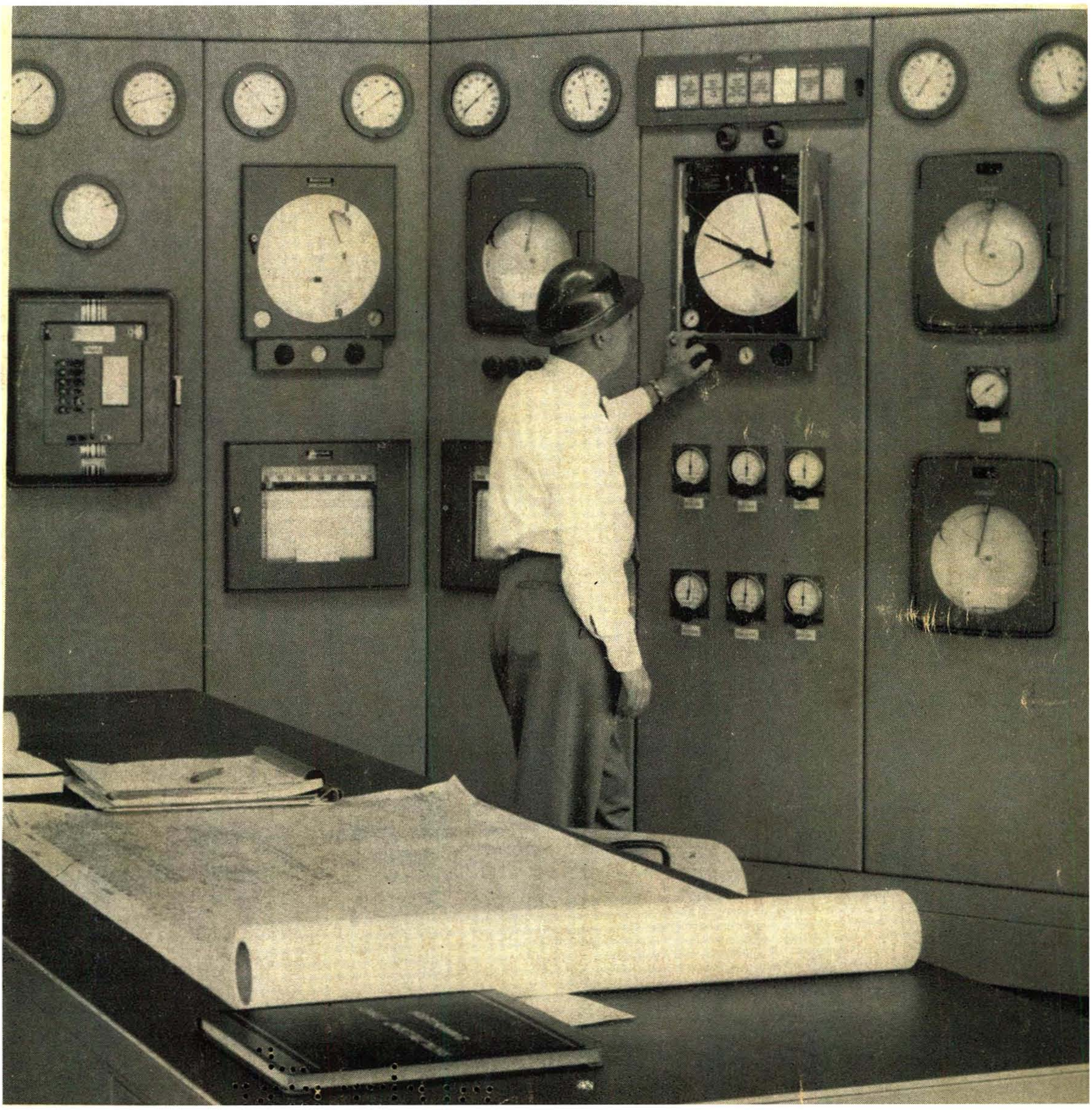
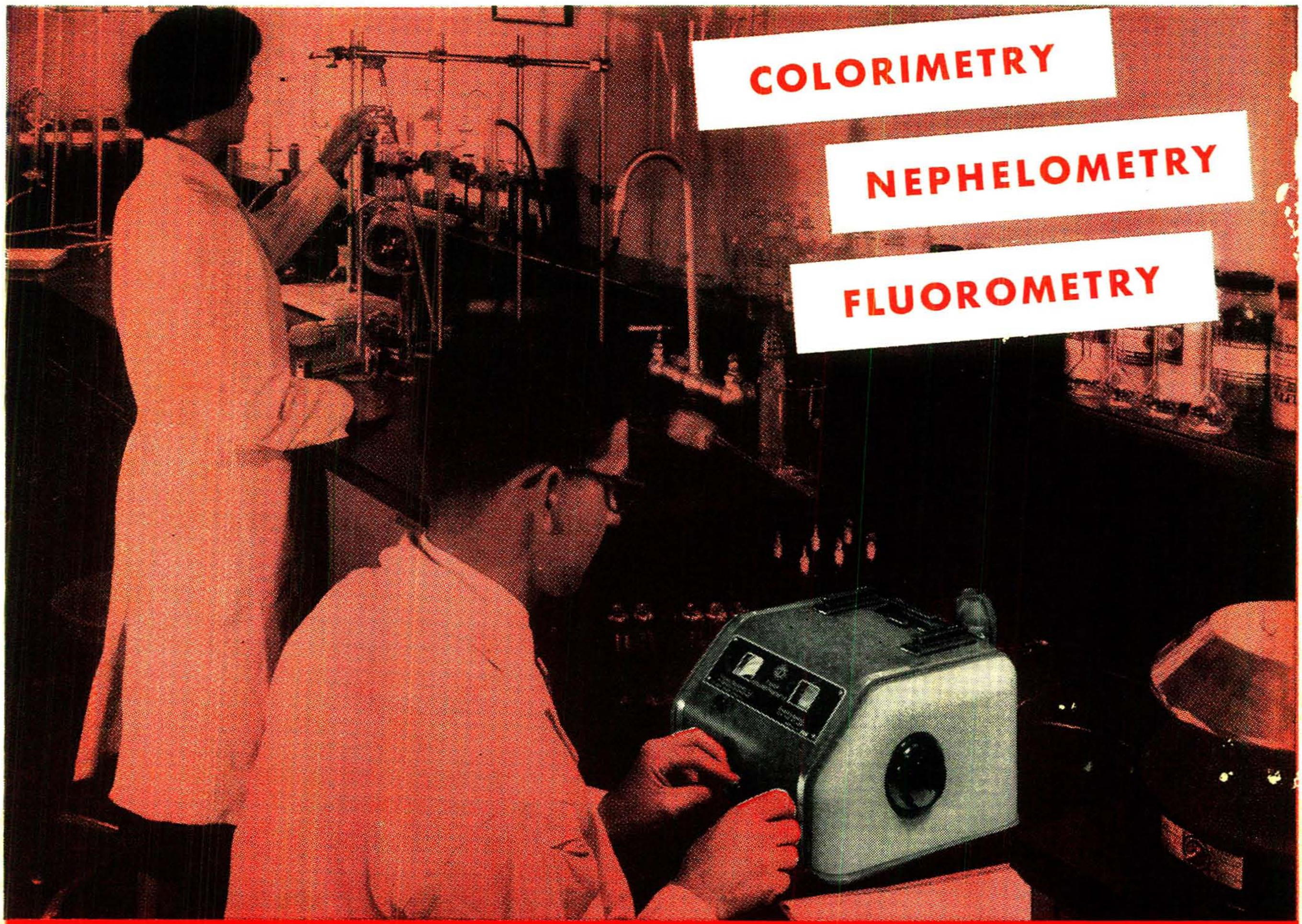
DECEMBER 1953

ANALYTICAL CHEMISTRY



Central instrument panel, control point in Shell Chemical's new ammonia plant at Ventura, Calif. Just put on stream; plant is one of most automatic of its type


COLORIMETRY
NEPHELOMETRY
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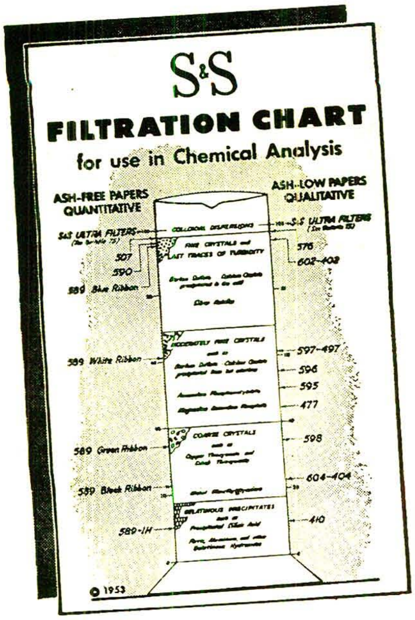
The FISHER
Nefluoro-Photometer

ANALYTICAL CHEMISTRY

Volume 25, No. 12
Issued December 22, 1953

This Month's Cover. Shell Chemical, firm believer in thorough instrumentation, put probably the most completely instrumented plant of its type on stream when it turned the valves of its new ammonia plant in Ventura, Calif., on Dec. 11. Hydrogen-nitrogen ratio, CO and CO_2 content (catalyst poisons), and inerts are control variables. Gas streams at seven key points are tapped and by-pass streams fed to an analyzer house. Here three infrared continuous analyzers monitor CO and CO_2 and methane content. A gas ionizer, completely designed and built by Shell Development, continuously determines argon and nitrogen content of the synthesis gas. Hydrogen content of synthesis gas, basic to process control, is determined by standard thermal conductivity instruments. Hydrogen content of the synthesis gas automatically determines flow rates of other major process gas streams.

(Continued on page 4 A)



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NOTES ON ANALYTICAL PROCEDURES

CRYSTALLOGRAPHIC DATA

AIDS FOR THE ANALYST

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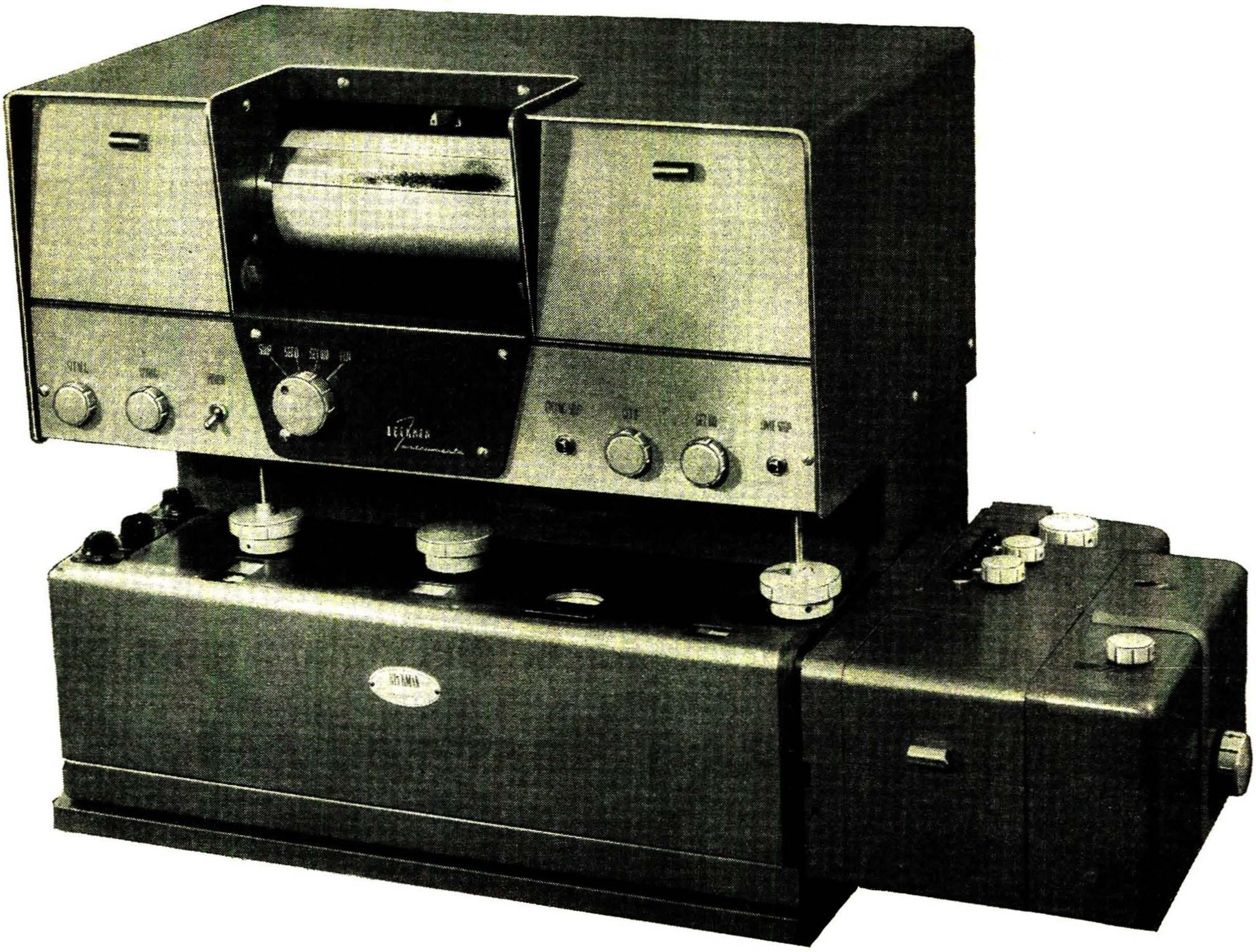

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Complete Flexibility of Operation

- *Operator can scan sample curve rapidly, then record any area of interest in greater detail.*
- *Records percent transmittance directly on convenient 8½" x 11" chart paper.*
- *Plots a complete spectrum from 220 to 1000 millimicrons.*

Here is the modern automatic spectrophotometer capable of handling the bulk of chemical analyses in any laboratory. Employing the unexcelled monochromator of the Beckman Model DU, this new recording spectrophotometer makes speedy, accurate analyses of one, two or three samples—simultaneously. Each sample curve is permanently recorded in a different color on a single chart. The recording system makes any Beckman "DU" quartz spectrophotometer an automatic instrument.

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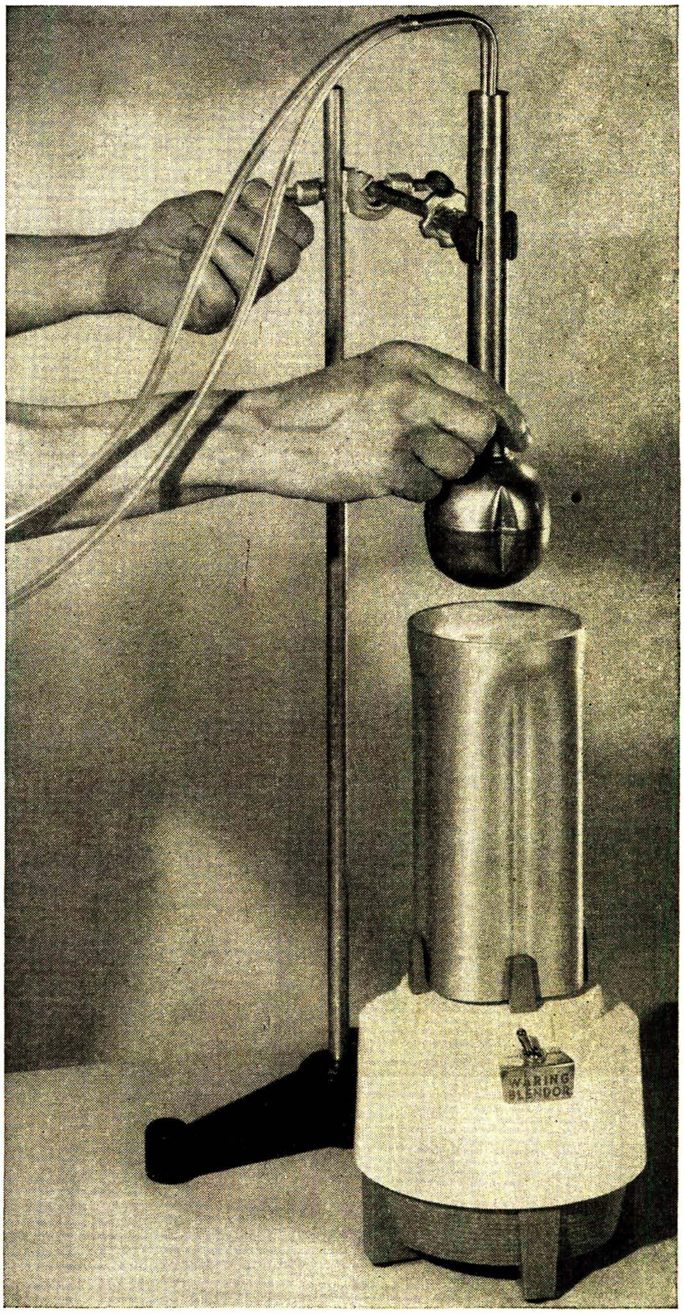
THIS NEW cenco®-*Pinto* SURFACE COOLER will double the usefulness of your Waring Blendor



This new device is designed to prevent the vaporization and splashing of liquids in a Waring Blendor, stabilizing the agitated liquid at its surface where cooling is most effective. During agitation the liquid is forced up and down outside the cooler bulb and contact soon cools the entire contents of the container. Temperature of the liquid will be maintained at 1 to 2°C. above the temperature of the circulating water. This reduces or eliminates vaporization of the solvent. It has been found excellent for rapid copra analysis.

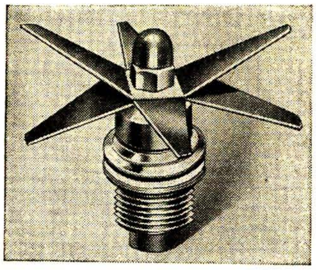
The Cenco-Pinto Surface Cooler is made of copper, attractively nickel plated and fitted with leads for attaching rubber tubing. It is designed so the cooler bulb conforms to the shape of the container, leaving just enough clearance to prevent particles from becoming entrapped. It will fit any standard Waring Blendor container of about 1000 ml capacity; bulb displacement one-half immersed is approximately 100 ml.

No. AD17249 Cenco-Pinto Surface Cooler, without support stand, each **$19.00**

No. AD17227 Waring Blendor, Two-speed Model PB-5, with "Pyrex" brand glass container of 1000 ml capacity, bakelite cover and stainless steel cutting blade assembly, for use on 115 volts, 25 to 60 cycles, AC or DC **$44.50**

Other models of Waring Blendors from **$33.20** to **$46.20.** All prices include Federal excise tax.

Our latest Bulletin No. AD86 gives full details about Waring Blendors and the Cenco-Pinto Surface Cooler as well as various types of containers and accessories. Write for your free copy today.



THE NEW cenco-*Pinto* BLADE ASSEMBLY

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No. AD17248 Cenco-Pinto Blade Assembly, fits standard Waring Blendor containers of 1000 ml capacity, each **$7.00**

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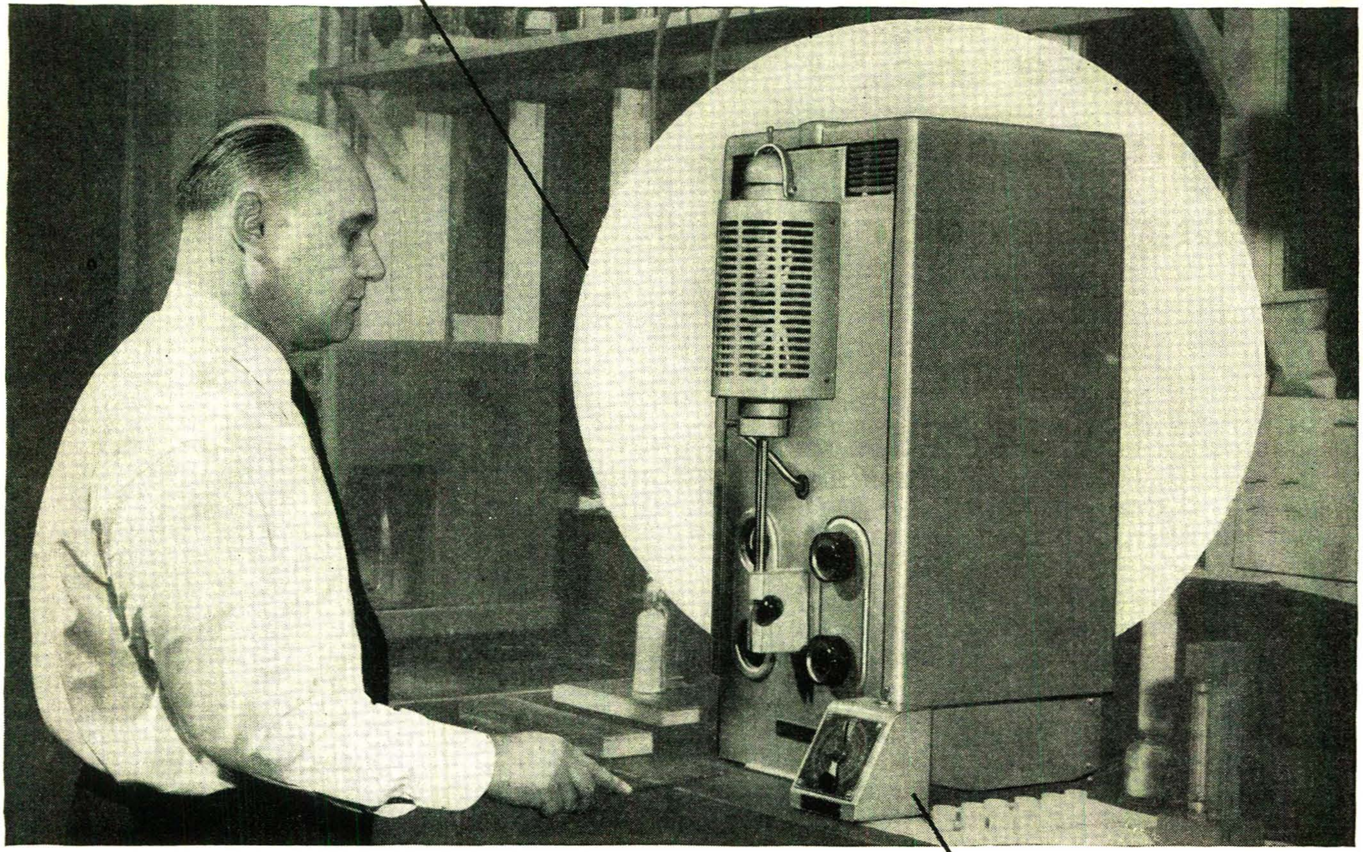

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HIGH FREQUENCY COMBUSTION UNIT

In Harrison, New Jersey, this single station Lindberg High Frequency Combustion Unit is being used with a gravimetric train for carbon analysis in the laboratories of Driver-Harris Company, nationally known manufacturer of wire, alloy fixtures and furnace strip parts sold under their registered trade-mark "NICHROME."

There are many good reasons why Driver-Harris and hundreds of other companies in all parts of the United States and Canada are using Lindberg High Frequency Combustion Units for the ignition of iron-steel samples in carbon and sulfur determinations.

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Lindberg "HF" combustion units are ready for instant use . . preliminary warm-up only once a day requires 57 seconds.

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National Lead Company Reports:

FASTER, MORE ACCURATE DETERMINATIONS with NATIONAL PRE-FORMED SPECTROSCOPIC ELECTRODES

TRADE-MARK

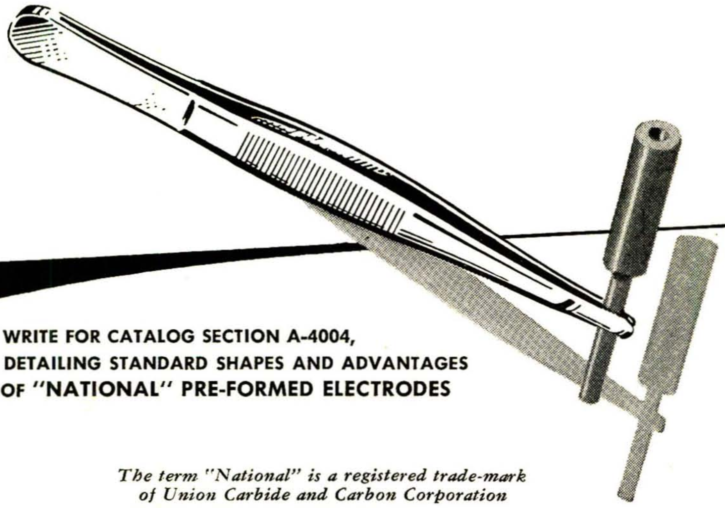
It may pay you -- in time, material and accuracy -- to investigate National Carbon Company's new PRE-FORMED spectroscopic electrodes for all types of analyses.

For example, National Lead Company, a major producer of battery-manufacturers' lead compounds and alloys, now uses "National" PRE-FORMED cup and pedestal shapes in preference to machining their own. As a result, they report savings in electrode material as well as preparation time.

Pre-formed Cups, smaller and deeper than those formerly made by National Lead from high-purity electrode stock, also give what they believe to be more sensitive readings on lead-oxide briquette samples.

Antimonial lead, another National Lead product, is regularly tested spectrographically by the spark method, using "National" counter electrodes of standard purity grade.

Uniform in quality and machined to close tolerances, "National" Pre-formed Electrodes come to you in a spectroscopically pure, rigid, air-tight container having adequate provision for maintaining purity from first to last electrode used.



WRITE FOR CATALOG SECTION A-4004, DETAILING STANDARD SHAPES AND ADVANTAGES OF "NATIONAL" PRE-FORMED ELECTRODES

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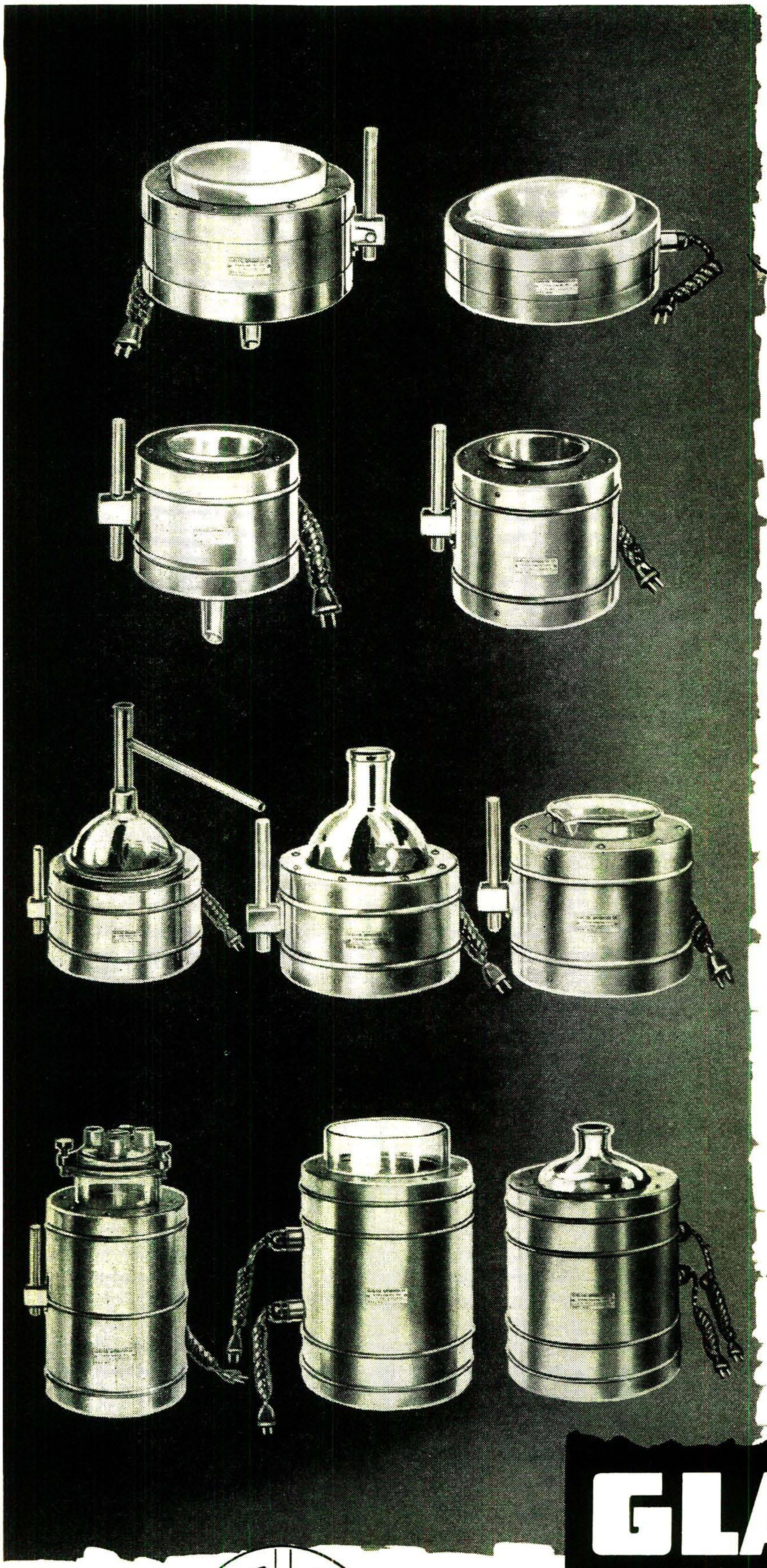

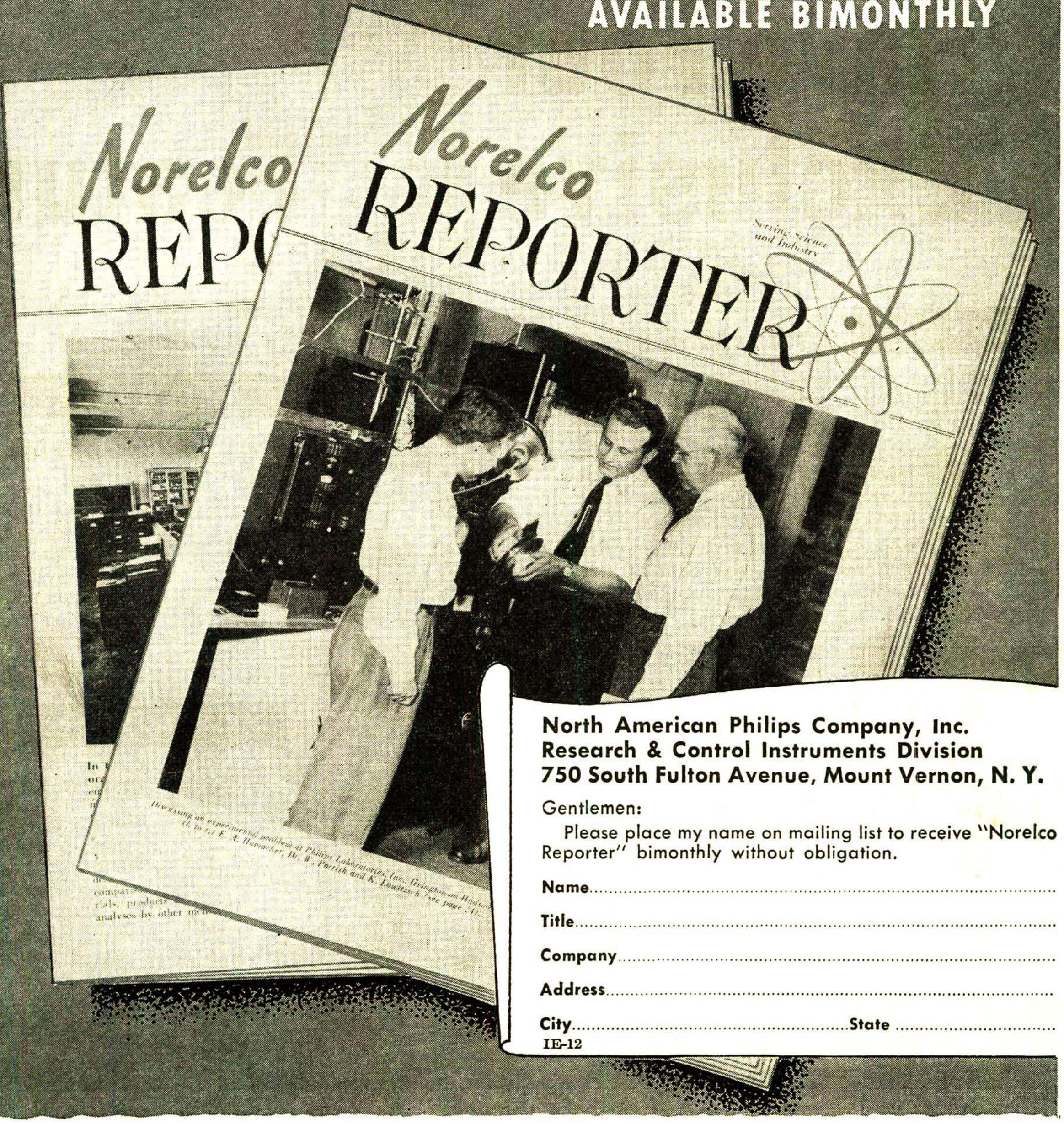
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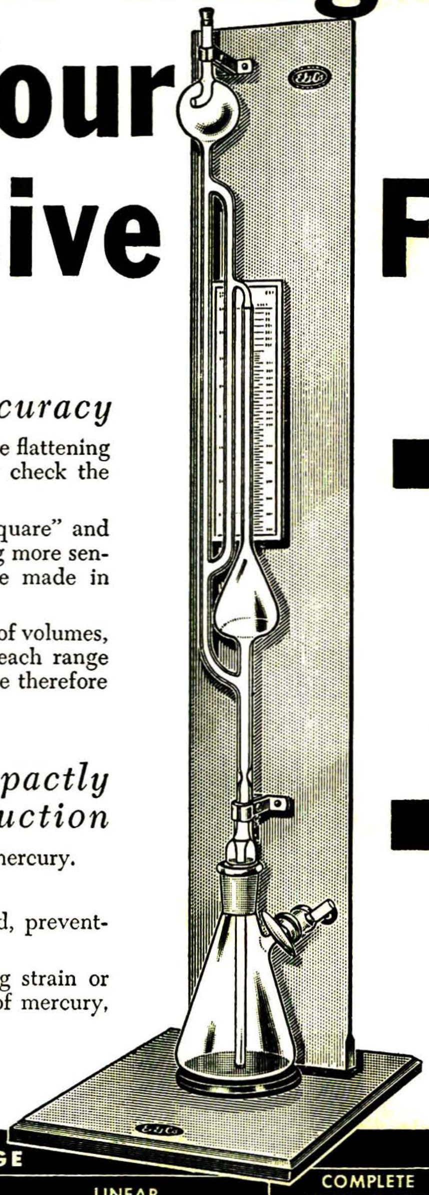
McLeod Gauges Have Four Exclusive Features



EXCLUSIVE *intrinsic accuracy*

Uniformity of bore of capillary and the flattening of upper end are made to precisely check the zero point.

Scales are graduated in both the "square" and "linear" system, the former permitting more sensitive observations. Readings can be made in microns and equivalent mm. values.

Individual precise calibration of ratio of volumes, permits *uniformity of all scales* for each range to conform with glass parts, which are therefore replaceable.

EXCLUSIVE *sturdy, compactly short, simple construction*

Trap at top preventing overflow of mercury.

Extra heavy wall mercury reservoir.

3-way stopcock with tail outlet tilted, preventing overflow of mercury in tube.

Constriction of main tube preventing strain or breakage of flask by sudden return of mercury. *All glass parts interchangeable.*

EXCLUSIVE *superior control*

Needle valve control assures precise setting of mercury level, and specially designed air filter prevents contamination of system. (Valve and absorption tube not shown in illustration are on back of panel.)

EXCLUSIVE *wide range*

For maximum sensitivity this series of gauges offers adequate selection of working range desired. See charts below.

GAUGE NO.	CATALOG NO.	SCALE RANGE		PRICES		
		SQUARE	LINEAR	COMPLETE WITH STAND	GLASS REPLACEMENTS GAUGE ONLY	GLASS REPLACEMENTS FLASK ONLY
1	G10701	.005 - 200 μ*	.005 - 0.20 MM	$87.50	$49.50	$12.50
2	G10702	.05 - 1000 μ	.02 - 1.0 MM	87.50	49.50	12.50
3	G10703	0.1 - 4000 μ	0.1 - 4.0 MM	87.50	49.50	12.50
4	G10704	.01 - 15 MM	0.1 - 15 MM	87.50	49.50	12.50
5**	G10704A	.01 - 20 MM	0.1 - 20 MM	87.50	49.50	12.50

*μ = MICRONS = $\frac{1}{1000}$ MM. HG

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The EMIL GREINER Co.
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the analyst's column

An innovation at the Chemical Show held this month in Philadelphia was a series of lectures given by experts in the chemical industries. David E. Pierce spoke the first day on progress made in engineering design during the past few years. He mentioned that while elaborate instrumentation for process control and analysis has been an outstanding development during the past few years, we may have reached a point where because of the high cost of complete instrumentation for central control, we should be thinking in terms of more manpower and less costly installations. Because scientists and engineers often become unduly fascinated by their own costly creations to the point of diminishing returns, it is well to pause and give some thought to this less glamorous point of view and to all factors involved in cost and maintenance of instrumentation programs.

Mentioning glamorous, there are certain unglamorous fundamentals in analytical chemistry which are often overlooked in our age of instrumentation and cause many of the troubles experienced by the analyst—especially if his work requires collaboration with other analytical laboratories. The commonest source of error, especially in commercial materials, is the taking of a representative sample by the production department and likewise by the analyst, himself, who assumes the sample in the bottle to be homogeneous. The second source of error is the standardization and development of an analytical procedure with an impure sample which unwittingly is assumed to be pure. Unfortunately, the analyst learns about these pitfalls, not in college, but often in his first job, where his mistake causes much consternation, if not loss of time and money, to his company.

Our first paper this month is one

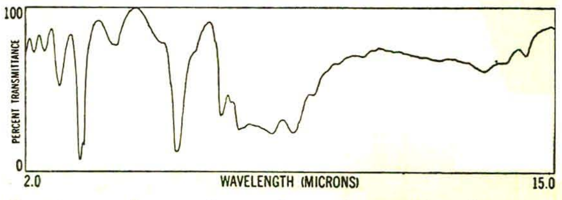
facts on Infrared *for the laboratory*

Do your coatings stand up?

The use of organic films as protective coatings for metallic containers is becoming increasingly widespread. Determining how these coatings react under different conditions is a difficult problem, but is often solved by infrared analysis.

The spectrum reproduced below demonstrates one approach to the problem. It was taken on a Perkin-Elmer Model 21 Infrared Spectrophotometer equipped with a pair of **specular reflectance attachments,** as shown in column at right. A section of the coating to be examined is placed in the attachment. The radiation passes through the coating and is reflected from the metal section back through it into the spectrometer. The resulting curve is the absorption spectrum of the coating. Differences in spectra taken before and after usage often provide valuable information on the performance of the coating. The same spectra can be recorded on any Perkin-Elmer spectrometer using the same accessory.

The determination of coating characteristics is just one example of the many problems that may be tackled by infrared analysis, provided a sufficiently versatile instrument and the proper accessories are available. Only Perkin-Elmer can provide both.



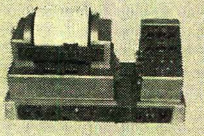
Spectrum of organic coating taken with reflectance attachment and Model 21 Spectrophotometer.

The Perkin-Elmer Corporation, 800 Main Avenue, Norwalk, Conn.
Southern Regional Office: New Orleans, Louisiana

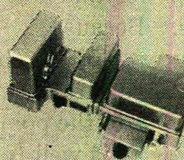
The ONLY complete range of infrared instruments and accessories to meet <u>all</u> research needs!

Model 12-C Infrared Spectrometer—a tested single beam instrument for routine control applications.

Model 112 Double Pass Infrared Spectrometer—single beam, high resolving power, excellent for structural analysis. Suitable attachments for all spectral ranges. *Write for Bulletin 102.*



Model 21 Double Beam Infrared Spectrophotometer—The fastest and most efficient instrument commercially available for the recording of large numbers of spectra. Records directly in percent transmission. *Write for Bulletin 104.*



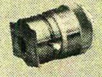
Model 13 Ratio Recording Infrared Spectrophotometer—Records double or single beam with many features of both the Model 112 and 21. Ideal for the small laboratory with a wide variety of problems. *Write for Bulletin 110.*

ACCESSORIES:

Demountable Cell—may be assembled to any thickness—for liquids, capillary films, mulls of solids—and films and powders.

Fixed Thickness Liquid Cell—for nearly all liquids and solution work. Standard thickness between 0.025 and 1 mm—others to order.

Standard Gas Cells—2, 5, 10 cm and 1 and 10 m path lengths for various absorption studies.



Microcell—obtains good spectra with 25-100 micrograms—available in thicknesses from 0.025 to 3 mm.

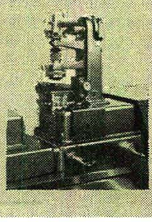
Variable Space Liquid Cell—has accurately variable path length of 0 to 5 mm to bring out features of a spectrum and to compensate for solute absorption in double beam spectrometry.

Reflectance Attachment—for specular reflectance studies of solid materials.

Pressure Cells—for liquids or gasses; path lengths from 0.025 to 50 mm, pressures up to 300 psi. Useful in trace absorption or overtone work. Long and short sizes.

Heating Stage and Heating Stage Power Supply—used with above attachment; permits heating to about 300° C. For studying polymerizations and oxidations. Cycling feature of the Model 21 automatically records reaction stages taking place.

Polarizer—for studies of crystal and molecular orientations. Rotable Cell—To assist in sample orientation for polymerization studies.



Microscope—For analysis of samples of few microns in size as single fibers, tissues, crystals, etc.

Write for Product Bulletin A-3 for more information about these accessories.

WORLD'S LEADING MANUACTURER OF INFRARED ANALYTICAL EQUIPMENT

PERKIN ELMER

on the analysis of tobacco leaves. In a field in which there is so little carefully planned scientific study, this paper strikes us as a good example of what can be done from a purely technical point of view, and it is hoped others may be spurred to undertake similar studies in this and related fields.

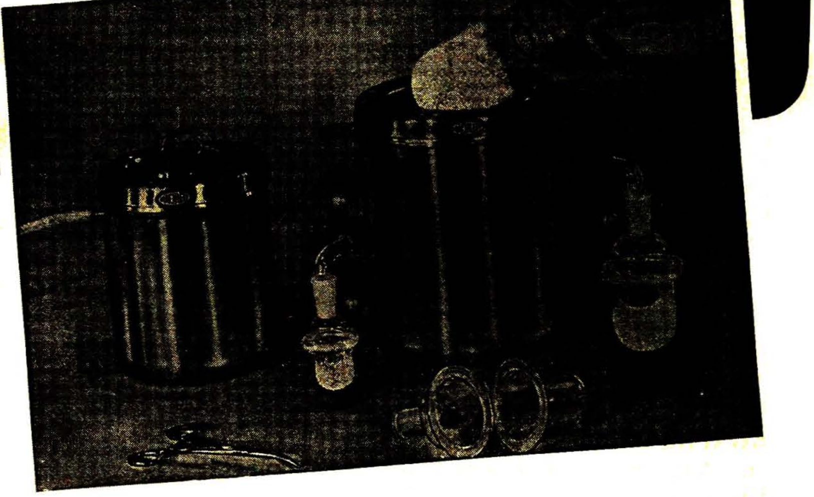
THE Society of Public Analysts and Other Analytical Chemists has provided abstracts of the literature on analytical chemistry for readers of *The Analyst* ever since the journal was first published in 1876. For the past four years these abstracts were prepared by the Bureau of Abstracts and supplied to members of the society and to subscribers to *The Analyst* under the title *British Abstracts C*. The Bureau of Abstracts will cease to exist at the end of 1953, and *British Abstracts* will not appear in their present form thereafter.

In these circumstances, the council of the society has decided to resume publication of abstracts of analytical literature. *British Abstracts C* will be replaced in the new year by a new publication to be called *Analytical Abstracts*. This will be edited by Norman Evers, B.Sc., Ph.D., F.R.I.C., and published by W. Heffer & Sons, Ltd., for the society, and it will cover the whole range of analytical literature in the same way as did *British Abstracts C*.

Analytical Abstracts will appear each month bound separately from *The Analyst* and in a format similar to that of the abstracts included in *The Analyst* prior to 1950. The two journals, *The Analyst* and *Analytical Abstracts*, will be available in 1954 to members of the society and to subscribers on the same terms as obtained for *The Analyst* and *British Abstracts C* in 1953. They will also be sold separately at 50s. ($7.00) per annum, including the index, or at 60s. ($8.40) per annum, printed on one side only, without the index.

Science Editor

FREEZE-DRYING?



TRY THE MACHLETT WAY

A completely self-contained unit, this new, VIRTIS, stainless steel apparatus does not require the conventional dry ice chamber or condenser, etc. Its inner 2.8 liter cooling chamber holds enough dry ice and solvent for unattended overnight operation. And, furthermore, because of its large capacity, it lends itself readily to shell freezing (see illustration).

Expertly made of polished 18-8 stainless steel the apparatus is fitted with 12 take-off taps capable of removing a minimum of 100 ml of water.

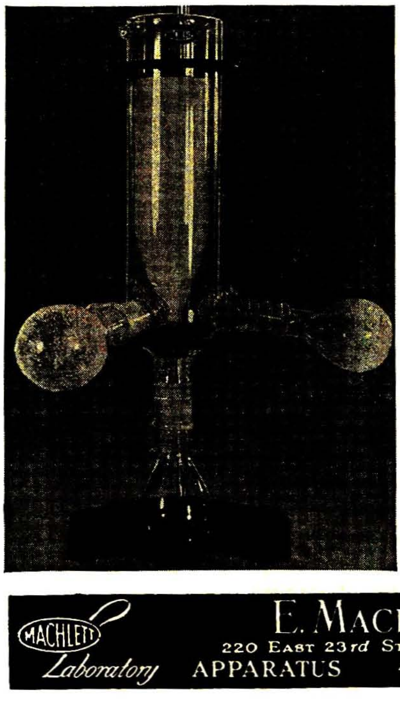
For added simplicity, freeze-drying flasks are designed with a wide mouth to facilitate easy removal of dried materials.

E36-693 Stainless Steel Drying Unit as illustrated, but without trap and flasks	$130.00
E36-683A Stainless Steel Trap	35.00

Specially Designed Freeze-Drying Flasks

E36-693B Flask, 25 ml	$ 8.00
E36-693C Flask, 50 ml	8.60
E36-693D Flask, 100 ml	8.80
E36-693E Flask, 250 ml	12.50
E36-693F Flask, 500 ml	14.00
E36-693G Adapters for flasks	2.50

Machlett All-Glass Freeze-Drying Apparatus



This economical self-contained unit is ideally suited for average freeze-drying operations. Standing only 21" high, it has a built-in dry ice and solvent chamber and three conveniently located ground, standard taper drying ports around its lower periphery. Standard size for joints is 34/45, but special sizes can be ground to your specifications.

E36-695 Machlett All-Glass Freeze-Drying Unit with support stand and rings, four flasks, 100, 200 or 250 ml capacity	$64.00
E36-695A Machlett Freeze-Drying Unit only	$42.00
E36-695B Freeze-Drying Flasks 100, 200, or 250 ml	3.00
E36-699F Support Stand and rings	10.00

All prices F.O.B. New York, N. Y.
For further information write for Bulletin F-D.

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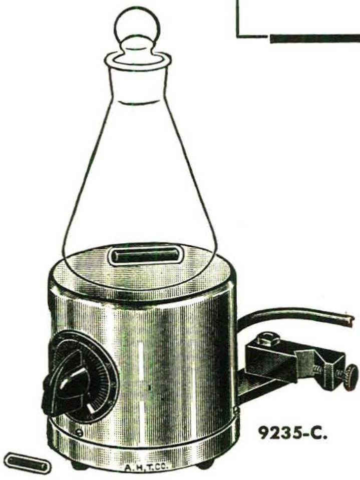
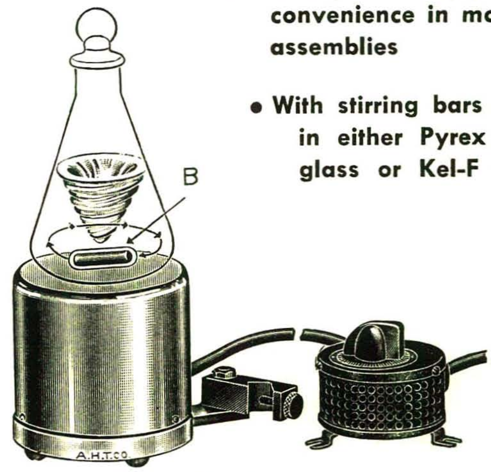

9235-C.

- **Now offered either with enclosed rheostat or with separate rheostat for control at a distance**
- **Swivel joint clamp provides convenience in mounting assemblies**
- **With stirring bars sealed in either Pyrex brand glass or Kel-F plastic**



9235-R.

MAGNETIC STIRRING APPARATUS, A.H.T. Co. Specification. A compact, quiet-running apparatus which utilizes a rotating field of magnetic force to induce variable speed stirring action *within either closed or open vessels.* Now offered in two executions, i.e. with either enclosed rheostat, which will facilitate manipulation in some assemblies; or with separate rheostat for control of speed at a distance of approximately 30 inches from stirrer.

Stirring is accomplished by means of a small magnetized bar, sealed either in heavy wall Pyrex brand glass or in Kel-F plastic, which is placed in the liquid to be stirred and which is rotated by magnetic force applied beneath the container. This force consists of a permanent bar magnet attached to the shaft of an electric motor and mounted in an aluminum housing with flat top 4-⅜ inches diameter and 4-½ inches high, on cast metal base. Can be used either on the table or on a support rod, attached by means of a clamp with swivel joints.

Suitable for any stirring operation which involves 1 ml to 1 liter of liquids with viscosities up to that of a 50% glycerol solution. Particularly convenient for use in closed systems. Any type of vessel of glass, porcelain or non-magnetic metal can be used. A ring-type burner can be used when stirring at elevated temperatures.

9235-C. Stirring Apparatus, Magnetic, with enclosed rheostat, A.H.T. Co. Specification, as above described. Rheostat, with graduated dial, is mounted in stirrer housing. Complete with two magnetized Stirring Bars, i.e. one Kel-F coated, 7/8-inch long x 1/4-inch diameter; the other Pyrex brand glass coated, 1-3/4 inches long by 7/16-inch diameter; also 5 ft. 3-wire connecting cord and conventional 2-prong plug cap and directions for use. Power consumption 7 watts; for 115 volts, 60 cycles, a.c. only. Without glass vessel **36.80**

9235-G. Ditto, but without Stirring Bars **32.00**

9235-R. Stirring Apparatus, Magnetic, Original Model, A.H.T. Co. Specification, similar to 9235-C but with separate rheostat for control of speed at a distance of approx. 30 inches. Complete with two Stirring Bars **38.50**

9235-S. Ditto, but without Stirring Bars **33.40**

MAGNETIZED STIRRING BARS

STIRRING BARS, MAGNETIZED, Kel-F Coated, Vacuum Tested, A.H.T. Co. Specification, for use with above Magnetic Stirrers. Consisting of Alnico Type V, cylindrical, permanent magnet sealed in transparent, durable shell of Kel-F, a polymer of trifluorochloroethylene resin, specific gravity 2.1, which has negligible water vapor transmission. This non-inflammable thermoplastic is suitable for use at temperatures from −200° to +200°C., and is unaffected by prolonged exposure to weak and strong acids, including concentrated sulfuric, hydrofluoric and hydrochloric acids, fuming nitric acid, aqua regia and other powerful oxidizing agents, concentrated alkalies and mineral oils. It is equally resistant to most organic solvents but is swelled slightly by highly halogenated materials and some aromatics. **9235-U7. Stirring Bars, Magnetized Kel-F Coated,** as above described.

Overall length, approx. inches	7/8	1-5/8	2
Diameter, approx. inches	1/4	5/16	5/16
Each	**2.20**	**2.55**	**2.75**

10% discount in lots of 12 or more, one size or assorted

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ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

Advisory Board Appointments

THE idea of rotation in the make-up of the Advisory Board of ANALYTICAL CHEMISTRY is a sound one, yet it is always with keen regret that we part with those who complete their terms. Those whose formal services on the board terminate in 1953 are P. J. Elving, Wayne A. Kirklin, and Edward Wichers. They have been a tower of strength to the editorial staff.

Two important board innovations will take place with the start of the new year. The number of board members will be increased from twelve to fifteen. Each member will serve three years instead of four. This plan will make it possible to have more fields of specialization represented on the board.

At this time, six new members instead of the customary three are being appointed. At the first meeting of the board, the new members will draw lots to determine terms of service of one, two, and three years.

The editors are happy to report that the following have accepted invitations to become board members: Hugh F. Beeghly, Jones and Laughlin Steel Corp.; George E. Boyd, Carbide and Carbon at Oak Ridge; A. Q. Butler, Mallinckrodt Chemical Works; Robert Kunin, Rohm & Haas; Philip W. West, Louisiana State University; and John H. Yoe, University of Virginia.

Those who continue to serve are S. E. Q. Ashley, Gordon MacKinney, and Grant T. Wernimont (1951–54); N. H. Furman, David N. Hume, and Stewart S. Kurtz, Jr. (1952–55); James J. Lingane, Wayne W. Hilty, and Vernon A. Stenger (1953–56).

Glances Backward and Forward

FORTUNATELY, there is a period in the year when it is customary to pause, if only for a few hours, in order to reflect on the blessings we have received and to thank our relatives, friends, neighbors, and associates for the many services and acts of kindness they have performed for us.

In that spirit, the editors take the opportunity to thank the Advisory Board members, reviewers, authors, and readers. We want to also thank those who are responsible for the mechanical side of the journal. There are many detailed steps that must be taken after a manuscript has been accepted before a publication reaches the subscriber. The printer is the unsung hero, given attention only when errors occur.

The past 12 months indeed have been fruitful ones for the field of analytical chemistry. Scientifically, many new and significant advances have been reported. Meetings of analysts in various parts of the country have attracted record numbers. The Division of Analytical Chemistry of the AMERICAN CHEMICAL SOCIETY continues to grow and prosper. Last, but certainly not least, the prestige of the analyst continues to increase in a very satisfactory manner.

The year 1953 has witnessed a number of comparatively new trends expanding at a lively rate. This is specially true of plant "automation." The coming year will see many new developments in this general direction. Here is a golden opportunity for the analyst to increase still further his responsibilities and to demonstrate how essential his services are to industry.

One or two other trends warrant comment at this time. We are impressed by the continuing interest in analytical chemistry and its fields of specialization at the local section level and the increase in the number of sections with analytical chemistry subdivisions. We are also pleased to note the increase in the number and quality of analytical papers appearing on regional meeting programs such as the Southwest-Southeast Regional Meeting in New Orleans this month.

ANALYTICAL CHEMISTRY in 1953 is publishing 2090 editorial pages, as compared with 2158 in 1952. The Board of Directors has authorized a slight increase in the number of pages for 1954 which, it is hoped, will permit the editors to maintain the journal on a reasonably prompt publication basis.

Analytical Studies on Harvested Tobacco Leaves

W. G. FRANKENBURG, A. M. GOTTSCHO, SUZANNE KISSINGER, DORIS BENDER, AND MARGARET EHRLICH
Research Laboratory, General Cigar Co., Inc., Lancaster, Pa.

Because existing procedures for the analytical determination of the nitrogen-containing compounds in tobacco do not cover all its nitrogenous constituents, and because of the possible biochemical and industrial significance of these undetermined compounds, a scheme of analysis was devised to permit not only determination of the known constituents, but also classification of the unknown nitrogen compounds in tobacco leaves. This comprehensive scheme involves the stepwise removal of definite nitrogenous fractions from a water extract of a tobacco sample and the determination of the nitrogenous compounds in these fractions. The method is of value for the exploration of the nitrogen compounds contained in different types of tobacco at various stages of their industrial processing and gives a complete accounting of the changes in nitrogenous compounds that occur during these stages. The method may lend itself equally well for nitrogen determination in other plant material.

AMONG the chemical changes that occur during the industrial processing of cigar tobacco leaves, the conversion of the nitrogenous leaf components plays a significant role, both qualitatively and quantitatively (*23, 24*). In previous communications from this laboratory (*26–28, 30*) attention was drawn to some formerly unknown chemical changes of this kind, such as the transformation of nicotine into various other pyridine derivatives, and the change of some water-soluble nitrogenous leaf constituents into water-insoluble products (*25*). These specific reactions were investigated as a part of a more general study of the entire nitrogen catabolism in harvested tobacco leaves.

In the course of this work, it appeared essential to develop a comprehensive analytical procedure for determining the various nitrogenous components in tobacco leaves at various stages of their processing. After extensive investigation of different possible analytical methods, two independent procedures have been developed that give satisfactory information on the amounts of the different nitrogenous substances in tobacco leaves.

The first of these procedures is described elsewhere (*26, 30*); it serves specifically for the isolation and separation of large quantities of alkaloids and alkaloid transformation products and is based on a stepwise extraction of the tobacco leaves with organic solvents.

FRACTIONATION OF WATER-SOLUBLE NITROGENOUS LEAF COMPONENTS

In this publication, the second, more general, procedure is described. It starts with a water extraction of a given tobacco sample and continues with the successive separation and determination of all the nitrogenous components contained in this sample.

BALANCE OF TOTAL NITROGEN

The dependability and completeness of the stepwise analytical procedure can be tested by summing up all the individual increments of nitrogen found in the fractions of a given leaf sample, and by comparing this sum with the value found for the total nitrogen of the same sample as obtained by its direct kjeldahlization. Provided that none of the nitrogenous leaf constituents has escaped the analysis and that the determinations of the individual components were sufficiently accurate, both nitrogen values should check within the limits of the over-all analytical error.

In Table I are listed the analytical values for the total nitrogen content of various samples of powdered tobacco leaves, determined for each sample by three different procedures. The total nitrogen (TN) values were obtained by direct Kjeldahl digestions of the original samples. The sums of the total water-insoluble nitrogen and the total water-soluble nitrogen were determined by kjeldahlization. The Σ values were obtained by summing up the nitrogen of all the separate nitrogenous components determined by the stepwise analytical scheme described in this paper. Considering that in this scheme such divergent analytical procedures are employed as titrations, measurements of ultraviolet absorption spectra, Nessler determinations, and Kjeldahl digestions, the agreement among TN, S, and Σ appears satisfactory.

FLOWSHEET OF STEPWISE PROCEDURE

The flowsheet shown in Figure 1 illustrates the main steps of the analytical scheme. An aqueous extract, B, of the tobacco sample, A, is carried through a series of analytical operations to the final stage, P, by means of successive withdrawals of various fractions, each of which contains another class of nitrogenous leaf components.

Table I. Total Nitrogen of Cigar Tobacco Samples

(Comparison of three independent determinations)

Total Sample No.	Total Nitrogen Content, Mg./G. Dry Tobacco Weight			Deviation from TN, %	
	TN[a]	S[a]	Σ[a]	Of S	Of Σ
(1)	(*2a*)	(*2b*)	(*2c*)	(*3a*)	(*3b*)
1[b]	37.4	37.4	37.4	0.0	0.0
2[b]	38.0	38.0	37.4	0.0	−1.6
3[b]	37.8	38.1	37.6	+0.8	−0.5
4[b]	35.0	35.0	34.7	0.0	−0.9
5[b]	45.4	44.9	44.7	−1.1	−1.5
6[b]	46.0	45.9	45.3	−0.2	−1.5
7[b]	34.6	34.8	35.4	+0.6	+2.3
8[b]	55.9	55.9	55.3	0.0	−1.1
9[b]	51.9	51.9	51.5	0.0	−0.8
10[b]	36.2	36.1	35.3	−0.3	−2.5
11[c]	47.4	46.9	48.2	−1.1	+1.7
12[d]	41.9	42.2	41.3	+0.7	−1.4
13[d]	39.8	39.8	39.4	0.0	−1.0
14[e]	40.5	...	40.3	...	−0.5
Average deviation				±0.37	±1.23

[a] TN. Values obtained by direct kjeldahlization of total sample.
S. Sums of water-soluble and water-insoluble nitrogen, each found by Kjeldahl.
Σ. Sum of nitrogen of all separate components determined by stepwise analytical scheme.

[b] Fermented and unfermented Pennsylvania seedleaf tobacco, 1941 crop.

[c] Green, freshly harvested Pennsylvania seedleaf tobacco, 1950 crop. Leaves, before grinding, were scalded with boiling water for 30 seconds to deactivate enzymes. The midrib was removed and leaves were dried in a Freas-type forced draft oven at 100° C.

[d] Green Connecticut shade wrapper tobaccos 1950 and 1951 crops (treated in same manner as green Pennsylvania tobacco).

[e] Thoroughly fermented, dark fired tobacco used in manufacture of snuff, kindly supplied by W. B. Bennett, United States Tobacco Co.

These are the main steps:

From the dried and weighed sample of tobacco powder A, the water-soluble compounds are extracted with boiling 0.05*N* sulfuric acid. The nitrogen contained in the water-insoluble fraction, C, is determined. Work is in progress on the analytical differentiation of the water-insoluble nitrogenous compounds in fraction C.

The water extract, B, is analyzed as follows: Concentration at pH 3 to 3.5 leads to the formation of small amounts of a brownish amorphous "acid precipitate," E, whose nitrogen content is determined separately. The filtrate or "concentrated water extract," D, is brought to a pH of about 6.5, magnesium oxide is added, and a steam distillation is carried out to remove nicotine and ammonia that are determined in distillate G. The distillation residue, F, is filtered and the nitrogen content of the solid filtration residue, I (magnesium oxide–nitrogen), is determined by Kjeldahl. The filtered distillation residue, E, contains, besides other nitrogen compounds to be determined later, the less volatile alkaloids, certain alkaloid transformation products, and other nitrogenous substances that have the common property of yielding precipitates with silicotungstic acid (STA). Enough silicotungstic acid is added to precipitate these substances, and precipitate K is analyzed for its total nitrogen, for the less volatile alkaloids, and for alkaloid transformation products. The filtrate, J, of the "silicotungstic acid precipitate" is subjected to a steam distillation in the presence of a 5% sodium hydroxide solution, and the distillate, N, is analyzed for ammonia. The distillation residue, L, is neutralized, magnesium oxide and Devarda alloy (*13*) are added, and the mixture is subjected to another steam distillation.

By this procedure, the nitrates contained in L are reduced to ammonia and determined in the distillate, O. The distillation residue, N, contains a muddy solid phase, Q, consisting of magnesium oxide and particles of reacted Devarda alloy, together with small amounts of nitrogenous substances. This "Devarda mud" is filtered off and kjeldahlized. The filtrate, P, represents the final fraction of the scheme. It contains the nitrogen of amino acids and of all those soluble nitrogen compounds that have not been determined up to this point. The nitrogen content of this final fraction is determined by Kjeldahl.

An aliquot is taken from fraction P for the determination of the α-amino nitrogen of amino acids according to the method described by Van Slyke in 1929 (*66*). Another aliquot of P serves for the determination of nicotinic acid.

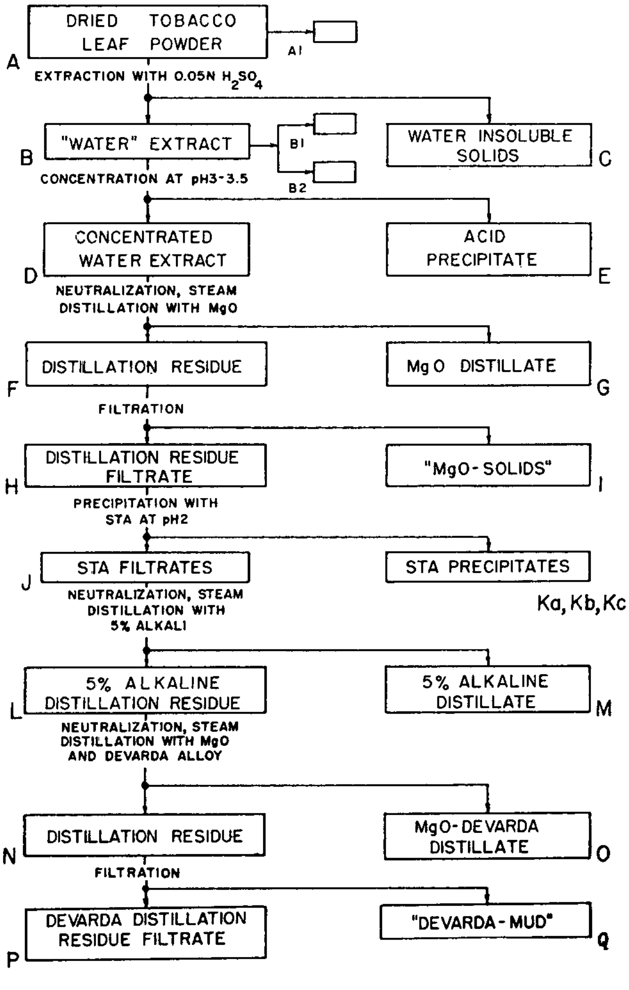

Figure 1. Flow Sheet of Stepwise Analytical Scheme

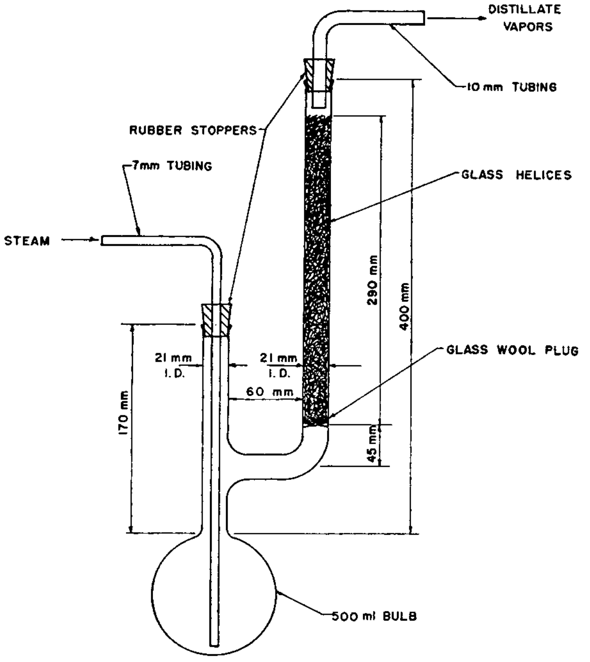

Figure 2. Special Claisen-Type Distillation Bulb

In this procedure the total ammonia found in distillates G and M represents the sum of the free ammonia present in the tissue as ammonium ion and of the "artifact" ammonia derived from (1) the amide nitrogen of glutamine, (2) the amide nitrogen of asparagine, and (3) unknown sources.

APPARATUS

All steam distillations are carried out in the conventional setup for steam distillation, except that the samples are distilled from a specially adapted Claisen flask fitted with a column densely packed with glass helices (helix diameter 5 mm., made of glass rod 1 mm. in diameter). The details of construction of this flask are shown in Figure 2. Spectrophotometric measurements are made with a Beckman DU spectrophotometer after calibration of the instrument and correction of the cells. A Beckman Model B pH meter is used for all pH measurements.

MATERIALS AND REAGENTS

Water is freed of analytically detectable traces of ammonia and other nitrogen compounds by redistillation in the presence of 0.5 gram of sulfuric acid and 1 gram of potassium permanganate per liter.

Devarda alloy, 20-mesh and finer.

Ethyl alcohol, 95%.

Ether, analytical reagent.

Hydrochloric acid (1 + 4). One part of hydrochloric acid, C.P., is diluted with 4 parts by volume of redistilled water.

Indicator, 125 mg. of methyl red and 82.5 mg. of methylene blue dissolved in 100 ml. of 95% ethyl alcohol (*64*). The solution is filtered if necessary.

Magnesium oxide, U.S.P., light.

Silicotungstic acid solution, 12%, 120 grams of silicotungstic acid ($SiO_2.12WO_3.26H_2O$, ACS) dissolved in 1 liter of redistilled water.

Sodium carbonate, anhydrous, analytical reagent.

Sodium hydroxide, pellets, analytical reagent, ACS.

Sodium hydroxide, 0.5*N* solution. Two hundred grams of sodium hydroxide pellets are dissolved in 9 liters of redistilled water. The exact titer of this base is determined by titration against standardized 0.5*N* sulfuric acid.

Sodium hydroxide, 0.05*N* solution. One liter of 0.5*N* sodium hydroxide is diluted to 10 liters, using redistilled water. The titer of this diluted base is determined by titration against standardized 0.05*N* sulfuric acid.

Sulfuric acid, 0.1000*N* solution, standardized acid purchased from General Chemical Co.

Sulfuric acid, 0.5*N* solution, 122 ml. of concentrated sulfuric acid, analytical reagent (low in nitrogen) diluted to 9 liters with redistilled water. This acid is standardized by titration with a standard sodium carbonate solution. The exact normality of the latter is calculated from the weight used and is checked with the purchased 0.1000*N* sulfuric acid. The titer of the 0.5*N* acid is then determined by titrating this acid with the standard sodium carbonate solution.

Sulfuric acid, 0.05*N* solution. One liter of 0.5*N* sulfuric acid is diluted to 10 liters with redistilled water. The titer of this solution is determined by titrating it with the standard sodium solution diluted tenfold.

Sulfuric acid, 0.005*N* solution. Sulfuric acid, 0.05*N*, diluted tenfold with redistilled water. No standardization is necessary.

DETAILED PROCEDURE

Preparation of Sample. The selection of the tobacco samples and the sampling procedure are described elsewhere.

In analyses of the leaf blades, the mid-ribs of the leaves are removed while the leaves are still pliable. After this "stripping" operation, the leaves are embrittled by first drying them at room temperature for 10 to 15 hours, followed by a second drying for 0.5 hour at 68° C. in a forced draft oven. They are then ground in an attrition mill to a powder (passing a 2-mm. mesh), which is thoroughly mixed and stored in screw-capped glass containers in a refrigerator at ca. 5° C. For analysis small portions are withdrawn as required from these larger samples.

The tobacco powder is dried prior to analysis. This final drying must be done in such a manner as to assure reproducible drying conditions regardless of the size of the sample being analyzed. Aluminum dishes 50 mm. in diameter and 23 mm. high are used for samples up to 1.5 grams. Samples up to 4.5 grams in size are dried in aluminum dishes 90 mm. in diameter and 20 mm. high. Samples of still larger size are dried in portions.

For a complete analysis, a sample of ca. 11 grams of tobacco powder is dried in four separate portions as described above for 0.5 hour at 68° C. in the forced draft oven. A sample of this size permits the withdrawal of several adequate aliquots for side determinations during the course of the stepwise analysis. At the same time two samples of ca. 800 mg. each are dried and weighed for the determination of total nitrogen in duplicate, and a third 500-mg. sample is used for the determination of ammonia by the method of Pucher, Vickery, *et al.* (*54, 71*) (subfraction A1, Figure 1).

Extraction and Filtration. Each of the four portions (two of about 1 gram each and two of about 4.5 grams each) of the dried 11-gram sample is weighed and carefully transferred into an Erlenmeyer flask. To each flask 0.05*N* sulfuric acid, 35 ml. for each gram of tobacco powder in the flask, is added and the mixture is refluxed over a low flame for 1 hour. From time to time the flasks are shaken to wash any particles adhering to the walls of the flask into the liquid.

At the end of the extraction, the flasks are cooled and the extracts filtered. Each of the four portions is filtered separately on a Büchner funnel fitted to a 1-liter suction flask because the four aliquots of the water-insoluble fraction, C, remaining on the filters are used for different determinations. The extracts and washings are combined. Each portion of the insoluble solids is washed with a minimum of 50 ml. of 0.005*N* sulfuric acid per gram of original tobacco sample. In these filtrations, the extracts tend to foam and spatter on entering the suction flask. Losses due to this effect are eliminated by lengthening the usually short stem of the Büchner funnel.

Water-Insoluble Solids, C. This fraction serves for the determination of the weight of water-insoluble solids from which the weight of water-soluble solids is calculated, and the determination by Kjeldahl of the insoluble, or, as it is usually called, the "protein" nitrogen. For the first determination, the filtration residue is weighed after it has been air-dried for 24 to 48 hours at room temperature, and then for 0.5 hour at 68° C. in a forced draft oven. To determine the insoluble nitrogen in duplicate samples, each of the wet residues of the 1-gram aliquots is transferred to a 500-ml. Kjeldahl flask, covered with 4*N* sulfuric acid, and immediately reduced with iron in preparation for kjeldahlization according to the procedure described later.

Water Extract B. The extracts may range in color from greenish yellow (green tobacco samples) to dark brown (fermented samples). The individual extracts and washings of the aliquots of the 11-gram sample are combined and made up to a volume of 1000 ml. From the combined extract, B, an aliquot is taken corresponding to 0.5 gram of tobacco powder for determining the total soluble nitrogen by the modified Kjeldahl procedure described later. Two more aliquots, B_1 and B_2, of the same size are withdrawn for the determination of glutamine amide nitrogen and of asparagine amide nitrogen by the method of Pucher, Vickery, *et al.* (*54, 71*).

The remainder of the water extract (at a pH of ca. 4 to 4.5), now corresponding to ca. 9.5 grams of tobacco powder, is brought to a pH between 3 and 3.5 with hydrochloric acid (1 + 4) and is concentrated to a volume between 180 and 200 ml. (18 to 20 ml. per gram of sample). The acid precipitate, E, is filtered off after the concentrated extract has stood overnight at 5° C., washed with a small quantity of water (10 to 20 ml.), and then kjeldahlized. The combined filtrate with the added washings is the concentrated water extract, fraction D.

Steam Distillation of D. After the pH of fraction D has been adjusted with sodium hydroxide to a value between 6 and 7, the solution (ca. 225 ml.) is placed in the distillation bulb (shown in Figure 2); magnesium oxide is added (250 mg. per gram of tobacco powder being distilled), and the apparatus is quickly assembled. The receiver is charged with a measured excess of 0.05*N* sulfuric acid. The distillation is started without the use of steam, and the sample is concentrated to 150 ml., at which time steam is introduced. The rate of distillation is adjusted by controlling the steam supply and the heating of the electrically heated distillation bulb so that approximately 400 ml. of distillate are collected per hour, keeping the volume in the flask at 150 ml. When ca. 600 ml. of distillate have been collected, the bottom heat on the distillation bulb is increased so as to concentrate the sample to approximately 75 ml. The distillation is then carried to completion—i.e., until a few drops of the distillate, on addition of 2 drops of hydrochloric acid (1 + 4) and 1 drop of silicotungstic acid (12%) show no signs of turbidity. The distillation residue, F, is cooled; the solids, I, are filtered off, washed, and kjeldahlized.

The Magnesium Oxide Distillate, G, is mixed thoroughly. Aliquots are withdrawn for determining ammonia and nicotine. The former is determined by the modified Nessler method described below; the latter, by ultraviolet spectrophotometry according to Willits *et al.* (*79*). The remainder of the distillate is titrated with 0.05*N* sodium hydroxide after addition of a few drops of indicator solution. Titration of a blank containing the same amount of 0.05*N* acid as that used for the distillate provides a standard with which the color shade of the distillate is compared at the end point of its titration. The difference between the amounts of 0.05*N* sodium hydroxide required for the neutralization of the blank and for the titration of the distillate is expressed

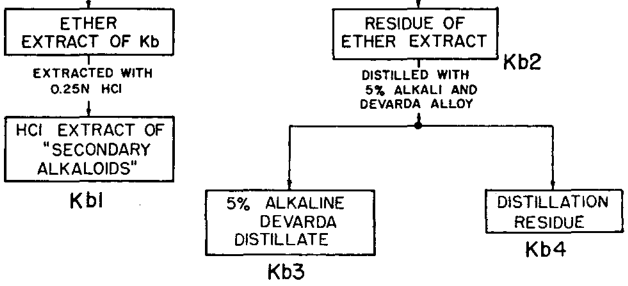

Figure 3. Detailed Analysis of Silicotungstic Acid Precipitate Kb

in milligrams of "total titratable" nitrogen. The latter represents the sum of the ammonia nitrogen and of one half of the nicotine nitrogen contained in the distillate.

The Filtrate of the Distillation Residue, H, light brown in color, is brought to a pH of 2 with hydrochloric acid (1 + 4), made to a volume of 250 ml., and then divided into three parts, two of 75 ml. each (*a* and *b*) and one of 100 ml. (*c*), corresponding to two aliquots of ca. 2.8 grams and one of ca. 3.8 grams of tobacco. To each of these three aliquots is added 1 ml. of silicotungstic acid solution (12%) per gram of tobacco powder. The samples are then placed in a refrigerator at 5° C. for at least 24 hours, after which time they are filtered, the two smaller precipitates (designated as Ka and Kb) on filter paper, the larger one (designated as Kc) on a weighed fritted-glass filter crucible. The three filtrates are returned to the refrigerator for an additional 24 hours, after which time any additional precipitates are filtered off. Each of the three precipitates is washed with 35 ml. of ice-cold dilute hydrochloric acid (1 ml. of concentrated acid made to 1 liter) and each washing is combined with its respective filtrate.

Silicotungstic Acid Precipitates, Ka, Kb, and Kc. Ka is kjeldahlized, yielding the total nitrogen contained in the silicotungstic acid precipitate.

Precipitate Kb is dissolved in 5% sodium hydroxide (w./v.). (See Figure 3 for the scheme of analysis of precipitate Kb.) From this solution the nonnicotine alkaloids are extracted in a liquid-liquid extractor with ethyl ether. The rate of extraction is adjusted so that about 4.5 ml. of ether pass per minute through the aqueous solution. After 21 hours of extraction, the substances in the ether extract are in turn extracted in a separatory funnel with hydrochloric acid (ca. 0.25*N*) and the alkaloid content of this acidic extraction (fraction Kb1) is determined by quantitative evaluation of its ultraviolet absorption spectrum. The results are expressed in terms of "secondary alkaloid nitrogen." After this removal of the nonnicotine alkaloids, the alkaline residue (KB2) of the ether extraction is allowed to stand for 24 hours at room temperature to remove most of the ether, whereupon it is transferred to a distillation flask (Figure 2). Two grams of Devarda alloy are added, and the sample is steam-distilled into 0.05*N* sulfuric acid, a constant volume being maintained in the distillation flask. The oxynicotine present in the solution is thereby reduced to nicotine. The nicotine and small amounts of ammonia contained in the distillate (Kb3) are analyzed in the same way as distillate G.

Precipitate Kc is dried to a constant weight at 110° C. and transferred from the glass crucible into a weighed platinum crucible. As this transfer from crucible to crucible is not completely quantitative (small particles of the precipitate adhere tenaciously to the glass walls and pores of the fritted-glass filter), the exact tobacco aliquot corresponding to the precipitate in the platinum crucible has to be recalculated. Precipitate Kc is then ignited for 1 hour at 650° ± 25° C. in a muffle furnace (*32*, *62*), cooled, and weighed again. The weights of Kc, after drying at 100° C. and after ignition, are expressed in milligrams per gram of tobacco powder.

Frequently during the ignition, the dried silicotungstic acid precipitate will fuse to the platinum crucible, making cleaning somewhat difficult. This can be avoided by covering the bottom of the crucible with a disk of ashless filter paper before the precipitate is placed in the crucible. If necessary, cleaning can be accomplished by adding a mixture of ca. 50% sodium carbonate (anhydrous) and 50% potassium carbonate (anhydrous) to the desired height in the crucible, fusing in an electric furnace, and quenching the hot melt in cold water. The crucible is then rinsed with distilled water. The use of a platinum Gooch crucible with an asbestos mat for drying and for igniting the precipitate may overcome these difficulties.

Silicotungstic Acid Filtrate J. Since for the subsequent determinations on the silicotungstic acid filtrate, a sample corresponding to 3 to 4 grams of tobacco powder is sufficient, the filtrate of precipitate Kc, corresponding to an aliquot of ca. 3.8 grams, is used. The two filtrates from precipitates Ka and Kb may be used for side analyses or for check determinations. Filtrate J is neutralized, 7.5 grams of sodium hydroxide pellets are added, and the sample is distilled with steam, maintaining the distillation volume at 150 ml. and thus establishing a 5% concentration (w./v.) of sodium hydroxide during the distillation. Completeness of the distillation is indicated by a negative check of a few drops of the distillate with phenolphthalein.

Distillate M collected in an excess of 0.05*N* sulfuric acid contains ammonia which is determined both by nesslerization of an aliquot of the distillate and by titration of the remainder in the manner described for distillate G.

Magnesium Oxide-Devarda Distillation. The distillation residue, L, is neutralized in the distillation flask. A gellike precipitate which had formed when the solution had been made alkaline for the previous distillation dissolves when the distillation residue is neutralized. One gram of magnesium oxide and 2 grams of Devarda alloy per gram of tobacco sample are added and the sample is steam-distilled into an exces sof 0.05*N* sulfuric acid. Under these conditions, the evolution of hydrogen occurs at a slow rate. Steam is introduced when the sample has been concentrated to a volume of 150 ml., or if the volume is 150 ml. after 0.5 hour of boiling, and the distillation continued until a negative check with phenolphthalein is obtained.

Distillate O is mixed and an aliquot withdrawn for determination of ammonia by the modified Nessler procedure described below, the remainder being titrated in the same manner as distillates G and M. The ammonia in distillate O has been formed from nitrates in the sample by the reducing action of the Devarda alloy.

Distillation Residue N is filtered and the Devarda mud, Q, is washed and kjeldahlized.

Filtrate P of Distillation Residue. Aliquots are taken for determinations of the "rest nitrogen" by Kjeldahl and for the determination of α-amino acid nitrogen. The remainder is used for the determination of nicotinic acid.

DISCUSSION OF METHODS

Drying of Samples. The procedure used for the drying of the samples is one of the conventional methods, recommended by Pucher, Vickery, *et al.* (*54*) for the preparation of tobacco for analysis. As shown by these authors, the main advantage of drying at the relatively low temperature of 68° C. is that at this temperature no detectable chemical changes occur in the samples, such as deamination of the labile glutamine. It cannot be claimed that this drying procedure yields better values for the actual dry weights and moisture contents of the samples than any of the numerous other drying methods (*3*, *22*, *48*, *75*, *76*). It serves very well, however, for obtaining reproducible reference weights in comparative analyses of tobacco samples, withdrawn at various stages of their processing.

The "dry weight" of a given tobacco sample is defined as the weight obtained from the powdered sample spread in a layer 2 to 4 mm. thick and dried for 0.5 hour in a forced draft oven at 68° C. Systematic drying tests showed clearly the necessity of keeping the layer of the drying powder at a standardized thickness regardless of the size of the samples. This is easily achieved by selecting drying dishes of the proper diameter as described in the procedure.

Extraction and Washing. Acidification of the water used for the reflux extraction of the dried powder samples to ca. 0.05*N* proved to be necessary to avoid any losses of ammonia or other volatile bases during the extraction. Such losses occur if insufficiently acidified water is used for extracting the samples, since the pH of the leaf tissue, particularly of fermented tobacco, can reach values as high as 7. After the samples have been extracted with 0.05*N* acid, reproducible and generally higher values are obtained for the nitrogen content of the water-insoluble leaf residues. These higher values for the insoluble nitrogen are more accurate than the insoluble nitrogen found after extraction with less acidic water. Under these conditions of extraction, all the glutamine contained in the sample is hydrolyzed to glutamic acid and ammonia. The original glutamine content of the sample is determined on subsamples A1 and B1 as outlined above.

To avoid any subsequent nitrogen losses from the water-insoluble fraction, it proved necessary to wash the extraction residue with 0.005*N* acid instead of with water. Furthermore, the washed extraction residues must be placed immediately in acid in preparation for their subsequent digestion for the Kjeldahl determination, because without this precaution the extraction residues will lose some of their nitrogen on standing. No indications were found of any solubilization of the insoluble nitrogen fraction by the acidified water used for the extraction of the samples.

Systematic tests have shown that all the soluble nitrogen components of the leaves have been quantitatively extracted when the washings become colorless. On the average, a minimum of 50 ml. of acidified wash water per gram of dry sample weight is needed to reach this point. An excess of wash water does not affect the quantities of soluble and insoluble nitrogen found in a given sample.

Distillations. After the investigation of various distillation units, the special distillation flask shown in Figure 2 was finally adopted as standard equipment. This flask effectively prevents the carry-over of any spray of liquid from the distillation residue into the distillate. This is particularly important for distillations made in the presence of Devarda alloy, because the hydrogen evolved creates an extremely fine spray of the alkaline liquid which, if transferred into the distillate, causes considerable titration errors. The insertion of "Iowa State" traps between ordinary distillation flasks and the condensers proved advantageous but did not solve the problem completely. Various other types of traps were found to be still less efficient. A complete retention of the spray was achieved by using the Claisen flask (Figure 2) with a tight packing of glass helices in its side tube. Its beneficial effect is illustrated by the data in Table II. The packed column of this distillation flask, because of its fractionating effects, permits the nearly quantitative separation of nicotine from the accompanying secondary alkaloids (see below).

DISCUSSION OF CONSTITUENTS

Survey on Components. The analytical scheme described here differs from the usual determinations of nitrogenous compounds in plant tissues in so far as it constitutes an attempt at recording all the nitrogenous substances in the tissues rather than a few selected compounds for which analytical methods are available.

Table III illustrates for three cigar tobaccos the summation of their nitrogen increments to give their Σ values of total soluble nitrogen. Several of the fractions employed for this additive evaluation are composite fractions and contain, in their turn, different types of nitrogen compounds. These fractions are further analyzed for their components with the results shown in Table IV, where the individual constituents are given in percentages of the Σ value of each of the three tobaccos. As can be seen, these percentages change considerably for some of the constituents with the kind of tobacco leaves investigated. According to the literature data (*12, 18, 59*) other tobacco types, particularly cigarette tobaccos, show a distribution of their soluble nitrogen constituents very different from those of the cigar tobaccos listed in Tables III and IV.

Table II. Comparison of Spray Retention in Two Distillation Units

(In all cases the receiving flask contained 10 ml. of 0.05*N* H_2SO_4 and was titrated after 1 hour of steam distillation with 0.05*N* NaOH. Titer for 10 ml. of acid was 10.35 ml. of base)

Contents of Distillation Flask	0.05*N* NaOH Required for Titration of Distillate, Ml. Claisen flask, helix packing	0.05*N* NaOH Required for Titration of Distillate, Ml. Ladenburg flask, Iowa State trap
100 ml. of 5%[a] NaOH	10.30	10.32
100 ml. of 5%[a] NaOH and 2 g. of Devarda alloy	10.38 10.04 10.25	8.51 9.20 9.25
100 ml. of 25%[a] NaOH	10.44	10.25

[a] Concentration of NaOH is on a weight-volume relationship—5 g. NaOH dissolved in 100 ml. water is a 5% NaOH solution.

Table III. Average Results Showing Summation of Soluble Nitrogen

(Values are expressed in milligrams of nitrogen per gram of dry tobacco. Same tobacco samples as listed in Table IV. Capital letters refer to fractions in which values were found, Figure 1)

Tobacco Type	Acid Ppt., E	Total NH_3, G + M	Nicotine, G	MgO, I	STA, K	Nitrate, O	Devarda Mud, Q	Rest Nitrogen P	Total Soluble Nitrogen, Σ	Total Nitrogen of Sample, A
1. Pennsylvania tobacco, 1941 crop unfermented	0.50	7.57	6.42	1.41	2.00	2.64	0.48	3.01	24.03	42.42
2. Pennsylvania tobacco, 1941 crop fermented	0.42	5.03	2.53	0.85	3.42	3.18	0.50	2.44	18.37	42.67
3. Connecticut shade-grown wrapper tobacco, 1950 crop fermented	0.38	7.42	2.09	1.28	1.02	5.00	0.45	7.03	24.67	37.39

As there are still considerable gaps in our knowledge of the nitrogenous substances in plant tissues, including tobacco, several fractions of an unknown nature are unavoidably encountered in the comprehensive analysis described here. Depending on the tobacco type analyzed and its state of industrial processing, roughly 67 to 78% of the total soluble nitrogen can be allocated to identifiable compounds. These compounds and the fractions in which they are obtained and determined are listed in the upper half of Table IV. The remaining approximately 22 to 33% of soluble nitrogen belongs to substances or groups of substances that have not yet been definitely identified as known chemical compounds. They are found in the fractions listed in the lower half of Table IV. Inasmuch as their chemical composition is unknown, their nitrogen is determined by kjeldahlization.

This emergence of fractions containing unknown nitrogen compounds in the comprehensive analytical procedure is a challenge to investigate these unidentified leaf components further, particularly those fractions that contain substantial quantities of "unknown nitrogen," and those that show drastic changes during the processing of the leaves. It was precisely such changes in the unknown nitrogen that instigated the study in this laboratory that led to the recognition of the profound chemical changes of the tobacco alkaloids that occur during the fermentation of the leaves. It is hoped that further work will help to clarify the nature of the remaining unknown nitrogen compounds.

Known Nitrogenous Leaf Components. NICOTINE NITROGEN. The distillation of the concentrated water extract, D, in the presence of the mildly alkaline magnesium oxide through the packed column of the Claisen flask yields more accurate values for the actual nicotine nitrogen content of the tobacco samples than does a distillation with sodium hydroxide as the alkalinizing agent (*8, 13, 46*) in other types of apparatus (*4, 9, 32*). This higher accuracy is due to a better retention in the distillation residue of the secondary alkaloids, and to the suppression of decomposition reactions that yield nicotine from nicotine transformation products present in fermented tobaccos.

In regard to the retention of the secondary alkaloids, model distillations were carried out with solutions of pure nornicotine, and with nicotine-nornicotine mixtures, using magnesium oxide and the Claisen flask. Some of the results of these tests are listed in Table V. The distillations of pure nornicotine 1 to 4 show that none of this compound distills under the conditions of distillation, and that the distillation residue on analysis gives a satisfactory recovery of nornicotine. The model distillations of nicotine-nornicotine mixtures 5 to 7 agree with these results in so far as the amounts of alkaloid nitrogen found by spectrophotometry in the distillates and in the distillation residues correspond, respectively, to the amounts of nicotine nitrogen and to the quantities of nornicotine nitrogen contained in the original synthetic mixtures.

As earlier results of Porter *et al.* (*52*) and some recent chromatographic work in this laboratory indicated the presence of anabasine as an additional alkaloid in cigar tobaccos, model distillations were carried out with this substance and also with nicotine-anabasine mixtures. The results obtained were less favorable than those found for the nicotine-nornicotine mixtures. Under the conditions required for the complete distillation of nicotine, from 25 to 40% of the total anabasine present was found in the distillates. This effect could possibly be avoided by the use of more efficient fractionating columns. Since, however, the total amounts of anabasine present in average tobacco samples probably do not exceed 3 to 5% of the

Table IV. Classification and Distribution of Soluble Nitrogen for Three Tobacco Types

Chemically Identified Leaf Components

Name	Found in Fraction	Approx. % of Total Soluble N 1[a]	 2[a]	 3[a]
Nicotine	G	26.7	13.8	8.5
Free ammonia	Subfraction of A	23.4	17.3	13.3
Amide of glutamine	Subfraction of B	1.9	3.4	1.3
Amide of asparagine	Subfraction after hydrolysis	2.1	3.1	13.7
Ammonia from unknown sources	Calculated, partly in G, partly in M	4.1	3.6	1.6
Nitrates	O	11.0	17.4	20.3
Nonnicotine alkaloids as nornicotine	Kb1	1.8	1.9	0.5
Oxynicotine	Kb3	0.5	3.0	0.1
α-Amino acid	Subfraction of P	6.6	3.8	18.3
Nicotinic acid	Subfraction of P	0.3	1.8	0.1
Total		78.4	69.1	77.7

Chemically Unidentified Leaf Components

Characteristic Behavior in Analysis	Found in Fraction	1[a]	2[a]	3[a]
Forms water-insoluble ppt. on concentrating original extract at pH 3 to 3.5	E	2.1	2.3	1.5
Combines with, and is adsorbed on MgO during steam distillation	I	5.9	4.6	5.2
Precipitates with STA along with oxynicotine and "nornicotine"	Ka − (Kb1 + Kb3)	6.0	13.7	3.6
Combines with and is adsorbed on MgO and reacted Devarda alloy	Q	2.0	2.7	1.9
Rest, not distillable with MgO, MgO and Devarda, nor 5% alkali. Does not fall with STA	P − (α-amino N) + nicotinic acid N	5.6	7.7	10.1
Total		21.6	31.0	22.3

[a] These figures refer to tobacco types listed in Table III.

nicotine contained in these samples, the quantities of anabasine erroneously determined as nicotine in distillate G correspond at the most to only about 2% of the nicotine. On the other hand, 100% of the nornicotine and at least 60% of the anabasine—i.e., at least a major part of the secondary alkaloids—are retained in the distillation residue.

The suppression of decomposition reactions that yield nicotine from other compounds during the distillation, is dealt with below in connection with the discussion of oxynicotine.

The quantities of nicotine in distillate G are determined by means of ultraviolet spectrophotometry, correcting for optical impurities present in the distillate in a way similar to that described by Willits *et al.* (*79*). Ammonia, the second main component of distillate G, is quantitatively determined by a modified Nessler method (see below). Provided there are no additional volatile bases or volatile acids contained in distillate G, the analytical values found for nicotine and ammonia can be checked by the simultaneous titration of both these bases. Since only one of the two basic nitrogen atoms of the nicotine molecule (*14, 70*) is titrated in the presence of the indicator (pH range 4.0 to 6.0), the "total titrable nitrogen" in the distillate should equal the sum of ammonia nitrogen plus one half of the nicotine nitrogen.

Model distillations of synthetic mixtures of ammonia and nicotine confirmed this and particularly the fact that under these conditions exactly one half of the total nicotine nitrogen is titratable. In the analyses of tobacco samples, the titration values of distillate G usually agree well with the total titratable nitrogen calculated from the independently determined quantities of ammonia and nicotine nitrogen in the distillate.

Occasionally the values found by direct titration deviate from the calculated values by ±5 to 8%. A positive deviation in which the titration result exceeds the calculated titrable nitrogen may indicate the presence in the distillates of small amounts of volatile bases other than ammonia or nicotine. In cases of negative deviations, the distillates may contain small amounts of volatile acids. Because of the smallness and erratic occurrence of these deviations, no special investigations have been made to detect their actual causes. However, Kjeldahl determinations made of the total nitrogen in distillates G of several tobacco samples proved beyond any doubt that ammonia and nicotine are the main components of these distillates.

SECONDARY ALKALOID NITROGEN. The evaluation of the secondary alkaloid nitrogen in fraction Kb1 (Figure 3) is based on the fact that nornicotine and anabasine, the main components of this fraction, have very similar absorption spectra, as shown by Table VI.

That the values for the secondary alkaloid nitrogen obtained by the ultraviolet absorption measurements are correct within the limits of analytical errors was repeatedly confirmed by Kjeldahl determinations and by determinations of the alkaloid content of fraction Kb1 by the silicotungstic acid method (*6, 15*).

As a rule, no efforts were made in the procedure described in this paper to differentiate analytically between nornicotine and the other secondary alkaloids present in the samples. Similarly, no quantitative determinations were made of the alkaloid transformation products 3-pyridyl methyl ketone and 2,3′-dipyridyl that are present in very small amounts in the secondary alkaloid fraction obtained from fermented tobacco samples. The separation and determination of these pyridine derivatives are better achieved by the "solvent extraction method" (*30*).

OXYNICOTINE NITROGEN. Oxynicotine is a major component (*29*) of the transformation products of nicotine that have been identified in fermented samples of tobacco. In fermented Pennsylvania tobacco, for example, the quantities of oxynicotine nitrogen average 20% of the nicotine nitrogen content, and in very thoroughly fermented samples can even amount to a multiple of the nicotine nitrogen (*28*) of this tobacco. In the present scheme of analysis, oxynicotine is obtained in fraction Kb2. It is easily and accurately determined by its quantitative reduction to nicotine.

Table V. Steam Distillation of Nornicotine and of Nicotine-Nornicotine Mixtures in Presence of Magnesium Oxide in Claisen Flask, Packed with Glass Helices

(All values are milligrams of nitrogen)

No.	Nicotine in Original Solution	Nornicotine in Original Solution	Found in Distillate	Found in Distillation Residue
1	Nil	1.70	Nil	1.46
2	Nil	1.70	0.01	1.60
3	Nil	0.17	Nil	0.16
4	Nil	0.17	Nil	0.17
5	2.00	0.35	1.99	0.36
6	2.00	1.76	1.96	1.70
7	2.00	0.09	2.00	0.12

Table VI. Secondary Alkaloid Nitrogen

Compound	Location of Minimum mμ	 Maximum mμ	Molecular Extinction[a] at Maximum
Nornicotine	228	259	5610
Anabasine	226	259	5570

[a] Measured in $0.25N$ aqueous HCl solutions in cells of 1-cm. thickness.

If the proper precautions are not observed during the distillation of the water extract, D, some of the alkaloid transformation products present in this extract may be decomposed with a simultaneous formation of artifact nicotine. This will result in a too high value for the nicotine nitrogen found in distillate G. Oxynicotine can yield nicotine when subjected to a steam distillation in the presence of sodium hydroxide and to a much smaller extent in the presence of magnesium oxide. Therefore, this compound is a potential source of artifact nicotine in the distillation of aqueous extracts of tobacco, particularly of fermented cigar tobacco. For this reason, the authors have endeavored to minimize any decomposition of oxynicotine by employing the mild conditions described in the distillation of the concentrated water extract, D.

Investigations by the solvent extraction procedure (*26*, *30*) have shown that other nicotine transformation products besides oxynicotine are present in fermented cigar tobaccos. In the procedure described here, a major part of these substances is present in fraction Kb2, and after the oxynicotine has been removed by its reduction to nicotine, in fraction Kb4. These additional alkaloid transformation products are being investigated further.

Nicotinic Acid Nitrogen. Nicotinic acid is another major product formed from nicotine during the fermentation of tobacco (*27*), and is contained in easily detectable amounts in aqueous extracts made from fermented samples. In contrast to most of the other fermentation products of nicotine, nicotinic acid is not precipitated by silicotungstic acid under the conditions of this analytical procedure, and is, therefore, contained in the final fraction, P. Its determination in this fraction is described below.

Ammonia Nitrogen. The ammonia found in distillates G and M represents the "total ammonia" of the sample and consists of free ammonia and ammonia resulting from the hydrolysis of amides and other leaf components.

The methods of Pucher, Vickery, *et al.* (*54*, *71*) are used for classifying this total ammonia into (1) the free ammonia present in the tissues; (2) the ammonia derived from the easily hydrolyzable glutamine; and (3) the less easily hydrolyzable asparagine. Specifically, the free ammonia nitrogen is determined in a 0.5-gram subsample, A1, of the unextracted tobacco powder, A, the glutamine amide nitrogen in an 0.5-gram aliquot, B1, of the freshly made water extract, B, and the asparagine amide nitrogen in a second 0.5-gram aliquot, B2, after its hydrolysis with 4*N* sulfuric acid. The sum of these three determinations when subtracted from "total ammonia nitrogen" leaves a small quantity of additional ammonia nitrogen. This is "decomposition ammonia" arising from an unknown source, possibly related to the "extra ammonia nitrogen" described by Vickery *et al.* (*72*).

Nitrate Nitrogen. Because the determination of the nitrate nitrogen involves its reduction to ammonia by Devarda alloy in the presence of the mildly alkaline magnesium oxide and determination of this ammonia in distillate O, it is essential as is done in this procedure to remove, prior to the nitrate determination, all the ammonia that might arise from other sources.

Unidentified Nitrogenous Leaf Components. Acid Precipitate Nitrogen E, Magnesium Oxide Nitrogen I, and Unidentified Silicotungstic Acid Precipitate Nitrogen. It is likely that the unidentified nitrogenous substances contained in these three fractions are of a similar chemical composition. The nitrogen found in these fractions is associated with an amorphous brown material that is present in colloidal dispersion in aqueous tobacco extracts. Efforts to obtain precise information on the chemical nature of this amorphous material have so far met with little success. Various observations indicate the possibility that the nitrogen of these substances originates from the formation of complexes between amino acids (or peptides) and nonnitrogenous leaf constituents.

It has been reported that reactions occur in various materials of vegetable origin between amino acids and carbohydrates (*1*, *20*, *45*, *57*) and also between amino acids and oxidized phenolic compounds (*5*, *36*, *38*, *39*), and that amorphous brown substances are produced in these reactions. Conversions of this kind are easily possible in the tissues of tobacco leaves that contain all the components mentioned in considerable amounts. The hypothesis that α-amino nitrogen is involved in the formation of the high molecular nitrogenous substances that are precipitated by silicotungstic acid is supported by the following observation: If an aliquot of fraction H of a given tobacco sample is subjected to a mild hydrolysis with 5% sodium hydroxide at room temperature, this aliquot compared with an untreated aliquot shows an increase of free α-amino nitrogen and a roughly equivalent decrease of the unidentified nitrogen in silicotungstic acid precipitate made subsequently in this fraction H.

The experimental values for the weights of the silicotungstic acid precipitates dried at 100° C., W', for the weights of the ignited silicotungstic acid precipitates, W'', for the total nitrogen of the precipitates, N_t, and for the nitrogen of the secondary alkaloids, N_s, and oxynicotine, N_o, can be used for obtaining some information on the unknown substances, U, that are precipitated together with the secondary alkaloids and oxynicotine by silicotungstic acid.

In the following calculations all quantities are expressed in milligrams per gram of dry tobacco weight:

The nitrogen of the unknown substance is evaluated as

$$N_u = N_t - (N_s + N_o) \tag{1}$$

The weight of the unknown substance is obtained as

$$W_u = (W' - W'') - \left(\frac{100}{18.9} N_s + \frac{100}{15.8} N_o\right) \tag{2}$$

where 18.9 is the per cent of nitrogen in the secondary alkaloids (when calculated as nornicotine) and 15.8 is the per cent of nitrogen in oxynicotine.

The per cent of nitrogen in the unknown compound is:

$$\frac{N_u}{W_u} \times 100 \tag{3}$$

In Table VII are listed the experimental values W' and W'' for the same three tobacco types for which the nitrogen data are presented in Tables III and IV. The values for the nitrogen, N_u, and the weight, W_u, of the unknown substances calculated from Equations 1 and 2, respectively, are also listed, together with the per cent nitrogen in the unknown substance. This nitrogen percentage, amounting to approximately 10% for all three tobacco samples, is not in contradiction to the hypothesis that the unknown substances might consist largely of complexes formed between amino acids and polyphenols or carbohydrates. Further work is needed to clarify the nature of these unknown nitrogenous substances.

The magnesium oxide residue, I, may contain not only high molecular complexes of the type discussed above, but also fragments formed from nucleic acids. This is indicated by the fact that considerable amounts of phosphorus as well as nitrogen and carbonaceous materials are contained in this fraction.

Table VII. Experimental Values

Tobacco Type	W'	W''	N_u	W_u	$N_u/W_u \times 100$
1	76	57	1.45	16	9.1
2	129	99	2.42	25	9.7
3	25	16	0.88	8	11.0

Devarda Residue Nitrogen Q. The small amounts of nitrogen associated with the solid residue, Q, of the Devarda distillation appears to be also derived from amino acids. In this case, however, no complex formation seems to occur. Rather, the retention of small amounts of nitrogen in the solid phase seems to be caused by a direct reaction between free amino acids and some components of the residue (magnesium oxide, aluminum oxide, and metallic copper). This is borne out by model experiments with aspartic acid which showed that some aspartic acid nitrogen was retained in the Devarda mud. Since, according to recent results obtained in this laboratory by chromatographic analysis, aspartic acid is predominant among the amino acids present in aqueous tobacco extracts, it is conceivable that a considerable part of the unidentified nitrogen in fraction Q originates from this compound.

Rest Nitrogen. Approximately 30 to 65% of the "rest nitrogen" found by kjeldahlization of fraction P can be identified by means of the Van Slyke method as α-amino acid nitrogen, and some 1 to 14% as nicotinic acid nitrogen, depending on the sample being analyzed. The remainder may consist chiefly of the basic nitrogen of arginine, histidine, and possibly the methylated nitrogen of nondistillable compounds. It is likely that fraction P contains also some heterocyclic nitrogen of the purine and pyrimidine type. More detailed investigations are required for elucidating the chemical nature of unidentified nitrogen components in fraction P.

DETERMINATIONS OF TOTAL NITROGEN, AMMONIA, NITRATE, AND NICOTINIC ACID

A description is given here of several specific procedures that are used in conjunction with the analytical scheme described above.

These procedures are, A, a specifically adapted Kjeldhal method for the determination of total nitrogen in a sample of tobacco powder or in fractions thereof; B, a modified Nessler procedure for determining ammonia in distillates; C, a variation of the Devarda method for reducing nitrate nitrogen to ammonia nitrogen; and D, a method for determining nicotinic acid by its selective extraction and spectrophotometric evaluation.

A. KJELDAHL METHOD FOR DETERMINATION OF NITROGEN IN TOBACCO SAMPLES

Like other plant materials, tobacco contains nitrogen in various forms, including ammonia, amino acids, and nitrates. Of these, the presence of nitrates requires special precautions for their quantitative determination in the Kjeldahl method for total nitrogen. Furthermore, considerable quantities of nitrogen are present in tobacco leaves as a part of heterocyclic ring compounds; their main representatives are nicotine, related alkaloids, and their transformation products (*27, 29, 30*). These heterocyclic compounds are among the most difficult to determine by the Kjeldahl procedure (*41, 49, 58, 77*). Correct Kjeldahl determinations of tobacco samples require, therefore, a special procedure designed to overcome the complication caused by the simultaneous presence of nitrates and of heterocyclic nitrogen compounds.

Reduction of Nitrates. Pucher, Vickery, *et al.* (*53, 69*) confirmed the finding of Ranker (*56*) that the zinc reduction of nitrates recommended as an official method (*2*) prior to Kjeldahl digestion, if applied to aqueous solutions, yields low nitrogen values. The same authors recommended, as a better procedure, reduction of the solutions with "reduced iron powder" prior to the Kjeldahl digestion. This and related methods are discussed by Street (*63*).

In analytical studies on tobacco, this method proved to be indispensable for the Kjeldahl determination of nitrogen in samples that contain nitrates or substances containing nitrogen-oxygen linkages. Specifically, the preliminary reduction with reduced iron powder was found to be necessary to obtain correct total nitrogen values for the original tobacco samples, their aqueous extracts, and their extraction residues [fractions A, B, and C, respectively, of the analytical scheme (Figure 1)], even though the presence of nitrates in fraction C appears unlikely. On the other hand, a series of tests showed that the preliminary iron reduction can be omitted in the Kjeldahl determination of the nitrogen contained in other fractions of the analytical scheme, specifically in fractions E, I, Kc, P, and Q. It is evident from the analytical scheme that none of these fractions contain nitrate nitrogen.

In using the reduction by iron, it was found essential not to concentrate the solutions prior to reduction. Without this precaution, substantial losses of nitrogen occur during the concentration of the unreduced solutions. Presumably, this is due to a loss of nitric acid by volatilization, or to a formation of molecular nitrogen from amino compounds and nitric acid in the presence of reducing substances.

Table VIII shows how the concentration of an acidified tobacco extract before its reduction with iron powder diminished the nitrogen values found for this extract. The values listed for Methods I and II were obtained for water extracts of the same tobacco powder, and correspond to aliquots of fraction B—i.e., the "total soluble nitrogen" of the analytical scheme. Method I involves (1) acidification of the aqueous extract to a strength of $2N$ with sulfuric acid, (2) concentration to about one half of its original volume, (3) reduction at this volume with "reduced iron powder," and (4) kjeldahlization as described in the experimental procedure. In Method II, the extracts were made $4N$ with sulfuric acid, immediately reduced, concentrated, and then kjeldahlized.

The total nitrogen contained in the same tobacco fraction B was independently evaluated as the difference between the total nitrogen (Kjeldahl value) of the original tobacco powder and its insoluble nitrogen (Kjeldhal value). It was found to equal 21.45 mg. per gram of the dry tobacco weight. As this difference value obviously represents the actual quantity of soluble nitrogen, it follows that the results obtained by Method I are low by about 17%, whereas the average of the results of Method II differ from the true value by only 1.4%, an agreement that is satisfactory within the limits of analytical and sampling errors (about ± 2.5%).

That the low values obtained by Method I show among themselves no appreciably greater deviation than do the correct values found by Method II is an indication that the nitrogen losses occurring during the concentration of the acidified unreduced tobacco extract are fairly well reproducible. This proves once again that reproducibility in itself has no bearing on the accuracy of analytical results.

Table VIII. Influence of Treatment of Extract, Prior to Kjeldahl Digestion on Nitrogen Content of Water Extracts of One Sample of Tobacco Powder

	Method I		Method II	
	Acidified Extract Concd. before Reduction, then Kjeldahlized		Acidified Extract Reduced before Concn., then Kjeldahlized	
	Value, mg./g.	% deviation from av.	Value, mg./g.	% deviation from av.
	17.93	1.5	21.32	1.4
	17.99	1.9	20.91	0.5
	16.90	4.3	21.41	1.9
	17.40	1.5	21.10	0.4
	17.88	1.2	21.15	0.6
	17.88	1.2	20.38	3.0
	...	...	21.10	0.4
	...	...	21.73	3.4
Av.	17.66	±1.9	21.14	±1.5

Digestion of Heterocyclic Nitrogen Compounds. The second difficulty in Kjeldahl determinations of tobacco powders and tobacco extracts is caused by the resistance of heterocyclic nitrogen compounds such as nicotine, against the usual digestion procedure.

In 1943, Chibnall, Rees, and Williams (*16*), working with protein-containing samples, pointed to the necessity of employing prolonged digestion periods after clearance of the digesting solutions to obtain complete recovery of the total nitrogen of the protein samples. In 1945 Shirley and Becker (*58*) stated that the same applies to an even greater extent to Kjeldahl determinations of heterocyclic nitrogen compounds and recommended the use of mercury, or of a combination of mercuric oxide and selenium oxychloride as digestion catalysts. Later Ogg, Brand, and Willits (*49*) published the results of a thorough investigation of the optimal duration of the digestion, and of the most effective catalysts for the digestion procedure when heterocyclic compounds such as nicotinic acid are kjeldahlized. These authors recommended a digestion period of 4 hours, and a catalyst combination of mercuric oxide and selenium oxychloride. In a subsequent paper Willits, Coe, and Ogg (*77*) modified this earlier recommendation by favoring a digestion time of 3 hours and the use of mercuric oxide and potassium sulfate in a fixed ratio, pointing out that the use of selenium or its compounds can lead to appreciable nitrogen losses. This opinion was supported by Lake (*43*), who claimed that any catalysts other than mercury, potassium sulfate, and sulfuric acid are superfluous and may even be harmful. On the other hand, Patel and Sreenivasan (*50*) concluded that the best results in kjeldahlizing heterocyclic compounds such as nicotinic acid are obtained by using digestion periods of 4 hours after clearance of the solution and selenium-mercury combination as catalyst.

During the development work on tobacco catalysis in this laboratory (started prior to 1945) studies were made on the factors influencing the Kjeldahl procedure, particularly the duration of digestion, and the catalysts used. It was concluded that only by prolonged digestion periods and by using a combination of selenium and mercury as digestion catalysts are reliable and reproducible results obtained for the nitrogen content of tobacco samples and of analytical fractions derived from them.

Using the procedure described below, various individual nitrogenous compounds either known to be present or suspected of being present in tobacco were kjeldahlized, with the results shown in Table IX. The agreement between the experimental and theoretical values for each of the compounds is of the same order as that reported by the authors quoted above.

Table X lists values found in repeated analyses for the total nitrogen (column 2), the insoluble nitrogen (column 4), and the soluble nitrogen (column 6) of two typical cigar tobacco samples (Pennsylvania Seedleaf U. S. Type 41). The good agreement of the values in column 2 with the calculated values in column 8 indicates that the nitrogen determinations listed in columns 2, 4, and 6 are dependable. All the values for soluble nitrogen in column 6 of Table X were obtained by immediate reduction with iron, prior to concentrating and digesting the solutions.

Table IX. Determination of Nitrogen Content of Various Compounds, Including Substances Containing Heterocyclic Nitrogen

Substance	Nitrogen by Theory, %	Nitrogen Found		% Deviation from Theory	
		%	Av. %	Single detns.	Averages
Trigonelline acid sulfate[a]	5.98	5.92		−1.0	−1.0
Nicotinic acid	11.38	11.51	11.43	+1.1	+0.5
		11.50		+1.1	
		11.36		−0.2	
		11.46		+0.7	
		11.11		−2.4	
		11.23		−1.3	
		11.57		+1.8	
		11.71		+2.9	
Glutamic acid	9.52	9.64	9.61	+1.3	+0.9
		9.59		+0.7	
Nicotine[b]	17.27	16.91	17.17	−2.1	−1.0
		17.41		+0.8	
		17.15		−0.7	
		17.21		−0.3	
Nornicotine[b]	18.90	19.52	19.42	+3.3	+2.8
		19.33		+2.3	
Average deviation					±1.2%

[a] Sample kindly supplied by J. W. Huff, Sharp and Dohme, Inc.

[b] Nicotine and nornicotine determined in aqueous solutions. Theoretical nitrogen content determined by silicotungstic acid precipitation of alkaloids and by weighing ignited precipitates, according to method of Bertrand and Javillier (*6*).

Table X. Total, Soluble, and Insoluble Nitrogen of Powdered Leaf Blades of Pennsylvania Seedleaf Tobacco, 1941 Crop

(Mg. per gram of dry tobacco weight)

	Direct Determinations, Modified Kjeldahl Procedure						By Calculation	
Sample No.	Total N	Deviation from av., %	Insoluble N	Deviation from av., %	Sol. N	Deviation from av., %	Total N	Deviation from av., %
1.	45.66	−0.1	24.32	+0.2	21.32	+0.9	45.64	+0.5
	45.49	−0.5	23.41	−3.5	20.91	−1.1	44.32	−2.4
	45.66	−0.1	24.27	0.0	21.41	+1.3	45.68	+0.6
	46.06	+0.8	24.29	+0.1	21.10	−0.2	45.39	−0.0
	45.84	+0.3	24.82	+2.3	21.15	0.0	45.97	+1.3
	45.59	−0.3	24.91	+2.7	20.38	+3.6	45.29	−0.2
	...	...	24.48	+0.9	21.09	−0.2	45.57	+0.4
	...	...	23.66	−2.5	21.73	+2.8	45.39	0.0
Average	45.72	±0.4	24.27	±1.5	21.14	±1.3	45.40	±0.4
2.	40.26	+0.8	17.85	+1.1	21.63	−1.6	39.48	−0.4
	40.34	+1.0	17.47	−1.0	21.91	−0.3	39.38	−0.7
	39.93	0.0	17.65	0.0	21.17	−3.7	39.82	+0.3
	39.90	−0.1	17.75	+0.6	22.17	+0.9	39.92	+0.6
	39.28	−1.7	17.51	−0.8	22.11	+0.6	39.62	−0.1
	39.96	+0.1	17.59	−0.3	22.12	+0.6	39.71	+0.1
	...	...	...	...	22.75	+3.5	...	...
Average	39.94	±0.6	17.65	±0.6	21.98	±1.6	39.66	±0.4

[a] Sum of fourth and sixth columns.

As can be seen from a comparison of the percentage deviations listed in Tables IX and X, the analytical limits of error in nitrogen determinations of tobacco samples are of the same order of magnitude as those incurred in determining the nitrogen content of well-defined single compounds, including heterocyclic compounds.

Reagents. Sulfuric acid, concentrated, low nitrogen, C.P. analytical reagent.

Sulfuric acid, 4*N* solution.

Sulfuric acid, 0.05*N* standard solution, standardized against a primary standard of sodium carbonate.

Sulfosalicylic acid, 1 gram of salicylic acid (analytical reagent grade) dissolved in 20 ml. of concentrated sulfuric acid.

Sodium thiosulfate, analytical reagent.

Potassium sulfide (Mallinckrodt) (potash, sulfurated, N.F. IX) 4% solution, prepared freshly for each set of distillations.

Sodium hydroxide, purified scales, 11*N* solution.

Sodium hydroxide, 0.05*N* standard solution, prepared from sodium hydroxide pellets, analytical reagent.

Mercury, U. S. P., redistilled.

Reduced iron powder (iron by hydrogen from City Chemical Corp.).

Selenized Hengar granules (Hengar Co.).

Indicator (*64*), 125 mg. of methyl red and 82.5 mg. of methylene blue dissolved in 100 ml. of 95% ethyl alcohol, and filtered if necessary.

Procedure. For reliable Kjeldahl analyses, aliquots containing from 2 up to 20 mg. of nitrogen are taken. When the sample contains nitrates (fractions A, B, and C of the scheme of analysis), the following procedure is used: Add to the sample, in a 500-ml. Kjeldahl flask, sufficient sulfuric acid, either concentrated or dilute, depending on whether the sample is in solution or not, to make the solution 4*N*, and 2 grams of reduced iron powder. As soon as the speed of the reaction diminishes, heat and swirl the flask intermittently, until the evolution of hydrogen ceases. Cool and add 10 ml. of concentrated sulfuric acid and concentrate until white fumes appear. Cool again and add 20 ml. of sulfosalicylic acid, add 5 grams of sodium thiosulfate, and allow to stand 4 hours. Heat to boiling. As soon as white fumes appear, add 3 selenized Hengar granules. Boil for 4 hours, and then add ca. 0.5 gram of mercury and 10 ml. of concentrated sulfuric acid. Continue the digestion for 2 more hours; then add one selenized Hengar granule, and after 2 more hours, add another one. The reagents, including the catalysts, are added during the digestion with extreme caution and always with concurrent mixing of the contents of the flask. It is also advisable to let the samples cool slightly prior to the addition of the reagents.

Finally, after a total of 12 hours of digestion, cool the solution, add 200 ml. of water, and cool again. Add 30 ml. of 4% potassium sulfide solution and mix thoroughly. Make alkaline by adding ca. 110 ml. of 11*N* sodium hydroxide solution slowly down the side of the flask, and connect immediately to the trap (*78*) and condenser of the distillation unit. Mix well and distill into an excess of standard 0.05*N* sulfuric acid, containing 4 drops of indicator. Titrate the distillate with standard 0.05*N* sodium hydroxide. Correct the resulting titer by subtracting the predetermined blank found by making a determination on the reagents alone. For the reagents used in this procedure, the blank amounts to about 0.2 mg. of nitrogen and to about 0.1 mg. of nitrogen when no reduction with iron is made. If the sample to be kjeldahlized includes filter paper, a special blank, including the same amount of filter paper, should be determined.

If the sample does not require reduction, it is acidified, concentrated, and digested according to the above procedure, omitting the reduction step.

B. DETERMINATION OF AMMONIA IN DISTILLATES BY A MODIFIED NESSLER PROCEDURE

Ammonia has to be determined in each of distillates G, M, and O of the stepwise analytical scheme and also in the distillates obtained from subfractions A1, B1, and B2. Depending on the specific tobacco samples under investigation, and on the stage of industrial processing at which the leaves are taken for analysis, some of these distillates contain very small amounts of ammonia, in some cases as little as 0.3 γ of ammonia nitrogen per milliliter. Moreover, in most of these distillates, varying amounts of nicotine are present besides ammonia. Thus, any method for determining the ammonia in these distillates must be sensitive, accu-

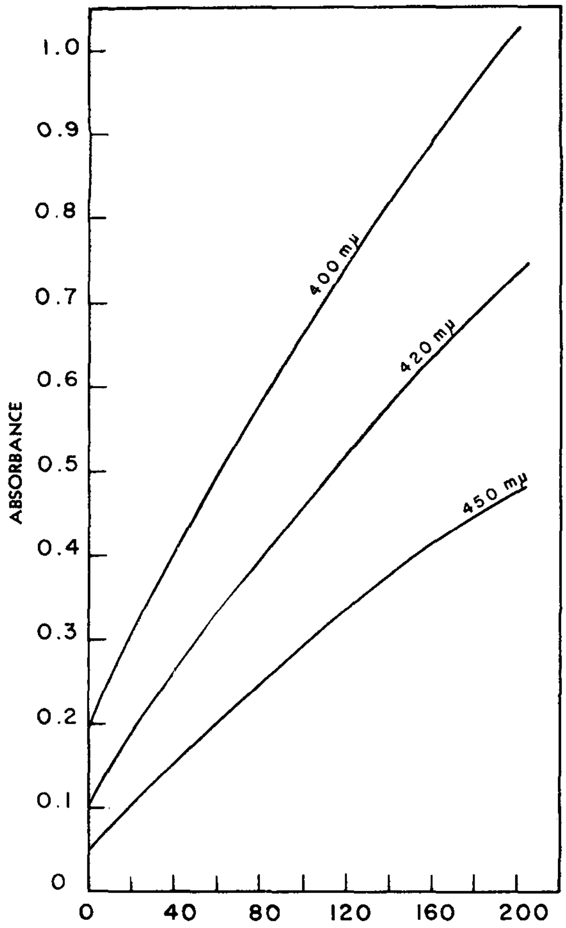

Figure 4. Calibration Curves for Determination of Ammonia Nitrogen Using Beckman Spectrophotometer

Absorbances of solutions determined in 1-cm. cells, read against redistilled water as a blank

rate, and free of interference by the presence of other volatile bases, particularly nicotine.

A simple titration of the distillate is convenient and is commonly employed when the only base present is ammonia, but its lack of specificity excludes its use for distillates containing additional bases. Of the more specific methods recommended for the quantitative determination of ammonia, two procedures were investigated in detail—the sodium phenate and hypochlorite colorimetric method described by Van Slyke and Hiller (*67*) and modified by Borsook (*7*), and the well-known Nessler procedure. According to the literature, both methods appeared to meet the requirements set forth above.

Borsook's Method. According to Borsook, ammonia concentrations of about 0.5 γ per milliliter can be determined with an accuracy of ±2%. Following this author's recommendations, ammonia solutions of concentrations between 0.5 and 5 γ per milliliter were treated with phenate-hypochlorite reagent. Solutions containing 0.5 γ of ammonia per milliliter, if measured in 1-cm. cells, yielded a color of a sufficiently high absorbancy to permit quantitative evaluation by spectrophotometry. However, this method yielded reproducible results only when carried out under the strictest temperature control. A second and decisive drawback of Borsook's method is that it yields erratic results with ammonia solutions containing nicotine, even if the concentration of the latter does not exceed 0.2 γ of nicotine nitrogen per milliliter. The colors obtained for the ammonia content of such nicotine containing solutions show, on optical evaluation, deviations of ±30%, far beyond the permissible limits of analytical error. Therefore, the phenate-hypochlorite color reaction is unsuitable for the determination of ammonia in the distillates obtained in the stepwise analysis of tobacco samples.

Nessler's Colorimetric Method. Relatively few exact data are given in the literature on the sensitivity and accuracy of Nessler's method. Pucher, Vickery, and Leavenworth (*54*) studied this method in considerable detail to determine the ammonia content of distillates obtained from plant extracts, including those of tobacco. They found that ammonia nitrogen concentrations between 1 and 10γ per milliliter could easily be determined in nesslerized solutions with an accuracy of about ±3% when a Pulfrich spectrophotometer with light filter S43 was used. On the other hand, in this laboratory, working with an immersion-type colorimeter, a similar accuracy in the optical evaluation of the ammonia contents of nesslerized solutions was unattainable, in spite of repeated efforts to improve the method. The main difficulty was that the nesslerized solutions of ammonium sulfate, serving as color standards, showed as a rule shades of color differing from those of the nesslerized distillates of tobacco fractions, probably because of the presence in some of these distillates of finely dispersed droplets of volatile oils that impart a grayish shade to the yellow color of the complex formed by the reaction of Nessler's solution with ammonia.

Measurement of Light-Scattering Capacity of Nesslerized Solutions. Searching for a less subjective and more sensitive way of evaluating the "color" of nesslerized solutions, the authors turned to the Beckman spectrophotometer. As was to be expected, the nesslerized solutions, being colloidal in nature, show no well defined absorption maximum, but exert a pronounced effect of light scattering. Since the degree of light scattering by a colloidal solution depends on the number of particles in the unit volume as well as on the sizes and size distribution of the particles, a dependable analytical method can be developed only when these variables are effectively controlled.

By systematic investigations it was found that the colloidal character of a nesslerized solution and hence its degree of light scattering can be sufficiently well reproduced if certain precautions are taken: adding gum arabic as a protective colloid, thorough mixing of the samples while the Nessler solution is being added, and permitting the samples to stand while they attain a practically stationary color value.

Ammonia solutions when nesslerized in this way show an increase of light-scattering capacities with increasing ammonia concentration. This fact can be used to construct a calibration curve for evaluating samples of unknown ammonia contents. Figure 4 shows a typical set of calibration curves obtained by measuring the absorbances of a series of nesslerized solutions in the Beckman spectrophotometer. These solutions in 50 ml. contain from 0 to 200 γ of ammonia nitrogen (0 to 4 γ of ammonia nitrogen per milliliter). The opacities of the solutions measured in 1-cm. cells at the wave lengths 400, 420, and 450 mμ proved to be reproducible within rather narrow limits for a given solution of Nessler's reagent.

This applies for measurements made in short intervals (within 6 successive days). Reproducibility of determinations carried out over a longer period is discussed below.

Table XI shows the average and maximum deviations encountered for each of eleven ammonia standard concentrations used for constructing the calibration curve. It is evident that the highest accuracy is attained with ammonia concentrations in the range of 1.0 to 4.0 γ of ammonia nitrogen per milliliter.

The absorbance of any nesslerized solution containing unknown amounts of ammonia is read at 400, 420, and 450 mμ. Two aliquots of a given sample are used for each determination, yielding altogether six measurements of absorbances for each unknown sample. These six readings require approximately 1 minute and usually agree with each other closely (within the limits listed in Table XI). The method is sensitive, fairly rapid, and sufficiently accurate. A disadvantage can be seen in the necessity of determining a new calibration curve every time a fresh Nessler solution

Table XI. Sensitivity and Accuracy of Ammonia Determinations Based on Measurements of Light-Scattering Effect Produced by Nesslerized Solutions[a]

Ammonia Nitrogen Concn., γ/Ml.	No. of Readings	Mean Deviations, % of Average	Maximum Deviations, % of Average
0.1	18	±6	±10
0.2	18	±9	±11
		Av. ±7.5	±11
0.3	18	±3.5	±4.7
0.6	24	±2.7	±5.4
		Av. ±3.1	±5.2
1.0	18	±1.4	±2.6
1.6	12	±1.4	±2.3
2.0	18	±1.1	±2.2
2.4	12	±2.2	±3.0
3.0	24	±1.3	±2.0
3.6	12	±1.1	±2.2
4.0	21	±1.6	±3.0
		Av. ±1.1	±2.5

[a] Optical values obtained are for solutions of a final volume of 50 ml. each containing 2 ml. of gum arabic solution and 1 ml. of Nessler reagent.

is put into use. The useful lifetime of Nessler reagent was found in this laboratory to be approximately 3 months, during which time it served for some 1000 determinations.

Reagents. Ammonium Sulfate Stock Solution, containing 100 mg. of ammonia nitrogen per liter. Approximately 0.48 gram of c.p. ammonium sulfate is dissolved in 1 liter of redistilled ammonia-free water. The exact ammonia concentration of this solution is determined by steam-distilling aliquots of 50 and 100 ml. in the presence of magnesium oxide, collecting the distillates in an excess of standard acid, and titrating them with standardized base. The average result of four or five such determinations, expressed in milligrams of ammonia nitrogen in 1 liter of ammonium sulfate solution, defines the concentration of this ammonium sulfate solution.

Ammonium Sulfate Standard Solution, prepared by diluting tenfold with redistilled water the solution containing 100 mg. of ammonia nitrogen per liter. The concentration of the diluted standard is 10 γ of ammonia per milliliter.

Nessler Reagent, Jackson's Modification (*61*). This solution should be stored at room temperature in the dark. It can be used 4 days after preparation.

Gum Arabic Solution (*60*). In purifying the crude gum arabic solution by adding Nessler solution, it was found preferable to let the mixture stand to settle for at least 4 weeks instead of the recommended (*60*) 10 to 14 days. Any sediment becoming visible in the purified gum arabic must be removed by filtration. It is advisable to prepare 2 liters of this gum arabic solution for each liter of Nessler reagent prepared for use in the following procedure.

Apparatus. A Beckman Model DU spectrophotometer.

Procedure. Construction of Calibration Curve. Pipet into 150-ml. beakers freshly rinsed with dilute hydrochloric acid and then with redistilled water each of the following aliquots taken from the ammonium sulfate standard solution: 0.5, 1.0, 1.5, 3.0, 5.0, 8.0, 10.0, 12.0, 15.0, 18.0, and 20.0 ml. These aliquots in their final volumes of 50 ml. give the ammonia nitrogen concentrations listed in column 1 of Table XI. Add to each aliquot in these eleven beakers and also to a twelfth empty beaker, redistilled water until the total volume is ca. 40 ml. Stir each of the solutions, and while stirring vigorously add 2 ml. of the gum arabic solution and then 1 ml. of the Nessler solution. Continue stirring for about 10 seconds and then transfer each nesslerized sample to a 50-ml. glass-stoppered volumetric flask. Make each solution up to volume by adding water used previously to rinse out the beaker. Stopper each flask, shake well, and allow to stand for 3 hours at room temperature. Measure the absorbances of these solutions in 1-cm. cells using the Beckman spectrophotometer at wave lengths 400, 420, and 450 mμ against redistilled water as a blank. For a calibration curve at least six density readings for each concentration are made and their averages used to plot the curves, a typical set of which is shown in Figure 4.

Determination of Ammonia Contents of Distillates. Take for the analysis duplicate aliquots of the distillates containing preferably between 50 and 150 γ of ammonia nitrogen. Place each aliquot in a 150-ml. beaker previously rinsed as described, make up to ca. 40 ml. with ammonia-free water, and proceed as described in the preceding paragraph. If the aliquot taken is larger than 40 ml., concentrate to that volume before proceeding with the determination. Three aliquots containing 50, 100, and 150 γ of ammonia nitrogen, *respectively, are taken from the* ammonium sulfate standard solution. These three aliquots are carried through the procedure simultaneously with the unknown samples and serve for the correction of the calibration curve for all the samples analyzed at that same time (see below).

The ammonia content of the unknown aliquots is evaluated by observing the absorbance at each of the three wave lengths, reading the ammonia concentrations from the calibration curves, and applying corrections as described below. All the ammonia concentrations found in this way for the duplicate aliquots are averaged, and this average is used to calculate the total ammonia content of the distillates.

Corrections of Calibration Curve for Individual Groups of Analytical Samples. Once a calibration curve has been set up for a given solution of Nessler's reagent, it remains usable as long as the Nessler solution shows no signs of rapid deterioration, such as the formation of large amounts of sediment. To obtain exact ammonia values, however, it has proved necessary at the start of any new determination of a group of samples on a given date to recheck the calibration curve. The method of correcting by these rechecks is illustrated by the following example.

Duplicate aliquots of distillate 1, fraction G, Table XII, were taken for ammonia determination on January 30. Following the procedure described above, these aliquots, some additional unknown samples, and three aliquots of the standard ammonia solution containing 50.3, 100.5, and 150.8 γ of ammonia nitrogen, all were nesslerized and their respective absorbances determined. From the calibration curve, the apparent ammonia nitrogen contents of the three standard ammonia solutions were evaluated as 57.3, 107.3, and 160.3 γ, respectively. The deviations between these values and the true ammonia nitrogen contents of the standards were +7.3, +6.8, and +9.5 γ respectively, the average deviation being +7.9 γ. (A deviation as high as this is not normally encountered except when a different gum arabic solution is substituted for the one used in compiling the data for the construction of the calibration curve.) This average of +7.9 γ is applied in the form of a correction of −7.9 γ to each of the ammonia values being determined for samples in the same group on the same day, January 30. For distillate 1, the value read from the calibration curve was 189.7 γ, which after correction becomes 181.8 γ of ammonia nitrogen. From this value, the ammonia nitrogen content of the distillate was calculated and found to be 4.22 mg. per gram of dry tobacco weight, the value listed in Table XII for January 30.

Table XII. Reliability of Calibration Curve Corrections as Shown by Reproducibility of Ammonia Determinations in Three Distillates

(All values expressed in milligrams per gram dry tobacco weight)

		Distillates Representing Fraction G of Two Tobacco Samples				Distillate Representing Fraction O	
		Distillate 1[a]		Distillate 2[a]			
Date of Detn.	Calibration Corrections[a], γ/50 Ml.	Ammonia N	Dev. from av., %	Ammonia N	Dev. from av., %	Ammonia N	Dev. from av., %
Jan. 30[b]	−7.9	4.22	1.2	3.82	1.8	...	...
Feb. 6[b]	−7.0	4.23	1.7	3.83	1.5	4.67	3.3
Feb. 10[b]	−6.2	4.10	1.4	3.83	1.5	4.43	2.0
Feb. 13	−3.0	4.16	0.0	3.89	0.0	4.67	3.3
Feb. 23	−3.8	4.07	2.2	3.83	1.5	4.33	4.2
March 3[c]	0.0	4.17	0.2	3.96	1.8	4.52	0.0
March 6	0.0	4.17	0.2	3.97	2.1	4.53	0.2
March 11	+3.3	...	...	3.96	1.8	...	...
Averages		4.16	±1.0%	3.89	±1.5%	4.52	±2.0%

[a] Distillates 1 and 2 contained nicotine nitrogen besides ammonia nitrogen in the ratio 1:3 and 1:1.4, respectively.

[b] On these dates solution of gum arabic used was different from that used for compilation of data for calibration curve.

[c] On March 3 and later dates a new Nessler solution and a new calibration curve were used.

Discussion. Effectiveness of Calibration Curve Corrections. By following this procedure on each of the dates listed in column 1 of Table XII and applying the specific daily corrections listed in column 2, the results listed in column 3 were compiled. They and the values obtained for two other distillates (columns 5 and 7) show clearly that reproducible values are obtained. The accuracy of this procedure is illustrated by Table XIII, in which are shown the ammonia nitrogen contents of three distillates containing ammonia as the only base. The values in brackets are the results of replicate determinations. The agreement between the Nessler values and titration values is satisfactory for all three samples.

Table XIII. Ammonia Content of Distillates Containing Ammonia as Only Base

Distillate No.	Ammonia Content, Mg. N			Correction Applied in Nessler Detn., γ NH$_3$N
	Titration	Nessler	Average	
	5.04	5.03 5.00 4.96	5.00	+4.0
2	4.84	4.94 4.84 4.87	4.88	+3.4
3	4.98	4.99 4.94 4.99	4.97	+3.4

The reasons for the varying light-scattering capacities of solutions that are nesslerized on different days under apparently equal conditions are not completely clear. Miller and Miller (*47*) as well as Thompson and Morrison (*65*) have discussed the variables that affect the color development in nesslerized ammonia solutions, and have pointed out that the temperature, the method of mixing, and the length of time for color development are important factors.

In experiments in which these variables were kept rigidly constant, the "daily variations" discussed above could not be eliminated. Hence, it seems that certain, not easily controllable changes in the Nessler reagent and in the colloidal gum arabic solution are responsible for the irregularities observed.

ACCURACY OF DETERMINATIONS OF AMMONIA IN DISTILLATES OF ANALYTICAL SCHEME. Of the fractions and subfractions analyzed for ammonia in following the stepwise scheme of tobacco analysis, distillates G and O and the distillates obtained from A1, B1, and B2 give satisfactory values, deviating in the average by not more than 2 to 3% from the correct ammonia contents. This can be shown by independently calculating the ammonia concentrations of these distillates from their contents of total volatile bases (found by titration) and from their contents of alkaloidal bases (found by spectrophotometry).

Only the ammonia content of fraction, M, which is obtained by a 5% alkali distillation, frequently shows a less satisfactory agreement (discrepancies up to ±5%) between the values based on titration and Nessler determination. Furthermore, this same fraction M, contrary to the other ammonia-containing distillates, shows a slight anomaly, as the ammonia values obtained from the three readings at 400, 420, and 450 mμ do not agree as well with each other as do the corresponding values obtained with other distillates and with the standard ammonia solutions. These characteristics seem to indicate that fraction M contains, besides ammonia, small amounts of amines. This conclusion is supported by the observation that aqueous solutions containing small amounts of methylamine and dimethylamine yield, on nesslerization, yellow colored solutions that are similar but not equivalent in their light-scattering capacities to nesslerized ammonia solutions of corresponding molar concentrations. This was also observed by Liebhafsky and Bronk (*44*).

C. DEVARDA METHOD FOR DETERMINATION OF NITRATES

During steps A to M, inclusive, of the analytical scheme, all the distillable ammonia, including that derived from glutamine and asparagine, has been removed from the aqueous tobacco extract. Hence, any ammonia formed on reducing the extract after step M can originate only from nitrates, especially since no indications were found of the presence of amounts of nitrites or of hydroxylamine in the aqueous tobacco extracts. Of the various methods for reducing nitrates to ammonia, the reduction in an alkaline medium by Devarda alloy was preferred to the reduction by metals, or by other reducing agents in an acidic medium. In the Devarda method (*19*), the newly formed ammonia is immediately removed by distillation, whereas the reduction in acid requires subsequent steps for the neutralization, alkalinization, and distillation of the ammonia derived from the nitrates. Moreover, in the usually employed reduction of nitrates with metallic iron and subsequent alkalinization of the solution, voluminous precipitates of iron hydroxide are formed that interfere both with the ammonia distillation and with the final analysis of the distillation residue, P.

The reason for discussing here the well-known reduction of nitrates by Devarda alloy is that two precautions were found to be essential with this method for obtaining accurate values for the nitrates in tobacco extracts.

The first precaution is to perform the reduction at a very low alkalinity; the second is to use a large excess of Devarda alloy. Without these precautions, the reduction of the nitrates in fraction L tends to be incomplete.

Alkalinity during Devarda Reduction. That the reduction of nitrates to ammonia by Devarda alloy can yield low values if carried out in a 5% (w./v.) sodium hydroxide solution, as usually recommended, has been reported by Brabson and Karchmer (*10*) and by Drouineau and Gouny (*21*). According to these authors, the interference of the nitrate reduction is not caused by the alkali itself, but by small amounts of silica that are leached by the boiling alkali from the walls of the distillation bulb in amounts sufficient to poison the surface of the Devarda alloy for the reduction of the nitrates. These same authors found that these difficulties can be overcome by substituting magnesium oxide for the caustic alkali solution.

Use of Large Amounts of Devarda Alloy. Even in the presence of magnesium oxide, the reduction of the nitrates in tobacco extracts does not always proceed smoothly and to completion, in spite of the fact that the amounts of hydrogen generated in the reaction mixture are obviously sufficient to reduce all the nitrates. Inasmuch as the nitrate reduction occurs catalytically on the metallic surface of the alloy, the insufficient reduction is obviously caused by the presence in fraction L of unknown tobacco components that poison the surface of the Devarda alloy.

The possibility that sulfur-containing compounds are responsible for this poisoning effect was excluded by model experiments with potassium nitrate solutions containing added hydrogen sulfide, cystine, and other sulfur-containing compounds. None of these compounds retarded the reduction of the nitrate to ammonia. The type of interfering substances was indicated, however, by other model experiments with potassium nitrate solutions to which had been added asparagine, a compound that is present (as shown by the analytical results for fractions B1 and B2 and by some recent work with paper chromatography) in appreciable amounts in most tobacco extracts. In the presence of asparagine, the reduction of nitrate to ammonia was considerably retarded, and failed to go to completion. This effect is identical with that observed in the Devarda reduction of fraction L. Moreover, it was found that analytically detectable quantities of nitrogen were retained in the Devarda mud (fraction Q), and that a corresponding quantity of α-amino nitrogen of the added asparagine disappeared. All this indicates that asparagine and possibly related substances, such as aspartic acid and glutamic acid, act as poisons for the nitrate reduction.

The difficulties described above can be overcome by using a large excess of Devarda alloy which, together with magnesium oxide, is added to fraction L prior to distillation. Specifically, it was found that a complete and sufficiently fast reduction of the nitrates was achieved for all the tobacco samples so far analyzed in this laboratory by adding 2 grams of the alloy for every gram of tobacco present in fraction L.

This is illustrated by the data listed in Table XIV. That the higher values for nitrate nitrogen obtained in using the large amounts of Devarda alloy are correct has been proved for a considerable number of samples by checking the method described here with the analytical method for nitrate determination in tobacco described by Vickery and Pucher (*55*, *69*). Excellent agreement was obtained between the values found by both methods (*68*). The reagents and procedure are presented above.

Table XIV. Influence of Amount of Devarda Alloy Used on Nitrate Determinations

(All values expressed in milligrams per gram of dry tobacco weight)

	Nitrate Nitrogen Found as Ammonia in Distillates after Distilling Fraction L in Presence of	
Sample No.	Insufficient Devarda alloy	2 g. of Devarda alloy per 1 g. tobacco
I	2.52	3.61
	2.25	3.47
	2.40	3.39
	2.39	3.48
	2.04	
	Av. 2.42	3.49
II	0.72	1.62
		1.51
	Av. 0.72	1.57
III	0.81	2.77
	0.64	2.63
	0.72	
	Av. 0.79	2.70
IV	2.20	3.30
		3.44
	Av. 2.20	3.37

D. DETERMINATION OF NICOTINIC ACID

Because of the general interest in nicotinic acid or niacin as a component of the vitamin B complex, an extensive literature exists on the analytical determination of this compound. Handler in 1948 reviewed the literature on niacin, including the analytical methods used for determining this compound (*33*). Essentially two kinds of analytical procedures have been developed, based on either biological or chemical methods.

Of the chemical methods, the most frequently employed procedure involves the color reaction, first observed by König (*42*) among nicotinic acid, cyanogen bromide, and an aromatic amine. As Handler points out, in almost each publication on this method a new modification is recommended, principally of the aromatic amine employed for the color development. In spite of their popularity, these procedures based on the König reaction have not proved to be very practical, because a number of variables have to be strictly controlled to obtain dependable results (*31*, *33*, *37*, *51*).

A second chemical method makes use of the fact that on reaction with pyridine compounds under certain conditions, 2,4-dinitrochlorobenzene forms products which on alkalinization yield a purple color (*40*, *73*, *74*). The instability of the colors obtained by this method and the time-consuming procedures have combined to make this analytical method unattractive. For several years this procedure was used in this laboratory for semiquantitative work. It was finally given up in view of its inherent disadvantages.

Isolation and Determination of Nicotinic Acid. Both colorimetric methods when employed for the determination of nicotinic acid in tobacco respond not only to nicotinic acid, but also to other pyridine derivatives, such as nicotine, related alkaloids, and their transformation products. As these other pyridine derivatives are present in most tobacco samples in very much larger quantities than nicotinic acid, the colorimetric method can obviously be used only for fractions from which all the pyridine derivatives other than nicotinic acid have been quantitatively removed. Fraction P of the analytical scheme, an aliquot of which is used for the determination of nicotinic acid, fulfills this condition, because, in the previous step of the analytical scheme, this fraction has been freed from alkaloids and from those alkaloid transformation products that are precipitated by silicotungstic acid. There is, however, always the possibility that some unknown pyridine compounds are still present associated with nicotinic acid in fraction P.

A way out of this difficulty is making use of the fact that nicotinic acid can be selectively extracted with ethyl ether from its aqueous solution at pH 3 and that the amounts of the extracted acid can be easily and accurately determined by its transfer into aqueous solution, and measurement of its ultraviolet absorption spectrum in this medium.

By employing this procedure interfering substances are eliminated and one of the principal objections against the colorimetric methods—namely, the necessity of strict control of the numerous variables affecting the results—is overcome.

Dann and Huff (*17*) have shown that nicotinic acid, although

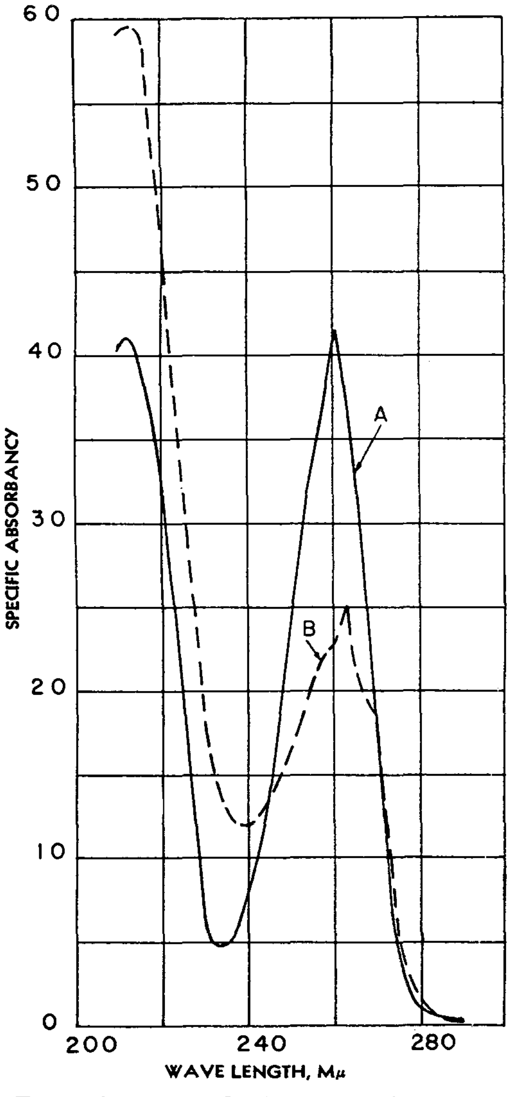

Figure 5. Ultraviolet Absorption Spectrum of Nicotinic acid

A. Aqueous acid. B. Aqueous alkali
Specific absorbancies plotted are those of a solution containing 1 gram of nicotinic acid per liter in a 1-cm. cell measured against pure solvent

sparingly soluble in ethyl ether, can be extracted by that solvent in a continuous liquid-liquid extractor from its aqueous solution at pH 3. Some data on the ultraviolet absorption spectrum of nicotinic acid have been reported by Hunecke (*34*), by Hughes, Jellinek, and Ambrose (*35*), and by Brode (*11*).

The ultraviolet absorption spectrum of nicotinic acid in aqueous acid and in aqueous alkali has been quantitatively measured in this laboratory. The spectra are shown in Figure 5. From these measurements, a value of 41.6 was determined for the specific absorbancy of nicotinic acid at its absorption maximum (261 mμ in an acidified solution). This laboratory also confirmed the findings of Hughes *et al.* (*35*) that Beer's law is valid for strongly acid solutions (pH $\eqslantless$ 1) of the compound, and that the absorbancies of such strongly acid solutions, for every concentration of nicotinic acid, are at a maximum, and not affected by further decreases of the pH.

Application of Method. To verify the observation of Dann and Huff concerning the extractibility by ether of nicotinic acid and to determine the accuracy of its quantitative determination by measuring its ultraviolet absorption spectrum, a number of experiments were undertaken using pure nicotinic acid. Table XV shows typical results of these tests in which known amounts of pure nicotinic acid were used. In no case is the recovery of the nicotinic acid less than 96%, a result which can be considered satisfactory for the assay of this compound in tobacco samples.

Table XV. Recovery of Nicotinic Acid by Extraction with Ether at pH 3 and Measurements of Ultraviolet Absorption Spectrum

(Values are in milligrams of nicotinic acid nitrogen[a])

Nicotinic Acid Nitrogen in 100 Ml. Water	Nicotinic Acid Nitrogen Found: In extract	In extraction residue	Recovery, %
0.119	0.114	nil	95.7
0.119	0.115	nil	96.6
0.598	0.586	nil	98.0
0.598	0.585	nil	97.8
1.195	1.163	nil	97.3
1.195	1.170	nil	97.9

[a] Multiply by 8.79 to convert into milligrams of nicotinic acid.

Table XVI lists the nicotinic acid contents of some typical fermented cigar leaf tobaccos. In each case the nicotinic acid was determined in fraction P of the stepwise analytical scheme by following the procedure described below. Samples 1a, 1b, and 1c are triplicate aliquots of the same fraction P, while samples 2a and 2b are duplicate aliquots of another fraction P. The results for the three aliquots of sample 1 and for the two aliquots of sample 2 illustrate that the method is sufficiently reproducible.

Table XVI. Nicotinic Acid Contents of Some Typical Fermented Cigar Tobacco Samples[a]

(All values are in milligrams of nicotinic acid nitrogen[b] per gram dry tobacco weight)

Fraction C of Sample No.	Aliquot	Content of Nicotinic Acid Nitrogen
1	a	0.40
	b	0.40
	c	0.39
2	a	0.31
	b	0.33

[a] Pennsylvania Seedleaf, U. S. Type 41.
[b] Multiply by 8.79 to convert into milligrams of nicotinic acid.

As there is still a remote possibility that the pyridine compound determined by this method is not nicotinic acid, but an unknown substance of very similar properties, a reliable identification of nicotinic acid appeared desirable.

As described in previous papers (*27*, *30*), substantial amounts of nicotinic acid were isolated from tobacco, using a special solvent extraction method that included, as an important step, an ether extraction of the acid from an aqueous solution at pH 3. After subsequent purification of the extracted substance by fractionated vacuum sublimation, it was identified as nicotinic acid by its melting point (no depression of melting point on admixture with authentic nicotinic acid) and by its ultraviolet absorption spectrum which was identical with that shown in Figure 5.

In other experiments the niacin contents of several tobacco extracts were determined by microbiological assay. The same extracts were analyzed for niacin by the method described below. In every case the results found by microbiological assay agreed well with those found by the method described here.

Apparatus. A Model DU Beckman spectrophotometer and a continuous liquid-liquid extraction apparatus (No. 6840, Ace Glass, Inc., Vineland, N. J.).

Reagents. Ethyl ether, analytical reagent grade.

Hydrochloric acid, 2.5*N*. One part of concentrated hydrochloric acid, ACS, diluted with 4 parts of optically pure distilled water.

Distilled water, spectroscopically pure.

Procedure. Take an aqueous solution in which the nicotinic acid content is estimated to be at least 1 mg. (0.11 mg. of nicotinic acid nitrogen). In the stepwise scheme of analysis the fraction taken for this purpose is fraction P. Adjust the pH of the solution to ca. 3 (pH test paper may be used) and place in the extraction chamber of a liquid-liquid extractor. Extract the aqueous solution (ca. 100 ml.) with ethyl ether. Adjust the boiling rate of the ether in the boiling flask so that 4 to 5 ml. of ether pass per minute through the aqueous solution for 21 hours. With quantities of nicotinic acid up to 5 mg., 21 hours should be sufficient for complete extraction under the stated conditions. Nevertheless, the completeness of the extraction must be checked by continuing the extraction after the 21 hours have elapsed, using a second portion of ether in order to make sure that all of the niacin has been extracted.

When the extraction is complete, separate the ether layer from the aqueous phase in the extraction chamber by decanting it into the ether extract contained in the boiling flask, then remove the aqueous extraction residue from the apparatus, taking care not to allow any of the aqueous solution to run into the side arm of the extractor. Wash this side arm with spectroscopically pure distilled water and place these washings in a distillation bulb. This precaution is advisable because minute quantities of nicotinic acid may be deposited in the side arm of the extractor owing to its very low solubility in ethyl ether. Add more distilled water to make the total volume in the distillation bulb 20 to 30 ml. and place the ether extract from the boiling flask on top of this aqueous phase. Rinse the boiling flask with ca. 10 ml. of distilled water and add this water to the distillation bulb. Cautiously distill the ether off. When all of the ether has been removed from the aqueous solution, transfer this solution, containing the nicotinic acid, to a glass-stoppered volumetric flask and make to volume. Pipet an aliquot of the aqueous extract containing ca. 1 mg. of the compound into a 50-ml. glass-stoppered volumetric flask, add 5 ml. of hydrochloric acid (ca. 2.5*N*), and make to volume. Determine the ultraviolet absorption spectrum of this solution in the Beckman spectrophotometer. Calculate the nicotinic acid content of the sample from the absorption curve, after applying a correction in a way corresponding to that described by Willits *et al.* (*79*) for nicotine.

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Differential Method for Precision Colorimetric Analysis

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COLORIMETRIC and spectrophotometric analyses are now carried out almost exclusively by means of photoelectric instuments, and the sensitivities of the photoelectric cells are as a rule remarkably high. However, if absolute methods are used—i.e., if the transmittance of a solution is measured—the full sensitivities of the cells are only seldom utilized. Only a small fraction of the maximum possible photocurrent passes through the galvanometer, the intensity of the light source is considerably reduced, or the devices for balancing the photocurrent are not sufficiently accurate. Colorimetric comparison methods, on the other hand, are theoretically more accurate, but they are not very convenient, as they demand very careful work, and hence have not acquired much popularity.

Recently, Hiskey (*5*), Bastian (*2*, *3*), and coworkers have shown that the precision can be considerably enhanced by using a differential method in which the transmittance of the unknown solution is measured in relation to a standard solution and not, as is usual, in relation to the solvent. Hiskey, Rabinowitz, and Young (*6*, *7*) have elucidated the theoretical basis of this method and calculated the precision attainable with some types of commercial instruments.

Compared with the usual methods this differential method has undoubtedly many advantages, but it seems questionable whether it is always possible to utilize fully the sensitivity of the photoelectric instruments. First, the method demands reference standards with a high absorbance; if the sample solution has a medium or low absorbance it is not possible to take full advantage of the sensitivity of the instrument. Secondly, very carefully calibrated cuvettes are required; if only one cuvette is used in order to dispense with calibration, the emptying, refilling, and

A differential method for colorimetric analysis makes it possible to utilize the sensitivity of photoelectric instruments more effectively than is usual. The precision of the method and the most favorable experimental conditions have been elucidated on the basis of theoretical considerations. When analyzing copper perchlorate and iron *o*-phenanthroline solutions, results accurate to within 1 to 2 parts per thousand were obtained. The method avoids errors caused by imperfect construction and positioning of the cuvettes and does not demand standard solutions of very high absorbance as are usually required in differential precision methods. If the standard is suitably chosen, the derived equations give satisfactory results even if Beer's law is not valid; relatively simple filter photometers can therefore be used.

repositioning of the cuvette can lead to considerable error, especially if barrier layer cells are used (fatigue and recovery phenomena). Thirdly, the method assumes validity of Beer's law. It is true that Hiskey and Young (*7*) have developed a method for correcting the results when Beer's law is not valid, but the method of correction is not very simple and takes considerable time.

In order to avoid the disadvantages mentioned, a new type of differential method has been developed. This method offers several advantages compared with the method of Hiskey. It always takes full advantage of the sensitivity of the photocell, and no high absorbance reference standards are required. The maximum precision is attained with medium absorbance standards. Furthermore, the method gives correct results independent of the validity of Beer's law. If the reference standard is suitably chosen, the equation used in the calculations is valid for white light as well as for monochromatic light; relatively simple filter photometers can therefore be used. The method avoids errors due to imperfect cuvettes and to the positioning of the cuvettes.

On the other hand, in performance the method is not so simple as the method of Hiskey. Moreover, the attainable precision is diminished by the fact that the entire volume of the standard is not replaced by the unknown.

COLORIMETRIC COMPARISON METHODS

There is an important difference between absolute methods and comparison methods. Accurate absolute methods in which the absorbance of an unknown solution is measured demand monochromatic light. Polychromatic light causes deviations from the absorption law, and hence empirical calibration curves are required. It is difficult to attain a high precision under such conditions. Comparison methods are based on other principles. The use of monochromatic light offers little advantage, whereas the use of polychromatic light can lead to a considerably enhanced precision.

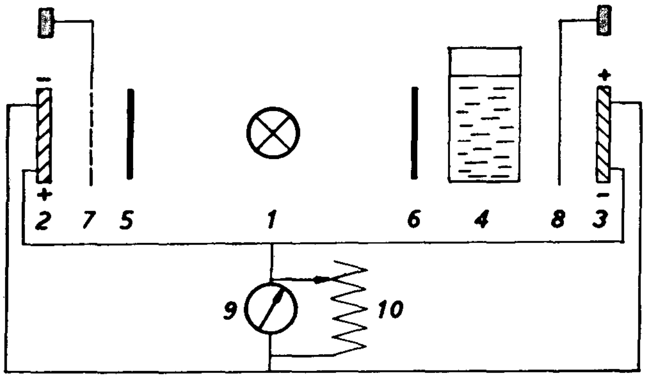

Figure 1. Two-Cell Filter Photometer

1. Lamp
2,3. Barrier layer cells
4. Cuvette
5,6. Color filters
7. Iris diaphragm
8. Shutter
9. Galvanometer
10. Resistor

In order to make the theoretical principles clear, they can be applied with reference to a two-cell instrument of the type illustrated in Figure 1. The principles can, of course, be extended also to other types of instruments.

When the absorbance of a solution is measured with the two-cell instrument, the scale units of the galvanometer (scale 0 to 100) indicate directly the percentage absorption. The 0 point is adjusted by means of the iris diaphragm, the 100 point by means of the shutter and the galvanometer resistor. The experimental conditions most favorable for comparing two almost identical solutions when one solution is substituted for the other can be defined. It is assumed that the lamp intensity is always the same.

A high precision is attained if the relative error per scale unit is as low as possible. In other words, if the photocurrent is designated by i and the concentration by c, the quotient $\frac{\mathrm{d}c}{c} \Big/ \mathrm{d}i$ should be as small as possible. The considerations should not be based on Beer's law, and hence the sole assumption is that the photocurrent is proportional to the intensity, I, of the light reaching the photocell. Consequently

$$i = k_\lambda I \tag{1}$$

where k_λ is a constant that depends on the composition of the light used. Each color filter of a filter photometer has thus a k_λ value of its own.

For simplicity, I is expressed in percentage transmittance and the photocurrent, i, in galvanometer units at maximum sensitivity; k_λ is then the number of scale units of the galvanometer per 1% transmittance. Since $\mathrm{d}i = k_\lambda\,\mathrm{d}I$,

$$\frac{\mathrm{d}c}{c} \Big/ \mathrm{d}i = \mathrm{d}\ln c / k_\lambda \mathrm{d}I \tag{2}$$

or

$$\frac{\text{Error, \%}}{\text{Scale unit}} = \left(\frac{\Delta c}{c} \times 100\right) \Big/ \Delta i = 230 \Big/ \left(k_\lambda \times \frac{\mathrm{d}I}{\mathrm{d}\log c}\right) \tag{3}$$

In order to determine the minimum error, it is thus necessary to know the value of k_λ and the differential quotient. These two quantities can easily be determined in the following way (*9*).

The cuvette is filled with water, and the optical scale of the instrument is adjusted so that the galvanometer deflection indicates the percentage absorption. Small amounts of a dissolved colored substance are then added to the cuvette until the percentage absorption of the solution is, for example, 1%. If the resistor of the galvanometer is then adjusted to give the maximum sensitivity, the galvanometer needle will indicate k_λ scale units. The differential quotient $\mathrm{d}I/\mathrm{d}\log c$ can be determined graphically by plotting the transmittance of a solution as a function of the logarithmic concentration of the colored compound. Figure 2 and Table I illustrate this method. The optimal conditions for determining cobalt on the basis of the red color of cobalt sulfate solutions (*10*) were determined using an instrument of the type given in Figure 1 (Lange photometer with galvanometer sensitivity of 9×10^{-9} A. per scale unit). The inflection points of the curves correspond to the optimal concentrations. The color filters used were far from monochromatic.

The results show that the error per scale unit attains its smallest value when no color filters are used and the concentration is about 15 grams of cobalt per liter. However, these arguments are purely theoretical, and in practice other factors must also be considered. Above all, the high intensity of the light when no filters are used results in a poor time stability of the photocurrent owing to the rapid fatigue of the photocells. Moreover, the inflection point of the curve occurs at a rather large concentration. As the difference in the error when working without filters and when working with green filters is not very large, an experienced chemist would prefer to use green filters in a comparison method.

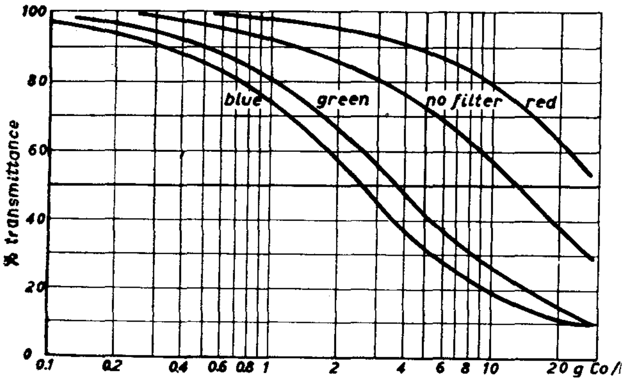

Figure 2. Transmittance of Cobalt Sulfate Solutions as a Function of Logarithmic Concentration

These considerations are not based on Beer's law. If the law were valid, $dI/d \log c$ would reach its maximum value of 84% transmittance per logarithm unit when $I = 36.8\%$ transmittance, and the minimum error would be $2.7/k_\lambda\%$ per scale unit (*1*, *9*).

It might seem that a very high degree of precision could be attained with a titration technique in which a standard solution is diluted until its transmittance is the same as the transmittance of the sample solution. However, many difficulties may be encountered when such a method is used. The time required for a determination is so long that the state of the photocells may alter. If different cuvettes are used for the standard and for the unknown solution, the calibration of the cuvettes must be very carefully performed; to use the same cuvettes for the two solutions is inconvenient, takes time, and may lead to errors. On the whole, the precision attainable would be considerably lower than expected.

NEW DIFFERENTIAL METHOD

An analysis with an instrument of the type shown in Figure 1 illustrates the principle of the new differential method. However, the method does not presuppose an instrument of this type; any instrument which allows the balancing of the photocurrent is suitable, provided that the galvanometer is sufficiently sensitive and the cuvette is of such size that the volume above the light beam is fairly large. The titration cuvettes described by Walter (*11*), by Osborn, Elliott, and Martin (*8*), and by Goddu and Hume (*4*) seem suitable.

A carefully measured volume of the standard solution is transferred to the cuvette. By means of the iris diaphragm the photocurrent is then balanced until the galvanometer needle at maximum sensitivity attains the end point of the scale corresponding to the largest absorption. A small volume of pure water is added from a calibrated pipet. The dilution causes a change in the galvanometer deflection of α scale units. In this way the relationship between the galvanometer deflection and the change in concentration is obtained. The water volume should be so small that the galvanometer scale is not exceeded, but large enough so that α is more than half the scale units.

By means of the iris diaphragm, the galvanometer is again adjusted to the end point of the scale. Which of the two end points is used depends on whether the unknown solution has a greater or lesser absorption than the diluted standard. A known volume of the sample solution is then added to the cuvette containing the diluted standard solution, and the corresponding change in galvanometer deflection, β scale units, is measured.

Table I. Calculation of Minimum Error per Scale Unit from Logarithmic Absorption Curve of Cobalt Sulfate Solutions

Filter	k_λ[a]	$-dI/d \log c$[b]	Optimal Concentration, Grams of Cobalt per Liter[b]	Error per Scale Unit, %[b] $\left[\frac{230}{k_\lambda \times dI/d \log c}\right]$
No filter	162	57	15	0.025
Green	86	65	3	0.041
Red	56	About 65	>20	About 0.06
Blue	19	65	2	0.19

[a] Scale units per 1% transmittance.
[b] At the inflection point.

The concentration of the unknown solution c_x can now be calculated from the equation

$$c_x = c_2\left(1 + K\frac{\beta}{\alpha}\right) \tag{4}$$

where c_2 is the concentration of the diluted standard and K is a constant. It is possible to choose the standard solution so that K can be calculated solely from the volumes added. This will be clear from the derivation given below in which the following symbols are used:

	Solution 1 (Standard Solution)	Solution 2 (Solution 1 + v_0 Ml. of Water)	Solution 3 (Solution 2 + v_x Ml. of Sample Solution)
Volume	V_1	$V_2 = V_1 + v_0$	$V_3 = V_2 + v_x$
Concentration	c_1	$c_2 = \frac{V_1}{V_2} c_1$	$c_3 = c_2 + \Delta c_2$
Transmittance, %	I_1	$I_2 = I_1 + \Delta I_1$	$I_3 = I_2 + \Delta I_2$
Absorbance	A_1	A_2	A_3
Change in galvanometer deflection	..	α	β

From the way in which the analysis is conducted, it follows that

$$V_3(c_2 + \Delta c_2) = v_x c_x + V_2 c_2 \tag{5}$$

All the quantities in this equation are known except c_x and Δc_2. In order to express Δc_2 in known quantities, the following equation is used.

$$\frac{dI/dc}{c} = \frac{dI}{d \ln c} \tag{6}$$

Taking into account the transmittance changes, ΔI_1 and ΔI_2, the followed equation is obtained.

$$\frac{\Delta I_1}{\Delta I_2}\,\frac{\Delta c_2}{c_2} \times \frac{c_1}{\Delta c_1} = \left(\frac{dI}{d \ln c}\right)_1 \Big/ \left(\frac{dI}{d \ln c}\right)_2 \tag{7}$$

As the photocurrent was assumed to be proportional to the light intensity,

$$\alpha/\beta = \Delta I_1/\Delta I_2 \tag{8}$$

Furthermore

$$\Delta c_1 = -\frac{v_0}{V_2} \times c_1 \tag{9}$$

The relationship between the differential quotients in Equation 7 can be expressed in Briggsian logarithms, and from Equations 7, 8, and 9

$$\frac{\alpha}{\beta} \times \frac{\Delta c_2}{c_2} \times \frac{V_2}{v_0} = -\left(\frac{dI}{d \log c}\right)_1 \Big/ \left(\frac{dI}{d \log c}\right)_2 \tag{10}$$

Equations 5 and 10 give

$$c_x = c_2 \left[1 - \frac{V_3}{V_2} \times \frac{v_0}{v_x} \times \left(\frac{dI}{d \log c}\right)_1 \Big/ \left(\frac{dI}{d \log c}\right)_2 \times \frac{\beta}{\alpha}\right] \tag{11}$$

and the constant of Equation 4 is consequently

$$K = -\frac{V_3}{V_2} \times \frac{v_0}{v_x} \times \left(\frac{dI}{d \log c}\right)_1 \Big/ \left(\frac{dI}{d \log c}\right)_2 \tag{12}$$

If the two differential quotients of this equation are equal, the constant K can be calculated from the volumes only—i.e.,

$$K = -\frac{V_3}{V_2} \times \frac{v_0}{v_x} \tag{13}$$

The question arises whether such a simplification is actually possible. It can be seen in Figure 1 that close to the inflection points of the curves the differential quotients change very slowly, and in this region the simplification is justified. In other words, the standard solution should have a concentration not far from that corresponding to the inflection point. The use of such a standard offers two advantages: Maximum accuracy is attainable and the mathematical equation required in the calculation is simpler.

The derivations made above are not based on Beer's law, and consequently they are valid for polychromatic light as well as for monochromatic light. It can easily be shown (*1*, *5*, *9*) that when Beer's law is fulfilled, the following relationship is valid:

$$\left(\frac{dI}{d \log c}\right)_1 \Big/ \left(\frac{dI}{d \log c}\right)_2 = \frac{I_1 A_1}{I_2 A_2} \tag{14}$$

Consequently

$$K = -\frac{V_3}{V_2} \times \frac{v_0}{v_x} \times \frac{I_1 A_1}{I_2 A_2} \tag{15}$$

It might not be possible to choose a standard solution with the most favorable concentration; this is the case if very low concentrations are measured. If the Beer's law is valid, K can then be calculated from Equation 15, but if polychromatic light is used K must be computed from Equation 12—i.e., the value of the quotient $\left(\frac{dI}{d \log c}\right)_1 \Big/ \left(\frac{dI}{d \log c}\right)_2$ must be known.

This quotient can be determined graphically from curves like those in Figure 1 as shown by Ringbom (*9*). However, this procedure is not very accurate, and the following more accurate procedure is suggested.

V_1 ml. of the standard solution are added to the cuvette and the galvanometer needle is adjusted to the point of largest absorption by means of the iris diaphragm. Then v_0 ml. of water are added from a pipet. The corresponding change in galvanometer deflection is α_1 scale units. The galvanometer needle is again adjusted to the end point of the scale, and v_0 ml. of the solution are withdrawn and replaced by an equal volume of water using the same pipet. The change in galvanometer deflection is α_2 scale units.

Since $\Delta c_1/c_1 = \Delta c_2/c_2$ it is obvious that

$$\frac{\Delta I}{\Delta \log c_1} \Big/ \frac{\Delta I}{\Delta \log c_2} = \frac{\Delta I_1}{\Delta I_2} = \frac{\alpha_1}{\alpha_2} \tag{16}$$

Consequently

$$K = -\frac{V_3}{V_2} \times \frac{v_0}{v_x} \times \frac{\alpha_1}{\alpha_2} \tag{17}$$

In this way constant K can be precisely determined for a standard solution of arbitrary concentration.

The simplest expression for K—i.e., Equation 13—can be used within a certain concentration range, and if this range is to be determined the above procedure may be repeated.

By successively adding v_0 ml. of water to V_1 ml. of solution, the volume and the galvanometer sensitivity always being kept constant, a series of α values—i.e., α_1, α_2, α_3—can be obtained. The maximum value of α corresponds to the optimal standard solution, and α remains almost constant in a range on the both sides of this value (Table II).

The exact error per scale unit or per percentage transmittance can be determined very precisely from these α values, since it can readily be shown that

$$\frac{\text{Error, \%}}{1\% \text{ transmittance}} = \Delta c/c \times 100/\Delta I = \frac{v_0}{V_2} \frac{k_\lambda}{\alpha} \times 100 \tag{18}$$

It is assumed that the maximum sensitivity of the galvanometer is used. However, for the determination of the maximum α value and of the optimal concentration range such a high accuracy is not required. For this purpose the sensitivity of the galvanometer and the volume v_0 can thus be chosen at will.

MODIFICATION OF THE METHOD

The differential method described can be slightly modified if the volume of the cuvette is not sufficiently large by removing part of the solution before adding v_x. It is convenient to remove exactly v_x ml. In this case $V_2 = V_3$, and Equation 17 will change into

$$K = -v_0/v_x \tag{19}$$

The equation for calculating c_x is then simply

$$c_x = c_2 \left[1 - \frac{v_0}{v_x} \times \frac{\beta}{\alpha}\right] \tag{20}$$

The pipet used when removing v_x should not be dry—i.e., the outflow volume should be measured.

PRECISION OF THE METHOD

The attainable precision depends, of course, on the instrument used and on the experimental conditions, but the error can be calculated on the basis of some general considerations. Calculation of the relative error when two solutions are compared was described earlier. However, the differential method described here presumes two readings, and the sample solution and the standard are not directly compared. The accuracy, therefore, depends on the volumes used. The volume V_0 should be chosen so that $-\alpha$ is as large as possible. On the other hand, a small value of β and a large value of v_x are advantageous.

As an example, the theoretical accuracy of the experimental work can be calculated. The simple Equation 20 was used, and the volumes were: $V_1 = 120$ ml., $V_2 = V_3 = 125$ ml., $V_0 = 5$ ml., $V_x = 50$ ml. It is assumed that the errors in reading α and β are ± 0.5 scale unit and that they cause errors in the same direction. The results are presented graphically in Figure 3. It can be seen that the theoretical maximum error will not exceed $\pm 0.2\%$ if α is more than 60% of the scale.

The errors caused by inaccurate measurements of the volumes can easily be calculated. If the volumes mentioned above were measured with an accuracy of ± 0.01 ml., the errors from this source can be neglected, but if very small cuvettes are used the volumes will have to be measured with a higher degree of accuracy.

It may be advantageous, although not necessary, to dilute the unknown solution before the analysis to a concentration approximately equal to the standard solution or preferably to a slightly lower concentration. If no preliminary dilution was made, v_x should be added not from a pipet but from a sufficiently accurate buret in order to prevent β from exceeding the scale. If β is smaller than α and of opposite sign, no adjustment of the galvanometer is necessary before adding v_x.

A constant lamp current is necessary for obtaining accurate results. The temperature of the standard and of the sample solution should not be allowed to differ considerably.

Many of the equations used were obtained by replacing differentials by differences rather arbitrarily. This is not strictly correct from a mathematical point of view. However, appreciable errors will arise only if the sensitivity of the galvanometer is poor and if the transmittance of the standard is very far from the optimum value.

EXPERIMENTAL

A number of experiments were performed in order to determine whether the theoretical accuracy of the method could be attained. Copper was determined from the blue color of the copper aquo ion in copper perchlorate solutions, and iron was determined from the red color of iron(II) o-phenanthroline solutions. A colorimeter of the Lange type was used. The maximum sensitivity of the galvanometer used was 9×10^{-9} ampere per scale unit.

When copper was determined, red color filters were used. Beer's law was valid at transmittances above 50%. The value of $-dI/d \log c$ reached a maximum at about 40% transmittance, which corresponds to about 2 grams of copper per liter. The results of a series of α measurements are given in Table II.

Table II. Evaluation of Analytical Error by Successive Dilution of a Copper Perchlorate Solution[a]

Transmittance, %	α for $\begin{cases} V_1 = 120 \text{ Ml.} \\ v_0 = 5 \text{ Ml.} \end{cases}$	Error per 1% Transmittance, % $\left(\frac{v_0 k_\lambda}{V_2 \alpha} \times 100\right)$
15.0	−56.2	4.03
16.0	−59.7	3.80
18.1	−64.2	3.53
20.4	−67.7	3.35
22.8	−71.0	3.19
25.1	−75.2	3.01
27.7	−77.2	2.94
30.5	−78.7	2.88
33.3	−80.2	2.83
36.1	−80.0	2.83
38.9	−80.5	2.82
41.7	−80.2	2.83
44.5	−79.5	2.85
47.3	−79.0	2.87
50.0	−78.2	2.90
52.7	−76.0	2.98
55.3	−74.0	3.06
57.9	−72.0	3.15

[a] Red color filters; $k_\lambda = 56.5$.

Table III. Determination of Copper and Iron Using Differential Method[a]

	Standard Solution, Copper	Transmittance, %	α	β	Error, %
1	1.080[b]	59.0	−56.6	23.0	−0.02
2	1.176	56.5	−56.8	22.5	−0.14
3	1.176	56.5	−59.0	24.7	+0.08
4	2.480	32.5	−66.5	27.5	−0.04
5	2.480	32.5	−66.0	27.2	−0.05
	Iron				
6	0.980[c]	58.0	−58.8	24.3	−0.06
7	0.980	58.0	−59.7	25.0	+0.01
8	2.160	37.0	−50.3	21.2	−0.08

[a] Volumes. $V_1 = 119.38$ ml.; $V_2 = V_3 = 124.37$ ml.; $v_0 = 4.99$ ml.; $v_x = 49.91$ ml.

[b] Grams per liter.

[c] Milligrams per liter.

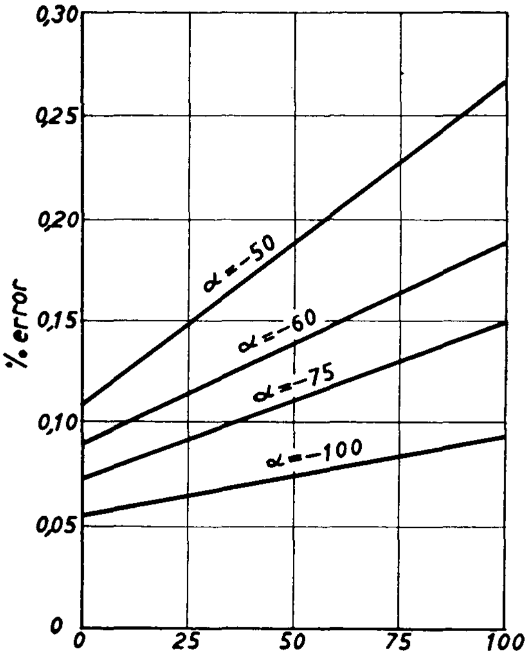

Figure 3. Percentage Error When Error in Reading α and β Is ±0.5 Scale Unit

It can be seen that α is practically constant at transmittance values from about 32 to 45%. If the transmittance of the standard is between these two values, the maximum accuracy will be attained and K can be calculated from Equations 13 or 19. The values obtained are presented graphically in Figure 4, which also contains the theoretical curve calculated from the absorption law.

A similar series of measurements was conducted in order to find the experimental conditions most favorable for determining iron, and curve 3 in Figure 4 illustrates the results. When light blue color filters were used, α and the percentage error were sufficiently constant in the range of 50 to 60% transmittance, which corresponds to about 1 to 1.5 mg. of iron per liter. Thus, a standard in this concentration range is suitable. At low transmittances the deviations from Beer's law are considerable, owing to the nonmonochromatic filters. The constant k_λ was about 80 scale units for the filters in question.

The results of copper and iron determinations are presented in Table III.

The procedure used was the modified method in which part of the diluted standard is replaced by an equal volume of the sample solution. Carefully calibrated pipets were used. The solutions were thoroughly mixed with a stirrer after adding v_0 and v_x. The undiluted standard was used as sample solution.

The iron phenanthroline color was developed at pH 3.5 (sodium

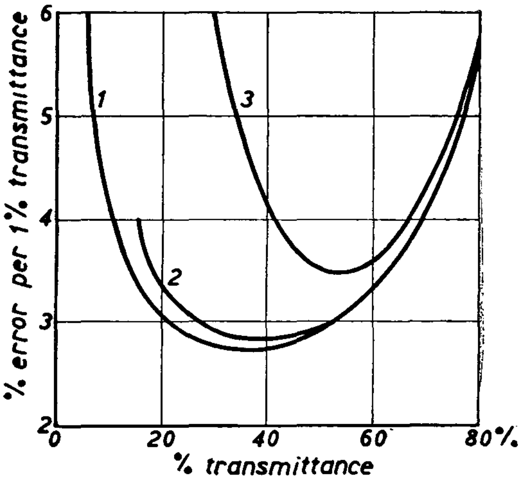

Figure 4. Error per 1% Transmittance as a Function of Transmittance

1. Error when Beer's law is valid
2. Error in determining copper
3. Error in determining iron

citrate buffer solution), and the reagent concentration was about 0.06 gram of phenanthroline per liter. The iron was reduced to the iron(II) state by means of ascorbic acid. The lamp used was not the same as that used in the experiments in Table II, hence the different α values.

As the standards in experiments 4 through 7 were sufficiently close to the optimum values, the results were calculated using Equation 20, whereas Equation 15 was used for experiments 1 through 3, and Equation 17 was used for experiment 8. The agreement is satisfactory.

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Ultraviolet Spectrophotometric Determination of Tantalum with Pyrogallol

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In a search for a more rapid method for the determination of tantalum in rocks and minerals, an intensive study was made of the tantalum-pyrogallol reaction recommended by Platanov and Krivoshlikov, and a better modified spectrophotometric procedure is given. The improved method consists in measuring the absorbancy of the tantalum-pyrogallol complex at 325 mμ in 4*N* hydrochloric acid and a fixed concentration (0.0175*M*) of ammonium oxalate. Beer's law is followed for the concentration range up to 40 γ per ml. Sensitivity in terms of molar absorbancy index is 4775. Most interferences are additive in character and readily correctable. Separations or major corrections are required in the presence of significant amounts of molybdenum, tungsten, antimony, and uranium. The method has been successfully applied to three ores previously analyzed by gravimetric techniques. The method affords greater speed, sensitivity, and reproducibility in the determination of tantalum in rocks and minerals. A more reliable technique for preparing standard solutions of tantalum has been developed.

KNOWLEDGE of the analytical chemistry of tantalum and niobium has improved considerably since the recent publication of a critical analysis of the existing methods by Atkinson, Steigman, and Hiskey (*2*). Since that time, among other methods, chromatography on cellulose has been successfully applied to the separation and determination of the individual earth acids from complex mixtures and ores by Burstall *et al.* (*3*), Mercer (*19*), and Williams (*29*); ion exchange separations using synthetic resins have been applied to selective mixtures by Huffman, Iddings, and Lilly (*7*) and Kraus and Moore (*11, 12*); a solvent extraction procedure by Leddicotte and Moore (*17*) has resulted in the separation of the earth acids from one another; neutron activation analysis has been applied by Eichholz (*4*) and Long (*18*) to the estimation of tantalum in the presence of several other elements.

Although three sensitive colorimetric methods now exist for the determination of niobium (*5, 16, 25*), no satisfactory colorimetric method was available for tantalum until recently. Soon after the work described in this paper was completed a simultaneous spectrophotometric method for the determination of tantalum and niobium was reported by Palilla, Adler, and Hiskey (*20*). They found that by working with concentrated sulfuric acid it was possible to shift the spectra of the peroxy tantalates from the ultraviolet cutoff and thus make possible the determination of tantalum. Titanium and iron give serious interference in this determination, however. If they are not removed by the chloride volatilization technique (*2*) or other means, their effect would have to be circumvented by the usual series of calculations involving absorbancy measurements at several wave lengths.

The use of the color-forming reaction between tantalum and pyrogallol as the basis for a colorimetric determination of tantalum was proposed by Platanov, Krivoshlikov, and Marakaev (*15, 23*) in 1936. Several studies and numerous applications of the method have since been made (*1, 6, 8–10, 13, 14, 21, 22, 26, 27*). The reported methods are lacking in sensitivity, however, and suffer from interferences caused by elements usually associated with the earth acids in rocks and minerals. Tedious separations are almost always necessary. In addition, most of the work is reported in Russian journals and is not readily accessible to English-speaking chemists except in the form of abstracts.

Thanheiser (*26*) studied the effect of sulfuric acid concentrations as high as 1*N* on the absorbancies of the tantalum-, niobium- and titanium-pyrogallol complexes. The present investigation extends the range of the hydrochloric acid concentrations studied to beyond 7*N*. The improved absorbancy relationships found at the higher acidities form, in part, the basis for the improved analytical procedure described later.

Selection of Acid. Sulfuric acid was the medium used by others in previously reported work. All of the experimental work with hydrochloric acid described later was also performed using sulfuric acid for comparison. Sulfuric acid was found to result in greater interference from niobium and titanium.

Nitric acid oxidized the pyrogallol to a yellow compound, which made measurements of the tantalum-pyrogallol complex impossible. Phosphoric acid depressed the absorbancy of the complex. Perchloric acid caused precipitation of salts from solution.

Hydrochloric acid was therefore found to be the best of the common mineral acids for use in this work.

GENERAL PROCEDURE

The following procedure for the analysis of a typical tantalum-bearing rock was adopted on the basis of the experimental work later described:

A 0.050- to 1.000-gram sample is fused with 10 grams of potassium bisulfate; 50 ml. of the ammonium oxalate extracting solution are then added to the cooled melt and the mixture is stirred continuously on a steam bath. The resulting solution is cooled and made up to volume with water. An aliquot, estimated to contain approximately 1 mg. of tantalum dioxide, is then added to a 50-ml. volumetric flask containing the following reagents: 25 ml. of hydrochloric acid solution, 10 ml. of pyrogallol solution, and sufficient dilute ammonium oxalate solution to make the final ammonium oxalate concentration 0.125 gram per 50 ml. The flask is then made up to volume, mixed well, and a portion is used for the determination of the absorbancy at 325 mμ, compared with the absorbancy of a blank set at 100% transmittancy.

STANDARDS, REAGENTS, AND APPARATUS

Standard Tantalum Solution. The purest grade of tantalum pentoxide available, containing less than 0.10% niobium pentoxide and less than 0.01% titanium dioxide, was used for the preparation of this solution. It was ignited at 1000° C. for 2 hours before use. An oxide obtained from another source contained more than 8% volatile matter and was quite hygroscopic even after ignition at 1000° C. for 48 hours.

A standard solution of the oxide, containing 0.20 mg. per ml., was prepared by the method outlined in the general procedure described above. Solutions thus prepared are stable for several months.

Continuous stirring has been found to be of critical importance in the preparation of a true solution of tantalum. The literature on tantalum commonly available does not stress the need for continuous stirring during an ammonium oxalate leach of a bisulfate melt. Its importance was indicated only after an intensive investigation was undertaken to determine the source of variations which were encountered during an attempt to prepare standard solutions giving the same absorbancy.

Numerous methods for the preparation of standard tantalum solutions were tried without success. In the common technique involving fusion of the oxide with potassium bisulfate and extraction of the fused melt with ammonium oxalate solution, variations were made in the temperature and duration of fusion, mole ratio of flux to oxide, and temperature and duration of extraction. Dissolution of the oxide in a mixture of hydrofluoric and sulfuric acids and evaporation of the solution to fumes of sulfur dioxide and dilution with water was also tried. Variations were made in the duration of fuming, mole ratio of sulfuric acid to oxide, temperature and duration of heating the final diluted solution, and reagent solutions used for the final dilution.

All of the variations listed above yielded solutions which gave absorbancies which could not be duplicated on retrial. In general, the absorbancy could be increased by maintaining the solution at 90° C. for greater lengths of time. The absorbancies produced by such aging, however, were not capable of being reliably duplicated. Moreover, some solutions were found to yield lower absorbancies when aged at elevated temperatures.

Dissolution of a bisulfate fusion of the oxide with either tartaric or oxalic acid frequently resulted in a turbid solution which gave a very low absorbancy, even when continuous stirring was applied.

The final procedure adapted—viz., fusion of the oxide with potassium bisulfate and dissolution of the fused melt with ammonium sulfate solution with continuous stirring—was found to allow considerable latitude in the permissible mole ratio of flux to oxide. Solutions giving the same absorbancy could be prepared consistently from mole ratios varying from 5000 to 1 to less than 5 to 1. Duration of time of heating the extracting solution was also not critical. Stirring the solution for 30 minutes or until the fused cake was completely dissolved was adequate; stirring for several additional hours had no further effect.

Aliquots of 11 different solutions prepared by stirring ammonium oxalate extractions gave absorbancies with a standard deviation of 0.003 absorbancy unit. This was only slightly greater than the standard deviation of 0.002 obtained using 10 aliquots from the same solution; it was also only slightly greater than the deviations caused by slight contaminations on the absorption cells.

The criterion of an adequate solution of tantalum oxide is usually implied to be a clear solution. A clear solution can be obtained, however, which gives only 1% or less of the expected absorbancy. Observation of the solutions for Tyndall effects showed a rough inverse correlation between the number of colloidal particles observed and the absorbancy of the solution. The cause of the colloidal particles can be ascribed to surface phenomena acting at the interface of the melt and the extracting solution.

Reagent Solutions. PYROGALLOL SOLUTION, 200 grams of pyrogallol, 100 ml. of concentrated hydrochloric acid, and 10 ml. of 2 *M* stannous chloride per liter. The presence of hydrochloric acid and stannous chloride increases the stability of the solution so that it can be kept for at least a month without decomposition. The period of usefulness of a solution prepared without these two reagents is about 2 days. Stannous chloride also serves to reduce the ferric ion usually present in the samples being analyzed.

HYDROCHLORIC ACID, 8 *N*.

AMMONIUM OXALATE EXTRACTING SOLUTION, 50 grams per liter.

DILUTE AMMONIUM OXALATE, POTASSIUM BISULFATE SOLUTION, 12.5 grams of ammonium oxalate and 50 grams of potassium bisulfate per liter. Because of a gradual increase in absorbancy, a fresh solution was prepared every 2 weeks.

Apparatus. The absorbancy measurements were made with a Beckman DU spectrophotometer equipped with ultraviolet attachments, and a set of matched 1.000-cm. silica cells. Water followed by acetone proved adequate as wash solutions.

All the data were rechecked using a photomultiplier attachment with a 10,000-megohm resistor in the spectrophotometer circuit. This permitted much higher sensitivities to be obtained and smaller band widths to be used; the necessary slit widths were reduced from approximately 1.5 mm. to less than 0.1 mm.

EXPERIMENTAL WORK

Effect of Variables on Absorbancy. The effect of the different variables on the absorbancy of the tantalum-pyrogallol complex was measured at 325 mμ.

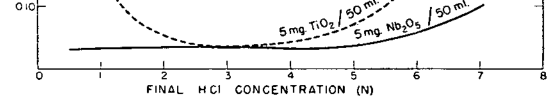

Figure 1. Effect of Hydrochloric Acid on Absorbancy of Tantalum-, Niobium-, and Titanium-Pyrogallol Complexes

HYDROCHLORIC ACID. The effect of varied concentrations of hydrochloric acid was studied in an effort to determine the optimum concentration to be used.

Varying amounts of the hydrochloric acid solution were pipetted into a series of 50-ml. volumetric flasks; 10 ml. of the pyrogallol solution were then added. This was followed by the addition of 8 ml. of the dilute ammonium oxalate solution and 5 ml. of the tantalum standard solution (1.00 mg. of tantalum pentoxide). The solutions were made up to volume and mixed well. For each concentration of hydrochloric acid used, a blank containing no tantalum was prepared immediately afterwards in a similar manner. Measurements were made on each pair of solutions with the tantalum-free solution serving as the blank. Similar measurements were made on solutions containing 5 mg. each of titanium dioxide and niobium pentoxide. These data are plotted in Figure 1.

The absorbancy of the tantalum-pyrogallol complex was increased by increase in the hydrochloric acid concentration, whereas the absorbancy of the niobium complex remained quite low below 5*N* hydrochloric acid. The absorbancy of the titanium complex was found to reach a minimum between 2.5 and 4*N* hydrochloric acid.

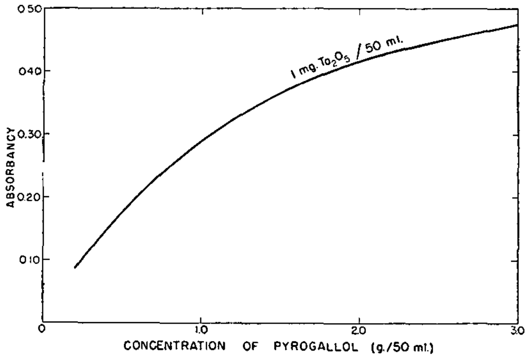

Figure 2. Effect of Pyrogallol on Absorbancy of Tantalum-Pyrogallol Complex

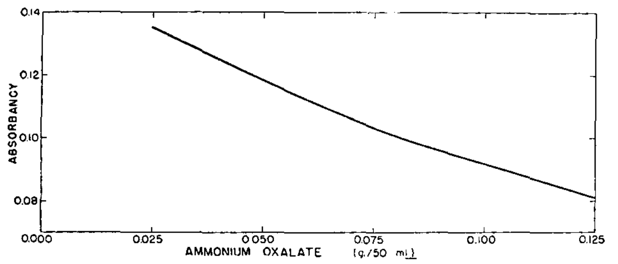

Figure 3. Effect of Ammonium Oxalate on Absorbancy of Tantalum-Pyrogallol Complex

A concentration of 4*N* hydrochloric acid was selected as the optimum compromise because it allowed a minimum interference from the two troublesome elements, titanium and niobium, while permitting a moderately high absorbancy of the tantalum complex.

PYROGALLOL. A similar study was made of the effect of pyrogallol concentration on the absorbancy of the complex. The procedure used was identical with the hydrochloric acid measurements except that the amount of pyrogallol added to each set of flasks was varied and the hydrochloric acid concentration was held constant at 4*N*.

The variations of absorbancy with pyrogallol concentration are shown in Figure 2.

The absorbancy of the tantalum-pyrogallol complex was found to increase with pyrogallol concentration. A limit was set on the permissible amount of pyrogallol, however, because of the high absorbancy it produced in the blank reference solution and the correspondingly larger slit widths required.

The amount of pyrogallol was fixed at 10 ml. of the pyrogallol solution (2 grams) in subsequent work.

AMMONIUM OXALATE. The effect of ammonium oxalate was studied in a similar manner by holding the hydrochloric acid concentration fixed at 4*N*, the pyrogallol concentration constant at 2 grams per 50 ml., the tantalum pentoxide concentration fixed at 0.2 mg. per 50 ml., and varying the ammonium oxalate concentration.

These data, shown in Figure 3, show the pronounced effect of ammonium oxalate on the absorbancy of the complex. The ammonium oxalate concentration in all the work performed was fixed at the arbitrary concentration of 0.125 gram per 50 ml. The relatively high concentration was chosen so as to enable greater amounts of tantalum solution to be used when desired; it also permitted taking larger aliquots in the analysis of samples low in tantalum pentoxide concentration.

Ammonium oxalate solution, measured against water in the reference cell, was also found to have an appreciable absorbancy in hydrochloric acid solution; this again necessitated the strict regulation of its concentration.

The dilute ammonium oxalate solution previously described was prepared for use in bringing solutions up to the fixed ammonium oxalate concentration.

POTASSIUM BISULFATE. Similarly, the effect of varying concentrations of potassium bisulfate was studied by holding the concentrations of all other reagents constant. It was found to have no measurable effect on the absorbancy of the tantalum-pyrogallol complex. It did, however, affect the absorbancy of the blank slightly; 0.5 gram per 50 ml. caused an increase in absorbancy of the blank of 0.01 absorbancy unit. For this reason, potassium bisulfate was also added to the dilute ammonium oxalate solution and its concentration was controlled along with that of ammonium oxalate.

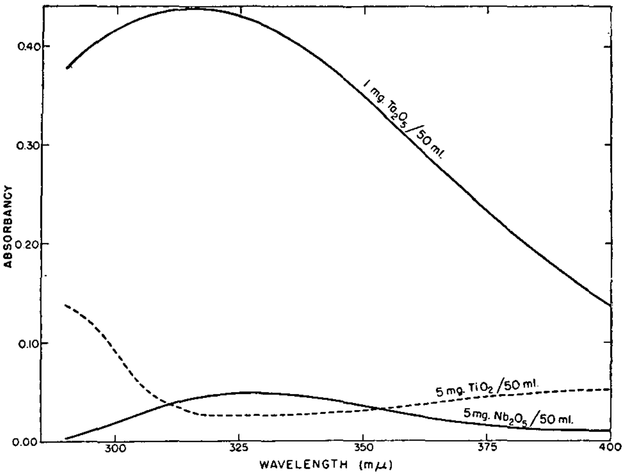

Figure 4. Absorption Curves of Tantalum-, Niobium-, and Titanium-Pyrogallol Complexes

Absorption and Standardization Curves. The absorption curve for the tantalum-pyrogallol complex was measured at the concentration level of 1.00 mg. of tantalum pentoxide per 50 ml. Curves for the niobium and titanium complexes were measured at five times this concentration in order to moderate the effects of any slight contamination of the absorption cells. The color development procedure outlined previously was followed and the absorbancies were measured against the absorbancy of a reference blank containing the same constituents with the exception of tantalum. The DU spectrophotometer and ultraviolet attachments were used for measurements below 350 mμ. For convenience, the Model B was used for measurements between 350 and 400 mμ. The data are shown in Figure 4.

The maximum absorbancy of the tantalum-pyrogallol complex was found at 315 mμ; 325 mμ was chosen as the operating wave length so that it would fall within the wave length range offered by the Model B spectrophotometer.

The standardization curve was found to follow Beer's law up to the concentration of 2.00 mg. of tantalum pentoxide per 50 ml.

The absorbancy index under the optimum conditions used was found to be 0.0215 ml. per microgram cm. Slight but significant variations from this value were sometimes caused by variations in the concentrations of hydrochloric acid or pyrogallol. For accurate work it is recommended that the same reagent solutions and pipets be used for the determination of the absorbancy index and the analysis of unknown solutions.

The molar absorbancy index, with concentration expressed as gram atoms of tantalum per liter, is 4775.

Factors Affecting Color Stability. TIME OF STANDING. A reference blank solution and a solution containing 1.0 mg. of tantalum pentoxide in addition to the recommended concentrations of reagents were permitted to stand at room temperature for 7 hours. Samples for absorbancy measurements were taken at regular intervals. The absorbancy of the reference blank measured against water was found to increase 0.015 absorbancy unit in the full 7 hours. No measurable difference was noted in 1 hour. The absorbancy of the tantalum-pyrogallol complex measured against a reference blank of similar age was found to decrease by 0.010 absorbancy unit. No measurable change was noted, however, after only 1 hour. Measurements could thus be made immediately or up to 1 hour from the time of mixing the solutions without a significant change in absorbancy.

The temperature coefficient for this reaction in the range 20° to 35° C. was found to be negligible.

The order of addition of reagents is immaterial.

INTERFERENCES. In the study of the effects of diverse ions on the absorbancy of the tantalum-pyrogallol complex those elements were considered which have been reported to be constituents of the many tantalum-bearing rocks and minerals.

Each of the elements, usually taken as 0.100 gram of the oxide, was treated as outlined in the general procedure.

Two series of measurements were made. In the first series the absorbancy of the reagent solution containing the diverse ion together with 1 mg. of tantalum pentoxide was measured. Comparison between this measurement and the absorbancy of a reagent solution containing 1 mg. of tantalum pentoxide alone showed the effect of the ion on the absorbancy of the tantalum-pyrogallol complex. Comparison of the difference between these two measurements and a second measurement showing the absorbancy of a solution of the ion alone, with no tantalum present, indicated whether the effect was additive or otherwise.

The data, expressed as apparent milligrams of tantalum pentoxide, are presented in Table I. Within detectable limits, in practically all cases the effect of the various ions was additive in character and readily correctable.

The elements that were found to require only moderate corrections for their presence in significant amounts were titanium, niobium, zirconium, chromium, vanadium, bismuth, and copper. The elements necessitating large corrections or separations were uranium, molybdenum, tungsten, and antimony. These elements, however, are rarely present in tantalum-bearing minerals in troublesome concentrations; if encountered, they should be separated from the earth acids. Iron did not interfere in the presence of stannous chloride.

In addition to the ions mentioned above, tartrate was found to depress the absorbancy of the complex moderately; fluoride bleached the color when present even in trace concentrations. Oxidizing agents oxidized the pyrogallol to black opaque solutions. Traces of platinum gave a very high absorbancy, probably because of the stannous chloride present; fused silica crucibles were used for the fusions for this reason. With the exception of fluoride, however, none of the interferences in this group are generally present in the rocks and minerals encountered, and their presence can usually be avoided.

APPLICATION OF METHOD TO MINERALS AND ROCKS

The method as described in the general procedure was applied to the analysis of three minerals. The results of the analysis of two of the minerals by eight cooperating laboratories (*24*) were recently republished (*2*). They appear as *a* and *b* in the frequency diagram shown in Figure 5, labeled gravimetric analyses. The third mineral, a columbite containing more than 60% niobium pentoxide, had been analyzed gravimetrically by two skilled rock analysts (*28*). The colorimetric analyses were performed by two analysts over a period of 4 months using several standard and reagent solutions. Corrections for the niobium content were made for mineral *c*, Figure 5. No separations were performed. The time required for a series of analyses was approximately one working day.

Table I. *Effect of Diverse Ions*

Expressed as	Apparent Wt. Ta_2O_5[a], Mg., 5.0 Mg. Diverse Oxide/50 Ml. Reagent	Permissible Amount, Mg., Giving <2% Error in Determination of 1.00 Mg. of Ta_2O_5
Nb_2O_5	0.12	0.80
TiO_2	0.07	1.4
ZrO_2	0.04	2.5
Na_2O	<0.01	>5.0
ThO_2	<0.01	>5.0
SiO_2	<0.01	>5.0
SnO_2	<0.02	>5.0
FeO	<0.01	>5.0
MnO	<0.01	>5.0
CaO	<0.01	>5.0
MgO	<0.01	>5.0
U_3O_8	0.25	0.60[b]
Y_2O_3	<0.01	>5.0
Ce_2O_3	<0.01	>5.0
MoO_3	2.5	0.04[b]
WO_3	0.75	0.15[b]
Cr_2O_3	0.04	2.5
V_2O_5	<0.02	>5.0
Co_3O_4	<0.01	>5.0
Al_2O_3	<0.01	>5.0
BaO	<0.01	>5.0
Sb_2O_3	0.50	0.20[b]
Bi_2O_3	0.03	3.5
CuO	0.04	2.5
Ni_2O_3	<0.01	>5.0
PbO	<0.02	>5.0
BeO	<0.02	>5.0
Sc_2O_3	<0.01	>5.0
B_2O_3	<0.01	>5.0
Li_2O	<0.01	>5.0
Rb_2O	<0.01	>5.0
K_2O	<0.01	>5.0

[a] Based on measurements made in the presence and absence of 1.00 mg. of Ta_2O_5.

[b] Based on measurements made in the presence of only 1 mg. of diverse oxide.

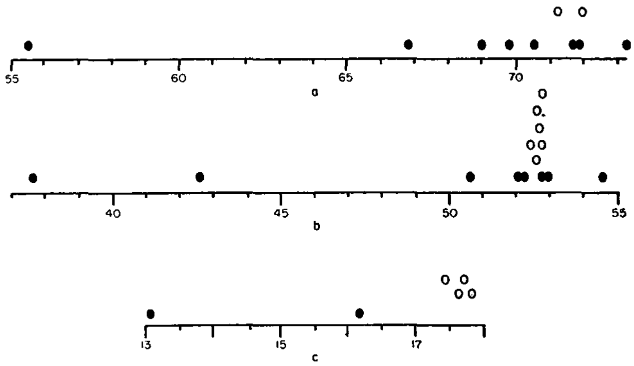

Figure 5. Comparative Analyses of Three Ores

Per cent of tantalum pentoxide
○ Colorimetric
● Gravimetric

ACKNOWLEDGMENT

The author would like to acknowledge the contributions of Mrs. Ambhai Anubunyong, of the Royal Department of Mines, Thailand, who patiently rechecked all of the data and performed many of the analyses. Leonard Shapiro, of the U. S. Geological Survey, suggested the application of continuous stirring to the preparation of true solutions of tantalum.

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Ultraviolet Spectrophotometric Determination of Niobium in Hydrochloric Acid

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The analytical methods available for the determination of niobium are not well suited for routine analysis because of critical or time-consuming operations. A hitherto unreported absorption peak at 281 mμ serves as the basis for a spectrophotometric determination of niobium. In the absence of large amounts of iron and certain other elements, the sample is obtained in a concentrated hydrochloric acid solution and the absorbance measured at the specified wave length. In the presence of interfering elements, the method may be used following a preliminary separation. It is rapid, accurate, and suitable for routine analysis.

TWO essentially different spectrophotometric methods for the determination of niobium have been reported in the literature: methods based upon the peroxy complex (*3, 5*), and those which make use of the thiocyanate complex (*2, 4*).

The peroxy methods lack sensitivity and suffer from the inconveniences inherent in the hydrogen peroxide—concentrated sulfuric acid reagent employed—i.e., the corrosive nature of the solutions and the annoyance of oxygen bubble formation. The lack of sensitivity of these methods is aggravated by the limited solubility of many materials in concentrated sulfuric acid, for this often establishes a practical upper limit to the sample size.

The procedures utilizing the thiocyanate complex, while possessing adequate sensitivity, offer other disadvantages. The complex is unstable in aqueous media. Freund and Levitt (*2*) stabilize the complex by the addition of a miscible organic solvent, acetone. The resulting solutions show increasing absorption with time, making it necessary to specify accurately the time interval from the addition of the reagent to the measurement of absorbance. Lauw-Zecha, Lord, and Hume (*4*) extract the colored species with ether, a process further complicated by the necessity for accurate control of the acidity.

Desesa and Rogers (*1*) report a method for the determination of iron using $6M$ hydrochloric acid, and give the absorption curves of other elements in the same solvent. Wernet (*6*) has shown that niobium hydrous oxide is appreciably soluble in highly concentrated hydrochloric acid.

A hitherto unreported absorption peak of a niobium species present in concentrated hydrochloric acid serves as the basis for a method for the determination of niobium. The interfering concentrations of certain ions are listed.

APPARATUS AND SOLUTIONS

Apparatus. All absorbance measurements were made with a Beckman Model DU spectrophotometer. Matched 1-cm. silica cells were employed; all solutions were measured against a hydrochloric acid solution of the same concentration as the sample.

Hydrochloric Acid. The concentrated hydrochloric acid used (Baker and Adamson, ACS specification grade) was $11.8M$. The concentration of hydrochloric acid solutions was determined by specific gravity. Chloride concentrations were determined by a potentiometric titration with standardized silver nitrate solution, using a silver indicating electrode. Acid concentrations were determined by titration with carbonate-free standard sodium hydroxide solutions to the phenolphthalein end point.

Standard Niobium Solution. A 0.5 mg. per ml. standard niobium solution was prepared by fusing 0.0715 gram of niobium pentoxide (A. D. MacKay Co.) with potassium bisulfate. The melt was dissolved in sulfuric acid, and the niobium precipitated as the hydrous oxide with ammonia. The gelatinous precipitate thus obtained was centrifuged, washed with distilled water, and centrifuged again. This freshly precipitated hydrous oxide dissolved easily in concentrated hydrochloric acid, and the clear solution thus obtained was diluted to exactly 100.0 ml. with the same reagent.

EXPERIMENTAL

In order to investigate the effect of the hydrochloric acid concentration on the absorption of the niobium complex, samples containing 8 γ of niobium per ml. were prepared by diluting the standard niobium solution with hydrochloric acid of different strengths. The absorption curves for these samples are shown in Figure 1.

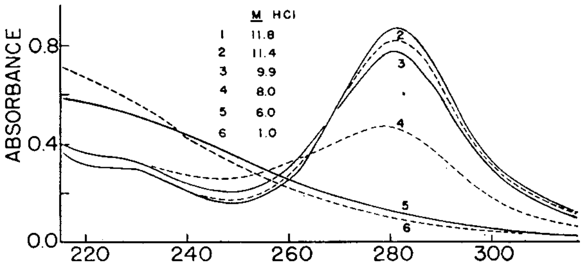

Figure 1. Absorption Spectra of Niobium Solutions

1-cm. cells
8 γ of niobium per ml.

It is clear that the hydrochloric acid concentration must be above 6*M* before the analytically important absorption at 281 mμ becomes useful. As the absorbance increases while the rate of change of absorbance decreases with increasing hydrochloric acid concentration, the maximum concentration of this reagent conveniently obtainable should be used for maximum sensitivity and accuracy of the method.

The effect of the independent variation of hydrogen ion and chloride ion is shown in Figures 2 and 3. The chloride ion concentration was maintained constant by dilution with 11.8*M* lithium chloride (Figure 2). The hydrogen ion concentration was kept nearly constant by dilution with 72% perchloric acid (Figure 3). In the latter case, the limited solubility of hydrogen chloride in perchloric acid caused the loss of some hydrochloric acid.

The niobium complex concentration depends, in a complicated manner, on both the hydrogen ion and the chloride ion concentrations. These curves cannot be quantitatively interpreted in terms of present knowledge, but it is hoped that investigations now in progress will provide the additional information necessary to assign structures to the complex species present. It is not the purpose of this paper to elucidate structures, but rather to present a rapid method for the determination of niobium. The fact that the exact structure of the complex used is not known does not impair its usefulness in analytical work.

The significance of Figures 2 and 3 is that hydrogen chloride is required for the formation of the niobium complex under consideration; neither hydrogen ion nor chloride ion alone will suffice.

An analytical curve was prepared by proper dilution of the standard niobium solution with concentrated hydrochloric acid. Beer's law was followed up to 10 γ of niobium per ml. The absorbance of the latter solution was 1.05. No attempt was made to verify linearity of absorbance with concentration at higher values.

The reproducibility of the method was determined by measuring six runs of five samples each, prepared and measured over a period of one week (Table I). Five supposedly identical volumetric flasks were used for all six runs. For each run, an aliquot of standard niobium solution was introduced, such that the final concentration of niobium would be 5 γ per ml. when the flask was filled to the mark with concentrated hydrochloric acid. The data were treated by the analysis of variance as a two-way classification with single replication. In this way the variance due to the differences among the flasks could be separated from the variance due to differences among runs, the latter being of interest as an estimate of the reproducibility of the method. The mean absorbance was 0.522, and the standard error of the runs was equal to 0.003. The obvious downward trend in the results is attributable to the fact that the hydrochloric acid used was kept in a ground-glass bottle from which hydrogen chloride could escape. Unless standards are to be carried along with the samples, the hydrochloric acid used as a reagent for this method should be protected from loss of strength by storage in a pressure-tight bottle.

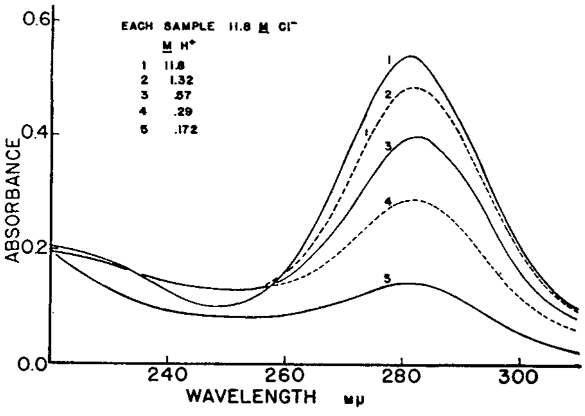

Figure 2. Effect of Variation of Hydrogen Ion Concentration on Ultraviolet Absorption of Niobium in Hydrochloric Acid

5 γ of niobium per ml.

Table I. Reproducibility Data

Run No.	Flask No. 1	2	3	4	5
1	0.530	0.525	0.525	0.523	0.525
2	0.533	0.524	0.523	0.521	0.522
3	0.528	0.524	0.521	0.518	0.517
4	0.523	0.523	0.516	0.519	0.519
5	0.523	0.518	0.514	0.514	0.515
6	0.538	0.518	0.515	0.515	0.516

Interferences were studied by measuring the absorption curves of a large number of cations in concentrated hydrochloric acid. The weights of the elements which caused an absorbance equal to 5% of the absorbance of 5 γ of niobium per ml. were calculated

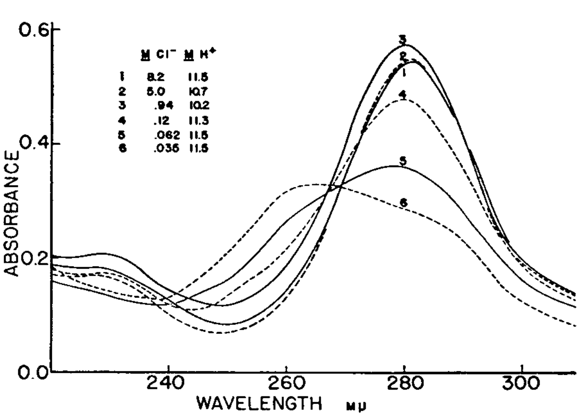

Figure 3. Effect of Variation of Chloride Ion Concentration on Ultraviolet Absorption of Niobium in Hydrochloric Acid

5 γ of niobium per ml.

Table II. Weight Ratios of Elements Causing 5% Error

Element	Ratio (M/Nb)	Element	Ratio (M/Nb)
Sn(II)	10	Pb(II)	0.2
Sn(IV)	7	Fe(III)	0.06
Co(II)	50	Cu(II)	0.04
Ni(II)	35	Bi(III)	7
Mn(II)	35	Ta(V)	2
Cd(II)	80	Th(IV)	500
Hg(II)	40	Mo(VI)	0.2
V(V)	0.6	W(VI)	3
V(IV?)	2	Si(IV)	50
Cr(III)	0.1	Ti(IV)	0.15
		Zr(IV)	>500

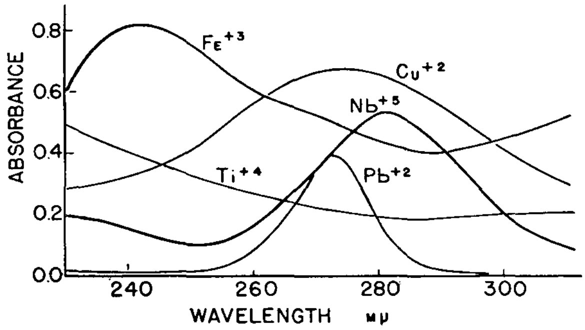

Figure 4. Absorption Spectra of Interfering Cations in Concentrated Hydrochloric Acid

5 γ of metal per ml.

from the absorbance of several different concentrations of each ion at 281 mμ. These calculated weight ratios, given in Table II, are presented as guides to the relative errors caused by the presence of the various ions.

The absorption curves of several interfering elements are shown in Figure 4. The interference due to the presence of copper may be eliminated by addition of a small measured excess of stannous chloride dissolved in concentrated hydrochloric acid to both the sample and blank solutions. The interference of iron can be reduced temporarily by the addition of a five- to tenfold excess of stannous chloride, but measurements must be made almost immediately, since the iron is rapidly reoxidized by air. Titanium may be determined independently by the peroxide method and its contribution to the absorbance at 281 mμ subtracted from the total absorbance. The remainder is due to niobium. Comparatively simple means of separation are available to reduce the concentrations of the other ions below the interfering ratio. The one exception is found in tantalum. The permissible weight ratio of tantalum to niobium was increased to 10 by the addition of sufficient hydrofluoric acid to make the solution about 0.01M in fluoride.

Sulfate does not interfere up to 0.1M concentration, introduced as potassium bisulfate. Sulfuric acid tends to decrease the solubility of the hydrogen chloride.

SUMMARY

The chloride complex which occurs in concentrated hydrochloric acid forms the basis for a simple, accurate, and rapid spectrophotometric method for the determination of niobium, in which absorbance is measured at 281 mμ. The method may be applied to any material that may be obtained in a hydrochloric acid solution. Interfering ions are vanadium(V), chromium(III), lead(II), iron(III), copper(II), molybdenum, and titanium(IV). The interferences from moderate amounts of iron(III) and copper(II) may be eliminated by reduction to the lower valence with tin(II). Tantalum up to ten times the amount of niobium measured can be tolerated.

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Spectrophotometric Determination of Arsenic and Tungsten as Mixed Heteropoly Acids

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IT HAS been known for years that a yellow color is formed on adding an excess of molybdate and vanadate solutions to one containing orthophosphate. The colored component is assumed to be molybdovanadophosphoric acid, a mixed heteropoly complex. Misson (*4, 5*) first proposed this reaction as a colorimetric method for the determination of phosphorus in steel. The color reaction and its analytical application were investigated spectrophotometrically by Kitson and Mellon (*2*). Wright and Mellon (*11*) studied the analogous mixed complex, tungstovanadophosphoric acid. Its use for the determination of vanadium was proposed by Sandell (*7*).

It seems reasonable that other mixed acids might have useful analytical applications. More specifically, since the addition of vanadate enhances the color of molybdophosphoric acid, it was of interest to study such a possibility with the colorless molybdoarsenic acid. At the same time tungstovanadophosphoric acid was restudied with the aim of using this complex for the determination of tungsten.

EXPERIMENTAL WORK

Apparatus and Solutions. Absorbance measurements were made in 1-cm. cells with a Beckman DU spectrophotometer.

Incidental reagents were prepared as follows: 5% sodium molybdate dihydrate, $Na_2MoO_4.2H_2O$, in water; 1% sodium metavanadate, $NaVO_3$, in dilute sodium hydroxide, subsequently neutralized with hydrochloric acid; 1.7N hydrochloric acid (1 to 6).

A standard solution of arsenic was prepared by dissolving, with heating, arsenic pentoxide in water and diluting to a concentration of 100 p.p.m. of arsenic. The standard solution of tungsten, containing 200 p.p.m., was made by dissolving sodium tungstate dihydrate in water.

All solutions were stored in polyethylene bottles to avoid contamination by silicate.

Color Reactions. Although molybdovanadophosphoric acid has been known for some years, the exact nature of the complex is not clear. It seems probable that other mixed heteropoly acids are of the same type.

Kitson and Mellon (*2*) applied the method of Vosburgh and Cooper (*9*) to determine the ratio of phosphorus to vanadium in molybdovanadophosphoric acid. The results indicated that the ratio is 1 to 1. Their data were redetermined in this work, with the same result. Using the same procedure on molybdovanadoarsenic acid indicated a ratio of 1 to 1 of vanadium to arsenic. Analogous determinations with tungstovanadophosphoric acid also gave a 1 to 1 ratio of vanadium to phosphorus. These latter results are less certain because the solutions were measured immediately after mixing to prevent precipitation of tungsten trioxide. In this case it is possible that measurement was not made under equilibrium conditions.

It has been postulated that molybdovanadophosphoric acid is formed by the substitution of both molybdenum and vanadium oxide radicals for the oxygen in the phosphate. By analogy, other mixed heteropoly compounds have been assumed to be similar. However, if this were the case, the ratio of vanadium to phosphorus should be at least 2 to 1. The theoretical interpretation of the observed evidence is not apparent from present knowledge of heteropoly compounds. Fortunately, the obscure nature of the mixed heteropoly acids does not impair their analytical applications.

Table I. Absorbance Readings for Reagent for Arsenic Determination *vs.* Distilled Water

Wave Length, mμ	Absorbance
460	0.015
440	0.055
420	0.116
400	0.226
380	0.370

DETERMINATION OF ARSENIC

The most sensitive colorimetric method for determining arsenic is based on the reduction of molybdoarsenic acid to molybdenum blue. This method usually requires preliminary distillation of the arsenic from interfering constituents. A procedure avoiding this separative operation would be preferable.

Since molybdoarsenic acid solution is colorless, it was hoped that the addition of vanadate to form molybdovanadoarsenic acid would shift the absorption to the visible region. Under suitable conditions a mixture of arsenic, molybdic, and vanadic acids does have a yellow hue. The analytical possibilities of this system, assumed to be a mixed heteropoly acid, are reported herein.

Effect of Variables on the Color Development. In order to study possible effects of variation in the several reactions concerned in the color reaction, preliminary experiments were made to select conditions for a starting system.

The following mixture of reactants seemed appropriate: 10 ml. of arsenate solution (100 p.p.m. arsenic), 7.5 ml. of vanadate solution (1%), 6 ml. of hydrochloric acid (1.7*N*), and 10 ml. of sodium molybdate solution (5%) were mixed in that order. The solution was diluted to 50 ml. in a volumetric flask and measured at 400, 420, 440, and 460 mμ. A reagent blank containing the same concentration of reagents except the arsenate was used as the reference system. Considerable color develops in the blank solution, as shown in Table I. Since a spectrophotometric curve between 340 and 500 mμ shows no maximum absorption, the wave lengths were chosen arbitrarily.

The study reported concerns the formation of two heteropoly complexes, molybdovanadoarsenic acid and tungstovanadophosphoric acid, and their colorimetric applications to the determination of arsenic and tungsten. The effects of the following variables were determined for each method: acidity, concentrations of reagents and of the desired constituents, order of adding reagents, stability, and 52 diverse ions. A procedure for the determination of arsenic is recommended, together with its application to the analysis of Paris green. Likewise, a procedure is recommended for the determination of tungsten, with application to tungsten steels from the National Bureau of Standards. The method for arsenic is rapid, convenient, and reliable for the range of 1 to 30 p.p.m. Determination of arsenic in Paris green proved feasible without separating the element from the copper. The method for tungsten is useful for the range of 10 to 120 p.p.m.

Using these experimental conditions, each of the several variable factors was then studied separately to find optimum conditions.

Acidity. The optimum amount of acid appears to be 6 ml. Additional acid decreases the color of the heteropoly acid. Less acid causes an extremely high blank and necessitates a prohibitively wide slit.

Sulfuric and nitric acids of the same normality give the same absorbances.

Vanadate Concentration. The color steadily increases as vanadate concentration increases up to 7 ml. of 1% sodium vanadate, even when a reagent blank containing the same amount of vanadate and molybdate is employed. The absorbance values then level out, regardless of the vanadate concentration. Vanadate concentration is critical, however, and carefully controlled concentrations should be used in the blank and sample solutions.

Molybdate Concentration. Molybdate must be in excess in order to have complete color development. The color increases up to 8 ml. of 5% sodium molybdate. Ten milliliters (±1) are recommended to ensure complete color development.

Order of Adding Reagents. The reagents should be added to the arsenic solution only in one order: acid, vanadate, and molybdate. If the acid is added last, a positive error results because of the deeper yellow color formed between vanadate and molybdate at higher pH values. This color does not disappear readily upon addition of acid. If the arsenate and molybdate are mixed before the addition of vanadate, molybdoarsenic acid forms. It is not readily converted to the mixed acid.

A composite reagent can be added directly to the arsenate solution. The absorbance readings are the same as if the recommended amounts, previously given, are added separately. The order of addition of the components of this reagent seems to be unimportant. Twenty-five grams of sodium molybdate dihydrate are dissolved in 70 to 80 ml. of distilled water. Sodium metavanadate (3.75 grams) is dissolved in 50 ml. of dilute sodium hydroxide and then neutralized with hydrochloric acid. These two solutions are mixed with 50 ml. of concentrated hydrochloric acid, cooled. and diluted to 250 ml. Five milliliters of this reagent will give optimum color in the arsenic determination. The blank contains 5 ml. of this reagent diluted to 50 ml. The reagent is stable for at least a week.

Arsenic Concentration. Beer's law applies from 1 to 30 p.p.m. for all wave lengths used. Greatest sensitivity is obtained at 400 mμ. Table II shows representative absorbance values. The molar absorptivity at 400 mμ is 2540 liters per mole per centimeter.

Stability of Color. The color is stable for at least 24 hours.

Diverse Ions. To study the effect of diverse ions, 10 ml. of a solution containing 100 p.p.m. of arsenic was added to a 50-ml. volumetric flask. To it was added 5 ml. of a solution containing 10 grams per liter of the diverse ion, followed by 5 ml. of reagent. The solution was diluted to 50 ml. and measured at the designated wave lengths.

In cases of interference, a more dilute solution of the diverse ion was tested until the relative error in measurement was 2% or less of the amount being measured.

The cation constituents were added as chlorides, nitrates, or sulfates. The anionic constituents were added as their sodium or potassium salts.

The error does not exceed 2% for amounts up to 1000 p.p.m. of any of the following cations: aluminum, ammonium, barium, beryllium, cadmium, calcium, cobalt(II), copper(II), lithium, magnesium, manganese(II), mercury(II), potassium, strontium, uranium(VI), and zinc.

The error is 2% or less for amounts of the following anions in concentrations up to 1000 p.p.m.: acetate, bromide, chloride, fluoride, formate, iodate, nitrate, nitrite, oxalate, perchlorate, periodate, selenate, and sulfate.

Bismuth(III), lead, thorium, and zirconium(VI) interfere because basic salts precipitate at the pH of the determination. Silver interferes because of precipitation of the chloride.

Ions such as dichromate, nickel, and permanganate interfere because of their color.

Reducing agents such as thiosulfate and thiocyanate reduce the heteropoly acid to heteropoly blue.

Other ions, such as borate, citrate, and tartrate, give a negative error because they prevent formation of the mixed heteropoly acid through complexation of the reagent.

Ions such as germanate, phosphate, silicate, tungstate, and vanadate give positive errors because of the formation of additional heteropoly acids.

A summary of the effect of interfering ions is given in Table III.

RECOMMENDED METHOD

Apparatus. The spectrophotometer must be capable of measurement at 400 mμ. The band width should be narrow for most accurate readings in this region of the spectrum.

Table II. Absorbance Readings for 20 P.P.M. of Arsenic at Various Wave Lengths Using a Reagent Blank

Wave Length, mμ	Absorbance
460	0.205
440	0.293
420	0.435
400	0.645
380	0.901

Table III. Effect of Interfering Ions

(Each solution contained 20 p.p.m. of arsenic; measurement was made at 400, 420, 440, and 460 mμ in 1-cm. cells using a reagent blank)

Ion	Maximum Permissible Amount[a], P.P.M.
Cr^{+++}	200
Au^{+++}	25
Fe^{+++}	0
Ni^{++}	25
Citrate^{---}	200
$Cr_2O_7^{--}$	0
GeO_3^{--}	0
I^{-}[b]	0
MnO_4^{-}	0
PO_4^{---}	0
SiO_3^{--}	0
SO_3^{--}	0
Tartrate^{--}	200
$S_2O_3^{--}$	0
WO_4^{--}	25
VO_3^{-}	0

[a] For an error (relative) not exceeding 2%.

[b] Oxidizes to iodine.

Reagents. MIXED COLORANT. Dissolve 25 grams of sodium molybdate dihydrate, $Na_2MoO_4.H_2O$, in 70 to 80 ml. of distilled water. Dissolve 3.75 grams of sodium metavanadate, $NaVO_3$, in dilute sodium hydroxide and neutralize with hydrochloric acid. Mix these two solutions with 50 ml. of concentrated hydrochloric acid, cool, and dilute to 250 ml. Store the solution in a polyethylene bottle.

STANDARD ARSENIC SOLUTION. Dissolve 0.0750 gram of arsenic pentoxide, As_2O_5, in 300 ml. of water. Heat until dissolution is complete, cool, and dilute to 500 ml. This solution contains approximately 100γ of arsenic per ml. Store in a polyethylene bottle.

Preparation of Calibration Curve. With a pipet transfer 0, 2.00, 4.00, 6.00, 8.00, and 10.00 ml. of the standard arsenic solution into each of six 50-ml. volumetric flasks. To each flask add 5 ml. of the molybdate-vanadate–hydrochloric acid reagent, dilute to 50 ml., and measure at the desired wave length. Use the sample containing 0 ml. of arsenic as a blank. Plot a curve of absorbance against weight of arsenic.

Table IV. Analysis of Paris Green Samples for Arsenic Using Molybdovanadoarsenic Acid

Sample No.	Arsenic Found, %					
	Colorimetric			Titrimetric		
	Detns.	Av.	Range	Detns.	Av.	Range
1	8	7.95	±0.25	3	7.92	±0.05
2	3	11.45	±0.13	3	11.30	±0.05
3	3	15.11	±0.14	3	15.32	±0.08
4	3	17.39	±0.19	3	17.71	±0.09

Procedure. Select a sample containing up to 30 p.p.m. of arsenic(V). If the concentrations of the other ions exceed the amounts permissible, as shown in Table III, separate the arsenic as the trichloride by the method of Scherrer (*8*). Adjust the pH to approximate neutrality, using dilute hydrochloric acid or sodium hydroxide. Add 5 ml. of reagent, dilute to 50 ml. in a volumetric flask, and determine the absorbance at the same wave length used for the calibration curve. Use a reagent blank for this measurement. Refer to the calibration curve to find the amount of arsenic in the sample.

ANALYSIS OF PARIS GREEN SAMPLES

To test the method, samples of Paris green were analyzed for arsenic content. A sample of 0.1 gram was used. Ten milliliters of a 5% solution of ammonium peroxydisulfate was added. The solution was boiled for 5 minutes to ensure arsenic(V). This also brings about complete sample dissolution. Heating destroys the excess oxidizing agent. The solutions were cooled and made up to 50 ml. These solutions were diluted 10 to 25 times depending on the arsenic concentration. Measurement was made at 400 mμ. The arsenic concentration was calculated from the following equation:

$$\%\ \text{As} = \frac{A \times 3.80}{0.100}$$

This equation is valid for an initial dilution to 50 ml. which is then diluted 2 ml. to 50 ml. The value of 3.80 was calculated from the best straight line for Beer's law plot. Table IV shows the results obtained on four samples.

CONCLUSIONS

The procedure is a rapid, convenient method for the determination of arsenic in a concentration range of 1 to 30 p.p.m.

The sensitivity is less than that of the heteropoly blue method or the extraction method recommended by Wadelin and Mellon (*10*). The absorptivity for the heteropoly blue procedure given by Boltz and Mellon (*1*) is 25,400 liters per mole per centimeter at 840 mμ and 5100 liters per mole per centimeter at 370 mμ in the extraction procedure recommended by Wadelin and Mellon. The absorptivity of this procedure is 2540 at 400 mμ.

The procedure is reproducible and accurate for mixtures containing arsenic as Paris green.

Measurement can be made at any desired wave length between

370 and 460 mμ. The lower wave lengths may sometimes be desirable to avoid interference from colored ions which absorb in the visible.

DETERMINATION OF TUNGSTEN

Sandell (*6*) mentions the use of tungstovanadophosphoric acid for the determination of tungsten. Although Lennard (*3*) used this means for the determination of tungsten, he did not study the optimum conditions. Thus, acidity is important in affecting the extent of color development, but he does not specify the amount of acid used in the determination. His reported sensitivity is much less than that which can be obtained under optimum conditions.

The general objective of this work, then, was to investigate the practicability of the use of tungstovanadophosphoric acid for the determination of tungsten. It was hoped that this procedure might eliminate or minimize the interferences inherent in other methods for tungsten.

Effects of Variables on the Color Development. In order to study possible effects of variation in the several variables concerned in the color reaction, preliminary experiments were made to select conditions for the starting system.

The following mixture of reactants was used; 40 p.p.m. of tungsten as tungstate, 1 ml. of phosphoric acid (Baker's 85%), and 3 ml. of neutral solution of 1% sodium vanadate were mixed. The solution was diluted to 50 ml. in a volumetric flask and measured at 400, 420, 440, and 460 mμ. The reagent blank contained the same concentrations of reagents except tungstate. Table V shows typical blank readings. Since a spectrophotometric curve between 260 and 500 mμ shows no maximum absorption, these wave lengths were chosen arbitrarily.

Table V. Absorbance Readings for the Reagent for Tungsten Determination *vs.* Distilled Water

Wave Length, mμ	Absorbance
460	0.030
440	0.036
420	0.057
400	0.077
380	0.100
360	0.130

Using these experimental conditions, each of the several variable factors was then studied separately to find optimum conditions.

Acid and Vanadate Concentrations. Varying amounts of phosphoric acid and sodium vanadate were added to a solution containing 40 p.p.m. of tungsten(VI). The optimum concentrations of vanadate and phosphoric acid are not independent variables. It is advisable to use enough acid to give a colorless blank with the amount of vanadate used. If 0.5 ml. of phosphoric was used with 2 ml. of a neutral solution of 1% sodium vanadate, optimum results were obtained.

Although the pH was important, excess phosphate had no effect on the color. The common mineral acids, hydrochloric, nitric, and sulfuric, can be used to adjust the acidity. The optimum pH is 1.8 as measured on a Beckman pH meter. If less acid is used, a highly colored yellow blank results. If more acid is used, there is a decrease in the sensitivity of the method.

The ratio of the two reagents is very critical. It was decided to prepare a mixed vanadate-phosphate reagent to add to the solutions containing tungsten. This reagent is made up as follows: 20 ml. of phosphoric acid and 80 ml. of 1% sodium vanadate solution were mixed in a 100-ml. volumetric flask. The reagent was then made up to 100 ml. with distilled water. The reagent is stable for about three days; but if it is kept longer, a precipitate develops and low results are obtained. Two and one half milliliters of reagent are used in 50 ml. of solution.

Tungsten Concentration. Beer's law applies from 10 to 120 p.p.m. for all wave lengths used. The molar absorptivity at 400 mμ is 621 liters per mole per centimeter. Absorbances for several wave lengths are given in Table VI.

A spectrophotometric curve was run in the ultraviolet down to 260 mμ and no absorption maximum occurs at any wave length. The wave lengths used for measurement were chosen arbitrarily. It might be desirable to shift the wave length for measurement in order to eliminate interferences which do not absorb at another wave length.

Stability of Color. The color is stable for at least 24 hours.

Table VI. Absorbance Readings for 40 P.P.M. of Tungsten at Various Wave Lengths Using a Reagent Blank

Wave Length, mμ	Absorbance
460	0.049
440	0.070
420	0.104
400	0.136
380	0.179

Diverse Ions. To study the effect of diverse ions, 10 ml. of a solution containing 200 p.p.m. of tungsten were added to a 50-ml. volumetric flask. To it was added 5 ml. of a solution containing 10 grams per liter of the diverse ion and 2.5 ml. of reagent. The solution was diluted to 50 ml. and measured at the designated wave lengths.

If a given ion interfered, a more dilute solution of diverse ion was tested until the relative error in the measurement was 2% or less.

The cations were added as chlorides, nitrates, or sulfates. The anions were added as their sodium or potassium salts.

The error does not exceed 2% for amounts up to 1000 p.p.m. of any of the following cations: aluminum, ammonium, barium, beryllium, cadmium, calcium, cobalt(II), copper(II), lithium, magnesium, manganese(II), mercury(II), potassium, strontium, uranium(VI), and zinc.

The error is 2% or less for amounts of the following anions in concentrations up to 1000 p.p.m.: acetate, borate, bromide, formate, iodate, nitrate, perchlorate, phosphate, selenate, silicate, sulfate, and thiocyanate.

Bismuth, lead, thorium, and zirconium(VI) interfere because basic salts precipitate at the pH of the determination. Silver interferes because of the precipitation of the chloride.

Ions such as dichromate and permanganate interfere because they themselves are colored.

Thiosulfate interferes because it reduces the heteropoly acid. Oxidizing agents interfere because of the formation of the peroxide of vanadium.

Ions such as oxalate and citrate cause a negative error because they destroy the heteropoly acid formed.

Ions such as arsenate and germanate give negative errors because of the formation of less colored heteropoly compounds.

A summary of the effect of the interfering ions is given in Table VII.

RECOMMENDED PROCEDURE

Apparatus. The spectrophotometer must be capable of measurement at 400 mμ. The band width should be narrow for the most accurate readings in this region of the spectrum.

Reagents. PHOSPHORIC ACID–SODIUM VANADATE SOLUTION. Dissolve 0.8 gram of sodium metavanadate, $NaVO_3$, in 50 ml. of dilute sodium hydroxide, neutralize, and dilute to 80 ml. in a graduate cylinder. Mix with 20 ml. of sirupy phosphoric acid (Baker's 85%) in a 100-ml. volumetric flask. Cool and dilute to 100 ml. Store in a polyethylene bottle.

STANDARD TUNGSTATE SOLUTION. Dissolve 0.3587 gram of sodium tungstate dihydrate, $Na_2WO_4.2H_2O$, in a liter of water. This solution contains 200 p.p.m. of tungsten. Store in a polyethylene bottle.

Preparation of Calibration Curve. With a pipet transfer 5, 10, 15, 20, and 25 ml. of the standard tungstate solution into five 50-ml. volumetric flasks. Add 2.5 ml. of the vanadate–phosphoric acid reagent, dilute to 50 ml., mix, and measure at the desired wave length. Use a sample containing 0 ml. of tungstate as a blank. Plot a curve of absorbance against weight of arsenic.

Procedure. Select a sample containing from 10 to 120 p.p.m. of tungsten. If the concentrations of other ions exceed the amounts permissible (see Table VII), separate the tungsten as tungsten trioxide. Filter this off in a sintered-glass crucible, discard the filtrate, redissolve the precipitate in sodium hydroxide, and neutralize the solution. Add 2.5 ml. of mixed reagent to the neutral solution containing the tungsten, dilute to 50 ml. in a volumetric flask, and determine the absorbance at the same wave length used for the calibration curve. Use a reagent blank for this measurement. Refer to the calibration curve to find the amount of tungsten in the sample.

Table VII. Effect of Interfering Ions

(Each solution contained 40 p.p.m. of tungsten; measurements were made at 400, 420, 440, and 460 mμ in 1-cm. cells using a reagent blank)

Ion	Maximum Permissible Amount[a], P.P.M.
Cr^{+++}	0
Au^{+++}	25
Fe^{+++}	0
Ni^{++}	25
Citrate^{---}	0
$Cr_2O_7^{--}$	0
F^-	25
GeO_3^{--}	200
I^-[b]	0
NO_2^-	10
Oxalate^{--}	25
IO_4^-	0
MnO_4^-	0
SO_3^{--}	0
Tartrate^{--}	0
$S_2O_3^{--}$	0
AsO_4^{---}	0
VO_3^-	0

[a] For an error (relative) not exceeding 2%.
[b] Oxidizes to iodine.

Analysis of Standard Samples. Samples of steel from the National Bureau of Standards were treated in the following manner: to 0.1-gram samples add 10 ml. of water and 25 ml. of concentrated hydrochloric acid. Heat the solutions on the hot plate until hydrogen is no longer evolved. Add concentrated nitric acid dropwise to the hot solutions until dissolution is complete. Cool the samples and add 25 ml. of distilled water. Let the samples stand for 2 hours and then filter through a fine-pore, sintered-glass crucible, without trying to remove quantitatively the precipitate of tungsten trioxide. Wash the beaker and precipitate with 2*N* nitric acid. Discard the filtrate and dissolve the precipitate in the beaker in dilute sodium hydroxide solution. Treat the precipitate in the crucible with three portions of 1*N* sodium hydroxide and collect the filtrate. Neutralize the solution with hydrochloric acid and make up to 100 ml. in a volumetric flask. Make a suitable dilution in a 50-ml. volumetric flask to bring the tungsten concentration within the range of the method. Add 2.5 ml. of the vanadate-phosphoric acid reagent, dilute the solution to 50 ml., and measure at 400 mμ.

Table VIII. Analysis of Two National Bureau of Standards Samples for Tungsten Using Tungstovanadophosphoric Acid

Sample No.	Tungsten, % Found	 NBS[a]
50 A	17.6	18.2
	18.5	..
	17.8	..
	18.2	..
75	75.4	75.2
	74.0	..
	76.8	..

[a] Average of several determinations carried out by bureau and contributing analysts.

Although it is known that the tungsten trioxide precipitate obtained in this manner is contaminated with small amounts of impurities, these quantities are too small to interfere with the determination. If no previous separation is made, results are 3 to 5% high. Table VIII shows results obtained on two samples.

CONCLUSIONS

The procedure is a rapid convenient method for the determination of tungsten in the concentration range of 10 to 120 p.p.m.

The sensitivity of the method, although not high, compares favorably with that of colorimetric methods for tungsten. The absorptivity is 621 liters per mole centimeter at 400 mμ.

The procedure is reproducible and accurate. It has been applied to samples of known tungsten content of the National Bureau of Standards.

Measurement can be made at any desired wave length between 360 and 460 mμ. Wave lengths other than 400 mμ may sometimes be desirable to eliminate interference from diverse ions.

ACKNOWLEDGMENT

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Analysis of Sodium Pyro- and Tripolyphosphate Mixtures

By X-Ray Diffractometer Using an Internal Standard

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Because commercial tripolyphosphate occurs in two polymorphic forms of the anhydrous salt, and may also contain some hexahydrate, a physical method of analysis can add materially to the information obtainable by chemical methods. A quantitative method has been developed based on x-ray diffraction, using magnesium oxide as an internal standard. Calibration involves application to mixtures of crystalline sodium pyrophosphate ($Na_4P_2O_7$), the two anhydrous phases of sodium tripolyphosphate ($Na_5P_3O_{10}$ I and $Na_5P_3O_{10}$ II), and the hexahydrate of sodium tripolyphosphate ($Na_5P_3O_{10}.6H_2O$). X-ray spectrometers have been used to measure intensities of x-ray diffraction peaks. Preferred crystallite orientation has been avoided by special methods of sample preparation. The average deviation of the results is of the order of 3%, based on analysis of mixtures of known composition. Such analyses go beyond chemical methods and enable one to determine the amount of each crystalline phase present. The total tripolyphosphate by x-ray diffraction will be identical to the chemical result for a fully crystallized sample, but lower than the chemical result, if some of the tripolyphosphate is amorphous.

If the x-ray diffraction pattern of a typical commercial sodium tripolyphosphate is examined, it is often found to be a mixture of the two anhydrous crystalline phases, $Na_5P_3O_{10}$ I and $Na_5P_3O_{10}$ II, sometimes is partially hydrated to the hexahydrate, $Na_5P_3O_{10}.6H_2O$, and usually is contaminated with sodium pyrophosphate, $Na_4P_2O_7$. Because chemical methods of analysis do not distinguish between polymorphic forms, a physical method, such as x-ray diffraction, must be used for a more complete characterization of such a sample.

Alexander and Klug (*1*), of Mellon Institute, have described the theoretical background for the quantitative analysis of polycomponent crystalline mixtures by x-ray powder diffraction, using an internal standard. They have shown that the relationship between the weight fraction, x_i, of the i^{th} component and the ratio of intensities of a diffraction line of the i^{th} component, I_i, to a diffraction line of an internal standard, I_s, is given by:

$$x_i = k\, I_i/I_s$$

where k is a constant as long as the weight fraction of internal standard is unchanged. Others have used this method for the analysis of crystalline mixtures. As one example, Gross and Martin (*4*) have used sodium chloride as an internal standard in mixtures of calcium fluoride, magnesium oxide, and quartz. In their case intensities were obtained from film patterns with the aid of a densitometer.

The availability of commercial direct-recording diffractometers of high precision in angular and intensity measurements makes this method all the more attractive. Its speed is also an advantage. With certain limitations, discussed later, the results are an important supplement to those obtained by chemical methods, especially when the latter may not give reliable results for all compositions of interest.

The internal standard method of analysis was, therefore, applied to mixtures of sodium pyrophosphate, the two anhydrous phases of sodium triphosphate, and the hexahydrate of sodium triphosphate, covering the composition range 0 to 100% for each component in mixtures with the others.

APPARATUS AND TECHNIQUES

Diffraction patterns were taken on both the General Electric XRD-3 and the North American Philips high angle diffractometers. Either instrument is capable of sufficient resolution of diffraction lines for these particular patterns. Intensities can be measured with sufficient precision in reasonable time with either instrument. As far as their application to this analysis is concerned, therefore, the two instruments perform equally well. In both cases corrections to intensities were made for nonlinear response of Geiger tubes and associated rate meter, scaler; and recorder circuits. Nickel-filtered CuKα radiation was used throughout.

Intensities of diffraction lines were determined as the difference between the intensity at the peak of the line and an average back-

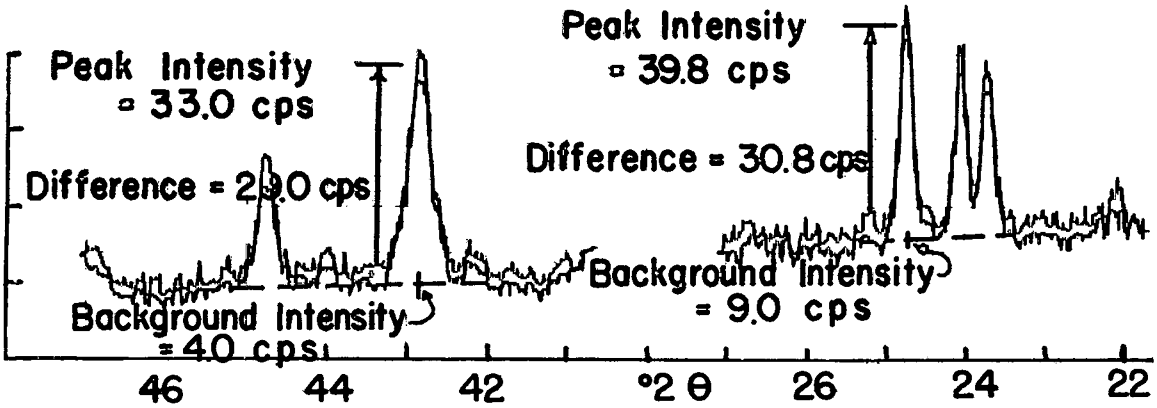

Figure 1. Measurement of Intensities

24.9° line intensity 30.8 counts per second
MgO line intensity 29.0 counts per second
Ratio of intensities 30.8 ÷ 29.0 = 1.06

ground reading calculated for the same angular position. This background figure was determined by interpolation between readings made on either side of the diffraction line, in areas where no diffraction lines occurred. Figure 1 shows typical recorder curves for a component and the magnesium oxide internal standard, with the intensity determination indicated. The actual intensity readings, in counts (x-ray quanta) per second, were determined either by manual counting using the scaler or by conversion from recorder chart reading to counts per second by appropriate calibration curves. More accurate results are obtained by the manual method if sufficient time is spent in counting to reduce statistical error in the measurement to the required level.

Magnesium oxide was chosen as an internal standard. Its strong (200) reflection, occurring at 43.0° (angles are degrees 2*θ*), is at a position where no phosphate lines occur, and was therefore chosen as the standard line. The amount of magnesium oxide used is 10% by weight of the unknown sample.

MATERIALS

The phosphates and magnesium oxide used in preparing mixtures of known composition were made as follows:

Sodium pyrophosphate, $Na_4P_2O_7$, by dehydrating the C.P. decahydrate.

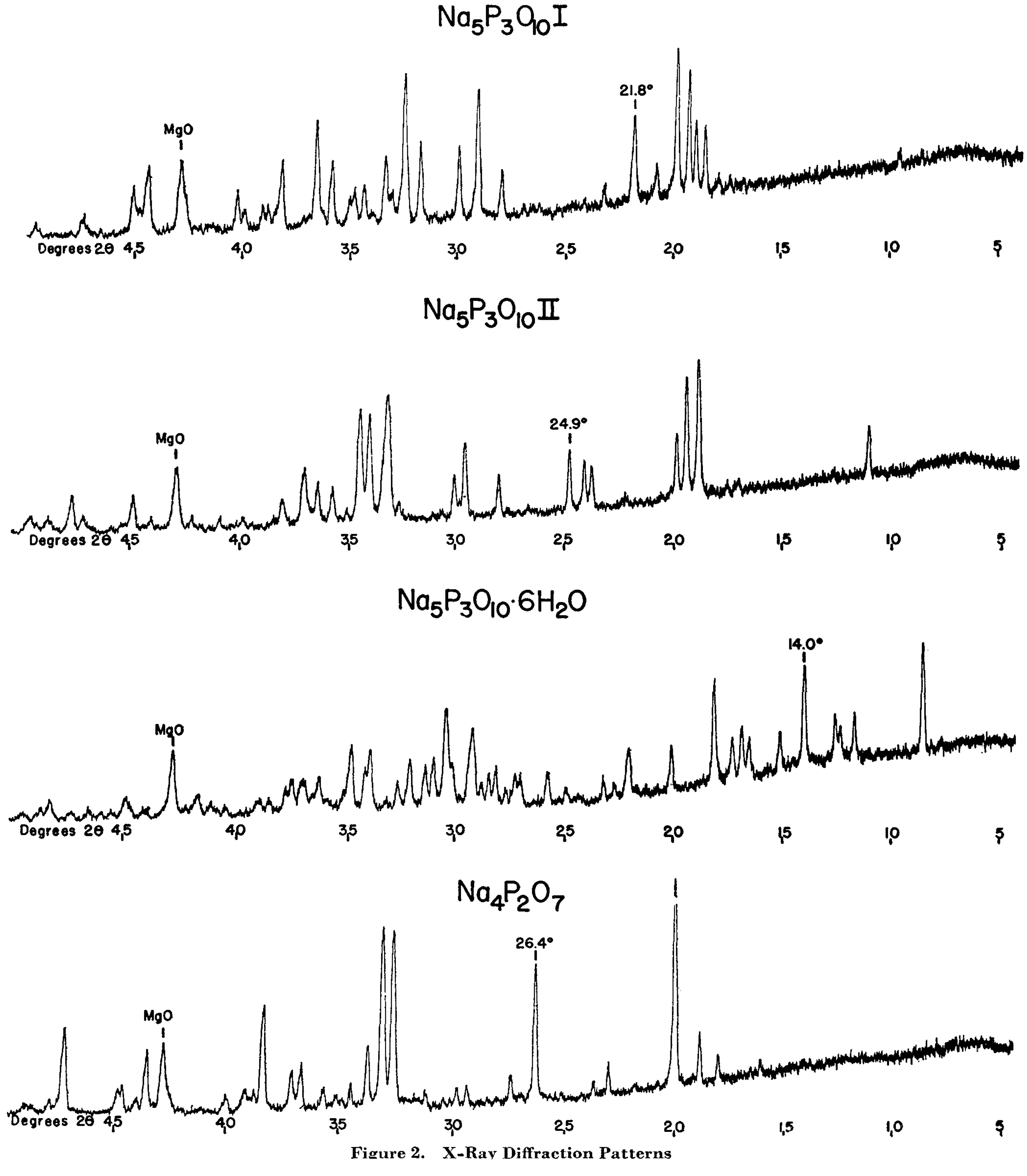

Figure 2. X-Ray Diffraction Patterns

Sodium triphosphate phase I, $Na_5P_3O_{10}$ I, by dehydrating and tempering the hexahydrate of sodium triphosphate, $Na_5P_3O_{10}.6H_2O$, at 550° C. for 2 hours.

Sodium triphosphate phase II, $Na_5P_3O_{10}$ II, by dehydrating and tempering pure hexahydrate at 450° C. for 2 hours. The pure hexahydrate for the above two preparations was made by six recrystallizations of a commercial phase II triphosphate from aqueous solution by precipitating the hexahydrate with ethyl alcohol.

Sodium triphosphate hexahydrate, $Na_5P_3O_{10}.6H_2O$. Pure phase II prepared above was ground and screened through a 400-mesh screen. This material was then vapor hydrated at 100% relative humidity at room temperature. The resultant hydrate contained 22.73% water (theoretical for the hexahydrate is 22.71%).

Magnesium oxide, MgO. C.P. magnesium oxide was used after drying at 100° C. It was found desirable to store the dried material over a desiccant.

PREPARATION OF MIXTURES OF KNOWN COMPOSITION

Before mixtures of known composition were prepared, the coarse components were ground with mortar and pestle until they would pass through a 400-mesh screen. The magnesium oxide was finer than 400-mesh and was used without grinding. The method of preparation gave triphosphate hexahydrate which would pass through a 400-mesh screen without further grinding. Mixtures were made so that the percentage of each component was varied systematically over the range from 0 to 100%, both in binary and in more complex mixtures of the four components.

Both these phosphate mixtures and their mixtures with 10% added magnesium oxide were made uniform by four brushings through a 200-mesh screen, a procedure described by Carl (*3*). This mixing technique is adequate, as further mixing did not change the relative intensities in the diffraction patterns. Care must be taken to avoid loss of magnesium oxide dust by excessive handling of the sample, as this will cause results to be too high.

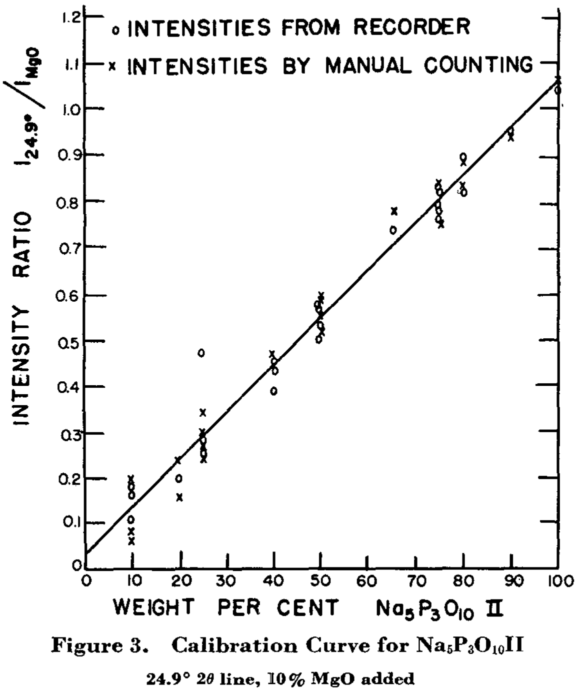

Figure 3. Calibration Curve for $Na_5P_3O_{10}$II

24.9° 2θ line, 10% MgO added

ELIMINATION OF PREFERRED ORIENTATION

The preparation of the powder sample for the diffractometer requires care in order to avoid preferred orientation of the phosphate crystals, especially at the surface of the sample. This is especially true for the platelets of hexahydrate.

The preferred procedure is to sift the powdered sample into a windowed holder from the back side, close the back with a section of a microscope slide, then gently place the sample in position on the diffractometer. This procedure is very much like that described by Beatty (*2*). Practice allows one to prepare a sample by this method that will neither "sag" nor "crack" away from the front or top of the holder when placed in a vertical position on the diffractometer. The sample holders used are made of aluminum 1/16 inch thick, 1 1/8 × 1.5 inches in dimensions. A rectangular hole 5/8 × 1 inch is cut in the center of this flat piece of aluminum.

Table I. Composition of Mixtures Used for Calibration

Sample No.	$Na_5P_3O_{10}$ I, %	$Na_5P_3O_{10}$ II, %	$Na_5P_3O_{10}.6H_2O$, %	$Na_4P_2O_7$, %
1	...	100	...	...
2	...	90	10	...
3	20	80	...	...
4	...	75	...	25
5	...	75	25	...
6	35	65	...	...
7	...	50	50	...
8	...	50	...	50
9	50	50	...	...
10	...	40	40	20
11	...	25	75	...
12	...	25	...	75
13	80	20	...	...
14	...	10	90	...
15	10	10	...	80
16	100	...	...	...
17	...	...	60	40
18	...	...	...	100
19	...	...	100	...

The "windows" for these holders were thin sheets of plastic, made as follows:

> Two or three drops of a thin solution of Duco cement in ethyl acetate is poured onto a quiet water surface in a large beaker or crystallizing dish. This solution will spread until thin, and will become a plastic film as the solvent evaporates. When nearly dry (several minutes), a flat metal ring is raised from below the water surface through the film, which adheres to the edges of the ring. The fingers may then be used to smooth wrinkles from the film by pulling gently at the edges. When relatively smooth, the film is further dried a few minutes in a 100° C. oven. The result is a plastic film thin enough to show interference colors, but possessing enough mechanical strength to hold the weight of the powdered samples. The film is glued to the front face of the aluminum sample holders with a water-soluble cement such as polyvinyl alcohol. The ring on which the film was formed is a convenient means for handling the film until it is glued to the sample holder

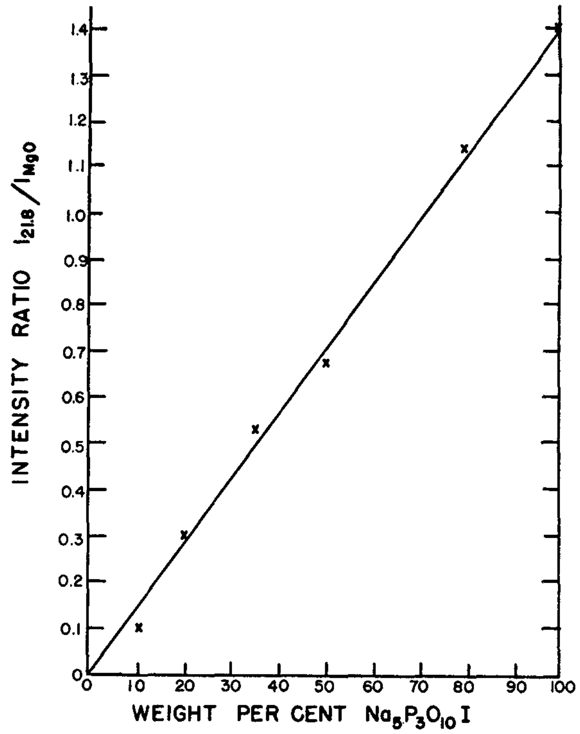

Figure 4. Calibration Curve for $Na_5P_3O_{10}$ I

21.8° 2θ line, 10% MgO added

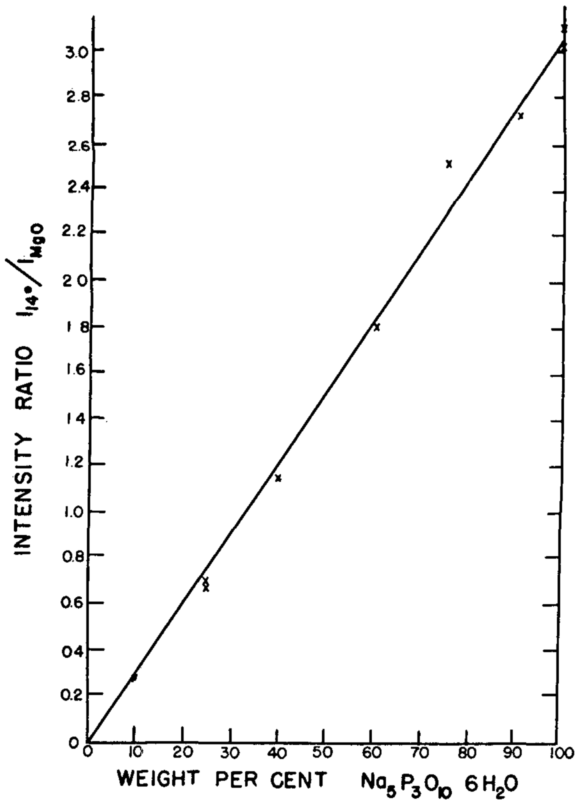

Figure 5. Calibration Curve for $Na_5P_3O_{10}.6H_2O$

14° 2θ line, 10% MgO added

after which it can be trimmed around the edges of the holder with a razor blade. Such a thin plastic film is practically nonabsorbent to x-rays; no change in intensities could be detected on patterns made with and without the plastic film over the sample.

Other methods of sample surface preparation were tried, but none gave results as reproducible as did the one just described. For example, samples were packed against the following surfaces: ground glass, emery paper, sandpaper, the sticky side of Scotch tape, or friction tape. It was expected that upon removal of the ground glass, Scotch tape, etc., a roughened sample surface would be left essentially free of preferred orientation. Although none of these methods was entirely satisfactory, packing against ground glass was second only to the preferred method and might be satisfactory for crystal mixtures less sensitive to orientation effects than the phosphates.

DISCUSSION OF RESULTS

A discussion of the determination of the calibration curve for phase II sodium triphosphate, for which the most data have been taken, will serve as an example of the treatment of the experimental data. Figure 2 includes the diffraction pattern for phase II, as well as those for the other three phosphates included in this study. The line spacings and intensities have not been tabulated, as they are not needed for this discussion. Thilo and Seemann (*5*) have recently published x-ray powder data for some of the sodium phosphates. The phase II diffraction line at 24.9° was chosen as the one whose intensity would be measured for this component. This is not the strongest line of the phase II pattern, but it is one of moderate strength which does not overlap diffraction lines of other phosphates likely to be present at the same time.

Mixtures of varying phase II content, with various combinations of the other components, were made up, including 10% by weight magnesium oxide. Table I is a tabulation of the compositions used for calibration of all the components. The diffraction patterns for these mixtures were determined, and on each of them the intensities of the 24.9° phase II line and of the (200) magnesium oxide line were measured as described earlier and illustrated in Figure 1. The ratio of the intensities of these two diffraction lines was then calculated for each mixture, and these ratios were plotted against weight per cent of phase II in the sample (computed before addition of the internal standard).

A plot of such data is shown in Figure 3, where crosses represent data obtained by manual counting of intensities, and circles represent data obtained from automatic recorder charts. The average deviation of the manual points from the best straight line, is 2.75% absolute—for example, at a composition of 50% results will, on the average, lie between 47.25% and 52.75%. The same figure for the automatic points is about 3%. The best straight line through the calibration points does not go through the origin, as it should theoretically. This is due to the fact that a good many of the known mixtures of phase II were made up with hexahydrate as one of the other components. There is a weak diffraction line at 24.9° in the hexahydrate pattern, which causes the intensity ratios to be high, especially at low phase II contents. An obvious remedy for this situation would be to choose another diffraction line of phase II for measurement. None could be found that were not more seriously affected by interference from other components. A correction to the intensity of the phase II line, proportional to the amount of hexahydrate present, should be made. As this has not been done, the calibration curve as drawn is a compromise. Results from it will be a little high when hexahydrate is present, and a little low when it is absent, and the effect will be noticed only at low phase II contents.

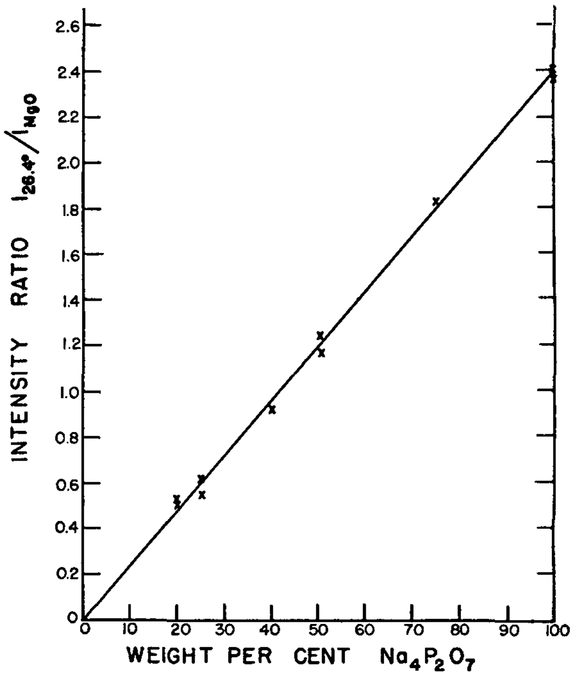

Figure 6. Calibration Curve for Sodium Pyrophosphate

26.4° 2θ line, 10% MgO added

Similarly, the calibration curves for the other components were obtained. Only the more accurate intensity ratios determined by manual scaling are plotted. In the case of phase I, whose calibration curve is given in Figure 4, the 21.8° line was measured (see Figure 2). The 29.1° line is sometimes useful also. In the phase I case the manual points (crosses) have a smaller average deviation from the mean than was the case for phase II.

Table II. Analysis of Unknowns

Sample Type	Sample No.		$Na_5P_3O_{10}$ I, %	$Na_5P_3O_{10}$ II, %	$Na_5P_3O_{10}.6H_2O$, %	$Na_4P_2O_7$, %
Mechanical mixture	10	Actual	....	40	40	20
		Found	....	42.5	37	21
	9	Actual	50	50	...	...
		Found	48.5	51	...	...
Commercial triphosphate	A	Found	65.5	29	None	5.5
	B	Found	9.5	83	None	7.5
	C	Found	27.5	58.5	None	12
Hydrated triphosphate	D	Found	None	45	47	7

The calibration curves for the hexahydrate of triphosphate and sodium pyrophosphate are given in Figures 5 and 6. The 14° line (see Figure 2) is measured for the hexahydrate; the stronger 8.7° line must be avoided, because it is very sensitive to orientation effects. In the case of the pyrophosphate, the diffraction line at 26.4° is measured.

A glance through Table I, the tabulation of compositions of standard mixtures, shows that samples of all combinations of the components were not prepared. This is not necessary, however, for as Alexander and Klug (*1*) point out, the matrix (that part of the sample other than internal standard and component being measured) does not affect the ratio of intensities of the component and internal standard, since the mass absorption coefficient for the matrix, determined by its composition, affects the intensities of diffraction lines for both the internal standard and the component by the same factor.

It is intended to extend the method to include other sodium phosphates. Sodium trimetaphosphate, phase I, has been investigated briefly, and its diffraction line at 26.2° will probably be chosen for measurement. Although this line is close to the 26.4° pyrophosphate line, the two are adequately resolved to allow intensity measurement except when the pyro content is very high and the trimeta content is very low.

ACCURACY OF RESULTS

An idea of the accuracy of these analyses may be obtained by looking at the consistency of the calibration data. As mentioned above, the average deviation of points from the calibration line for phase II is 3% or less. The results for phase I and pyrophosphate are better than this, while those for the hexahydrate are not so good. Another way of looking at the consistency of results is to choose any known mixture as an unknown, analyzing *it by using* the calibration curves determined from the other standard mixtures. Table II includes such a treatment for two of the known mixtures. The results found are obtained by applying the calibration curves to the intensity ratios obtained experimentally for these samples, and are typical of those to be expected on unknowns. Some analyses of typical commercial sodium tripolyphosphates and a partially hydrated sample are also given in Table II.

There are several possible sources of error in this method of analysis. The most serious one, because it is so subtle, is the possible presence of amorphous material in a sample. When such is the case, there cannot be good agreement between results by this method and chemical methods. This method actually measures the weight fraction of crystalline components. Lack of reproducibility in sample preparation, chiefly due to crystal orientation, is probably the next most serious cause of error, or lack of precision in results. For example, while the average deviation of results of repeat runs on a single sample ranges from 0.5 to 1%, the average deviation of runs on a number of preparations of the same sample is roughly 2%, nearly as great as the average deviation of the calibration data as a whole. Statistical errors in the determination of intensities may be appreciable if intensities are low and sufficient time is not taken to measure them. Intensity measurements can probably be made more precise by measuring diffraction line areas, rather than diffraction peak heights.

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Determination of Naphthalene in Wash Oil and Coke Oven Gas

Infrared and Ultraviolet Spectrometry

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IN PROCESSING and purifying coke oven gas by wash oil scrubbing it is desirable to have rapid methods for the determination of naphthalene in both the gas and the wash oil. The method most generally applied to wash oil involves volatilization of the naphthalene with a stream of hot air into a solution of picric acid (*2*), thereby precipitating the picrate. Another method (*3*) requires distillation of the wash oil and isolation of the fraction boiling between 195° and 250° C. This fraction is then chilled and the solid naphthalene filtered and weighed.

More recently (*4*), Reichardt and White reported a modification of the volatilization procedure in which the analytical manipulations were reduced to a minimum. Their method utilizes a titration of the excess picric acid after formation of the naphthalene picrate. Good accuracy and precision were obtained for the specific wash oil used. Schubert (*5*) reports an optical method, in which the freezing point of the sample is compared with that of a naphthalene–wash oil standard.

Naphthalene in gas (*1*) is usually determined by passing the latter through a picric acid solution, filtering off the picrate, and estimating the naphthalene either gravimetrically or volu-

The classical picrate methods for determining naphthalene in wash oil and coke oven gas were time-consuming and not wholly quantitative. Poor recovery was experienced in tests where known amounts of naphthalene were added to wash oil. Spectrophotometric methods were developed for determining naphthalene in wash oil by infrared, and in coke oven gas by ultraviolet, techniques. Naphthalene concentrations in coke oven gas as low as 0.001% can readily be measured, as can concentrations up to 4% in wash oil. Accuracy of the methods is 0.5% relative. The spectrophotometric methods are direct, simple in application, and specific, and offer a definite improvement in sensitivity, accuracy, and analysis time. No significant interferences are encountered from the more common compounds associated with naphthalene. The methods have been applied successfully to routine plant samples.

metrically. In general these methods are time-consuming and not readily adaptable to routine plant control.

In this laboratory rapid methods were developed and successfully applied for determining naphthalene in wash oil by infrared absorption and in coke oven gas by ultraviolet techniques.

The method utilizes the intensity of the specific naphthalene absorption band at 12.77 microns as a quantitative measure of the naphthalene content of the wash oil. Compensation is made for the slight absorbance exhibited by wash oil itself at this wave length. 2,2,4-Trimethylpentane (iso-octane) is used as the solvent for the wash oil samples, and in this medium the background absorbance of the oil is fairly linear with its concentration. The amount of naphthalene in an unknown oil sample can be determined by comparing it to a standard solution of naphthalene–wash oil in iso-octane. The standard naphthalene solution is prepared to contain a weight of wash oil approximately equal to the weight of the sample being tested. This makes it unnecessary to use an accurately calibrated cell in the analysis.

The procedure is designed to cover a concentration range of 0.50 to 4.00% naphthalene in wash oil. A sample weight is selected which will place the naphthalene content of the iso-octane solution between 0.005 and 0.10%. In this range the best linearity is obtained between absorbance and concentration.

Apparatus. Spectrometer. Perkin-Elmer model 12-C, with sodium chloride optics.

One 0.5-mm. sodium chloride cell.

Reagents. Iso-octane, Phillips 66 pure grade, or comparable, free of absorption peaks between 12.50 and 13.25 microns.

Sodium chloride, U.S.P.

Naphthalene, C.P.

Fresh wash oil, free of naphthalene absorption peaks.

NAPHTHALENE IN WASH OIL BY INFRARED ABSORPTION

Procedure. PREPARATION OF STANDARDS. Weigh accurately 0.0800 ± 0.0002 gram of pure naphthalene into each of eight 100-ml. volumetric flasks. Add to the flasks 2.000, 2.500, 3.000, 3.500, 4.000, 6.000, 8.000, and 16.000 ± 0.001 grams, respectively, of naphthalene-free wash oil. Dilute each flask to volume with iso-octane and mix thoroughly. Transfer and store these standards in well-stoppered glass bottles, labeling each with the exact weight of naphthalene and wash oil added.

PREPARATION OF SAMPLE. Weigh 4.000 ± 0.001 grams of wash oil sample into a clean 100-ml. volumetric flask. This weight of sample is usually sufficient for wash oil containing from 1.50 to 2.00% naphthalene. (See Table I for sample sizes of wash oil containing more or less naphthalene. The absorbance should fall between 0.090 and 0.360.) Dilute the sample to volume with iso-octane and mix thoroughly. Dry a portion of this solution by shaking with 0.5 gram of sodium chloride in a 25-ml. volumetric flask. This step is taken to protect the sodium chloride cell from any water present in the sample.

INFRARED MEASUREMENT. Set the slit schedule to obtain a slit width of 0.222 mm. at 12.50 microns. Adjust the gain to place the base line in the high transmission region and balance the instrument (0.0 at 12.62 microns) by means of the lithium fluoride shutter. Make all measurements with a speed setting of 4, and a response setting of 3.

Fill a 0.5-mm. cell with the dry wash oil–iso-octane solution and scan over the range of 12.50 to 13.02 microns. Remove the cell, empty, and clean with iso-octane. Refill the cell with a portion of the wash oil–naphthalene–iso-octane standard containing a weight of wash oil nearest to the sample weight taken. Scan the standard over the same spectral range of 12.50 to 13.02 microns.

CALCULATIONS. Draw a straight base line across the 12.77-micron absorption band on both the sample and standard scans. Measure and record the absorbance of the band from base line to peak for each scan and calculate the naphthalene content of the sample as follows:

$$\text{Naphthalene, } \% = \frac{A}{A_1} \times \frac{W_n}{W_s} \times 100$$

where A = absorbance of sample
A_1 = absorbance of standard
W_n = weight of naphthalene, grams in standard
W_s = weight of sample, grams

Interferences. The interfering compounds which were studied were divided into two classes (Table II): compounds whose presence might be expected in wash oil and those related structurally to naphthalene but not likely to be present.

Table I. Approximate Size of Wash Oil Sample for Naphthalene Determination

Concentration Level, %	Approximate Sample Weight, Grams[a]	Approximate Sample Volume, Ml.
0.50	16.00	18.4
1.00	8.000	9.2
1.50	6.000	6.9
2.00	4.000	4.6
2.50	3.500	4.0
3.00	3.000	3.5
3.50	2.500	2.9
4.00	2.000	2.3

[a] These weights will give absorbance values of approximately 0.300 at their respective concentration levels.

Table II. Interferences[a]

Interfering Compound	Weight Added, Mg.	Relative Error, %	Maximum Concn. for 2% Relative Error, Mg.
Compounds Likely to Be Present			
Benzene	879	1.0	879
Phenol	536	1.0	536[b]
Toluene	866	0.6	866
Xylene	43	1.0	43[b]
Compounds Not Likely to Be Present			
Anthracene[c]	1000	1.3	...
1-Methylnaphthalene	10	1.2	10[b]
2-Methylnaphthalene	497	1.9	497[b]
1-Naphthol	10	1.0	10[b]
2-Naphthol[c]	1000	0.9	...

[a] All solutions contained, in addition, about 2.800 grams of wash oil and 0.0850 gram of naphthalene diluted to a final volume of 100 ml. in iso-octane.

[b] Any additional amount of the interfering compound would be likely to give an error greater than 2%.

[c] Only slightly soluble in iso-octane.

The interfering compound was added to a 100-ml. flask (containing wash oil and naphthalene) in an amount about 10 times the weight of naphthalene in the sample. The flask was diluted to volume with iso-octane and the absorbance of the solution measured in the manner already described. If more than a 2% relative error in the calculated absorbance was observed, another solution was prepared containing half as much of the interfering compound. This process was repeated until an absorbance error not exceeding 2% relative was obtained.

In some cases—i.e., phenol, xylene, and the two alpha-substituted naphthalenes tested—it was found that when the amount listed in Table II under maximum concentration for 2% relative error was doubled, a three- to sevenfold increase in relative error was observed. The data in Table II show the magnitude of the interference of these compounds.

Of the interfering compounds that might be present in the wash

Table III. Accuracy and Precision Data for Naphthalene in Wash Oil Samples

Accuracy Test[a]	Naphthalene, % Added	Naphthalene, % Found
1	0.51	0.51
2	1.01	1.01
3	1.50	1.51
4	2.00	2.02
5	2.49	2.49
6	2.98	2.99
7	3.48	3.46
8	3.99	4.04

Precision Test	Naphthalene, %
1	2.54
2	2.45
3	2.44
4	2.53
5	2.49
6	2.49
7	2.50
8	2.48
9	2.43
10	2.44
Average	2.48
Standard deviation	0.04
95% confidence limits	2.48 ± 0.08

[a] Test solutions were prepared by adding known weights of C.P. naphthalene to a definite weight of wash oil.

oil, xylene can be tolerated least. Its absorption bands lie close to either side of the 12.77-micron naphthalene band, thus distorting the normal base line of the latter. Benzene, phenol, and toluene cause much less trouble and can be tolerated in greater concentrations.

The compounds that are not likely to be present, but which are of interest, interfere considerably more. The 1-substituted naphthalenes, in particular, depress the naphthalene absorption band about equally, and consequently neither one can be present if the parent compound is to be measured. The 2-substituted isomers cause less difficulty and may occur in concentrations approximately five times that of the naphthalene. Anthracene and 2-naphthol are only slightly soluble in iso-octane and no toleration limits are given for them in Table II. The errors noted, therefore, are for iso-octane solutions saturated with respect to those two compounds.

Accuracy and Precision. The results from testing in duplicate a series of eight samples, each containing a known weight of naphthalene in wash oil, show the method to have 95% confidence limits of ±0.04%. These samples included a naphthalene range from 0.50 to 4.00%. By testing 10 separate portions of a standard containing 2.49% naphthalene the precision of the method was shown to be 3.2% relative (2 sigma or 95% confidence).

The results of the analyses for accuracy and precision are tabulated in Table III.

NAPHTHALENE IN COKE OVEN GAS BY ULTRAVIOLET ABSORPTION

In determining naphthalene in coke oven gas advantage is taken of the greater sensitivity of ultraviolet measurements as compared to infrared. The naphthalene content of coke oven gas is determined by scrubbing the gas with cyclohexane and measuring the ultraviolet absorption of the cyclohexane-naphthalene solution at 309, 311.5, and 318.5 mμ. Naphthalene exhibits an absorption maximum at 311.5 mμ and minima at 309 and 318.5 mμ in cyclohexane.

The true absorbance for naphthalene at 311.5 mμ is obtained by subtracting from the observed reading the corrected base line absorbance calculated from the absorbances observed at 309 and 318.5 mμ. This correction is calculated according to the theorem which states that the coordinates of a point, P_3, on a straight line, P_1P_2, where P_3 divides P_1P_2 in the ratio m_1m_2 may be represented as $\left(\frac{m_1X_2 + m_2X_1}{m_1 + m_2}, \frac{m_1Y_2 + m_2Y_1}{m_1 + m_2}\right)$. Knowing the absorptivity, a, for the system and the volume of gas sampled, the naphthalene content can be calculated.

Apparatus and Reagents. Beckman model DU photoelectric quartz spectrophotometer, with ultraviolet light source.

Two matched 10-cm. silica cells.

Calibrated wet test meter.

Dewar flask, pint or quart capacity.

Scrubber tube, borosilicate glass, 20- to 25-mm. inside diameter, 7 to 8 inches long, and of approximately 100-ml. capacity, fitted with a cork through which are passed a gas outlet tube several inches long, and a gas inlet tube on the bottom of which is a medium grade, fritted-glass sparger.

Cyclohexane, any grade, free of absorption peaks between 305 and 320 mμ when referred to distilled water.

Procedure. CALIBRATION OF SYSTEM. The naphthalene-cyclohexane system conforms to Beer's law over the concentration range of 0.001 to 0.005% naphthalene. The system was calibrated by measuring the absorbances of a series of naphthalene standards at 309, 311.5, and 318.5 mμ and calculating the absorptivity, a. These standards were prepared from a 0.01% stock solution of pure naphthalene in cyclohexane by diluting 10-, 20-, 30-, 40-, and 50-ml. aliquots to 100 ml. with cyclohexane in separate 100-ml. volumetric flasks. The true absorbance, A, for naphthalene at 311.5 mμ was determined by the procedure outlined below for sample analysis. The data are tabulated in Table IV. An a value of 9.15 was obtained ($a = A/bc$ where b is the cell length in centimeters, and c is the concentration in grams of naphthalene per 100 ml. of cyclohexane).

COLLECTION AND PREPARATION OF SAMPLE. Place 100 ml. of cyclohexane in the scrubber tube and close with the stopper carrying the gas inlet sparger tube and the gas outlet tube. Place the assembled scrubber tube in a Dewar flask containing an ice-water slurry, and maintain the temperature of the cyclohexane at about 10° C. (The freezing point of cyclohexane is approximately 6.5° C.)

Open the valve of the gas sampling line and purge sufficiently to ensure collecting a representative sample. Close the valve. Attach the inlet sparger tube to the sample line valve with a suitable length of rubber tubing and similarly connect the gas outlet tube to a calibrated wet test meter. Open the sample line valve cautiously and adjust the flow of gas through the sparger tube to give a rate of about 1 cubic foot per hour. Pass about 2 cubic feet of gas through the cyclohexane scrubber, close the sample line valve, and record the exact volume of gas measured by the wet test meter. Disconnect the sparger tube.

ULTRAVIOLET MEASUREMENT. Carefully transfer the cyclohexane from the scrubber tube into a clean 100-ml. volumetric flask and dilute to volume with cyclohexane. Fill the reference

Table IV. Absorptivity, a, from Naphthalene Standards[a]

Naphthalene in Cyclohexane, Mg. per 100 Ml.	Absorbance 309 mμ	Absorbance 311.5 mμ	Absorbance 318.5 mμ	A	a
1.0	0.102	0.174	0.017	0.095	9.50
2.0	0.196	0.335	0.025	0.183	9.15
3.0	0.292	0.493	0.040	0.267	8.90
4.0	0.371	0.652	0.050	0.364	9.10
5.0	0.462	0.812	0.062	0.454	9.08
			Average		9.15

[a] 0.40-mm. slit; 10-cm. silica cells; 0.002 cell correction.

Table V. Accuracy and Precision Data for Naphthalene in Coke Oven Gas[a]

Accuracy Test	Naphthalene, Mg. Added	Naphthalene, Mg. Found
1	10.6	10.6
2	15.8	15.8
3	20.3	20.2
4	24.6	24.7
5	30.4	30.9

Precision Test	Naphthalene, Mg.
1	10.6
2	10.7
3	10.7
4	11.1
5	10.4
6	10.8
7	10.9
8	10.5
9	10.8
10	10.5
Average	10.7
Standard deviation	0.21
95% confidence limits	10.7 ± 0.42

[a] Test solutions were prepared by adding known weights of C.P. naphthalene to cyclohexane.

and sample cells with cyclohexane and determine the cell absorbance corrections at 309, 311.5, and 318.5 mμ with the spectrophotometer using a slit width of 0.40 mm. Empty the sample cell and fill it with the sample solution. Measure the absorbance at 311.5 mμ and 0.40-mm. slit width. If the absorbance is greater than 0.600, dilute 50 ml. of the solution to 100 ml. with cyclohexane. Using the same slit width, measure the absorbance of the final sample solution at 309, 311.5, and 318.5 mμ.

CALCULATIONS. Correct the observed sample absorbances for the cell absorbance corrections previously made. Using the corrected sample absorbances, calculate the base line absorbance of the naphthalene at 311.5 mμ by means of the following formula:

$$A_B = (0.74 \times A_{309}) + (0.26 \times A_{318.5})$$

Subtract this absorbance from that read at 311.5 mμ to give the absorbance from naphthalene only:

$$A = A_{311.5} - A_B$$

$$G = \frac{1543\ A}{9.15\ V}$$

where G = grains of naphthalene per 100 cubic feet of gas and V = volume of gas sampled, cubic feet

If dilution of the initial 100-ml. sample volume was necessary, the proper dilution factor must be incorporated into the above calculation.

Interferences. No adverse effects on the determination of naphthalene by this method were observed from the presence of light oils (benzene, xylene, and toluene) when added to naphthalene-cyclohexane solutions. These light oils are the most likely interfering compounds present in coke oven gas.

Accuracy and Precision. The accuracy of measuring naphthalene in cyclohexane by this method was determined by analyzing five samples, each containing a known weight of naphthalene in cyclohexane. The 95% confidence limits as determined from this data are ±0.42%.

Precision was determined by analyzing 10 separate portions of one of the standard samples which contained about 10 mg. of naphthalene per 100 ml. of cyclohexane. The results show a relative error for precision of 1.6%.

The data for the accuracy and precision tests are tabulated in Table V.

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Flame Spectrophotometric Determination of Sodium and Potassium

In Viscous Solutions or Plant Extracts

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A new method was needed for the routine determination of sodium and potassium in plant extracts, using the Beckman Model DU spectrophotometer and its flame attachment. The principle of operation of this instrument is such that viscosity and other characteristics of the fluid under test affect the flame color intensity. When properties of the fluid carrier alter the flame color intensity, this instrument is ideally suited to the application of a lithium internal standard method. Synthetic organic mixtures in solutions containing known quantities of the elements in question were tested. The apparent concentrations of sodium and potassium found spectrophotometrically could be corrected by use of concentration correction factors. Based on this work, a controlled analytical method is developed for solutions having characteristics differing from water.

THE instrument used in all the following experimental work was the Beckman Model DU spectrophotometer and the Model 10300 flame attachment, described by Gilbert *et al.* (*6*). It is set to measure the intensity of one of the characteristic wave lengths of light produced by exciting the element in question in an oxygen–natural gas flame. In this instrument the rate of introduction of the solution containing the element is controlled by causing it to pass through a glass capillary tube under a constant pressure differential. When the unknown solution has the same physical characteristics as those of a known standard solution, the rate of introduction will be the same, and the line intensities can be directly related to concentrations (*3*).

Often the material to be tested will have various kinds of organic matter as contaminants. The resultant changes in viscosity and surface tension may cause the unknown sample to be introduced at a rate different from that of the known standard solution (*4, 10*). Various authors (*1, 2, 7, 9, 11–13*) have proposed wet or dry ashing of the sample to remove organic matter or have proposed to ignore its presence. To avoid ashing, other workers (*4, 8, 10*) have proposed that the same amounts of organic matter be added to the standard solutions as were contained in the unknown solution. If this method is used, the samples must contain a relatively constant concentration of the contaminants.

When the unknown solutions have variable physical characteristics, particularly in regard to viscosity, the rate of introduction varies and a problem presents itself, as the concentrations are not directly correlated to line intensities. Such a problem, which becomes acute in the case of certain plant extracts, can be solved by the introduction into the solution of a rarely occurring element, in this case lithium. Lithium has been used as an internal standard in other types of flame photometers for a number of years (*4, 10*). Its use, however, with the Beckman instrument for this purpose has been discouraged (*6*) because of uncompensated errors that may arise. As these errors may be significant in precise work, they should be appropriately compensated by the means of the conditions stipulated below.

Errors due to interference caused by the presence of ions other

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Table II. Instrument Settings Used to Prepare Calibration Curves

Element	Concn. of Std. *vs.* 100% *T*	Selector Switch	Sensitivity, Turns from Full Clockwise	Slit Width, Mm.	Wave Length, Mμ	Pressures		
						Oxygen, inches water	Air, lb./sq. inch	Natural gas (950 B.t.u.), cm. isopropyl alcohol
Li	375	0.1	2.5	0.030	670.8	36	25	4.0
Na	200	0.1	2.5	0.065	589	42	25	4.0
K	150	0.1	2.5	0.037	767	36	25	4.0

than sodium and potassium are not considered here. This phase has been covered (*1, 3–7, 9, 12*).

This laboratory has had the project for the past 3 years of determining sodium and potassium in solutions with slightly varying sugar content. This problem was partially overcome by adding sugar to the standards in amounts equal to the average sugar content of the solutions to be tested and standardizing the instrument under these conditions. This method was satisfactory until it was decided to analyze some sugar factory juices of widely varying organic content. The problem was even more difficult when alcohol was present. If the direct method employing compensated calibration curves were used, the preparation of calibration curves to fit all concentrations of sugar and alcohol would be a practically impossible task. A search of the literature indicated that, with care to avoid noncompensated errors, an internal standard might be used with the Beckman instrument (*2*), although no actual experimental work has been reported which uses the method described here. An investigation of this problem was undertaken and a controlled precision method has been developed which is unusual in that the emission of the unknown ion is not related to the emission of the internal standard ion. Rather, concentration factors are used. The method has been successfully applied to beet root water extracts clarified with lead acetate, factory diffusion juice, carbonated factory juices, and sulfured factory juices preserved by the addition of alcohol. The method has not been applied to, but would seem equally pertinent for, any plant extract material not containing large particles of suspended matter. The concentrations of the sodium and potassium should be adjusted to be in the upper range of the calibration curves, by proper selection of the highest standard solution for setting the 100 *T* point of the calibration curve. This laboratory has had better success when the concentrations range between 50 and 500 p.p.m.

Table I. Concentrations of Elements in Solutions Used to Prepare Calibration Curves

Standard Solutions	Final Concentrations, P.P.M. Element		
	Li	Na	K
1	0	0	0
2	75	40	30
3	150	100	75
4	300	140	105
5	375	200	150

EXPERIMENTAL

The reagents used in the following work were made up from materials of the highest purity obtainable, with the exception of ethyl alcohol. Solox, a specially denatured ethyl alcohol, was employed, as a suitable substitute for the pure alcohol, because of its low cost. The other reagents were lithium chloride, potassium chloride, sodium chloride, and sucrose. The manufacturer's assay reported 0.02% sodium in the potassium chloride, 0.01% potassium in the sodium chloride, and 0.13% other alkali salts as chlorine in the lithium chloride. The sucrose contained about 0.001% sodium. All these amounts of trace impurities are unconsequential except the sodium in the sucrose, which would amount to less than 1 p.p.m. in the final solution. Lithium chloride is a difficult reagent to use for a standard concentration solution. The chloride was chosen to eliminate interference effects that result from a second anion. The errors due to the presence of secondary ions are not considered here. Small errors in lithium concentration are of no consequence if the same lithium stock solution is used throughout a test series.

PREPARATION OF CURVES

To study the effects of varying viscosity, eighteen curves, six for each element, were prepared as determined by five points for each curve. To do this six sets of solutions were prepared, each composed of five standard solutions having final concentrations as shown in Table I. These were made by using appropriate amounts of stock solutions of lithium, sodium, and potassium in 100-ml. volumetric flasks.

Table III. Emission (Transmittance Dial Readings) Obtained from Solutions Used to Prepare Calibration Curves

(Transmittance units)

Concn., P.P.M.	Element Only	Element plus 4% Sucrose	Element plus 8% Sucrose	Element plus 10% Alcohol	Element plus 4% Sucrose, 10% Alcohol	Element plus 8% Sucrose, 10% Alcohol
			Lithium			
0	0.1	0.0	0.0	1.0	1.0	1.0
75	51.3	35.2	28.2	57.1	43.2	36.2
150	71.2	54.2	45.3	78.5	63.5	56.2
300	92.7	75.1	66.0	103.1	85.9	76.0
375	100.0	82.3	73.0	111.0	93.2	84.0
			Sodium			
0	0.0	2.2	2.6	2.3	2.2	2.0
40	46.2	34.0	28.2	53.5	40.2	35.0
100	73.7	55.8	48.8	84.2	65.8	57.2
140	85.8	67.0	59.1	96.9	78.1	68.0
200	100.0	78.8	70.0	112.0	91.2	80.8
			Potassium			
0	0.3	0.3	0.3	0.5	0.7	0.8
30	26.5	16.8	11.3	35.8	20.8	16.8
75	62.7	41.2	31.7	75.3	51.0	41.2
105	81.0	55.3	45.8	92.3	66.8	57.2
150	100.0	73.8	60.3	116.0	86.2	75.1

The physical characteristics of each of the six sets were made different by introducing varying amounts of sucrose and alcohol according to the following directions: To set 1, add 10 ml. of alcohol to each of the five flasks comprising it; to set 2 add nothing; to set 3 add 4 grams of sucrose and 10 ml. of alcohol to each flask; to set 4 add 8 grams of sucrose and 10 ml. of alcohol; to set 5 add 4 grams of sucrose only; and to set 6 add 8 grams of sucrose only. Finally, make all solutions to 100-ml. volume with distilled water.

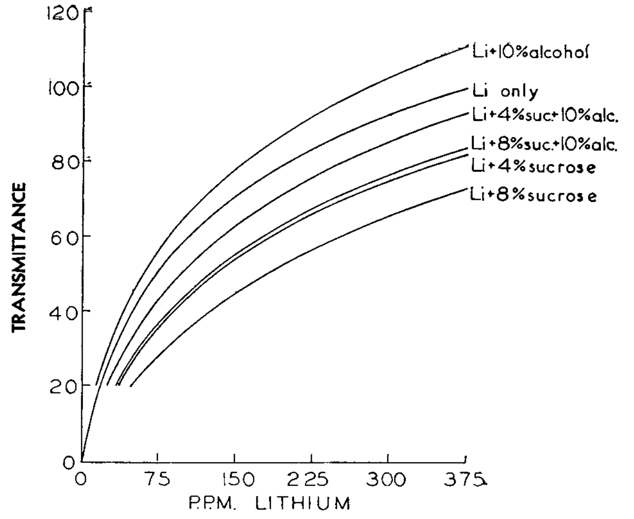

Figure 1. Lithium Curves

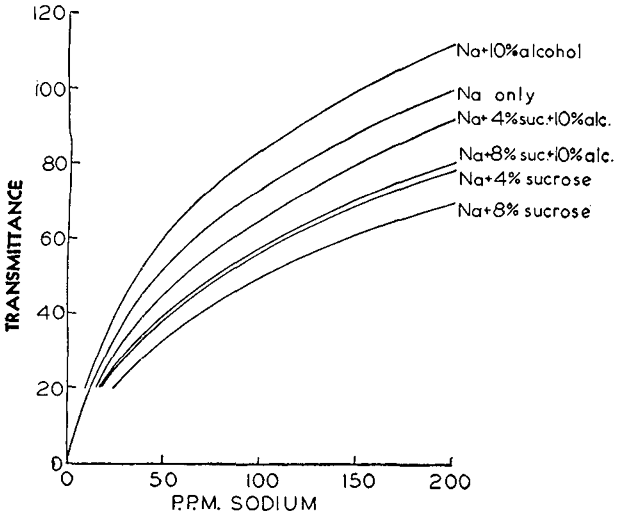

Figure 2. Sodium Curves

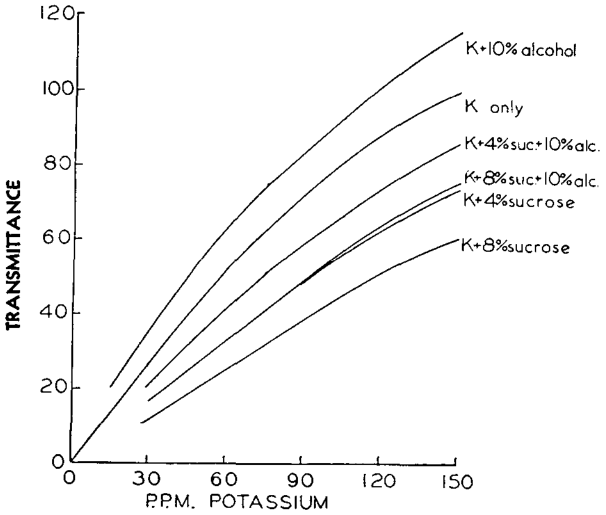

Figure 3. Potassium Curves

Lithium, sodium, and potassium were then read on the flame spectrophotometer, using the instrument settings as shown in Table II. The amount of light emitted by the characteristic spectral line is reported here as per cent transmittance or transmittance units, as it is the reading taken from the transmittance dial on the colorimeter portion of the instrument.

The initial 100% transmittance setting on the instrument is always made using the solution containing 375 p.p.m. of lithium only, 200 p.p.m. of sodium only, and 150 p.p.m. of potassium only, respectively. The transmittance units found are recorded in Table III. Each value represents an average of five readings. These values are not absolute. Among other things, they depend upon the capillary tube used. All transmittance units shown in Table III are plotted in Figures 1, 2, and 3.

DISCUSSION

In a solution containing unknown amounts of organic material, accurate determination of sodium and potassium using the Beckman flame spectrophotometer presents a problem due to the different rates at which the samples are introduced into the flame. This problem has been satisfactorily overcome by the addition of a known amount of lithium to the solution. Alcohol and sugar were added to known solutions to determine whether a correction factor could be derived, which, when applied to the apparent sodium and potassium concentrations found, would give the true concentrations of these elements. This correction factor value is calculated as a ratio value obtained by dividing the actual parts per million of lithium added by the parts per million of lithium found from the transmittance reading. This ratio value is referred to as the concentration correction factor.

To use the curves for calculating the experimental values, the values of the ratios of concentrations at comparable transmittance readings are first calculated. Examination of the lithium only curve (Figure 1) at transmittance 100 shows that the concentration of lithium is 375 p.p.m., having been so set. Following the 100 transmittance line across to the lithium + 10% alcohol curve, the corresponding concentration of lithium is 277.5 p.p.m. The concentration correction factor is obtained by finding the value of the ratio 277.5/375, which is 0.74. This factor will convert the apparent parts per million found in the 10% alcohol solution to the true concentration. It will be shown that this factor, 0.74, holds very closely, regardless of what transmittance reading is chosen, provided that the lithium only and the lithium plus 10% alcohol are the curves under con-

Table IV. Calculated Ratios or Concentration Correction Factors

Curves	Transmittance	Ratio	Value of Ratio	Average Value of Ratio
	A. Lithium			
Li plus 10% alcohol/Li only	100	277.5/375	0.74	
	70	112.5/147	0.77	0.75
Li plus 4% sucrose, 10% alcohol/Li only	92	367.5/295	1.25	
	62	142.5/112.5	1.27	1.26
Li plus 8% sucrose, 10% alcohol/Li only	82	359/217.5	1.65	
	60	172.5/105	1.64	1.65
Li plus 4% sucrose/Li only	82	372/217.5	1.71	
	62	193/111	1.74	1.72
Li plus 8% sucrose/Li only	72	368.5/157.5	2.34	
	52	190/79	2.41	2.37
	B. Sodium			
Na plus 10% alcohol/Na only	100	151/200	0.75	
	72	72/96	0.75	0.75
Na plus 4% sucrose, 10% alcohol/Na only	90	195/155	1.26	
	66	100/79.5	1.26	1.26
Na plus 8% sucrose, 10% alcohol/Na only	80	197/126	1.65	
	64	124/75	1.65	1.65
Na plus 4% sucrose/Na only	76	185/107.5	1.72	
	54	95/54	1.76	1.74
Na plus 8% sucrose/Na only	70	200/90	2.22	
	54	120/52	2.31	2.26
	C. Potassium			
K plus 10% alcohol/K only	100	118/150	0.79	
	60	55/70	0.79	0.79
K plus 4% sucrose, 10% alcohol/K only	86	149.5/117	1.28	
	52	77/60	1.28	1.28
K plus 8% sucrose, 10% alcohol/K only	74	147.5/93.0	1.59	
	56	103/64	1.61	1.60
K plus 4% sucrose/K only	72	146/89.5	1.63	
	52	98/59	1.66	1.65
K plus 8% sucrose/K only	60	149/70	2.13	
	32	75/35	2.14	2.14

Table V. Comparison of Calculated Ratios

Curve	Average Value of Ratio			Largest Deviation	Largest Deviation, %	% T 375 P.P.M. Lithium from Table III
	Li	Na	K			
10% alcohol/element only	0.75	0.75	0.79	0.04	5.2	111.0
4% sucrose plus 10% alcohol/element only	1.26	1.26	1.28	0.02	1.6	93.2
8% sucrose plus 10% alcohol/element only	1.65	1.65	1.60	0.05	3.1	84.0
4% sucrose/element only	1.72	1.74	1.65	0.09	5.3	82.3
8% sucrose/element only	2.37	2.26	2.14	0.23	10.2	73.0

sideration. Likewise, the concentration correction factor should be nearly 0.74 when similarly calculated from either the sodium only and sodium plus 10% alcohol curves, or the potassium only and potassium plus 10% alcohol curves.

In Table IV a tabulation of concentration correction factors is found. Arbitrary but high transmittance readings were used in order to improve the accuracy of the calculations of the factors. A comparison of the average ratios for the three elements is given in Table V. As the concentration of the various organic matters is altered, so that the rate of introduction and flame temperatures become less similar to the conditions holding during introduction of the standard solutions, the error increases significantly.

The last column in Table V shows the transmittance values obtained for the 375 p.p.m. lithium plus the organic mixtures, after the instrument has been previously set at 100 transmittance units with the 375 p.p.m. lithium only solution. Such values, established by preparing known solutions similar to the unknown solutions, can be used as the criteria for establishing the accuracy of analysis that may be anticipated when working with organic mixtures of unknown composition. In sample analysis it is preferable to bring the lithium reading of the sample as close to the standard lithium reading of 100 as possible.

The principle of operation of the ordinary internal standard technique presupposes that the emission-concentration curve of the internal standard element will be similar in shape to the curve of the unknown element. That this is not true has been shown by many workers and can be seen by reference to Figures 1, 2, and 3. If emissions are used to calculate the correction factors rather than using concentration correction factors, gross errors will be found.

A calculated example will make this point clear. Assume 375 p.p.m. lithium is added to a sample and that it then contains 8% sucrose and 10% Solox. The instrument would first be set on 100 *T*, using a 375 p.p.m. lithium only solution. Reference to the appropriate curve of Figure 1 shows that the sample would report 84.0% *T*. The emission factor would be 100/84.0 = 1.19. If the sample contained 150 p.p.m. of potassium, it would report 75.1% *T* (Figure 3). 75.1 × 1.19 = 89.4. Reference to the potassium only curve (Figure 3) shows 89.4% *T* is 123 corrected p.p.m. of potassium, an error of 27.0 p.p.m. or 18%. The method proposed here would use a concentration correction factor of 1.65 as obtained from Table IV. 75.1% *T* is 92 p.p.m. 92 × 1.65 = 151.8 corrected p.p.m. K, an error of 1%.

The values found in Table V are not absolute values, but depend upon relative concentrations and settings of the instrument. They are comparable within themselves, but would be different if other instrument settings or other concentrations of either positive or negative ions were used, in part because of ion interaction.

PROCEDURE

Place a measured amount of the unknown solution in a 100-ml. flask, add 25 ml. of a 1500 p.p.m. lithium stock solution to produce 375 p.p.m. lithium in the final solution, then make to the mark with water. Having set the instrument at 100% *T*, using the standardization procedure with a solution of 375 p.p.m. lithium only, read the per cent *T* for lithium in the sample. If this per cent *T* is farther from 100 than will give the required degree of accuracy (Table V) a further dilution of the sample is necessary or either sucrose or alcohol is to be added, until a transmittance reading indicating adequate accuracy is obtained. The instrument is now set to measure sodium, then potassium flame color intensities. The apparent concentrations obtained from these readings are then corrected by means of the concentration correction factor already described.

Analyses of Actual Sample. Fifty milliliters of an unknown solution was transferred to a 100-ml. volumetric flask, 25 ml. of the 1500 p.p.m. lithium stock solution was added, and water was used to make to the mark. Another solution containing only 375 p.p.m. lithium was first used to set the instrument at 100% *T*, and the unknown solution containing lithium was then read. The transmittance was found to be 91.2%. The 91.2% *T* indicates (Table V) that the error of the analyses will be less than 2.5%, which is considered satisfactory, so the analyses were continued by finding the per cent *T* for sodium and potassium, which were 77.2 and 69.3, respectively. Referring to the sodium only curve (Figure 2) 77.2% *T* is 112 p.p.m. sodium, and referring to the potassium only curve (Figure 3) 69.3% *T* is 85 p.p.m. potassium. The concentration correction factor is obtained by reference to the lithium only curve (Figure 1). The lithium transmittance is 91.2%, which corresponds to 300 p.p.m. lithium. Thus the concentration correction factor is 375/300 or 1.25. Correcting sodium, the true concentration of sodium in the solution is 112 × 1.25 or 140 p.p.m., and the true concentration of sodium in the sample is 280 p.p.m., owing to the 1 to 1 dilution previously made. Following this through for potassium, the true potassium concentration in the solution is 85 × 1.25 or 106 p.p.m., which multiplied by 2 gives 212 p.p.m. potassium in the sample.

For sample analyses it is not necessary to duplicate all the curves shown. These curves were developed only to justify the technique and to show what magnitude of error may be anticipated under these experimental conditions. Three curves are necessary for the analysis of unknown solutions: the lithium, sodium, and potassium only curves in Figures 1, 2, and 3.

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Improvements in Flame Photometric Determination of Sodium in Portland Cement

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The sodium content of portland cement is routinely determined by flame photometry. Some flame photometers were found consistently to indicate sodium contents of less than zero for certain cements. In addition, different brands of flame photometers almost never gave identical results for any cement. A study of the method showed that flame photometers having wide effective band widths gave low, often negative results, because of the inhibiting effect of silica on the emission of calcium oxide in the region of the sodium line. Increased accuracy was obtained by using an auxiliary multilayer interference filter to narrow the effective band width of the instrument or by removing the silica from the cement prior to the determination of the sodium. Both techniques together converted the current routine procedure into one which gave results substantially identical with those obtained by the gravimetric J. Lawrence Smith method, when using the Barclay, the Beckman DU, or the Perkin-Elmer flame photometer.

MOST of the portland cements manufactured in this country contain from 0 to perhaps 1% each of sodium and potassium oxides. There was little interest in these minor constituents until it became known that the alkali in cement can react with certain types of aggregate to give unsound concrete (*15*). Since that time there has been a continuing interest in these constituents and a corresponding interest in the development of more rapid and accurate methods for their determination.

Until very recently, gravimetric procedures were invariably used, notably modifications of the Glaze (*8*), Berk-Roller (*4*), and J. Lawrence Smith (*9*) methods. Even the more rapid of these procedures were very time-consuming, and the most accurate required much patience and skill on the part of the analyst. In general, the precision and accuracy to be expected from ordinary analyses were not good.

In 1948, Pritchard (*13*) first proposed the use of flame photometry for the determination of sodium and potassium in portland cement. This suggestion received ready acceptance, and additional development work was carried out by Pritchard, Bogue, and Bean. This resulted, in 1949, in the adoption by the American Society for Testing Materials of a tentative standard method (*1*) and a study of the subject by Eubank and Bogue (*7*). The 1952 revision of the federal specification on methods of testing cement included essentially the same procedure (*10*).

All this work was done with the various models of the Perkin-Elmer flame photometer. In addition, the requirements of both the 1949 ASTM and the 1952 federal specifications are fulfilled only by the use of one of the Perkin-Elmer Model 52 series of instruments. There has long been a feeling, however, that it would be desirable to have methods available which would permit the use of other instruments. In 1951, Diamond and Bean (*6*) modified the ASTM Perkin-Elmer method to adapt it to the Beckman DU spectrophotometer with the Model 11300 flame photometry attachment. This method has been incorporated in the U. S. Army Corps of Engineers' "Handbook of Concrete and Cement" (*14*).

Extensive use of these methods at the National Bureau of Standards for research, routine acceptance testing, and the analysis of comparative samples disclosed several anomalous facts about the determination of sodium. First, at least two brands of low sodium cement, when analyzed by any one of three model 52A Perkin-Elmer flame photometers, using the ASTM procedure, showed negative sodium contents. Second, consistent differences between the results obtained by several Perkin-Elmer instruments were noted when a series of comparative samples were analyzed. Third, one particular Perkin-Elmer Model 52A instrument consistently gave results several hundredths of 1% lower for sodium than the Beckman instrument with the old Model 11300 flame photometry attachment (*6*), as well as with the new Model 9200 attachment.

This investigation was undertaken to try to explain these phenomena. It was hoped that knowledge of the basic factors relevant to the analysis would be increased and the accuracy of the method improved to the point where its use as a referee method would be warranted. Moreover, it was hoped that a procedure could be developed that would permit the use of at least some of the other flame photometers now available commercially in addition to the Model 52 Perkin-Elmer.

EXPERIMENTAL WORK

In current flame photometric procedures for the determination of sodium in cement, the sample solution contains all the constituents of the cement, except the negligibly small acid-insoluble residue, dissolved in 5 to 95 hydrochloric acid. The standard solutions contain, in addition to the alkalies, 6300 p.p.m. of calcium oxide in 5 to 95 hydrochloric acid. The other constituents of portland cement are not included in the standards. Eubank and Bogue (*7*) showed that these standards give reasonably good results, and several years of experience by the cement industry have confirmed this.

The assumption was made, however, that the errors in which this laboratory was interested were caused by interference by one or more of the other constituents of cement. Since the silica content of cement is about 21%, next in importance to the 63% of calcium oxide present, it received the greatest attention. The assumption was also made that some instrument characteristics were involved because any error due to solution interferences would otherwise be identical with all instruments.

To determine the effect of silica on the emission of sodium and calcium at the sodium wave length, four solutions were prepared. Silica was introduced into two of these solutions by the hydrolysis of tetraethyl orthosilicate. Since ethyl alcohol was the other product of the hydrolysis, an equal amount was added to the other two solutions to eliminate it as a variable. One solution contained 50 p.p.m. of sodium oxide; a second 50 p.p.m. of sodium oxide plus 2100 p.p.m. of silicon dioxide; a third, 6300 p.p.m. of calcium oxide; and a fourth, 6300 p.p.m. of calcium oxide plus 2100 p.p.m. of silicon dioxide. All four solutions contained the same amounts of ethyl alcohol and were made up in 5 to 95 hydrochloric acid.

The emissions of these four solutions at the sodium wave length (589 mμ) were compared with that of the 100-p.p.m. standard routinely used in the current ASTM and federal specification methods. This solution contained 100 p.p.m. each of sodium and potassium oxides, plus 6300 p.p.m. of calcium oxide in 5 to 95 hydrochloric acid. The solutions were compared on a Perkin-Elmer Model 52A instrument with air-propane flame and on a Beckman Model 9200 instrument with oxyhydrogen flame and a 2-mμ band width, with the results shown in Table I. The silica depressed the calcium emission by about 40% and enhanced the sodium emission slightly. The same 40% depression was noted at the adjacent wave length for maximum calcium emission, indicating the same effect at all wave lengths in that band. The silica stock solution was examined flame photometrically and was found to be sodium-free and to have no emission of its own at the sodium wave length.

Table I. Effect of Silica on Emission of Sodium and Calcium at 589 mμ

Solution	Flame Photometer, Scale Reading	
	Perkin-Elmer	Beckman
100 p.p.m. standard	100	100.0
50 p.p.m. of sodium	58	67.0
50 p.p.m. of sodium, plus silica	59	68.0
Calcium	12	5.2
Calcium plus silica	7	3.2
Distilled water	0	0

A study was then made of the effect of varying the effective band width of the light measured by an instrument. This was done by determining the apparent sodium content of four cements using the Beckman DU flame photometry attachment (Model 9200) at three different slit widths. Since it was recognized that flame temperature might be important, both the oxyacetylene and the oxyhydrogen flames were used. The cements used in the study were ASTM comparative samples, having a range of sodium contents. Their chemical compositions, as determined by a single analysis of each using routine federal specification methods, are given in Table II. Their sodium and potassium contents were determined by the J. Lawrence Smith procedure (*9*), with the results shown in Table III.

Two sets of samples were prepared as follows.

One-gram samples of each cement were dissolved in 5 ml. of concentrated hydrochloric acid, filtered, and diluted to 100 ml. as specified in the current routine methods. For the second set, 1-gram samples were dissolved in hydrochloric acid, the silica was removed by a single dehydration and the cement was dissolved in 2.5 ml. of concentrated hydrochloric acid, filtered, and diluted to 100 ml. These two sets of samples were designated as SiO_2 in and SiO_2 out, respectively. The standard solutions used were the lime-acid standards, as specified in the ASTM and federal specification methods.

Table II. Chemical Composition of Cement Samples[a]

Constituents	Cement, %			
	A	B	C	D
SiO_2	21.3	22.3	21.1	22.3
Al_2O_3	6.0	5.2	6.3	5.8
Fe_2O_3	2.8	4.0	2.8	2.5
CaO	65.1	64.3	63.9	65.4
MgO	1.3	1.1	1.8	1.2
SO_3	2.1	1.6	2.0	1.8
Ignition loss	1.2	0.8	0.9	1.0
Insoluble residue	0.2	0.1	0.1	0.1

[a] See Table III for alkali values.

Table III. Alkali Content of Four Cements Determined by J. Lawrence Smith Method

Na₂O in Cement, %			
A	B	C	D
0.14	0.27	0.65	0.062
0.16	0.24	0.58	0.051
0.12	0.25	0.56	0.057
0.12	0.26	0.56	0.042
0.12	0.24	0.54	0.041
0.11	0.27	0.57	0.055
0.12	..	..	...
Av. 0.13	0.26	0.58	0.051
	K₂O in Cement, %		
0.30	0.63	1.03	0.14
0.29	0.64	1.06	0.18
0.33	0.66	1.06	0.16
0.31	0.64	1.03	0.15
0.30	0.63	1.03	0.16
0.29	0.64	1.06	0.16
0.29	..	..	...
Av. 0.30	0.64	1.04	0.16

Table IV. Effect of Varying *Slit Width* on Apparent Sodium Oxide Content of Four Portland Cements[a]

Cement	Effective Band Width, Mμ					
	1	10	20	2	10	20
	Na₂O Determined, %					
	Oxyhydrogen burner			Oxyacetylene burner		
A SiO_2 in	0.12	0.080	−0.002	0.10	0.052	−0.029
SiO_2 out	0.15	0.14	+0.12	0.14	0.14	+0.10
B SiO_2 in	0.27	0.20	+0.074	0.24	0.16	+0.027
SiO_2 out	0.28	0.27	+0.25	0.27	0.25	+0.22
C SiO_2 in	0.59	0.50	+0.31	0.54	0.40	+0.17
SiO_2 out	0.60	0.57	+0.54	0.59	0.52	+0.47
D SiO_2 in	0.048	0.020	−0.038	0.040	0.008	−0.069
SiO_2 out	0.051	0.044	+0.042	0.048	0.023	+0.018

[a] Beckman DU spectrophotometer with Model 9200 flame photometer; lime-acid standards.

Table V. Effect of Varying Slit Width on Apparent Sodium Oxide Content of Four Portland Cements[a]

Cement	Effective Band Width		
	2 Mμ	10 Mμ	20 Mμ
	Apparent Na₂O determined, %		
A	0.14	0.15	0.15
B	0.28	0.27	0.28
C	0.59	0.60	0.62
D	0.048	0.049	0.052

[a] Beckman DU spectrophotometer with model 9200 flame photometer; synthetic cement standards; oxyhydrogen burner.

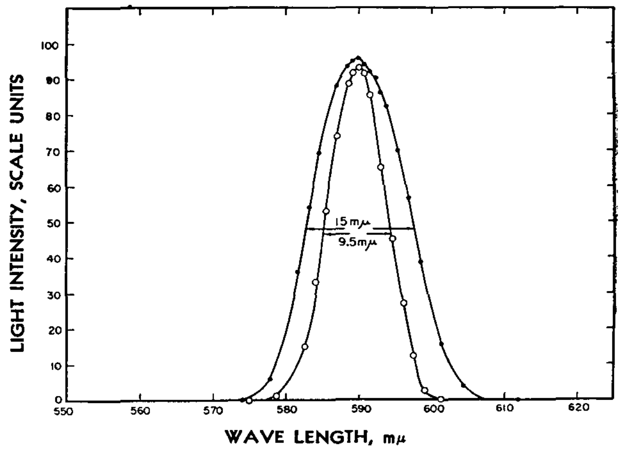

Figure 1. Wave Length *vs.* Intensity Curves for Two Perkin-Elmer Flame Photometers Used to Determine Effective Band Width at 590 mu

○. Perkin-Elmer No. 446, model 52C
●. Perkin-Elmer No. 72, model 52A

Since the effective band width of the Beckman DU spectrophotometer was known to be 33 mμ per mm. slit width at 590 mμ (*2*), slit widths were selected to give band widths of 1 or 2, 10, and 20 mμ. The photocell, range, and sensitivity settings were made as needed to balance the instrument.

From an examination of the results shown in Table IV, several conclusions may be drawn. It is evident that the greater the band width, the lower the apparent sodium content, and that the elimination of silica minimizes the effect of slit width but does not eliminate it. Both effects are more pronounced for the oxyacetylene flame than for the somewhat cooler oxyhydrogen flame.

In order to assess the importance of the constituents of cement other than lime and silica, a set of synthetic cement standards was made up. These were made from reagent grade chemicals and contained typical concentrations of silica, alumina, ferric oxide, magnesium oxide, and sulfur trioxide in addition to lime and acid. The samples were made up with silica in, and alcohol was added to compensate for that in the standards. The results shown in Table V indicate that the effect of slit width can be essentially eliminated by the use of such synthetic standards. However, the values obtained with the synthetic standards are substantially identical with those obtained at small slit widths for samples with SiO_2 out.

Since the Perkin-Elmer flame photometer does not have a variable slit width, these experiments could not be duplicated with it. However, various attempts were made to confirm them indirectly. Some of the results of these attempts are shown in Tables VI and VII. One difference between the models is that the 52A uses a motor-driven chopper unit to interrupt the light beam from the flame, and the 52C and the modified 52A use a vibrating reed for the same purpose.

The slit widths were measured directly by means of feeler gages or by a precision glass-tubing gage. They were found to be substantially identical in all instruments. However, direct measurement of the effective band widths of the instruments showed a range of 8 to 16 mμ. These measurements were made as follows.

A solution containing sodium, but no calcium, was atomized into the flame. Data on intensity, or meter reading, *vs.* wave length were obtained on the resulting light.

A divided circle was substituted for the wave-length dial to permit more precise reading of wave-length increments, and this divided circle was calibrated in terms of wave length in the region of the sodium line by measuring in terms of its units the 5800- and 6000-A. marks on the instrument panel. The wave length *vs.* intensity data were plotted on cross-sectional paper, as illustrated in Figure 1. The two points on the curve where the intensity values were one half the maximum were then noted. The wave-length interval between these points was taken as the effective band width.

From Table VI it can be seen that these instruments of different band width gave different apparent sodium contents for cement D. The sodium values were obtained flame photometrically by the current federal specification method (*10*).

Table VI. Characteristics of Four Perkin-Elmer Flame Photometers

Serial No.	Model	Entrance Slit Width, Inch	Exit Slit Width, Inch	Effective Band Width, Mμ	Apparent Na_2O in Cement D, %
72	52A	0.030	0.019	15.2 tight 16.0 loose	+0.01 −0.02
255	52A modified	0.029	0.020	10.7	0.00
466	52C	0.029	0.018	9.5	+0.03
259	52A modified	0.029	0.020	8.3	+0.04

Table VII. Apparent Sodium Oxide Content of Four Portland Cements Determined under Various Conditions by a Perkin-Elmer Flame Photometer[a]

Condition	Na_2O in Cement, % A	B	C	D
Specification method				
Instument loose	0.06	0.20	0.49	−0.02
Instrument tight	0.10	0.22	0.51	+0.01
SiO_2 out, instrument tight	0.125	0.26	0.60	+0.04
Synthetic standards				
Instrument loose	0.12	0.26	0.59	+0.035
Instrument tight	0.13	0.26	0.58	+0.055
No alcohol in sample	0.10	0.23	0.52	+0.025
Special filter				
SiO_2 in	0.11	0.26	0.58	+0.05
SiO_2 out	0.13	0.28	0.58	+0.045
Gravimetric average	0.13	0.26	0.58	+0.051

[a] Model 52A, serial No. 72.

At the start of this investigation the Model 52A instrument (Serial No. 72) consistently gave values of −0.02% of sodium oxide for sample D, and two similar instruments in other laboratories also gave negative results. Several attempts were made to improve the performance of instrument 72, first by fastening a metal plate over the exit slit so as to decrease its width by one half or more. This attempt did not succeed, mainly because the instrument became unstable, the meter needle fluctuating over a range of perhaps 15 scale divisions. Because of this instability, the next step of proportionately decreasing the width of the entrance slit was not taken, and the exit slit was restored to its original state.

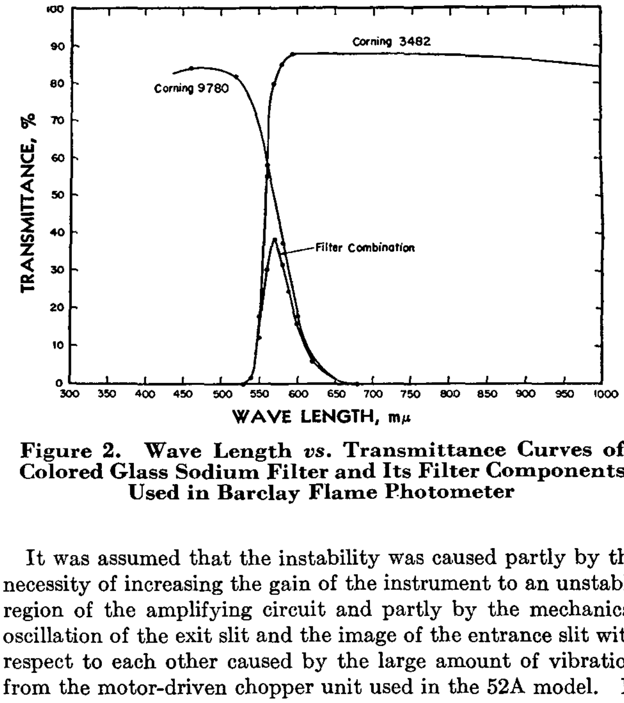

Figure 2. Wave Length *vs.* Transmittance Curves of Colored Glass Sodium Filter and Its Filter Components Used in Barclay Flame Photometer

It was assumed that the instability was caused partly by the necessity of increasing the gain of the instrument to an unstable region of the amplifying circuit and partly by the mechanical oscillation of the exit slit and the image of the entrance slit with respect to each other caused by the large amount of vibration from the motor-driven chopper unit used in the 52A model. It was possible to decrease appreciably this vibration by recementing one of the rubber motor mountings that had apparently broken loose and tightening all the others. This resulted in an appreciable improvement in performance as shown by determination of the sodium content of samples A, B, C, and D (Table VII). However, only a slight decrease in effective band width accompanied this improvement (Table VI).

Table VII shows that the results obtained on the Perkin-Elmer instruments parallel those obtained on the Beckman (Tables IV and V). The elimination of silica increases the apparent sodium content. The same improvement is obtained by the use of synthetic standards. In addition, the use of synthetic standards essentially eliminates the effect of vibration on the results just as their use eliminates the similar effect of change in slit width with the Beckman instrument. Finally, the effect of alcohol is appreciable, and it must be added to the sample solution when silica from tetraethyl orthosilicate is used in the standard.

From the data presented it is evident that in order to give acceptable results for the determination of sodium in cement, a flame photometer must be so designed that the sodium light impinging on the photocell is not appreciably contaminated by light from the adjacent calcium band. It was known to the authors that several unsuccessful attempts had been made to determine the sodium content of portland cement using optical filter type flame photometers. Because of the insight into the problem obtained from the data presented above, it seemed possible that this situation could be corrected by relatively simple modifications of the instruments or the procedure.

Accordingly, experiments were performed on a Barclay flame photometer, designed primarily for use as an internal standard instrument for the analysis of biological materials. This instrument, which uses an air-propane flame, was selected as typical of the group of optical filter, barrier layer photocell instruments now available. For the isolation of sodium light it comes equipped with a combination of the Corning colored glass filters 3482 (heat-resistant lantern shade yellow) and 9780 (colorimeter blue-green medium). The transmittance curves of these filters are shown in Figure 2, together with their transmittance when used in combination. They transmit a maximum of 38.5% at 570 mμ. They have an effective band width of 41 mμ and transmit about 24.5% at 590 mμ and 6% at 620 mμ.

Comparative samples A, B, C, and D were analyzed on the Barclay flame photometer using the direct intensity method. It was difficult to use the instrument in this way because of erratic needle fluctuation. This behavior is predicted by the manufacturer, who strongly recommends use of the internal standard method for quantitative work. The results are shown in Table VIII. Using the specification procedure (*2*), the results for sodium are low by 0.35 to 0.51%. Eliminating the silica interference raises all sodium results by about 0.30%, but they are still not generally acceptable.

Eliminating all other interferences by the use of the synthetic cement standards seems to overcompensate slightly, and the results for sodium, except for sample D, are 0.07% high. The high results might involve experimental error caused by the poor performance of the instrument when using the direct intensity method, or they might be due to incomplete solution of the silica in the cements invariably noted when these comparative samples (which had been stored for some time) were dissolved.

An attempt was then made to improve the performance of the Barclay and the Perkin-Elmer 52A (No. 72) by adding a Fabry-Perot multilayer interference filter (*5*), obtained from Baird Associates, Inc. This filter has a maximum transmittance of 78% at 591.7 mμ and a band width of about 5 to 8 mμ. A typical wave length *vs.* transmittance curve is shown in Figure 3.

The Barclay flame photometer was modified by replacing its sodium filter with a combination consisting of the interference filter with Corning 3486 and 9780 colored glass filters to eliminate the side bands. This resulted in a remarkable increase in needle stability and improvement in performance. The results for cements, both with silica in and out, are included in Table VIII and are substantially identical with those obtained with the Perkin-Elmer instruments, without filters, especially No. 255 (Table IV).

The interference filter was added to the Perkin-Elmer 52A (No. 72) by taping it over the exit slit. Because this is a monochromator instrument, no auxiliary glass filters were needed to eliminate the side bands. The filter caused some increase in needle flicker, but improved the results obtained analytically, as shown in Table VII. The removal of silica still resulted in a slight, but negligible, increase in sodium value.

This filter did not have to be removed for the determination of potassium with the Perkin-Elmer instrument. Because of the sideband present (Figure 3), it has no effect other than an insignificant decrease in sensitivity at the potassium wave length (768 mμ). None of the variations in technique mentioned in this paper seemed to have any significant effect on the potassium determination. However, this was not investigated in detail.

DISCUSSION

There has been a tendency in investigations on flame photometry to obtain a relatively large number of flame photometer results and then compare these against single gravimetric values. Sometimes the single gravimetric value has been an average of a number of somewhat scattered values, perhaps with one or two stray values excluded from the average. At other times a single determination has been reported. Because gravimetric values, especially by the Smith procedure, are difficult and costly to obtain, one is generally content with a few values. Furthermore, occasional wild values are the rule, generally accompanied by high blank values. These considerations have made flame photometry popular for alkali determinations. Wild values seldom occur, and precision is much better when using the flame photometric procedure.

In this study it was felt that a number of determinations by the best known gravimetric procedure were required in order to establish a criterion by which the various flame photometric techniques could be compared. The results shown in Table III are believed to be typical of what can be obtained by an experienced, competent analyst.

In flame photometry calcium has a twofold interference with sodium. First, it radiates at the same wave length as sodium. This is a true calcium radiation (the lower wave-length region of the calcium oxide bands with wave length maxima at 603 and 622 mμ) and is not due to contamination with sodium salts of low alkali reagent calcium carbonate. The second calcium interference is its depressant effect on the emission of sodium. These two interferences are adequately compensated for in the present routine methods for sodium in cement by the use of standard solutions containing 6300 p.p.m. of calcium oxide equivalent to the average amount in portland cement. It has been shown that no appreciable error is introduced by the normal variation in the amount of calcium in cement (*7*).

The calcium interference discussed here is due to the depressant effect of the silica in cement on the emission of the calcium oxide band. Alumina has long been known to have a similar

Table VIII. Apparent Sodium Oxide Content of Four Portland Cements Determined under Various Conditions by Barclay Flame Photometer[a]

	Na_2O in Cement, %			
	A	B	C	D
Lime-acid standard				
SiO_2 in	−0.23	−0.10	0.05	−0.36
SiO_2 out	+0.09	+0.18	0.38	−0.07
Synthetic standard	+0.19	+0.32	0.63	+0.06
Special filter				
SiO_2 in	+0.085	+0.195	0.49	0.00
SiO_2 out	+0.13	+0.27	0.53	+0.05
Gravimetric average	+0.13	+0.26	0.58	+0.051

[a] Values obtained by direct intensity method. Manufacturer prescribes use of internal standard method for quantitative work.

Table IX. Apparent Sodium Oxide Content of Four Portland Cements as Determined on Different Instruments Using Two Different Methods and Lime-Acid Standards

Instrument	Na_2O in Cement, %							
	A	B	C	D	A	B	C	D
	SiO_2 in				SiO_2 out			
Perkin-Elmer 52A (No. 72)	0.10	0.22	0.51	0.01	0.125	0.26	0.60	0.04
Perkin-Elmer 52A modified (No. 255)	0.07	0.19	0.50	0.00	0.125	0.26	0.58	0.045
Perkin-Elmer 52A modified (No. 259)	0.105	0.23	0.54	0.04	0.125	0.275	0.59	0.06
Beckman DU, oxyacetylene burner	0.10	0.24	0.54	0.04	0.14	0.27	0.59	0.048
Beckman DU, oxyhydrogen burner	0.12	0.27	0.59	0.048	0.15	0.28	0.60	0.051
Barclay[a]	−0.23	−0.10	0.05	−0.36	0.09	0.18	0.38	−0.07
Filter + Barclay[a]	+0.085	+0.195	0.49	0.00	0.13	0.27	0.53	+0.05
Filter + Perkin-Elmer (No. 72)	0.11	0.26	0.58	+0.05	0.13	0.28	0.58	0.045
Gravimetric average	0.13	0.26	0.58	0.051	0.13	0.26	0.58	0.051

[a] Values obtained by direct intensity method. Manufacturer prescribes use of internal-standard method for quantitative work.

effect (*12*), but in this study its effect has been lumped with those of the elements other than calcium and silicon. Since the emission of the calcium in the cement sample is depressed about 40% by the silica present and this effect is not compensated for in the lime-acid standards, the net result is an apparently lower sodium content.

The amount of error depends on the ratio of light caused by calcium to that caused by sodium that is permitted to strike the measuring photocell. Since the light transmitted by a slit is directly proportional to its width for the sodium line and is proportional to the square of its width for the calcium oxide band (*3*), it is evident that the ratio of calcium to sodium light will increase with increasing slit width or effective band width. The variable slit-width experiments performed with the Beckman flame photometer showed this directly. The results were most nearly correct when the band width was narrowest.

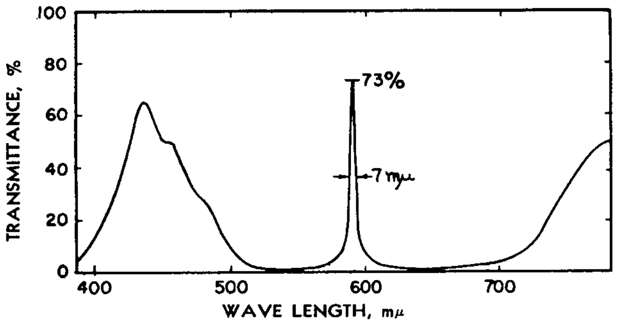

Figure 3. Typical Wave Length *vs.* Transmittance Curve for Multilayer Interference Filter

The greatest error results from the use of instruments having a combination of colored glass filters as the sole means of isolating the sodium light. Figure 2 shows that the Barclay instrument transmits at 620 mμ about 25% as much light as at 589 mμ. So much calcium light is transmitted by these filters that the light from the calcium in the cement, as inhibited by the silica present, even when added to light from the sodium present, is still considerably less than that caused by the calcium alone in the lime-acid standard containing no sodium. Hence the negative results found for most cements.

For cement analysis purposes, a colored glass filter combination is a sodium-plus-calcium filter, rather than a sodium filter. Fortunately, filter-type instruments can be adapted to the determination of sodium in cement by the use of a narrow-band-pass multilayer interference filter plus colored glass filters to cut out the side bands.

The following explanations are suggested as consistent with the data obtained on the various Perkin-Elmer instruments. The effective band widths obtained by direct measurement ranged from about 8 to 16 mμ, even though the slits themselves were identical in width. This could occur if there were slight differences between instruments in the bowing of the spectral lines, in their tilt with respect to the exit slit, in the placement of the prisms, or the focus of the entrance slit. The actual cause or causes have not been determined.

The observation that decreasing the vibration in a model 52A Perkin-Elmer instrument improved its performance considerably, while decreasing its measured effective band width only slightly, is explained as follows. The sodium line and the calcium oxide band coincide in a wave-length region of the wave length *vs.* emission curve for calcium where the emission is low, increasing with increasing wave length, and where the slope of the curve is continually increasing. In other words, the calcium curve in this region is concave upward.

Where there is no vibration of the spectrum and the exit slit with respect to each other, the wave-length interval passed by the slit will include the sodium light and some of the calcium light for some definite total of light. When the image and the slit do vibrate with respect to each other, the slit will pass a somewhat smaller amount of calcium light at the lower wave-length end of its travel across the spectrum since the calcium curve has a fairly low slope in that region.

At the higher wave-length end of its travel, it will pass a greatly increased amount of calcium light because of the fairly high slope of the curve. The net result of the vibration is an increase in the average amount of calcium light transmitted by the slit, and as far as the analytical results are concerned, vibration gives the same result as increased band width.

The effective band width as measured, however, is affected very little by moderate vibration. The picture can be simplified by assuming that the image of the sodium line at the exit slit is a rectangle of uniform light intensity and is being scanned by the rectangular slit opening of the same or a slightly narrower width. At the wave length where the slit is half filled with light, the average light transmitted will be the same whether the image and slit are in relative motion or at rest, unless the vibration caused relative motion equal to more than half the slit width. This is also true for most other settings, where the slit is partly filled with light.

At the wave-length maximum, at rest, the slit would be full of light. If there were enough vibration so that at parts of its travel the slit would be only partly filled with light, the average light passed would be lower and a slightly lower maximum emission and hence greater effective band width would be measured. This is apparently the case for the particular instrument investigated. The vibration decrease caused by tightening the motor mount was enough to decrease appreciably the ratio of calcium to sodium light but to decrease the effective band width only slightly.

The limited data obtained on the effect of the flame used indicate that the hotter the flame the greater the interference of silica and the narrower the slit required for acceptable results. The interference was greatest for oxyacetylene, less for oxyhydrogen, and least for air-propane.

CONCLUSIONS

The results obtained in this study are summarized in Table IX. From it, and from the data detailed elsewhere, the following conclusions can be drawn.

Silica inhibits the emission of calcium in the region of the sodium wave length, 589 mμ. This effect of silica introduces an error in the flame photometric determination of sodium in cement when silica, calcium, and sodium are present in the sample and only calcium and sodium are present in the standard. The magnitude of the error is a function of the ratio of light caused by calcium to that caused by sodium which is permitted to strike the measuring photocell. Therefore, the greater the effective band width of the monochromator device, the greater the error.

The error can be decreased by removing the silica from the cement prior to the determination of the sodium or by narrowing the effective band width of the instrument by adding to its optical system a multilayer interference filter. The use of both techniques together converts the current routine procedure into one which gives results substantially identical with those obtained by the gravimetric J. Lawrence Smith method. The use of the modified procedure for referee analyses therefore seems to be warranted.

When silica was eliminated from a cement sample before the flame photometric determination of sodium, excellent results were obtained with the three instruments tried: the Barclay with special filter, the Beckman DU with either oxyacetylene or oxyhydrogen flame, and the Perkin-Elmer 52 either with or without additional filter.

When silica was not eliminated from the cement before the flame photometric determination of sodium (as in the present specification method) excellent results were obtained using the

Beckman DU with oxyhydrogen flame and the Perkin-Elmer 52 when an auxiliary multilayer interference filter was added to its optical system. Results which were low, but perhaps acceptable for most routine purposes, were obtained with the Perkin-Elmer instrument without added filter, the Beckman DU with oxyacetylene flame, and the Barclay with special sodium filter incorporating a multilayer interference filter instead of its regular sodium filter.

A Perkin-Elmer Model 52A instrument of the motor-driven chopper type was improved in performance by adjusting it to decrease its vibration.

It seems reasonable to assume that filter instruments similar to the Barclay—namely, the Process and Instruments, the Janke developed by Fox (*11*), and the Baird developed by White (*16*)—could be used for cement analysis if a special sodium filter were provided and if the instruments were otherwise suitable. The Baird instrument is supplied with such a filter as optional equipment. The Process and Instruments apparatus may require modification of its stainless steel atomizer because of the corrosive effect of strong hydrochloric acid solutions.

ACKNOWLEDGMENT

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Rapid Photometric Determination of Cobalt in the Presence of Iron

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When cobalt is determined by use of nitroso R salt in the presence of iron, a depression of absorbance is observed. This phenomenon is successfully eliminated by the addition of suitable alkali fluorides to the sample, subsequent to the complete oxidation of ferrous iron with bromine. Several interferences are discussed and data are presented to substantiate and illustrate the techniques employed. Typical working curves are included along with complete analytical details. The precision of the method has been established throughout its range. The standard deviation varies from ±2% in samples containing less than 20 micrograms of cobalt to less than ±1% in samples containing more than 30 micrograms of cobalt. The procedure is suitable for the routine analysis of large numbers of both solid and liquid samples. It has been found suitable for the determination of cobalt in steel, and has been used at the U. S. Naval Radiological Defense Laboratory for a great number of other samples.

ALTHOUGH nitroso R salt has been used for many years in the detection and determination of cobalt (*7*), the exact nature of the reaction is not definitely known. This reagent forms colored complexes with many cations. The cobalt nitroso R salt complex is stable to nitric acid, while all other cation complexes are destroyed by it (*9*).

Iron, copper, cyanide, peroxides, persulfates, and reducing agents interfere in the determination of cobalt; small quantities of manganese, chromium, nickel, titanium, and vanadium are without effect (*4, 7, 9*). The exact mechanism of these interferences has not been reported. It has been observed that iron depresses the absorbance of the cobalt nitroso R salt complex ordinarily exhibited in its absence. Shcherbov claims that he can remedy this phenomenon by increasing the quantity of nitroso R salt (*6*). Low results were observed in the presence of ferrous iron originally in solution and with ferrous iron formed by the reduction of ferric iron by nitroso R salt.

Because iron is almost always associated with cobalt in nature and is the principal interfering element in its determination, nearly all photometric procedures employ methods for the removal of iron. The most common methods utilize zinc oxide or phosphoric acid (*1–3, 8, 9*). Both procedures are rather lengthy and involve so much handling that chances of mechanical and chemical losses are increased.

Overholser and Yoe (*5*), studying the use of *o*-nitrosoresorcinol as a reagent for the determination of cobalt, observed that, although citrates and tartrates did not completely eliminate the interference of ferric iron, potassium fluoride could be used for this purpose. Nevertheless, these investigators did not recommend the use of *o*-nitrosoresorcinol for cobalt in the presence of iron.

The method described here may be adapted to the determination of cobalt in steel, soils, minerals, and water after the samples have been dissolved by the appropriate methods.

Table I. Effect of Fluorides on Determination of Cobalt

Co, γ/25 Ml.	Average Absorbances × 10^2 [a]					
	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6
5	..	..	..	8.5	..	..
10	16	14.5	17.0	17.0	17.3	17.0
20	31.5	27.5	32.5	33.2	33.0	32.5
30	45.5	41.5	47.3	47.0	48.2	47.3
40	58.5	52.5	62.0	62.2	62.3	62.0
50	71.5	65.0	75.0	76.0	76.0	75.0
60	84.0	76.0	88.0	87.7	88.0	88.0
70	96.0	88.0	99.7	99.7	99.7	99.7

[a] Set 1. Cobalt only
Set 2. 200γ of Fe^{+++}
Set 3. 200γ of Fe^{+++} and KF
Set 4. 200γ of Fe^{+++} and NH_4F but no sodium acetate
Set 5. NH_4F but no Fe^{+++} or sodium acetate
Set 6. KF but no Fe^{++}

REAGENTS AND APPARATUS

Sodium acetate, 33%, c.p.
Potassium fluoride, 33%, c.p.
Concentrated hydrochloric acid, specific gravity 1.187, c.p.
Concentrated nitric acid, specific gravity 1.410, c.p.
Nitroso R salt, 0.5 gram per 100 ml.
Liquid bromine, c.p.
Photoelectric filter photometer using a 525-mμ filter or equivalent instrument.

EXPERIMENTAL METHOD

Triplicate solutions known to contain from 5 to 70γ of cobalt were treated with nitroso R salt, sodium acetate, and nitric acid and their absorbances were measured against a similarly treated blank (Table I).

Interferences. Duplicate solutions containing 40γ of cobalt and varied amounts of permanganate, ferric, and cupric ions were treated with nitroso R salt and ammonium fluoride. The results are shown in Table II.

Table II. Effect of Ferric, Cupric, and Permanganate Ion on Determination of 40γ per 25 Ml. of Cobalt in Presence of Ammonium Fluoride

Interfering Ion	Concentration, γ/25 Ml.	Average Absorbance × 10^2
Cu^{++}	0	62.5
	200	62.4
	400	62.3
	600	57.0
	800	49.5
MnO_4^-	0	62.4
	438	62.3
	876	61.8
	1314	48
	1758	8.8
Fe^{+++}	0	62.5
	200	62.0
	400	62.0
	600	62.4
	800	62.3
	1400	62.1
	1800	62.4
	3000	62.4

Preparation of Sample. Samples containing refractory oxide of cobalt are ground, mixed, weighed, and transferred to a crucible. Six to ten times the sample weight of potassium pyrosulfate are added, the mixture is heated gently until dry, and then the heat is gradually increased until no more sulfur trioxide is evolved. After the crucibles have cooled, the fused material is transferred to a 150-ml. beaker and dissolved in water (heat may be required). If the sample is liquid or acid-soluble, it need only be neutralized.

Procedure. Two or 3 drops of bromine are added to neutral samples and the solution is boiled until bromine fumes are no longer evolved. After addition of 2 ml. of 33% sodium acetate, potassium fluoride is added dropwise until the amber color of ferric acetate disappears; then 2 or 3 more drops of 33% potassium fluoride are added. One or 2 drops of concentrated hydrochloric acid and 1 ml. of nitroso R salt are added and the solution is heated to boiling. If the solution turns green, indicating the presence of ferrous iron, the bromination procedure is repeated and 1 ml. of nitroso R salt is added. One milliliter of concentrated nitric acid is added and any solids present are filtered or centrifuged. The sample is cooled and diluted to 25 ml., and its absorbance is measured using a 525-mμ filter against a blank prepared in the same manner.

Samples containing from 0 to 500γ of iron may be spiked with 200γ of iron and the quantity of cobalt determined from a reproducible working curve in which 200γ of iron are added to the standard cobalt solutions buffered with sodium acetate as shown in Table I and Figure 1.

DISCUSSION AND RESULTS

When present in concentrations greater than 150γ per 25 ml., uncomplexed iron interferes with the determination of cobalt in two ways: In large concentrations it increases the absorbance of the final solution, and in low concentrations where the first phenomenon does not occur, a depression of the absorbance due to the formation of ferrous-nitroso R salt takes place as shown in Figure 1.

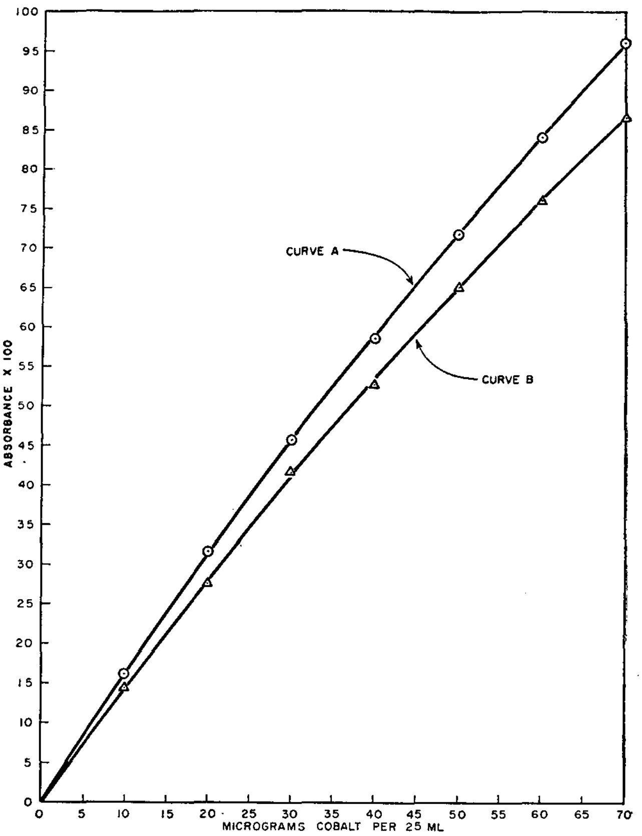

Figure 1. Effect of Uncomplexed Iron on Absorbance of Cobalt Nitroso R Salt

A. Cobalt nitroso R salt in absence of iron against reagent blank
B. Cobalt nitroso R salt formed in presence of 200γ of uncomplexed iron against reagent blank

Fluorides form stable colorless complexes with ferric iron in solution, which prevent the formation of the deep green ferrous-nitroso R salt complex, or precipitate the iron in the form M_3FeF_6 where M can be sodium, ammonium, or potassium. Ferrous iron does not form stable fluoride complexes and, therefore, must be completely oxidized before the addition of the complexing agent. (Bromine may be used for this purpose, if it is subsequently removed.)

Of the fluorides studied, ammonium fluoride is unsatisfactory because it etches glass badly and precipitates excess iron too slowly. Potassium fluoride, because of its high solubility in water is satisfactory as a complexing agent, as shown in Figure 2, for samples that do not require filtration of other solids. This result confirms the observation of Overholser and Yoe (*5*).

Curve *A* in Figure 2 is identical to working curves produced from cobalt in buffered solutions containing ammonium fluoride with and without iron and solutions of potassium fluoride without iron. This is also evident from Table I.

Excess potassium fluoride etches glass absorption cells; therefore, solid sodium fluoride may prove more satisfactory in solutions containing unknown quantities of iron. Consider

$$\underset{\text{solid}}{6\text{NaF}} \rightleftharpoons 6\text{Na}^+ + 6\text{F}^- + \text{Fe}^{+++} \longrightarrow \text{FeF}_6^{---}$$

As sodium fluoride is only slightly soluble in water, fluoride ion does not exist in sufficient concentration to cause etching. The quantity of sodium fluoride consumed is controlled by the quantity of ferric ion present.

It appears from Figure 2 that solutions treated with alkali fluorides follow Beer's law more closely than those which contain no fluorides. While ammonium fluoride effectively eliminates the interference of ferric iron, it has apparently less, if any, effect on the interferences of cupric or permanganate ion. Table II shows that copper interferes above 400γ and permanganate interferes above 900γ, while even 3000γ of iron does not interfere. Other experiments show that even 300 mg. of iron will not interfere with determination of cobalt in this procedure.

PRECISION OF METHOD

The method was tested throughout the range by two operators (Table III).

Table III. Precision of Method

No. of Detns.	Co Present, γ	Standard Deviations[a], %
3	5	No deviation
6	10	2.17
12	20	1.08
10	30	1.13
46	40	0.45
12	50	0.66
11	60	0.22
12	70	0.47

[a] Calculated by the following equation:

$$\text{Sigma} = \sqrt{\frac{(x - \bar{x})^2}{n}} = \sqrt{\frac{x^2 - (x)^2/n}{n}}$$

where x is a numerical result, $\bar{x}$ is the mean value of x, and n is the number of determinations.

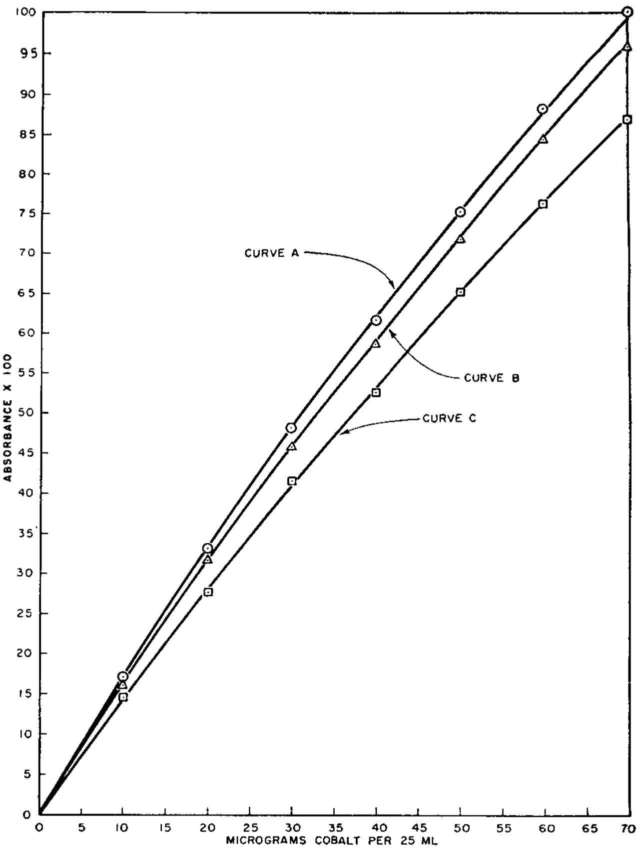

Figure 2. Effect of Fuorides on Absorbance of Cobalt Nitroso R Salt in Samples Containing Iron

A. **Cobalt nitroso R salt formed in presence of 200γ of iron complexed with potassium fluoride and sodium acetate and measured against reagent blank**

B. **Nitroso R salt in absence of iron and fluorides against reagent blank**

C. **Cobalt nitroso R salt in presence of iron against reagent blank**

SUMMARY

A rapid photometric procedure using nitroso R salt for the determination of microgram quantities of cobalt in the presence of macro quantities of iron has been established. Its precision ranges from a standard deviation of greater than ±2% with 10γ samples to less than ±1% for samples containing larger quantities of cobalt.

Alkali fluorides were effective as complexing agents for ferric iron and precautions are described to prevent etching of absorption cells. In the presence of fluorides, working curves conform very closely to Beer's law in both the presence and absence of iron. This method makes possible the rapid determination of cobalt in steels.

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Amperometric Titration and Voltammetric Determination of Iodide

With Rotated Platinum Wire Indicator Electrode

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The determination of 10^{-6} to $10^{-3}M$ iodide, by measurement of its anodic diffusion current at the rotated platinum wire electrode, was carried out at a potential of +0.65 volt *vs.* SCE in a supporting electrolyte of $1M$ sulfuric acid plus $0.01M$ hydrocyanic acid. Application of the voltammetric characteristics of the iodine-iodide system and various oxidizing and reducing agents led to the development of several amperometric titration methods for the determination of iodide in very dilute solutions. Iodide can be titrated with standard potassium iodate or permanganate in sulfuric acid solution to an iodide or an iodine cyanide end point at a potential of +0.65 volt *vs.* SCE. Titrations at a potential of +0.2 volt *vs.* SCE with ceric cerium in the presence of hydrocyanic acid and with permanganate in the presence of either acetone or hydrocyanic acid yield good results, but are limited in applicability because other halides interfere. Two precise methods, effective for extremely small concentrations of iodide, are based on the oxidation of iodide to iodate and the titration of iodate. Using the amperometric titration methods, a precision and accuracy of 0.2% were attained in the 10^{-5} to $10^{-3}M$ concentration range and of 1 to 5% at concentrations as low as 10^{-7} to $10^{-6}M$.

IT HAS been shown (*4*) that the anodic diffusion current of iodide at the rotated platinum wire electrode under certain conditions is proportional to concentration. From the knowledge of the voltammetric characteristics of the iodide-iodine system, combined with a study of the behavior of commonly used oxidizing and reducing agents, it is possible to predict suitable conditions for an accurate, rapid, and simple determination of iodide at high dilutions by amperometric titration, using the rotated platinum wire electrode as indicator electrode. Several oxidimetric and reductimetric reagents have been investigated with this purpose in view. Methods have been developed for the direct titration of iodide with iodate, cerium(IV), or permanganate at concentrations greater than $10^{-6}M$. The sensitivity range of the amperometric titration can be extended to trace concentrations of iodide of the order of 0.3 to $0.4 \mu M$ by conversion of the iodide to iodate and titration of the latter.

At the rotated platinum wire electrode the reaction $I_2 + 2e \rightleftharpoons 2I^-$ proceeds reversibly in acid solution (*4, 12*). A solution containing both iodine and iodide yields a cathodic and an anodic wave with well defined diffusion currents as illustrated in Figure 1. It is seen (Figure 1, curve I) that a solution of $5 \times 10^{-5}M$ iodine and $10^{-4}M$ iodide in $1M$ sulfuric acid gives a composite wave with a half-wave potential of +0.5 volt. (All potential values reported are referred to the saturated calomel electrode.) The cathodic diffusion current of iodine extends over a potential range between 0 and +0.35 volt *vs.* SCE.

Because of the marked volatility of iodine in aqueous solution in the absence of iodide (or much bromide or chloride), it is not practicable to determine iodine voltammetrically unless a complex former is present to decrease the volatility. With proper precautions against losses, the diffusion current of iodine in water (in the absence of complex formers) is proportional to concentration. In the presence of an excess of iodide practically all the iodine is converted into the triiodide ion, the diffusion current of which is equal to that of an equimolar concentration of free iodine (*4*).

The anodic diffusion current of iodide (oxidation to iodine) extends over the potential range between +0.60 and +0.75 volt *vs.* SCE. At still more positive potentials, an ill-defined second anodic wave is obtained corresponding to the irreversible electro-oxidation of iodide (or iodine) to I^+. Iodide can be determined at 25° C. by measurement of its anodic diffusion current in $0.1M$ perchloric acid at a potential of +0.70 volt in a range of concentrations between 10^{-5} and $10^{-3}M$, the precision being of the order of 2% in a $10^{-4}M$ solution (*4*).

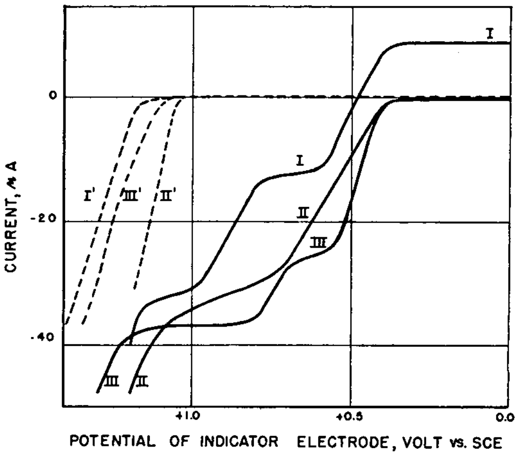

Figure 1. Current-Voltage Curves Obtained at Rotated Platinum Wire Electrode

- **I.** $5 \times 10^{-5}M$ **iodine +** $10^{-4}M$ **potassium iodide in** $1M$ **sulfuric acid**
- **II.** $2 \times 10^{-4}M$ **potassium iodide in** $0.1M$ **hydrochloric acid +** $1M$ **potassium chloride**
- **III.** $2 \times 10^{-4}M$ **potassium iodide in** $0.1M$ **hydrochloric acid**
- **I′, II′, III′. Residual current curves**

In the present work it was found that the voltammetric determination is more precise (1% in a $10^{-4}M$ solution) when carried out in $1M$ sulfuric acid (instead of $0.1M$ perchloric acid) as supporting electrolyte, the diffusion current being measured at a potential of +0.65 volt. Furthermore, the range of the method was extended to iodide concentrations as low as $10^{-6}M$ by using an indicator electrode of an area about 10 times larger than that of the platinum wire electrode previously described.

Bromide does not interfere, even if present in a concentration of $2M$. In the presence of chloride, high and erratic results are obtained. This is due to the fact that in the presence of much

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chloride (1*M* and up), iodide yields an anodic wave with a poorly defined diffusion current (*4*) corresponding to the electrode reaction $I^- + 2Cl^- \rightarrow ICl^-_2 + 2e$. An example of a wave of this type (in 1*M* potassium chloride plus 0.1*M* hydrochloric acid) is shown in Figure 1 (curve II) together with a current-voltage curve of $10^{-4}M$ iodide obtained in the presence of 0.1*M* hydrochloric acid only (curve III). The first of the two diffusion currents obtained in 0.1*M* hydrochloric acid with no additional chloride present ($2I^- \rightleftarrows I_2 + 2e$) is ill defined; the second ($I_2 + 2Cl^- \rightarrow 2ICl + 2e$) is well defined, but its magnitude varies considerably with changes in chloride concentration.

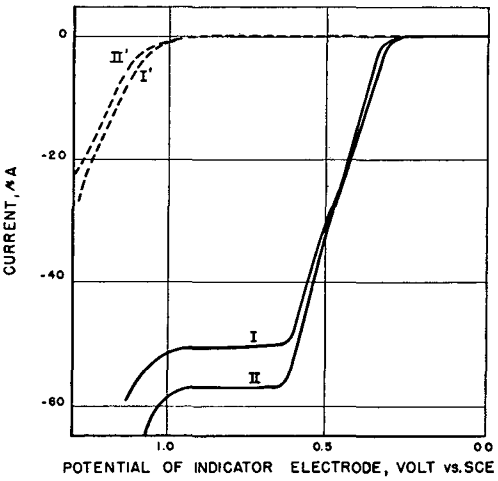

Figure 2. Current-Voltage Curves of 2 × $10^{-4}M$ Potassium Iodide Solutions at Rotated Platinum Wire Electrode

I. In *M* sulfuric acid + 0.01*M* potassium cyanide
II. In 1*M* sulfuric acid + 20% v./v. acetone
I', II'. Residual current curves

Iodide can be determined voltammetrically in the presence of chloride if hydrocyanic acid is added to the supporting electrolyte. A current-voltage curve of $10^{-4}M$ iodide in 1*M* sulfuric acid plus 0.01*M* hydrocyanic acid is shown in Figure 2 (curve I). A single anodic wave is obtained which corresponds to the two-electron transfer

$$I^- + HCN \rightleftarrows ICN + H^+ + 2e \quad (1)$$

and which is not affected by the presence of chloride.

Current-voltage curves of iodide in the presence of acetone have not been reported previously. It was found in this investigation that iodide yields a well defined anodic wave in 1*M* sulfuric acid plus 20% v./v. acetone. An example is plotted in Figure 2 (curve II). It is seen that the diffusion current in the presence of acetone extends over the same range of potentials as in the presence of hydrocyanic acid (Figure 2, curve I)—i.e., from +0.65 to +0.95 volt. The diffusion current in 20% acetone is proportional to iodide concentration in the range between 10^{-5} and $10^{-3}M$. The oxidation of iodide in the presence of 20% acetone and 1*M* sulfuric acid occurs quantitatively according to the over-all equation

$$I^- + CH_3COCH_3 \rightleftarrows CH_2ICOCH_3 + H^+ + 2e \quad (2)$$

The diffusion current in the presence of 20% acetone is about one sixth larger than that obtained in 0.01*M* hydrocyanic acid at the same iodide concentration. Acetone has a similar effect on the wave heights of permanganate and cerium(IV). This effect cannot be accounted for by changes in the viscosity of the solution. While an increase in diffusion current would normally correspond to a decrease in viscosity, it was found the viscosity at 25° C. of 1*M* sulfuric acid in water and in 20% acetone is equal to 1.08 and 1.38 centipoises, respectively. The waves in acetone and in hydrocyanic acid have a half-wave potential of about +0.45 to +0.50 volt and are not affected by the presence of even 2*M* bromide or chloride. Iodoacetone and iodine cyanide yield no cathodic waves at the rotated platinum electrode within the working range of potentials.

Iodate, permanganate, and cerium(IV) were considered as possible reagents for the direct oxidative titration of iodide to an amperometric end point. Iodate in 1*M* sulfuric acid (also in the presence of 0.01*M* hydrocyanic acid or of 20% acetone) yields no reduction current at the rotated platinum wire electrode at potentials more positive than +0.3 volt.

Current-voltage curves of $10^{-4}M$ cerium(IV) (one-electron reduction) and of $10^{-4}M$ permanganate (five-electron reduction) in 1*M* sulfuric acid (not reported previously in the literature) are shown in Figure 3. Both solutions yield cathodic waves with diffusion currents extending over a potential range between +0.5 and 0.0 volt. The cathodic diffusion currents, measured at a potential of +0.2 volt, are proportional to the concentration of cerium(IV) and permanganate, respectively, between $2 \times 10^{-6}M$ and $10^{-3}M$. The half-wave potential is +0.85 volt for the cerium wave and +0.82 volt for permanganate. The presence of 0.01*M* hydrocyanic acid does not affect the waves.

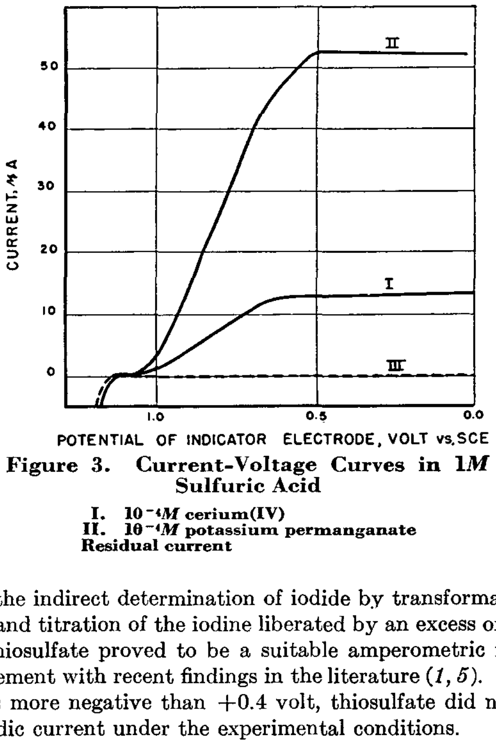

Figure 3. Current-Voltage Curves in 1*M* Sulfuric Acid

I. $10^{-4}M$ cerium(IV)
II. $10^{-4}M$ potassium permanganate
Residual current

For the indirect determination of iodide by transformation to iodate and titration of the iodine liberated by an excess of iodide (*11*), thiosulfate proved to be a suitable amperometric reagent in agreement with recent findings in the literature (*1*, *5*). At potentials more negative than +0.4 volt, thiosulfate did not give an anodic current under the experimental conditions.

OUTLINE OF METHODS

The iodide-iodine cyanide diffusion current is ideally suited for the voltammetric determination of iodide at the rotated platinum wire electrode. It is recommended that the current be measured at a potential of +0.65 volt at 25° C. and that an aqueous solution of 1*M* sulfuric acid plus 0.01*M* potassium cyanide be used as supporting electrolyte.

To select suitable conditions for the amperometric titration of iodide, application was made of the classical stoichiometric oxidation reactions with iodate, cerium(IV), and permanganate. The direct oxidation of iodide must be carried out in acid solution and involves one or two equivalents. In the presence of acetone or cyanide, iodide is oxidized to the unipositive state (*7–10*). In the absence of acetone or cyanide, cerium(IV), permanganate, or iodate in acid solution oxidize iodide to elementary iodine. However, the amperometric titration of iodide in dilute solutions to an iodine end point could not be carried out satisfactorily under the

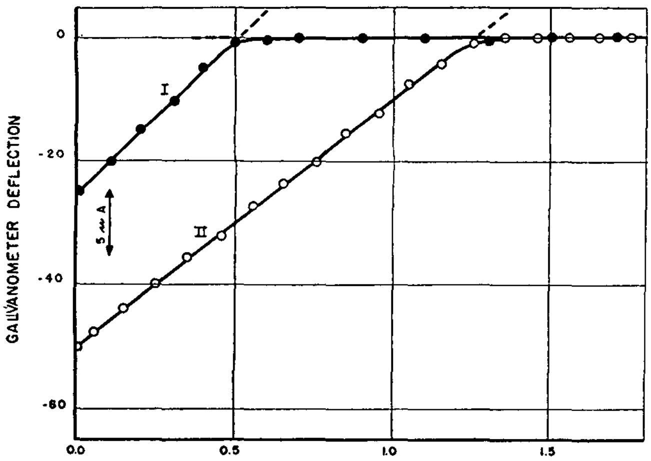

Figure 4. Amperometric Titration Curves of 50 Ml. of $10^{-4}M$ Potassium Iodide Solution with $2 \times 10^{-3}M$ Potassium Iodate at +0.65 Volt *vs.* SCE

- I. In 1*M* sulfuric acid
- II. In 1*M* sulfuric acid + 0.01*M* potassium cyanide

experimental conditions with cerium(IV) or permanganate because the reaction rates were too slow. Good results were obtained using standard potassium iodate as titrant and 1*M* sulfuric acid as supporting electrolyte.

The reaction proceeds according to the equation

$$5I^- + IO_3^- + 6H^+ \rightarrow 3I_2 + 3H_2O \qquad (3)$$

The titration was carried out at a potential of +0.65 volt corresponding to the iodide-iodine diffusion current. A titration curve of this type is shown in Figure 4 (curve I). If bromide is also present, it reacts with the excess iodate to form elementary bromine which yields a cathodic current at the working potential. In the presence of chloride, the titration line of iodide in 1*M* sulfuric acid is distorted, because of the effect of chloride on the current-voltage curve of iodide (Figure 1, curves II and III). These interferences can be eliminated by making the supporting electrolyte 0.01*M* in hydrocyanic acid. In a medium of 1*M* sulfuric acid plus 0.01*M* potassium cyanide, the oxidation of iodide proceeds to the unipositive state

$$2I^- + IO_3^- + 3HCN + 3H^+ \rightleftharpoons 3ICN + 3H_2O \qquad (4)$$

A titration curve of iodide with iodate in the presence of hydrocyanic acid is plotted in Figure 4 (curve II). In this case, bromide (in concentrations as high as 2*M*) does not interfere because it is oxidized by excess iodate to bromine cyanide which does not yield a cathodic current under these experimental conditions. Chloride has no effect on the titration line, because iodine cyanide is considerably more stable than iodine chloride. Under the same conditions (in 1*M* sulfuric acid plus 0.01*M* potassium cyanide and at a potential of 0.65 volt) permanganate and cerium (IV) can also be used as titrants, even though their diffusion currents are not attained at the working potential of the indicator electrode. During the titration the anodic diffusion current of iodide decreases as in Figure 4, curve II. At the end point the current is 0. After the end point a cathodic reagent current is obtained which, however, is not proportional to the concentration of the excess titrate. The titration curve with permanganate becomes entirely similar to curve II in Figure 4 when the medium is 0.01*M* in hydrocyanic acid, 0.5*M* in potassium bromide, and 1*M* in sulfuric acid. Excess permanganate is rapidly reduced by the bromide, with bromine cyanide being formed. Cerium(IV) is not a satisfactory reagent in this titration because it reacts too slowly with bromide.

In the absence of bromide, at chloride concentrations below 0.1*M* and in the presence of 0.01*M* hydrocyanic acid, iodide can be titrated with either permanganate or with cerium(IV) at a potential of +0.2 volt, where the diffusion current of excess reagent is measured and where iodide does not yield an oxidation current. Illustrations of the corresponding titration curves are given in Figure 5 (curves I and II). However, since bromide and much chloride interfere, the titration at +0.65 volt with permanganate in the presence of bromide is much to be preferred because of its general applicability.

Iodide in 1*M* sulfuric acid can also be titrated with permanganate in the presence of 20% v./v. acetone at a potential of +0.2 volt (Figure 5, curve III); this procedure, too, fails in the presence of bromide or more than 0.1*M* chloride. The use of cerium(IV) as reagent instead of permanganate is not practicable on account of a relatively slow reaction rate.

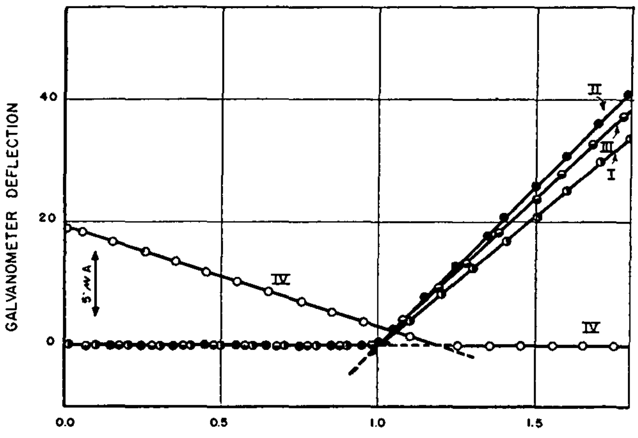

Figure 5. Amperometric Titration Curves at a Potential of +0.2 Volt *vs.* SCE

- I. 50 ml. of $10^{-4}M$ potassium iodide in 1*M* sulfuric acid + 0.01*M* potassium cyanide titrated with $2 \times 10^{-3}M$ potassium permanganate
- II. 50 ml. of $10^{-4}M$ potassium iodide in 1*M* sulfuric acid + 0.01*M* potassium cyanide titrated with $10^{-2}M$ cerium(IV)
- III. 50 ml. of $10^{-4}M$ potassium iodide in 1*M* sulfuric acid + 20% v./v. acetone titrated with $2 \times 10^{-3}M$ potassium permanganate
- IV. 50 ml. of $2 \times 10^{-5}M$ potassium iodate in 0.02*M* sulfuric acid + excess potassium iodide titrated with $5 \times 10^{-3}M$ sodium thiosulfate

Reaction 3 was used in an indirect determination of iodide. The iodide was converted into iodate using an excess of chlorine water as oxidant. Subsequently, the iodate was titrated amperometrically by one of the following methods:

Titration with standard 'potassium iodide solution in 1*M* sulfuric acid at a potential of +0.65 volt. The corresponding titration curve is the converse of the one obtained in the titration of iodide by standard iodate in the same medium (Figure 4, curve I). The end point is determined by the excess reagent line of iodide—i.e., by the iodide-iodine anodic diffusion current.

Reduction of iodate to iodine by an excess of potassium iodide. The liberated iodine is titrated with standard thiosulfate solution at a potential of +0.2 volt corresponding to the iodine-iodide cathodic diffusion current. An example of a titration curve of this kind is plotted in Figure 5 (curve IV).

EXPERIMENTAL

Materials. Reagent grade chemicals were used throughout. Solvents (conductivity water and acetone) were redistilled immediately before use. Cerium(IV) solutions were prepared by standard methods from ceric ammonium sulfate obtained from the G. Frederick Smith Chemical Co.

Apparatus and Technique. A simple electrolysis cell, made of a 100-ml. borosilicate glass beaker (*3*) and a Hume-Harris saturated calomel reference electrode (*2*) were used in all the experiments. Current-voltage curves were recorded with a Sargent Model XXI polarograph in a thermostated water bath at 25°

Table I. Concentration Range, Precision, and Accuracy of Iodide Determinations

Medium	Method	Approximate Sensitivity Limit: C_{I^-}, Moles per Liter, A[b]	Approximate Sensitivity Limit: C_{I^-}, Moles per Liter, B[c]	Approximate Sensitivity Limit: Iodide, γ per 10 Ml. of Sample, A[b]	Approximate Sensitivity Limit: Iodide, γ per 10 Ml. of Sample, B	Precision[a], %, A[b]	Precision[a], %, B[c]	Accuracy[a], %, A[b]	Accuracy[a], %, B[c]	Maximum Tolerance: To chloride	Maximum Tolerance: To bromide
1M H_2SO_4, 0.01M HCN	Measurement of i_d at +0.65 volt	10^{-5}[d]	10^{-6}[e]	13	1–2	1	5	1	5	∞	∞
1M H_2SO_4	Amperometric titration with iodate at +0.65 volt	2×10^{-5}	2×10^{-6}	25	2–3	0.4	2	0.5	3	$5 \times 10^{-4}M$	None
1M H_2SO_4, 0.01M HCN	Amperometric titration with iodate at +0.65 volt	10^{-5}	10^{-6}	13	1–2	0.2	1	0.2	1	∞	∞
1M H_2SO_4, 0.01M HCN	Amperometric titration with Ce(IV) at +0.2 volt	10^{-5}	10^{-6}	13	1–2	0.3	3	0.3	3	0.1M	$10 \times [I^-$
1M H_2SO_4, 20% acetone	Amperometric titration with permanganate at +0.2 volt	10^{-5}	10^{-6}	13	1–2	0.2	1	0.1	1	0.1M	None
1M H_2SO_4, 0.01M HCN	Amperometric titration with permanganate at +0.2 volt	10^{-5}	10^{-6}	13	1–2	0.2	1	0.2	1	0.1M	None
1M H_2SO_4, 0.01M HCN, 0.5M KBr	Amperometric titration with permanganate at +0.65 volt	10^{-5}	10^{-6}	13	1–2	0.1	2	0.2	1	∞	∞
1M H_2SO_4	Oxidation to iodate and amperometric titration of IO_3^- with iodide at +0.65 volt	4×10^{-6}	4×10^{-7}	5	0.5	0.4	4	1	2	$5 \times 10^{-4}M$	None
1M H_2SO_4, 0.1M KI	Oxidation to iodate, addition of excess iodide, and titration of liberated iodine with thiosulfate at +0.2 volt	3×10^{-6}	3×10^{-7}	4	0.4	0.2	4	0.2	4	∞	∞

[a] In $5 \times 10^{-5}M$ solution for electrode A and in 5×10^{-6} solution for electrode B.
[b] Small electrode
[c] Large electrode.
[d] Upper limit of concentration range is $10^{-3}M$.
[e] Upper limit of concentration range is $10^{-4}M$.

± 0.02° C. Diffusion currents were measured at the same temperature, using a manual setup described previously (*6*).

Amperometric titrations were carried out at room temperature with a simple circuit. Potentials were applied using an uncalibrated slide-wire potentiometer which was adjusted to the desired value by compensating against a Leeds and Northrup type K1 potentiometer at the electrodes. The potentiometer can be eliminated by short-circuiting the electrolysis cell with a half cell of suitable potential.

The current was measured with a General Electric portable-type mirror and scale galvanometer which had a maximum sensitivity of about $10^{-3}\mu$a per mm. The working sensitivity of the current measuring instrument was adjusted with an Ayrton shunt and calibrated by measuring the potential drop across a standard resistance connected in series. Whenever current readings were taken, the cell circuit was closed for a brief period of time with a tap key. Between readings the circuit was left open to avoid depletion of the solution by electrolysis. It has been shown (*3*) that significant errors may be caused by depletion upon continued electrolysis, especially when large electrodes and small volumes of solution are used.

Two indicator electrodes made of platinum wire about 0.5 mm. in diameter but of different area were used. They were approximately 4 and 35 mm. in length and yielded diffusion currents of 251 ± 1 and 2232 ± 20μa. per millimole per liter, respectively, for the thallous-thallic oxidation in 0.1M sodium hydroxide. The electrodes were sealed into glass, and electrical contact was made with the aid of mercury. The platinum-glass joints were carefully annealed, because irregularities at the metal-glass interface give rise to abnormally large residual currents.

The electrodes were stored in 10M nitric acid when not in use. They were rinsed with water shortly before the start of an experiment and then immersed for 10 minutes in the experimental solution before a potential was applied. On applying an initial potential 2 to 3 minutes elapsed before a steady current could be measured; thereafter the electrode responded instantaneously to changes in applied potential (when measuring current-voltage curves) and concentrations (in amperometric titrations). A synchronous motor was used to rotate electrodes at 600 r.p.m. The electrode wire was bent eccentrically to provide sufficiently constant stirring for rapid mixing during titrations.

Residual currents appeared to be negligible in the working range of potentials. Standard solutions of titration reagents in the 10^{-3} and $10^{-4}N$ concentration range were prepared shortly before use by dilution of $10^{-2}N$ solutions. The 0.01N solutions were standardized daily by classical procedures. The cerium(IV) solutions were made up in 1M sulfuric acid. Standard potassium iodide solutions were made oxygen-free by deaeration with purified Linde nitrogen and were stored under nitrogen

Procedure. In each titration, 25 or 50 ml. of sample was used. Normalities of reagents used for titration were about 50 times those of the samples and were dispensed from 5-ml. precision microburets. For direct titrations with permanganate and cerium(IV) an aliquot of the sample was made 1M in sulfuric acid and 0.01M in potassium cyanide (or 20% v./v. in acetone) and titrated immediately.

For the determinations involving the oxidation of iodide to iodate, the sample solution was first neutralized with sulfuric acid to a methyl orange end point and then made 0.02M in sulfuric acid. Chlorine water was added until a permanent yellow color was obtained. The solution was boiled for 3 minutes, cooled, and deaerated for 30 minutes with nitrogen to remove excess chlorine. Subsequently, an aliquot was either acidified to 1M in sulfuric acid and titrated with standard potassium iodide, or excess potassium iodide was added (to make the solution about 0.1M in iodide) and the liberated iodine titrated with standard thiosulfate.

When using the smaller electrode, the reagent blanks proved to be negligibly small. In titrations with the large electrode, blanks were equal to about 5% of the reagent required for the sample of the smallest concentrations determined. These blanks were applied as a correction to the experimental results.

RESULTS AND DISCUSSION

The methods were tested over a wide range of iodide concentrations from $10^{-3}M$ down to extreme dilutions where the sensitivity of the current measuring instruments became the limiting factor. The lower limit to the applicability of the various amperometric titration procedures was set at concentrations at which the maximum current reading became less than a full scale deflection of the galvanometer at maximum sensitivity (about 2μa). The large electrode carried the range of all methods to iodide concentrations smaller by one order of magnitude as compared to the small electrode.

All results presented in the figures were obtained using the small indicator electrode. Concentration ranges, precision, and accuracy as determined with both electrodes are summarized in Table I. Interferences tested for were bromide and chloride. The limit of tolerance towards these halides is also given in the table.

The voltammetric determination of iodide by the measurement of its diffusion current in the presence of cyanide can be carried out without interference from bromide and chloride. The method is limited to iodide concentrations between 10^{-6} and $10^{-3}M$ and its precision and accuracy are relatively poor. The titration methods give considerably better accuracy and precision. From the viewpoint of applicability in the presence of bromide and chloride, the titration at +0.65 volt in a supporting electrolyte of 0.01M hydrocyanic acid plus 1M sulfuric acid with iodate as reagent is recommended. Permanganate can be used as well when the titration medium is made also 0.5M in bromide. The determination of iodide after oxidation to iodate and titration of the liberated iodine with thiosulfate has the additional advantage that for a given amount of iodide the required amount of reagent equivalents is three times as large as in previous methods.

Although the titration of iodide in concentrations greater than $10^{-3}M$ has not been discussed herein, the methods described can be used with necessary changes for larger concentrations.

ACKNOWLEDGMENT

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Titration of Weak Bases in Acetic Anhydride Solvent Mixtures

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The purpose of this investigation was to study the effect of titrating bases in organic solvents from which the last traces of water have been removed by the addition of excess acetic anhydride. For most tertiary amines and alkali metal salts, this increases considerably the sharpness of the break and rise in potential at the end point. A very large excess of acetic anhydride does not further increase the sharpness of the end point. The method proposed gives excellent results for tertiary amines including nitrogen heterocyclics of the purine, pyridine, pyridone, and thiazole type. Primary, secondary, and a few heterocyclic amines cannot be titrated. This method offers increased accuracy and broadens the scope of titrations in nonaqueous solvents to include weaker bases than formerly could be titrated.

IT HAS been recognized (*3–5*) that the presence of water decreases the sharpness with which bases can be titrated in acetic acid and other nonaqueous solvents. Because of this, the water in perchloric acid from which the titrant is prepared is often removed by addition of a calculated amount of acetic anhydride. Some water still remains, however, since reagent grade glacial acetic acid commonly contains 0.3 to 0.5% water.

The purpose of the present investigation was to study the titration of weak bases in nonaqueous solvents completely free from water. These conditions are practically obtained by titrating in solvents such as acetic acid or nitromethane containing 5 to 20% acetic anhydride. Titrations of tertiary amines in acetic acid containing acetic anhydride have been carried out (*1, 7*), but no data are available concerning the effect of the acetic anhydride on the titration curve.

REAGENTS AND SOLUTIONS

Acetic acid, ACS grade glacial.

Acetic anhydride, ACS grade.

Nitrobenzene, Matheson.

Nitromethane, Eastman or Matheson.

1,5-Diphenyl-3-pentadieneone (dibenzalacetone). A 0.1% solution in acetic acid.

1-Naphtholbenzein. A 0.1% solution in acetic acid.

Neutral red, 0.1% solution in acetic acid.

Perchloric acid. A 0.1*N* solution is prepared by mixing 8.5 ml. of 70 to 72% perchloric acid with enough acetic anhydride to remove all of the water. Let this mixture stand overnight, then dilute to 1 liter with acetic acid. Standardize against potassium acid phthalate.

Triphenylmethanol (triphenylcarbinol). 0.1% solution in nitromethane.

Samples were analyzed as received. Most were 98 to 100% pure.

PROCEDURE

Dissolve a 0.3- to 0.8-meq. sample in 20 ml. of 4 to 1 nitromethane–acetic anhydride. Substances not readily soluble in nitromethane at room temperature are dissolved in a small amount of acetic acid, heating if necessary to effect solution. Titrate from a 10-ml. buret using a pH meter or other direct-reading titrator equipped with glass and fiber-type calomel electrodes to determine the end point. For visual titrations, use 2 drops of a suitable indicator. For macrotitrations using a 50-ml. buret, multiply the quantities of sample and solvent used by 5.

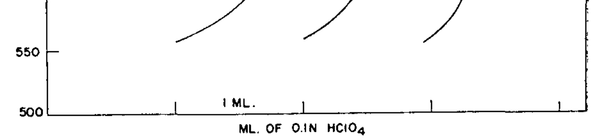

Figure 1. Titration of Lithium Nitrate

A. In acetic acid–acetic anhydride
B. In acetic acid
C. In acetic acid with titrant in *p*-dioxane

A blank is run to correct for the small amount of acid required to bring the solvent mixture to the equivalence point potential or to the corresponding indicator color. This usually amounts to 0.01 to 0.04 ml. and is subtracted from the final buret reading.

EFFECT OF ACETIC ANHYDRIDE

Several weak bases were dissolved in acetic acid, acetic acid–acetic anhydride, nitromethane, and nitromethane–acetic anhydride. Each solution was then titrated with perchloric acid in glacial acetic acid. Figures 1 and 2 show that the rise in potential at the end point is considerably greater for the titrations where acetic anhydride is present. Acetic anhydride mixed with the solvent also causes a sharper end point, as shown by the plot in Figure 3.

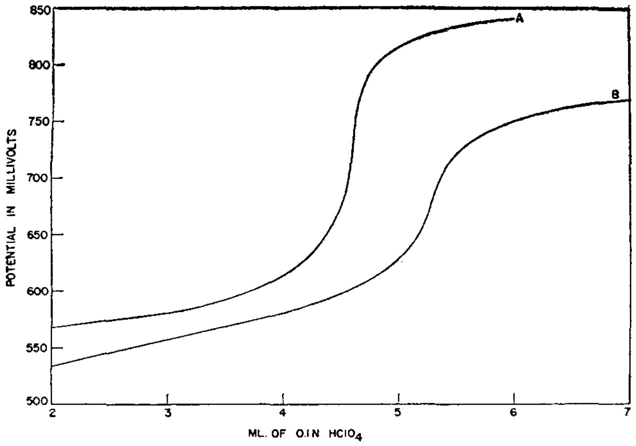

Figure 2. Titration of Caffeine

A. **In nitromethane–acetic anhydride**
B. **In nitromethane**

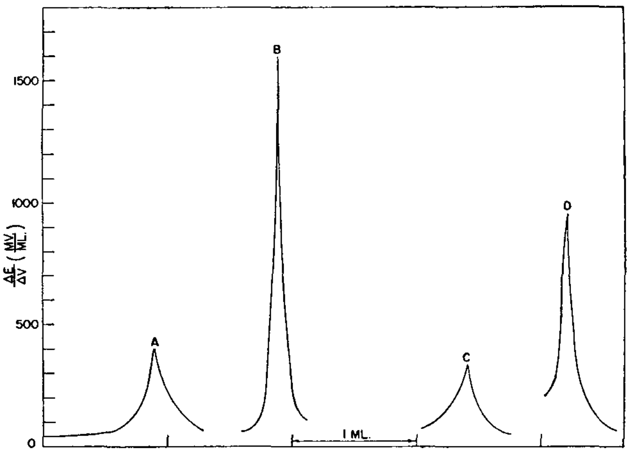

Figure 3. Differential Plots

A. **Lithium nitrate in acetic acid**
B. **Lithium nitrate in acetic acid–acetic anhydride**
C. **Caffeine in nitromethane**
D. **Caffeine in nitromethane–acetic anhydride**

It is believed that the complete removal of water by acetic anhydride causes the considerable improvement in end points noted above. When water is present, at least some of the H_2Ac^+-ClO_4^- is probably converted to $H_3O^+ClO_4^-$, which is a considerably weaker acid. The data plotted in Figure 4 give further experimental support to this argument. Here it will be noted that the addition of a small amount of water to a water-free solution of perchloric acid causes a considerable drop in potential. Also, in the titration of caffeine almost identical curves were obtained in nitromethane containing 5, 20, and 50% (volume) acetic anhydride. This infers that 5% acetic anhydride removes all of the water, and more acetic anhydride therefore causes no further improvement in the end point.

SCOPE

As excess acetic anhydride is present during the titration, it is evident that this method cannot be used for primary and secondary amines. (The latter, however, do not interfere in the titration of other bases if they are completely acetylated by heating with acetic anhydride beforehand.) It is a valuable method for tertiary amines and for the alkali metal or tertiary amine salts of most acids. Included with tertiary amines are numerous important nitrogen heterocyclics of the purine, pyridine, pyridone, thiazole, and other types. Figures 5 and 6 show titration curves for several typical bases. Table I gives quantitative data.

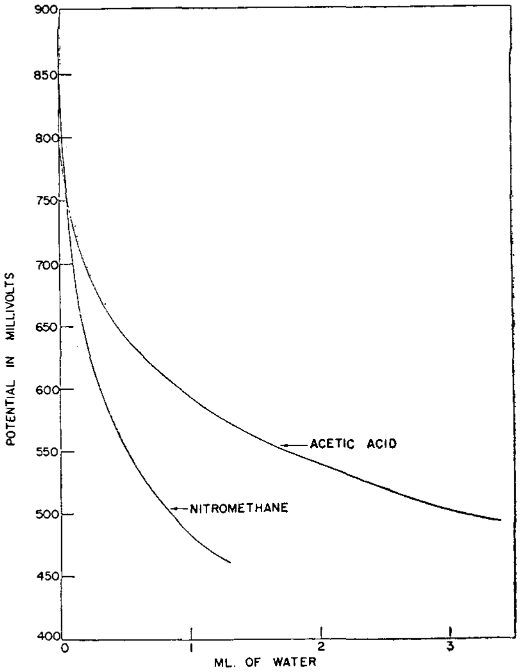

Figure 4. Addition of Water to Perchloric Acid Solutions

An interesting compound which can be quantitatively titrated is triphenylmethanol (Figure 5). This is perhaps the first example reported of a quantitative titration of an alcohol as a base.

Although many nitrogen heterocyclic compounds can be titrated, acetic anhydride appears to enter into disturbing side

Table I. Potentiometric Titrations in 4 to 1 Nitromethane–Acetic Anhydride

Compound	Purity Found, %
Benzothiazole	97.8 97.7
Caffeine	99.8 99.6
5,7-Dichloro-8-quinolinol	95.8 96.2
1-Methyl-2-pyridone	95.1 96.1
Nicotinamide	99.8 99.6
8-Nitroquinoline.	95.1 94.5 94.5
Quinoxaline	97.8 97.5
Theobromine	99.3 100.0 100.0
Theophylline	99.2 99.2
Triphenylmethanol	100.5 100.2 100.0

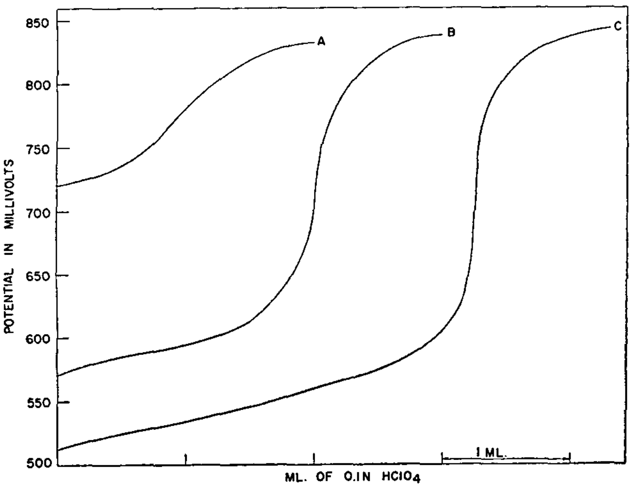

Figure 5. Titrations in 80% Nitromethane–20% Acetic Anhydride

A. Triphenylmethanol. *B.* Quinoxaline. *C.* Benzothiazole

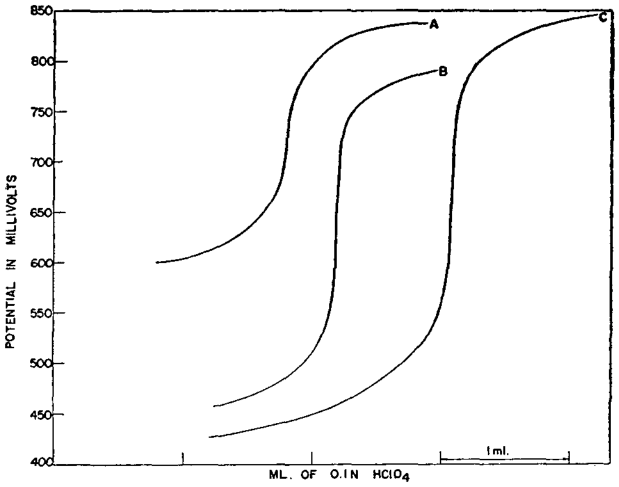

Figure 6. Titrations in Various Solvents

A. 1-Methyl-2-pyridone in nitromethane–acetic anhydride
B. Sodium acetate in acetic acid–acetic anhydride
C. 8-Nitroquinoline in nitromethane–acetic anhydride

Table II. Titrations in 4 to 1 Nitromethane–Acetic Anhydride Using Visual Indicators

Compound	Indicator	Purity, %	
		Indicator	Potentiometric
Benzothiazole	Triphenylmethanol	97.1	97.7
Caffeine	Neutral red	99.4	99.7
1-Methyl-2-pyridone	Neutral red	95.2	95.6
Nicotinamide	Triphenylmethanol	99.6	99.7
Quinoxaline	Triphenylmethanol	97.8	97.7

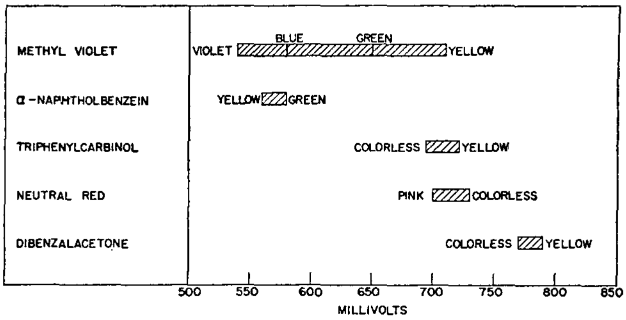

Figure 7. Approximate Transition Ranges of Indicators in 80% Nitromethane–20% Acetic Anhydride

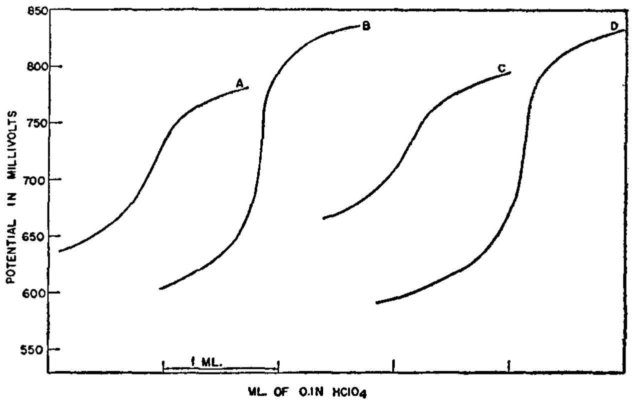

Figure 8. Titration Curves for 1-Methyl-2-Pyridone and Caffeine

A. 1-Methyl-2-pyridone in acetic acid–acetic anhydride
B. 1-Methyl-2-pyridone nitromethane–acetic anhydride
C. Caffeine in acetic acid–acetic anhydride
D. Caffeine in nitromethane–acetic anhydride

reactions with some. Quantitative results with benzotriazole, 3-methoxypyrazole, phenazine, pyrimidine, or pyrrole could not be obtained.

INDICATORS

Although potentiometric titrations are usually better for colored compounds and for very weak bases, use of visual indicators is often very convenient. Methyl violet, 1-naphtholbenzein, neutral red, triphenylmethanol and dibenzalacetone are all useful indicators for titration of bases in nitromethane–acetic anhydride. A potentiometric curve should first be carried out to establish the equivalence potential and thus choose the proper indicator.

Figure 7 shows approximate transition potentials for these indicators. These data were obtained by titrating magnesium acetate with $0.1N$ perchloric acid; also by titrating various weak organic bases with $0.1N$ perchloric acid. For titrations carried out using different ionic strengths than those reported here, the transition point of the indicator may be appreciably different (*2, 6*). Data for visual titrations of several amines are given in Table II.

DISCUSSION

Nitromethane was widely used in the present work for several reasons. It is readily available and is practically water-free. Its high dielectric constant makes it possible to obtain steady potentials using a fiber-type calomel electrode.

Use of nitromethane–acetic anhydride often gives sharper end points than are obtainable with acetic acid–acetic anhydride. Figure 8 illustrates this effect for caffeine and 1-methyl-2-pyridone. Salts such as lithium nitrate and sodium acetate gave just as good results in acetic acid–acetic anhydride.

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Direct Extraction-Pycnometer Method for Oil Content of Refinery Effluents

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Existing methods for determining the oil content of waste water are not entirely satisfactory. If rapid. they are inaccurate; if accurate, they are time-consuming. This report describes a rapid and accurate method, which is especially applicable to waste waters having appreciable quantities of volatile material. The oil is extracted with carbon tetrachloride. The extract, after filtration and separation from entrained water, is run into a two-armed pycnometer and weighed. The oil solution will weigh less than an equal volume of pure solvent. From this difference in weight and an assumed value for the density of the oil (based on prior measurements) the oil content of the sample is computed. A nontechnical man using two extraction units can do two determinations in 1.5 hours With all types of samples, including a gasoline with initial boiling point of 100° F., the average deviation from known values is less than 6%.

THE disposal of waste from manufacturing and refining processes into waterways always has been a serious problem to industry and adjacent communities. Many industries have converted their wastes into marketable by-products. Despite the great progress made in this direction, the problem is of ever-increasing interest to communities using water for recreation, transportation, and human consumption.

In the petroleum industry, refineries have set up elaborate installations to minimize the amount of waste products getting into neighboring streams. The efficiency of such installations is checked at regular intervals by analyzing the waste water leaving the refineries and the water in adjacent streams. An important phase of this program is to determine the oily material content in these waters. For this purpose, numerous methods have been suggested. These fall into four general categories:

Methods in which the oil is adsorbed on a fresh precipitate, usually ferric hydroxide, extracted from the precipitate with a volatile solvent, and weighed after evaporation of the solvent (*2, 6, 9, 10, 12*).

Methods in which the oil is extracted from the water with a volatile solvent and weighed after evaporating the solvent (*3, 5, 12*).

Methods in which volatile constituents are separated by boiling the water sample, after which the nonvolatile oil is extracted from the remaining sample with a volatile solvent. The oil is weighed after evaporation of the solvent (*1, 2, 8*).

Methods in which the oil is separated from the water by solvent extraction or gas stripping. The solution or gas stream is then analyzed with an instrument such as an infrared spectrophotometer (*11*) or a mass spectrometer (*7*).

Of the methods reported, the American Petroleum Institute has recommended two procedures in its latest publication (*2*). These are the flocculation–extraction U-tube method and reflux distillation–extraction method.

The U-tube method uses ferric hydroxide to collect the oil and diethyl ether to extract it from the precipitate. The procedure is rapid, but if volatile material is present, it gives low results.

In the reflux distillation–extraction method a sample is boiled to remove volatile material and the remainder is extracted with benzene. The solvent is evaporated—the last traces in a special flask—and the residue weighed. The method is more accurate than the U-tube method and determines both the volatile and nonvolatile oil present. However, the procedure requires about 2 hours for a determination and the use of bulky equipment such as 3-liter distillation flasks, and 4-liter separatory funnels. Apparatus of this size requires considerable bench space and is awkward to handle when it has to be picked up and shaken. A detailed and extensive discussion of the nonvolatile oil phase of this procedure can be found in a recent article by Musante (*8*).

Thus, although many methods have been recommended for this analysis, no method is available that is rapid, accurate (whether the oil has little or a high percentage of volatile constituents), sensitive to about 1 p.p.m., and simple enough to be run by nontechnical personnel using inexpensive equipment.

The purpose of this investigation was to develop a procedure that has these characteristics to a greater degree than other methods. However, this method is not sensitive to 1 p.p.m. of oil but only to 10 p.p.m.

THEORY

This method is based on the fact that carbon tetrachloride containing a small quantity of oil will weigh less than an equal volume of pure carbon tetrachloride. The difference in weight is the difference between the weight of the oil dissolved in the carbon tetrachloride and the weight of a volume of solvent equal to that of the oil.

If x represents the grams of oil in a sample and d the assumed density of the oil, then x/d is equal to the volume of oil and to the volume of carbon tetrachloride displaced by the dissolved oil. Since the density of the water-saturated carbon tetrachloride is 1.59, the weight of the carbon tetrachloride displaced by the oil is 1.59 x/d. The net loss in weight represents the difference between the weight of carbon tetrachloride displaced and the weight of the oil. Hence,

$$1.59\,x/d - x = W_2 - W_1$$

where

W_1 = weight in grams of solution of oil in solvent
W_2 = weight in grams of an equal volume of solvent

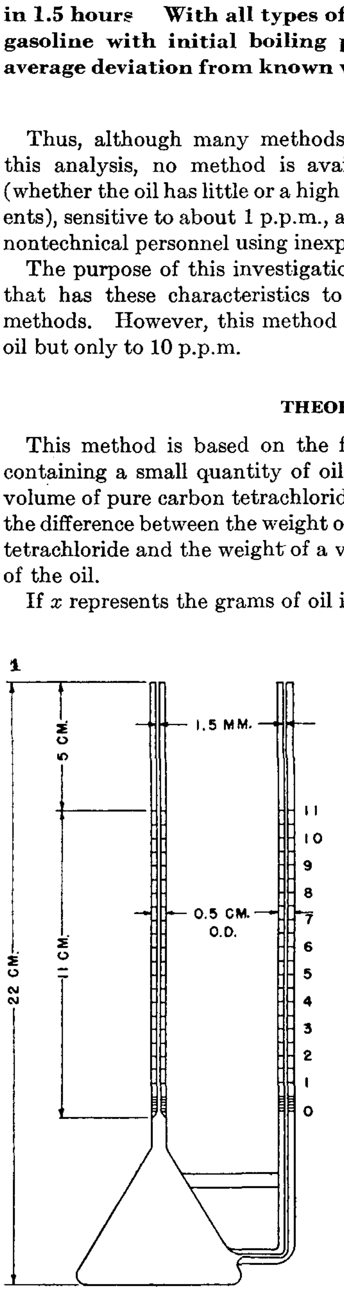

Figure 1. Pycnometer for Determination of Oil in Refinery Effluents

This equation involves two assumptions: (*a*) formation of an ideal solution when the oil dissolves in the carbon tetrachloride, and (*b*) a value for the density of the oil. While the latter assumption is a disadvantage of the method, the same assumption is made in calculating volatile oil contents in the API distillation-extraction method (*1*) and the API reflux distillation–extraction procedure (*2*). In these methods a value of 0.88 is assumed. In this procedure the value used is 0.87, the density of oil taken from an oil separator.

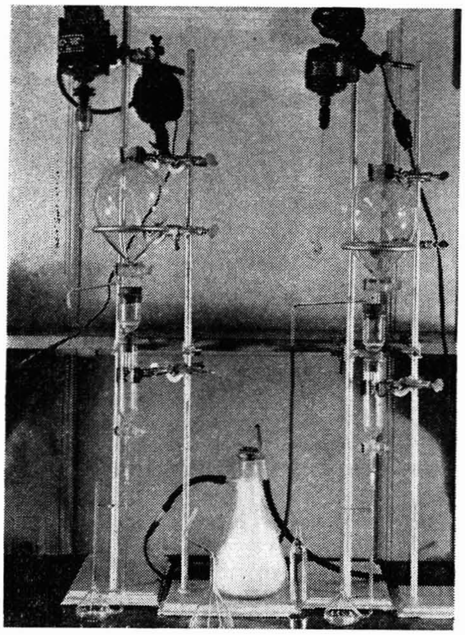

Figure 2. Assembled Extraction Apparatus

Any laboratory using this method should not assume that the oil in its waste water has this density. Wherever possible, each laboratory should determine its own density values. This may be done by skimming some oil from the surface of an oil separator, centrifuging, and measuring the density of a portion of the oil phase.

The weight of carbon tetrachloride equal in volume to the solution of oil in carbon tetrachloride is measured with a two-armed pycnometer (Figure 1), which is an adaptation of one described by Lipkin, Davison, Harvey, and Kurtz (*6*). Actual volumes of pure solvent or solutions are not measured, but a curve relating weight of carbon tetrachloride in the pycnometer to the sum of the heights of solvent in the arms of the pycnometer is prepared. Thus, when the pycnometer is filled with a solution of oil in carbon tetrachloride, the weight of an equal volume of the pure solvent is that weight, read off the curve, that corresponds to the sum of the heights of the sample solution in the arms of the pycnometer.

ANALYTICAL METHOD

Apparatus. Constant temperature bath, a conventional bath used for determining the viscosity of oils. This consists of a circular glass tank having a capacity of about 5 gallons, an ASTM Saybolt viscosity thermometer (17 F-51), 66° to 80° F. range, electric stirrer, and suitable thermostatic controls and heaters to maintain the bath temperature at 77° ± 0.08° F. The bath is equipped with cooling coils through which cold water may be run when necessary. A copper or brass platform, with a surface 6 × 4 inches and a height of about 3.5 inches is placed in the bath. The water level in the bath is adjusted so that when the pycnometer is on the platform, its side-arm markings are immersed.

Separatory funnels, one globe type having a capacity of 1 liter (all lubricant must be removed from the stopcock before use); one cylindrical open-top type having a capacity of approximately 60 ml.

Stirrer, any glass or metal stirrer that can fit into the globe separatory funnel. A paddle type has been found suitable. It should extend to within 1.5 inches from the bottom of the funnel. A variable high-speed motor, clamped to a separate ring stand to minimize vibrating the rest of the apparatus, drives the stirrer which should be rotating at about 2400 r.p.m.

Fritted-glass filtering funnel, a coarse porosity funnel having a capacity of approximately 30 ml.

Filtering flask, 2-liter capacity. The side arm is connected to the fritted-glass filtering funnel inlet tube with rubber tubing. The flask is filled with glass wool and closed with a two-hole cork stopper. A long glass tube extending almost to the bottom of the flask through one hole is connected by means of rubber tubing to a source of compressed air. The other hole remains open and is closed with a finger when it is desired to apply pressure to the mixture in the fritted-glass funnel.

Pycnometer, constructed as shown in Figure 1. It may be purchased from the Otto Greiner Co., 221 High St., Newark, N. J.

Reagents. Carbon tetrachloride, C.P. Several gallons of the solvent should be mixed in one container to minimize variations in purity and used for this determination.

Water-saturated carbon tetrachloride. Mix about 100 ml. of carbon tetrachloride with 10 ml. of water in a 1-liter separatory funnel and agitate the mixture vigorously for 5 minutes. After the phases separate, run the lower layer through a clean, dry filter paper into a 200-ml. flask and stopper with a metal foil–covered cork stopper.

Phosphoric acid solution. Dilute 10 ml. of concentrated acid to 100 ml. with distilled water.

Paper pulp. Whatman ashless tablets are suitable.

Preparation of Apparatus. A photograph of the apparatus assembly is shown in Figure 2. The 1-liter separatory funnel is supported by a metal ring and a clamp around its neck. The funnel stem, about 2 inches in length, passes through the center hole of a two-hole cork stopper, grooved down its side, which fits into the fritted-glass funnel. A glass air inlet tube, just passing through the second hole of the stopper, connects when necessary to the 2-liter filtering flask filled with glass wool.

The stem of the fritted-glass funnel fits into a cylindrical funnel through a one-hole cork stopper also slotted down the side. Any lubricant on the stopcock must be removed. The stem of the cylindrical separatory funnel is cut to about 1 inch and tapered down to about 5 mm. outside diameter—the diameter of the pycnometer arms.

A piece of Tygon tubing in which a plug of qualitative filter paper is inserted connects the funnel to the pycnometer. The plug is made by rolling up, fairly tightly, a piece of filter paper about 0.75 inch square and folding it once. Use a new plug for each determination. The paper plug removes any water droplets that may be suspended in the solution passing into the pycnometer.

Pycnometer Calibration. Fill the pycnometer with potassium dichromate–sulfuric acid cleaning solution and allow to stand for about an hour. Pour out the cleaning solution and wash the pycnometer thoroughly with distilled water. Rinse it three to four times with C.P. acetone and dry by suction, connecting the center arm of the pycnometer to the suction source. Dip the pycnometer in a beaker of acetone, dry carefully with a clean, lintless cloth and weigh to the nearest 0.1 mg. using a tare. (The bulb of a 50-ml. pipet sealed at both ends and supported by inserting one end into a rubber-lined metal or wooden stand makes a suitable tare.)

Add about 150 ml. of water and 150 ml. of carbon tetrachloride to the large separatory funnel. Stir the mixture for 15 minutes, adjusting the stirring until the mixture is uniformly opaque. Allow the phases to separate. Close the cylindrical funnel stopcock, open that of the large separatory funnel and run solvent into the fritted glass filter until it is about two thirds full.

When the carbon tetrachloride filters into the lower funnel, open the stopcock and permit the solvent to flow into the pycnometer at a rate of about 5 to 10 ml. per minute until about 0.5 ml. remains in the separatory funnel. Close the stopcock and continue drawing solvent from the large separatory funnel in the manner just described until the pycnometer arms are filled to the top of the graduations. The side arm of the pycnometer is always connected to the extraction apparatus when being filled to avoid trapping air.

Dip the pycnometer in acetone, dry with a clean lintless cloth, and weigh to the nearest 0.1 mg. using the tare. Place the pycnometer in the constant temperature bath. When the level of the solvent in the pycnometer arms becomes constant, record the sum of the heights of the solvent in the arms, reading to the nearest 0.5 mm.

Pour a drop of solvent out of the center arm of the pycnometer and repeat the weighing as described above. Continue to do this until three or four sets of readings are obtained. Empty the pycnometer. Repeat the filling, weighing, and bath immersion steps until a total of six to eight sets of readings are obtained.

Calculate the weight of carbon tetrachloride in the pycnometer by subtracting the weight of the empty pycnometer. Plot the weight of solvent against the corresponding sum of the heights of the solvent in the pycnometer arms on cartesian coordinate paper. Draw the best straight line through the points.

Procedure. Collect the sample in a quart bottle according to API recommended procedure (*1*) until the bottle is nearly full. Make a crayon mark on the bottle to indicate sample volume. Add 25 ml. of carbon tetrachloride to the sample, stopper with a metal foil-covered cork, and shake the mixture well for about 2 minutes.

Transfer the contents of the bottle to the 1-liter separatory funnel. This can be done without lifting out the stirrer by using a glass funnel having two right-angle bends in the stem (Figure 2). Add 3 ml. of the phosphoric acid solution and about 0.5 gram of paper pulp (one fourth of a compressed tablet broken into small pieces) to the sample to minimize the formation of stable emulsions. Start stirrer and adjust motor speed so that the mixture is uniformly opaque. While the mixture is being stirred, add 20 ml. of carbon tetrachloride to the sample bottle, stopper, and shake vigorously for 2 minutes.

Stop the stirrer after 15 minutes, and allow the two phases to separate. Run the lower phase into the fritted-glass filter, making certain that the lower separatory funnel stopcock is closed. Allow any emulsion in the carbon tetrachloride phase as well as a little of the paper pulp to run into the filter. Close the stopcock. Add the 20 ml. of solvent in the sample bottle to the large separatory funnel and repeat the agitation of the mixture as described above.

Meanwhile, let the first extract go through the fritted-glass filter into the lower separatory funnel. If filtration is slow, apply a little pressure to the solution. To do this, connect the air inlet tube in the fritted-glass filter stopper to the side arm of the filtering flask and close the open hole with a finger. For best results, minimize the time pressure used to push the solution through the filter. Although it is necessary to process the carbon tetrachloride solution out of the fritted-glass funnel before running in the next extract, it is not necessary to have all the water accompany the first extract going through. If pressure is used to aid in filtering the carbon tetrachloride solution, the pressure should be cut off when the water layer reaches the fritted-glass surface. Open the stopcock of the lower funnel. Adjust the flow rate so that 3 to 5 minutes are required for all but about 0.5 ml. of the lower phase to flow into the pycnometer.

Repeat the separation of phases and add a third 20-ml. portion of carbon tetrachloride to the sample bottle, which is then processed through the apparatus in the same manner. When the third extract is in the lower funnel, run enough of it into the pycnometer to fill the latter to the desired height. The height depends on the room temperature. If room temperature is around 25° C., fill the pycnometer to about the middle of the arms. If around 20° C., fill it to the bottom of the graduations, and fill to the top of the graduations if room temperature is near 30° C. A little experience will enable an operator to fill the pycnometer to the proper height.

When the pycnometer is filled to the desired height, there should be a few milliliters of solvent remaining in the lower separatory funnel. If more carbon tetrachloride is needed to fill the pycnometer, add 5 to 10 ml. of the water-saturated solvent to the 1-liter separatory funnel and carry it down through the extracton apparatus. Agitation is not needed.

Dip the pycnometer in acetone, wipe with a lintless cloth, and weigh to the closest 0.1 mg. using the tare. Subtract the weight of the empty pycnometer to get the weight of the solution. Place the pycnometer in the bath. When the level of the liquid in the arms becomes constant, note the height in millimeters in each arm to the nearest 0.5 mm. and add them. Read from the calibration curve the weight of carbon tetrachloride corresponding to this sum.

Fill the sample bottle to the crayon mark with tap water. Pour the water into a 1-liter graduated cylinder. The volume of water in the graduated cylinder equals the volume of sample analyzed. The original sample volume can not be measured directly without risking loss of oil.

Blanks. When a new batch of carbon tetrachloride is used, make a blank run exactly as described under pycnometer calibration, omitting the repeated weighings and taking the weight at only one liquid level. If the weight of the reagent is the same for the sum of the liquid level heights as that from the calibration curve, the new batch of solvent has no blank.

If the weight of new solvent in the pycnometer is greater than that read off the curve, the blank on the new solvent batch is positive and the difference between the two weights is added to all subsequent weights read off the curve. Conversely, if the weight of the solvent in the pycnometer is less than that read off the curve, the blank is negative and is subtracted from subsequent weights read off the curve. However, with C.P. carbon tetrachloride, the blanks from one batch to another should be small.

Table I. Results on Synthetic Samples

Type Oil	Oil Added, Mg.	Oil Found, Mg.	Recovery, %
SAE No. 10	31.5	31.3	99
	33.0	32.0	97
	62.6	63.3	101
	74.0	74.0	100
	97.7	94.3	96
	109.0	105.0	96
	205.5	192.0	94
		Average recovery	97.5
Separator oil	21.2	24.0	113
	43.4	44.2	102
	62.5	63.7	102
	95.6	90.1	94
	200.0	194.7	97
	226.4	205.6	91
		Average recovery	99.8
Kerosine (initial boiling point 330° F.)	29.0	25.4	88
	57.0	59.0	104
	84.0	84.0	100
	92.0	81.2	88
	110.2	115.0	104
	140.2	118.0	84
	200.8	184.7	92
		Average recovery	94.3
Gasoline (initial boiling point 100° F.)	25.0	25.0	100
	36.9	26.8	73
	51.0	39.0	77
	63.2	48.7	77
	80.1	73.0	91
		Average recovery	83.6

Calculations. The oil content of the sample is calculated by means of the following equation:

$$\text{Oil, p.p.m.} = 10^6 \times \frac{(W_2 \pm B - W_1)}{V\,(1.59/d - 1)}$$

where

d = assumed value for the density of the waste oil

V = volume of sample taken for analysis, milliliters

W_1 = weight of carbon tetrachloride plus oil in the pycnometer, grams

W_2 = weight of carbon tetrachloride from the calibration curve, grams

B = blank correction, grams

Report oil content of the sample to the nearest part per million.

RESULTS OBTAINED

The method has been tried on samples prepared by shaking known amounts of oil with tap water and on a large number of refinery effluent samples. Table I shows typical results obtained with an SAE 10 oil, oil reclaimed from separators, a kerosine, and a gasoline.

The recovery of the SAE 10 and separator oil is good—97.5 and 99.8%, respectively. Kerosine recovery is satisfactory, while that for gasoline is somewhat low. However, considering the volatility of this product, the recovery is fair. A factor tending to lower recovery of the gasoline is the difficulty met in weighing out milligram amounts of the gasoline and transferring them to the sample bottle. About 84% is the recovery achieved on a regular gasoline. In waste water, the bulk of the low boiling material weathers away, so that such samples will never contain the quantities of light material put into these synthetic samples.

Three oils, blended for American Petroleum Institute cooperative work on oil-in-water methods, were also used to prepare synthetic samples. Table II shows the results obtained with these oils.

As is the case with other synthetic oil-in-water samples, those made with the SAE 70 oil and the mixture of this oil with kerosine showed good recovery. The percentage recovery on sample 3 runs were not quite as good as for those made with samples 1 and 2, but considering the volatility of the gasoline they are satisfactory. However, if one recalls that the percentage recovery on a straight-run gasoline was 83.6% (Table I), better recovery on this sample is believed possible. This was verified by the cooperative test results obtained with this method and summarized by Headington (*4*). His data show that with six independent laboratories reporting the average recovery on sample 3 was 101%.

Table II. Analysis of Cooperative Samples

Type Oil	Oil Added, Mg.	Oil Found, Mg.	Recovery, %
SAE 70 (No. 1)	18.6	16.1	87
	49.5	47.4	96
	55.7	53.6	96
	110.0	99.5	90
	110.0	102.5	92
	112.1	115.8	102
		Average recovery	94
2/3 SAE 70 + 1/3 kerosine (No. 2)	56.3	54.5	97
	59.6	56.4	95
	60.7	59.1	98
	61.8	61.8	100
		Average recovery	98
1/3 SAE 70 + 1/3 kerosine + 1/3 gasoline (No. 3)	103.0	86.4	84
	105.4	93.0	88
		Average recovery	86

A large number of refinery effluent samples were run to test the method in routine operation. The results were compared with values obtained on duplicate samples by the API U-tube method (*2*). The work was done by a nontechnical operator and Table III shows some of the comparison values obtained. On the average, results by the direct extraction method are about twice those by the U-tube method. Of course, the more volatile material in the sample, the greater will be the difference.

Another factor that must enter into such a comparison is sampling. The samples for this comparative work were taken at the same time and every effort made to get duplicate samples. Yet, as shown in Table IV, some results can only be due to poor sampling.

Such data emphasize the fact that sampling must be done with great care to obtain significant results.

DISCUSSION

A basic advantage of the method is its applicability to samples with volatile constituents. Several factors make this possible.

1. No heating steps are involved.
2. No suction is required; this is important because it is the step involving the drying of the ferric hydroxide precipitate by suction that promotes the inaccuracy of the U-tube method with samples containing volatile constituents. It has been found that even with relatively nonvolatile oil, some is lost if suction is applied for 10 to 15 minutes to the precipitate on which the oil is adsorbed.
3. In the use of pressure to force the carbon tetrachloride through the fritted-glass filter, the compressed air strikes a thin layer of water and not the carbon tetrachloride. This minimizes the loss of volatile matter by the use of the compressed air.

One factor in this method is the need to assume a value for the density of the oil; other oil-in-water methods make a similar assumption and an average value is not hard to obtain. The largest error that could result from an incorrect assumption, if 0.87 were assumed and the density was really 0.80, would be results that are 10% too high. If 0.87 were assumed and the density was 1.00, results would be 18% low. However, these are extremes. In practice the range of density is much less. A more realistic range would be 0.81 to 0.89.

In special cases it might be desirable to eliminate altogether the need for making this assumption. One way to accomplish this is to run duplicate samples by this method using carbon tetrachloride on one sample and carbon disulfide as the solvent on the duplicate. Thus, two equations are obtained in which the unknowns are the density and the oil content of the sample. By solving the two equations simultaneously, the density and oil content are obtained. Since this scheme requires two determinations, it is not practical for routine work. The method assumes, of course, that the two solvents extract the same kinds and quantity of substances out of the sample.

Tests showed that three extractions, each of 15 minutes' duration, are enough to extract the oil quantitatively from a water sample. The first extraction removes about 75% of the oil, and the second one removes all but traces of the rest. The third extraction picks up traces of oil that may still remain and washes down any oil-bearing solvent on the sides of the fritted-glass filter and lower separatory funnel. The ability of carbon tetrachloride to extract quantitatively the oil from water samples is also proved by Headington and coworkers in their infrared method (*11*).

While this investigation was concerned with a method for determining the total oil content of refinery effluents, others may wish to determine the volatile and the nonvolatile oil content. This method can easily be adapted for this purpose.

After determining the total oil, the pycnometer contents are poured into a weighed beaker. The solvent is evaporated on a steam plate and the residue dried to constant weight. The weight of oil in the beaker is the nonvolatile oil. The difference between this value and the total oil content is the volatile oil present.

Of course, the nonvolatile oil is not all oil, but may include other material suspended or dissolved in the water that is partially or wholly extractable with carbon tetrachloride. Some refineries are interested in an estimation of the acidic content of the extracted oil. In the API distillation-extraction method (*1*) and the reflux distillation-extraction method (*2*), the oil remaining after evaporating the solvent is dissolved in a suitable solvent and titrated with standard alkali. In a similar fashion, the nonvolatile oil obtained above may be titrated for its acidic content.

For best results, a few precautions must be taken in cleaning the apparatus after a determination and before starting another. The large separatory funnel needs only rinsing with water. The lower separatory funnel, after remaining solvent and water have been poured out, should be rinsed twice with carbon tetrachloride. It is not necessary to wipe it dry and the use of acetone to clean it is not advisable.

Table III. Comparison of Results by Direct Extraction-Pycnometer Method and API U-Tube Method

	Oil, P.P.M.										
Direct extraction-pycnometer method	49	38	46	57	52	64	43	49	28	34	148
API U-tube method	14	9	28	14	28	42	23	42	9	19	150

Table IV. Comparison of Results on Apparently Nonrepresentative Samples

	Oil, P.P.M.			
Direct extraction method	2379	83	152	950
U-tube method	42	140	187+	103

The fritted-glass filter is cleaned by removing the layer of paper pulp, rinsing twice with carbon tetrachloride, and wiping dry. Then strong suction is applied to its stem until bubbles of air are no longer visible on the underside of the fritted glass. The filter is then wet with a little carbon tetrachloride. This treatment is important in maintaining the rapid filtering characteristics of the fritted glass. If filtration is slow, it usually means the fritted glass is wet with water. Drying with suction will improve its operation. Acetone should not be used to clean the filter, as it appears to be difficult to get all the acetone out of the fritted glass with suction.

The pycnometer is cleaned by pouring out its contents, rinsing twice with C.P. acetone, and applying suction to the center arm for about 2 minutes. The acetone is added from a buret or separatory funnel connected to the side arm of the pycnometer by means of Tygon tubing. It must be perfectly clean for the next run.

An important feature of any method for determining oil in refinery effluents is the ease with which it can be handled on a routine basis. A nontechnical operator working with two extraction units can analyze two samples in 1.5 hours of over-all time. This figure includes the time required to clean up the apparatus for the next determination. The operator time required is about 40 minutes, which also includes cleaning time.

Nevertheless, the method is not as rapid as the U-tube method, since the same nontechnical operator can do six determinations in an over-all time of 2.5 hours, of which only 50 minutes is operator time. However, results by the direct extraction method are much more accurate.

Some samples, after standing for several days, required the use of compressed air to force the bulk of the carbon tetrachloride solution through the fritted glass. Duplicate samples analyzed within a few hours of receipt gave no difficulty at all. It is therefore recommended that samples be analyzed as soon as possible after they are received.

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Uncombined Calcium Oxide or Hydroxide in Lime and Silicate Products

Volumetric Determination

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The methods for the volumetric determination of uncombined calcium oxide and hydroxide extracted from lime and silicate products by glycerol, ethylene glycol, and acetoacetic ester have been studied. The best results are obtained by conductometric titration with a strong acid. Reasonable values can also be obtained using an indicator: methyl red for glycerol and ethylene glycol extracts and bromophenol blue for the acetoacetic extracts. When an accelerator such as strontium nitrate is used in the glycerol extraction, the only convenient method is titration with an indicator.

ONE of the most important determinations in the lime and cement industry concerns the percentage of lime occurring as calcium oxide or hydroxide in the products, the so-called free or uncombined lime. Much effort has been expended in attempts to find suitable methods for this determination. A summary is found in modern handbooks on the lime industry (*3*). In connection with some investigations on pozzuolanas the authors required a tolerably accurate method for the determination of uncombined lime, but after a scrutiny of the methods described in the literature and after some experiments it was found that some of them are unsuitable and most of them need improvement. Thus the first step in this investigation was to find the conditions under which the determinations were sufficiently accurate and the analytical work could be best adapted.

There are two chief groups of methods. The first involves the measurement of ignition loss (determination of calcium hydroxide) or of the heat of hydration of an anhydrous or an ignited sample at well-defined humidification conditions. The second group includes methods based on an extraction of the uncombined lime with a suitable solvent. The former methods cannot be used in the presence of kinds of pozzuolanas usually encountered and are not discussed here.

The extraction methods are suitable only when the solvent is sufficiently selective, completely dissolving calcium oxide or hydroxide without decomposing hydrated silicates or aluminates. Several solvents have been proposed and investigated. The most acceptable are glycerol, acetoacetic ester, and ethylene glycol. The extracted lime can be determined volumetrically or gravimetrically, but in view of the difficulties encountered during the precipitation of calcium from organic solvents, volumetric methods have been exclusively employed.

The problems involved are of such embracing nature that it was necessary to limit the scope of this investigation. An examination of the volumetric determination of calcium oxide or hydroxide dissolved in the solvents has now been undertaken, while the actual extraction methods for pozzuolanas must be treated separately.

REAGENTS AND APPARATUS

The titration solutions used in the experiments described were:

Hydrochloric acid (aqueous solution), 1 ml. equivalent to 10 mg. of calcium oxide.

Sulfuric acid (ethyl alcohol solution), 1 ml. equivalent to 10 mg. of calcium oxide.

Benzoic acid (ethyl alcohol solution), 1 mg. equivalent to 1 mg. of calcium oxide.

Ammonium acetate (ethyl alcohol solution), 1 ml. equivalent to 2 mg. of calcium oxide.

These solutions were calibrated in aqueous solution according to standard methods—that is, they were not standardized by carrying out a control titration of a known amount of calcium oxide under the conditions of the determinations. Usually a microburet was used for the acids.

The concentrations of the indicators used were:

Methyl red, 1%.

Mixed indicator of equal parts of 1% phenolphthalein and 1.5% naphtholphthalein.

Bromophenol blue, 1%.

The titrations were followed by two physicochemical methods: conductometric titration and titration under measurements of the potential difference between the solution and an antimony electrode.

The electrical conductivity of the solution was measured with the aid of a commercial resistance instrument (Philoscop). The measuring instrument was used together with a frequency modulator for very high resistances. The resistance determination was accurate within about 1% of the measured value, which was sufficiently accurate for the purpose. No thermostat for regulation of the temperature of the reaction liquid was used. As the conductivities of the different solutions varied over a considerable range, it was found necessary to employ three resistance cells with constants 1.2, 2.5, and 3.5 reciprocal ohms. The resistance measured (reciprocal of the conductivity) is plotted in the lower half of Figures 1, 2, and 3.

For the measurement of the potential difference between the antimony electrode and the solutions a saturated calomel electrode was used as reference electrode. The potential difference was converted into normal pH values and is referred to hereafter as pH. Glass electrodes as well as a hydrogen electrode [palladium (*10*)] were also tried, but they became poisoned rapidly by the organic solvents used. The antimony electrode, on the other hand, retained its correct potential during a titration under the conditions involved. Before it was used it was cleaned by gentle polishing with fine emery paper. If absolute values are required, the surface must be kept clean—e.g., by rotating brushes—but for this investigation this was not necessary. As the proportion between the solvents in the reaction mixture cannot be kept constant, an accuracy greater than ±0.02 volt has no significance. The calomel electrode was connected with the solution to be titrated by an agar-agar saturated potassium chloride siphon.

The reaction mixture was thoroughly stirred by a mechanical stirrer. A magnetic stirrer cannot be used because of the influence of its electromagnetic field on the high-frequency current in the conductivity measurements and on the pH measurement instrument and its antimony-calomel electrodes. With certain indicators no sharp change of color was obtained, and when this was the case the color was compared with that of a reference solution.

EMLEY METHOD, DENNIS MODIFICATION

The uncombined calcium oxide or hydroxide is extracted with a boiling mixture of glycerol and ethyl alcohol and the boiling solution is titrated with ammonium acetate, using phenolphthalein as indicator. Since the first publication of the method (*6*) some modifications have been described (*1*, *2*, *8*, *9*). One of the inconveniences of the method is that the rate of extraction of the calcium oxide is very slow and that of the calcium hydroxide is still slower. To avoid this, Brandenburg (*4*) recommended the addition of an acid-base neutral salt (barium chloride), which by ion exchange could form a more suitable glycerate. Dennis (*5*) investigated a large number of such possible accelerators and considered strontium nitrate to give the best results. Accurate values can be obtained by this method only if the experimental conditions are ideal, which is often not the case.

The procedure was carried out in accordance with the directions of Lerch and Bogue (*8*) and Dennis (*5*), except that the ammonium acetate solution was standardized by analysis, and was not titrated against calcium oxide under the experimental conditions. Some values from the titrations are listed in Table I.

The method has some disadvantages: The solution must be boiled to remove the ammonia; the change of color of the indicator is dependent on the temperature of the solution and fading also occurs as the end point is approached; the ammonium acetate must be added successively with controlled boiling for some minutes between the portions added; and the values are at least 5 to 6% too low. The method has some advantages: The extraction of calcium oxide is fairly rapid and it is not always necessary to remove the extracted residue.

It is not possible to have an independent control of the method in any way other than a repeating of the determination. The error in the obtained values, however, seems to be constant; it cannot be due to impurities in the chemicals used, which are of analytical reagent standard and which give correct values in determinations performed in other ways. The error must arise from the failure of the theoretical end point of the reaction of calcium glycerate and calcium acetate to coincide with the color change of red-violet to colorless under the given conditions. The ammonium acetate solution could be calibrated by carrying out control experiments with known amounts of calcium oxide.

Table I. Emley-Lerch-Bogue-Dennis Method[a]

Calcium Oxide, Mg.		
Weighed	Recovered	%
41.5	39.4	95
45.1	42.9	95
44.4	41.5	93
39.9	37.9	95

[a] Titration of calcium oxide dissolved in glycerol–ethyl alcohol in the presence of 2 grams of strontium nitrate; titration solution, ammonium acetate in ethyl alcohol; indicator, phenolphthalein.

Table II. Glycerol–Ethyl Alcohol Extraction with Strontium Nitrate Accelerator[a]

Acid	Indicator	Calcium Oxide, Mg.		Error, %
		Present	Found	
HCl	M[b]	31.8	32.5[c]	+ 2
HCl	M	62.4	60.3[c]	− 3
HCl	M	40.1	39.3[c]	− 2
HCl	M	50.0	49.3[c]	− 1
H_2SO_4	M	38.1	36.4[c]	− 4
HCl	M	49.2	48.6[d]	− 1
HCl	M	61.6	57.7[e]	− 6
HCl	M	46.4	43.4[e]	− 6
HCl	D[f]	33.0	24.9[e]	−25
Benzoic acid	D	36.4	29.2[e]	−20

[a] Glycerol–ethyl alcohol ratio of 1 to 4 (volume).

[b] Methyl red.

[c] With comparison solution.

[d] With comparison solution and addition of calcium chloride.

[e] Without comparison solution.

[f] Mixed indicator.

In order to obtain correct values the titrations must be performed under identical experimental conditions, such as the concentration of calcium oxide in the extract and the proportion of ethyl alcohol–glycerol, so that the method is thus a purely empirical one. This method would be more useful if another indicator were used, if boiling of the solutions could be avoided during the titration by cooling down the extracts to room temperature, and if another titration solution could be substituted for the ammonium acetate.

OTHER METHODS BASED ON GLYCEROL EXTRACTION

The other methods employing glycerol as extraction solvent differ from Emley's original method in the use of acids for the titrations instead of ammonium acetate. Rathke (*11*) and Schindler (*12*) used tartaric acid and Bessey used (*1*, *2*) benzoic acid, both acids in ethyl alcohol solution with phenolphthalein as indicator. It is, of course, possible to extract the calcium oxide or hydroxide with or without accelerator, and the titrations can be performed at room temperature. They can be determined by other methods (pH and conductivity measurements). Since a decomposition of calcium silicate, aluminates, or their hydrates in the sample could be brought about by an excess of the titration liquid, the residue must be filtered off after the extraction.

Extraction with Accelerator. Samples used for testing the method were 0.030 to 0.050 gram of calcium oxide. The extraction mixture consisted of 2 grams of strontium nitrate, 20 ml. of glycerol, 30 ml. of ethyl alcohol; 105 ml. of ethyl alcohol was used for washing and diluting the extract afterwards. The indicators used were the mixed indicator phenolphthalein–naphtholphthalein as described by Schläpfer-Bukowski (*13*), and methyl red.

The pH curves (Figure 1) show that the potential between the antimony and the calomel electrode is lowered successively with increasing amounts of acid. The curves are shaped as a double wave when hydrochloric and sulfuric acids are used, and the end points of the reaction are at the passage between the two parts of the double wave, about at the same pH measured. The double wave is very marked, and it recurs in other experiments. The benzoic acid gives only a slow lowering of the pH with an extended single wave, and the true neutralization point is far from the inflection point of the curve.

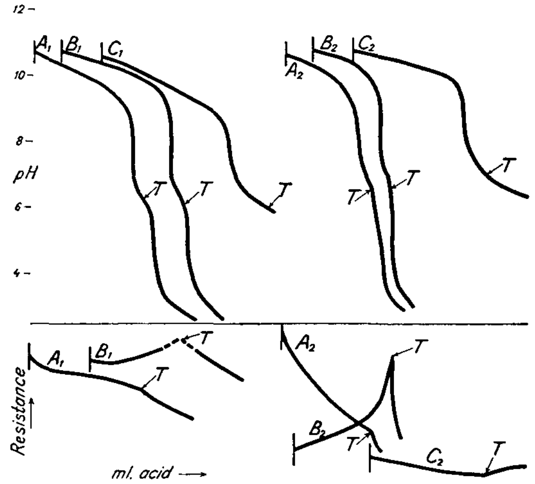

Figure 1. Titration Curves of Glycerol Extracts

Left. Extraction with strontium nitrate accelerator
Right. Without accelerator
A. Hydrochloric acid
B. Sulfuric acid
C. Benzoic acid
T. Calculated end point of reaction

The conductivity measurements gave no distinct information concerning the neutralization point because of the large amount of accelerator present in the solutions (Figure 1). If a more sensitive bridge were used for the resistance determination it would probably be possible to find the neutralization point, but much more rigorous control of the experimental conditions would then be necessary.

Since the measurement of pH can also lead only to an approximate result, the methods suitable for the determination are limited to those in which the titration is carried out with an indicator. The results obtained with different indicators were not in complete agreement with the results mentioned in the literature. In Table II are recorded some of the results from these determinations. Only one of the indicators examined, methyl red, showed a distinct change of color close to the theoretical end point of the reaction when hydrochloric or sulfuric acids were used. This indicator can therefore be used in the determinations; it is not sensitive to benzoic acid in the solvents used. The mixed indicator as well as phenolphthalein showed considerable errors with the strong acids as well as with benzoic acid (10 to 20% too low).

Extraction without Accelerator. This method has the disadvantage that calcium oxide or hydroxide dissolves slowly. The substance must be treated repeatedly and the completeness of the extraction controlled carefully, with the result that the analysis is laborious and time-consuming. However, some determinations were made by this method for comparison. The titrations were performed as described above and the change of color of the indicators was studied.

The titrations with hydrochloric and sulfuric acids were followed by pH measurements. The same double wave curve found earlier is observed, and the end point of the reaction of calcium glycerate and sulfate or chloride is at the part of the curve between the two waves (Figure 1). The titration with benzoic acid shows a single wave which is rather extended, and because of this the inflection point is difficult to determine. The end point of the reaction of calcium glycerate and benzoate, however, is far from the inflection point of the curve.

The conductivity measurement curves are of two types (Figure 1). Hydrochloric acid gives a curve, having two branches, and showing a conductivity increasing with increasing acid added. The break between the branches is distinct and corresponds exactly with the end point (Table III). The sulfuric acid lowers the conductivity because of the low solubility of the calcium sulfate formed. When the neutralization point is passed, the conductivity increases rapidly. The end point of the reaction appears as a very sharp peak on the conductivity curve, corresponding exactly to the calculated value. Benzoic acid, too, has a conductometric curve with two branches, between which the reaction end point appears as a break. The agreement between the calculated and the found values showed that no objections could be raised concerning the quality of the chemicals. The indicator methyl red also gave the best values here, while the mixed indicator and phenolphthalein gave results which were 10 to 30% too low.

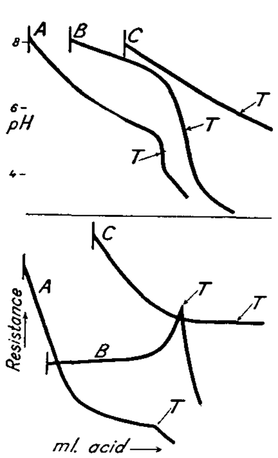

Figure 2. Titration Curves of Acetoacetic Ester–Isobutyl Alcohol Extracts

A. Hydrochloric acid
B. Sulfuric acid
C. Benzoic acid
T. Calculated end point of reaction

If this extraction method is to be used, the most accurate determination is accomplished by a conductometric titration with sulfuric acid. Methyl red is the best indicator, and if the change of color is used for finding the neutralization point the most accurate procedure appears to be that described below.

Extract the sample for 1 hour under reflux conditions with 50 ml. of glycerol–ethyl alcohol mixture (ratio of 1 to 2 parts by volume) in a 100-ml. round-bottomed flask heated on a steam bath or in a heating jacket. Cool, filter, and wash with small portions of ethyl alcohol. As the extraction is not always complete after 1 hour, retain the residue for repeating the extraction. Titrate the solution with hydrochloric acid (1 ml. equivalent to 10 mg. of calcium oxide) using a microburet and methyl red as indicator.

To obtain the exact end point, compare the sample solution with a solution containing the same portions of ethyl alcohol, glycerol, and methyl red together with 0.05 ml. of the same hydrochloric acid. Titrate the sample solution to the same color as the comparison solution. Then add to the comparison liquid a calcium chloride solution containing the weight of calcium chloride calculated to be present in the titrated solution and having approximately the same volume as the hydrochloric acid added. Continue the titration of the sample solution until it is the same color as the comparison solution.

Read the volume of hydrochloric acid consumed by the sample and correct for the 0.05 ml. in the comparison solution. The

Table III. Glycerol-Ethyl Alcohol Extraction without Accelerator

		Calcium Oxide, Mg.				
			Titration with Indicator		Conductometric Titration	
Acid	Indicator	Present	Found	Error, %	Found	Error, %
HCl	M[a]	34.7	32.9	− 5	34.4	1
H_2SO_4	M[a]	45.3	45.3	0	...	...
HCl	D[b]	30.8	20.8	−32	...	...
HCl	D[b]	20.3	14.2	−32	...	...
HCl	D[b]	31.5	25.9	−18	32.5	+3
HCl	D[b]	25.6	19.8	−22	26.0	+1
Benzoic Acid	D[b]	38.0	34.0	−11	36.0	−5

[a] Methyl red.
[b] Mixed indicator.

error seldom exceeds 1% of the correct calcium oxide value. If an accuracy of about ±3% of the calcium content is sufficient, the corrrection for the calcium chloride dissolved in the sample solution can be omitted. For control, repeat the extraction of the retained residue until calcium can no longer be extracted.

EXTRACTION WITH ACETOACETIC ESTER AND ISOBUTYL ALCOHOL

The instructions given by Franke (*7*) were followed. The sample was placed in a flask in an electrically heated jacket and extracted under reflux conditions for 1 hour with a mixture of acetoacetic ester and isobutyl alcohol. The solution was cooled, filtered, and washed with isobutyl alcohol, and the filtrate was titrated with hydrochloric acid using bromophenol blue as indicator. It was observed during the investigation that the calcium compound of acetoacetic ester was readily deposited on the walls of the flask during boiling. The indicator has a rather indefinite and faint color change, so that there was some difficulty in discerning the exact end point of the reaction. In some of the determinations ethyl alcohol was used for washing and diluting the filtrated solutions without any disadvantage.

The pH curves for the titration with hydrochloric and sulfuric acids show a single wave, the inflection points of which are very near the end point of the reaction of the organic calcium compound with inorganic salt (Figure 2). The benzoic acid gives a curve of very slight slope without any inflection point. The conductometric titrations with hydrochloric and sulfuric acids show very distinct breaks at the neutralization points quite as was described with regard to the glycerol–ethyl alcohol extraction. The benzoic acid curve has no break (Table IV).

Although the indicator bromophenol blue recommended by Franke has an indefinite color change, the reaction end point was normally found to be within 2% of the theoretical value. The other indicators gave no color change.

The conductometric titration gives accurate results and takes only a little more time than the indicator titration, especially if the greater part of the solution is first titrated approximately with bromophenol blue and only the final titration up to the end point of the reaction followed by conductivity measurements.

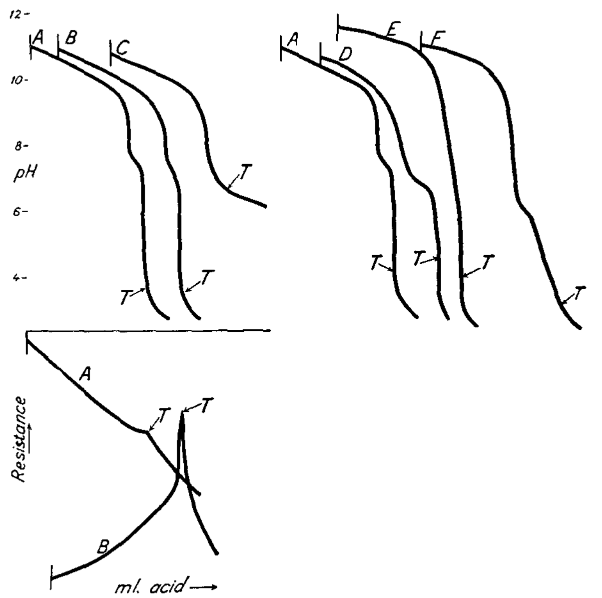

Figure 3. Titration Curves of Ethylene Glycol Extracts

A, *B*, and *C* diluted with 96% ethyl alcohol; *D* diluted with absolute ethyl alcohol; *E* pure ethylene glycol; *F* diluted with water 1 to 1

A. Hydrochloric acid
B, *D*, *E*, *F*. Sulfuric acid
C. Benzoic acid
T. Calculated end point of reaction

Table IV. Extraction with Acetoacetic Ester and Isobutyl Alcohol[a]

		Calcium Oxide, Mg.				
			Titration with Indicator		Conductometric Titration	
Acid	Indicator	Present	Found	Error, %	Found	Error, %
HCl	B[b]	51.8	50.9	−2	51.5	−0.6
HCl	B[b]	52.8	51.8	−2	51.5	−2
HCl	B[b]	34.0	33.6	−1	34.0	0
HCl	B[b]	41.7	41.7	0	41.7	0
H_2SO_4	B[b]	35.4	35.0	−1	35.6	+0.5
Benzoic acid	B[b]	32.9	No color change[b]		Indeterminable	
H_2SO_4	D[c]	37.3	No color change[c]		37.3	0

[a] Titration solution 6 ml. acetoacetic ester diluted with 100 ml. isobutyl alcohol.
[b] Bromophenol blue.
[c] Mixed indicator.

EXTRACTION WITH ETHYLENE GLYCOL

Schläpfer, Bukowski, and Esenwein recommend the extraction of calcium oxide and hydroxide with pure ethylene glycol at 75° to 85° C., the extraction being complete after 30 minutes (*13*, *14*). In some of the experiments pure ethylene glycol was used, but extractions were also attempted with ethylene glycol diluted with methanol, ethyl alcohol, and ethylene glycol monoethyl ether in order to try the use of a heating jacket instead of the 75° C. bath. When diluted ethylene glycol was used, calcium oxide, but not calcium hydroxide, could be dissolved completely within reasonable time. All the diluted solutions, however, showed that the solubility of calcium glycolate has a negative temperature coefficient.

Table V. Extraction with Ethylene Glycol

		Calcium Oxide, Mg.				
			Titration with Indicator		Conductometric Titration	
Acid	Indicator	Present	Found	Error, %	Found	Error, %
HCl	M[a]	35.0	33.5[b]	− 4	...	...
HCl	M[a]	36.7	35.6[b]	− 3	36.8	0
H_2SO_4	M[a]	42.9	41.6[c]	− 3	42.2	−1.5
HCl	M[a]	42.1	32.9[d]	−22	...	...
HCl	D[e]	57.8	52.6[f]	− 9	58.0	+0.3
HCl	D[e]	34.0	30.0[c]	−12	33.8	−0.6
HCl	D[e]	36.7	35.4[g]	− 3	...	...
H_2SO_4	D[e]	35.1	31.4[b]	−10	...	...
H_2SO_4	D[e]	35.5	31.8[b]	−10	35.0	−1.5
H_2SO_4	D[e]	43.5	39.9[c]	− 8	43.8	+0.7
Benzoic acid	D[e]	34.4	31.0[b]	−10	...	...

[a] Methyl red.
[b] Ethylene glycol extract diluted 1 to 4 with ethanol.
[c] Ethylene glycol extract diluted 1 to 1 with ethanol.
[d] Ethylene glycol extract diluted 1 to 1 with water.
[e] Mixed indicator.
[f] Ethylene glycol extract diluted 1 to 0.5 with ethanol.
[g] Pure ethylene glycol.

After the extraction with pure ethylene glycol the solutions may be filtered and diluted with one of the solvents mentioned. Therefore, in cases where the sample contains calcium hydroxide, the solvent should be pure ethylene glycol and the extraction should be performed at about 75° C. The ethylene glycol used in these experiments was an Eastman Kodak product labeled "For Chemical Purposes" (*9*).

The pH curves for hydrochloric and sulfuric acids show the double wave (Figure 3), earlier mentioned in connection with the glycerol extraction. The end points of the reaction of calcium glycolate to chloride or sulfate are on the lower part of the second wave. As this curve branch has a steep slope, the position of the end point of the reaction can be determined rather accurately. The titration with benzoic acid gives a rather extended single wave curve with the theoretical end point of the reaction far from the inflection point of the curve.

The glycerol extracts as well as the ethylene glycol extracts have pH titration curves containing a double wave. A brief investigation into the cause of this phenomenon was made. A solution containing pure glycerol is viscous and difficult to handle, while the ethylene glycol solutions are more suitable for experiments. Some titrations were therefore performed in ethylene glycol solutions, since it was assumed that the characteristics of the glycerol and ethylene glycol curves probably have the same explanation. The results and the curves from these titrations are given in Table V and Figure 3.

Two titrations were performed in pure ethylene glycol; for one of them hydrochloric acid (aqueous solution) was used, for the other sulfuric acid (ethyl alcohol solution) was used. These two titrations gave the same curve; a single wave of the same shape (only one of them is drawn in Figure 3). They contain, however, a very slight flexure in the middle of the wave, an indication of the double wave. The kind of acid and the small amount of water and ethyl alcohol added during the titration have no significance for the shape of the curve.

Another titration was carried out after dilution of the ethylene glycol extract with absolute ethyl alcohol. The curve obtained has a very marked double wave. The titration curve after dilution with 96% ethyl alcohol has a double wave with a less marked part between the two waves. The curve after dilution of the extract with an equal part of water also shows a double wave. The part of this curve at lower pH values has a less steep slope, and there is a large volume of acid required between the point for the color change of the methyl red indicator and the theoretical end point for the reaction. This shows the importance of defined experimental conditions. Similar curves containing double waves were obtained when titrations of glycerol–ethyl alcohol extracts were followed by measuring the pH with a glass electrode instead of the antimony electrode.

From these experiments it can be concluded that the double wave is caused by the dilution of the ethylene glycol solution, which apparently involves a partial decomposition of the calcium glycolate. However, this explanation cannot be accepted definitely until new experiments are conducted which do not lie within the scope of this investigation.

The conductometric titrations gave curves similar to those described above in connection with the glycerol and acetoacetic ester extracts. Hydrochloric and sulfuric acids gave a distinctive end point for the reaction (Figure 3).

The indicators showed very different points for the change of color. Methyl red gave very good values with hydrochloric or sulfuric acid when the solutions were in pure ethylene glycol or were diluted with ethyl alcohol; a dilution with water caused considerable errors. The mixed indicator, which is recommended by the authors of the original description of the method (*13*, *14*), showed correct values when ethylene glycol alone was the solvent, but showed values which were 10 to 20% too low if the solution was diluted with an equal part of ethyl alcohol. The mixed indicator gave an indistinct change of color with benzoic acid after dilution with ethyl alcohol; when the extract was diluted with water the color change was highly dependent on the proportions in the mixture.

If this extraction method is to be used, the best results will be achieved if pure ethylene glycol is used for the extraction and the titration is performed conductometrically with hydrochloric or sulfuric acid after thorough cooling and dilution of the extracts with ethyl alcohol. It is also possible to obtain reliable values correct within 1 to 2% of the dissolved calcium compound by using methyl red as indicator in the extracts diluted with ethyl alcohol. A correction for the calcium chloride formed during the titration can be applied as described in connection with the titration of the glycerol–ethyl alcohol extracts.

DISCUSSION OF RESULTS

A method in practical use should give sufficiently accurate results, be easy to handle experimentally, and not be too time-consuming. When several methods exist, their advantages and drawbacks must be taken into consideration from these points of view. The greatest degree of accuracy in the calcium determination which could be achieved in the experiments described above was obtained by conductometrical titration of the ethyl alcohol–diluted extracts. It is absolutely objective, without errors of the kind which occur, for example, in the indicator titrations. The best titration fluid used is sulfuric acid in a solution of ethyl or any other alcohol. The low solubility of the calcium sulfate formed increases the control of the end point of the reaction of organic calcium compound with the acid added, so that this point can be established very distinctly and the titration is very easy to perform. The titration can be applied in the majority of cases.

In the method where the substance is extracted with glycerol in the presence of an accelerator, the conductivity measurements are not suitable, however. The end point of the reaction must then be referred to the change of color of an indicator. Only a few indicators were examined in connection with this investigation, but a suitable one must be chosen carefully because its color change is dependent on the properties of the solvents. Some of those recommended in the literature do not meet the requirements, particularly when water is used for the dilution. The best indicator for the titration of the glycerol–ethyl alcohol extracts containing strontium nitrate seems to be methyl red, giving an end point very close to the theoretical one.

The extracts from the other methods can be titrated conductometrically very rapidly and accurately, but indicators may be used in these cases for approximate determinations and for following the reactions during the titration. The pH measurements cannot generally be used for direct determinations, as they do not give accurate values but only an orientation with regard to the end point.

The methods described are generally easy to perform. The extraction with glycerol without the accelerator seems to be more difficult and more time-consuming than the others. The extraction in the presence of the accelerator is preferred, if it can be shown that the accelerator does not influence the calcium silicate hydrates or aluminates considerably. However, it would appear to be advisable to titrate the filtered cooled extracts with a strong acid and methyl red as indicator, instead of titrating the boiling extract with ammonium acetate solution.

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Quantitative Determination of Isomers of *O,O*-Diethyl Ethylmercaptoethyl Thiophosphate

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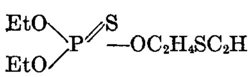
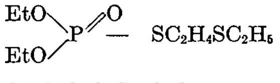
Pure *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate (I) prepared in the laboratory showed different physical and toxicological characteristics from those of the active constituent of the insecticide Systox, which is stated to be this compound. In view of the fact that many compounds of the S=P—O type—e.g., parathion—are known to be thermally unstable, it was presumed that the discrepancies were due to partial isomerization of the commercial product. Concurrent partition chromatography of a mixture of radioactive preparations (^{32}P) of pure *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate and *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate (II), with the active ingredient isolated from Systox demonstrates the presence of both I and II in Systox. It is also shown that I isomerizes to II on heating. A chromatographic method of analysis is described for determination of the two active ingredients in *O,O*-diethyl (*O,S*)-ethylmercaptoethyl thiophosphate preparations. The analytical identification technique described—concurrent partition chromatography of the unknown sample with radioactive components of established structure and purity—may find more general application, particularly to preparations of the S=P—O and O=P—S— types.

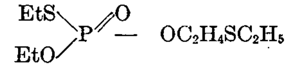
THE active constituent of the insecticide Systox (*3, 5, 20*) is stated to be *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate:



Systox, however, is virtually insoluble in water (*21*), which is inconsistent (*9*) with its systemic properties (*2, 11*); and its toxicity to insects and mammals (*5, 17, 20, 21*) was greater than the toxicity of *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate synthesized in these laboratories. Parathion (*O,O*-diethyl *O-p*-nitrophenyl thiophosphate) is known to isomerize to *O,S*-diethyl *O-p*-nitrophenyl thiophosphate on heating (*24*), and the reaction S=P—O— → O=P—S— appears to be fairly general (*6, 20*). Parathion normally contains a few per cent of the *S*-ethyl isomer, which is more toxic to mammals (*24*) than the parent compound and is a much more potent inhibitor of cholinesterase. Consequently, as a reliable method of analyzing parathion had not been worked out, much of the early work on the toxicity and cholinesterase-inhibiting power of parathion has been proved incorrect (*1, 4*). By analogy it was natural to suspect that Systox contained either or both of the two isomers



O,O-Diethyl *S*-ethylmercaptoethyl thiophosphate



O,S-Diethyl *O*-ethylmercaptoethyl thiophosphate

or even that the active constituents might consist wholly of these isomers; for the patents describing the methods of preparation (*7*) contain no proof of constitution, and the isomerization may go to completion under the reaction conditions used in manufacture. A method for determining the various isomers of Systox was therefore prerequisite of further work; the method should also distinguish between the methyl and ethyl homologs, for example, as a mixture of *O,O*-diethyl- and *O,O*-dimethyl *O-p*-nitrophenyl thiophosphates was for a time distributed by one concern as parathion (*14*), the name normally given only to the diethyl analog.

This paper describes the development of an analytical technique for determining the insecticidal constituents of Systox on lines which are of general application to the analysis of such mixtures.

O,O-DIETHYL *S*-ETHYLMERCAPTOETHYL THIOPHOSPHATE

Preparation. Diethoxychlorophosphine oxide, $(EtO)_2POCl$, was prepared from phosphorus trichloride containing the radioactive isotope of phosphorus, ^{32}P, by a modification of the method of McCombie, Saunders, and Stacey (*12*). 2-Hydroxyethylethyl sulfide, $HOC_2H_4SC_2H_5$, was prepared by the method of Meyer (*15*) from ethylene chlorohydrin and sodium mercaptide. The fractionated product was a typical alcohol, with a sulfur content, determined after sodium peroxide fusion, of 30.0%, against 30.2% theoretical. This was converted to 2-mercaptoethylethyl sulfide, $HS.C_2H_4SC_2H_5$, by the method of Frank and Smith (*8*). The —SH groups in the product after fractionation were determined by iodine titration (*22*), and gave a titer 98% of theory.

The sodium salt of the mercaptan was reacted with diethoxychlorophosphine oxide in toluene at 60° C. for 1 hour,

$$(EtO)_2POCl + NaSC_2H_4SC_2H_5 \longrightarrow (EtO)_2PO(SC_2H_4SC_2H_5) + NaCl$$

The product, washed twice with water, was fractionated; 107–110° C. at about 0.5 mm. Twenty grams of phosphorus trichloride yielded 10 grams of fractionated product. This contained a few per cent of phosphorus compounds, extractable by water from iso-octane, which seem to be produced by oxidation. These were removed by dissolving in iso-octane, washing twice with water, and sucking off as much iso-octane as possible at 100° C. under 1-mm. pressure. This product was used for further work. The photometric method of Sumner (*23*) showed it to contain 70% product, the remainder being iso-octane.

Proof of Structure. The product can be hydrolyzed in alkaline solution to give a high yield of 2-mercaptoethylethyl sulfide. Neither the *O-S*-diethyl *O*-ethylmercaptoethyl thiophosphate nor *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate can liberate this compound, whereas *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate can in principle yield either 2-mercapto- or 2-hydroxyethylethyl sulfide, according to which of the two bonds in the P–S–C system is broken during hydrolysis. The liberation of the mercapto compound is thus positive proof of structure, and incidentally shows which bond is broken. The test was so designed that ethyl mercaptan (ethanethiol) could be determined also, were it liberated, as this similarly would indicate the presence of the *O-S* diethyl *O*-ethylmercaptoethyl thiophosphate. The purity of the product was also tested chromatographically.

Liberation and Determination of Mercaptans. A weighed sample, approximately 1 gram, was hydrolyzed in 100 ml. of 1*N* sodium hydroxide overnight. The hydrolysis rate is rapid, 0.81 (OH^-) min.$^{-1}$ at 25° C., but was slowed somewhat in this instance by the presence of iso-octane, which provided a second phase in which *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate is markedly more soluble than in 1*N* sodium hydroxide. The solution was acidified to about pH 3 with concentrated hydrochloric acid, and extracted three times with 30 ml. of chloroform. Any mercaptans liberated by hydrolysis were now in chloroform solution. This was carefully fractionated, and the first 10 ml. collected in methanol. As ethyl mercaptan is much more volatile than chloroform, any ethyl mercaptan would appear in this fraction, while any 2-mercaptoethylethyl sulfide, boiling point 188° C., would remain in the residue. The residue was further concentrated to 10 ml.

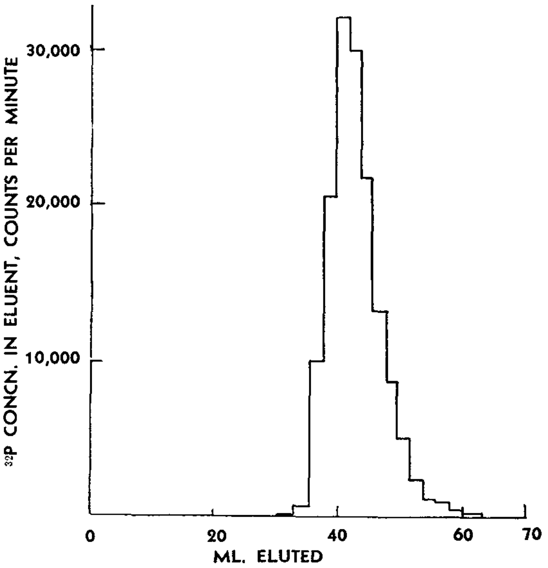


Figure 1. Chromatogram of *O-O*-Diethyl *S*-Ethylmercaptoethyl Thiophosphate

It was shown in a preliminary experiment that less than 5% of the 2-mercapto-compound would evaporate in this process. The —S—H groups in the distillate and residue were then determined by the iodometric method described by Siggia (*22*), with the modification that sufficient acetone-free methanol was introduced to homogenize the two-phase system produced by running standard iodine into chloroform. This modification was shown not to affect the results with pure thiol.

The distillate contained no mercaptan, but the residue contained high boiling point mercaptan equivalent to 94% of the thiophosphate present in the original sample. The discrepancy of 6% is doubtless partly accounted for by some evaporation during the concentration of the chloroform residues, but may also indicate that the S—C bond is ruptured to some extent during the hydrolysis.

Chromatographic Test of Purity. A small quantity of product in iso-octane was subjected to partition chromatography, using methanol–iso-octane (*18*) on kieselguhr (*13*). The iso-octane eluent was assayed for radioactivity in a Geiger-Müller liquid counting tube with the usual electronic high tension ond scalar setup. All the phosphorus appeared in one sharp band, with no sign that more than one compound was present. The chromatogram is reproduced in Figure 1.

These tests, therefore, proved the compound to be *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate.

O,O-DIETHYL *O*-ETHYLMERCAPTOETHYL THIOPHOSPHATE

This was prepared from thiophosphoryl chloride containing ^{32}P by the reactions:

$$PSCl_3 + 2NaOEt \longrightarrow (EtO)_2PSCl + 2NaCl \quad (1)$$

$$(EtO)_2PSCl + NaOC_2H_4SC_2H_5 \longrightarrow (EtO)_2PS(OC_2H_4SC_2H_5) + NaCl \quad (2)$$

The second reaction was performed by refluxing in xylene for 4 hours. After two water washings the product was fractionated: (boiling point 92° C. under about 0.5 mm. of mercury pressure) yield approximately 60%; on thiophosphoryl chloride.

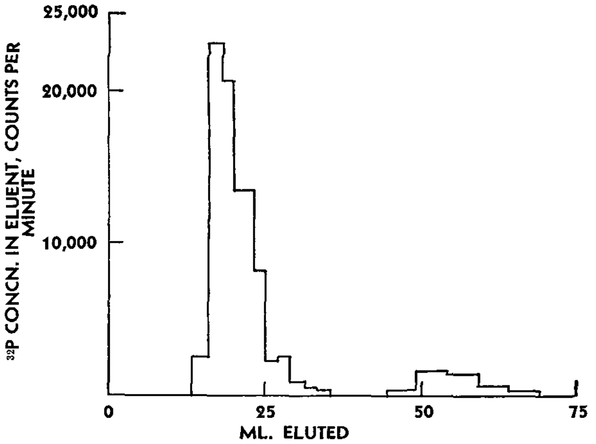


Figure 2. Chromatogram of *O-O*-Diethyl *O*-Ethylmercaptoethyl Thiophosphate

Proof of Structure. The product was very much less soluble in water, by a factor of about 100, than *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate. The *S*-ethyl isomer is likely to have physical properties similar to *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate, especially as regards solubility in water. An infrared spectrograph showed absorption in the wave-length band expected for P=S.

Chromatographic Test of Purity. A partition chromatogram, performed as above, showed only one major band, with a small subsidiary band, corresponding to about 5% of the total phosphorus, which was due to *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate. The chromatogram is reproduced in Figure 2. All the evidence therefore pointed to the product of Reaction 2 being *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate.

Isomerization of *O,O*-Diethyl *O*-Ethylmercaptoethyl Thiophosphate. It appeared likely that *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate would isomerize on heating. When some *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate was heated to 130° to 145° C. for 2.5 hours, and the product was hydrolyzed, it was found by the methods described earlier, that high boiling point mercaptan equivalent to 68% of the original sample was liberated, but no ethyl mercaptan could be detected. Only 78% of the sample after heating and prior to hydrolysis could be extracted from water by iso-octane, so under these conditions other reactions besides isomerization must occur. The production of high boiling-point mercaptan is not in itself a proof of the isomerization of *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate to *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate, as it may have been formed after the thermal decomposition of the parent substance. It was therefore necessary to confirm the result by some other method.

At this stage of the investigation a suitable method of chromatographic analysis had not been devised. A heated sample of *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate was therefore subjected to a sequence of successive partitioning (*10*) between iso-octane and 90% methanol–10% water (by volume), the partition ratios of the radioactive phosphorus being determined after each extraction. If only one compound is present, all the ratios will be the same; if two or more of different partition coefficients are present, the observed over-all partition ratios will change in a systematic fashion. From the changes observed, the quantities and partition coefficients of the individual compounds present can be estimated by arithmetical trial and error.

It was found that the partition ratios observed could be accounted for on the assumption that two compounds were present, 29.2% with an iso-octane–aqueous methanol partition ratio of

1.06, and 70.8% with one of 0.101. These compare with 1.08 and 0.101 determined independently for *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate and *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate, respectively. The small difference between 1.08 and 1.06 is not significant, as the *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate coefficient is highly sensitive to the proportion of water used in the methanol, and it is difficult to keep this precisely constant during the numerous transfers.

It is therefore established that *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate on heating is converted substantially to *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate. The errors in the observed partition coefficients should not exceed 2.5%. Some of the calculated values differ from the observed ones by more than this, and in a systematic fashion, suggesting that a small proportion of the activity, not exceeding 5%, is present in other compounds.

Experimental. A sample of pure radioactive *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate was heated to 125° to 130° C. for 3 hours. The product was dissolved in iso-octane, and the iso-octane extracted successively with an equal volume of 90% methanol–10% water (v./v.) mixture. The ^{32}P count in both layers was determined as before after each extraction. The first methanol extract was similarly extracted with iso-octane. The observed partition ratios, and those calculated for a mixture of 29.2% *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate and 70.8% *O,O*-diethyl S-ethylmercaptoethyl thiophosphate, are compared in Table I. The percentages and the iso-octane–aqueous methanol partition ratio of 1.06 for *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate were chosen as best fitting the observed results.

Table I. Partition Ratios for Heated *O,O*-Diethyl *O*-Ethylmercaptoethyl Thiophosphate between Aqueous Methanol and Iso-octane

No. of Extract	Partition Ratio, Iso-octane–Methanol: Iso-octane Extracted with 90 MeOH/10 H_2O v./v., Obsd.	Calcd.	First Methanol Extract Extracted with Iso-octane, Obsd.	Calcd.
1	0.274	0.274	4.94	4.96
2	0.593	0.629	6.11	6.34
3	0.92	0.94	7.47	7.55
4	1.03	1.04	8.05	8.47
5			8.85	9.10
6			9.39	9.44

PHYSICOCHEMICAL PROPERTIES OF *O,O*-DIETHYL *O*-ETHYLMERCAPTOETHYL AND *O,O*-DIETHYL *S*-ETHYLMERCAPTOETHYL THIOPHOSPHATE

Some typical physicochemical properties of the compounds were determined to aid later identification and in a search for methods of analysis. They are given in Table II.

PROOF OF CONSTITUTION OF SYSTOX

To show that the active ingredients of Systox are *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate and *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate, pure radioactive samples of *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate and *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate were chromatographed on a kieselguhr methanol–iso-octane column concurrently with a sample of the active ingredients of Systox separated from wetting agent. The radioactive phosphorus and total phosphorus in the eluent fractions were estimated. The method of separation from wetting agent and the method of estimating total phosphorus are given in detail below. As the quantities of the radioactive samples placed on the column were much smaller than the Systox sample, the total phosphorus was used to estimate the latter and the radioactivity the former. Two chromatograms were thus run simultaneously on the same column, and the identity in shape and position of the total phosphorus and radiophosphorus bands was evidence that the radioactive and nonradioactive compounds were the same.

Experimental. A column was prepared from 20 grams of kieselguhr (Hyflo Super-cel, Johns-Manville & Co., Artillery Row, London E. 13) mixed with 12 ml. of methanol, packed in 1.2-cm. tubing according to the method of Martin (*16*). Standard solutions of radioactive *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate, *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate, and the constituents of Systox freed from wetting agent were prepared in iso-octane, and mixed in known proportions. Then 1 ml. of the composite iso-octane solution was placed on the column. This aliquot contained 1.16 mg. of radioactive *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate, 0.26 mg. of radioactive *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate, and extracts from 20.36 mg. Systox. The last contained phosphorus equivalent to 48% *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate according to the authors' analysis, against the 50% stated on the label.

Table II. Properties of *O,O*-Diethyl *O*-Ethylmercaptoethyl Thiophosphate (*a*) and *O,O*-Diethyl *S*-Ethylmercaptoethyl Thiophosphate (*b*)

	(*a*)	(*b*)
Solubility in water	Very low	Circ. 2000 p.p.m.
Solubility in methanol	Misc.	Misc.
Partition ratios		
Iso-octane–water	Very large	Approx 15[a]
Chloroform–water	Very large	Very large
Cyclohexane–90% MeOH, 10% H_2O v./v.	1.63 (18° C.)	0.161 (18° C.)
Iso-octane–90% MeOH, 10% H_2O v./v.	1.08 (18° C.)	0.101 (18° C.)
Half life		
1*N* NaOH	75 min. (25° C. in 50% EtOH)	0.85 min. (25° C.
1*N* HCl	330 min. (84.5° C. in 50% EtOH)	550 min. (82.3° C.)

[a] The partition ratio of *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate between iso-octane and water proved difficult to measure, because the compound in the aqueous layer tended to stick to the vessel. Values ranging from 8 to 20 have been obtained by radiotracer assay, the lower values being found for very dilute solutions. It is probable that the partition coefficient is highly sensitive to concentration.

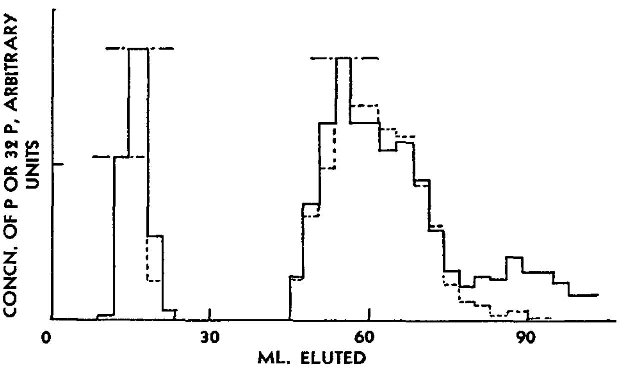


Figure 3. Chromatogram of Systox Plus Radioactive *O-O*-Diethyl *O*-Ethylmercaptoethyl Thiophosphate and *O-O*-Diethyl *S*-Ethylmercaptoethyl Thiophosphate

— Total phosphorus
- - - ^{32}P
—·—· Both indistinguishable

Total phosphorus and radioactive phosphorus were determined in 3-ml. eluent fractions. The errors on the former were about ±1.5% and of the latter less than ±3% (1000 counts total) except near the feet of the peaks, where the errors were greater, as the quantities were too small for accurate analysis. The chromatogram is reproduced in Figure 3. The concentration units are arbitrary, for it has been arranged that the radioactive and total phosphorus bands are of equal height at the points of highest concentration to bring out the similarities most clearly.

Interpretation of Chromatogram. The phosphorus recovered from the column totaled 1.27 mg., while 1.34 mg. were introduced. The peaks occur in the same position, and their shape is in fairly good agreement, especially for *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate. In both bands the ^{32}P falls off faster than the total phosphorus, owing to traces of impurity in the commercial product. This agreement is strong evidence, but not proof, that Systox is predominantly *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate and *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate. The solubilities of isomers containing P—S—C bonding are likely to be very similar, so that the chromatograph may not distinguish between *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate and the *O-S*-diethyl *O*-ethylmercaptoethyl thiophosphate. The substitution of methyl for ethyl groups changes the solubility heavily in favor of the polar phase, so, while a single chromatogram is not sufficient to prove the absence of the S-ethyl isomer, it is sufficient to show that the radioactive and commercial compounds do not differ in any other way. The subsidiary band appearing only on the total phosphorus chromatogram may be due to other such compounds, but the small proportion of total phosphorus this band represents suggests that it is due to an impurity accidental from the method of manufacture.

In the study of partition coefficients of Systox between several pairs of solvents, no pair suitable for partition chromatography was found dissimilar enough from methanol–iso-octane to lend any hope that the radioactive and nonradioactive peaks would be separated, even were they of different compounds. Further evidence had therefore to be adduced from other sources. The Bayer patents (*7*) associated with Systox describe reactions which are formally identical with those used by the authors to produce *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate and *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate, which have been shown to produce the stated products. This, combined with the chromatographic findings above, establishes with reasonable certainty the constitution of the insecticide.

ANALYSIS OF *O,O*-DIETHYL *S*-ETHYLMERCAPTOETHYL AND *O,O*-DIETHYL *O*-ETHYLMERCAPTOETHYL THIOPHOSPHATE

The analysis may be performed by a slight modification of the chromatographic technique, used for the identification of the phosphorus-containing compounds in Systox. The active ingredients are first separated from wetting agent and chromatogramed, and the eluent fractions analyzed for phosphorus.

Separation from Wetting Agent. The active ingredients of B8169 could be extracted by three extractions with isopropyl ether, the active compounds appearing, virtually free of wetting agent, in the ether. Systox samples cannot be separated in this way, but may be treated as follows:

Dilute with 10 volumes of water, shake the sample with 30 volumes of iso-octane, and centrifuge. Remove the iso-octane with a pipet, and extract successively with five more 30-volume quantities of iso-octane. Evaporate the iso-octane under vacuum to a volume suitable for chromatography. The iso-octane extract seems to be free of wetting agent. This stage is omitted when the material to be analyzed contains no wetting agent or other additive.

Chromatographic Analysis. Pack a column of 1.2-cm. diameter with 20 grams of kieselguhr mixed with 12 ml. of methanol under iso-octane. Run with iso-octane approximately $^4/_5$ saturated with methanol only. If the iso-octane is fully saturated, methanol tends to be squeezed from the column, and the bands are not so clear. Figure 3 shows that, for an approximate analysis, it is necessary to take only three fractions: one to contain the *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate, one between the bands to check the procedure, and a large final fraction. If an accurate analysis is required, it is necessary to take several eluent fractions on the descending side of the *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate band, to obtain separation from the minor ingredients which follow *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate through the column. The eluent fractions should be concentrated carefully to about 10 ml. and treated as below before proceeding to the analysis for phosphorus (*19, 23*).

Method of Estimating Fractions. REAGENTS. Bromine water; sulfuric acid, 7.5*N*; hydrogen peroxide, 100 volume; saturated sulfur dioxide water; ammonium molybdate, 66 grams per liter; ferrous sulfate, 5 grams of ferrous sulfate heptahydrate plus 1 ml. of 7.5*N* sulfuric acid to 50 ml., freshly prepared.

To the eluent fraction add 3 to 4 ml. of bromine water and 5 ml. of 7.5*N* sulfuric acid. Remove the iso-octane by careful evaporation on the hot plate, adding bromine water dropwise to maintain an excess. Continue the evaporation until fumes of sulfuric acid appear, cool, and add 2 to 3 drops of hydrogen peroxide to destroy any organic matter. Boil to decompose most of the hydrogen peroxide. Cool, dilute with water to about 10 ml., add a few drops of sulfur dioxide water, and boil to remove excess sulfur dioxide. Transfer the cooled solution to a standard 50-ml. flask, dilute to 40 ml., and add 5 ml. of ammonium molybdate with shaking, followed by 4 ml. of ferrous sulfate. After shaking, make up the solution to 50 ml., and measure absorption against a reagent blank on a spectrophotometer at 720 mμ. (A potassium dihydrogen phosphate calibration curve showed only slight deviation from linearity up to 80γ of phosphorus in 50 ml.)

DISCUSSION

The isomers of *O,O*-diethyl (*O,S*)-ethylmercaptoethyl thiophosphate have been separated and determined using partition chromatography. This type of technique is very useful for the analysis of preparations of the S=P—O— type, which are often thermally unstable and therefore extremely difficult to prepare in a state of high purity.

In particular, the technique of concurrent partition chromatography of the "unknown" sample with radioactive compounds of established structure and purity may find more general application in the identification of isomers of the S=P—O— and O=P—S— types.

Discussion. In analyses on commercial products Bayer 8169 and Systox were found to contain approximately 15% *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate, 15% *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate, and 18% *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate, 30% *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate, respectively. The presence of such high proportions of *O,O*-diethyl *S*-ethylmercaptoethyl thiophosphate, which is about ten times more toxic to mammals (*20*) and insects (*17*) than *O,O*-diethyl *O*-ethylmercaptoethyl thiophosphate, adequately explains the insecticidal and toxicological properties of these formulations.

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Determination of Zinc with Special Reference to Separation from Cobalt and Copper

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The extraction of zinc from citrate solutions at pH 5 to 10 with di-2-naphthylthiocarbazone and sodium diethyldithiocarbamate is described. At pH 9.8 negligible amounts of zinc are extractable with sodium diethyldithiocarbamate. In presence of both reagents, zinc is extracted practically entirely in the form of zinc di-2-naphthylthiocarbazone from pH 7 to 9.5. Extraction of copper as the complex of di-2-naphthylthiocarbazone or sodium diethyldithiocarbamate from pH 1 to 10 showed irregularities which were considered due to nonattainment of equilibrium conditions. Extraction of cobalt with di-2-naphthylthiocarbazone and sodium diethyldithiocarbamate demonstrated that equilibrium is attained very slowly with this metal. The use of sodium diethyldithiocarbamate is unnecessary in preventing the interference of copper and cobalt in the estimation of zinc. The implications of these findings are discussed and a tentative method for determining zinc in dilute digests containing sulfuric acid is described. Satisfactory recoveries of zinc (97 to 102%) can be effected from citrate solutions at pH 9.8 in the presence of copper, cobalt, iron, phosphate, and titanium and in combinations of these in concentrations likely to be encountered in soils.

THIS work was undertaken as part of a project designed to investigate the status of zinc in black soils on the Darling Downs, Queensland, as for some time certain pasture species have been showing symptoms characteristic of zinc deficiency (*1*). An essential prerequisite of this program was the development of a reliable method for the determination of total zinc in soils, based on a wet digestion procedure employing a sulfuric-perchloric acid mixture.

Colorimetric methods for zinc are usually based on dithizone (Dz) and more recently on di-2-naphthylthiocarbazone (Dn) (*5, 20*), despite the fact that many other transition metals interfere. Turbidimetric methods using diethyldithiocarbamate (*2*) or ferrocyanide (*4*) show a greater lack of specificity. The use of the dropping mercury electrode affords the most specific and sensitive method for zinc over a wide range of concentrations. With careful choice of the basal solution most interferences can be eliminated. This is a great advantage when concentration of metals as dithizone or di-2-naphthylthiocarbazone complexes into organic solvents is undertaken, a procedure which is known to be only weakly selective.

Cobalt is the most serious interference in the polarographic determination of zinc, since the half-wave potentials of these two metals are too close to permit accurate wave-height measurements. Although Prajzler (*18*) found that zinc could be determined polarographically in the presence of nickel, cobalt, and manganese by using a nearly saturated solution of ammonium oxalate as supporting electrolyte, trials conducted during the present study showed that the wave form was ill defined and difficult to measure with precision. Further work indicated that basal solutions composed of potassium citrate (acid or alkaline) plus fuchsin, neutral barium chloride plus gelatin, neutral calcium chloride plus methyl red, or the basal solution employed by Walkley (*22*) were unsatisfactory with regard to the successful resolution of the zinc and cobalt waves. In other respects, Walkley's solution is satisfactory as good separation of the zinc and nickel waves can be obtained.

Furthermore, both zinc and cobalt are extracted from ammoniacal citrate solutions with either dithizone or di-2-naphthylthiocarbazone. Cobalt must therefore be separated chemically from zinc before the final polarographic determination. This is particularly important in analyses of soils or rocks in which the content of cobalt may be of the same order as the zinc content. [Kidson, Askew, and Dixon (*12*) report a range of 0.3 to 41 p.p.m. in New Zealand soils.] The ratio of zinc to cobalt in plants is normally large enough to preclude great errors in wave-height measurements.

Holland and Ritchie (*8*) and Cowling and Miller (*7*) described procedures in which zinc could be determined in the presence of a number of other transition metals by the use of diethyldithiocarbamate (De) combined with dithizone. Cholak, Hubbard, and Burkey (*5*) modified the method of Cowling and Miller by employing di-2-naphthylthiocarbazone, which gives 100% recovery of zinc at pH 9 to 10 in a single extraction compared with 70 to 90% using dithizone. Quantitative extraction at high pH is important in the removal of zinc from silica (*22*).

However, the results of Cholak *et al.* for extraction of the zinc-dithizone complex exaggerate this difference, probably because of the high concentration of citrate present (0.286*M*) in the solutions. The data of Walkley (*21*) refer to recoveries from 0.05*M* citrate solutions, a concentration more usually employed. A further advantage of di-2-naphthylthiocarbazone is shown by its apparent insensitivity to ferric iron at high pH. Dithizone is partially oxidized by Fe(III) in alkaline citrate solutions according to Sandell (*19*), although it is claimed that hydroxylamine removes this effect by reduction of the carbodiazone.

The data of Cholak *et al.* indicate that interference by copper, mercury, lead, bismuth, and cobalt can be eliminated in the final zinc estimation. The extent to which cobalt can be removed is not clear from their results, although one polarogram obtained in their investigation failed to show the presence of this metal in the final solution. In view of the importance of suppressing cobalt and the possibility that a clean separation of copper could be obtained (enabling its separate determination) their results were examined in more detail.

As far as interference by cobalt and copper with recovery of zinc is concerned, the use of diethyldithiocarbamate and di-2-naphthylthiocarbazone together is based on the following considerations:

The di-2-naphthylthiocarbazone complex of zinc is formed in preference to the diethyldithiocarbamate complex and is unstable to dilute acid, the zinc appearing completely in the aqueous layer.

The complexes of cobalt and copper with diethyldithiocarbamate are formed in preference to those with di-2-naphthylthiocarbazone.

The complexes of cobalt and copper with diethyldithiocarbamate (but not necessarily those of di-2-naphthylthiocarbazone) are stable towards dilute acid.

EXPERIMENTAL

It was recognized that the concentration of citrate recommended by Cholak *et al.* was five to six times greater than that employed by Walkley. Marston and Dewey (*14*) have demonstrated in the case of cobalt that increasing citrate concentration markedly diminishes recovery, and Walkley (*23*) has observed a similar effect with regard to zinc. Preliminary experiments supported this conclusion. The final concentration of citrate was therefore limited to 1% as citric acid ($0.048M$).

In the recovery of zinc from the organic phase the procedure was further modified by omitting the washing of the di-2-naphthylthiocarbazone layer with water in order to reduce manipulations. Evaporation of the final hydrochloric acid layer disclosed small amounts of ammonium citrate which had been entrained in the chloroform phase; this was likely to occur in reduced quantities even after one water washing. This residue must vary in amount and thus cause variation in diffusion current. It was decided to submit the residue to wet oxidation with a nitric-sulfuric-perchloric acid mixture followed by ignition at 300° to 350° C. to remove the last traces of sulfuric acid. In this way the final zinc residue was obtained free from ammonium salts and traces of carbon arising from citric acid.

The final procedure was envisaged as involving a wet digestion of the soil sample, the diluted digest containing 2 ml. of concentrated sulfuric acid. In all recovery trials, therefore. this quantity of acid was present in a total volume of 100 ml.

Considerable difficulty was experienced at first in getting agreement between duplicates. This was caused partly by insufficient attention to the complete cleaning of glassware but also by the presence of traces of diethyldithiocarbamate in the final solution. Diethyldithiocarbamate appears to give a marked catalytic hydrogen wave starting in the region of −1.0 volt. Treatment of the evaporated chloroform solutions with the acid mixture was found to eliminate any of these traces, provided the digestion was slow and even. To offset possible contamination, the concentration of diethyldithiocarbamate employed was reduced to one tenth of that recommended by Cholak *et al.* Diethyldithiocarbamate and its immediate decomposition product were found to be fairly resistant to wet oxidation; one sample also contained traces of zinc, but this could probably be removed by repeated extraction of the aqueous solution with chloroform.

REAGENTS

All reagents, including distilled water, were purified by redistillation in borosilicate glass apparatus or recrystallization. All solutions were stored in borosilicate glass stoppered reagent bottles with covers. Dip pipets were employed throughout for reagent transfer.

Ammonium Hydroxide, approximately $10N$. Scrubbed cylinder ammonia was passed into 750 ml. of cold redistilled water in a flask surrounded by cracked ice until the volume of the solution reached 1000 ml. The final solution was standardized by titration before use.

Citrate Solutions, pH 1 to 10. Five grams of citric acid were dissolved in about 350 ml. of water containing 10 ml. of concentrated sulfuric acid. Ammonium hydroxide, $10N$, was added with stirring until the pH recorded was within 0.05 of the desired value. The solution was then cooled and diluted to 500 ml. Before use, each solution was extracted with di-2-naphthylthiocarbazone in chloroform until metal free, excess reagent removed with pure chloroform, and the last drop of solvent allowed to evaporate.

Chloroform was redistilled and stored in the refrigerator with addition of 1% absolute ethyl alcohol. Chloroform was recovered from residues by the method of Bambach and Burkey (*3*).

Standard Metal Solutions. Working standards containing 10γ of zinc per milliliter were prepared from a more concentrated solution of zinc sulfate containing 22 grams of zinc sulfate heptahydrate per liter of 0.5 v./v. sulfuric acid. The zinc content of this solution was checked gravimetrically as zinc pyrophosphate. Standards for cobalt and copper were obtained by dilution of solutions containing accurately weighed quantities of the metal sulfates.

Basal Solution ($0.1M$ ammonium chloride, $0.02M$ potassium thiocyanate, containing 0.0002% methyl red). This solution was prepared as described by Walkley (*21*).

Sodium Diethyldithiocarbamate. Aqueous solutions of convenient strength were made by dissolving the required quantity of Merck Pro Analysi reagent in distilled water, filtering, and diluting to volume. Reagent solutions were stored at 5° C. when not in use and discarded after 2 days.

Di-2-naphthylthiocarbazone. Synthesis of this compound, as described by Hubbard and Scott (*9*), is tedious, and the commercial product may be very impure. One proprietary sample proved only 18% pure; this caused low recoveries of zinc for some time before detection. Spectrophotometric determinations were made on three samples in chloroform solution using a Beckman spectrophotometer and 1-cm. cell. The absorption curve produced in each case was similar to that given by Cholak *et al.* with an absorption maximum at 650 mμ. Table I gives values for the molar absorptivity (ϵ) and purity, assuming the sample prepared in this laboratory was 100% pure.

Table I. Spectra of Di-2-Naphthylthiocarbazone Solutions in Chloroform

Sample	Concentration, Mg./Liter	ϵ	Purity, %
Commercial	10.0	8,440	18.5
Purified commercial	10.0	40,300	95.1
Synthetic specimen	10.8	45,690	100.0

Interpolated values from Cholak's curve give $\epsilon = 44{,}160$. It appears, therefore, that the synthetic specimen represents the purest that can be obtained by ordinary purification methods. Cooper and Kofron (*6*) have obtained larger yields of di-2-naphthylthiocarbazone by the use of dry ice and acetone in the final stages of purification with but little increase in ϵ. However, from results obtained by a modified reversion technique it is suggested the true value of ϵ is about 67,000.

In most of the succeeding trials, the di-2-naphthylthiocarbazone samples employed were purified by the method of Hubbard and Scott (*9*) by reprecipitation from chloroform solution with absolute ethyl alcohol at 5° C. An improved method of purification, analogous to that commonly used for dithizone, was employed in the later stages of this work. Extinction data were obtained on a crude sample of di-2-naphthylthiocarbazone by spectrophotometric measurement at 650 mμ, as described above, and its purity was calculated.

A weighed amount of the crude compound containing the required quantity of pure compound was dissolved in 8 to 10 ml. of 5% ethanolic potassium hydroxide in a 50-ml. beaker. The deep red solution was transferred to a 1000-ml. separatory funnel containing 500 ml. double-distilled water. The beaker was washed two or three times with ethyl alcohol and the washings added to the funnel. The solution, which appeared colloidal at this stage, was extracted several times with carbon tetrachloride to remove oxidation products and traces of heavy metals until the solvent layer became only faintly colored. The solution was then stripped of excess carbon tetrachloride with two 5-ml. portions of chloroform, acidified with purified $0.5N$ hydrochloric acid, and the precipitated di-2-naphthylthiocarbazone extracted into the required quantity of redistilled chloroform. The organic layer was then washed once with redistilled water and filtered through a dry 11-cm. Whatman No. 41 fluted filter paper into a reagent bottle.

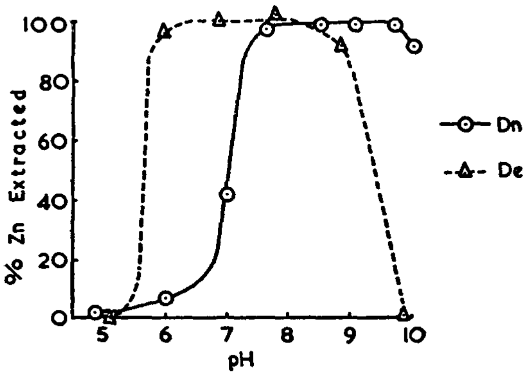

Figure 1. Extractability of Zinc with Di-2-naphthylthiocarbazone (Dn) and Diethyldithiocarbamate (De) at Various pH Values

Provided the chloroform is freshly distilled after standing over calcium oxide and anhydrous calcium chloride and that it contains 1% by volume of absolute ethyl alcohol, di-2-naphthylthiocarbazone solutions are stable for some weeks in a refrigerator without any apparent change. The use of chloroform which has stood at room temperature or in diffuse light, even in the presence of ethyl alcohol, results in rapid deterioration.

CLEANING OF GLASSWARE

All glassware used in the procedures was of borosilicate glass. Separatory funnels of 125-ml. capacity, with all but 0.5 inch of the lower stem removed, were completely dismantled, cleaned thoroughly to remove all traces of grease, and immersed overnight in chromic-sulfuric acid mixture. After thorough rinsing with hot water the components were steeped in $2N$ nitric acid, washed thoroughly with distilled water, and dried in an oven. After cooling and reassembly, each funnel was rinsed once with purified $0.5N$ hydrochloric acid and twice with redistilled water.

All other glassware was subjected to the same treatment. When in continuous use, rinsing with hydrochloric acid and redistilled water was found satisfactory before each day's determinations.

APPARATUS

Nearly all the polarographic data recorded were obtained by means of a Cambridge Voltamoscope. All measurements were undertaken in a constant temperature room at 25° C. Dissolved oxygen was removed by commercial nitrogen which was purified by passing the gas through $0.1M$ vanadous sulfate solution. The use of this reagent, described by Meites and Meites (*16*), provides a more convenient method than alkaline pyrogallol for deoxygenation.

Calibration of the dropping electrode in the range 5 to 300γ of zinc per milliliter of basal solution gave a mean value of i_D/C of 8.208 μa. per millimole per liter (standard error = 0.066) at 25° C. The electrode characteristics were: $t = 3.08$ seconds, $m^{2/3}t^{1/6} = 2.176$ mg.$^{2/3}$ sec.$^{-1/2}$ on open circuit.

EXTRACTION OF ZINC FROM CITRATE SOLUTIONS

Purified 100-ml. volumes of $0.048M$ citrate solutions, at different pH values and containing 2 ml. of concentrated sulfuric acid and 50γ of zinc, were extracted for 1 minute with 10-ml. portions of a solution of di-2-naphthylthiocarbazone in chloroform (200% theoretical excess). The organic layer was removed and each solution extracted with two 10-ml. portions of chloroform to remove excess reagent. In another series, extraction of the colorless zinc diethyldithiocarbamate complex at different pH values was carried out by a similar method, except that 5 mg. of diethyldithiocarbamate were present in the aqueous layer and pure chloroform was employed as extractant, using three separate 5-ml. portions of solvent and shaking each for 1 minute.

The combined di-2-naphthylthiocarbazone extracts were collected in beakers, evaporated to dryness, and submitted to wet oxidation with nitric-sulfuric-perchloric acid mixture before ignition at 350° C. The residue was treated with 1 ml. of basal solution and diffusion currents were measured. In the diethyldithiocarbamate series the extracts from each solution were combined and diluted to 25 ml., and zinc was determined in 10-ml. aliquots after oxidation and ignition. The aqueous citrate solutions from each series were removed from the funnels and their pH values determined with the glass electrode at 25° C.

Figure 1 shows the relation between pH and extractability of zinc. The di-2-naphthylthiocarbazone (Dn) curve is similar to that observed by Cholak *et al.*, except that recoveries in the region of pH 10 dropped significantly below 100% in spite of the lower citrate concentration employed. Further, recoveries at pH 6 to 7 were higher than those reported by these authors. Chloroform solutions of di-2-naphthylthiocarbazone impart a pink tinge to $0.1M$ citrate solutions at pH 10.8; the lowered recoveries at pH 10 may therefore be due to a significant increase in the solubility of the reagent in the aqueous phase at these high alkalinities. Cholak *et al.* found recovery of zinc unimpaired between pH 8.2 and 10.5. In the diethyldithiocarbamate series it is seen that quantitative extraction occurs within the narrow range of pH 6 to 9 while at pH 9.8 (the extraction pH suggested by Cholak *et al.*) extraction of zinc as the diethyldithiocarbamate complex is negligible.

RECOVERY OF ZINC IN PRESENCE OF BOTH REAGENTS

This series represents a combination of the two extraction techniques. Each citrate solution contained 5 mg. of diethyldithiocarbamate and 50γ of zinc; extraction was carried out by shaking with 10 ml. of the chloroform solution of di-2-naphthylthiocarbazone (200% theoretical excess) for 1 minute.

After separation of the phases, 1 ml. of solvent layer from each funnel was pipetted into a 50-ml. graduated flask and diluted to the mark with chloroform. Transmittances were recorded photometrically employing a 2-cm. cell and an Ilford No. 605 filter ($E_{\text{max.}} = 550$ mμ), and were compared with a standard curve. Further 5-ml. aliquots of the solvent layers containing the extracted zinc were evaporated, digested, and polarized as previously described. The final pH value of each aqueous layer was also recorded.

The standard absorption curve for zinc di-2-naphthylthiocarbazone was prepared as follows. Twenty-five milliliters of di-2-naphthylthiocarbazone solution were used to extract a citrate solution at pH 8.0 containing a threefold excess of zinc. The red organic layer was transferred to another funnel, washed three times with redistilled water, and filtered through a dry No. 41 Whatman filter paper into a clean beaker. Suitable volumes of this solution were transferred to a set of 10-ml. stoppered measuring cylinders and diluted to the 10-ml. mark with original di-2-naphthylthiocarbazone solution. Each solution was mixed thoroughly, 1 ml. withdrawn for photometric reading after dilution to 50 ml., and a further 5 ml. taken for polarographic zinc determination. A standard curve relating light absorbance with zinc content was then prepared. The same solution was used for the standard curve and the extraction of zinc in presence of diethyldithiocarbamate so that the photometric readings are directly comparable with the polarographic data.

Table II gives details of the recoveries of zinc extracted in both forms. Errors in the colorimetric determination are likely to be high because of the large dilution factor and the shallow slope of the calibration curve. Nevertheless it is apparent that from pH 8.5 to 10.0 zinc is present in the extract wholly as the di-2-naphthylthiocarbazone complex. It is noteworthy that in the range pH 6 to 7 extraction of the zinc complex is enhanced by the presence of diethyldithiocarbamate (compare Table II and Figure 1). Later work confirmed this effect (which was variable but consistently positive) and demonstrated qualitatively that the proportion of zinc present as the di-2-naphthylthiocarbazone increased with time of contact with aqueous citrate solutions at about pH 6. It is possible that the extractability curve in Figure 1 for the complex does not represent true equilibrium and that in the presence of both reagents, diethyldithiocarbamate affords a bridge whereby zinc, extracted mainly as zinc diethyldithiocarbamate into the chloroform phase, participates in an exchange reaction in which the metal is transferred to the di-2-naphthylthiocarbazone molecule.

RECOVERY OF COPPER IN PRESENCE OF DIETHYLDITHIOCARBAMATE AND OF DI-2-NAPHTHYLTHIOCARBAZONE

The procedure followed closely that described for zinc. Twenty micrograms of copper were added to the citrate solutions; 2 mg. of diethyldithiocarbamate only were added in one series to give a comparable excess of this reagent; in the other series a 200% excess of di-2-naphthylthiocarbazone was employed. After evaporation and oxidation of the extracts the copper residues, in presence of traces of sulfuric acid, were diluted and transferred to 25-ml. stoppered measuring cylinders. Copper was estimated as the diethyldithiocarbamate complex in ammoniacal solution; the complex was shaken into 10 ml. of 3-methyl-1-butanol (isoamyl alcohol) before absorptiometric measurement at 430 mμ (Ilford No. 601 filter, 2-cm. cell). Copper estimations were evaluated from a standard curve.

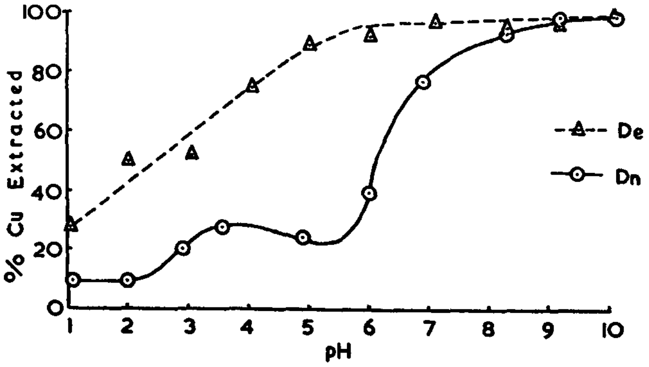

Figure 2. Extractability of Copper with Di-2-naphthylthiocarbazone (Dn) and Diethyldithiocarbamate (De) at Various pH Values

The extraction of copper as diethyldithiocarbamate complex at pH 1 to 5 was at first found to be low and variable. Repeated determinations were made in this pH range under conditions which minimized the decomposition of the complex—i.e., addition of metal before reagent and determination of copper directly on the chloroform extracts. The results from this series have been incorporated into the diethyldithiocarbamate curve in Figure 2, which shows the relation between pH and extractability of both complexes.

The shapes of the curves suggest that true equilibrium had not been established, particularly in the case of di-2-naphthylthiocarbazone. The position is further complicated in the case of diethyldithiocarbamate in that other data (*15*) show that it is unstable in acid solution and the rate of its decomposition is inversely proportional to pH. The amount of copper diethyldithiocarbamate extracted between pH 1 to 6 represents a balance between rate of chelation and decomposition of reagent, and it is not possible to predict which complex is likely to be preferentially formed by comparison of the two curves. Owing to the close proximity of the absorbance maxima of the copper complexes, the measurement of the proportion of each by a method similar to that employed for zinc would be difficult.

RECOVERY OF COBALT IN PRESENCE OF DIETHYLDITHIOCARBAMATE AND OF DI-2-NAPHTHYLTHIOCARBAZONE

Twenty micrograms of cobalt were extracted from citrate solutions into di-2-naphthylthiocarbazone and the extracted metal was determined by a modified method of Pickering and described by Piper (*17*). Recoveries after 1-minute shaking were low at pH values from 5 to 8, rising to 67% at pH 9 and 10.

A further series of recoveries at pH 10.0 using a mechanical shaker for increasing periods, showed that a time factor is involved in the extraction of cobalt similar to that observed by Marston and Dewey (*14*) for dithizone. Recovery of 20γ of cobalt reached 85% in 5 minutes and was virtually complete beyond 30 minutes.

Table II. Extraction of Zinc from Citrate Solutions in Presence of Both Diethyldithiocarbamate (De) and Di-2-naphthylthiocarbazone (Dn)

Final pH	Total Zinc Extracted, γ[a]	Zinc Extracted as $ZnDn_2$, γ[b]	Fraction of Total Zinc Extracted as $ZnDn_2$
5.09	2.4	3.6	...
5.60	23.3	7.2	0.31
6.16	104.0	19.2	0.18
6.98	95.6	50.4	0.53
7.53	99.6	72.0	0.72
7.91	100.8	80.0	0.80
8.53	105.2	98.4	0.93
8.99	Not determined	100.0	...
9.51	100.4	100.0	1.00
9.95	90.8	96.8	1.07[c]

[a] Determined polarographically.

[b] Determined colorimetrically.

[c] High value associated with limitations to accuracy of colorimetric determination.

The extraction of cobalt by the two reagents separately at three pH values was investigated, using a shaking period of 2 hours; the solutions were contained in stoppered reagent bottles. At pH 9 to 10 complete recovery of cobalt (20γ) was obtained with di-2-naphthylthiocarbazone but only 20 to 30% with diethyldithiocarbamate; recoveries were rather variable but it appears that the cobalt complex of the former may be preferentially formed at pH 9 to 10. However, qualitative tests performed on citrate solutions at pH 9.8 containing 50 γ of cobalt, in the presence and absence of diethyldithiocarbamate showed that the latter markedly depresses the reddish purple color imparted to the organic phase.

This observation, which qualitatively confirms those of Cholak *et al.*, conflicts with the tentative conclusion reached from preceding results that the di-2-naphthylthiocarbazone complex is more stable. The possibility of exchange reactions occurring within the organic phase, discussed above in connection with the extraction of zinc, must not be overlooked and seems likely to be facilitated by entrainment of aqueous phase in the chloroform layer.

STABILITY OF COBALT AND COPPER COMPLEXES OF BOTH REAGENTS TO DILUTE ACID

Twenty milligrams of di-2-naphthylthiocarbazone (ϵ = 44,000, approx.) were dissolved in about 80 ml. of chloroform and shaken with previously purified citrate solution at pH 8.5 containing 200γ cobalt or copper. The organic phase was removed and washed three times with redistilled water, then filtered through a dry Whatman No. 41 paper into a dry 100-ml. flask, and diluted to the mark with chloroform.

Two-hundred-microgram quantities of cobalt or copper in 100 ml. of previously purified citrate solution at pH 8.5 containing 2.7 mg. of diethyldithiocarbamate (200% theoretical excess) were shaken with chloroform, which was washed, filtered, and diluted to 100 ml. as before.

Ten-milliliter portions of the chloroform solution of each complex were shaken with 50 ml. of purified dilute hydrochloric acid of increasing strength for 90 seconds in 125-ml. separatory funnels. The organic layer was removed and the acid layer stripped of excess reagent by washing with three successive 2-ml. portions of chloroform. The acid extracts were transferred quantitatively to 100-ml. glass evaporating dishes and evaporated to low bulk on a waterbath. Copper was determined with diethyldithiocarbamate and cobalt with nitroso-R-salt, as previously described. Copper and cobalt contents of the original chloroform solutions were determined prior to the stability determinations.

Results are shown in Table III; they are the means of closely agreeing duplicate determinations.

The removal of cobalt from chloroform solutions of either complex is negligible and independent of hydrochloric acid concentration up to 1.0*N*. The copper complexes are more affected by acid. The di-2-naphthylthiocarbazone complex is relatively stable against acid concentrations up to 0.8*N*, but the diethyldithiocarbamate complex shows significant decomposition with even 0.2*N* acid, although in no case did the amount of copper extracted exceed 5%.

A further check was performed on the stability of the cobalt complex of di-2-naphthylthiocarbazone by comparing a polarogram of a decomposed chloroform solution containing known amounts of zinc and cobalt di-2-naphthylthiocarbazones with that of the corresponding evaporated 0.2*N* hydrochloric acid extract of these solutions. Figure 3 shows one pair of polarograms so obtained and demonstrates the very sharp cutoff of cobalt. The recovery of zinc in this instance was unimpaired.

Table III. Stability of Di-2-naphthylthiocarbazone (Dn) and Diethyldithiocarbamate (De) Complexes of Cobalt and Copper to Dilute Hydrochloric Acid

Complex	Normality of HCl	Metal Added as Complex, γ	Metal Recovered in HCl, γ	Recovery, %
CoDn_2	0.19	15.1	0.0	0
	0.39	15.1	0.15	1.0
	0.58	15.1	0.05	0.3
	0.78	15.1	0.0	0
	0.97	15.1	0.0	0
CoDe_2	0.19	13.8	0.05	0.3
	0.39	13.8	0.0	0
	0.58	13.8	0.15	1.1
	0.78	13.8	0.4	2.9
	0.97	13.8	0.0	0
CuDn_2	0.19	34.8	0.35	1.0
	0.39	34.8	0.0	0
	0.58	34.8	0.35	1.0
	0.78	34.8	0.6	1.7
	0.97	34.8	1.4	4.0
CuDe_2	0.19	22.5	1.0	4.4
	0.39	22.5	0.85	3.8
	0.58	22.5	0.6	2.7
	0.78	22.5	0.75	3.3
	0.97	22.5	1.1	4.2

CONCLUSIONS

It is apparent that the use of diethyldithiocarbamate is superfluous in the prevention of interference by cobalt and copper in the determination of zinc. Not only is the di-2-naphthylthiocarbazone complex of zinc formed in preference to the diethyldithiocarbamate complex at pH 9.8, but the di-2-naphthylthiocarbazone complexes of cobalt and copper are reasonably stable to an acid concentration up to 1.0*N* so that they are not reextracted along with the zinc on shaking with dilute acid. One of the factors determining whether either of the complexes of copper and cobalt will be preferentially formed in citrate solution is the initial concentration of diethyldithiocarbamate since the concentration of di-2-naphthylthiocarbazone in the aqueous phase is likely to be small but constant because of its relative insolubility in water. The results presented do not represent equilibrium conditions as defined by Irving and Williams (*10*), but they have the advantage of analytical application to the present problem. Further work would be needed to verify the conclusions of Cowling and Miller in regard to the proportions of zinc extracted as dithizone or diethyldithiocarbamate complex. However, by analogy with the present results it is likely that the total zinc extracted under their conditions is unaffected, although that present as zinc dithizone is probably dependent on extraction pH.

These considerations demonstrate the danger of the indiscriminate use of the concept of competitive complex formation in eliminating interferences. The work of Irving *et al.* and of Maley and Mellor (*13*) have shown certain regularities in the stability constants of the complexes of one ligand with different metals and of those of different ligands with one metal. Thus if, under given conditions, di-2-naphthylthiocarbazone complexes of a metal are more stable than those of diethyldithiocarbamate, this property will be manifest whatever metal is employed, unless steric hindrance or other side effects alter the value of the stability constant—e.g., cobalt with the nitrosonaphthols (*11*).

The results of this investigation do not necessarily invalidate those of Cholak *et al.*, since these workers employed a tenfold increase in diethyldithiocarbamate concentration. According to the work of Irving and Williams, this would shift the pH of 50% extractability down one unit and increase the stability-constant of the diethyldithiocarbamate complex; these considerations would not, however, affect the subsequent behavior of the complex towards dilute acid.

TENTATIVE METHOD FOR DETERMINATION OF ZINC IN DILUTED DIGESTS IN PRESENCE OF COPPER AND COBALT

A tentative method for the determination of zinc in acid extracts has been devised. The extracts investigated corresponded to those obtained from the digestion of soil with sulfuric acid or from the oxidation of biological material by wet digestion. and the method is therefore likely to have wide application. Certain difficulties are inherent in the quantitative separation of zinc from acid digests of soils; one of the main sources of interference may be silica. Results presented below, however, show that interference from some common micro and macro constituents is likely to be small.

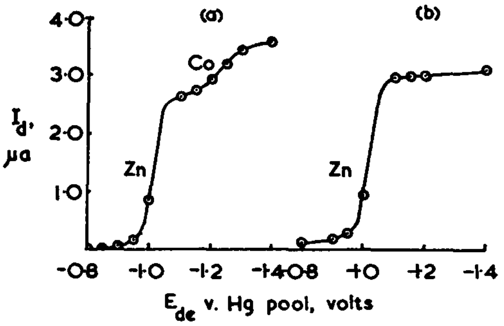

Figure 3. Polarograms of Residue

From (*a*) di-2-naphthylthiocarbazone (Dn) solution containing zinc and cobalt complexes of di-2-naphthylthiocarbazone and (*b*) 0.2*N* hydrochloric acid extract of this solution

Reagents. The following solutions are required in addition to those already described.

HYDROCHLORIC ACID, 0.5*N*. Dilute 82 ml of constant-boiling hydrochloric acid, which has been redistilled, to 1000 ml. with redistilled water. It is not necessary to standardize this solution.

DILUTE NITRIC ACID. Dilute 150 ml. of concentrated redistilled nitric acid to 250 ml. with redistilled water.

DI-2-NAPHTHYLTHIOCARBAZONE REAGENT. A concentration of 250 mg. per liter ($\epsilon \sim 45{,}000$ at 650 mμ) in chloroform was found to be convenient for extraction of 50- to 100-γ quantities of zinc.

AMMONIUM CITRATE. Dissolve 20 grams of recrystallized or analytical reagent citric acid in redistilled water, add 400 ml. of 10*N* ammonium hydroxide, cool, and dilute to 1000 ml. This solution has a pH value of about 10.8. Immediately before use, transfer the required quantity of citrate solution to a 1000-ml. separatory funnel and extract repeatedly with a solution of di-2-naphthylthiocarbazone in chloroform until the original color of the reagent is maintained. Fifty milliliters of ammonium citrate solution, when diluted to 100 ml. with water containing 2 ml. of concentrated sulfuric acid, give a 0.048*M* solution in citric acid with a pH of 9.8. The use of thymolsulfonephthalein to indicate the correct final pH was not found satisfactory.

Procedure. Transfer quantitatively the solution or digest, containing 2 ml. of concentrated sulfuric acid, to a 125-ml. separatory funnel and dilute the solution and washings to about 50 ml. Add 50 ml. of 0.096*M* ammonium citrate solution (pH 10.8), mix thoroughly, and allow to cool. Add 5 ml. of di-2-naphthylthiocarbazone reagent, shake for 1 minute, allow the phases to separate, and draw off the solvent layer into a second 125-ml. funnel. Continue extraction with the reagent until the organic phase retains its original color on two successive extractions. Strip excess reagent from the solution by extracting with 2 ml. of chloroform and transfer the washing to the second funnel.

Add 20 ml. of 0.5*N* hydrochloric acid to the contents of the second funnel and extract the zinc from the solvent layer by shaking vigorously for 1 minute. Allow the phases to separate and reject the lower layer. Strip the acid extract with two successive 2-ml. portions of chloroform, again rejecting the organic phase. Keep the chloroform extracts for subsequent recovery of solvent.

Transfer the acid extract quantitatively to a 50-ml. tall-shaped beaker (or preferably a 50-ml. weighing bottle with an externally ground cap), using 20 ml. of redistilled water for transfer. Evaporate the solution to low bulk over a hot plate and complete the evaporation to dryness on a water bath. Cool, add 5 ml. of dilute nitric acid, and heat gently over a water bath until the reaction subsides. Add 2 drops of concentrated sulfuric acid and 0.5 ml. of 72% perchloric acid and evaporate to dryness on a hot plate. When perchloric acid fumes have disappeared, remove the last traces of sulfuric acid from the beaker by igniting at 300° to 350° C. under an aluminum shield in a muffle furnace.

To the cooled residue in the beaker, add 1 ml. of basal solution (0.1*M* ammonium chloride, 0.02*M* potassium thiocyanate, 0.0002% methyl red), allow the zinc salts to dissolve, transfer to a polarizing cell, and measure the diffusion current between −0.8 and −1.3 volts *vs.* SCE.

RESULTS

Recoveries of Zinc from Citrate Solutions with Di-2-naphthylthiocarbazone. Quantities of zinc ranging from 10 to 300γ were added to purified citrate solutions at pH 9.8. The metal was extracted with di-2-naphthylthiocarbazone in chloroform, removed from the solvent layer, and determined as described. The mean recovery of zinc under these conditions was 98.8% (standard error = 0.80).

Recoveries of Zinc in Presence of Other Substances. Several series of recovery tests were carried out in presence of copper, cobalt, and iron in quantities likely to be present in soils. Further recoveries were also made in presence of iron, titanium, and phosphorus, since it was considered likely that precipitated iron or titanium phosphates might occlude zinc. It was found that in the presence of 80 mg. of titanium dioxide, 10 mg. of iron, and 10 mg. of phosphorus no precipitate was formed, indicating that sufficient citrate was present to coordinate with the iron and titanium.

Recoveries in presence of calcium sulfate were also attempted, since many workers attribute low recoveries of trace metals to absorption on this material. Recoveries in presence of calcium sulfate were carried out by adding known amounts of zinc to 0.1 to 0.3-gram quantities of precipitated calcium carbonate and submitting the suspension to a wet digestion with 2 ml. of concentrated sulfuric acid before transferring to the funnels for extraction. Table IV gives a summary of the results of this series of tests.

Table IV. Recovery of Known Amounts of Zinc in Presence of Copper, Iron, Cobalt, Titanium Dioxide, and Phosphate

Zn Added, γ	Other Elements Present	Zn Recovered (Less Blank), γ	Recovery, %
50	. . .	48.3	96.6
50	Cu, 32 γ	49.1	98.2
50	Cu, 64 γ	49.2	98.4
20	Fe, 5 mg.	19.3	96.5
20	Fe, 50 mg.	19.8	99.0
20	Fe, 100 mg.	20.5	102.5
50	Co, 50 γ + Cu, 50 γ	51.2	102.4
50	Co, 100 γ + Cu, 100 γ	51.0	102.0
50	Fe, 10 mg. + P, 2 mg.	50.8	101.6
50	Fe, 10 mg. + P, 10 mg.	48.6	97.1
50	TiO_2, 20 mg.	48.7	97.3
50	TiO_2, 20 mg. + P, 2 mg.	49.3	98.6
50	TiO_2, 80 mg. + Fe, 10 mg. + P, 10 mg.	51.1	102.2
50	$CaSO_4$, 136 mg.	47.6	95.2
50	$CaSO_4$, 272 mg.	46.4	92.8
50	$CaSO_4$, 408 mg.	48.1	96.2

Assuming that 1 gram of air-dried soil is employed in the digestion, it is apparent that the recovery of zinc is satisfactory in the presence of maximum amounts of possible interferences. The recovery in the presence of calcium sulfate is variable but higher than expected. The suspensions of calcium sulfate were allowed to stand overnight with crystallization, and it is likely that higher recoveries would be obtained if digests containing this substance were extracted immediately.

ACKNOWLEDGMENT

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Reactions of Aromatic Nitro Compounds with Active Methyl, Methylene, Methine Groups in Presence of Base

Determination of Creatinine with Alkaline 3,5-Dinitrobenzoate

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Utilization of alkaline sodium 3,5-dinitrobenzoate for the colorimetric determination of creatinine has been limited, owing to poor stability of the reaction color. The creatinine–sodium 3,5-dinitrobenzoate reaction was therefore studied extensively for conditions of optimum sensitivity and color stability. Greater sensitivity with decreased stability was obtained with progressively increasing concentrations of sodium 3,5-dinitrobenzoate or sodium hydroxide. Maximum stability was observed at pH values up to 11.7 in 0.025*M* sodium 3,5-dinitrobenzoate, applicable to creatinine concentrations of the order of $1 \times 10^{-3}M$. Maximum sensitivity and minimum stability were found at approximately 0.2*M* sodium hydroxide and 0.2*M* sodium 3,5-dinitrobenzoate, applicable to creatinine concentrations approximating $1 \times 10^{-5}M$. A method which provides a suitable compromise between the extremes of instability and sensitivity has been developed. With 0.025*M* sodium 3,5-dinitrobenzoate and 0.025*M* sodium hydroxide Beer's law is obeyed in the range 1×10^{-4} to $1 \times 10^{-3}M$ creatinine. The error of the procedure is less than 1%. The method can be extended to other nucleophilic substances, particularly ketones.

DURING an investigation of the color reaction between creatinine and sodium 3,5-dinitrobenzoate (NaDNB), which had been discovered independently in 1936 by three groups of investigators (*1*, *2*, *10*), it became apparent that the reaction represented a specific instance of a very general reaction involving aromatic nitro compounds on one hand and compounds containing methyl, methylene, or more rarely, methine groups adjacent to electronegative groups, on the other. For the elucidation of the creatinine–sodium 3,5-dinitrobenzoate reaction, therefore, a broader study, designed to include the various types of compounds capable of such reactions with each other, was undertaken. The present paper deals with the application of this general reaction to the determination of creatinine. A more detailed treatment is to be found in the author's thesis (*3*).

In 1886 Jaffe (*9*) discovered that orange to red colors are produced upon the addition of creatinine to an alkaline solution of sodium picrate. This reaction was utilized by Folin (*6*, *7*), and later by many others, for the quantitative estimation of creatinine. Although the alkaline picrate solution is probably the most sensitive known reagent for creatinine, it is not completely satisfactory. It does not meet the requirements for a system suitable for photometric analysis. Because the absorbance of the reagent blank approaches that of the test solution in the vicinity of the absorption maximum (*5*), the wave length usually employed for absorbance readings lies in a rather steep region near the foot of the absorption curve. For satisfactory results, a very narrow wave band must be employed. Even at 510 mμ, the absorbance of the reagent itself is greater than desired. Considerable error may result when small amounts of creatinine are to be determined. The concentration of alkali is fairly critical (*4*)—when higher concentrations of alkali are used, the absorbances of the reagent blanks increase proportionately, while the absorbances of test solutions, corrected for blank readings, decrease.

An alkaline solution of sodium 3,5-dinitrobenzoate was proposed as a new reagent for the determination of creatinine by Bolliger (*2*), Benedict and Behre (*1*), and Langley and Evans (*10*). Attractive features are the production of a brilliant garnet red color in the presence of creatinine, coupled with a practically colorless solution in its absence. Unfortunately, neither the intensity nor the hue of the developed color could be adequately stabilized. Lehnartz (*11*) attempted to minimize these undesirable color changes by employing sodium acetate to lower the effective alkalinity due to sodium hydroxide. Although Lehnartz reports that by means of his modified technique the absorbance remains constant over a period of 28 minutes, the present author observed a regular decrease in absorbance of the order of 2% per minute during the first 30 minutes, when the procedure outlined by Lehnartz was followed.

In view of the potential application of this reaction to the analysis of a wide variety of carbonyl compounds, particularly aldehydes and ketones, it was decided to investigate the nature of the color or colors produced and to determine the most suitable set of conditions for the quantitative determination of creatinine. Essentially the same factors are responsible for the production of initial and secondary colors with creatinine and with other carbonyl compounds, when alkaline 3,5-dinitrobenzoate is used as the reagent.

COLORS PRODUCED BY MIXTURES OF SODIUM 3,5-DINITROBENZOATE AND CREATININE WITH SODIUM HYDROXIDE

The rates of development and of fading of the initial violet-red color were found to vary with the concentrations of each of the three reactants. In the following typical experiment qualitative observations of color were made in the presence of fixed concentrations of creatinine (0.007*M*) and 3,5-dinitrobenzoate (0.007*M*) while the concentration of sodium hydroxide was varied from 0.006 to 0.800*M*.

In the presence of low concentrations of sodium hydroxide the typical violet-red color develops slowly, reaches a comparatively low maximum intensity, and is reasonably stable. Upon acidification with mineral acid, these solutions become colorless. With progressively increasing concentrations of sodium hydroxide the initial color is deeper and develops more rapidly, but it is correspondingly less stable and changes continuously towards orange or yellow colors. At least two components appear to be responsible for the color of these solutions—the violet-red addition product, which becomes colorless upon acidification, and a yellow component which is not decolorized in acid solution. It is apparent that the attainment of a color of suitable intensity and stability for photometric analysis must involve a compromise between high concentration of sodium hydroxide, high intensity, and poor stability of color on one hand, and low concentration of sodium hydroxide, low intensity, and considerable sta-

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bility of color, on the other. A similar effect was observed when varying concentrations of sodium 3,5-dinitrobenzoate were used in the presence of fixed concentrations of sodium hydroxide and creatinine.

ABSORPTION SPECTRUM OF ADDITION PRODUCT

In order to determine the optimum wave length for photometric analysis of the colored addition product, conditions permitting a sufficient degree of color stability over a reasonable period of time were ascertained by preliminary trials and incorporated in the following procedure.

Into each of two cuvettes there was transferred 53 mg. (0.25 millimole) of 3,5-dinitrobenzoic acid, which was then dissolved in 5 ml. of 0.100*M* sodium hydroxide solution. To one tube (blank) 5 ml. of water was added. To the other tube 0.5 ml. of an 0.01*M* creatinine solution and 4.5 ml. of water were added. Absorbance readings were taken at various wave lengths, beginning at 400 mμ 37 minutes after the reactants were mixed and ending at 700 mμ 61 minutes after mixing.

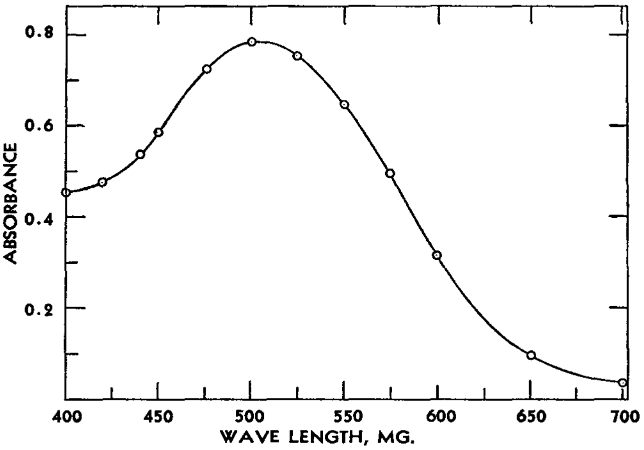

Figure 1. Absorption Spectrum of Creatinine-Dinitrobenzoate Complex

$5.0 \times 10^{-4}M$ creatinine, $2.5 \times 10^{-2}M$ NaDNB, $2.5 \times 10^{-2}M$ NaOH, Coleman Junior spectrophotometer, 20-mm. cuvette

The results, corrected for blank readings, are shown in Figure 1. A broad maximum in the vicinity of 500 mμ is clearly evident. Absorbance readings obtained after a shorter waiting period reveal a maximum at a slightly higher wave length (approximately 510 mμ), whereas a maximum is obtained at a slightly lower wave length when later readings are taken. The absorbance of the blank solution is negligible at 500 mμ.

RATE OF COLOR DEVELOPMENT

As a reasonably stable color is required over a sufficient period, the variation of absorbance at 500 mμ with respect to time was studied, employing various concentrations of sodium dinitrobenzoate and sodium hydroxide.

In the first experiment, 53 mg. of 3,5-dinitrobenzoic acid, contained in each of two cuvettes, was dissolved in 5 ml. of 0.100*M* sodium hydroxide solution, 5 ml. of water was added to the blank tube, and 4.5 ml. of water, followed by 0.5 ml. of an 0.01*M* solution of creatinine, was added to the other. The solutions were mixed at once and absorbance readings of the test solution, corrected for blank readings, were taken at fixed intervals. In the second experiment, 5 ml. of 1.00*M* sodium hydroxide solution was used in place of the 0.100*M* solution.

The curves in Figure 2 show that the conditions of experiment 1 are better suited to the desired objective. Curve I is characterized by a fairly flat region in which the absorbance remains reasonably constant for approximately 60 minutes. Obviously the conditions of Experiment 2 are unsuitable. The maximum of curve II is considerably higher than that of curve I. Despite the presence of a fiftyfold excess of sodium hydroxide over creatinine in experiment 1, further addition of sodium hydroxide results in an even higher absorbance. Similar experiments, not presented here, show that the same effect is produced by increasing the concentration of sodium dinitrobenzoate in the presence of a fixed concentration of sodium hydroxide. Obviously, the reaction leading to the production of the colored addition product is not a complete one.

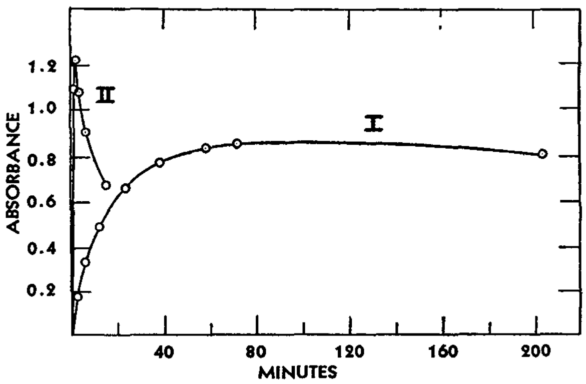

Figure 2. Rate of Color Development in Presence of Large Excesses of Sodium Dinitrobenzoate and Sodium Hydroxide

λ = 500 mμ, Coleman Junior spectrophotometer, 20-mm. cuvettes

I. $5.0 \times 10^{-4}M$ creatinine, $2.5 \times 10^{-2}M$ NaDNB, $2.5 \times 10^{-2}M$ NaOH

II. $5.0 \times 10^{-4}M$ creatinine, $2.5 \times 10^{-2}M$ NaDNB, $4.75 \times 10^{-1}M$ NaOH

ABSORBANCE

At Relatively Low pH Values. In an effort to obtain conditions even more favorable to color stability, the variation of absorbance with time was studied in solutions of relatively low pH. Although the color obtained in such solutions is red, rather than violet red, the absorption maximum is located at the same wave length, approximately 500 mμ. All absorbance readings were therefore taken at this wave length.

SOLUTIONS. Sodium dinitrobenzoate solution (0.1*M*) prepared by dissolving, with the aid of heat, 2.121 grams of 3,5-dinitrobenzoic acid in 100 ml. of water with an exact equivalent of sodium bicarbonate (0.840 gram).

Creatinine solution, 0.005*M* (56.6 mg. per 100 ml. of solution).

Glycinate buffer mixtures, prepared by dilution of a stock buffer mixture (0.10*M* sodium glycinate, 0.10*M* sodium chloride) according to Sorensen and Walbum (*8*).

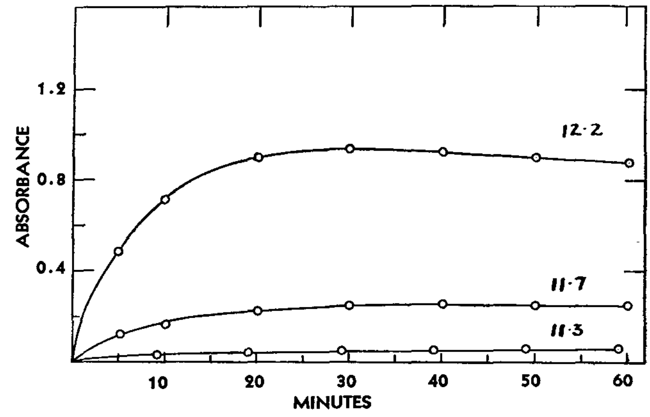

Figure 3. Effect of pH on Absorbance of Creatinine-Dinitrobenzoate Solutions

λ = 500 mμ, Beckman Model DU spectrophotometer, glycinate buffer $1.25 \times 10^{-3}M$ creatinine, $2.5 \times 10^{-2}M$ NaDNB

Color was developed by mixing 1 ml. of creatinine solution, 1 ml. of sodium dinitrobenzoate solution, and 1 ml. of one of the prepared buffer mixtures, and transferring it immediately to a tube, measuring 2 × 1/2 inch. The tube was promptly stoppered and the absorbance of the solution was compared at suitable intervals in a Beckman Model DU spectrophotometer with that of a blank prepared by substituting an equal volume of water for the solution of creatinine.

The results of this experiment are shown in Figure 3. For fixed concentrations of creatinine and sodium dinitrobenzoate both the absorbance and instability of color vary directly with pH. Although greater sensitivity is attained at a higher pH, more reliable determinations of creatinine can be performed at lower pH values—for example, at or below pH 11.7 the absorbance remains constant after reaching a maximum value. All colors obtained in this experiment were less stable when exposed to air; atmospheric carbon dioxide neutralized a sufficient amount of base to produce this effect (*3*).

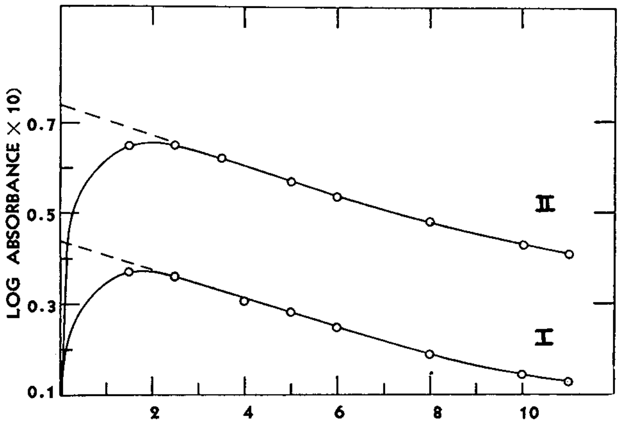

Figure 4. Absorbance at High Concentrations of Sodium Dinitrobenzoate and Sodium Hydroxide

λ = 500 mμ, t = 28.1° C., Beckman Model DU spectrophotometer, 10-mm. quartz cell
0.157*M* NaDNB, 0.200*M* NaOH

At High Concentrations of Sodium Dinitrobenzoate and Sodium Hydroxide. As the absorbance values for a fixed concentration of creatinine increased with increasing concentrations of both sodium dinitrobenzoate and sodium hydroxide, even when already present in very large excess, it was decided to measure the absorbance obtainable with considerably higher concentrations of these reagents than those already employed.

Accordingly, reaction mixtures were prepared by adding 2 ml. of a 1.50*M* sodium hydroxide solution and 5 ml. of an 0.470*M* sodium dinitrobenzoate solution (prepared by dissolving 10.0 grams of 3,5-dinitrobenzoic acid with 2.5 grams of sodium carbonate in 100 ml. of water) to solutions containing 0.113 and 0.226 mg. (1 and 2 micromoles), respectively, of creatinine in 8 ml. of water. Absorbances were compared at 500 mμ against that of a blank prepared by substituting 8 ml. of water for the solutions of creatinine.

Log absorbance is plotted as a function of time in Figure 4. As expected, when higher concentrations of reagents are used with a given amount of creatinine, considerably higher absorbances are obtained. Again, however, the rate of decomposition of the initial product is accelerated by these same conditions. The decomposition process is apparently first order, since the plot of log absorbance against time is a straight line.

CONSTANCY OF ABSORBANCE-CONCENTRATION RATIO

Into each of seven tubes, measuring 2 cm. in diameter, 53 mg. (0.25 millimole) of 3,5-dinitrobenzoic acid and 5 ml. of 0.100*M* sodium hydroxide solution were introduced. After complete solution of the nitro compound, 5, 4, 3, 2, 1, 0, and 4 ml. of water were added, followed by 0, 1, 2, 3, 4, and 5 ml. of an 0.001*M* solution of creatinine and 1 ml. of an 0.01*M* solution of creatinine, respectively. These solutions were mixed at once and the absorbances of those containing creatinine were read against that of the blank (first solution) at 500 mμ in a Coleman Junior spectrophotometer in the time interval of 60 to 70 minutes after preparation. Absorbances of these same solutions were then compared at the same wave length in a Beckman Model DU spectrophotometer in 10-mm. quartz cells, during the time interval of 95 to 110 minutes.

Table I. Linearity of Absorbance-Concentration Curve

Creatinine in 10 Ml. Solution, Mg.	Coleman Absorbance, *D*	Creatinine[a] Calcd. (*D* × 0.784), Mg.	Error, %
0.113	0.152	0.119	+5.3
0.226	0.292	0.229	+1.3
0.339	0.430	0.337	−0.6
0.452	0.576	0.452	0.0
0.566	0.721	0.565	−0.2
1.131	1.38	1.08	−4.4

[a] Factor 0.784 is reciprocal of slope of Figure 5, *C*.

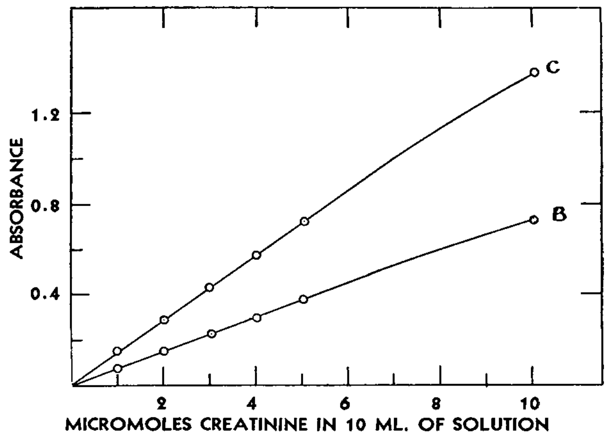

Figure 5. Linearity of Absorbance *vs.* Concentration of Creatinine

2.5 × $10^{-2}M$ NaDNB, 2.5 × $10^{-2}M$ NaOH
1 micromole of creatinine = 0.113 mg.
B. Beckman Model DU spectrophotometer, 10-mm. quartz cell
C. Coleman Junior spectrophotometer, 20-mm. cuvette

Adherence to the Lambert-Beer law is evident from the linearity of each absorbance-concentration curve shown in Figure 5. The accuracy of this procedure is demonstrated in Table I. The error of the method does not exceed 1% when 0.25 to 0.60 mg. of creatinine is to be determined in a final volume of 10 ml.

Table II. Apparent Molar Extinction Coefficients of Creatinine-Sodium Dinitrobenzoate Complex

NaDNB, *M*	NaOH, *M*	Creatinine, *M*	*E*
0.025	0.003 (pH 11.5)	5.0×10^{-3}	78
0.025	0.025	5.0×10^{-4}	760
0.157	0.200	6.67×10^{-5}	4110 (zero time)
0.157	0.200	13.3×10^{-5}	4120 (zero time)

OPTIMUM CONDITIONS FOR DETERMINATION OF CREATININE

A comparison of the apparent molar extinction coefficients at 500 mμ, based upon the concentration of creatinine, is presented in Table II for some of the experimental conditions described above. Utilization of high concentrations of reagents might be desirable when very small amounts of creatinine, of the order indicated at the bottom of Table II, are to be determined. Figure 4 shows that peak absorbance values are obtained very rapidly under these conditions. The absorbance values at zero time, obtained by extrapolation, are proportional to the concentrations of creatinine tested. A determination could be completed within a few minutes, but great care would be required, as the absorbance values change rapidly with respect to time. Readings at two or three exactly specified time intervals would suffice for the extrapolation of log absorbance to zero time. The creatinine content would be obtained by comparison with a standard curve, prepared at the same time.

At a comparatively low pH—i.e., 11.5—conditions for color stability are ideal. Unfortunately, owing to the very low extinction at this pH, a procedure utilizing these conditions would be applicable only to relatively high concentrations of creatinine, such as those in urine.

As the most generally applicable procedure for the determination of creatinine, intermediate conditions were selected as a suitable compromise between sensitivity and stability. As Figure 5 demonstrates, Beer's law is obeyed under these conditions.

Table III. Summary of Dinitrobenzoate Procedures for Determination of Creatinine

	Final Concentration of Reagent, M				
Reagent	Benedict-Behre (*1*)	Bolliger (*2*)	Langley-Evans (*10*)	Lehnartz (*11*)	Present method
NaDNB	0.0088	0.040	0.226	0.0566	0.025
NaOH	0.085	0.154	0.100	0.0500	0.025
Creatinine	App. 3×10^{-4}	App. 7×10^{-4}	App. 2.5×10^{-4}	App. 8×10^{-5}	5×10^{-4}
Sodium acetate	...	...	...	0.40	...
Ethanol	17.8%	...	...	...	...
Time of reading, min. after mixing	10–20	15–25	10–30	5	60
Temp., ° C.	Room temp.	App. 0–5	Room temp.	Room temp.	25

Recommended Procedure for Determination of Creatinine. REAGENTS. Sodium hydroxide solution, 0.300M, prepared from a saturated solution of reagent grade sodium hydroxide.

Sodium 3,5-dinitrobenzoate, 0.300M, prepared by dissolving 6.36 grams of 3,5-dinitrobenzoic acid, Eastman Kodak brand recrystallized according to Langley and Evans (*10*), in a final volume of 100 ml. with the aid of 1.59 grams of reagent grade anhydrous sodium carbonate.

Alkaline dinitrobenzoate reagent, freshly prepared, as needed, by mixing equal volumes of each solution.

APPARATUS. A Beckman Model DU spectrophotometer and a Coleman Junior spectrophotometer were used for measurement of absorbance. Other spectrophotometers or photoelectric colorimeters capable of transmission at 500 mμ are probably satisfactory.

PROCEDURE. To 10 ml. of a neutral solution of the sample to be analyzed 2 ml. of the alkaline dinitrobenzoate reagent are added. A blank is prepared by adding 2 ml. of the reagent to 10 ml. of water. A comparison standard is prepared by adding 2 ml. of the reagent to 10 ml. of a standard solution containing 0.6 mg. of creatinine. After mixing, the tubes are stoppered and allowed to stand for 1 hour, preferably in a water bath maintained at 25° C. Absorbances of both standard and unknown solutions are then read promptly against that of the blank, at 500 mμ. The creatinine concentration of the unknown is obtained from the ratio of the two absorbances.

This method is suitable for the determination of 0.1 to 1.0 mg. of creatinine in 10 ml. of solution. Although under these selected conditions absorbance readings remain practically constant over a period of time which is sufficient to permit accurate determinations, both standard and unknown solutions should be read at nearly the same time after the addition of reagent. A standard should be used with each set of determinations, as the intensity of developed color is sensitive to differences in temperature.

COMPARISON WITH OTHER DINITROBENZOATE PROCEDURES

A comparison of the present method with the techniques recommended by other investigators employing alkaline dinitrobenzoate for the determination of creatinine is presented in Table III. The stability of color produced by the Benedict-Behre technique approximates that of the present method (*3*). These authors, however, measure color intensity at 10 to 20 minutes after mixing, whereas maximum stability is attained at 30 to 50 minutes. At the temperature employed by Bolliger, color development is excessively slow (*3*). Furthermore, because the reaction is temperature-dependent, the intensity of color will change when the cold solution is allowed to stand at room temperature during the process of measuring absorbance. The present author has found that under conditions very similar to those employed by Langley and Evans, maximum color is obtained at 7 minutes, with steadily decreasing values thereafter (*4*). The color is therefore unstable during the 10- to 30-minute interval recommended by Langley and Evans for color measurement. When the method of Lehnartz is followed, a regular decrease in absorbance is observed. These conditions are, therefore, not suitable for the determination of creatinine.

The practice of diluting the reaction mixture prior to measurement of color (*1, 10, 11*) is not supported by the finding that absorbance decreases rapidly with respect to time immediately after dilution (*3*).

COMPARISON WITH JAFFE TECHNIQUE

The principal advantage of the Jaffe reaction for the determination of creatinine consists in greater sensitivity, combined with a constancy of photometric readings after 30 minutes. Readings are sometimes taken at 10 or 20 minutes after mixing, when they have not yet attained their maximum values (*4*). The apparent maximum extinction coefficient at 510 mμ of the creatinine picrate complex, based upon the concentration of creatinine, was calculated from the data of Clark and Thompson (*5*) as 8600, a considerably larger value than that obtained in the selected dinitrobenzoate method. At high concentrations of sodium dinitrobenzoate and sodium hydroxide, half of this value is obtained (Table II). Although the creatinine picrate complex appears to be the more stable of the two addition products, a sufficiently stable color is obtained by the recommended procedure with dinitrobenzoate to permit accurate and reproducible photometric readings.

The necessity for reading the absorbance of the creatinine picrate complex in an unsuitable region of its absorption spectrum and the undesirably high absorbance of the reagent blank have been referred to. The proposed dinitrobenzoate procedure is free from these objections. Readings are taken at the wave length corresponding to maximum absorbance, at which the absorbance of the reagent blank is negligible. The absorbances obtained by both the alkaline picrate and the alkaline dinitrobenzoate procedures are sensitive to variations in temperature.

Both the alkaline picrate and the alkaline dinitrobenzoate reagents are capable of reacting with a large number of nucleophilic reagents, particularly with aldehydes and ketones. If present, these interferences usually may be removed by distilling a portion of the solution before determination of creatinine.

DISCUSSION

Details concerning the nature of the reactions which take place when creatinine, sodium 3,5-dinitrobenzoate, and sodium hydroxide are permitted to react with each other in aqueous solution are to be found in the author's thesis (*3*).

In general, aromatic nitro compounds are capable of producing color reactions with a variety of carbonyl compounds in the presence of base. Positive reactions are obtainable with mononitro compounds only under special conditions. A red color is obtained, for example, upon the addition of an aqueous solution of tetraethylammonium hydroxide to a solution of nitrobenzene in acetone. Of the polynitro compounds, those containing nitro groups in positions meta to each other are the most reactive.

Trinitro compounds, such as 1,3,5-trinitrobenzene, are unsuitable because a red color appears merely upon the addition of alkali, in the absence of other reactants. . Alkaline picrate solutions are less deeply colored and are therefore less objectionable. Alkaline solutions of *m*-dinitro compounds readily produce colors with many carbonyl compounds, while exhibiting very little color in the presence of base alone. As a rule, ortho-substituted nitro compounds are less reactive than unsubstituted nitro compounds. Multiple ortho substitution serves to inhibit the reactivity of a nitro compound even more strongly—for example, 2,4-dinitromesitylene fails to produce any color in the presence of acetone and sodium hydroxide. For aqueous systems, an alkali-soluble *m*-dinitro compound, such as 3,5-dinitrobenzoic acid, is a suitable reagent for producing color reactions. In nonaqueous systems, *m*-dinitrobenzene is probably the most appropriate reagent (*12*).

The reaction leading to the formation of the violet-red creatinine-dinitrobenzoate addition compound is apparently reversible. Color stability is affected by irreversible changes which occur at the expense of the addition compound. In this process the nitro compound is reduced, largely to azoxy compounds, while creatinine is oxidized to glyoxylate, oxalate, and other products. Another type of irreversible change is produced concurrently by the introduction of hydroxyl groups into the aromatic ring of the addition product, resulting in destruction of the complex and the production of nitrophenols, which are probably responsible for the yellow color that appears when a reaction mixture is allowed to stand for some time.

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Colorimetric Determination of Vanadium(V) and Its Separation from Copper

Use of Cupferron

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This work was undertaken as part of a larger project on the analytical chemistry of vanadium and the use of cupferron with complexing agents. A new colorimetric method was developed based on the green color formed by vanadium cupferrate in acetone. Vanadium was separated from copper by precipitation with cupferron at a pH less than 1. This furnishes a good colorimetric method for vanadium in the presence of copper and a convenient separation from copper.

CUPFERRON has been reported (*3, 4*) as a precipitating agent for vanadium(V) and vanadium(IV) from an acid solution. In most instances the precipitate was filtered, ignited to the oxide, and weighed as such. A procedure has been suggested (*1*) in which the vanadium cupferrate precipitate is dissolved in chloroform and determined by colorimetric means. It was found in this investigation, however, that the color faded on standing. For the determination of relatively large amounts of vanadium this might not be serious, but for the determination of small amounts the error was large. Among other organic solvents tested, acetone was found to give satisfactory results. Iron. titanium, molybdenum, tungsten, tantalum, niobium, palladium, zirconium, antimony(III), bismuth, thallium(III), rare earths, copper, and chromium(VI) interfered.

Cupferron was selected as the precipitating agent for vanadium, since it was found to form a relatively stable complex. This complex was not as stable as those formed with iron, titanium, or molybdenum, indicating the possibility that cupferron might serve the dual purpose of first removing other elements, under controlled conditions, and then serving as the precipitating agent for vanadium.

APPARATUS AND MATERIALS

The absorption spectra measurements were made on a Beckman Model B spectrophotometer. A Beckman Model H2 pH meter, the glass electrode of which was calibrated frequently against potassium dihydrogen phosphate buffers, was used for all pH measurements. The colorimetric determinations were carried out with the Evelyn photoelectric colorimeter.

A solution of ammonium vanadate was prepared, 1 ml. of which contained 0.56 mg. of vanadium. From 1 to 25 ml. of this stock solution was used, and the final volume of the acetone solution of vanadium cupferrate was 100 ml. A 6% solution of cupferron was prepared, filtered from dark, insoluble material, and stored in a refrigerator. The acetone and acids were C.P. grade.

EXPERIMENTAL

Choice of Organic Solvent. Previous investigators (*1*) used ether or chloroform to extract the vanadium cupferrate. Most of

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the solutions in these and other solvents faded on standing. The color produced in ethyl alcohol solution was stable, but the absorption in this solution was not satisfactory.

When the vanadium cupferrate is dissolved in acetone at about 20° C. the first color produced is a yellow to brown. Within a few minutes this slowly changes to green. At 30° to 35° C. the change is rapid, but at 10° C. it may require several days. All measurements reported here have been based on the intensity of the green color. It is believed that the color measured is the result of a reaction between the cupferron and the acetone, and constitutes an indirect method for the determination of vanadium. Therefore, it is absolutely necessary to wash the vanadium cupferrate precipitate free of any excess cupferron with 10% sulfuric acid in order to assure accurate results. No difficulty has been encountered in this respect. After the development of the color, the temperature of the solution in the volumetric flask can be adjusted to the temperature selected in the preparation of the standards and the solution can be diluted to volume.

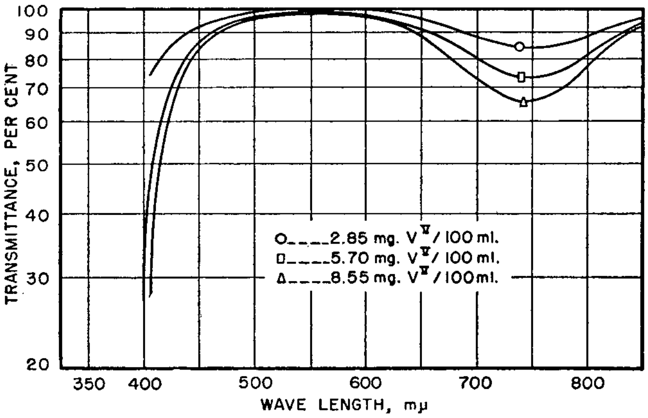

Figure 1. Spectral Transmittance Curves for Acetone Solutions of Vanadium Cupferrate

It does not appear to make any difference when the color is allowed to develop or under what conditions. Studies have been carried out under many different conditions and the results agree regardless of method employed. Solutions have been held at a temperature of 10° C. for 2 days with no tendency to develop the green color, and the volumetric flask was then placed in a water bath at 50° C. and the color allowed to develop. The results check with those in which the color was developed immediately. After the color change begins the reaction is complete in a few minutes.

Effect of Concentration of Cupferron. Precipitations carried out with different amounts of cupferron in excess did not affect the results, since the excess was readily washed out of the precipitate with 10% sulfuric acid solution. The wash solutions from the vanadium cupferrate were tested, and washing was continued until the absence of cupferron was assured.

pH. Other workers (*4*) have stated that complete precipitation is obtained from solutions in which the pH varied from 0 to 3. In all this work the pH was less than 1 and complete precipitation was obtained.

Temperature. Since cupferron solutions are not stable at elevated temperatures (*3*), all precipitations were carried out at 0° to 10° C. The precipitates were filtered immediately to prevent any decomposition of the precipitate or of the excess cupferron. When this procedure was followed, a clean precipitate was obtained. If this precipitate was allowed to remain on the filter paper for over an hour at 38° to 43° C., there were definite signs of decomposition, but at temperatures of 21° to 27° C. this was not serious. After the precipitate was dissolved in acetone, temperature had no effect.

Filtration. When the cupferron precipitate was separated by centrifugation or by use of a filtering crucible, it was impossible to be certain that the precipitate was free of excess cupferron. If filter paper pulp was added to the solution before precipitation and the solution filtered through a double layer of No. 40 Whatman paper, a clean filtrate was obtained which was free of vanadium. The use of filter paper pulp facilitated the washing of the precipitate.

Stability. No variations in the transmittance were observed over a period of 48 hours. Data for transmittancy–wave-length curves were obtained by measuring the transmittancy in a 1-cm. cell, at room temperature, against an acetone blank, over the range from 350 to 1000 mμ (Figure 1). The curves showed a broad band of minimum transmittancy against concentration of vanadium at 745 mμ, and showed good conformity to Beer's law over the range of concentrations determined (0.0056 mg. to 0.14 mg. per ml.).

PROCEDURE

The vanadium solution was diluted to 50 ml. and cooled in an ice bath to 0° to 10° C., and the pH was lowered to less than 1. Filter paper pulp was added along with a calculated excess of freshly prepared 6% solution of cupferron. The pH was again adjusted to less than 1° and the solution allowed to stand for 5 to 10 minutes in the ice bath. The solution was then filtered through a double layer of No. 40 Whatman filter paper and washed with 10% sulfuric acid solution until the washings showed no test for cupferron.

A 100-ml. volumetric flask was then placed under the funnel and acetone poured over the precipitate on the filter paper. The filter paper was washed clean of the vanadium cupferrate and the final volume diluted to 100 ml. This was allowed to stand for 20 to 30 minutes for the development of the color.

The results are shown in Table I. The operations should be carried out without undue delay, especially if the room is unusually warm, because cupferrate precipitates tend to decompose on standing.

COLORIMETRIC DETERMINATION OF VANADIUM IN THE PRESENCE OF COPPER

In addition to precipitating vanadium from an acid solution, cupferron causes precipitation of many metal complexes insoluble in acids. The stability of these complexes varies widely. A method has been developed for the separation of copper from vanadium(V), depending upon the fact that when a salt of ethylenediaminetetraacetic acid (Versene) is added to a mixture of copper(II) and vanadium(V), chelation occurs. The copper complex is sufficiently stable so that copper(II) will not precipitate with cupferron, but vanadium cupferrate will precipitate quantitatively. The vanadium can then be determined colorimetrically

Table I. Determination of Vanadium

Vanadium(V), Mg.		
Taken	Found	Error
0.57	0.57	0.00
0.57	0.59	+0.02
2.85	2.94	+0.09
2.85	2.88	+0.03
2.85	2.96	+0.11
2.85	2.83	−0.02
6.27	6.27	0.00
6.27	6.13	−0.14

Table II. Determination of Vanadium(V) with Cupferron in Presence of Copper[a]

Vanadium(V), Mg.		
Taken	Found	Error
2.9	3.0	+0.1
2.9	3.1	+0.2
5.7	5.7	0.0
5.7	5.8	+0.1
8.5	8.5	0.0
8.5	8.3	−0.2

[a] 3.2 mg. of copper were present in each determination.

by the method already described with an average error of ±2.5%. The use of Versene for the separation of vanadium from chromium, iron, and other elements will be reported later.

APPARATUS AND MATERIALS

Copper sulfate solution. A solution of copper sulfate was prepared containing 0.63 mg. of copper per ml.

Ethylenediaminetetraacetic acid, tetrasodium salt (Versene). A 10% solution prepared from a 34% solution was used in these determinations. The Versene was obtained from the Bersworth Chemical Co.

EXPERIMENTAL

Effect of Concentration of Versene Solution on Precipitation of Vanadium. It has been reported that vanadium precipitates in solutions with a pH of 3 to less than 0 (*1*, *3*, *4*). In the presence of Versene there was no precipitation of the vanadium cupferrate until the pH was below 1.5, and at a pH of 0.5 precipitation was complete.

From 2 to 20 ml. of 10% Versene solution was added to solutions containing 0.0255 mg. of vanadium(V) per ml. The vanadium(V) precipitated quantitatively with cupferron even in the presence of a large excess of Versene, and the excess could be washed readily from the precipitate.

Effect of Versene Solution on Precipitation of Copper with Cupferron. In solutions containing 5 ml. of the standard copper solution and from 2 to 10 ml. of the 10% Versene solution, no precipitation of the copper was obtained by the addition of 10 ml. of the 6% cupferron solution, even when the pH was less than 0.1.

Precipitation of Vanadium in Presence of Copper. In solutions containing varying amounts of vanadium and copper, the precipitation of the vanadium was carried out at a pH less than 1.0, and the vanadium cupferrate precipitate was first washed with a 2% Versene solution, adjusted to a pH of 1 to 1.5, and finally washed with 10% sulfuric acid. The results obtained for the vanadium checked well with the work on solutions which contained vanadium alone (Table II). The acetone solution of vanadium cupferrate was checked for the presence of copper by the dithizone method as described by Sandell (*2*) and no copper was found.

PROCEDURE

To a solution containing the vanadium and copper salts was added 10 ml. of 10% Versene solution, and the procedure described was followed.

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Automatic Measurement of Light Absorption and Fluorescence on Paper Chromatograms

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Previous automatic devices for scanning paper strip chromatograms have either been of the intermittent, nonrecording type or have required the use of an automatic recording spectrophotometer. The attachment described is designed for use with a commercially available monochromator employing a stabilized light source such as the Beckman Model DU spectrophotometer. An innovation in the assembly described is the use of interference filters to permit the measurement of fluorescence on paper strips. Strip chart records, both of absorption *vs.* strip length and of fluorescence *vs.* strip length, have been obtained. The reproducibility of results appears to be better than that obtained with other scanning devices. By plotting per cent transmittance rather than absorbancy, greater sensitivity is observed in evaluating low concentrations of absorbing materials on the strips.

THE application of the paper chromatographic technique to the quantitative as well as qualitative evaluation of complex mixtures continues to receive considerable attention. Recent studies have resulted in the construction of several devices for the scanning of paper strips from the standpoint of light absorption measurements (*3–6*).

The present work represents a continuation of some preliminary observations reported earlier (*2*) and is an attempt to broaden the utility of the automatic technique, by providing for the measurement of fluorescence on paper strips and by incorporating components which are more accessible to the average laboratory.

DESCRIPTION OF APPARATUS

The attachment for automatic scanning consists of five major parts—a spacer, a scanning chamber, a strip transporting mechanism, a photomultiplier tube detector with amplifier, and a strip chart recorder.

Since paper strip chromatograms can be prepared most conveniently on strips 0.5 inch wide (*1*), the first requirement in designing a scanner was to provide a beam of light at least that wide through which the strip could be transported. Examination of the characteristics of the light beam emitted by the Beckman DU monochromator used in this work showed that in the vicinity of the absorption cells as normally placed in the cell compartment the beam is a vertical rectangle about $^1/_8 \times ^3/_8$ inch. With the hydrogen lamp as a source, this beam diverges in the horizontal dimension and converges in the vertical dimension as the distance from the exit slit increases, and at a distance of about 8 inches it forms a slightly diffuse horizontal rectangle about $^3/_{16} \times ^5/_8$ inch. With the tungsten lamp as the source, the image at the 8-inch distance is a vertical ellipse about $^5/_8$ inch wide and 1 inch high but with the brightest portion concentrated in a diffuse horizontal rectangle about $^3/_{16} \times ^5/_8$ inch. Thus, it appeared feasible to transport the strip vertically through the beam at a distance of 8 inches from the block supporting the filter slide and exit slit. An alternate method would be to place the scanner closer to the block and alter the beam with lenses but this appeared to be an unnecessary complication. The use of the beam at the 8-inch point has at least two advantages. First, the chamber and scanner are located where there is no interference with the light source housing; secondly, it allows for the transporting of the strip vertically through the beam. Thus, provision is

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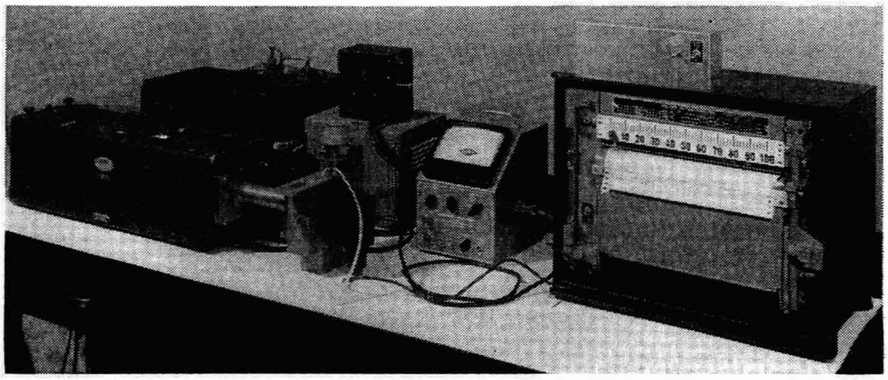

Figure 1. General View of Complete Instrument

easily made for shifting the strip out of the beam and for inserting a reference specimen, by mounting the mechanism on a sliding top to the light chamber. If the strip were transported in any direction other than vertical, the shifting of the strip would present a more difficult mechanical problem.

The positioning of the scanner chamber at the correct distance was accomplished with the spacer. This consists of two identical brass plates $5\,^3/_8 \times 3\,^5/_8 \times {}^3/_8$ inch brazed to a length of brass pipe 1.25 inch in inside diameter by 1.5 inch in outside diameter in such a manner that the over-all length of the spacer was $6\,^{11}/_{16}$ inches. The plates were drilled so that they could be fitted to the existing filter slide block guide pins and threaded holes on the optical bench of a Beckman Model DU spectrophotometer. For convenience, both plates were drilled identically. The plates were machined after the brazing so that the faces were parallel to each other, perpendicular to the pipe, and fitted precisely to the filter slide block and scanner chamber.

The chamber was constructed of an easily workable and extremely light weight magnesium alloy. The two identical sides are 6.75 inches high, 7 inches wide, and 0.25 inch thick, with a notch $1\,^{11}/_{16}$ inches wide and 1.75 inches high cut out of the lower corner to provide room for the cables so that a standard Beckman photomultiplier attachment could be mounted if desired. The ends and bottom of the chamber were made from pieces of the alloy 1.5 inches wide and $^3/_8$ inch thick. Each of the parts of the box were precisely machined and the parts overlapped sufficiently to provide a sturdy light-tight chamber. The parts were assembled with 38 countersunk machine screws. The sides were drilled, one to fit the spacer plate and the other to accommodate either the Beckman or the Aminco (American Instrument Co., photomultiplier microphotometer) phototube housing. The lid is a piece of the same alloy 8 inches long, 2 inches wide, and $^3/_8$ inch thick, with 0.25×0.25 inch rabbits on each side. The sides of the chamber extend 0.25 inch above the ends, the lid fitting between these extensions of the sides and overlapping the top to make a light-tight fit. The lid is free to slide.

The insides of both the spacer and the chamber were painted with a flat black paint to minimize reflections.

The scanner is similar to one described by Parke and Davis (*5*) as modified by Brown (*1*). It incorporates one solid brass roller, roughened to eliminate slippage of the strip, which is geared to a synchronous motor. The brass frame holding the gears and roller is mounted on a brass block $^3/_8$ inch thick which is in turn mounted on the underside of the lid. The brass block contains the aperture which is a hole 0.5 inch in diameter; over this are mounted two movable plates to provide an adjustable slit. The brass block also contains an adjustable track to guide and retain the paper strip and a spring-loaded companion roller to the driving roller so that a slight pressure is exerted on the paper strip as it is being driven. Provision is made for mounting a stationary reference strip of paper with an identical aperture and adjustable slit. The dimensions of the scanner and its placement are such that when one end of the lid is flush with the end of the chamber the reference specimen aperture is located in the beam and when the other end is flush with the other end of the chamber the main aperture is in the beam. Adjustable stops were placed on the ends of the chamber to limit the travel of the sliding top. All of the parts were constructed so that the apertures were in a line centered at the exit slit and perpendicular to the filter slide block on the monochromator. The position of the light beam can be shifted somewhat with the focusing adjustments provided on the monochromator; the final alignment of the beam to cover the aperture was accomplished in this manner. The depth of the chamber was made as large as possible to provide space for the strip to collect after it had passed the aperture. The synchronous motor is commercially available (R. W. Cramer Co., Centerbrook, Conn.). It is of a high torque design with an electric clutch which disengages when the power is off so that the strip can be moved by hand. Its output shaft speed is 4 r.p.m. and the strip travels about 7 inches per minute.

As mentioned by Parke and Davis (*5*), the light, after passing through the paper, is essentially all scattered. This light passes through the $^3/_8$-inch thick hole in the body of the scanner and this may result in some collimation. The scanner was placed as close as possible to the exit side of the chamber and when the Beckman phototube housing is mounted on the chamber the phototube is located close enough to the scanner so that an adequate amount of light strikes it. This is not true when the Aminco photomultiplier tube housing is used since there is a sizable chamber built into the photomultiplier tube housing to accommodate filters; therefore, an inadequate amount of light strikes the phototube unless the beam is further collimated. This was done by mounting a piece of stainless steel tubing of 0.5-inch inside diameter and 1.5-inch length inside the nipple connecting the phototube housing to the scanner chamber, using a suitable brass bushing, so that one end was as close as possible to the scanner. The interior surface of the tube was highly polished. All of the light from the strip passes into this tube and practically all is collimated into a beam which strikes the phototube. Only enough space was left between the collimating tube and the phototube to insert the thin interference filter which is necessary when fluorescence measurements are being made.

The detecting element is an RCA 1P28 photomultiplier tube. The assembly shown in Figure 1 utilizes the Aminco photomultiplier tube housing and line-operated direct current amplifier and power supply (American Instrument Co.). It has also been found possible to install the photomultiplier tube in the standard photocell housing of the Beckman spectrophotometer and to employ the internal amplification system of the instrument itself, the take-off to the recorder being in the null meter circuit. In the latter case, an external battery power supply for the photomultiplier tube is necessary. The Beckman photomultiplier attachment includes a power supply and operates satisfactorily for this application (*2*).

The recorder selected for use in these studies was a Brown Instrument Co., fast-acting strip-chart recorder. The pen speed is one second for full-scale travel and the voltage range is 0 to 1 mv. For most applications a recorder with a 0- to 10-mv. span would be satisfactory but a high-speed pen travel is mandatory. In order to secure the proper input potential, the output of the amplifier was supplied to a suitable voltage dividing resistor wired in parallel with the recorder.

Figure 1 shows a photograph of the complete apparatus. Figure 2 is a close-up exploded view which shows the various parts and their approximate relationship to each other.

OPERATION

Absorption Measurements. The proper operating range of the instrument is determined by choice of slit width, load resistor, and high-voltage setting. The general specifications for photomultiplier tubes indicate that maximum stability and reproducibility of output is obtained when the high voltage is kept as low as possible. It has seldom, if ever, been necessary to increase the high voltage above the 680-volt minimum of the Aminco amplifier. To reduce the noise output, the load resistor should be as low as possible.

It is necessary to fix the 0 and 100% ends of the recorder pen travel corresponding to zero and maximum amplifier output, respectively. The zero end is fixed first, with the scanner in a mid-position in the chamber so that no light falls on the phototube, by adjusting the zero adjust (dark current compensating) knob on the amplifier. The 100% end is fixed by shifting the reference section of the scanner which contains a typical specimen of the paper being used into the beam and adjusting the slit on the optical bench. It is often desirable to fix the 100% point at a small distance from the end of the chart so that the pen may travel above 100% because of normal fluctuation of the background absorption. The sample strip is backed out of the beam by rotating the clutch mechanism of the synchronous motor; then, after setting the transport mechanism in the sample position, the motor is started and at the first deflection of the pen, indicating entrance of the strip into the beam, the chart motor is started, thus synchronizing the chart record with strip position. Settings at both ends of the recorder scale are checked and readjusted if necessary before each strip is run through or whenever the wave-length setting is changed. The principal precaution in making these settings is to make them approximately the same for all strips of a given series since the height and thus the area of a peak is dependent on the total span of the pen.

Fluorescence Measurements. For the measurement of fluorescent zones on paper strips, a mercury vapor lamp (available from Beckman Instruments, Inc. and recommended by them for calibration of the wave-length scale of the DU spectrophotometer) is placed in the normal position for the light source. The wave-length dial is set at 365 mμ which effectively isolates the fluorophor-activating line. An interference filter having a peak transmittance at a wave length near the wave length maximum of the emission spectrum of the fluorophor is placed in the light beam between the paper strip and the photomultiplier tube. Suitable filters having essentially complete cutoff at 365 mμ and 25 to 35% transmittance at 450 mμ (blue fluorescence) or 580 mμ (yellow fluorescence) have been purchased from Baird Associates and Farrand Optical Co. With this assembly, light strikes the photocell only when a fluorescent zone is passing through the beam. This provides a recording of emission versus strip length. It is necessary to employ a standard fluorescence for reference with each sample series studied since a reference light intensity is not otherwise obtainable. This can be conveniently done by placing a spot of the material being tested or of any stable fluorescent substance having a similar emission spectrum, on a piece of paper strip and placing it in the reference aperture of the scanner. Then, before each strip is scanned, the pen is adjusted to a fixed reference point on the chart with the reference specimen in the beam by adjusting the slit. The only other adjustment necessary is that to compensate for zero drift.

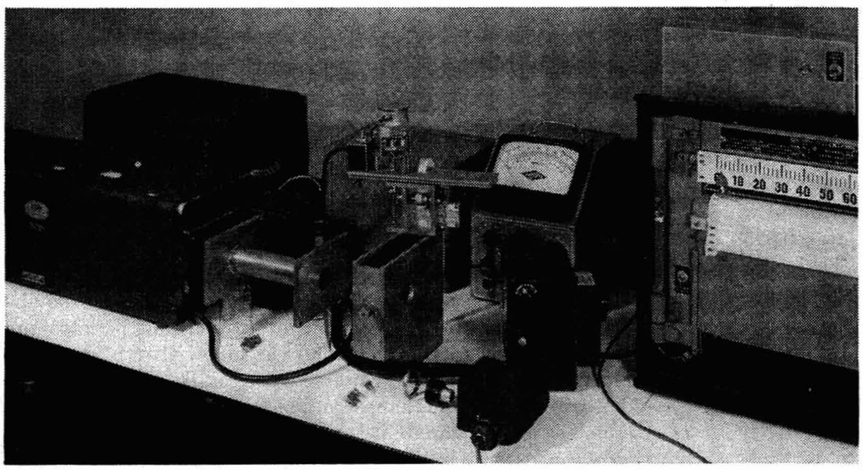

Figure 2. Exploded View Showing Relationship of Attachments

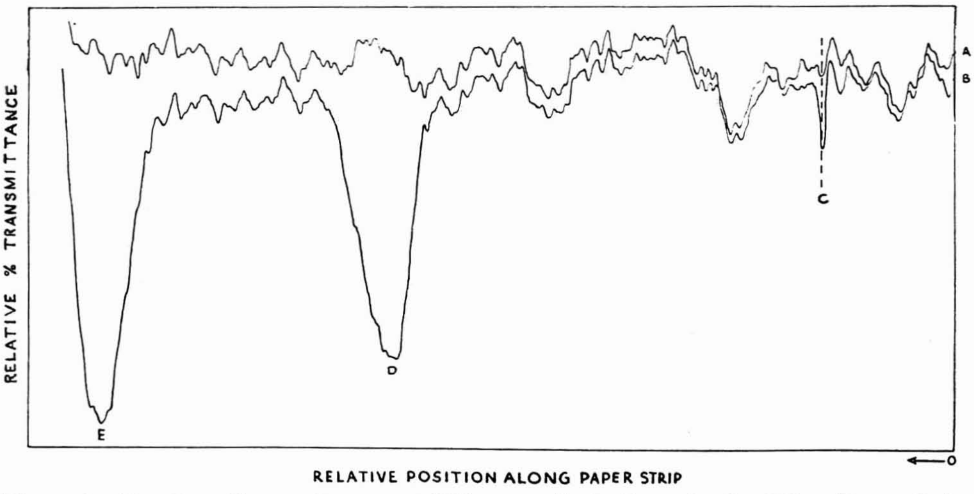

Figure 3. Per Cent Transmittance at 297 mμ *vs.* Strip Length of a Filter Paper Strip Containing Pyridoxine Hydrochloride

A. Base line (scan of the strip before applying the sample)
B. Scan of the strip after development
C. Point of sample application
D. Pyridoxine hydrochloride band
E. Solvent front

EXPERIMENTAL

Paper strips 0.5 × 22.5 inches were used in developing chromatograms according to the method described previously (*2*). After drying, the strips were scanned at the appropriate wave lengths using the described apparatus.

RESULTS

Figure 3 shows a typical graph of relative per cent transmittance of a pyridoxine hydrochloride band versus a linear function of strip length. Figure 4 shows a typical graph of relative fluorescence intensity of a riboflavin band versus a linear function of strip length. Table I and Figure 5 show the quantitative results of measuring the areas of the percent transmittance peaks from a series of concentrations of pyridoxine hydrochloride, and Table II and Figure 6 show similar data from the fluorescence peaks from a series of concentrations of riboflavin.

DISCUSSION

In Figure 3 the typical background absorption of a paper

Table I. Concentration-Area Data for Various Concentrations of Pyridoxine Hydrochloride

(Four strips at each concentration. Wave length = 297 mμ)

Concentration, γ per strip	0.625	1.25	1.875	2.5	3.125
Area of absorption zone. sq. inch	0.83	1.46	2.23	2.94	3.69
	0.81	1.53	2.24	3.00	3.52
	0.77	1.55	2.23	2.94	3.64
	0.81	1.52	2.16	2.86	3.83
Average	0.80	1.51	2.21	2.93	3.67
Max. dev. from average, %	+3.7 −3.7	+2.6 −3.3	+1.4 −2.3	+2.4 −2.4	+4.4 −4.1

Table II. Concentration-Area Data for Various Concentrations of Riboflavin

(Four strips at each concentration)

Concentration, γ per strip	2.6	5.2	7.8	10.4	13.0
Area of fluorescent zone, sq. inch	3.30	5.24	7.02	7.40	8.16
	3.46	5.30	6.64	7.66	8.43
	3.34	5.41	6.72	7.75	8.22
	3.44	5.30	6.73	7.32	8.24
Average	3.39	5.31	6.78	7.53	8.26
Max. dev. from average, %	+2.1 −2.7	+1.9 −1.3	+3.5 −2.1	+2.9 −2.8	+2.1 −1.2

strip is shown and it is evident that the variation in absorption is considerably more than is shown in the previous work (*2*), where absorbance versus strip length was plotted instead of per cent transmittance versus strip length as in the present study. This is to be expected since, in the plotting of per cent transmittance, small variations in transmittance are magnified in relation to large variations as compared to an absorbance (logarithmic) plot. The plot of per cent transmittance is on a highly expanded scale for low transmittance values as compared to the absorbance scale on the Cary Recording spectrophotometer used in (*2*). The disadvantage of having a more variable base line is offset by the greatly increased sensitivity of the present instrument which allows, for example, the determination of as little as 0.625γ of pyridoxine hydrochloride on a strip as shown in Table I. The ability of the instrument to reproduce a trace, including all of the background peaks, is well demonstrated in Figure 3.

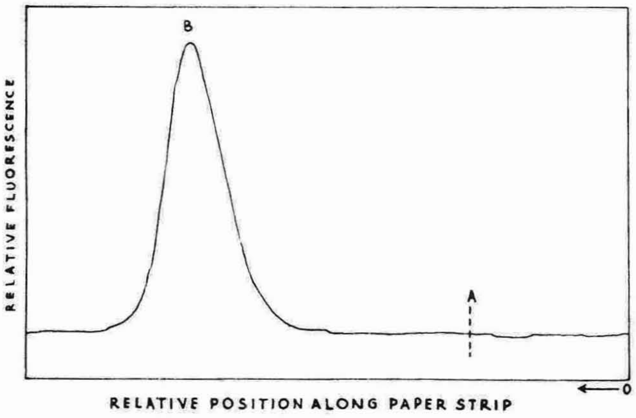

Figure 4. Fluorescence *vs.* Strip Length of a Developed Filter Paper Containing Riboflavin

A 580-mμ interference filter was used in front of the phototube
A. Point of sample application
B. Riboflavin band

It might be expected that with the more variable base line, less precision would be attained in quantitative work. This apparently is not necessarily the case, as shown in Table I. One reason for this may be the faster pen response of the recorder as compared to that of the Cary instrument used in the previous work (*1, 2*).

While the present instrument is subject to zero drift, light fluctuations, etc., these apparently are of little consequence. The zero setting was checked before and after each strip was scanned and occasionally small discrepancies were noted, but the only changes which would have significant effect are those which might occur while an actual band was passing the beam, since gradual range changes are compensated for when superimposing the base line.

In Figure 4 it is evident that the background variation is no problem with fluorescence measurements since it represents an infinite value in terms of absorption. Thus, no prescanning of the strips is necessary when only fluorescence measurements are to be made. Any background fluctuation which does occur can be attributed to instrument noise and is not reproducible.

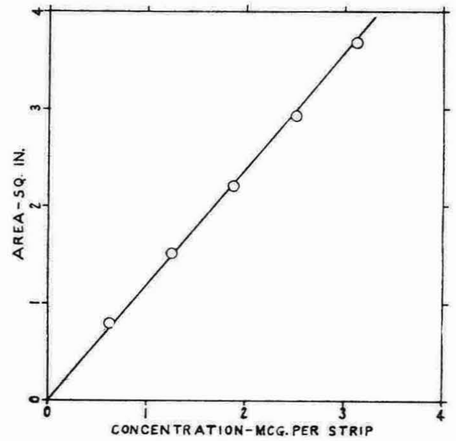

Figure 5. Plot of Areas of Absorption Peaks of Pyridoxine Hydrochloride *vs.* Concentration

The precision obtained in quantitative measurement of fluorescence as shown in Table II is somewhat better than that from absorption measurements. This might be attributed to the absence of the variable background absorption and perhaps the comparision shows how much the precision of absorption measurement is affected by the variable background. Measurements of both absorption and fluorescence of bands on strips are subject to some error and variability, because of the imperfect distribution of the material in the band, the finite dimensions of the beam of light passing through the strip, the fact that only approximately 94% of the width of the strip is covered by the beam, etc. These factors affect both fluorescence and absorption measurements.

The forementioned factors also affect the form of the concentration-area relationship curves as do such things as amplifier and phototube linearity, etc. Figure 5 indicates that the concentration-area curve for per cent transmittance measurements is

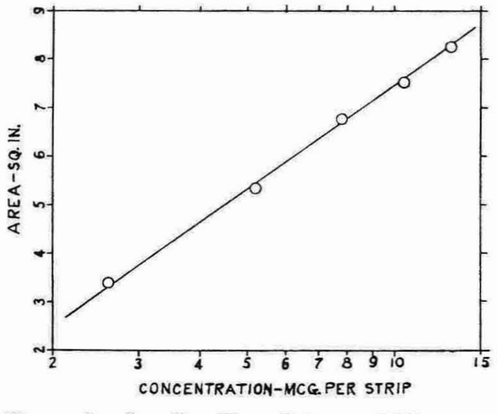

Figure 6. Semilog Plot of Areas of Fluorescence Peaks of Riboflavin *vs.* Concentration

essentially a straight line over the range used, which is contrary to Beer's law and the usual experience with solutions. There is no ready explanation for this except to say that it is probably due to compensating effects of the various factors mentioned. The concentrations used in this series were chosen so that the maximum deflection of the pen was not over about 50% on the transmittance scale. Obviously, the closer to 0% transmittance the deflection becomes, the less deflection there is per unit of concentration increase and the sensitivity decreases rapidly. Therefore, the linear relationship shown in Figure 5 could not hold for larger concentrations. It appears to be best, for quantitative work, to limit the maximum deflection to around 50%. The semilogarithmic relationship indicated in Figure 6 for fluorescence measurements again is the result of all of the various factors. Since, in quantitative work, the analytical results are always based on results from standards run under the same conditions, the form of the calibration curve is not particularly important.

If the Beckman photomultiplier attachment is used, and the output to the null-point meter is supplied to the recorder, the instrument is operated in a state of unbalance (a state for which it was not designed) and the nonlinearity of the amplifier under these conditions becomes a factor. For qualitative work this is not important and for quantitative work it appears that the only effect would be on the shape of the concentration-area calibration curve.

ACKNOWLEDGMENT

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Dichromate Oxidation of Diethylene Glycol

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An oxidation method for resolving organic mixtures containing nonvicinal hydroxyl and/or ethereal oxygen, as in glycol mixtures, requires a strong oxidant acting differentially. The behavior of dichromate as a differential oxidant is indicated by a study of the literature. Oxidation of diethylene glycol by dichromate in sulfuric acid solution at 100° C. results in three well-defined stages or levels of oxidation indicative of definite stoichiometric reaction mechanisms. These levels are attained in solutions which are 50, 25, and 12.5% by volume in sulfuric acid and are independent of the glycol and dichromate concentrations, provided that a moderate excess of dichromate over that stoichiometrically required for the oxidation of the glycol to the respective level is provided. Application of the differential oxidation method for the resolution of the binary mixture, diethylene glycol and ethylene glycol, was successful over a limited composition range with an accuracy within ±5% of the glycol content. The accuracy limitation was due to the lack of differential oxidation behavior of ethylene glycol under the conditions, coupled with the relatively small oxidation level change of the diethylene glycol. Further study providing additional data on the dichromate oxidation characteristics of these and other organic compounds should result in considerable improvement of the differential oxidation method.

IN THE analysis of either pure compounds or organic mixtures the use of dichromate as an oxidant is not limited to vicinal hydroxyl compounds as is periodic acid in the Malaprade reaction (*1*). Dichromate oxidation has been employed as a means of analytically resolving organic mixtures by one of three general approaches: (1) total oxidation by dichromate and subsequent correction for the oxidizable components (*2*) which are determined separately by independent methods, such as the Malaprade reaction, (2) removal or destruction of the more easily oxidizable components by a prior treatment (*10*) such as the Malaprade reaction and subsequent total oxidation of the remaining components with dichromate, and (3) differential dichromate oxidation (*9, 20*), which embodies independent oxidations under separate and different oxidizing conditions. In the latter case only the component in question varies in behavior.

Selection of the conditions necessary to effect complete oxidation of the organic compound or compounds by dichromate in the first two methods is of fundamental importance, but an examination of the literature discloses considerable latitude in the conditions used (*2, 4, 9, 10, 12, 20*). In the the third method usually one of the oxidation conditions selected is the highest level of oxidation—i.e., oxidation to carbon dioxide and water. Since only the differential in oxidizability is required, assurance of complete oxidation is not so critical as in the first two methods. The chief objection to the differential method lies in the use of different oxidizability. This has proved to be satisfactory for the diethylene glycol but represents only an arbitrary experimental behavior under the prescribed conditions. Under these circumstances, the method is open to the criticism of being empirical. In order to circumvent this objection, this investigation had as its purpose the determination of the dichromate oxidation characteristics of organic compounds (specifically, diethylene glycol) which are not empirical levels of oxidation, but which are represented by stoichiometric oxidation mechanisms.

CHEMICALS AND SOLUTIONS

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

Glycolic Acid, Matheson Co., No. 5770, assay 99±% and containing only water as impurity.

Sodium Oxalate, Bureau of Standards No. 40e.

All other chemicals have been described (*5*).

OXIDATION AND DETERMINATION PROCEDURE

The oxidation procedure and the spectrophotometric method for following the oxidation have been described in detail previously (*5*). For some portions of the work the iodometric method was employed (*2, 9*). In this case, the major portion of the sulfuric acid was neutralized with sodium hydroxide after the oxidation step had been completed or stopped, as in many cases the acidity was very high and could not be sufficiently decreased by simple dilution. Iodometric blanks were run and calculated in a manner analogous to the spectrophotometric blanks (*5*).

y = potassium dichromate blank, meq.
blank = x − (standard sodium thiosulfate blank, ml.) × (normality of sodium thiosulfate)
x = potassium dichromate taken, meq.

EFFECT OF SULFURIC ACID CONCENTRATION

The apparent oxidation potential of dichromate redox reactions is greatly influenced by the hydrogen ion concentration (activity) (*15*). The nature of the acid media should also be an influence, as the activities of equal molar concentrations may be significantly different. Some investigators have employed phosphoric acid (*3, 4*), phosphoric-sulfuric acid (*13, 18, 19*), acetic acid (*6, 16, 17*), and acetic-perchloric acid (*6*) media. In this work, however, only the sulfuric acid medium was employed.

Elkins *et al.* (*9*) studied the effect of sulfuric acid concentration upon the oxidation of methyl Cellosolve and Cellosolve and obtained higher oxidation numbers (factors) at the higher acidities for oxidations conducted at reflux temperatures for 4 hours. Thus, in 12.5% sulfuric acid solution, oxidation numbers of 9.3 and 9.0, respectively, were obtained, whereas in 33% sulfuric acid solution, the values obtained were 15.5 and 13.2 (theoretical values, 13.0 and 14.0).

The effect of sulfuric acid concentration upon the oxidation of diethylene glycol is shown in Table I. In obtaining these data, an attempt was made to hold all other variables constant. Two series of experiments were performed, one series of oxidations being followed iodometrically and the other spectrophotometrically. In each experiment, 10.42 mg. of diethylene glycol was taken for oxidation and 5.00 meq. (0.5*N* potassium dichromate used) of potassium dichromate was added. The dichromate additions provided a 2.5-fold excess over that which would be required for complete oxidation of the diethylene glycol to the carbon dioxide level.

Table I. Effect of Sulfuric Acid Concentration on Dichromate Oxidation of Diethylene Glycol[a]

H_2SO_4, Vol. %	Oxidation Number	
	Iodometric[b]	Spectrophotometric[c]
60	20.3	20.0[d]
55	20.2	20.0
50	20.3	20.0
45	20.0	...
40	20.1	19.6
35	18.8	18.6
30	17.8	17.0
25	17.0	16.0
20	15.6	15.1
15	14.4	14.3
10	13.7	13.5
5	12.8	12.6[e]

[a] 4-hour oxidations at 100° C.
[b] Average of triplicate determinations.
[c] Average of duplicate determinations.
[d] Single value.
[e] Average of five determinations.

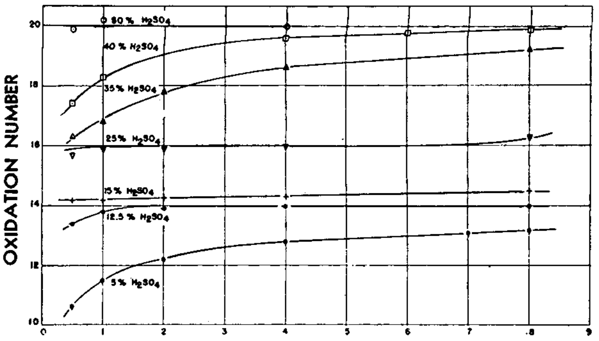

Figure 1. Effect of Sulfuric Acid Concentration and Time upon Dichromate Oxidation of Diethylene Glycol at 100° C.

Oxidations were conducted at 100° C. because of the ease with which this temperature is fixed and controlled. Also, consideration of the work of Werner and Mitchell (*21*) on related compounds showed that this temperature would provide a sufficiently rapid reaction rate. Reflux temperatures were not used, as these vary over a wide range depending principally upon the acid concentration. The only foreseeable variable not controlled was the redox ratio, which increases as the oxidation proceeds because of the decrease in the dichromate concentration and increase in the chromium(III) concentration. This change in the redox ratio could be minimized by initially adding chromium(III) to the system. However, Kolthoff and Laitinen (*14*) state that in dichromate reactions, trivalent chromium ions apparently have no influence upon the apparent oxidation potential, hence chromium(III) additions were not made.

In analogous agreement with the results of Elkins *et al.*, the results in Table I show a progressive decrease in the values of the oxidation numbers with decrease in sulfuric acid concentration. This expected decrease might be interpreted as indicative of the incompleteness of oxidation of the diethylene glycol to the carbon dioxide level. However, if lower oxidation levels exist, they must theoretically by definition be represented by oxidation numbers which are whole and even. The value of 16.0 in Table I obtained in 25% sulfuric acid solution (spectrophotometric) meets those criteria. In addition, this value agrees with the calculated value of 15.5 obtained by Francis (*10*), who found it necessary to use a reproducible but lower conversion factor than the theoretical in terms of complete oxidation of diethylene glycol to carbon dioxide.

No significant evidence of overoxidation at the carbon dioxide level is indicated by the results shown in Table I, such as were observed by Werner and Mitchell (*21*) in their oxidation study of methyl Cellosolve in 55% sulfuric acid solution. Observations of free oxygen loss have been reported when the acidity is extremely high (*4, 8*), which might account for the oxidation numbers greater than theoretical obtained by Werner and Mitchell.

The values in Table I obtained by the two methods agree well except in the 20 to 30% sulfuric acid concentration range, and it is not yet possible to explain satisfactorily the reason for the difference. It is significant that the values obtained by the spectrophotometric method are lower, for in a previous study (*5*) it was shown that the possible presence of unoxidized diethylene glycol caused no interference and that if compounds other than carbon dioxide were to be formed as end products any interference from these compounds should result in higher oxidation number values.

The reproducibility or precision measure of the oxidation numbers obtained by both methods was on the order of ±0.05 (deviation from the mean), except for the 5% sulfuric acid concentration determinations where the values were somewhat more erratic.

TIME–SULFURIC ACID CONCENTRATION STUDY

If the results shown in Table I are actually due to incomplete oxidation to the carbon dioxide level, then the effect of oxidation time should result in an increase of the oxidation number (even if only a very slow increase). On the other hand, if the oxidation is actually seeking some lower level or levels, then the effect of oxidation time should have very little influence, provided only that the lower oxidation level be an essentially stable one.

Oxidations were therefore performed as before in which the sulfuric acid concentration was again varied over the range of 50 to 5%. In this study, however, each experiment was conducted for the time interval as shown in Figure 1 and 11.65 mg. of diethylene glycol was taken for the oxidation. Only the spectrophotometric method was used for following the progress of the oxidations. The results are shown in Figure 1, where all the values represented are the average of duplicate determinations.

Complete (theoretical) oxidation of diethylene glycol to carbon dioxide and water occurs in 50% sulfuric acid solution within 30 minutes and is uninfluenced by longer oxidation time. This carbon dioxide level of oxidation represented by an oxidation number of 20 is then the maximum or most stable oxidation level. The plot of oxidation number *vs.* time for solutions which are 40 and 35% in sulfuric acid indicates that the reaction rate decreases with decreasing sulfuric acid concentration. Complete oxidation to the carbon dioxide level is attained after 8 hours in the case of the 40% sulfuric acid solution but is only 96% complete after 8 hours in the case of the 35% acid solution. Since at these acid concentrations the oxidations asymptotically approach the carbon dioxide level, it can be deduced that the (apparent) oxidation potential is still high enough to drive the reactions to this level.

Analogously, two lower oxidation levels at 25% (oxidation number 16.0) and 12.5% sulfuric acid (oxidation number 14.0) are indicated, since they are again independent of time. It takes somewhat longer to attain these levels, approximately 1 and 2 hours, respectively. Again, this can be attributed to the lower reaction rates at the lower acid concentrations. However, this increase in the time required to reach the oxidation level in question is also explainable if it is assumed that as the oxidation potential decreases, the reaction rate also decreases (*7*). The similarity of the 5% sulfuric acid curve to the 35 and 40% curves is apparent, but the asymptotic limit is now an oxidation number of 14.0, which is a further indication of a stable 14.0 level. The results of the 15% acid solutions are interesting in that a stable level of fractional value is indicated. This is interpreted as overoxidation of the 14.0 level because of a higher oxidation potential than is required.

CONCENTRATION OF REACTANTS

In order to be analytically useful, it is important that the facts represented in Figure 1 be proved to be definite stoichiometric relationships and not empirical functions holding only for the particular experimental conditions obtaining. The reactant concentrations (glycol and dichromate concentrations) should be flexible over a broad range if any subsequent application is to be of a versatile nature.

Three series of oxidations were made as previously described containing, respectively, 50, 25 and 12.5% sulfuric acid. The amounts of glycol taken for oxidation in each series were, respectively, 40.20, 21.97, and 5.487 mg. Each oxidation was conducted for a definite time interval with the maximum time used in any series being only that required for completeness of oxidation or indicating the point of leveling off. The amount of dichromate taken in any experiment was calculated so as to represent a minimum excess of approximately 1.0 meq. over that required to oxidize completely the amount of glycol taken to carbon dioxide and water.

The results are shown in Table II where, for the sake of continuity, the appropriate data shown in Figure 1 for the 11.65-mg. glycol experiments are included. In every case, the expected oxidation numbers of 20.0, 16.0, and 14.0 were obtained. The larger amounts of glycol taken for oxidation to any of the oxidation levels did not require longer oxidation times. Maximum oxidation was obtained in 1 hour except for the 12.5% sulfuric acid oxidation of 11.65 mg. of the glycol, where a 2-hour oxidation time was preferable. Longer oxidation times had no significant effect. In some of the experiments (3 to 7, 10 to 14, and 19 to 23), although only the glycol taken is decreased as shown in Table II, the dichromate concentration of 5.000 meq. represents an actual increase, since proportionately less dichromate is required. However, in the cases where the dichromate concentration was directly varied (15 to 18), the same results were obtained.

Results were also obtained for the oxidation of approximately 5 mg. of the glycol at the various acidities. They are not shown in Table II, as the results were somewhat erratic because of the small amount of glycol taken for the oxidation, but in general the same trend in the results was obtained as for the other glycol concentrations. The precision measure of the results as shown in Table II was good, being on the same order as indicated in the preceding acidity study.

Table II. Effect of Glycol and Dichromate Concentrations and Time upon Oxidation of Diethylene Glycol at Acidities Producing Specific Oxidation Levels

No.	H_2SO_4, Vol. %	Oxidation Time, Hr.	Glycol Taken, Mg.	K_2CrO_7 Taken, Meq.	Oxidation No.[a]
1	50.0	0.5	40.20	8.495	19.7
2	50.0	1.0	40.20	8.495	19.9
3	50.0	0.5	21.97	5.000	19.4
4	50.0	1.0	21.97	5.000	20.0
5	50.0	2.0	21.97	5.000	20.0
6[b]	50.0	0.5	11.65	5.000	19.9
7[b]	50.0	1.0	11.65	5.000	20.2
8	25.0	0.5	40.20	8.495	15.9
9	25.0	1.0	40.20	8.495	16.0
10	25.0	0.5	21.97	5.000	15.9
11	25.0	1.0	21.97	5.000	16.0
12[b]	25.0	0.5	11.65	5.000	15.7
13[b]	25.0	1.0	11.65	5.000	15.9
14[b]	25.0	2.0	11.65	5.000	15.9
15	12.5	0.5	40.20	8.495	13.5
16	12.5	1.0	40.20	8.495	14.1
17	12.5	0.5	40.20	6.500	13.5
18	12.5	1.0	40.20	6.500	14.0
19	12.5	0.5	21.97	5.000	13.4
20	12.5	1.0	21.97	5.000	14.0
21[b]	12.5	0.5	11.65	5.000	13.4
22[b]	12.5	1.0	11.65	5.000	13.8
23[b]	12.5	2.0	11.65	5.000	13.9

[a] Average of duplicate determinations.
[b] From data as shown in Figure 1.

CONCLUSIONS

Dichromate oxidation of diethylene glycol in 50, 25, and 12.5% sulfuric acid at 100° C. proceeds in a stoichiometric manner to the oxidation levels represented by the oxidation numbers, 20.0, 16.0, and 14.0, respectively. These oxidations are independent of the glycol and dichromate concentrations if the amount of dichromate employed provides an excess over that required by the glycol for the level desired. However, for the most satisfactory results the amount of glycol taken for oxidation should be from 10 to 40 mg. (per 50-ml. volume) which will require a 1-hour oxidation time for solutions 50 and 25% in sulfuric acid and preferably 2 hours for a solution which is 12.5% in sulfuric acid.

OXIDATION MECHANISM

A limited amount of work was done in an attempt to explain the nature of the oxidation mechanisms responsible for these observed stoichiometric oxidation levels. Only an indirect approach was tried—namely, the examination of likely compounds which could be predicted as end products, on the basis of recognized oxidation theory under the same oxidation conditions. The behavior of formic, oxalic, and glycolic acids postulated on the basis of the hydroxylation theory (*11*) is shown in Table III. (In the hypothetical mechanism, it was assumed that the reactions are those of diethylene glycol and not of a compound produced by the action of sulfuric acid.) These compounds were completely oxidized to carbon dioxide and water even in 12.5% sulfuric acid solution and were therefore eliminated as possible end products (or intermediates). One mechanism remains, however, as represented by the over-all reaction:

$$HOCH_2CH_2OCH_2CH_2OH + 8[O] \rightarrow 2CO_2 + 2CO + 5H_2O$$

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

No.	Compound	Compound Taken, Mg.	$K_2Cr_2O_7$ Taken, Meq.	H_2SO_4, Volume %	Oxid. Time, Hr.	Oxidation Number	
						Exptl.[a]	Theory
1	Formic acid	19.29	5.000	25.0	0.5	2.0	CO_2; 2.0
2					1.0	2.0	
3					2.0	2.0	
4				5.0	0.5	0.55	
5					1.0	0.81	
6					2.0	1.42	
7	Oxalic acid (as sodium oxalate)	70.41	3.000	50.0	1.0	2.0	CO_2; 2.0
8[b]					2.0	2.0	
9				25.0	1.0	2.0	
10					2.0	2.0	
11				12.5	1.0	1.9	
12					2.0	2.0	
13[c]	Glycolic acid	40.00	5.000	50.0	1.0	6.0	CO_2; 6.0
14[c]					2.0	6.0	
15				25.0	1.0	6.0	
16					2.0	6.0	
17				12.5	1.0	5.6	
18					2.0	5.9	
19	Ethylene glycol	47.39	8.495	50.0	1.0	10.0	CO_2; 10.0
20[c]					2.0	10.0	
21		23.70	5.000		1.0	10.0	
22		47.39	8.495	25.0	1.0	9.9	
23					2.0	9.9	
24		23.70	5.000		1.0	9.9	
25		47.39	8.495	12.5	1.0	9.6	
26					2.0	9.8	
27					4.0	9.9	
28				14.0	2.0	9.8	
29		23.70	5.000	12.5	1.0	9.4	

[a] Average of duplicate determinations.
[b] Single value.
[c] Average of triplicate determinations.

This possibility is of interest because it represents a case where no reaction end products would remain in the system after the oxidation process is completed. As yet, no carbon dioxide–carbon monoxide determinations have been made.

The oxidation of formic acid in 5% sulfuric acid solution is only 71% complete (carbon dioxide level) after 2 hours. The asymptotic limit is obviously the theoretical oxidation number value of 2.0. However, formaldehyde under the same conditions (*5*) is completely oxidized to the carbon dioxide level in a half hour or less. This indicates that formaldehyde does not pass through a formic acid intermediate step since this would require that the formic acid intermediate be in an activated form more susceptible to oxidation than formic acid in its normal state.

APPLICATION

Analysis of pure compounds by dichromate oxidation involves only the customary quantitative principles once the oxidation number (or numbers) has been found, but for the analytical resolution of mixtures the method of calculation is slightly more complex. For use with mixtures, it has been found expedient to employ the following expression which is analogous to those employed in mass and infrared spectrometry.

$$PQa + PRb + PSc + PTd + \dots = 100\,K$$

where

P = sample mixture taken, mg.
Q,R,S,T = percentage of components q, r, s, t.
a,b,c,d = calibration coefficients for pure components, q, r, s, t. = oxidation number of pure component for particular level divided by molecular weight of component
K = potassium dichromate consumed by the mixture, meq. This is a measured value peculiar to a particular mixture under the experimental conditions.

The K values can be measured by any of the analytical methods—e.g., spectrophotometry, titrimetry. If such expressions are employed for each experimental condition wherein characteristic oxidation is observed, one expression for each component of the mixture, it would be possible to solve them simultaneously and obtain the percentage composition of the mixture. All that is required are the values of the calibration coefficients of each component for each experimental oxidation condition or level. (In the general case, the assumption is made that each component of the mixture oxidizes to its respective oxidation level independently of the presence of the other components. This assumption must be experimentally verified for each component system in question.)

OXIDATION OF ETHYLENE GLYCOL

In order to resolve the ethylene glycol–diethylene glycol mixture, the calibration coefficients of ethylene glycol were determined in 50, 25, and 12.5% sulfuric acid solutions under conditions the same as those described for diethylene glycol. The results are shown in Table III.

In every case, ethylene glycol oxidized to the carbon dioxide level, the rate of reaction being rapid in 50 and 25% sulfuric acid solutions and requiring 1 hour. In 12.5% acid solution, however, the reaction rate was slow and at least 4 hours were required. Increasing the sulfuric acid content to 14% did not significantly alter the results. The values of the calibration coefficients for ethylene glycol in 50, 25, and 12.5% sulfuric acid solutions are then the same. Thus, this is a mixture which fits the special differential oxidation case—one component, ethylene glycol, oxidizing in a constant manner and the other, diethylene glycol, oxidizing differentially but stoichiometrically.

ETHYLENE GLYCOL–DIETHYLENE GLYCOL MIXTURES

A series of five synthetic solution mixtures of ethylene and diethylene glycol was prepared. Weighed mixed portions of the glycols were diluted to volume in a 1-liter volumetric flask at 25° C. The percentage of each glycol in the mixture was calculated on the basis of the total glycol weighed out and was varied so that the percentage compositions ranged from approximately 90 to 10% and 10 to 90%, respectively. The strength of the mixed glycol solutions was approximately 4 mg. per ml. in total glycol. The exact percentage compositions of the binary solution mixtures are shown in Table IV.

Ten-milliliter aliquots of the solution mixtures were taken for oxidation. Oxidations were conducted in 50, 25, and 12.5% sulfuric acid solutions for 2 hours. Some of the oxidations in 12.5% sulfuric acid solution were given a 4-hour oxidation time, since ethylene glycol under these conditions had shown a tendency to be incompletely oxidized. (In 50 and 25% sulfuric acid solutions, an oxidation time of 1 hour would have been ample.) The amount of dichromate added was calculated so as to leave 1.0 to 2.0 meq. in excess after the oxidation process was complete. The determination of the milliequivalents of dichromate consumed by the sample mixture (K) was made both spectrophotometrically and iodometrically. The results are shown in Table V.

The experimental determination of K for the mixtures by the spectrophotometric method in solutions 50 and 25% in sulfuric acid, respectively, was in almost every case within the experimental accuracy of the spectrophotometric method for the determination of dichromate—i.e., ±0.3% of the dichromate content (*5*). The precision measure of K was very good; the maximum deviation of any single value from its mean was ±0.3% and was usually closer to ±0.1%.

Solutions which were 12.5% in sulfuric acid gave K values which were in error by as much as 3% but generally somewhat less. The precision measure, however, was still ±0.1 to 0.3%. These results in 12.5% sulfuric acid solution were not unexpected in view of the tendency toward incomplete oxidation of ethylene glycol even after 4 hours.

Apparently the results obtained by the iodometric method were more accurate than those obtained by the spectrophotometric method. (The precision was somewhat inferior.) However, these results are compared against K values calculated using calibration coefficients determined by the spectrophotometric method. This is not quite fair, for it has already been seen that the results obtained by the two methods (Table I) are not in complete agreement. If the iodometric K values are compared against K values calculated using the oxidation numbers for diethylene glycol according to Table I, the results are more concordant.

CALCULATION OF PERCENTAGE COMPOSITION

The general expressions for oxidations at the different levels are:

$$P_1Qa_1 + P_1Rb_1 + \ldots = 100K_1 \quad (50\% \text{ sulfuric acid}) \quad (1)$$

$$P_2Qa\_ + P_2Rb_2 + \ldots = 100K_2 \quad (25\% \text{ sulfuric acid}) \quad (2)$$

$$P_3Qa_3 + P_3Rb_3 + \ldots = 100K_3 \quad (12.5\% \text{ sulfuric acid}) \quad (3)$$

Although there are available three expressions for this two-component system, only two are needed. Mathematically, three pairs, the 1-2, 2-3, and 1-3 could be employed. Only the 1-2 pair is analytically useful when it is recalled that the oxidation of ethylene glycol in 12.5% sulfuric acid solution was comparatively slow and resulted in a larger error in the measurement of K than desired. Although oxidation of the sample mixture at the respective levels can be made on samples of different sizes, the simplest case results when the same sample size is used. For such a case, substitution of the respective calibration coefficients results in the expressions:

$$0.1611\,PQ + 0.1885\,PR = 100K_1 \quad (50\% \text{ sulfuric acid}) \quad (4)$$

$$0.1611\,PQ + 0.1508\,PR = 100K_2 \quad (25\% \text{ sulfuric acid}) \quad (5)$$

where Q is the percentage of ethylene glycol and R is the percentage of diethylene glycol in the mixture. Simultaneous solution yields:

$$Q = \frac{100K_1 - 500.0\,(K_1 - K_2)}{(0.1611)\,(P)}$$

$$R = \frac{(K_1 - K_2)\,(100)}{(0.0377)\,(P)}$$

These expressions were used for calculating the percentage compositions of the mixtures from the data in Table V. The results are shown in Table IV.

An accuracy good to within ±5% of the respective glycol contents was realized except when the glycol content of either component was less than 30%; in this case the accuracy dropped sharply. The accuracy of these results is not equal to the expectations in view of the accuracy with which the K values may be determined. Of course, two independent determinations are made with the possibility that the error in each measurement may be additive. Even so this would only account for an over-all maximum error of approximately 0.6%.

The explanation lies in the consideration of the expression for R and the term 0.0377 therein. This constant, 0.0377, is fundamentally a reflection of the difference in the oxidation of diethylene glycol at the two oxidation levels, 1 and 2—that is, a change of oxidation number from 20 to 16. This difference in oxidizability is only a change of one fifth, yet this measured difference must represent the whole (for ethylene glycol exhibits constant oxidation behavior). Obviously, then, an additive error in the K values of 0.6% when magnified fivefold results in an error in the percentage composition of the magnitude observed.

Thus, it is clearly seen that although the oxidation characteristics of the binary mixture of ethylene and diethylene glycol turned out to be the simple case type (involving differential oxidation of only one of the components), it is not necessarily the system which is the most accurately analytically resolved. Good results are obtained only when the differentially oxidizable component shows a large change in oxidation number at the respective oxidation levels. Whether the accuracy of the differential oxidation method can be improved by employing

Table IV. Determination of Percentage Composition of Ethylene Glycol–Diethylene Glycol Mixtures by Spectrophotometic Method

	Solution Mixture					Compos., Expt. 1–2, %		Total Glycol Recovered, %	Compos. Deviation, %	
No.	Mixture (P) Mg.	% Ethylene glycol (Q)	% Diethylene glycol (R)	K_1	K_2	Q	R		Ethylene glycol (q)	Diethylene glycol (r)
1	45.66	11.45	88.55	8.434	6.961	14.5	85.6	100.1	+26.1	−3.3
2	45.80	34.23	65.77	8.172	7.066	35.8	64.0	99.8	+ 4.7	−2.8
3	42.81	49.45	50.55	7.518	6.704	50.0	50.4	100.4	+1.0	−0.2
4	42.44	69.13	30.87	7.225	6.704	67.6	32.6	100.2	−2.2	+5.5
5	41.98	89.64	10.36	6.891	6.710	88.5	11.4	99.9	−1.2	+9.6

Table V. Oxidation of Ethylene Glycol–Diethylene Glycol Mixtures at 100° C.

No.	H_2SO_4, Vol. %	Oxid. Time, Hr.	$K_2Cr_2O_7$ Taken, Meq.	K (exp.)[a] Spectro.	K (exp.)[a] Iodom.	K Calcd.	Deviation K, % Spectro.	Deviation K, % Iodom.
			Solution 1: 45.66 mg. of mixed glycols taken					
1[b]	50	2	10.000	8.434	8.472	8.464	−0.35	+0.09
2	25	2	8.500	6.961	6.933	6.939	+0.32	−0.09
3	12.5	4	8.500	6.358	...	6.175	+3.0	...
4	12.5	2	8.500	...	6.303	6.175	....	+2.1
			Solution 2: 45.80 mg. of mixed glycols taken					
5[b]	50	2	10.000	8.172	8.200	8.204	−0.27	−0.05
6	25	2	8.500	7.066	7.067	7.068	−0.03	−0.01
7	12.5	4	8.500	6.654	...	6.499	+2.4	...
8	12.5	2	8.500	6.625	6.577	6.499	+1.9	+1.2
			Solution 3: 42.81 mg. of mixed glycols taken					
9	50	2	8.500	7.518	7.476	7.489	+0.39	−0.17
10	25	2	8.500	6.704	6.664	6.673	+0.46	−0.14
11	12.5	4	8.500	6.366	...	6.264	+1.6	...
12	12.5	2	8.500	6.331	6.294	6.264	+1.1	+0.48
			Solution 4: 42.44 mg. of mixed glycols taken					
13	50	2	8.500	7.225	7.196	7.196	+0.40	0.00
14	25	2	8.500	6.704	6.682	6.702	+0.03	−0.30
15	12.5	2	8.500	6.454	6.429	6.455	−0.02	−0.40
			Solution 5: 41.98 mg. of mixed glycols taken					
16	50	2	8.500	6.891	6.890	6.882	+0.13	+0.12
17	25	2	8.500	6.710	6.689	6.718	−0.12	−0.43
18	12.5	4	8.500	6.619	...	6.636	−0.26	...
19	12.5	2	8.500	6.558	6.563	66.36	−1.2	−1.1

[a] Average of duplicate determinations; all values corrected for blank.

[b] 60 ml. oxidation volume; 20.0 ml. of 0.5N $K_2Cr_2O_7$.

oxidation conditions such that both components are differentially oxidized, preferably to specific oxidation levels, is still to be determined.

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Chromatographic Separation of Glycols and Monohydric Alcohols

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A rapid separation procedure was required for analyzing decigram quantities of glycol mixtures and alcohol mixtures. Distillation procedures for effecting these separations required large samples and were not applicable to all glycol mixtures. The current work was undertaken to modify a procedure for the separation of alcohols by partition chromatography and to extend this technique to the separation of glycols. The separations were performed on silicic acid and silicic acid–Celite columns. In both cases the immobile phase was water; the alcohols were resolved with mixtures of carbon tetrachloride and chloroform, the glycols with butanol and chloroform. The procedures were found suitable for the separation of mixtures of C_1 to C_5 normal alcohols and the C_2 to C_4 glycols. Isoalcohols were not distinguished from the normal alcohols, and only those glycols containing vicinal hydroxyl groups could be measured. In general, the separations were well defined and gave recoveries of 90 to 110%.

RAPID and accurate methods have not been available for the separation of mixtures of either monohydric alcohols or glycols. Although precision distillation is often employed for such separations, this exacting technique requires relatively large amounts of material. In the case of certain glycols, the vapor pressure relationships are such that efficient resolution of mixtures is difficult if not impossible. The procedures developed in this laboratory employ partition chromatography for the quantitative separation of the lower monohydric alcohols and also the lower glycols.

These methods are an extension of the recent work by Neish (*3, 4*), who reported the analysis of 2- to 3-mg. quantities of alcohol and glycol mixtures by partition chromatography on Celite-water columns. Upon repeating Neish's work it was found that preparation of suitable Celite columns was difficult even with the greatest care. Also, the procedure was applicable only to small samples and in this laboratory gave variable recoveries when tested with known alcohol mixtures.

In this investigation separation procedures were developed for mixtures of the lower alcohols and glycols on silicic acid and silicic acid–Celite columns containing water as the immobile aqueous phase. Carbon tetrachloride–chloroform and chloroform–*n*-butyl alcohol, respectively, were used as developing solvents. Contrary to expectations, only small amounts of the various hydroxy compounds studied were irreversibly retained on these supports.

APPARATUS

Chromatographic apparatus, the assembly shown in Figure 1, is used to carry out the separations of alcohol mixtures and glycol mixtures. Except for some modifications, this equipment is that described by Marvel and Rands (*2*). The head assembly is designed to permit adsorbent packing and continuous operation under air or nitrogen pressure. By manipulation of appropriate stopcocks, different solvents may be added successively to the head reservoir without interruption of flow through the column.

Both reservoirs are large enough to permit addition of the necessary volume of a given solvent at one time. A spherical joint connects the column to the head assembly and simplifies packing and cleaning. A manometer near the pressure inlet and a stopcock at the bottom of the column permit close control of the flow rate.

REAGENTS

Silicic acid, Mallinckrodt's chromatographic grade (100 mesh), analytical reagent.

Carbon tetrachloride, analytical reagent, extracted once with an equal volume of water before use.

Chloroform, analytical reagent. This material contains ethyl alcohol as a preservative, which is removed by extracting five times with an equal volume of water. Washed chloroform is unstable and should be prepared as needed.

n-Butyl alcohol, analytical reagent. Free acids in this material are removed by simple distillation from sodium carbonate.

Sodium metaperiodate ($NaIO_4$), analytical reagent, 0.05*M* aqueous solution.

Sodium hydroxide, 0.05*N* aqueous solution, standardized.

Potassium dichromate, 0.04*N* in 18*N* sulfuric acid solution.

Sodium thiosulfate, 0.02*N* aqueous solution, standardized.

Starch indicator solution, 1% aqueous solution.

Alizarin Yellow R indicator solution, 0.2% in 50% aqueous ethyl solution.

Ice, crushed and washed.

PROCEDURE FOR MONOHYDRIC ALCOHOL MIXTURES

Separation. To 20 grams of silicic acid in a 250-ml. beaker are added 12.5 ml. of water. The mixture is stirred until it appears homogeneous. Approximately 80 ml. of carbon tetrachloride are added, and the mixture is stirred until a smooth slurry results. More or less than the 12.5 ml. of water may be required to produce a smooth slurry. The correct volume is determined by trial. A cotton pledget is placed in the constriction of the apparatus and the adsorbent slurry poured into the tube. Enough pressure is applied to give a rapid flow rate. The column should be tapped frequently during this operation to ensure uniform settling of the adsorbent.

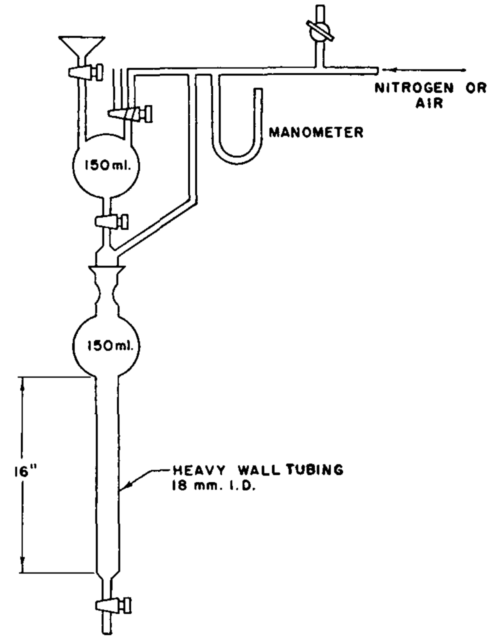

Figure 1. Partition Chromatography Apparatus

When the top of the adsorbent column is almost dry the lower stopcock is closed, and 50 ml. of carbon tetrachloride are added. The solvent is percolated through the adsorbent at a fast rate. Care should be taken not to disturb the surface of the column when the solvent is added. When the last of the carbon tetrachloride just enters the top of the column, the lower stopcock is closed and the pressure released. A satisfactory column results when no cracks or voids are visible and the top of the adsorbent column appears firm. The head assembly is removed, and a circle of filter paper is placed on the column.

An accurately known weight of the alcohol mixture (about 100 mg.) in 1 to 2 ml. of carbon tetrachloride is pipetted onto the column. The sample solution is allowed to enter the adsorbent by gravity. The sample is washed into the column by the successive addition of two 2-ml. portions of the solvent. When the last wash has just entered the column the lower stopcock is closed, the head assembly attached to the column and the alcohols eluted with the following sequence of solvents:

100 ml. of carbon tetrachloride
100 ml. of 1 to 3 chloroform–carbon tetrachloride
100 ml. of 1 to 1 chloroform–carbon tetrachloride
175 ml. of chloroform
200 ml. of 1 to 9 acetic acid–chloroform

Each of the solvents (except the last) is shaken with an equal volume of distilled water before use. A flow rate of 2 to 3 ml. per minute is maintained using air or nitrogen pressure. Each succeeding solvent is added when about 10 ml. of the preceding solvent are left on the column. The percolate is collected in 25-ml. volumes and transferred to 125-ml. Erlenmeyer flasks. Each is analyzed for its alcohol content by dichromate oxidation.

Table I. Oxidation Equivalents of Monohydric Alcohols by Dichromate Oxidation

Alcohol	Molecular Weight	Equivalent Weight	Equivalents of Dichromate per Mole of Alcohol
Methyl	32.0	5.3	6.04
Ethyl	46.1	11.7	3.94
n-Propyl	60.1	12.2	4.92
Isopropyl	60.1	31.1	1.93
n-Butyl	74.1	16.0	4.63
Isobutyl	74.1	13.8	5.36
n-Amyl	88.2	19.3	4.56
n-Hexyl	102.2	30.3	3.38

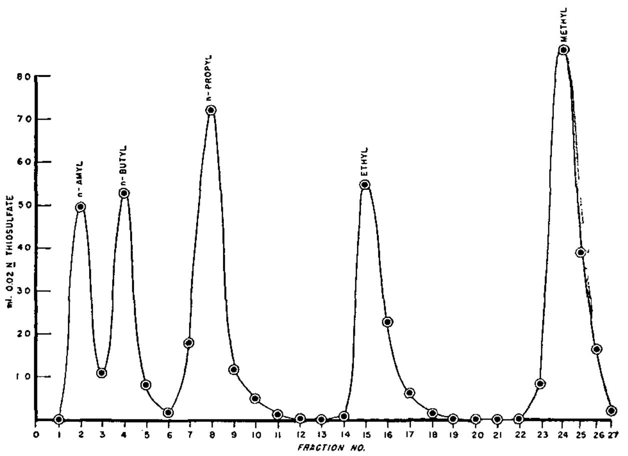

Figure 2. Separation of C_1 to C_5 Monohydric Alcohols by Partition Chromatography

Analysis of Fractions. To each of the 25-ml. percolate fractions are added 10 ml., or some multiple of this volume, of the 0.04*N* dichromate reagent from a pipet. As much as 40 ml. of the reagent may be needed to provide an excess if a fraction contains 15 to 20 mg. of an alcohol. An excess of dichromate is present if a yellow-green color persists in the aqueous layer. The mixture is allowed to stand for 25 minutes with occasional swirling. About 100 ml. of water and 2 to 3 grams of potassium iodide are added. The solution is vigorously swirled and allowed to stand for 5 minutes, after which it is titrated with 0.02*N* thiosulfate to the usual starch end point.

The highest nearly constant titration value or the appropriate multiple of it is taken as the blank from which all other values are subtracted. A plot of the net titration values against fraction number should closely approximate Figure 2. The amount of each alcohol present in the original sample is calculated from the dichromate oxidation equivalent of the alcohol corresponding to a given percolate fraction. The alcohol content of a given fraction is calculated using the equivalent weights given in Table I.

Alcohol mg. = (thiosulfate for blank ml. − thiosulfate for sample, ml.) × normality of thiosulfate × eq. wt. of alcohol.

PROCEDURE FOR GLYCOL MIXTURES

Separation. The adsorbent is prepared by thoroughly mixing 15 grams of a 1 to 2 mixture of Celite–silicic acid with 9.5 ml. of water which has been saturated with 1 to 4 *n*-butyl alcohol in chloroform. As in the alcohol separation, the resulting mixture is worked into a smooth slurry with 80 ml. of chloroform and packed into the column under pressure. An additional 50 ml. of chloroform are forced through the adsorbent to complete the packing.

Two milliliters of 1 to 4 *n*-butyl alcohol in chloroform, containing about 200 mg. of the glycol mixture accurately weighed, are pipetted onto the column. When the sample has just entered the adsorbent, two 2-ml. portions of the same solvent are added to wash the sample into the column. Elution of the glycols is carried out with the following order of solvents:

Table II. Analysis of Known Alcohol Mixtures by Partition Chromatography

	1		2		3		4	
Alcohol	Added, mg.	Rec., %	Added, mg.	Rec., %	Added, mg.	Rec., %	Added, mg.	Rec., %
Methyl	17.7	97.0	17.7	87.0	20.4	82.8	..	...
Ethyl	18.2	89.4	18.2	91.7	18.7	102.1	..	...
n-Propyl	17.0	90.7	17.0	96.1	25.1	103.2	..	...
n-Butyl	15.1	89.9	15.1	101.9	18.9	111.1	20.9	100.9
n-Amyl	..	..	..	..	19.4	104.7	20.7	94.7

Table III. Analysis of Glycol Mixtures by Partition Chromatography

		Composition, %		
Mixture No.	Glycol Components	Known	Found	Recovery
1	1,2-Butylene	2.0	2.2	110
	2,3-Butylene	2.3	2.3	100
	1,2-Propylene	2.2	2.8	126
	Ethylene	93.4	..	...
2	1,2-Butylene	8.3	8.3	100
	2,3-Butylene	8.8	8.0	91
	1,2-Propylene	9.8	9.7	99
	Ethylene	73.2	..	...
3	1,2-Butylene	19.0	20.2	106
	2,3-Butylene	18.1	18.6	103
	1,2-Propylene	19.9	20.0	101
	Ethylene	43.0	..	...

110 ml. of 1 to 4 *n*-butyl alcohol–chloroform
100 ml. of 1 to 1 *n*-butyl alcohol–chloroform
60 ml. of *n*-butyl alcohol

Each of the solvents is equilibrated with an equal volume of water before use and added to the column when about 10 ml. of the previous solvent remains. Successive 10-ml. fractions are collected using a flow rate of 2 to 3 ml. per minute. Normally, pressure is not required to attain this rate. When the last fraction is collected, the column stopcock is closed and the column retained until it has been established that all of the ethylene glycol (if present) has been eluted. If some ethylene glycol remains on the column, it is removed by percolating more *n*-butyl alcohol through the adsorbent, collecting several additional fractions.

Analysis of Fractions. Each percolate fraction is transferred to a 500-ml. wide-mouthed Erlenmeyer flask and 10 ml. of 0.05*M* sodium metaperiodate solution are added. The solutions are allowed to stand 25 minutes with occasional swirling. One hundred milliliters of water, 2 ml. of Alizarin Yellow R indicator solution, and washed, crushed ice are added. Enough ice should be present to maintain the temperature of the solution at about 0° C. during the subsequent titration. Excess periodate is titrated with 0.05*N* sodium hydroxide to the indicator change from yellow to orange.

The first five fractions may be discarded. The next three fractions are collected and used as blanks for the titration. The glycols appear in subsequent fractions as shown in Figure 3. The glycol content of each fraction is calculated from the equation:

$$\text{Glycol, mg.} = (\text{NaOH ml. blank} - \text{NaOH ml. sample}) \times \text{normality NaOH} \times \text{mol. wt. of glycol}$$

DISCUSSION

Monohydric alcohols are determined in the chromatographic fractions by means of dichromate oxidation. Oxidation equivalents of a number of alcohols are given in Table I. As can be seen from these values, between 2 and 6 equivalents of dichromate are involved in oxidation. Isopropyl alcohol is oxidized to acetone. Although most of these oxidation equivalent weights are empirical, they are reproducible.

Identification of a chromatographic peak is made from its location on a chromatogram such as Figure 2. In this experiment, approximately 20 mg. of each alcohol were added to the column and separated by the recommended procedure. Excellent resolution of the mixture was obtained, the alcohols appearing in this order: *n*-amyl, *n*-butyl, *n*-propyl, ethyl, and methyl. Attempts were made to improve the separation between amyl and butyl alcohols by the use of less polar solvents, particularly hydrocarbons, but without success. When mixtures of isopropyl and *n*-propyl alcohols were subjected to the separation procedure, no resolution was obtained. Similarly no separation occurred between *n*-butyl and isobutyl alcohols. In each case both alcohols appeared in the same fractions as the normal alcohols.

Mixtures of *n*-hexyl and *n*-amyl alcohols could not be adequately separated by the recommended procedure. Some resolution was obtained with petroleum ether as solvent, but the chromatographic peaks were not distinct. In view of these results the procedure in its present form is suited only to the separation of the C_1 to C_5 alcohols without distinction between isomers. Some analyses of known mixtures are reported in Table II. In all cases the alcohols were the best obtainable commercially.

The recoveries from a number of separations similar to those in Table II ranged from 83 to 111%. Methyl alcohol generally gave the lowest recovery, possibly because of some irreversible adsorption on the silicic acid. This recovery range could be improved by combining all fractions for a single alcohol (or glycol) and performing only one analysis rather than the three to seven separate analyses as in the present procedure.

Upon applying the alcohol separation procedure to mixtures of glycols it became evident that these compounds were much more strongly retained in the immobile aqueous phase than the simple alcohols. Consequently a solvent sequence of greater polarity was required. Solutions of *n*-butyl alcohol in chloroform, similar to those used to separate dibasic acids (*2*), were most effective. The high proportion of butyl alcohol in these developing solvents resulted in greatly decreased flow rates through the silicic acid–water columns. This difficulty was obviated by the use of 1 to 2 mixtures of Celite and silicic acid which permitted excellent throughput without use of pressure.

The degree of separation achieved by the recommended procedure is shown in Figure 3. In this case the mixture consisted of 10 mg. each of 1,2-butylene, glycol (1,2-butanediol), 2,3-butylene glycol (2,3-butanediol), and 1,2-propylene glycol (1,2-propanediol) with 170 mg. of ethylene glycol. This mixture and those shown in Table III were prepared to simulate impure samples of ethylene glycol; therefore this glycol was not collected.

1,2-Butylene glycol was eluted with 1 to 3 *n*-butyl alcohol in chloroform, 2,3-butylene glycol and 1,2-propylene glycol were removed with 1 to 1 *n*-butyl alcohol in chloroform, and ethylene glycol could be completely eluted with *n*-butyl alcohol. Results obtained with three known mixtures are shown in Table III. In each case approximately 200 mg. of the mixture were analyzed.

The analytical procedure used for the analysis of the chromatographic fractions is that of Dal Nogare and Oemler (*1*). This method employs periodate cleavage of vicinal hydroxyl groups and is specific for glycols which have hydroxyl groups on adjacent carbons. Consequently only glycols containing this grouping were studied in this work.

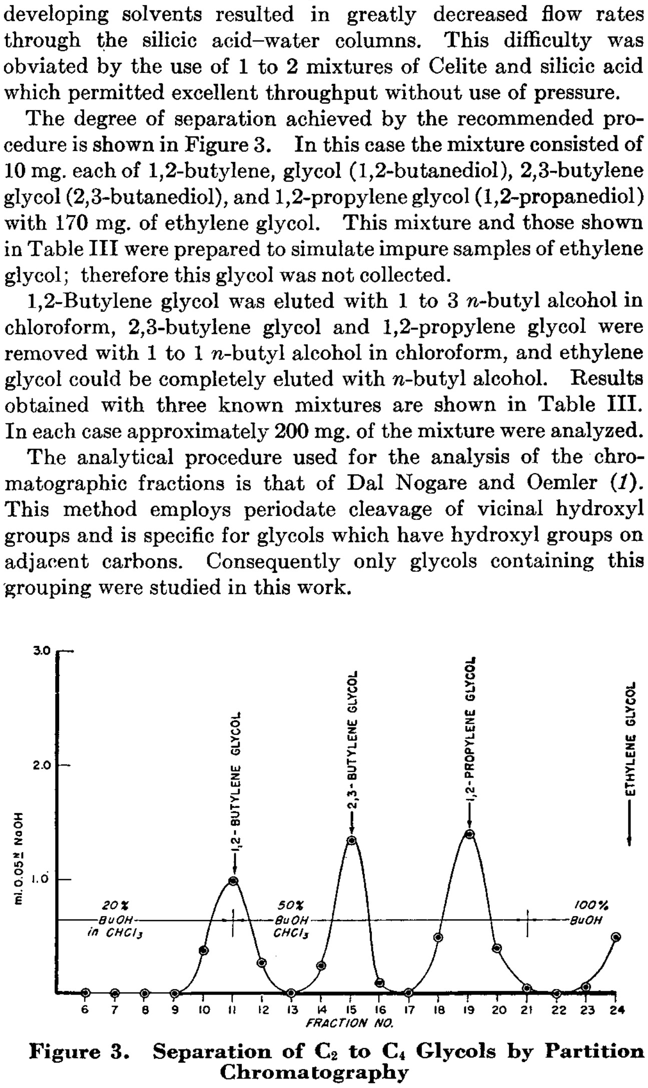

Figure 3. Separation of C_2 to C_4 Glycols by Partition Chromatography

Compounds such as diethylene glycol and 1,4-butylene glycol (1,4-butanediol) do not interfere with the separation and detection of the glycols studied in this work. It is possible that these and other nonvicinal glycols can be separated by partition chromatography, but this study will require the development of an accessory method for their determination. Dichromate oxidation was considered for this purpose but could not be used because of the *n*-butyl alcohol present in the developing solvents.

ACKNOWLEDGMENT

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Determination of Elemental Fluorine

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In connection with an investigation of halogen fluorides, it appeared practical to adapt the bromine-fluorine reaction as an analytical method for fluorine. Bromine, dissolved in bromine trifluoride, can be titrated with fluorine gas at room temperature. The reaction is quantitative and the end point is readily detected by the discharge of the bromine color. Using this procedure, the purity of several fluorine samples has been determined to ±2%. The uncertainty of the titration, which is limited by the error in reading the fluorine pressure, should be reducible to about 0.1% by use of a more sensitive pressure-measuring device. It is possible to employ the titration to determine bromine, or substances which yield bromine on reaction with bromine trifluoride. These include metals, oxides, and halides other than fluorides. The titration can also be adapted for the determination of bromine pentafluoride.

FLUORINE is usually prepared by the electrolysis of a molten potassium fluoride–hydrogen fluoride bath, using a steel cathode and a carbon anode (*1*). Fluorine so produced contains hydrogen fluoride from the bath, carbon fluorides from reaction with the anode, and air due to leakage. The hydrogen fluoride is easily removed from fluorine by treatment with sodium fluoride. The other contaminants, however, are very difficult to remove, and laboratory-generated or commercial tank fluorine will contain variable amounts of these almost unavoidable impurities. For many purposes it is important to know the purity of the fluorine, but many investigators have neglected fluorine purity because of the difficulties of analysis.

At present two methods of fluorine determination are commonly used. The first consists of reaction of a known volume of fluorine with mercury and measurement of the residual gas after fluorine has been converted to mercury fluoride (*7*). The second consists of reaction of a known volume of fluorine with sodium chloride and determination of the chloride by absorption in alkaline arsenite (*5*). The first method has several drawbacks, most important of which is the formation of a mercury fluoride scum on the surface of the mercury. This makes readings of the mercury level difficult and inaccurate, especially where fluorine is a major constituent. This method is primarily useful for the determination of small amounts of fluorine in other gases inert to mercury. The second method is complicated and involves a rather large number of chemical operations. Loss of some of the residual gases in the various absorbents can lead to error in analysis of the inert components. Bibliographies and additional methods of analysis have recently appeared in the literature (*3*, *6*).

In the course of other work, a procedure has been developed which can readily be applied to fluorine determination. This method depends on the fact that elemental fluorine reacts smoothly and quantitatively with bromine in the liquid phase according to the reaction $3F_2 + 2Br_2 \rightarrow 2BrF_3$. Bromine trifluoride (boiling point 127° C.) itself is an excellent solvent for carrying out this reaction. The method involves the titration of a known amount of bromine dissolved in bromine trifluoride with the fluorine gas to be analyzed. A visual end point, the disappearance of the red-brown bromine color to give the light yellow of a fluorine-saturated bromine trifluoride solution, is easily discernible. The inert components can then be collected if desired for further analysis. This titration analysis is especially useful where fluorine is the major constituent.

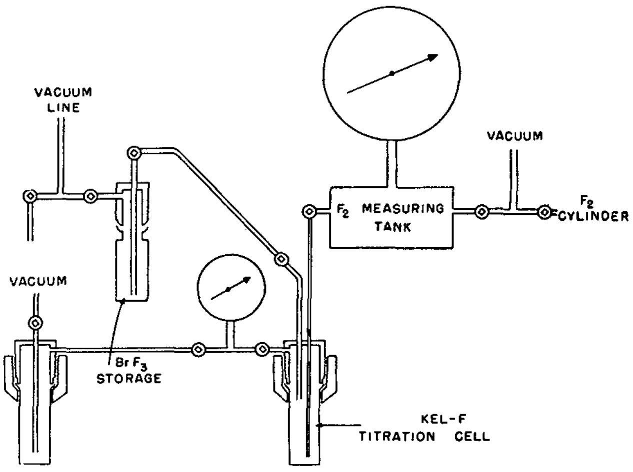

Figure 1. Diagram of Apparatus

MATERIALS

The bromine trifluoride was obtained from the Harshaw Chemical Co. and purified by vacuum distillation in a nickel still. Bromine, bromine pentafluoride, hydrogen fluoride, and nonvolatile fluorides are the major impurities. The fraction used (boiling point 95–95.5° C. at 250 mm.) was pale yellow in color and was stored in a welded nickel container. The fluorine was obtained from the Pennsylvania Salt Co. and was used directly from the tank. The purity of the fluorine in two cylinders employed in this work was greater than 99%. Another cylinder which was analyzed contained 95% fluorine, and an isolated shipment of two cylinders has been found to contain less than 80% fluorine.

APPARATUS

The titrations were carried out in the nickel apparatus shown in Figure 1. The fluorine-measuring tank is a welded nickel tank fitted with Hoke nickel-alloy diaphragm valves (Nos. 411 and 413). An $8^1/_2$-inch K-Monel Bourdon tube Helicoid gage (No. 462), 0 to 1500 mm. absolute, was used to measure the fluorine pressure in the tank. The volume of the tank and gage was determined at several pressures by expanding gas from a standard volume into the tank and noting the pressure change. After correction for the connecting tubing, the volume was found to be 1125 ± 2 ml. at 25° C. The tank was enclosed in a Lucite box to minimize temperature fluctuations.

The titration cell is a molded Kel-F (chlorotrifluoroethylene high polymer) (*8*) tube, $^3/_4$ inch in outside diameter, with a standard S.A.E. $^3/_4$ inch flare. These tubes are transparent and have been made in several ways. Machining from commercially available 1-inch rod is simplest, but gives the least transparent tubes. The Argonne Plastics Shop has produced reasonably clear tubes by molding directly from Kel-F powder, but with only intermittent success. Molding bottoms and flares on lengths of commercially available extruded tubing, $^3/_4$ inch in outside diameter, gives the clearest tubes, but the technique is not yet fully developed. Directions for preparation of suitable tubes will be published when the methods have been fully explored. The fluorine delivery tube is an $^1/_8$ inch nickel tube, long enough to dip below the surface of the solution in the cell.

TITRATION PROCEDURE

The bromine used in the titration was measured either by weighing, or from micropipets calibrated "to deliver." The bromine–bromine trifluoride solution can be prepared in various ways. For example, bromine trifluoride may first be placed in the titration vessel, either by pipetting or from the storage vessel. The authors have found a volume of about 8 ml. to be convenient. The bromine trifluoride is then pumped on for a short time at room temperature to remove any traces of volatile impurities such as bromine or bromine monofluoride (boiling point 15° C.) which may have formed since the initial purification. The titration vessel is then filled with dry nitrogen and removed momentarily from the line, and a known volume of bromine is added by pipet. The titration vessel is then replaced on the line, the contents are frozen in liquid nitrogen, and the system is evacuated. After warming to room temperature, fluorine is added from the fluorine reservoir until the deep bromine color is discharged. The end point is sharp and is readily detected by visual comparison of the reaction mixture with pure bromine trifluoride in a similar Kel-F tube.

Alternatively, bromine may be weighed into the empty titration vessel, which is then attached to the line and frozen with liquid nitrogen, and the vessel is evacuated. Bromine trifluoride is then delivered from the storage vessel by application of helium pressure. The volume between the two valves is about 8 ml., and after this portion of the line has been filled with bromine trifluoride, the lower valve is opened and the proper amount of bromine trifluoride allowed to flow into the titration vessel. The titration can then be carried out as before. This procedure was used in samples 1 and 4.

When the fluorine contains large amounts of inert gases, it may be necessary to bleed the inert gases from the titration cell in order to add enough fluorine to reach the end point. This may be done by interrupting the titration, venting the gases, and continuing the titration until the end point is reached. The inert gases may readily be allowed to expand into an evacuated container and submitted for mass spectrographic analysis.

The reaction of fluorine with bromine dissolved in bromine trifluoride is rapid, smooth, and complete at room temperature. At temperatures above 50° C., the reaction of fluorine with bromine trifluoride to yield bromine pentafluoride becomes appreciably fast. To avoid error as a result of this side reaction, it is important to carry out the titration at temperatures below 30° C., where the rate of bromine pentafluoride formation is negligibly small.

RESULTS AND DISCUSSION

The results of a number of determinations are given in Table I. The accuracy of the titration is limited by the 2- to 5-mm. error in reading the fluorine pressure. With a fluorine consumption corresponding to a 300-mm. change in pressure, an uncertainty of about 2% may be anticipated. When greater accuracy is desired, a balancing device such as a differential pressure transmitter or a Booth-Cromer gage (*2*, *4*) may be employed. A sensitive gage or manometer may be used to measure the inert gas side and determine the fluorine pressure to ±0.1 mm. Using such equipment and bromine samples of about 1 gram, the uncertainty in the titration should be readily reducible to about 0.1%.

To illustrate a complete analysis of commercial fluorine, the inert gases from a sample of the 95% fluorine were collected and analyzed mass spectrographically by the Consolidated Engineering Corp., Pasadena, Calif., with the following results: nitrogen 62.8%, oxygen 28.0%, carbon monoxide 3.2%, argon 0.6%, hydrogen <0.11%.

Table I. Determination of Fluorine by Titration with Bromine in Bromine Trifluoride Solution

Fluorine Sample[a]	Bromine, Grams	Fluorine Equivalent to Bromine, Cc. (STP)	Fluorine Sample Used, Cc. (STP)	% F_2 in Sample
1	1.520	638	631	101.0
2	0.609	255	259	98.5
3	0.609	255	256	99.6
4	0.609	255	261	97.7[b]
5	0.609	255	258	99.0
6	0.609	255	271	93.7
7	0.609	255	264	96.5
8	0.609	255	267	95.5

[a] Samples taken from three different fluorine cylinders obtained from Pennsylvania Salt Manufacturing Co. Samples 1, 2, and 3 came from one cylinder, 4 and 5 from a second, and 6, 7, and 8 from a third.

[b] A sample of fluorine from this tank was also analyzed by the mercury absorption method and found to contain >99% fluorine (*9*).

The bulk of the inert impurity is obviously air. The presence of carbon monoxide is rather surprising, and illustrates the desirability of detailed information about fluorine used in experimentation.

OTHER ANALYTICAL APPLICATIONS

Using fluorine of known purity, it is possible to employ the titration to determine bromine, or substances that yield bromine by reaction with bromine trifluoride. These include metals, oxides, and halides other than fluorides, all of which yield bromine on reaction with bromine trifluoride. Some of these reactions liberate substantial amounts of heat and are best performed in a metal (nickel or Monel) titration tube. When the titration is carried out in metal tubes, an alternative to the disappearance of bromine color as a criterion of the end point is used. In this procedure, fluorine is added until uptake ceases. The excess fluorine is then pumped out, and the cell is again filled with fluorine. This latter amount of fluorine is then an overtitration correction, and by subtraction from the first amount of fluorine used, gives the fluorine actually used in the titration. The fluorine must be of such purity that the back-pressure of the inert gases does not exceed the fluorine pressure in the measuring tank.

The titration can also be adapted for the determination of bromine pentafluoride. It has been shown that bromine will react quantitatively with bromine pentafluoride at temperatures above 150° C. It should be possible, therefore, to add excess bromine to the sample in a nickel titration tube which is frozen and evacuated as described. The tube is then heated at 150° to 200° C. to complete the reaction

$$3BrF_5 + Br_2 \rightarrow 5BrF_3$$

The residual bromine is back-titrated at room temperature with fluorine. Presence of bromine trifluoride, a common impurity in bromine pentafluoride, obviously will not interfere with the analysis, and this procedure then becomes particularly useful in determining bromine pentafluoride in bromine trifluoride.

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Determination of Maleic Hydrazide Residues in Plant and Animal Tissue

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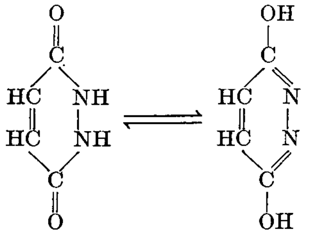
Maleic hydrazide, 1,2-dihydropyridazine-3,6-dione, is a growth regulant or retardant. Because it is useful on certain food crops, it is important to determine the residues in a large number of plant and animal tissues as a prerequisite to registration of the chemical for agricultural use. A method is presented whereby the hydrazide is reduced and hydrolyzed in water, alkali, and zinc, to split off hydrazine, which is then distilled and determined colorimetrically using *p*-dimethylaminobenzaldehyde. Residue determinations of below 1 p.p.m. are possible. The procedure can probably be used without major modifications for other compounds that will form hydrazine under the conditions of this method.

MALEIC hydrazide (*8*) (1,2-dihydropyridazine-3,6-dione) is a growth regulant or retardant. Because it is useful on certain food crops, it is important to determine the residues in a large number of plant and animal tissues as a prerequisite to registration of the chemical for agricultural use. Maleic hydrazide(I) probably exists in tautomeric equilibrium.

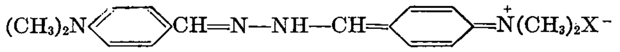


I

It is stable to both acidic and basic hydrolysis, as evidenced by the fact that heating for several hours at temperatures up to 200° C. in $18N$ sulfuric acid or concentrated sodium hydroxide causes no breakdown. Some decomposition does occur in concentrated sodium hydroxide at higher temperatures. However, after reduction, ring opening by hydrolysis readily occurs, yielding a quantitative amount of hydrazine which can be distilled out under favorable conditions. Many reduction-hydrolysis systems were evaluated, resulting in the present selection of zinc plus alkali in water.

Of the possible quantitative methods for determining small amounts of hydrazine, the colorimetric method of Pesez and Petit (*5*) was selected because of minimum interference from naturally occurring substances. A recent publication by Watt and Chrisp (*7*) also describes use of this method. According to Pesez and Petit, hydrazine reacts with *p*-dimethylaminobenzaldehyde to give an azine, the acid salts of which are an intense red (yellow in dilute solution). Solutions of these water-soluble salts obey Beer's law and are stable after maximum color is developed in 10 to 15 minutes.

The *p*-dimethylaminobenzalazine formed in alkaline solution gives canary yellow crystals, insoluble in water and slightly soluble in alcohol, melting point 264–6°. In an acid, HX, isomerization to a *p*-quinone structure(II) occurs, giving dark red crystals, melting point 224°; it is a reversible reaction. This method is sensitive to 0.1γ per ml. Based on this information, absorption and concentration curves were made from purified hydrazine monosulfate and *p*-dimethylaminobenzaldehyde.



II

REAGENTS

Reagent grade *p*-dimethylaminobenzaldehyde was further purified by the procedure described by Adams and Coleman (*1*). The purified product was washed free of chloride and dried to a constant weight in a vacuum desiccator over calcium chloride. If passed through a 60-mesh screen prior to final drying, the resulting product will dissolve more rapidly. The dried compound or dissolved reagent should be stored in the dark. The reagent should be prepared fresh daily by dissolving 0.20 gram in 5 ml. of $2N$ sulfuric acid.

Purified maleic hydrazide available from Naugatuck Chemical, Division of U. S. Rubber Co., Naugatuck, Conn.

Hydrazine monosulfate, c.p., freshly recrystallized from water.

p-Dimethylaminobenzaldehyde, practical grade, purified according to (*1*).

Sodium hydroxide, analytical reagent grade pellets.

Sulfuric acid, analytical reagent grade.

Nitrogen, oil pumped.

Benzene, analytical reagent grade.

Oxygen-free water, freshly boiled distilled water, used while still hot.

Zinc, analytical reagent grade, 30-mesh granular.

An excellent discussion (*6*) on the stability of *p*-dimethylaminobenzaldehyde in analytical work is of interest.

CHOICE OF WAVE LENGTH

Curves of wave length *vs.* per cent transmittance are shown in Figure 1.

All readings were taken on a Model DU Beckman quartz spectrophotometer using 1-cm. Corex cells. Curve *A* was obtained by developing maximum color under optimum conditions, using a solution of 0.5 ml. of reagent plus 5.5 ml. of distilled water which contained 6γ of hydrazine monosulfate. Distilled water was used as a blank. The solution for curve *B* contained 0.5 ml. of reagent plus 5.5 ml. of distilled water, using distilled water as a blank. Curve *C* was obtained from the solution for curve *A* using the solution for curve *B* as a blank. The preferred wave length of 455 mμ was thus chosen.

EFFECT OF ACID NORMALITY ON COLOR INTENSITY

The effect of acid normality on degree of color obtained with a fixed quantity of hydrazine monosulfate (7.5γ) and reagent was determined using both hydrochloric and sulfuric acids. A blank containing an equal acid concentration and 0.5 ml. of reagent was used as a reference source. The curves in Figure 2 were obtained by plotting acid normality against per cent transmittance at 455 mμ. For a given increase in normality, a greater deviation in per cent transmittance occurs with hydrochloric acid than with sulfuric acid. The normal working normality used is about 0.17, obtained by adding 0.5 ml. of reagent to 5.5 ml. of approximately neutral distillate sample. Decreasing the normality much below 0.17 precipitates the reagent and the azine.

EFFECT OF REAGENT CONCENTRATION ON COLOR DENSITY

To determine the optimum concentration of reagent for maximum color development, samples containing 7.5γ of hydrazine monosulfate with varying amounts of reagent were run against blanks containing equal amounts of reagent. From the curve in Figure 3, 0.02 gram of reagent in the 6-ml. sample was chosen as the most desirable concentration for normal use.

PREPARATION OF STANDARD CONCENTRATION CURVES

Aliquots of a standard solution (10γ per ml.), representing from 1 to 12γ of hydrazine monosulfate, were brought to a volume of 5.5 ml. with water and the azine color was developed with 0.5 ml. of reagent. A curve plotting hydrazine monosulfate concentration against per cent transmittance at 455 mμ is shown in Figure 4, *A*. Excellent conformity to Beer's law is evident.

A recovery curve obtained by using aliquots of a standard solution of maleic hydrazide (10γ per ml.) put through the entire analytical procedure is shown in Figure 4, *B*. This curve lies

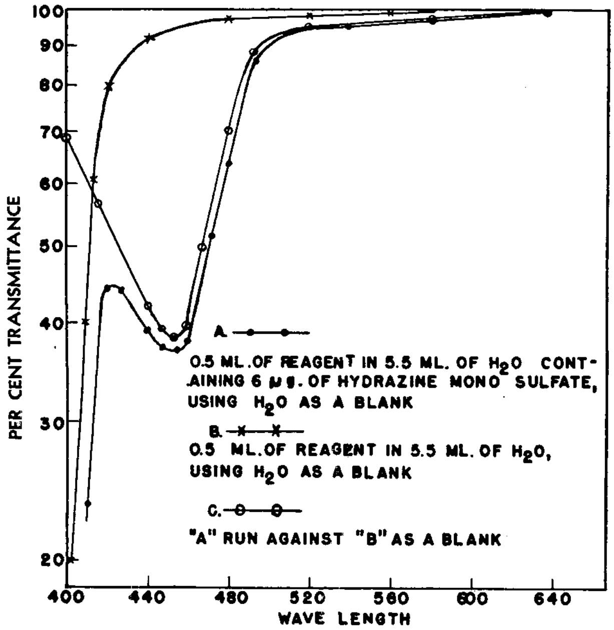


Figure 1. Absorption Curves for Choice of Wave Length

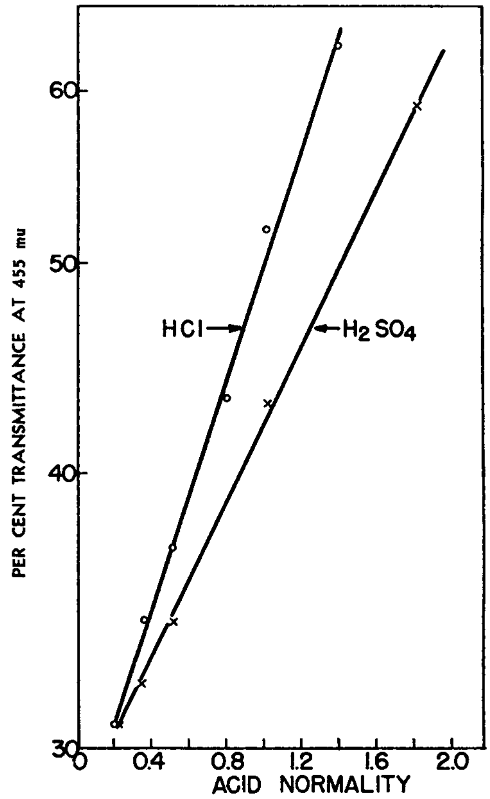


Figure 2. Effect of Acid Normality on Color Intensity

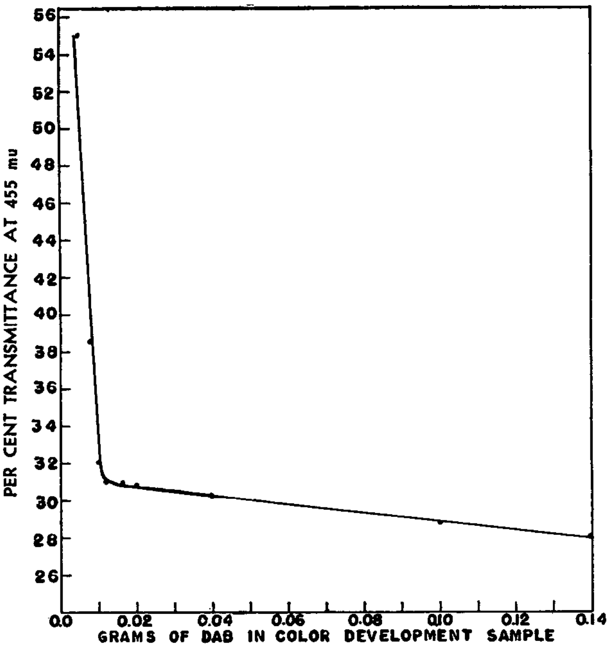


Figure 3. Standard Concentration Curves

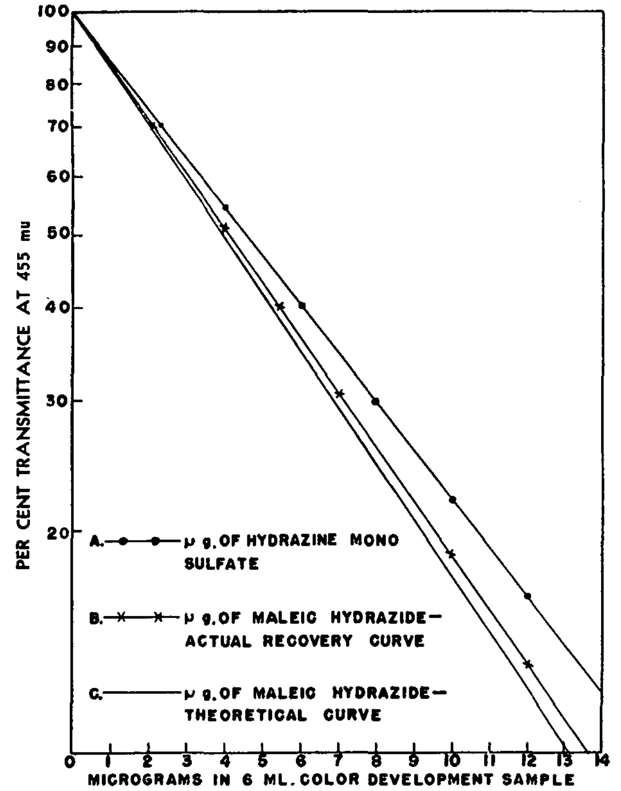


Figure 4. Effect of *p*-Dimethylaminobenzaldehyde Concentration on Color Density

slightly above the theoretical recovery curve, *C*. The actual recovery curve might vary slightly with changes in the distillation cycle but is usually at least 95% of the theoretical curve.

APPARATUS

From early development work on this analytical method it was evident that a special reduction-distillation apparatus would be useful. A detailed drawing of the apparatus in use in this laboratory at the present time is shown in Figure 5. A complete apparatus or any part may be purchased from Macalaster Bicknell, 181 Henry St., New Haven, Conn. The main feature is the thick-walled flask made necessary by the corrosive action of concentrated sodium hydroxide at high temperatures.

The vapor line of the apparatus should be wrapped with asbestos tape. A metal valve from an ordinary rubber suction bulb placed in the tube connecting the funnel and flask inlet tube is helpful in preventing steam from blowing back into the inlet tube.

A free flame was found to be most satisfactory for heating. However, a 200-ml. heating mantle may be used. A smaller mantle does not have the required heating capacity.

Tap water for condensing may be adequate in winter months, averaging below 10° C., but ice water should be used when water temperature is above this value.

PROCEDURE FOR OBTAINING STANDARD MALEIC HYDRAZIDE CURVE

Place approximately 1 ml. of oxygen-free water and 1 drop of concentrated sulfuric acid in the distillate receiver and immerse the condenser tube. Place 30 grams of sodium hydroxide in the 125-ml. flask and add the maleic hydrazide standard sample (1γ and up) and water to make a total volume of 15 ml. of water added. Heat on a hot plate until a well temperature of 180° C. is reached. Silicone oil has proved a safe fluid for the temperature well. Remove the flask from the hot plate, wipe the ball joint dry, and apply silicone grease, being careful to keep it out of the neck of the flask. Add through a funnel 15 grams of 30-mesh granular zinc metal. Swirl the flask once to distribute the zinc evenly and quickly clamp the flask in place. Start the flow of nitrogen to remove oxygen from the system, which might cause autooxidation of the hydrazine formed. A bubble rate of 100 to 150 per minute as counted through the condenser tip is sufficient.

Reduction of maleic hydrazide and hydrolysis to hydrazine begins at low temperatures. However, hydrazine does not begin to distill from concentrated sodium hydroxide until a temperature of approximately 140° C. is reached. Therefore, to avoid low results due to side reactions of the liberated hydrazine, it is important to reach the distillation temperature as quickly as possible.

This is best accomplished by heating with a free flame, played over the bottom of the flask. Throughout the distillation, adjust

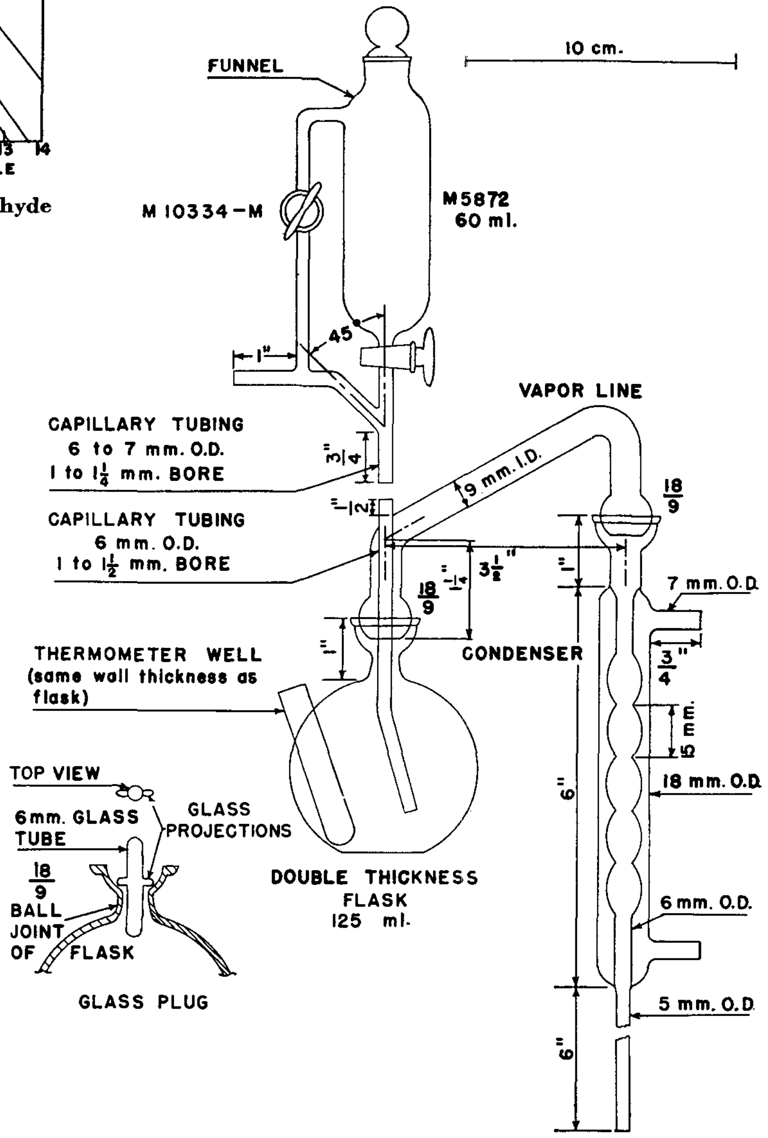


Figure 5. Reduction-Distillation Apparatus for Determination of Maleic Hydrazide

the flow of nitrogen for a bubble rate of 100 to 150 per minute. During a typical run, the caustic solution starts to boil within 1 or 2 minutes. Within approximately 5 minutes the temperature rises to 180° C. When this temperature is reached, remove the flame and add approximately 10 ml. of oxygen-free water slowly through the dropping funnel. Apply heat and continue distilling until a temperature of 180° C. is again reached. The whole operation should not take more than 15 minutes.

A preheater heating mantle can be used if desired, but the distillation cycle should correspond closely to the cycle using a free flame. Applying about 65 to 80 volts to a 200-ml. mantle is usually sufficient.

After the distillation, disconnect and wash the condenser by sucking the distillate up into the condenser several times, using a rubber suction bulb with an 18/9 male ball joint attached. Rinse with only a few milliliters of water to keep the distillate volume at a minimum. For initial work it may be well to connect another condenser in place to collect an additional 5 ml. to run separately to be sure that all the hydrazine has been collected. At times, the one drop of concentrated sulfuric acid added to the distillate receiver may not be sufficient to keep the distillate acid. Until the proper amount to add has been determined, check the pH of the distillate with pH paper several times during the distillation. Use the minimum amount of sulfuric acid to keep the distillate acid, to prevent a serious normality change in the final 6-ml. color development sample.

Sometimes it may be more convenient to add an appropriate amount of reagent (0.5 ml. for every 5 ml. of distillate) in place of the water and acid. The reagent serves as a pH indicator, in that it will begin to precipitate before the distillate becomes neutral.

The 125-ml. flask should be washed as soon as possible after each distillation or the caustic mass will solidify. Wash the flask by slowly adding a small stream of water directed to run down the side of the flask. When the spattering stops, rinse with tap water. Before the flask is re-used, rinse with concentrated sulfuric acid to be sure that no zinc remains.

To a 5.5-ml. or smaller aliquot of the distillate add 0.5 ml. of reagent and adjust the total volume to 6 ml. A 10-ml. precision mixing cylinder is useful for this work. After 15 minutes determine the per cent transmittance at 455 mμ, reading against a blank prepared by adding 0.5 ml. of reagent to 5.5 ml. of distilled water. Plot per cent transmittance figures, using varying amounts of maleic hydrazide, against concentration to obtain the standard maleic hydrazide curve. In case the reagent is added to the distillate receiver, merely adjust the volume accordingly prior to determining the per cent transmittance.

EFFECT OF ADDED SUBSTANCES

Compounds which before or after zinc-alkali reduction give the Ehrlich test (*4*) give a characteristic pink interference which is an additive error. Such compounds as pyrrole, pyrogallol, resorcinol, pyrazoles, and piperidine give a positive test without reduction, while tryptophan and nicotine do so only after reduction.

Compounds such as diethanolamine, urea, ethylamine, and formaldehyde give no interference when added to the zinc-alkali reduction mass, unless several grams are added. Ammonium hydroxide and sodium cyanide have no effect. Several milligrams of copper sulfate had no effect, but large quantities should probably be avoided (*2, 3*). Sodium bisulfite and other compounds which break down to sulfur dioxide interfere with the color development.

DETERMINATION OF MALEIC HYDRAZIDE IN PLANT AND ANIMAL TISSUES

All samples should be reduced by conventional methods to approximately 0.5 pint (0.3 liter) in volume. This laboratory uses a heavy-duty potato French-fry cutter for this work. The reduced sample is then finely ground and blended. An Osterizer made by the John Oster Manufacturing Co., Racine, Wis., has proved very satisfactory for this final grinding and blending. Some samples require an addition of $^1/_2$ part or more of water to facilitate grinding.

An aliquot of the final blend representing from 0.5 to 3 grams of original sample is then placed in the 125-ml. reaction flask. Samples having high starch content, such as potatoes and sugar beets, present a serious foaming problem unless a sample weight of 1 gram or less is used. The sensitivity of the method is such that 1 gram of sample is usually sufficient for an analysis. In some cases a larger sample weight only increases the complexity of the analysis. Adding the sample through a funnel will aid in directing it to the bottom. Wash the sample through the funnel with 15 ml. of water, add 30 grams of sodium hydroxide, and heat on a hot plate until a well temperature of 180° C. is reached. This caustic precooking step removes most of the interference encountered from plant and animal tissues.

When working with a new type of tissue, a recovery curve should be obtained by adding known amounts of maleic hydrazide to an untreated check sample in the concentration range expected. Recoveries from 90 to 100% are usually obtained. If recoveries with certain samples are not in this range, vary the amounts of water and caustic used, as well as the temperatures involved.

INTERFERENCE

When plant and animal tissues are carried through the reduction-hydrolysis procedure, the distillate sometimes contains materials which interfere with the colorimetric test for hydrazine by developing a pink color in contrast to the yellow azine. This interference is additive; however, it can be compensated for or eliminated where precise or low concentrations of maleic hydrazide are encountered.

For approximate data it may only be necessary to run several untreated blank samples of the particular tissue to be analyzed to determine the average error due to interference, which can then be subtracted from the total reading. In most cases, the absolute value of interference is independent of sample size. Therefore, a 1-gram sample will show approximately one third the interference encountered from a 0.3-gram sample, as in the former case the interference is divided by 3 to obtain parts per million.

Table I. Typical Data on Residues

Maleic Anhydride, P.P.M.	
Added to blank onion sample	Recovered (including interference)
0	0.24–0.26
0.50	0.75–0.80
1.00	1.30–1.26
5.00	5.25–5.27

The situation may arise in which it is not known whether maleic hydrazide is actually present in a sample, and any color obtained could all be due to interference, or mostly to interference and only a small amount to maleic hydrazide. In such cases, an absorption curve should be run to detect the presence of the characteristic dip at 455 mμ.

INTERFERENCE REMOVAL BY BENZENE WASHING

When the caustic precook does not remove essentially all of the interference, it may be further reduced by washing with benzene.

Increase the acidity of the distillate by adding 1 drop of concentrated sulfuric acid for every 5 ml. collected, and then vigorously wash two or three times with approximately 10-ml. portions of benzene. After each wash allow the two layers to separate by centrifuging and remove the benzene layer with a vacuum line. After the last washing, bubble nitrogen through the distillate for several minutes to remove the last traces of benzene (heating to remove the benzene causes low results). Add 0.5 ml. of reagent to an aliquot of the distillate. Add potassium hydroxide dropwise until the precipitated reagent is just persistent. Add just enough $1N$ sulfuric acid to redissolve and adjust the total volume to 6 ml. In this way free hydrazine in a basic solution never occurs.

LOW RESIDUE WORK

If the maleic hydrazide residue in a sample is of such a level that the distillate collected contains much less than 1γ of hydrazine monosulfate in a 5-ml. aliquot, the per cent transmittance will be above 90% on the concentration curve. Therefore, for the most accurate work the hydrazine in the distillate should be concentrated. This cannot be accomplished by heat evaporation of the water, because this method produces low results. A satisfactory method, consisting of precipitating the hydrazine as *p*-dimethylaminobenzalazine and redissolving in a smaller volume of water, has been worked out.

Add 0.5 ml. of reagent for each 5 ml. of distillate to be concentrated. After complete color development, add dropwise a saturated solution of potassium hydroxide until precipitation is complete. Filter through a 15-ml. medium or fine fritted-glass funnel and wash the precipitate and funnel with a few milliliters of water. Also wash inside the funnel stem. Place the funnel in a micro bell jar vacuum filtration apparatus, using a 10-ml. beaker as a receiver. Add 6 ml. of water containing only a sufficient quantity of concentrated sulfuric acid (5 to 10 drops) to dissolve the precipitate completely. Because of the slope of the curve in Figure 3, it is necessary to make a standard curve similar to curve *B* in Figure 4, using the same reagent concentration in the blank.

TYPICAL DATA ON RESIDUES

The data in Table I were obtained using 1 gram of onion sample throughout. All samples were run using the standard procedure and the distillate concentrating method.

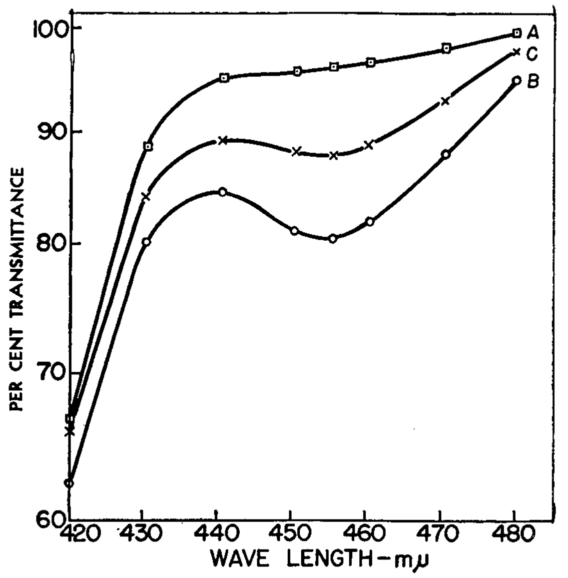


Figure 6. Use of Absorption Curves to Estimate Residue

A. Blank 1-gram sample of onion
B. Blank 1-gram sample of onion with 1 p.p.m. of maleic hydrazide
C. Unknown with 0.5 p.p.m. of maleic hydrazide

Interference in the blank could be reduced by increasing the sample size or using the benzene washing method, thus permitting analysis below 0.5 p.p.m.

When only an estimate of the residue in the 1 p.p.m. range is necessary—i.e., whether it is above or below 1 p.p.m.—a great saving in time is realized by omitting special concentrating and interference removal steps. This is illustrated graphically in Figure 6. The relative dip in the curves at 455 mμ indicates that the unknown obviously contains less than 1 p.p.m.

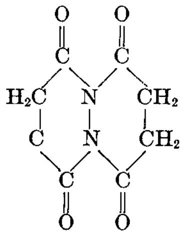
DISCUSSION

Because the determination of maleic hydrazide actually involves the determination of microgram quantities of hydrazine, it may be well to investigate general precautions necessary for quantitative work with this compound. Two excellent references (*2*, *3*) are given for this reason.

Solutions containing hydrazine or *p*-dimethylaminobenzalazine should not be filtered through filter paper or any other absorptive filters. Fritted-glass filters are *satisfactory*.

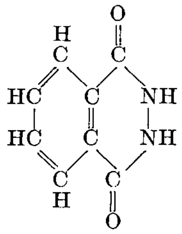
Various plant samples handled by this laboratory include onions, white and sweet potatoes, sugar and table beets, turnips, parsnips, carrots, celery, spinach, apples, pears, tomatoes, tobacco, grasses, cottonseed oil, and various seeds. Animal samples include muscle, stomach, spleen, pancreas, gonad glands, liver, kidney, small and large intestine, feces, urine, and milk. Samples of soil have also been run. Duplicate determinations on final blended samples have checked to within 10% or better.

Some work has been done on the possibility of extending this analytical method to other compounds. In general, this procedure can probably be used without major modifications for compounds which will hydrolyze, or be reduced and hydrolyzed under these conditions to give hydrazine. For example, bicyclodisuccinhydrazide (III),



III

yields hydrazine under these conditions, while phthalhydrazide (IV),



IV

does not. Phthalhydrazide probably could be analyzed if it were first reduced by other means, but this would be classed as a major modification.

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Differential Determination of Vitamin B_6 Group

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A new differential assay of individual components of the vitamin B_6 group with *Sacch. carlsbergensis* 4228 has been studied. The general method consists of a combination of the separation of pyridoxamine with synthetic cation exchanger, alkali-acetone treatment for the decomposition of pyridoxal, and microbiological assays of the fractions. The results of experiments with natural substances were similar to those of Rabinowitz and Snell. The method is useful for the differential determination of the vitamin B_6 group in natural materials.

RABINOWITZ and Snell (*5*) reported that pyridoxal, pyridoxamine, and pyridoxine may be separately assayed using *Saccharomyces carlsbergensis*, *Streptococcus faecalis*, and *Lactobacillus casei.*

The author studied a new microbiological differential determination of the vitamin B_6 group, which involves the combined use of an adsorption procedure with a cation exchanger and a microbiological assay with *Sacch. carlsbergensis*. *Sacch. carlsbergensis* 4228 has been most widely used for the determination of the total vitamin B_6 group. Pyridoxal, pyridoxamine, and pyridoxine are almost equally active with this organism (*8*).

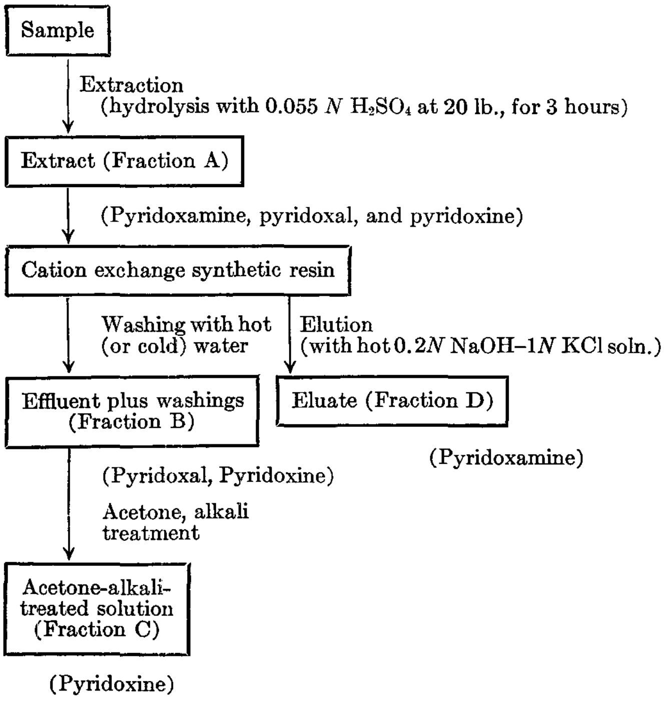
Shimizu and Shiba (*6*) have suggested that pyridoxamine, because it is more basic than other forms of vitamin B_6, can be separated by adsorption on the R—COONa type of cation exchange resin. After the separation of pyridoxamine, pyridoxal can be inactivated by treatment with acetone and alkali (*7*).

The method for determining the three members of the vitamin B_6 group with *Sacch. carlsbergensis* after fractionation by these treatments has been tested and found useful, inasmuch as it does not require so much reagent for the basal medium as the previous method.

PROCEDURE

The general method is shown in Scheme I.

Scheme I. General Method



Extraction of Vitamin B_6. For most materials, acid hydrolysis with $0.055N$ sulfuric acid at 20 pounds pressure for 3 hours was used. The transformation of one form of vitamin B_6 to the other has been shown to be negligible under these conditions (*3*).

Separation of Pyridoxamine. As an adsorbent, the following two resins were found useful for the separation of pyridoxamine from other forms of the vitamin B_6 group.

KH4B (sodium form), Oda Laboratories, Department of Industrial Chemistry, Kyoto University. The resin was synthesized by the condensation polymerization of phenoxyacetic acid and formaldehyde (*2*).

Duolite C 60 (sodium form), a phosphonic cation exchanger of the Chemical Process Co., Redwood City, Calif.

The exchange tube, made of brown glass 0.7 to 0.8 cm. in diameter, was filled with 1.5 grams of 40- to 60-mesh resin. After the extraction, the filtrate was adjusted to pH 5.0 and the volume was made to 200 ml. (Fraction A).

An aliquot—for example, 50 ml.—containing 0.05 to 50 γ of each form of vitamin B_6, was allowed to pass through the resin column at the rate of 1 to 2 ml. per minute at pH 6 to 7. Then the column was washed down with about 50 ml. of cold water in the case of KH4B or hot water (80° C.) in the case of Duolite C 60. All effluents (effluent and washing) were mixed together

Table I. Calculation of Values of Three Forms of Vitamin B_6

Fraction	Total (Fraction A)	Acetone-Alkali Treated Soln. (Fraction C)		Eluate (Fraction D)	
Standard used	Pyridoxal HCl	Pyridoxine HCl	Pyridoxal HCl	Pyridoxamine 2HCl	Pyridoxal HCl
Value measured	a	b	c	d	e

Pyridoxine hydrochloride = b
Pyridoxamine 2 hydrochloride = d
Pyridoxal hydrochloride = $a - (c + e)$

Table II. Separation of Pyridoxamine from Pyridoxine and Pyridoxal by Adsorption with Sodium Salt of Duolite C 60

[50 ml. of an aqueous solution of vitamin B_6, containing 1 to 10γ of pyridoxamine dihydrochloride, pyridoxal hydrochloride, and pyridoxine hydrochloride, was passed through the resin column (resin 1.5 grams), washed with 50 ml. of hot water, and eluted with 50 ml. of a hot $0.2N$ NaOH-N KCl solution.]

Vitamin B_6	Vitamin, γ	Vitamin Recovery, %: Effluent plus washing	Vitamin Recovery, %: Eluate
Pyridoxamine 2HCl	10	0	98.0
	5	0	101.0
	1	0	98.0
Pyridoxal HCl	10	100.0	0
	5	101.2	0
	1	101.0	0
Pyridoxine HCl	10	100.0	0
	5	98.5	0
	1	99.0	0

Microbiological assays were run with 5, 10, and 20 millimicrograms of the vitamin.

and made up to a definite volume (Fraction B). The solution was considered to contain pyridoxal and pyridoxine.

Pyridoxamine adsorbed on the resin was eluted with 50 or 100 ml. of a hot 0.2*N* sodium hydroxide–1*N* potassium chloride solution at 80° C. at the rate of 1 ml. per minute. The eluate was neutralized with 1*N* hydrochloric acid and made up to a definite volume (Fraction D).

Destruction of Pyridoxal. According to Snell (*7*), pyridoxal is inactivated in solution by treatment with equal volumes of acetone and 5*N* sodium hydroxide.

In this experiment, an aliquot of the effluent (Fraction B), containing more than 20 millimicrograms of pyridoxine and appropriate amounts of pyridoxal, was mixed with 0.5 volume of acetone and 5*N* sodium hydroxide, respectively, allowed to stand for 4 hours at room temperature, neutralized with hydrochloric acid, and made up to a definite volume, after removal of acetone under vacuum (Fraction C).

Microbiological Assay of Vitamin B$_6$. The vitamin contents of the fractions were determined with *Sacch. carlsbergensis* according to the method of Atkin *et al.* (*1*), modified by Rabinowitz and Snell (*4*).

The sample solution for microbiological assay was prepared from each fraction to contain 1 to 20 millimicrograms.

The averages of the values measured with the sample solutions diluted in three ways from each fraction were indicated as the amounts of the vitamin in the fraction.

Because the responses of *Sacch. carlsbergensis* to pyridoxal pyridoxamine, and pyridoxine are not exactly identical, standards for the three forms must be run. The values of the three forms of vitamin B$_6$ were calculated as shown in Table I.

Table III. Separation of Pyridoxal from Pyridoxine by Acetone-Alkali Treatment

Vitamin B$_6$	Amounts of Vitamin Treated, γ	Destruction of Pyridoxal, %	Pyridoxine Recovery, %
Pyridoxal HCl	5	97.5	...
	1	99.3	...
	0.1	100.0	...
	0.05	100.2	...
Pyridoxine HCl	5	...	95.0
	1	...	100.0
	0.1	...	105.1
	0.05	...	102.0
Pyridoxal HCl plus Pyridoxine HCl	5 + 5	102.5	97.5
	1 + 1	103.0	97.0
	0.1 + 0.1	97.5	102.5
	0.05 + 0.05	101.0	99.0

Microbiological assays were carried out with 2 and 4 millimicrograms of vitamins.

Table IV. Recoveries of Pyridoxal, Pyridoxamine, and Pyridoxine

Fraction	Vitamins Expected	Recoveries of Vitamins Added, %	
		Mean	Standard Deviation
Effluent (Fraction B)	Pyridoxal, pyridoxine	104.3	5.3
Acetone-alkali-treated solution (Fraction C)	Pyridoxine	99.8	7.5
Eluate (Fraction D)	Pyridoxamine	98.2	3.0

Tests were run at concentrations of 1, 0.2, 0.1, 0.02, and 0.01γ per ml. of sample solution.

Recovery Test. To an aliquot of the extract (Fraction A), definite amounts of pyridoxal, pyridoxamine, and pyridoxine were added. Assays were run just as for the sample solution.

RESULTS AND DISCUSSION

The results of the tests for the separation of pyridoxamine from pyridoxal and pyridoxine by the adsorption are shown in Table II.

Pyridoxamine is adsorbed on the resin, while neither pyridoxine nor pyridoxal is adsorbed. It would be expected that pyridoxamine could be separated from the others by this procedure. When applied to natural products, however, some loss of pyridoxamine may occasionally be observed. This might be accounted for by interferences of adsorbed contaminating substances. In such cases, better results should be obtained by passing the influent through the two resin columns successively.

Table VI. Distribution of Pyridoxal, Pyridoxamine, and Pyridoxine in Natural Materials

Sample	Vitamin B$_6$[a]		
	Pyridoxal	Pyridoxamine	Pyridoxine
Rice bran (air-dried)	9.68	12.9	26.2
Spinach (fresh)	0.40	0.20	0.42
Baker's yeast (fresh)	2.28	4.59	0.45
Fodder yeast (fresh)	4.56	9.33	2.49
Beef liver (fresh)	2.05	5.98	1.04
Beef heart (fresh)	0.92	0.62	0.13
Hog liver (fresh)	3.97	7.38	0.57
Sake	0.13	0.17	0.57

[a] Free vitamin B$_6$ base.

All three compounds were adsorbed similarly, by the COOH type of KH4B and Duolite C 60 resins. More leakage of pyridoxamine was observed with amberlite IRC 50.

The results of experiments on the decomposition of pyridoxal are given in Table III. By acetone-alkali treatment, pyridoxal was completely decomposed; pyridoxine was not affected.

The recoveries of the three members of vitamin B$_6$ during the total process are shown in Table IV. The distributions of pyridoxal, pyridoxamine, and pyridoxine in some natural materials are given in Tables V and VI.

For extraction, 1 gram of rice bran and 20 grams of fresh spinach were autoclaved with 0.44*N* sulfuric acid at 20-pound pressure for 3 hours; 2 grams of pressed yeast, beef liver, beef heart, and hog liver was hydrolyzed with 0.055*N* sulfuric acid at 20-pound pressure for 3 hours; 100 ml. of sake was mixed with concentrated sulfuric acid and water, made up to 180 ml., and after adjustment of the acid concentration to 0.055*N*, was autoclaved as usual.

For the recovery tests, 5γ of pyridoxal hydrochloride, 5γ of pyridoxamine hydrochloride, and 10γ of pyridoxine hydrochloride were added to 50 ml. of the extract.

The contents of the three forms of the vitamin were indicated

Table V. Differential Assay of Vitamin B$_6$ in Natural Materials

Sample	Fraction A, Pyridoxal HCl		Fraction C			Fraction D					
			Pyridoxine HCl		Pyridoxal HCl,	Pyridoxamine 2HCl		Pyridoxal HCl,			
	Assay value (*a*)	Recovery[a], %	Assay value (*b*)	Recovery, %	assay value (*c*)	Assay value (*d*)	Recovery, %	assay value (*e*)	Pyridoxal HCl (*a*) − (*c* + *e*)	Pyridoxine HCl (*b*)	Pyridoxamine 2HCl (*d*)
Rice bran	54.3	96	31.5	98	28.3	19.1	97	14.3	11.7	31.5	19.1
Fresh baker's yeast	8.07	103	0.54	108	0.50	6.42	95	4.83	2.74	0.54	6.42
Fresh beef liver	9.80	101	1.25	101	1.06	8.37	92	6.28	2.46	1.25	8.37
Fresh beef heart	1.79	100	0.16	107	0.14	0.87	98	0.65	1.00	0.16	0.87

Vitamin content, micrograms per gram of sample.

[a] Amounts of pyridoxine HCl and pyridoxamine 2HCl added were converted to pyridoxal HCl.

as the amounts of free bases (micrograms per gram of material, for sake, milliliters) (Table VI).

The distributions of pyridoxal, pyridoxamine, and pyridoxine in the natural materials so far tested are similar to the results of Ribinowitz and Snell. Consequently, the method has been considered practicable, inasmuch as less reagents are required for basal medium than in the previous method.

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Quercetin as Colorimetric Reagent for Determination of Zirconium

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Methods described in the literature for the determination of zirconium are generally designed for relatively large amounts of this element. A good procedure using colorimetric reagent for the determination of trace amounts is desirable. Quercetin has been found to yield a sensitive color reaction with zirconium suitable for the determination of from 0.1 to 50γ of zirconium dioxide. The procedure developed involves the separation of zirconium from interfering elements by precipitation with *p*-dimethylaminoazophenylarsonic acid prior to its estimation with quercetin. The quercetin reaction is carried out in $0.5N$ hydrochloric acid solution. Under the operating conditions it is indicated that quercetin forms a 2 to 1 complex with zirconium; however, a 2 to 1 and a 1 to 1 complex can coexist under special conditions. Approximate values for the equilibrium constants of the complexes are $K_1 = 0.33 \times 10^{-5}$ and $K_2 = 1.3 \times 10^{-9}$. Seven Bureau of Standards samples of glass sands and refractories were analyzed with excellent results. The method described should find considerable application in the analysis of minerals and other materials for macro as well as micro amounts of zirconium.

ALTHOUGH numerous excellent reagents and procedures are available for the determination of macro amounts of zirconium, the situation is far from satisfactory with respect to the determination of microgram amounts. Only a few reagents have been developed for trace analysis and the reactions are not altogether ideal from the standpoints of sensitivity and selectivity.

Alizarin (or Alizarin Red S) (*4–7, 9, 10, 13, 14*) is probably the most important reagent for the colorimetric determination of zirconium, but the color reaction is not too sensitive. *p*-Dimethylaminoazophenylarsonic acid (*8, 11, 12*) is second in importance to Alizarin Red S. The procedures are indirect, light absorption measurements being made on the dye solution obtained by decomposing the zirconium azo-arsonate with ammonia. Stehney and Safranski (*12*) determined microgram amounts of zirconium in this manner. Flavonol, introduced by Alford and coworkers (*1*), is important in zirconium analysis by fluorescence.

This study, made in part on behalf of the Atomic Energy Commission, was undertaken with two objectives in mind: First to find a colorimetric reagent sensitive to small concentrations of zirconium and second, to apply it to the determination of microgram amounts of zirconium in siliceous materials. Quercetin was selected from more than 100 compounds tested, because of its high sensitivity, a nearly colorless blank, stable color over a wide acidity range, and availability in a pure state.

EXPERIMENTAL

Factors Affecting the Zirconium-Quercetin Color System. When quercetin is added to an acid solution of zirconium an intense yellow color is obtained. Various factors affecting the zirconium-quercetin color system were studied to establish optimum working conditions. Preliminary experiments indicated that a certain amount of alcohol was necessary to prevent the precipitation of quercetin and this variable was included in the study. In the experiments, all the solutions were made to a total volume of 25 ml. The order of addition of the reagents was always the same. The zirconium solution was added first, acid second, alcohol third, and an alcoholic solution of quercetin last. Absorbancies were determined with a Beckman spectrophotometer, Model DU, using 1-cm. cells and distilled water as reference solution. The slit width was 0.05 mm. except for the spectral transmittancy data below 420 mμ where 0.1 mm. was used. Unless otherwise indicated all solutions were $0.5N$ in hydrochloric acid and contained 3 mg. of quercetin and 8 ml. of alcohol.

Spectral transmittancy data for the reagent blank and 30γ of zirconium dioxide are given in Figure 1. The optimum wave length was taken as 440 mμ because at this wave length the absorption given by the blank is small and that by zirconium still large. In the work that follows, all absorbancies were measured at 440 mμ.

Figure 2 illustrates the effect of alcohol concentration on the absorbancy of 54.4γ of zirconium dioxide. A precipitate of quercetin was obtained almost immediately from solutions containing less than 6 ml. of alcohol; with 6 ml. of alcohol some quercetin precipitated after 10 hours. It is desirable to keep the alcohol concentration to a minimum because of the smaller solubility of salts in alcoholic media. For this reason 8 ml. of alcohol in 25 ml. of solution was taken as optimum.

The effect on acid concentration on the absorbancy given by 54.4γ of zirconium dioxide is illustrated in Figure 3. The absorbancy is almost independent of acid concentration in the region from 0.1 to $1N$. The reagent blank increases sharply in absorbancy for acid concentrations above $1N$ and reaches a value of more than 0.2 absorbancy unit at $3N$. On the basis of the foregoing the optimum acidity was taken as $0.5N$.

The reaction can also be carried out in nitric or perchloric acid solution, as comparable data are obtained. Hydrochloric acid is preferable to perchloric acid because of the greater stability of zirconium in hydrochloric acid solution (*3*). For example, it was found that standard perchloric acid solutions of zirconium lose strength rapidly if the acid concentration is below 20% by volume. Nitric acid is inferior to hydrochloric acid because of its tendency to oxidize quercetin. At elevated temperatures this effect is serious for solutions of greater acidity than $0.5N$. The sensitivity of the zirconium-quercetin color reaction is greatly diminished in sulfuric acid medium, probably because of the complexing action of sulfate with zirconium.

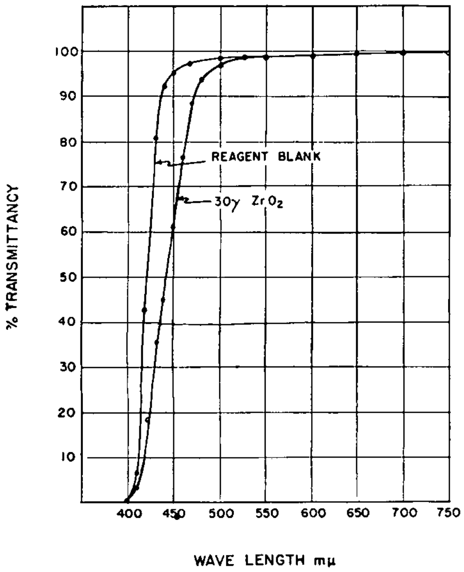


Figure 1. Spectral Transmittancy Curve

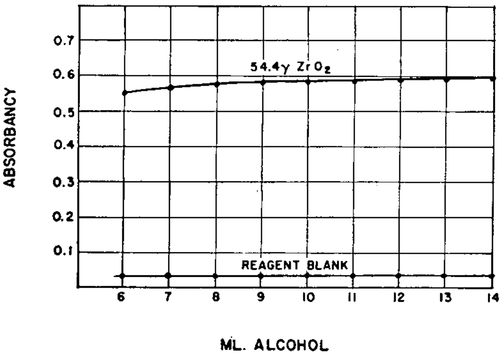


Figure 2. Effect of Alcohol Concentration

The data in Figure 4 were obtained by keeping the zirconium concentration fixed at two levels (20 and 54.4γ of zirconium dioxide) and varying the quercetin concentration in each series. The absorbancies shown are corrected for the absorbancies of blanks containing the same amounts of quercetin. The optimum concentration of quercetin was taken as 3 mg. of quercetin for 25 ml. of solution. In Figure 5 the quercetin concentration was kept fixed at two levels (150 and 300γ of quercetin) and the zirconium concentration was varied.

Data for the working curve are given in Figure 6. These data were obtained using the optimum conditions previously established. The solutions were $0.5N$ in hydrochloric acid and contained 8 ml. of alcohol and 3 mg. of quercetin in a total volume of 25 ml. A straight-line relationship between absorbancy and micrograms of zirconium dioxide is shown up to 60γ of zirconium dioxide. Solutions containing from 60 to 150γ of zirconium dioxide deviate slightly from the Beer-Lambert-Bouguer law but their absorbancies are reproducible. The region from 0 to 10γ of zirconium dioxide was examined in detail for any deviation from the straight-line relationship, but none was found.

The solutions, containing as much as 150γ of zirconium dioxide, were allowed to stand for 24 hours, after which time the absorbancies were redetermined. The maximum change in absorbancy was found to be 0.003. The spectrophotometric sensitivity of the zirconium-quercetin color reaction is 0.004γ zirconium dioxide per square centimeter.

Theoretical Analysis of the Zirconium-Quercetin Reaction. The relationship between absorbancy and mole ratio of quercetin to zirconium, curves (1) and (2), Figure 4, is linear up to a mole ratio of 1 to 1. At ratios where the molar concentration of the quercetin is greater than that of the zirconium the absorbancy increases in a nonlinear fashion with increase in quercetin (zirconium concentration fixed) and reaches a nearly constant value for very high mole ratios.

Various hypotheses were tested as possible explanations for the

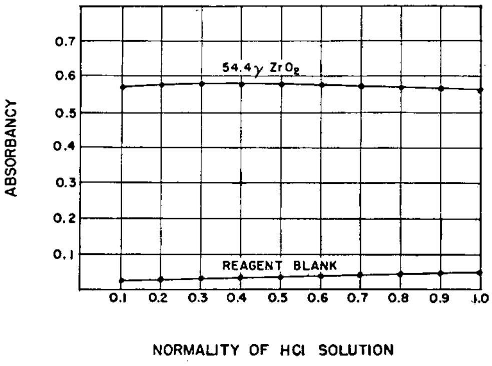


Figure 3. Effect of Acidity

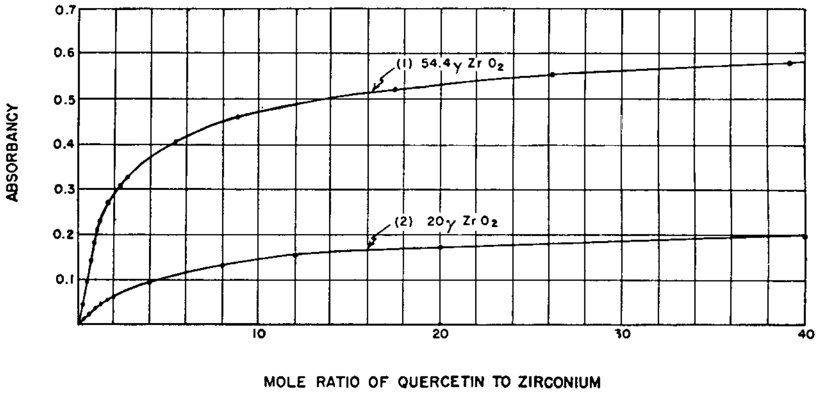


Figure 4. Effect of Quercetin Concentration with Zirconium Concentration Fixed

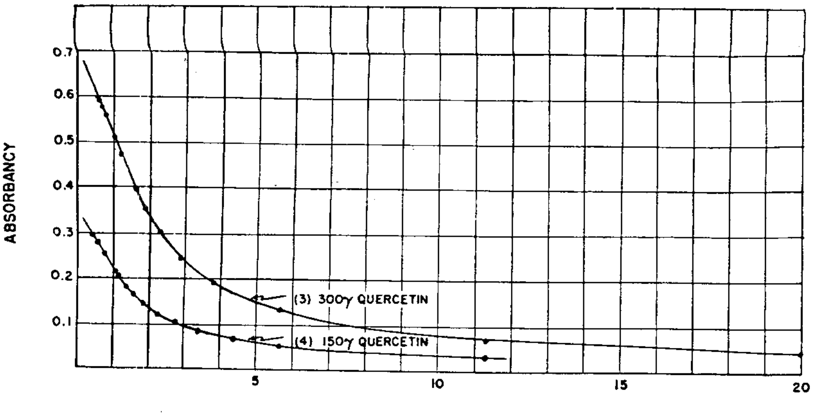


Figure 5. Effect of Zirconium Concentration with Quercetin Concentration Fixed

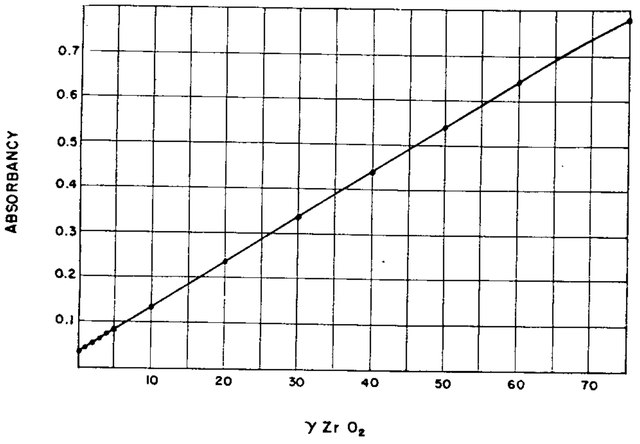


Figure 6. Working Curve

nature of the curves plotted from these data. The only consistently logical explanation is based on the hypothesis that two zirconium-quercetin complexes coexist in the test solutions. The zirconium forms only the 1 to 1 complex in dilute solutions of quercetin. This complex is largely dissociated (α being constant in the straight line portion of the curves), and as the quercetin concentration is increased (zirconium concentration fixed) a greater fraction of the zirconium forms the 1 to 1 complex. As the quercetin concentration is increased still further a point is reached where nearly all the zirconium is in the form of 1 to 1 complex and the 2 to 1 complex makes its appearance. The relative amount of the 2 to 1 complex increases as more quercetin is added until all the zirconium is present in the form of the 2 to 1 complex. At this point the absorbancy of the solution should remain constant.

The equilibria involved are

$$ZrOClQ + H^+ = ZrO^{++} + QH + Cl^- \qquad (1)$$

$$ZrOQ_2 + 2H^+ = ZrO^{++} + 2QH \qquad (2)$$

and

$$\frac{(ZrO^{++})(QH)(Cl^-)}{(ZrOClQ)(H^+)} = K_1$$

$$\frac{(ZrO^{++})(QH)^2}{(ZrOQ_2)(H^+)^2} = K_2$$

For both complexes coexisting in equilibrium

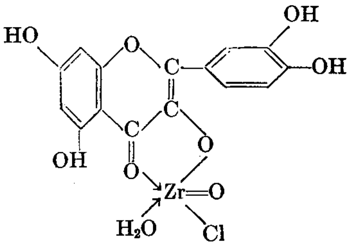
$$\frac{(ZrOClQ)(QH)}{(ZrOQ_2)(H^+)(Cl^-)} = \frac{K_2}{K_1} = k_3$$

and at constant hydrochloric acid concentration

$$\frac{(ZrOClQ)(QH)}{(ZrOQ_2)} = k$$

It is possible, from the spectrophotometric data given, to test the hypothesis by calculating k for the various test solutions in which the two complexes coexist and noting if these values are reasonably constant. For these calculations the absorbancy indexes of the pure 1 to 1 and 2 to 1 complexes are required. The absorbancy index of the 1 to 1 complex may be calculated from the absorbancies obtained on extrapolation of the curves, Figure 5, to zero mole ratio. A reasonably close average value of the absorbancy index of the 2 to 1 complex may be calculated from the absorbancies at mole ratio 40 to 1, Figure 4. The calculations for k are simplified if it is assumed that in solutions where both complexes coexist the concentration of uncombined zirconium is negligible and that no complexes of zirconium other than the quercetin complexes are formed. The values of k were found to be reasonably constant and lend weight to the foregoing interpretation. Approximate values of the equilibrium constants for the 2 to 1 and 1 to 1 complexes were determined, $K_1 = 0.33 \times 10^{-5}$ and $K_2 = 1.3 \times 10^{-9}$.

The method of Bent and French (*2*) is especially applicable to the determination of formulas of dissociable complexes. This method indicated that in the dilute region only the 1 to 1 complex is formed. It was possible to isolate this complex and chemical analysis indicated the probable formula to be:



Reactions of Quercetin with Other Elements. Nearly all of the elements were tested to determine their behavior with quercetin under the optimum conditions used for the zirconium-quercetin reaction. Each element was tested in the absence of zirconium and at two levels of zirconium (5 and 25γ of zirconium dioxide) from a total volume of 25 ml. The absorption given by colored ions in 0.5N hydrochloric acid was also determined. A density difference of 0.003 unit (equivalent to 0.3γ of zirconium dioxide) was taken as the cutoff for reporting interference. Elements not discussed were not tested.

The following cations do not interfere in the maximum amounts taken (expressed as oxides):

0.5 gram: Na, K, and NH_4
0.1 gram: Rb, Cs, Mg, Ca, Ba, Sr, Zn, Cd, Hg^{++}, Ag, Pb, Tl^+, Mn^{++}, and As^{+++}
0.05 gram: La and Y
0.004 gram: Eu, Yb, Gd, and Dy

Acetate (0.5 gram) and bromide (0.2 gram) ions do not interfere.

Some elements interfere by decreasing the color intensity given by zirconium. These elements probably consume reagent or their anions react with zirconium. Some elements interfere because of the natural color of their ions in hydrochloric acid solution. Some elements produce unstable colors with quercetin, whereas other elements react to increase the color intensity of the solution. The maximum amount of each element that can be tolerated and the nature of the interference are given in Table I. Serious interference is given by the following ions: oxalate, fluoride, phos-

Table I. Maximum Permissible Amounts of Various Anions and Cations in the Colorimetric Determination of Zirconium with Quercetin

(Amounts calculated as oxides unless otherwise indicated)

Compound	Maximum Amt. at 5γ ZrO_2 Level	Maximum Amt. at 25γ ZrO_2 Level	Nature of Interference
Citric acid	10 mg. acid	1 mg. acid	Neg., quenches
Tartaric acid	10 mg. acid	1 mg. acid	Neg.
$(NH_4)_2HPO_4$	>100γ P_2O_5	65γ P_2O_5	Neg.
NaF	10γ F^-	5γ F^-	Neg.
Na_2SO_4	13 mg. SO_3	3.5 mg. SO_3	Neg.
LiCl	> 50 mg.	16 mg.	Neg.
$HAuCl_4$	0.25 mg.	0.25 mg.	Pos., colored ion
$CuCl_2$	5 mg.	5 mg.	Unstable soln.
$Be(NO_3)_2$	3 mg.	3 mg.	Pos.
$Ce(NO_3)_3$	25 mg.	5 mg.	Neg.
$Sm(NO_3)_3$	10 mg.	10 mg.	Pos.
$Pr(NO_3)_2$	10 mg.	10 mg.	Pos., colored ion
$Nd(NO_3)_3$	25 mg.	25 mg.	Pos.
$Sc(NO_3)_3$	20γ	20γ	Pos.
H_3BO_3	8 mg.	8 mg.	Pos.
$AlCl_3$	0.6 mg.	0.6 mg.	Pos.
$Ga(NO_3)_2$	15γ	15γ	Pos.
$In(NO_3)_3$	7 mg.	7 mg.	Pos.
$HgNO_3$	150γ	150γ	Unstable soln.
$TiCl_2(OAc)_2$	15γ	15γ	Pos.
$HfOCl_2$	0.75γ	0.75γ	Pos.
$Th(NO_3)_4$	40γ	40γ	Pos.
$GeCl_4$	75γ	75γ	Pos.
$SnCl_4$	0.4γ	0.4γ	Pos.
NH_4VO_3	200γ	200γ	Unstable soln.
$BiCl_3$	5 mg.	5 mg.	Pos.
$CrCl_3$	250γ	250γ	Pos., colored ion
$K_2Cr_2O_7$	1γ Cr_2O_3	1γ Cr_2O_3	Pos.
$(NH_4)_6Mo_7O_{24}$	0.2γ	0.2γ	Pos.
Na_2WO_4	0.5γ	0.5γ	Pos.
$FeCl_3$	6γ	6γ	Pos., unstable soln.
$Fe(NH_4)_2(SO_4)_2$	50γ	50γ	Pos., unstable soln.
$CoCl_2$	5 mg.	5 mg.	Pos., colored ion
NaI	5 mg. I^-	5 mg. I^-	Pos., I_2
Na_2HAsO_4	...	3 mg. As_2O_5	Neg.
H_2SeO_3	> 0.2 g. SeO_2	50 mg. SeO_2	Neg.
H_2TeO_3	10 mg. TeO_2	10 mg. TeO_2	Pos.
$NiCl_2$	8 mg.	8 mg.	Pos., colored ion
$UO_2(NO_3)_2$	3 mg.	3 mg.	Pos.
Na_2SiO_3	...	40 mg.	Neg.
$SbCl_3$	3γ	3γ	Pos.
Nb_2O_5 in H_2SO_4	1γ	1γ	Pos.
Ta_2O_5 in H_2SO_4	2γ	2γ	Pos.
H_2PdCl_4	60γ Pd	60γ Pd	Pos., colored ion
H_2RhCl_5	100γ Rh	100γ Rh	Pos., colored ion
H_2RuCl_5	4γ Ru	4γ Ru	Pos., colored ion
H_2PtCl_6	500γ Pt	500γ Pt	Pos., colored ion

phate, scandium, gallium, mercurous mercury, titanium, hafnium, thorium, germanium, tin, vanadium, chromium, molybdenum, tungsten, iron, antimony, niobium, tantalum, and the platinum metals tested. Although the interfering elements are many, only scandium, titanium, hafnium, thorium, iron, and possibly antimony, niobium, and tantalum would interfere after a potassium hydroxide separation of zirconium under oxidizing conditions.

Some additional observations are to be noted. Tungsten, antimony, niobium, and tantalum form precipitates with quercetin. As quercetin does not precipitate titanium and zirconium, it may prove valuable for the separation of niobium and tantalum from titanium and zirconium.

The sensitive color reaction given by tin with quercetin is being investigated for the colorimetric determination of tin. Quercetin may also prove useful as a colorimetric reagent for fluorine (by quenching of the zirconium-quercetin color), and for hafnium, molybdenum, antimony, and hexavalent chromium.

PROCEDURE

In the determination of zirconium by means of quercetin provisions must be made for the removal of interfering ions, especially iron and titanium, which are common constituents in most naturally occurring materials. Precipitation of zirconium by means of either propylarsonic acid or mandelic acid would be an effective means of accomplishing this, but no carrier for zirconium could be found that would be precipitated by these reagents. At this point it seemed that the best approach was to find a zirconium reagent that would quantitatively precipitate microgram amounts (5γ of zirconium dioxide) of this element without carrier and that would at the same time separate zirconium from interfering elements. The reagent *p*-dimethylaminoazophenylarsonic acid offered a means of accomplishing this if it were possible to prevent the precipitation of titanium with hydrogen peroxide. Preliminary experiments indicated that hydrogen peroxide could not be used because of its rapid and complete oxidation of the azoarsonic acid. Attempts to prevent the precipitation of titanium with the azoarsono dye by employing media of high acidity were not completely successful. At the same time it was observed that the titanium formed no precipitate when the solutions were hot, a precipitate being obtained only during the cooling process. Experiments indicated that only a few micrograms of titanium were coprecipitated with zirconium if the zirconium precipitate was formed in (15 + 85) hydrochloric acid solution and the solution was filtered hot (70° to 90° C.). In this manner microgram amounts of zirconium could be separated from at least 10 mg. of titanium dioxide (see Table II). The maximum amount of titanium dioxide coprecipitated was 25γ when 500γ of zirconium dioxide were used. Because only a few micrograms of zirconium dioxide may be determined with quercetin, even this slight interference of titanium dioxide is removed by dilution. Other elements usually precipitated by the azoarsonic acid were found to be more soluble under these conditions. Of the elements that would interfere seriously in the determination of zirconium by means of quercetin only tungsten, tantalum, niobium, scandium, and thorium were precipitated by the azoarsonic acid in hot (15 + 85) hydrochloric acid solution. The amount of scandium precipitated is small and possibly 0.5 mg. of the oxide can be tolerated in the solution used for precipitating zirconium. The interference of thorium is negligible, as only 5γ of thorium dioxide was precipitated when 10 mg. were tested. No precipitates were obtained for molybdenum, bismuth, beryllium, gallium, chromium(III), uranium(VI), platinum, tin(IV)—50 mg. of each oxide tested; cerium (III), yttrium, praesodymium, gadolinium—20 mg. of each oxide tested; 15 mg. of germanium dioxide; or 5 mg. of auric oxide. Vanadium and other strong oxidizing agents destroy the azoarsonic acid and should be absent.

Table II. Coprecipitation of Titanium

Combination		
TiO_2, Mg.	ZrO_2, Mg.	TiO_2 Coprecipitated, Mg.
7	0.5	0.025
7	0.25	0.020
7	0.15	0.020
10	0.10	0.015
10	0.05	0.015
0.2	0.05	Not detected
0.05	0.02	Not detected

The precipitation of zirconium in microgram amounts by *p*-dimethylaminoazophenylarsonic acid is seriously hindered by microgram amounts of fluoride ion. Fluoride should be avoided in the preparation of the solution for analysis because it cannot be completely removed even by prolonged fuming with perchloric acid, especially if aluminum is present. It is possible, however, to separate fluorine when present in the original sample by fusing the sample with an alkaline flux and filtering the water leach. Small amounts of sulfate (70 mg. of SO_3 maximum tested) do not interfere. Phosphate in amounts under 0.6 mg. of P_2O_5 (maximum tested) was also without effect.

The detailed procedure calls for the addition of ferric iron and potassium hydroxide to the leach of the carbonate melt prior to the filtration of the solution. This ensures that a carrier is present for small amounts of zirconium; the high alkalinity serves to destroy soluble complex carbonates of zirconium and allows most of the silica to remain in solution.

The precipitate of zirconium with the pararsonic acid tends to leak through the filter unless a very tight filter, such as Whatman No. 42, and paper pulp are used in the filtration. The precipitate should be heated slowly (preferably in a small muffle furnace) and in the absence of drafts to prevent dusting losses. The temperature should not exceed 500° C. or the residue becomes difficult to dissolve in sulfuric acid. After solution in sulfuric acid, the excess sulfuric acid is removed because sulfate quenches the zirconium-quercetin color.

Reagents and Apparatus. To prepare a standard stock solution of zirconium (1 ml. contains approximately 2 mg. of zirconium dioxide), add 100 ml. (1 + 1) hydrochloric acid to 2.17 grams of zirconyl nitrate dihydrate and boil gently until 75 ml. remains. Make to 500 ml. in a volumetric flask with (1 + 1) hydrochloric acid. Standardize by taking 20-ml. portions and precipitating with redistilled ammonium hydroxide and igniting to oxide. Zirconium dioxide prepared from reagent grade zirconyl nitrate contained less than 0.3% hafnium dioxide by spectrographic tests.

To prepare standard zirconium solution in (1 + 4) hydrochloric acid (1 ml. contains 10γ of zirconium dioxide), take 5 ml. (or appropriate amount) of stock solution, add 395 ml. (1 + 1) hydrochloric acid, and make to 1 liter with distilled water. Tests indicated that zirconium solutions containing more than 2% by volume of hydrochloric acid are stable for at least 6 months.

p-Dimethylaminoazophenylarsonic acid solution [0.3% in (1 + 1) hydrochloric acid] was prepared in the following way: Powder the pararsonic acid in an agate mortar and dissolve 3 grams in hot (50° C.) (1 + 1) hydrochloric acid and make to 1 liter with (1 + 1) hydrochloric acid.

The alcoholic solution of quercetin (1 ml. contains 1.00 mg. ot quercetin) was made up in the following way: Dissolve 0.500 gram of quercetin in 300 ml. of 95% alcohol, warming if necessary. Make to 500 ml. with alcohol. The solution should be filtered if necessary. (The quercetin used was obtained from Delta Chemical Co., 23 West 60th St., New York, N. Y. Quercetin of suitable quality is also available from other sources including S. B. Penick Co., 50 Church St., New York, N. Y., and T. Schuchardt, Ltd., Leopoldstrasse 4, München 23, Germany.)

Potassium hydroxide solution was prepared by dissolving 100 grams of potassium hydroxide in 100 ml. of distilled water.

To prepare ferric chloride solution (1 ml. contains 1 mg. ferric oxide), dissolve 0.1 gram pure iron wire in hydrochloric acid, using a small amount of hydrogen peroxide to oxidize the iron. Evaporate the solution to dryness on the steam bath. Add 1 ml. of (1 + 1) hydrochloric acid and 20 ml. of water, digest the solution, cool, and make to 100 ml.

The carbonate-borate flux was made by mixing 3 parts of anhydrous potassium carbonate with 1 part of borax.

Redistilled hydrochloric acid was used for all work, including the preparations of reagents. Sulfuric acid was reagent grade. Beckman spectrophotometer, Model DU, was used.

Detailed Procedure. Weigh 0.200 gram of sample, ground to an impalpable powder, into a platinum crucible. Burn off organic matter if present in the sample.

Add 3 grams of the carbonate-borate flux and mix thoroughly. Cover the crucible and heat gently at first and then fuse the sample at high heat over a burner for 30 minutes. The fusion period can be greatly shortened for samples that are not refractory. Cool.

Leach the melt with about 50 ml. of water. Remove crucible and the cover, scrubbing and rinsing them before setting them aside. The total volume at this point should be about 70 ml.

Add 10 ml. of potassium hydroxide solution, 5 ml. of ferric chloride solution, stir, and digest the solution on the steam bath for 30 minutes to an hour.

Filter the solution on Whatman No. 40 paper and wash the precipitate with 1% potassium hydroxide solution. Drain the paper and stem of the funnel thoroughly.

Dissolve the precipitate with 10 ml. of hot (1 + 1) hydrochloric acid (use pipet), collecting the filtrate in a 100-ml. beaker. Wash the paper with water.

Add 5 ml. of *p*-dimethylaminoazophenylarsonic acid solution, adjust the volume to 50 ml. and digest the solution on the steam both for at least an hour.

Add a generous amount of paper pulp and filter the precipitate of zirconium while hot. In order to prevent precipitation of titanium the solution must not be allowed to cool below 70° C. during the filtration.

Wash the precipitate thoroughly with a hot wash solution of the dye (made by taking 10 ml. of the azo-arsonic acid solution, 70 ml. of concentrated hydrochloric acid, and diluting to 500 ml. with water).

Transfer the paper and precipitate to a platinum crucible and remove most of the water by drying in an oven. Then gently ignite the precipitate in a small muffle furnace at 500° C., starting with a cold furnace.

Add 10 drops of sulfuric acid, cover the crucible, and heat on the hot plate for about half an hour to dissolve the zirconium residue. A small amount of silica usually follows zirconium up to this point, so that a complete solution of the residue may not result. However, the zirconium is dissolved by this treatment.

Remove the cover and allow the sulfuric acid to evaporate on the hot plate until no more fumes of acid appear.

Holding the crucible with platinum-tipped tongs, remove the last traces of sulfuric acid by gently heating below 500° C. over a low burner.

Add 5.2 ml. of (1 + 4) hydrochloric acid by a pipet. Cover the crucible and warm the solution a few minutes to dissolve the zirconium. Rinse the cover with water, adding the washings to the crucible. Transfer the solution to a 25-ml. volumetric flask. An aliquot of the solution should be taken here if the sample is known to contain more than 25 to 50γ zirconium dioxide. Additional acid will then be required to bring the acidity up to 0.5*N* hydrochloric acid used in the determination of zirconium with quercetin.

Adjust the volume to about 15 ml. with water. Cool. Add 5 ml. of alcohol and 3 ml. of quercetin using pipets. Make the solution to the mark, mix, and obtain the absorbancy of the solution in the spectrophotometer at 440 mμ using a slit width of 0.05 mm.

Determine the amount of zirconium by reference to a standard curve. A blank should be run in the procedure. For example, 1γ zirconium dioxide was found in the combined residue of the filter paper and pulp used for the filtration of the azoarsono precipitate.

Test of the Procedure. The procedure was applied to the determination of zirconium in glass sands, clays, and refractories. Bureau of Standards standard samples were used. The results obtained for zirconium by the quercetin procedure are compared with certified analyses in Table III. The agreement is satisfactory.

Table III. Comparison of Results of Zirconium Analysis

Bur. Standards Sample No.	Zirconium Dioxide, % Certified analysis (av.)	(Quercetin) procedure
B.S. 76	0.07	0.072
B.S. 77	0.09	0.089
B.S. 78	0.12	0.112 0.114
B.S. 81	0.031	0.033 0.029
B.S. 91^a	0.0095	0.0100 0.0104
B.S. 97	0.25	0.245
B.S. 98	0.041	0.0409

^{a} This sample contained 5.72% F.

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Vacuum Fusion Analysis

Apparatus and Determination of Oxygen in Chromium

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This report describes the apparatus designed and constructed for research in vacuum fusion analysis. The work was undertaken to provide methods for the determination of oxygen, nitrogen, and hydrogen and nonferrous metals. This is exemplified by an investigation for determining oxygen in chromium. Blank rates and results for National Bureau of Standards samples of cooperative steels compare favorably with those of other laboratories. Blank rates were indistinguishable, whether measured for 10 or 20 minutes. The presence of stopcock grease gave a measurably higher blank rate than when the grease was absent. The small increase should interfere only with obtaining extremely low blanks. The recovery of oxygen from mixed powders containing chromium and chromic oxide was fitted to an equation of the form: $R = a + c_1C + c_2C^2 + t_1T + t_2T^2 + kCT$, where a, c_1, c_2, t_1, t_2, and k are the fitted constants, R is the recovery in per cent, C is the weight per cent of chromium in the bath, and T is the operating temperature. Much better results were obtained by using a molten bath of 75% iron and 25% tin by weight than by using one of pure iron. At 1600° C. the method with the alloy bath gave about 85% recovery when no chromium was present. When consecutive samples had increased the chromium to about 5% of the bath, the oxygen recovery dropped to about 70%. The results agree with those obtained by modification of an acid-solution method. Oxygen may be determined in chromium by the vacuum fusion method with certain limitations and using occasional standards to check recovery.

ALTHOUGH the workers introducing the vacuum fusion method for determining gases in metals (*12*) considered nonferrous metal samples, the early use was limited to iron and steel (*28*). Recently, however, a number of general articles have appeared discussing applications of this method to nonferrous metals. Work by Sloman (*21–25*) in England and by Mallett (*14*) in the United States should be mentioned. While a remarkable experimental and theoretical account of work covering a number of metals has been given by Sloman and coworkers (*25*), many articles have appeared discussing single metals, especially for the determination of oxygen in titanium (*7, 29*), in zirconium (*26*), and in copper (*11*). It appears that the best results are obtained from each type of apparatus using procedures developed for that specific apparatus. There is ample indication (*27*) that varying results among investigators are influenced by the different experimental arrangements. For that reason the apparatus is rather carefully described in this paper. This description of the apparatus and its characteristics and an experiment with stopcock grease is followed by a report of an investigation for determining oxygen in chromium with the described apparatus.

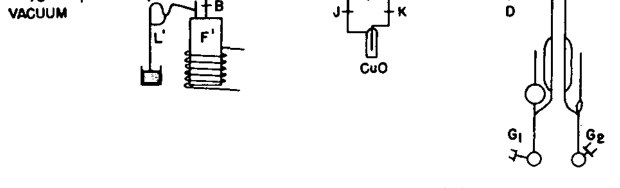

Figure 1. Schematic Diagram of Apparatus

2. U-type cutoff
I, II. Calibrated volumes, 1 and 4 liters, respectively
A, B, C, M. Glass-mercury solenoid valves
F'. Vacuum furnace
G_1, G_2. McLeod gages, 500μ and 10 mm., respectively
G_3. Thermocouple gage
L'. Mercury lift for the introduction of samples
P. Mercury diffusion circulation pump, also used for gas extraction
T_1, T_2. Cold traps

APPARATUS, CHARACTERISTICS, AND EFFECT OF STOPCOCK GREASE

General Description. For the apparatus described it was desired to attain a low blank rate, to be able to handle a wide range of oxygen composition among the samples, and to use no stopcocks. The first two goals were imposed by the expected nature of the analysis requests from other sections of the laboratory. The last requirement was imposed in order to be able to test the influence of stopcock grease on the blank.

The apparatus is represented schematically in Figure 1. Except for certain sections of the furnace, *F'*, the lines are constructed of borosilicate glass with an outside diameter of 1 inch from *F'* to the main loop and clockwise to the circulating pump, *P*, and an outside diameter of $^1/_2$ inch elsewhere. A 5-kw. General Electric Model 4HL2A5 electronic induction heater is used to heat the furnace. Temperatures in excess of 2500° C. have been attained with this heater, although this has been unnecessary in any work done to this date. Volumes *I* and *II* are calibrated by the expansion method (*16*) to within 1%. Closing valves *D, E,* and *F* forces the collected gas into the smaller volume of approximately 1 liter. Closing valves *D, E,* and *G* instead utilizes both volumes or a total of about 5 liters. The maximum collectable pressure is about 1.4 mm. of mercury. Since a sample of 0.5 gram is the normal size used in this apparatus, materials up to 1.2% oxygen can be handled. Homogeneous materials, for which small samples of the order of 20 mg. may be used, can be handled up to 30% oxygen. In practice 10-mg. samples of material containing about 30% oxygen have been analyzed.

Components. The vacuum furnace is similar to that of Beach and Guldner (*3*) and is shown in detail in Figure 2.

The large standard-tapered ground joint used at the bottom by them is replaced here by a butt-type joint which is sealed on the outside with Apiezon Wax W. This greatly facilitates changing crucibles and avoids the occasional freezing of the tapered joint. The glass-enclosed iron slug used by Beach and Guldner as a shield for the optical window is omitted. The standard-tapered joint used for the window was probably first employed by Walter (*29*). The connections to the system and the sample loader are made through ball joints to facilitate removal for cleaning, repair, or replacement. The ball joints are waxed on the outside. A Heinze, 3400-r.p.m., continuous-duty air blower is used to cool the outside of the furnace. The graphite crucible and funnel are made of AUC grade material purchased from the National Carbon Co. This assembly is placed in a quartz tube $5^{1}/_{2}$ inches long by 2 inches in diameter and is surrounded by graphite powder, 100 to 200 mesh. The powder is made by filing or machining AUC stock. National Carbon Co. powder No. 38 may also be used. The quartz tube is then suspended from the hooks in the furnace by 0.050-inch platinum wire.

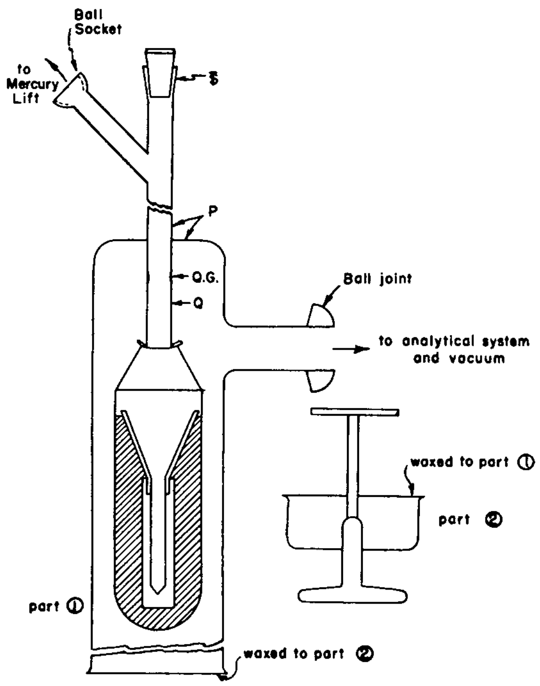

Figure 2. Schematic Diagram of Furnace

P. Borosilicate glass
Q.G. Borosilicate glass to quartz grade
Q. Quartz

The mercury lift, used to introduce samples and which seems to have been suggested first by Derge and coworkers (*8*), avoids being restricted to a given set of determinations or a given order as generally occurs with the usual loading tree. It also avoids lengthy storage under vacuum which tends to remove hydrogen from steels. Nonmagnetic samples may be wrapped with iron wire; the type used as standard chemical iron is satisfactory. Powders may be placed inside small iron or nickel capsules.

To collect and circulate gases the mercury diffusion pump described by Naughton and Uhlig (*17*) is used. After it was installed in the apparatus, the pumping speed was found to be about 12 liters per minute. The highest pressure that the pump would collect at this speed was about 1.4 mm. of mercury. The main evacuation pump is a DPI three-stage glass, oil diffusion pump backed by a Welch Duo Seal mechanical pump.

In place of stopcocks and U-type mercury cutoffs, a glass-mercury solenoid value was designed. Although sketches of these valves appear in the literature (*13*), dimensions and other design data are given in this report and in Figure 3, as they may prove useful to others. The coil, developed by the electrical products group of the General Electric Research Laboratory, has the following characteristics:

Inductance	1.25 henries, iron slug out 1.55 henries, iron slug in
D.c. resistance	285 ohms
Identification No.	W.S.F. 3241580

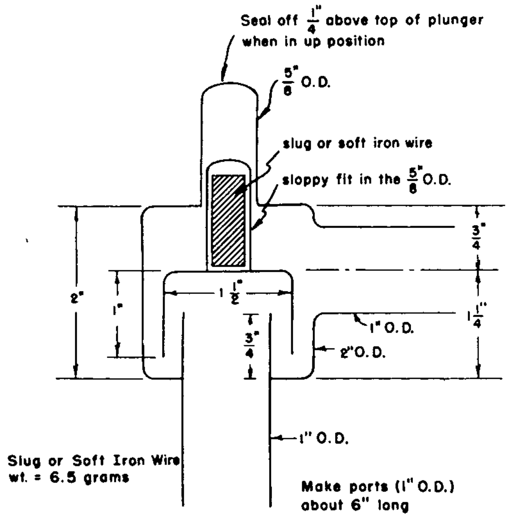

Figure 3. Large Mercury-Glass Solenoid Valve

A small valve is also used and has $^{1}/_{2}$-inch ports instead of 1-inch. The solenoid, not shown here, is described in the text.

With about $^{1}/_{4}$ inch of mercury, the valve provides a closure for pressure differentials up to about 2 mm. Greater differentials tend to blow the inverted cup open or to hold it down against the pull of the pickup coil. Since the installation of these glass-mercury solenoid valves no accidents attributable to them have occurred. Previously the U-type cutoff had been involved in two or three accidents and had always presented a safety problem because of the slender glass tubes used for mercury risers. Additional advantages of the valves are ease and speed of operation. Assembly of the controlling switches with indicating lights on a panel provides a quick indication of the opened parts of the system.

To make possible 24-hour continuous operation of the main evacuating and circulating pumps, water safety switches (Figure 4) were designed to shut off the heating current in case of water failure.

The cooling water from the diffusion pump enters the pressure head tube. If the stopcock is partly open, the water in the tube will seek a level such that the pressure head causes a rate of flow out the bottom drainage tube which equals the rate of flow inward. Adjustment of the stopcock will bring this level to about the middle of the tube. Sufficient mercury is placed in the side tube to connect the contacts. When the water fails, the level in the pressure head tube drops because of drainage through the bottom tube. With loss of the pressure head, the mercury assumes an almost level position opening the contacts and shutting off the heater current. The top drainage tube provides an exit for water in case of sudden pressure surges in the water system. In order to reduce these surges, a constant flow regulator (Fisher Scientific Co. catalog No. 15-529) is placed directly on the line supplying the cooling water. A General Electric electromagnetic relay, catalog No. CR-2790-E100A2 (115 volts, 60 cycles) is interposed between the contacts and the power to the heater to reduce sparking. Of course, an electronic relay would practically eliminate this trouble.

Copper oxide is prepared by the method of Murdock, Brooks, and Zahn (*15*), resulting in a copper oxide containing 1% iron oxide. The caked precipitate is chopped with a spatula and the 6- to 30-mesh material is separated and used.

For trap T_1 methanol is previously cooled to its freezing point by successive small additions of liquid nitrogen. The alcohol must not be entirely frozen for two reasons: Temperatures cold enough to trap carbon dioxide in the wrong trap may be attained and their is some danger of breaking the Dewar flask containing the alcohol because of expansion on freezing. The more usual dry ice–acetone mixture permits a water vapor pressure of 0.5 micron of mercury and some transfer to the carbon dioxide when the latter is collected.

Temperatures are read with a Leeds and Northrup optical pyrometer (catalog No. 8622-C) calibrated with a National Bureau of Standards tungsten lamp.

Procedure. Prior to each analysis the crucible is degassed by heating to about 200° C. higher than the temperature to be used in the analysis. For iron and steel samples operation is at about

1650° C. and degassing at 1850° C. Degassing is continued until the blank rate at the operating temperature drops to the desired value. This is determined by closing valves M, D, E, and F and collecting gas for 10 minutes.

When a satisfactory blank rate has been obtained, a cleaned and weighed sample is introduced through the mercury lift with the assistance of a magnet, and the small air bubble which generally accompanies it is pumped out. Valves M, D, E, and F are closed and the magnet is used to bring the sample over the vertical tube of the furnace, where it is dropped into the crucible. The evolved gases are pumped clockwise (Figure 1) by P into the calibrated volume. After measurement of the pressure on G_1 or G_2, E, J, and K are opened and B and C are closed; thus the gases are circulated over the trap containing the copper oxide which is heated to about 400° C. Traps T_1 and T_2 are cooled with the cold methanol previously described and liquid nitrogen, respectively. As the oxidation proceeds, water and carbon dioxide are condensed in T_1 and T_2, respectively, and the pressure decrease is conveniently followed on the thermocouple gage. When the rate of decrease is zero as checked on the McLeod gage, the residual gas is collected in the calibrated volume by closing E; the pressure is measured on the McLeod gage and recorded as nitrogen. Valves J and K are closed and the nitrogen is discarded through E, M, and the vacuum lines. After evacuation, M is closed and C opened; the liquid nitrogen is removed from T_2, the re-evolved carbon dioxide is circulated around the main loop for a minute or two to remove any traces of water that may have distilled over from T_1, and is collected in the calibrated volume by closing E, and is measured and discarded. The methanol is removed from T_1 and the entire system evacuated in preparation for another analysis. The difference between the original total pressure and the sum of nitrogen and carbon dioxide pressures is attributed to hydrogen.

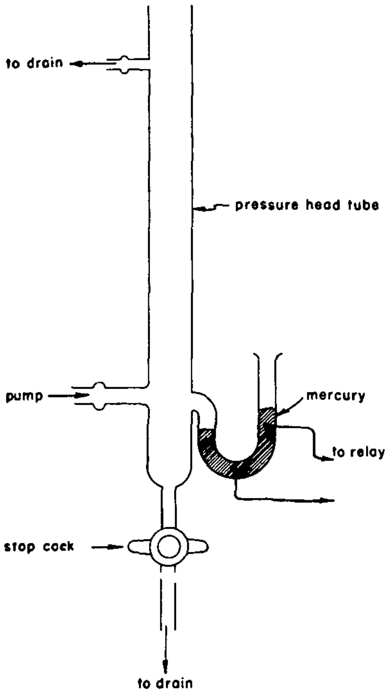

Figure 4. Diagram of Water Safety Switch

At the point marked 2 (Figure 1) the usual U-type mercury cut-off is employed because another type of analysis, expected to be possible in the same apparatus, requires a valve capable of holding a larger pressure difference than the mercury-glass solenoid valves used elsewhere in the apparatus. During evacuation of gases from the calibrated volumes or the entire apparatus, it was found best to close this cutoff.

When the larger volume is required for the gases evolved from the sample the valves D, E, and G are closed and F and H are opened. After measuring the total pressure in this case, it is convenient to close F and to continue the analysis with the smaller part of the sample using the small volume. The excess gas is discarded at the end during the general evacuation of the apparatus.

System Characteristics. With the circulation pump operating the average pumping speed for evacuation of the entire system is about 3.0 liters per minute in the range 200 to 0.2 microns pressure.

In order to determine the pumping speed of the circulating pump, P (Figure 1), about 300 liter-microns of nitrogen were admitted and collected in the large volume. With G and F closed the system was evacuated to at least 0.1 micron. Valves B, D, E, F, G, J, K, and M were now kept closed and at a recorded time G was opened. The pressure collected in the small volume was measured at various time intervals. If P_s is the pressure at time, t, in the small volume, the total loss of pressure in the large volume, ΔP_L, during time $t - t_0$ is given by

$$\Delta P_L = P_s \frac{V_s}{V_L} \tag{1}$$

where t_0 is the initial time of opening valve G, V_s is the known size of the small volume, and V_L is for the large volume. The volume ratio is known from the calibration data or can be obtained from the initial pressure in the large volume and the final pressure in the small volume. At time t, therefore,

$$P_L = P_{L0} - \Delta P_L = P_{L0} - P_s \frac{V_s}{V_L} \tag{2}$$

This pressure, P_L, now approximately follows the exponential law, so that by choosing one time near the beginning and another near the end, an apparent average pumping speed may be calculated from

$$S = [2.303 V_L \log (P_1/P_2)]/(t_2 - t_1) \tag{3}$$

The numerical subscripts indicate the early and late values of P and t.

In the apparatus described herein this determination was made for various amounts of heater power supplied to the circulating pump. The speed was found to be a maximum of 12 liters per minute at about 265 watts. No doubt this will vary as a function of the rate of cooling provided and the amount of insulation placed around the pump.

The maximum pressure collectable by the pump was measured by admitting various quantities of nitrogen and noting the maximum value for which the thermocouple gage showed less than 0.1 micron remaining on the low pressure side. At 265 watts this pressure was found to be 1.4 mm. of mercury. By increasing the heater power to 300 watts 1.87 mm. of mercury could be collected, but a drop to 10 liters per minute was noted for the pumping speed. The lower power is now used in normal operation.

A commonly achieved blank rate in this apparatus is 0.1 to 0.2 liter-micron per minute. This is compared with that of other laboratories in Table I.

Table I. Comparison of Blank Rates

Blank Rate, Ml./Hour(STP)	Reference
0.60	(*14*)
0.045 ± 0.015	(*2*)
0.006	(*21*)
0.004	(*23*)
0.9 ± 0.1	(*19*)
0.75 ± 0.25	(*18*)
1.0	(*4*)
1.9	(*28*)
0.55 ± 0.11	(*6*)
0.012 ± 0.004	This laboratory

The preliminary results of analyses performed with the apparatus are summarized in Table II for oxygen and nitrogen determined in the eight cooperative steels prepared by the National Bureau of Standards and used in an international cooperative study (*27*). Because of the small size of sample (0.5 gram) and the probable concentration gradient along a radius of the cylindrical bar of the steels, pie section–shaped pieces were used in order to obtain representative samples of the cross section.

The arithmetic averages for the eight steels listed in Table II are slightly different than the so-called best values assigned by

Table II. Results on Cooperative Steels

	Oxygen					Nitrogen			
Steel	n[a]	Found, p.p.m.	C[b]	s[c]	s_c[d]	Found, p.p.m.	C	s	s_c
1	3	154	181	35	33	17	26	4.5	4.5
2	3	118	135	29	33	31	39	18	11
3	3	161	168	28	40	133	135	24	24
4	7	25	26	6.2	15	17	50	5.6	23
5	25	93	92	22	25	26	40	8.9	14
6	3	48	56	1.0	16	19	44	1.0	11
7	3	937	1060	129	69	28	50	12	17
8	4	120	174	13	26	33	38	9	10

[a] n, Number of determinations.

[b] C, Arithmetic average of the results reported by all of the laboratories that participated in the study for the given steel.

[c] s, Estimated standard deviation for the present results.

[d] s_c, Estimated standard deviation for the results of the different cooperators.

the committee reviewing the results of the cooperative study. In general, s is less than s_c since s is for one laboratory and s_c for 15 laboratories. This would be in agreement with the general feeling that variance within one laboratory ought to be less than variance between laboratories. However, the comparison is obscured by the fact that s_c is for the means of the 15 cooperators while s is for the individual results of one laboratory. Steel 5 has generally been used in this laboratory as a standard to detect unsatisfactory operation.

To test the effect of stopcock grease on the blank rate it was decided to compare alternate blank rates with and without the presence of the grease in the region near the furnace. It also appeared desirable to determine the effect of length of time for blank rate measurements. In order to test the two effects simultaneously, and with increased precision, a 2^2 factorial experiment (*5*) was chosen as a useful design. The treatments and levels were as follows:

Treatments	Levels
Grease	Present, absent
Blank collection time	10 minutes, 20 minutes

Apiezon grease was placed in a small nickel cup and placed in the upper section of the mercury lift. Both treatments at each level were tested in combination with one another. The data obtained are listed in Table III.

Table III. Effect of Stopcock Grease on Blank Rate

Set	Run Description[a]	Blank Rate, μ/Min.
II	N-10	0.177
	G-20	0.182
	G-10	0.177
	N-20	0.157
III	N-10	0.201
	G-20	0.236
	G-10	0.227
	N-20	0.190

[a] N, no grease present; G, grease present; 10, 10-minute blank determinations; 20, 20-minute blank determinations.

Set I (not shown) was invalid, since it was performed in a different manner and had resulted in grease being present when not desired. Set II was expected to be relatively insensitive to the effects looked for, since temperatures more erratic than usual were observed. Set III appeared to be satisfactory. The sets were performed on different days. Analysis of variance performed on set III alone indicates that time has no significant effect on the blank rate. The presence of stopcock grease, however, increases the blank rate, with the chances being only about 1 to 21 that the difference could have been obtained by chance alone. Sets II and III can be analyzed together with the effect of days (or sets) removed. This analysis also gives no significant time effect. The grease shows an increased blank rate again, this time with the odds being about 1 to 32 that chance could have given these results. It appears safe to conclude, therefore, that Apiezon M grease causes an increased blank rate. This increase —about 0.024 liter-micron per minute in this case—may not be of practical significance, however, unless extremely low blanks are being attempted. On the other hand, in the low pressure combustion method for carbon determination (*16*, *30*), the effect might be greater because of combustion of the grease vapors and probably should be checked by users of that method.

DETERMINATION OF OXYGEN IN CHROMIUM BY VACUUM FUSION METHOD

The approach to the problem in this laboratory differs somewhat from the excellent one that Sloman and coworkers (*25*) recently described. Their method for determining oxygen involves the following steps. The minimum operating temperature is determined for the oxide of the metal being investigated. An experiment is made to determine the maximum concentration of metal allowable in the iron bath or flux used in the graphite crucible. Then the gas content of the sample of metal used for the preceding step is determined accurately. Repeated determinations are made to check the procedure and to approach the maximum concentration determined earlier to discover at what point low results become apparent. As a check the metal of known gas concentration is then used to repeat the first step.

The following work done at this laboratory on chromium (a metal not included in the recent Sloman experiments) involved the following steps:

1. A synthetic mixture of oxide and metal powder was analyzed for oxygen at a variety of temperatures using an iron or other type of bath. Careful account was kept of all materials added to the graphite crucible—for example, samples, bath material, and wrappings or capsules for samples.
2. The chromium metal powder used in step 1 was analyzed for oxygen by an independent method. The known amount of oxygen in the synthetic chromium–chromic oxide mixtures could then be computed, and the recoveries determined in step 1 could also be computed according to

$$R = \frac{\text{oxygen found}}{\text{oxygen expected}} \times 100 \qquad (4)$$

3. The values of R were next fitted by the method of least squares to an equation of the form

$$R = a + c_1C + c_2C^2 + t_1T + t_2T^2 + kCT \qquad (5)$$

where a, c_1, c_2, t_1, t_2, and k are constants, C is the weight per cent of chromium in the molten bath into which a given sample is dropped, and T is the centigrade temperature at which the recovery was determined. This form of equation was chosen because it is simple and allows interacting and conflicting effects to be expressed.

4. Steps 1 and 3 were repeated for other bath materials in an attempt to improve recovery.

Materials. CHROMIUM. Metal powder purchased from the Fisher Scientific Co. was used. This was labeled "about 98% pure." The major impurities as determined in this laboratory were found to be 1.3% iron, about 0.6% silicon, and 0.2% carbon. According to this research, it contained about 0.5% oxygen. Other minor impurities amounted to about 0.3%.

CHROMIC OXIDE. Baker's analyzed powder containing 0.4% unidentified substances not precipitated by ammonium hydroxide was used.

IRON. National Research Corp. High purity iron was used as a flux material.

TIN. Baker's analyzed tin (20-mesh granules) was used as an additive to the iron flux.

ALUMINUM. Foil as purchased from Fisher Scientific Co. in roll form was used as a wrapping for powder.

WIRE. Standardizing iron wire from J. T. Baker was used to wind around aluminum-wrapped powders.

NICKEL. Commercial Grade A nickel was used to fabricate small nickel capsules.

Procedure. Four series of recovery determinations were made using a synthetic mixture of chromium and chromic oxide containing 10% by weight of the oxide. Two more series of determinations were made using the chromium powder from which the

Table IV. Recovery of Oxygen from Synthetic Chromium–Chromic Oxide Mixtures with Iron Bath

C, Wt. %	T, ° C.		R, %
00.0	1648		39.8
10.3	1751		23.2
14.9	1854		15.1
18.9	1854		11.4
20.9	1751		18.5
24.5	1648		31.6
13.5	1544		31.9
15.0	1544		27.8
19.3	1544		29.4
20.7	1544		27.3
21.9	1544		28.1
23.2	1544		24.6
		Av.	25.7
		Variance	61.3

Table V. Recovery of Oxygen from Synthetic Chromium–Chromic Oxide Mixtures with Iron-Tin Bath

C, Wt. %	T, ° C.	R, %	L[a]
3.7	1544	68.7	1
10.2	1544	65.6	1
26.2	1544	61.5	1
14.7	1648	77.8	1
19.0	1648	72.2	1
24.3	1648	75.0	1
2.4	1751	72.2	1
8.1	1751	68.9	1
23.3	1751	73.6	1
5.0	1854	79.9	1
12.2	1854	76.1	1
25.3	1854	71.7	1
1.06	1751	86.8	2
2.07	1802	76.9	2
3.05	1802	78.3	2
4.01	1854	59.0	2
5.86	1802	72.0	2
6.74	1854	56.1	2
7.82	1751	48.6	2
8.86	1854	40.0	2
10.73	1751	56.1	2
11.58	1802	36.5	2
12.43	1751	41.2	2
4.38	1802	58.5	3
8.65	1854	66.2	3
11.22	1802	63.5	3
	Av.	65.5	
	Variance	211	

[a] All results with $L = 1$ were obtained with one crucible, those with $L = 2$ with another, etc.

synthetic mixtures were made. At first iron was used as the flux material in the graphite crucible. In the remaining work an iron and tin mixture containing 20 to 25% tin by weight was used. The purpose of the first four series was to compare the recoveries as a function of the bath material and to attempt to determine the best operating conditions utilizing Equation 5 as an empirical description. This procedure also permitted the alternation of recovery determinations with unknown sample determinations as a service to other sections of the laboratory. This was done with some degree of confidence by using the recovery determinations as correction factors for the unknowns. The last two series, in addition to furnishing data for the chromium powder, made possible certain comparisons which will be discussed later.

The procedure followed was essentially that described previously with minor modifications. The various powders were wrapped with aluminum foil and iron wire, except as indicated in Table VII, where nickel capsules were used. The quantities generally used were 100 mg. of powder, 30 mg. of aluminum, and 20 mg. of iron wire. The powder was wrapped with the foil and then wound with the wire; this could then be introduced through the mercury lift. A record was carefully kept of the various materials and samples introduced into the crucible so that a reasonable knowledge of the chromium content was available. From prior determinations made upon the wire and foil, it was known that these materials contributed negligible amounts of gas to the gas obtained from the synthetic metal-oxide mixtures. For these mixtures also, a few prior analyses showed that the nitrogen and hydrogen content of the gas was sufficiently small to be neglected. Therefore, only a determination of total gas as carbon monoxide was required of the mixtures.

The determinations involving the pure chromium powder (Tables VI and VII) were carried out at 1800° C. In one series, aluminum foil and iron wire were used as already described. The other series was performed using small nickel capsules to contain the powder. These capsules, fabricated of Grade A sheet nickel, each consisted of a top and bottom part, the cap and the can. Each part was shaped like a right circular cylinder and the cap was constructed to fit snugly over the can. The can had a $^3/_{16}$-inch diameter and height. A No. 80 drill was used to make a hole in the cap. When used, the powder was placed in the can and the cap squeezed on. When the vacuum fusion apparatus was ready for a sample, the capsule was introduced through the mercury lift. The capsule was permitted to rest in the upper part of the lift until the few liter-microns of air thus introduced were pumped out. The hole in the cap, of course, permitted any residual air inside the capsule to be withdrawn also. In both series, analyses of chromium were alternated with analyses of blanks and of sample containers (aluminum-iron combination or nickel capsule).

It was desired to check the determination of oxygen in chromium by the independent method mentioned by Short (*20*), which was apparently taken from an earlier but incomplete description in the article by Adcock (*1*). Essentially, Short's method was to anneal the chromium in vacuum at 800° C. for 2 hours, cool, dissolve the metal in 1.2*N* hydrochloric acid, and filter, wash, ignite, and weigh the residue as chromic oxide. Since the interest of this work was in relatively small amounts of oxygen and in small samples, the weighing part of this procedure appeared to be a drawback. For this reason some effort was spent in replacing this part of the method by a colorimetric determination. As finally worked out, the procedure decided upon was the same as Short's, except that the washed filter paper and residue were ignited in a nickel crucible. Then 3 grams of potassium persulfate and 5 grams of potassium hydroxide were added and the mixture was fused. When the cooled fusion mixture is dissolved in water, any chromic oxide present appears as potassium dischromate in a basic solution. Measurement of the filtered and diluted solution on a Beckman Model B spectrophotometer at 370 mμ gave the amount of chromium by reference to a standard curve previously prepared. Prior experiments with ignited chromic oxide had shown that this fusion procedure coupled with the colorimetric determination gave about 99% recovery. The missing 1% may be attributed either to impurity in the oxide or to loss as a result of the analytical method. In either case, the magnitude involved is insignificant. Calculation to chromic oxide and hence to oxygen is straightforward. The only chromium analyzed and reported here was that previously described. All impurities would either be separated by the basic nature of the final solution or would not affect the color measured by the spectrophotometer. The results of these measurements will be treated later. Future work is planned on this method for determining oxygen in chromium.

Results. The recoveries determined experimentally with the synthetic chromium–chromic oxide mixtures are shown in Tables IV and V. Tables VI and VII give quantities of gas obtained from chromium powders. The data from these two tables are treated further in Tables XI and XII. Symbols T, C, and R have the meanings previously assigned in connection with Equation 5.

In Tables IV and V the indication of temperatures to within 1° C. should not be construed as an assertion of precision or accuracy of measurement. The original readings were at even 50° C. intervals probably good to within 10° C. The odd figures of the tables were the result of calibration corrections. In least squares computations it is wise to carry a little more precision in the data than is warranted. The rounding is performed later. Pressure measurements of Tables VI and VII were indicated more precisely than warranted for computation reasons also.

Table VI. Analysis of Chromium Powder by Vacuum Fusion with Iron-Tin Bath at 1777° C.[a]

(Aluminum-iron wrapping)

Run No.	Sample Weight[b], Mg.		CO₂[c]		Blank Gas,[c] μ	Cr in Bath Wt. %
	Cr	Al-Fe	μ	%		
1	...	..	....	59.25	..	0
2	...	54.0	43.60	...	9.80	0
3	100.3	55.5	117.8	...	1.28	0
4	...	..	....	58.44	..	0.68
5	...	53.2	48.73	...	6.45	0.68
6	...	..	....	58.15	..	0.68
7	100.5	51.6	115.22	...	1.42	0.68
8	...	..	....	58.23	..	1.34
9	...	50.9	38.76	...	4.81	1.34
10	...	..	....	58.97	..	1.34
11	100.1	52.9	113.38	...	0.85	1.34
12	...	..	....	60.18	..	1.99
13	...	52.3	44.05	....	4.08	1.99
14	...	..	....	50.02	..	1.98
15	99.9	52.4	118.61	...	1.11	1.98
16	...	..	....	51.57	..	2.61
17	...	52.8	45.05	...	6.00	2.61
18	...	..	....	38.53	..	2.60
19	100.2	51.5	119.00	...	0.88	2.60
20	...	..	....	44.12	..	3.22
21	...	51.4	39.73	...	7.56	3.22
22	...	..	....	44.81	..	3.21
23	100.4	52.4	118.61	...	0.84	3.21
24	...	..	....	46.18	..	3.82
25	...	49.8	....	...	..	3.82
26	...	..	....	40.39	..	3.81
27	...	51.0	40.43	..	6.12	3.81
28	...	..	....	34.63	..	3.79
29	100.3	50.0	118.61	...	0.86	3.79
30	...	..	....	41.82	..	4.39

[a] All gas samples were measured in the small volume (0.951 liter) of the apparatus (called *I*), except those for samples of chromium powder. These latter were measured in the combined volumes (4.90 liters, *I* and *II*). The blank pressures for the chromium samples were measured in *I* and corrected to the large volume by the factor 0.194 (= 0.951/4.90).

[b] Where no weight is given for either chromium or aluminum-iron, the run was a blank determination, for which only the relative composition is needed.

[c] For samples or wrapping materials, the pressure of carbon dioxide is given. The accumulated pressure of blank gas is determined by blank rate × collection time. As explained in connection with Tables XI and XII, the percentage of carbon dioxide in the blank multiplied by the accumulated pressure of blank gas gives the blank correction.

The error of pressure reading is in the neighborhood of 0.09 times the square root of pressure.

Table VIII shows the recoveries of chromic oxide as given by the basic presulfate fusion described previously.

Table IX gives the oxygen content of the chromium powder used to make the chromium–chromic oxide mixtures which were used to obtain the data given in Tables IV and V, as determined by the modified acid solution method of Short.

Discussion. For reasons already given, it was decided to attempt to fit the vacuum fusion recovery data to a power series equation in *C* and *T*, the chromium content of the bath and the operating temperature, respectively. As indicated by Equation 5, this would involve evaluation of six constants. The method of least squares is probably the best method of curve fitting, although in this case it involves excessive labor in computing the extensions and solving a linear system of six simultaneous equations. At first this was attempted with a desk-model electric calculator. The lengthy work involved and the uncertainty of checking for errors led to the use of IBM computing machines resulting in a substantial reduction of the computation time. The equations so obtained are listed in Table X.

In Table X the variance of fit has been included for purposes of making statistical decisions. This is defined as

$$V = \frac{\Sigma\,(R_c - R_e)^2}{n - q} \tag{6}$$

where R_e is an experimentally determined recovery, R_c the correspondingly calculated recovery for the given set of constants, n the number of experimental points, and q the number of constants. The summation is taken over all the experimental points. The value of computing the variance lies in the possibility of showing that Equation 5 with the constants indicated in Table X is a more satisfactory representation of the data than is the arithmetical average of results without regard to temperature or chromium content of the molten bath. For the iron bath (data from Table IV) the variance ratio for the arithmetic average and the equation is 6.61 (61.3/9.28). The odds are about 1 to 50 that this could happen by chance if there were no real improvement due to the equation. For the iron-tin bath this ratio is 1.42 (211/156) and the odds on this being a chance happening are approximately 1 to 4. These two facts taken together appear to justify the equation as an approximate empirical description of the results.

It is important to observe that the data for Table IV and Table V, even when considered merely by the average, indicate a significant increase in recovery when tin is added to the molten bath. The approximately 20% difference gives a *t* (difference/standard error of the difference) of 5.44 which by Student's test (*10*) is very highly significant. This can be taken as sufficient evidence that tin in the iron bath improves the recovery of oxygen. This effect is probably dependent upon the characteristics of the apparatus. The General Electric Research Laboratory (*9*) made the observation that tin improved the recovery of oxygen from one of the NBS cooperative steels but that the use of tin

Table VII. Analysis of Chromium Powder by Vacuum Fusion with Iron-Tin Bath at 1777° C.[a]

(Nickel capsules)

Run No.	Sample Weight[b], Mg.		CO₂[c]		Blank Gas[c], μ	Cr in Bath, Wt. %
	Cr	Ni Capsules,	μ	%		
1	...	...	...	36.82	..	0.00
2	...	415.4	20.87	...	6.30	0.00
3	99.8	408.1	80.37	...	0.75	0.00
4	...	...	...	72.95	..	0.65
5	...	437.3	13.90	...	3.77	0.65
6	100.2	434.6	103.00	...	0.70	0.63
7	...	...	....	52.44	...	1.22
8	...	427.8	15.14	...	5.07	1.22
9	99.7	401.3	89.90	...	0.64	1.19
10	...	...	....	32.68	..	1.73
11	...	428.0	19.60	...	3.77	1.73
12	100.4	425.6	72.30	...	0.73	1.69
13	...	...	...	45.35	..	2.19
14	...	422.3	7.08	...	5.40	2.19
15	100.4	436.7	77.16	...	0.12	2.14
16	...	...	....	14.79	..	2.61
17	100.5	422.4	73.90	...	1.07	2.61
18	...	...	....	12.96	..	3.05
19	...	425.9	2.01	...	2.51	3.05
20	100.2	417.8	72.30	...	0.12	2.98

[a] All gas samples were measured in the small volume (0.951 liter) of the apparatus (shown as *I* in Figure 1), except those for samples of chromium powder. These latter were measured in the combined volumes (4.90 liters, *I* and *II*). The blank pressures for the chromium samples were measured in *I* and corrected to the large volume by the factor 0.194 (= 0.951/4.90).

[b] Where no weight is given for either chromium or aluminum-iron, the run was a blank determination, for which only the relative composition is needed.

[c] For samples or wrapping materials, the pressure of carbon dioxide is given. The accumulated pressure of blank gas is determined by blank rate × collection time. As explained in connection with Tables XI and XII, the percentage of carbon dioxide in the blank multiplied by the accumulated pressure of blank gas gives the blank correction.

Table VIII. Recovery of Chromic Oxide by Basic Persulfate Fusion

Cr_2O_3 Taken, Mg.	Cr_2O_3 Found, Mg.	Recovery, %
18.4	18.20	98.91
17.6	17.44	99.09
18.2	17.98	98.79

Av. 98.93 ± 0.71
(95% confidence limit)

Table IX. Oxygen Content of Chromium Powder by Modified Method of Short

Wt. of Cr Taken, G.	Oxygen Wt. Mg.	Oxygen, Wt. %
1.015	5.21	0.513
1.003	5.18	0.517
1.018	5.91	0.580
0.992	4.93	0.497
1.000	5.35	0.535
1.001	5.49	0.549

Av. 0.532 ± 0.031%
(95% confidence limit)

Table X. Constants for Equation 2 as Determined by Least Squares

Bath	a	c_1	c_2	t_1	t_2	k	Variance
Iron	−387.97	−2.689	+0.0325	+0.56626	−0.00018601	+0.000834	9.28
Iron-tin	−287.19	−6.453	+0.1217	+0.46940	−0.00014735	+0.001752	155.86

Table XI. Analysis of Chromium Powder

(From data of Table VI)

Sample Wt., Mg.	Cr, Wt. %	CO_2, μ	Blank Correction, μ	Wrapping Correction, μ	CO_2 Sample, μ	Oxygen, P.P.M
100.3	0.00	117.8	0.8	8.1	108.9	4576
100.5	0.68	115.2	0.8	7.6	106.8	4479
100.1	1.34	113.4	0.5	7.8	105.1	4425
99.9	1.98	118.6	0.6	7.7	110.3	4652
100.2	2.60	119.0	0.4	7.6	111.0	4669
100.4	3.21	118.6	0.4	7.7	110.5	4640
100.3	3.79	118.6	0.4	7.3	110.9	4661
					Av. found	4586

Table XII. Analysis of Chromium Powder

(From data of Table VII)

Sample Wt., Mg.	Cr, Wt. %	CO_2, μ	Blank Correction, μ,	Capsule Correction, μ	CO_2 Sample, μ	Oxygen, P.P.M.
99.8	0.60	80.37	0.44	3.00	76.93	3248
100.2	0.63	103.00	0.36	3.07	99.57	4187
99.7	1.19	89.90	0.28	2.54	87.08	3681
100.4	1.69	72.30	0.27	2.26	69.77	2928
100.4	2.14	77.16	0.04	1.80	75.32	3161
100.5	2.61	73.90	0.27	1.07	72.56	3043
100.2	2.98	72.30	0.02	0.45	71.83	3021
					Av. found	3324

was unnecessary for this sample after the apparatus was changed to improve the pumping speed at the outlet of the furnace.

The equations show decreasing recoveries as the chromium concentration begins to increase in the iron-tin bath. This can be seen by computing the partial derivative $(\partial R/\partial C)_T$, which by consideration of Equation 5 must be equal to $c_1 + 2c_2C + kT$. For 1750° C. and 0% chromium in the bath this derivative becomes for the iron bath −1.23 and for the iron-tin bath −3.39. The significance of these numbers as usable figures is questionable but the fact that both are negative appears to bear out the contention that additional chromium samples added to the same crucible reduce the recovery of oxygen. The square term indicates only very slight alleviation of this problem by showing a tendency to increase the recovery and the alleviation is so small that questioning its validity is justifiable.

Another interesting effect is observed by computing the temperature coefficient for recovery, $(\partial R/\partial T)_C$. Recourse to Equation 5 again shows this to be $t_1 + 2t_2T + kC$. At 0% chromium this becomes $t_1 + 2t_2T$. Both equations show a maximum at some temperature. These temperatures for maximum recovery (at 0% chromium) are ($T_m = -t_1/2t_2$) 1522° and 1593° C. for the iron bath and the iron-tin bath, respectively. The maximum for the iron bath at a temperature outside the range of experiments should not be considered dependable; rather, it shows ever-decreasing recoveries as the temperature increases.

In order to check these results and to answer a few other questions, the two series of determinations were carried out using the chromium powder. The data of Table VI were first analyzed for dependence of blank on the chromium content of the bath. This dependence was highly significant with odds in its favor greater than 1000 to 1, and it may be expressed in the form $y = 62.88 - 5.497\ C$, where C is the weight per cent chromium and y is the volume per cent carbon dioxide in the blank. Using this equation, the oxygen results for the aluminum-iron wrapping were corrected for their blanks. Since the ratio of aluminum to iron was kept practically the same throughout the experiment, it is possible to determine the wrapping correction as a function of the total weight of wrapping. The earlier statement that blanks need not be considered for the recovery determinations may be checked at this point. The chromium–chromic oxide mixtures contain 3.16% oxygen. This gives about 700 microns in a 5-liter volume. Blank correction of about 1 micron is obviously insignificant. Up to about 1% error (caused by blanks, etc.) can be admitted before corrections are needed. The corrected results showed no dependence upon chromium content and an average 0.756-micron pressure of carbon dioxide per milligram of wrapping contributed to volume *I*. Having both a blank correction and wrapping correction, these could be used for the chromium powder analyses. Table XI shows the final result of these calculations.

One of the purposes of this series was to compare the oxygen found by the vacuum fusion method to that found by the method of Short. Table IX shows the results by Short's method, and the recovery by method of vacuum fusion may now be computed by 0.459/0.532 × 100 = 86.3%. The constants listed in Table X for the iron-tin bath when used with Equation 5 at 1777° C. and C = 1.94 (the average value of C in Table XI) give 75.6% recovery. The agreement within limits of experimental error suggests that the equation is a reasonable description of effects of temperature and chromium concentration. While 100% recoveries would be desirable, analyses may be performed by occasionally running chromium powder as a standard.

The data of Table VII were taken in an attempt to compare nickel capsules with the aluminum-iron wrapping technique. These data were analyzed in the same manner as those of Table VI although it was noted that the nickel capsule oxygen contribution varied with the square of the chromium concentrations. The significant corrections were found to be $y = 59.73 - 13.11\ C$ and $z = 0.734 - 0.07062\ C^2$, where y and C have their previously described significance and z is the capsule pressure contribution to the large volume in microns per 100 mg. of capsule. The computations and results are now given in Table XII.

The result for Table XI is significantly different from that for Table XII. Student's t for the difference between the averages is 7.20, whereas t significant at 5% is 2.18. The fact that the chromium dependence of the blank is greater for Table XI and that the nickel capsule contributes less and less, points to a deleterious effect of nickel. It appears wise to avoid the use of nickel.

CONCLUSIONS

Because of the work reported here, it is believed that the vacuum fusion method using an iron bath containing 25% tin and operating in the neighborhood of 1600° C. is satisfactory for determining oxygen in chromium. As samples are added to the bath, the recovery of oxygen under these conditions begins at about 85% and drops to the neighborhood of 70% when the bath concentration has reached 5% chromium. During a series of determinations, the use of occasional standards to check the recovery is advisable. Wrapping material of aluminum foil and iron wire for powders is superior to nickel.

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Comparison of Spectrochemical and Semimicromethods in Analysis of Petroleum Ashes

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Because of the great importance attached to the quantitative determination of trace elements in petroleum fractions, and because either a spectrochemical or a semimicromethod of analysis must be employed, it is essential to know whether or not these two methods yield comparable results. Samples of ashes from various petroleum fractions have been analyzed by spectrochemical and semimicroprocedures and the data obtained have been subjected to a statistical evaluation. The results of this study have indicated that, although there are some differences between the two procedures, in general, one method is as effective as the other. The coefficient of variation for the various determinations is of the order of +10%. Statistics can be a powerful aid to one's judgment when a choice between two methods of analysis must be made. In the analysis of residua from petroleum stocks, the present procedures should be improved, in order to obtain an increase in precision.

A KNOWLEDGE of the quantity and composition of the residue remaining after the combustion of a petroleum crude is important to establish economic refining practices and utilization of petroleum products. During the processing of crudes, this noncombustible material becomes concentrated or distributed in certain of the fractions. The extent of this distribution varies according to the type of processing and the peculiar nature of the metal-bearing material, which may be either suspended salts or oil-soluble metal containing organic complexes. Although the ashes of petroleum stocks often contain a wide variety of elements, sodium, vanadium, nickel, and iron are those most universally found, and therefore, they are of greatest interest. The quantitative determination of these elements found in the ashes from catalytic feed stocks and burner fuels is among the more frequent analyses performed in a petroleum analytical laboratory.

In view of the limited amount of ash obtained from many of the samples submitted for analysis, spectrochemical and/or semimicro wet chemical techniques must be employed in the analysis of these ashes.

The evaluation of a method of analysis as applied to a given type sample is based on the precision and accuracy attainable. Statistics offer a powerful tool for comparing several methods of analysis and are an aid to one's judgment in choosing an economical and effective method of analysis.

This paper presents a statistical evaluation of the results obtained for the analysis of four petroleum ashes by a spectrochemical and semimicromethods of analysis.

EXPERIMENTAL

Spectrographic Procedure. The wide variation in major constituents of the ashes from various petroleum stocks dictates the use of a common matrix system. Here, all samples are given the same major constituent, a flux, thus reducing the concentrations of the sought elements to a level of minor constituents or traces in a common matrix. A number of common matrix-type techniques using a variety of compounds and mixtures have been described in the literature (*2, 7, 11-14, 17-20, 25, 26*). The use of lithium carbonate as described by Weaver and Brattain (*25*), however, appeared to be most suited to the problem. These authors describe a common matrix system using lithium carbonate as a buffer and the direct current arc as a source of excitation, burning the sample to completion. The success that this laboratory has had in using the alternating current arc, with its high analytical accuracy and negligible spectral background, led to the investigation of the possibility of using this excitation source. Preliminary work showed that this type of excitation did not have sufficient spectral sensitivity to give spectrograms of suitable density. This deficiency was overcome by using an equal part of powdered graphite with the lithium carbonate. The powdered graphite serves to enhance the spectral lines sufficiently to give the required spectral line density.

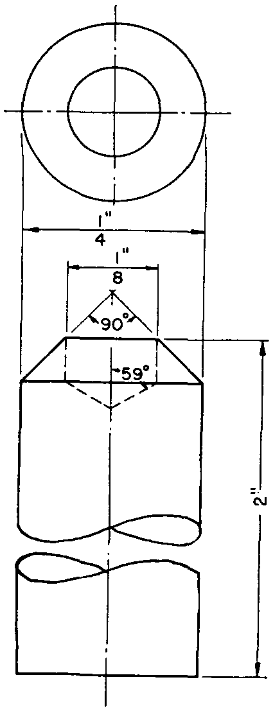

Figure 1. Diagram of Electrode

The paucity of lithium lines in the region of interest (3000 to 3400 A.) led to the use of cobalt as an internal standard. This choice was governed mainly by the similarity in excitation characteristics of cobalt to those of the sought elements—iron, nickel, and vanadium. Its arc behavior parallels that of sodium to a fair degree so that a single internal standard line (Co 3044.0 A.) can be used as a reference line for the four elements. Originally, the number of elements determined was limited to these four because they were considered to be major contaminants, and it was hoped that by establishing conditions for a limited number of components per sample it would be possible to obtain a more favorable error factor. However, it was found that the method could be applied to a wide variety of samples and in most cases without a sacrifice in accuracy. The method described has been satisfactorily applied to the following types of samples: ashes of crudes, bunker fuels, feed stocks, or other petroleum fractions; fireside boiler deposits; combustion chamber and other engine deposits; and used crankcase lubricants.

Apparatus. The following apparatus was used in the development of the method. Comparable equipment may be substituted if more readily available.

Spectrograph. ARL-Dietert No. 2060 1.5-meter grating spectrograph.

Excitation. ARL-Dietert No. 2040 alternating current arc unit.

Densitometer. ARL-Dietert No. 2250 projection comparator densitometer.

Developing Unit. ARL-Dietert temperature controlled rocking developing machine.

Film. Eastman Kodak SA No. 1.

Electrodes. National Carbon special spectrographic graphite $^1/_4$- and $^1/_8$-inch electrodes.

The sample electrode is prepared by scoring the $^1/_4$-inch rod at 2-inch intervals and breaking at the score mark. The electrode is shaped by cutting a crater into the end with a $^1/_8$-inch drill, $^1/_{16}$ inch in depth, and the outside wall of the crater is tapered at a 45 degree angle to give a feather edge. The cutting device consists of a slotted cylindrical tube for shaping the tapered edge and a $^1/_8$-inch drill for cutting the crater. Diagrams of the sample electrode and cutting tool used are shown in Figures 1 and 2.

The counterelectrode is prepared by scoring the $^1/_8$-inch rod at 2-inch intervals and breaking at the score mark. The raw surface at the break is used without any shaping.

Sample Preparation. Inasmuch as the majority of samples received in a petroleum laboratory are organic in composition, it is first necessary to ash a sufficient quantity of hydrocarbon to produce at least 10 mg. of residue. In ashing, the sample should be burned at the lowest possible temperature to maintain combustion and then ignited in a muffle at 1000° F. The per cent ash is calculated when the final results are to be related to the original sample.

All ashes and dry solid samples must be ground and mixed using a mullite (or equivalent) mortar and pestle.

Preparation of Common Matrix Mixture. Dissolve 0.2000 gram of cobalt metal (nickel and iron-free, Fisher Scientific Co., c.p. reagent No. 363 or equivalent) in 10 ml. of 8*N* nitric acid. Add 25.0 grams of spectroscopically pure graphite powder (National Carbon Co. SP No. 2) and sufficient distilled water to produce a pasty mixture. Heat this mixture on a hot plate, stir continuously until dry, then increase the heat to expel the oxides of nitrogen completely. A temperature of 700° to 750° F. is required. By this procedure, the cobalt oxide will be uniformly deposited on the powdered graphite.

The finished common matrix mixture is prepared by thoroughly mixing and grinding together one part of the cobalt-impregnated graphite powder and one part of spectroscopically pure lithium carbonate.

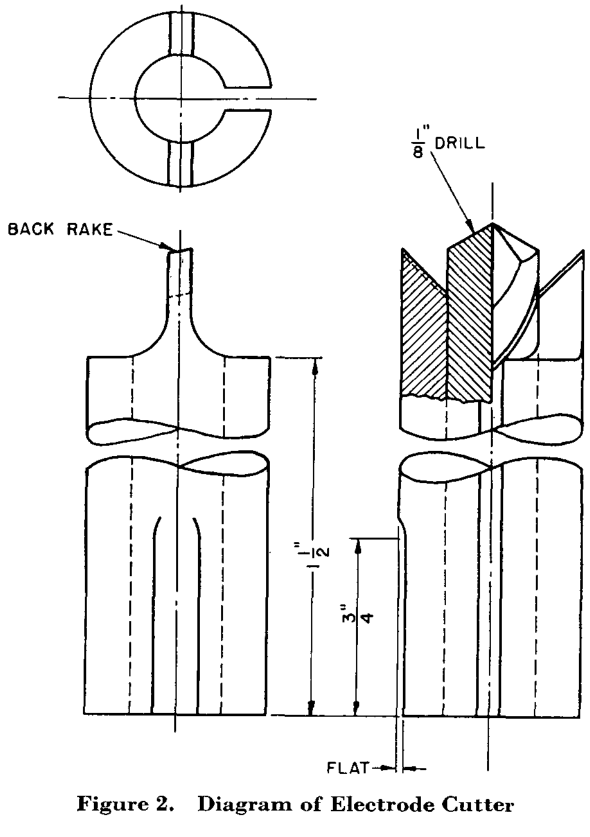

Figure 2. Diagram of Electrode Cutter

Calibration. The standards to be used to establish the analytical curves may either be synthetically prepared mixtures of c.p. chemicals comparable to the samples to be analyzed or a number of the actual samples which have been chemically analyzed a sufficient number of times to establish firmly the concentration of the elements of interest. The standard or analyzed samples should cover the range of concentration which is to be encountered, so that extrapolation will be avoided.

Weigh exactly 10 mg. of the synthetic or analyzed standards and 90 mg. of the common matrix mixture; mix together thoroughly by grinding. In order to extend the range of concentrations, the weight of standard may be varied from 2.5 to 15 mg. The cavity of the sample electrode is packed level full of the standard matrix mixture.

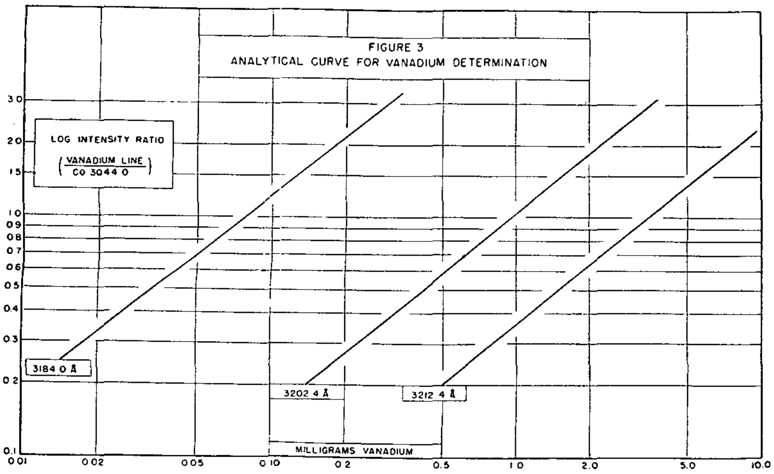

FIGURE 3
ANALYTICAL CURVE FOR VANADIUM DETERMINATION

Place the prepared sample electrode in the lower electrode holder of the arc-spark stand and adjust the counter electrode to a 6-mm. gap; the optical axis is at the midpoint of the gap. The sample is then arced under the conditions found by experiment to yield spectrograms suitable for densitometering. The conditions found to be satisfactory are as follows: type of excitation, alternating current arc; voltage, 5 kv.; current, 2 amperes; slit width, 50 microns; and arcing time, 75 seconds. (When this method is applied to the analysis of used railroad Diesel oils, two additional electrodes of the sample mixture must also be excited in the direct current arc for the determination of silicon. The conditions of excitation and the required filter in the optical path must be determined by experiment.)

In order to read the analytical lines at the optimum transmittance, the energy passed by the 25, 50, and 100% steps of a rotating four-stepped sector is photographed.

The details of the photographic processing are as follows:

Developer	E.K. D19 developer, 5 minutes, 68° C.
Shortstop	5 ml. glacial acetic acid in 400 ml. water, 15 seconds
Fixer	G.E. x-ray fixer, 1 minute
Water wash	2 minutes
Drying	A.R.L. film dryer, 2 minutes

The processed film is placed in the densitometer and the transmittance of the selected analytical lines is determined. The densitometer is adjusted to 100% transmittance for the background adjacent to the line.

The intensity ratio of the analytical lines to the cobalt internal standard line is determined by applying the transmittance readings to a film calibration curve. The intensity ratios for each concentration are averaged and the milligrams of metal versus average ratio are plotted on log-log coordinates, as illustrated in Figure 3 for vanadium.

The analytical lines of the sought elements and the internal standard found to be satisfactory in the analysis of the different types of samples are given in Table I.

In the analysis of a sample, the procedure is the same as that described for the calibration. It is important that all samples be ground and mixed thoroughly. Two spectrograms are prepared for each sample and the intensity ratio is averaged to obtain a result.

Calculations. To complete the analysis the transmittance readings of the element lines and internal standard line are applied to a gamma curve (film calibration curve) and the intensity ratio is determined.

These intensity ratios are then applied to the analytical curves and the concentrations of the elements sought are determined. In instances when the sample weight is other than 10 mg., the results taken from the analytical curve must be adjusted for the weight of sample taken.

When the final results are to be reported as related to the original sample, the per cent of element found in the ash is multiplied by the per cent ash in the sample.

$$\% \text{ element in sample} = \frac{\% \text{ element in ash} \times \% \text{ ash}}{100}$$

Preparation and Analysis of Synthetic Ashes. Three synthetic ashes were prepared from C.P. chemicals to establish the accuracy of the method. Ten grams of each were prepared by weighing the dried chemicals onto a tared watch glass making all weighings to the nearest milligram. The chemicals were then transferred to a piece of glazed paper and rolled to effect complete mixing. The mixtures were then ground and mixed using a mullite mortar and pestle. The ground portions were placed in a large weighing bottle and rolled and shaken before use. The composition by synthesis of the simulated ashes is shown in Table II along with the spectrochemical results.

Semimicroprocedure. Past experiences in this laboratory with analyses of this type have given the impression that not all methods of putting the sample into solution were equally efficacious. Therefore, before comparing the spectrochemical and chemical methods of analysis, it became necessary first to study the wet methods in order to choose the best method for making the comparison. Three methods of dissolving the sample were chosen for study. These methods are as follows:

I. The ash sample is fused with sodium carbonate, the melt is leached with water, and the insoluble residue is digested with concentrated hydrochloric acid.

Table I. Internal Standard and Element Lines of Different Types of Samples

Type of Sample	Internal Standard Line, A.	Element Line, A.
Ashes of crudes, bunker fuels; feed stocks or other petroleum fractions; fireside boiler deposits	Co-3044.0	V-3202.4 3212.4 3184.0 Fe-3020.6 2966.9 3008.1 3009.6 Ni-3414.8 3391.1 Na-3302.3 3303.0 2853.0 Ca-4302.5 4318.7 3179.3 Mg-2795.5 2779.8
Combustion chamber and other engine deports	Co-3044.0	Pb-2833.1 2663.2 Fe-3020.6 2966.9 3008.1 3009.6 Cu-3274.0
Used crankcase lubricants	Co-3044.0	Ba-3501.1 3071.6 2335.3 Ca-4302.5 3179.3 Fe-3020.6 2966.9 Pb-2833.1 2614.2 Ag-3280.6 Cu-3274.0 Cr-4254.3 3021.6 Al-3944.0 3082.2
	Co-3048.9	Si-2881.6 2514.3

Table II. Composition and Analysis of Simulated Ashes

Sample	Na_2SO_4 (as % Na)		V_2O_5 (as % V)		NiO (as % Ni)		Fe_2O_3 (as % Fe)	
	Syn.	Spec.[a]	Syn.	Spec.[a]	Syn.	Spec.[a]	Syn.	Spec.[a]
Synthetic ash, I, (high sodium)	27.5	28.4	2.80	2.75	3.93	4.58	3.50	3.78
Synthetic ash II, (high vanadium)	1.62	1.56	33.6	37.5	7.86	7.74	17.5	18.5
Synthetic ash III, (high iron)	6.48	6.2	8.40	9.8	3.93	3.7	42.0	39.2

[a] Typical spectrochemical results, 10-mg. sample weight, average of two electrodes.

II. The sample is digested with 1 to 1 sulfuric acid followed by a potassium bisulfate fusion of the insoluble residue.

III. The sample is water leached and the insoluble residue is fused with sodium carbonate. The carbonate melt is dissolved in water and any remaining insoluble residue is fused with potassium bisulfate. (The purpose of the water leaching of the original sample was to ascertain whether or not the sodium in the samples could be completely extracted. The data obtained showed that it was not possible to extract all the sodium from these petroleum ashes by this method.)

In this report, these three treatments are referred to as modifications I, II, and III, respectively.

Although a sulfuric-hydrofluoric acid digestion of the sample has been successfully used in this laboratory, it was not tested in this particular program for two reasons: In the past, some question has arisen as to the possibility of losing vanadium as a volatile fluoride compound (*4, 15, 16*). In certain routine laboratories where primarily nontechnical employees perform analyses, there is a reluctance to use hydrofluoric acid unless it is absolutely necessary.

PROCEDURES OF ANALYSIS

Ash sample weights ranging from 5 to 15 mg. were used and the vanadium, iron, and nickel were determined by colorimetric methods as follows: vanadium as the soluble yellow phosphotungstovanadic acid (*5, 23, 27*); iron as the reddish ferrous complex of *o*-phenanthroline; and nickel as the red-brown nickelic complex of dimethylglyoxime. The procedures followed were those described by Sandell (*23*). A Coleman Model 11 spectrophotometer was used in making this investigation. Description of the sample-treating procedures follow.

Modification I. Weigh approximately 10 mg. of ash into a small platinum dish, add 0.5 to 1 gram of sodium carbonate, mix thoroughly, cover with a platinum cover, and fuse over a blast burner. Swirl the dish occasionally while the mixture is in a molten condition. Continue heating for 5 to 10 minutes. Remove from the flame and cool. After cooling, dissolve in about 30 ml. of distilled water. When the melt has completely dissolved, filter into a beaker and wash the residue thoroughly with hot water. Acidify the filtrate with dilute sulfuric acid and make up to a convenient volume (100 ml.). Reserve this solution for the determination of vanadium.

Return the filter paper containing the insoluble residue to the platinum dish and char the paper on a hot plate. Finally, ignite the dish and residue in a muffle furnace at 550° C. until no more carbonaceous material remains. Cool the dish and add 5 to 10 ml. of concentrated hydrochloric acid, then warm on a hot plate to effect solution. Transfer this solution to a suitable sized volumetric flask, make up to volume, and reserve for the determination of iron and nickel.

The colorimetric determinations are carried out as follows:

Vanadium. Take a suitable sized aliquot (1 to 5 ml.) of the appropriate solution and add enough sulfuric acid to make the final solution about $0.5N$. Add 5 drops of bromine water, place on a hot plate, and boil until the yellow color of the bromine disappears. Develop the yellow phosphotungstovanadic acid color as described in (*23*).

Iron. Take a suitable sized aliquot of the appropriate solution, adjust the pH to 3.5 with $2N$ sodium acetate, and develop the color as described in (*23*).

Nickel. Take a suitable sized aliquot of the appropriate solution, add 2 ml. of 10% sodium citrate, and carry out the procedure as described in (*23*).

Modification II. Weigh approximately 10 mg. of sample into a small beaker. Add 5 ml. of 1 to 1 sulfuric acid, and heat on a hot plate for 2 to 3 hours. Cool and dilute to about 25 ml. with distilled water. Filter into a volumetric flask and wash the residue thoroughly with hot distilled water, adding the washings to the solution in the volumetric flask. Cool and make up to volume. Ignite the paper and residue as described in modification I. Fuse the insoluble residue with about 0.5 gram of potassium bisulfate. Dissolve the melt in water and make up to a convenient volume. (Do not add this solution to the acid-soluble portion.)

For the separations and analysis, proceed as follows:

Vanadium. Dilute a suitable sized aliquot of the acid solution to about 30 ml. and electrolyze on a mercury cathode for 1 hour to remove the iron, nickel, etc. Determine the vanadium as previously described.

Iron and Nickel. Dilute a suitable sized aliquot of the acid solution to about 30 ml. Make decidedly alkaline to litmus with sodium hydroxide (pellets or flakes are preferable to a solution), add about 0.5 gram of sodium peroxide, and heat to boiling. Filter and wash the precipitate thoroughly with hot water. The precipitate contains the iron and nickel. Dissolve the precipitate with a small amount of hot 1 to 1 hydrochloric acid and wash the paper thoroughly with hot water. Make this solution up to a convenient volume. Determine the iron and nickel as previously described. The iron and nickel in the solution from the bisulfate fusion must also be determined and added in the calculations.

Modification III. This treatment is similar to modification I, except that the hydrochloric acid digestion is replaced by a potassium bisulfate fusion.

Statistical. In order to determine which of the three methods was the best one, it was necessary to subject the data obtained to a statistical evaluation (*3, 6, 9, 24, 28*). Statistical terms used in this treatment are standard deviation, *t* test, variance ratio, and interaction.

Standard deviation, *s*, is usually the best measure of the spread or variability of the data. The significance of the standard deviation is such that, on the average, two out of three measurements will deviate from the average by less than one standard deviation. It is a measure of the precision of the analysis (*28*).

$$s = \sqrt{\frac{\Sigma(X - \bar{X})^2}{n - 1}}$$

where X = individual result, $\bar{X}$ = average value, and n = number of determinations.

The *t* test is a measure of the ratio of the difference between averages to the standard deviation of this difference. It is a means of determining whether two average values are significantly different.

$$t = \frac{\bar{X} - \bar{Y}}{s}\sqrt{\frac{nm}{n + m}}$$

where $\bar{X}$ and $\bar{Y}$ are the averages to be compared, n = number of determinations used to determine $\bar{X}$, and m = number of determinations used to determine $\bar{Y}$.

$$s^2 = \frac{\Sigma X^2 - (\Sigma X)^2/n + \Sigma Y^2 - (\Sigma Y)^2/m}{(n - 1)(m - 1)}$$

where X and Y are individual results (*28*).

The variance ratio, *F*, is the ratio of two variances, used to determine whether or not significant differences are obtained between two variances (*28*).

The existence of an interaction in an experiment of the type being discussed is an indication that either the estimate of the replicate variance (experimental error) is overoptimistic or else the relationship between the variables is not independent.

In order to minimize the bias that is present when duplicates are run simultaneously (giving rise to overoptimistic estimates of the precision), replicate determinations were not made in the usual manner; instead, two analysts working independently made single analyses on several different days.

Table III. Determination of Vanadium in Petroleum Ashes

Modification	Sample			
	A	B	C	D
	Vanadium (as V_2O_5), % Found			
I	65.04	35.36	35.67	2.69
	62.27	34.57	33.33	2.78
	68.68	36.74	33.86	2.74
II	57.49	36.50	34.45	3.21
	54.22	36.43	35.36	3.04
	56.60	35.89	33.76	3.20
III	69.73	37.70	35.95	2.63
	61.67	37.06	36.19	2.87
	63.46	38.19	35.16	2.50

Table IV. Data of Table III Converted to Log_{10} Values

Modification	Sample			
	A	B	C	D
I	1.813	1.549	1.522	0.430
	1.794	1.539	1.523	0.444
	1.837	1.565	1.530	0.438
II	1.760	1.562	1.537	0.507
	1.734	1.561	1.549	0.483
	1.753	1.555	1.528	0.505
III	1.843	1.576	1.556	0.420
	1.790	1.569	1.559	0.458
	1.803	1.582	1.546	0.398

Table V. Analysis of Variance Table for Transformed Data of Table III

Source of Variance	Corrected Sum of Squares	Degrees of Freedom	Variance	Variance Ratio (*F*)
Modifications	0.00033755	2	0.00016878	<1
Samples	9.71947844	3	3.23982615	12.250[a]
Samples × modifications	0.01869223	6	0.00311537	11.78[a]
Replicates	0.00634267	24	0.00026428	.....
Total	9.74485089	35	......	.....

[a] Significant at 1% significance level.

When making tests of significance, any effect that was found to be significant at the 5% significance level was judged to be real.

Since it was intended to learn what could be expected from this analysis under normal working conditions, no extraordinary precautions were taken in handling these samples, nor were any modifications of the methods, as presented in the literature, introduced to improve either the accuracy or the precision of the determinations.

RESULTS

Table III presents the results obtained for the triplicate determination of vanadium in four samples of ash. The replicate variances obtained for the high and low concentrations were subjected to the *F* test, and the variance ratio was found to be highly significant—i.e., the precision is dependent upon the concentration of the measured variable. For this reason, no valid analysis of variance of these data can be made. However, by transformation of the data, a solution can be made. Transformation of the data to logarithmic values was made and the transformed data are shown in Table IV (*10*). Application of the *F* test to these data has indicated that now no significant difference is evident between the two precisions. [A more elegant method for making this test is Bartlett's test for homoscedasticity (*6, 10*).] An analysis of variance can now be performed on the transformed data. Table V presents this analysis of variance for the transformed data and shows that there is a significant variance attributable to samples and that a significant interaction (samples × modifications) is also present. Although the variance caused by modifications has not been shown to be significant, the fact that the interaction variance is significant indicates that all methods of dissolving the sample are not equally efficacious on all the samples. Inspection of the data indicates that possibly the combination of modification II with sample A is the cause of this interaction. Rerunning these statistical tests on all the data except modification II results in no significant variation being attributable to either the modifications or the interaction. Hence it was concluded that modification II was not generally applicable to the determination of vanadium in all types of samples.

Table VI presents the results of triplicate determinations of iron on each of the four samples. Here again the standard deviation is dependent upon the magnitude of the measured variable and it is necessary to make the logarithmic transformation before performing an analysis of variance. Table VII presents the analysis of variance table for the transformed data; these data show that all the effects are significant, indicating that the modification used determines the observed value for the per cent iron in the sample. Here, too, as with the determination of vanadium, a significant modification × sample interaction is observed, indicating that the efficiency of the various modifications is dependent upon the type of sample. Further investigation leads to the conclusion that modification I is not suitable for determining iron in all types of samples.

Similar treatment of the nickel data shown in Table VIII indicated that the only significant variable was the modification used and in this case, modification I was not a satisfactory method of dissolving the samples.

As a result of this investigation, modification III was chosen as the best general method for attacking this type of sample. At this point, a synthetic sample was run using modification III. The results of triplicate determinations on this synthetic sample

Table VI. Determination of Iron in Petroleum Ashes

Modification	Sample			
	A	B	C	D
	Iron (as Fe_2O_3), % Found			
I	2.60	6.38	21.18	15.90
	2.71	6.20	22.18	13.30
	2.47	7.51	20.91	15.21
II	2.55	9.71	22.17	16.03
	2.65	9.21	22.73	15.56
	2.51	8.82	21.28	15.19
III	3.01	9.65	22.13	15.70
	2.70	9.85	21.52	15.51
	2.68	8.97	22.76	15.32

Table VII. Analysis of Variance Table for Transformed Data of Table VI

Source of Variance	Corrected Sum of Squares	Degrees of Freedom	Variance	Ratio (*F*)
Modifications	0.020352	2	0.010176	18.502[a]
Samples	4.354627	3	1.45154	2639+[a]
Samples × modifications	0.026755	6	0.004459	8.107[a]
Replicates	0.013220	24	0.000550	...
Total	4.414954	35	....	...

[a] Significant at 1% significance level.

Table VIII. Determination of Nickel in Petroleum Ashes

Modification	Sample			
	A	B	C	D
	Nickel (as NiO), % Found			
I	1.75	1.67	2.11	0.82
	3.28	2.62	1.79	0.95
	1.65	1.70	2.52	0.75
II	2.63	3.67	2.44	0.86
	2.14	3.31	2.57	0.88
	2.55	3.11	2.70	1.01
III	3.03	3.74	2.90	0.65
	2.44	2.89	2.49	1.02
	2.70	3.57	2.75	0.82

Table IX. Average Results of Colorimetric Analysis of Synthetic Sample 66

Constituent	Synthesis	Found, %	Stand. Deviation[a], s	Coeff. of Variation[b]	Relative Bias[c],
V_2O_5	59.91	58.88	5.52	0.0938	−0.0189
Fe_2O_3	25.01	23.62	0.75	0.0318	−0.0588
NiO	10.32	9.12	0.65	0.0713	−0.1316
Na_2O	2.18	2.25[d]	0.15	0.0667	+0.0311

[a] Calculated from three determinations.

[b] Coefficient of variation = $\frac{s}{\%\ \text{found}\ (1)}$

[c] The term "relative bias" as herein used is synonymous with the term "relative accuracy" used by Mitchell (*21*).

[d] Gravimetric determination.

are shown in Table IX. These data demonstrate the type of precision and accuracy which could be expected from an analysis of such samples.

DISCUSSION

Since the aim of this experimental program was to compare the spectrochemical and the semimicromethods of analysis with regard to both their precision and accuracy, it was necessary to have a true value to use for determining the accuracy of the methods. Inasmuch as the samples studied were actual ash samples and not synthetics, no true value could be used. Instead, determination was made of a value termed "the most probable value," and this was used to determine the amount of bias existing in the determinations. This most probable value was determined by running quintuplicate determinations of each element by standard macromethods of analysis which are described in (*8*). Solution of the samples was effected either by a sulfuric-hydrofluoric acid digestion or by the described modification III. The data obtained for these values are shown in Table X.

In order to reduce the possibility of personal bias and to obtain a reasonable estimate of the precision of the methods being tested, two or more analysts made the required determinations but no duplicates were run on the same day (*10, 28*). None of the data

Table X. Results Obtained for Replicate Macrodeterminations of Ash Constituents

					Sodium Determination[b]	
	Method used[a]	V_2O_5	Fe_2O_3	NiO	Na_2O	Method used[a]
			SAMPLE A			
	1	69.08	2.77	3.36	7.01	1
	2	69.95	2.63	2.93	7.30	3
	2	69.65	2.65	3.38	7.24	3
	1	69.40	2.69	2.96	7.48	3
	1	69.70	2.76	3.17	7.17	1
Av.		69.56	2.70	3.16	7.24	
Stand. dev.		0.33	0.06	0.21	0.18	
			SAMPLE B			
	2	40.22	9.29	4.30	5.52	1
	1	40.47	9.48	3.85	5.45	1
	1	41.65	10.39	4.10	5.60	1
	2	40.73	9.92	4.08	5.67	3
	2	40.48	10.17	4.27	5.61	3
Av.		40.71	9.85	4.12	5.57	
Stand. dev.		0.55	0.46	0.21	0.09	
			SAMPLE C			
	2	39.58	23.27	2.88	4.02	3
	2	40.05	25.04	2.89	4.03	3
	2	40.33	23.30	3.18	4.49	3
	1	40.18	24.26	3.01	4.31	1
	1	40.11	24.18	3.14	4.60	1
Av.		40.05	24.01	3.02	4.29	
Stand. dev.		0.28	0.74	0.14	0.26	
			SAMPLE D			
	1	3.49	15.27	0.71	1.20	3
	1	3.54	15.30	0.75	1.04	1
	1	3.54	16.15	0.78	1.43	3
	2	3.57	16.46	0.84	1.15	1
	2	3.71	16.87	0.82	1.33	3
Av.		3.57	16.01	0.78	1.23	
Stand. dev.		0.08	0.71	0.05	0.15	

[a] 1, H_2SO_4-HF digestion; 2, modification III; and 3, J. Lawrence Smith.

[b] Sodium determination made on separate samples.

Table XI. Results Obtained for the Spectrochemical and Semimicrodeterminations of Vanadium

	Sample			
	A	B	C	D
		V_2O_5, %		
		SPECTROGRAPHIC METHOD		
	77.0	33.0	50.9	3.65
	80.0	31.7	48.2	3.80
	83.5	34.3	46.0	3.83
	76.2	34.3	42.0	3.83
	65.9	37.7	39.6	3.57
	66.2	33.3	37.7	3.67
	68.8	35.5	36.3	4.05
	76.0	33.0	38.9	4.00
	62.4	35.6	33.7	4.18
Av.	72.9	34.3	41.5	3.84
		SEMIMICROMETHOD		
	69.7	37.7	36.2	2.63
	61.7	39.2	36.0	2.50
	61.9	34.6	35.6	3.08
	60.3	37.7	35.5	2.87
	61.0	38.2	34.2	2.58
	59.5	35.7	35.2	2.08
	72.0	39.7	35.5	2.78
	63.5	40.7	37.6	2.96
	60.9	38.5	32.0	2.29
Av.	63.4	38.0	35.3	2.64

Table XII. Analysis of Variance of Transformed Data from Table XI

Source of Variance	Corrected Sum of Squares	Degrees of Freedom	Variance	Variance Ratio (F)
Methods	0.068400	1	0.068400	48.997[a]
Samples	18.831009	3	6.277003	High[a]
Method × samples	0.099607	3	0.033202	23.784[a]
Replicate	0.089401	64	0.001396	...
Total	19.088417	71	....	...

[a] Highly significant.

Table XIII. Summary of Data Obtained by Spectrochemical and Semimicromethods of Analysis

	Sample			
	A	B	C	D
		Vanadium (as V_2O_5), %		
Most probable value	69.56 ± 0.23	40.71 ± 0.37	40.05 ± 0.19	3.57 + 0.05
		SPECTROCHEMICAL METHOD		
Average % found	72.9	34.3	41.5	3.84
Relative bias[a]	+0.0453	−0.1866	+0.0337	+0.0703
Stand. dev., s	7.25	1.80	5.77	0.20
Coeff. of variation, %	9.99	5.25	13.90	5.21
		SEMIMICROMETHOD		
Average % found	63.4	38.0	35.3	2.64
Relative bias[a]	−0.0978	−0.0711	−0.1756	−0.3523
Stand. dev., s	4.41	1.90	1.54	0.32
Coeff. of variation, %	6.96	5.00	4.36	12.12
Variance ratio[b], F	2.705	1.121	14.12[c]	2.549
Calcd. t.	3.356[c]	4.240[c]	3.111[c]	9.493[c]

[a] The term "relative bias" is synonymous with the term "relative accuracy" as used by Mitchell (*21*).

[b] Ratio of large variance to smaller variance.

[c] Significant at 5% significance level.

Table XIV. Results Obtained for the Spectrochemical and Semimicrodeterminations of Iron

	Sample			
	A	B	C	D
	Fe_2O_3, %			
	Spectrochemical Method			
	3.44	10.0	31.8	16.8
	3.71	9.62	31.1	18.3
	3.71	10.1	30.4	17.1
	3.54	9.75	29.7	16.0
	3.19	9.92	28.6	16.8
	3.18	10.4	26.6	17.0
	3.30	10.2	25.6	17.0
	3.50	10.2	26.5	17.5
	3.05	10.3	26.6	17.6
	3.02	11.5	27.0	17.2
Av.	3.36	10.20	28.39	17.13
	Semimicromethod			
	3.01	9.65	21.5	15.7
	2.62	8.07	22.0	15.5
	2.70	10.75	22.1	17.0
	3.29	8.97	20.7	15.3
	2.55	9.20	21.1	15.6
	2.85	9.52	22.8	15.4
	2.69	9.21	24.2	14.8
	2.77	9.31	22.2	15.3
	3.57	11.31	23.3	15.7
	2.68	9.85	23.9	16.3
Av.	2.87	9.58	22.38	15.66

Table XV. Summary of Data Obtained by Spectrochemical and Semimicromethods of Analysis

	Sample			
	A	B	C	D
	Iron (as Fe_2O_3), %			
Most probable value	2.70 ± 0.04	9.85 ± 0.31	24.01 ± 0.50	16.01 ± 0.49
	Spectrochemical Method			
Average % found	3.36	10.20	28.39	17.13
Relative bias	+0.1964	+0.0343	+0.1543	+0.0650
Stand. dev., s	0.25	0.52	2.22	0.60
Coeff. of variation, %	7.44	5.10	7.82	3.50
	Semimicromethod			
Average % found	2.87	9.58	22.38	15.66
Relative bias	+0.0592	−0.0282	−0.0728	−0.0223
Standard dev., s	0.33	0.91	0.49	0.48
Coeff. of variation, %	11.50	9.50	2.20	3.07
Variance ratio, F	1.653	3.071	20.08[a]	1.553
Calcd. t	1.776	1.978	8.816[a]	6.357[a]

[a] Significant at 5% significance level.

obtained were rejected. The first nine vanadium results and the first ten iron and nickel results obtained by each method of analysis were used to make this study. The reason for making this number of runs was to improve the statistical sensitivity of the experiment. Any effect found to be significant at the 5% significance level was judged to be real. Whenever a significant interaction (methods × samples) was found, the data were treated individually by samples; then the average values were compared by means of the t test (*28*) and the precisions compared by means of the F test (*28*).

Presented in Table XI are the data obtained for the determination of vanadium by both methods of analysis. As in the previous data, it was determined that the standard deviation was dependent upon the magnitude of the measured variable and therefore it was necessary to make a logarithmic transformation of the data before performing the analysis of variance. Table XII presents the analysis of variance table for the transformed data and indicates that both the main effects and the interaction (methods × samples) are significant. The fact that the interaction is significant can be interpreted to mean that the bias between the two methods is not constant and that it varies with the nature of the sample. Whether this bias is always with one method or whether it shifts from one method to the other as the composition varies has not yet been ascertained. Treating each sample separately and testing the average values by the t test, it is found that the two methods give average values that are significantly different at the 5% significance level. When the precisions were tested by means of the F test, it is found that only sample C shows a significantly different precision, the colorimetric method being more precise for this one sample. It has not been possible to attribute this difference to any assignable cause.

Table XIII is a summary of pertinent vanadium data. From this table and with the information learned from the foregoing analysis of variance, it can be stated that, in general, the precisions of the semimicro and spectrochemical methods for determining vanadium are comparable, but the spectrochemical method yields results that are less biased than the semimicromethod.

Similar treatment of the iron and nickel data (shown in Tables XIV, XV, XVI, and XVII) has led to the following conclusions: In the determination of iron there is a significant difference between the methods of analysis and also a significant method × sample interaction, indicating that one method tends to rate certain samples differently from the other method. A study of Table XV reveals that the spectrochemical method has a tendency

Table XVI. Results Obtained for the Spectrochemical and Semimicrodeterminations of Nickel

	Sample			
	A	B	C	D
	NiO, %			
	Spectrochemical Method			
	4.72	5.22	4.22	1.21
	5.15	4.57	4.85	1.10
	5.28	4.91	4.55	1.07
	4.81	5.20	4.65	1.12
	4.19	5.02	4.08	1.24
	4.21	5.40	4.08	1.02
	4.18	4.84	3.85	1.10
	4.72	5.00	4.16	1.10
	4.16	5.06	3.69	1.15
	3.82	5.03	3.95	1.16
Av.	4.52	5.03	4.21	1.13
	Semimicromethod			
	3.03	2.89	2.90	0.65
	3.37	3.74	2.49	1.02
	1.74	5.12	2.75	0.82
	2.44	2.88	3.25	1.02
	2.70	2.44	4.05	0.88
	3.39	3.57	3.05	0.90
	2.18	4.46	3.58	0.75
	1.79	3.82	2.43	0.76
	2.73	4.23	2.27	0.71
	2.40	3.11	3.01	0.90
Av.	2.58	3.63	2.98	0.84

Table XVII. Summary of Data Obtained by Spectrochemical and Semimicromethods of Analysis

	Sample			
	A	B	C	D
	Nickel (as NiO), %			
Most probable value	3.16 ± 0.14	4.12 ± 0.14	3.02 ± 0.09	0.78 ± 0.03
Average % found	4.52	5.03	4.21	1.12
Relative bias	+0.3009	+0.1809	+0.2827	+0.3036
	Spectrochemical Method			
Stand. dev., s	0.48	0.32	0.37	0.07
Coeff. of variation, %	10.62	6.36	8.79	6.25
Average % found	2.58	3.63	2.98	0.84
Relative bias	−0.2248	−0.1350	−0.0134	+0.0714
	Semimicromethod			
Stand. dev., s	0.58	0.82	0.56	0.13
Coeff. of variation, %	22.48	22.59	18.79	15.48
Variance ratio, F	1.472	6.730[a]	2.199	3.708[a]
Calcd. t	8.588[a]	5.288[a]	6.233[a]	6.637[a]

[a] Significant at 5% significance level.

to yield results that are higher than the results of the semimicromethod. With the exception of sample C, the two methods have comparable precisions, but, in general, the semimicromethod is less biased than the spectrochemical method.

Table XVIII. Summary of Data Obtained by Spectrochemical Analysis

(Sodium as Na_2O)

Sample	Most Probable Value	Average % Found[a]	Standard Deviation, *s*	Coeff. of Variation, %	Relative Bias
A	7.24 ± 0.12	14.26	0.82	5.75	+0.4923
B	5.57 ± 0.06	11.30	0.39	3.45	+0.5071
C	4.29 ± 0.18	8.25	0.68	8.23	+0.4806
D	1.23 ± 0.10	1.03	0.03	2.91	−0.1942

[a] The spectrographic analytical curves were prepared from synthetic samples with no regard to the analyses of actual samples.

Furthermore, the fact that in both the determination of iron and vanadium, sample C does not appear to behave normally led to the performance of another statistical test on these data. The results of this test, although inconclusive because of the limited amount of data available, hint that here may possibly be a significant iron × vanadium interaction in the spectrochemical determination—i.e., the apparent amount of iron found is dependent upon the vanadium concentration.

Statistical evaluation of the nickel determination has shown that again there is a significant difference between the methods of analysis. However, the data shown in Table XVII indicate that neither method appears to be very satisfactory. Although the spectrochemical method appears to be somewhat more precise than the semimicromethod (note samples B and D), the semimicromethod in all cases yields results that are nearer the most probable value. It is possible that by using another oxidizing agent in place of bromine (*22*), carefully adjusting the pH, and removing large amounts of free acid before adjusting the pH, the semimicromethod for determining nickel can be made more precise and more accurate.

Inasmuch as the semimicromethod for determining the sodium was the same as that used for obtaining the most probable value, these data are not reported here. Instead, Table XVIII shows the data obtained by the spectrographic procedure as compared to the most probable value. From these data it can be seen that although the precision of the spectrochemical procedure is good (of the order of ±5%), in general, the average results are about double the most probable value.

This phenomenon may be due to the nature of the synthetic samples. An earlier report appearing in the literature (*9*) states that when mixtures of pure compounds were used in preparing spectrochemical calibration curves, the analysis of actual samples by means of these curves usually led to erroneous results. A study of these data showed that in almost all cases the error introduced was one of yielding high results for the elements determined. Since the calibration curves of this work were based on synthetic mixtures of sodium sulfate and various oxides, this may be the reason for some of the high results obtained.

CONCLUSIONS

The results of this study, comparing spectrochemical and semimicromethods of analyzing petroleum ashes, have led to the conclusion that, in general, the precisions of the two methods are comparable. If better precision and accuracy are desired, two alternatives are available: Gain improvement in precision by running replicate analyses of each sample; either improve the present methods of semimicro or spectrochemical methods or devise new methods for performing these analyses.

In view of the rapidity of the spectrochemical method of analysis, it is the preferable method for analyzing ash samples. In this regard, it might very well be profitable to try to establish good standard samples for use in calibrating spectrographic procedures for this type of analysis.

SUMMARY

A statistical study of the results obtained in the analysis of four petroleum ignition residues by spectrochemical and semimicromethods indicates that not all methods of dissolving these ashes, preparatory to making the semimicroanalysis, are equally effective. When a suitable semimicromethod is used, the precisions of the two methods are comparable. Although there are some differences between the two methods, the advantages of precision and accuracy are about evenly distributed between them. Therefore, the rapidity of the spectrochemical method was the deciding factor which led this laboratory to adopt it as the method for analyzing these petroleum ashes.

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Infrared Identification of Materials in the Fractional Milligram Range

By Means of a Beam Condensing System and Pressed Potassium Bromide Pellets Containing the Sample

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Infrared records on samples weighing less than 1 mg. frequently are needed. Previously available macrotechniques are completely unsuited to examining these small samples. A pair of silver chloride lenses was arranged to form a beam condensing system which has a cross-sectional area of less than 4 sq. mm. Provision was made to place the unit quickly and precisely in the sample beam of a Baird infrared double-beam spectrophotometer. Simple microdies were designed and fabricated so that potassium bromide tablets weighing only 5 mg. can be pressed. These may contain as little as 10γ of the compound under test. Only a few seconds are required to convert from macro to micro scale operation. The manipulation techniques employed are simpler and easier than any previously available.

IN WORKING with photographic goods, foreign particles sometimes need to be identified and it is usually not possible to collect enough particles to make macro infrared records. The use of a reflecting microscope system was first considered in order to make infrared measurements on tiny samples. Reflecting microscope systems are, however, expensive; they must be aligned very precisely and, most important, have not as yet been used with double-beam instruments. It was therefore decided to see if some infrared-transmitting material could be used to construct lenses for a beam-condensing system that would substantially reduce the area of the sample beam.

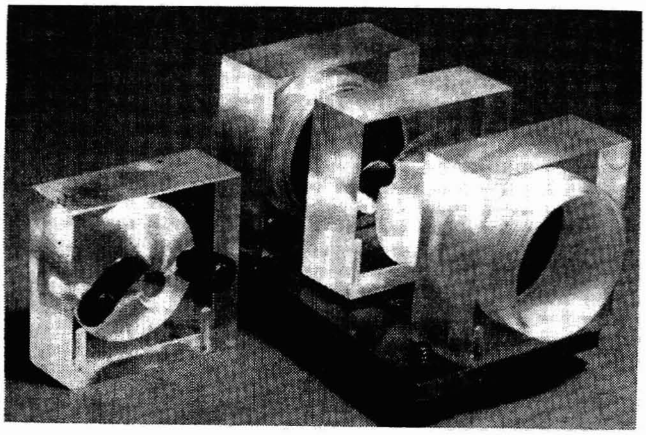

Figure 1. Silver Chloride Lenses Mounted on Base Plate with Sample Holders

Kremers (2) reviews the properties and usefulness of silver chloride as a lens material for infrared work. With the techniques outlined, satisfactory lenses were formed and a beam-condensing unit was constructed. While it does not provide the degree of sample reduction provided by a reflecting microscope system, a great many problems do not require testing samples smaller than 10γ. The reflecting microscope system can, however, be used for samples at least as small as 1γ. The lens system is not expensive and does not require any instrumental adjustments in going from macro to micro scale work with the Baird double-beam spectrophotometer.

BEAM-CONDENSING SYSTEM

The beam-condensing system (*1*) consists of a pair of plano-convex, silver chloride lenses coated with an antihalation and antireflection coating, and a reference plate similarly coated. The lenses have diameters of 28 mm. and radii of curvature of 22

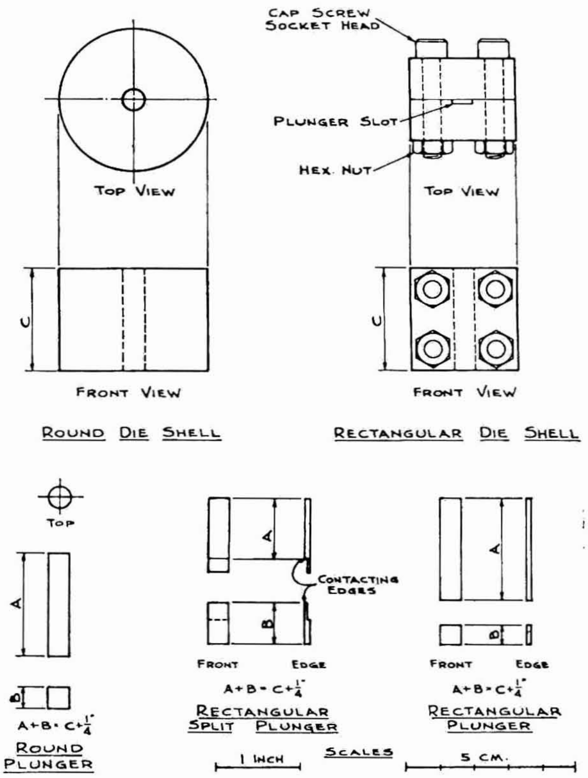

Figure 2. Dies and Plungers Used to Prepare Round and Rectangular Potassium Bromide Pellets

Over-all dimensions are not critical. Plungers fit apertures closely but can be pushed through with firm hand pressure alone.

Material.
Die shell. AISI Type 440C stainless steel or methacrylate plastic
Plunger. AISI Type 440C stainless steel. The round plunger can be cut from a steel dowel pin

mm. They are assembled on a holder, shown in Figure 1, which has locating pins so that it can be placed precisely in position in the instrument in a rapid, reproducible manner. The beam-condensing unit is placed between the source and the focusing mirror in the sample beam. The sample under test is then placed between these lenses at the point where the beam has the minimum cross-sectional area by means of plastic blocks that fit over locating pins. It requires only seconds to convert the Baird double-beam spectrophotometer from macro to micro scale work and all the advantages of double-beam operation are retained.

The sample area covered is usually 1.5 by 3.5 mm. Occasionally preparations half this width are used. When enough sample is available, a potassium bromide disk about 6 mm. in diameter is used. Strips even narrower than these can be used when necessary because the slit on the Baird varies from about 0.06 mm. (0.002 inch) at 2 microns to 2.5 mm. (0.1 inch) at 16 microns. As the dimensional reduction with the beam-condensing system is about $^1/_3$, the reduced slit image varies from about 0.02 to 0.8 mm. (0.0007 to 0.03 inch). It is therefore possible to fill completely the slit image between the lenses over a considerable wave-length range by strips even smaller than the half-width strips reported.

As sample size is reduced, more attention must be paid to holding and working with the samples as well as to positioning them in the instrument. Sample positioning, using a mechanical stage as sold for use with a microscope, is described by Anderson and Miller (*1*). Since the initial disclosure was made, it has been possible to dispense with the mechanical stage for holding most samples. The sample holder in current use is a plastic block with a small central aperture and two guide holes that fit over pins in the base plate carrying the silver chloride lenses. This permits placing the samples accurately in the beam. This system is used with the 6-mm. potassium bromide disks and the narrow potassium bromide strips as well as film or oil samples that require micro testing. The block is shown in position between the lenses in Figure 1.

PREPARATION OF SAMPLE

Sample preparation techniques have developed to the point where good records can be obtained with only 10γ of a chemical. It is known that this can be pushed with some sacrifice in resolution to at least 5γ, but for the authors' work this has not yet been necessary. The development of potassium bromide pressings (*3*, *4*) has been of great help in furthering this micro work. The greatest advantage of the technique is in the increase in sample bulk obtained by mixing the inert potassium bromide with the sample.

Ordinarily 100 to 200 parts by weight of potassium bromide are mixed with the sample under test and in this way it is possible to handle and manipulate the material conveniently. The mixing is carried out in mullite mortars with hand grinding. The potassium bromide is ground fresh each morning by hand or with a mechanical mortar grinder to a fineness of about 100 mesh. After the sample to be tested is added, further grinding reduces the mixture to a fineness of about 200 mesh. It has not been necessary to dry the potassium bromide in this laboratory, where the air is maintained at about 40% relative humidity and 74° F. A 25-γ sample is mixed with about 5 mg. of potassium bromide and pressed in a micro die to form a strip about 0.75 by 6 mm. Only about 3.5 mm. of this strip are actually on the beam; the rest of the strip is used in holding it in position.

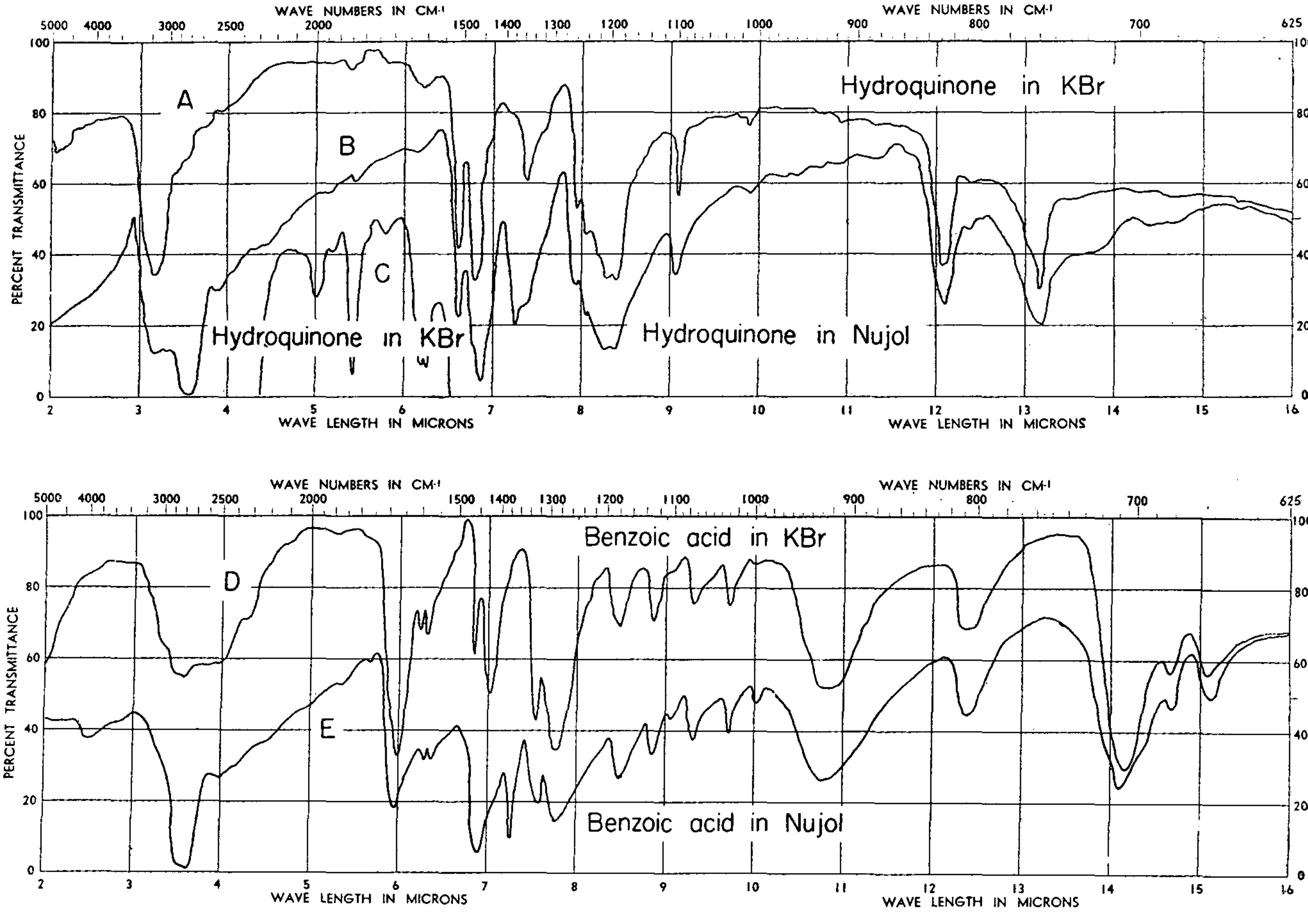

Figure 3. Typical Records Comparing Mineral Oil Mulls and Potassium Bromide Pressed-Plate Preparations

Hydroquinone concentrations 1% in potassium bromide for record A and 10% in potassium bromide for record C. Benzoic acid concentration 1% in potassium bromide. Curves displaced vertically for sake of clarity

The strips obtained by the technique reported are not optically clear. They are, however, translucent, and pencil marks or lettering can ordinarily be read through them when they are laid over the writing.

Because of the small sample apertures to be covered, microdies were constructed. These were initially made of AISI Type 440 C stainless steel which can be hardened after machining. In this laboratory, dies are now available for making 6-mm. (0.25-inch) round disks, strips 1.5 by 6 mm., and strips about 0.75 by 6 mm. The first dies that were used for pressing micropellets had the body of the die bolted to a base plate and the plunger pressed the pellets directly against the base plate. This die was moderately successful, but the disks and strips were frequently uneven because it was so difficult to spread the powder uniformly at the bottom of the hole in the die, which was about 1 inch deep.

The current procedure is to have a 3-piece die as shown in Figure 2, consisting of a body or shell about 30 mm. (1.25 inches) long and two plungers of AISI Type 440 C steel, the combined length of the plungers being about 6 mm. (0.25 inch) longer than the die shell. Experience to date is that methacrylate-type plastic bodies will yield pressings as good as those from the stainless steel dies. They are particularly convenient to use because they are lightweight and one can observe changes occurring during pressing. The plastic bodies do not last so long as those of stainless steel, but they are less expensive to fabricate. Because of their transparency they are easier to use and clean. The potassium bromide has a corrosive effect on steel parts and this is another reason for using plastic dies. To fill the dies, the longer plunger is inserted until it is within a few millimeters of the bottom of the die shell, which is held upside down. For the 6-mm. round die, a normal charge consists of 25 to 50 mg. of potassium bromide thoroughly ground, containing 0.1 to 0.5 mg. of the chemical under test, which is distributed uniformly over the end of the plunger. Then the short plunger is inserted and the assembly pushed until the base of the short plunger is flush with the base of the shell of the die. Pressure is then applied by means of a small hydraulic press or a small portable arbor press having a 14-inch handle. In preparing the half-width strips using the plungers shown in Figure 2, very little force is required. The ends of the half-width plungers are of different lengths and for convenience in loading, the upper plunger has the shorter narrow section. Pressing times are short, frequently less than a minute even for the round disks. Pressures used on the 6-mm. (0.25-inch) round disks have been usually between 1000 and 2000 pounds dead weight.

It has not been found necessary to evacuate the salt to remove entrapped air, although a more transparent pellet would probably result if this were done. The entire procedure of preparing these pellets is simple and direct. During the actual pressing, the die rests on a steel base plate to protect the press platen and a brass strip is placed over the top of the plunger to protect it and the top press platen. When the pressing is completed, the flat plate under the die is shifted, so the die is over an area at the edge of the plate having a hole in it and the top plunger is pressed through until the lower plunger and pellet are expelled. Brass pieces slightly smaller than the plungers are sometimes used to aid in removing the plungers.

With the mineral oil technique previously used, it was customary to prepare the oil dispersions just before recordings were to

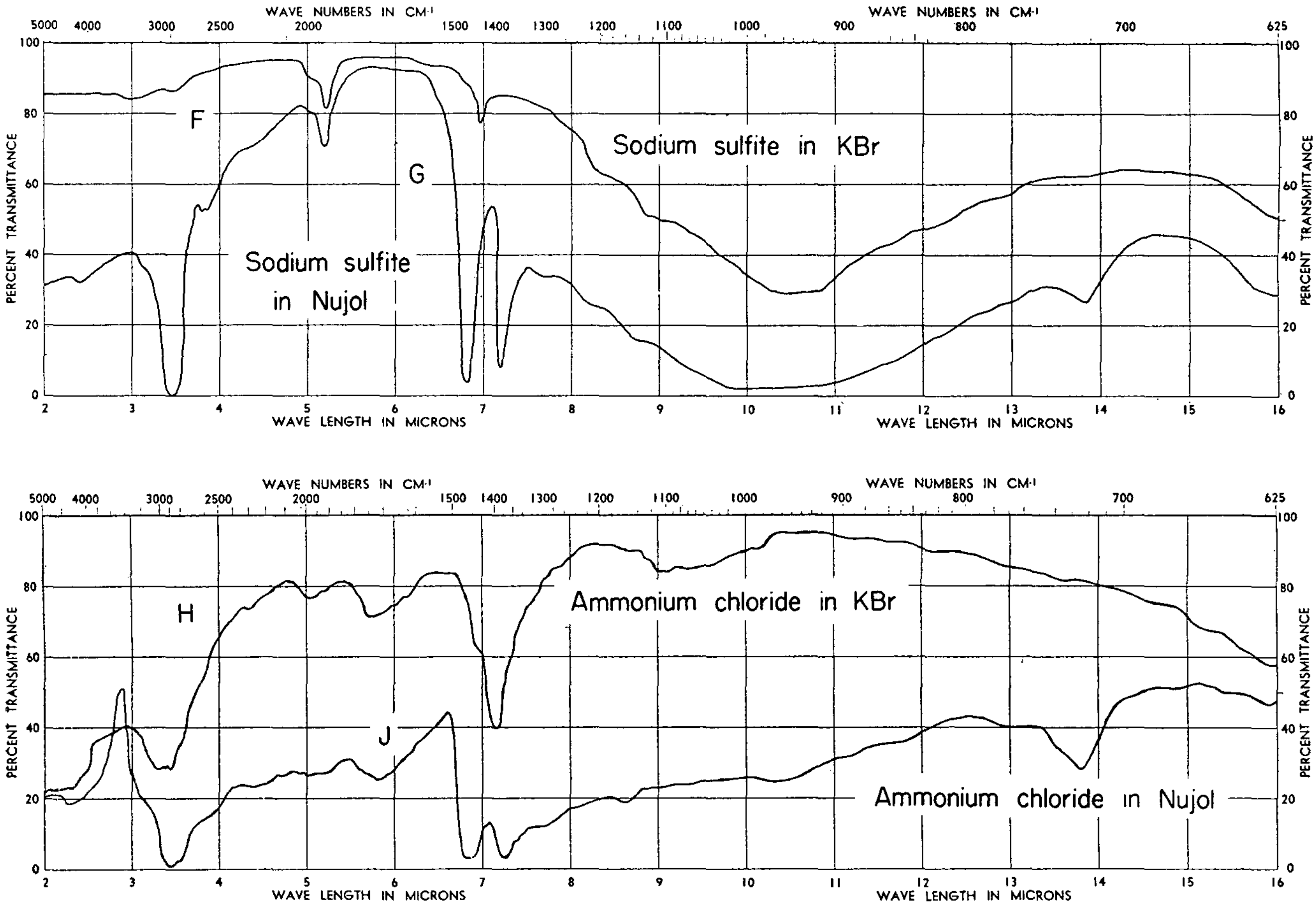

Figure 4. Mineral Oil and Potassium Bromide Preparations of Sodium Sulfite and Ammonium Chloride

Concentrations are 1% in potassium bromide. Curves displaced vertically for sake of clarity

be made. Now, however, as oil separation and other preparation difficulties are not encountered, the pressings are often prepared in advance and held until instrument time is available.

Some typical results are given in Figures 3 and 4 to show the marked superiority of the pressed salt technique over conventional macro mineral oil mulls which invariably show bands at 3.5, 6.8, 7.2, and 13.8 microns. The potassium bromide curves are useful over their entire length, as potassium bromide itself shows no absorption bands. This is clearly evident when the potassium bromide and mineral oil records of the chemicals shown in Figures 3 and 4 are compared. Curves *A*, *B*, and *C* are of hydroquinone. The available data in the 5- to 6-micron region are increased in curve *C* where 10% of hydroquinone was present in the disk. In this laboratory it has never been possible to secure records of this quality at this concentration using mulling techniques. Curves *D* and *E* are of benzoic acid at concentrations of about 1% in potassium bromide and mineral oil. The marked increase in record quality around 7 microns is an important gain. Curves *F* and *G* in Figure 4 are of sodium sulfite. The minor band that can be seen at about 7 microns was previously hidden by the mineral oil bands. Curves *H* and *J* are of ammonium chloride, and again the record in potassium bromide is an improvement over that in mineral oil. In one instance, 5 p.p.m. of ammonia in a water sample were detected and pictured by means of the micro infrared recordings.

The ability to retain standards and samples of importance is particularly facilitated by the potassium bromide technique. The disks can readily be filed for future use as evidence; they are also valuable in case of controversy or for use as reference standards. For example, a standard hydroquinone disk has been run as an instrument performance check many times over a period of several months with no change in the curves indicative of decomposition or alteration of the material.

This technique appears to be almost universal in its application to dry samples. As a matter of fact, only one compound so far has been found to give a poorer record in potassium bromide than in mineral oil. This effect in itself is interesting and will be investigated further as time permits, to allow correlating changes in structure or crystallinity that take place under pressure.

The majority of this work has been confined to potassium bromide as the carrier salt, but work currently going on indicates that a wide selection of carrier salts will soon be available. The work reported has also been confined to solids and nonvolatile liquids and only semiquantitative results have been attempted so far. The quantitative results are, however, most encouraging and all indications are that the microtechniques reported here will be readily adaptable to quantitative analysis.

CONCLUSIONS

A combination of the pressed salt technique using potassium bromide and a silver chloride lens beam-condensing system produces records of excellent quality from microgram quantities of organic and inorganic chemicals. It has extended the use of the Baird double-beam spectrophotometer to the examination of microgram quantities of material, and it accomplishes these results without basically altering the instrument. All the advantages of double-beam operation are retained and it requires only seconds to convert from macro to micro infrared work. In the authors' laboratory, the use of mineral oil mulls, even for macro work, has almost entirely disappeared because of the ease with which potassium bromide records can be prepared using this system.

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Polarography of Zirconium Salts in Methanol

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LITTLE fundamental work on the thermodynamic and electropotential properties of zirconium salts has been reported to date (*4*). The exact nature of quadrivalent zirconium in perchloric acid aqueous solutions, ordinarily considered noncomplexing, is still under active consideration (*1*, *2*). Quadrivalent zirconium complexes readily with many anions (*1*) and undergoes hydrolysis except in strong acid solution or when highly complexed. This pronounced tendency toward complexing and hydrolysis makes it difficult to characterize zirconium species in any investigation.

Laubengayer and Eaton (*5*) have reported the polarographic reducibility of quadrivalent zirconium in aqueous solutions. Solubility considerations in the useful range of acidity limit the analytical applications of the polarographic method in this case. Furthermore, the reported reduction of quadrivalent zirconium is obtained as a relatively small polarographic wave on top of a large hydrogen decomposition wave. The polarographic results in methanol reported have eliminated some of the difficulties encountered in aqueous solutions. Well defined reproducible polarographic waves are obtained in methanol and are not complicated by the simultaneous appearance of hydrogen decomposition waves.

MATERIALS

The methanol and lithium chloride were Baker and Adamson's reagent grade. The zirconium sulfate tetrahydrate (zirconium oxide: theoretical, 34.67; found, 34.88 and 34.86) and zirconyl chloride octahydrate (per cent zirconium oxide: theoretical, 38.24; found, 38.24 and 38.36) were prepared in this laboratory. Purity of these salts was substantiated by gravimetric ignition procedures at 850°. The pure zirconium sulfate and zirconyl chloride used in the polarographic investigation were prepared by a method described by Falinski (*3*). The purified zirconium oxide used in these preparations was obtained by igniting at about 360° a purified zirconyl chloride sample obtained from A. D. McKay, Inc.

POLAROGRAPHIC APPARATUS

Polarographic data were obtained at 25.0° ± 0.1° using a Leeds and Northrup Electro-Chemograph Type E and an H-cell. Characteristic polarographic properties were: drop time, 4.02 seconds per drop; *m* for the capillary, 1.79 mg. per second; and 1.86 mg.$^{2/3}$sec.$^{-1/2}$ (open circuit) for $m^{2/3}t^{1/6}$ at $h = 50$ cm. The supporting electrolyte employed in all cases reported was $0.1M$ lithium chloride in methanol. Oxygen was removed satisfactorily by rapid degassing with tank nitrogen for 5 minutes.

RESULTS

Slope analysis results [plot of E_{de} *vs.* log $I/(I_d - I)$] for zirconium sulfate are in the range 0.12 to 0.16, indicating an irreversible reduction. *E* values were corrected for the small *iR* drop across the cell.

The two waves reported for zirconium sulfate are attributed to two different quadrivalent species of zirconium in solution, as the total I_d/C values are constant over a tenfold concentration range. Apparently, these two species are present in substantially equal amounts in the most dilute solutions (compare I_d/C values for waves I and II at $1 \times 10^{-3}M$ and below).

Table I. Polarographic Results in Methanol

Salt, $M \times 10^3$	I_d/C, $\mu a./mM$			$-E^1/_2$ volt *vs.* SCE	
	Wave I	Wave II	Total	Wave I	Wave II
$Zr(SO_4)_2.4H_2O$					
5.0	5.6	1.6	7.2	1.25	1.56
2.5	5.2	2.0	7.2	1.24	1.55
1.0	3.7	3.4	7.1	1.25	1.55
0.5	3.6	3.5	7.1	1.25	1.54
$ZrOCl_2.8H_2O$					
5.0	6.0	...	...	1.43	....
2.5	5.6	...	...	1.41	....
1.0	5.0	...	...	1.39	....
0.5	4.0	...	...	1.37	....

I_d/C values for wave I are seen to decrease with concentration of zirconyl chloride similar to the behavior observed with zirconium sulfate. Unfortunately, wave II for zirconyl chloride merges with the solvent-supporting electrolyte decomposition wave. Thus I_d/C values are not ascertainable for wave II in this case.

Diffusion current data for zirconium sulfate reduction waves at various drop times between 3.0 and 6.0 seconds were analyzed. I_d values obtained were in accordance with the Ilkovič equation, showing that kinetic currents are not involved in the present investigation.

The data presented in Table I indicate the possible application of the polarographic method to the analysis of hydrated quadrivalent zirconium salts such as zirconium sulfate tetrahydrate. The addition of a few tenths of 1% of water to the anhydrous methanol did not appreciably alter the polarographic data. Polarographic results obtained in a mercury pool cell were no better than those obtained in the H-cell, if the polarogram was recorded within about 5 minutes. In this way, diffusion of water through the agar plug in the H-cell was maintained below interfering quantities.

ACKNOWLEDGMENT

The authors are indebted to E. L. Anderson of this laboratory for providing these compounds.

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Simultaneous Titration of Iron and Copper with Ethylenediaminetetraacetic Acid

Spectrophotometric End Points

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Several recent papers have pointed out the advantages of photometric end points in certain titrations (*1*, *2*, *7*, *8*). Sweetser and Bricker (*8*) have shown that the principal disadvantage of ethylenediaminetetraacetic acid (Versene, Sequestrene) as a volumetric reagent for various cations—viz., lack of suitable indicators—may be overcome in certain cases by this technique. Studies in this laboratory have indicated that the photometric approach permits the simultaneous titration of two or more cations provided: (*a*) the stability constants of their complexes with ethylenediaminetetraacetic acid are sufficiently large, (*b*) the constants differ sufficiently, and (*c*) the spectra of the complexes permit selection of suitable wave lengths. Many of the stability constants have been measured, and a convenient compilation has been given (*4*). Although these constants may be expected to vary considerably with the medium, they may be used as rough guides in predicting the feasibility of titrating certain metals simultaneously. Proviso *c* may easily be checked experimentally.

Sweetser and Bricker have reported good photometric titrations of ferric and cupric ions, separately, with ethylenediaminetetraacetic acid (*8*). The stability constants reported for the complexes of these ions are large, and differ sufficiently to indicate the possibility of obtaining two consecutive end points when a mixture of the two is titrated with an ethylenediaminetetraacetic acid solution [ferric complex, log $K = 25.1$ (*6*); cupric complex, log $K = 18.3$ (*5*)]. Furthermore, the cupric complex absorbs strongly at a wave length (745 mμ) where the ferric complex exhibits no absorption. Since the ferric complex is the most stable, the formation of the cupric complex with the attendant increase in absorbancy serves as indicator for the titration of the iron. The copper end point is indicated, of course, by a plateau representing maximal formation of the cupric complex. This paper reports results obtained in this fashion, and includes application to an aluminum alloy containing low percentages of iron and copper.

REAGENTS AND APPARATUS

Ethylenediaminetetraacetic acid under the trade name Sequestrene AA was obtained from the Alrose Chemical Co., Providence, R. I. About 14.6 grams of this material was suspended in distilled water and treated with sodium hydroxide to dissolve the acid. The solution was diluted to 500 ml. with distilled water to give a $0.1M$ reagent, and standardize against the standard cupric solution, using the spectrophotometric end point.

As emphasized by Sweetser and Bricker (*8*), careful control of pH is essential for successful titrations with ethylenediaminetetraacetic acid. The buffer used in this work, 94.5 grams (1 mole) of monochloroacetic acid (Eastman Kodak Co. practical grade, distilled to remove dark-colored impurities), was dissolved in distilled water, adjusted to pH 2.0 with sodium hydroxide, and diluted to 1 liter.

The standard cupric solution was about $0.01M$, prepared by dissolving accurately weighed electrolytic copper foil in nitric acid, boiling the solution to remove oxides of nitrogen, and diluting with distilled water. The standard ferric solution of about the same molarity was prepared from Baker's analyzed ferric ammonium sulfate. It was analyzed by passage through a Jones reductor, followed by titration with standard dichromate solution, and was kept sufficiently acidic to prevent hydrolysis of the iron.

The Beckman Model DU spectrophotometer was adapted as follows: The titration cell, which was a rectangular cuvette with a capacity of about 90 ml. designed for a Lumetron colorimeter, was mounted inside a Beckman test tube attachment with the test tube bracket removed. The cell, whose base projected beyond its sides, could be positioned and held securely by means of the setscrews designed to hold the test tube bracket. The cover for the test tube attachment was replaced by a flat wooden cover; molding around its edges gave a light-tight fit. Two holes in the cover, equipped with felt gaskets, admitted the glass stirrer and the 2-ml. microburet. The cover was painted with a flat black enamel. The exterior of the cell was likewise painted, leaving only small windows for the light beam. The stirrer and the buret were painted for a distance several centimeters above the cover down nearly to the tips, which were immersed in the solution. This arrangement was essentially light-tight; moving a strong light source about the exterior of the cell compartment had no noticeable effect on the galvanometer. The light path (about 3 cm. in length) was near one end of the cell; the stirrer, operating near the opposite end, did not affect absorbancy readings.

PROCEDURE

The results described below were obtained with the standard ferric and cupric solutions.

Aliquots of the standard solutions furnishing the desired quantities of iron and copper were pipetted into the titration cell, followed by 25 ml. of the chloroacetate buffer solution. The pH was checked, and in cases where the acidity from the iron solution could not be handled by the buffer, sodium hydroxide solution was added to bring the solution to a pH of about 2. The total volume was adjusted to about 50 ml. with distilled water, and the solution was allowed to stand for at least 1 hour. The cell was then positioned in the instrument, and at a wave length of 745 mμ, the absorbancy was set at zero, using the slit width and sensitivity controls in the usual manner. The absorbancy was then measured after the addition of appropriate increments of titrate, and the titration curve plotted.

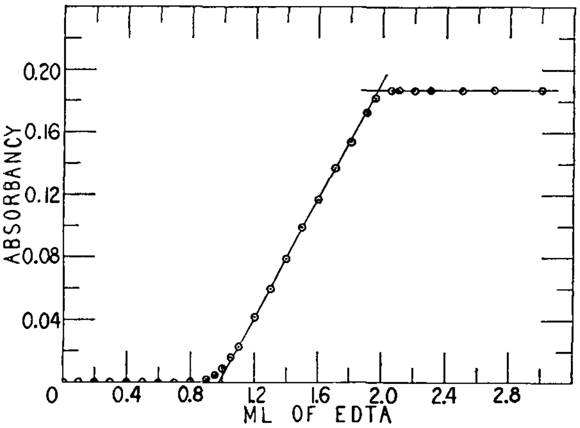

Figure 1. Titration of an Iron(III)–Copper(II) Mixture with 0.1*M* Ethylenediaminetetraacetic Acid

5.40 mg. Fe, 6.36 mg. Cu; 745mμ

To show a practical application of this method, an aluminum alloy obtained from the National Bureau of Standards (standard sample 603, wrought alloy), certified to contain 0.29% copper and 0.21% iron, was analyzed as follows:

A sample of about 2 grams was dissolved in dilute hydrochloric acid, and the bulk of the aluminum was precipitated as aluminum chloride, following the procedure of Hillebrand and Lundell (*3*). Great care was taken to wash the precipitate thoroughly. The resulting solution was evaporated to 15 to 25 ml., adjusted to a pH of about 2 with sodium hydroxide, buffered with 25 ml. of the chloroacetate buffer, diluted to about 50 ml., and titrated as described above.

DISCUSSION

Figure 1 shows a typical curve for the titration of a ferric-cupric mixture with ethylenediaminetetraacetic acid. The curve is linear with no change in absorbancy during the titration of the iron, because neither ferric ion in the chloroacetate medium nor its complex with the titrant absorbs appreciably at 745 mμ. It has been observed, however, that strongly acid ferric solutions approach zero absorbancy slowly after the pH has been raised to 2. Presumably a slow hydrolysis reaction is responsible for this phenomenon. In any case, it is desirable to wait at least an hour after raising the pH before starting the titration.

During the titration of the iron, each increment of titrant causes an increase in absorbancy, followed by a slow return to zero. Apparently cupric ion is complexed as a result of temporary high local concentrations of ethylenediaminetetraacetic acid, in spite of the stirring. However, this drift in the absorbancy persists for only 5 to 10 minutes (somewhat longer in the vicinity of the end point). As only a few points are required to define the curve, this does not represent a serious defect in the method. Inconveniences of this type must be evaluated in the light of the fact that determinations of two metals accrue from each titration. The presence of cupric ion is necessary to indicate the iron end point. In cases where the sample to be analyzed contains no copper, cupric ion might be added by the analyst, or alternatively, the technique of Sweetser and Bricker based on the color of the ferric complex with salicylate might be used (*8*).

Table I. Results of Experiments

Standard Solutions

	Iron, Mg.			Copper, Mg.		
	Taken	Found	Error, %	Taken	Found	Error, %
1	2.70	2.61	3.3	3.18	3.23	1.6
2	5.40	5.42	0.37	4.45	4.48	0.67
3	8.10	8.03	0.86	5.09	5.08	0.20
4	10.81	10.83	0.19	6.36	6.41	0.79
5	10.81	10.78	0.28	3.18	3.20	0.63
6	8.10	8.14	0.49	4.45	4.43	0.45
7	5.40	5.41	0.19	5.09	5.10	0.20
8	2.70	2.65	1.9	6.36	6.38	0.31

Aluminum Alloy, NBS Certificate: 0.21% Iron, 0.29% Copper[a]

	Iron found, %	Error, %	Copper found, %	Error, %
1	0.20	4.8	0.27	6.9
2	0.20	4.8	0.28	3.4
3	0.21	0.0	0.30	3.4
4	0.21	0.0	0.29	0.0
5	0.20	4.8	0.28	3.4

[a] Other elements certified: Mg, 1.01%; Si, 0.52%; Cr, 0.24%; Ti, 0.037%.

That portion of the curve representing the titration of the cupric ion is similar to the copper curves reported by Sweetser and Bricker (*8*). As there is no drift during the titration of either pure ferric or pure cupric solutions, mixtures containing only these two ions exhibit no drift during the titration of the copper. Thus this portion of the titration can be performed as rapidly as one can manipulate the apparatus, if complex-forming ions other than ferric and cupric are absent. When titrations were attempted on solutions of the aluminum alloy which had been subjected to no prior separations, however, the drifting was very troublesome during the titration of the copper, and an inordinate length of time was required to complete the titrations. With sufficient patience it was possible to determine iron and copper even under these conditions. Presumably aluminum ions compete effectively with cupric ions until stirring has dissipated the high local concentration of ethylenediaminetetraacetic acid about the tip of the buret; a slow return to equilibrium conditions, favoring formation of the cupric complex, follows. (This effect is not so pronounced during the titration of the iron. As ferric ion forms a stronger complex than cupric ion, aluminum perhaps does not compete so effectively with the former. There is some drift in this part of the curve, due to the presence of cupric ions.)

The maximum quantity of aluminum which can be present without slowing down the titration in this way has not been determined. Sweetser and Bricker (*8*) have shown that small amounts of aluminum have little effect. It was found in the present study that removal of the bulk of the aluminum from the aluminum alloy solution by the precipitation of aluminum chloride [by passing hydrogen chloride gas into the solution to which ether has been added (*3*)] eliminated the aluminum interference completely, although this precipitation is known not to be quantitative. As about 2 grams of aluminum was being precipitated in the presence of only a few milligrams of iron and copper, the precipitate was washed more scrupulously than might ordinarily be necessary. When care was exercised in this regard, there were no appreciable losses of iron or copper. The troublesome drifting during the titration of the copper was completely eliminated by this separation.

The results shown in Table I indicate that the method is sufficiently accurate for many purposes. As pointed out by Sweetser and Bricker (*8*), interferences from other cations would be expected to be small because of the large stability constants of the ferric and cupric complexes with ethylenediaminetetraacetic acid. Furthermore, such interferences are minimized by working at a fairly low pH. This method is especially attractive for the determination of small quantities of iron and copper in aluminum alloys, because the bulk of the aluminum is so easily removed, and the small remainder does not affect the results appreciably.

Inspection of the stability constants of the metal ion complexes with ethylenediaminetetraacetic acid suggests other possibilities for the simultaneous determination of metals in various mixtures.

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Paper Chromatography of Some Alkoxy Acids

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In connection with a pharmacological investigation being conducted in this laboratory, it became desirable to determine the presence and concentration of some alkoxy acids. Paper chromatography with its high sensitivity and simple procedure offers a straightforward approach to the qualitative and quantitative determination of small quantities of acids (100 to 500γ). This paper covers a number of compounds not elsewhere reported and is offered in the interest of increasing the knowledge of this useful analytical tool.

Many of the procedures described in the literature have been concerned with column chromatography. Elsden (*2*) used silicic acid columns for the separation of acids, but the quantity of starting material was greater than was available in this laboratory. Most of the papers applied to paper chromatography have been developed for the separation and detection of amino acids. Lugg and Overell (*7*) have described a method of separation of nonvolatile organic acids, but volatile acids did not lend themselves to the procedure, as the material was lost during the process owing to heating. In addition, a swamping acid, acetic or formic, was included with the solvent and as this study concerned monocarboxylic acids, the procedure obviously was unsuitable.

The work of Fink and Fink (*3*) was investigated in which the acids were converted to nonvolatile hydroxamic acid derivatives. This method yielded excellent results qualitatively but, during the several steps, variable amounts of starting material were lost and quantitative analyses were impossible.

The technique of Kennedy and Barker (*5*) offered a reasonable approach and their general method was applied to the acids under consideration. In supplement, some aspects of the procedures of Brown and Hall (*1*) and Hiscox and Berridge (*4*) were incorporated in the work. Some of the solvent systems employed by Long, Quale, and Stedman (*6*) were used to extend the scope of the separation procedures.

EXPERIMENTAL

Whatman No. 1 filter paper was used for this study. Large sheets 18.25 × 22.5 inches were trimmed to 30 × 45 cm. before use. The sheets were washed with oxalic acid, following Kennedy and Barker, before use. It was found convenient to place the papers in a large tray and then add the wash. After sufficient rinsing, the papers were dried by placing an infrared light underneath the tray at a distance of 12 to 18 inches and heating overnight. This technique obviated any necessity for handling the wet paper, which tears easily.

The acids in the form of a solution of their ammonium salts containing 10 to 40 mg. per ml. of free acid were applied in 0.01-ml. quantities on a line 3 cm. from the bottom of the paper, spaced 3.5 cm. apart, and developed by the "ascending" technique in a cylindrical tank after the paper was stapled in the form of a cylinder. The developing solution was contained in a large Petri dish to which a handling rod of glass had been fused. The tank was sealed with a flat glass plate lubricated with stopcock grease.

After the development of the chromatogram had proceeded 6 to 8 hours, the paper was dried in an oven at 105° to 110° C. for 5 minutes, then placed against a large glass plate and sprayed with bromophenol blue (50 mg. of bromophenol blue plus 200 mg. of citric acid per 100 ml. water). The acid spots appeared blue against a yellow background with little or no "tailing." This spraying agent appeared to be the most satisfactory for general use. When several different concentrations of the same acid are spotted along the paper, the size of the spot and depth of color allow the ready estimation of the acid concentration of unknown quantities of the same acid, provided the original spot on the starting line was the same diameter as the known acid concentrations.

Table I. R_f Values × 100 of Some Semivolatile Acids

		Solvent						
Acid	M.W.	*A*	*B*	*C*	*D*	*E*	*F*	*G*
Methoxyacetic	90	10	8	18	..	8	28	6
Ethoxyacetic	104	19	17	17	..	17	36	9
n-Propoxyacetic	118	28	27	9	46	28	48	18
n-Butoxyacetic	132	42	39	18	52	39	54	26
3-Ethoxypropionic	118	22	26	4	46	28	44	17
4-Methoxy-*n*-butyric	118	19	24	4	46	29	48	14
n-Hexanoic	116	58	57	39	68	65	68	48
2-Ethyl-*n*-hexanoic	144	70	74	55	72	79	84	71

Solvent composition:

A. 1-Butanol saturated with 1.5*N* aqueous ammonia.
B. 1-Butanol, 75 ml.; 1-propanol, 25 ml.; 1.5*N* aq. ammonia, 10 ml.
C. 1-Hexanol saturated with 1.5*N* aqueous ammonia.
D. 95% Ethyl alcohol, 99 ml.; ammonium hydroxide, sp. g. 0.9, 1 ml.
E. 1-Butanol, 50 ml., abs. ethyl alcohol, 50 ml.; ammonium hydroxide, sp. g. 0.9, 1 ml.
F. Same as *D* but unwashed filter paper was used.
G. Iso-octane, 40 ml.; acetone, 30 ml.; 95% ethyl alcohol, 30 ml.; ammonium hydroxide, sp. g. 0.9, 1 ml.

SUMMARY

The R_f values for a number of acids not yet listed in the literature have been reported. The values obtained were duplicable within 2 R_f units. Two aliphatic acids possessing the same molecular weight range and moderate volatility were included for purposes of comparison. It will be seen that in a homologous series, the R_f values have increased linearly with molecular weight in several of the solvent systems. This fact might find application in predicting the molecular weights and identities of unknown acids of a similar class.

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Rapid Interferometric Analysis of Germane-Hydrogen Mixtures

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THE Rayleigh-Zeiss interferometer, fitted with a 500-mm. double-chamber gas cell, has been used for assaying germane-hydrogen mixtures. The method depends upon measuring the difference in refractive index between hydrogen and the germane-hydrogen mixture, and so involves an initial calibration with germane-hydrogen mixtures of known composition. This method of analysis was used for determining the amount of germane (germanium hydride, GeH_4) in the hydrogen evolved at the cathode during the electrolysis of solutions of various germanium compounds.

In the Rayleigh-Zeiss interferometer light from a narrow slit is rendered parallel by a lens, split into two beams by a double slit, passed through two identical tubes containing the gases to be compared, and then through two identical glass plates. The emergent beams are recombined to form interference fringes. Where the gases in the two tubes differ in refractive index, the band system is displayed sideways. This displacement can be compensated by changing the inclination of one of the glass plates which is attached to a micrometer screw with a measuring drum. The point for exact compensation is found by comparing this first band system with a second system formed by two beams which have traveled identical paths. The construction and operation of the interferometer have been fully described (*1, 2*). The only innovation was the thermal insulation of the main body of the instrument to prevent it from being affected by rapid room-temperature fluctuations. The light source used was a tungsten filament lamp.

The gas-handling apparatus is shown schematically in Figure 1.

The dotted lines enclose that part of the apparatus used for making up known germane-hydrogen mixtures. The germane used was purified by trap-to-trap distillation under high vacuum until its vapor pressure at two different temperatures corresponded to Paneth's values (*3*). A compressed supply of electrolytic hydrogen (assayed 99.8% H_2 with water as major impurity) was used, and was dried by passing it through anhydrous calcium sulfate, barium oxide, and two traps immersed in liquid nitrogen. The difference in refractive index between dried hydrogen and spectroscopically pure hydrogen was measured and found to be negligible—i.e., a few divisions on the measuring drum of the micrometer screw.

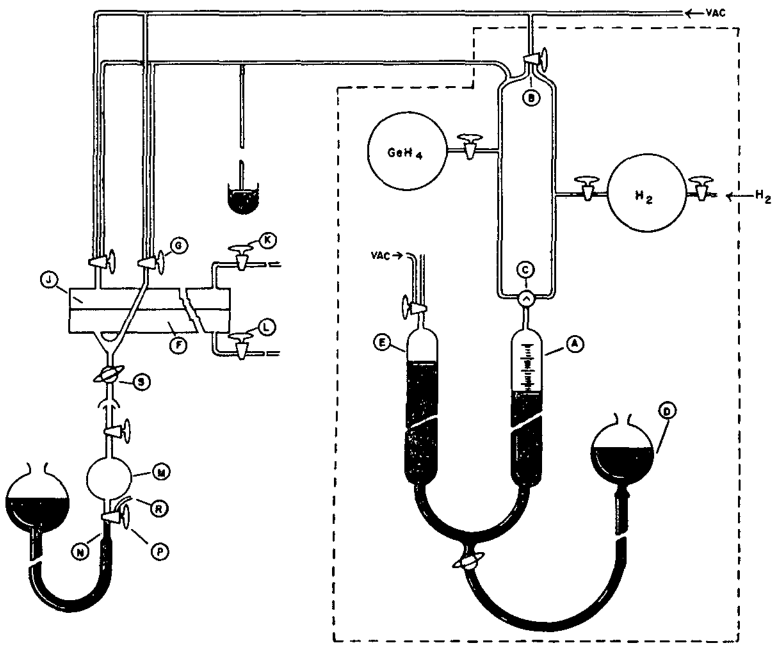

Figure 1. Gas-Handling Apparatus

CALIBRATION OF INTERFEROMETER

The procedure used for obtaining mixtures of known composition was as follows: With mercury completely filling the graduated limb, *A*, all the lines were evacuated, and stopcock *B* was closed. The required amount of germane was admitted into *A* and the three-way stopcock, *C*, was then closed; the mercury levels in the U-tube were lowered by drawing mercury into reservoir *D;* limb *E* was evacuated, and the volume and pressure of germane in *A* were measured. The difference between the mercury levels in the limbs of the U-tube was a direct measure of the pressure in *A*, and was measured using a cathetometer. Hydrogen was admitted to *A* and, with stopcock *C* closed, the new pressure and volume were measured. With the pressure and volume data obtained, the composition of the mixture could be calculated. The mixture was pipetted into the interferometer chamber, *F*, by raising the mercury level in *A* until the pressure of gas was a little in excess of atmospheric pressure; stopcock *G* was closed and the gas lines were evacuated.

The interferometer chamber, *J*, was filled with hydrogen to a pressure slightly greater than 1 atmosphere. Each interferometer chamber was opened to the atmosphere through an 80-cm. length of 1-mm. bore capillary tubing, which effectively prevented the diffusion of air into the chambers when stopcocks *K* and *L* were opened. The pressure of gas in the chambers fell to atmospheric pressure. The number of divisions on the measuring drum of the micrometer screw, which corresponded to a null-point reading for the mixture, was read. The temperature and pressure were noted and the null-point value corrected to 0° C. and 76 cm. of mercury.

Fifteen different mixtures varying from 0.64 to 12.58 mole % germane were used to obtain a calibration curve. The slope of the calibration curve, which was assumed to be linear, was obtained using the method of least squares, applying the restriction that the errors arise from uncertainty in the composition of the mixtures and not from the interferometer readings. It was found that 100 divisions ≡ 0.506% germane (the null-point reading with both chambers evacuated was 30 divisions, and this must be subtracted from the number of divisions read before using the above equivalent).

ANALYTICAL PROCEDURE

The dried gaseous mixture was collected over mercury in pipet *M*. One end of the pipet was attached to the interferometer chamber and the other end to a mercury reservoir, as shown in Figure 1. The air trapped in the mercury line at *N* was bled out through tube *R*. Stopcocks *P* and *S* were opened and the mixture was pipetted into the evacuated chamber until the pressure of gas was a little over 1 atmosphere. Chamber *J* was filled with hydrogen and the refractive index difference measured, as described.

The germane content of the mixture was calculated using the relation:

$$V = 0.141 \times d \times T/P$$

where

V = mole percentage of GeH_4 in H_2
d = null-point reading, divisions, after subtracting the zero reading
T = temperature, degrees Kelvin
P = barometric pressure, mm. of mercury

The accuracy of this method, with the germane-hydrogen mixture made up as described and using the 500-mm. cell, is ±0.059 mole % germane, and the range is 0 to 15% germane. The main advantage of this method is the rapidity with which analyses can be performed, about 10 minutes per determination, an important matter when a large number of samples have to be analyzed.

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Mass Spectrometric Analyses of Some Six- and Seven-Carbon Alcohols

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RECENT advances in the design of commercial mass spectrometers—e.g., close temperature control of the source, suppression of metastable ions, fabrication of metal inlet systems that eliminate the use of stopcock lubricant, and accurate measurement of pressure in the 0- to 100-micron range (*12*)—permit the examination and analysis of compounds that, heretofore, could not be studied. The last-mentioned improvement, particularly, has widened the scope of mass spectrometers considerably. Previously, more cumbersome micromanometers (*14*) or calibrated micropipets (*3*) were required, which often were not entirely dependable for mixtures with vapor pressures less than 50 microns.

APPARATUS AND PROCEDURE

A Consolidated mass spectrometer Model 21-101, converted to Model 21-103, was used for all analyses. The samples were introduced into the instrument through a mercury orifice (*2*), which was modified in these laboratories by M. L. McTeer and G. E. Mellen to the design presented in Figure 1. A capillary micropipet (*3*), pictured in Figure 2, was used to introduce the samples through this mercury orifice into the preleak bulb. When a sample was to be introduced, the Teflon plug was removed, and the container holding the liquid to be analyzed was inserted in the orifice. The Teflon plug was broken away from the vacuum seal by the disk threaded to the shaft of the plug. The pressure of the mercury above forced the liquid out of the pipet into the inlet system of the spectrometer. The size of the micropipet (about 0.0005 ml.) was such that, upon complete expulsion and expansion of the liquid into the 3-liter, preleak bulb, the desired pressure (about 30 microns) was attained. From two viewpoints this system had a decided advantage over the older technique which employed a sintered-glass disk under a mercury seal (*10*): The time of introduction was shortened considerably; and difficulties due to adsorption in the sintered glass and loss from fractionation, both of which are characteristic of the sintered glass disk, were greatly minimized. The sample passed directly from the orifice to the 3-liter, preleak bulb, in which the pressure was measured with a micromanometer (*12*).

Compounds containing a hydroxyl group tend to be adsorbed on the walls of the inlet system through hydrogen bonding. For samples containing alcohols, therefore, the system had to be saturated several times with the mixture in question. This was accomplished by introducing the sample into the mass spectrometer, leaving it there for 2 minutes, and then pumping the sample out again. Usually, after several such "saturation" treatments, the mixture could be reproducibly analyzed. A "pump-out" period of 10 minutes between runs is adequate.

RESULTS

The analyses of mixtures of oxygenated compounds—e.g., alcohols, ketones, aldehydes, etc.—have been reported (*4–6, 9, 11, 13*). The present paper describes a method developed for the analysis of mixtures of six- and seven-carbon alcohols. To test that method, its accuracy, and its reproducibility, three mixtures containing known amounts of 3-heptanol, 2-heptanol, 4-heptanol, 2-ethyl-1-butanol, and 1-hexanol were analyzed. These results are summarized in Table I. The reproducibility is within ±5% of the value of the contained component. The average deviation of a given component from its known value (for all three mixtures) is 8.2%. The maximum single deviation is 19.3% of its known value. The mass patterns for these five alcohols are presented in Table II.

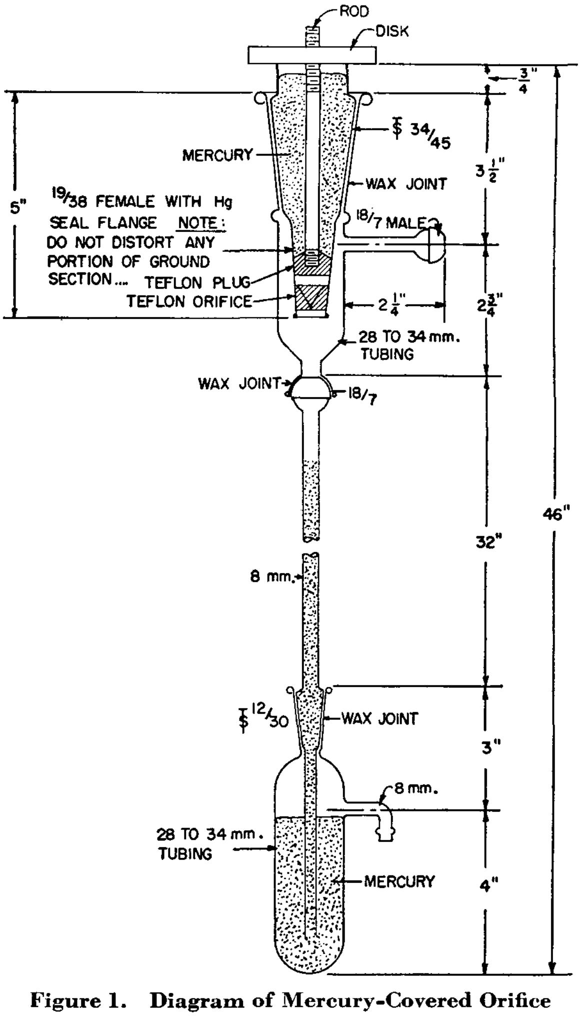


Figure 1. Diagram of Mercury-Covered Orifice

Table I. Mass Spectrometric Analyses of Six- and Seven-Carbon Alcohols

(Weight per cent)

Component	I Known	I Measured	I Deviation	II Known	II Measured	II Deviation	III Known	III Measured	III Deviation
3-Heptanol	8.0	7.6	−0.4	30.7	32.5	+1.8	12.4	10.9	−1.5
4-Heptanol	8.0	8.9	+0.9	11.5	11.9	+0.4	4.1	4.7	+0.6
1-Hexanol	67.9	66.8	−1.1	30.7	39.9	−0.8	16.6	13.4	−3.2
2-Ethyl-1-butanol	8.1	8.4	+0.3	11.7	12.8	+1.1	63.3	67.6	+4.3
2-Heptanol	8.0	8.4	+0.4	15.4	12.9	−2.5	3.6	3.4	−0.2
Average deviation			0.62			1.32			1.96

Table II. Mass Patterns for Some Six- and Seven-Carbon Alcohols

Mass to Charge Ratio	1-Hexanol	2-Ethyl-1-butanol	2-Heptanol	4-Heptanol	3-Heptanol	n-Butane
14	1.75	3.12	0.85	1.19	1.19	15.63
15	8.58	14.91	5.59	8.44	7.40	85.88
16	0.40	0.60		0.44	0.36	1.72
19	1.72	3.84		3.26	1.21	0.41
26	5.46	10.22	1.55	3.23	4.34	72.28
27	46.72	95.65	17.32	41.82	39.62	358.13
28	12.42	19.18	3.26	5.29	8.95	278.03
29	44.61	98.55	15.09	26.76	37.74	351.25
30	1.97	3.59	0.47	1.03	2.55	7.50
31	51.38	77.54	4.67	28.26	41.51	0.31
32	1.47	1.65	0.12	0.42	0.45	1.38
33	0.44	1.68	0.05	0.41	0.14	
38	2.15	4.58	0.65	1.42	1.14	23.75
39	24.45	51.68	4.98	16.61	15.43	141.56
40	4.82	6.59	1.44	2.22	2.37	18.81
41	56.77	94.38	16.22	23.95	50.00	257.19
42	51.24	39.18	7.10	4.48	5.96	100.63
43	77.29	289.13	19.48	60.75	28.55	798.75
44	5.64	11.61	8.83	13.05	6.38	26.72
45	4.23	10.61	100.00	8.12	14.91	0.50
			2.17	8.21	0.35	
53	3.20	8.71	1.10	2.11	2.16	7.50
54	3.54	3.68	0.65	1.38	0.82	1.81
55	56.26	91.85	16.71	139.78	13.74	8.53
56	100.00	43.42	8.74	9.10	6.17	6.56
57	9.47	14.47	4.72	8.09	17.04	20.06
58	0.53	0.83	1.18	1.06	9.21	100.00
59	0.63	4.22	0.08	1.05	100.00	4.03
69	24.26	24.67	2.50	2.32	67.74	
70	3.40	100.00	4.15	1.11	6.23	
71	2.64	82.79	1.27	2.16	2.31	
72	0.36	5.13	0.28	10.72	0.77	
73	1.56	1.82	0.48	100.00	2.25	
83	1.28	0.76	7.33	0.23	0.63	
84	9.04	30.76	0.78	0.11	0.88	
87	0.00	0.00	0.00	0.07	29.28	
Molecular weight	102.17	102.17	116.20	116.20	116.20	58.12
Sensitivity, div./micron	62.84	31.12	177.76	82.04	75.93	10.53 (43 sensitivity = 84.11)

Ionizing voltage. 70 volts
Ionizing current. 10 microamperes
Ion source temperature. 250° C.
Magnet current. 0.605 ampere
Basis of pressure measurement. Micromanometer

DISCUSSION

Simultaneous equations were utilized for the analysis of mixtures of 1-hexanol, 2-ethyl-1-butanol, 2-heptanol, 3-heptanol, and 4-heptanol. The contribution of a given component to a selected mass peak was obtained by the use of determinants. Five general inverse solutions were set up which, once solved, permitted the resolution of these six- and seven-carbon alcohol mixtures in less than an hour. The pattern coefficients used for the analyses presented herein are given in Table III.

Table III. Pattern Coefficients

m/e	1-Hexanol	2-Ethyl-1-butanol	2-Heptanol	3-Heptanol	4-Heptanol
56	1.0000	1.0000	1.0000	1.0000	1.0000
70	0.0340	2.3031	0.5130	1.0097	0.1220
45	0.0423	0.2674	12.3610	2.4165	0.8923
87	0.0000	0.0000	0.0000	4.7455	0.0077
73	0.0156	0.0419	0.0593	0.3647	10.9890

Another, somewhat less accurate but much faster, method is to use the 87 and 73 mass peaks to remove the major portion of the contribution of 3-heptanol and 4-heptanol to the other peaks. These two peaks are virtually free of interference from the other constituents. Simultaneous equations involving the 56, 70, and 45 peaks next are employed to separate 1-hexanol, 2-ethyl-1-butanol, and 2-heptanol, and a correction for their small contribution to the 73 and 87 peaks then is made.

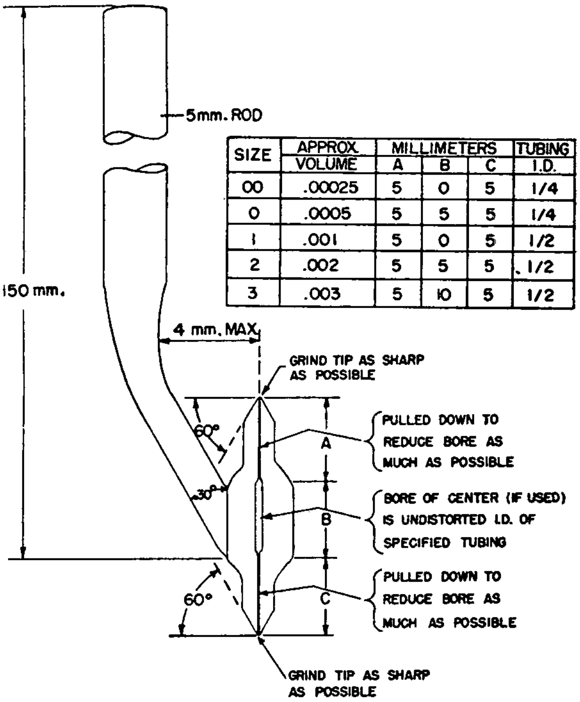
Under electron-beam bombardment, secondary alcohols rupture preferentially at the carbon-to-carbon bond immediately adjacent to the carbon to which the hydroxyl group is attached. The resulting galvanometer deflection is not always the most intense in the mass spectrum, but is characteristic for a specific secondary alcohol. 1-Hexanol is characterized by large 56 and 31 peaks; 2-ethyl-1-butanol, on the other hand, exhibits a large 70 peak as well as substantial galvanometer deflection at a mass-to-charge ratio of 84. The following equations represent plausible explanations for the formation of some of these fragments:

$$CH_3(CH_2)_4CH_2OH + e \longrightarrow [C_4H_8]^+ + CH_3CH_2OH + 2e \quad (1)$$

$$CH_3(CH_2)_4CH_2OH + e \longrightarrow [CH_2OH]^+ + [C_5H_{11}] + 2e \quad (2)$$

$$(C_2H_5)_2CH{-}CH_2OH + e \longrightarrow [C_6H_{12}]^+ + H_2O + 2e \quad (3)$$

$$(C_2H_5)_2CH{-}{-}CH_2OH + e \longrightarrow [C_5H_{10}]^+ + CH_3OH + 2e \quad (4)$$



SIZE	APPROX. VOLUME	MILLIMETERS A	MILLIMETERS B	MILLIMETERS C	TUBING I.D.
00	.00025	5	0	5	1/4
0	.0005	5	5	5	1/4
1	.001	5	0	5	1/2
2	.002	5	5	5	1/2
3	.003	5	10	5	1/2

Figure 2. Micropipet

The analyses of other oxygenated compounds, and of aromatic and aliphatic hydrocarbons, have been discussed (*1, 7–9, 11, 13*). The use of the mercury orifice (*2*) and micromanometer (*12*) described herein, in conjunction with a metal inlet system, however, makes such analyses much easier to carry out with accuracy and precision.

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Reference Electrode for Potentiometric Titrations in Glacial Acetic Acid

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THE titration of nitrogen bases in glacial acetic acid with a standard solution of perchloric acid in the same solvent has proved to be an indispensable technique in the analysis of petroleum (*7*), shale oil (*7*), coal hydrogenation oils (*10*), pharmaceuticals (*8*), and many other organic nitrogenous compounds (*1–4, 6*).

In the titration of colorless compounds the end point may be readily determined by use of color indicators, but with colored compounds it must be determined potentiometrically.

The choice of electrodes for making potentiometric titrations in nonaqueous media, however, presents a problem. The glass electrode, in conjunction with a sleeve-type calomel reference electrode, both with and without a salt bridge, has been used by several workers (*6–9*), but not without difficulties resulting from the high sensitivity of the system to stray currents and from contamination of the cell by the solution being titrated.

To eliminate the use of a salt bridge and the difficulties encountered in the use of the calomel electrode, Fritz (*2*) substituted a silver wire coated with silver chloride for the sleeve-type calomel reference electrode.

This paper reports further on the use of the silver–silver chloride electrode in conjunction with the glass electrode and how the instability of the electrode pair in the region of the end point may be obviated.

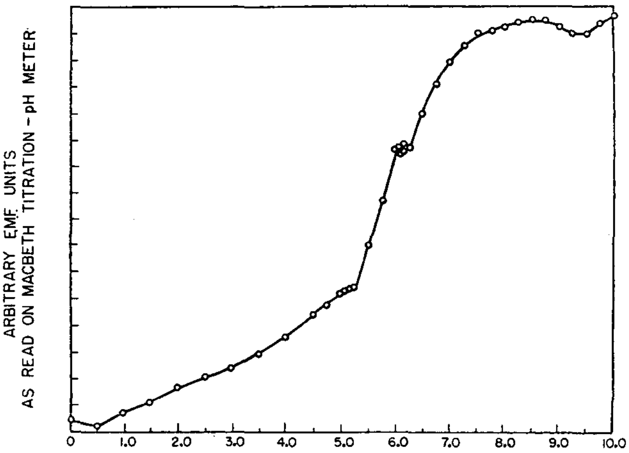

Figure 1. Manual Titration Curve

Silver–silver chloride electrode used in conjunction with glass electrode
Sample. Coal hydrogenation neutral distillate oil

When the silver–silver chloride electrode is used in conjunction with the glass electrode and the two are immersed directly into the solution of the sample being titrated potentiometrically, very erratic e.m.f. readings are observed in the region of the end point (see Figure 1), thus making its determination doubtful. When the same electrodes are used in a continuous automatic titration using the apparatus of Katz and Glenn (*5*), a reversal in the change of the observed e.m.f. occurs in the region of the end point (see curve *A*, Figure 2), which again renders its location difficult. This reversal in the change of the observed e.m.f. results from the fact that the potential of both the glass and the silver–silver chloride electrodes in the region of the end point changes markedly but in the opposite direction.

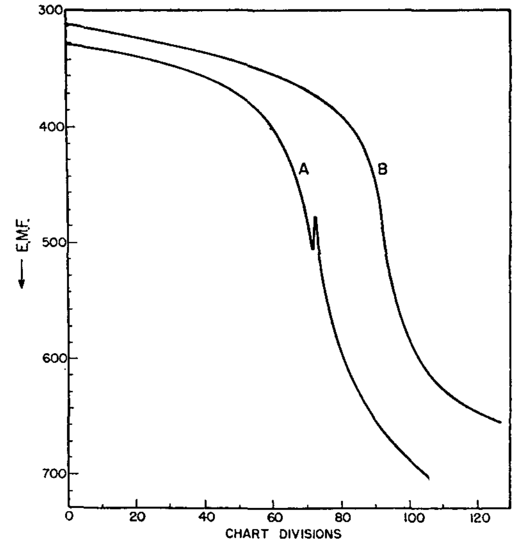

Figure 2. Continuous Automatic Potentiometric Titration Curves

A. Silver–silver chloride electrode dipping directly into solution being titrated. Sample, quinoline
B. Silver–silver chloride electrode placed in isolation cell. Sample, coal hydrogenation distillate oil

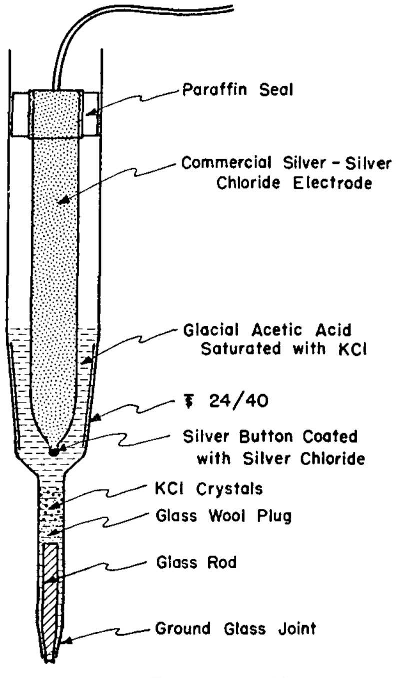

Figure 3. Reference Electrode Isolation Cell

By isolation of the silver–silver chloride electrode in a separate compartment, which makes electrical contact with the solution being titrated by means of a liquid-liquid junction through a ground-glass sleeve (see Figure 3), the reversal in the e.m.f. curve is eliminated and a normal titration curve is obtained (see curve *B*, Figure 2). The silver–silver chloride electrode used is one that is readily available commercially. The liquid in the isolation compartment is the same solvent as used for the sample—i.e., glacial acetic acid—but it is saturated with potassium chloride to increase its conductivity.

So far approximately 100 continuous automatic titrations have been made without difficulty using the silver–silver chloride electrode isolated in the separate compartment. The observed e.m.f.'s are essentially reproducible and thus permit the shutting-off of the automatic titration apparatus by means of a relay operated by the Brown recorder-controller.

ACKNOWLEDGMENT

The writer wishes to thank Joy S. Wolfarth for making the titration with the Macbeth titration-pH meter.

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Photometric Determination of Copper in Gasoline

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Copper appears in gasoline in very minute quantities, usually less than 0.5 mg. per liter and, because of its pro-oxidant effect, is detrimental to gasoline stability. In order to handle the problem of copper contamination of gasolines intelligently, it is necessary to be able to measure accurately the amount of copper present.

A review of the literature reveals that only a few methods deal with the determination of copper in gasoline. A qualitative test was described several years ago (*4*). Another method (*5*), designed for the estimation of copper in gasoline by visual matching of yellow copper diethyldithiocarbamate solutions, was adapted later (*2*) for use with a photoelectric colorimeter but is not sufficiently sensitive or accurate for many purposes. This appears to be due principally to incomplete extraction of the copper from the gasoline and the high transmittancy range at which measurements are made.

A rapid photometric method for the quantitative determination of copper in gasoline has been developed that is applicable to concentrations of 0.025 to 5 mg. of copper per liter of gasoline with an accuracy within 10% when using a 200-ml. sample of gasoline.

It was found during the development of this method that the normality of the hydrochloric acid solution had a considerable effect on the completeness of the extraction of the copper from the gasoline. It was found that 0.1*N* acid removed the copper much more readily than the 4*N* acid recommended in earlier methods. The use of multiple extractions also improved the recovery of the copper. Furthermore, if the transmittance measurements were made on solutions of the copper complex in an organic solvent such as carbon tetrachloride, as suggested by Sandell (*3*), more consistent results were obtained. The use of chloroform to remove residual color from the alkaline solution of copper is an improvement over the use of the previously recommended *tert*-amyl alcohol, as chloroform does a more effective job. Throughout the method the transferring of the solutions containing the copper from one container to another is minimized in order to reduce the possibility of the loss of copper.

APPARATUS

The transmittance measurements were made with a Lumetron Model 402-E photoelectric colorimeter equipped as follows:

Picture projection lamp, G.E. T-8, 100-watt.

Filter, monochromatic, narrow band, No. 440 (transmittance peak = 440 mμ).

Absorption cell, 50 mm., 70-ml. capacity. (Any change in light path from the 50 mm. used here will result in changes in precision and accuracy.)

REAGENTS

Hydrochloric acid, 0.1*N*. Dilute 8.3 ml. of concentrated c.p. hydrochloric acid (37%) to 1000 ml. with copper-free water, double-distilled from glass distillation apparatus.

Sodium diethyldithiocarbamate solution. Dissolve 1 gram of sodium diethyldithiocarbamate in copper-free water, double-distilled from glass distillation apparatus, and dilute to 1000 ml.

Standard copper solution for preparing the standard curve (1 ml. = 0.005 mg. of copper). Dissolve 1.9645 grams of c.p. copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), which contains 0.50 gram of copper, in copper-free water, double-distilled from glass distillation apparatus, and dilute to 1000 ml. Dilute a 10-ml. aliquot of this solution to 1000 ml.

Ammonium hydroxide, concentrated, c.p. (28% minimum).

Chloroform, c.p.

Carbon tetrachloride, c.p.

PROCEDURE

Extraction with Acid. Pipet 200 ml. of the gasoline to be tested into a 500-ml. separatory funnel and add 30 ml. of 0.1*N* hydrochloric acid. Shake the solution vigorously for 3 minutes,

Table I. Recovery of Copper Added to Iso-octane

Copper[a], Mg.	
Added to 200 ml. of iso-octane[b]	Found in final 100 ml. of of CCl_4 solution
...	...
...	...
0.005	0.005
0.005	0.005
0.025	0.025
0.025	0.025
0.050	0.051
0.050	0.050
0.050	0.050
0.050	0.051
0.075	0.074
0.075	0.074

[a] Copper-2-ethyl hexoate ($C_{16}H_{30}O_4Cu$), assay 17.46% copper, dissolved in acetone to obtain gasoline-soluble copper standard.
[b] Iso-octane (2,2,4-trimethyl pentane) treated with three 30-ml. portions 0.1*N* HCl before copper addition.

Table II. Precision

	Operator A					Operator B				
	% *T*	Absorbance	Minus absorbance of blank	Mg. copper/ 100 ml. CCl_4	Mg. copper/ liter	% *T*	Absorbance	Minus absorbance of blank	Mg. copper/ 100 ml. CCl_4	Mg. copper/ liter
Blank	95.8	0.019	...	...	...	97.2	0.012	...	...	...
	95.8	0.019	...	...	...	97.2	0.012	...	...	...
Sample (gasoline contaminated with copper by addition of copper-2-ethyl hexoate to 3 liters)	42.1	0.376	0.357	0.0405	0.203	42.4	0.373	0.361	0.0408	0.204
	42.6	0.371	0.352	0.0398	0.199	41.9	0.378	0.366	0.0414	0.207
	42.2	0.375	0.356	0.0403	0.202	42.5	0.372	0.360	0.0407	0.204
	42.0	0.377	0.358	0.0407	0.204	42.2	0.375	0.363	0.0411	0.206
	41.9	0.378	0.359	0.0408	0.204	42.5	0.372	0.360	0.0407	0.204
	41.7	0.380	0.361	0.0410	0.205	42.3	0.374	0.362	0.0409	0.205
Average	42.08	...	0.357	0.0405	0.203	42.3	...	0.362	0.0409	0.205
Repeatability	0.52	...	0.005	0.0007	0.004	0.4	...	0.004	0.0005	0.002
			Grand average, mg. copper/liter			0.204				
			Reproducibility, mg. copper/liter			0.005				

Table III. Analysis of Different Types of Commercial Gasolines

Sample	Copper Added (as Hexoate), Mg./Liter	Copper Found, Mg./Liter
Thermal reformed, gasoline A	...	0.02
	0.20	0.22
Catalytically cracked, gasoline B	...	0.02
	0.20	0.22
Straight-run, gasoline C	...	0.01
	0.20	0.20
Aviation, gasoline D	...	0.01
	0.20	0.22

Table IV. Effect of Various Substances

Copper Taken, Mg./Liter	Material Added		Copper Found, Mg./Liter
	Description	Mg./ liter	
0.050	Sulfur (as dibutyldisulfide)	700[a]	0.050
0.050	Iron (as naphthenate)	1	0.050
0.050	Iron (as naphthenate)	2	0.023
0.050	Tetraethyllead	1315[b]	0.050
0.050	Tetraethyllead	2191[c]	0.051
0.050	*N*,*N'*-disalicylidene-1,2-diaminopropane	140	0.050
0.050	*N*-butyl-*p*-aminophenol	70	0.051
0.050	*N*,*N'*-di-*sec*-butyl-*p*-phenylenediamine	70	0.051
0.050	2,6-di-*tert*-butyl-4-methylphenol	350	0.050
0.050	Du Pont Oil Yellow N	70	0.050
0.050	Du Pont Oil Orange	70	0.050
0.050	Du Pont Oil Red	70	0.050
0.050	Du Pont Oil Blue A	70	0.050

[a] 0.10 weight %.
[b] 3 ml./gallon.
[c] 5 ml./gallon.

allow to settle, and drain the lower layer into a 500-ml. separatory funnel. Repeat the acid extraction for a total of three times.

Removal of Residual Color. Pipet 5 ml. of concentrated ammonium hydroxide into the second separatory funnel and swirl to mix. Remove any residual color in the alkaline solution by treating with successive 40-ml. portions of chloroform until the aqueous layer is colorless; then follow the chloroform extractions with one 40-ml. carbon tetrachloride wash. Discard the chloroform and carbon tetrachloride layers.

Development of Color. Pipet 10 ml. of sodium diethyldithiocarbamate solution into the separatory funnel and swirl to mix. Add 40 ml. of carbon tetrachloride and shake the solution vigorously for 2 minutes. Allow the solution to settle and drain the lower layer into a 100-ml. volumetric flask. Add another 40 ml. of carbon tetrachloride to the separatory funnel and shake the solution vigorously for 1 minute. Allow the solution to settle and drain the lower layer into the 100-ml. flask, make up to volume (100 ml.) with carbon tetrachloride, and mix the solution well. Fill a 70-ml. (50-mm.) absorption cell with solution from the flask and determine the per cent transmittance within 5 minutes (the solutions gradually became darker on standing in the open absorption cell, owing to evaporation of solvent) at a wave length of 440 mμ, the Lumetron having been balanced at 100% transmittance against carbon tetrachloride. If the yellow carbon tetrachloride solution is too dark in color and does not give a reading on the standard curve, dilute an aliquot of not less than 10 ml. with carbon tetrachloride and make up to 100 ml. If a satisfactory reading is not obtained by this means, repeat the analysis using a smaller sample of gasoline.

A blank should be run daily and with each new supply of reagents. Determine the blank by starting with 90 ml. of $0.1N$ hydrochloric acid and proceeding as outlined under "Development of Color." Convert the per cent transmittances obtained to absorbance (log $100/T$). Subtract the absorbance of the blank from the absorbance of the sample and read the milligrams of copper equivalent to the corrected absorbance from the standard curve.

Standard Curve. Prepare the standard curve by adding various volumes of the standard copper solution to 90 ml. of $0.1N$ hydrochloric acid and analyzing as outlined above. Then plot the absorbance against milligrams of copper in the final 100 ml. of carbon tetrachloride.

Concentration Range and Accuracy. Inspection of a standard curve indicates that the system conforms to Beer's law since the standard curve is a straight line. When per cent absorbtancy is plotted against logarithm of concentration (*1*), the concentration range for best accuracy for the conditions and technique used is 0.027 to 0.074 mg. of copper in the final 100 ml. of carbon tetrachloride; in this range the relative analysis error is 3% per 1% absolute error in transmittancy. The relative analysis error will not exceed 10% in the concentration range of 0.005 to 0.098 mg. of copper in the final 100 ml. of carbon tetrachloride.

Precision. The expected limit of deviation of test results from their mean value based on data in Table II is:

	Repeatability	Reproducibility
Mg. copper in final 100 ml. CCl_4	0.0008	0.0012
Mg. copper per liter (200-ml. sample)	0.004	0.006
% transmittance	0.5-0.6	...

Statistical analysis of the data obtained in establishing precision shows the standard deviation to be 0.0025 mg. of copper per liter.

This method is satisfactory for the analysis of different types of commercial gasolines as is illustrated in Table III.

Sulfur up to at least 0.1 weight % as dibutyl disulfide and tetraethyllead up to at least 5 ml. per gallon do not interfere in this method. Iron naphthenate at 1 mg. per liter of iron did not interfere in the method, but at 2 mg. per liter it gave high results. Gasoline additives (such as antioxidants, deactivators, and dyes) at normal dosages also do not interfere (Table IV).

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Water Vapor Saturation in Gas-Measuring Burets

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IN THE common volumetric or Orsat analysis of gas mixtures, in which the various components are determined by successive absorption and/or combustion, the gas sample must have the same percentage of water vapor present each time it is measured if the results are to be correct. The most convenient way to accomplish this is to keep the gas sample saturated with water vapor. In the commonly used procedure, saturation of the gas sample is obtained by keeping a few drops of water on the surface of the mercury used as the confining fluid. This water is spread in a thin film over the buret walls as the mercury level is lowered. If a salt solution were used as a confining fluid, water vapor would be available from the solution at a constant, but reduced, partial pressure. Although it has been recognized that saturation of the gas sample may take considerable time, no figures or estimates are available. The question of how long it takes to saturate the gas is discussed in this paper.

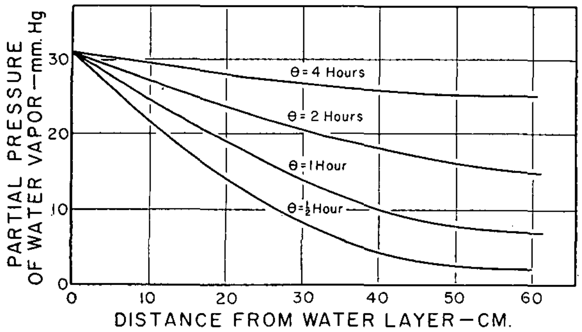

Figure 1. Partial Pressure of Water Vapor in Buret When Sides Are Not Wetted

An example will show the importance of saturating the gas before volume measurements are made.

Consider a typical gas analysis. A 70-cc. sample of dry gas containing 10% carbon dioxide is drawn into a measuring buret over mercury covered with a few drops of water. The water jacket is kept at 25° C. If the gas sample were to be completely saturated with water vapor it would contain (23.7/760)(100) = 3.1% water vapor and the measured volume would equal (70/0.969) = 72.3 cc. However, if the gas were only 50% saturated, it would contain 1.55% water vapor and a volume of (70/0.9845) = 71.1 cc. would be measured, introducing an error of (72.3 − 71.1) = 1.2 cc. When the carbon dioxide in the gas sample is absorbed in dilute base, the residual gas which is returned to the buret, saturated with water vapor (neglecting, for purpose of the example, the decrease in vapor pressure of water in caustic solutions), has a volume equal to (63/.969) = 65.1 cc.; thus the carbon dioxide percentage reported would be (71.1 − 65.1)/(71.1) × (100) = 8.4% when the correct percentage equals (72.3 − 65.1)/(72.3) × (100) = 10%.

Calculations are given in this paper to show that a clean buret whose walls are covered with a thin film of water will saturate the gas very rapidly, whereas in a buret whose sides are not wetted by water it is virtually impossible to saturate the gas in any reasonable length of time.

Considering the latter case first, a buret of length L has a layer of water in the bottom. It is desired to calculate the partial pressure of water vapor along the length of the buret at different time intervals, assuming the gas is stagnant and all mass transfer occurs by molecular diffusion. Let x represent the distance up the buret from the liquid water, P the partial pressure of water vapor, and θ the time. Then by Fick's second law of diffusion

$$\frac{1}{D}\frac{\partial P}{\partial \theta} = \partial^2 P/\partial x^2 \tag{1}$$

where D = diffusivity of water vapor into air, set $\Delta = (P - P_o)/(P_i - P_o)$, and the boundary conditions then become

$$\Delta = 1, \theta = 0$$
$$\Delta = 0, \theta = \infty$$
$$\Delta = 0, x = 0$$
$$\Delta = 0, x = 2L$$

Where

P = partial pressure of water vapor at any value of x at any time θ
P_o = vapor pressure of water in water layer
P_i = initial partial pressure of water vapor at $L = 0$

the solution is of the general form

$$\Delta = \frac{4}{\pi}\sum_{1}^{\infty}\frac{1}{n}e^{-\frac{Dn^2\pi^2\theta}{4L^2}}(\sin nx/2L) \tag{2}$$

$$n = 1, 3, 5 \ldots\ldots$$

The time required to reach saturation in the case of a buret whose walls are covered with a film of water is found to be considerably less.

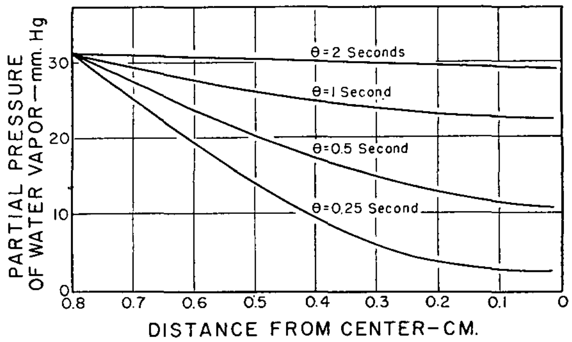

Figure 2. Partial Pressure of Water Vapor in Buret When Sides Are Wetted

If a buret whose radius is R has a film of water on its sides, then the relation between the radius, R, time θ, and the partial pressure of the water vapor, P, is:

$$\frac{\partial^2 P}{\partial r^2} + \frac{1}{r}\frac{\partial P}{\partial r} = \frac{1}{D}\frac{\partial P}{\partial \theta} \tag{3}$$

Inserting the dimensionless parameter Δ as before, with the boundary conditions:

$$\Delta = 0, \theta = \infty, r \leqq R$$
$$\Delta = 1, \theta = 0, \quad r < R$$
$$\Delta = 0, \theta = \theta, \quad r = R$$

the solution is of the form,

$$\Delta = \frac{2}{R}\sum_{1}^{\infty}\frac{e^{-k_n^2 D\theta}J_0(k_n r)}{k_n J_1(k_n R)} \tag{4}$$

where k_n is defined by the roots of the Bessel function

$$J_0(k_n R) = 0 \tag{5}$$

A typical example has been solved using these equations. A buret 61 cm. in length and 16 mm. in diameter is considered to be filled initially with dry air. The water available to saturate this air is at 30° C. where the vapor pressure, P_o, equals 31.8 mm. of mercury and the diffusion coefficient of water vapor into air, D, equals 0.2 sq. cm. per second.

Figure 1 illustrates the case where the sides are not wetted and water is present only at the base of the buret, and Figure 2 shows the other extreme where the sides of the buret are covered by a film of water. A time of 2 to 3 seconds suffices to saturate the air in the clean buret, whereas in the buret whose sides are not wetted, the air is not saturated even after 4 hours.

Because most burets become dirty or nonwettable because of a layer of oil or grease on the glass, it is suggested that the water used to saturate the gas contain a small amount of some neutral detergent. This procedure should keep burets cleaner and consequently decrease any analytical error from insufficient water vapor saturation.

The calculations presented in this paper are valid only for the limiting cases where all water vapor is transported through the gas in the buret by molecular diffusion. In any actual case, the gas entering the buret creates a large amount of turbulence which will aid in equalizing any water vapor pressure gradients. Thus the time required for saturation will be less than calculated; the amount less will depend on the actual case under consideration.

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Spectrophotometric Determination of Copper in Ores with 2,2′-Bipyridine

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Blau (*1*) described the properties of 2,2′-bipyridine (2,2′-bipyridyl) along with its method of preparation. Tartarini (*10*) in discussing new color reactions involving cuprous salts reported that it forms a cupric complex which can be reduced with hydroxylamine in ammoniacal solution to give a highly colored cuprous complex. Moss and Mellon (*8*) developed a colorimetric method for the determination of iron with 2,2′-bipyridine and Mehlig and Shepherd (*7*) applied 2,2′-bipyridine to the spectrophotometric determination of iron in ores. In a study of the 1,10-phenanthroline–cuprous system Moss and Mellon (*9*) stated that 2,2′-bipyridine, which contains the same cyclic —N—C—C—N— grouping, is not as sensitive as 1,10-phenanthroline in the formation of a colored cuprous complex, but made no copper determinations with it.

The purpose of the work described in this paper was to obtain further proof that macro constituents may be satisfactorily determined spectrophotometrically by application of 2,2′-bipyridine to the determination of copper in ores.

APPARATUS AND SOLUTIONS

All spectrophotometric measurements were made with a Beckman Model B spectrophotometer.

2,2′-Bipyridine. A solution made by dissolving 1 gram in 0.2*M* hydrochloric acid and diluting to 1000 ml. with distilled water.

Hydroxylamine Hydrochloride. An aqueous solution containing 10 grams per 100 ml.

Methyl Carbitol (Diethylene Glycol Monomethyl Ether). Commercial grade.

Standard Copper Solution. One gram of electrolytically pure copper pellets was dissolved in 10 ml. of concentrated hydrochloric acid and 5 ml. of concentrated nitric acid and the solution was transferred to a 1000-ml. volumetric flask. The solution was neutralized with 6*M* ammonium hydroxide until the first indication of the formation of the blue cupric ammonia complex, diluted to the mark at 20° C. with distilled water, and thoroughly shaken. By means of a microburet 5 ml. of this solution were transferred to a 100-ml. volumetric flask, diluted to the mark at 20° C. with distilled water, and thoroughly shaken. Each milliliter of this solution contained 0.05 mg. of copper.

THE COLOR REACTION

To produce the color system the volume of the standard copper solution required to give the desired concentration of copper was measured with a microburet into a 50-ml. volumetric flask. Then, in order, were added 2 ml. of 6*M* ammonium hydroxide to form the cupric-ammonia complex, 10 ml. of 2,2′-bipyridine solution, 1 ml. of hydroxylamine hydrochloride solution to reduce the copper to the cuprous state, and 20 ml. of methyl carbitol as a stabilizer. The mixture was diluted to the mark at 20° C. with distilled water and thoroughly shaken. The orange-brown color developed immediately. All transmittance measurements were made with a 1-cm. Corex glass cell at a wave length of 430 mμ, the wave length of maximum absorption, after adjustment of the instrument so that the transmittance of the blank solvent containing the reagents was 100%.

That Beer's law is obeyed by the color system was proved by the straight line which resulted when the extinctions for six solutions containing 1, 2, 3, 4, 5, and 6 mg. of copper per liter were plotted against the respective concentrations. Above 6 mg. per liter the line began to curve.

Table I. Results Obtained with 2,2′-Bipyridine

Sample No.	Nature	Copper by Iodide Method, %	Copper by 2,2′-Bipyridine Method, %	Difference, %	Error, %
1	Ore	10.43	10.48	+0.05	+0.48
2	Ore	11.16	11.16	0.00	0.00
3	Ore	12.04	12.00	−0.04	−0.33
4	Ore	20.33	20.37	+0.04	+0.20
5	Ore	19.40	19.31	−0.09	−0.46
6	Ore	18.63	18.61	−0.02	−0.11
7	Oxide	15.02	15.05	+0.03	+0.20
8	Oxide	14.00	14.03	+0.03	+0.21
9	Oxide	13.23	13.22	−0.01	−0.08
10	Oxide	22.31	22.30	−0.01	−0.05
11	Matte	21.61	21.63	+0.02	+0.09
12	Matte	14.09	14.02	−0.07	−0.50

DETERMINATION OF COPPER IN ORES

An accurately weighed sample of ore, varying from 0.1 to 0.2 gram depending upon the copper content, was heated with a mixture of 10 ml. of concentrated hydrochloric acid and 5 ml. of concentrated nitric acid until solution was complete or only a white siliceous residue remained. Iron was removed by double precipitation with 15*M* ammonium hydroxide. The filtrate in a 1000-ml. volumetric flask was acidified with 6*M* hydrochloric acid, then neutralized with 6*M* ammonium hydroxide to the first appearance of the cupric ammonia complex, diluted to the mark at 20° C. with distilled water, and thoroughly shaken.

An aliquot of 4 ml. of this solution was measured with a microburet into a 50-ml. volumetric flask and the procedure from this point for the determination of the transmittancy at 430 mμ was the same as that described above. The percentage of copper was calculated by use of the extinction coefficient, which had been determined by obtaining the transmittancy at 430 mμ of a series of solutions containing 1, 2, 3, 4, 5, and 6 mg. of copper per liter.

RESULTS

The method was applied to the determination of copper in six ores, four oxides, and two mattes. Duplicate determinations were made for each sample and at least two aliquots were analyzed for each duplicate. The results are shown in Table I along with the values obtained by the iodide titrimetric method (*2*).

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Table II. Effect of Diverse Ions

Ion	Added as	Concn., Mg./Liter	Effect	Approx. Limiting Concn., Mg./Liter
Aluminum	$Al_2(SO_4)_3$	500	Precipitate	0
Ammonium	NH_4Cl	500	Negligible	...
Bismuth	$Bi(NO_3)_3$	20	Precipitate	15
Cadmium	$CdCl_2$	25	Fading	20
Chromic	$CrCl_3$	25	Change in hue	20
Cobaltous	$CoCl_2$	20	Change in hue	5
Ferric	$FeCl_3$	20	Change in hue	0
Lead	$Pb(NO_3)_2$	200	Precipitate	100
Nickelous	$Ni(NO_3)_2$	20	Change in hue	5
Potassium	KNO_3	500	Negligible	...
Sodium	$NaNO_3$	500	Negligible	...
Zinc	$ZnSO_4$	20	Fading	10
Chloride	NH_4Cl	500	Negligible	...
Cyanide	KCN	5	Fading	0
Nitrate	$NaNO_3$	500	Negligible	...
Oxalate	$K_2C_2O_4$	500	Fading	300
Sulfate	Na_2SO_4	500	Negligible	...
Thiosulfate	$Na_2S_2O_3$	10	Fading	5

In no case was the difference between the two methods greater than ±0.09% and the average difference was 0.03%. The percentage error ranged from −0.50 to +0.48% with an average of −0.03%. Results were duplicated on the same sample with a precision of ±0.01 to ±0.11%. The results also checked closely with those of the ammonia (*3*), triethanolamine (*5*), and 1,10-phenanthroline (*6*) spectrophotometric methods, giving ample proof that the method can be applied to ores containing at least as much as 22% of copper.

DISCUSSION

Order of Addition of Reagents. Just as in the determination of copper with 1,10-phenanthroline (*6, 9*), it is very important that the reagents be added in the given order. The cupric-ammonia complex must first be formed and then be reduced in the presence of 2,2′-bipyridine by the hydroxylamine hydrochloride. A large excess of the bipyridine has no harmful effect.

Adjustment of pH Value. The ammonia concentration is an important factor, easily controlled by adding 2 ml. of 6*M* ammonium hydroxide to the aliquot taken for analysis. An excess must be avoided, as it has been found (*4, 11*) that ammonia solutions show an appreciable absorption of light in the visible region. No particular advantage is gained by buffering with ammonium acetate.

Stability. The color system is stabilized for 30 minutes by the addition of methyl carbitol.

Sensitivity. Although 2,2′-bipyridine is a more sensitive reagent toward copper than are ammonia and triethanolamine, it is less sensitive than 1,10-phenanthroline.

Effect of Diverse Ions. In an interference study of 18 of the more common diverse ions, transmittancy measurements were made at 430 mμ on solutions containing 1 mg. of copper per liter and varying concentrations of the ion in question up to a maximum of 500 mg. per liter. Unless the added ion caused a variation of more than 0.1% in the transmittancy, it was assumed that there was no interference. The results which are listed in Table II are similar to those obtained by Moss and Mellon in their studies of the 1,10-phenanthroline–copper system (*9*) and the 2,2′-dipyridine–iron system (*8*). Cadmium, chromic, cobaltous, ferric, nickelous, and zinc ions interfere, apparently by complex formation with the reagent. Of the cations which precipitate in ammoniacal solution and are therefore removed in the course of the procedure, only iron is normally found in copper ores. Cyanide and thiosulfate are the only common anions which interfere.

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Europium Determination in Rare Earth Mixtures

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SMALL amounts of europium are present in all rare earth ores, and partially separated rare earth mixtures will contain amounts of europium varying from traces to several per cent. Because of interference from other rare earth absorption bands, europium concentrates cannot be analyzed conveniently by spectrophotometric methods. McCoy (*2*) determined europium by passing a sample of europium chloride solution through a Jones reductor into an excess of standard iodine in a carbon dioxide atmosphere and titrated the excess iodine with standard sodium thiosulfate solution. This procedure is satisfactory for samples containing fairly high europium concentrations, but it has not been found satisfactory in this laboratory for samples of low europium concentrations.

This investigation has resulted in the development of a method which yields satisfactory results at nearly any concentration of europium, making possible a rapid europium determination at any stage of rare earth purification. The method consists of passing a rare earth chloride solution containing the europium through a Jones reductor into an excess of ferric chloride. An amount of ferric chloride equivalent to the amount of europous chloride formed by reduction is reduced to ferrous chloride, and the latter is titrated with standard potassium dichromate solution. There is no interference from other rare earths such as samarium and ytterbium which can exist in the divalent state, as these rare earths are not reduced by zinc.

REAGENTS

Standard potassium dichromate, 0.04*N*.

Ferric chloride, ca. 0.04*N*, containing just enough hydrochloric acid to prevent hydrolysis.

Hydrochloric acid, ca. 0.05*N*.

Sodium diphenylamine-*p*-sulfonate, 0.3% aqueous solution.

Amalgamated zinc is prepared by stirring 300 grams of reagent grade 20-mesh zinc with 300 ml. 2% mercuric nitrate solution containing 2 ml. of concentrated nitric acid. The amalgamated zinc is washed well with water by decantation, and is used to fill a Jones reductor 2 cm. in diameter and 23 cm. high.

PROCEDURE

The sample size must be varied according to the concentration of europium in the rare earth mixture in order to obtain a suitable titration for a microburet. Suitable amounts of europium are in the range 0.1 to 0.4 meq., which will require 2.5 to 10 ml. of 0.04*N* potassium dichromate.

The rare earth sample must be purified to remove materials reducible by zinc. This is done by oxalate precipitation of the rare earths and ignition of the oxalate to oxide.

Dissolve the sample of rare earth oxide in hydrochloric acid, evaporate to a sirup under an infrared lamp to remove excess acid, and dilute to about 20 ml. with distilled water. Flush the Jones reductor column with 150 ml. of 0.05*N* hydrochloric acid. Add 20 ml. of 0.02*N* ferric chloride solution to a 500-ml. Erlenmeyer flask. Attach the flask to the Jones reductor with a three-holed rubber stopper fitted with a glass tube for introducing carbon dioxide gas below the liquid level in the flask. Bubble carbon dioxide through the solution in the flask for 7 minutes to remove air. Add the 20-ml. sample solution to the top of the reductor, and follow with 150 ml. of 0.05*N* hydrochloric acid wash solution; 10 minutes are required to pass the sample and wash solution through the reductor. Remove the flask, add 4 ml. of concentrated hydrochloric acid, 5 ml. of 85% phosphoric acid, and 3 drops of 0.3% sodium diphenylamine-*p*-sulfonate solution, and titrate with standard 0.04*N* potassium dichromate solution.

DISCUSSION

Kolthoff (*1*) states that if ferrous iron is titrated in the presence of hydrochloric acid with diphenylamine compounds as indicators, the end point is poor because the indicator is oxidized before the end point is reached. The addition of phosphoric acid lowers the oxidation potential of the ferric-ferrous iron system by forming a complex ferric phosphate ion, thereby avoiding premature oxidation of the indicator. By trial and error, the quantities of phosphoric acid and hydrochloric acid used were found to give a very sharp end point.

The method is designed as a control method for europium purification techniques. The wide range of the method and its reliability are shown by the following typical experiment.

A sample of samarium acetate analyzed by the method was found to contain 2.21% Eu_2O_3 in the rare earth oxide. A solution of 315 grams of the samarium acetate containing 159.7 grams of rare earth oxide was extracted nine times with liquid sodium amalgam to separate europium by the method of Marsh (*4*). The rare earths from the extracts and residue were recovered by precipitation as oxalates, and analyzed for europium.

Table I. Separation of Europium from Samarium

Extract	Rare Earth Oxide, Grams: In extract	Rare Earth Oxide, Grams: Sample for analysis	0.0424*N* $K_2Cr_2O_7$, Ml.	Eu_2O_3 in Rare Earth Oxide, %	Eu_2O_3 in Extract, Grams
1	2.40	0.1419	13.80	72.55	1.74
2	1.90	0.1814	13.77	56.62	1.10
3	1.19	0.1775	4.83	20.30	0.24
4	5.27	0.4621	0.61	0.98	0.05
5	1.80	0.1872	0.12	0.48	0.008
6	3.56	0.2551	0.12	0.35	0.012
7	30.65	0.4509	0.05	0.08	0.024
8	2.03	0.1716	0.07	0.30	0.006
9	3.40	0.2719	Nil	Nil	Nil
Residue	98.00				
Total Recovered	150.20				3.18

Not all of the rare earths taken were recovered, because of unavoidable incomplete precipitation of rare earth oxalate and mechanical losses in the extractions. The data are given in Table I.

The precision of the method is adequate. With rare earth oxide samples containing 2 to 3% Eu_2O_3, analyses can be duplicated to within 1% precision, and with essentially pure europium oxide the difference between duplicate samples is less than 0.5%.

The method was checked against pure europium oxide prepared by McCoy (*3*) and results were consistently 3.3 to 3.5% low. Europium materials prepared in this laboratory known to contain a maximum of 0.2% impurities by spectroscopic tests also analyzed about 3% low. The cause of these low results could not be found, but this does not prevent the procedure from being a very useful tool for following a europium separation. It is possible to standardize the potassium dichromate solution empirically against pure europium compounds.

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Polarographic Analysis of Lead Driers

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LEAD soaps are widely used in the paint industry as driers, and numerous methods of determining the lead content of such materials have been reported. In general, these methods require a preliminary separation of the metallic ion from the organic acid, followed by the determination of the lead by one of the standard procedures. In the American Society for Testing Materials method (*1*), the sample is wet oxidized with nitric and sulfuric acids, followed by determination of the lead as sulfate. Gracis (*4*) recommends separation of the lead by hydrolysis of the soaps with nitric acid. The lead is then determined in the aqueous solution by titration with molybdate. Castiglioni (*2*) hydrolyzed lead naphthenates in acetic acid and then removed the naphthenic acid by extraction with ether. The lead was then determined as the chromate. Gavarret (*3*) analyzed lead driers by dissolving in benzene or trichloroethylene and extracting the lead with nitric acid. The lead was determined in the extract by the ferrocyanide method.

All of these procedures are time-consuming and tedious, and in the search for a simpler method the polarographic behavior of lead soaps dispersed in aqueous medium by means of a detergent was investigated. Preliminary experiments showed that most of the common lead driers could be readily suspended in aqueous dodecylamine acetate to give clear solutions. Such dispersions gave typical polarographic waves for lead, and it was found that the wave heights were directly related to the concentration of the lead in the driers. These experiments led to the development of a very simple and rapid procedure for the analysis of lead driers which does not require the preliminary isolation of the metallic ion from the organic substrate. This paper gives a detailed description of the method and the important variables affecting the results obtained by the procedure.

MATERIALS AND APPARATUS

Lead Soaps. Several lead driers were obtained from commercial sources. These included lead naphthenate, octoate, linoleate, and resinate from Frederic A. Stresen-Reuter, Inc., Chicago, Ill.; and lead naphthenate, octoate, and linoresinate from Advance

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Solvents and Chemical Corp., New York, N. Y. These were analyzed for lead content by the ASTM method (*1*) for liquid driers.

Dodecylamine Acetate, 0.1*M*. Solutions were prepared from dodecylamine or dodecylamine acetate obtained from Armour and Co., Chicago, Ill., under the trade names of Armeen 12D and Armac 12D, respectively. To prepare a 0.1*M* solution from the dodecylamine acetate, 25 grams of the solid were dissolved in hot water and made up to 1 liter. Solutions were also prepared from the dodecylamine by adding 19 grams of the reagent to 5.8 ml. of glacial acetic acid and diluting to 1 liter. The solutions were clear when first prepared; however, with standing a haze slowly developed. This did not appear to affect the results in any way.

Lead Acetate Solutions. A standard 0.02*M* lead acetate solution was prepared for calibration purposes by dissolving 6.5 grams of the reagent in water and diluting to 1 liter. This solution was analyzed for lead gravimetrically as lead sulfate and then used for preparation of more dilute standards by accurate dilutions with the dodecylamine acetate solution.

Apparatus. Most of the current-voltage measurements were made with a Fisher Elecdropode. The galvanometer scale was calibrated at the various sensitivities, and where half-wave potentials were to be determined, voltages were measured with an auxiliary potentiometer and corrected for the *IR* drop of the cell. A few curves were obtained with a Sargent Model XXI polarograph.

The polarographic cell was an H-type with a sintered-glass plate in the cross arm. One side of the cell was a saturated calomel electrode which was used as the reference electrode. The cell temperature was maintained at 25° ± 0.3° C.

Table I. Half-Wave Potentials for Reduction of Lead Soaps

(Solutions 0.1*M* in dodecylamine acetate)

Lead Compound	$E_{1/2}$ vs. Saturated Calomel Electrode
Lead acetate	−0.484
Lead naphthenate	−0.494
Lead octoate	−0.505
Lead linoleate	−0.497
Lead linoresinate	−0.484
Lead resinate	−0.484

EXPERIMENTAL

All of the driers investigated, with the exception of lead resinate, were rapidly dispersed in 0.1*M* solutions of dodecylamine acetate to give clear stable solutions with no undispersed residues. In the case of the lead resinate, dispersion was incomplete and small particles could be detected even after long agitation and heating. These particles, when removed by filtration, were found to contain lead, and it was concluded that polarographic analysis of this particular soap from detergent solutions would lead to low results. This was subsequently confirmed.

The solutions containing the dispersed driers were found to yield well-defined polarograms for lead. Current maxima were never encountered, and no suppressors were required in any of this work. The half-wave potentials for the lead wave varied slightly for the different soaps, as is illustrated in Table I. However, the potentials are the same as, or only slightly more negative than, the half-wave potential for lead acetate in 0.1*M* dodecylamine acetate solution. Furthermore, the slopes of the waves for lead soaps were found to be the same as for the lead acetate solutions. These data and observations indicate that the lead soaps are appreciably dissociated in the detergent media and that the dissociation equilibrium is rapidly attained.

An investigation of the effect of detergent concentrations on the lead polarograms was undertaken. It was found that sufficient amounts of the driers for analytical purposes could be dispersed even in 0.005*M* solutions of the dodecylamine acetate. However, the more dilute detergent solutions gave high values for limiting currents because insufficient electrolyte was present to eliminate the migration current. This is shown in Table II. Constant currents are obtained for detergent concentrations above 0.05*M*. Attempts were made to reduce the migration current in the more dilute detergent solutions by addition of such electrolytes as sodium acetate and potassium nitrate. However, coagulation of the dispersed soaps occurred and these experiments were abandoned.

The diffusion currents for lead from 0.1*M* detergent solutions were found to be directly proportional to the lead concentration; the proportionality constant was the same for lead acetate and the various lead soaps investigated with the exception of the lead resinate. This is illustrated in Table III, where the values for the diffusion current over concentration for lead acetate and lead naphthenate are compared at different lead concentrations. Similar values were found with the other soaps studied. Thus, standard solutions of lead acetate can be used for calibration purposes for the analysis of any of the common lead driers.

RECOMMENDED PROCEDURE

Weigh out samples of 0.05 to 0.1 gram into 50-ml. glass-stoppered Erlenmeyer flasks. Measure exactly 25 ml. of the 0.1*M* dodecylamine acetate into the flask with a pipet, and shake the mixture vigorously until dispersion is complete and a clear solution is obtained. Rinse the polarographic cell with this solution and finally fill the cell. Remove the oxygen from the solution by bubbling with nitrogen. During this process, foaming will occur and some of the solution may be lost. However, this does not alter the results in any way. After the oxygen has been removed and the solution brought to constant temperature, a polarogram is obtained between −0.2 and −1.0 volt versus the saturated calomel electrode. The height of the lead wave at −0.5 volt is determined and blank correction is made by running a solution of 0.1*M* dodecylamine acetate under the same conditions. The lead concentration in the solution is calculated from the diffusion current by reference to calibration data obtained with a standard solution of lead acetate in 0.1*M* dodecylamine acetate or from samples of driers of known lead concentration which have been treated by the above procedure. The diffusion current is directly proportional to the concentration of lead.

RESULTS AND DISCUSSION

A number of commercial driers were analyzed by the above procedure and the results are given in Table IV. The data are compared with results by the American Society for Testing Materials procedure (*1*) involving wet ashing and gravimetric estimation of the lead as lead sulfate. The results compare favorably in every case but the lead resinate. This material could not be completely dispersed in the medium and the method cannot be applied satisfactorily to this type of sample.

The main advantage of the procedure lies in its simplicity and speed. A single analysis can be completed in 20 minutes and when

Table II. Effect of Dodecylamine Acetate Concentration on Limiting Currents for Lead

($2 \times 10^{-3}M$ solutions of Pb^{++})

Dodecylamine Acetate Concn., *M*	Limiting Current, μa.
0.005	20.5
0.010	18.7
0.050	16.9
0.080	16.7
0.090	16.6
0.100	16.7

Table III. Relationship between Diffusion Current and Concentration for Lead Acetate and Lead Naphthenate in Dodecylamine Acetate Solutions

Lead Acetate		Lead Naphthenate	
Lead concn., *C*, millimoles/liter	i_d/C	Lead concn., *C*, millimoles/liter	i_d/C
0.99	8.29	2.01	8.50
1.98	8.34	4.23	8.25
3.96	8.21	5.73	8.21
4.95	8.38	7.45	8.37
7.92	8.40	8.53	8.49
9.90	8.47	10.08	8.53
		10.44	8.34
Av.	8.35		8.38

Table IV. Analysis of Lead Driers

Type of Drier	Lead Analysis, %		Deviation from Gravimetric Method, Rel. %
	Polarographic	Gravimetric	
Naphthenate	24.6	24.8	−0.8
	23.5	23.8	−1.3
	23.8	23.7	+0.4
	17.1	16.9	+1.2
Octoate	23.4	23.4	0.0
	23.5	23.3	+0.9
Linoresinate	24.6	24.6	+0.0
Linoleate	27.9	27.7	+0.7
Resinate	19.6	21.4	−8.4

several analyses are to be done, an even smaller time per sample is needed. The procedure should be particularly useful in the control of dilute driers (4 to 6% lead) where the errors of the method would not be serious.

The naphthenates of several other metals were investigated polarographically to determine whether this technique might not have wider application. These included iron, cobalt, manganese, copper, and zinc naphthenates. Although a wide variety of conditions was tried, only copper gave a satisfactory wave. Apparently the remainder are so slightly dissociated in aqueous suspensions that no reduction occurs below the decomposition potential for the supporting electrolyte. Further work on the polarographic analysis of copper soaps is now in progress and the results of this work will be published subsequently.

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Calculation of Amount of Tracer Carried with Precipitates of Its Radioactive Parent

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IN A recent note Kirby (*1*) showed how to calculate the proportion of measured radioactivity due to each nuclide, when genetically related radioisotopes are employed to follow a chemical reaction.

A similar type of reasoning can be used to study carrying of genetically related radioisotopes when they are in equilibrium before fractionation by precipitation.

Consider the case of the following radioactive disintegration:

$$E_1 \rightarrow E_2 \rightarrow E_3 \rightarrow$$

Suppose that a suitable absorber is used to screen out all radiations except that from the first decay product, E_2. The activity of nuclide E_2 at time t is (*3*):

$$A_2 = A_1^0\, m(e^{-\lambda_1 t} - e^{-\lambda_2 t}) + A_2^0\, e^{-\lambda_2 t} \qquad (1)$$

where

A_1^0 = activity of the parent at zero time
A_2^0 = activity of the daughter at zero time
λ_1 and λ_2 = decay constants of parent and daughter, respectively
$m = \lambda_2/(\lambda_2 - \lambda_1)$

"Zero time" is taken as the time when separation is complete—i.e., the precipitate is no longer in contact with the solution

Suppose that λ_1 is much smaller than λ_2 and that E_1 and E_2 are in secular equilibrium and consequently $A_2 = A_1$, where A_1 is the activity of the parent at time t. Suppose that element E_1 is precipitated and that some E_2 is entrained with the precipitate. We wish to calculate the amount of E_2 carried with E_1. Rather than measure the activity of E_2 before and after precipitation, we shall use Equation 1 and measure the activity of the precipitate only. Since $A_2/A_1 = 1$ (or is equal to m if equilibrium is not secular) before precipitation, the deviation from unity (or from m), of A_2/A_1, measured in the precipitate, will indicate the percentage of E_2 carried with E_1. We shall see that it is not necessary to measure A_1; it is sufficient to measure A_2 at two different times.

Equation 1 can be written alternatively as

$$A_2 = A_1^0\, [m(e^{-\lambda_1 t} - e^{-\lambda_2 t}) + e^{-\lambda_2 t}\, A_2^0/A_1^0] \qquad (2)$$

Defining $A_2^0/A_1^0 = a$ as "entrainment," we can write

$$A_2 = A_1^0\, [m(e^{-\lambda_1 t} - e^{-\lambda_2 t}) + a\, e^{-\lambda_2 t}] \qquad (3)$$

Since, from now on, only the activity of element E_2 will be considered, we shall use A as meaning the activity, A_2, measured at time t after separation of precipitate and filtrate. Measurement of A at two different times t' and t''—i.e., A' and A''—and substitution in Equation 3 will give two equations and two unknowns, A_1^0 and a. Solving for the entrainment a, one gets:

$$a = m\left[1 - \frac{A'e^{-\lambda_1 t''} - A''e^{-\lambda_1 t'}}{A'e^{-\lambda_2 t''} - A''e^{-\lambda_2 t'}}\right] \qquad (4)$$

In a special case where $\lambda_2 \gg \lambda_1$, $m \cong 1$; and if t is sufficiently small so that $-e^{-\lambda_1 t}$ is very nearly equal to unity, for the times ts of measurement, Equation 4 will simplify to

$$a = 1 - \frac{A' - A''}{A'e^{-\lambda_2 t''} - A''e^{-\lambda_2 t'}} \qquad (5)$$

In fact, absolute activities are not measured, and all values for A should be multiplied by a constant of proportionality; however, since counting of a sample, at two different times, is done under the same conditions the constant of proportionality will cancel out.

Kirby (*2*) called attention to the fact that the condition of equilibrium before fractionation or precipitation is not stringent and that the ratio of parent to daughter in a nonequilibrium mixture could be calculated with the same Formula 4 by counting an unfractionated aliquot of the mixture. Also, he showed (*2*) that Equation 4 could be applied when the parent had not been quantitatively precipitated or carried. In this last case the required algebraic transformations are:

$$A_2^0/A_1^0 = (A_2^0/A_1) \times (A_1/A_1^0) \qquad (6)$$

where A_1 is the total activity of parent at the time of fractionation. A_1/A_1^0 is calculated from Equation 8 of Kirby's work (*1*):

$$A_1/A_1^0 = \frac{\alpha' - \alpha''e^{-\lambda_2(t'' - t')}}{A'' - A'e^{-\lambda_2(t'' - t')}} \qquad (7)$$

where α' and α'' are the activities of an unfractionated aliquot of the mixture at times $(t' + \Delta t)$ and $(t'' + \Delta t)$, respectively; Δt is supposed to be very small compared with the half life of the parent. A_2^0/A_1^0 is calculated from Equation 4.

Equation 4 is being used to study carrying of bismuth at tracer level, with insoluble salts of lead; the precipitation of lead in the experimental case under study is complete and secular equilibrium existed before precipitation, so simplifications mentioned before are applied; the radioisotopes being used are Ra*D* and Ra*E*—i.e., lead-210 and bismuth-210.

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Copper–Ethylenediaminetetraacetic Acid Complex in Alkaline Solution

Qualitative Comparisons with Benedict's Reagent

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ETHYLENEDIAMINETETRAACETIC acid (ethylenebisiminodiacetic acid) and its salts have recently been studied by Pfeiffer and Offermann (*6*), Schwarzenbach, Biedermann and Bangerter (*9*), Klemm (*4*), and Schwarzenbach and Ackermann (*8*), as chelating agents for various cations, like cupric, zinc, calcium, magnesium, and nickel. Schwarzenbach and Ackermann (*8*) found the cupric ion complex very stable. Plumb, Martell, and Bersworth (*7*) reached the same conclusion by spectrophotometric studies; at high pH values there is an insignificant amount of copper ions in equilibrium with the complex. This chelating agent has the ability to chelate copper ion over a broad pH range.

It therefore was of interest to see how the cupric ion complex with ethylenediaminetetraacetic acid would act with various organic compounds (reducing and nonreducing) encountered in the biological chemistry laboratory, and to compare this action qualitatively with the action of these organic compounds on the Benedict qualitative reagent.

SOURCES OF CHEMICALS

The complexing agents used in this investigation were kindly furnished by the Bersworth Chemical Co. and the Alrose Chemical Co. Qualitatively, the reagents supplied by these companies gave similar results. The ethylenediaminetetraacetic acid supplied by the Alrose Chemical Co. is described as "technically pure."

All other reagents met ACS specifications.

PREPARATION OF REAGENTS

As it was planned to compare the action of a solution containing copper ethylenediaminetetraacetic acid with Benedict's qualitative reagent, the same quantities of copper sulfate and sodium carbonate were used in preparing both reagents. One such solution contained 100.0 grams of sodium carbonate, 17.3 grams of copper sulfate pentahydrate, 25.8 grams of the disodium salt of ethylenediaminetetraacetic acid, and water to make 1000.0 ml. The quantity of ethylenediaminetetraacetic acid used was that found by experiment to dissolve the copper carbonate precipitate. The pH of this solution was approximately 10.2, while that of Benedict's qualitative reagent was 10.1. All pH values were obtained with the Beckman Model H pH meter using a Type E glass electrode.

Solutions of copper ethylenediaminetetraacetic acid containing sodium hydroxide instead of sodium carbonate were prepared. The composition of one such solution was: 17.3 grams of copper sulfate pentahydrate, 25.8 grams of disodium ethylenediamine tetraacetate, 7.5 grams of sodium hydroxide, and water to make 1000.0 ml. The approximate pH was 11.0.

As the tetrasodium salt of ethylenediamine tetraacetate was available (Alrose's compound), a solution of this compound was made as follows: 17.3 grams of copper sulfate pentahydrate, 29.2 grams of tetrasodium ethylenediamine tetraacetate, 5.05 grams of sodium carbonate (or sufficient 1*M* sodium hydroxide solution), and water to make 1000.0 ml. pH = 10.1.

Another modification was to take an equivalent quantity (27.5 grams) of the cupric complex of disodium ethylenediamine tetraacetate (Alrose's compound) and add to it about 500 ml. of water. The pH was adjusted to about 11.4 with 1 *M* sodium hydroxide solution or saturated sodium carbonate solution.

EXPERIMENTS ON REDUCING AND NONREDUCING COMPOUNDS

To 5 ml. each of Benedict's qualitative reagent and copper ethylenediaminetetraacetic acid in separate test tubes were added about 10 mg. of the compounds listed below. Each mixture was boiled in an actively boiling water bath for 5 minutes, and then removed to cool spontaneously.

No significant differences were noticed between the action of Benedict's qualitative reagent and copper ethylenediaminetetraacetic acid complex with the exception of chloroform and chloral hydrate. With both reagents positive tests (yellow to reddish brown precipitates) were obtained with the following reducing compounds: glucose, galactose, maltose, lactose, fructose, arabinose, xylose, and ascorbic acid. Compounds which gave no reduction of Benedict's qualitative reagent and copper ethylenediaminetetraacetic acid were: sucrose, raffinose, starch, urea, formaldehyde, egg albumin (three times crystallized), bovine albumin (three times crystallized), trypsin, casein, creatinine, uric acid, gelatin, cellulose, and thrombin.

As for the difference in the action of chloroform and chloral hydrate, Benedict's qualitative reagent gave slight but positive tests with each (*1*), while the copper ethylenediaminetetraacetic acid reagent containing sodium carbonate gave no precipitate with either compound. However, the copper ethylenediaminetetraacetic acid reagent containing sodium hydroxide gave a positive test with chloroform, but not with chloral hydrate.

On standing, the tubes containing creatinine and Benedict's qualitative reagent or copper ethylenediaminetetraacetic acid develop a whitish turbidity, but give no evidence of reduction.

NATURE OF PRECIPITATE FORMED

The precipitate was filtered and was dissolved in dilute ammonia water. The solution was colorless initially, but gradually turned blue, starting from the air-liquid interface down as time progressed. Addition of hydrogen peroxide caused the solution to turn completely to the characteristic blue of the copper-ammonia complex.

Another precipitate was treated with phosphomolybdic acid according to the details given by Folin and Wu (*2*), yielding a blue solution.

Another precipitate was added to dilute sulfuric acid. The presence of copper and copper sulfate was experimentally determined (*3*).

The colors and the properties of the precipitates obtained with both copper ethylenediaminetetraacetic acid and Benedict's qualitative reagent were the same.

Thus there is little doubt that the precipitate resulting from the action of reducing sugars on copper ethylenediaminetetraacetic acid is cuprous oxide.

OPTIMUM CONDITIONS FOR REDUCTION OF COPPER ETHYLENEDIAMINETETRAACETIC ACID BY GLUCOSE SOLUTIONS

In connection with the effect of pH on the amount of precipitation, solutions of the chelating agent were prepared by dissolving 17.3 grams of copper sulfate pentahydrate and 29.2 grams of ethylenediaminetetraacetic acid in water and making the solution up to 500 ml. A series of various pH values from 3.0 to 13.0 was obtained by adding different amounts of a saturated solution of sodium carbonate or 1*M* sodium hydroxide solution. Each aliquot was then diluted with water to 50 ml. To two test tubes containing 5 ml. of each copper ethylenediaminetetraacetic acid solution of different pH were added 8 drops of 0.2 and 0.4% glucose, respectively, and tests were made in the usual manner. No reactions were observed in the solution with a pH less than 9, while solutions of pH 9 and 9.5 gave small quantities of greenish yellow precipitates.

Because differences in the size of the precipitate were difficult to distinguish accurately, the procedure was varied at this point. A modified Folin-Wu method (*2*) was adopted in which 2 ml. of reagent were mixed with 1 ml. of 0.04% glucose solution, boiled for 5 minutes in a boiling water bath, and cooled in an ice bath. After cooling, 5 ml. of phosphomolybdic acid reagent were added and the volume of the solution was made up to 25 ml. Comparative readings of the per cent transmittance were made in a Leitz Rouy photometer at 580 mμ.

It can be generalized that the higher the pH the greater the extent of precipitation. In the case of copper ethylenediaminetetraacetic acid containing sodium carbonate, a suitable operating range was pH 10 to 10.5, with best results at 10.5. A suitable pH range for reduction of copper ethylenediaminetetraacetic acid containing sodium hydroxide was found to be between 10.3 and 12.8. However in the range between pH 11.5 and 12.5 there was only a slight change in sensitivity. A pH of 12 was chosen as a working standard.

In general, at a pH of 10.0 to 10.5 precipitates obtained with the chelating agent in sodium carbonate solution are larger than in sodium hydroxide solution. Benedict (*1*) states that "the hydroxides of the alkali metals have a greater destructive action upon dextrose and various other carbohydrates than have the carbonates, and in accordance with this fact, a copper-containing solution in which the alkalinity is secured by sodium carbonate makes a more delicate and specific test for the detection of dextrose than does a copper solution which contains sodium hydroxide." The precipitates formed with copper ethylenediaminetetraacetic acid in the presence of sodium hydroxide appear faster and are more reddish, while precipitates formed in the presence of sodium carbonate take a longer time to form and settle more slowly.

It was then considered of value to determine at a satisfactory pH (10.5 for the solutions containing sodium carbonate and 12.0 for the sodium hydroxide solutions) that amount of tetrasodium ethylenediamine tetraacetate required to give optimum precipitation with the 17.3 grams of copper sulfate pentahydrate. Amounts of tetrasodium ethylenediamine tetraacetate varying between 20 and 60 grams per liter were used. Using 20 grams of tetrasodium chelating compound under the above conditions did not prevent the precipitation of either copper hydroxide or copper carbonate upon the addition of sodium hydroxide or sodium carbonate. The optimum amounts of the tetrasodium ethylenediamine tetraacetate found both in the sodium hydroxide and sodium carbonate solutions were between 40 and 45 grams per liter. This information was obtained by dissolving the precipitates in phosphomolybdic acid (*2*) and comparing the resulting blue solutions in a colorimeter.

The copper ethylenediamine tetraacetate reagent was generally a deeper blue than Benedict's qualitative reagent.

The time for reaction in the boiling water bath was studied—5 to 6 minutes were necessary to complete the reaction.

The solutions containing the ethylenediamine tetraacetate appear to be very stable on standing. One solution 4 months old and another 15 months old gave good results when tested in the usual manner.

However, Benedict's solution shows no sign of reduction or other alteration upon heating for 24 hours in a boiling water bath (*1*), whereas Fehling's solution shows a marked precipitation of cuprous oxide after 3 hours' heating (*1*) and the preparations containing ethylenediaminetetraacetic acid begin to form cuprous oxide in 2.5 to 3 hours when subjected to this treatment. When the amount of ethylenediamine tetraacetate was increased from 0.09 to 11.6 moles keeping the quantity of copper sulfate constant (0.07 mole), no precipitate appeared until 4.5 hours of heating.

COMPARISON WITH BENEDICT'S REAGENT

The copper ethylenediamine tetraacetate reagent prepared with sodium carbonate at a pH of 10.5 was more sensitive than the complex containing sodium hydroxide solution at the same pH for qualitative determination of glucose in aqueous solutions, but was less sensitive than Benedict's reagent in the qualitative estimation of glucose in urine. With aqueous solutions of 0.12% dextrose both Benedict's solution and copper ethylenediamine tetraacetate sodium carbonate gave visible precipitates of cuprous oxides, although the precipitate obtained with Benedict's solution was larger. In urine the copper ethylenediamine tetraacetate sodium carbonate reagent did not give significant yellow precipitates until 0.5% glucose solution was added to normal urine. However, Benedict's reagent can give a positive test with 0.1 to 0.2% glucose in urine.

The lesser sensitivity of the ethylenediamine tetraacetate reagent compared with Benedict's reagent for the determination of glucose in aqueous solutions could be due partly to the presence of a smaller concentration of cupric ions in the former case. Schwarzenbach and Ackermann (*8*) found the copper ethylenediamine tetraacetate complex very stable, with a log *K* of 18.2, while Meites (*5*) obtained a value of 14.2 for the copper citrate complex. West and Todd (*10*) discussing the Fehling and Benedict tests state that these complexes dissociate sufficiently to provide a continuous supply of readily available cupric ions for oxidation. From this it would follow that the more stable complex in allowing fewer cupric ions to be present would result in less reduction.

ACKNOWLEDGMENT

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Separation and Concentration of Trace Metals from Natural Waters

Partition Chromatographic Technique

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THE determination of many of the dissolved constituents of natural waters requires the processing of relatively large volumes of sample by techniques which concentrate the test substances and separate them from interfering elements. This processing is required because many of the biologically and catalytically active elements are so extremely diluted. In addition, the presence of other substances in much higher concentrations often interferes with the analytical processes. Sea water, for example, which is grossly a 3% salt solution, is composed of at least 44 dissolved constituents (*4*). However, the six elements, chlorine, sodium, magnesium, sulfur, calcium, and potassium, make up 94.2% by weight of the dissolved solids. Of the remaining 38 elements, 13 are in the range 10^{-4} to 10^{-6}%, 17 are in the range 10^{-6} to 10^{-8}%, and 8 are less than 10^{-8}%. Forty-three elements have not been detected in sea water. Thus, all dissolved substances, with the exception of the six major constituents, are in concentrations below 0.01%, a figure suggested by Sandell (*3*) as "indicating the approximate upper limit of a trace constituent."

The direct determination of minor constituents in sea water is possible in only a few cases. Methods have been devised for the determination of dissolved nitrate, nitrite, and inorganic phosphate, substances which have received considerable attention because of their nutrient role during photosynthesis, and direct spectrophotometric methods for several trace metals have also been reported. Nevertheless, our knowledge of the distribution of most of the trace elements and of their importance in biological and geological processes is extremely limited and is likely to remain so until concentrating and separating techniques and analytical procedures are developed that can be made to function efficiently under the severe conditions normally encountered in the field operations in oceanography, limnology, and sanitary engineering.

REQUIREMENTS

The following general requirements for a satisfactory concentrating technique will be common to many field uses. Exposition of detailed requirements will depend upon the nature of specific studies.

Equipment must be simple in both construction and operation.

Test substances must be concentrated by factors up to 10,000 with a precision that will not limit the precision and accuracy of modern analytical methods.

For many studies, especially in oceanography, specificity of the technique should be limited to a separation of as many of the minor elements as possible from all of the major elements—that is, the concentrate should contain none of the major constituents initially present in the sample and all of as many of the remaining elements as possible. In some cases this requirement may necessitate further separation prior to analysis.

EXPERIMENTAL

The operation of the extraction column described here depends upon the reactivity of metallic ions in aqueous solution with dithizone in carbon tetrachloride solution. In principle, the device operates as a partition chromatographic column in which the carbon tetrachloride–dithizone solution is the immobile phase, being held in the column on a cellulose acetate support, and the aqueous solution (the sample) is the mobile phase.

Seventeen elements are reported to form dithizonates (*3*). Of these, lead, zinc, manganese, cadmium, cobalt, and copper have been studied and are completely removed from solution by the column when operated under the conditions described herewith. Recovery tests were made by passing standard solutions and natural water samples containing known added quantities of test substance through the column. Recovery was considered "complete" when the analysis of the concentrate agreed with the analyses of standard solutions prepared with the same quantity of test substances as in the column influent but in a volume equal to that of the concentrate.

Column. The construction and operation of the extraction column are shown in Figure 1. The dimensions are those of the column used to obtain the results reported below. No attempt was made to study the effects produced by major dimensional changes.

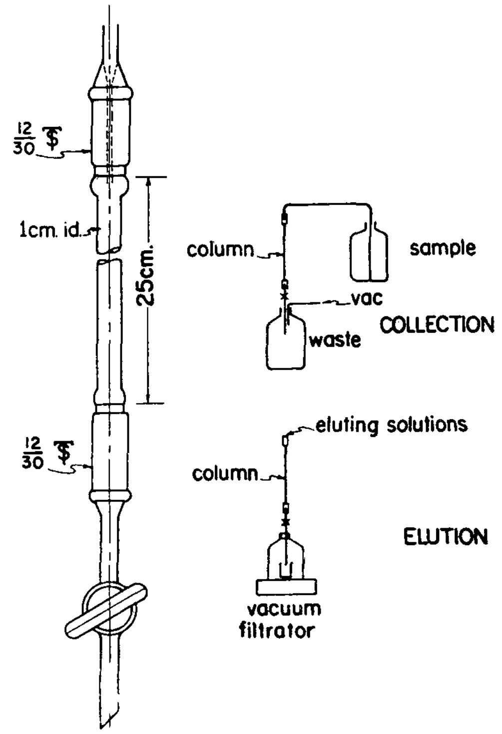

Figure 1. Extraction Column

Column Packing Material. The column packing consisted of a granular cellulose acetate support carrying a carbon tetrachloride solution of dithizone. The cellulose acetate was prepared by crumbling and sieving a commercial product (Fisher Scientific Co., No. C-215) through a set of well cleaned standard sieves. Two fractions gave satisfactory results—that passed by sieve No. 18 (1000-micron opening) and retained on No. 25 (750 microns), and that passed by No. 25 and retained on No. 35 (500 microns).

An attempt to use powdered filter paper (Whatman, cellulose powder, standard grade) was unsuccessful, as the dithizone–carbon tetrachloride was rapidly stripped from the support by passage of the sample.

Ten grams of sized cellulose acetate were treated with 100 ml. of a carbon tetrachloride solution of dithizone (0.5 gram per liter) and the slurry was heated with constant agitation in a 500-ml. beaker on a hot plate to evaporate the carbon tetrachloride. Heating was discontinued when the bulk of the material had passed from a deep blackish green to a light green, a transition which is marked, and which produces an essentially dry product that can be readily poured and packed into a column.

A column was prepared by adding and tamping firmly into place approximately 2 grams of untreated cellulose acetate followed by 3 grams of treated cellulose acetate. Glass wool plugs at both ends of the column served to hold the packing in the column. Three milliliters of carbon tetrachloride were then added to the top of the column and drawn into the packing by momentary application of vacuum. This "wet" approximately three quarters of the length of the treated column packing. A column to which carbon tetrachloride had not been added gave practically no recovery and permitted rapid stripping of the dithizone from the support. Excessive wetting with carbon tetrachloride permitted migration of the immobile phase out of the column along with the sample and eluting solutions.

The column was washed with 100 ml. of *M* hydrochloric acid and 250 ml. of metal-free water and was then ready for use.

Sample Pretreatment. Solutions from which the recovery of lead, zinc, cadmium, cobalt, and copper were to be studied were adjusted to pH 7.0 ± 0.1, this being the only pre-extraction treatment required.

The recovery of manganese was studied using the divalent element. Complete recovery was obtained in experiments in which air oxidation of the manganese was minimized. In several experiments poor recovery was obtained and in each case the test solutions inadvertently had been made alkaline while adjusting the acid test solution to pH 7.0. As the air oxidation of manganese(II) in alkaline solution proceeds rapidly and, in fact, is the controlling reaction in the Winkler determination of dissolved oxygen, the observed behavior on the column suggests that only manganese(II) reacts on the column or that dithizonates of higher oxidation states cannot be removed from the column by the methods described below. The reduction of manganese prior to column extraction has not been studied.

Column Operation. After pretreatment, the sample was drawn through the column with vacuum. In the tests described here the flow rate has been controlled solely by the pressure drop of the packing material. Flow rates up to 6 liters per hour have had no effect on recovery. Most tests, however, were at a flow rate of approximately 2 liters per hour. Rapid fluctuations in pressure, such as those produced by a slow-speed vacuum pump, tend to strip the dithizone–carbon tetrachloride from the support. This can be prevented by inserting a large free volume between the pump and column.

Collection of reactive substance can be observed by the color change produced in the column as the sample is drawn through. The capacity of the column, as estimated by the color change, is approximately 3 mg. of dithizone reactive elements. This has not yet been confirmed by breakthrough studies.

Elution from Column. Dithizonates were removed from the column and partial separation of the reactive elements was achieved by elution with the proper reagents. Of the six elements tested, lead, zinc, cadmium, and manganese(II) were completely removed with 50 ml. of hydrochloric acid *M*. Copper and cobalt were removed with 50 ml. of concentrated ammonia. Elution with ammonia destroyed the column, whereas an acid-washed column could be re-used, provided sufficient dithizone–carbon tetrachloride remained in the column.

Analysis of Elutants. In the studies reported here, all elements except manganese were determined by the polarographic method, using a Sargent Model XXI recording polarograph. Manganese was determined spectrophotometrically using the persulfate oxidation (*3*) to permanganate, and a Beckman Model DU spectrophotometer with 10-cm. Corex cells.

The acid elutant was prepared for analysis by adding 0.5 ml. of sulfuric acid, evaporating to fumes of sulfuric acid, adding 1 ml. of nitric acid, and evaporating to dryness.

For the simultaneous analysis of lead, cadmium, and zinc, 5 to 7 ml. of *M* potassium chloride containing 0.001% gelatin as a maximum suppressor were added to the residue, warmed to ensure solution of the residue, cooled, washed into a volumetric flask, and diluted to volume with *M* potassium chloride. The solution was then polarogramed.

Molar sodium hydroxide containing 0.001% gelatin was used as supporting electrolyte for the polarographic determination of lead and zinc when subsequent spectrophotometric analysis for manganese was to be performed on the same sample. When this was to be done, the sample was removed from the cell after polarograming and neutralized with sulfuric acid, and the persulfate oxidation to permanganate was performed without further treatment.

The ammonia elutant was evaporated to near dryness to remove the ammonia, 0.5 ml. of sulfuric acid was added and evaporated to fumes of sulfuric, 2.0 ml. of nitric acid were added, and the solution was evaporated to dryness. For the polarographic determination of copper and cobalt in the residue a supporting electrolyte, 1*M* in ammonia and ammonium chloride and containing 0.001% gelatin, was added, warmed, washed into a volumetric flask, and diluted to volume, and the solution was polarogramed.

Recovery Tests. Column efficiency was determined by passing solutions containing singly, and in combinations, known quantities of zinc, lead, cadmium, manganese(II), cobalt, and copper through the column. Sample volumes were 0.5, 1.0, or 10 liters. Column elutants, after treatment, were made up to 10 ml. for polarographic analysis.

All six substances were tested individually by passing 1 liter of solution containing 1 mg. of test element through the column. Recovery was complete in all cases.

Combinations of zinc, cadmium, and lead were studied by passing 0.5-liter quantities of solution containing not over 1 mg. of total test elements through the column. Complete recovery was obtained.

A 10-liter sample containing 10 γ each of zinc and copper was passed through the column. The recovery of zinc was 114% and of copper 102%.

Recovery from a sample of Chesapeake Bay water, salinity = $12^0/_{00}$, was determined by a comparison of zinc analyses of an aliquot to which 10 γ of zinc had been added, with one to which no zinc had been added. Liter samples were used in both cases. Recovery of the added zinc was 93%.

Blank corrections were made for all analyses and residual current correction applied to all polarograms.

DISCUSSION

The use of a "solid" extraction column has several advantages over liquid-liquid techniques, especially over batchwise liquid-liquid extraction. It permits the use of extremely simple apparatus which requires little or no attention during operation and in the form described here is well suited for many field studies, thus satisfying one of the requirements mentioned. In addition, the column technique provides a means of attaining much greater efficiency of operation, for its performance approaches that of a system composed of a series of theoretical plates in which equilibrium is maintained between the mobile and immobile phases.

The recovery of manganese(II) is perhaps the best indication of the efficiency of the column, for the dithizonate of this element is reported to be unstable and only 50% recovery is attainable by batchwise liquid-liquid extraction (*2*).

The dithizone system complies with the specificity requirements, as none of the major constituents appear in the concentrate.

Concentration factors up to 1000 have been achieved. An increase to 10,000 would undoubtedly be possible if the residue from digestion of the elutant were to be diluted to 1 ml. rather than to 10 ml. as reported here. Polarographic analysis of samples of 1-ml. volume and less has been described (*1*) and absorption cells with 5-cm. path and volume approximately 0.5 ml. for the Beckman DU spectrophotometer are available from Microchemical Specialties Co., Berkeley, Calif.

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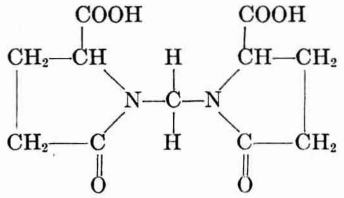
CRYSTALLOGRAPHIC DATA

76. Methylene-bis-(*N*-pyrrolidone-2-carboxylic Acid)

Contributed by FRANCIS T. JONES, KENNETH J. PALMER, AND DALE R. BLACK,
Western Regional Research Laboratory, Albany, Calif.

During an investigation of acids present in sugar-beet processing liquors the previously unreported acid, methylene-bis-(*N*-pyrrolidone-2-carboxylic acid), was discovered and identified (*1*).



Structural Formula of Methylene-bis-(*N*-pyrrolidone-2-carboxylic Acid)

Methylene-bis-(*N*-pyrrolidone-2-carboxylic acid) is sparingly soluble in water and in alcohol, the solubility increasing markedly with temperature. When a hot saturated aqueous solution is cooled, elongated prismatic or bladed crystals of an unstable modification (Figure 1) sometimes appear initially, especially if the solution is unseeded and undisturbed by scratching, etc. These unstable crystals transform spontaneously into opaque pseudomorphs if allowed to stand dry for a few hours (Figure 1), but if left in solution the long crystals crumble into a mass of granular crystals of the stable phase (Figure 2). When grown in a drop on a slide, both phases are likely to appear (Figure 1) if the slide is not scratched. Scratching at slight supersaturation gives the best crystals. Many poorly formed crystals, in difficultly recognizable orientations, will result if the supersaturation is too great.

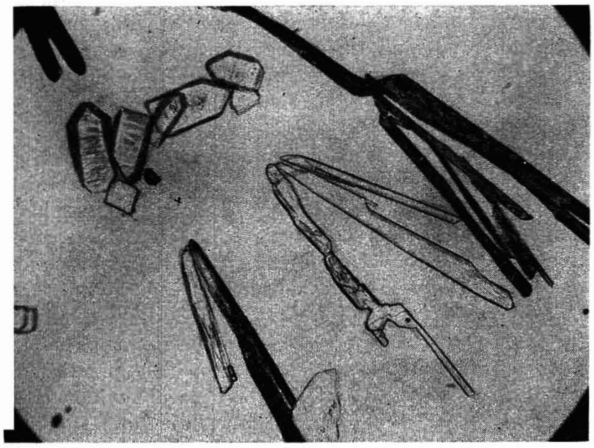


Figure 1. Methylene-bis-(*N*-pyrrolidone)-2-carboxylic Acid

Unstable phase. Clear blades
Stable phase. Clear prismatic crystals and opaque blades

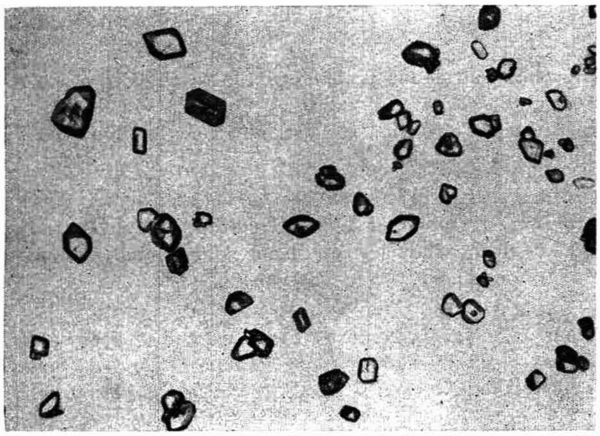


Figure 2. Methylene-bis-(*N*-pyrrolidone)-2-carboxylic Acid

Stable phase grown on microscope slide from water

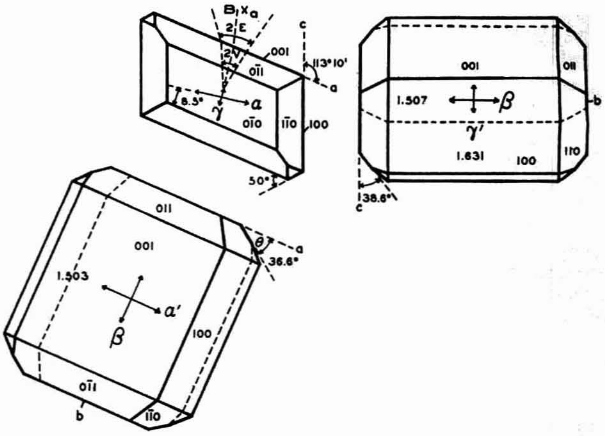


Figure 3. Orthographic Projection of Methylene-bis-(*N*-pyrrolidone)-2-carboxylic Acid

The optical properties and x-ray data were determined on crystals grown to convenient size in a beaker. The crystals were either picked out and blotted or allowed to dry slowly after the solution had been poured off. Floating crystals unsuitable for use usually appear.

CRYSTAL MORPHOLOGY. Stable phase (Figures 1, 2, and 3).

Crystal System. Monoclinic, Class 4 (only a 180° axis of symmetry).

Form and Habit. Although crystals grown from water may lie on any face and be nearly equidimensional (Figure 2), they usually develop as short prismatic rods or tablets elongated parallel to *b*, lying on {001} or {100}, terminated by {110}, {011}, and sometimes {010} (Figure 3). Cleavage is perfect parallel to {100} and good parallel to {001}.

The crystals of the stable phase shown in Figure 1 appear to lack a plane of symmetry, but symmetrical growth (Figure 3) occurs just as often under the conditions used. Crystals etched by brief immersion in alcohol show unsymmetrical etch figures on {001} or {100} and crystals (of the same phase) grown from alcoholic solution are predominantly pointed on one end and blunt on the other. The optical activity of this compound requires that the crystals should be hemimorphic and pyroelectric. Their pyroelectric character was proved by heating a 3 × 1 mm. crystal, carried on a slide, to approximately 150° C., discharging it while hot with a polonium foil, then touching the ends of the cooled crystal to the probe of an x-ray dosimeter electroscope. Opposite ends of the crystal showed opposite charges.

Axial Ratio. $a:b:c = 0.9956:1:0.8566$.

Interfacial Angles (Polar). $110 \wedge 010 = 47° 31'$, $011 \wedge 010 = 51° 47'$.

Beta Angle. 113° 10'.

X-RAY DIFFRACTION DATA

Cell Dimensions. $a = 9.24$ A., $b = 9.28$ A., $c = 7.95$ A.; $\beta = 113°10'$.

Space Group. $C_2^1 - P2$.
Formula Weights per Cell. 2.
Formula Weight. 270.2.
Density. 1.440 (flotation), 1.43 from x-ray data.

Principal Lines

d	I/I_1	d	I/I_1	d	I/I_1
8.53	22	3.24	7	2.21	5
7.31	8	3.14	11	2.09	6
6.27	19	3.08	4	2.04	4
5.73	43	3.02	10	2.00	5
4.64	100	2.82	21	1.937	4
4.24	8	2.72	17	1.906	3
4.07	39	2.62	4	1.875	4
3.87	59	2.55	5	1.849	3
3.65	6	2.50	10	1.823	4
3.55	6	2.43	6	1.794	4
3.40	7	2.36	7	1.715	4
3.31	7	2.28	7		

OPTICAL PROPERTIES

Refractive Indices (5893 A.; 28° C.). $\alpha = 1.500 \pm 0.001$. $\alpha' = 1.503 \pm 0.001$. $\beta = 1.507 \pm 0.001$. $\gamma = 1.642 \pm 0.001$. $\gamma' = 1.631 \pm 0.001$.

Optic Axial Angles (5893 A.; 28° C.). $2E = 41°$, $2V = 27°$ (by stage goniometer). $2V = 27°$ (calcd. from β and $2E$). $2V = 27°20'$ calcd. from $\sin^2 V = \frac{\gamma^2 (\beta^2 - \alpha^2)}{\beta^2 (\gamma^2 - \alpha^2)}$.

Dispersion. Inclined, weak.

Optic Axial Plane. 010.

Optical Character. Positive.

Acute Bisectrix. $\gamma \wedge c = 14°40'$.

Extinction. Views showing parallel extinction with either γ' or α' crosswise are most common, but occasional crystals growing on or nearly on 010 (Figure 2) will be found showing oblique extinction with α making an angle of about 8° with a.

Molecular Refraction (5893 A.; 28° C.). $R_{\text{calcd.}} = 62.1$, $R_{\text{obsd.}} = 59.6$.

FUSION DATA. The melting point is approximately 310°: with decomposition. Too much decomposition accompanies melting to make any sublimate or crystals from the brown colored melt useful in the characterization of this compound.

SPECIFIC ROTATION. $(\alpha)_D^{27} = +83.1°$ (C 0.5, water).

ACKNOWLEDGMENT

The authors wish to acknowledge the help of J. B. Stark and A. E. Goodban who furnished us with the material used, and Frank E. Young who determined the specific rotation.

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(1) Stark, J. B., and Goodban, A. E., *J. Am. Chem. Soc.*, 74, 4966 (1952).

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Section, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

Merck Graduate Fellowship

Applications are being received for the Merck Graduate Fellowship in Analytical Chemistry, financed by Merck & Co., Inc., and administered by the AMERICAN CHEMICAL SOCIETY. The annual stipend is $2500. The place of study must be an institution whose undergraduate course of instruction in chemistry is approved by the Society. In Canada the institution must be approved by the Chemical Institute of Canada and the ACS.

A fellowship will be awarded to the applicant believed capable of contributing most to the advancement of the theory and practice of analytical chemistry during the fellowship and in the future. It will be voted contingent upon the successful candidate's obtaining acceptance from the institution and professor selected for the study program sponsored.

Application blanks may be obtained from the AMERICAN CHEMICAL SOCIETY, 1155 Sixteenth St., N.W., Washington 6, D. C. They should be completed and returned with letters of recommendation and transcripts of credits before February 1, 1954.

Book Reviews

Papierchromatographie. *Friedrich Cramer.* 2nd edition. 118 + xviii pages. Verlag Chemie, GMBH, Weinheim/Bergstr., Germany, 1953. Price, DM12.80.

There is growing evidence that the appearance of small monographs on paper chromatography will soon reach epidemic proportions. The urge to write one is understandable. Contributions to this field now number in the thousands, and last year's Nobel Award to Martin and Synge for their work in this and closely related techniques is ample evidence of its importance.

In preparing this revision of an earlier edition, Dr. Cramer of the Chemisches Institut, Heidelberg, has done better than most. In choice of material, selected references, and condensation of details, the organization is incomparable. Almost half of the text is devoted to fundamental principles, apparatus, and techniques. Specific applications to amino acids, sugars, and practically every class of naturally occurring and synthetic organic substances are dealt with in concise economical fashion. Most of the practical and useful information relating to R_f values in various solvents is collected in tables. The bibliography contains 362 well-chosen references with cross reference to an author index. Subject index is also provided.

The German text is elegant, direct, and uninvolved in style. Quite a few Anglicisms are evident, which is mute testimony to the fact that so much of the subject itself is British in origin.

Binding, format, and typography are excellent—reminiscent of prewar German monographs. There are 68 well-drawn figures and 4 striking color plates. A folded insert gives a large scale "map" of the two-dimensional pattern for amino acids. Errors are negligible, but the reviewer cannot forego the trivial observation that the correct address of Messrs. H. Reeve Angel & Co., Ltd., is 9 Bridewell Place, London E.C. 4, England. This is a hospitable establishment, headquarters for Whatman papers and technical information. Incidentally, it is also publisher of an excellent monograph on the same subject by J. N. Balston and B. E. Talbot.

It is regrettable that so much of this subject remains empirical. In many respects, chromatography is a cookbook practice. The subject has not been without able and resourceful Brillat-Savarins and Escoffiers, but too few have been interested in the mechanism of the process or in a detailed investigation of these curious and bewildering phenomena. These things will be understood in the future. In the meantime, Cramer, and the others we have mentioned, are doing chemists a great service by keeping them acquainted with advances in this most useful technique.

RALPH H. MÜLLER

Reviews of Petroleum Technology 1951. *F. H. Garner, E. B. Evans,* and *George Sell,* editors. Vol. 13. viii + 360 pages. Institute of Petroleum, 26 Portland Place, London W. 1, England. Price, 50s.

Of interest to analysts is a 19-page chapter on "Analysis and Testing" by J. Scott and A. G. Meredith, which covers the progress made during the past year in the field of analysis and testing of the major petroleum fractions.

Ferrous Analysis. Modern Practice and Theory. *E. C. Piggott.* xxvii + 690 pages. John Wiley & Sons, 440 Fourth Ave., New York, N. Y., 1953. Price, $12.50.

The text is divided into five sections: Analytical Techniques, Constituents of Iron and Steel, Microchemical Analysis of Iron and Steel, Alloys and Ores, and Refractory Materials. An appendix contains a ready reference to methods for the rapid analysis of bath samples, literature references from 1930 (including books published in English), a glossary of chemical, physical, and metallurgical terms, and lists of British chemical standards. Section I contains chapters on available techniques and their choice, photoelectric absorptiometry, spectrographic procedure, and organic reagents for the analysis of iron and steel. Section II considers methods for the determination of 31 different elements in steel. For each element, a brief resume is given of its reduction from the ore, its functions in iron and steel, its chemical properties, methods for its detection, one or more methods for its determination, and the theory on which each method is based. Included are methods for hydrogen, nitrogen, and oxygen. A statement is made concerning the accuracy of each method and the time required to carry out the procedure. A similar arrangement is used for the remaining three sections.

The book emphasizes applied analysis, with somewhat more emphasis on English practice than that of other countries. With its bibliographies, it will provide a convenient and valuable reference and a good source of specific procedures for ferrous analysis.

H. F. BEEGHLY

Regional Conclave

AT THE Regional Conclave of the AMERICAN CHEMICAL SOCIETY, held December 10 to 12 in New Orleans, 51 papers on analytical chemistry were presented. Abstracts of most of these are given below.

Volumetric Determination of Sulfate. JAMES L. KASSNER, ESTELLE O. JACKSON, AND MYRON E. CALKINS, University of Alabama, University, Ala.

A rapid and accurate volumetric method for the determination of moderate quantities of sulfate has been developed. The method involved only three steps: the removal of metallic ions by passing the sample through a bed of a strong acid cation exchange resin; the precipitation of the sulfate ions by the addition of an excess of a standard lead nitrate solution; the titration of the excess lead ions with a standard tri-sodium phosphate solution. A special mixed indicator prepared from chlorophenol red and alphazurene dye was used to adjust the pH of the solution before the lead nitrate was added and to indicate the end point. The method has been applied successfully to synthetic samples containing 50 to 200 mg. of sulfate.

Volumetric Determination of Alumina in Bayer Process Liquors. ROBERT R. PHILLIPS AND ALTON E. MCLAUGHLIN, Kaiser Aluminum and Chemical Corp., Baton Rouge, La.

The gravimetric determination of alumina in Bayer process liquors is lengthy and usually of no great accuracy. Volumetric methods involving precipitation of K_3AlF_6 are not acceptably accurate for plant liquors containing silica. The following method, a synthesis of methods already known, was convenient, rapid, and acceptably accurate. Silica and phosphate interference was inappreciable if these substances were not present in rather large amount, but sulfate should be absent or low.

A solution of alumina in excess hydrochloric acid was sampled in duplicate aliquots. To one aliquot was added a large excess of neutral potassium oxalate; titration with standard base then determined the free acid. Under proper conditions, the pH of the end point varied only slightly, and the end point was readily noted by the use of a mixed indicator. To the duplicate aliquot was added an excess of neutral barium chloride and Rochelle salt solution. The result was the apparent liberation of three protons per aluminum atom. The acid thus produced was conveniently titrated to a sharp end point with phenolphthalein. The difference between the two end points was stoichiometrically related to alumina content. Caustic soda and total soda, quantities of interest in Bayer process control, were also easily determined.

Determination of Epoxides with Sodium Sulfite. J. DONALD SWAN. Jefferson Chemical Co., Austin, Tex.

A method for determining epoxides based on their reaction with aqueous sodium sulfite was described. Titration of the liberated caustic provided a convenient measure of epoxide concentrations. A number of different epoxides have been analyzed successfully by this procedure. Several solutions containing from 0.5 to 80% epoxide have been analyzed with an accuracy of ±0.5%. Glycols, alcohols, and chlorohydrins did not interfere, but aldehydes did. A procedure was described for determining both epoxide and aldehyde in mixtures.

Determination of Oxygen in Purified Argon, Carbon Dioxide, Helium, Hydrogen, and Nitrogen. Improved Winkler Method. WANDA BRADSHAW AND LOUIS SILVERMAN, North American Aviation Co., Downey, Calif.

The careful present-day studies of metal corrosion necessitated the preparation and analysis of inert gas blankets free, or nearly free, of oxygen. Micro amounts of oxygen may be determined in the inert gases and in nitrogen, carbon dioxide, and hydrogen by a modification of the Winkler method. The oxygen was absorbed in manganous hydroxide and converted to iodine, which was extracted into *o*-xylene and determined colorimetrically.

The analysis took place at low pressures, from 350 to 760 mm. of mercury. Either small volumes of gas (200 ml.) or large quantities such as those obtained from a flow of gas or from pressurized gas tanks may be analyzed by this method. A reproducibility of 0.5 p.p.m. was obtained over a range from 0 to 150 p.p.m. (microliters per liter). The method could be extended to higher ranges by titrating iodine with thiosulfate.

Improved methods of removing dissolved oxygen from the analytical reagents resulted in a constantly low blank which permitted the high degree of precision obtained.

***N*-Bromosuccinimide as an Organic Classification Reagent.** PAUL F. KRUSE, JR., AND THOMAS A. MCCOY, Samuel Roberts Noble Foundation, Ardmore, Okla.

N-Bromosuccinimide served in a simple test for differentiating saturated primary, secondary, and tertiary alcohols. Three drops of the alcohol, or an equivalent amount of solid, were dissolved in 1 ml. of carbon tetrachloride, treated with an amount of *N*-bromosuccinimide on the tip of a small spatula (15 to 30 mg.), and boiled over a microburner. Primary alcohol tests yielded an orange color within 3 minutes, which was permanent on cooling. Tests with secondary alcohols gave an orange color which disappeared rapidly on cooling, and an orange color formed in the precipitate of unreacted bromoimide. Tertiary alcohols gave no observable change even after boiling for 5 minutes. Succinimide was present at the top in all tests in which reaction occurred. Thirty saturated aliphatic and alicyclic alcohols were tested, ranging from methanol to 1-octadecanol.

Twenty-six additional tests indicate that the method was useful in characterizing the nature of the hydroxyl group in compounds such as hydroxy esters, ether alcohols, and others. The test was being applied to a series of glycols, amino alcohols, amines, and substituted benzenes and naphthalenes.

N-Chlorosuccinimide, *N*-iodosuccinimide, and dibromodimethylhydantoin were also being investigated as classification reagents.

Analytical Studies of Some Acyloin Oximes. HANS A. SUTER AND PHILIP W. WEST, Louisiana State University, Baton Rouge, La.

The unusual solubility relationships of the copper salts of the alpha isomers of acyloin oximes have been investigated. Based on these studies, a polymeric structure for these copper salts was proposed.

The following alpha-isomers of acyloin oximes were examined: benzoin oxime, 2,2′-furoin oxime, phenylbenzoin oxime, methylbenzoin oxime, 2,2′-thenoin oxime, α-hydroxyacetophenone oxime, and α-hydroxyisobutyrophenone oxime. Of these compounds only benzoin oxime had been previously studied from the standpoint of their use in analytical chemistry.

Microscopic Fusion Study of 2,4,5-Trichlorophenoxyacetic acid and Related Compounds. CLAUDE J. ARCENEAUX, Ethyl Corp., Baton Rouge, La.

Microscopic fusion techniques were applied to a study of 2,4,5-trichlorophenoxyacetic acid and six related compounds. This was done in order to obtain fundamental crystallographic data and rapid methods of characterization for the following compounds: 2,4,5-trichlorophenoxyacetic acid; 5-methoxy-2,5-dichlorophenoxyacetic acid; 2,4,5-trichlorophenol; the sodium and potassium salts of trichlorophenoxyacetic acid; 2,3,6-trichlorophenoxyacetic acid; and

2,5-dichlorophenoxyacetic acid. Furthermore, simple and rapid qualitative fusion methods of detection have been developed for the sodium and potassium salts of 2,4,5-trichlorophenoxyacetic acid in the commercial product.

Theory of Electrolysis at Controlled Current in Unstirred Solution. Experimental Study of Potential-Time Curves. Paul Delahay and Calvin C. Mattax, Louisiana State University, Baton Rouge, La.

Experimental methods for the precise recording of potential-time curves are described and applied to various electrode processes. The theoretical treatment of reversible electrode processes is verified in the cases of the reduction of ferricyanide, thallous, and cadmium ions. The properties of potential-time curves obtained by reversing the current at the transition time are also in good agreement with theory. The features of experimental potential-time curves for the irreversible reduction of oxygen, nickel(II), and cobalt(II) in various electrolytes also agree well with theory. The analysis of potential-time curves for the reduction of chromate ion in 1 M sodium hydroxide indicates the occurrence of a stepwise process. Potentialities of the constant current method in the study of electrode processes are discussed, and applications to analytical determinations are briefly mentioned. Experimental data determined by the present method are used in the verification of the theory of irreversible polarographic waves previously developed.

Determination of Moisture in Salts by the High Frequency Method. Fred W. Jensen and M. B. Burton, Jr., A. & M. College of Texas, College Station, Tex.

The purpose of this research was to develop a rapid, simple, and accurate method for the determination of moisture in salts. The apparatus consisted of a 10-megacycle Clapp oscillator with a sample column placed in series with a diode rectifier and a d.c. microammeter. The device was equipped with a temperature compensator. Studies were made on sodium chloride and ammonium nitrate. Fifty-gram samples of the salt mixtures containing varying amounts of moisture were agitated for 5 minutes with a mixture of 1,4-dioxane and methanol. After settling or centrifuging, the liquid was introduced into the sample column and the readings were made on the microammeter.

During the stirring process an equilibrium is set up between the moisture in the salt and the moisture in the solvent. With increase in moisture content in the salt the amount of moisture extracted by the solvent also increases. Since the solvent is in all cases saturated with salt, the conductance of the solvent increases with increase in moisture content. With the correct concentration of 1,4-dioxane and methanol the response is linear.

The instrument showed very good accuracy and reproducibility, excellent time stability, and temperature compensation. The time required for a single moisture determination was on the order of 10 minutes.

Determination of Water in Alcohols by an Indirect Conductivity Method. C. Kinney Hancock, Charles M. Hudgins, Jr., and Ralph E. Zerwekh, Jr., Texas Engineering Experiment Station, College Station, Tex.

Using methyl and ethyl alcohols, studies of alcohol-acetone-water–saturated sodium chloride systems show that the conductivity is nearly linear with the water content over the range of 0 to 10% water. Linearity can be approached more clearly by increasing the acetone concentration, but a parallel decrease in sensitivity occurs. The optimum acetone concentration is about 30%. The procedure requires about 15 minutes and may be used to determine water in methyl and ethyl alcohols. The application of the method to the determination of water in other materials is of considerable interest and encouraging results have been obtained in the determination of water in soils and in sucrose solutions.

Acetic Anhydride in Nonaqueous Titrimetry and Its Role in the Determination of Sulfuric Acid Catalyst in Ethanol Esterification Mixtures. A. F. Gremillion, Carbide and Carbon Chemicals Co., Texas City, Tex.

The use of acetic anhydride in nonaqueous titrimetry has been extended beyond its employment as a dehydrating agent. It has been successfully used in the determination of sulfuric acid catalyst in ethanol esterification and a study of the influence of other solvents in the mixture conducted.

In addition, acetic anhydride has been used as a solvent for the titration of a number of weak bases. Some pyridines, amines, and ureas have been titrated with considerable sharpness of end point and in some cases large potential changes at the end point. A technique enabling the use of this solvent when titrating weak primary and secondary amine bases was described.

Coordination of Instrumental Analysis with Other Analysis Methods. John T. Wiley, Texas Co., Port Arthur, Tex.

In laboratories where analytical instruments are in use questions arise as to what instrument or instruments should be used for a particular sample or if instrumental methods are desirable. This is especially true of samples that are submitted with only the notation "identify" and without additional information as to source or possible constituents. A fairly definite program of analysis for such samples has been established that expedites their analysis by taking advantage of modern instrumentation and at the same time not losing sight of the value of other analytical methods.

Samples are divided roughly into two classes, hydrocarbon and nonhydrocarbon samples, except in the case of gases, where no division is made. The samples are then redivided as to whether liquid or solid. The samples are then observed for general appearance and in the case of solids examined microscopically. Procedure from this point is dependent on the results of their examinations and other factors which were discussed.

Apparatus for Determination of Sulfur in Petroleum Products by the Lamp Method. Howard Hopkins, Pawhuska Research Laboratory, Pawhuska, Okla.

An apparatus has been developed which will determine the sulfur content of petroleum products, ranging from liquefied petroleum gases through Diesel fuels, simultaneously and with equal facility. Very precise control over the sample combustion can be obtained throughout the determination, and once started the samples require a minumum of attention. The sulfur is determined volumetrically, and these values are closely comparable with the gravimetric values. The results obtained with this apparatus equal or surpass those obtainable with similar apparatus and methods, and the degree of control over sample combustion possible with this apparatus far surpasses that obtainable upon other lamp sulfur apparatus.

Radioactive Tracers as Adsorption Indicators. R. B. Escue and Newman P. Bulloch, North Texas State College, Denton, Tex.

A progress report on the use of P^{32} and I^{131} tracers to follow the course of precipitation titrations. The adsorption of tracers is affected by excess titrant, and the radioactivity of the tracer is followed by a G-M tube in contact with the solution. The specific case of adsorption on barium sulfate and the possibility of determining sulfate ion by volumetric precipitation were discussed.

Use of Nuclear Techniques in Analytical Chemistry. G. W. Leddicotte and S. A. Reynolds, Oak Ridge National Laboratory, Oak Ridge, Tenn.

This paper introduced the application of the unique properties of nuclear particles to analytical chemistry problems. Of primary interest are the properties of these nuclear particles and their interactions with matter. Some of these interactions, especially those involving neutrons, have been found useful as techniques for chemical analysis by induced radioactivity (activation analysis), neutron transmission, and neutron absorption. A general discussion of each method, the theoretical sensitivities, and present applications were presented. In addition to these techniques, the method of analysis by radioactive tracers was discussed.

Determination of Trace Elements in Crude Oils and Distillation Products by Radioactivation Analysis. G. W. Leddicotte and H. A. Mahlman, Oak Ridge National Laboratory, Oak Ridge, Tenn.

The nuclear reactions which occur in the Oak Ridge National Laboratory graphite reactor now form the basis of a unique analytical service for determining trace element concentrations in many different materials. The method of radioactivation analysis, its sensitivities of detection, and applications were reviewed. Special attention was directed to the determination of trace element concentrations in crude oils and distillation products by neutron activation analysis. Trace amounts of arsenic, copper, chromium, iron, mercury, nickel, sodium, and vanadium in crude oils and distillation products have been determined by this method of analysis. Nuclear data, method of irradiation, processing of samples after irradiation, and type results obtained in the assay of these materials were presented.

Determination of Trace Elements in Grass and Grain by Radioactivation Analysis. H. A. MAHLMAN AND G. W. LEDDICOTTE, Oak Ridge National Laboratory, Oak Ridge, Tenn.

The usefulness of nuclear techniques involving induced radioactivity by neutron irradiation—i.e., neutron activation analysis—in determining trace elements in grass and grain was discussed. Pertinent nuclear data, method of irradiation, processing of samples after irradiation, and typical results obtained in the assay of samples were reviewed. So far, neutron activation analysis has been applied to the determination of trace concentrations of cobalt, copper, iron, manganese, molybdenum, strontium, and zinc in rye grass and corn.

Isotope Effect Study of the Pinacol Rearrangement. WAYNE CARRICK AND ARTHUR FRY, University of Arkansas, Fayetteville, Ark.

1,1,2,2-Tetraphenylethylene glycol-1-C^{14} has been prepared and allowed to rearrange to 1,1,1-triphenylacetophenone under several different sets of reaction conditions. The ketone was degraded to triphenylmethane and benzoic acid. In all cases these two compounds were found to have the same molar activity within experimental error, indicating that there had been no intramolecular isotope effect in the rearrangement. These results show the improbability of some of the detailed reaction mechanisms which have been considered for the reaction, but are consistent with the postulation that the pinacol rearrangement is a completely concerted reaction.

Use of Carbon-14 in a Competitive Reaction Rate Study. CHARLES T. LESTER AND GUS A. ROPP, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Maleic-1-C^{14} anhydride(I) was synthesized from sodium cyanide-C^{14} by a modification of the method of Nystrom, Loo, and Leak [*J. Am. Chem. Soc.*, **74**, 3434 (1953)]. Samples of the anhydride were used in three Diels-Alder reactions at 25° C., with the dienes 1-phenyl-1,3-butadiene(II), 1-(*p*-methoxyphenyl)-1,3-butadiene(III), and 1-(*p*-nitrophenyl)-1,3-butadiene(IV). The three reaction mixtures were of the following compositions in benzene solutions:

I with a large excess of an equimolar mixture of II and III
I with a large excess of an equimolar mixture of II and IV
I with a large excess of an equimolar mixture of III and IV

By analysis of the product mixtures using carbon isotope dilution technique, the relative values of the specific reaction rate constants for the three dienes were shown to be $k_{-OCH_3} > k_H > k_{-NO_2}$. Although the measured ratios did not show a close fit to the Hammett equation, the results are in accord with an ionic mechanism for these Diels-Alder reactions. In view of the high polarizability of such dienes as those used here, a close fit to a Hammett equation should, perhaps, not be expected.

Synthesis of 1,2-Dihydro-3,6-pyridazinedione-3,6-C^{14}. J. GRAY DINWIDDIE, JR., AND JOHN A. PORTER, Clemson Agricultural College, Clemson, S. C.

The process was described by which barium carbonate containing carbon-14 is converted into 1,2-dihydro-3,6-pyridazinedione-3,6-C^{14}, the cyclic hydrazide of maleic acid. Intermediate compounds in the synthesis include potassium cyanide, succinic acid, dibromosuccinic acid, fumaric acid, maleic acid, and maleic anhydride.

One-Step Preparation of C^{14}-Cyanide from Barium Carbonate-C^{14}. J. K. JEANES, Texas Research Foundation, Renner, Tex.

The importance of cyanide in the synthesis of organic compounds labeled with isotopic carbon requires that it be made available in high yields by simple and inexpensive procedures starting with barium carbonate. Methods have been described for the conversion of alkali carbonates to cyanide, but each has its own limitation. A procedure has been developed whereby barium carbonate can be converted to sodium cyanide in one step by heating barium carbonate, zinc dust, and metallic sodium in a stream of anhydrous ammonia which had passed over hot iron. Iron seemed to have an important role, in that it catalyzed the decomposition of ammonia to give nitrogen gas and other "active" forms of nitrogen which were essential in the reaction. The yields in this laboratory were quantitative. The specific activity of the labeled cyanide remained unchanged from that of the carbonate which was used.

Electromagnetic Isotope Separation of the Palladium, Platinum, and Rare Earth Elements. C. P. KEIM, Oak Ridge National Laboratory, Oak Ridge, Tenn.

The electromagnetic separation of isotopes has now been accomplished with most of those elements having naturally occurring isotopes. In recent months, in addition to maintaining a stock of enriched isotopes for research, progress has been made in processing some of the more difficult elements. For example, the palladium and platinum elements do not have stable compounds which can be vaporized in the ion source. Also, the rare earth elements must be chemically separated to provide charge material for the mass spectrograph isotope separations.

Palladium and platinumlike metals have been heated by electron bombardment to as high as 2800° C. to provide sufficient vapor in the calutron ionization chamber for isotope separation. The rare earths are being separated chemically in tributyl phosphate–nitric acid exchange columns to provide mass spectrograph charge materials.

Determination of Tritium by Radioautography. D. E. BEISCHER, U. S. Naval School of Aviation Medicine, Pensacola, Fla.

The isotope hydrogen-3 offers a convenient way to label many inorganic and organic compounds. The possibilities and limitations of a quantitative determination and localization of the labeled molecules by radioautography were discussed. Effective use was made of tritium-labeled stearic acid monolayers as secondary radiation standards. Based upon results of this study recommendations for the radioautographic procedure and the choice of the photographic material were made. Examples of the use of the method in surface chemistry were presented.

Gamma-Ray Spectroscopy in Analytical Radiochemistry. BERND KAHN AND W. S. LYON, Oak Ridge National Labratory, Oak Ridge, Tenn.

A thallium-activated sodium iodide gamma-ray spectrometer has been calibrated with respect to gamma-ray energy and efficiency between the energies of 30 k.e.v. and 2.76 m.e.v. This calibration makes it possible to solve many quantitative problems in analytical radiochemistry simply, quickly, and often without any chemical separation or chemical treatment of the sample. The gamma spectrometer goes beyond radiochemical analysis in that a particular radioisotope rather than just the element is identified. Several types of problems to which this technique has been applied were described. These include the checking of analytical methods for specificity, monitoring of ORNL radioisotope products for gamma contaminants, isotopic analysis, and identification of unknown activities in solid, liquid, and air samples.

Causes for Abnormal Mass 30 Values in Nitrogen Isotope Analyses with the Mass Spectrometer. ROBERT L. ORY, J. M. PRESCOTT, AND CARL M. LYMAN, Texas Agricultural Experiment Station, College Station, Tex.

While conducting biological studies involving N^{15} as a tracer, an abnormally high mass 30 (M_{30}) peak was often encountered in the mass spectrometer curves for samples oxidized by the Dumas method. Of the two equations which may be used to compute atom per cent excess N^{15}, erratic values were obtained with the equation involving experimental values for M_{30} as well as M_{28} and M_{29} as compared to the alternative equation which represents M_{30} in terms of M_{28} and M_{29}.

It appeared that the source of the error might be traces of nitric oxide escaping reduction by the copper in the combustion tube. Evidence in support of this hypothesis was obtained by shaking the gas samples with alkaline potassium permanganate, which oxidizes nitric oxide. This procedure completely removed the abnormally high M_{30} peaks.

Composition of the Basic Ferric Formate Precipitate. Effect of Final pH. C. T. KENNER, Southern Methodist University, Dallas, Tex.

The composition of the basic ferric formate precipitate varies with the final pH of the precipitation medium. X-ray diffraction patterns prove the presence of FeOOH and chemical analyses indicate a general formula of $(FeOOH)_x(FeOCl)_{1.7}(FeOCHO_2)_{1.0}$ with x varying from 8.4 to 15.3 as the final pH is raised from 2.95 to 4.65. The presence of a small amount of some crystalline material which is slightly soluble in benzene and which contains iron and chlorine is indicated by transmission of light in the dark field of a polarizing microscope and by qualitative tests upon benzene extracts of the precipitates.

Principles of Precision Colorimetry. Two New Methods. Charles N. Reilly, University of North Caroline, Chapel Hill, N. C.

This study was made to investigate the effect of the "dark-current" knob on spectrophotometric precision with a view toward the development of improved high-precision methods. Utilization of the knob for this purpose requires the use of a reference solution for setting the instrument zero. In this way, apparent positive deviation from Beer's law is achieved, with its attendant increase in precision. An expression for the relative error is derived and the conditions for its minimization were discussed under assumptions sufficiently general both to include and extend previous techniques. Four methods are distinguished, of which two are new. One of the new methods gives the best precision obtainable, using two reference solutions and other conditions selected to make this statement true. The selection procedure was described. The other new method is applicable to trace analysis, and represents the best compromise possible when solutions sufficiently concentrated to permit use of optimum conditions are not available. Both methods promise substantial improvement in precision over former methods at small extra cost in time and effort.

Effects of Time and Temperature on the Reaction of HCHO with a Modified Schiff's Reagent. Richard Hatfield and E. C. Smith, Southwest Research Institute, San Antonio, Tex.

The colorimetric test using Schiff's reagent as modified by Denigès was studied over a range of conditions of 0 to 25 hours using temperatures of 20°, 25°, and 30° C. at varying concentrations of HCHO. A color development time of 10 minutes at 25° C. was established as a standard for routine analysis. Experiments with both basic fuchsin and rosaniline hydrochloride showed that the form of the fuchsin dye produced differences in color intensities, and Coleman and Bell's rosaniline hydrochloride was selected as a standard. When the dichromate ion was present in concentrations less than 100 p.p.m. it was found that a change of wave length from the standard value of 580 to a value of 650 mμ would give results in which all values were within 10% of the mean. Experimental work also showed that HCHO-derived acetals could be completely hydrolyzed when 1 ml. of sulfuric acid was added to 5 ml. of the acetal solution and allowed to stand 15 minutes at approximately 65° C. The resulting HCHO could then be determined by the modified Schiff's test. A qualitative test was developed to determine whether an acetal is derived from HCHO or a higher aldehyde by hydrolyzing the acetal solution and then preforming the modified Schiff's reagent to see if HCHO is present.

Colorimetric Determination of Small Amounts of Uranium in the Presence of Bismuth. Pentaether Thiocyanate Method. Lavada Moody and Louis Silverman, North American Aviation, Inc., Downey, Calif.

Bismuth, in microgram quantities, interferes in the colorimetric determination of uranium by the better known reagents such as thiocyanate and 8-quinolinol. The usual chemical procedures for the preliminary separation of bismuth from uranium are ineffective, as small portions of uranium accompany the bismuth. This has been noted for cupferron. The preliminary bismuth oxychloride separation or the electrodeposition of bismuth leaves residuals of bismuth (micrograms) which tend toward high uranium results.

It was shown that the residual bismuth in the uranium solution may be extracted with dithizone, and the uranium determined photometrically as thiocyanate, in the 350 to 400 mμ range.

Determination of Low Hydrocyanic Acid in Acrylonitrile. Robert L. Maute and M. L. Owens, Jr., Monsanto Chemical Co., Texas City, Tex.

The need for a precise but simple method for the determination of free and combined hydrocyanic acid in the range of 0 to 100 p.p.m. in acrylonitrile led to a study of four different methods. It has been found that simple potentiometric procedures give sufficient precision and accuracy for the determination of either free hydrocyanic acid or total (free and combined) hydrocyanic acid on routine samples. Where a higher precision is required, a simplification of the phenolphthalein colorimetric procedure has been found more satisfactory. The visual end point of the Liebig-Denigès titration method for total cyanide is useful for concentrations down to about 20 p.p.m., but is not recommended for routine application when lower concentrations are to be encountered.

Determination of Free Gossypol in Mixed Feeds. R. W. Storherr and K. T. Holley, Georgia Agricultural Experiment Station, Experiment, Ga.

Common methods for the determination of free gossypol are inapplicable to mixed feeds because of various interfering substances. A study of other reagents and solvent systems showed that phloroglucinol is a satisfactory reagent and 2-butanone azeotrope a suitable extracting solution. Gossypol-time extraction curves of raw, hydraulic, screw-pressed, and solvent-extracted meals show a plateau after an initial rapid extraction rate. The values at a point just past the sharp break are in close agreement with those by the *p*-anisidine method. These values are defined as free gossypol.

The color developed at room temperature by phloroglucinol-gossypol in the presence of strong acid is estimated spectrophotometrically at 550 mμ. A small amount of aniline in the extracting solution checks the reaction of gossypol with substances in other components of the feedstuffs. Theoretical values are obtained for meals mixed with alfalfa, minerals, ground corn, barley, oats, wheat, and blackstrap molasses, either individually or in various formulations. As low as 0.004% free gossypol in mixed feeds is readily determined.

Photometric Titrations in Nonaqueous Solvents. Charles N. Reilley and Barbara Schweizer, University of North Carolina, Chapel Hill, N. C.

Photometric titrations have proved valuable in circumventing difficulties and inadequacies experienced with other end-point detection techniques. For this reason a study of the application of the photometric method in the general field of acid-base titrations in nonaqueous media seemed desirable. Glacial acetic acid was employed as the solvent and a glacial acetic acid solution of perchloric acid was used to titrate several weak bases. The titration cell was placed directly in the light path of a Beckman DU spectrophotometer and the absorbancy at an experimentally determined wave length was plotted against the corrected volume of the titrant. In this manner bases such as *o*-chloroaniline, *m*-chloroaniline, and quinoline gave distinct breaks at the equivalent point (agreeing well with potentiometric end points) and were therefore successfully titrated. The possibility of titrating substances was attempted for cases where neither acid nor base forms show absorbancy in the ultraviolet region. This was accomplished by addition of an absorbing species of weaker basicity. Successive end-point breaks indicate the volume equivalent to the quantity of each component.

Spectrophotometric Determination of Copper after Solvent Extraction of the Copper-Pyridine-Thiocyanate. Stephen S. Baird, University of Texas, Austin, Tex., and David W. Sanshuck, Naval Powder Factory.

In the determination of less than 0.2% copper in aluminum, the electrolytic method was found to give erratic results. The usual colorimetric copper determination using sodium diethyl dithiocarbamate required a preliminary separation, thereby making the determination time-consuming. A solvent extraction method was sought, as it was felt that a simultaneous separation and determination could be accomplished by choice of the proper reaction. The method chosen for study is the reaction between copper, thiocyanate, and pyridine to yield the insoluble compound $Cu(C_6H_5N)_2(CNS)_2$.

The dependence of various solution factors and foreign ions upon the absorbance of the solution was shown, as well as the effect of various solvents used to extract the colored compound.

Influence of Perrhenate Ion on Flame Photometric Determination of Potassium. A. D. Melaven and A. J. Chadwell, University of Tennessee, Knoxville, Tenn.

Rhenium in the form of the perrhenate ion, ReO_4^-, will cause low results in the flame photometric determination of potassium using both the direct intensity and the internal standards methods. A Model 52-C Perkin-Elmer flame photometer was used in the work, covering the range 0 to 100 p.p.m. of potassium and 0 to 5000 p.p.m. of rhenium as perrhenate ion.

The internal standard method is subject to less error than the direct intensity method. In addition to the error due to perrhenate ion, hydrogen ion also depresses the direct intensity readings but not those by the internal standard method. In the use of either method, the greatest relative error was found with the smaller potassium concentrations. In the case of the internal standard method, an almost linear relationship exists between instrument reading and concentration of rhenium present for a given concentration of potassium.

In both methods, a given ratio of rhenium to potassium by weight causes a constant relative error over the concentration range used. This relationship was first noticed in the case of pure potassium perrhenate and was found to be true for other ratios.

Flame Photometric Determination of Calcium and Magnesium. Florence B. Strohmeyer, Byron E. Leach, and Robert G. Heath, Tulane University, New Orleans, La.

The determination of calcium and magnesium by flame photometry was undertaken in order to study the variations encountered in biological specimens. The instrument used was a Beckman DU spectrophotometer with flame attachment and a photomultiplier. A number of conflicting substances are present in these materials and must be taken into consideration. A series of studies was carried out on the effects of sodium, potassium, and phosphate and the mutual effect of calcium and magnesium on each other. The wave length used for calcium was 554 $m\mu$ and 371 $m\mu$ for magnesium.

The literature reported that 40% isopropyl alcohol enhanced calcium, so this was the first effect studied. It was also found that the isopropyl was necessary for the magnesium determination.

As a result of all these effects a diluting fluid containing sodium, potassium, and phosphate was used and made up to contain 40% isopropyl alcohol. It was found that calcium has no appreciable effect on magnesium but that magnesium has a depressing effect on calcium. To a series of solutions containing a known quantity of calcium, varying quantities up to equivalent amounts of magnesium were added. The most feasible concentration appears to be a close approximation to that found in biological fluids.

Effects of Certain Alkali Compounds in the Burning of Powdered Graphite Samples. E. L. Grove and J. A. Norris, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Effects of fluoride, chloride, carbonate, and sulfate compounds of lithium, sodium, and potassium were studied in the burning of powdered graphite standards containing oxides of silicon, aluminum, iron, and carbonates of barium and calcium. The procedure consisted of burning a 1-mg. graphite-oxide standard mixed with 9 mg. of various ratios of the compound and graphite powder. Center-drilled $^3/_{16}$-inch diameter lower electrodes with very thin walls were used and the samples burned to completion in a 5-ampere, 2400-volt a.c. arc. Moving plate studies of the various standard elements in the mixtures gave information on the smoothness of burning, time required, and completeness of burning.

Potassium and sodium fluoride gave cleaner and sharper burn-out of the elements silicon, iron, and aluminum than did the same weight of the other compounds. Fluorides of lithium, sodium, and potassium produced spectrograms with less background than the other salts. Also, the lithium fluoride and sodium fluoride enhanced the arc lines more and suppressed the spark lines more than the other compounds.

Ratios of 1 part salt to 7 parts graphite did not produce effective CN band suppression; however, a ratio of about 2 to 5 yielded good results. Lithium fluoride appears to be the most effective. The use of equivalent weights of lithium for the different salts did not produce equal suppression effects.

Polarographic Determination of Vanadium and Molybdenum in the Presence of Ethylenediaminetetraacetic Acid. Robert D. Feltham and Ernest L. Martin, University of New Mexico, Albuquerque, N. M.

The use of salts of ethylenediaminetetraacetic acid (Versene) as the supporting electrolyte and complexing agent in the determination of vanadium(V) and molybdenum(VI) was described. The half-wave potentials of vanadium(V) Versenate and molybdenum have been reported, but no suitable method for their determination in the same solution has been found in the literature.

The concentration of the vanadium Versene complex is proportional to the diffusion current up to a concentration of 1.3×10^{-3} formal, when measured at -1.560 volt *vs.* the S.C.E., and a pH of 10.0. Results of the measurements of vanadium in the presence of molybdenum and tungsten were given. Molybdenum gives a double wave in acid solution at a pH 2.8. The half-wave potentials are -0.244 and -0.608 volt *vs.* the S.C.E. The heights of the waves are proportional to the molybdenum concentration up to a concentration of 1.1×10^{-3} formal, when measured at -0.450 or -0.850 volt *vs.* the S.C.E.

Analyses of various test solutions containing vanadium, molybdenum, and tungsten were reported.

High-Frequency Measurements as Applied to Titrimetry. H. W. Hamme, E. L. Grove, and J. L. Kassner, University of Alabama, University, Ala.

The high-frequency conductance and susceptance were measured for certain coil-type containers used in "high-frequency techniques" over a range of frequencies. At a certain narrow frequency band, or bands depending on the coil, there appears a sharp maximum, or maxima, of conductance accompanied by a sudden drop, then rise of the susceptance values. If pure water is added to the container (which represents an increase in the over-all capacitance of the coil) the conductance and capacitance maxima shift to a lower frequency. If the distilled water is replaced with a dilute electrolyte the peak, or peaks, is shifted to a still lower frequency. This narrow band represents a region of high sensitivity and appears to be a function of the type and size of the coil, the solvent and its concentration, and the distributive capacity between the coil and its case.

Different electrolytes caused the high-frequency conductance maximum to shift different amounts. Thus, the shape of various titration curves can be predicted, depending on whether the high-frequency conductance drops or rises to the salt value and then rises or drops as an increasing amount of the titrant is added.

Preliminary Studies of the Separation and Determination of Bismuth, Antimony, and Tin by Controlled Cathode Electroanalysis. John A. Dean and S. A. Reynolds, University of Tennessee, Knoxville, Tenn., and Oak Ridge National Laboratory, Oak Ridge, Tenn.

A preliminary study has been made of the separation and determination of bismuth, antimony, and tin by electrochemical means, using controlled cathode potential. The effects of electrolyte composition, cathode potential, and temperature were reported, with respect to separations of the three metals and the degree of recovery of each. The use of radioactive tracers in testing separations was described. Best results were obtained by use of an electrolyte containing sulfate and citrate with hydrazine as depolarizer. A procedure was reported which will serve for approximate determination of bismuth, antimony, and tin in alloys and other mixtures.

Peak Volume-Concentration Concept in Organic Acid Chromatography Using Progressively Changing Solvents. Charles Mader, Oklahoma A. & M. College, Stillwater, Okla.

A general volume-concentration equation was derived and proved experimentally. The peak volume-concentrations of the acids were found to be described by characteristic equations. The use of the interrelation of the peak volume-concentrations of the acids and the factors influencing the choice of a system for separating a particular group of acids was shown to have theoretical basis.

Chromatographic Fractionation of Wood Carbohydrates. Robert Roy Bridges, Masonite Corp., Laurel, Miss.

The complex mixture of water-soluble carbohydrates, commercially known as Masonoid, resulting from the hydrolytic degradation of wood hemicelluloses in the production of fiber by the Masonite process has been partially resolved into 20 individual simple sugars and oligosaccharides by the application of a combination of the charcoal column chromatography of Whistler and Durso and large sheet paper chromatography. Ten of the compounds are known and have been identified by paper chromatographic comparison of the original or its hydrolysis products or by physical constants. The remaining ten compounds are not definitely identified except with respect to their size and the simple sugar units of which they are composed, both of which are obtained from paper chromatographic evidence.

The simple sugars isolated are xylose, arabinose, glucose, galactose, and mannose and the oligosaccharides are made up of units of these sugars (except arabinose) arranged either in polymers of different sugar units or the same sugar units ranging in size from two to six units.

Oligosaccharides larger than six units are not readily separated by charcoal column chromatography or resolved by paper chromatography. That portion of Masonoid that is unresolved is in this classification.

Analysis by Alumina Adsorption of the Aromatics Boiling Above 400° F. in Jet Fuels. C. M. McKinney and R. L. Hopkins, U. S. Bureau of Mines, Bartlesville, Okla.

To provide information regarding the composition of fuels in the jet-engine fuel (JP-4) boiling range, partial analysis by alumina adsorption was attempted of the material boiling above 400° F. Fuels from 11 crude oils from California, Illinois, Nebraska, Oklahoma, and Texas were prepared and analyzed. The crude oils were selected to provide fuels with considerable variation in composition.

The aromatic portion of each fuel was separated from the paraffin-naphthene portion using a conventional silica gel adsorption technique. A high-dilution alumina adsorption technique using selective desorption for separation of the aromatic portion into fractions was described. Tables of density and refractive index data compiled from various literature sources for compounds in the boiling range studied were given for comparison with similar data determined on each of the aromatic fractions from which adsorptograms were plotted to illustrate the degree of separation obtained.

Smoke point determinations were made on the original fuels, the original fuels less the aromatics boiling above 400° F., and the original fuels less the polycyclic aromatics boiling above 400° F.

Spectroisotopy. Applications of High Resolution Spectroscopy. J. Rand McNally, Jr., Oak Ridge National Laboratory, Oak Ridge, Tenn.

This paper covered recent applications of high resolution spectroscopy in the fields of isotope spectrum shifts and nuclear hyperfine structure. The former give information concerning nuclear volume effects in the heavy elements and also lend themselves to isotopic assay applications. Nuclear hyperfine structures are used to interpret problems concerning the mechanical, electric, and magnetic properties of the nucleus. Slides were shown on the use of high resolution concave grating spectrographs, echelle spectrographs, and Fabry-Perot interferometers in conjunction with hollow cathode and gaseous discharge sources, as well as the more common spectrochemical type of light sources.

Mass Spectroscopy. B. W. Thomas, Humble Oil & Refining Co., Baytown, Tex.

Notable recent advancements toward development of the mass spectrometer into a widely used and highly respected laboratory analytical tool were reviewed. New applications and improvements in analysis techniques of mass spectrometry were described. Needs for more rapid method of obtaining correlation spectra and achieving per cent compositional results was discussed. Production model process mass spectrometers were described briefly, and mention was made of a few potential applications of these new version instruments to flowing gaseous and liquid streams. Relative merits of laboratory versus process mass analysis instruments were set forth and opinions were offered concerning indicated future trends in the field of mass spectroscopy and rapid multicomponent analysis by instrumentation.

Emission Spectroscopy. E. E. Creitz, U. S. Bureau of Mines, University, Ala.

A review of several new applications of emission analysis with some emphasis on methods useful for inorganic, nonferrous samples.

Raman Spectroscopy. Forrest F. Cleveland, Illinois Institute of Technology, Chicago, Ill.

When molecules scatter monochromatic light, the Raman spectrum so obtained is characteristic of the molecules and therefore may be used in qualitative and quantitative analysis of the sample. The Raman spectrum, in conjunction with the infrared spectrum, also may be used to determine the structure of the molecules. A reliable determination of the depolarization factors of the Raman lines, and a careful normal coordinate treatment, are of importance in the determination of the fundamental frequencies and their degeneracies. Once these are known, the heat capacity, free energy, heat content, and entropy can be calculated from the spectral data.

Nuclear Magnetic Resonance Spectroscopy. James N. Shoolery, Varian Associates, Palo Alto, Calif.

The fundamental principles of nuclear magnetic resonance spectroscopy were developed, and typical instrumentation was described. Two general techniques were discussed. First, a measure of the total number of nuclei of a given isotope can be obtained from studies of broad lines, using rapid scanning and narrow-band coherent detection. The second technique, high resolution nuclear magnetic resonance spectroscopy, is capable of separating very narrow signals from chemically nonequivalent protons or fluorine nuclei in liquid samples, using an extremely homogeneous polarizing magnetic field and slow scanning. Typical results were displayed and discussed in terms of present theories of molecular structure.

Applications of Ultraviolet Spectroscopy. Norman D. Coggeshall, Gulf Research & Development Co., Pittsburgh, Pa.

Ultraviolet absorption generally occurs only in systems involving chemical unsaturation called chromophores. An example of a chromophore is the benzene ring. Changes in structure involving substitution on a chromophore through saturated bonds produce secondary alterations of spectra. Thus the various alkyl benzenes possess spectra which are generically the same. The joining of two chromophores in such a way that each may influence the other will give rise to a new type of absorption. Thus the substituted naphthalenes possess fundamentally different spectra from the substituted benzenes. These effects define the applications and limitations of ultraviolet absorption to molecular structure determination and to analytical applications. The secondary alterations of spectra will in some cases allow analysis for individual members of a homologous series. On the other hand, the basic similarities of spectra for members of a class characterized by the same chromophore will at times allow determination of total functional group type concentration. Particular cases of the various applications were discussed.

Society of Public Analysts

An ordinary meeting of the Society of Public Analysts and Other Analytical Chemists was held in London on October 7, when the destruction of organic matter was discussed.

Preparation of Biological Material for the Determination of Trace Metals. Method for the Destruction of Organic Matter in Biological Material. G. Middleton and R. E. Stuckey, The British Drug Houses Ltd., Graham St., London N. 1.

The satisfactory destruction of biological tissues before the determination of trace metals was often difficult, particularly when the quantities of such metals were relatively small and large amounts of organic material have to be taken. In such circumstances dry ashing presents difficulties and methods of destruction with chlorine oxy acids were unsatisfactory. Procedures involving sulfuric and nitric acids often produced compounds resistant to oxidation, and a method was developed in which nitric acid was the only reagent used in quantity. On heating biological material such as liver with diluted nitric acid it was dispersed in a short time with the evolution of a comparatively small quantity of oxides of nitrogen. On further heating this solution evaporated or boiled until, at a certain concentration, it became sirupy and began to evolve a gas, at first colorless, later brown; the mass then darkened and left a black "char." If this residue was treated with a small amount of nitric acid, brown fumes were evolved, and the mixture swelled into a froth and ultimately left a hard black residue. Successive repetitions of this treatment with further small quantities of nitric acid and heating on a hot plate at 300° to 350° C. resulted in the production of a white residue that did not char on heating. Variations of the procedure necessary for different materials were discussed. Advantages claimed for the method were that relatively small amounts of reagents were used and the temperature did not exceed 350° C. The application to the destruction of a number of organic products was described.

Determination of Lead in Foodstuffs. H. C. Lockwood, Cadbury Brothers Ltd., Bournville, Birmingham 30.

The reagent diethylammonium diethyldithiocarbamate used by the subcommittee of the Society of Public Analysts and Other Analytical Chemists for the extraction of zinc [*Analyst*, **73**, 304 (1948)] was found quantitatively effective with lead at a pH of 7.0. This meant that lead can be extracted from solutions prepared from the ash of foodstuffs rich in phosphates at a pH at which phosphates were not precipitated. This applied particularly when magnesium was also naturally present, as with cocoa products. The lead complex was decomposed with acids and adjusted to a pH of 9. Finally, the lead was determined by extractive titration with a 0.0035% solution of dithizone in chloroform, 1 ml. being approximately equivalent to 0.5 p.p.m. of lead in a 10-gram sample.

The procedure offered greater accuracy than was attainable with the usual sulfide technique or with the Spekker absorptiometer on dithizone solutions. Tests on several commodities to which 2 or sometimes 3 p.p.m. of lead were added indicate 80 to 95% recovery. Results for a number of foodstuffs were given.

On October 23 the Physical Methods Group met in Southampton to discuss paper electrophoresis.

Analysis of Inorganic Compounds by Electromigration and Electrochromatography. F. H. POLLARD, Department of Physical and Inorganic Chemistry, The University, Bristol.

The subject was introduced by reference to early experiments of O. Lodge and J. Kendall, and the conditions affecting electromigration and the types of cells used for inorganic separations were described. Examples of separation achieved on paper and in agar jelly were given. The effect of complex-forming agents and the use of columns were described.

The combination of electromigration with solvent flow chromatography was described. The types of cell used for discontinuous and continuous separations, the principles involved in continuous and discontinuous procedures, and examples of the separations attained by such processes were given. One-way, two-way, and three-way separations and the use of complex-forming reagents were described.

The methods have been applied to the separation of rare earths and radioactive elements from other inorganic material. The use of ion exchange resins in electromigration was also described.

Use of Paper Electrophoresis in the Study of Nucleic Acids. ROY MARKHAM, Agricultural Research Council, Plant Virus Research Unit, Molteno Institute, Cambridge.

The degradation products of nucleic acids produced by chemical or enzymatic hydrolysis were extremely difficult to separate by chromatographic methods. This was largely because they occur as families of compounds having very similar structures and differing only in their purine and pyrimidine composition. Fortunately, these bases contained specific ionizing groups, and by taking advantage of their differing pK values it was possible to effect the separation of the various compounds by electrophoresis on paper.

Although to some extent empirical, the method had the great advantage that predictions as to the relative rates of movement of various compounds could be made with some confidence, and conversely the method could be used to detect the presence or absence of certain groupings in the molecules by the use of very small amounts of material. This was analogous to electrometric titration, which required many times the quantity of the substance.

Besides its purely analytical use, the paper electrophoresis method could be used in the preparation of reasonably large amounts of pure materials, and by the use of high voltage gradients, combined with adequate cooling, such separations could be carried out in 1 to 3 hours. The design of an inexpensive high-voltage paper electrophoresis apparatus was described.

Paper-Strip Electrophoresis of Serum Proteins. A. L. LATNER, Department of Pathology, Royal Victoria Infirmary, Newcastle-on-Tyne 1.

The apparatus and technique for paper-strip electrophoresis were briefly described. A brief account was given of the methods of staining and scanning used by the author, including a simple visual method of scanning. The procedure has proved quantitative and evidence for this was given. The method cannot be strictly compared with the classical Tiselius procedure on serum. This also estimated lipoid material in combination with proteins, which did not affect the paper-strip method.

Results obtained with the sera of different animals were discussed, as interesting differences between species have been observed. Some applications of the technique to problems of human disease were given.

At an ordinary meeting of the Society held November 4 in London, the following papers were read:

Absorptiometric Determination of Niobium in Some African Low-Grade Minerals and Mineral Dressing Products. G. W. C. MILNER AND A. A. SMALES, Atomic Energy Research Establishment, Harwell, Dr. Didcot, Berks.

A method was described for the determination of between 0.05 and 16% of niobium pentoxide in head samples and mineral dressing fractions produced during treatment of Sukulu soils (mainly magnetite, apatite, and quartz) and Nigerian granite (mainly quartz and cryolite). After chemical attack on the sample, the niobium was separated from the bulk of the other materials by precipitation with tannic acid and cinchonine with silica as carrier, and was finally determined absorptiometrically with potassium thiocyanate. The separation steps need not be quantitative because radiometric correction for losses was made by incorporating niobium-95 tracer in the procedure.

Absorptiometric Determination of Niobium in Some African Low-Grade Ores. A. E. O. MARZYS, Research Division, Uganda Development Corp., Ltd., P.O. Box 18, Entebbe, Uganda.

The absorption of light in a region near to the ultraviolet by the reduced niobium-thiocyanate complex in an organic medium was made a basis for the determination of niobium in low-grade ores and minerals. The merits of a mixture of acid acetone and of ether extract as a solvent were described. The method was adapted for use with a Spekker absorptiometer provided with a mercury vapor lamp. Four modifications of the main procedure were described for use in the presence of certain interfering elements. Results for a variety of African soils and minerals were compared with those obtained by gravimetric methods. The precision of the method was as good as, and probably better than, that of gravimetric methods when applied to complex soils and rocks of low niobium content. The speed and ease of manipulation were greatly increased. By suitable adjustment of the amounts of material used, the method can be extended to all niobium minerals. The ratio of niobium to tantalum in mixed oxides can be rapidly found by determining niobium and titanium colorimetrically and calculating the tantalum content by difference.

Spectrographic Determination of Niobium and Tantalum in Sukulu-Type Soils. C. S. CAMPBELL AND D. NICHOLAS, Fulmer Research Institute, Ltd., Stoke Poges, Bucks.

A solution method for the spectrographic determination of niobium and tantalum in a low-grade ore was described. After an initial hydrochloric acid extraction, the sample residue was taken up in hydrofluoric acid. Molybdenum was added as internal standard and the solution was fed into a condensed arc discharge from a porous cup electrode. The lines Nb 2698 A. and Mo 2688 A. give a standard deviation of about 2%; tantalum is present in much smaller concentration and is estimated visually by using the line Ta 2685 A.

Inorganic Chromatography on Cellulose. Shortened Method for Determination of Niobium and Tantalum in Minerals and Ores. R. A. MERCER AND R. A. WELLS, Chemical Research Laboratory, Teddington, Middlesex.

A chromatographic procedure for the extraction of the mixed oxides of tantalum and niobium from minerals and ores was described. A solution of the sample in hydrofluoric acid containing ammonium fluoride was absorbed on cellulose and transferred to a 3-inch column of cellulose. Complete extraction of the niobium and tantalum was attained by passing 400 ml. of methyl ethyl ketone, containing 15% v./v. of 40% hydrofluoric acid, through the column. The separation was complete from all metals other than tungsten. The two oxides, recovered from this solvent, are subsequently separated by further chromatography or estimated without separation by a suitable colorimetric procedure.

Colorimetric Estimation of Niobium and Tantalum with Pyrogallol. E. C. HUNT AND R. A. WELLS, Chemical Research Laboratory, Teddington, Middlesex.

Colorimetric procedures for the estimation of niobium and tantalum in mixtures of the oxides of two metals were described. The method was applied to the estimation of the two metals in mixtures obtained by their chromatographic extraction from minerals and ores. The determinations were based upon the colored complex formed between tantalum and pyrogallol in acid solution and between niobium and pyrogallol in alkaline solution. Both systems obey Beer's law and, with 1-cm. cells, the optimum limits of concentration were 0 to 20 p.p.m. for niobium and 0 to 80 p.p.m. for tantalum. The effect of variation in pH and the interference of a number of cations and anions was recorded.

Inorganic Chromatography on Cellulose. Rapid Method for Determination of Niobium in Low-Grade Ores. E. C. HUNT AND R. A. WELLS, Chemical Research Laboratory, Teddington, Middlesex.

A rapid and simple method was described for the determination of niobium in a hydrofluoric acid solution of an ore; it makes use of upward diffusion on a paper strip. The niobium was detected as a yellow band by spraying the strip with tannic acid. An accurate estimation of niobium can be made by direct visual comparison of the band with standard strips. Alternatively, a simple densitometer, which was described, permitted a direct estimation to be made of the niobium on the strip. The chromatographic separation took 20 minutes, and a simple technique for running 10 separations simultaneously was described. An accuracy of 10% was attained for ores containing 0.16 to 0.6% of niobium pentoxide.

The Analyst's Calendar

LSU Symposium on Analytical Chemistry

The seventh annual Louisiana State University Symposium on Modern Methods of Analytical Chemistry will be held February 1 to 4, 1954 at Baton Rouge, La. A group of internationally recognized speakers have been secured to discuss their fields of specialization and a broad selection of important topics will be considered. The program will include discussions of both instrumental and noninstrumental methods.

In addition to the formal program, time will be provided for informal discussions. Representatives of scientific supply houses will be present, and exhibits of instruments are planned. Special time will be allotted for the discussion and demonstration of new instruments, and tours will be scheduled through local industrial plants and university laboratories.

The following program has been arranged:

Organic Reagents. Charles V. Banks, Iowa State College, Ames, Iowa.

Mass Spectroscopy. Willard H. Bennett, Naval Research Laboratories, Washington, D. C.

Extraction. Lyman C. Craig, Rockefeller Institute for Medical Research, New York, N. Y.

Applications of Newer Physical Methods to High Polymer Analysis. Peter Debye, Cornell University, Ithaca, N. Y.

Complex Compounds. Hans Jonassen, Tulane University, New Orleans, La.

Ion Exchange. Robert Kunin, Rohm & Haas Co., Philadelphia, Pa.

X-Ray. William Parrish, North American Phillips Co., Inc., New York, N. Y.

High Frequency and Coulometric Titrations. Charles N. Reilley, University of North Carolina, Chapel Hill, N. C.

Raman and Infrared Spectroscopy. E. J. Rosenbaum, Sun Oil Co., Marcus Hook, Pa.

Preregistration is requested where possible. The registration fee is $10, including ticket for the mixer and banquet. The registration fee for students is $2 (without banquet ticket).

The university has established facilities for use as a continuation education center and has reserved rooms on the campus. The charge is $2 per day per person. Room reservations on the campus can be made at the time of preregistration. Those desiring to stay in the downtown area should write directly to the Hotel Heidelberg.

All correspondence should be addressed to: Philip W. West, Coates Chemical Laboratories, Louisiana State University, Baton Rouge, La.

New York Meeting-in-Miniature

The New York Section of the American Chemical Society has planned an all-day Meeting-in-Miniature on February 12 at Hunter College, New York, N. Y. There will be separate sessions in the following divisions: analytical chemistry, biological chemistry, organic chemistry, inorganic chemistry, physical chemistry, high polymers, microchemistry, chemical engineering, and chemical education.

Scientists from New York and contiguous areas are invited to submit papers. Titles and abstracts of about 200 words should be sent to Nathan Weiner, Chairman, Endo Products, Inc., 84–40 101 St., Richmond Hill 18, N. Y. December 28 has been set as the *deadline for receipt* of papers.

Symposium on Recent Advances in Science

The First Annual Symposium on Recent Advances in Science, Physics, and Applied Mathematics, will be sponsored by the Division of General Education, New York University, during a 15-week course to be given Monday evenings from February 8 to May 24. Designed for technically qualified persons who wish to appreciate contemporary scientific developments, the symposium will present a leading man of science each week, with ample time for discussion. Information may be obtained from Sidney G. Roth, Division of General Education, New York University, 3 Washington Square North, New York, N. Y.

Corrections

Spectrographic Determination of Trace Elements in Lubricating Oils

In the article on "Spectrographic Determination of Trace Elements in Lubricating Oils" [Anal. Chem., **25**, 151 (1953)] the gravimetric factor was inadvertently used for converting chromium trioxide (CrO_3) to chromium, rather than the factor for chromic oxide (Cr_2O_3). Thus the percentage of chromium in the master mixture shown in the first table in column 2, page 152, should be 7.14 rather than 5.43.

R. F. Meeker

Determination of Germanium

In the article on "Determination of Germanium" [Anal. Chem., **23**, 1023 (1951)] on page 1024, line 6 in the paragraph on "Effect of Hydrogen Peroxide" should read: "1 mg. of hydrogen peroxide to 3 mg. of hematoxylin."

Hanna Newcombe

In the paper "Fractionation of Barium-Radium Mixtures as Chromates by Precipitation from Homogeneous Solution" [Salutsky, M. L., Stites, J. G., Jr., and Martin, A. W., Anal. Chem., **25**, 1680 (1953)], Figures 2 and 3 were interchanged. The captions are correct as printed. The ordinate of Figure 2 is Logarithmic Distribution Coefficient and the ordinate of Figure 3 is Limiting Distribution Coefficient.

In the article on "Titrimetric Determination of Thorium" [Fritz, J. S., Anal. Chem., **25**, 1640 (1953)], the name of the author is James S. Fritz.

Fifth Annual Southeastern Symposium on Industrial Instrumentation. University of Florida, Gainesville, Fla., February 1 to 3

Instrument Society of America. Ninth Annual Regional Conference. Hotel Statler, New York, N. Y., February 4

Meeting-in-Miniature, New York Section, American Chemical Society. Hunter College, New York, N. Y., February 12

American Chemical Society. 125th National Meeting, Kansas City, Mo., March 24 to April 1, 1954

Symposium on Instrumentation. University of Michigan, Ann Arbor, Mich., May 24 to 27, 1953

AIDS FOR THE ANALYST . . . . .

Rapid Filtration and Drying Technique.

Raymond H. Pierson, Analytical Chemistry Branch, U. S. Naval Ordnance Test Station, Inyokern, China Lake, Calif.

Solutions containing partially hydrolyzed cellulosic solids or other gelatinous precipitates are frequently difficult to filter through ordinary folded filter papers, Gooch crucibles, or sintered glass. A simple apparatus for achieving rapid filtration of such solutions and quantitative washing has been widely used but is often overlooked. Most of these "difficult" solutions will filter easily through a paper thimble supported in a filter tube similar to Corning Glass Works item 9480. Moderate suction may be applied if the paper thimble is cushioned with a plug of glass wool.

The filtration step is sometimes to be followed by extraction in a Soxhlet with a solvent different from the one employed during filtration. This means evaporation of all the first-used solvent from the paper. Complete removal of water or organic solvents may require 4 hours or even longer by the procedure of drawing air through the assembly at room temperature or by oven drying. Especially in the case of thimbles which contain large cellulosic precipitates, removal of water or even low boiling solvents such as methylene chloride is very tedious. Thorough drying may be accomplished in half an hour merely by placing the tube on its side in an oven and drawing heated oven air through it by means of a rubber suction line run into the oven through a small hole and attached to the small end of the filter tube.

The determination of the oil content of water samples by one of the commonly used procedures may be considered a typical application of the technique described. In essence the method depends on collecting the oil on precipitated ferric hydroxide, filtering, drying, and extracting with ethyl ether. The oil collected in this way from boiling solution is not low-boiling-point material and may be dried by the oven-suction procedure

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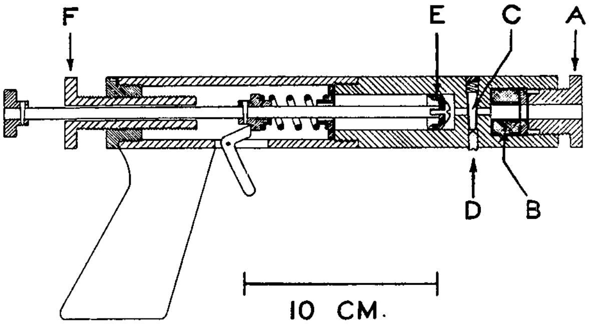
Sampling Gun for Spectrographic Pin Samples.

H. H. Grossman, C. B. Pratt, and W. S. Myers[1], Spectroscopy Laboratory, The Dow Chemical Co., Midland, Mich.

A sampling gun for drawing molten magnesium alloys into borosilicate glass tubing for spectrographic pin samples has been in use at the Magnesium Alloy Plant, Dow Chemical Co., since June 1946. A cross section of a cocked gun is shown in the diagram.



In operation, a piece of tubing between 5.0 and 5.4 mm. in inside diameter and 20 cm. long is placed in the end of the gun and secured by compressing the rubber bushing, *B*, around it by the thumb nut, *A*. The sample is drawn into the tubing by dipping it approximately 0.5 inch into molten metal from which the flux has been temporarily cleared, and triggering the gun. The trigger releases the spring-loaded rod, which causes motion of the leather-cup piston, *E*, thereby creating the partial vacuum for sampling.

Initial gun adjustments are made by adjusting length of piston travel by screw *F*, so that water is drawn into a sampling tube to a height of 7 ± 0.5 cm. Further adjustments are made by adjusting screw *D*, which controls rate of draw-up by determining the amount of clearance around the taper pin, *C*. During draw-up, air flows from the sampling tube to the cylinder through a small opening formed by this clearance. For a satisfactory sample, the rate of draw-up depends upon the aluminum concentration in the magnesium alloy as well as upon other factors such as temperature of the molten metal.

The rate of draw-up can be adjusted to operate satisfactorily in one of three groups of aluminum concentrations in the alloys: 4% and over, between 4% and 1%, and 1% and below. Initially the draw-up rate is adjusted using water; the proper draw-up times for the three groups are approximately 0.5, 1, and 2 seconds. Final valve adjustments are made when sampling metal, with the object of obtaining solid samples about 7 cm. long. The Magnesium Alloy Plant has guns adjusted for use in each of the three groups. If the rate of draw-up is appreciably less than optimum, a short sample will be obtained which cannot be made into two electrodes and sparked with convenience. Samples drawn appreciably faster than optimum are usually hollow or porous. The degree of tolerance required in the draw-up rate for each group was small enough to prevent successful application of a rubber bulb for drawing the metal.

After service, some guns will begin to draw samples shorter than standard, owing to partial plugging of the valves by dirt. To correct these guns it is more convenient to adjust plunger travel to correct sample length than to alter the sensitive valves. Periodically guns are cleaned and reset with water as described.

It is believed that this type of gun can be used for sampling alloys of other base metals.

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Glass Atomizer for Use in Chromatography.

W. J. Wingo, Biochemistry Department, The University of Texas, M. D. Anderson Hospital for Cancer Research, Houston, Tex.

A nonmetallic atomizer is often needed in paper chromatographic analysis—for example, the method of Berry (*1*) requires that the chromatogram be sprayed with sodium hypochlorite, and several other methods require the spraying of corrosive agents. Commercially available glass atomizers are rather expensive, and a previously published design (*2*) for an atomizer to be constructed in the laboratory requires a ground-glass joint.

About a year ago, a glass atomizer was built in this laboratory to use in detection of urea on paper chromatograms by the method of Berry (*1*); it performed so well that the author made several of them, and has since that time used them in all his chromatographic spraying. It is easy to make (a fair amateur glass blower can make one in about half an hour, including the time required for cooling at several intermediate stages and the time for final annealing); it delivers a good volume of a spray having a small droplet size, and requires only a few pounds per square inch of air pressure for operation. (An atomizer typical of this design will begin to spray when supplied by air at 7 pounds per square inch; it works very well at 10 pounds per square inch, spraying at this pressure about 5 ml. per minute.)

The construction of the atomizer is shown in Figure 1.

A bulb, *A*, is blown on the end of a piece of 3-mm. borosilicate glass tubing. The tubing is cut about 1.5 inches below the bulb, and the cut end is lightly fire-polished. The bulb is sealed into a piece of 9-mm. tubing, *B*, and a bulge, *C*, is blown in the larger tubing just below the ring seal. While the seal is still plastic, the tubing is manipulated so that the inner and outer tubes are concentric; the assembly is allowed to cool and the outer tube is constricted, *D*, leaving a clearance of about 1 mm. between the inner and outer tubes.

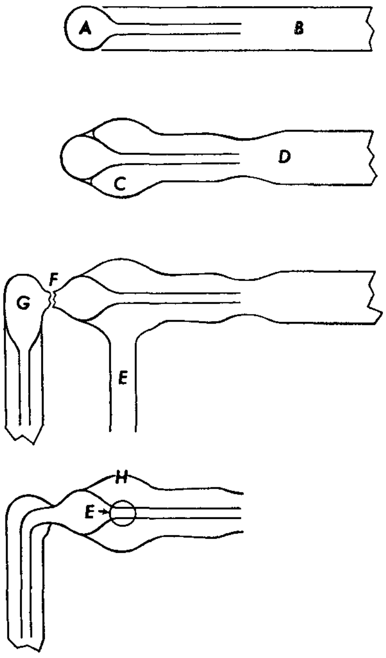

Figure 1

A side arm for the air hose, *E*, of 7-mm. tubing, is now attached, and a hole about 5 mm. in diameter is blown in the bulb, *F*. The liquid feed tube, *G*, is prepared as shown from capillary tubing 2 mm. in inside diameter and sealed to *F* as shown. Finally, a control hole, *H*, 5 mm. or larger in diameter, is blown in the top of the bulge, and the outer tube is cut off and ground down to be flush with the end of the inner tube. Inner and outer tubes of the atomizer jet are now made concentric by warming in a flame and adjusting as needed, and the assembly is annealed. If the clearance between inner and outer tubes is found to be too large, it can be reduced by careful fire-polishing.

The atomizer is usually used by fixing the fluid feed tube in a two-hole rubber stopper, so that the fluid feed reaches nearly to the bottom of a 250-ml. Erlenmeyer flask; if desired, the liquid feed can be ring-sealed through a ground-glass cap for a flask or bottle to give an all-glass system.

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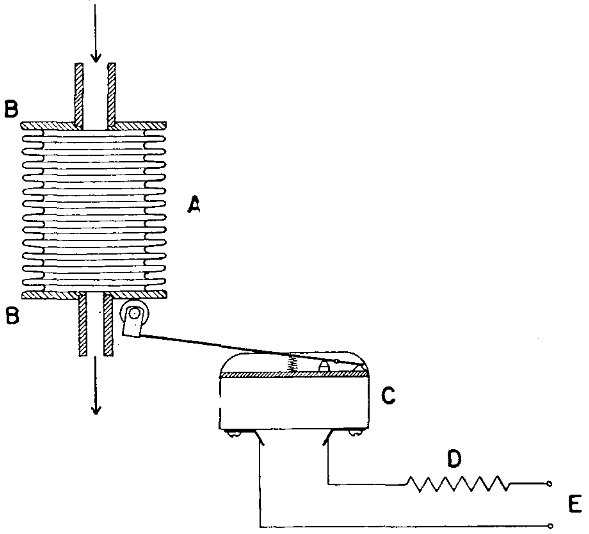
Safety Switch for Water-Cooled Systems. Walter Roth, Walker Laboratory, Rensselaer Polytechnic Institute, Troy, N. Y.

A simple safety switch has been designed for use with any electrically heated apparatus which requires circulation of cooling water. It is being used successfully in this laboratory as a safety control on a water-cooled, mercury diffusion pump which operates unattended through the night. For some unknown reason, water pressure has fallen off during the night on several occasions. Without a safety switch to shut off the heating current to the pump, there existed the danger of distilling out large quantities of mercury. With water-cooled, oil diffusion pumps, there is the additional danger of pyrolysis of the oil. A switch of this type would also be useful on distillation or reflux apparatus.

The control element consists of a double-ply brass bellows, *A* (supplied by The Robertshaw Fulton Co., Fulton Sylphon Division, Knoxville, Tenn.) having twelve corrugations, outside diameter of 1.5 inches, and an over-all length of 2 inches, in each end of which has been soldered a circular brass plate, *B*, $^1/_{16}$ inch thick. The top plate has a counterbored hole $^1/_4$ inch in diameter drilled through its center, into which a 2-inch length of copper tubing $^3/_8$ inch in outside diameter has been soldered. In like manner, the bottom plate has a $^1/_8$-inch hole at its center and a copper tube $^1/_4$ inch in outside diameter leading from it. The upper tube is clamped to a rigid support. A Type W, lever arm microswitch, *C*, with contacts rated at 15 amperes at 125 volts, is clamped to a vertical rod and mounted so that the end of the lever arm is below the bottom plate of the bellows. The switch is connected in series with the heater to be controlled, *D*, and the voltage source, *E*. A rubber tubing connection is made between the cooling water outlet from the apparatus and the $^3/_8$-inch tube at the top of the bellows. The $^1/_4$-inch tube at the bottom of the bellows is led to a drain.



When water is flowing through the apparatus, the bellows expands as a result of the flow restriction offered by the smaller outlet tube. The height of the microswitch is adjusted so that the switch is depressed to the on position when the desired rate of coolant flow is obtained. A decrease in the rate of flow causes the bellows to contract toward its normal position and results in the interruption of the heating circuit. The microswitch height can be adjusted to give cut-off at any desired flow or at complete cessation of flow.

The use of a similar type of instrument in conjunction with a pressure-protective circuit for vacuum systems has been described [Amdur, I., *Rev. Sci. Instr.*, **18**, 66 (1947)].

Vapor Pressure Ebulliometer for Milliliter Samples. Sam R. Hoover, Harry John, and Edward F. Mellon, Eastern Regional Research Laboratory, Philadelphia 18, Pa.

In studies of the separation of amino acids by distillation of the *N*-acetyl amino acid esters, the determination of the boiling point–vapor pressure relationship of a number of samples was required. Measurements on small samples (<2 ml.) at temperatures of 75° to 250° C. and pressures of 2 to 100 mm. were the essential conditions which had to be met. A survey of the literature revealed no technique that fulfilled these requirements. After numerous unsuccessful attempts to employ the micro-method of Garcia (*1*), the apparatus shown in Figure 1 was developed. It is a simple adaptation of the Cottrell principle. The larger and more elaborate apparatus described by Willingham *et al.* (*6*) undoubtedly can be used to produce results of great accuracy in the characterization of organic compounds available in quantity. Willard and Crabtree have described a Cottrell-type apparatus which more closely approaches the present design than others, but its use at reduced pressures was not reported (*5*). Ebulliometric measurements and vapor-pressure tensimeter stills have been reviewed recently (*4*).

APPARATUS AND METHOD

The boiling pot and column are of 15-mm. borosilicate glass tubing. The reflux bell is about 35 mm. in diameter. The reflux return tube is 4 mm. in diameter. The side arm, of 10-mm. tubing, has a slight downward slope for about 25 mm., then slopes upward for 50 mm., and has a 25-mm. horizontal portion (out of the photograph) on the end. The 10/30 standard-taper borosilicate glass joint takes a commercial 125-mm. immersion thermometer. The only critical dimension is that which places the thermometer bulb just below the lip of the boiling pot. A 10- to 15-mm. length of 0.5-mm. tungsten wire is sealed through the bottom of the pot. The completed apparatus should be thoroughly annealed.

Apparatus is supported by a clamp around the rubber tubing of the vacuum line on the side arm. The lower portion of the side arm serves as a receiver and the upward sloping portion serves as a condenser. A stream of water is run onto a cloth which is placed over the upward sloping portion of the arm and is drained off through a funnel placed underneath. The tungsten wire projects through a sheet of asbestos paper which is supported immediately below the apparatus. This wire is heated by a microburner, and serves as a point source of heat which minimizes bumping. A partial shield of asbestos around the apparatus may be required at temperatures of 200° C. and above, especially if the apparatus is operated in an air-conditioned or drafty room.

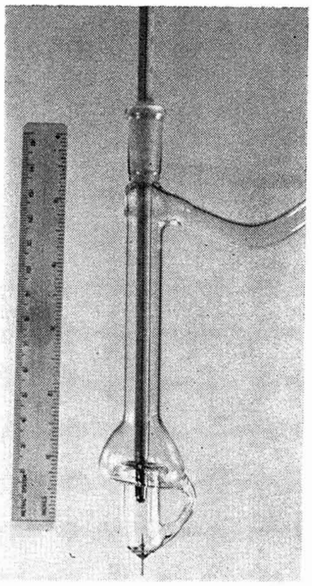


Figure 1. Semimicro-ebulliometer

The sample is added to the pot to a depth of about 10 mm. It should not touch the thermometer bulb even after expansion on heating. Silicone stopcock grease on the thermometer joint is used to lessen the possibility of contaminating the sample. The system is pumped down to the required vacuum, which is maintained by a manostat. The manostat described by Ratchford and Fein (*3*) was used successfully to control the pressure, which was measured by a Zimmerli gage (*7*) to 0.1 mm. A series of determinations at increasing pressures is made. A 10- to 15-minute period in which the reflux is maintained about half-way up the tube between the bell and side arm is sufficient to establish stable pressure and temperature readings. If the purity of the sample is questionable, about 20% of it is distilled into the side arm and the measurements are repeated.

The thermometer is easily calibrated by determining the boiling points of pure compounds which are accepted standards for thermometer calibration. This calibration can be made at atmospheric pressure. The temperatures, reported here, were read to 0.1°.

RESULTS

The ability of this ebulliometer to function as desired was checked by comparing the boiling point curve of a sample of pure capric acid with the data obtained on larger samples by Pool and Ralston (*2*). A log P versus $1/T$ plot of the results is shown in Figure 2. The line was obtained as the least square straight line, using all the points obtained by use of the new ebulliometer. Its equation is $\log P = 10.3002 - 3937/T$; 95% of the observed pressures fell within 4% of the pressure values given by the curve. The corresponding equation calculated from the data of Pool and Ralston is $\log P = 10.3719 - 3970/T$; 95% of their observed pressures fell within 9% of the pressure values given by the least square line for their data. Both sets of data are in excellent agreement. The slight difference in slopes and the better precision of the new data are probably due to the larger number of data obtained in this study and to the better control of pressure by modern pressure regulators.

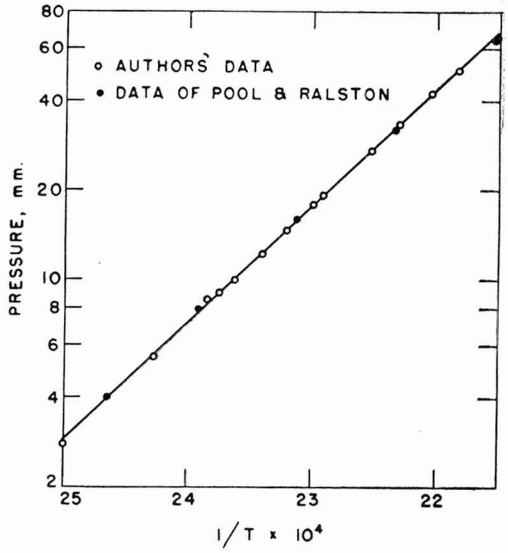


Figure 2. Vapor Pressure of Capric Acid

○ Authors' data
● Data of Pool and Ralston

This ebulliometer has also been used to determine the vapor pressure–temperature curves of nine acetylated amino acid esters; 70% of the experimental values are within 3% of the values calculated from the equation for the least squares line through the data in the range of 2 to 100 mm. These data will be described in detail at a later date.

This ebulliometer, therefore, is a simple and precise apparatus for the determination of the vapor pressures of milliliter quantities of materials by the boiling point method at all pressures from atmospheric downward.

ACKNOWLEDGMENT

The authors are indebted to W. S. Port of this laboratory for supplying the sample of capric acid, and to M. C. Audsley for the photograph of the apparatus.

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Constant-Pressure Device for Gas Flow System.

Alban Charnley and Geoffrey L. Isles[1], Chemistry Department, Manchester University, Manchester, England.

When gas is flowing through a system to a region of lower pressure, as in a condensation process, the higher pressure must often be maintained at a constant value.

Constant-pressure devices such as rubber membranes (*1*) or buoyancy manometers (*2*) depend not only on the backing pressure but also on the rate of gas flow. A solenoid-controlled plunger has been used by Clow and Shand (*1*), but the actual cutoff was made of glass which would clearly not stand up to fast vibrations for long periods.

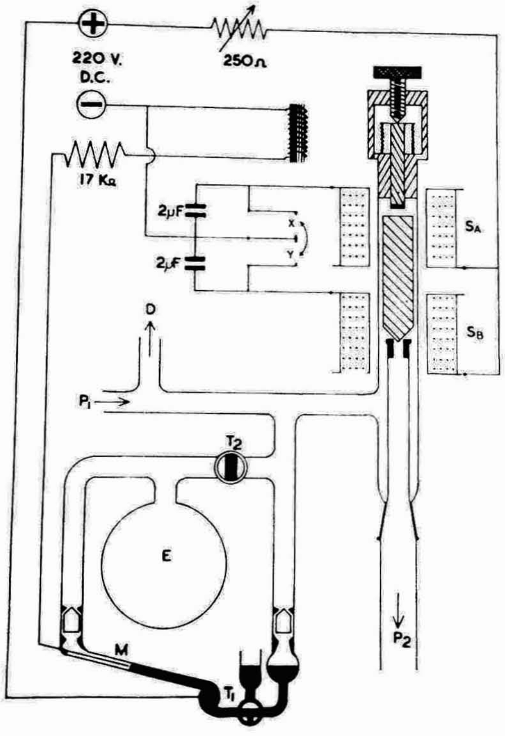

Figure 1

Instead of this the authors used a cone-tipped iron plunger and a seating made from a soft pencil eraser having a hole made by a standard (No. 2) cork borer. At the beginning of an experiment the relay is in position *Y* (Figure 1), and the bottom solenoid is then actuated. As soon as tap T_2 is opened, gas is allowed to flow into the 250-cc. bulb, *E*, until the desired working pressure is reached. T_2 is then closed. When any further pressure increase occurs in the system, the rising mercury in the sensitive manometer, *M*, makes contact and completes the circuit, causing the solenoid, S_A, to lift the plunger. When the pressure falls sufficiently, contact is broken and the bottom solenoid, S_B, pulls the plunger back.

An ordinary barometer control (*1*) is subject to violent oscillations even under steady flow conditions. In the controlling manometer the oscillations are damped by partially closing tap T_1 and the control is further improved by having the capillary (2 mm. in internal diameter) at a small angle to the horizontal. The sensitivity of such a device is clearly dependent upon the flow rate, backing pressure, and size of the hole in the cutoff. The effective size of the hole can be altered by varying the height of the plunger oscillation with a rubber-tipped brass bar, which can be externally adjusted using flexible copper bellows.

The rubber seating and the copper bellows unit are both held in position by vacuum wax. A 5-liter ballast bulb, *D*, is used with the higher flow rates and the manometer unit is well lagged; thermostating is essential if reproducibility is required over long periods.

The plunger is 10 cm. long with a diameter of 1 cm. and a taper of 45°. The solenoids are controlled by a telephone relay and sparking is reduced by 2 μf. condensers. Since the controlling solenoids are identical, solenoid S_B helps to pull the plunger back with an acceleration of at least 2*g* and so reduces the time lag as well as giving a better cutoff. Both solenoids have 10,000 turns, a resistance of 300 ohms, and internal diameters of 2.5 cm.

When P_2 equals 100 mm. of mercury and air is flowing through at the rate of 4 liters per minute at pressure P_1, the amplitude of the plunger vibration is about 1 mm. This gives 100 to 150 vibrations per minute, according to the fineness of the adjustment. The pressure variation measured in a mercury U-tube is a barely perceptible movement of the meniscus. The plunger always seats itself properly, as the clearance in the tube is only about 1 mm. The maximum gas flow used is 5 liters per minute.

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[1] Present address, Gas Research Department, Fuel Department, Leeds University, Leeds, England.

Simple Multiunit Magnetic Stirrer.

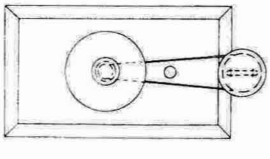
L. A. Wollermann and R. G. Tischer, Iowa State College, Ames, Iowa.

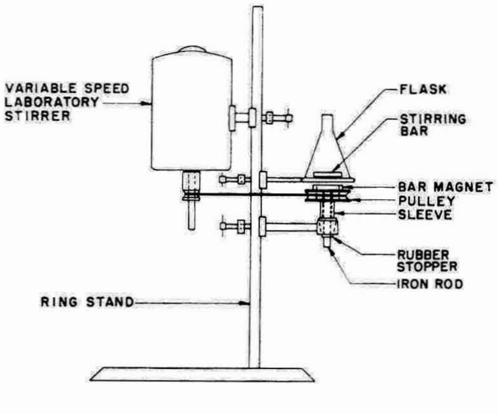
Having an immediate need for an additional magnetic stirrer, the authors assembled the apparatus from items commonly available in a chemical laboratory.

A small stirring motor, preferably one with a variable-speed drive, serves as the source of power and a small bar magnet wired across a pulley activates the stirring bar.

The shaft for the pulley is a short iron rod on which the pulley may turn freely. This rod is inserted in a rubber stopper and the stopper is mounted in a buret clamp with a sleeve or a number of washers between the stopper and the pulley to allow free rotation of the pulley.

The motor shaft may be grooved or, if not, a pulley may be mounted on it. A belt of rubber bands or heavy cord will be sufficient to turn the assembly, and the low speed at which magnetic stirring is commonly done obviates the need for bearings.





As only the pulley assembly need be mounted under the material to be stirred, a considerable saving of space is affected; moreover, the material is not subjected to the heat that would arise from the motor directly under the container. A number of such assemblies may be operated from one motor, thus enabling one source of power to serve in the handling of several samples simultaneously.

Journal Paper No. J-2283, Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1123.

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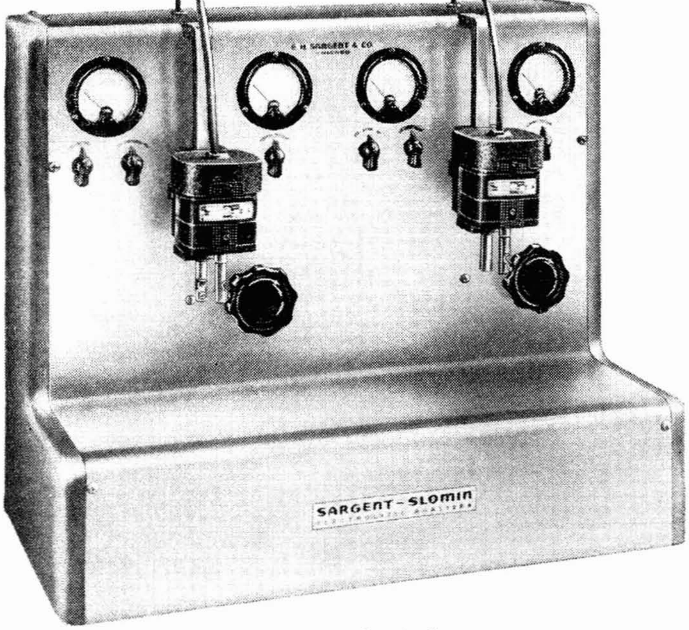

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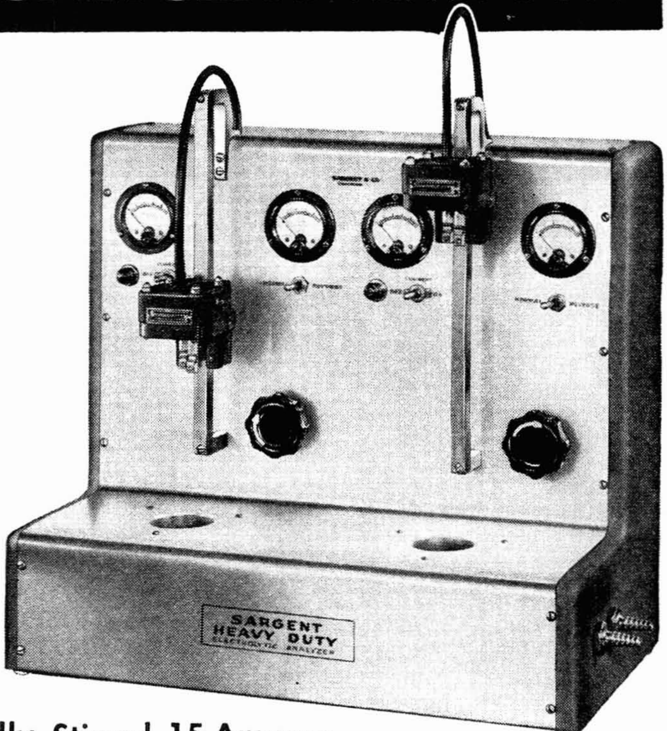

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Designed for continuous trouble-free performance, these electrolytic analyzers, manufactured by E. H. Sargent & Co., are durably constructed of the highest grade materials and component parts, including stainless steel front panel, cast aluminum end castings and stainless steel fittings.

Completely line operated, the Sargent analyzers employ self-contained rectifying and filter circuits. The deposition voltage between the electrodes is adjusted by means of autotransformers, with meters indicating volts and amperes and controls on the panel. An easily replaceable fuse guards against circuit overload.

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The Sargent Heavy Duty Analyzer provides efficient stirring by the interaction between the cell current and the field established by a permanent magnet, tubular in shape and coaxial with the cell holder.

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INSTRUMENTATION

Improved optical components, phototubes, and electronic circuitry facilitate use of spectrophotometric techniques, important in analytical determinations

by Ralph H. Müller

SPECTROPHOTOMETERS are indispensable tools for identification of light-absorbing substances and for analytical determinations in which characteristic light-absorbing entities can be formed in definite quantitative proportion by suitable reagents. The use of spectrophotometric techniques is widespread; indeed, it accounts for a considerable portion of all analytical determinations.

In recent years, exhaustive tests and intercomparisons have been made to estimate the reproducibility and calibration accuracy of commercial spectrophotometers. Most recent among such tests are results from the National Bureau of Standards [Brode, W. R., Gould, J. H., Whitney, J. E., and Wyman, G. M., *J. Opt. Soc. Amer.*, **43,** 862 (1953)]. This study was a cooperative effort involving 15 laboratories and afforded a comparison of 12 Cary recording spectrophotometers and 11 Beckman DU instruments. We quote the conclusions arrived at in this study:

> Ultraviolet and visible spectral absorption curves obtained on the Cary or the Beckman DU instruments are adequately reproducible.
>
> At wave lengths longer than 235 mμ there are random variations, usually not exceeding 0.02 unit, in the absorbance values obtained on the various instruments. Individual instruments give consistently slightly higher or lower readings (compared to the median value) at all wave lengths. These discrepancies are too small to be noticeable on spectra published in technical journals.
>
> The shape of the spectral absorption curve (which is of utmost importance in identification work) is adequately reproduced by instruments of both types.

In the body of the paper is another statement which we believe is still more significant:

> It must be emphasized that results obtained under these conditions are not truly representative of instrumental reproducibility, but are, in addition, subject to discrepancies introduced by the instrumental environment and the different operators. Enviromental effects may consist of such irregularities as fluctuations in line voltage, temperature changes, etc., while the operator errors are due to the individual differences in the filling and cleaning of absorption cells, and the balancing and checking of instruments, etc. It was, however, considered desirable to include all of these possible sources of nonreproducibility in the present comparison, in order to enable the research worker to estimate the value and the usefulness of the absorption spectra published in the scientific literature.

These, and related tests, of which there have been many, will afford the analyst much comfort, whether he be a routine or occasional user of spectrophotometers. He can rest assured that the array of "black boxes" conceals no optical or electronic deviltry conspiring to foul up his results.

To our way of thinking the most significant result of all such tests is the suggestion that Beckman and Cary have known what they were doing from the beginning. After all, the fundamental design principles of spectrophotometry have been known for a long time. The advent of improved optical components, better phototubes, and improved electronic circuitry have all signalized progressive improvement. Theoretical advances have not been lacking in such matters as stray light, slit width effects, signal to noise ratio, and the significant details are all incorporated in these instruments. Of necessity, an instrument manufacturer must establish tolerances and standards. Modern machine tool practice enables him to hold to established tolerances within very narrow limits. These tolerances have been established by original design and research. As a rule, instrument performance reflects the contemporary status of research and design knowledge. Radical advances arise only as totally new concepts or techniques are discovered. At each stage of progress, it is not too difficult to secure reproducibility. One strongly suspects that Cary and Beckman, as well as other reputable instrument designers, have established checking procedures, calibration techniques, and over-all inspections which are far more reliable than many that have appeared in the literature. In a business sense, this is almost a necessity. Otherwise, the complaint department might well require a staff exceeding research, development, production, and sales combined.

Calibration Procedures

Calibration procedures have been worked out in great detail in terms of standard solutions and glasses. The preferred recommendations are covered by National Bureau of Standards Circular 484. Although precise calibrations can be made with these systems, they are not too versatile for the entire range of useful wave lengths.

A most versatile and precise technique has been described recently which involves simple physical principles for both wave-length and absorbance calibration [Heidt, L. J., and Bosley, D. E., *J. Opt. Soc. Amer.*, **43,** 760 (1953)]. The technique for wave-length calibration is based on the locations of the maxima and minima of the absorbance wave of a pair of transparent closely spaced parallel plates. Calibration for absorbance is based on the absorbances of properly orientated screens of square-woven single strands of brass wire. Both principles are very old and have been used in related problems before, but Heidt and Bosley have made skillful use of them for calibrating spectrophotometers.

As a means of covering the entire ultraviolet and visible range, the authors use quartz plates half aluminized on the surfaces facing each other. Silvered glass plates may be used for the visible region only. The plates must be at least 0.25 inch thick to avoid distortion. They are separated by rolled gold foil spacers, the thickness being 8, 10, and 40 times 10^{-4} cm. for use with the Hardy-G.E., Beckman, and Cary spectrophotometers, respectively. The periodic amplitude change in the absorbance wave is produced by interference effects arising from the thin film of air between the plates. The wave

INSTRUMENTATION

lengths of the centers of the absorbance maxima are given by the equation:

$$\lambda_N = 2d/N$$

where N is the order of interference and d is the distance between the plates. More conveniently expressed, the distance between adjacent maxima or minima is given by:

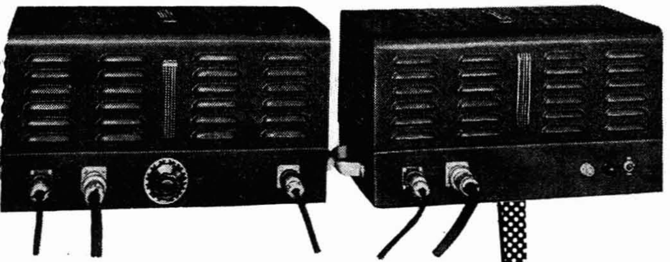
$$1/\lambda' - 1/\lambda'' = n/2d$$

where n is unity for adjacent maxima or minima. The latter equation is also useful for a precise estimation of d because a plot of reciprocal wave length against successive integers is a straight line of slope $1/2d$ or $1/4d$, depending upon whether maxima or minima have been chosen with respect to the running index. If an absorbance wave is recorded through the interference pack and compared with a recording of the hydrogen spectrum in the band region (ca. 3600 to 4400 A.), the curves may be used to determine the wave lengths of the maxima and minima of the absorbance wave.

The authors also record careful studies on the absorbance of wire screens with considerably more attention to orientation factors than has been given by earlier investigators. Their data show close agreement with the absorbance calculated from the structure of the screen for which the per cent open area $= 100[1 - mS \times (2 - mS)]$ where m is the mesh (wires per inch) and S is the diameter of the wire in inches.

High Speed Cathode-Ray Indicating Spectrophotometer

In 1935, we demonstrated a high speed cathode-ray indicating spectrophotometer [Müller, R. H., and Garman, R. L., *Mikrochemie,* **21,** 302 (1936)]. At that time it was hoped that the fullest resources of electronics might be applied to the problem and that the young television industry could undertake improvements. Despite the tremendous developments since then, there is no cathode ray spectrophotometer embodying even a small fraction of television know-how. However, the lapse of 18 years has brought at least one commercially available instrument which can afford, as we originally showed, an almost instantaneous and repetitive indication of absorption, reflectance, or emission spectra. A good description of the American Optical Co.'s instrument has been given by R. C. Beitz [*J. Opt. Soc. Amer.,* **43,** 773 (1953)]. The curves are presented on a 5-inch scope with a linear wavelength scale and linear per cent transmission scale. An ingenious nonlinear sweep circuit achieves the linear wave-



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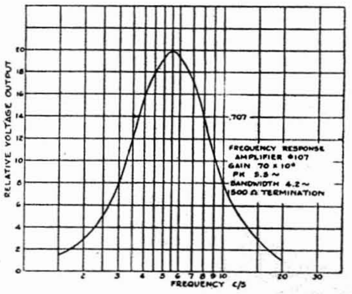


The frequency characteristic of the complete Amplifier when operating from a generator impedance equal to an average thermocouple.

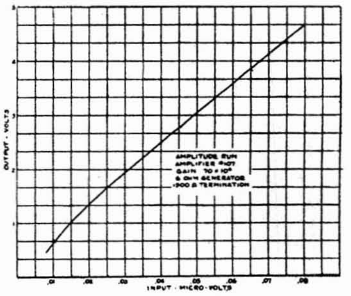
The Farrand Low Frequency High Gain Amplifier and associated Regulated Power Supply, although primarily developed for use with Farrand Hornig Type Thermocouples, can be utilized for other thermocouples, thermopiles and similar type detectors.

The amplifier operates on frequencies between 2 and 20 cycle signals. However, amplifiers are peaked for frequency response desired or most suitable for a particular application, therefore, any intermediate peak frequency response can be specified. With lower peak frequency amplifiers, e.g., 5 or 7½ cy., band width and gain are altered; gain increases and band width narrows, both, obviously desirable characteristics.

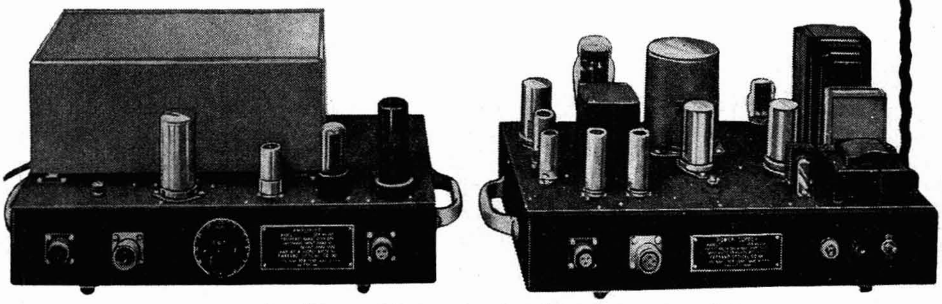
The gain provided is generally sufficient to produce full scale deflection on a balancing type recorder requiring 10 M.V. full scale signal when the amplifier input is 0.001 microvolt.

With full amplifier gain, it is then possible to observe the Johnson noise of typical thermocouples at an approximate output level of .1 volt across the 1500 ohm output load resistor.

The Power Supply to operate this Amplifier has sufficient regulation and freedom from noise which permits detection of the first stage tube noise and, thereby, enables observation of lowest thermocouple noise.



An amplitude curve as obtained from a generator of impedance representing the average thermocouple.



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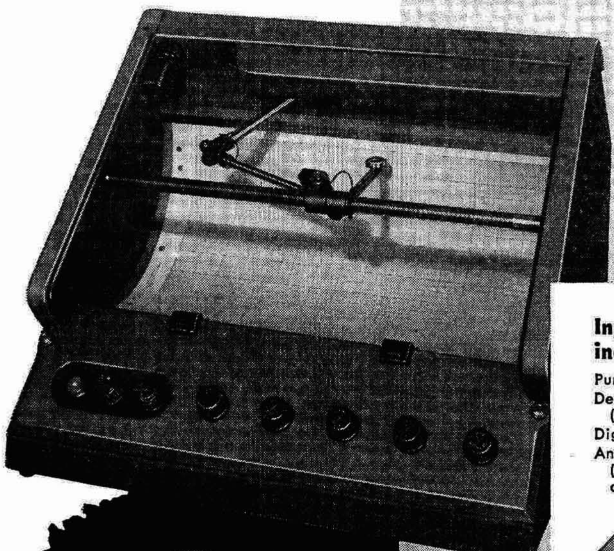

length scale despite the nonlinear property of the spectrometer prism. Optical means correct for light source energy distribution and the selectivity of the photomultiplier tube. The resultant output is an essentially equal energy line for white light (no sample). Ordinate and abscissa expansion can be used for closer examination of portions of the spectrum. These are achieved by horizontal and vertical gain controls.

This is an unusual instrument and should find many special and unique applications. It is likely that the principal applications will be in rapid and automatic inspection and scientifically in gaining more intimate information about reaction kinetics. In the latter connection, we showed motion pictures at the 1936 Baltimore ACS meeting illustrating the kinetics of the hemoglobin-oxyhemoglobin conversion. Britton Chance of the University of Pennsylvania has made elegant extensions of this technique and applied them to numerous biochemical problems.

It is to be regretted that there has been so much delay in producing such an instrument. More regrettable, perhaps, is the fact that the achievement represents electronic and television practice of the vintage of 1940. It is ironical that the research chemist has but one choice of instrument to view spectra, whereas in his home he can view scenes on a 20-inch screen, a process which intrinsically, is far more complex that the spectrophotometer.

A faint notion of what can be accomplished in these times by the complete utilization of modern electronic techniques can be learned from a paper by Brehm and Fassel [*J. Opt. Soc. Amer.*, **43,** 886 (1953)]. This stimulating paper deals with an entirely new approach to direct-reading spectrochemical analysis. Whereas existing direct readers employ a separate photomultiplier for each analytical spectral line, these authors use a single tube and read each of the lines sequentially in a rapid scanning spectrometer. Each line in turn is presented on a scope. The pulse heights are "read" and converted to equal amplitude "bursts" whose number is proportional to the magnitude of the original spectral line pulse. The separate lines which are of interest are sorted by electronic gates. High speed scalers and mechanical registers indicate the time integrals of the spectral line intensities. Intensity ratios are automatically computed after the exposure and recorded on a strip chart recorder empirically calibrated to read directly in per cent. Preliminary results indicate an analytical precision of ±1%.

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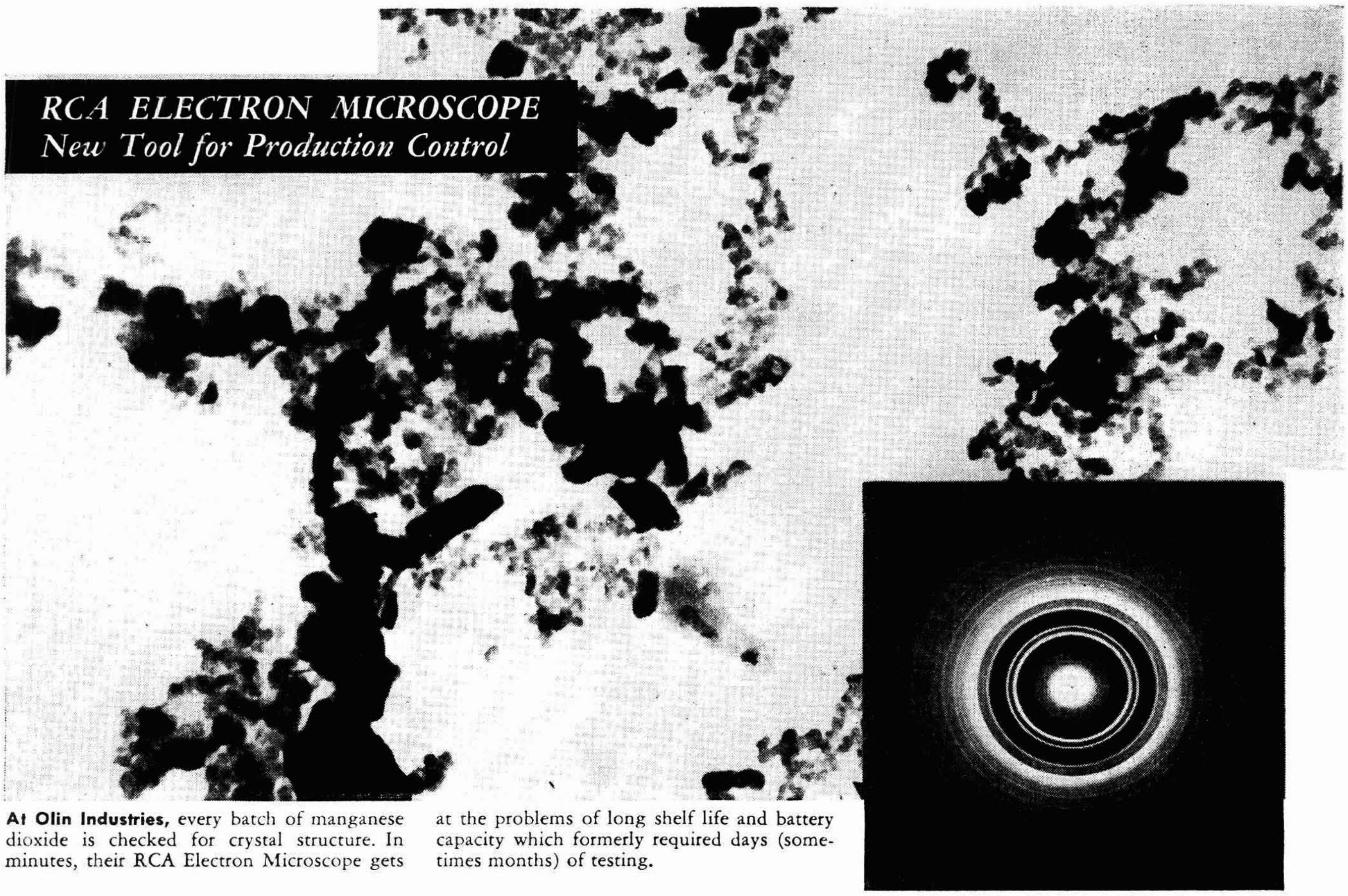

At Olin Industries, every batch of manganese dioxide is checked for crystal structure. In minutes, their RCA Electron Microscope gets at the problems of long shelf life and battery capacity which formerly required days (sometimes months) of testing.

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Olin Industries institutes new quality-control technique

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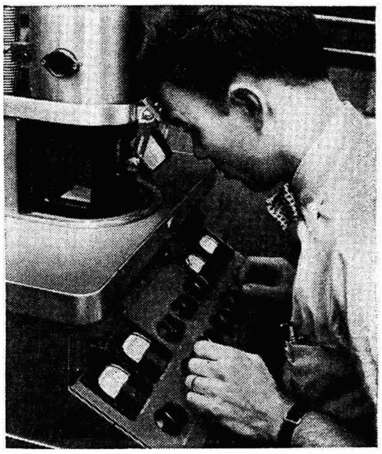
For information on the RCA Electron Microscope and RCA's training assistance program, write Scientific Instruments, Dept. 11X, Radio Corporation of America, Camden, N. J.



Micrographs and diffraction patterns produced in the RCA Electron Microscope permit identification of various types of MnO_2 crystals.

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The Chevron

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The camera provides the size advantage of 2¼″ x 2¼″ negatives or color transparencies, yet is used at the eye-level position preferred by many. It has the world's fastest between-the-lens shutter, with rotating blades for smoother, more accurate exposure. There are ten speeds from 1/800 second to one second, plus "B." Flash synchronization is continuously adjustable for top efficiency at all shutter speeds with both Class F and Class M flash bulbs and electronic flash equipment like the Kodatron Speedlamp.

The lens is a 78mm *f*/3.5 Kodak Ektar Lens—a finer one we doubt you can buy. To provide the focusing accuracy such a lens merits, there is a split-field rangefinder operating from 3½ feet to infinity and based on such advanced design features as double V-bearings for moving its mirror, ball-bearing mounting for the focusing tube, and cams almost eight inches long machined to accuracy within .0005″ throughout their length. The rangefinding operation automatically applies the proper parallax correction to the adjacent viewfinder. An adapter is available to take No. 828 film for the larger 28 x 40-mm Kodachrome slides. Weight: 2½ pounds. Price: no fortune but a sensible $215 for a piece of equipment of strictly professional calibre. (Worth keeping in mind, for example, as a retirement gift for someone who respects noble instrumentation.)

Superlatives in print tire the eyes. If you want to see a well-built camera, go down to your Kodak dealer and ask him to show you the Kodak Chevron Camera. *Since quantities are still limited, there may be a little wait for delivery.*

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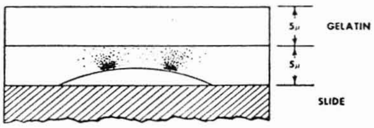

Not to be confused with it is *Kodak Autoradiographic Stripping Film, Type NTB.* This consists of a 10-micron emulsion on a thin cellulose ester film which can be split off from a carrier sheet of ordinary film base. The impermeable cellulose ester layer tends to eliminate chemical and abrasion artifacts and prevents direct contact between the photographic developer and the radioactive section. It also prevents stain in the tissue section from staining the emulsion. The material comes as 4″ x 5″ sheets.

For work where low radiation flux necessitates sacrifice of fine-grain qualities for higher sensitivity, we can supply 25-micron-coated *Kodak Autoradiographic Plates, Type A.* An even greater sacrifice in that direction is represented by *Kodak Autoradiographic Plates, Type No-Screen.*

If you know which autoradiographic material you want, just call up your Kodak Industrial Dealer and order it. If you have questions, write Eastman Kodak Company, Industrial Photographic Division, Rochester 4, N. Y., and find out if we can answer them.

Three books

These three fellows of ours are smiling for the photographer in celebration of the publication of their book, "Principles of Color Photography." In the book they don't smile at all. Soberly and with an abundance of illustrations, Messrs. Ralph M. Evans, W. T. Hanson, Jr., and W. Lyle Brewer (pictured r. to l. in that order) present the theory behind visual and photographic sensitometry, analyze the methods of obtaining colorant images, and tell how they are combined to give color processes. It's sold by Kodak dealers for $11, and no inventor of new systems of color photography should be without a copy.

No user, or prospective user, of the photographic emulsion as a scientific instrumentality should be without a copy of "Kodak Photographic Plates for Scientific and Technical Use." Besides giving the detailed information implied by the title, it contains just about the most concise précis of the scientific theory of photography to be found. The new edition has been extensively revised. Kodak dealers sell it for 50¢.

Still lower on the price scale, namely free, is another newly revised booklet, "Kodak Materials and Accessories for Industrial Radiography." This one tells how to pick the best type of film for any non-medical job involving exposure to x-rays and describes various devices to make life more pleasant in the x-ray laboratory. You can get a copy by writing Eastman Kodak Company, X-ray Division, Rochester 4, N. Y.

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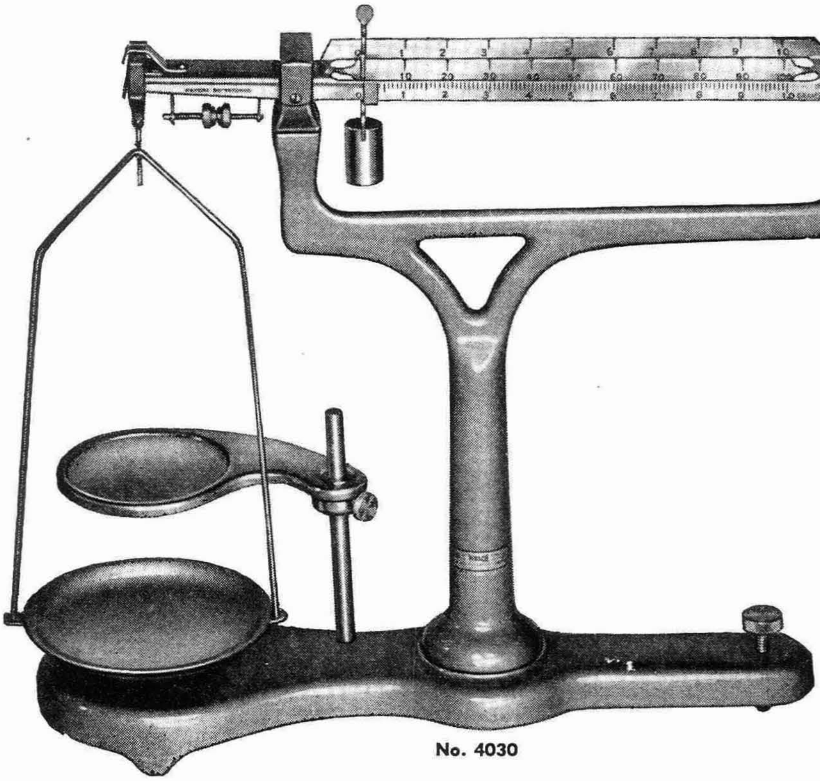

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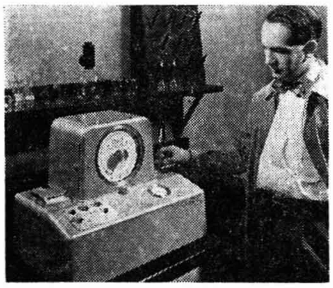
Hydrogen Determination

A new instrument for measuring the percentage of hydrogen in liquid hydrocarbons by absorption of β-rays from strontium-90, rather than through the use of a combustion chain, has been announced by Central Scientific Co.



Cenco predicts that its Beta-Ray H/C Meter, developed by Standard Oil Co. (Indiana), will reduce control test time for petroleum products from four hours to five minutes while increasing accuracy 100%. Individual determinations can be made with a statistical probable error of 0.02 weight % hydrogen, it is claimed. Corrections can be made for oxygen, nitrogen, sulfur, lead, or other elements present. Designed for control of production operations in petroleum refining, the apparatus has potential applications in process control of pharmaceuticals, chemicals, foods, and other products.

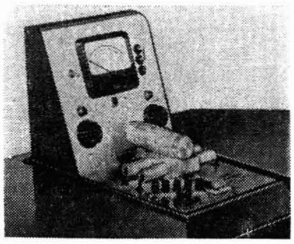
The instrument's β-ray source is constructed to radiate equally in two directions. One beam traverses a fixed absorber and proceeds to an ionization chamber β-ray detector. An equal β-ray traverses the sample cell and a wedge-shaped absorber before entering another detector. A galvanometer indicates the difference in magnitude of the electric currents produced in the two ionization chambers. The density of the liquid sample is measured simultaneously by the weight displacement method.

In use, the sample cell of 10-cc. capacity is filled with the test hydrocarbon. The wedge is then moved until the galvanometer indicates equal currents in the ion chambers. At this point absorption of the sample plus that of the wedge equals the absorption of the fixed absorber, and the position of the wedge is recorded as a measure of its absorption. **1**

Fluorimeter

A fluorimeter based on a design which eliminates the need for air or water cooling systems and optical meters is now in production by Jarrell-Ash Co. The instrument consists of an ultraviolet light chamber and sample slide, a detecting and measuring unit, and a power supply. All components except the power supply are mounted in a metal base to form a compact, portable unit.

The ultraviolet light chamber of the fluorimeter consists of an aluminum block which holds the ultraviolet lamps and contains an opening for the sample slide. Lamps used developed little heat, so that no special cooling is required. With the cooling system eliminated, the sample slide, light source, and detecting unit can be mounted in close proximity to make optical systems unnecessary and permit lower voltage across the dynode of the photomultiplier tube. The sample slide contains compartments for a small or large sample melt and a fluorescent standard.



The detecting unit, located above the light chamber, has a photomultiplier tube which is separated by a set of filters that screen out unwanted light. Current developed by the photomultiplier tube is measured by a stable vacuum tube voltmeter. Because of the dependence of the photomultiplier tube on voltage, a vacuum tube regulated power source is used. Line voltage variations between 90 and 120 volts will produce no change in output and the ripple voltage is less than 0.03 volt a.c. at full power output of 640 volts d.c. **2**

Gas Flow Meter

The Model 30 Vol-O-Flo dial-indicating gas flow meter offered by National Instrument Laboratories, Inc., consists of a differential pressure gage across a linear pressure drop flow element. It is designed for measuring the rate of flow of gases at pressures to 20 pounds per square inch gage or vacuums as low as $^1/_2$ pound per square inch absolute.

The indicator can be used several

Circle desired items for further information on items listed in this section

Inquiry Card
Is Valid Six Months from
Publication Date

feet distant from the flow element and neither indicator nor flow element require leveling. Use of a mechanical differential pressure gage makes operation at temperatures from −60° to 180° F. possible. Instruments are available with full scale flow ranges from 0.003 to 30 cubic feet per minute for most gases. **3**

Recording Spectrophotometer

Beckman Instruments, Inc., has developed an automatic recording spectrophotometer which can run analyses practically unattended, yet with accuracies said to match the best manual operation. This new routine instrument is built around the single-beam quartz monochromator of Beckman's DU spectrophotometer to combine the accuracy of the Model DU with rapid automatic operation.

The Model DR spectrophotometer measures and records per cent transmittance in the 210 to 1000 millimicron region of the spectrum. As many as three samples can be run simultaneously, with each curve plotted in a different color. It takes 8 seconds to record a point for one sample, 10 seconds for two samples, and 12 seconds for all three.

Operation of the Model DR is always at highest sensitivity or resolution of which the instrument is capable for optimum signal-to-noise ratio. Once its controls have been set, the instrument can run by itself for a whole day; an operator is needed only to change samples, chart paper, and scanning regions. The automatic unit of the equipment can be attached to any standard Model DU spectrophotometer with no change in monochromator. **4**

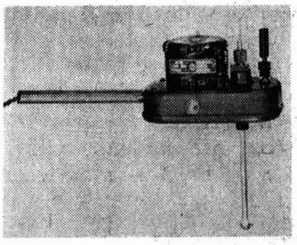
Polyethylene Beakers

One-liter beakers of polyethylene molded in one piece and with reinforcing rings for added strength are available from the Technicon Co.

The beakers are flexible and unbreakable. They are inert, nonconducting, and, in addition, translucent, so that the inside liquid level is visible. Molded construction is said to offer a more durable yet less expensive product. **5**

Synchronous Rotator

Designed for use in amperometric titrations with rotating metallic microelectrodes, E. H. Sargent & Co.'s synchronous rotator incorporates a hollow shaft chuck to hold the electrode securely in line with the axis of rotation and a constant speed drive, compactly assembled with a terminal connecting post and line switch.



The rotator is driven by a heavy-duty capacitor-type synchronous electric motor which, when connected to a 60-cycle power line, drives the chuck at a constant speed of 600 r.p.m.—a speed found to provide reproducible results in amperometric titrations. The chuck will accommodate electrodes $^1/_4$ inch in diameter and has sufficient range to hold normal variations in the diameter of 6-mm. commercial tubing. **6**

Laboratory Partitions

For flexibility in laboratory construction, Virginia Metal Products, Inc., has designed movable steel partitions of 6-inch thickness that include internally housed utilities. VMP Mobil-Lab-Walls offer partition post and panel construction to provide access to and support for mechanical and electrical services. Up to 20 feet of services and utilities, in one section, can be removed without disturbing work in the adjacent laboratory. Twenty-foot sections, according to the manufacturer, result in fewer joint connections and minimum leakage potential, and also reduce installation and maintenance costs.

Shelving and wall cabinets can be mounted on metal hangers secured behind the cornice by mechanical fasteners, and can be easily installed, changed, or removed. **7**

Explosion Chamber

Tenney Engineering, Inc., has added an explosion chamber to its line of environmental test equipment. The apparatus can be used to determine the effect of explosions on materials and assemblies or the explosion hazards of equipment operated in an explosive atmosphere. The equipment comprises a cylindrical shell, devices to cause an explosion, and control instruments. It can be furnished in a variety of sizes and with auxiliary equipment. A vacuum pump permits simulation of high altitude conditions.

Explosions are caused by igniting a mixture of butane and air by means of a spark plug, or equipment on test can be operated in the butane atmosphere to determine whether it will set off an explosion. Two adjustable pressure relief valves located on top of the chamber allow expanded gases to escape. A scavenging valve can be opened to release burnt fumes.

The door of the chamber is gasketed for vacuum runs and is provided with a 12-square-inch window for observations. **8**

Plastic Tubing

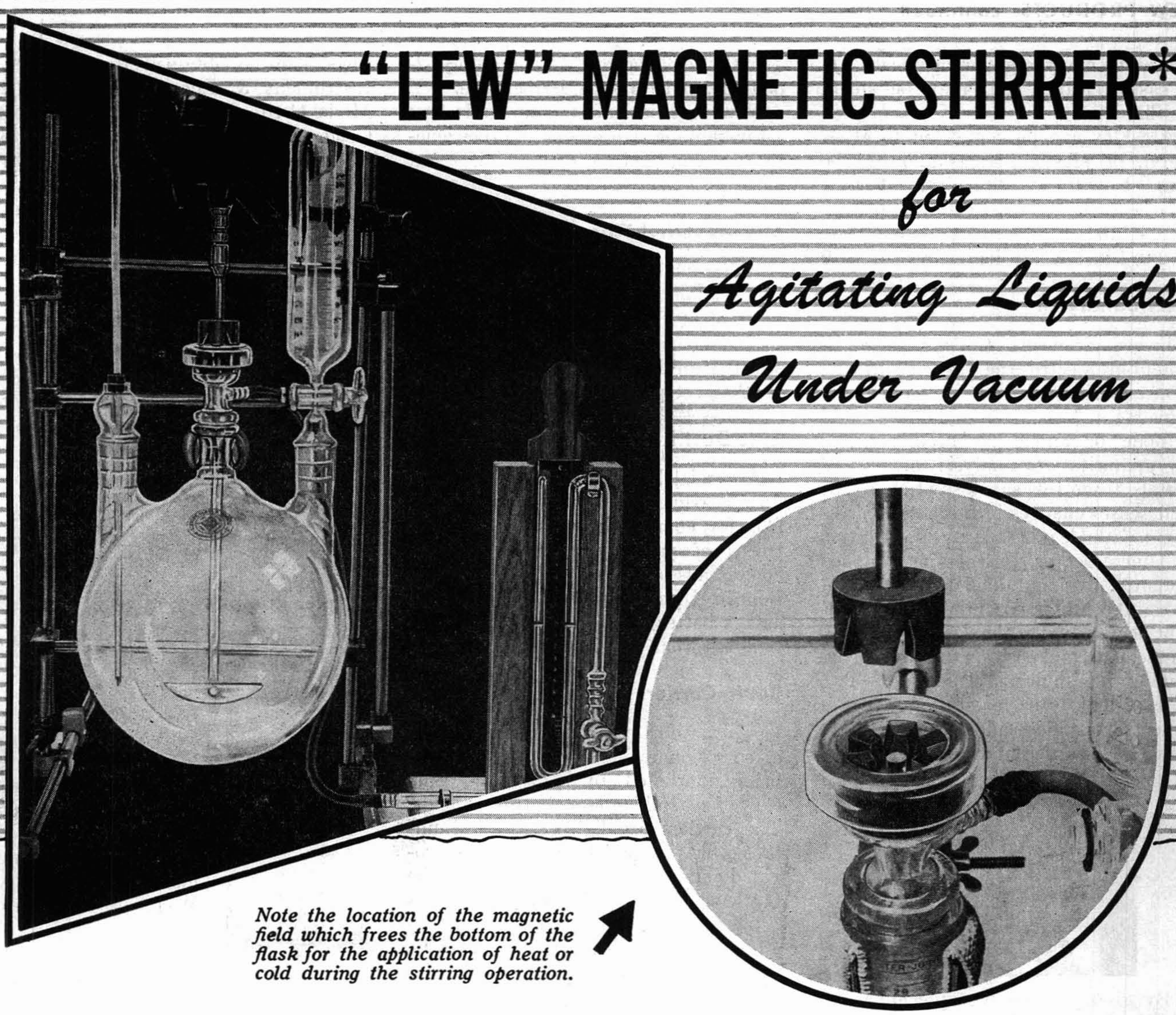
A flexible, transparent plastic tubing for temporary or permanent fluid lines has been announced by American Hard Rubber Co. Ace-Flex is inert to most inorganic acids and alkalies and is re-

(*Continued on page 30 A*)

Use this handy return card to save yourself time. It will bring information of use to chemists and engineers in laboratory, pilot plant, and production. The items listed in this special section have been selected by the editors of ANALYTICAL CHEMISTRY for their value and timeliness in helping you to keep abreast of the latest developments in the field.

"LEW" MAGNETIC STIRRER*

for Agitating Liquids Under Vacuum



Note the location of the magnetic field which frees the bottom of the flask for the application of heat or cold during the stirring operation.

Now, you can mix liquids in a closed container *under vacuum* while simultaneously heating or cooling. That's because the magnetic field in the "Lew" Stirrer is above the vessel proper, leaving the underside entirely free. This design also prevents glass breakage caused by friction between the stirring magnet and the "Pyrex" glass flask.

Two permanent magnets made of Alnico V and an anti-magnetic "Pyrex" glass cover comprise the powerful magnetic field. The upper magnet, attached directly to the motor shaft, is entirely outside the vessel and may be swung to one side *even during operation!* The lower magnet, connected to the "Pyrex" glass stirring rod and blade, is mounted in a "Pyrex" glass adapter and fits into the neck of the flask on which the glass cover rests. As the contact between the rotor and motor is indirect — achieved only through the magnetic field — a hermetically sealed vessel is obtained.

There are two "Lew" Magnetic Stirrers available for agitating liquids up to a consistency of glycerin — Model A for vessels up to 5 liter capacity; Model B for vessels up to 50 liter capacity. *Descriptive circular sent on request.*

No. J-2165X **6-Pole Magnetic Stirrer, Model A, as described. (Please specify flask capacity):**

₮	24/40	29/42	34/45	40/50	45/50	55/50
Each	**$28.50**	**$28.80**	**$29.20**	**$29.70**	**$30.25**	**$30.75**

No. J-2165-1X **8-Pole Magnetic Stirrer, Model B, similar to Model A but with more powerful magnets. (Please specify flask capacity):**

₮	24/40	29/42	34/45	40/50	45/50	55/50
Each	**$69.60**	**$29.70**	**$70.00**	**$70.50**	**$71.00**	**$71.60**

* Patent Applied For

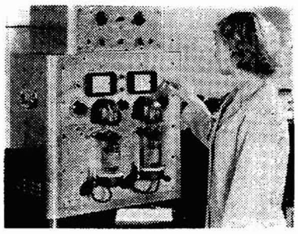
SCIENTIFIC GLASS APPARATUS CO., INC.
BLOOMFIELD - NEW JERSEY
LABORATORY APPARATUS - INSTRUMENTS - CHEMICALS - GLASSWARE

sistant to many organic chemicals. It is easily cleaned and may be steam sterilized.

Ace-Flex can be connected to glass, metal, or plastic fittings and can be coupled without difficulty. Ketone type cement can be used to weld permanent joints. Standard sizes range from 0.12 to 1 inch inside diameter. **9**

Electroanalyzer

Fisher Scientific Co.'s Controlled-Potential Electro-Analyzer features an electronic unit atop the instrument designed to make constant readjustment of cathode or anode potential required for quantitative analysis of several metals from the same solution. The unit senses changes in the potential between cathode or anode and the solution and increases or decreases the applied potential to maintain the desired electrode-to-solution potential. The operator need only dial the desired potential on the panel and position the beaker containing the multimetal sample. Two independent sample stations permit simultaneous determinations of either duplicate or different samples.



By giving successive determinations of several metals in one sample automatically, the apparatus speeds up routine metallic assays. It may also be used to study the electrolytic oxidation of organic compounds through addition of increments of potential and observation of the resulting current flow. **10**

Reagents

Two new reagents are available from Distillation Products Industries.

N',N'-Diethylsulfanilamide acts as a colorimetric reagent for thyroxine. It provides an assay based on diazotization of the reagent, formation of a strongly colored reaction product with thyroxine, and extraction in *n*-butanol.

m-Toloxyacetic acid is a reagent for separating thorium from the rare earths of monazite sands and from uranium. **11**

API Standard Compounds

A number of hydrocarbons and organic sulfur compounds are newly available as American Petroleum Institute Standard samples.

Hydrocarbons now offered include: 3-methyl-*cis*-3-hexene, 2,3-dimethyl-2-pentene, and 1,3,5-triethylbenzene.

New organic sulfur compounds are: 4-thiaheptane, 1-propanethiol, benzenethiol, 2-methyl-1-propanethiol, and methanethiol. **12**

Methoxyethyl Thioglycolate

Water-white ester available from Evans Chemetics, Inc., has a boiling point of 112° to 113° C. at 23 mm. It is recommended for use in making substituted amides of thioglycolic acid or in synthetic steps requiring alcoholysis. **13**

Zinc Dust Reagent

A special low-nitrogen grade of zinc dust is being sold by Fisher Scientific Co. for completely reducing the last traces of partially reduced nitrogen compounds to ammonia after acid digestions in Kjeldahl analyses. The reagent assays better than 95% zinc and contains less than 0.001% nitrogen. **14**

Bottle Carriers

Agilene bottle carriers made entirely of corrosion resistant polyethylene are being marketed by American Agile Corp. for handling large bottles of acids. The carriers are lightweight and unbreakable. A bail type handle is provided and a snug fitting cover holds the bottle safely in position, even when it is tilted for pouring.

Agilene bottle carriers may also be used to transport iced sample bottles containing monomers or reagents which boil below room temperature. **15**

Homogenizer

For emulsions, solutions, electron microscope preparations, and biochemical and biological research, E. Machlett & Son has introduced a laboratory homogenizer suitable for micro or macro work.

The apparatus has a rate adjustment knob for easy speed control from 100 to 27,000 r.p.m. Three specially designed fluted homogenizing flasks made of stainless steel or borosilicate glass handle volumes ranging from 0.2 to 50 ml. Efficient cutting blades of stainless steel give rapid homogenizations. Changing of flasks and blade assemblies can be accomplished quickly. **16**

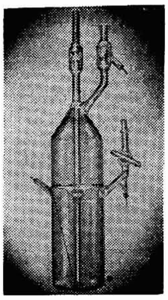
Radioactivity Absorber Set

A packaged set of radioactivity absorbers, consisting of 24 lead and aluminum disks mounted in uniform plastic rings, can be obtained from Nuclear Instrument & Chemical Corp. The Model C-101 absorber set is used with a Geiger tube mount and a scaler or count-rate meter to determine the energies of photons and beta radiations, check isotope purity, identify radioactive species, study decay schemes, or count a radioisotope in the presence of another.

Activity readings are taken with various absorbers placed between the Geiger counter and the radioactive source. Successively heavier absorbers are positioned one at a time over the radioactive sample until there is no longer any measured activity. In this manner a characteristic absorption curve is obtained for the isotope in question.

The absorbers are $1^7/_8$ inches in diameter and $^3/_{16}$ inch thick. Each is individually calibrated. **17**

Toepler Pump



A Toepler pump designed by Grant Urry of the University of Chicago for use in the quantitative transfer of gases and volatile liquids for gas analysis, reactions in vacuum line apparatus, and analysis of absorbed gas in solids is being manufactured by Wakefield Industries, Inc.

The new design is said to be more compact and to give better support for the mercury used. It can be made with a large piston volume within a reasonable size, yet with short movement of mercury. The minimum mercury movement allows the pump to be operated at high speeds. A pump with a piston volume of 800 cc. and an operating piston cycle of 35 seconds can evacuate a 2-liter system to 0.15% of original gas in approximately ten minutes. **18**

Electronic Timer

For timing requirements, such as in viscosity determinations, Hallikainen Instruments has a timer that provides accurately regulated 60-cycle voltage and eliminates errors due to fluctuating frequency. The device is designed to furnish power for one to 36 odometer counters and will register time intervals from 0.1 to 1000 seconds accurate to 0.1 second. Power output is 115 volts, 60 cycles, 150 watts.

The unit is constructed in three parts and contained in a single cabinet. The upper section houses a tuning fork unit and a fork amplifier. Mounted on the

NEW PRODUCTS, continued

panel is an electric clock which is powered by the amplifier to provide for adjustment of the tuning fork. The center section houses the power amplifier. Located on the panel of this section is an output voltmeter. The lower section contains a high-voltage power supply. **19**

Magnetic Stirrer

Fisher Scientific Co.'s Flexa-Mix stirrer is designed for use in limited space. It is $2^{3}/_{4}$ inches high, $4^{1}/_{4}$ inches wide. Maximum speed of the motor at full load is 1650 r.p.m.

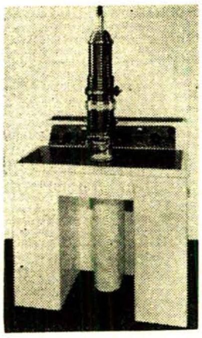
The Flexa-Mix assembly includes a stirring motor in an aluminum housing, a cast iron support plate with an integral clamp, and two Alnico stirring bars—one sealed in glass and the other in polyethylene. The base plate has four holes into which the motor's rubber feet fit, making it possible to use the stirrer in a variety of positions and tilts and on stands or frames. **20**

Scintillation Scaler

A scaler suitable for alpha, beta, and gamma scintillation counting through use of easily changed crystal phosphors is being produced by Reed-Curtis Scientific Instrument Co., Inc. The unit is provided with external connectors for accessory Geiger tubes and scintillation counters.

The instrument features binary type scales up to 256 and uses a mechanical register in collecting these units up to 10,000. Count can be preset from 40 to 256,000. High-voltage power supply is continuously variable from 750 to 1500 volts d.c. **21**

Electron Microscope



North American Philips Co., Inc., has a new electron microscope that has approximately half the power of Philips' larger EM-100 model and is less expensive. The EM-75 instrument utilizes an electron-optical system having condenser, objective, and projector lenses. Magnification is continuously adjustable between 1500 and 15,000 diameters. Resolving power is better than 100 A. The instrument is 74 inches high, 37 inches wide, and 25 inches deep.

The electromagnetic deflection method is used for beam alignment and

For further information, see coupon on page 27 A

focusing is facilitated by a beam wobbler. Specimen carriers for $^{1}/_{8}$-inch diameter grids and plates are available. The specimen carrier can be tilted 15°, without breaking the vacuum, for stereoscopic exposures. Diameter of the image is $3^{1}/_{8}$ inches and a supplemental 2× optical magnifier can be fitted into the column.

A camera using 35-mm. film is employed. Vacuum obtainable is 10^{-4} mm. of mercury, using a rotary prevacuum pump and an oil diffusion unit. **22**

Voltmeters and Amperemeters

A line of rack-mounted vacuum tube voltmeters and amperemeters is being sold by Millivac Instrument Corp. The meters can be equipped with terminals for connection of external indicating instruments and recorders.

Included in the new line of meters are a d.c. millivoltmeter having a lowest range from 0 to one millivolt, 6 megohms input, and an a.c. voltmeter with a lowest range of 0 to 3 millivolts, 20 cycles to 250 kilocycles per second. **23**

Laboratory Ovens

Two series of ovens have been announced by Labline, Inc. Force-Aire ovens for temperatures to 950° F. have either horizontal or vertical mechanical flow of air provided by a high pressure blower. They are available in two temperature ranges and four sizes ranging from 19 × 19 × 19 inches to 25 × 50 × 37 inches.

Labline's Bench-Saver ovens feature controlled temperatures to 520° F. with three-way variable air flow. Blower is of the high pressure type and provides uniform air velocity through the working chamber by a series of baffled perforated walls. Air velocity can be regulated by a damper from 75 to 250 feet per minute. The ovens are made in three sizes, all floor models. **24**

MANUFACTURERS' LITERATURE

Ethylenediaminetetraacetic Acid. An 8-page bibliography covers the 1952 literature on this sequestering agent, including its analytical applications. Alrose Chemical Co. (*Sequestrene*). **25**

Platinum Crucibles. Notes on the care and use of platinum crucibles and dishes are available in 8-page bulletin. J. Bishop & Co. **26**

Pressure Reaction Apparatus. Six-page bulletin describes an improved model of shaker-type pressure reaction apparatus for catalytic reduction and other reactions with hydrogen at pressures to 5 atmospheres. Parr Instrument Co. (*Spec. 3910*). **27**

Can Seam Inspection. A 4-page folder covers apparatus and accessories for examining double seams on cans by projecting magnified image of the cross section of the seams. Wilkens-Anderson Co. **28**

First Aid Kits. A 6-page folder describes first aid kits available from Mine Safety Appliances Co. (*Bull. 0401-2*). **29**

Abrasion Testing Set. A folder of 6 pages describes an instrument for measuring the abrasion resistance of surfaces of various materials. Taber Instrument Corp. (*Abraser*). **30**

Absorptive Lenses. Information on absorptive glass lenses for eye protection in glass blowing and other operations where an intense yellow flame is encountered is presented in a 2-page sheet. Fish-Schurman Corp. (*Neophan*). **31**

Mass Spectrometers. A 12-page booklet on the components and use of mass spectrometers for chemical analysis. General Electric Co. (*GEC-587A*). **32**

Valves and Pressure Regulators. A 66-page catalog contains engineering data on small valves, pressure regulators, steel cylinders, flow gages, torches,

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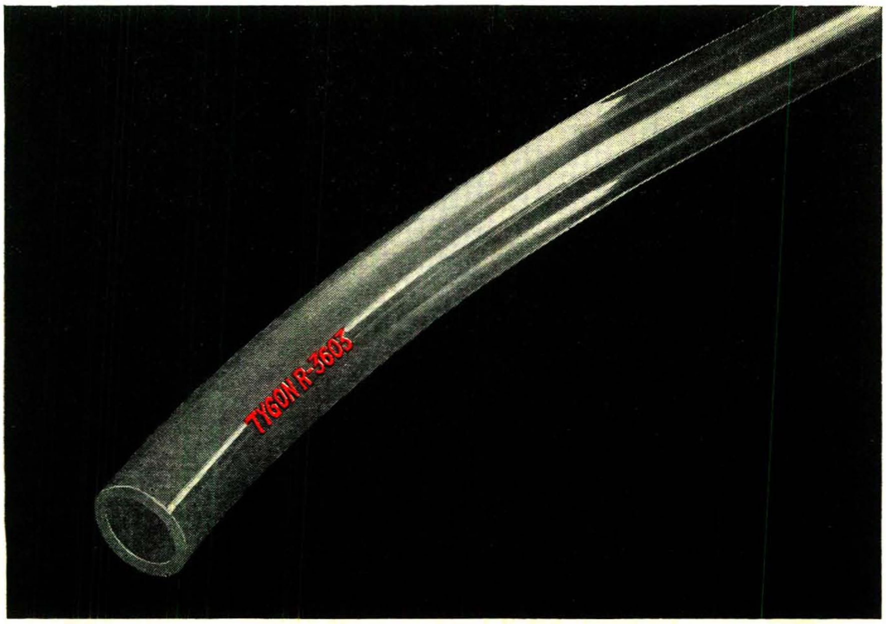

LITERATURE, continued

fittings, and accessories. Hoke, Inc. (*Cat. 2*). **33**

Thermometers. Recommended specifications for fractional degree and general laboratory-grade thermometers available in a 2-page announcement from Scientific Apparatus Makers Assoc. **34**

Balances. Series of descriptive bulletins on German-made analytical and special-purpose balances is available from C. A. Brinkman & Co. (*Sartorius Balances*). **35**

Electrophoresis-Diffusion Instrument. Instrument featuring double sensitivity in an optical system based upon double passage through the cell is described and illustrated in 8-page pamphlet. Specialized Instruments Corp. (*Form 8H853*). **36**

Tetraethyllead Analyzer. Theory, operation, and test methods for direct-reading, push-button polarographic analyzer for tetraethyllead in gasoline discussed in 10-page booklet. Fisher Scientific Co. (*TEL-Meter*). **37**

Polyglycol Ethers. Technical data on a series of monomethyl and monooctyl ethers of polyethylene and polypropylene glycols are available from The Dow Chemical Co. **38**

Vermiculite. Eight-page report summarizes chemical and physical properties of vermiculite and lists its uses. Zonolite Co. **39**

Organic Chemicals. Revised booklet of 20 pages on physical properties of synthetic organic chemicals tabulates data on over 330 products of Carbide and Carbon Chemicals Co. (*Booklet F-6136*). **40**

Hardness Tester. Description of shear hardness and diamond scratch tester for determining the resistance of plastics and plastic-coated surfaces to scratches, scrapes, and similar damage is presented in 4-page leaflet. Taber Instrument Corp. (*Bull. 5002*). **41**

Ketones. A 44-page bulletin on commercial ketones covers uses, specifications, and shipping data and presents detailed information on test methods, as well as tables and charts of physical properties and a bibliography. Carbide and Carbon Chemicals Co. (*F-4767*). **42**

Vacuum Equipment. Illustrated catalog of 114 pages covers vacuum pumps, diffusion pumps, vacuum evaporators, coaters, glass blowing equipment, rubber tubing, ovens, and other equipment. Research Vacuum Supply Co. (*Cat. 13-K*). **43**

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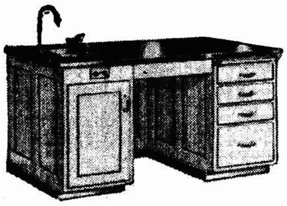

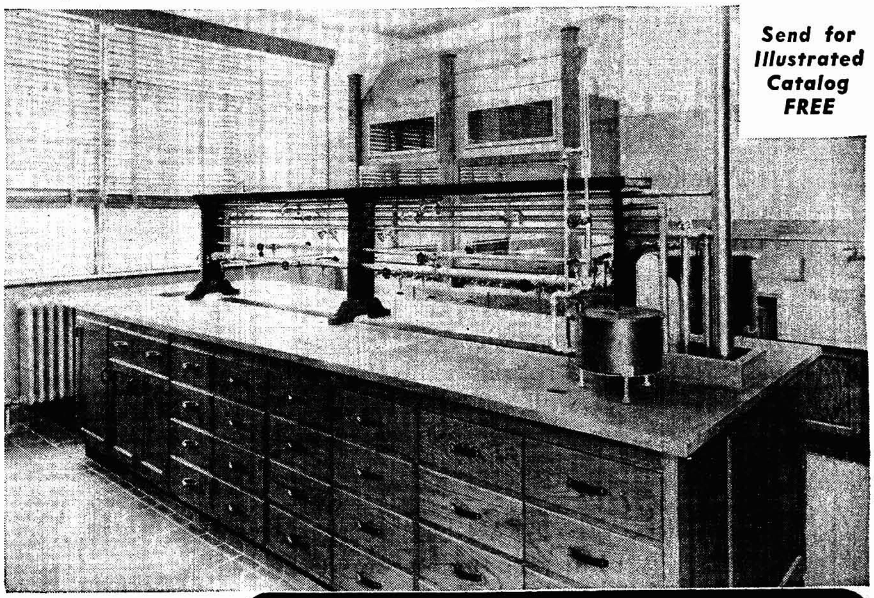

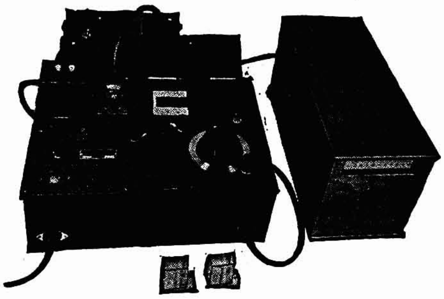

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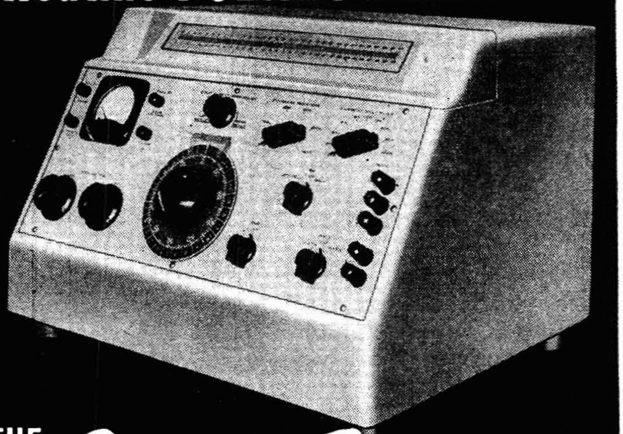

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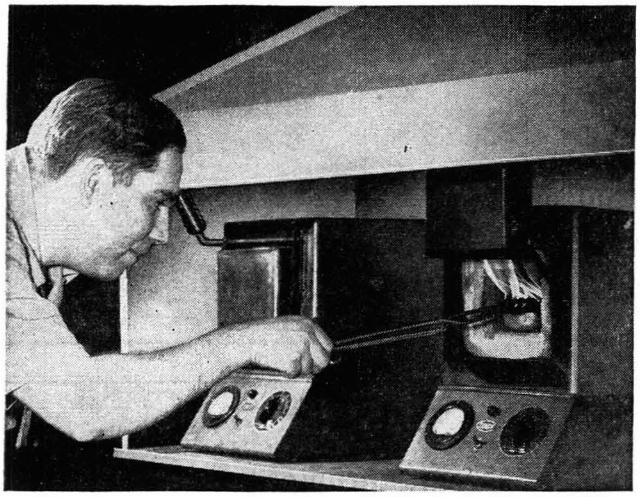


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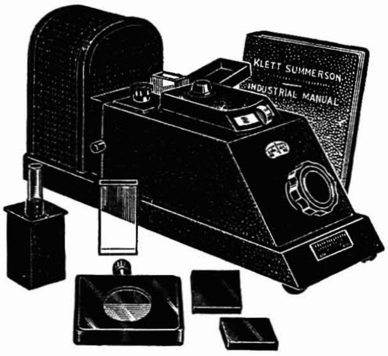
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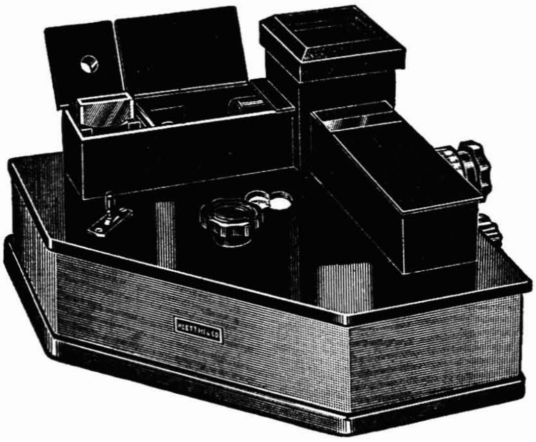

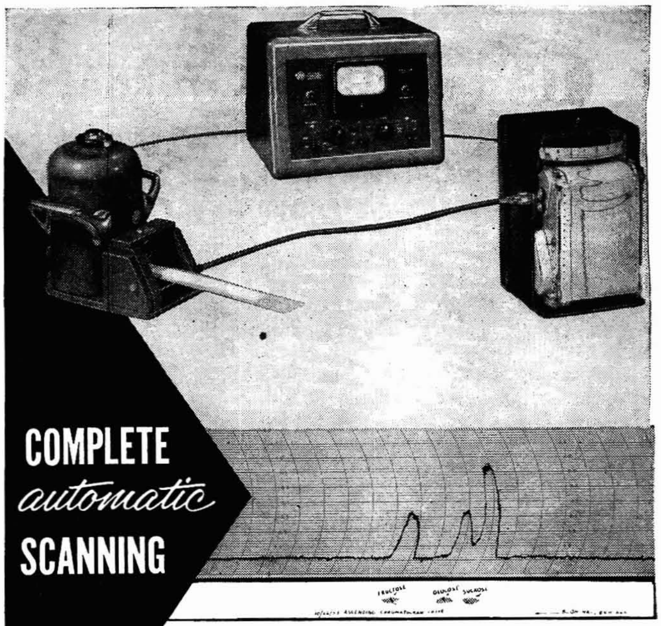


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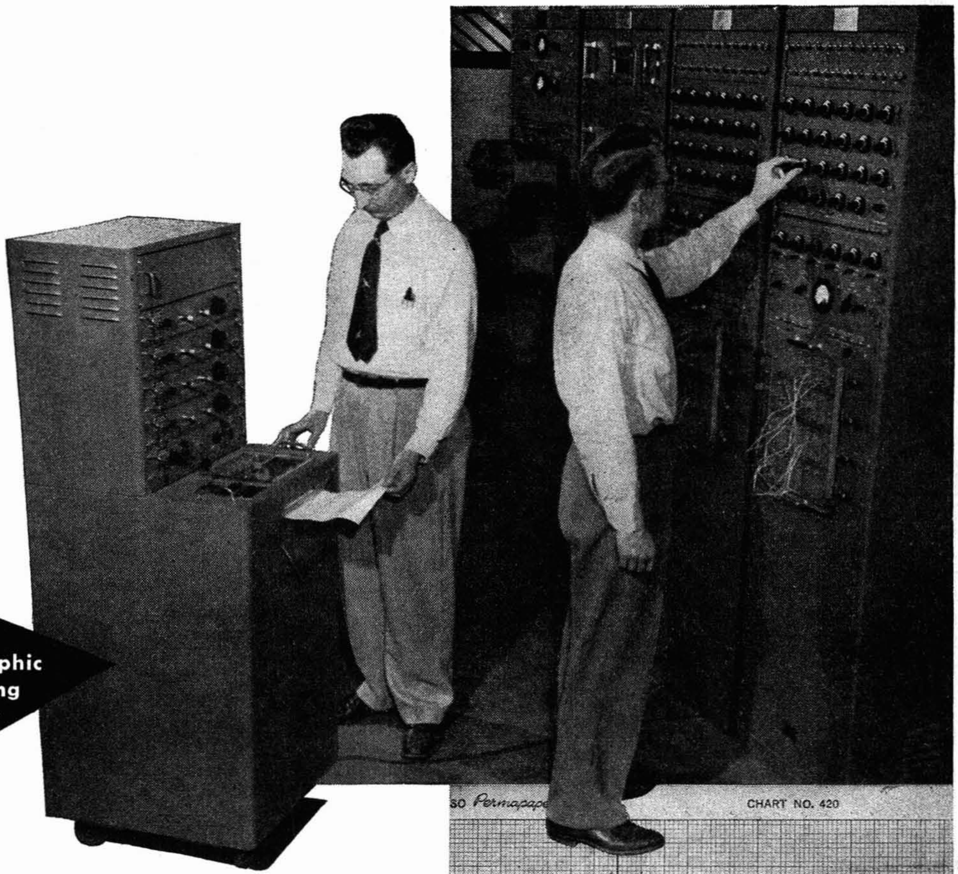

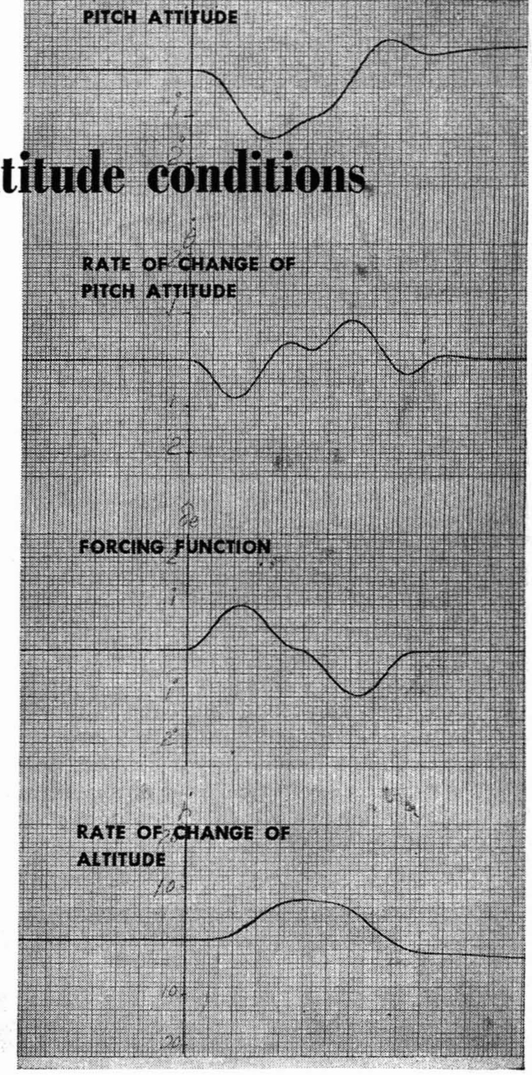
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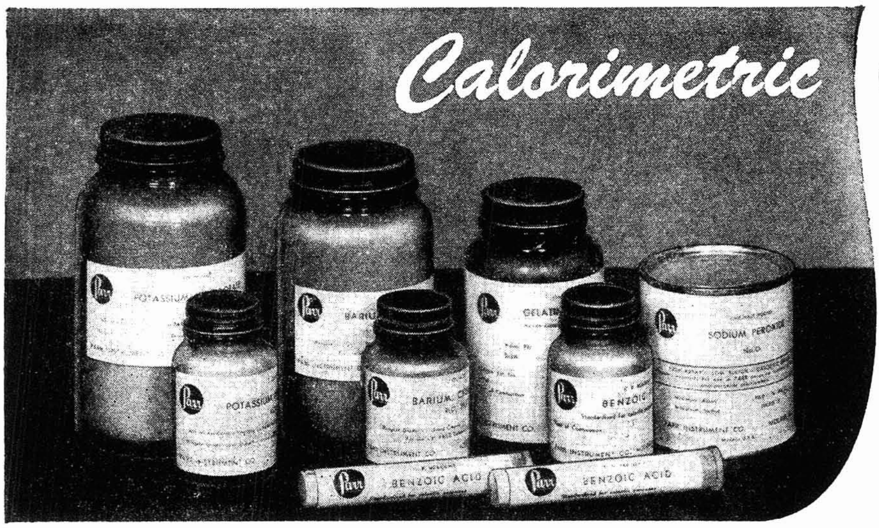

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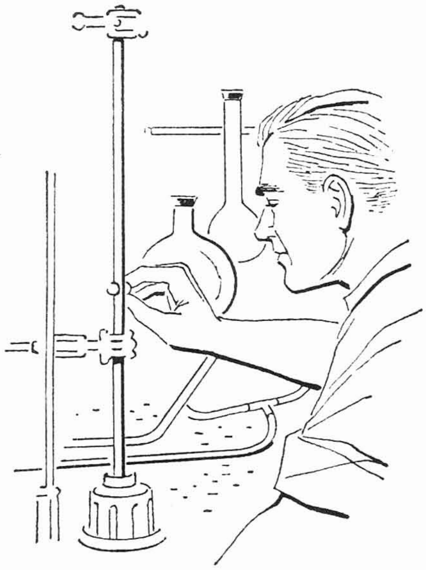

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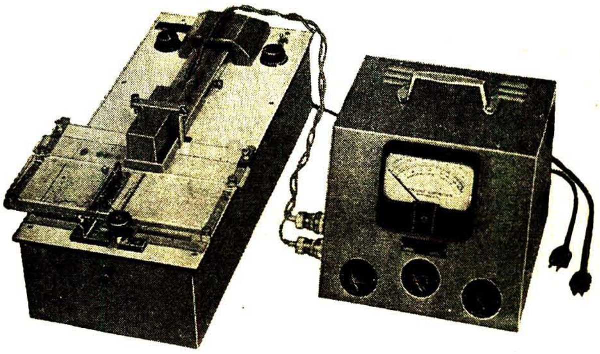

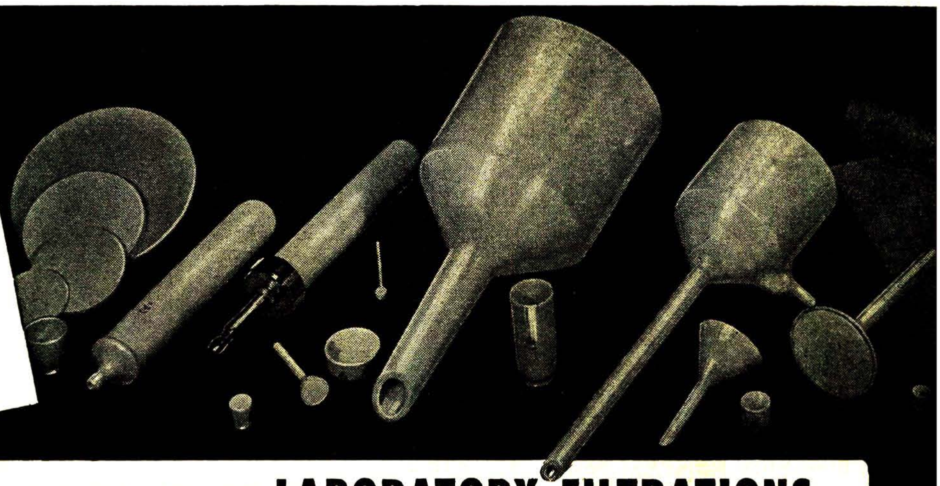


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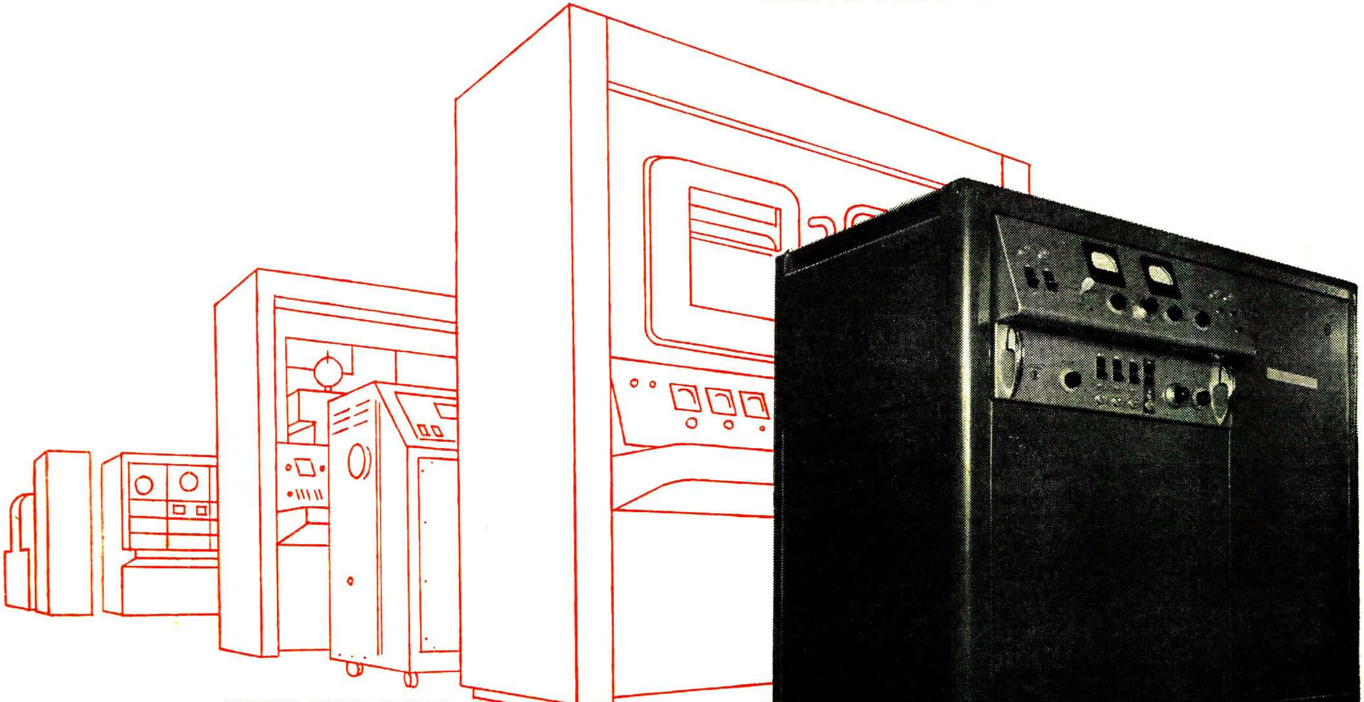

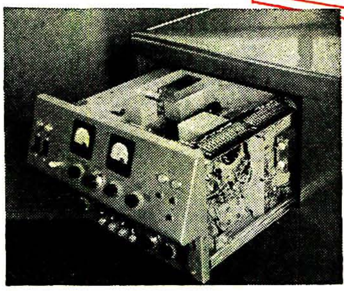

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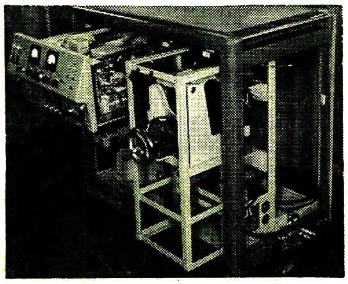

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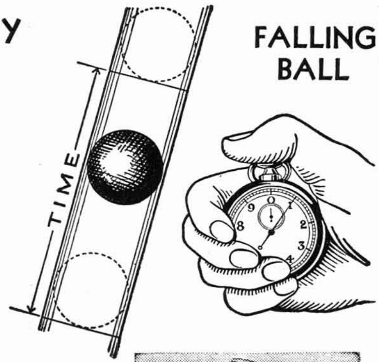
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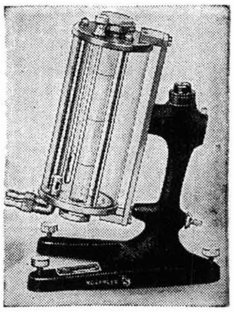

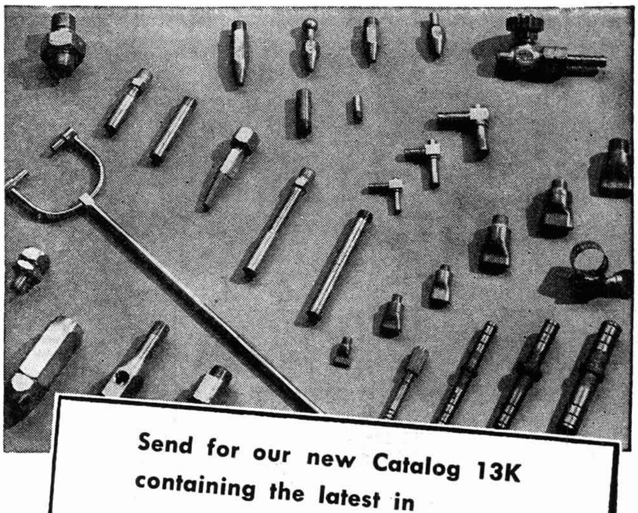

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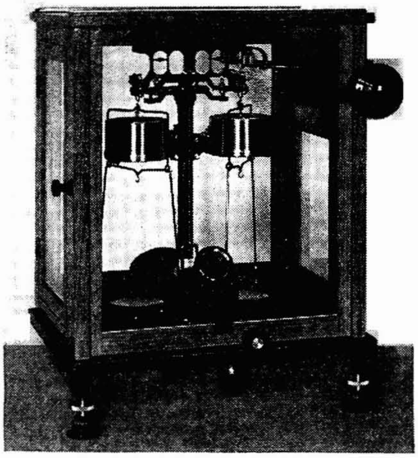

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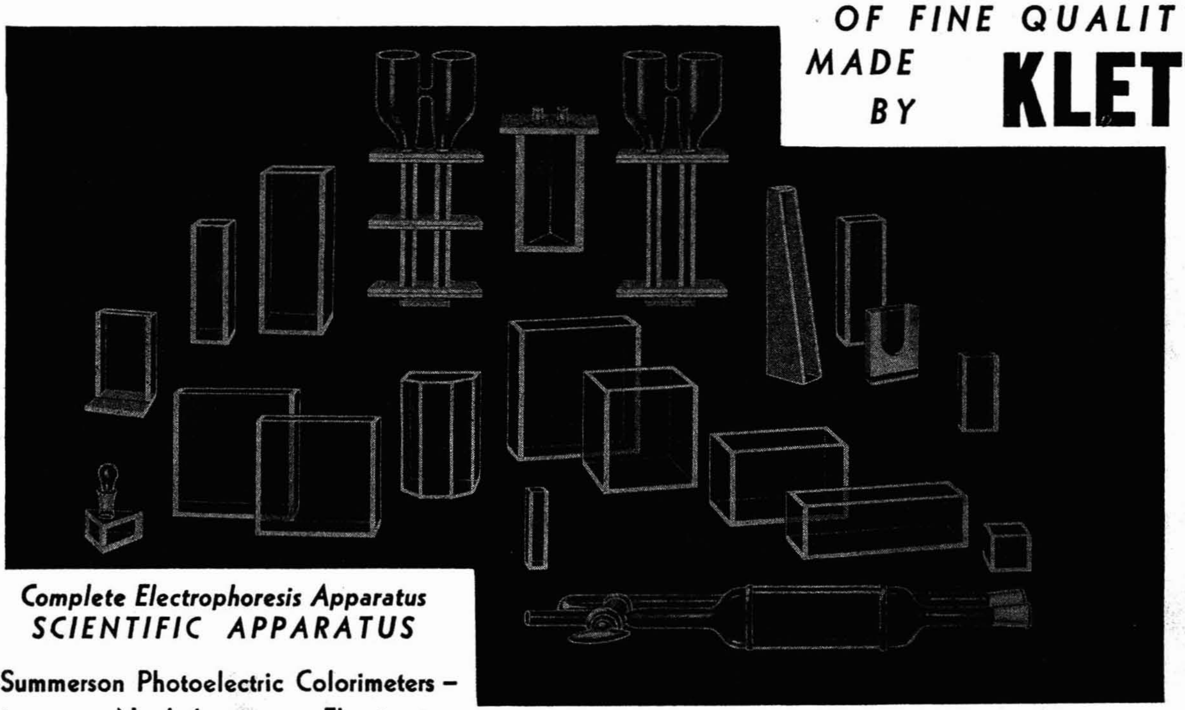

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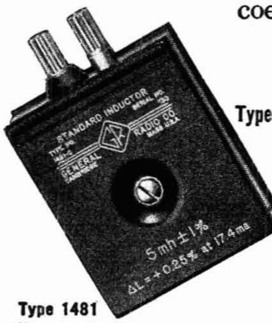
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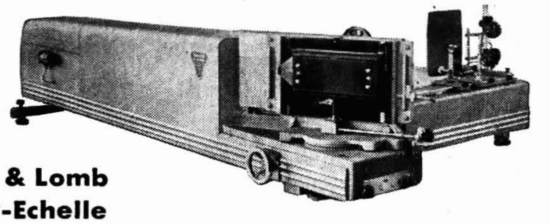

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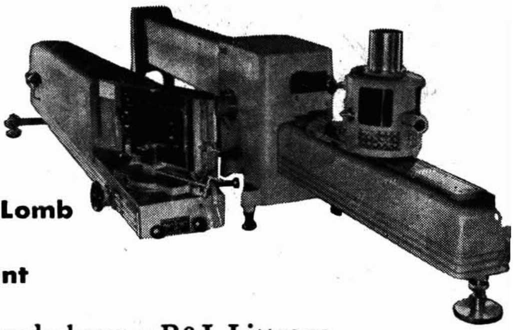

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