pyrometer Supplies

A series of new pyrometer supplies has been developed by Minneapolis-Honeywell Regulator Co. One of these products, a cast iron, closed-end protecting tube for molten aluminum applications, features a service life several times that of former models. The tube, which is built of dense cast iron having a smooth outer skin, has a 1.94-inch outside diameter and 0.81-inch inside diameter, and comes in lengths of 12, 18, 24, 30, and 36 inches. First shipments are scheduled for about September 15.

A two-conductor, 24-gage copper-constantan thermocouple wire has been developed for refrigeration applications. It is insulated with moistureproof polyvinyl chloride. Over-all dimensions are approximately 0.05 × 0.09 inch. Field tests show that the thermocouple has a temperature range from −20 to +225° F., and where flexing does not occur, the low limit is below −30° F.

A portable immersion, removable-tip thermocouple has

been developed for the measurement of molten steel temperatures. The new model is credited with accurate readings in 15 to 25 seconds of immersion time in molten steel having a carbon content of 2.5% or more, through a range from 2400° to 2900° F.

Another development is a 20-gage JX and KX two-conductor insulated extension wire, with standard I.S.A. wire color coding in both iron-constantan and Chromel-Alumel components. Each wire is insulated with enamel and covered by moisture- and flame-resistant, wax-impregnated asbestos. The two conductors are covered with a heavy Fiberglas braid.

A 1-inch, I.P.S. protecting tube is being made as a standard product for immediate shipment. The new tube has been satisfactorily tested for thermocouple protection in heat treating furnaces, salt and lead hardening baths, and gas burners. A temperature of 1800° F. is the recommended high measuring temperature for this tube. **8**

Dehumidifier

The Abbeon Supply Co. has announced the development of a new dehumidifier, Model DMS 4. This unit will dehumidify any closed area of tight construction up to about 8000 cubic feet. The principle used is that of an electric refrigerator. Moisture-soaked room air is drawn into the dehumidifier by a fan located inside the unit. This air is then passed over cold coils, where its moisture is deposited much as droplets of water form on the outside of a cold pitcher on a hot, humid day. This water then drips into a pan for removal. The moisture collection pan may be emptied either by hand or by a hose or copper pipe connected for automatic discharge of the condensate through a floor drain or sewer.

The dehumidifier can handle air flows of 110 cubic feet per minute. It is 17 inches long, 13 inches wide, and 15.5 inches high, weighing 55 pounds. Price, $149.50. In large areas, more than one dehumidifier or possibly larger units may be employed to ensure the proper control of moisture. **9**

Manometer

A new, large-range absolute and differential manometer designed to cover more than one atmosphere of pressure has been introduced by the Emil Greiner Co. This large-range instrument, said to possess the simplicity and high precision of the company's small manometer, employs a silk screened scale on saran. The temperature coefficient of expansion of saran compensates for changes in mercury density caused by room temperature variations. Other features of the instrument include two rods for easy mounting on a frame, a protective bracket for the stopcock, a metal rod to carry the vernier, and a rigid aluminum casting to support the glass assembly. **10**

Cathodic Etcher

High-vacuum equipment for cathodic etching and for studying metals by this technique has been developed by Distillation Products Industries, a division of Eastman Kodak Co. The unit is expected to be particularly useful for investigating cold flow lines. The sample of the metal to be studied is bombarded with a glow discharge at low pressures. The discharge is produced by positive ions passing between an aluminum anode and the sample of metal under test.

The advantage of this technique is that the etching is produced physically rather than chemically, so that there is less danger of oxides and other compounds being formed. Under ion bombardment, the grain boundaries are attacked at a different rate than the main body of the metal. When the cathodic etcher is used, the crystal shapes and other characteristics of the metal are often brought out with greater clarity than is possible in an acid etch. The new apparatus will be of value both in pure research and in production sampling. **11**

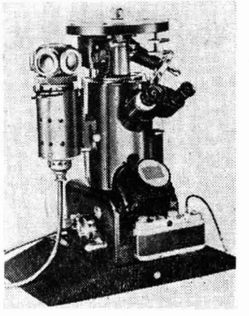
Portable Electric Oven

The Grieve-Hendry Co. has added Model PL-1 to its line of portable electric ovens. Inside measurements are 29 × 24 × 20.5 inches. The oven's four shelves provide room for eight 2 × 12 × 23 inch drying pans. Products or materials can be placed directly on the shelves if desired. The portable feature, permitting the use of these ovens close to machines, will eliminate considerable handling and hauling. The oven operates on 110 volts.

Construction of the oven is all steel, with air-cell asbestos insulation. Uniform temperature is provided throughout the oven by forced air circulation. A motor-driven fan draws in fresh air and drives out the stale air through specially located vents. This prevents stratification and makes the oven practical for any dehydration or baking process. The oven is capable of heating to 225° F. in 15 minutes. Price, $92.50. Other temperature and shelving arrangements available from $79.50. **12**

Metallograph

The metallograph offered by F. T. Griswold Manufacturing Co. makes possible the rapid structural analysis of metal samples and other substances. A large assortment of objective, ocular, and projective lenses are available. These are parfocal, parcentered, and coated on all air-glass surfaces. The range of magnification is from 25 to 2000 diameters. The instrument occupies a table space of less than 12 × 12 inches. Over-all height is 18 inches. The staging table is approximately 8 inches in diameter, so that fairly large samples can be examined. The table has tee slots for clamping the sample if required.



The technician can observe the sample through binoculars or on a ground-glass screen without changing his position. Photographs may be taken on 35-mm. roll film by merely swinging the image from the ground glass to the camera. **13**

Warning Label

A convenient means of labeling radioactive hazards, chemicals, and containers is announced by Nuclear Instrument and Chemical Corp. This is a pressure-sensitive tape on which the radioactivity symbol and the word "radioactivity" have been printed in magenta on a yellow background (the approved radiation warning colors) The tape is arranged for small pieces to be torn off and attached to any surface. Each complete design is 1.5 inches long. The roll is 1 inch wide and can be used on a standard holder. **14**

Thermometer

A new thermometer, Model 8689, is now available from the manufacturer, Tagliabue Instruments Division, Weston Electrical Instrument Corp. Its corrosion-resistant stainless steel case imparts to the new model a degree of protection unusual in an instrument of this kind. The scale of the instrument is large, clear, and easy to read. It can be

obtained in five different ranges extending from −40° to +400° F. The arched "bows" and flanged edges of the case provide the greatest possible angle of vision consistent with the best protection of the glass tube. The open spaces around the bulb ensure maximum air circulation for true-value readings. A special feature of the thermometer is the removable scale and tube unit for easy replacement of a broken tube. This feature also permits the convenient interchange of various scales even though only one case is used. **15**

MANUFACTURERS' LITERATURE

Liquefier. Technical bulletin 20-A discusses the properties of Azite 900, a liquefier which is of particular interest to manufacturers and converters of paper because of its capacity for reducing the viscosity of colloidal solutions of certain materials such as starches and proteins, its tendency to stabilize the viscosity of these same materials, its effect in retarding the growth of bacteria, and its ability to improve the heat stability or performance of acidic paper or paper exposed to acid conditions. Booklet is revision of one originally prepared in 1948. American Cyanamid Co. **16**

Testing Instruments. A 32-page booklet gives details on recording gravitometers (with or without controller), dead weight gages, gas gravity balances, high pressure consistometers, moisture tester for gases, vapor pressure bombs, manometers, and thermometers. Refinery Supply Co. **17**

Insulated Panels. New prefabricated insulated panels suitable for the erection of hot or cold walk-in rooms are subject of 2-page bulletin. Panels may be used in building rooms with controlled temperatures from −100° to +200° F. and relative humidities from 20 to 95%. Bowser Inc. **18**

Laboratory Apparatus. New issue of company house organ, *Precisionomics*, describes automatic distillation apparatus, mercury relays, water baths, burners, heaters, stills, and a B.O.D. cabinet for low temperature testing of sewage and waste. Precision Scientific Co. **19**

Electrochemical Measurements. Measurement of pH, oxidation-reduction potential, and electrolytic conductivity in industrial control systems is covered in 24-page bulletin. Fundamental principles of electrochemical measurements, as well as final control elements for automatic control systems, are outlined. Minneapolis-Honeywell Regulator Co. **20**

pH Control. New 12-page catalog entitled "Precise pH Control and Water Tests" describes company's glass color standards, pocket and standard comparators, testers, replacement parts, and accessories. Hellige Inc. **21**

Laboratory Equipment. Spring issue of *Cenco News Chats* describes laboratory trip scales, rain gages, centrifuges, temperature recorders, optical cells, sodium arc, immersion oils, and other apparatus. Central Scientific Co. **22**

Resins for Adhesives. An 18-page booklet evaluates resins for use in rubber, cellulosic, and water-soluble adhesives. Properties are given for 17 resins. Acid number, bulking value, index of refraction, specific gravity, viscosity, and other properties are listed. Hercules Powder Co. **23**

Analytical Balances. An 8-page pamphlet illustrates and describes analytical balances, Westphal specific gravity balance, and pan balances manufactured by Reyers and Zoon. Livingston Commercial Corp. **24**

New Products. Publication gives details on helium and hydrogen liquefiers, oxygen plants, helium refrigerators, liquid oxygen pumps, low pressure gas holders, air coolers, and research electromagnets. Company's engineering, research, and development services are discussed. Arthur D. Little, Inc. **25**

Infrared Microspectrometer. Spring issue of *Instrument News* features articles on infrared microspectrometer and new transverse panoramic camera. Perkin-Elmer Corp. **26**

Hexachlorophene. Comprehensive bibliography on hexachlorophene contains abstracts of 51 scientific and trade articles and 19 patents. Compound is used as a germicide in soap and synthetic detergents. Sindar Corp. **27**

Respirator. A 2-page bulletin CR-26 describes redesigned respirator featuring a new type of mineral wool filter which requires less than half the filter area and offers only half the breathing resistance of previous models having the same dust-collecting efficiency. The cushion-type face piece is soft and pliable and assures a gas-tight seal for a wide range of facial contours. Mine Safety Appliances Co. **28**

Photochlorinations. An 8-page illustrated booklet provides information on photochlorinations and a proposed system for continuous-flow operation. Hanovia Chemical and Manufacturing Co. **29**

Optical Parts. Lenses, prisms, reflectors, and miscellaneous optical parts are discussed in 16-page brochure. Crystal quartz lenses and condensers, as well as lenses, prisms, and plates of fused quartz, are described. Bausch & Lomb Optical Co. **30**

Research Laboratory. The laboratory of the Aluminum Co. of America at East St. Louis, Ill., is described in 14-page illustrated booklet, which also discusses the products that Aluminum Ore Co. provides for industry. Aluminum Co. of America. **31**

Instrumentation. A nontechnical account of the role of instrumentation in everyday life is provided in 12-page pamphlet. Aviation, pasteurization, fire control, communication, and atomic energy are briefly covered. Weston Electrical Instrument Corp. **32**

Chart Recorder. A 4-page bulletin explains how up to six permanent records of industrial processes may be obtained on a single strip chart recorder. The accuracy of the deflection-type measuring circuit and the simple chart-printing mechanism are described. An open view of the instrument illustrates the arrangement of the basic recorder assemblies. Wheelco Instruments Co. **33**

Metallograph. Well-illustrated 20-page booklet covers the operation of the Balphot metallograph. The photographic unit, motor-driven carbon arc, ribbon filament, zirconium oxide concentrated arc, visual light source, filters, and Magna-Viewer are discussed. Bausch & Lomb Optical Co. **34**

Viscosity Measurement. New brochure describes instruments that provide an instantaneous method of measuring viscosity. Featured are the single-float, two-float, auto-sampling, cylindro-plunger, close-coupled, and remote-recording instruments. Fischer and Porter Co. **35**

Shock Resistance. The basic concepts involved in designing for shock resistance are explained in comprehensive bulletin. The influence of weight, energy storage, stress concentration, and residual compressive stress is discussed, and information is given on means of preventing functional difficulties caused by shock. The Barry Corp. **36**

Plastic Tubing. Pamphlet provides information on Alanol plastic tubing that is said to have a flex life 14 to 16 times that of rubber and may be used within a temperature range of −30° to +190° F. The tubing, available in sizes ranging from 0.12- to 1.0-inch inside diameter, is clear, nontoxic, and will withstand steam sterilization. Couse and Bolten Co. **37**

THE LABORATORY SUPPLY CENTER

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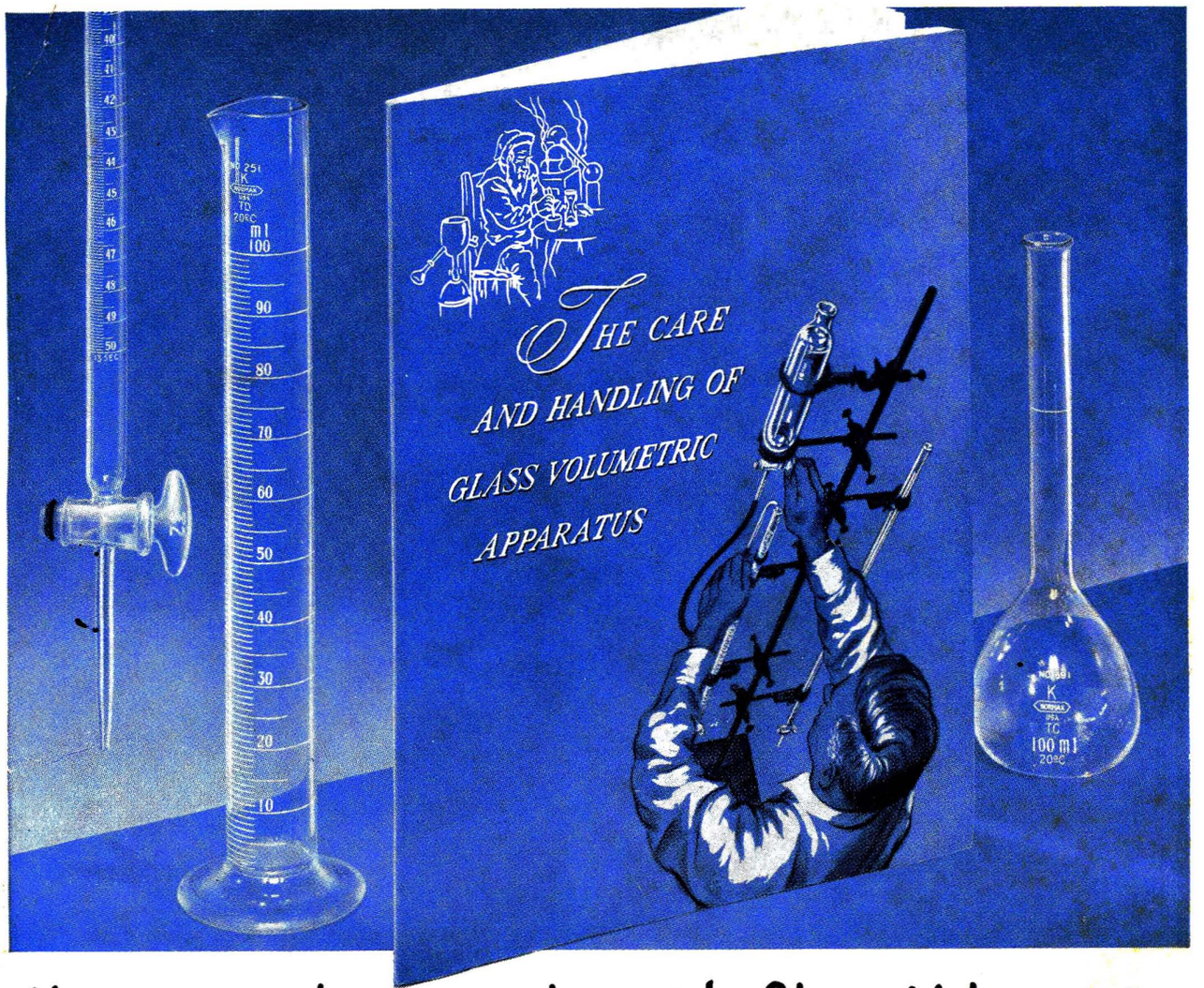

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