ANALYTICAL CHEMISTRY

MAY 1951

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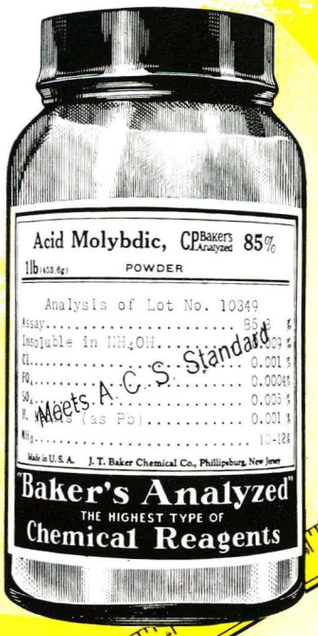

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ANALYTICAL CHEMISTRY

Volume 23, No. 5

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*Gilmont, R., *Anal Chem.* **20** 474, (1948)

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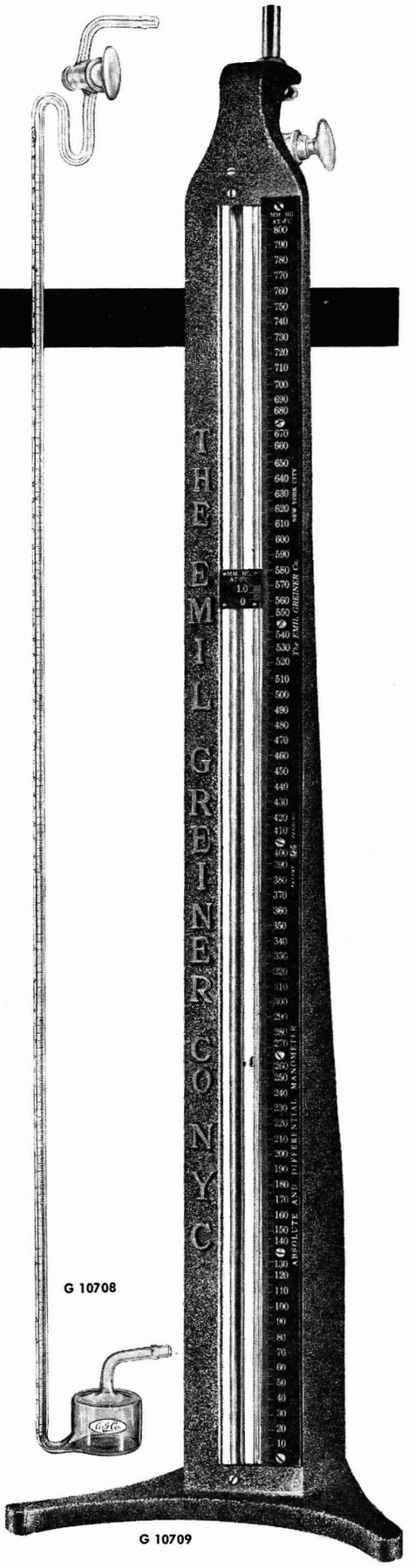

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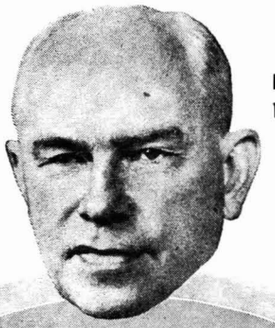

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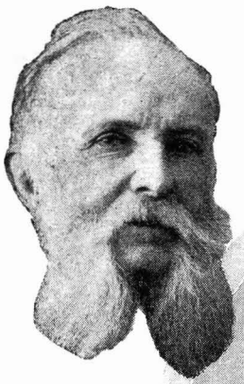

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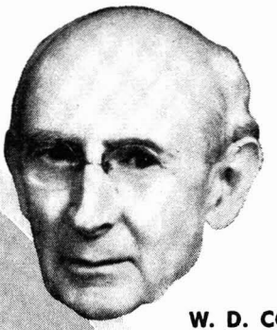

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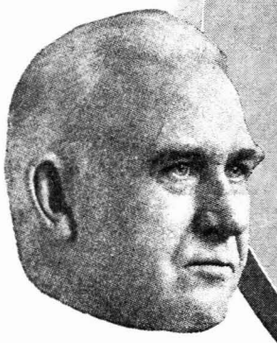

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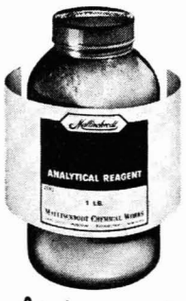

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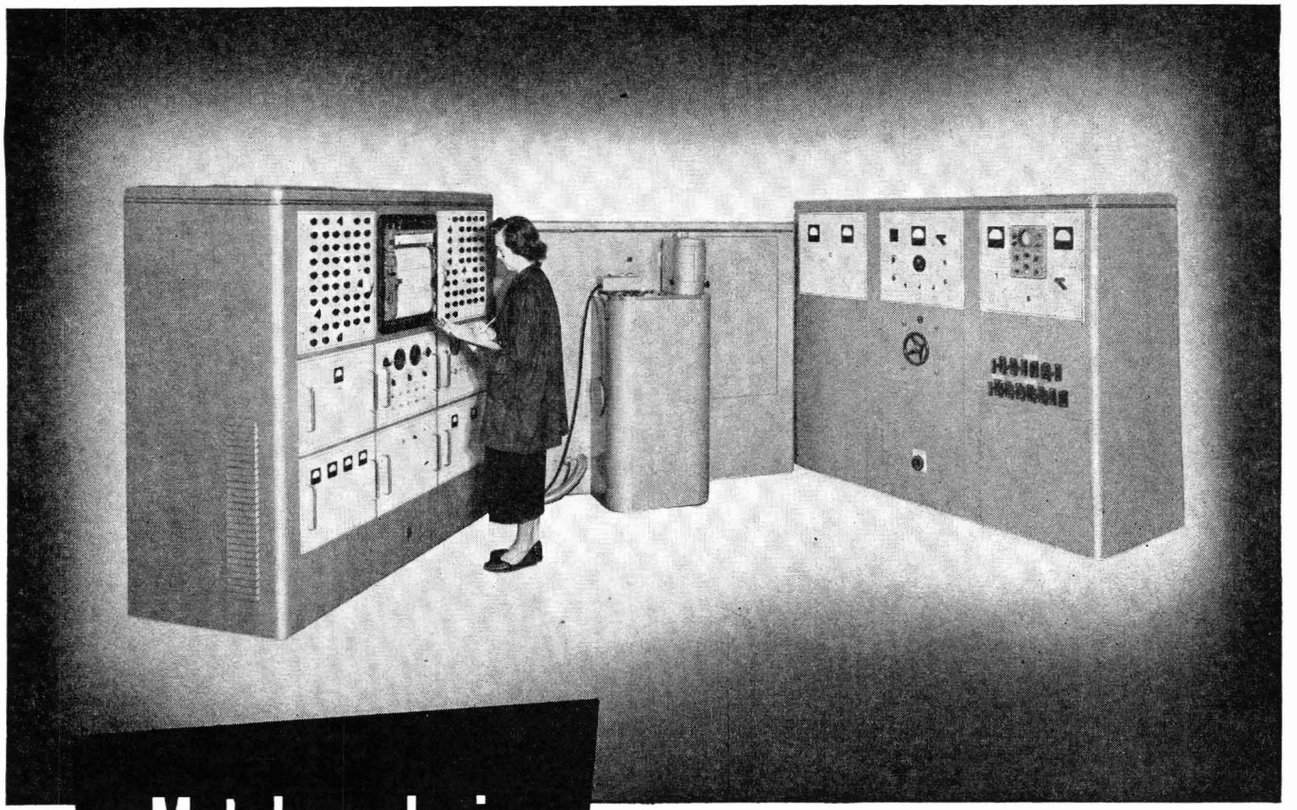

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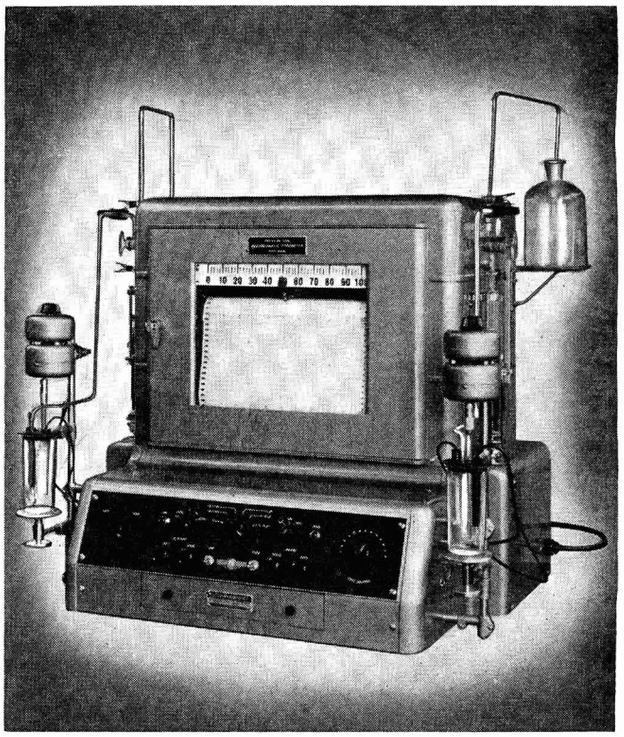

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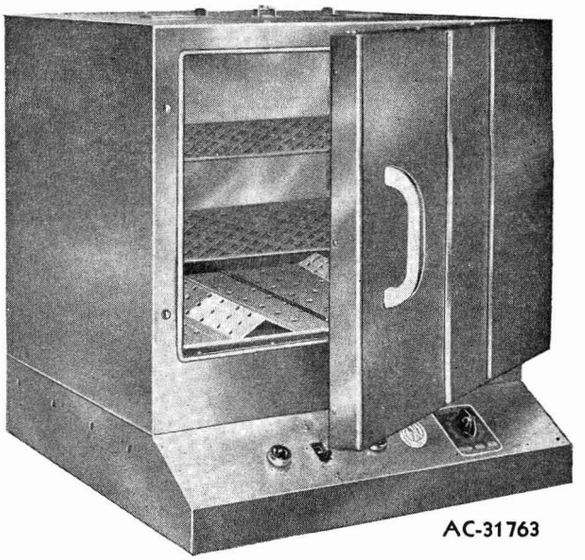

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Cat. No.	I. D. Inches W. × H. × D.	Temperature Range	Voltage A.C.	Each
AC-31759A	8 × 8 × 8	37.8-204°C.	115	**75.00**
AC-31759C	8 × 8 × 8	37.8-204°C.	230	**85.00**
AC-31761A	12 × 12 × 12	37.8-232°C.	115	**117.50**
AC-31761C	12 × 12 × 12	37.8-232°C.	230	**127.50**

Cat. No.	I. D. Inches W. × H. × D.	Temperature Range	Voltage A.C.	Each
AC-31763A*	19 × 15 × 18	37.8-288°C.	115	**225.00**
AC-31763C*	19 × 15 × 18	37.8-288°C.	230	**235.00**

* These models are also available with enameled steel exteriors at prices of 175.00 and 185.00 respectively.

NOTE: Built-in timer and switch can be furnished for any of the above ovens. Add $11.00 to above prices. Prices for D.C. current or special sizes, on request.

CONSTRUCTION: Double walled housing and door of polished stainless steel (Type 302, 18-8) inside and out.

INSULATION: All six sides insulated with thick blanket of Fiberglas wool; fireproof, moisture-proof, will not settle nor deteriorate with age.

HEATING ELEMENT: Heavy Nichrome V resistance wire spread over entire width of interior—assures positive temperature uniformity throughout entire working chamber. Helically coiled wire will actually withstand 2150°F. Refractories can stand 2600°F. Elements or refractories are easily replaced.

HEAT CONTROL: A sensitive, powerful, hydraulic, electric thermostat is conveniently mounted on front of base panel to allow easy setting of any temperature. Fine, extra large silver contacts are totally enclosed. The dial plate is graduated for both °F and °C readings. Approved by Underwriters Laboratory.

SHELVES: Two bright stainless steel electro polished wire shelves are furnished with larger models. Adjustable to five positions. Small model has one perforated stainless steel shelf.

ON-OFF SWITCH: Of ample capacity to place ovens into quick operation.

INTAKE PORTS: At bottom allows fresh air to enter. Exhaust ports at top, with stainless steel sides, gives control of exhausted fumes.

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The screw syringe is designed to facilitate the precise measurement and control of fluid in the capillary tube of a micropipet (0.001 to 0.1 cc). It makes possible highly accurate dilutions as, for example, in diluting blood for cell counting or micro chemical analysis. It also has outstanding use in the field of radioactive materials, bacteriology, etc., where there is the danger of sucking noxious or toxic materials into the mouth when regular macropipets are being used. Its small size and convenience of operation makes it an ideal instrument for extracting the extreme precision available in micropipet techniques for all types of fluid measurements.

Made with macro and micro controls, it is versatile and readily finger-tip controlled. The macro control plunger handles like a regular syringe while the finger-tip micro adjustments are made with the screw control. It is also equipped with an air vent that simply opens or closes with the twist of a collar. The vent permits the removal of the micropipet without displacement of the fluid in the capillary, or permits drainage by gravity in the larger pipets.

The syringe is made entirely of stainless steel with neoprene rubber seals for resistance to all forms of corrosion. The pipet is safely and firmly held in place by compression type rubber collet, of which several sizes are supplied to fit micro and macro pipets.

H-43015—Model MSS-3 Micro Screw Syringe—capacity, 3 cc; control accuracy: 1-2%. Price, including collets in four sizes **$15.00**

H-43020—Model MSS-15 Micro Screw Syringe—For use with large volume pipets, this is a large version of the 3 cc screw syringe described above. Capacity, 15 cc. Price **$30.00**

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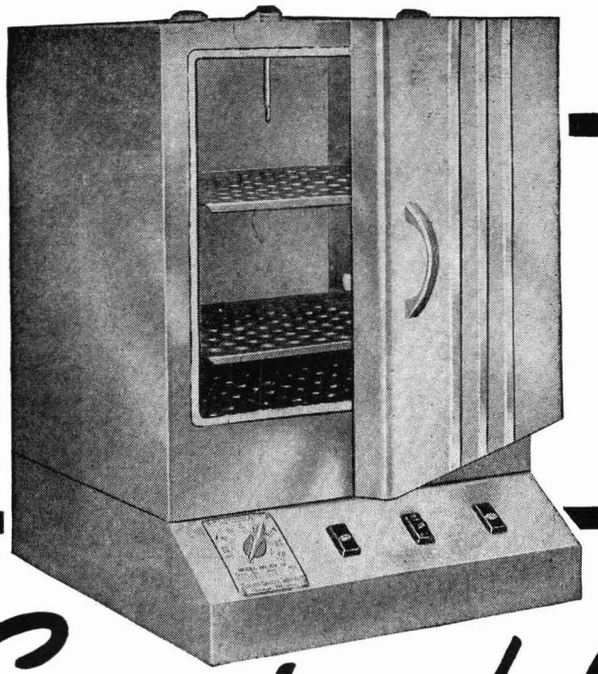

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Evenheat Ovens

These De-Luxe ovens are the result of superb engineering, the finest of workmanship and materials. Designed to withstand maximum abuse, they are ideal for analytical laboratory and general chemical work involving baking, drying, conditioning, pre-heating, and many other laboratory jobs.

CONSTRUCTION—Outside housing, door and base are made of beautiful shining No. 4 finish Stainless Steel (Type 302, 18-8). The inside working chamber is made of No. 2 finish Stainless Steel (Type 302, 18-8). Two adjustable shelves of electro-polished Stainless Steel Wire.

INSULATION—All six sides insulated with 1½" layer of fiber glass wool in Models AN-4440 and AN-4400, and 2½" layer in Models AN-4500 and AN-4480. Keeps heat loss at a minimum and outer surfaces cool even after prolonged use.

VENTILATION—Intake ports at bottom allow fresh air to enter. Double exhaust ports at top, with Stainless Steel slides, control exhaust fumes.

HEAT CONTROL—Sensitive, powerful, automatic hydraulic electric thermostat conveniently mounted on front allows easy setting of temperatures. Graduated for Fahrenheit and Centigrade readings; response sensitivity ± ½° C.

HEATING ELEMENT—Heavy Nichrome V resistance wire, spread over entire base of inside chamber. Positive temperature uniformity assured.

DOOR—Fully insulated, hung on a continuous hinge and fitted with a fool-proof latch designed to insure safety. Exclusive dovetail design gives positive seal.

PILOT LIGHTS—Red neon light denotes unit operation, green neon light for thermostat operation.

MODEL AN-4440 — A most popular oven, 12" x 12" x 12" inside. Temperatures to 232 deg. C. (100 to 450 deg. F. range). Two adjustable Stainless Steel shelves, 6-foot cord and plug. For 110 volt, 60 cycle, single phase A. C. current. 800 watts **$117.50**

MODEL AN-4460 — Same as AN-4440, but 110 volt D. C. **$127.50**

MODEL AN-4400 — New, small size — only 8" x 8" x 8" inside. Temperatures to 204 deg. C. (100 to 400 deg. F. range). One Stainless Steel shelf, thermometer, 6-foot cord and plug. For 110 volt, 60 cycle, single phase A. C. current. 500 watts **$75.00**

MODEL AN-4420 — Same as AN-4400, but 110 volts, D. C. **$85.00**

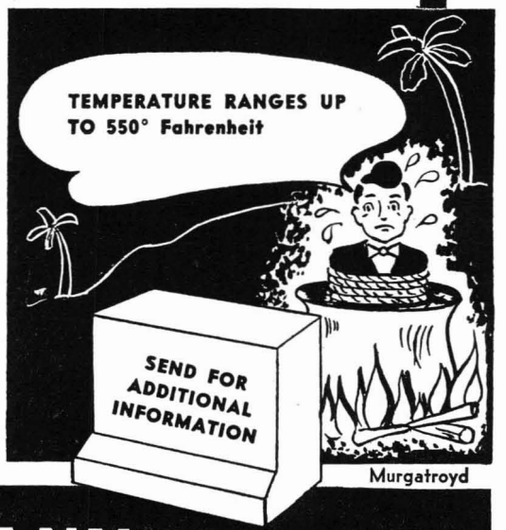
MODEL AN-4480 — A DeLuxe, large size, high temperature oven, 18⅞" x 17¾" x 14¾" high inside. Temperatures up to 288 deg. C. (100 to 550 deg. F. range). Two adjustable Stainless Steel shelves, thermometer, terminal block for electrical connections. For 110 volt, 60 cycle, single phase A. C. current. 1750 watts **$225.00**

MODEL AN-4490 — Same as AN-4480, but for 220 volt, 60 cycle, single phase, A. C. current **$235.00**

MODEL AN-4500 — Similar to AN-4480. Stainless Steel interior but baked enamel exterior, and aluminum coated shelves. For 110 volt, 60 cycle, single phase A. C. current. 1750 watts **$175.00**

MODEL AN-4510 — Same as AN-4500, but for 220 volt, 60 cycle, single phase A. C. current **$185.00**

All Models FURNISHED WITH BUILT-IN TIMER SWITCH (1 MINUTE TO 1 HOUR) $10 additional.



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the analyst's column

TECHNICAL Information Service, 732 Woodward Building, 15th and H Sts., Washington 5, D. C., is compiling a bibliography covering chemicals and allied products, abstracted in the "Bibliography of Scientific and Industrial Reports," now known as "Bibliography of Technical Reports" and published by the Office of Technical Services, U. S. Department of Commerce, Washington, D. C. The bibliography is divided into twelve parts covering the following subjects:

Agricultural Chemicals
Analytical Chemistry
Chemical Engineering and Equipment
Detergents
Drugs and Pharmaceuticals
Dyes
Inorganic Chemicals
Ordnance Chemicals
Paint, Varnishes, and Lacquers
Plastics and Plasticizers
Miscellaneous Chemicals
Patents on Chemicals and Allied Products

The first two have been issued and are priced at $5 each. Parts 3 and 4 are nearing completion.

Part 2, Analytical Chemistry, covers all Publication Board Reports so classified in Volumes 1 to 13, January 1946 to June 1950. This book is planned for the use of directors of research and development departments, technical librarians, chemists and chemical engineers, physicists, biologists, patent searchers and researchers, laboratory technicians, analysts, and schools of chemistry in colleges and universities offering research service to industry. It is an index guide to:

Captured German-language reports
Reports released by various agencies of the U. S. Government
Reports released by British government agencies
Reports prepared by a large number of laboratories in colleges and in chemical industries of the United States
Final reports prepared as a result of special investigations in Germany by teams sponsored by certain American and British industries

The five sections into which the index is divided offer a complete coverage of these important documents.

Section 1, Subject Index, contains 121 pages and consists of 4835 entries, to which there are approximately 16,000 annotations, covering 402 basic reports. The entries are arranged in a straight alphabetical classification. Data are cross-indexed wherever they seem to add to the usefulness of this publication.

THE Institute of Statistics of the University of North Carolina is offering courses in statistics at its summer session, June 11

(*Continued on page 19 A*)

THE ANALYST'S COLUMN

to July 19. Increasing interest by chemists and chemical engineers in this subject makes intensive training in this field over a short period necessary for those who have completed their formal education.

Progress in the theory, application, and teaching of statistics has been rapid during recent years. This development has been stimulated not only by the recognition of the scope, usefulness, and efficiency of statistical methods when applied in various fields of scientific research, but also by advances in the theory of statistics which have resulted in more powerful tools for the collection, analysis, and interpretation of data. 1951 summer courses include:

Basic Experimental Statistics
Applied Mathematical Statistics
Design of Experiments
Sample Survey Designs
Statistics for Chemists
Advanced Experimental Statistics
Seminar for Statistical Problems
Mathematics of Design of Experiments
Statistical Inference

Of particular interest to analytical chemists in the above list is the course on "Statistics for Chemists" to be given by W. J. Youden, assistant chief, Statistical Engineering Laboratory, National Bureau of Standards. He has applied statistical techniques in his own experimental work for over 20 years and has a wide experience in collaboration with chemists, physicists, and engineers. The course will deal with precision and accuracy, statistical units, evaluation of precision, and comparison of averages; resolution of sampling and analytical errors; determination of number of samples and analyses per sample; locating sources of error in analytical procedures; requirements data should meet; statistical techniques for evaluating data; and schemes for improving the precision of comparisons.

Fees and living expenses amount to about $125 for the 6-week period. For additional information write to Mrs. Sarah Carroll, Institute of Statistics, North Carolina State College, Raleigh, N. C.

YOUR attention is also directed to page 812 where the week-long Symposium on Statistics at the Gordon Research Conferences is outlined.

WHILE on the general subject of where to spend the summer, Boston College has again announced its special 2-week intensive course in modern industrial spectrography at Chestnut Hill, Boston, Mass., from July 23 to August 3. The course is particularly designed for chemists and physicists from industries in the process of installing spectrographic equipment. Information can be obtained from James J. Devlin, Physics Department, Boston College, Chestnut Hill, Boston 67, Mass.

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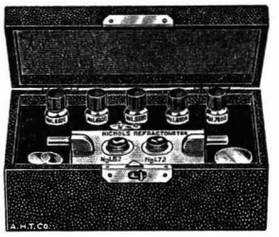
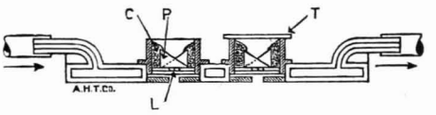
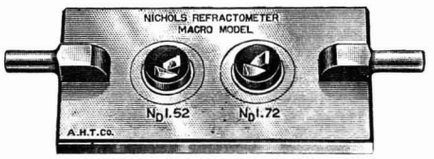
For determination, on the stage of a microscope, of the refractive indices of liquids and solids within a wide range



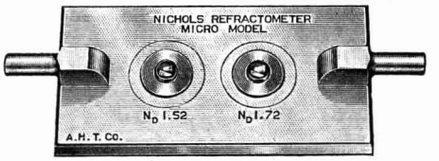
8664.
Complete Outfit, in case



Cross-section of Nichols Refractometer, indicating water circulation within jacket. P, prisms; C, cement; L, engraved line; and T, cover glass



Nichols Refractometer, Macro Model



Nichols Refractometer, Micro Model

NICHOLS REFRACTOMETER, Improved Model, with metal water jacket. For determination of the refractive indices of liquids on the stage of a microscope. The range for liquids is practically unlimited. Potential accuracy under controlled conditions of temperature and light is $n_D = \pm 0.0005$ and, under usual laboratory conditions, is $n_D = \pm 0.001$. See Lyman Nichols, *National Paint Bulletin, Vol. 1 (Feb., 1937), p. 12, and Mar., 1937, p. 14;* and Herbert K. Alber and James T. Bryant, *Industrial and Engineering Chemistry, Anal. Ed., Vol. 12, No. 5 (May 15, 1940), p. 305.*

A determination consists of a simple measurement under the microscope of the distance between two lines and reference to a calibration graph — prepared in accordance with the method outlined below — which permits conversion of the distance directly into n_D without further calculations. The two lines observed in the microscope are refractions of the single line L, 0.0001 inch wide, engraved on the glass base of the cell beneath the prisms and are produced because of the difference between the refractive index of the prisms and that of the sample placed in the cell. The n_D of solids can also be determined by indirect methods as described in the directions for use.

The instrument is offered in two models, i.e. Macro and Micro, each having a nickel plated brass water jacket, 76 × 38 × 4.5 mm, to permit precise temperature control and two cells marked n_D 1.52 and n_D 1.72, respectively, to provide for convenient measurement of a wide variety of liquids. Each model is supplied with two cover glasses to prevent evaporation of liquids with high vapor pressure. Macro cells are 11 mm outside diameter and require 100 to 200 cu. mm (2 to 4 drops) of sample; Micro cells are 5 mm outside diameter and require only 6 to 8 cu. mm of sample, of which 5 to 6 cu. mm can be recovered, depending upon the physical properties of the liquid.

Method of Calibration. To calibrate the instrument in accordance with the individual characteristics of the cell and microscope set-up used, the cell is filled with a liquid of known refractive index, covered with a cover glass and placed under a microscope with a magnification of approximately 100×. Then, using the special eyepiece micrometer disc on the diaphragm of the microscope eyepiece, the distance between the two lines observed is measured. This is repeated for each of the five standard liquids supplied with the outfit, and a graph prepared on cross section paper by entering the refractive indices as ordinates and the measured distances in scale divisions as abscissae. The points plotted for the standard liquids will form a line which is nearly straight and from which observed distances for liquids of unknown refractive index can be converted directly into terms of refractive index provided the conditions of test are identical.

8664. Refractometer, Nichols, Improved Macro Model, complete outfit, as above described, with cells 11 mm outside diameter, with two cover glasses, eyepiece micrometer disc and set of five standard liquids, n_D 1.4000, 1.4500, 1.5500, 1.6500 and 1.7000, respectively, in leatherette velvet-lined case, with detailed directions for use.. **84.00**

8665. Ditto, Micro Model, with cells approximately 5 mm outside diameter..... **104.00**

More detailed information sent upon request.

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ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

Again We Favor "Improved Service"

AN EDITORIAL entitled "Improved Service" in the January 1929 issue of *Industrial and Engineering Chemistry* announced the ANALYTICAL EDITION of that journal. Its purpose was to segregate articles dealing with analytical methods and apparatus, which up to that time had been part of the *Industrial Edition*. Mention was made of the greater ease with which they would now be available to the analyst, and of the wider utility they might have in "every field of chemistry." Publication began on a quarterly basis and continued so until 1933, when a bimonthly was announced, again editorially and in *Industrial and Engineering Chemistry*. This more frequent appearance of the ANALYTICAL EDITION was proclaimed as the happy result of greater popularity than had been anticipated, and of a growing demand for the journal by those in industrial plants and laboratories.

By 1937 it was time for a monthly issue, the important change being announced in the journal itself by means of an editorial entitled "Improved Service to Analysts," the first to appear in the ANALYTICAL EDITION. It was not until ten years later that editorials became an established feature. The January 1937 editorial pointed out that the journal had developed its service to the point where "its position in the field of chemical literature is unquestioned and is to be envied." This was strikingly emphasized by a statement to the effect that contributions now dealt with new analytical methods and procedures, including physical testing and new apparatus, and that papers dealing with microchemistry were to be set apart in a special section.

During the initial nine-year period the number of published pages increased from 233 in 1929 to 598 in 1937. With such growth it was logical that progress would not halt, pagewise or purposewise, and by 1947 the January editorial discussed "A New Name and an Expanded Program," and promised to try to "meet the changing and expanding needs of its readers and as far as possible to anticipate such needs." That year marked the beginning of ANALYTICAL CHEMISTRY as an independent journal, and introduction of the monthly editorial, the "Aids for the Analyst" section, and the Analyst's Column. The text pages for the year totaled 1046.

Now in its fifth year as an independent journal and definitely established as the world's leader in its field, ANALYTICAL CHEMISTRY looks back on a record of 1582 pages published in 1950 and strives daily to keep in step with the times and with the increasing volume of contributed articles. Readers cannot help noticing that the range of subjects discussed in the journal has changed considerably in the past several years. Today a strict adherence to the original criteria for articles would seriously impair the usefulness of the journal. It is necessary to publish contributions which in earlier days would have been considered more appropriate for other journals. Indeed, in some cases the broadening of the analytical horizon has been so seemingly radical that readers have questioned our judgment in interpreting the analyst's interests. However, despite the few who still favor a restricted field for the analytical chemist, the editors plan to continue efforts to mirror through ANALYTICAL CHEMISTRY the modern—sometimes ultramodern—developments in analysis, both in industry and in academic institutions. As in recent times, papers will sometimes be purposely selected because of their value in revealing trends and anticipating needs.

Perhaps the greatest recent changes have taken place in emphasis on the physical and on the application of physics and physical chemistry to the solution of analytical problems. This again has been necessary to meet the growing needs of a growing group. Doubtless most of our space will continue to be used for articles of strictly analytical content, but we cannot overlook newer techniques, regardless of basic principle, if in our judgment they can be applied to the solution of analytical problems. Only by such adaptability can ANALYTICAL CHEMISTRY continue to flourish and live up to its initial promise of "Improved Service" for analysts.

In Appreciation

THE long-awaited appearance of the book "Reagent Chemicals, American Chemical Society Specifications 1950" marks a milestone—and an important one—in the work of the Society's Committee on Analytical Reagents. Analytical chemists the world over have reason to be grateful to the members of this committee, who for years have worked on the perfection of these specifications, which are intended to serve for reagents to be used in precise analytical work. These men have tested and retested in their laboratories, met frequently for discussions, and more recently have spent long hours checking the galley and page proofs of the book. Other chemists who have not been members of the committee have also furnished valuable assistance in the preparation of these specifications—in particular, in laboratories of organizations with which committee members are connected.

The original committee was appointed in 1917, and as early as 1921 published four specifications in *Industrial and Engineering Chemistry*—for hydrochloric, nitric, and sulfuric acids, and ammonium hydroxide. Other specifications were published from time to time, more recently in ANALYTICAL CHEMISTRY, and the work of the committee has gone on continuously. The specifications included in the present book are the result of the work on reagents done since 1917, but these specifications are in large measure due to the efforts of those holding membership since 1925. The 1950 committee is responsible for the preparation of the present edition. W. D. Collins, a member of the committee since 1920 and its chairman for several years, has done yeoman work in assembling and editing the manuscript. Special thanks are also due to Edward Wichers, chairman of the committee since 1943.

The responsibilities of the committee have not come to an end with the appearance of this book, but its work will continue, in the expectation of extending the list of reagent specifications, modifying existing specifications as better reagents become available, and improving methods of test.

To all those who have served on the committee, and to those not on the committee who have helped in preparing and checking specifications for reagent chemicals, the chemical profession and the chemical industry, including all who depend in any way on authoritative chemical standards, owe a lasting debt of gratitude.

X-Ray Diffraction Patterns of Solid Aromatic Hydrocarbons

L. J. E. HOFER AND W. C. PEEBLES

Office of Synthetic Liquid Fuels, Bureau of Mines, Bruceton, Pa.

For the positive identification of solid crystalline aromatic hydrocarbons, x-ray diffraction analysis is proposed. X-ray powder diffraction patterns of 59 hydrocarbons have been obtained using iron-target radiation. Impurities sufficient to lower the melting point by as much as 15° C. do not appreciably modify the diffraction patterns. The patterns are very characteristic; those of closely related compounds and even isomers are unique and can be readily identified.

INVESTIGATIONS into the basic aspects of coal hydrogenation at this laboratory have led to the preparation of a series of highly purified solid aromatic hydrocarbons that are of interest to the coal tar, dye, plastics, fuel, and other industries. The well-known relationship of such compounds to carcinogenesis makes them especially interesting in the field of medicine. Aromatic hydrocarbons of high molecular weight are often difficult to purify, resulting in tedious and difficult identification by melting point. In some instances—for example, periflanthene—the melting point is unattainable. X-ray diffraction patterns of relatively impure aromatic hydrocarbons, however, are characteristic. Even minor modifications in structure produce almost completely different powder diffraction patterns. To take full advantage of the possibilities that x-ray diffraction offers as a method of identification of micro samples, a device for preparing extruded specimens from very small samples (2 to 3 mg.) using no permanent binder has been developed (*12*). Thus, x-ray diffraction offers a completely nondestructive method of microanalysis.

PROCEDURE

A modified form of the technique employed by McKinley, Nickels, and Sidhu (*14*) was used. A finely ground sample con-

Table I. Melting Points and Three Most Intense Diffraction Lines of Aromatic Hydrocarbons

(Arranged in order of first lines)

Pattern No.	Three Strongest Lines 1st	2nd	3rd	Melting Points, ° C. Literature	Found[a]	Compound
54	10.9	3.96	8.0	257 (*7*)	255.7[b]	Dibenzo[*cd,jk*]pyrene[c]
50	10.5	4.01	4.61 4.31	91.4 to 92.4 (*21*)	91.4 to 92.4	5-Ethylchrysene[d]
22	8.4	5.3	3.63	135.2 to 135.7 (*23*)	135.2 to 135.7	4,5-Dihydropyrene[e]
38	8.3	5.85	3.71	100.5 (*11*)	101.6 to 101.9	Bimesityl[f]
29	8.1	3.27	4.12	147.5 to 148.5 (*2*)	148.8 to 149.4	4-Methylpyrene[e]
58	6.0	6.6	4.70	...	142	2,2′,7,7′-Tetramethyl-1,1′-binaphthyl[e]
17	5.6	4.51	5.4	76 (*1*)	77.2 to 78.2	1,4-Di-*tert*-butylbenzene[g]
49	5.4	3.68	10.9	128.6 to 129.8 (*21*)	128.6 to 129.8	5,6-Dimethylchrysene[d]
5	5.3	4.15	3.98 4.59 4.97	46 to 47 (*13*)	45.6 to 45.8	2,2′-Dimethyldicyclopentyl[h]
12	5.3	3.45	9.3	45 (*7*)	44.8 to 45.6	9-Methylfluorene[c]
31	5.2	5.8	4.31	67.5 to 68.5 (*6*)	68.9 to 70.4	1-*o*-Tolyl-naphthalene[h]
16	5.2	3.98	4.18	73 to 74 (*7*)	72.0 to 73.0	1,2,3,4,5,6,7,8-Octahydroanthracene[c]
28	5.1	3.67	3.33	124.5 (*7*)	126.8 to 127.4	3,4-Benzofluorene[c]
2	5.1	3.49	3.63 7.0	95 (*7*)	93.2 to 93.8	Acenaphthene[i]
33	5.0	12.1	3.93	159.5 to 160.5 (*7*)	158.0 to 159.0	1,2-Benzanthracene[i]
42	5.0	3.98	12.1	215.5 to 216.0 (*18*)	217.0 to 217.4	8,9-Benzofluoranthene[h]
55	5.0	3.39	13.6	364 (*3*)	366.0 to 367.0	Picene[h]
56	4.97	13.4	3.65	251.6 to 252.2 (*22*)	254.5 to 255.0	Methylpicene[d]
9	4.93	3.36	9.2 4.02	100.7 to 101 (*7*)	98.8 to 99.8	Phenanthrene[i]
40	4.88	4.51	3.87	161.0 to 161.4 (*20*)	161.0 to 161.4	6-Methylchrysene[d]
48	4.88	3.95	4.625	196.6 to 197.2 (*22*)	196.6 to 197.2	2-Phenylphenanthrene[e]
26	4.88	3.39	4.10	189 to 190 (*7*)	185.4 to 186.0	1,2-Benzofluorene[f]
30	4.86	4.62	3.68	55.5 (*11*)	54.3 to 55.3	2-Benzylnaphthalene[h]
32	4.86	4.33	11.75	45.7 to 48.0 (*8*)	45.7 to 48.0	2-*o*-Tolyl-naphthalene[h]
39	4.80	3.81	10.7	117.2 to 117.8 (*21*)	117.2 to 117.8	5-Methylchrysene[d]
7	4.76	3.77	9.8	46 to 47 (*10*)	53.8 to 54.2	2-Isopropenylnaphthalene[g]
45	4.74	4.38	3.84	79 to 80 (*11*)	76.4 to 77.4	1,2′-Binaphthyl[c]
15	4.72	3.48	4.12	103 to 105 (*7*)	99.8 to 100.6	1,2,3,4-Tetrahydroanthracene[c]
23	4.71	3.86	3.23	102.5 (*16*)	102.2 to 103.8	2-Phenylnaphthalene[h]
10	4.68	5.1	7.3	108.5 (*7*)	108.6 to 109.6	9,10-Dihydroanthracene[i]
27	4.68	4.11	3.36	208 to 209 (*7*)	213.0 to 214.2	2,3-Benzofluorene[k]
25	4.67	5.4	4.25	46 (*5*)	47.0 to 47.8	1-(1-Cyclohexen-1-yl)-naphthalene[h]

[a] Corrected.
[b] Decomposed.
[c] Synthesized by M. Orchin, Bureau of Mines.
[d] Donated and synthesized by M. S. Newman, Ohio State University.
[e] Donated by M. S. Newman, Ohio State University.
[f] Synthesized by L. Reggel, Bureau of Mines.
[g] Donated by J. E. Nickels, Mellon Institute.
[h] Donated by Bureau of Mines.

taining no binder was partly extruded from a 0.75-inch tube of 19-gage, stainless-steel tubing having an inside diameter of 0.7 mm. The detailed method of sample preparation is described in another paper (*12*).

Each specimen was exposed in a 114.6-mm. diameter, Debye-Scherrer camera for 6 hours in order to obtain the long spacings (up to 19 A.) in the low angle region; the sample was overexposed in a 57.3-mm. diameter, Debye-Scherrer camera for 2 hours to bring out the shorter spacings in the larger angle region of the diffraction pattern. Long wave length radiation (FeK_α, $\lambda = 1.937$ A.) was obtained from a commercial sealed-off x-ray tube equipped with an iron anode, beryllium windows, and manganese oxide filters.

In choosing a method for measuring the diffraction patterns of organic crystalline compounds for identification purposes, the following considerations are important. In general, the patterns of these compounds exhibit strong reflections only at small Bragg angles ($\theta \lesssim 45°$) even when long wave-length radiation (CrK_α or FeK_α) is used in producing the powder patterns. This effect is related to the fact that the amplitudes of the thermal vibrations of the atoms in organic molecules are a large fraction of the crystal spacing, d, when d is small—that is, when θ is large. The width of the diffraction lines is exactly equal to the specimen width if parallel radiation is used and if little or no absorption takes place in the specimen (*24*). But neither of these conditions is actually met generally. Divergent radiation broadens the lines in the region $\theta < 45°$ and narrows the lines in the region $\theta > 45°$. Inasmuch as the patterns studied herein were found only in the region $\theta \lesssim 45°$, only the broadening effect need be considered. The effect of finite focus size is to broaden the lines in all regions. Because of the efficient centering system, the broadening effect resulting from eccentricity is so small as to be negligible. Finally there is the absorption effect which tends to shift the point of maximum density toward larger values of θ and toward smaller values of d. The absorption effect increases with the product μr where μ is the absorption coefficient and r is the specimen radius. In the present study $\mu r = 10$ reciprocal cm. and $r = 0.035$ cm. Thus, μr is 0.35 and is large enough to produce a small but definite shift of density maximum (*24*).

The following are several methods of measuring the arc corresponding to the angle 4θ: (*a*) measurement of the distance between the densest portions of corresponding lines; (*b*) measurement of the distance between the outside edge of one line to the inside edge of the corresponding line; (*c*) measurement of the distance between the outsides of the two corresponding lines and subtraction of the specimen width from the distance. Method (*a*) produces maximum systematic errors in the region $\theta < 45°$ owing to absorption. These systematic errors follow a definite law but cannot be evaluated without indexing the patterns. Inasmuch as indexing is in general impossible, this method is not acceptable. Methods (*b*) and (*c*) avoid the difficulty in method (*a*), because the positions of the edges with respect to the true center of the line are determined only by the specimen diameter, the divergence of the radiation, and the form of the focus. However, the location of the edges is difficult; the edges of the dense lines tend to be located farther from the center than the edges of light lines. Furthermore, the outside edge (large θ edge) is more readily located than the inside edge because outside half of the line is denser than the inside half whenever there is appreciable absorption as is the case here. Thus, method (*c*), which uses the outside edges only, has some advantage. Furthermore, the diffraction patterns of organic com-

Table I. (*Continued*)

(Arranged in order of first lines)

Pattern No.	Three Strongest Lines 1st	2nd	3rd	Melting Points, ° C. Literature	Found[a]	Compound
3	4.62	4.24	3.98	71 (*11*)	68.5 to 69.4	Biphenyl[l]
46	4.605	3.98	3.31	187 to 188 (*11*)	186.4 to 187.4	2,2′-Binaphthyl[c]
35	4.60	12.1	2.135	341 (*7*)	333.0 to 334.0	Naphthacene[e]
4	4.60	9.9	3.92	66 (*11*)	63.3 to 64.8	2-Vinylnaphthalene[g]
36	4.60	4.36	3.85	213 (*11*)	206.0 to 208.0	1,4-Diphenylbenzene[g]
6	4.59	3.35	4.15	116 to 117 (*7*)	114.8 to 115.4	Fluorene[c]
8	4.53	9.0	3.03	216.2 to 216.4 (*7*)	216.0 to 218.0	Anthracene[i]
1	4.53	7.2	3.39 3.49	80.06 (*17*)	80.2	Naphthalene[m]
57	4.51	3.59	10.8	203.6 to 204.4 (*22*)	203.6 to 204.4	13-Methylpicene[d]
41	4.44	5.3	3.67	131 to 132 (*9*)	132.0 to 133.0	1-Methyl-3,5-diphenylbenzene[g]
24	4.09	4.87	3.73	152.5 (*11*)	152.8 to 153.7	*trans-trans*-1,4-Diphenyl-1,3-butadiene[f]
14	4.03	4.33	7.0	125 (*11*)	120.8 to 122.0	4,4′-Dimethylbiphenyl[f]
52	3.98	4.57	6.4	...	140.2 to 141.2	3,4,3′,4′-Tetrahydro-1,1′-binaphthyl[e, i]
20	3.95	5.1	4.88	110 (*7*)	110.6 to 111.0	Fluoranthene[j]
19	3.92	4.83	9.0	90 to 91 (*7*)	90.9 to 91.4	9-Methylphenanthrene[n]
18	3.87	5.3	8.2	116 (*7*)	115.5 to 116.3	4-Cyclopenta[*def*]phenanthrene[o]
11	3.86	5.25 5.0	8.5	63 (*7*)	70.4 to 71.2	4-Methylfluorene[c]
37	3.82	8.6	7.7	...	74.8 to 75.3	4-Ethylpyrene[e]
13	3.81	4.53	5.1	124 (*11*)	123.2 to 125.1	*trans*-Stilbene[j]
21	3.78	8.3	7.5	156 (*7*)	151.4 to 152.0	Pyrene[i]
43	3.78	3.91	10.15	273 to 274 (*7*)	274.0 to 276.0	Perylene[c]
51	3.77	9.9	6.6	126.4 to 126.8 (*20*)	126.4 to 126.8	6-Ethylchrysene[d]
47	3.685	5.0	5.4	160.5 (*11*)	159.5 to 160.0	1,1′-Binaphthyl[c]
59	3.59	9.7	13.45	does not melt by 360 (*4*)	[b]	Periflanthene[h]
44	3.54	11.5	5.05	173 to 173.5 (*7*),	169.5 to 170.5	Cholanthrene[e]
53	3.46	8.3	5.5	179.5 to 180 (*7*)	179.4 to 180.0	20-Methylcholanthrene[p]
34	3.35	4.76	4.14	254.1 to 254.4 (*7*)	254.0 to 256.0	Chrysene[i]

[i] Purified by M. Orchin, Bureau of Mines.
[j] Purified by E. O. Woolfolk, Bureau of Mines.
[k] Purified by M. Orchin, L. Reggel, and E. O. Woolfolk, Bureau of Mines.
[l] Purified by L. Reggel, Bureau of Mines.
[m] Eimer & Amend Co., C.P.
[n] Synthesized by I. Wender, Bureau of Mines.
[o] Donated by W. E. Bachman, University of Michigan.
[p] Donated by B. D. Polis, Eastern Regional Research Laboratory, Department of Agriculture.

Table II. Powder Diffraction Data[a]

1. $C_{10}H_8$, Naphthalene

d/n	I/I1
7.9	w
7.2 (2)	S
5.0	M
4.53 (1)	vS
4.14	S
3.86	w
3.70	M
3.49 (3)	S
3.39 (3)	S
3.01	S
2.77	M
2.685	vw
2.55	w
2.43	M+
2.275	vvw
2.06	w
1.99	vw
1.97	vw
1.92	w
1.88	vw
1.85	M
1.80	vvw
1.755	vw
1.67	vw
1.63	w
1.57	vvw
1.54	vvw
1.40	vvw
1.36	vvw
1.23	vvw
1.21	vvw
1.16	vvw
1.13	vvw

2. $C_{12}H_{10}$, Acenaphthene

d/n	I/I1
8.4	M
7.9	w
7.0 (3)	S
5.6	M
5.1 (1)	vS
4.59	w
4.36	vw
4.15	S
3.99	M
3.87	M
3.63 (3)	S
3.49 (2)	vS
3.32	w
3.21	M
3.01	vw
2.85	w
2.74	w
2.68	w
2.59	M
2.49	w+
2.32	M
2.20	w
2.13	M[b]
2.09	w
2.04	w
1.97	M[b]
1.79	M[b]
1.75	vw
1.62	vvw
1.56	vvw
1.43	vvw
1.35	vvw
1.30	vvw
1.24	vvw
1.22	vvw
1.20	vvw
1.18	vvw
1.125	vvw
1.09	vvw
1.07	vvw
1.04	vvw

3. $C_{12}H_{10}$, Biphenyl

d/n	I/I1
9.5	S
5.1	M
4.78	M
4.62 (1)	vS
4.24 (2)	vS
4.08	w
3.98 (3)	vS
3.63	w
3.52	w
3.40	w
3.30	w
3.17	vS
3.05	M
2.96	vvw
2.83	vvw

3. $C_{12}H_{10}$, Biphenyl (*Contd.*)

d/n	I/I1
2.67	M
2.59	w
2.42	S
2.37	S
2.31	vw
2.28	vw
2.23	w
2.12	w[b]
2.07	w
2.04	w
1.97	w
1.94	w+
1.90	w
1.86	w
1.825	w
1.80	vvw
1.78	vvw
1.70	M
1.59	w[b]
1.58	vvw
1.56	vvw
1.31	vvw[b]
1.28	vvw
1.22	vvw
1.21	vvw
1.19	vvw
1.17	vvw

4. $C_{12}H_{10}$, 2-Vinylnaphthalene

d/n	I/I1
9.9 (2)	vS
4.95	M
4.60 (1)	vS
4.34	w
4.12	w
3.92 (3)	S
3.74	M
3.60	w
3.49	vw
3.28	S
3.16	M
2.97	w
2.85	w
2.76	w
2.39	M
2.14	vvw
2.04	vvw
1.96	w
1.91	w
1.85	w
1.315	vvw
1.09	vvw

5. $C_{12}H_{22}$, 2,2′-Dimethyldicyclopentyl

d/n	I/I1
8.0	S
6.2	S
5.9	w
5.3 (1)	vS
4.97 (3)	S
4.59 (3)	S
4.15 (2)	S
3.98 (3)	S
3.72	S
3.32	w
3.21	w
2.92	M
2.65	w
2.505	M
2.41	vvw
2.36	vw
2.27	w
2.19	vw
2.11	vvw
2.06	w
1.97	vw
1.90	vw
1.86	w
1.68	w
1.17	vvw
1.11	vvw

6. $C_{13}H_{10}$, Fluorene

d/n	I/I1
9.3	S
5.1	M
4.78	M
4.59 (1)	vS
4.24	S
4.15 (3)	S
3.86	w

6. $C_{13}H_{10}$, Fluorene (*Contd.*)

d/n	I/I1
3.77	w
3.70	w
3.53	w
3.35 (2)	S
3.21	w
3.13	vw
3.00	w
2.87	w
2.69	w
2.59	S
2.45	w
2.35	w
2.28	vw
2.13	w+
2.01	M
1.98	M
1.92	vw
1.85	w
1.83	w
1.70	w
1.67	w
1.49	vvw
1.46	vvw
1.38	vvw
1.31	vvw
1.265	vvw
1.17	vvw
1.11	vvw
1.09	vvw
1.07	vvw

7. $C_{13}H_{12}$, 2-Isopropenylnaphthalene

d/n	I/I1
9.8 (3)	vS
6.2	w
5.3	M
4.76 (1)	vS[b]
4.41	S
4.17	w
4.03	M
3.77 (2)	vS
3.46	M
3.37	M
3.21	S
3.01	M
2.93	w
2.76	w
2.62	w
2.575	w
2.47	vw
2.41	M
2.33	w+
2.19	w
2.14	w
1.99	w
1.95	w
1.89	vw
1.80	M
1.74	vw
1.61	vw
1.55	vvw
1.505	vvw
1.35	vvw
1.31	vvw
1.13	vvw
1.12	vvw

8. $C_{14}H_{10}$, Anthracene

d/n	I/I1
9.0 (2)	vS
5.4	w
5.1	M
4.88	M
4.53 (1)	vS
4.17	S
3.81	w[b]
3.53	S
3.43	S
3.03 (3)	S
2.85	w
2.76	w
2.515	vw
2.44	M
2.30	w
2.14	w
2.04	M
1.99	w
1.97	w
1.925	w
1.85	M

8. $C_{14}H_{10}$, Anthracene (*Contd.*)

d/n	I/I1
1.80	vw
1.755	vw
1.69	w
1.64	w
1.60	vw
1.56	vvw
1.53	vvw
1.42	vvw
1.39	vvw
1.36	vw
1.31	vw
1.24	vw
1.22	vw
1.20	vw
1.15	vw
1.09	vvw
1.04	vvw
1.01	vvw

9. $C_{14}H_{10}$, Phenanthrene

d/n	I/I1
10.4	w
9.2 (3)	vS
5.8	w
5.5	M
4.93 (1)	vS
4.53	S
4.24	M
4.02 (3)	vS
3.72	w
3.48	M
3.36 (2)	vS
3.11	w
2.945	w+
2.80	w
2.73	w
2.54	S
2.46	w
2.35	M
2.19	w
2.13	w
2.07	w+
1.98	w
1.93	w
1.86	w
1.815	w
1.74	w
1.69	w
1.46	vvw
1.31	vvw
1.27	vvw
1.20	vvw
1.15	vvw
1.13	vvw
1.09	vvw

10. $C_{14}H_{12}$, 9,10-Dihydroanthracene

d/n	I/I1
0.3	M
8.0	w
7.3 (3)	S
5.1 (2)	S
4.68 (1)	vS
4.34	vw
3.91	S
3.72	w
3.56	S
3.29	M
3.10	S
2.87	M
2.72	w
2.39	w
2.23	w
2.14	vw
2.09	M
1.99	M
1.90	w
1.815	w
1.72	w
1.55	vvw
1.50	vvw
1.44	vw
1.38	vw
1.31	vvw
1.26	vvw
1.20	vvw
1.18	vvw
1.11	vw[b]
1.09	vvw
1.065	vvw
1.03	vvw
1.02	vvw
1.00	vvw

11. $C_{14}H_{12}$, 4-Methylfluorene

d/n	I/I1
9.5	w
8.5 (3)	vS
7.5	vw
6.6	vw
5.8	w
5.25 (2)	vS
5.0 (2)	vS
4.62	M
4.24	M
3.86 (1)	vS
3.72	M
3.49	S
3.39	M
3.18	w
3.01	vw
2.85	vvw
2.73	w
2.46	S
2.13	w+[b]
2.05	vw
1.98	vw
1.94	vw
1.89	w+
1.73	w[b]
1.53	vvw
1.485	vvw
1.31	vw[b]
1.23	vvw
1.11	vvw
1.065	vvw

12. $C_{14}H_{12}$, 9-Methylfluorene

d/n	I/I1
9.3 (3)	S
7.0	w
5.9	M
5.65	w
5.3 (1)	vS
4.76	M
4.41	w
4.09	S
3.98	S
3.81	w[b]
3.65	w
3.45 (2)	vS
3.04	M
2.91	vw
2.74	S
2.63	w
2.48	w
2.40	w
2.33	w
2.25	vw
2.16	S
2.12	vvw
2.08	vvw
2.01	w
1.95	w
1.88	w[b]
1.81	w
1.74	w
1.545	w
1.45	vvw
1.37	vvw
1.32	vvw
1.09	vvw

13. $C_{14}H_{12}$, *trans*-Stilbene

d/n	I/I1
7.3	M
6.35	w
5.5	S
5.1 (3)	S
4.53 (2)	vS
4.24	M
4.09	w
3.81 (1)	vS
3.67	M
3.26	M
3.18	M
3.09	w
2.94	w
2.75	M
2.68	w
2.54	w
2.41	w
2.34	w
2.23	vw
2.11	w[b]
2.06	vw
2.02	w
1.88	w
1.85	vw
1.80	vw
1.25	vvw
1.09	vvw

14. $C_{14}H_{14}$, 4,4-Dimethylbiphenyl

d/n	I/I1
10.5	S
8.0	S
7.0 (3)	S
5.9	M
5.4	S
5.1	w
4.78	w
4.59	w
4.33 (2)	S
4.03 (1)	vS
3.82	w
3.51	w+
3.37	w+
3.14	S
3.03	w
2.93	M
2.65	w+
2.58	vw
2.505	vw
2.44	w
2.21	w+
2.15	w
2.12	w
2.05	w+
1.99	vw
1.95	w
1.91	w
1.85	w
1.74	vw
1.63	vw[b]
1.38	vvw
1.31	vvw
1.22	vww
1.12	vvw

15. $C_{14}H_{14}$ 1,2,-3,4-Tetrahydroanthracene

d/n	I/I1
9.15	S
5.6	w
5.3	M
5.1	M
4.72 (1)	vS
4.59	w
4.12 (3)	vS
3.87	w+
3.82	vw
3.48 (2)	vS
3.21	w
3.09	S
2.945	w
2.79	vw
2.64	vw
2.46	M
2.43	w
2.31	w
2.10	M
2.04	M
1.96	vw
1.90	w
1.85	M
1.77	vvw
1.70	w
1.65	vvw
1.60	vw
1.57	vw
1.50	vvw
1.405	vvw
1.38	vw
1.31	vvw
1.27	vvw
1.24	vvw
1.22	vw
1.16	vvw
1.14	vvw
1.09	vvw
1.06	vvw
1.02	vvw

16. $C_{14}H_{18}$, 1,2,3,4,5,6,7,8-Octahydroanthracene

d/n	I/I1
9.3	w
8.4	S
5.8	w
5.2 (1)	vS
4.97	w
4.64	w
4.41	M
4.18 (3)	vS
3.98 (2)	vS
3.88	vw
3.55	M

16. $C_{14}H_{18}$, 1,2,3,4,5,6,7,8-Octahydroanthracene (*Contd.*)

d/n	I/I1
2.91	S
2.78	M
2.66	vw
2.60	w
2.49	w
2.34	M
2.28	vw
2.22	M
2.14	S
2.03	w
1.96	w
1.92	vw
1.84	vw
1.74	vvw
1.70	vvw
1.48	vvw
1.45	vvw
1.31	w
1.18	vvw[b]
1.12	vw
1.10	vvw
1.07	vvw

17. $C_{14}H_{22}$, 1,4-Di-*tert*-butylbenzene

d/n	I/I1
7.75	w
7.1	vS
6.2	w
6.0	w
5.6 (1)	vS
5.4 (3)	vS
4.93	vS
4.51 (2)	vS
3.98	M
3.78	vw
3.54	S[b]
3.20	M
3.10	w
3.00	w
2.91	vw
2.82	M
2.70	w
2.61	vw
2.55	vw
2.46	vw
2.31	w+
2.22	vw
2.16	w
2.10	M
2.04	M
1.99	M
1.95	M
1.89	vvw
1.80	vw
1.77	vw
1.67	vvw
1.63	vvw
1.57	vvw
1.52	vvw
1.35	vw
1.31	vw
1.22	vvw
1.16	vvw
1.035	vvw

18. $C_{15}H_{10}$, 4-Cyclopenta-[*def*]phenanthrene

d/n	I/I1
9.3	w
8.2 (3)	vS
5.9	w
5.3 (2)	vS
5.0	S
4.82	M
4.31	M
4.14	w
3.87 (1)	vS
3.73	S
3.51	S
3.41	S
3.30	vw
3.18	w
3.03	vw
2.88	vvw
2.75	w
2.47	S
2.14	M[b]
1.96	w
1.90	M
1.83	vvw
1.74	w[b]
1.54	vvw
1.31	vvw
1.12	vvw
1.07	vvw

19. $C_{15}H_{12}$, 9-Methylphenanthrene

d/n	I/I1
9.0 (3)	vS
5.5	w
5.1	M
4.83 (2)	vS
4.56	M
4.37	w
4.18	w
3.92 (1)	vS
3.76	S
3.62	M
3.42	vw
3.18	S
3.07	S
2.885	vvw
2.81	w
2.57	w
2.46	vvw
2.38	vvw
2.28	w[b]
2.11	M
2.065	vvw
2.01	w
1.96	w
1.94	vw
1.89	vvw
1.77	w
1.62	vvw
1.31	vvw

20. $C_{16}H_{10}$, Fluoranthene

d/n	I/I1
16.0	w
11.05	w
9.5	S
7.9	vw
6.65	vw
5.9	w
5.7	w
5.1 (2)	vS
4.88 (3)	S
4.50	S
4.34	M
3.95 (1)	vS
3.70	S
3.55	S
3.34	w
3.17	w
2.99	S[b]
2.86	vw
2.73	w
2.66	w
2.365	vw
2.26	vvw
2.21	vvw
2.13	vw
2.04	vvw
1.99	vw
1.94	vw
1.875	vw
1.81	vw
1.68	vvw
1.59	vvw
1.56	vvw
1.52	vvw
1.31	vvw

21. $C_{16}H_{10}$, Pyrene

d/n	I/I1
9.2	w
8.3 (2)	vS
7.5 (3)	S
5.8	S
5.45	S
4.86	M
4.23	M[b]
3.78 (1)	vS
3.58	S
3.34	w
3.12	S
2.805	M
2.72	M
2.50	w
2.40	vw
2.14	M
2.03	M
1.90	M
1.76	w
1.69	w
1.62	vw
1.57	vw
1.32	vvw
1.24	vvw
1.19	vvw
1.12	vvw
1.08	vvw

[a] *d/n* = interplanar spacing in Angstroms; *I/I*1 = estimated relative ntensity; S = strong; M = medium; w = weak; v = very; (1) strongest line; (2) 2nd strongest line; (3) 3rd strongest line.

[b] Broad line probably due to partial coincidence of two or more lines.

Table II. (*Continued*)

22. $C_{16}H_{12}$, 4,5-Dihydropyrene

d/n	I/I1
9.4	w
8.4 (1)	vS
6.1	w
5.3 (2)	vS
4.76	vw
4.43	vw
4.245	vw
4.11	S
3.96	S
3.85	M
3.63 (3)	S
3.48	w
3.34	vvw
3.20	vvw
2.87	vw
2.60	M
2.54	M
2.23	vvw
2.13	w
2.00	vvw
1.95	vvw
1.83	vvw
1.30	vvw
1.11	vvw

23. $C_{16}H_{12}$, 2-Phenylnaphthalene

d/n	I/I1
11.8	S
6.4	w
5.9	S
5.3	M
4.71 (1)	vS
4.49	S
4.37	w
4.02	w
3.86 (2)	S
3.74	w
3.57	w
3.32	w
3.23 (3)	S
3.14	w
2.94	vw
2.63	w[b]
2.54	vvw
2.41	M
2.355	vw
2.305	vw
2.14	vw
2.08	w
1.97	w
1.88	vw
1.81	vw
1.705	vvw
1.61	vvw
1.54	vvw
1.315	vvw
1.14	vvw

24. $C_{16}H_{14}$, *trans-trans* 1,4-Diphenyl-1,3-butadiene

d/n	I/I1
8.5	w
6.7	w
6.3	w
5.7	S
5.4	M
4.87 (2)	vS
4.52	M
4.09 (1)	vS
3.73 (3)	S
3.55	w
3.43	S
3.21	w
3.11	S
2.80	w[b]
2.65	w
2.545	w
2.38	w
2.23	M
2.135	w+
2.015	vvw
1.96	vw
1.885	vvw
1.85	vvw
1.79	w
1.72	w
1.62	vvw
1.56	vvw
1.31	vvw
1.12	vvw
1.085	vvw

25. $C_{16}H_{16}$, 1-(1-Cyclohexen-1-yl)-naphthalene

d/n	I/I1
11.7	w
6.9	S
6.3	w
5.8	M
5.4 (2)	S
5.1	w
4.67 (1)	vS
4.56	M
4.25 (3)	S
3.89	S
3.70	w+
3.40	S
3.27	w
3.14	S
3.03	S
2.86	w
2.78	w+
2.68	w+
2.61	vw
2.545	vw
2.43	vw
2.34	w
2.18	vvw
2.12	vvw
2.09	vvw
2.05	vvw
2.00	vvw
1.97	vvw
1.93	vvw
1.90	vvw
1.79	vvw
1.765	vvw
1.72	vw
1.65	vw
1.22	vvw
1.08	vvw

26. $C_{17}H_{12}$, 1,2-Benzofluorene

d/n	I/I1
11.3	vS
7.4	vw
6.3	vw
6.0	M
5.4	M
4.88 (1)	vS
4.70	M
4.53	vw
4.36	w
4.10 (3)	vS
3.78	w
3.66	vw
3.39 (2)	vS
3.14	w
2.87	w
2.72	w
2.53	M
2.47	vvw
2.35	vvw
2.25	vvw
2.13	M
2.04	w+
1.96	w
1.86	w
1.72	w
1.45	vvw
1.31	vw[b]
1.17	vvw
1.12	vvw
1.07	vvw

27. $C_{17}H_{12}$, 2,3-Benzofluorene

d/n	I/I1
11.75	vS
10.6	vw
10.0	vvw
7.9	vvw
6.5	vvw
5.9	M
5.25	S
4.68 (1)	vS
4.11 (2)	vS
3.95	M
3.71	M
3.36 (3)	vS
3.26	w
3.12	w
2.945	M
2.77	M
2.51	S
2.38	w[b]
2.29	vw
2.22	vw
2.14	vw
2.02	M
1.95	M
1.90	vw
1.70	w
1.44	vvw
1.175	vvw

28. $C_{17}H_{12}$, 3,4-Benzofluorene

d/n	I/I1
10.15	S
5.7	M
5.1 (1)	vS
4.72	M
4.18	S
3.67 (2)	vS
3.33 (3)	vS
3.24	M
3.11	vw
2.90	w
2.76	vvw
2.70	vvw
2.54	w
2.37	S
2.305	vw
2.14	w
2.08	w
2.02	w
1.95	vw
2.84	vw
1.82	w
1.745	vvw
1.67	w
1.47	vvw
1.39	vvw
1.31	vvw
1.17	vvw
1.12	vvw

29. $C_{17}H_{12}$, 4-Methylpyrene

d/n	I/I1
9.1	w
8.1 (1)	vS
6.3	M
6.0	w
5.4	M
4.59	w
4.39	vw
4.12 (3)	vS
3.95	S
3.78	S
3.62	M
3.27 (2)	vS
3.14	vvw
2.97	vw
2.76	w
2.71	M
2.24	vvw
2.13	M
2.08	vw
2.02	M
1.87	M
1.81	vw
1.68	vw
1.64	vw
1.31	vvw
1.18	vvw
1.11	vvw
1.09	vvw

30. $C_{17}H_{14}$, 2-Benzylnaphthalene

d/n	I/I1
10.9	w
9.9	M
6.7	S
5.85	w
5.4	M
5.2	M
4.86 (1)	vS
4.62 (2)	vS
4.28	S
4.10	w
3.86	S
3.68 (3)	vS
3.48	S
3.36	S
3.22	S
3.11	vw
2.99	vw
2.91	w
2.81	M
2.71	w
2.67	w
2.575	M
2.43	w
2.33	w
2.26	w+
2.15	vw
2.09	w
2.04	w+
2.01	w
1.97	vw
1.93	w
1.875	w
1.845	vw

30. $C_{17}H_{14}$, 2-Benzylnaphthalene (*Contd.*)

d/n	I/I1
1.81	w
1.76	vw
1.73	w
1.68	vvw
1.63	vvw
1.60	vvw
1.57	vvw
1.53	vvw
1.18	vvw
1.14	vvw

31. $C_{17}H_{14}$, 1-*o*-Tolylnaphthalene

d/n	I/I1
12.7	S
7.7	S
6.5	w
5.8 (2)	vS
5.2 (1)	vS
4.74	w
4.31 (3)	S
4.20	M
3.94	S
3.83	M
3.59	M[b]
3.36	S
3.26	M
3.16	w
3.02	w
2.88	w
2.82	w
2.715	M[b]
2.62	w
2.48	M
2.275	w
2.22	w+
2.13	w+
2.09	w+
2.03	vvw
1.97	w+
1.90	vw
1.83	vw
1.80	w
1.74	vvw
1.68	vw
1.58	vvw
1.50	vvw
1.43	vvw
1.40	vvw
1.31	vvw
1.265	vvw
1.24	vvw
1.18	vvw
1.12	vvw
1.11	vvw
1.065	vvw

32. $C_{17}H_{14}$, 2-*o*-Tolylnaphthalene

d/n	I/I1
19.15	w
11.75 (3)	S
7.65	w
6.4	w
6.1	S
5.35	M
4.86 (1)	vS
4.64	M
4.33 (2)	vS
3.72	M
3.495	S
3.40	S
3.28	M
3.17	M
3.03	w
2.94	w
2.83	M
2.64	S
2.47	w
2.25	vvw
2.21	vvw
2.16	w
2.09	w+
2.00	vw
1.95	M
1.85	w
1.77	w
1.65	vvw
1.56	vvw
1.41	vvw
1.31	vvw
1.24	vvw

33. $C_{18}H_{12}$, 1,2-Benzanthracene

d/n	I/I1
12.1 (2)	vS
6.0	M
5.5	w
5.00 (1)	vS
4.79	M
4.45	M
3.93 (3)	S
3.74	w
3.37	S
3.12	vw
2.89	vvw
2.47	M
2.39	vvw
2.26	vvw
2.14	w
2.04	w+
1.915	vw
1.70	vvw
1.435	vvw
1.17	vvw

34. $C_{18}H_{12}$, Chrysene

d/n	I/I1
11.05	vS
6.1	w
5.7	M
5.35	M
4.76 (2)	vS
4.62	w
4.28	w
4.14 (3)	vS
3.73	M
3.48	M
3.35 (1)	vS
3.28	w
3.22	w
3.14	w
3.01	w
2.87	vw
2.74	w
2.64	w
2.55	vw
2.49	S
2.35	w
2.27	w
2.14	M
2.07	w
2.02	w
1.95	w
1.89	w+
1.83	w
1.755	w
1.73	w+
1.685	w+
1.46	vw
1.44	w
1.31	vw[b]
1.27	vvw
1.25	vvw
1.145	w

35. $C_{18}H_{12}$, Naphthacene

d/n	I/I1
12.1 (2)	vS
7.3	M
6.05	M
5.05	M
4.60 (1)	vS
4.45	w
3.95	M
3.66	S
3.38	w
3.22	S
3.10	S
2.86	vw
2.76	vw
2.355	w
2.135 (3)	S
2.03	M
1.93	vvw
1.85	vw
1.43	vvw
1.305	w
1.17	vvw
1.12	vvw
1.07	vvw

36. $C_{18}H_{14}$, 1,4-Diphenylbenzene

d/n	I/I1
13.95	S
6.85	M
5.1	M
4.88	w
4.60 (1)	vS
4.36 (2)	vS
3.85 (3)	vS
3.54	M
3.30	w
3.18	vS
3.01	M
2.81	vvw
2.73	vvw
2.66	w
2.60	w
2.39	M
2.32	w
2.275	w
2.21	w
2.07	w
2.01	w
1.93	w
1.90	w
1.82	w
1.78	vw
1.70	w+
1.59	w
1.32	vvw
1.28	vvw
1.20	vw
1.17	vw
1.09	vvw

37. $C_{18}H_{14}$, 4-Ethylpyrene

d/n	I/I1
9.7	w
8.6 (2)	vS
7.7 (3)	vS
7.1	M
6.6	M
5.7	S
5.0	w
4.53	S
4.24	S
4.09	S
3.82 (1)	vS
3.58	vw
3.41	M
3.24	M
3.10	M
2.93	S
2.81	w
2.56	w
2.49	vw
2.35	w
2.28	w
2.23	w
2.17	vw
2.12	M
2.03	M
1.99	w
1.92	w
1.89	w
1.82	vw
1.75	vw
1.72	vw
1.67	M
1.61	vvw
1.58	vw
1.53	vvw[b]
1.48	vvw
1.41	vvw
1.37	vvw
1.24	vvw
1.21	vvw
1.17	vw
1.14	vvw
1.11	vvw
1.08	vvw
1.03	vvw

38. $C_{18}H_{22}$, Bimesityl

d/n	I/I1
10.9	M
9.15	w
8.3 (1)	vS
6.8	S
6.3	w
5.85 (2)	S
5.60	S
5.4	S
5.1	S
4.82	w
4.33	M
4.12	M
3.98	w
3.87	w

38. $C_{18}H_{22}$, Bimesityl (*Contd.*)

d/n	I/I1
3.71 (3)	S
3.62	w+
3.40	M
2.97	S
2.81	w
2.69	w
2.54	w
2.34	w
2.26	w
2.12	w
2.03	w
1.96	w
1.84	w
1.70	w
1.67	vvw
1.62	vvw
1.525	vvw
1.49	vvw
1.31	vvw
1.11	vvw
1.07	vvw

39. $C_{19}H_{14}$, 5-Methylchrysene

d/n	I/I1
12.3	w
10.7 (3)	vS
9.6	S
6.5	M
5.9	S
5.4	vS
4.80 (1)	vS
4.53	w
4.20	S
3.81 (2)	vS[b]
3.63	M
3.53	w
3.40	M
3.31	S
3.16	S
2.99	M
2.76	w
2.64	M
2.47	vw
2.34	w
2.13	S
2.02	M
1.85	vvw
1.76	vvw
1.69	vvw
1.30	w
1.16	vvw
1.11	vw
1.06	vvw

40. $C_{19}H_{14}$, 6-Methylchrysene

d/n	I/I1
10.6	S
9.6	w
7.6	w
6.4	vw
5.9	S
5.3	M
4.88 (1)	vS
4.51 (2)	vS
4.28	S
4.06	S
3.87 (3)	vS
3.67	vS
3.41	vS
3.22	w
3.13	M
2.79	M
2.64	M
2.44	w
2.35	w
2.14	S
2.02	w[b]
1.95	vvw
1.89	vw
1.84	vw
1.775	vw
1.70	vw
1.54	vvw
1.48	vvw
1.42	vvw
1.30	vvw
1.17	vvw
1.11	vvw

41. $C_{19}H_{16}$, 1-Methyl-3,5-diphenylbenzene

d/n	I/I1
11.9	S
9.8	vvw
8.1	vvw
6.85	vvw
6.3	w
5.9	M
5.3 (2)	vS
4.99	M
4.44 (1)	vS
4.11	M
4.02	M
3.67 (3)	vS
3.55	S
3.44	S
3.34	S
3.07	S
2.945	vw
2.86	w
2.75	w
2.66	M
2.54	w
2.43	w
2.36	M
2.25	M
2.18	w
2.11	w
2.08	w
2.04	S
2.00	w
1.82	w+
1.785	M
1.76	vw
1.71	w
1.60	vvw
1.565	vvw
1.365	vvw
1.33	vvw
1.31	vvw
1.28	vvw
1.24	vvw
1.20	vvw
1.12	vvw
1.05	vvw
1.01	vvw

42. $C_{20}H_{12}$, 8,9-Benzofluoranthene

d/n	I/I1
19.3	w
16.15	w
12.1 (3)	S
6.6	vw
6.0	S
5.5	w
5.0 (1)	vS
4.74	w
4.34	S
4.21	M
3.98 (2)	vS
3.57	S
3.47	M
3.27	w
3.04	w
2.94	S
2.61	vw
2.29	vvw
2.14	w
2.09	w
2.03	vw
1.99	vw
1.93	w
1.78	vvw
1.71	vvw
1.64	vvw
1.31	vvw
1.115	vvw

[b] Broad line probably due to partial coincidence of two or more lines.

(*Concluded on next page*)

Table II. (*Concluded*)

43. $C_{20}H_{12}$, Perylene

d/n	I/I1
10.15	(3) vS
8.65	w
7.8	S
6.6	M
5.3	M
4.93	S
4.74	w
4.20	w
3.91	(2) vS
3.78	(1) vS
3.52	S
3.37	vw
3.27	vw
3.15	vw
3.05	S
2.97	w
2.82	vw
2.715	vvw
2.50	w
2.46	w
2.39	vw
2.13	w
2.07	vw
1.99	vw
1.92	w
1.82	vvw
1.73	vvw
1.70	vvw
1.68	vvw
1.645	vvw
1.27	vvw
1.20	vvw
1.18	vvw

44. $C_{20}H_{14}$, Cholanthrene

d/n	I/I1
11.5	(2) vS
7.8	w
7.15	vS
6.6	S
5.8	S
5.05	(3) vS
4.85	M
4.44	w
4.23	w
4.08	S
3.88	S
3.68	w
3.54	(1) vS
3.26	w
3.05	S
2.88	vw
2.785	w
2.60	M
2.43	w
2.26	w
2.14	M
2.03	M
1.97	vw
1.92	w
1.79	M
1.76	vvw
1.71	vvw
1.65	vvw
1.61	vvw
1.50	vvw
1.20	vvw
1.18	vvw

45. $C_{20}H_{14}$, 1,2′-Binaphthyl

d/n	I/I1
12.4	S
6.8	w
6.1	S
5.5	S
5.2	w
4.74	(1) vS
4.38	(2) S[b]
4.17	M
3.84	(3) S
3.64	M
3.43	S
3.33	M
3.18	S
3.05	M
2.87	M
2.76	vvw
2.67	w
2.59	w
2.39	w
2.32	vw
2.24	w+
2.03	w
2.01	w
1.95	vw
1.875	w
1.805	vvw
1.73	vvw
1.67	vw
1.32	vvw
1.17	vvw
1.09	vvw

46. $C_{20}H_{14}$, 2,2′-Binaphthyl

d/n	I/I1
13.95	S
7.5	w
6.8	S
5.4	vw
5.1	w
4.88	M
4.605	(1) vS
3.98	(2) S
3.845	w
3.60	S
3.31	(3) S
3.09	S
3.03	w
2.75	M[b]
2.62	vw
2.575	vw
2.47	vvw
2.37	M
2.25	w[b]
2.17	vw
2.13	vw
2.09	w
2.05	vw
2.00	w
1.96	vw
1.92	w+
1.815	w+
1.77	vw
1.72	vvw
1.68	vvw
1.61	w+
1.54	vvw
1.47	vvw
1.395	vvw
1.35	vvw
1.26	vvw
1.22	vw
1.20	vw
1.12	vvw
1.07	vw
1.045	vw
1.00	vvw

47. $C_{20}H_{14}$, 1,1′-Binaphthyl

d/n	I/I1
10.2	M
6.0	w
5.4	(3) S
5.0	(2) S
4.66	w
4.39	vw
4.11	M
3.98	M
3.685	(1) vS
3.52	vvw
3.20	w
3.00	M
2.945	w
2.81	w+
2.68	vw
2.56	w+
2.49	w+
2.35	w
2.29	w
2.25	w
2.18	w
2.14	w
2.08	w+
2.04	vw
1.99	w+
1.95	w
1.89	w
1.85	w
1.72	vw
1.68	vw
1.57	vvw
1.42	vvw
1.31	vvw
1.28	vvw
1.21	vvw
1.185	vvw
1.16	vvw
1.09	vvw

48. $C_{20}H_{14}$, 2-Phenylphenanthrene

d/n	I/I1
13.6	S
6.8	M
5.4	M
4.88	(1) vS
4.625	(3) S
4.38	w
4.15	w
3.95	(2) S
3.66	w
3.57	w
3.42	w
2.325	S
3.05	w
2.85	vvw
2.71	vvw
2.61	vvw
2.53	vw
2.48	M
2.34	w
2.26	w
2.13	S
2.03	w
1.98	vw
1.94	vw
1.86	w[b]
1.80	vw
1.66	vvw
1.58	vvw
1.43	vvw
1.31	vvw
1.12	vvw
1.07	vvw

49. $C_{20}H_{16}$, 5,6-Dimethylchrysene

d/n	I/I1
13.8	w
10.9	(3) vS
9.45	w
8.65	M
6.8	M
6.0	w
5.4	(1) vS
5.1	w
4.88	vvw
4.43	S
4.11	M
3.68	(2) vS
3.52	w
3.23	vw
3.08	M
2.81	vvw
2.65	vvw
2.51	vw
2.37	vvw
2.33	vvw
2.24	vvw
2.13	S
2.03	M
1.85	w
1.43	vvw
1.31	vw
1.17	vvw
1.11	vvw
1.07	vvw
1.00	vvw

50. $C_{20}H_{16}$, 5-Ethylchrysene

d/n	I/I1
10.5	(1) vS
6.8	M
6.1	S
5.85	S
5.4	M
5.1	S
4.93	M
4.61	(3) S
4.31	(3) S
4.01	(2) vS
3.84	M
3.47	S
3.21	M
3.05	w
2.95	w
2.88	M
2.785	w
2.51	vvw
2.39	vvw
2.13	M
2.02	w
1.86	vvw
1.74	vvw
1.30	vvw
1.17	vvw
1.11	vvw
1.07	vvw

51. $C_{20}H_{16}$, 6-Ethylchrysene

d/n	I/I1
9.9	(2) vS
7.1	w
6.6	(3) S
5.7	M
4.82	S
4.625	S
4.29	S
4.02	M
3.77	(1) vS

51. $C_{20}H_{16}$, 6-Ethylchrysene (*Contd.*)

d/n	I/I1
3.47	w
3.24	M
3.14	w
3.05	S
2.81	M
2.62	vvw
2.48	vvw
2.42	vvw
2.32	w
2.13	S
2.01	vw
1.85	w
1.74	vvw
1.63	vvw
1.31	vw
1.23	vvw
1.17	vvw
1.11	vw
1.07	vvw

52. $C_{20}H_{16}$, 3,4,3′,4′-Tetrahydro-1,1′-binaphthyl

d/n	I/I1
8.1	S
6.8	M
6.4	(3) S
6.0	S
5.5	S
4.98	S
4.57	(2) vS
3.98	(1) vS
3.81	w
3.61	S
3.53	M
3.45	w
3.16	w
3.09	M
2.95	w
2.83	M
2.71	w
2.60	vw
2.50	M
2.44	vw
2.365	w
2.26	vw
2.145	w
2.10	M
2.04	M
1.97	vw
1.91	w
1.81	vvw
1.60	vvw
1.31	vvw
1.12	vvw
1.09	vvw

53. $C_{21}H_{16}$, 20-Methylcholanthrene

d/n	I/I1
12.7	w
10.3	M
9.4	w
8.3	(2) vS
7.5	vvw
6.8	vvw
6.2	S
5.5	(3) vS
5.2	S
4.90	S
4.39	M[b]

53. $C_{21}H_{16}$, 20-Methylcholanthrene (*Contd.*)

d/n	I/I1
4.12	M
3.81	vS
3.71	w
3.46	(1) vS
3.30	S
2.87	w+
2.76	w
2.64	w+
2.47	w+
2.38	w
2.32	w
2.25	w
2.15	w+[b]
2.09	vw
2.04	vw
1.99	w
1.95	w
1.85	w
1.82	w
1.74	w
1.70	w
1.61	vvw
1.52	vvw
1.305	vvw
1.21	vvw
1.19	vvw
1.07	vvw

54. $C_{22}H_{12}$, Dibenzo[*cd,jk*]-pyrene

d/n	I/I1
10.9	(1) vS
8.0	(3) S
6.5	M
5.3	S[b]
4.79	M
4.39	w
4.17	vw
3.96	(2) vS
3.77	S
3.63	S
3.41	vw
3.21	w+
3.09	S
2.845	vw
2.56	vw
2.35	vw
2.14	S
2.04	vvw
1.97	w
1.86	w
1.30	w
1.11	vw
1.07	vvw

55. $C_{22}H_{14}$, Picene

d/n	I/I1
13.6	(3) vS
6.8	M
5.5	M
5.0	(1) vS
4.70	S
4.50	vw
4.28	vw
4.05	S
3.74	M
3.51	M
3.39	(2) vS
3.10	w
2.81	vw
2.53	M
2.43	vw
2.34	w
2.27	w

55. $C_{22}H_{14}$, Picene (*Contd.*)

d/n	I/I1
2.14	M
2.04	w[b]
1.99	w
1.93	vw
1.83	w[b]
1.74	vvw
1.69	vw
1.60	vvw
1.31	vw
1.23	vvw
1.17	vvw
1.11	vw
1.07	vvw

56. $C_{23}H_{16}$, 5-Methylpicene

d/n	I/I1
13.4	(2) vS
10.0	M
8.3	w
7.3	w
6.6	S
6.3	vw
5.5	M
4.97	(1) vS
4.70	vS
4.46	w
4.26	M
3.96	S
3.73	M
3.65	(3) vS
3.42	vw
3.24	M
3.10	M
2.99	vvw
2.82	M
2.68	vvw
2.53	vw
2.29	vvw
2.225	vw
2.14	vw
2.01	vw
1.89	vvw
1.86	vvw
1.81	vvw
1.74	vvw
1.30	vvw
1.25	vvw
1.21	vvw
1.18	vvw

57. $C_{23}H_{16}$, 13-Methylpicene

d/n	I/I1
10.8	(3) S
7.1	M
6.3	w
5.7	S
5.4	vw
5.2	S
4.95	S
4.51	(1) vS
4.11	S
3.96	w
3.82	vw
3.59	(2) vS
3.31	S
3.14	vvw
2.98	vvw
2.85	w
2.53	w
2.47	w
2.33	w
2.23	w
2.13	M
2.03	w

57. $C_{23}H_{16}$, 13-Methylpicene (*Contd.*)

d/n	I/I1
1.97	vw
1.85	w
1.74	w
1.44	vvw
1.30	w
1.22	vvw
1.17	vvw
1.11	vvw
1.07	vvw

58. $C_{24}H_{22}$, 2,2′,7,7′-Tetramethyl-1,1′-dinaphthyl

d/n	I/I1
10.5	M
9.5	w
7.25	w
6.6	(2) vS
6.0	(1) vS
5.65	M
5.2	M
4.70	(3) S
4.50	M
4.15	S
4.01	w
3.77	M
3.60	w
3.43	S
3.28	M[b]
3.06	w
2.81	M
2.70	M
2.48	vvw
2.43	vvw
2.27	vvw
2.21	w
2.15	vw
1.91	vvw
1.875	vvw
1.76	vvw
1.72	vvw
1.61	vvw
1.55	vvw
1.31	vvw
1.09	vvw

59. $C_{32}H_{16}$, Periflanthene

d/n	I/I1
19.0	w
13.45	(3) vS
9.7	(2) vS
9.15	w
8.0	w
6.65	M
4.72	M
3.85	w
3.59	(1) vS
3.32	S
3.03	vw
2.24	vvw
2.14	vw
2.04	w
1.74	vvw

(diffuse pattern)

[b] Broad line probably due to partial coincidence of two or more lines.

pounds are often very complex so that all the reflections are not resolved. Under these conditions, method (*b*) gives a *d* value corresponding to neither of the component lines, whereas method (*c*) gives at least the *d* of the outer line accurately. (Especially broad lines are designated by a superscript *b* in Table II.) For these reasons, method (*c*) was chosen for the present study. Direct measurement of the width of several very dense lines showed none of them to be broader than 0.9 mm. as compared with the specimen width of 0.7 mm. Thus, the broadening resulting from divergent radiation and finite focus is minor under the conditions employed herein.

In general, the diffraction data presented were obtained with the 114.6-mm. camera, but the additional short interplanar spacings which were found with the small camera are also included. The error in *d* because of an error in θ is

$$\Delta d = (-d/\lambda)\sqrt{4d^2 - \lambda^2}\,\Delta\theta.$$

The error in θ is probably about 0.0035 radian in the small camera and 0.0017 radian in the large camera (corresponding to a 0.1-mm. error on the films of the small and large cameras, respectively). The results obtained with the large and small cameras checked within the error as calculated. This error varies from 0.003 A. at d = 1.5 A. to 0.044 A. at d = 5.00 A. to 0.40 A. at d = 15.00 A. for the large camera.

The order of intensity of the three most intense reflections was determined by the microdensitometer where these lines were isolated; in those cases where the reflections tended to merge, visual estimates were made.

DISCUSSION

Table I lists the hydrocarbons studied, their literature and observed melting points, and the sources. Table II presents the x-ray powder diffraction data listed by formula.

Table III. Names and Melting Points of Impure and Commercial Products Whose Diffraction Patterns Are Identical to Those of Pure Compounds

Compound	Melting Point, ° C. Found	Literature (7)
Anthracene (90% pure)	210 to 215	216.2 to 216.4
Chrysene	237 to 243	254.1 to 254.4
Fluorene	114.6 to 118.8	116 to 117
Pyrene	140.2 to 147.8	156
Fluoranthene	108.8 to 110.0	110
Phenanthrene (90% pure)	99.6 to 103.2	100.7 to 101
Acenaphthene	93.2 to 94.4	95

Comparison of x-ray diffraction patterns of compounds which are isomeric or closely related reveals that in every instance they are entirely different—for example, no relationship can be found among the patterns of anthracene, 8,10-dihydroanthracene, 1,2,3,4-tetrahydroanthracene, and 1,2,3,4,5,6,7,8-octahydroanthracene. The same can be said of the series, fluorene, 4-methylfluorene, and 9-methylfluorene, and of the series, 1,1′-binaphthyl, 1,2′-binaphthyl, and 2,2′-binaphthyl. Even naphthalene, anthracene, and naphthacene, though closely related chemically and crystallographically (*19*), have powder patterns that are unique.

The compounds studied herein were exceedingly pure; in some cases, purification was made by chromatographic methods. To test the practicability of x-ray diffraction analysis, some impure, commercial products were examined. A list of these products, together with their melting points and the melting points of the corresponding pure compounds, is given in Table III. The manufacturer estimated the purity of the phenanthrene and anthracene to be 90%. The diffraction pattern of each of these products was indistinguishable from that of the carefully purified compound. This observation is in line with the study of Matthews and Michell (*15*).

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Index of Refraction as Supplement to X-Ray Crystal Analysis of Solid Solutions

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IN A series of papers (*2*, *4*) the composition of a solid solution of cesium chloride and cesium bromide, as determined by the method of x-ray crystal analysis, has been used in a study of the reciprocal system,

$$CsCl + KBr \rightleftharpoons CsBr + KCl$$

When a mixture of powdered cesium chloride and potassium bromide or cesium bromide and potassium chloride is heated at temperatures not too far below the fusion point (*6*), solid solutions of cesium chloride–cesium bromide and potassium chloride–potassium bromide begin to form. The compositions of these solid solutions have been determined by x-ray diffraction and in this way it has been determined when the reciprocal system reaches equilibrium. At equilibrium the x-ray diffraction pattern for the cesium chloride–cesium bromide solid solutions was the same regardless of the direction from which the equilibrium was approached. Before equilibrium is reached the solid solution is rich in cesium chloride when approached from one direction and rich in cesium bromide when approached from the other. Furthermore, until equilibrium has been reached, the mixture of cesium chloride and cesium bromide would be expected to consist of a whole series of solid solutions of continuously varying composition. In this case the lines of the x-ray pattern would be broad, each line in reality consisting of a series of overlapping lines, and the results of the x-ray analysis would represent average values.

On the other hand, the immersion method for the measurement of the index of refraction makes it possible to examine individual particles. Differences in composition between particles of solid solution of cesium chloride–cesium bromide can be detected

Table I. Interplanar Distances for 50 to 50 Mixture of Cesium Chloride and Cesium Bromide after 24 Hours at 400° C.

(Compared with those of a 50 mole % CsCl-CsBr solid solution film GR-106)

Line No.	Relative Intensity	Mixture	Aluminum Standard[b]	Solid Soln.
1	2	4.20	..	4.200 (100)[a]
2	10	2.98	..	2.970 (110)
3	5	2.33	2.3332 (111)	..
4	2	2.11	..	2.100 (200)
5	3	2.025	2.0206 (200)	..
6	1 (Diffuse)	1.88	..	1.878 (210)
7	7	1.715	..	1.715 (211)
8	2 (Diffuse)	1.488	..	1.485 (220)
9	3	1.428	1.4288 (220)	..
10	4	1.327	..	1.328 (310)
11	5	1.219	1.2185 (311)	..
12	(Very faint)	1.213	..	1.213 (222)
13	1 (Diffuse)	1.167	1.1666 (222)	1.165 (320)
14	3 (Broad)	1.126	..	1.122 (321)

[a] Numbers in parentheses represent Miller indexes.
[b] Relative to unit cell edge of NaCl taken as 5.628.

because the index of refraction varies with the composition. It should be possible to detect the very beginnings of solid solution formation resulting from ion migration, even before the number of such particles is sufficient for detection by x-ray analysis. If the composition of only a few particles falls outside the principal composition range, this circumstance could also be detected by the index of refraction method (even for one particle). In 1944 it was reported (*5*) that ions had been found to migrate across crystal boundaries between a salt pair, the migration being detected by means of the immersion method for the index of refraction. This work was interrupted by the war but has now been continued and the results reported below show how the immersion method for the determination of the index of refraction can be used as a supplement to the x-ray crystal analysis method in a study of ion migration as crystals of solid solution are formed.

APPARATUS, MATERIALS, AND METHODS

The cesium salts used in this work were of the highest quality obtainable and were carefully tested for impurities. These salts were fused and ground to pass a 200-mesh sieve before weighing and mixing. The salt mixtures were heated in a well-insulated electric oven controlled by a shift-phase photoelectric circuit control to about ±0.25° C., the temperature being read with a Type K potentiometer. A General Electric multiple-diffraction apparatus was used for the x-ray analysis of the crystals. For the measurement of the index of refraction the Becke line immersion method was used. A sodium vapor lamp was used as a source of monochromatic light.

The index of refraction of a crystal was determined by comparing it with a series of certified diffraction liquids which varied in steps of 0.002 index units. When a liquid was found with an index near that of the crystal, the microscope slide was placed in a water jacket on the microscope stage and the temperature was raised or lowered until a match was obtained. Because the index of each oil was known at 25° C. as well as the temperature coefficient of the index (both furnished by the supplier), the index could be determined within a narrow range. With the temperature rising (or falling) slowly the point was observed at which the crystal disappeared into the oil and the point at which it began to reappear as the index of the oil became lower (or higher) than the crystal. Under favorable circumstances this temperature range was sometimes as little as 0.5° C. which corresponded to a difference in index of 0.00025 to 0.0003. The index of the oil corresponding to the middle of this temperature range was taken as the index of the crystal. Successive determinations made in this way frequently did not vary more than 0.0001, even when two different oils were used, one having an index above the crystal and the other having an index below the crystal. The absolute index values of several of the oils were checked by comparison with standard crystals of known index values and by direct measurement of the index by means of an Abbe-56 refractometer. For the latter purpose the water chambers of the Abbe were connected in series with the water jackets on the stage of the microscope. Using ammonium chloride as a standard crystal with a reported index of 1.6392, the crystal index corresponded to that of the 1.638 oil at 22.45° C. on one day and on the following day it corresponded to the same oil at 22.40° C. which gave an index value a little greater than 1.639 ($-\frac{dn}{dt}$ for oil 1.638 = 0.00044). All of the oils checked with the Abbe refractometer gave values very close to those assigned to them (±0.0001).

The temperature of the water passing through the water jacket could be varied from 20° to 30° C. and controlled within about 0.1° C. Inasmuch as the temperature coefficient of the index of refraction for any oil in the index range used for these measurements varied from about 0.0005 to 0.0006, the index of refraction of any oil on the slide could be increased or decreased about 0.003 starting at 25° C. This represented an important advantage because a given portion of a field could be examined over an index range of 0.006 without changing the oil. An estimate could be made of the per cent of crystals having an index of refraction below (or above) any desired index within this range. For this work the crystals were mounted on a slide with Neubauer rulings in such a way that markings on the slide would permit a more accurate count.

EXPERIMENTAL

Mixtures of cesium chloride and cesium bromide were heated for varying lengths of time and were then analyzed by the method of x-ray diffraction and by the immersion method for the index of refraction of light. In Table I the observed interplanar spacings for a 50 mole % mixture of cesium chloride and cesium bromide which was heated at 400° C. for 24 hours are compared with the known corresponding interplanar spacings for a 50 mole % solid solution of cesium chloride and cesium bromide obtained by melting the mixture. Comparing columns 3 and 5 shows that the pattern for the 24-hour heat taken from film Gr 106, agrees closely with the known solid solution pattern; from this it would be concluded that the ions of the cesium chloride and cesium bromide have migrated, at a temperature below the fusion point, into each other completely and formed a solid solution having a composition of 50 mole % cesium chloride and 50 mole % cesium bromide. However, the lines of this pattern were broad, diffuse, and poorly defined when compared with standard aluminum lines which were sharp and easily readable. The wide and diffuse nature of the lines is best explained on the assumption that, after 24 hours, there is present a whole series of solid solutions. If it be assumed that the compositions of these solid solutions begin at somewhat less than

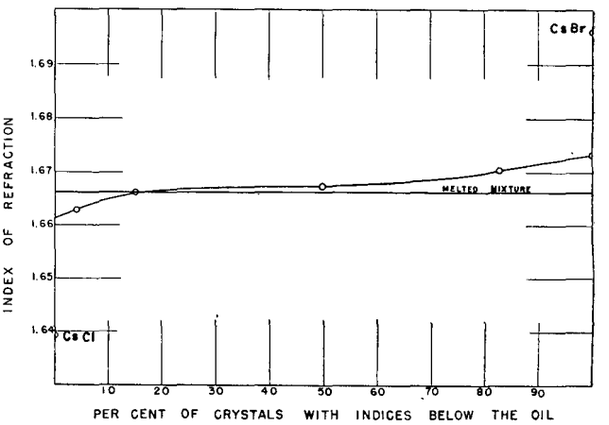

Figure 1. Index of Refraction of Crystals of Cesium Chloride–Cesium Bromide Solid Solution after 24 Hours at 400° C.

The lines of a series of similar x-ray patterns, such as are frequently formed by a continuous series of solid solutions, may overlap and form one pattern of wide lines; the measurements from the x-ray analysis will then give an average result and the mixture will appear to be homogeneous. Furthermore, a certain minimum number of particles in a crystal mixture are required to give x-ray interference lines of sufficient intensity for detection. In this paper index of refraction by the immersion method is used to supplement the results of x-ray crystal analysis. Because the index of refraction method examines individual crystals, no particles can escape examination and heterogeneity is readily revealed.

50 mole % cesium chloride and extend to somewhat more than 50 mole % cesium chloride, the broad, diffuse lines of the x-ray pattern would result from the overlapping of lines that lie close to each other. The centers of these composite lines could easily lead to a 50% molar solid solution.

Table II. Index of Refraction of Cesium Chloride, Cesium Bromide, and a Mixture, 1 to 1 Molar Ratio, of Both

	Refractive Index of Oil at 25°C.	$-\frac{dn}{dt}$	Temp., °C.	Corrected Index
CsCl, source 1	1.640	0.00045	26.9	1.6391[a]
	1.640	...	27.2	1.6390[b]
CsCl, source 2	1.640	...	25.9	1.6396[c]
	1.640	...	26.3	1.6394
	1.640	...	26.5	1.6393[d]
CsBr, lot 1	1.698	0.00044	28.8	1.6957[e]
lot 2	1.698	...	28.3	1.6960[f]
Mixt. of CsCl and CsBr, unheated	1.668	...	25.0 (approx.)	
Mixt. of CsCl and CsBr, melted	1.668	...	21.0	1.6704[h]
	1.668	...	29.4	1.6654[i]
	1.668	...	28.0	1.6662[k]

[a] Two different lots tested on same slide gave idential results.
[b] Index of one of the lots described in a determination on the following day.
[c] Index of CsCl from a second source was determined several weeks later.
[d] The average value 1.6393 should be compared with 1.6418 reported by Sprockhoff (*3*), with 1.63961 ± 0.00005 reported by Wulff (*7*), and with 1.63966 ± 0.00003 reported by Wulff and Heigl (*8*).
[e] Crystals nearly invisible in the oil at 28.8° C.
[f] Determination made several weeks after the one described in [e]. The average value 1.6959 should be compared with 1.6984 reported by Sprockhoff. The authors indexes for CsBr and CsCl are 0.0025 lower than the Sprockhoff values.
[g] Index of 60.5% of the crystals is far above the oil (CsBr), and the index for 39.5% is far below the oil (CsCl).
[h] Index of all of sample definitely below the oil.
[i] Nearly all (about 96%) of the sample was above the oil. A few crystals were close to the oil.
[k] Index of nearly all of the sample was close to this value.

This possible explanation was tested by measuring the index of refraction of a mixture of cesium chloride and cesium bromide which had been heated for 24 hours as shown in Table I. The results of these observations are given in Figure 1. Nearly all of the particles in the field of the microscope had an index near 1.6662 which was the index found for a 50 mole % solid solution prepared by melting the mixture (Table II). This is a little lower than the average value of the indexes of cesium chloride and cesium bromide, which is 1.6675 (calculated from Table II) from which it appears that the variation of the index with the composition of this solid solution is almost but not quite linear. Figure 1 shows that about 15% had an index less than 1.6662; 50% had an index less than 1.6674 whereas 83% had an index less than 1.6704. It is clear then that the sample was almost but not quite homogeneous because approximately 70% of the particles had indexes falling in a narrow range between 1.6662 and 1.6704. It seems likely that these particles are responsible for the wide x-ray interference lines which correspond to a 50 mole % solid solution whereas the particles varying far above or below this range were too few in number to be detected by means of the x-ray method.

Some light may be thrown on the position of the curve in Figure 1 by a consideration of the values of the unit cell volumes of cesium chloride and cesium bromide. The unit cell edge of cesium chloride is 4.1231 A. and that of cesium bromide is 4.2939 A. (*5*); thus, the unit cell volume for cesium chloride is 70.09×10^{-24} ml. and that for cesium bromide is 79.17×10^{-24} ml. On a volume basis the cesium bromide will occupy 53% of the volume of the equimolar mixture or if the particle sizes of cesium chloride and cesium bromide are the same (they were both passed through a 200-mesh sieve) the cesium bromide particles will make up 53% of the total. This was found by actual count to be 60.5% (Table II).

The results obtained by heating this same mixture of cesium chloride and cesium bromide for 0.5 hour and for 4 hours are shown in Figures 2 and 3 and are compared with the 24-hour results in Table III. At first there is a rapid ion migration but this rate decreases and becomes slow before solid solution is complete. This finding is entirely in agreement with the suggestion of Jander and Hoffman (*1*) that there is a "reaction layer" in which the reaction may proceed rapidly at first but that as the layer becomes thicker more time is required for movement through the layer and the reaction rate becomes much less. Figure 2 would seem to indicate that a considerable amount of solid solution has been formed after heating at 400° C. for 0.5 hour and Table IV seems to confirm this conclusion. The x-ray lines are, for the most part, broad and diffuse, and do not correspond to a 50 mole % solid solution. On the other hand, it is not possible to recognize patterns for pure cesium chloride and cesium bromide. It is not certain whether lines 4 and 5 are actually two lines or merely one wide line. Line 5 appears

Table III. Variation of Index of Refraction for 50 Mole % Mixture of Cesium Chloride and Cesium Bromide after Heating for Varying Lengths of Time at 400° C.

After 30 Min.		After 4 Hours		After 24 Hours			
Index of oil	Crystals below oil, %	Index of oil	Crystals below oil, %	Index of oil	Crystals below oil, %	After 2 Weeks Index of Oil	After 2 Months Index of Oil
1.6473	5	1.6590	3	1.6629	4	...	...
1.6550	37	1.6637	10	...	...	...	...
..	..	1.6661	15	1.6662	15	8% below 1.6662 (est.)	100% near 1.6662
..	..	...	..	1.6674	50	...	...
..	..	1.6704	43	1.6704	83	95% below 1.6705 (est.)	...
1.6771	65	1.6738	92	...	...	...	...
1.6830	97	1.6787	100	1.6730	100	...	...
1.6873	99	..	..	..	..	...	...

Table IV. X-Ray Diffraction Lines

(Comparison of 50 Mole % CsCl–CsBr mixture heated for 0.5 hour with pure CsCl and CsBr, and a 50 mole % CsCl–CsBr solid solution)

Line No.	Relative Intensity	Mixture	Aluminum Standard[a,b]	CsCl	CsBr	Solid Soln.
1	2	4.14	..	4.113 (100)[a]	4.287 (100)	4.200 (100)
2	10	2.98	..	2.908 (110)	3.031 (110)	2.970 (110)
3	4	2.33	2.3332 (111)	2.375 (111)	2.419 (111)	2.397 (111)
4	4|2	2.11	..	2.057 (200)	2.144 (200)	2.100 (200)
5	4/lines?	2.08				
6	2	2.02	2.0206 (200)	..	..	..
7	1|2	1.886	..	1.839 (210)	1.917 (210)	1.878 (210)
8	1/lines?	1.858				
9	4 Wide Diffuse	1.716	..	1.679 (211)	1.750 (211)	1.715 (211)
10	0.1 Wide Diffuse	1.491	..	1.454 (220)	1.516 (220)	1.485 (220)
11	2	1.429	1.4288 (220)	1.371 (221)	1.429 (221)	1.400 (221)
12	0.1 Wide Diffuse	1.329	..	1.301 (310)	1.355 (310)	1.328 (310)
13	1	1.219	1.2185 (311)	1.240 (311)	1.293 (311)	1.267 (311)

[a] Numbers in parentheses represent Miller indexes.
[b] Relative to unit cell edge of NaCl taken as 5.628.

to be a 200-diffraction line for a solid solution relatively rich in cesium chloride and poor in cesium bromide whereas line 4 appears to correspond to the 200 line for a solid solution relatively rich in cesium bromide and poor in cesium chloride. Lines 7 and 8 may be two lines that are close to each other or they may make up one broad diffuse line. A similar uncertainty exists in the case of lines 9, 10, and 12. The best interpretation of the film seems to be that two patterns are present—namely, a cesium chloride pattern with a considerable amount of cesium bromide in it and a cesium bromide pattern with a considerable amount of cesium chloride in it. Figure 2 shows that a considerable amount of ion migration occurred at 400° C. in only 30 minutes although the mixture was heterogeneous compared to the 24-hour heat of Figure 1. The mixture was carefully examined in oil 1.696 (refractive index) at 25.0° C. for pure cesium bromide and in oil 1.640 at 26.0° C. for pure cesium chloride. No pure cesium bromide or cesium chloride was found although a few crystals were found that had reacted only a little. A careful examination of the mixture in oil 1.668 at 28.0° C. (n = 1.6662) revealed that only a small per cent (about 5%) of the crystals had an index close to that of the melted sample. Such a mixture of solid solution crystals might easily give the indefinite series of x-ray interference lines described in Table IV.

When the cesium chloride–cesium bromide mixture was heated at 400° C. for 4 hours the x-ray diffraction lines given in Table V were obtained. The lines are still broad and diffuse and it seems probable that there are still lines of two solid solutions but that the compositions of these are approaching each other from each side of the 50% molar solution. This interpretation of the film is entirely consistent with the 4-hour curve of Figure 3 and with the measurements in Table V. According to this curve about 68% of the particles had a composition between 50 and 55% cesium bromide whereas about 16% of the particles had a cesium bromide content below this range and 16% had a cesium bromide content above this range. The data in Table V do not indicate with certainity whether there is one diffraction pattern or whether there are two patterns. The index of refraction data do indicate that certainly the sample is not homogeneous.

The 24-hour curve (Figure 1) represents a nearly homogeneous solid solution. The horizontal part of the 4-hour curve (Figure 3) dropped toward the 1.662 ordinate upon increasing the heating time to 24 hours, and upon increasing the time to 2 weeks (not shown in the figures) it came practically into coincidence with this ordinate. Even after 2 weeks, however, small per cents of crystals still had indexes somewhat less and somewhat more than 1.662. These percentages and index values changed only a small amount after the first 4 hours of heating. It seems possible that the slow rate at which the horizontal portions of the curves moved downward was due to increasing thickness of the reaction layer of Jander and Hoffman (*1*). The slow rate at which the end portions of the curves came into coincidence with the 1.6662 ordinate may have been largely due to unfavorable orientation of some of the crystals of cesium chloride and cesium bromide with respect to each other.

The curve in Figure 2 supports the reaction layer theory. It shows that all of the crystals are affected in the incredibly short heating time of 30 minutes while the index of some 4 to 5% of these crystals approached the 1.6662 ordinate of the melted sample. Presumably these were the small crystals with favorable orientation. It seems evident that with continued heating the low end of the curve moves upward and the high end moves downward and the vertical portion becomes undetectable in Figures 1 and 3. The ordinate values of the points could be

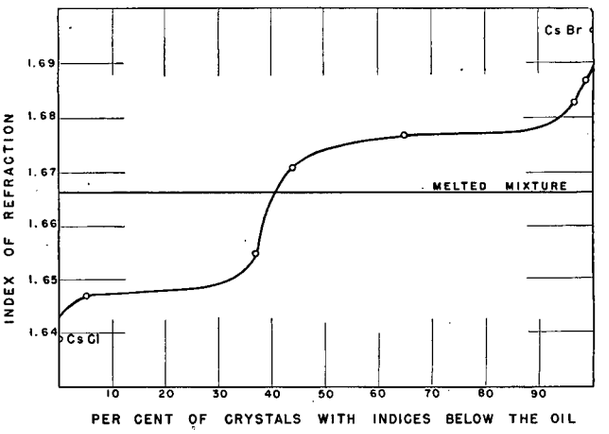

Figure 2. Index of Refraction of Cesium Chloride–Cesium Bromide Solid Solution after 0.5 Hour at 400° C.

Table V. X-Ray Diffraction Lines

(Comparison of 50 mole % CsCl–CsBr mixture heated for 4 hours with 50 mole % CsCl–CsBr solid solution)

Line No.	Relative Intensity	Mixture	Aluminum Standard[a,b]	Solid Soln.
1	1	4.20	..	4.200 (100)[a]
2	10	2.99	..	2.970 (110)
3	9	2.34	2.3332 (111)	..
4	2	2.11		2.100 (200)
5	5	2.03	2.0206 (200)	..
6	1	1.864	..	1.878 (210)
7	8	1.718	..	1.715 (211)
8	0.2|2	1.493	..	1.485 (220)
9	0.5/lines?	1.471		
10	8	1.429	1.4288 (220)	..
11	1 Broad Diffuse	1.328	..	1.328
12	8	1.218	1.2185 (311)	(310)
13	1	1.166	1.1666 (222)	..
14	0.5|2	1.130	..	1.122 (321)
15	0.5/lines?	1.122		

[a] Numbers in parentheses represent Miller indexes.
[b] Relative to the unit cell edge of NaCl taken as 5.628.

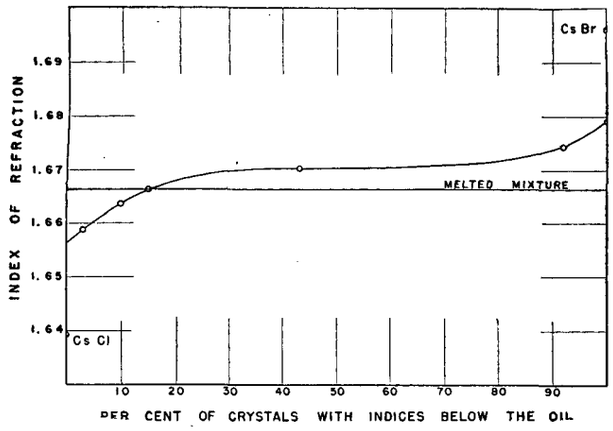

Figure 3. Index of Refraction of Cesium Chloride–Cesium Bromide Solid Solution after 4 Hours at 400° C.

observed with little uncertainity but the uncertainity in the abscissa was much larger, part of which was undoubtedly due to a lack of complete uniformity of the composition in going from one edge to the other edge of the solid solution particles (these differences would be much greater for the short heating periods than for the long). Hence the heights of the horizontal parts of the curves are accurately known but the exact locations of the breaks in the curves are not so accurately known. The beginning and end of each curve could be determined accurately.

The reaction layer and unfavorable orientation hypotheses were further tested by heating another sample of the mixture at 400° C. for 4 months. A sample of this mixture was then compared on the same microscope slide with the 24-hour sample, thus eliminating largely the uncertainity in the percentage counts. The 4-month sample was almost completely uniform and was definitely lower than the 24-hour sample. The 4-month sample was then compared directly with the melted mixture and the two samples were found to have the same index, both near 1.6662. The variation above and below at the ends of the curve could no longer be observed. Another mixture was heated at 400° C. for 72 hours but was stirred at 6-hour intervals. As nearly as could be determined it was uniform throughout and had the same index as the melted mixture.

SUMMARY

The immersion method for the determination of the index of refraction of powder particles of solid solutions has proved a valuable supplement to the x-ray crystal analysis method. Because the index of refraction method can be used for the examination of individual particles it is possible to distinguish between particles of solid solutions that would give an average value when examined by the x-ray method. Individual particles of widely varying composition but not sufficiently numerous for detection by the x-ray method can be readily studied by the index of refraction method. When a 50 mole % mixture of cesium chloride and cesium bromide powders was heated at 400° C. (well under the fusion point) the following conclusions were reached:

1. At first there was a rapid formation of solid solutions resulting from ion migration.
2. After a preliminary period the rate of ion migration fell off rapidly.
3. Unfavorable orientation of some of the crystals appeared to have an important influence on the rate of migration.
4. Frequent stirring of the mixture resulted in complete solid solution in a much shorter time than was required for an unstirred mixture.
5. The index of refraction for cesium chloride agreed well with the latest literature value whereas the index for cesium bromide did not.

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Determination of Sulfur in Petroleum Fractions by X-Ray Absorption

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QUANTITATIVE chemical analysis by means of x-ray absorption has been receiving an increased amount of attention recently because of the availability of improved x-ray generating and detecting equipment. The advantages of x-ray absorption analysis are speed, economy, and nondestruction of sample.

Two means of analysis are being used. In one method the absorption of a given element is measured at two wave lengths on each side of an absorption peak (*7*). The method is specific for a given element in a base stock containing unknown contaminants. In the other method the absorption of a given element is measured in a base stock containing an insignificant quantity of contaminants or a known quantity of contaminants (*3*, *5*, *6*, *11*). This second method has the advantages of speed, simplicity of equipment, and relatively good accuracy.

One important analytical problem that falls into the second classification is the determination of total sulfur in crude petroleum and its distillate fractions. A large percentage of samples coming into the petroleum analytical laboratory for total sulfur analysis contain insignificant quantities of contaminants in a hydrocarbon base stock.

Inasmuch as x-ray absorption is an atomic property, the physical form or chemical combination of the sulfur will not affect the results. The measured quantity will be the total elemental sulfur.

DERIVATION OF METHOD

The procedure and calibrations are based on equations derived from the well-known law of absorption of monochromatic elec-

tromagnetic energy passing through a homogeneous absorber. This law states that

$$\ln I/I_0 = -\mu'\rho X \quad (1)$$

where I_0 is the intensity of the beam entering the absorber, I is the intensity of the beam after passing through the absorber, μ' is the mass absorption coefficient at a given wave length, ρ is the density of the absorber, and X is the length of the absorber through which the beam passes.

For more than one element in the absorber

$$\mu' = \sum_i \mu_i' F_i \quad (2)$$

where μ_i' is the mass absorption coefficient of the ith element and F_i is its mass fraction. When the absorber is a pure hydrocarbon, Equation 2 becomes

$$\mu_{CH}' = \mu_C' F_C + \mu_H' F_H \quad (3)$$

It is a property of carbon and hydrogen that they have the same mass absorption coefficients (*10*) at a wave length of 0.53 A. Equation 3 at this wave length becomes

$$\mu_{CH}' = \mu_C' \text{ or } \mu_H' \quad (4)$$

and the absorption of a pure hydrocarbon is independent of the carbon to hydrogen ratio of that hydrocarbon. If sulfur is present in the hydrocarbon base stock, Equation 2 combined with 4 gives

$$\mu_{CHS}' = \mu_{CH}' F_{CH} + \mu_S' F_S \quad (5)$$

and F_S, the mass fraction of sulfur in the carbon hydrogen base, is the quantity to be measured.

Substitution of Equation 4 and then Equation 5 in Equation 1 will give

$$\ln I_{CHS}/I_0 = -(\mu_{CH}' F_{CH} + \mu_S' F_S)\rho_{CHS} X \quad (6)$$

and

$$\ln I_{CH}/I_0 = -\mu_{CH}' \rho_{CH} X \quad (7)$$

If $\rho_{CH} = \rho_{CHS}$ is chosen as a condition and Equation 7 is subtracted from Equation 6, the result is

$$\ln I_{CHS}/I_{CH} = -[\mu_{CH}'(F_{CH} - 1) + \mu_S' F_S]\rho_{HS} X \quad (8)$$

Because $F_{CH} + F_S = 1$, Equation 8 becomes

$$\ln I_{CHS}/I_{CH} = -(\mu_S' - \mu_{CH}')\rho_{CHS} X F_S \quad (9)$$

The above considerations are based on the absorption of monochromatic radiation. Polychromatic radiation will apply approximately if the radiation is characterized by its effective wave length (*4*). The effective wave length of polychromatic radiation is defined by comparison to monochromatic radiation. When both types of radiation have the property of being absorbed equally under identical conditions of measurement, the effective wave length is the same as the monochromatic wave length.

The sulfur calibration curves are based on Equation 9. Considering the terms in Equation 9, at a wave length of 0.53 A., μ_S' and μ_{CH}' are constants. The absorber length, X, may be made constant and ρ_{CHS} may be chosen as constant. Under these conditions Equation 9, when plotted on semilog paper, is a family of straight lines with all lines passing through $I_{CHS}/I_{CH} = 1$ for $F_S = 0$. The ratio, I_{CHS}/I_{CH}, is plotted on the log ordinate and the sulfur fraction, F_S, is plotted on the linear abscissa. Each calibration line represents constant density. Figure 1 is such a plot with weight fraction of sulfur written as per cent.

In the derivation of Equation 9 density comes into consideration twice: (*a*) in obtaining Equation 8 from 6 and 7, conditions were chosen such that the density of the hydrocarbon sample containing sulfur was equal to the density of the pure hydrocarbon; (*b*) in plotting Equation 9 density is held constant for variation in the sulfur fraction, F_S.

In the petroleum refinery analytical laboratory a significant portion of the work load is the quantitative determination of sulfur in petroleum fractions. Present chemical methods are relatively long, tedious, and therefore expensive. Because the x-ray absorption of sulfur is appreciably greater than that of pure petroleum, the application of x-ray absorption methods of analysis to the problem of sulfur in petroleum fractions was undertaken. Accuracy of analysis is ±0.02% sulfur and one operator can analyze thirty samples per day. By this method samples may be analyzed much faster than by chemical means and with comparable accuracy. It also has the advantages of economy and nondestruction of samples, and it may be applied to other analytical problems where proper conditions exist.

To carry out a sulfur analysis by use of Figure 1, it is required that I_{CHS}/I_{CH} be measured. This means that the x-ray transmittance of a hydrocarbon sample containing sulfur must be compared to the transmittance of a pure hydrocarbon of the same density. It would be impractical to prepare a pure hydrocarbon of the same density as the sample for each sample to be analyzed. One solution to the problem would be to compare some pure hydrocarbon such as toluene to several pure hydrocarbons covering a wide density range. The ratio of the transmittance of toluene to the transmittance of the various hydrocarbons, $I_{toluene}/I_{CH}$, would be plotted against density as shown in Figure 2. In an analysis the sample transmittance would be compared to toluene transmittance. From Figure 2, $I_{toluene}/I_{CH}$ would be read for the sample density. By multiplying $(I_{toluene}/I_{CH})(I_{CHS}/I_{toluene}) = I_{CHS}/I_{CH}$ would be obtained.

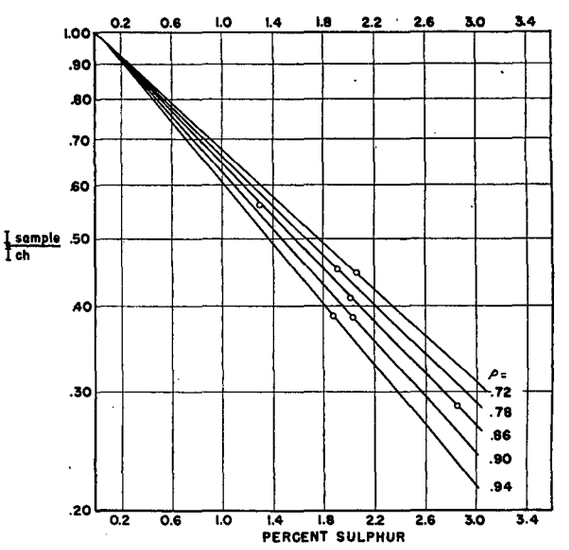

Figure 1. X-Ray Absorption of Sulfur in Hydrocarbons

This approach was investigated but the curve of Figure 2 shifted from day to day. Such a shift could not be tolerated in the analyses because a small error in I_{CHS}/I_{CH} would be magnified in obtaining the final sulfur result.

To eliminate the need of Figure 2, fifteen solid, pure hydrocarbon standard absorbers were constructed of polystyrene rod. The

polystyrene absorbers were cut to such a length that they had x-ray absorbancies equivalent to those of several liquid hydrocarbons. Since this work was done, another laboratory has described a somewhat similar use of polystyrene in x-ray absorption work (*3*). The liquid hydrocarbons were made to have density increments of 0.02 by mixing such compounds as hexane, hexadecane, and 1,2,3,4-tetrahydronaphthalene. The length of the x-ray absorption path of the liquid hydrocarbon was fixed by the sample cell length and was constant as required in the derivation of Equation 9.

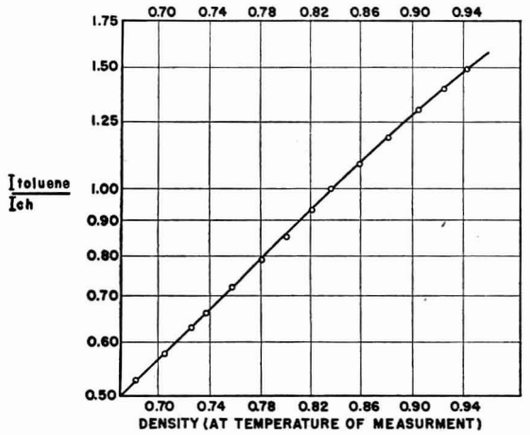

Figure 2. X-Ray Absorption of Pure Hydrocarbons

In carrying out an analysis a sample is compared to a polystyrene standard having the nearest density. Because the polystyrene standards have effective density increments of 0.02, the greatest difference in sample and standard density is 0.01. This difference in densities can be accounted for by interpolation.

To accomplish the interpolation the equation for Figure 2 was determined. It is best represented by

$$\ln (I_{\text{toluene}}/I_{\text{CH}}) = 3.90\ \rho_{\text{CH}} + b \qquad (10)$$

where b is some constant. This equation was then written for the density of the sample and the density of the polystyrene absorber as follows:

$$\ln (I_{\text{toluene}}/I_{\text{sample}}) = 3.90\ \rho_{\text{sample}} + b \qquad (11)$$

$$\ln (I_{\text{toluene}}/I_{\text{standard}}) = 3.90\ \rho_{\text{standard}} + b \qquad (12)$$

Subtracting Equation 11 from 12,

$$\ln(I_{\text{sample}}/I_{\text{standard}}) = 3.90(\rho_{\text{standard}} - \rho_{\text{sample}})$$

or

$$I_{\text{sample}}/I_{\text{standard}} = e^{3.90\Delta\rho} \qquad (13)$$

where $\Delta\rho$ is the difference in density between the polystyrene standard and the sample.

Using the power expansion of e^X, Equation 13 becomes

$$I_{\text{sample}}/I_{\text{standard}} = 1 + 3.90\ \Delta\rho + (3.90\ \Delta\rho)^2/2 + \ldots\ldots \qquad (14)$$

Since $\Delta\rho$ is always 0.01 or less, all terms after the first two may be neglected and

$$I_{\text{sample}} = (1 + 3.90\ \Delta\rho)\ (I_{\text{standard}}) \qquad (15)$$

To find what the transmittance of a pure hydrocarbon of the same density as the sample would be, the transmittance of the polystyrene standard is multiplied by the term $(1 + 3.90\ \Delta\rho)$.

The analysis of a sample containing sulfur is carried out by first taking its density and selecting the proper standard. The sample and standard are compared in the x-ray beam for transmittances, the standard is corrected for density interpolation by use of Equation 15, and the resulting ratio of $I_{\text{CHS}}/I_{\text{CH}}$ is applied to Figure 1 to obtain the sulfur content of the sample.

EQUIPMENT AND OPERATING CONDITIONS

The equipment consisted of a North American Philips Geiger-counter x-ray spectrometer. It was altered electrically to make possible a variable high voltage for the x-ray tube. This was accomplished by introducing a variable transformer at the input to the primary of the high voltage transformer as indicated in Figure 3. A Type 116 Superior Electric Co. Powerstat was used for the variable transformer. Use of a double pole, double throw switch, as shown in Figure 3, makes it possible to remove the variable transformer from the circuit so that the equipment may be changed back to diffraction work without changing the transformer setting.

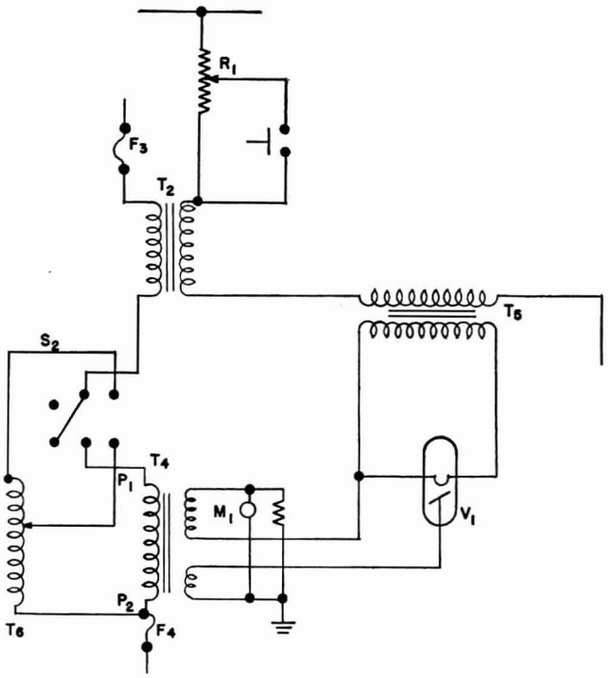

Figure 3. Alterations to X-Ray Unit

T_2. Reactor
T_4. High voltage transformer
T_5. Tube filament transformer
T_6. Variable autotransformer, Superior Electric Co., Powerstat Type 116
S_2. D.P.D.T. toggle switch
V_1. X-ray tube
P_1P_2. Terminals of primary on high voltage transformer
M_1. X-ray tube current meter

As indicated previously, it is necessary to obtain an effective wave length of 0.53 A. This is accomplished experimentally by adjustment of the variable transformer which varies the high voltage applied to the x-ray tube. A preliminary setting is made by calculation from the equations $\lambda_0 = 12{,}350/V_0$ and $\lambda = 1.3\ \lambda_0$. λ_0 is defined as minimum wave length at voltage V_0 and λ is the approximate effective wave length. For 0.53 A., λ_0 is 0.41 A. and V_0 is 30,000 volts. A setting of 95 on the variable transformer will give approximately 30 kilovolts peak on the x-ray tube. The North American Philips x-ray unit utilizes self-rectification and the applied voltage is sinusoidal. Because under the experimental condition the shorter wave lengths are the only ones that penetrate the absorbers to any appreciable degree, the near peak voltage is the only voltage that need be considered and 30 kilovolts peak is an approximation of the voltage required for 0.53 A. effective wave length. The final adjustment is made by comparing in the x-ray beam a known hydrocarbon sample containing approximately 2% sulfur with a pure hydrocarbon of the same

density. Applying Equation 9, the value of $(\mu'_S - \mu'_{CH})$ may be calculated. The tables of mass absorption coefficient given by Victoreen (*10*) show that at 0.53 A., $(\mu'_S - \mu'_{CH}) = 3.9$. The high voltage is readjusted and the ratios of the known sulfur sample and the pure hydrocarbon are measured until calculation of $(\mu'_S - \mu'_{CH})$ from Equation 9 gives a figure of 3.9. Once the correct setting for the variable transformer is found, it should not be changed.

Other electrical alterations include the installation of a variable resistor in the x-ray tube filament circuit with the adjusting control accessible on the front panel. Such a control makes possible the setting of the x-ray tube current to a constant value without shutting the equipment down. An electronic-type line voltage stabilizer, the Sorenson Model 2000S of 2-kv.-amp. capacity, was installed to give a constant voltage input to the x-ray and Geiger-counter units. The Sola line voltage stabilizer in the original equipment was removed.

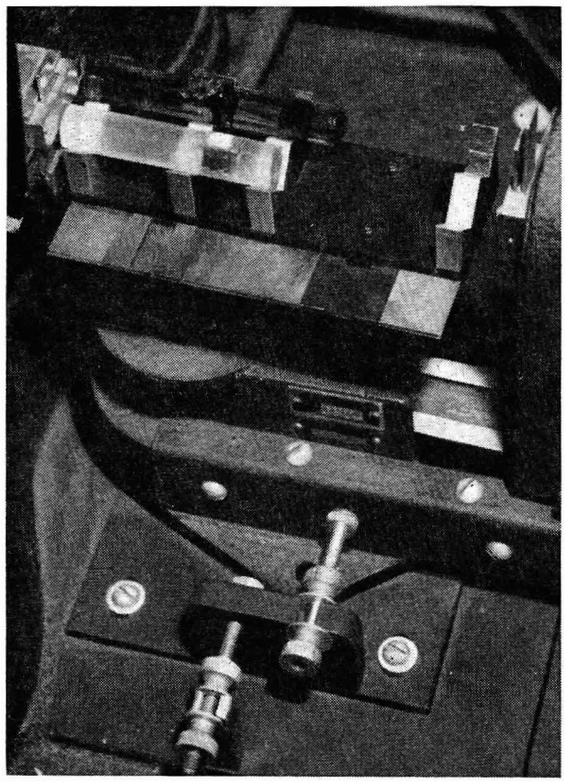

Figure 4. Sample and Standard Sliding Cradle

To facilitate comparison of standard and sample in the x-ray beam a sliding dual cradle was constructed as shown in Figure 4. It makes possible rapid and reproducible placement of the standard cell and the unknown sample in the x-ray beam. The cradle is mounted on a steel post that duplicates the one used in x-ray diffraction work. It is then a simple matter to change the equipment from absorption measurements to diffraction work.

Both iron and copper target tubes have been used in this work although other target materials may be used. Changing from an iron to a copper target tube has a small effect on the sulfur calibration curve. At 1.50% sulfur such a change causes a shift in the calibration curve of 0.06% indicating similar effective wave lengths for both target materials.

Polychromatic radiation is used instead of monochromatic for two reasons. A comparatively high beam intensity is obtained to give a high counting rate. This makes possible a short counting time with a low statistical error in counting. The use of polychromatic radiation eliminates the need of a diffraction crystal with its associated mounting and adjustment.

Sample cells are 10 cm. long and are made of 15-mm. diameter borosilicate glass tubing. Mica windows of 0.02-mm. thickness are attached with de Khotinsky cement by a special technique. This technique involves roughing the mica slightly with crocus cloth and working the de Khotinsky cement around the outer edge of the cell window. A mechanically strong seal is thus obtained.

A typical sample cell is shown in Figure 5. To compensate for differences in geometry of cells and mica window thickness all cells are filled with the same hydrocarbon and compared in the x-ray beam to a selected cell. A correction factor is calculated for each cell to make the absorption of each the same as a selected standard cell.

Liquid hydrocarbon standards used in the work were 2,2,4-trimethylpentane, hexadecane, toluene, and 1,2,3,4-tetrahydronaphthalene. Various proportions of each were blended to give mixtures of the desired densities. The 2,2,4-trimethylpentane was obtained from the Shell Oil Co. It was percolated through silica gel and a sulfur analysis of the purified fraction as determined by the A.S.T.M. lamp method (*2*) indicated less than 0.001%. The hexadecane was obtained from Du Pont. On dilution with 2,2,4-trimethylpentane and analysis by the A.S.T.M. lamp method (*2*), the sulfur content was 0.015%. This sulfur was not removed but an appropriate correction was made during calibration. The toluene was Baker, C.P. After percolation through silica gel the sulfur analysis was 0.002% for which a correction was made. The 1,2,3,4-tetrahydronaphthalene was Eastman practical grade. Purification was carried out by high efficiency distillation.

The scaler used is the one supplied with the original equipment except that line voltage stabilization is provided by the Sorenson electronic voltage regulator.

Operating conditions for the equipment are as follows:

1. X-ray tube voltage, 30 kilovolts peak
2. X-ray tube current, 5.8 ma. for iron and copper
3. Counting time per single measurement, 64 seconds
4. Scaling ratio, 64
5. Filters, manganese for iron and nickel for copper although the filters may be omitted
6. Collimating slit adjustments to give a counting rate of approximately 400 counts per second for a sample cell filled with toluene as the absorber
7. Sample cell length, 10 cm.

CALIBRATION

Geiger Counter Resolving Time. The calibration requires the determination of the resolving time or so-called "dead time" of the Geiger counter. This is a correction that must be made to all observed readings obtained from the Geiger counter to give a measure of the true x-ray beam intensity. The correction may be made by use of the formula

$$C_T = C_0 + C_0^2 \tau \qquad (16)$$

where C_T is the true count, C_0 is the observed count, and τ is the resolving time.

Most manufacturers give a resolving time for their Geiger tubes and this value is usually between 100 to 300 microseconds for single chamber counters. The resolving time is a function of counting rate (*8*), temperature, and tube age. For best results the resolving time of the tube should be determined under operating conditions.

Polystyrene Standards. Polystyrene standards are made of 15-mm. diameter rod. A sample cell is filled with a mixture of pure hydrocarbons having a density near 0.94. This density may be attained by mixing 1,2,3,4-tetrahydronaphthalene and hexadecane in proper proportions. A length of polystyrene rod is cut and the ends are polished with jeweler's rouge until its x-ray transmittance is within 5 counts of the liquid hydrocarbon in the sample cell. The exact equivalent density is calculated by use of the density interpolation equation, Equation 15.

This procedure is repeated for density increments of 0.02 through a range of 0.94 to 0.68. In this manner fourteen polystyrene standards are obtained having density increments of approximately 0.02. Figure 6 illustrates a set of such standards.

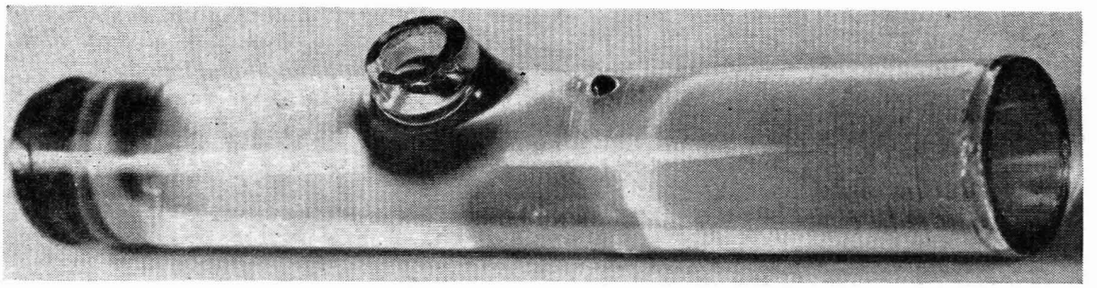

Figure 5. Sample Cell

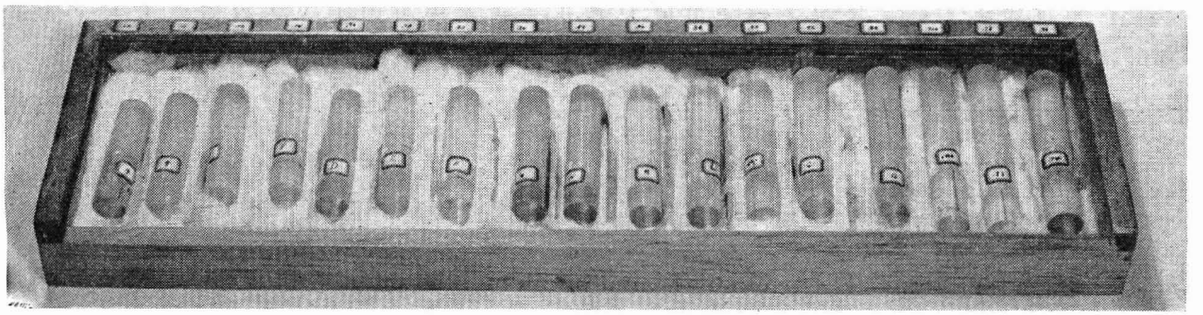

Figure 6. Polystyrene Standards

Inasmuch as the density of liquid and solid hydrocarbon is a function of temperature, correction must be made for change of density with temperature. The correction for polystyrene is small but significant. Calculation of the correction is made by considering the change of mass the cell presents to the x-ray beam with a change in temperature. Expansion in a radial direction is the only expansion factor that need be considered.

For polystyrene the linear expansion coefficient is 0.00004 per ° F. The coefficient for correcting the effective density of the polystyrene cell for temperature change is then $(1 + 0.00004)^2 - 1^2$ per ° F. or 0.00008 per ° F. When a polystyrene cell is calibrated for effective density as given above, the density determined is applicable at the temperature existent at the time of measurement. A table is made up giving the effective density of the polystyrene cells at all desired temperatures by correcting the measured density with the factor 0.00008—for example, if a cell were calibrated at 74° F. with an effective density of 0.7808, at 84° F. the effective density would be 0.7800.

Determination of Slope of Density Curve. To find the slope of the density curve of Figure 2—that is, the coefficient of $\Delta\rho$ in Equation 15—each polystyrene standard is compared to toluene in the x-ray beam. The ratio of $I_{\text{toluene}}/I_{CH}$ is plotted against density on semilog paper. The slope of this curve is the coefficient of $\Delta\rho$ required for density interpolations.

Sample Cell Calibrations. To enable an operator to load more than one sample cell at a time several sample cells are constructed. Because it is impractical to make the cells exactly the same, a correction factor must be assigned to each cell to give it the equivalent absorption of a selected standard sample cell.

This factor is determined by filling all the cells with toluene and comparing them in the x-ray beam. The correction factor for a cell is that number which will make the transmittance of the cell numerically equal to the transmittance of the selected standard cell.

Sulfur Calibration (Figure 1). Standards for the sulfur calibration are made by dissolving dipheny! disulfide in mixtures of pure hydrocarbons. The mixtures plus the disulfide are made to have final densities of increments of approximately 0.02. A sulfur value of approximately 2% is a convenient one to use in preparing standards.

A line of the family of curves is constructed by determining I_{CHS}/I_{CH} for a sulfur standard of known density and sulfur content. A line through this point and the point where $I_{CHS}/I_{CH} = 1$ for $F_S = 0$ represents constant density equal to that of the sulfur standard. This procedure is repeated for each of the sulfur standards. Samples having intermediate densities are interpolated.

PROCEDURE

To perform an analysis the value of I_{CHS}/I_{CH}, the ratio of the transmittance of the sample to a pure hydrocarbon of the same density, is required. This value when applied to the calibration curves of Figure 1 will give the sulfur content.

To obtain the value I_{CHS}/I_{CH}, the sample is compared in the x-ray beam to a polystyrene standard of the nearest effective density. The densities used are those existent at the temperature of the analysis. In order to minimize the effect of line voltage fluctuation the sample is placed in the beam and counted for 64 seconds after which the polystyrene standard is counted for 64 seconds. This cycle is repeated three times to give three individual readings for each absorber. An arithmetical average of the three readings for the sample and the three readings for the polystyrene standard is used to give the ratio I_{CHS}/I_{CH}.

Samples that contain more sulfur than is covered by the calibration curve may be analyzed by dilution with a pure hydrocarbon such as toluene. The probable error of analysis will then be greater in proportion to the dilution factor. Samples that are too viscous to pour into cells may be analyzed by solution in toluene as above with the attendant increase in error by the dilution factor.

CALCULATIONS

Several corrections must be made to the ratio obtained above. The corrections are listed as follows in the order in which they are made:

1. Resolving time correction is made to the observed counts for the sample and for the standard. This correction is made by use of Equation 16. Because counting with a scale of 64 for 64 seconds gives the reading directly in counts per second, the resolving time must be expressed in seconds.

Table I. Comparison of Sulfur Analysis by X-Ray Absorption and Chemical Means

Density of Sample	X-Ray Analysis	Chemical Analysis	X-Ray Minus Chemical
0.6988	−0.01	0.002[a]	−0.01
0.7256	0.02	0.027[a]	−0.01
0.7579	0.01	0.007[a]	0.00
0.7543	0.06	0.060[a]	0.00
0.7775	0.01	0.004[a]	+0.01
0.7805	1.27	1.30[b]	−0.03
0.8270	0.67	0.64[b]	+0.03
0.8368	0.38	0.382[a]	0.00
0.8676	1.57	1.58[c]	−0.01
0.8729	0.78	0.71[b]	+0.07
0.9094	1.68	1.66[b]	+0.02
0.9129	1.38	1.40[c]	−0.02
0.9194	2.70	2.72[b]	−0.02
0.9212	1.83	1.80[b]	+0.03
0.9365	0.88	0.89[b]	−0.01
0.9413	0.80	0.78[b]	+0.02

[a] Lamp method (*2*).
[b] Capillary combustion.
[c] Bomb method (*1*).

2. An interpolation correction is made for the difference in density between sample and polystyrene standard. The density of the sample is taken at room temperature and the equivalent density of the polystyrene standard is read from the chart mentioned previously. The difference in density is the $\Delta\rho$ used in equation 15. This value may be positive or negative depending on which density is the greater.

3. The cell factor correction is made to give the value used in obtaining the sulfur content of the sample from Figure 1.

Sample calculation:

Room temperature = 76° F.
Resolving time of Geiger tube = 125 microseconds
Sample cell factor = 0.994
Slope of density curve = 3.90
Density of sample = 0.8704 (at 76° F.)
Effective density of standard = 0.8780 (at 76° F.)
$\Delta\rho$ = 0.0076
I_{CHS} (sample) = 420 counts per second
I_{CHS} (corrected) = $C_0 + C_0^2\tau = 420 + (420)^2(125 \times 10^{-6})$ = 442 counts per second
I_{CH} (polystyrene standard) = 508 counts per second
I_{CH} (corrected) = $508 + (508)^2(125 \times 10^{-6})$ = 540 counts per second
Density interpolation correction (Equation 15) = $(1 + 3.90\ \Delta\rho) = 1 - (3.90)(0.0076) = 0.9704$
$I_{CHS}/I_{CH} = (442/540)(0.9704)(0.994) = 0.790$
From Figure 1, sulfur content of sample = 0.53%

ACCURACY AND REPRODUCIBILITY

The accuracy of the method has been checked by comparing results of x-ray analysis to chemical analysis. For 90 comparisons available the average difference was ±0.02%. Table I shows a comparison of x-ray analysis and chemical analysis on typical samples. The reproducibility of analyses has been found by running a single sulfur standard each day that samples are analyzed. The reproducibility is 0.013%.

Although crudes and still bottoms have ash contents considerably higher than those of distillate fractions, the only samples that gave erroneous results owing to high metal content have been Venezuela asphaltic crudes. The effect of contaminants should, however, always be considered.

The following table lists a few typical elements that may be present as contaminants with the amount necessary to give an error of 0.01% sulfur (calculated from data of Victoreen, *10*):

Oxygen	0.14%	Chlorine	0.01%
Nitrogen	0.32%	Iron	0.002%
Sodium	0.04%	Lead	0.0006%[a]

[a] Calculated from Sproul (*9*).

CONCLUSION

This rapid and accurate method for the determination of sulfur in petroleum fractions with x-ray absorption makes possible the analysis of a sample in 10 to 15 minutes with an accuracy of ±0.02%. Advantages of the method include independence of analysis on the carbon to hydrogen ratio of the base stock, and minimization of the effect of fluctuation in x-ray beam intensity and in sensitivity of the Geiger counter. Samples of all densities may be analyzed with a single set of calibration curves.

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Determination of Uranium in Solution by X-Ray Absorption

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Carbide and Carbon Chemicals Co., K-25 Plant, Oak Ridge, Tenn.

The determination of uranium in solution by x-ray absorption was investigated to determine its applicability to rapid, routine uranium determinations on a variety of solutions. With a new type of absorption cell having a solution depth of 19 mm., straight-line calibration curves indicated a sensitivity of 14 scale divisions per gram of uranium per liter. The absorption by 1% solutions of possible contaminating elements increased rapidly with increasing atomic number up to 42, molybdenum, and above 53, iodine. In the absence of contaminating elements, the precision, expressed as the 95% symmetric confidence interval, was ±0.05 gram per liter in the concentration range up to 10 gram per liter. The lower limit of detection was approximately 0.1 gram per liter. Moderate concentrations of substances of low atomic number, such as sodium, ammonium, and fluoride ions, did not interfere, but chemical separation of the uranium was necessary when appreciable quantities of most other elements were present.

ANALYTICAL methods based on absorption of polychromatic x-rays are rapid, nondestructive, and very sensitive when the desired constituent is of considerably higher atomic number than the other elements present. Probably its most serious disadvantage is nonspecificity, as all elements absorb x-rays to some extent. Other applications of x-ray absorption to analysis include the determination of tetraethyllead in gasoline (*1*, *9*, *10*), sulfur in hydrocarbons (*10*), chlorine in plastics (*6*), and a previous application to the determination of uranium (*5*).

INSTRUMENT

A General Electric, Model 5328350G1, x-ray photometer was employed. The instrument is similar to those described by Michel and Rich (*7*) and Vollmar, Petterson, and Petruzzelli (*10*). It is a split-beam instrument using the polychromatic radiation from a tungsten target x-ray tube. The transmitted intensities of the two portions of the x-ray beam are matched by adjusting an aluminum, wedge-type attenuator disk in one portion of the beam until the absorption by the aluminum is equivalent to the absorption by the sample in the other portion of the beam.

Several minor changes in the instrument have been made at this laboratory. A new attenuator disk, similar to the one originally present, has been installed, and, at the same time, the graduations on the reading drum were changed to an arbitrary 0 to 100 scale with small graduation marks representing 0.2 of the major scale divisions. As the new attenuator varies in thickness from 0.050 to 0.110 inch, 1 scale division corresponds to approximately 0.0006 inch of aluminum. To eliminate lost motion in the connection between the reading drum and the attenuator disk, the flexible shaft originally present was replaced with a solid shaft and 45° bevel gears.

Fixed-volume absorption cells and a special cell holder were designed and are illustrated in Figure 1.

These cells were constructed of 0.125-inch Lucite sheet, which has been found to be satisfactorily inert to most of the solutions encountered. Cells were made in 30- and 70-ml. sizes with x-ray path lengths of 19 and 38 mm., respectively. In order to make matched cells, the Lucite sheets were machined to a uniform thickness and gage blocks were used to establish the depth of the cells. This type of cell is more convenient than the divided cell supplied with the instrument, because the blank or standard solution may be left in the instrument while the test solution is changed. Furthermore, the necessity of adding a measured volume of test solution to the cell is avoided, as the solution is simply poured into the cell until the horizontal portion is completely filled.

Aluminum blocks with parallel, plane faces were constructed and used either to increase the available thickness of aluminum or to balance the minimum thickness of the attenuator. These blocks, one of which is shown in Figure 1, are notched to fit projections on the cell holder in order that they may be positioned reproducibly.

EXPERIMENTAL PROCEDURE

Preparation of Solutions. Standard uranium solutions were prepared by dissolving weighed amounts of uranyl nitrate hexahydrate in water and diluting in volumetric flasks. The uranium content of such solutions was checked by a ceric sulfate titration procedure (*4*) until it was established that the uranium content of the salt was in agreement with the formula, $UO_2NO_3.6H_2O$.

The solutions described in Table II were prepared from fresh, reagent-grade chemicals.

Operation of the Instrument. The electronic circuit was allowed to warm up for 45 minutes and the x-ray unit for 10 minutes before making any measurements. It was found desirable to have a sample or standard aluminum blocks in position and the attenuator adjusted to approximate balance during the warm-up period for the x-ray unit.

The instrument was operated at a primary voltage of 100 volts, and an emission current of 7.5 ma. The 30-ml. absorption cells were used and the cell containing the test solution was placed on the right and a second cell containing water on the left (attenuator) side.

PRECISION, SENSITIVITY, AND RANGE

The data in Table I are the results of replicate calibrations with the same solutions. For nine successive working days, readings were taken on aliquots of the nine uranium solutions, on water, and on an aluminum block. The widest 95% symmetric confidence interval (*2*) was ±0.63 scale division, which corresponds to ±0.05 gram of uranium per liter.

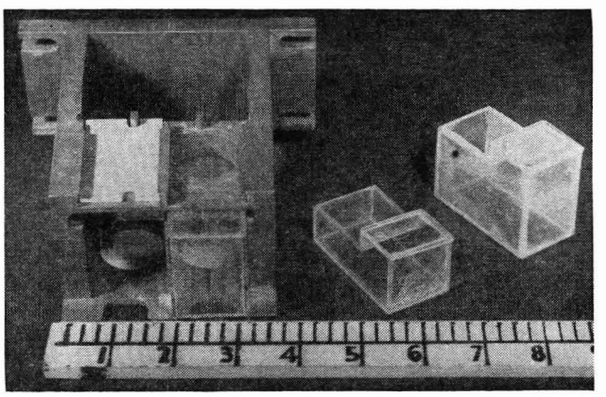

Figure 1. Absorption Cells and Cell Holder

Frequent recalibration was found necessary because of changes in response of the instrument. Comparison of readings obtained over a period of a month indicated a gradual drift of the readings, believed due to fatigue of the photocell. Occasionally, a more abrupt change in readings occurred, the reason for which is unknown. For example, a tenth set of readings, not included in Table I, was obtained with the solutions described above, but these readings were omitted from the statistical analysis because all were approximately 1 scale division higher than had been obtained in the previous nine readings.

Table I. Precision with Uranium Solutions

	Concentration of Uranium, Grams per Liter											Check Reading on 100-Mil Al Block
	0	1	2	3	4	5	6	7	8	9	10	
	Scale Readings											
	13.9	29.5	42.4	56.7	70.8	88.0	12.0	28.4	41.3	55.8	70.1	85.3
	13.5	29.3	42.3	56.3	70.6	87.4	11.4	28.3	41.2	55.6	70.2	84.9
	13.7	29.7	42.3	56.6	71.0	88.2	12.2	28.6	41.2	56.0	70.8	85.2
	13.9	29.6	42.4	56.6	70.9	87.8	12.2	28.5	41.2	56.0	70.5	85.0
	13.7	29.6	42.6	56.8	70.8	87.8	11.9	28.4	41.4	56.0	70.2	85.4
	14.0	29.4	42.5	56.7	71.2	87.8	12.2	28.6	40.6	56.4	70.2	85.5
	13.6	29.7	42.4	56.6	71.1	87.8	11.8	28.6	41.6	56.0	70.3	85.5
	13.6	29.4	42.4	57.0	71.0	88.0	12.0	28.6	41.4	55.8	70.3	85.4
	13.4	29.5	42.2	56.8	71.1	87.8	11.8	28.2	41.2	55.6	70.2	85.8
Mean	13.7	29.5	42.4	56.7	70.9	87.8	11.9	28.5	41.2	55.9	70.3	85.3
Precision[a]	0.46	0.32	0.27	0.44	0.43	0.50	0.60	0.35	0.63	0.57	0.50	0.63
	Uranium Found[b], Grams per Liter											
	−0.05	1.05	1.95	2.95	3.94	5.12	5.90	7.06	7.95	8.99	9.99	

Readings on solutions containing 0 to 5 grams of uranium per liter were made with 51-mil aluminum block on right side.

[a] 95% symmetric confidence interval about mean.
[b] Calculated from above means by use of calibration equations.

Table II. Interferences

Substance	Atomic Number	Compound Used	Formula	Apparent Uranium Content of 1% Solution of Substance, Grams/Liter
Fluoride	9	Ammonium fluoride	NH_4F	0.04
Sodium	11	Sodium acetate	$NaC_2H_3O_2.3H_2O$	0.3
Chloride	17	Ammonium chloride	NH_4Cl	0.7
Calcium	20	Calcium acetate	$Ca(C_2H_3O_2)_2.H_2O$	1.5
Iron	26	Ferric nitrate	$Fe(NO_3)_3.9H_2O$	2.7
Copper	29	Cupric acetate	$Cu(C_2H_3O_2)_2.H_2O$	3.8
Bromide	35	Sodium bromide	$NaBr$	5.0[a]
Strontium	38	Strontium nitrate	$Sr(NO_3)_2$	5.8
Molybdenum	42	Ammonium molybdate	$(NH_4)_2MoO_4$	6.1
Silver	47	Silver nitrate	$AgNO_3$	5.0
Iodide	53	Ammonium iodide	NH_4I	3.2
Barium	56	Barium acetate	$Ba(C_2H_3O_2)_2.H_2O$	3.4
Tungsten	74	Tungstic acid	H_2WO_4	7.0
Lead	82	Lead acetate	$Pb(C_2H_3O_2)_2.3H_2O$	8.8
Thorium	90	Thorium nitrate	$Th(NO_3)_4.4H_2O$	9.6
Ammonium	..	Ammonium hydroxide	NH_4OH	0.01
Acetate	..	Acetic acid	CH_3COOH	−0.003[b]
Nitrate	..	Nitric acid	HNO_3	0.05
Sulfate	..	Ammonium sulfate	$(NH_4)_2SO_4$	0.3
Phosphate	..	Monobasic Ammonium phosphate	$NH_4H_2PO_4$	0.2
Citrate	..	Citric acid	$C_3H_4(OH)(COOH)_3.H_2O$	0.02
Carbonate	..	Sodium bicarbonate	$NaHCO_3$	0[a]

[a] Corrected for absorption due to sodium.
[b] Scale readings decreased with increasing acetic acid content.

With the 30-ml. fixed-volume absorption cells, a change in uranium concentration of 1 gram per liter resulted in a change of approximately 14 scale divisions. This sensitivity is shown graphically by the slope of the calibration lines in Figure 2. The values plotted in Figure 2 are the means in Table I, but the lines were drawn by means of the following equations:

$$R = 14.33U + 14.44 \text{ (51-mil aluminum block on right)}$$
$$R = 14.24U - 72.05 \text{ (no aluminum block)}$$

where R is the scale reading and U is the concentration of uranium in grams per liter. These equations were calculated, by the method of least squares, as being the straight-line equations which best fit the calibration data.

In addition to the deviation of scale readings obtained on the same solution, there is error due to deviation of readings between solutions. This is illustrated by the final row of data in Table I, grams of uranium per liter found. The maximum error from this second source, 0.12, and the greatest sum of the two deviations, 0.15, occurred with the solution containing 5 grams of uranium per liter. The values were calculated from the mean and the highest of the readings on the solution, respectively.

The range of uranium concentration which may be used in the small absorption cells has been considered to be 0.1 to 90 grams per liter. The lower limit is the concentration which may readily be distinguished from water, while the upper limit is imposed by the limit of amplification. It is preferable to dilute solutions containing high concentrations of uranium to 20 grams per liter or less in order to avoid extensive calibration and because of unsteadiness of the balance-indicating needle at high amplification.

INTERFERENCES

The x-ray absorption of 22 elements and ions was measured and the extent of interference determined as apparent concentration of uranium by reading from a calibration curve. The compounds used to prepare the solutions are listed in Table II, and the apparent uranium content of 1% (w./v.) solutions of the elements and ions is presented both in Table II and in Figure 2. Water was the only solvent used, although the pH was increased by adding a small amount of ammonia in the case of ammonium molybdate and tungstic acid. The absorption by elements other than the one being investigated was neglected except for sodium compounds, when the absorption by sodium was subtracted from the absorption by the compound.

The apparent uranium content increased rapidly with atomic number up to 42 (molybdenum) and above 53 (iodine). The absorption by silver, atomic number 47, was between that of molybdenum and iodine. The location of the minimium in the curve of Figure 2 indicates that the "effective" wave length

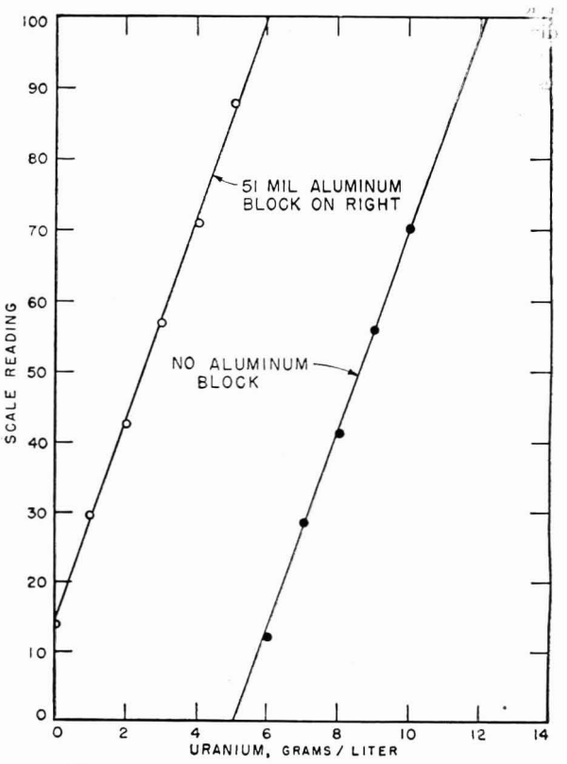

Figure 2. Uranium Calibration Curves

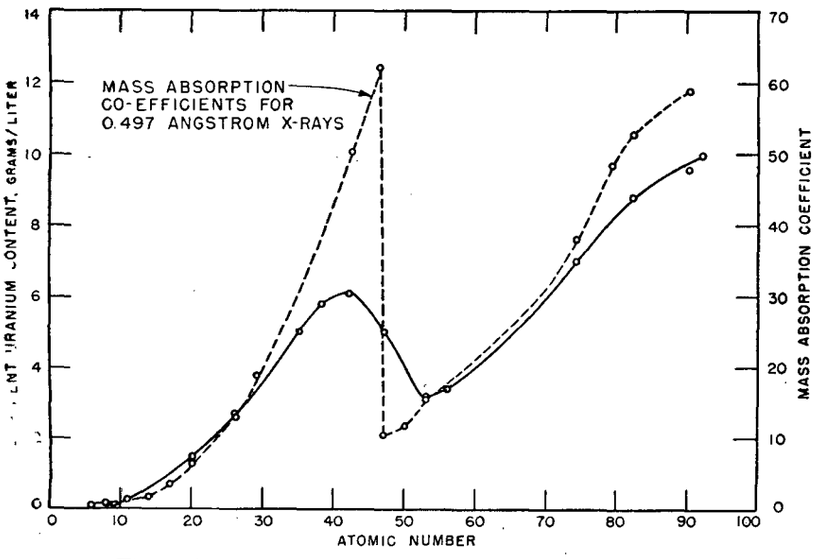

Figure 3. Apparent Uranium Content of 1% Solutions

of the x-ray beam is approximately 0.5 A. To illustrate this, the published values of the mass absorption coefficients for 0.497 A. x-rays (*3*) have also been plotted against atomic number in Figure 3.

The organic anions and those such as carbonate, nitrate, and fluoride, which contain only elements of very low atomic number, showed little interference. Sulfate, phosphate, and chloride were slightly more serious, while bromide, iodide, molybdate, and tungstate were very serious interferences.

Variation of the primary voltage in the range 60 to 120 volts had only a minor effect on the scale readings obtained with uranyl nitrate solutions and, generally, with the solutions described in Table II. However, in the cases of molybdenum, silver, and tungsten, a marked decrease of scale reading occurred when the primary voltage was decreased to 60 or 70 volts. This effect was probably due to the increase of effective wave length above the *K* critical absorption wave lengths of these elements.

Because interference by most elements may be materially reduced only by removal of the element, chemical separations have usually been necessary when determining uranium by means of the x-ray photometer. The choice of separation method depends upon the nature and the amount of the contaminating elements, the amount of uranium present, and the accuracy desired. Ether extraction from nitrate solution (*8*) separates uranium from a large number of elements, it is applicable to a wide range of uranium concentrations, and an aqueous solution, well adapted to use in the x-ray photometer, may be obtained by evaporation of the ether over water.

ACKNOWLEDGMENT

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Analysis of Uranium Solutions by X-Ray Fluorescence

L. S. BIRKS AND E. J. BROOKS, *U. S. Naval Research Laboratory, Washington, D. C.*

THE principle of the x-ray fluorescence method of chemical analysis as developed at the Naval Research Laboratory (*4*) is shown diagrammatically in Figure 1 of (*3*). With the present-day, high-intensity, sealed-off x-ray tubes and sensitive Geiger counter circuits, it is feasible to locate the specimen outside the x-ray tube and excite it to fluorescence with the direct x-ray beam. The fluorescent radiation characteristic of the elements in the specimen is collimated by an array of nickel tubings. The resulting parallel, polychromatic beam is analyzed by a single-crystal Geiger counter spectrometer in which each characteristic x-ray line from the specimen is diffracted at a particular angle according to Bragg's law.

In the past, the x-ray fluorescence method has been applied successfully to both solid (*2*) and liquid (*3*) specimens. It has an advantage over x-ray absorption analysis in that most impurities in the specimen do not affect the results. However, any impurity whose x-ray lines do interfere with those of the desired element, or whose absorption coefficient is high enough to affect the intensity, must be considered and the analysis corrected for its effect. In some liquid samples, the background scattering is so high that the accuracy is affected, and it becomes necessary to evaporate the liquid before making analysis.

SPECIMEN PREPARATION

When water solutions of uranium salts were examined in liquid cells, the background near the uranium $L\alpha$ line was high because of scattering by the hydrogen in the water. For a 1 gram per liter solution of uranium (as the nitrate), the background intensity from a tungsten-target x-ray tube operated at 50 kv. and 25 ma. was 35 counts per second; the uranium $L\alpha$ line was only 15 counts per second above background. With a molybdenum-target tube and the same operating conditions.

This paper is part of a series describing the application of x-ray fluorescence to the rapid quantitative analysis of various chemical systems. It was feasible to determine uranium in aqueous solution in concentrations as low as 0.05 gram per liter. Less than 10 minutes were required for each analysis, including preparation of the specimen. The accuracy for a uranium concentration of 1 gram per liter was approximately 5% of the amount present. The presence of other elements had no effect on the uranium analysis except for heavy elements such as lead, and then only when the impurity concentration exceeded 10% of the uranium concentration. The results indicate that x-ray fluorescence analysis is applicable to uranium solutions of low concentration without previous knowledge or separation of impurities present. The rapidity of the method makes it practical as an almost continuous check on the uranium concentration in a separation process.

the background was lowered to 20 counts per second and the uranium $L\alpha$ line was 30 counts per second above background. Because of the high background with either target, the limit of detection with ±50% accuracy would be about 0.5 gram per liter at best. The authors decided, therefore, to develop a rapid method of evaporating the water leaving just the uranium salt.

A shallow spherical dish 1 cm. across and 2 mm. deep was pressed into a strip of aluminum foil 0.001 inch (0.025 mm.) thick. The strip was then heated to about 100° C. across the terminals of a low-voltage transformer. Using a pipet, 1 ml. of the uranium solution was then slowly dropped into the cup and allowed to evaporate a drop at a time, about 5 minutes being required to complete the operation.

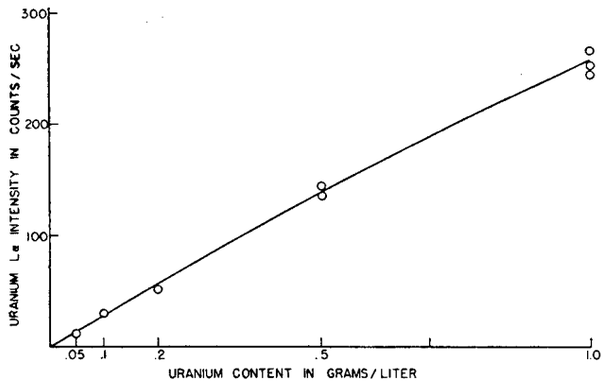

Figure 1. Calibration Curve for Aqueous Uranium Nitrate Solutions

With this technique, the dried specimen prepared from a 1 gram per liter solution gave a peak count at uranium $L\alpha$ of about 250 counts per second and a background of about 20 counts per second as compared with 30 counts per second peak over 20 counts per second background for the specimen in solution.

EXPERIMENTAL PROCEDURE AND RESULTS

To obtain a standard curve of counts per second *vs.* uranium concentration, solutions of uranium (as the nitrate) were prepared in concentrations of 0.05, 0.1, 0.25, 0.5, and 1.0 grams per liter. The intensity of the uranium $L\alpha$ line above background *vs.* concentration is plotted in Figure 1. For the 0.05 grams per liter concentration, the probable error in counting rate due to statistical fluctuations was about 6% of the amount of uranium present for a 30-second counting interval. For the 1 gram per liter specimen the probable error was less than 1% of the amount of uranium present. However, the average variation in 18 specimens of 1 gram per liter concentration was larger, being

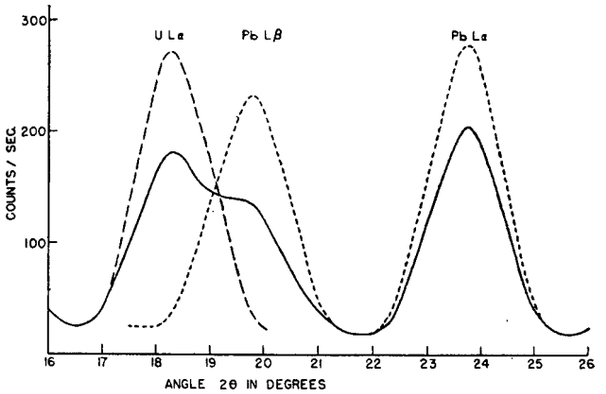

Figure 2. *L* Series Spectra for Uranium and Lead

- - - Uranium, 1 gram per liter
- - - - Lead, 1 gram per liter
——— Mixture of uranium, 1 gram per liter, and lead 1 gram per liter

about ±5% of the amount of uranium present. The maximum deviation of any one specimen from the mean of 18 specimens was about 15% of the amount present. (The accuracy could be improved by careful pipetting methods to ensure an equal amount of solution being evaporated each time.) These results should be compared with x-ray absorption determinations of uranium by Bartlett (*1*). He calculated that the lowest uranium concentration which could be detected was 0.07 gram per liter, but did not give any actual results on such a low concentration.

EFFECT OF IMPURITIES

Impurity elements which might affect the uranium determination fall into three classes: (1) elements from protactinium to tungsten, (2) elements from strontium to arsenic, and (3) elements from barium to silver. The characteristic L series lines from elements in class 1 lie close to those of uranium and the absorption coefficients of these elements for uranium radiation are high (comparable to the self-absorption coefficient of uranium). The K series lines of elements in class 2 lie near the uranium $L\alpha$ line. The second-order reflections of the K series lines of class 3 elements lie near the uranium $L\alpha$ line. Other elements such as iron, of interest because of their common occurrence, were also considered.

Lead was chosen as typical of elements in class 1. The spectra of uranium, of lead, and of a mixture of equal parts of uranium and lead are shown in Figure 2. Although the overlapping of the lead and uranium lines is not of consequence, there is an effect on the uranium peak intensity due to absorption of the uranium radiation by the lead. Several specimens were prepared with varying relative amounts of uranium and lead, and

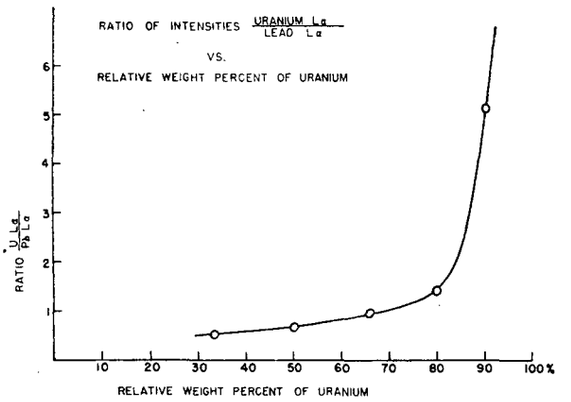

Figure 3. Calibration Curve for Establishing Relative Weight Per Cent of Uranium and Lead

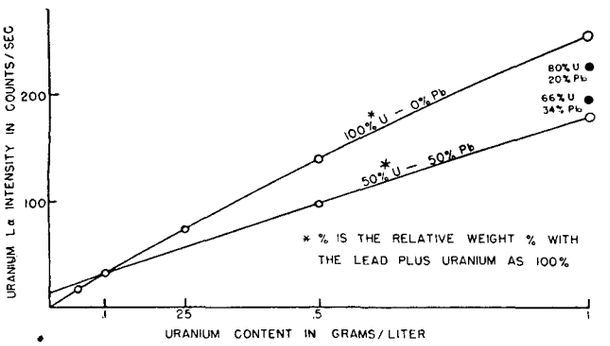

Figure 4. Family of Uranium Calibration Curves for Lead as an Impurity

the ratio of the uranium $L\alpha$ peak to the lead $L\alpha$ peak is plotted against weight per cent or uranium in Figure 3. These specimens were prepared from salt solutions of uranium and lead as usual but only the uranium and lead were considered as contributing to the weight per cent. Next, a set of standards with equal weights of lead and uranium was prepared for uranium concentrations ranging from 0.1 gram per liter to 1 gram per liter. These standards give the calibration curve in Figure 4, which also shows the uranium calibration curve from Figure 1. Similar curves would result from standards with different relative amounts of lead. However, for less than 10% of lead by weight, no correction of the pure uranium calibration curve was found necessary.

In practice, when heavy element impurities were suspected, the procedure would be first to determine the impurity element from its x-ray spectrum, then, from a curve similar to Figure 3, determine the ratio of $UL\alpha$ to, say, $PbL\alpha$. This value would indicate the appropriate curve of the family in Figure 4. The uranium content in grams per liter would then be obtained directly from the counting rate. About 2 minutes would be required to determine the ratio, once the impurity element was known. Reading the data from Figures 3 and 4 would require less than 1 minute.

Rubidium is typical of elements in class 2. However, because of the different separation in wave length of the α and β lines of the K series as compared to the L series, none of these elements overlaps both the $UL\alpha$ and $UL\beta$. Therefore, if overlapping of one uranium L line should occur, the other uranium L line would be used to determine the uranium content.

Silver was chosen as typical of the elements in class 3. The second-order K line from 1 gram per liter of silver was too weak to be detected under the experimental conditions, and it was concluded that no interference was to be expected from such elements.

Other elements of common occurrence, such as iron, have no effect on the standard 100% uranium calibration curve of Figure 1. For example, with equal weights of iron added to each of the uranium standards, the measured uranium content was within 5% of that for the pure uranium standards.

The effect of impurities on the x-ray fluorescence analyses should be compared with their effect on x-ray absorption analyses in Bartlett's work (*1*). Bartlett found that, in x-ray absorption analysis, the interference from impurities increased with atomic number because of the increasing absorption coefficient. He prepared 1% solutions of various elements and determined the apparent uranium concentration which each would indicate in the absence of uranium. For example, a 1% solution of calcium indicated 1.5 grams per liter of uranium; a 1% solution of bromine indicated 5.0 grams per liter; a 1% solution of lead indicated 8.8 grams per liter. With the fluorescence method, no elements in such low concentration would have any effect on the uranium determination.

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Characterization of Benzene Ring Substitution by Infrared Spectra

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FOR a number of years the authors have been employing infrared spectra for characterizing benzene ring substitution. The spectral interval from 5 to 6 microns is peculiarly useful for this purpose. Aromatic compounds have usually a much more intense and a richer absorption pattern in this region than do other kinds of compounds. Fortunately, application of the method is not limited to a few benzene derivatives, but has almost universal validity for aromatic compounds. The method has stood the test of experience with a large number and variety of compounds. Inasmuch as the certainty of the method is now well established by experience, the authors are bringing it to the attention of chemists generally who will, they believe, find it of considerable value.

INFRARED SPECTRA

Figures 1, 2, 3, and 4 are collections of spectral fragments in the 5- to 6-micron interval of compounds which illustrate the method. Three examples of each typical pattern have been chosen, for the

The general problem of determining the type of substitution in aromatic compounds is greatly simplified by the information obtainable from infrared spectra as described in this paper. The spectra of benzene derivatives exhibit absorption patterns in the 5- to 6-micron region which are characteristic of the number and location of substituent groups on the benzene ring. These patterns, rather than specific frequencies, offer the key to the interpretation of the substituent configurations. The atomic constitution or chemical functionality of the substituent groups has only a minor influence on the appearance of the patterns. This new method represents a useful addition to the analytical methods that are available for use by the structural organic chemist.

most part, in order to illustrate the essential similarity of typical patterns for a given substituent configuration.

The spectra were obtained on a Baird Associates double-beam spectrophotometer with a sodium chloride prism. Materials liquid at room temperatures were examined in a 0.1-mm. cell. Solids were dissolved in carbon tetrachloride at a concentration of 100 mg. per ml., and spectra were obtained in a 1.0 mm. cell. Weak bands of carbon tetrachloride were eliminated with a compensating cell of suitable thickness. A few compounds of low solubility were examined in thicker cells.

These methods of obtaining the spectra correspond to the authors' customary procedures in obtaining spectra. In practice this is more convenient than attempting comparison on an equivalent molar basis.

Although a double-beam instrument was employed for the spectra reproduced, single-beam instruments have been found to be perfectly satisfactory. Water vapor background causes no difficulty under ordinary conditions of operation of single-beam infrared spectrometers.

ANALYTICAL APPLICATIONS

If no distinction is made among various kinds of organic groups, considering each only in its property as a substituent attached to the benzene ring, there are twelve different configurations: one monosubstituted, three disubstituted (ortho, meta, para), three trisubstituted (1,2,3-, 1,2,4-, 1,3,5-), three tetrasubstituted (1,2,3,4-, 1,2,3,5-, 1,2,4,5-), and one each of penta- and hexasubstituted. Each configuration is identified by means of a unique spectral pattern between 5 and 6 microns.

Figure 5 is a chart of these typical patterns. The chart represents generalized conceptions of spectra which, although they correspond exactly to no specific compounds, are immediately identified with the appropriate spectra of Figures 1, 2, and 3.

For mono-substituted compounds, Figure 5 shows that the characteristic pattern for the phenyl group consists of a series of four bands showing a diminishing relative intensity with increasing wave length. In Figure 4, spectral patterns of monosubstituted compounds are to be found which do not follow the rule, at least closely enough to be entirely typical. Fluorobenzene and the ethers illustrated appear to form almost a special class. Their patterns resemble somewhat the more typical phenyl pattern of Figure 5, but only if the entire spectrum were shifted to shorter wave lengths. Styrene, in Figure 4, illustrates a different variation. Inasmuch as styrene contains the vinyl group, an intense band characteristic of this group appears at about 11 microns. The enhanced intensity of the 5.5-micron band hence occurs as the result of a superposition of the overtone of the 11-micron band on the usual phenyl pattern. Nitrobenzene, also illustrated in Figure 4, deviates in a marked way from the typical. For these variant compounds, there is no danger that the kind of substitution will be mistaken from the 5- to 6-micron pattern inasmuch as the variant patterns do not resemble any of the typical patterns. It is a general rule that if the pattern of a compound deviates from the normal type for a specific configuration as given in Figure 5, such a pattern will resemble none of the typical patterns. This simply means that on rare occasions compounds are encountered for which no information about substitutional configuration can be obtained by the method expounded herein.

Di-, tri-, and tetrasubstituted benzenes appear to be very regular with respect to typical patterns. Occasionally a more marked splitting of doublet bands may occur than is generally typical, but such effects of wave length shifts of the individual bands making up the typical pattern are not troublesome.

o-Nitrotoluene has a typical ortho pattern (Figure 1), although nitrobenzene is atypical. Generally, groups causing atypical mono-patterns do not, except possibly rarely, cause deviations when the degree of substitution increases.

The penta- and hexasubstituted compounds cannot be uniquely characterized by this method. In the first place, only a few examples of such compounds have been available to this laboratory. Consequently, what are listed as typical patterns in Figure 5 are not statistically significant. In the second place, the last three patterns of Figure 5 show that uniqueness of pattern tends to be lost with increasing substitution. The patterns of the penta- and hexasubstituted compounds have been included merely for the sake of completeness and for intercomparison with the patterns of the less substituted compounds.

Compounds with substituent groups which contribute a fundamental band in the 5- to 6-micron interval will be expected to mask seriously the substitution patterns. In practice, carbonyl compounds make up the only significantly large class for which the effect of a fundamental (carbonyl bands) is notable. If the 5.5- to 6-micron portion of Figure 5 is masked off, a fair picture is given of the rather severe limitations placed on the applicability of the method to carbonyl compounds.

THEORETICAL APPLICATIONS

There seems little question from the background picture contributed by normal mode treatments of the vibrational spectra of benzene and substituted benzenes, from existing information, that the spectral patterns in the 5- to 6-micron region for aromatic compounds are made up of overtone and combination bands. Also, empirical examination of infrared spectra confirms this spectral pattern composition. (Naturally, this does not apply to carbonyl bands, etc., which appear for compounds containing substituent groups giving rise to fundamentals in this region.)

It is doubtful that any convincing assignment of particular fundamental frequencies could be made generally at present which, together with appropriate selection rules, would place the interpretation of these patterns on a satisfactory theoretical basis. However, examination of Figure 5 elicits some interesting speculations. On the whole, the patterns simplify as substitution increases, or, in other words, as the number of benzene hydrogens decreases. Also, the more symmetrical para- and 1,3,5-trisubstituted forms have simpler patterns, suggesting the operation of more rigorous selection rules—at least, in respect to effect on relative intensities. The entire picture indicates the importance of ring hydrogen frequencies, but also shows that ring skeletal frequencies play as important a role in contributing to the patterns by way of higher harmonics.

The examples of nitrobenzene and fluorobenzene, which deviate from type, indicate that several perturbing effects can be operative. The regularity found generally is little short of amazing. Retention of the relative intensity pattern is perhaps the most unexpected feature of all. It is not, therefore, surprising that certain groups—for example, the vinyl group in styrene—may

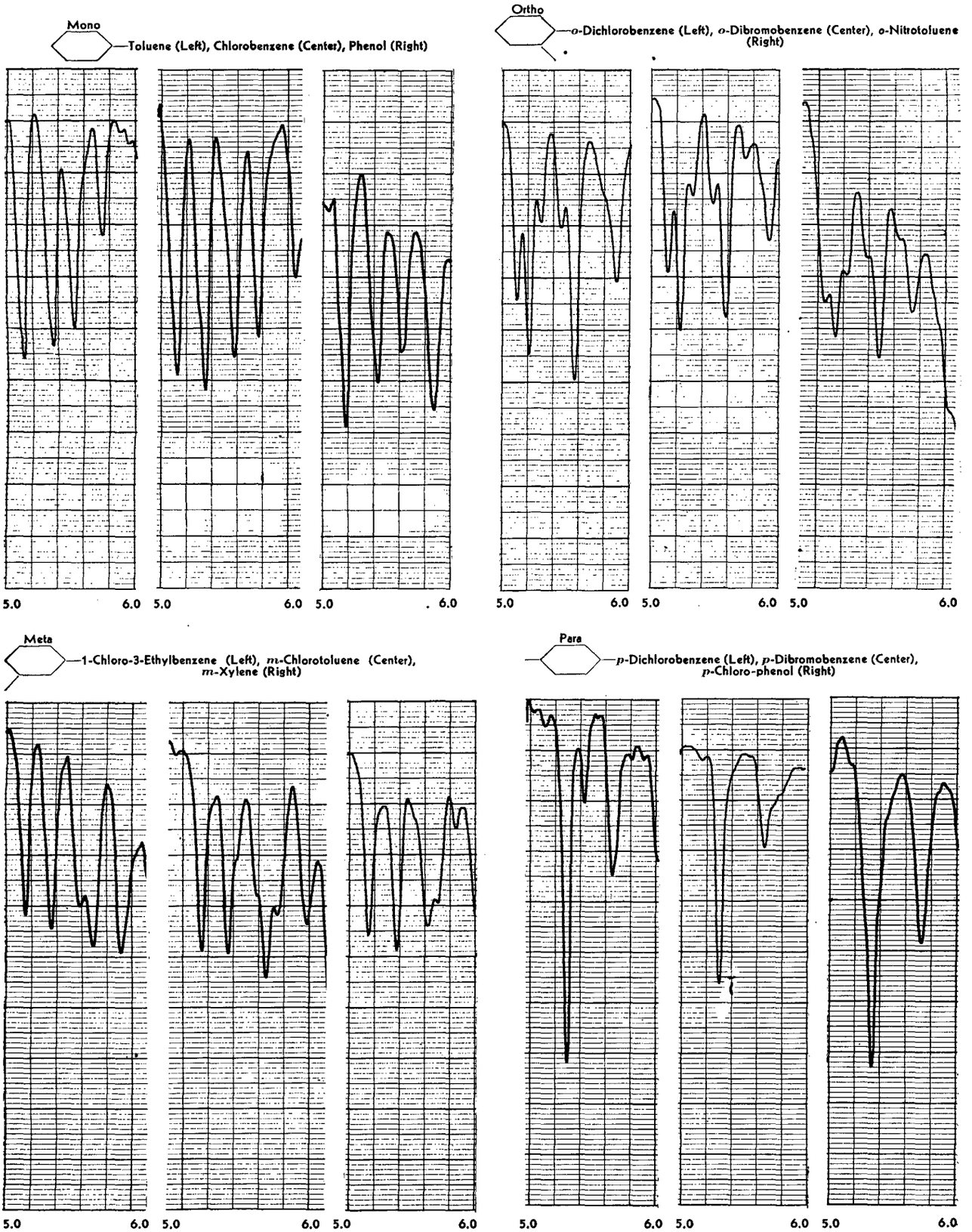

Figure 1. Infrared Spectra

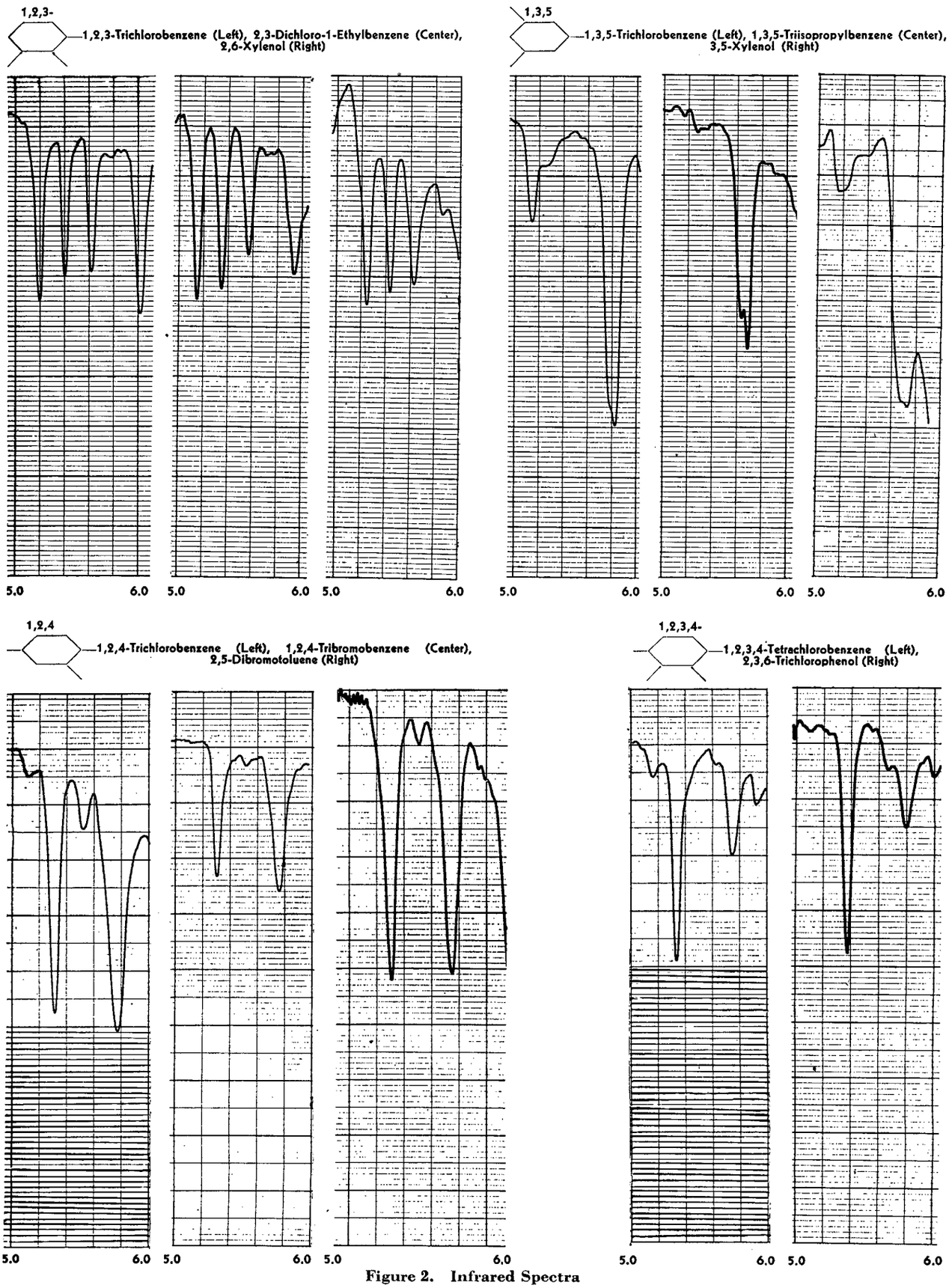

Figure 2. Infrared Spectra

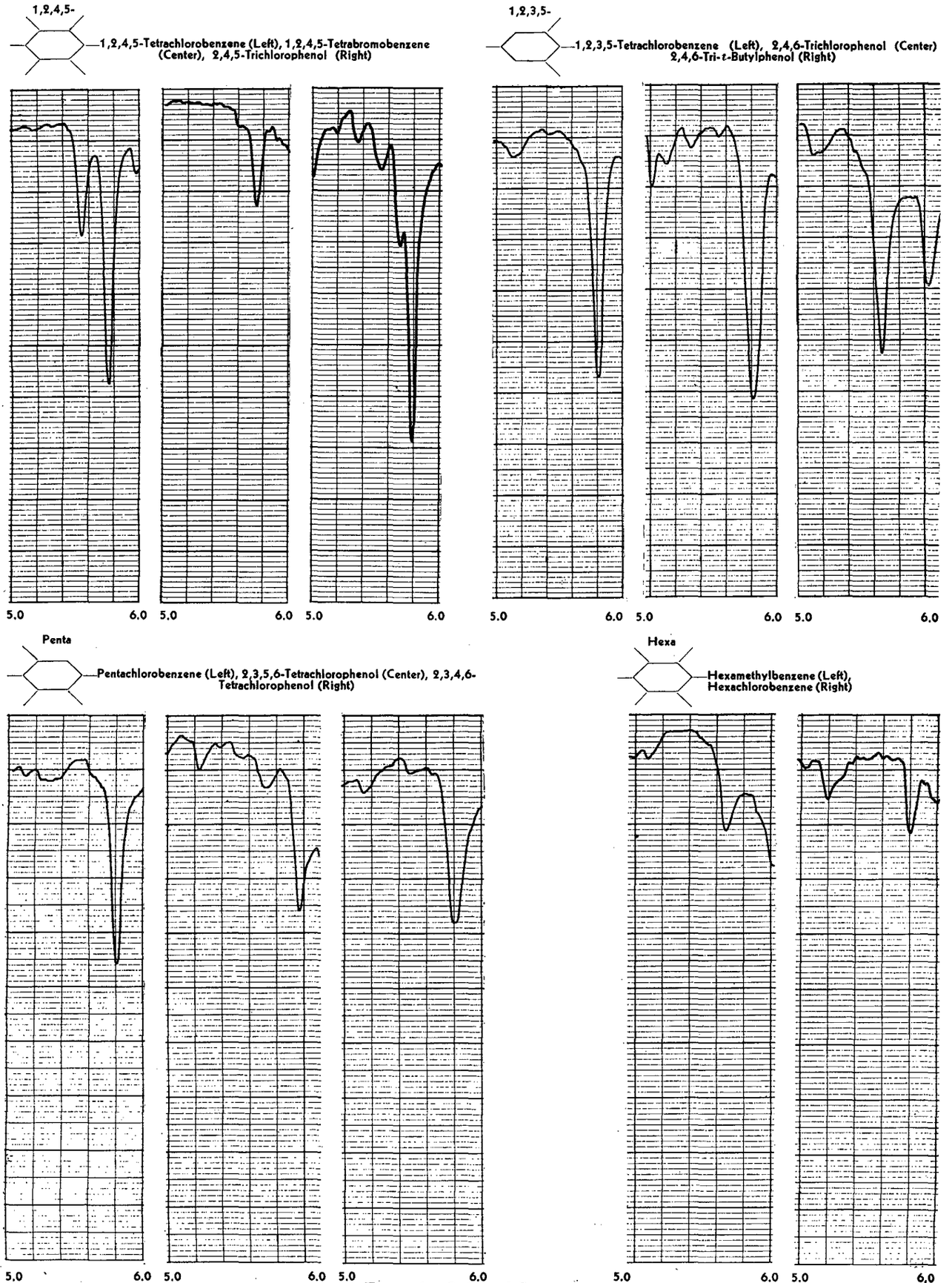

Figure 3. Infrared Spectra

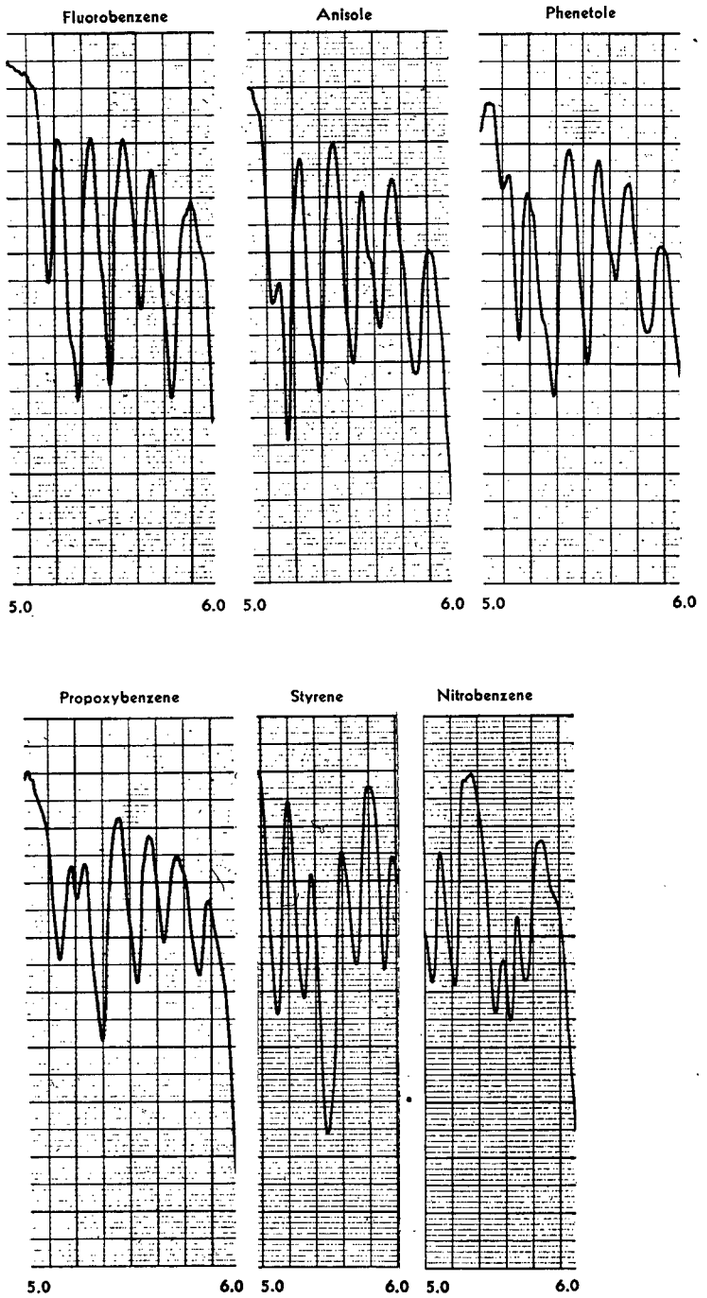

Figure 4. Infrared Spectra

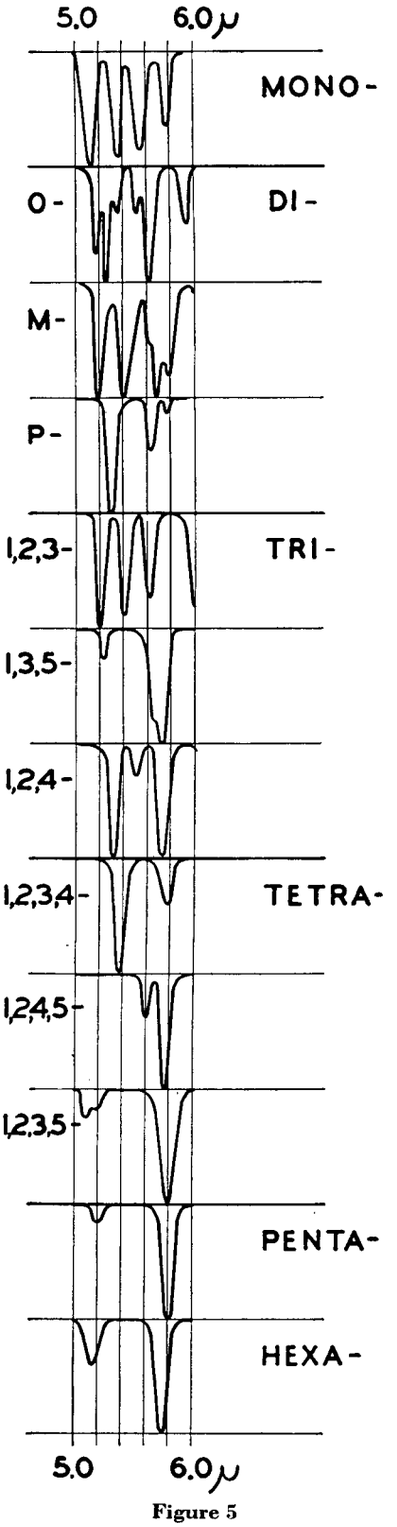

Figure 5

introduce superposing overtones on the typical pattern. Other substituent groups may at times be expected to introduce overtone or combination bands which disturb the typical patterns and which must be explained in less obvious ways (nitrobenzene). It is plausible to assume that certain groups may influence the electronic structure of the benzene ring so that normal mode irregularities would make themselves felt as disruptions of the typical patterns.

These typical patterns suggest an interesting side issue. Unless there are truly astounding coincidences, the 5- to 6-micron patterns must be explained as analogous transitions for similarly substituted benzenes. This affords an extra requirement on normal mode analyses of such compounds inasmuch as the assignments of fundamentals must lead to reasonable explanations of the typical patterns.

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Coordinate System for Electrophoresis

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The imposition of an orthogonal coordinate system on the scanning negatives obtained in electrophoresis studies permits a more convenient and accurate determination of the boundary positions and of the areas occupied by the shadows. The orthogonal coordinate system is produced on the negative by a combination of a millimeter scale etched on the face of the electrophoresis cell and one, normal to that on the cell, etched on a glass plate that is placed immediately before the unexposed plate in the plate holder. The advantage gained is the elimination of the microcomparator and of the planimeter measurements.

THE advantages experienced by the superposition of a coordinate system upon the scanning pattern obtained in electrophoretic work include a more convenient method than the one currently used for the determination of the position of the boundary, and a more precise and convenient method for the determination of the areas occupied by the shadows than the one involving the use of a planimeter. Certain errors are also reduced by the scheme presented in this paper, inasmuch as it is independent of optical defects in the camera, and the inaccuracies in determining the magnification factor are not important. The errors introduced because of mechanical movements between the camera and cell are also reduced.

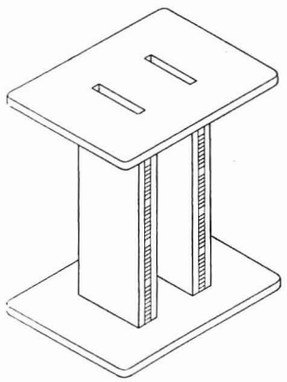

Figure 1. Millimeter Scale Engraved on 11-Ml. Tiselius-Type Electrophoresis Cell

A millimeter scale was engraved on the face of each leg of the center section of the Tiselius-type, 11-ml. electrophoresis cell, as shown in Figure 1. The method of engraving was entirely conventional. Molten paraffin was brushed over the face of each leg of the cell to give a smooth coating. The cell was then clamped to the table of a dividing engine and aligned with a machinist's square. The knife edge of the engine was drawn through the paraffin, making a mark on each leg of the cell. The cell was then advanced 1 mm. and an additional set of marks was made. This was continued throughout the entire length of the cell, except that every tenth line was omitted. The cell was then removed from the engine and inspected with a lens. A cotton swab, moistened with a 48% solution of hydrofluoric acid, was then brushed over the division marks, and after half a

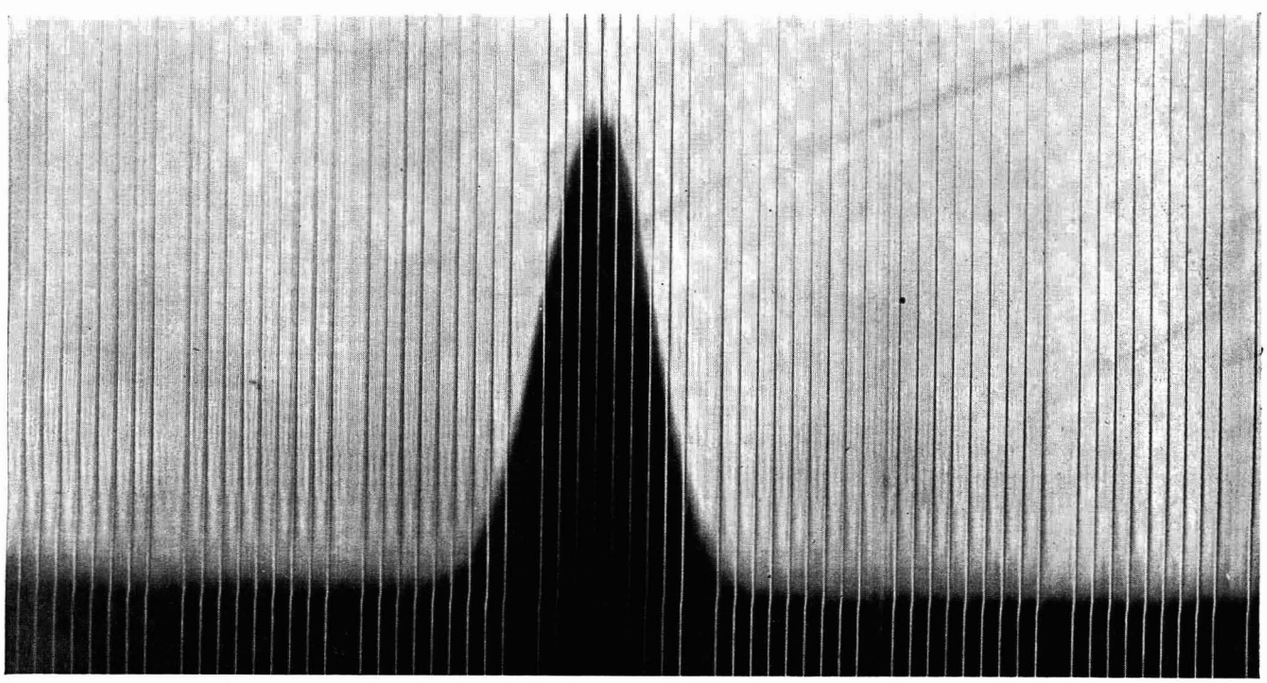

Figure 2. Pattern Obtained with Graduated Cell Using Longsworth Scanning Technique

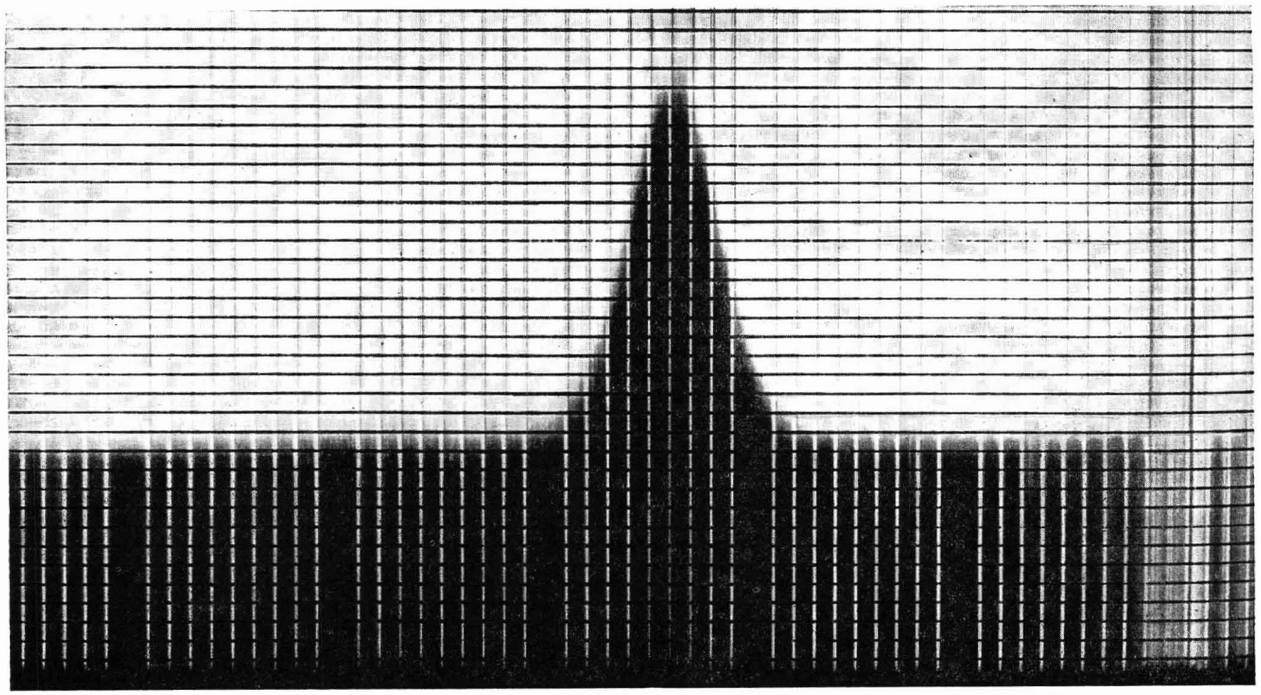

Figure 3. Superposition of Scanning Pattern upon Coordinate System Using Longsworth Technique

minute, the cell was thoroughly washed with water. The paraffin was subsequently removed with benzene. Finer graduations may be ruled with a diamond (*2*). The scale engraved on the face of the cell was checked with a microcomparator and was found to be accurate to ±0.003 mm.

The scale lines appearing in the photographs taken during the normal course of an electrophoresis experiment extend over the length of the plate. Because of the groove shape of the scale lines, the light passing through the lower portion of the groove is refracted upward, whereas the light passing through the upper portion is refracted downward. The effect is that in the schlieren method the dark part of the image of the scale lines is emphasized in the light areas of the photograph and the light part of the image is emphasized in the dark part of the photograph. One

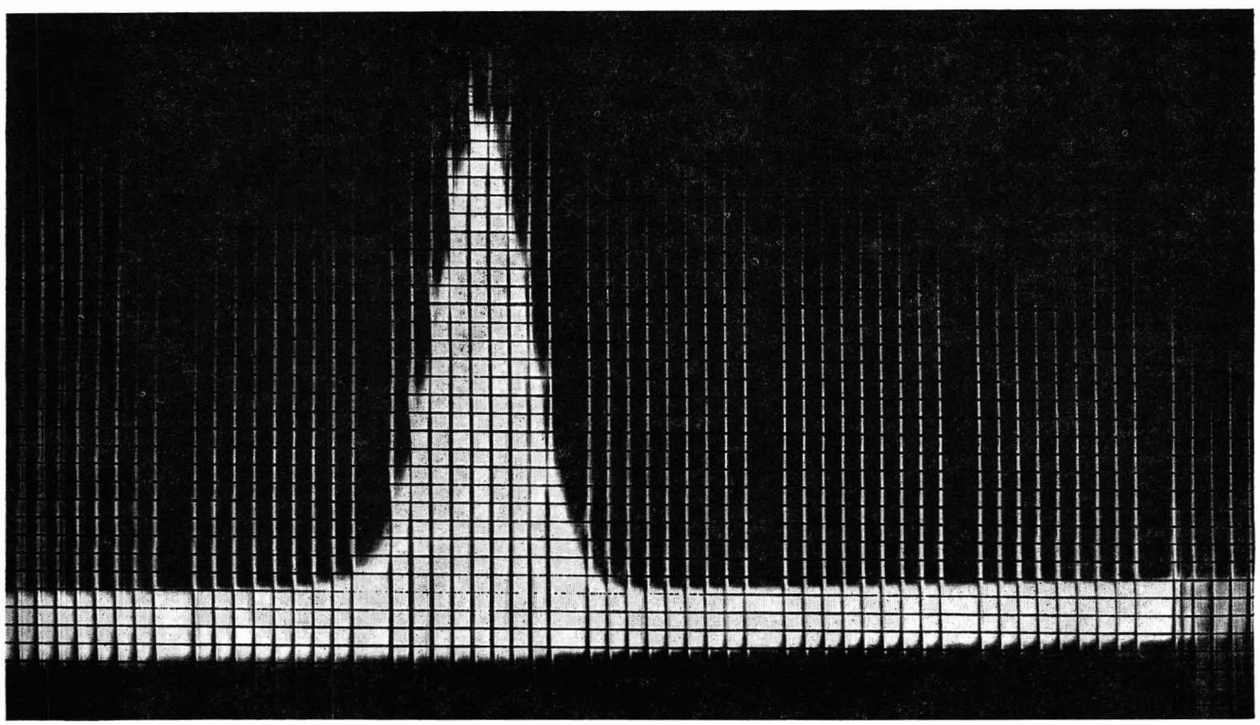

Figure 4. Philpot Scanning Pattern

Mask at focal plane before camera lens above, rather than below, light image graduated cell and millimeter plate used

obtains an illusion that the lines are displaced at the junction of the light and dark parts of the photograph. Actually, there is no displacement of the lines except in the schlieren shadow, as may be seen if the inner edges of the light and dark portions of the line images are considered. Measurements should be made from these inner edges.

In Figure 2 the scale lines appearing in the schlieren shadows are displaced in the direction of increasing concentration gradient in the cell, but the displacement of the lines is reduced to a minimum by virtue of the nearness of the scale to the solution in the cell. The displacement of the scale lines in the photograph, however, is of no importance in connection with the determination of the rate of boundary movement. When a particular boundary has moved five divisions, it has moved 5 mm.

In the actual operation, the cell is placed in the bath with the scale lines toward the camera. A photograph is taken at zero time after the boundaries have been moved into the center section, and the knife edge has been placed in a position to give the narrowest shadow possible. This operation records the original position of the boundary. The electrophoresis is then continued in the normal manner, and all measurements of the boundary positions observed in the photographs taken subsequently are referred to the original photograph. The number of divisions through which the boundary has moved is a measure of the distance moved, and it is independent of the camera system. The accuracy of any determination may be improved by interpolation with the aid of a microcomparator.

The scale lines may be used in determining the area occupied by the shadows in the electrophoresis pattern. The sum of the lengths of the lines in any given shadow, multiplied by the magnification factor of the camera system, is equal to the area. The determination of the lengths of the lines is greatly facilitated by superimposing the pattern upon a coordinate system. The engravings on the face of the cell effect the production of a scale in one direction in the photograph. A scale, normal to the first, is produced by placing a millimeter scale immediately before the unexposed plate in the plate holder. The scale used in this work was etched on a 9 × 12 cm. glass plate with hydrofluoric acid by the procedure employed with the cell. Thus, a coordinate system, as well as the scanning pattern, appears on the negative, as illustrated in Figure 3.

The patterns presented in Figure 3 were obtained using the Longsworth scanning technique (*1*), but similar results are obtained with the technique for obtaining electrophoresis patterns described by Philpot (*3*), and by Svensson (*4*). The pattern shown in Figure 4 was obtained by the Philpot technique. The graduated cell, together with the millimeter scale in the plate holder, were used in the manner indicated in the Longsworth scanning procedure. In this instance, however, the mask was set at 45° with the horizontal, and was placed above, rather than below, the light image immediately before the camera lens. Thus the pattern was reversed—that is, the pattern was light and the background dark.

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Fluorometric Determination of 2-Nitronaphthalene

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This work was undertaken to establish a rapid method for the estimation of small amounts of 2-nitronaphthalene in crude and refined 1-nitronaphthalene. The method depends upon sulfonation, reduction, and measurement of the blue fluorescent light intensity produced by the beta isomer when irradiated with ultraviolet light of 365 mμ wave length, using suitable filters to exclude the dull green light produced by the alpha. The procedure will detect 0.05% beta and covers the range up to 5% with a precision of about ±3% of the amount present. Naphthalene and dinitronaphthalene normally present in crude material do not interfere. A single determination can be run in an hour.

THERE are many references in the literature to the fluorescent properties of naphthalene derivatives (*1*), but none of them appeared to apply directly to the estimation of small amounts of 2-nitronaphthalene (beta) in refined and crude 1-nitronaphthalene (alpha). Preliminary tests showed that neither of these compounds fluoresces appreciably under light of 365 mμ wave length. It is well known, however, that under these conditions the corresponding amines do fluoresce, the beta much more strongly than the alpha. The investigation was, therefore, conducted along this line.

APPARATUS

A Coleman Model 14 Universal spectrophotometer with fluorometer attachment, operated through a constant voltage transformer, was used in the experimental work. It was provided with a Coleman UV-1 light filter for the mercury lamp and Corning Nos. 5433 and 3850 filters for the photoelectric cell. Fluorescence measurements were taken from a 20 × 40 mm. cuvette. To minimize undesired reflection of stray light, the entire surface of the cuvette carrier was roughened with emery cloth and it, as well as the inside of the light compartment of the spectrophotometer, was painted with a flat black paint.

REAGENTS

Monohydrate, 99 to 100% sulfuric acid.
Oleum, 58 to 60% free sulfur trioxide.
Charcoal, Nuchar W. Darco D-51 was tried and found unsatisfactory.
Wash water, 2 ml. of 95% sulfuric acid diluted to 1 liter.
Sodium acetate, 100 grams of sodium acetate trihydrate dissolved in 100 ml. of water.

STANDARD REFERENCE SOLUTION

Sulfonate 0.100 gram of purified 2-naphthylamine as directed below for the analysis of samples and dilute to 500 ml. This solution should be clear and colorless without charcoal treatment or filtration. Dilute a 5-ml. aliquot to 500 ml. Both solutions are stable for at least a month. Prepare the final reference solution daily as needed by diluting 5 ml. of the second solution plus 1 ml. of the sodium acetate buffer to 100 ml.

PROCEDURE

Weigh 0.100 gram of sample into a 6-inch test tube, add 1 ml. of monohydrate, heat for about a half minute in a water bath at 50° C. to effect complete solution, cool to 25° in a water bath, and while swirling the tube vigorously add rapidly 2 ml. of oleum.

Set the tube in the steam bath for 5 minutes, cool in ice, and add cautiously 2 to 3 ml. of water. Pour the reaction mass into a 100-ml. beaker containing 25 ml. of water, dilute with washings from the tube to 50 ml., add 0.5 gram of charcoal, boil 5 minutes, and filter hot with suction through a 2.5-inch Büchner funnel provided with two No. 5 Whatman filter papers. Wash with four 40-ml. portions of boiling acid wash water, transfer to a 250-ml. volumetric flask, cool, and dilute to volume. The solution should be a very light yellow color, but is sometimes brownish owing to slight peptization during the hot washing. Any such color must be removed by adding about 0.5 gram of Filter-Cel and refiltering by gravity enough of the solution for subsequent operations.

Transfer a 20-ml. aliquot to a 100-ml. volumetric flask, add 0.5 gram of zinc dust, heat on the steam bath for 5 minutes (final temperature about 75°), cool, dilute to 80 ml., add 4 ml. of sodium acetate solution, dilute to the mark, and filter through a folded paper into a substantially dry flask. The solution at this stage should be a very pale yellow color and the pH about 4.6. Measure 30 ml. of the reference solution into one cuvette and 30 ml. of the sample solution into the other. Mount the cuvettes in the carriage so that a narrow face is adjacent to the photoelectric cell and read the fluorescent light intensity of both the sample and standard solutions. Obtain the corresponding percentage of the beta isomer from the calibration curve.

EXPERIMENTAL

The materials used in this work were purified as follows:

2-Nitronaphthalene. One kilogram of crude 1-nitronaphthalene was crystallized from about twice its weight of methanol, the crystals were filtered off, and the methanol was evaporated from the filtrate. The residue was then distilled slowly through a small, well insulated fractionating column at a pressure of about 1 mm. of mercury. The first 50 to 60% of the distillate was discarded and the next 30% was repeatedly crystallized from methanol to a constant melting point of 76.8° C.

1-Nitronaphthalene. Some of the 1-nitronaphthalene crystals from the above operation were recrystallized from methanol or benzene until a freezing point of 56.4° C. was reached.

2-Naphthylamine. Commercial material was crystallized once from water as the hydrochloride, converted to the base, and crystallized from ethyl alcohol to a freezing point of 110.0° C.

1-Naphthylamine was purified through the same procedure as the beta isomer. Final freezing point, 48.7° C.

Solutions containing 2 micrograms per ml. of 2-naphthylamine were prepared in water and thirty-odd organic solvents and the relative fluorescence intensity was measured. The variation was great, from zero in cyclohexene or *n*-butylphthalate to 66 for dimethylformamide, with acetone, the lower alcohols, glycols, and water intermediate (12 to 40). The fluorescence in all cases was a blue-violet color. A solution of 1-naphthylamine in dimethylformamide gave a blue color of considerably lower intensity, but no light filters were found that would permit reading the beta color intensity in the presence of the alpha with the necessary degree of precision.

Various derivatives of the amines were then considered. Condensation products with aldehydes or carbon disulfide also yielded fluorescence colors too close together in the spectrum to be effectively separated, and the same was true of the naphthols. Acetylation reversed colors, making the alpha violet and the beta blue, but again the difference was not enough for quantitative purposes.

SULFONATION

Sulfonation was next explored. When 1- and 2-naphthylamines were sulfonated and diluted and the excess sulfuric acid was neutralized, both solutions fluoresced strongly in blue colors not distinguishable by the eye. When the nitro compounds were sulfonated, reduced, and neutralized, the alpha isomer was found to yield a dull green fluorescence and the beta a bright blue. These colors appeared sufficiently separated in the spectrum to afford quantitative estimation, as the peak of the alpha curve, as determined with a modified Beckman spectrophotometer, comes at about 510 mμ and the beta at 450 mμ. Doubtless the difference in the fluorescence colors produced by the alpha isomer when sulfonated before and after reduction of the nitro group results from different orientation of the sulfonic acid groups.

A study of light filters was next in order. The Coleman UV-1 (Corning 5874) was found satisfactory for the isolation of the 365 mμ line of the mercury vapor lamp, but the most suitable combination for separating the blue fluorescence of the beta from the green of the alpha was a more difficult problem. Many single filters and combinations of the Corning Glass and Eastman (Wratten) gelatin series were tried in addition to several interference filters. Three combinations were eventually found to function satisfactorily: Corning 5433 and 3850 or Eastman 2B plus either 34 or 34A. The latter two combinations give slightly greater distinction between the colors produced by pure alpha and alpha plus a small amount (up to 4%) of beta, but they are less stable than glass filters and their total transmission is inconveniently low. The Corning filters were therefore adopted, the function of the 3850 being to shut out stray 365 mμ light and of the 5433 to transmit the blue light from beta while absorbing most of the green alpha.

The intensity of fluorescence of solutions prepared from either isomer varies widely with the pH, being very feeble in the presence of mineral acids or strong alkalies and of maximum value near the neutral point when buffered with ammonia (pH 8) or sodium acetate–acetic acid (pH 4.6). The latter was chosen as being more easily controllable.

The conditions of sulfonation were next investigated. When sulfonated with monohydrate, the fluorescence produced by pure alpha was so strong as to mask that of a small amount of beta, but as the strength of the sulfonating acid was increased, the intensity of the alpha fluorescence progressively decreased, while the beta remained substantially constant. The diminution in alpha intensity appears to be due primarily to decomposition of 1-nitronaphthalene with increasing severity of sulfonation conditions, particularly concentration of free sulfur trioxide. Increasing the temperature from 25° to 150° C. had a similar effect but to a much lesser degree. Complete solution of the sample in either 95 or 100% sulfuric acid prior to addition of oleum was found essential to concordant results. Substitution of chlorosulfonic acid for oleum as the sulfonating agent produced high alpha values with no compensating advantage.

The most favorable conditions of sulfonation found were solution of the sample (0.1 gram) in 1 ml. of 99 to 100% sulfuric acid (this acid strength producing somewhat less alpha color than 95%), addition of 2 ml. of 58 to 60% oleum, and reaction for 5 minutes in the steam bath. In effecting solution, temperatures above about 50° C. produce some initial sulfonation that seems partially to stabilize the 1-nitronaphthalene, giving high and erratic fluorescence intensities. Cooling to room temperature before addition of the oleum yields more consistent results than higher temperatures. The rate of addition is not important, but the tube should be swirled vigorously to prevent local high concentrations. The time of heating is not critical, but should not be prolonged much beyond 5 minutes.

CLARIFICATION

Because of decomposition, when the sulfonation mass is drowned a considerable amount of tarry material separates, and the solution is a dark brown color. Both the precipitate and dissolved color must be removed at some stage before the fluorescence readings are taken, but there is a choice between clarification before or after reduction of the nitrosulfonates. In the early part of the work, the reduction was conducted immediately, charcoal added, and the mixture filtered hot, yielding solutions varying from practically colorless to distinctly yellow. Upon subsequent adjustment of the pH with either ammonia or sodium acetate, the yellow color was intensified. Because the intensity of the fluorescent light evolved is a function of the concentration

Table I. Light Intensities of Type AH-3 Lamps

Lamp Series No.	Fluorometer Reading after Lamp Had Burned 10 min.	20 min.	30 min.
8	61.1	60.0	58.8
13	96.5	94.0	92.8
14	77.3	76.0	74.9
68	30.8	34.1	35.8

Table II. Effect of Ultraviolet Light Intensity

(Fluorescence readings)

Sample No.	Lamp 8 Std.	Sample	Ratio	% β	Lamp 13 Std.	Sample	Ratio	% β	Lamp 68 Std.	Sample	Ratio	% β
1	57.5	355	6.17	3.74	16.5	100.5	6.09	3.67	42.0	263	6.26	3.79
13	58.0	356	6.14	3.71	16.5	99.5	6.03	3.63	41.2	249	6.04	3.64
24	57.3	55.3	0.97	0.11	91.8	86.3	0.93	0.08	41.8	40.8	0.97	0.11
26	57.3	98.9	1.73	0.62	95.8	148	1.55	0.50	40.2	64.8	1.61	0.54

of the 365 mμ light permeating the solution, any color that strongly absorbs ultraviolet light is objectionable. A yellow color also absorbs some of the blue beta fluorescence, thus reducing the measured intensity in two ways. For these reasons, either colorless or uniformly colored solutions are required for reproducible fluorescence values. With a view to eliminating or minimizing this extraneous color, reducing agents such as sulfite, thiosulfate, or dithionite (hydrosulfite, $Na_2S_2O_4$) were added before or after filtration. The color was thereby diminished, but highly erratic fluorescence readings were obtained. It was later found that treatment with charcoal and filtration before reduction yields much more consistent results and that under these conditions the use of a color suppressor is not only unnecessary but detrimental.

Various adsorbing agents were tested in the clarification step. These included activated silica, alumina, and two brands of powdered charcoal, Darco D-51 and Nuchar W, of which only the charcoals were effective. It was found, however, that these adsorb not only the colored materials but also a considerable proportion of the nitronaphthalene sulfonic acids and so strongly that a large amount of hot wash is required for substantially complete extraction. The efficiency of extraction depends upon the acidity of the wash; if the acid concentration is too low, some of the colored matter is peptized and passes through; if too acid, extraction is not quantitative. The concentration of 2 ml. of 95% sulfuric per liter finally chosen is on the borderline and sometimes washes through some color. It is for this reason that a second filtration, after the addition of Filter-Cel, is recommended. Of the two charcoals tested, Nuchar W is preferred because the Darco adsorbs so strongly that the sulfonates cannot be washed out sufficiently with a convenient quantity of solution.

REDUCTION

Aliquots of the nitrosulfonate filtrates were reduced with magnesium, aluminum, tin, stannous chloride, and sodium hydrosulfite (after making alkaline), but none of these offered any advantage over the zinc dust previously used. The reduction is not critical with respect to either time or temperature, identical results being obtained at the boiling point or after 5 minutes on the steam bath where the maximum temperature attained is about 75° C. Filtration after cooling, buffering (pH 4.6), and diluting to final volume is necessary, for although the supernatant liquid may appear clear, it sometimes contains traces of suspended zinc that lead to false fluorescence values.

FLUOROMETRY

Throughout the foregoing developmental work, the beta value of a solution prepared from a known mixture of 1- and 2-nitronaphthalenes was considered to be proportional to the difference between the fluorescence reading of this solution and one similarly processed from pure alpha. Now that the principal chemical causes of variation had been eliminated, there still remained disparities of as much as 10% in duplicate determinations. This was found to be due to one or both of two causes:

1. Gradual decrease in intensity of the light emitted by the high pressure mercury vapor light. Thus, readings taken at intervals on a stable fluorescent solution dropped gradually from an initial value of 93 5 minutes after the light was turned on to 84 after it had been in continuous operation for 7 hours.
2. A temperature effect in the galvanometer system and photoelectric cell. This was not due to change in temperature of the solution, for it was kept constant and subsequent experiments showed that the fluorescence intensity of a 2-naphthylamine sulfonic acid solution does not vary significantly between 20° and 50° C.

To compensate for these errors, a stable ultimate standard was sought in terms of which the beta values could be calculated as a ratio rather than a difference. If a reading were taken upon such a standard solution for each reading of a sample, the ratio of the two would be independent of changes in the ultraviolet light source or sensitivity of the galvanometer.

Five 0.100-gram portions of purified 2-naphthylamine were sulfonated through the previously established procedure, and diluted to 500 ml., giving clear and colorless solutions without charcoal treatment or filtration. Five milliliters of these were again diluted to 500 ml. and finally 5 ml. plus 1 ml. of the acetate buffer solution were diluted to 100 ml., yielding solutions containing the equivalent of 0.100 microgram of amine per ml. These gave readings of 59.7, 60.3, 61.7, 60.1, and 61.2 on the potentiometer scale, indicating a high degree of consistency in the sulfonation. The stability of these stock solutions (before addition of buffer and final dilution) was established by using them over a period of several weeks in the calibration experiments described below.

The use of this standard sulfonated 2-naphthylamine solution makes possible the calibration of the fluorometer without the necessity for the preparation of pure 2-nitronaphthalene. The calibration data presented later give the 2-nitronaphthalene values corresponding to various ratios of equivalent nitro readings to standard amine readings. These ratios should be constant for all fluorometers, irrespective of intensity of activating light or galvanometer sensitivity. This contention is substantiated in the following experiments.

The Coleman fluorometer uses the Hanovia Type AH-3 mercury vapor lamp as the source of ultraviolet light. Four of these bulbs were mounted in the instrument successively and fluorescence readings of a standard sulfonated 2-naphthylamine solution taken at intervals after the light was turned on. The data, in terms of potentiometer scale readings, show wide variation in light intensity.

A calibration curve, based on mixtures of pure 1- and 2-nitronaphthalene, was prepared using bulb 8 in the instrument. Samples of commercial 1-nitronaphthalene, both crude and refined, were then analyzed using successively bulbs 8, 13, and 68. In Table II, the fluorescence readings are given in terms of potentiometer scale, taken directly for the standard solution and refined samples and calculated from galvanometer scale readings, in the case of crudes, by multiplying by 3.41, the numerical relation found, for this particular instrument, between the two scales.

The readings with lamp 68 were taken after it had been in operation for over an hour, which accounts for the higher standard values than given for it in Table I. This lamp was abnormal in that its intensity increased for a considerable period, apparently reached a maximum, and then decreased, for after operating for about 16 hours its value had returned to 35.3. A modified procedure was required with lamp 13 for crude samples high in beta. Because of its high light intensity, these

Table III. Calibration Data for Fluorescence Readings, Potentiometer Scale

Beta, %	Series A			Series B			Series C		
	Std.	Sample	Ratio	Std.	Sample	Ratio	Std.	Sample	Ratio
0.00	...	...	...	58.7	47.5	0.81	58.2	50.2	0.86
0.25	60.3	69.1	1.27	58.7	76.0	1.29	57.2	65.0	1.14
0.50	60.3	93.3	1.60	58.7	94.3	1.61	57.7	80.9	1.40
0.75	60.0	114	1.95	58.8	117	2.00	57.8	105	1.82
1.00	60.0	134	2.29	58.8	138	2.35	57.5	129	2.24
2.00	59.5	224	3.83	58.0	226	3.90	57.7	217	3.76
3.00	59.0	297	5.08	58.0	303	5.22	57.5	295	5.13
4.00	58.5	385	6.58	58.0	384	6.62	56.0	362	6.46

Table IV. Effects of Impurities and Charcoal Type

Impurity Added	Charcoal Brand	Fluorometer Reading			Beta Found, %
		Std.	Sample	Ratio	
None	Darco D-51	58.5	354	6.05	3.64
Naphthalene	Nuchar W	58.5	387	6.64	4.06
DNN[a]	Nuchar W	58.5	377	6.44	3.92
Naphthalene and DNN	Nuchar W	58.5	375	6.41	3.90

[a] Dinitronaphthalene.

Table V. Analysis of Samples

[Fluorescence readings (potentiometer scale)]

Sample	Series A				Series B				Series C			
	Std.	Sample	Ratio	% β	Std.	Sample	Ratio	% β	Std.	Sample	Ratio	% β
Cr. 1	56.6	354	6.26	3.79	57.5	355	6.17	3.73	55.8	338	6.05	3.65
Cr. 13	57.0	346	6.07	3.66	58.0	356	6.14	3.71	57.2	358	6.26	3.79
Cr. 18	56.8	347	6.11	3.69	57.5	353	6.14	3.71	56.8	338	5.95	3.59
Cr. 21	56.7	325	5.73	3.42	57.5	339	5.89	3.53	56.8	319	5.62	3.34
Ref. 16	56.8	73.2	1.29	0.32	58.0	70.8	1.22	0.28	57.5	70.2	1.22	0.28
Ref. 24	56.4	57.8	1.02	0.14	57.3	55.3	0.96	0.10	57.1	53.2	0.93	0.08
Ref. 25	56.8	58.2	1.02	0.14	57.3	55.8	0.97	0.11	56.2	52.5	0.93	0.08
Ref. 26	57.0	96.9	1.70	0.60	57.3	98.9	1.72	0.62	56.1	88.3	1.57	0.52

samples gave off-the-scale values with the galvanometer at its customary highest sensitivity setting. The sensitivity was therefore reduced until suitable scale readings were obtained and then both standard and sample solutions were read at this setting on the galvanometer scale, the potentiometer being of advantage only for samples low in beta. It might be supposed that the same purpose can be accomplished by using smaller aliquots of both standard and sample solutions. This is not the case, however, because the standard solution is colorless and further dilution does not appreciably affect its absorption of ultraviolet light, but the sample solution because of its slightly yellow color does decrease in absorbancy with dilution. For this reason, determinations from 10-ml. aliquots gave beta values of 4.74 and 4.71%, respectively, for samples 1 and 13. The data of Table II establish that the ratio method of calculating results is independent of variations in activating light intensity and also of fluctuations in galvanometer and photocell sensitivity. All of the fluorescent solutions encountered in this work, both standard and sample, exhibit fatigue upon prolonged exposure to 365 mμ light. Readings should, therefore, be taken as quickly as possible.

CALIBRATION AND STABILITY OF SOLUTIONS

Using the procedure as detailed above, three series of runs were made on different days by two operators working independently, upon known mixtures of purified 1- and 2-nitronaphthalene (Table III).

Calibration curves were plotted from the averages of these values and used in the analysis of samples, discussed below. The curves are linear. The final solutions from Series A were kept overnight and the fluorescence readings again taken. The results, although concordant among themselves, averaged 10% lower than the above. Fresh aliquots of the acid filtrates of the same series were then processed and ratios practically identical with the above were obtained. Subsequent experiments showed that the buffered solutions are stable for 3 hours or more.

EFFECTS OF IMPURITIES AND CHARCOAL TYPE

Assuming efficient alkaline washing to remove nitronaphthols in the manufacturing process, the only impurities present in crude 1-nitronaphthalene in appreciable amounts are naphthalene and dinitronaphthalene, neither of which normally exceeds 2%. Runs were made upon 0.100-gram portions of a standard nitro sample containing 4.0% beta to which 5 mg. of naphthalene and/or dinitronaphthalene (mixed isomers) were added. Two types of charcoal for the purification were also tested. Typical results are set forth in Table IV.

ANALYSIS OF SAMPLES

The analytical procedure was applied to four samples each of crude and refined 1-nitronaphthalene (Table V). These analyses also were made on different days by two operators.

The charcoal filter cakes from some of these crudes were given an additional extraction by boiling for 15 minutes with 70 ml. of acid wash water, but the recovery of equivalent 2-nitronaphthalene was negligible. The standard washing procedure therefore effects substantially complete extraction.

FLUORESCENCE INTENSITIES

The specific intensities of fluorescence of the various materials dealt with in the course of this investigation were determined. One-tenth gram portions were treated as indicated, and appropriately diluted, the pH was adjusted to about 4.6 with acetate buffer, and the fluorescence was read, using the No. 8 mercury lamp in the fluorometer and only the 3850 filter to exclude stray ultraviolet light from the photoelectric cell (Table VI).

The slow sulfonation of the 1-nitronaphthalene was accomplished by heating in the steam bath for 10 minutes with 1 ml. of monohydrate, adding 1 ml. of 30% oleum, and again heating, then adding 1 ml. of 58% oleum and finally heating 10 minutes.

Table VI. Relative Fluorescence Intensities

Material	Treatment	Concn., γ/Ml.	Fluorometer Reading	Concn. for Readings of 100 γ/Ml.	Fluorometer Color
α-Amine	Dissolved in acetic acid	10	65.8	15.2	Dk. blue
β-Amine	Dissolved in acetic acid	4	63.5	6.3	Violet
α-Amine	Std. sulfonation	1	82.0	1.22	Lt. blue
β-Amine	Std. sulfonation	0.1	44.5	0.22	Lt. blue
α-Nitro	Std. sulfonation and reduction	80	71.3	112	Green
α-Nitro	Slow sulfonation and reduction	3.2	67.5	4.7	Green
β-Nitro	Std. sulfonation and reduction	0.2	42.3	0.47	Lt. blue

Very little decomposition was noted, which is substantiated by the above figures that indicate something over 95% decomposition in the standard sulfonation procedure. Without this high degree of decomposition, the estimation of small amounts of beta would be impossible. The effect of different orientation of the sulfonic groups is clearly shown by the pronounced difference in specific fluorescence of sulfonated 1-naphthylamine and sulfonated and reduced 1-nitronaphthalene. A similar effect is noted for the beta isomer, with the further inference that in this case there is little if any decomposition in the standard sulfonation. The great increase in fluorescence as the result of sulfonation of both isomers is also of interest.

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Separation and Determination of Ammonia in Methylamine Mixtures

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In connection with process control work it was necessary to determine ammonia accurately in mixtures of mono-, di-, and trimethylamine at all levels of concentration above a few tenths of a per cent. Existing methods based on use of mercuric oxide or sodium cobaltinitrite were found to be unreliable. Yellow mercuric oxide samples vary markedly in their ability to remove ammonia from solutions containing methylamines, and in the presence of ammonia the reagent adsorbs considerable amounts of amines. The sodium cobaltinitrite precipitation method has been modified and improved, so that ammonia in methylamines in concentrations above 5% can be determined with an average recovery of at least 99.7% and a standard deviation of less than 0.67% relative. At concentrations below 5% the error may approach 0.25% absolute. Methanol and water do not interfere. The modified procedure permits an accurate chemical determination of ammonia in the presence of amines.

IN THE production of methylamines, the mixtures obtained before purification contain from 10 to 60% ammonia with varying amounts of monomethylamine, dimethylamine, trimethylamine, water, and methanol. As part of an investigation of analytical methods applicable to such mixtures procedures for the determination of ammonia were studied.

Because of the similarity of physical properties and the existence of azeotropes, the separation of ammonia from methylamines by distillation is not practical in the control laboratory. The earliest chemical methods were based on the separation of the hydrochlorides, sulfates, platinochlorides, and periodides of the bases in suitable solvents. These methods have been criticized on the ground that separations were incomplete (*1*, *5*, *8*). More recently Egly and Smith have described a method based on the relative insolubility of ammonium chloride in butanol, but the method is inconvenient and necessitates an empirical correction for the solubility of ammonium chloride (*3*).

One of the classic procedures is that of Francois, which is based on the removal of ammonia from aqueous alkaline solutions on slightly soluble mercuric oxide, followed by distillation of the filtrate into standard acid in order to determine the residual volatile bases (*5*). The same principle has been employed by others (*4*, *10*, *13*), but a number of workers (*2*, *8*, *9*) have reported that mercuric oxide retains small amounts of monomethylamine and have applied the method of Leone (*8*) in which the ammonia is precipitated with sodium cobaltinitrite. Leone, who did not supply extensive data on methylamines, determined the amines in the filtrate. However, the determination of ammonia in the cobaltinitrite precipitate has been found more advantageous (*9*). In a modification reported more recently (*12*) methyl Cellosolve is used in the precipitating solution, apparently to decrease the solubility of ammonia as sodium diammonium cobaltinitrite. However, the authors report that their procedure is not applicable in the presence of methylamines, because of the insolubility of the methylammonium cobaltinitrites in the reagent used. The method of Mizuch and Savchenko (*9*) was examined, but recoveries of better than 98% of the ammonium sulfate taken could not be obtained by their procedure. A method based on partition chromatography has been described, but it does not appear to be applicable to routine control (*6*).

This investigation was devoted to a study of the mercuric oxide and cobaltinitrite procedures, in the hope of overcoming their shortcomings. This aim was not realized for the mercuric oxide method. A study of the effect of reagent concentration, temperature, and the composition of the wash solution in the separation of ammonia by the cobaltinitrite procedure led to the development of a satisfactory method.

APPARATUS AND REAGENTS

Funnel, Fisher Filtrator, Catalog No. 10-352, Fisher Scientific Co.

Kjeldahl Distillation Apparatus. A standard macro assembly was used.

Sampling Tube. A 100-ml. Foust centrifuge tube, A. H. Thomas Co. No. 3132-A, equipped with a rubber stopper and inlet and outlet tubes, was used.

Ammonium Sulfate Solutions. Standard ammonium sulfate solutions were made up from reagent grade ammonium sulfate which assayed 99.3% by the formalin method (*11*).

Mercuric Oxide, yellow, reagent grade. Several samples of Baker and Adamson and Merck material were used in this study.

Methylamine Salt Solutions. Samples of anhydrous Rohm and Haas monomethylamine, dimethylamine, and trimethylamine, each containing less than 0.3% of the other amines, were obtained in pressure cylinders. The gases were used to prepare saturated solutions at room temperature and pressure and the solutions were treated by the method of Francois (*5*) to remove traces of ammonia. One hundred grams of yellow mercuric oxide (Merck), 10 ml. of saturated sodium carbonate, and 10 ml. of 50% sodium hydroxide were added to 1 liter of saturated amine solution in a dark bottle and the solution was shaken for 2 hours. After standing overnight, the supernatant liquid was decanted and filtered through paper into a Kjeldahl flask. Twenty milliliters of 10 *N* sodium hydroxide were added to the flask and the amines were distilled into standard hydrochloric or sulfuric acid until the distillate solutions were just alkaline to methyl red. The amine solutions were then neutralized with the appropriate acid and diluted to the desired concentration.

Sodium Cobaltinitrite, 20% solution. Twenty grams of reagent grade compound (J. T. Baker Chemical Co.) were dissolved in 100 ml. of water immediately before use.

Sodium Diammonium Cobaltinitrite. To 50 ml. of ethyl alcohol were added 20 ml. of 40% sodium nitrite and 20 ml. of 20% sodium cobaltinitrite. The mixture was cooled to 5° C. and 5 ml. of water and 5 ml. of a 10% ammonium sulfate solution were added. After thorough mixing of the solution, the precipitate was allowed to settle out for 5 minutes, filtered by suction on a No. 5 Whatman paper, and then washed with a 1 to 1 ethyl alcohol–water solution containing 8% sodium nitrite (w./v.). The wet precipitate was stored in a refrigerator until used to prepare the saturated wash solution.

Sodium Nitrite, 40% solution. Forty grams of reagent grade material (J. T. Baker Chemical Co.) were dissolved in 100 ml. of water immediately before use.

Wash Solution. Eighty grams of sodium nitrite were dissolved in 500 ml. of water and 500 ml. of ethyl alcohol (Formula 2B) were added. The wash solution was saturated at 5° C. with sodium diammonium cobaltinitrite by shaking for 5 minutes with 500 mg. of the precipitate. The mixture was placed in a refrigerator for several hours and the suspension allowed to settle out. After the cold solution was filtered with suction through Whatman No. 5 paper, the excess precipitate was returned to the refrigerator for later re-use.

Table I. Recovery of Ammonia Adsorbed by Various Lots of Yellow Mercuric Oxide

Supplier	Lot No.	Color	Average Particle Size, Microns[a]	Ammonia Recovered, %
Baker & Adamson	33	Red-orange	2.4	2.4
Baker & Adamson	27	Yellow-orange	1.3	99.4
Merck	42116	Yellow-orange	1.3	99.9
Merck	41138	Yellow-orange	1.5	99.5
Merck	40529	Yellow-orange	1.2	99.8

[a] By Fisher subsieve sizer.

USE OF YELLOW MERCURIC OXIDE

In preliminary work with the Francois procedure (*5*) a solution containing monomethylamine and ammonium sulfates was treated with Baker and Adamson's mercuric oxide (lot 33) and Francois' alkali solution. The apparent recovery of methylamines from the filtrate was approximately 200%, indicating that most of the ammonia had not been removed by the mercuric oxide. A similar set of experiments with the Weber and Wilson procedure (*13*) resulted in the appearance of approximately 91% of the ammonia in the filtrate. Separate tests with monomethylamine sulfate and ammonium sulfate indicated that none of the amine and only 2.4% of the ammonia were removed by the mercuric oxide.

On the other hand, a sample of Merck's mercuric oxide (lot 42116) removed 99.9% of the ammonia present. A number of other samples of mercuric oxide were examined using the Weber and Wilson procedure, and the results obtained are listed in Table I. In subsequent work involving yellow mercuric oxide, the Merck reagent was used and each lot was checked before use. The maximum quantity of ammonia removed by 1 gram of mercuric oxide was found to be approximately 15 mg.

A series of experiments was made with mixtures of ammonia and amine sulfate solutions using a procedure adapted from Weber and Wilson. The results of this work, summarized in Table II, indicated that ammonia was almost quantitatively removed and that methylamines were not adsorbed in the absence of ammonia. However, in the presence of ammonia, 5 to 18% of the monomethylamines present were removed from the solution and could not be recovered in the filtrates. For this reason, the attempt to develop an analytical method based on the use of mercuric oxide was abandoned.

Table II. Recovery of Amines by the Mercuric Oxide Method

	Sample Composition, Millimoles				
NH_3	Mono	Di	Tri	No. of Detns.	Average Amine Recovery, %
....	4.079	...	...	2	99.9
....	6.120	...	...	3	99.2
....	....	0.621	...	2	100.3
....	....	3.107	...	3	99.9
....	....	...	1.380	2	100.0
5.872	....	...	...	4	0.5[a]
8.808	0.245	...	...	3	90.2
7.927	0.406	...	...	3	95.2
14.680	0.816	...	...	4	88.6
14.680	2.040	...	...	5	82.5
5.872	12.238	...	...	3	94.0

[a] Unadsorbed ammonia.

USE OF SODIUM COBALTINITRITE

In the method described by Leone (*8*) the precipitated sodium diammonium cobaltinitrite is allowed to stand for 24 hours at room temperature in contact with the reagents and is then filtered off. The volatile amine bases in the filtrate are determined by Kjeldahl distillation. This procedure was followed in the early work, but results using both the sulfate and hydrochloride salts of the bases indicated that some of the ammonia and less than 30% of the methylamines were recovered in the filtrate.

The low results obtained by the Leone procedure were believed to be due to the reaction of the amine with nitrous acid present and this was confirmed in a semiquantitative manner by collecting the gas formed overnight from 10 ml. of a reagent-monomethylamine mixture in an inverted graduate. The colorless and odorless gas, bubbles of which appeared shortly after the solutions were mixed, was presumably nitrogen and had a volume equivalent to almost 100% decomposition of the amine according to the reaction:

$$CH_3NH_2 + HNO_2 \longrightarrow CH_3OH + N_2 + H_2O$$

The pH of the solution rose from 5.5 to 6.6. The effect of pH was examined by substituting quantities of 0.1 *N* sodium hydroxide solution for a portion of the water used in the mixture. At a pH of 6.5, approximately 24% of the monomethylamine was decomposed and at a pH of 7.0 no gas was collected, indicating that no reaction had taken place. Above pH 7.0, the sodium cobaltinitrite reagent breaks down and a brownish-black gelatinous precipitate of cobalt hydroxide is formed. It was apparent from these experiments that a quantitative determination of ammonia based on the analysis of the residual volatile base in the filtrate might be difficult to achieve.

Table III. Effect of pH on Recovery of Ammonia by Cobaltinitrite Method

pH	Ammonia Taken, Mg.	No. of Detns.	Ammonia Recovered, %
5.5	39.7	2	90.6
5.5	79.4	2	90.4
6.5	39.7	3	86.4
7.0	39.7	2	17.8
7.0	79.4	2	31.0

The alternative procedure (*2*), involving determination of the ammonia in the precipitate of sodium ammonium cobaltinitrite, yielded recoveries of 90% of the ammonia taken and, in the hope of obtaining better accuracy, the effect of pH, reagent composition, time of reaction, and the composition of the wash solution was studied. The sulfate salts of ammonia and the amines were used in all this work.

The effect of pH was demonstrated by adjusting the pH with 0.1 *N* sodium hydroxide and then adding aliquots of a standard ammonium sulfate solution. After standing overnight at room temperature, the solution was filtered and the precipitate washed with 50% alcohol. The paper and contents were transferred to a Kjeldahl flask, water and sodium hydroxide were added, and the evolved ammonia was distilled into standard acid. The results (Table III) indicated that an increase of pH would decrease the recovery of ammonia, and all subsequent work was performed at the normal pH of the reagent.

A marked increase in recovery of ammonia was obtained at low temperatures, as summarized in Table IV. All subsequent determinations were run at 5° C., the temperature usually obtained when the solutions were kept in an ice bath or a refrigerator.

Some data reported by Leone (*8*) indicated that the solubility of sodium diammonium cobaltinitrite could be repressed by increasing the concentration of the reagents, and this work was repeated and extended. From the data of Table V it is apparent that in a solution containing 8 grams of sodium nitrite and 4 grams of sodium cobaltinitrite in a final volume of 100 ml. the recovery of ammonia is increased from 97.4 to 98.9% and the use of larger samples is possible.

The results in Table VI indicate that the amount of ammonia precipitated reaches a maximum in 1.5 to 3 hours and that overnight digestion is unnecessary.

In an effort to increase the recovery of ammonia by decreasing the solubility of the precipitate in the wash solution, a number of mixtures containing 50% alcohol and varying amounts of sodium

Table IV. Effect of Temperature on Recovery of Ammonia by Cobaltinitrite Method

Temperature, ° C.	Sample	No. of Detns.	Base Taken, Mg.	Average Recovery, %
25–30	NH_3	2	36.0	90.6
	NH_3	2	79.4	90.4
	CH_3NH_2	1	92.0	No precipitate
5–8	NH_3	6	39.7	97.3
	CH_3NH_2	1	92.0	No precipitate

Table V. Effect of Reagent Concentration on Recovery of Ammonia

Sodium Nitrite, G./100 Ml.	Sodium Cobaltinitrite, G./100 Ml.	No. of Detns.	Ammonia Taken, Mg.	Ammonia Recovered, %
2	2	6	39.7	97.4
2	2	2	79.5	88.4
3	2	4	39.7	97.9
4	2	4	39.7	98.5
5	4	2	79.5	98.1
8	4	2	99.3	98.9

nitrite were tested for this purpose. Only by saturating the wash solution with sodium ammonium cobaltinitrite was it possible to increase the recovery to nearly 100%. The wash solution also contains 8 grams of sodium nitrite per 100 ml.

The method finally adopted for gaseous methylamines is as follows, and applies equally to solutions of the amines, except that the use of a sampling tube is unnecessary.

PROCEDURE

Fifty milliliters of standard 5 *N* acid are run into the sampling tube, 2 drops of 0.1% methyl red are added, and the sampling assembly is weighed to the nearest milligram. The tube is immersed in an ice bath and the gas sample run slowly into the solution until the color just turns yellow. The rate of gas flow is adjusted so that no bubbles escape from the solution. After wiping and drying, the tube is reweighed; the difference in weight is the sample weight. The contents are then neutralized with a measured amount of standard 5 *N* acid, transferred to a volumetric flask, usually of 500-ml. capacity, and then diluted to volume. The total acid consumed provides a measure of the total amount of base in the sample.

Fifty milliliters of Formula 2B alcohol, 20 ml. of sodium nitrite, and 20 ml. of sodium cobaltinitrite solution are mixed in a 250-ml. wide-mouthed Erlenmeyer flask and the mixture is cooled to 5° C. in an ice bath. An aliquot of the sample solution, not exceeding 10 ml., containing not more than 100 mg. of ammonia, not more than 70 mg. of monomethylamine, and a total of not more than 10 milliequivalents of nitrogen bases, is pipetted into the flask. Water is added, if necessary, to bring the volume added to 10 ml. The solution is swirled as the sample is added and the flask is then stoppered and placed in a refrigerator or an ice bath, so that the temperature of the solution is maintained at approximately 5° C. for a minimum of 3 hours. The cold solution is decanted through an 11-cm. Whatman No. 5 paper and filtered by suction on a Filtrator funnel. The precipitate in the flask is washed twice with cold (5° C.) wash solution and then the entire precipitate is transferred to the paper, where it is washed about 10 times with 10-ml. portions of cold wash solution. After the precipitate has been sucked dry, the paper and precipitate are transferred to an 800-ml. Kjeldahl flask. Several pieces of pumice, 250 ml. of water, and 25 ml. of 10 *N* sodium hydroxide are added, the flask is attached to the distillation apparatus, and the ammonia is distilled into 75.00 ml. of 0.1 *N* standard sulfuric acid containing 2 drops of 0.1% methyl red indicator. A volume of approximately 150 ml. is usually collected. The excess acid is back-titrated with standard alkali and the ammonia in the sample is calculated in the usual way.

Table VI. Effect of Time of Digestion on Ammonia Recovery by Cobaltinitrite Method

Digestion Time, Hours	Ammonia Recovered,[a] %
1.0	96.9
1.5	97.2
3.0	98.2
5.0	98.0
65.0	98.0

[a] Average of two or more determinations.

RESULTS OF ANALYSIS OF SYNTHETIC MIXTURES AND PLANT SAMPLES

Mixtures were made up containing ammonia, methylamines, methanol, and water, and aliquots of these solutions were taken for analysis by the above procedure. Results, summarized in Table VII, A, indicate that an average of 99.7% of the ammonia present was recovered. The results listed in Table VII, B, were obtained in determining the lower limit of applicability of the method.

The ammonia content of a number of production samples from different parts of the process stream was determined and the total ammonia redetermined after the addition of ammonium sulfate. The ammonia present ranged from 5 to 65% and the mean of the recovery of the total ammonia present, as determined in 47 analyses by four analysts, was 100.0% with an over-all standard deviation of 0.62%.

DISCUSSION

The data concerning the behavior of mercuric oxide are of interest for a number of reasons. There has been some confusion in the literature as to the nature of the failure of this reagent in quantitative work, but it is now apparent that some preparations do not remove ammonia at all and that when ammonia is quantitatively removed considerable amounts of methylamine are carried along with the ammonia. Explanations for either of these phenomena are not at all obvious, and the entire matter would be worth investigating from the theoretical point of view. An x-ray diffraction study of two batches of yellow mercuric oxide differing in their ability to adsorb ammonia (lots 33 and 41138 of Table I) did not reveal any significant differences in structure. Both samples appeared to consist of identical orthorhombic crystals ranging in size principally between 1000 and 10,000 A. It is not likely that the differences in average particle size noted in

Table VII. Results of Analysis of Synthetic Samples

	Sample	Mono, %	Di, %	Tri, %	MeOH, %	NH_3, %	Sample Size, Mg.	No. of Detns.	Rel. Av. Deviation, Parts/1000	Av. Recovery of Ammonia, %
A.	I	5.52	8.22	...	27.96	58.30	170.3	4	±1.0	99.1
	II	12.99	4.05	...	45.74	37.22	173.1	7	±2.5	99.8
								2[a]	±0.8	98.7
	III	7.85	4.89	0.57	55.26	31.43	143.3	4[a]	±0.2	99.4
	IV	39.88	...	14.46	...	45.65	141.0	2	±2.8	100.6
								2	±1.2	99.9
	V	20.63	12.85	7.48	...	59.04	109.0	6	±1.2	100.4
	VI	15.16	12.44	14.48	...	9.14	281.5	2[a]	±1.1	98.5
									Mean	99.7
									Standard deviation	0.67
B.	IX	91.32	...	...	...	8.68	73.4	4	±0.05	100.1
	X	...	98.47	...	...	1.53	346.7	3	±0.02	91.2
	XI	...	94.81	...	...	5.19	483.6	4	±5.19	100.0
	XII	...	...	94.14	...	5.86	668.1	3	±0.00	100.1
	XIII	...	...	90.35	...	9.65	609.4	4	±0.01	99.9
	XIV	30.34	33.68	32.49	...	3.47	592.9	2	±17.1	102.0
	XV	31.15	34.59	33.37	...	0.90	577.5	4	±33.9	124.4

[a] Run by analyst previously unfamiliar with procedure.

Table I are sufficient to account for the marked dissimilarity in behavior. Methylamines are ordinarily not adsorbed by mercuric oxide, but possibly the presence of an ammonia adsorption complex promotes the retention of methylamines. The latter phenomenon does not invalidate the use of yellow mercuric oxide to remove ammonia from amines; however, each lot of mercuric oxide should be pretested for ammonia adsorptive capacity.

The results obtained in this investigation contradict those reported by Leone (*8*) on methylamines. It has been suspected by others that the loss of methylamines and consequently erroneously high ammonia results (obtained by difference) were due to the familiar reaction of the amines with nitrous acid. The occurrence of this reaction was confirmed as described in the experimental portion of this paper, and it is difficult to accept the results described by Leone. A possible explanation has appeared in recently published work (*7*) in which it was found that aliphatic primary amines do not react with nitrous acid below a pH of approximately 3. Leone does not supply data concerning the pH of his reagents and the authors have not had an opportunity to determine if quantitative separation of ammonia from methylamines can be obtained at such low pH.

The precipitation of the ammonia in the cobaltinitrite complex prevents decomposition of the ammonia by the nitrous acid and permits subsequent direct determination of the ammonia in the precipitate. Incomplete precipitation of ammonia occurs unless the solutions are kept cold; the necessity for cooling has been pointed out by Wagner *et al.* (*12*). At temperatures between 15° and 18° ammonia losses as high as 5% have been noted and at 30° the loss has been 15%. In the procedure described here the solubility of the sodium diammonium cobaltinitrite has been decreased by cooling without significantly affecting the solubility of the methylamine salts. Loss of precipitate on washing is eliminated by using a wash solution saturated with the precipitate. When wash solutions not saturated with sodium diammonium cobaltinitrite are used, the recoveries of ammonia are usually between 1.5 and 3% low.

As much as 130 mg. of ammonia may be determined quantitatively by the procedure given, but it is advisable that aliquots containing not more than 100 mg. of ammonia be taken for analysis. The ammonia forms a dense, finely divided precipitate and large amounts decrease the speed of filtration. In samples in which less than 5% of the total base is ammonia, the relative accuracy of the method decreases rapidly, but it is still useful for most purposes. At the 1% level the uncertainty is 0.25% absolute.

Methanol and methylamines do not ordinarily interfere in the determination of ammonia by this method. As much as 70 mg. of monomethylamine, 400 mg. of dimethylamine, or 700 mg. of trimethylamine may be present without causing precipitation of the corresponding methylammonium cobaltinitrites.

The determination of ammonia by this procedure requires perhaps 3 hours of an analyst's time and 6 hours' elapsed time.

ACKNOWLEDGMENT

The authors wish to express their appreciation to James Stroupe for the x-ray diffraction studies on mercuric oxide, to David Lentz, Henry B. Jones, and William E. Scanlon for some of the analyses reported here, and to the many members of the Rohm & Haas organization who have aided with advice and suggestions.

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Carbon on Cracking Catalyst

Determination by Combustion and Conductometry

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WORK recently reported by Schmitkons (*4*) revealed that in the determination of carbon on cracking catalyst by combustion, more or less unburned coke may be entrapped. The amount of carbon entrapped depends on the combustion temperature, nature of the catalyst, and nature and quantity of coke. Schmitkons pointed out that in borderline cases of entrapment, a slow, continuing evolution of carbon dioxide, or "straggling," occurs.

As an aid in the selection of proper burning times for individual types of catalysts, a method for following the progress of the combustion has been devised. It is based upon the change in conductivity of a solution of sodium hydroxide when carbon dioxide is absorbed (*2*). The replacement of highly conducting hydroxide ions by less mobile carbonate ions results in a marked increase in the resistance of the solution.

$$CO_2 + 2OH^- \longrightarrow CO_3^{--} + H_2O$$

Because the conductivity of the solution can be measured in a few seconds, the progress of the combustion can be followed closely. Presumably, a recording conductivity bridge would furnish a continuous record of the reaction.

EXPERIMENTAL

Apparatus. The combustion apparatus consisted of an 800-mm. quartz tube packed according to Lescher (*3*), and heated with three split-type heavy-duty furnaces. The first and second furnaces were 125 mm. long and were operated at 1100° and 1600° F., respectively. The third furnace was 250 mm. long and was kept at 1600° F. The carbon dioxide formed by the combustion

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Considerable variation in the burning rates of coke deposited on cracking catalysts has recently been observed. Carbon is frequently entrapped during the combustion, the amount depending upon the conditions of burning. An analytical method that would follow the progress of the combustion for use in the study of burning rates was devised, based on the decrease in conductivity of aqueous sodium hydroxide as the carbon dioxide produced by the combustion is absorbed. As the conductivity can be measured at intervals of a few seconds, the progress of the combustion can be followed closely. The conductometric method gave results for total carbon comparable to those obtained by the conventional gravimetric method, and has proved useful in the comparison of burning rates of catalysts and in the selection of optimum conditions of combustion for a particular catalyst.

was absorbed in the cell illustrated in Figure 1. The cell is designed to hold 75 to 100 ml. and a fritted plate is used to disperse combustion gases through the absorbing solution. A stopcock is provided at the bottom of the cell to allow removal of used solutions and a spherical joint inlet permits convenient addition of fresh solutions. Platinized platinum electrodes are located just below the fritted scrubber and are consequently unaffected by bubbles of scrubbing gases. The cell is water-jacketed to maintain a constant temperature. The cell constant was 2.94 reciprocal cm.

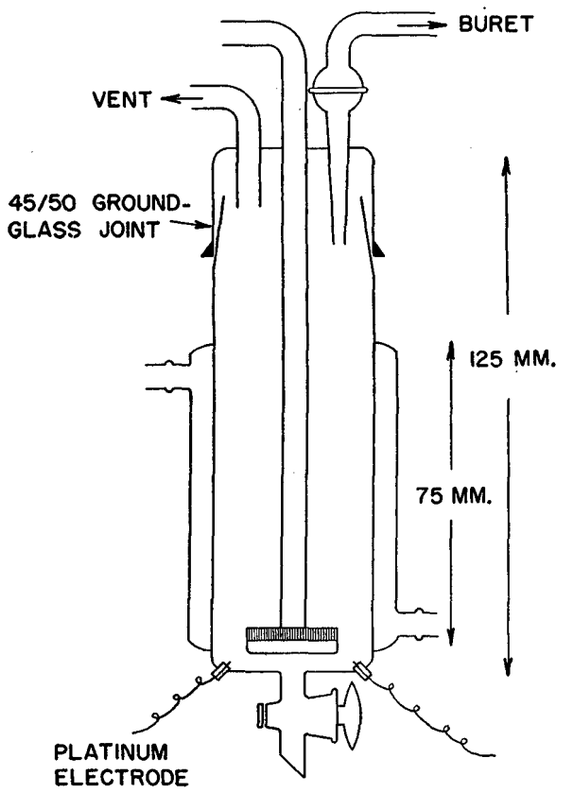

Figure 1. Cell for Conductometric Determination of Carbon

Instead of a dehydrant bulb before the absorption cell, a bubbler was inserted to saturate the combustion gases and, thus, prevent changes in the water content of the absorbing solution. The bubbler contained a dilute solution of sulfuric acid of approximately the same ionic strength as the sodium hydroxide in the absorption cell.

Resistances were measured with an Industrial Instruments conductivity bridge, Model RC-BC.

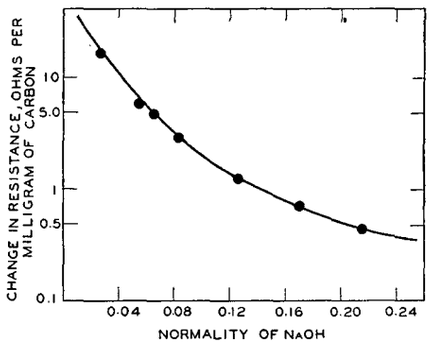

Figure 2. Effect of Sodium Hydroxide Concentration on Calibration Factor in Conductometric Determination of Carbon

Concentration of Sodium Hydroxide. The effect of the concentration of sodium hydroxide on the sensitivity of the method, as measured by the change in resistance per milligram of carbon burned, is shown in Figure 2. These data were obtained by burning samples of a standard catalyst, the carbon content of which was accurately known, and absorbing the carbon dioxide in various concentrations of sodium hydroxide.

As would be expected, the sensitivity is much greater for low concentrations of sodium hydroxide. To provide an adequate excess of hydroxide ions, small samples were taken for analysis. As a compromise between maximum sensitivity and reasonably sized samples to ensure against depletion of sodium hydroxide, a concentration of 0.04 *M* and a sample weight of 0.2 to 0.3 gram were chosen. Depletion of the sodium hydroxide should not exceed 50%.

Solutions of barium hydroxide and sodium carbonate were also investigated as absorbents but were found to be less satisfactory than sodium hydroxide. Excessive foaming was observed with the barium hydroxide, as also reported by Bennet, Harley, and Fowler (*1*).

Calibration. It is necessary to calibrate each batch of sodium hydroxide because the relationship between change in resistance and milligrams of carbon burned is dependent upon the concentration. In these experiments, 3-gallon quantities of sodium hydroxide were prepared and stored in borosilicate glass bottles, protected from the atmosphere by Ascarite absorbers.

A used cracking catalyst, the carbon content of which had been established as 3.50% by several hundred gravimetric analyses, was employed as a standard for calibration purposes. By measurement of the increase in resistance observed when known weights of this standard sample were burned, the relationship, milligrams of carbon per ohm change in resistance, was established for each fresh batch of sodium hydroxide. This value was checked at intervals of several days and was found to be constant for weeks. The relationship between milligrams of carbon burned and change in resistance of the sodium hydroxide was a straight line in the range of 0 to 100 mg. of carbon. A typical calibration factor found for a batch of approximately 0.04 *N* sodium hydroxide was 0.0884 mg. of carbon per ohm change in resistance.

Procedure. Pipet 75 ml. of the calibrated sodium hydroxide into the absorption cell with oxygen flowing through the combustion tube and cell at about 200 ml. per minute. Weigh 0.2 to 0.3 gram of catalyst into a porcelain boat. After measuring the resistance of the sodium hydroxide solution, remove the stopper from the front end of the combustion tube, and, by means of a wire, push the sample boat into the center of the first or second furnace, depending upon whether the combustion is to be made at 1100° or 1600° F. Replace the stopper and ignite the sample at the desired temperature, measuring the resistance of the sodium hydroxide solution at frequent intervals. When the resistance becomes constant, the total amount of carbon burned, or the carbon burned after any given length of time, is found by multiplying the corresponding change in resistance by the calibration factor of the sodium hydroxide. Burning rate curves are obtained by plotting the amount of carbon burned against the length of time of combustion.

Table I. Comparison of Conductometric and Gravimetric Methods for Determination of Carbon on Catalysts

Sample	Wt. % Carbon	
	Gravimetric	Conductometric
1	0.06	0.05
	0.06	0.10
2	0.25	0.25
	0.22	0.23
3	0.58	0.53
4	1.18	1.12
	1.14	1.09
	1.13	1.05
5	1.88	1.96
	1.94	2.07
6	2.67	2.87
	2.59	2.78
7	3.08	3.04
8	3.18	3.17
	3.18	3.21
9	3.57	3.77
	3.56	3.64
10	4.07	4.07
	4.06	4.04
11	4.50	4.52
	4.49	
12	5.22	5.30

BURNING RATE

Inasmuch as measurements can be made at intervals of only a few seconds, it is possible to follow the progress of the combustion closely. Figure 3 illustrates the burning rate of the carbon on two different cracking catalysts. Presumably, a recording conductivity bridge would furnish continuous burning rate curves. Burning rate curves of this type would be valuable in the study and comparison of various kinds of catalysts. The oxygen rate was kept constant at about 200 ml. per minute during all of these studies.

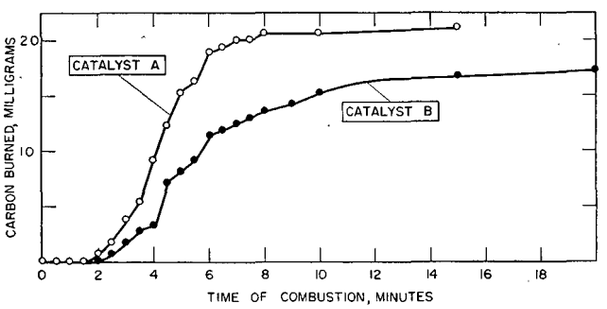

Figure 3. Burning Rate of Carbon on Catalysts
Temperature of combustion, 1100° F.

COMPARISON WITH GRAVIMETRIC METHOD

A comparison of the results of carbon determinations on twelve catalysts by both conductometric and gravimetric methods is shown in Table I and graphically in Figure 4 according to the method described by Youden (*5*). Assuming the errors by the standard gravimetric method to be small as compared to those by the proposed conductometric method, the slope of the line was calculated by Youden's system of computations to be 1.016 ± 0.011, and the intercept was calculated to be 0. This indicates the absence of a blank and that the conductometric method has a tendency to give 1.6% higher results than the gravimetric method. The standard deviation (*5*) of a single analysis was calculated to be ±0.08% carbon.

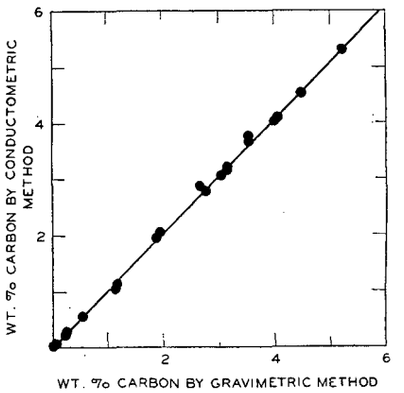

Figure 4. Comparison of Conductometric and Gravimetric Methods

The conductometric method is swifter than the conventional gravimetric method because the conductivity of the sodium hydroxide solution can be measured more rapidly than an Ascarite bulb can be weighed, and, if desired, a permanent record of the rate of combustion can be obtained. Another timesaving feature lies in the fact that the combustion can be discontinued as soon as the measurements show no additional evolution of carbon dioxide. The conductometric method has also been employed as a tool for finding the optimum conditions for the determination of burnable carbon by the gravimetric method. It was found that the optimum sample size, temperature, time, and rate of combustion vary with the type and history of the catalyst being investigated.

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Geochemical Field Method for Determination of Nickel in Plants

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The use of biogeochemical data in prospecting for nickel emphasizes the need for a simple, moderately accurate field method for the determination of nickel in plants. In order to follow leads provided by plants of unusual nickel content without loss of time, the plants should be analyzed and the results given to the field geologist promptly. The method reported in this paper was developed to meet this need. Speed is acquired by elimination of the customary drying and controlled ashing; the fresh vegetation is ashed in an open dish over a gasoline stove. The ash is put into solution with hydrochloric acid and the solution buffered. A chromograph is used to make a confined spot with an aliquot of the ash solution on dimethylglyoxime reagent paper. As little as 0.025% nickel in plant ash can be determined. With a simple modification, 0.003% can be detected. Data are given comparing the results obtained by an accepted laboratory procedure. Results by the field method are within 30% of the laboratory values. The field method for nickel in plants meets the requirements of biogeochemical prospecting with respect to accuracy, simplicity, speed, and ease of performance in the field. With experience, an analyst can make 30 determinations in an 8-hour work day in the field.

THE interest manifested in the chemical composition of plants as a means of prospecting for ore bodies (*5, 6, 14, 17, 21, 22*) has made apparent the need for quick tests to analyze plants for certain trace constituents. A geochemical study is expedited when results are readily available to guide further sampling of a specific area. A simple and rapid field test furnishes the explorer with daily results, and in this way the chemical analyses are a basis for further sampling. Some of the accuracy usually required in chemical analysis is sacrificed and an individual analysis cannot be considered out of context with its companion samples (*15*). However, with a field test for zinc in soils, Lakin *et al.* (*7*) have shown that if the number of analyses is sufficient, the differences in the over-all pattern are real.

Nickel is one of the metals to which biogeochemical prospecting has been applied. Plants normally contain small amounts of nickel. Ramage (*13*) found detectable amounts of nickel in most of the spices and herbs he analyzed, and Mitchell (*11*) found that the normal content of dry matter of plants is 0.0001 to 0.0005% nickel. Bertrand and Mokragnatz (*2*) analyzed fruits, vegetables, and cereals and found that the nickel ranged from less than 0.0001 to 0.0003% in the dry plant. In a study of the distribution of nickel in the biosphere, Malyuga (*9*) stated that nickel in dry tree leaves ranges from less than 0.0001 to 0.0004%, and in the dry fruits and seeds from less than 0.0001 to 0.0002%. All of these plants were grown in agricultural soils which, from the viewpoint of geochemical prospecting, are designated as normal or background soils and average 0.001% nickel (*1, 8, 9*).

Soils in mineralized areas, however, or soils on serpentine outcrops may contain as much as 1.0% nickel (*9, 16, 19*). Rankama (*14*) has collected samples of birch and willow from the immediate vicinity of an outcropping ore body and found nickel as high as 0.4% in the ash (0.02% in the dry plant, assuming 5% ash). Birrell and Wright (*3*) report that leaves of *Pancheria glabrosa* growing on serpentine soils contained as much as 0.009% nickel in the oven-dried plant. Furthermore, Malyuga (*10*) has shown that grasses of the same species, growing in an area of nickel mineralization and growing in soil low in nickel, had 0.1 and 0.0001% nickel, respectively, in the dry grasses. Thus nickel in plants may be expected to range from less than 0.0001 to 0.1%.

METHOD OF ANALYSIS

The pertinent features of the field test presented here are the ashing of fresh vegetable matter directly over a flame and the determination of nickel by a modified spot test technique. The ash is put in solution with hydrochloric acid, the solution buffered with sodium citrate, and the pH adjusted to 8.8 with ammonium hydroxide. Standard solutions of increasing nickel concentration are passed through the reagent paper impregnated with dimethylglyoxime to give a series of confined spots. The intensity of pink is a measure of the nickel concentration. The spots from the sample solutions are then compared with the standard series of spots to determine the amount of nickel present.

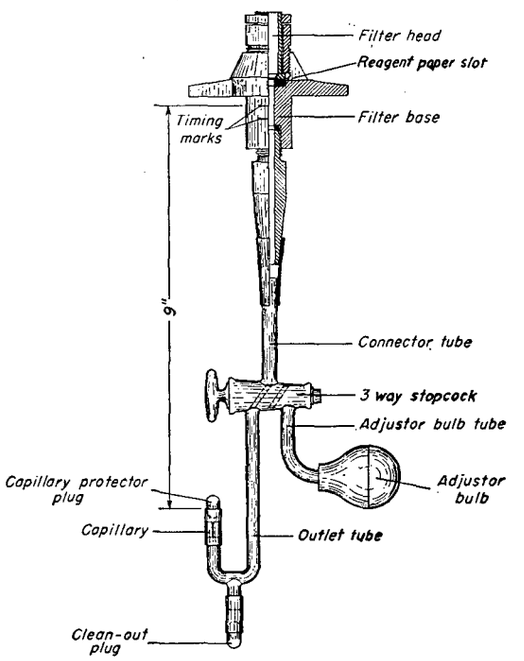

Figure 1. Chromograph Used to Make Confined Spots under Controlled Speed of Flow of Test Solution through Reagent Paper

The confined spot test was first proposed by Yagoda (*23*) as a technique for making quantitative measurements by means of spot tests. Stevens and Lakin (*19*) used a device called a chromograph for making spot tests, whereby confined spots were made

on a strip of reagent paper fed into the device, and the speed of flow of test solution through the reagent paper was automatically controlled. The chromograph is shown in Figure 1. The reagent paper is fed through a slot, the sample solution is placed on the paper in the filter head, and suction is applied by connecting a water column to pull the solution through the paper. The capillary plug in the water column controls the rate of flow of solution. The rate of flow is such as to permit maximum speed with essentially complete precipitation of nickel from solution.

APPARATUS AND REAGENTS

Apparatus. The chromograph is as shown in Figure 1.

A burner such as a Coleman GI pocket stove to ash the samples.

A balance such as Roller-Smith, Model C, 0 to 75 mg., to measure the sample. A metal-free (lucite) scoop, previously calibrated by weighing a measured amount of pulverized plant ash, can also be used.

Test tubes, 13 × 100 mm. calibrated at 2 ml.

Filter sticks, as those listed in Scientific Glass Co. catalog, Catalog No. M-2480C.

Micropipet, 0.5 ml. calibrated in tenths.

Rubber bulbs of the type used on large pipets or syringes, such as listed by Eimer and Amend, Catalog No. 14-070.

Reagents. Hydrochloric acid, constant boiling or 1 to 1.

Sodium citrate. Dissolve 50 grams of sodium citrate in 100 ml. water.

Ammonium hydroxide, concentrated and 1 to 1, freshly prepared.

Thymol blue indicator solution, 0.1%.

Molybdate solution. Dissolve 1 gram of molybdic acid in a few milliliters of dilute sodium hydroxide and dilute with water to 100 ml.

Water, distilled in an all-borosilicate glass still or passed through a resin demineralizer such as the Bantam manufactured by Barnstead Still and Sterilizer Co.

Standard nickel solution, 0.01%.

Dimethylglyoxime reagent paper. Dip sheets of Whatman No. 50 filter paper into a saturated solution (approximately 2%) of dimethylglyoxime in acetone. Insert the paper at a rapid, constant rate, as pauses in the immersion produce an uneven coating (*4*). When the paper is dry, it should be dipped again. The standard series and the tests should be made on the same batch of paper. Cut the reagent paper into strips $^7/_{16}$ inch wide. The edges which have been handled should be discarded.

Standard series of nickel dimethylglyoxime spots. A series of standard nickel dimethylglyoxime spots were prepared from 0.2-ml. portions of solutions containing 0.5, 1.25, 2.5, 3.75, 5.0, 7.5, and 10 micrograms of nickel per ml. (corresponding to 0.010, 0.025, 0.050, 0.075, 0.10, 0.15, and 0.20%, respectively, of nickel in the ash for a 10-mg. sample), each standard solution containing 0.2 ml. molybdate solution, 0.5 ml. sodium citrate, and 1 drop thymol blue in 2 ml., and each adjusted to pH 8.8.

PROCEDURE

The fresh plant material is placed in a platinum dish and ashed directly over the flame of the Coleman stove. The material should be stirred occasionally during the ashing to facilitate burning. After glowing ceases, it should be removed from the burner and the ash allowed to cool. The ash is pulverized and mixed thoroughly with a glass rod flattened on the end.

Ten milligrams of ash are placed in a calibrated test tube, and 0.5 ml. hydrochloric acid is added and the mixture is heated in a boiling water bath for 20 minutes. The test tube is transferred to a rack and 0.2 ml. molybdate solution, 0.5 ml. sodium citrate solution, and 1 drop thymol blue are added. The mixture should be shaken thoroughly and ammonium hydroxide added until the solution turns yellow. Then the freshly prepared 1 to 1 ammonium hydroxide is added carefully, one drop at a time, to just a true blue. If the flakes of carbon remaining from the ashing cause confusion in seeing the color of the solution the carbon should be allowed to settle out before adding more ammonium hydroxide. The volume is made up to 2 ml. with purified water, shaken thoroughly and let stand for about 15 minutes to allow most of the calcium citrate to precipitate before filtering. To filter, the air is squeezed out of a rubber bulb which is placed over the open end of a filter stick and the stick is inserted into the test tube. The vacuum draws the liquid into the filter stick rather rapidly.

A confined spot with 0.2 ml. of the solution is made on dimethylglyoxime reagent paper in the chromograph. When the spots are dry they are compared with the standard series to determine the amount of nickel in the sample of plant ash.

RESULTS

The samples used in testing this method were selected for their range of nickel and not for any geochemical significance, and therefore only a brief description of them need be given. Group A (see Table I) was collected from plants and trees growing in residual soil over serpentine rich in nickel in Fairfax County, Va. Group B was selected for negative contrast, some distance from the serpentine. Group C was plants from a Conowingo soil in Montgomery County, Md., known to be richer than normal in nickel (*16*). Group D is the negative contrast for the same plants taken on Chester loam and Penn silt loam also in Montgomery County.

Table I. Comparison of Field Test Results with Laboratory Results

Sample No.		% Ash, Av.	% Nickel: Dried plant, lab. detn.	% Nickel: Ash, Lab. (calcd.)	% Nickel: Ash, Field test	% Error
	Group A (Nickel-Rich Soil, Fairfax Co., Va.)					
1	Clover leaves	9.0	0.0012	0.013	<0.025	...
2	Honeysuckle leaves	8.0	0.0014	0.020	<0.025	...
3	Cedar needles	4.5	0.0026	0.06	0.05	−17
4	Cedar needles	4.5	0.0031	0.07	0.05	−30
5	Cedar needles	4.5	0.0032	0.07	0.05	−30
6	Honeysuckle	8.0	0.0054	0.07	0.05	−30
7	Cedar needles	4.5	0.0035	0.08	0.05	−37
8	Cedar needles	4.5	0.0038	0.08	0.075	− 6
9	Oak leaves	5.0	0.0047	0.09	0.075	−17
10	Pine needles	2.0	0.0026	0.13	0.15	+15
11	Dogwood leaves	5.5	0.0085	0.15	0.15	0
12	Pine needles	2.0	0.0037	0.18	0.15	−17
13	Pine needles	2.0	0.0038	0.19	0.20	+5
	Group B (Low Nickel Soil, Fairfax Co., Va.)					
14	Honeysuckle leaves	8.0	0.0004	0.004	<0.025	...
15	Cedar needles	4.5	0.0030	0.06	0.05	−17
	Group C (Nickel-Rich Conowingo Soil, Montgomery Co., Md.)					
16	Red clover	7.5	0.0008	0.01	0.01	...
17	Chicory	9.0	0.0008	0.01	0.01	...
18	Wild carrot	9.0	0.0010	0.01	0.01	...
19	Ragweed	13.0	0.0013	0.01	0.01	...
	Group D (Low Nickel Soil)					
20	Wild carrot	9.0	0.0001	0.001	0.003	...
21	Ragweed	13.0	0.0001	0.001	0.003	...
22	Chicory	9.0	0.0002	0.002	0.003	...
23	Red clover	7.5	0.0003	0.004	0.003	...
24	Wild carrot	9.0	0.0001	0.001	0.003	...
25	Ragweed	13.0	0.0001	0.001	0.003	...
26	Chicory	9.0	0.0001	0.001	0.003	...
27	Red clover	7.5	0.0002	0.003	0.003	...

The results obtained from the field test are compared in Table I with results from careful analyses of duplicate samples. These laboratory analyses (column 4, Table I) were made by a nitric-perchloric acid digestion of the dried plant followed by a colorimetric determination of the nickel with dimethylglyoxime (*18*). The figures for per cent nickel in the ash for the laboratory determinations are calculated from the per cent ash and nickel in the dried plant.

In comparing results of the laboratory determinations with those obtained by the field method, the laboratory results cannot be considered true values as the determination of traces by the laboratory procedure may normally be 5 to 10% in error (*20*), and an additional uncertainty is involved in calculating to percentages of nickel in the ash.

In the samples which are at the background level (less than 0.025% in the ash), the test spots are too faint for an accurate comparison with the standard. Calculating the per cent error in these samples would be meaningless; samples 1, 2, 14, and group D are in this classification. For the remaining samples, all results but one have an error of 30% or less compared with the

laboratory results. The amount of nickel in the C and D groups (0.01 to 0.001%) is very small. By increasing the sample size to 30 mg. of ash and holding the volume constant for these groups, the amount of nickel per milliliter in the sample solution was brought into the workable range. Increasing the sample size is also advantageous for samples containing a high percentage of ash, which is equivalent to dilution of nickel in the unit weight of the ash.

DISCUSSION OF PROCEDURE

Ashing. As the simplicity of this procedure depends on the analysis of ash from the fresh plant, it is convenient to report the nickel found in the ash. A serious criticism of this is based on the misleading results caused by contamination. Whereas the results that are on a dry-plant basis are little affected by soil contamination, this contaminant may represent a large part of the sample of ash and so affect the results to a great extent. If the samples are not contaminated, the ash basis for reporting results is satisfactory.

The individual project for which the test is used will determine the number of ash analyses needed in order to calculate the results on a dry-weight basis. In geochemical prospecting, the determination of ash on only a few samples for each species of plant from the same general location should suffice. When plants from different sources are properly ashed in a furnace at controlled temperatures, the results of ash determinations for a given species may differ by as much as 20% of the ash content. In replicate tests of one sample ashed in platinum dishes with no temperature control the ash content varied by 5 to 15%. For this work the percentage ash of each species of plant was averaged and the figure rounded off to the nearest 0.5%.

The fact that the field results tend to be low raises the question of whether nickel is lost in the ashing as nickel carbonyl, although elevated temperatures and high pressures in combination with high concentration of carbon monoxide have been considered necessary for its production. Rankama (*14*) considered the possibility of formation of nickel carbonyl. He concluded from his experimental data that no nickel is lost when an oxidizing atmosphere is maintained. It is more probable that the nickel is held in the complex silicates which may be formed in the ashing (*12*).

Effect of pH. The pH of the test solution should be close to 8.8. Spots made from solutions differing by only 0.4 pH unit differed noticeably. Although the spots at pH of 8.8 are not of maximum intensity, this value was used because, of the various ones tried, it could be duplicated most reliably.

Thymol blue indicator affects the hue of the spot somewhat, making it slightly more yellow than a solution in which no indicator is used. Approximately the same concentration of indicator should be used in the standard as in the sample solutions for greater ease in matching the colors. The intensity of the spot is not affected. Phenolphthalein would be a better indicator as it does not affect the hue, but, because of the solubility of dimethylglyoxime in alcohol, it is not recommended.

Silica and Citrate Precipitate. The silica in the plant ash solution forms a gel which prevents the passage of the solution through the chromograph. Molybdate was added to the solution to sequester the silica by the formation of soluble silicomolybdic acid. The amount of silica in an ash varies, depending on the species and source of the plant being analyzed. Samples to which the prescribed amount of molybdate has been added can still be passed through the chromograph after standing for 4 days. This indicates that the amount added was adequate to form a complex with the silica in solution.

If the solution is passed through the chromograph before the calcium citrate has had time to settle out, a white layer will probably form over the top of the nickel spot, making it appear paler than it actually is. The calcium citrate is a crystalline precipitate and does not occlude nickel; nickel added to sample solutions and to standard solutions with an excess of calcium was recovered quantitatively.

Modifier Ions. Stevens and Lakin (*19*) found that the induced precipitation of nickel dimethylglyoxime caused by the presence of modifier ions made it necessary to add soil solutions to their standards for correct results. In the case of plants the selection of a typical ash for this purpose is a problem. A synthetic plant solution was added in varying quantities to approximate various ashes but better results were obtained from standards without modifier ions added.

CONCLUSIONS

The ashing of the fresh plant and the determination of nickel with the chromograph provide a rapid test which can be used to advantage where extreme accuracy is not necessary. Accuracy of 30% or better can be obtained. By working with a set of 10 samples or more simultaneously one analyst can test a minimum of 30 samples per day.

ACKNOWLEDGMENT

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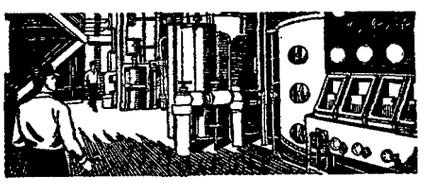

Specificity of Chromatographic Adsorbents

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The object of the present work was to determine what factors were responsible for the adsorption of a given substance on a given adsorbent. It was hoped that it would be possible to construct a mathematical function relating the interactions in the chromatographic system to the movement of the adsorptive zone. Preliminary work indicated that, for the compounds studied, the adsorption could be accounted for in terms of donor-acceptor and hydrogen-bonding interactions and that the carbon side chain acted to decrease adsorption. An arbitrary relationship was set up on this basis; donor values were assigned to oxygen and nitrogen atoms and hydrogen-bonding values to OH and NH hydrogens. The solvents were assigned arbitrary values as competitive agents, and the adsorbent strengths were experimentally evaluated. Rates of movement of chromatographic zones calculated on the basis of assigned values agreed reasonably well with experimental values. Using the methods proposed, it should be possible to evaluate the strength of adsorbents quantitatively, and to determine their specificity toward compounds, so that optimum conditions for separating compounds may be selected.

THIS work was undertaken with the purpose of discovering the forces responsible for reversible adsorption of chemical compounds as manifested in chromatographic behavior. It was hoped that it would be possible to devise a mathematical function relating the properties of adsorbent, developing solvent, and adsorbed compound [called "adsorptive" by Weil-Malherbe (*5*)] to the R value of the substance (see Nomenclature). The authors feel that these efforts have been successful to the extent of a first approximation.

As yet most of the theoretical treatments of the chromatographic process have been based on the adsorption isotherm of the adsorbed substance and have given relationships between the shape and rate of movement of zones and the isotherm constants. The authors have attempted to relate these constants to the structure of the adsorbed compound and to empirical donor, acceptor, and hydrogen-bonding strengths of the adsorbent and developer. Experiments have indicated that interactions involving the above variables may suitably account for the behavior of most common adsorbed compounds, and that it is possible in a large number of cases not only to calculate the order of magnitude of the R value, but even to achieve considerable accuracy in these calculations.

The authors hope that this approach to chromatography will provide a good method for expressing the strength and specificity of adsorbents, for translating results obtained with one lot of adsorbent to those obtained with another, and for discovering new adsorbents with special properties.

NOMENCLATURE

R = the ratio of rate of movement of the adsorbed compound in the column to the movement of the developing solvent in the column; R_L (*4*) [or R_F (*2*)] applies to the front edge of the zone, R_T applies to the rear edge
f = the proportionality factor in an adsorption isotherm such that the amount of substance adsorbed on the adsorbent in equilibrium with one unit volume of solvent is obtained by multiplying the concentration in solution by f. The value of f may vary with concentration
k = an equilibrium constant for the adsorption reaction
s = surface area of the adsorbent in terms of moles per unit of adsorbent as defined for f
T_s = statistical average time an adsorptive particle spends in solution between adsorptions
T_a = statistical average time an adsorptive particle spends on the adsorbent during each adsorption
M_{sc} = applies to the sum of the molecular weights of all side chains in an adsorptive molecule
D = donor strength of substance in respect to electron pair. D_a refers to the adsorbent; D_d, to the developer; D_s, to the adsorbed compound. These subscripts are also applied similarly to terms listed below
A = acceptor strength of a substance for an electron pair
D^H = donor strength in terms of an electron pair donated to a hydrogen atom in hydrogen bond formation
H = acceptor action of a hydrogen-bonding hydrogen for an electron pair.

DISCUSSION OF METHOD

The compounds used as adsorbed compound in this work were classified according to functional group as follows: electron donor, electron acceptor, hydrogen-bond hydrogen acceptor, hydrogen-bond hydrogen donor, odd-electron, ionic, polar, and miscellaneous. Practically all of the compounds which are stable and sufficiently soluble in organic solvents for chromatography belong to the electron donor, hydrogen donor or acceptor, polar, or miscellaneous groups. Experimental results indicate that of the adsorbents studied the last two classes are of minor importance.

The proportionality factor in the adsorption isotherm is a measure of adsorption affinity; in dilute solutions the following relations may be shown between this factor and other terms:

$$f = ks = T_a/T_s = (1 - R)/R$$

It may also be shown (*2, 3*) that $R = 1/(f + 1) = T_s/(T_a + T_s)$. The evaluation of T_s and T_a in terms of the variables affecting these terms will probably furnish the best approach to the accurate description of the chromatographic process since a considerable simplification of the problem is accomplished by the separation of variables. Preliminary examination indicates that this process will be time consuming and, although more accurate, will probably not displace the approximate treatment given below.

Both T_a and T_s as well as f are functions of the energy of adsorption which may be expressed as a donor, acceptor, and hydrogen-bond interaction between adsorbent and the adsorbed compound, modified by the developing solvent. Experimental results have indicated that on the adsorbents used here increased size of the side chain decreases the adsorption affinity; consequently, the hypothesis was made that the adsorption affinity could be expressed as:

$$f = \left(\frac{1}{M_{sc}}\right)\left[\left(\frac{A_a D_s}{D_d}\right) + \left(\frac{D_a A_s}{A_d}\right) + \left(\frac{D_s^H H_s}{H_d}\right) + \left(\frac{H_a D_s^H}{D_d^H}\right)\right] = \frac{(1 - R)}{R}$$

Terms for other interactions may be added as necessity indicates.

In using this approach to adsorption affinity it was necessary to

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Table I. Interaction Tendencies of Substances

	A	*D*	*DH*	*H*
Developers				
Petroleum ether	1[a]	1[a]	...	1
Benzene	25	5.8	...	4.3
Adsorbents				
Special Filtrol	14,000	1,333	1,300	...
Merck reagent silicic acid	4,800	2,570	120	...
Florisil	2,000	1,160	260	...
Merck heavy powder calcium carbonate	33	224	26	...
Calcium acid phosphate dihydrate	77	...	42	...
Magnesium oxide	47	3,350	190	...
Calcium hydroxide	40	11,500	23	...
Adsorbed compound				
Amino N	...	1.00[a]	...	...
Alkyl B	1.00[a]	...	...	...
Alcohol H	...	...	...	1.00[a]
Alcohol O	...	0.17	...	...
Acid or ketone O	...	0.20	...	...
Nitro group	...	0.04	...	...
Aromatic ring	...	0.002	...	...

[a] These values are arbitrarily assumed.

set up some rules for operating. It was first assumed that the electron pair on a tertiary amino nitrogen atom (triethylamine) represented one electron donor unit, an unfilled orbital on a boron-alkyl boron (tributyl boron) was taken to be one acceptor unit, and the hydrogen of an alcohol (ethyl alcohol) was taken to be one hydrogen-bond hydrogen donor unit.

After a consideration of the uncertainties in regard to the surface conditions on adsorbents it was decided to evaluate empirically the donor, acceptor, etc., strengths of these substances. Petroleum ether and benzene were used as developing solvents: here, arbitrary assignments of interaction values were made to give petroleum ether one unit value; the benzene value was determined by measurements of the R value of a given adsorbed compound on an adsorbent from both benzene and petroleum ether, in this way a ratio such as $D_{\text{benzene}}/D_{\text{petroleum ether}}$ could be obtained. For substances such as alcohols, ketones, etc., it was necessary to evaluate empirically donor strength in reference to the amines. In the assignment of acceptor strength to adsorbents it was not always possible to use the amines as these often were too strongly adsorbed; here a weaker donor such as a ketone could be used in the evaluation. Finally, it should be clearly understood that the scales for the adsorbed compound, the adsorbent, and the developer are different and that they are related only by the process of evaluation.

The following rules were needed for calculation of R values from the interaction tendencies assigned to the components of the system:

1. In aromatic amines the donor strength of the nitrogen was divided by the ratio of the total number of most important resonance forms to those showing the electron pair on the nitrogen atom.
2. An internally bound hydrogen (in hydrogen bond) was disregarded; this included the acid hydrogen in case of dimers.
3. The H_a interaction of the adsorbents was not isolated and is included in the A_a value; therefore, this term is dropped from adsorption affinity calculations.

Table I lists the values for interaction tendencies assigned in this work, and is subject to revision. These interaction tendencies were calculated on the basis of data in Table II as indicated there. These values apply only to the particular batches of adsorbents used in this work and must be evaluated for each new batch of adsorbent used, although a given adsorbent—viz., Merck reagent silicic acid—will not vary appreciably in order of magnitude of its interaction tendencies.

A comparison between experimental R values for some substances and R values calculated from data in Table I is shown in Table II for a petroleum ether developer and in Table III for a benzene developer. In general, the agreement is good as to order of magnitude, and in many cases accurate values were calculated. Refinements in these calculations may be made by considering

Table II. Calculated and Experimental R_L Values for Various Adsorbed Compounds on Certain Adsorbents Using Petroleum Ether as the Developer

Adsorbed Compound	Special Filtrol		Silicic Acid		Florisil		Calcium Acid Phosphate		Magnesium Oxide		Calcium Hydroxide		Calcium Carbonate	
	Calcd.	Exptl.	Calcd.	Exptl.	Calcd.	Exptl.	Calcd.	Exptl.	Calcd.	Exptl.	Calcd.	Exptl.	Calcd.	Exptl.
n-Butylamine	<0.01	<0.01	0.01	<0.01	0.02	<0.01	0.25	<0.01	0.12	0.45	0.40	0.48	0.40	0.64
Triethylamine	<0.01	<0.01	0.02	<0.01	0.04	<0.01	0.52	0.24	0.64	0.83	0.68	0.83	0.71	0.85
Aniline	0.01	<0.01	0.05	0.07	0.07	0.07[a]	0.43	0.48	0.16	0.43	0.58	0.58[a]	0.56	0.71
Ethylaniline	0.02	<0.01	0.07	0.16	0.12	0.13	...	...	0.35	0.59	...	...	0.76	0.89
Dimethylaniline	0.03	<0.01	0.08	0.27	0.17	0.17	0.83	1.00	0.90	0.84	0.91	0.95	0.93	0.99
Diethylaniline	0.04	<0.01	0.11	0.27	0.22	0.21	0.88	1.00	0.92	0.97	0.93	1.00	0.94	0.99
Diphenylamine	0.04	<0.01	0.16	0.24	0.22	0.20	0.75	1.00	0.45	0.39	0.84	0.68	0.96	0.91
Triphenylamine	0.61	0.15	0.82	0.54	0.53	0.49	0.99+	1.00	0.99+	1.00	0.99+	1.00	0.99	1.00
Methanol	<0.01	<0.01	0.02	<0.01	0.02	<0.01	0.21	0.24[a]	0.07	<0.04	...	...	0.32	0.32
Ethyl alcohol	<0.01	<0.01	0.03	0.03[a]	0.05	<0.01	0.35	0.84	0.15	0.13[a]	0.49	<0.01	0.48	0.48[a]
Phenol	0.02	0.02[a]	0.08	0.04[b]	...	...	0.58	0.14	0.28	<0.01	0.72	<0.01	0.70	0.44
Acetone	0.01	<0.01[b]	0.03	0.03	0.07	0.07[b]	0.66	0.66[b]	0.76	0.76[b]	0.79	0.79[b]	0.82	0.82[b]
Benzoic acid	...	...	...	...	0.10	0.06	...	...	...	...	...	...	...	...
Nitromethane	0.03	0.03	0.07	0.08	0.20	0.10	0.98	0.76	0.90	0.38	0.91	0.01	0.93	0.88
Nitrobenzene	0.12	0.09	0.29	0.31	0.50	0.18	0.99+	0.93	...	...	...	...	0.98	0.95

[a] This substance was used in assigning the D_a^b value to the adsorbent; each hydrogen was taken as one H_s unit.
[b] This substance was used in the assignment of acceptor strength to the adsorbent (D_s was taken as 0.20 for acetone).

Table III. Calculated and Experimental R_L Values for Various Adsorbed Compounds on Certain Adsorbents Using Benzene as the Developer

Adsorbed Compound	Special Filtrol		Silicic Acid		Florisil		Calcium Acid Phosphate		Magnesium Oxide		Calcium Hydroxide		Calcium Carbonate	
	Calcd.	Exptl.	Calcd.	Exptl.	Calcd.	Exptl.	Calcd.	Exptl.	Calcd.	Exptl.	Calcd.	Exptl.	Calcd.	Exptl.
n-Butylamine	0.02	<0.01	...	...	0.11	<0.02	0.63	<0.01	...	...	0.78	0.79	...	...
Aniline	0.06	<0.01	0.22	0.31	0.27	0.41	0.77	1.00	0.46	0.94	0.86	1.00	0.85	1.00
Dimethylaniline	0.14	<0.01	0.34	0.66	0.55	0.70	...	...	0.98	1.00	0.98	1.00	...	...
Diethylaniline	...	...	...	...	...	...	0.98	1.00	...	...	0.99	1.00	0.99	1.00
Methanol	0.02	0.01	0.08	0.13	0.11	0.11	...	...	0.17	0.49	...	...	0.68	0.75
Ethyl alcohol	0.04	0.06	...	...	...	...	0.71	0.64	0.39	0.58	0.82	0.35	0.80	0.80
Phenol	0.10	0.28	0.31	0.29	0.39	0.40	0.85	0.82	0.63	1.00	0.92	<0.01	0.92	0.83
Acetone	0.06	0.11	0.15	0.17	0.30	0.25	0.92	1.00	0.95	0.98	0.95	1.00	0.96	1.00
Acetic acid	0.02	0.08	0.04	0.03	0.11	0.14	...	...	...	...	...	...	...	...
Benzoic acid	0.08	0.09	0.23	0.14	...	...	...	...	...	...	...	...	...	...
Nitromethane	0.13	0.41	0.31	0.48	0.51	0.70	...	...	0.99	0.91	...	...	...	...
Nitrobenzene	0.43	0.66	0.70	1.00	0.85	0.72	0.99	1.00	...	...	...	...	...	...

steric effects, etc. In a few instances, where calculated values differ greatly from the experimental values, salt formation is an obvious explanation as illustrated by the failure of phenol to move on calcium hydroxide.

An example of the calculation of the R value for ethylaniline on Florisil (a synthetic magnesium silicate, Floriden Co., 200/300 mesh) is given below.

D_{NH_2} = 1. Fraction showing electron pair on nitrogen in resonance structures = 1/4; for ethylaniline use D_s = 0.25
H_s for one hydrogen = 1
M_{sc} = $C_6H_5 + C_2H_5 = 77 + 29 = 106$
D_d for petroleum ether is by definition 1
H_d for petroleum ether is by definition 1
A_a for Florisil was determined as 2000
D^H for Florisil was determined as 260

$$= \left(\frac{1}{M_{sc}}\right)\left(\frac{A_a D_s}{D_d} + \frac{D_s^H H_s}{H_d}\right) = \left(\frac{1}{106}\right)\left(\frac{2000 \times 0.25}{1} + \frac{260 \times 1}{1}\right) = 7.15$$

$$R = \frac{1}{f+1} = \frac{1}{7.15+1} = 0.12$$

EXPERIMENTAL

Extensive data concerning the flow rate, etc., of the adsorbents used here will be published elsewhere in connection with a survey of adsorbents. In general, the flow rates of the columns (75 × 9 mm.) were between 3 and 25 mm. per minute; however, this is relatively unimportant in this work since Austin and Shipton (*1*) have shown R to be independent of flow.

The R values were determined with 0.01 M solutions of the adsorptives, using an initial volume sufficient to form a layer of solution 1 cm. thick above the adsorbent in the chromatographic tube. In this region the rate of movement is nearly independent of concentration and initial volume (*3*).

The solvents were thiophene-free reagent grade benzene and specially purified Skellysolve B.

Substances used as adsorbed compounds were of the highest purity grade and were repurified where necessary.

The adsorbents are listed in Table I. They were used as obtained from the manufacturer.

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Effect of Side Chain on the Chromatographic Adsorption of Some Ketones

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A COMPLETE series of straight-chain methyl ketones from C_3 to C_{20} was used to determine the effect of the side chain on chromatographic behavior. Certain periodic increases in adsorption affinity were observed with increasing chain length, although the general trend was toward decreasing adsorption affinity.

Earlier work in these laboratories has indicated that adsorption on the usual adsorbents, excluding charcoal (*1*), is generally due to chemical-type interactions between the adsorbed compound and the adsorbent. These interactions appear to be explainable on the basis of the relative electron-donating and accepting tendencies of the adsorbent and of the functional group of the molecule adsorbed, and they have been represented by an equation of the type (*5*):

$$f = (1 - R)/R = (1/M_{sc})(k) = k(M_{sc})^{-1}$$

where f is the adsorption affinity; R, the ratio between the distance moved down the adsorbent column by the adsorbate zone and solvent, respectively; M_{sc}, the molecular weight of the side chain attached to a functional group such as hydroxyl, amino, or ketone; and k, the summation of the interaction tendencies between adsorbent and adsorbed compound.

As indicated by this equation it was at first assumed that there was a simple reciprocal relationship between the R value and the molecular weight of the members of a given homologous series (*4*). There was, however, little data on which to base this assumption and so it was decided to prepare and determine R values of several homologous series in order to provide data for determining the proper mathematical relationship between the R value and side chain molecular weight. Data will be presented for a series of aliphatic straight-chain methyl ketones from C_3 through C_{19}, and certain conclusions from these data will be pointed out. While, in general, the data support the type of equation postulated above, it was found that the molecular weight relationship is somewhat more complex than was assumed at first. These facts can probably best be brought out by a brief discussion of each of the accompanying graphs and tables of the experimental data.

DISCUSSION OF GRAPHS AND TABLES

Figure 1 is a plot of R_L (leading edge) values as ordinates versus the total number of carbon atoms in the various ketones as abscissa when calcium carbonate (Merck heavy powder) was used as the adsorbent and petroleum ether as the developing solvent. Only weak and apparently general adsorption results so that no conclusions can be drawn other than that this adsorbent and, presumably, others of its type such as lime, magnesium oxide, or alumina are of little value in effecting separations of the ketones tested even when a very weak developer is used. When a stronger developer such as benzene was used the R values were, of course, 1.00, indicating no adsorption.

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In connection with work in these laboratories on the specificity of chromatographic adsorbents it became desirable to determine the effect of the organic side chain on the behavior of homologous series of compounds. The first series of compounds selected for this study were the methyl ketones with straight-chain side chains. The complete series, from three to twenty carbon atoms, was studied and a few other miscellaneous ketones were included to indicate other trends. An unexpected result of this study was the observation of periodic increases in adsorption affinity with increasing length of side chain. No clear interpretation of the observed phenomena seems obvious at present, but it is suggested that the periodic increases in adsorption affinity may be due either to fit-patterns or to molecular configuration of the adsorbed molecule.

When using silicic acid (Merck heavy powder reagent) as the adsorbent and benzene as developing solvent a steady rise in R value was found with increasing side-chain length in the homologous series, resulting in an almost smooth curve (Figure 2, upper). There are slight irregularities in this curve, however, at C_9 and C_{14}, which are believed significant as will be discussed later. The so-called miscellaneous ketones were included in these plots to show that structural relationships must also be considered in predicting R_L values. While space will not permit a discussion of these ketones in detail, it will be seen that branched chain compounds are adsorbed less and cyclic aliphatic ketones more than the corresponding straight-chain methyl ketones. Both of these facts appear to point out the importance of steric effects in chromatographic adsorption.

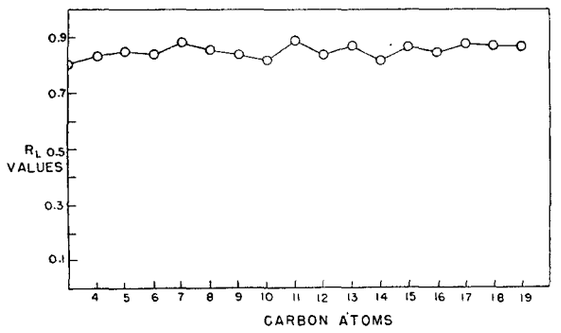

Figure 1. R_L Values of Straight Chain Methyl Ketones from 0.01 *M* Petroleum Ether Solutions on Calcium Carbonate

Figure 2, lower, shows that on Florisil (a synthetic magnesium silicate, Floridin Co., 200/300 mesh) there is again a steady, though slower, rise in R value as the side-chain molecular weight is increased. The deviations from a smooth curve which were noticed in the preceding figure are now seen to be much greater at C_{14} and, as will be shown in the succeeding figures, cannot be ignored as being within the limit of experimental error. The R_L value depressions found with Florisil are in about the same places as with silicic acid. On florisil the R_L values tend to approach a maximum value of about 0.45 so that for the higher members of the ketone series Florisil is a stronger adsorbent than silicic acid, while for the lower members the reverse is true. The miscellaneous ketones fall about the curve essentially as was noted for silicic acid.

Figure 3 shows a plot of the trailing edge R values (R_T) (uncorrected for the distance moved by the solvent during the time that the initial volume of solution was added to the column) for all three adsorbents studied. This graph is included here, although, in general, R_T values appear to be more sensitive to the "dips" in the R value curves than are the leading edge values and, moreover, appear to point to a periodicity of these dips—for example, silicic acid and Florisil both show depressions in R_T values at C_8, C_{11}, C_{14}, and C_{17}. In addition, work with an apparently impure sample of C_{20} (melting point, 46° to 49° C.; literature, 58° to 59° C.) indicated that another large dip probably occurs at this point but these data were not included here because of uncertainty as to the purity of this material. It appears, however, that there are rather large depressions in R value at C_8, C_{14}, and C_{20}, with smaller ones at C_{11} and $_{17}$.

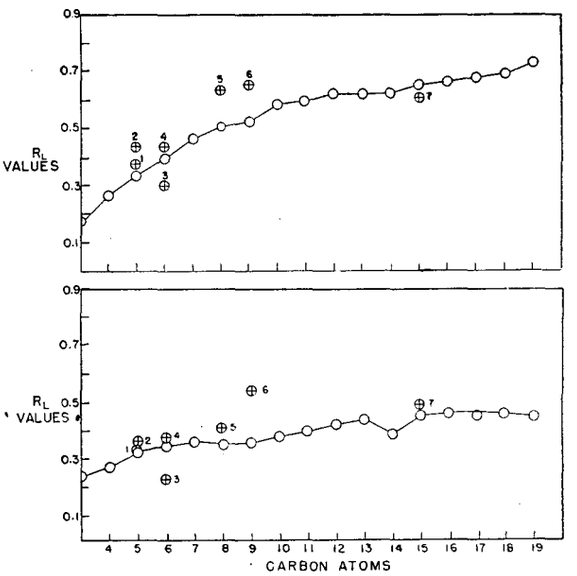

Figure 2. R_L Values of Ketones Form 0.01 *M* Benzene Solutions on Silicic Acid (*upper*) and on Florisil (*lower*)

○, straight-chain methyl ketones; ⊕, miscellaneous ketones: 1, methyl isopropyl; 2, diethyl; 3, cyclohexanone; 4, methyl isobutyl; 5, methyl phenyl; 6, ethyl phenyl; 7, dibenzyl

Figure 4 brings out the effect of drying (*3*, *6*) the adsorbents (130° C. for 72 hours) before using for R_L value determinations. The dips are accentuated by this treatment and dried silicic acid is a stronger adsorbent than dried Florisil throughout the homologous series. It seems apparent from these curves that water adsorbed on these materials greatly decreases their general strength as well as their ability to separate the homologous ketones.

In work of this sort the question naturally arises as to whether the small deviations from a smooth curve observed are significant, or whether they are simply due to experimental error. Therefore, statistical data were taken, Table I, which showed that the probable error of a single R_L value determination was about 3%, and that of an R_L determination about 6%. Since at least two values were taken for all points shown on the preceding curves and from 6 to 10 determinations made about the points where dips were noted, it will be seen that the data must be considered reproducible to at least 0.01 R value. Even so, however, only the large depressions in R_L value noted at C_{14} may be regarded as "statistically reliable" (*2*), but since these repeated depressions have also been observed in other homologous series now under in-

Table I. Statistical Treatment Showing Reliability of R Value Determinations

(0.01 M benzene solution of methyl dodecyl ketone)

Run No.	Zone Limits[a]	Column Length, Mm.	R Values R_L	R_T
1	19–30	80	0.38	0.24
2	20–31	79	0.39	0.25
3	24–35	85	0.41	0.28
4	24–33	84	0.39	0.29
5	19–33	79	0.42	0.24
6	18–32	80	0.40	0.23
7	23–34	82	0.42	0.28
8	24–35	86	0.41	0.28
9	26–37	96	0.40	0.28
10	22–31	82	0.38	0.27
		Average	0.40	0.26
		Probable error (2), r	0.011	0.016
		Probable % error	2.8	6.2

[a] Measured in millimeters from top of column.

vestigation, the authors have felt compelled to include these smaller R value depressions in their discussion.

As about half of the ketones studied in this work were synthetic samples specially prepared for this study, and the rest were commercial samples which were purified where necessary, it seemed unlikely that concentration effects could be the cause of the R_L value depressions noted. The data plotted in Figure 5 were taken, nevertheless, to be certain that small errors in making up the 0.01 M solutions used could not cause variations in R_L value of the order observed. The flatness of these curves would seem to eliminate this possibility. These curves remain fairly flat even at concentrations of 0.03 and 0.04 M as in future work it may be found desirable, or even necessary, to work in this range of concentrations.

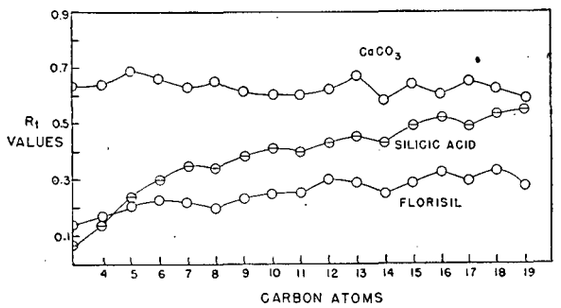

Figure 3. R_T Values for Methyl Ketones from 0.01 M Benzene Solutions on Florisil and Silicic Acid, and from 0.01 M Petroleum Ether Solutions on Calcium Carbonate

The main object of this study was to provide a mathematical formulation of the effect of side chain on chromatographic adsorption. The data in Figure 6 show the approximate linearity of a log-log plot of "adsorption affinity" $[(1 - R)/R]$ versus the molecular weight of the side chain of the straight-chain methyl ketones. The general equation of these straight lines are of the type predicted but it was found that the exponent of the M_{sc} is not -1 as was assumed in this first approximation. Instead, the value of this exponent depends on the adsorbent used and perhaps also upon the particular homologous series under study and other factors. The k values determined by this type of plot represent the summation of all of the interaction tendencies causing adsorption and should prove useful in assigning values of donor and acceptor strengths to adsorbents and adsorbed compounds in future work. The fact that there is a constant value for the homologous series seems to indicate the general correctness of the assumption that the functional group of a molecule is of primary importance in causing its adsorption.

Further work is necessary to establish the significance of the R value depressions revealed in this work. Preliminary work in these laboratories has indicated that the location of these dips is dependent only on the nature of the adsorbed molecule, although the magnitude of the dips is affected by the adsorbent. The authors are presently continuing work along these lines.

EXPERIMENTAL

All of the chemicals used as adsorbed compounds in this work were fairly well known compounds of which almost half were commercial samples. These were purified by standard techniques where necessary. Table II summarizes the sources of all of these materials. As indicated in this tabulation it was found necessary to synthesize most of the straight-chain methyl ketones used and

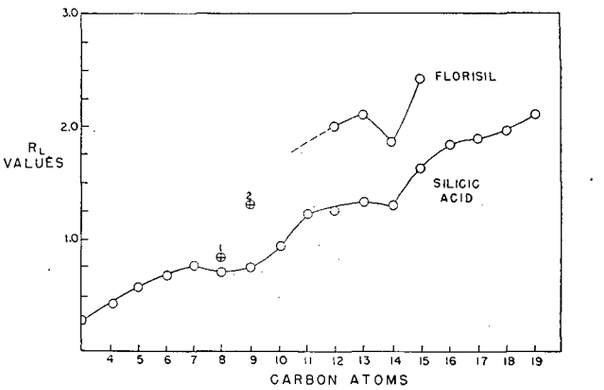

Figure 4. R_L Values of Ketones from 0.01 M Benzene Solutions on Dried Adsorbents

○, straight-chain methyl ketones; ⊕, miscellaneous ketones on silicic acid: 1, methyl phenyl; 2, ethyl phenyl

Table II. Source of Pure Ketones Used in This Work

No. Carbon Atoms	Ketone	Source of Pure Chemicals
3	Methyl methyl	Eastman Kodak White Label
4	Methyl ethyl	Eastman Kodak White Label
5	Methyl propyl	Eastman Kodak practical, redistilled
5	Methyl isopropyl	Eastman Kodak White Label
5	Diethyl	Eastman Kodak White Label
6	Methyl butyl	Synthetic from calcium valerate + calcium acetate
6	Methyl isobutyl	Eastman Kodak White Label
6	Cyclohexanone	Eastman Kodak practical, redistilled
7	Methyl pentyl	Eastman Kodak White Label
8	Methyl hexyl	Eastman Kodak practical, redistilled
8	Methyl phenyl	Eastman Kodak White Label
9	Methyl heptyl	Synthetic from heptyl magnesium bromide + acetyl chloride
9	Ethyl phenyl	Eastman Kodak White Label
10	Methyl octyl	Synthetic from octyl cadmium bromide + acetyl chloride
11	Methyl nonyl	Synthetic from calcium caproate + calcium acetate
12	Methyl decyl	Synthetic from decyl cadmium bromide + acetyl chloride
13	Methyl undecyl	Synthetic from calcium laurate + calcium acetate
14	Methyl dodecyl	Synthetic from dodecyl cadmium bromide + acetyl chloride
15	Methyl tridecyl	Synthetic from calcium myristate + calcium acetate
15	Dibenzyl	Synthetic from phenylacetic acid + acetic anhydride
16	Methyl tetradecyl	Synthetic from tetradecyl cadmium bromide + acetyl chloride
17	Methyl pentadecyl	Synthetic from methyl magnesium iodide + palmityl chloride
18	Methyl hexadecyl	Synthetic from hexadecyl cadmium iodide + acetyl chloride
19	Methyl heptadecyl	Synthetic from calcium stearate + calcium acetate
20	Methyl octadecyl	Synthetic from octadecyl cadmium bromide + acetyl chloride

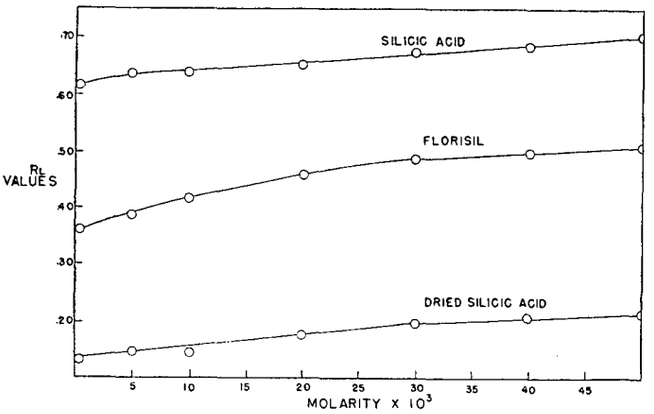

Figure 5. Effect of Concentration on R_L Values of Methyl Dodecyl Ketone Developed with Benzene

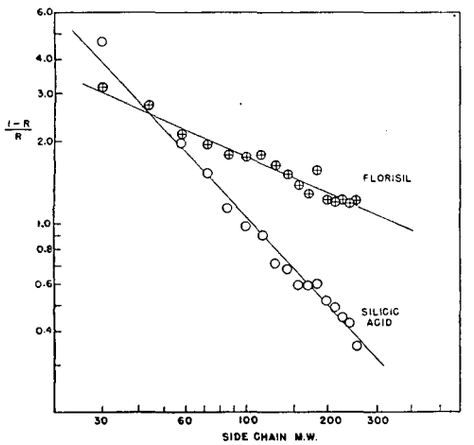

Figure 6. Adsorption Affinities of Straight-Chain Methyl Ketones

General equation. $(1 - R)/R = k\,(M_{SC})^n$
Fluorisil. $n = -0.45$, $k = 13.9$
Silicic acid. $n = -1.10$, $k = 170$

these materials were prepared by standard methods found in the literature. As only very small quantities of these materials were required most of them were prepared by the relatively inefficient Grignard method from the commercially obtainable straight-chain halides. Chromatographic methods were found very valuable in removing the by-product materials from the desired ketone. These by-products were generally the corresponding hydrocarbons which were removed by chromatographing on silicic acid using petroleum ether as solvent and developer. By this method the hydrocarbons were washed through the adsorbent into the filtrate leaving the almost pure ketone near the top of the chromatographic column. The ketones were then eluted and recovered in the pure state by recrystallization where practicable. In other instances the liquid ketones were developed farther down the adsorbent with benzene and obtained pure by elution and evaporation of the eluate. In all cases the physical properties, either boiling point or melting point, checked closely with the values given in the literature except as noted in the case of methyl octadecyl ketone (C_{20}).

The 0.01 M solutions used in the R value determinations were made up either by pipetting or by weighing. The greatest error possible by these methods was estimated to be about 5%. The R values were determined by pipetting πr^2 ml. (r = inside radius of the chromatographic tube in centimeters) of these standard solutions on to the top of the adsorbent in a No. 1 chromatographic tube (inside diameter, 9 mm.; length, 90 mm.) packed to a height of about 75 mm. with the dry adsorbent under full water pump vacuum. As the last of this initial volume of 0.01 M solution was entering the top of the adsorbent column, additional pure solvent was added until the leading edge of the solvent just reached the bottom of the packed tube. Development was then stopped by releasing the vacuum, the packed column extruded, and the leading and trailing edges of the zone located by streaking either with dilute alkaline permanganate solution or with a saturated solution of 2,4-dinitrophenylhydrazine in 2 M hydrochloric acid. The permanganate test showed up as a green zone against a purple background color, and the 2,4-dinitrophenylhydrazine gave an orange zone on a yellow background. The R values were calculated as the ratio between the distance moved by the leading or trailing edge of the zone from the top of the adsorbent column, to that distance moved by the solvent—i.e., the column length. Since the trailing edge of these zones could not begin to move from the top of the adsorbent until development with pure solvent was commenced, the apparent R_T values obtained in this way were, of course, lower than their actual rate of movement which approximated that of the leading edges.

As all of the important data obtained in this study are shown in the accompanying graphs with sufficient accuracy for anyone desiring the numerical values, no tabular data will be included here.

SUMMARY

The effect of side chain on the strength of chromatographic adsorption of some ketones on calcium carbonate, silicic acid, and Florisil has been studied. Several types of side chains were investigated as well as a complete series of straight-chain methyl ketones from C_3 to C_{20}. The rates of movement of the adsorbate zones down the adsorbent columns were used as a measure of adsorption strengths.

It was found that, in general, the ketones appeared to be adsorbed due to interactions between their carbonyl oxygen and the adsorbent, and that the heavier the side chain the smaller was the adsorption strength. Certain exceptions were noted, however, where the addition of a $-CH_2$ group to the side chain decreased the rate of zone movement appreciably. These decreases in R value seemed to be periodic within the homologous series and preliminary work has indicated that the location of these R-value depressions is dependent only on the nature of the adsorbed molecule, although their magnitude is affected by the adsorbent. Not only the mass of the side chain but also its nature is of importance in determining adsorption strength.

The effect of concentration on R value was studied and found to be slight up to 0.05 M, and data were given to show the statistical reproducibility of measuring the rate of zone movement (R values) as a measure of adsorption strengths.

ACKNOWLEDGMENT

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Determination of Traces of Water Vapor in Gases

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A general method for the trace determination of water vapor in condensable gases, particularly hydrocarbon gases, has been sought. The novel method here described is based on measurement, with a thermistor bridge, of the temperature rise produced when a stream of the test gas is passed over solid calcium hydride. The apparatus is readily assembled from normally available components, calibration is easily accomplished with a dynamic blending system, and a single determination requires but 15 minutes. The measurement is unaffected by (or readily compensated for) wide and rapid fluctuations in the composition of the matrix gas, and the method may be used for the determination of 0.001 to 0.1 volume % of water vapor. Water determinations formerly accomplished with difficulty can be conducted by this method; and the thermistor bridge technique should find important application in a number of analogous trace determinations based on thermometric measurements.

A GREAT variety of methods for the determination of the water content of gases has been developed. However, there is currently available no completely satisfactory method for the estimation of minute traces of water vapor in gases in general, and in hydrocarbon gases in particular. Yet the need for such a method has become ever more acute—for example, in connection with the measurement of the aqueous content of a number of gases involved in industrial processes based on the use of catalysts that are readily poisoned by water. The method described herein presents certain advantages in that (*a*) it is easily applied to condensable (as well as noncondensable) gases; (*b*) it is semispecific for water; (*c*) though primarily intended for the analysis of batch samples, it is at least potentially serviceable in semicontinuous automatic analysis of streaming gases; and (*d*) the apparatus is readily assembled from commonly available components.

It seemed desirable to base the determination on some specific or semispecific chemical property (reaction) of water that could be followed in terms of an easily measured physical property of the system. Thus, water enters as a reactant into a number of fairly selective and highly exothermic processes that involve the progressive destruction of solid reagents—for example, phosphorus pentoxide and metallic sodium. To be useful in the proposed determination it is essential that the reactivity of the solid reagent is not substantially impaired by its progressive exhaustion, and by the accumulation of solid reaction products. Solid calcium hydride appeared to be the most promising of a small number of possible reagents. At room temperature this material is substantially inert to most readily volatile materials (except for acids, alcohols, etc.), but it reacts rapidly and quantitatively with minute traces of water. Inasmuch as some 25,000 calories are released for each mole of water involved, the reaction is sufficiently exothermic to be followed by thermometric measurements. Of particular importance is the fact that the calcium hydroxide formed in the reaction is nonadherent; much of it is blown away by a rapid gas stream, and the activity of the surface is maintained until the hydride is practically exhausted (*3*).

To measure the small temperature differences expected in the proposed determination, multijunction couples and/or fairly elaborate electrical equipment would ordinarily be required. The use of a thermistor bridge appeared to be a more promising possibility. Such a bridge retains the desirable feature that the measurement of the temperature rise is unaffected by small changes in room temperature; it is, potentially, a much more sensitive instrument than a thermocouple, and it involves only the simplest and least expensive electrical equipment.

The resistance, R, of a thermistor at any temperature, T, is calculable from the relation, $R = a.e^{b/T}$, where a and b are constants (*2*). The constant, b, is characteristic of the material used in the thermistor and, in the authors' specimens, had a common value of 3700° K. If two thermistors (designated by the subscripts 1 and 2) are maintained at different temperatures,

$$\frac{R_1}{R_2} = \frac{a_1 e^{b/T_1}}{a_2 e^{b/T_2}} = \frac{a_1}{a_2}\, e^{b(T_2 - T_1)/T_1T_2} \qquad (1)$$

From this it follows that:

$$T_2 - T_1 = \frac{T_1T_2}{b} \ln \frac{a_2R_1}{a_1R_2} \qquad (2)$$

In the system proposed herein, T_1 and T_2 never differed by more than a degree or so from room temperature which, in turn, commonly fluctuated over a range of not more than a few degrees. Under these conditions, and noting that a_1, a_2, and b are all constants, it is plain that the difference of the temperatures prevailing around the thermistors is a simple function of the ratio of the resistances, which can easily be determined by placing the thermistors in a Wheatstone bridge circuit.

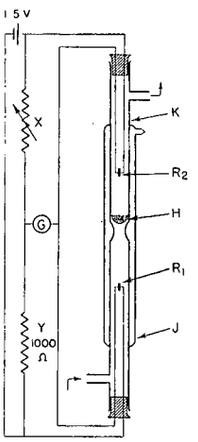

Figure 1. Reaction Cell and Thermistor Bridge

APPARATUS AND PROCEDURE

The heart of the apparatus shown in Figure 1 is based on the design of a related system described by Cohn (*1*).

The greater length of the inner reaction tube, K, was enclosed in a silvered vacuum jacket, J. Tube K was made from a 25-cm. length of 16-mm. tubing, with a median constriction of about 9 mm. in diameter. As a protection against drafts, the unjacketed projections of tube K were wound with two layers of sheet asbestos. The calcium hydride was used in the form of fragments with a mean diameter of about 1 mm. These fragments were easily selected from a partially crushed mass of the commercial (Metal Hydrides, Inc.) substance. One gram of the calcium hydride was introduced into the hemispherical cup, H, formed from 50-mesh platinum gauze. On insertion into the reaction tube, this cup was supported by the shoulders of the constriction. R_1 and R_2 were Western Electric 17A thermistors—disks about 1 mm. thick and 5 mm. in diameter, with a nominal resistance of 1000 ohms. The thermistors were supported, at a distance of about 3 cm. from the calcium hydride mass, by leads of heavy bell wire that passed through the rubber stoppers with which the ends of K were plugged. The rest of the measuring circuit consisted of a dry cell, a dial-decade resistance box, X, a precision wire-wound 1000-

ohm fixed resistance, Y, and a Rubicon box-type galvanometer, G, with a resistance of 10.27 ohms and a sensitivity of 0.002 μa per mm. scale deflection.

The stream of the test gas was passed through the reaction cell at a rate of 1.3 liters per minute, and the variable resistance, X, was set (usually only to the nearest ohm) to bring the bridge into balance.

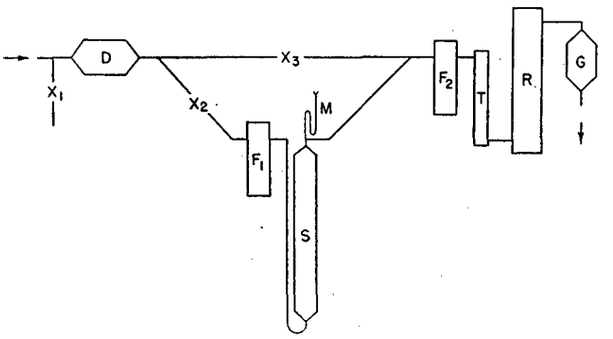

Figure 2. Calibration Train

The circuit design then requires that $R_2/R_1 = X/Y = X/1000$. The temperature rise must be a simple function of R_2/R_1; hence the temperature rise must also be a simple function of the value of X which, after calibration, can be taken as an indication of the water content of the test gas. The observations are essentially unaffected by the thermal effects of the thermistor bridge current. Under the authors' experimental conditions the electrical heat output is barely of the order of the heating expected from a gas containing 10^{-4} mole % of water; this concentration is well below the authors' present range of investigation.

CALIBRATION

The operating characteristics of this system were investigated in a series of trials with a number of gases containing various determinate concentrations of water vapor. These test mixtures were prepared with the aid of a dynamic blending system, shown in Figure 2, which is related to that described by Walker and Ernst (*4*). The gas enters at the left, under a positive pressure that can be controlled closely with the aid of the variable leak, X_1. The gas stream passes through vessel D, where it is dried by a packing of Dehydrite followed by calcium hydride. The stream then bifurcates; the major proportion of the gas is by-passed through tap X_3, whereas the rest is routed through tap X_2 to a capillary flowmeter, F_1, and thence to the water saturator, S. The two gas streams are then reunited, and the mixture flows successively through another flowmeter, F_2; through a tube, T, containing a thermometer; through the reaction cell, R (shown in detail in Figure 1); and through the desiccant contained in the guard tube, G, to the vent.

The aqueous content of the mixture delivered at R depends primarily on the aqueous tension in the saturator, which was maintained constant, and on the proportion of wet and dry gas entering the final mixture, which was controlled by suitable manipulation of taps X_2 and X_3. The saturator consisted of a long bead tower, partially filled with water, and immersed in an ice bath. The gas entered at the bottom of the tower, and was precooled during its passage through the ice bath. The column of beads above the water level in the saturator served as an effective spray trap. Walker and Ernst found (*4*) that the gas delivered from their saturator was only 93 to 96% saturated. However, the authors' saturator was more than twice as long as theirs and the maximum flow rate through the saturator described herein was approximately one fifth of their maximum rate. It seemed safe to conclude that the authors' saturating efficiency was close to 100%. With the saturator at 0° C. this corresponds to a concentration of about 0.60% water vapor in an effluent gas at a pressure close to 1 atmosphere.

Table I. Trials with Determinate Samples

Matrix Gas	Volume Percentage of Water	Resistance, X, Ohms	Temperature Rise, ° C.
Oxygen	0.0000	1080.0	0.000
	0.0028	1078.5	0.033
	0.0070	1076.5	0.077
	0.0084	1076.0	0.088
	0.0140	1072.5	0.166
	0.0393	1060	0.447
	0.060	1045	0.79
	0.097	1022	1.32
Butane	0.0000	1080	0.000
	0.0130	1073	0.155
	0.0286	1066	0.312
	0.0324	1063	0.381
	0.0467	1058	0.49
	0.061	1051	0.65
Carbon dioxide	0.0000	1079	0.000
	0.0169	1072	0.155
	0.0345	1063	0.359
	0.063	1050	0.65
Ethylene	0.0000	1080	0.000
	0.0156	1073	0.155
	0.063	1046	0.77
	0.104	1023	1.30
Hydrogen	0.0000	1080	0.000
	0.0355	1062	0.403
	0.0587	1049	0.695

The proportion in which the wet and dry gas streams were mixed, to form final mixtures of determinate low water concentrations, was measured with the capillary flowmeters, F_1 and F_2. Preliminary trials verified that, other things being equal, the reading of each flowmeter was a linear function of the rate of gas flow. Flowmeter F_2 was accurately calibrated with oxygen in a series of trials in which the gaseous throughput in unit time was measured volumetrically. Flowmeter F_1 was then calibrated in terms of the readings of F_2 by passing a stream of gas through the system with tap X_3 completely shut, so that the same quantity of gas flowed through F_1 and F_2. After this calibration it was possible to determine, from the relative readings of the two flowmeters, what proportion of any final mixture consisted of gas that had been routed through the saturator. The final mixture was blended from wet and dry gas streams at pressures equal to each other and to the pressure (calculable from the reading of the small, open-end manometer, M) at which the wet gas left the saturator. The aqueous concentration of the wet gas was determined from the equilibrium vapor pressure of water at 0° C. and the total gas pressure at the exit from the saturator. Hence, knowing the proportion in which the wet and dry gases were mixed, the concentration of water vapor in the final mixture could be calculated.

All the test materials used were C.P. grade tanked gases. With each new gas the same general procedure was followed, except that the total flow was adjusted until the ratio between the pressure drop indicated by the second flowmeter and the pressure drop that had been produced there by oxygen, flowing at a rate of 1.3 liters per minute, was equal to the ratio of the viscosity of the test gas to the viscosity of oxygen. The results obtained are shown in Figure 3 and Table I.

RESULTS

The temperature rises listed in the last column of Table I were calculated with the aid of Equation 2. When a perfectly dry gas is passed through the system there should be no rise in temperature—that is, $T_2 = T_1$—so that $a_2/a_1 = R_2/R_1$. But $R_2/R_1 = X/Y$; thus, in this system, $a_2/a_1 = 1080/1000 = 1.08$. For temperatures only slightly different from room temperature,

$$T_2 - T_1 = \frac{T_2 T_1}{b} \ln 1.08 \cdot \frac{R_1}{R_2} = \frac{(298)^2}{3700} \ln 1.08 \cdot \frac{1000}{X} = 55.2\,(3.0334 - \log X) \quad (3)$$

In the range of the authors' experimental data the difference $(T_2 - T_1)$ is an almost perfectly linear function of the resistance, X, as may be seen from the double graduation of the horizontal axis of Figure 3. This happy circumstance permits the preparation of undistorted calibration curves of water concentration as a function of the measured resistance, X, and there is no necessity to calculate the actual temperature rises corresponding to these measurements.

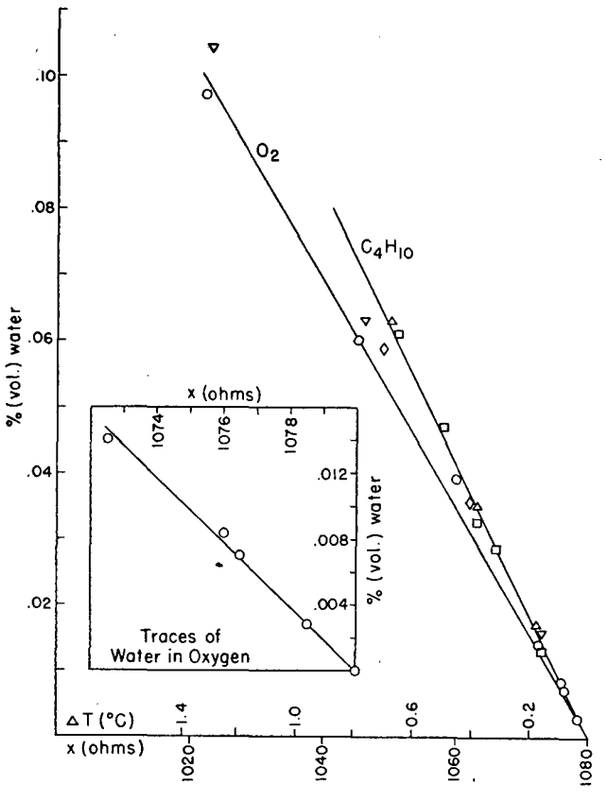

Figure 3. Graph of Calibration Data

◇ Hydrogen
○ Oxygen
□ *n*-Butane
△ Carbon dioxide
▽ Ethylene

The equipment was fairly rapid in its response. After a large change in water concentration a reading within 0.002% water of the final value could be obtained in 10 minutes, and the final value in 15 minutes; with smaller changes of water concentration the corresponding times were 5 and 10 minutes. The final readings were steadily maintained for periods of hours. Thus the progressive exhaustion of the calcium hydride charge, provided it did not proceed too far, was without effect on the experimental results. The water content of the gas under test determines the frequency with which the charge has to be replaced. For concentrations of the order of 0.1%, the maximum studied, the useful lifetime of the charge exceeded 1 hour—that is, the exhaustion of 15% of the hydride. The lifetime of the charge was greatly extended when lower concentrations of water vapor were involved; the same charge has been used for as long as 48 hours. The approach of effective exhaustion of the charge is unmistakably signalized by a pronounced upward drift of the resistance readings and a failure to settle down to any steady equilibrium value, as in normal operation. When a gas of invariant water content was passed through the train while an exhausted charge was replaced with a fresh one, the last steady reading obtained with the old charge and the first steady reading obtained with the new one were always the same. The results seemed to be unaffected by a change of as much as 10% in the total flow rate.

The accuracy of a calibrated method of analysis can best be judged from its reproducibility and sensitivity. This is particularly true when, as in the present case, it seems probable that the instrumental readings are better defined than the compositions of some of the test samples. The scatter of the points secured with a given matrix gas, observable in Figure 3, probably reflects an indeterminacy in the latter sense. Given occasional checks of the zero point, it appears that the sensitivity of this method approximates 0.0005 volume per cent of water, and that the reproducibility is of the same order of magnitude. The large scale plot of the results obtained with low concentrations of water vapor in an oxygen matrix, displayed as an inset in Figure 3, countenances such an evaluation. The nature of the measurements is such that the accuracy should be fairly uniform throughout the accessible range of concentrations.

Calculation of the thermal efficiency of the system, based on a comparison of the theoretical and observed temperature rises, yielded results which, although they varied with the nature of the matrix gas, were of the order of 35%. A thorough examination of the origin(s) of this relatively meager thermal recovery indicated that a major part of the heat leakage was due to a reverse flow of gas, partially cooled by contact with the upper, unjacketed portion of the reaction cell over the hot thermistor. When the upper (hot) thermistor was inserted more deeply, so that it was almost in contact with the calcium hydride mass, the thermal efficiency mounted from 35% to close to 60%, a reasonable approach to the 80% thermal efficiency reported by Cohn in a related application of similar apparatus. In his work the hot junction of a thermocouple was imbedded in the downstream end of the catalyst mass in which the heat was liberated, whereas in the authors' system the hot thermistor was normally some 3 cm. distant from the bed of calcium hydride.

The calibration data, plotted in Figure 3, indicate a relatively weak dependence of the experimental findings on the nature of the matrix gas. However, with a given water concentration, the various temperature rises should be inversely proportional to the heat capacities of the various matrix gases; Cohn observed at least a qualitative approach to such proportionality (*1*). However, the agreement was far from exact, and he attributed this failure to heat leakage from the reaction cell. The apparent heat leakage from the equipment described herein was much greater than that noted by Cohn. Furthermore, because of the character and magnitude of the heat losses in this system, the measured temperature rise with any matrix gas cannot be a simple (inverse) function of its specific heat but, rather, a complex (and undetermined) function of its specific heat, heat conductivity, viscosity, etc. On the not unreasonable assumption that this function is such that all the test gases behave much the same, the close similarity of the calibration curves obtained with different matrix gases is readily understood.

The thermal economy of the system could easily be improved by using small thermistors that could be imbedded in the calcium hydride, by extending the vacuum jacket to cover the upper part of the reaction cell, and/or by using a top-to-bottom direction of gas flow, the plan adopted by Cohn, so that natural convection would not tend, as it did in the authors' system, to cause a recirculation over the hot thermistor. These changes might be advantageous if it were desired to extend this method to lower concentrations of water vapor, if a matrix gas of perfectly stable composition were involved, or if the autocompensatory system described below were used. However, these changes seemed decidedly disadvantageous in connection with the batch analyzer already described, because it seemed plain that the existent heat losses were chiefly responsible for the similarity of all the calibration curves. Thus, in accepting a relatively poor thermal economy, there is obtained a favorable situation in which only limited calibration, especially in the range of low water concentra-

tion ranges, is required; and in which, in all accessible concentration ranges, the results are relatively insensitive to fluctuations in the composition of the matrix gas.

For routine semicontinuous analysis of the water content of streaming industrial gases, a relatively simple apparatus can be used. A linear train of the following components promises to furnish the desired results.

1. A flow controller, consisting of a diaphragm valve, to control the gas pressure at the inlet, and an orifice or capillary to govern the rate of flow under this pressure.
2. A small flowmeter to show that the flow is of the correct order of magnitude. The flow need not be closely controlled.
3. A reaction cell and measuring bridge, as shown in Figure 1.
4. A saturator, consisting of an intimate mixture of a salt hydrate and its anhydride, held in a constant temperature (ice) bath. The chosen hydrate should furnish an aqueous tension of the same order of magnitude as that to be measured. The output of the saturator must be accurately reproducible, but it need not be a perfect equilibrium mixture. The actual percentage of water in the hydrated gas can be determined finally by an absolute (gravimetric) method.
5. A second cell exactly like the first.
6. A guard tube containing a desiccant and leading to the vent.

The null readings of both cells ought first to be determined by passing a perfectly dry gas through the train with the saturator temporarily by-passed. The train can then be reconstituted and used without any further calibration; the data supplied by the second cell serve as a reference standard in terms of which the readings of the first cell can be interpreted. The calibration curves are essentially linear. Therefore the reading of the second cell with the gas of known water content delivered from the saturator, taken together with the null reading of that cell, serves to define the calibration line appropriate to the composition and flow rate of the gas passing through the system. With this calibration line the original water content of the test gas can be easily and uniquely determined from the reading of the first cell.

ACKNOWLEDGMENT

Much of the preliminary work in the development of this analytical system was carried out by J. A. and Peter Kafalas.

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Precision Multiple Sorption-Desorption Apparatus

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A precision multiple apparatus has been constructed which is capable of determining fifteen sorption-desorption isotherms or isobars simultaneously, thus permitting the rapid accumulation of accurate sorption data. The apparatus is based on the gravimetric principle. Weight changes are followed by silica spring balances. A micrometer slide and microscope are moved into successive spring balance positions by means of a hand-operated or motor-driven screw. The reproducibility of the readings is independent of the screw mechanism. Extensive evacuation and degassing ensure removal of air from the samples prior to the introduction of the sorbate. Equilibrium points are attained at a temperature constant to better than 0.001° C. and at pressures determined to within 0.007 mm. of mercury. Isotherms and isobars are usually obtained in the temperature range −20° to 60° C. and at pressures from 10^{-6} to 400 mm. of mercury. The apparatus is especially designed for the examination of hydrous oxide–water systems but is not limited to these materials. Adsorption and desorption isobars and isotherms have been obtained for over 500 samples, representing 15,000 equilibrium point readings.

THE determination of the amount of adsorption of gases or vapors on solids has usually been carried out by volumetric methods. Gravimetric methods possess the advantage of permitting the simultaneous examination of several samples, and do not require a determination of the volume of the adsorbent. Since the classic experiments of McBain and Bakr (*3*) who first employed fused silica spring balances, the gravimetric method has come into widespread use. A multiple apparatus consisting of six McBain-Bakr balances has been described by Stamm and Woodruff (*12*). In this device, each of six silica spring balances may be rotated in turn into position before a common measuring microscope. Harris, Ott, and Arnold (*2*) have discussed a similar apparatus.

In this laboratory there has been constructed a multiple sorption-desorption apparatus capable of following fifteen samples simultaneously. Silica spring balances are used, and the measuring microscope is moved from balance to balance by means of a translatory motion, employing a screw. The apparatus is designed to yield accurate data in quantity with speed and convenience in over-all operation.

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APPARATUS

Fifteen fused silica springs are mounted in vertical positions in individual borosilicate glass tubes suspended in a thermostatically controlled bath. The springs employed vary in sensitivity from 0.3 to 40 mm. per mg. Each sample tube is approximately 1 meter in length, in order to allow the use of the high sensitivity springs, and also to allow the springs to operate at room temperature.

This latter feature is essential to prevent the continuous sensitivity variations observed when silica springs are exposed to high pressures of water vapor at elevated temperatures. Also, the springs exhibit a small negative temperature coefficient; in this apparatus, temperature corrections are employed. Figures 1

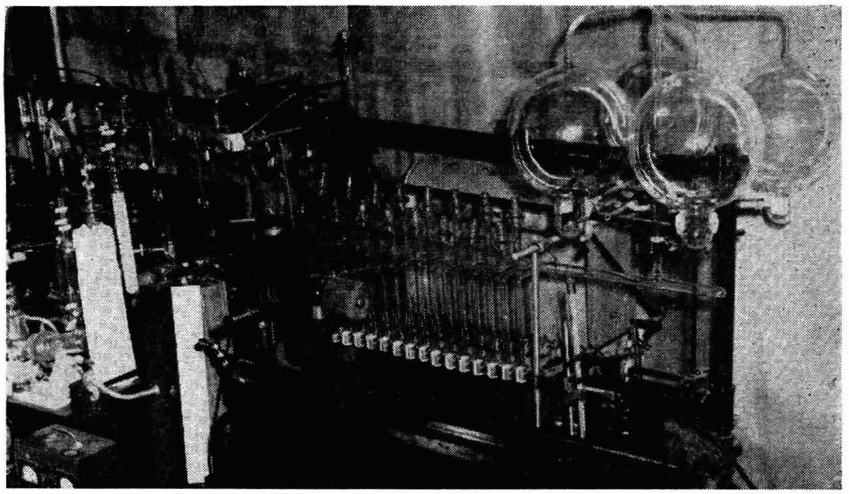

Figure 1. Sorption-Desorption Apparatus

and 2 illustrate photographically and diagrammatically the form of the apparatus.

The silica springs are suspended from glass hooks attached to hand-ground spherical joints, and the aluminum or platinum buckets are suspended by fused silica fibers. Changes in sample weight, and hence the amounts of sorption or desorption, are determined by measuring the change in the length of the spring. At times a reference rod, consisting of a silica fiber suspended from the upper hook, is used to make possible exact measurement of spring lengths in excess of the 100-mm. scale on the measuring microscope. The spring lengths may be determined to slightly better than ±0.001 mm., using a Gaertner Model M-342 micrometer scale.

In the determination of spring lengths, the microscope cross hair is adjusted tangent to the uniformly curved silica hooks comprising the ends of the springs. This procedure is considered much superior to methods involving setting the cross hair on vertical silica fibers drawn to a sharp point. Measurement of the entire length of the spring or the use of the reference rod obviates the necessity of establishing a zero base point of reference. The measuring microscope may be moved to successive balance positions by means of a hand-operated or motor-driven precision screw. A locking device prevents movement of the microscope during readings at a particular balance position. The accuracy of the spring length measurements depends on the calibration of the micrometer slide scale, and not upon the accuracy of the screw mechanism employed to shift the microscope from one balance position to another.

Pressures are measured by means of a differential manometer filled with either mercury or Apiezon-B oil, depending on the gas or vapor used as adsorbate. The manometer is maintained at 25° ± 0.01° C. in a water bath with glass windows. Manometer readings are made with an Ehrbach cathetometer. In most of the work carried out in this laboratory, water vapor is used as the adsorbate, and the oil manometer makes possible determination of pressure to 0.007 mm. of mercury.

The vacuum system consists of a liquid nitrogen trap, a trap filled with phosphorus pentoxide spread within layers of borosilicate glass wool when water vapor is employed, or with charcoal or

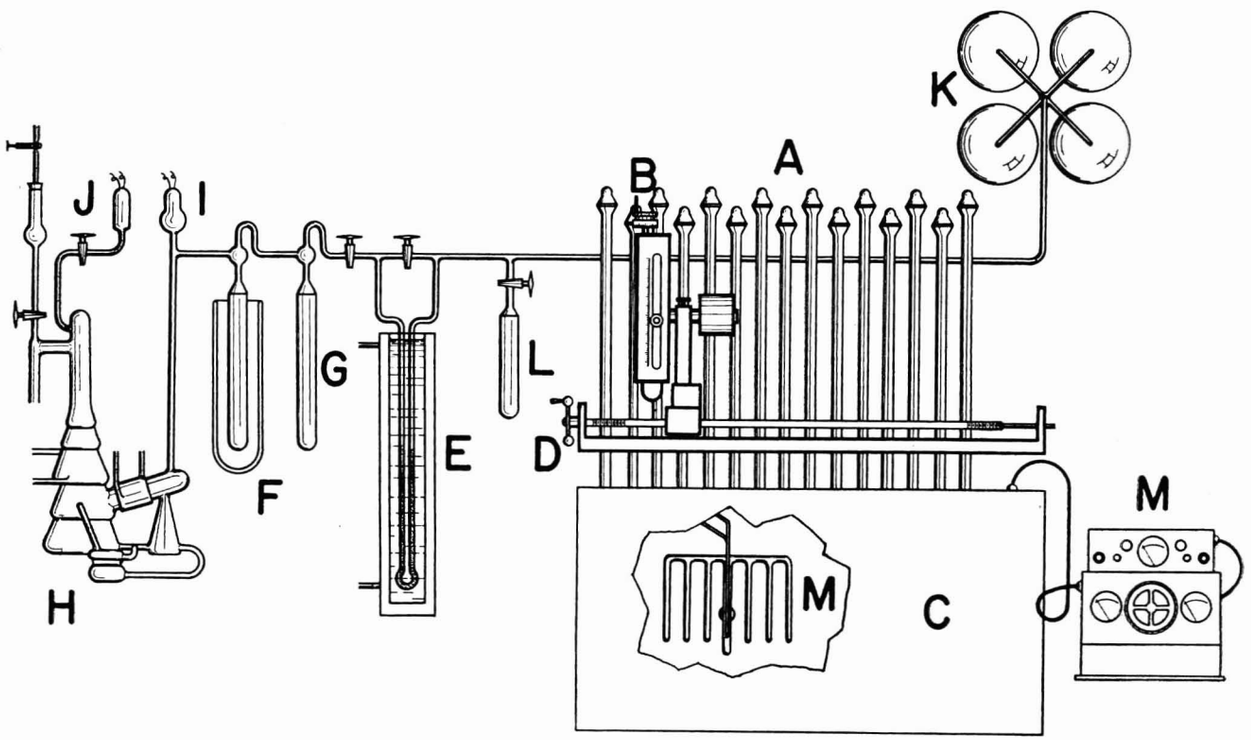

Figure 2. Sorption-Desorption Apparatus

A. Individual mountings for silica spring balances
B. Measuring microscope
C. Thermostatically controlled bath
D. Precision screw to move *B*
E. Differential manometer
F. Liquid nitrogen trap
G. Drying trap
H. Diffusion pump
I. Ionization gage
J. Pirani gage
K. Ballast volume
L. Adsorbate source tube
M-M. Thyratron controller-thermoregulator

other adsorbents when adsorbates such as hydrocarbons are in use, a Distillation Products, Inc., water-cooled glass diffusion pump, two-stage, filled with Octoil-S, and a mechanical fore pump. The ultimate high vacuum is measured with an ionization gage tube with a control circuit similar to that of Ridenour (*10*). The ionization tube is calibrated with a McLeod gage. The low vacuum is registered by a standard Pirani gage.

The apparatus is constructed entirely of glass, and contains numerous stopcocks and spherical joints, all of which are hand-ground for high vacuum use. Special lubricants such as Apiezon-L grease are used. Preliminary tests were made using mercury cutoff stopcocks, but experience shows that the amount of grease vapor present in the apparatus is no more harmful than mercury vapor.

In studying the phenomenon of sorption-desorption hysteresis, it is known (*9*) that the volume of the apparatus should be large compared with the volume of the samples, in order to provide essentially isobaric increments or decrements of sorbate. A 20-liter ballast volume is available in this apparatus, which permits an apparatus-volume to sample-volume ratio of the order of 10,000.

Gas or vapor adsorbate is introduced into the system through a stopcock from a tube which can be either heated or cooled; this tube is fitted to the system by a spherical joint. The pure water vapor used in most of the work carried out in this laboratory is obtained from the thermal decomposition of barium chloride dihydrate crystals placed in the source tube and carefully degassed at liquid nitrogen temperatures before heating. The spherical joint and the ease of heating and cooling the adsorbate source make it readily adaptable to other gases and vapors.

The constant temperature bath is especially designed for operation in the temperature range of $-20°$ to $+40°$ C. The tank consists of a double-walled, 15-gallon copper container with double glass windows to permit visual examination of the buckets during operation. The space between the copper walls is filled with Silocel insulation and a layer of drying agent, and is hermetically sealed with soft solder. Additional desiccant material is available between the double-glass windows to prevent deposition of moisture; this precaution is especially important when working at the lower temperatures. The copper tank also has 1 inch of external cork, sealed with an insulating paint. Temperatures below that of the room are obtained by a 0.75-horsepower Freon-12 compressor with a copper expansion coil within the bath. The compressor runs continuously and the cooling effect is balanced against adjustable fixed heaters. A smaller heater (5 to 250 watts) is in the anode circuit of an FG-57 Thyratron tube controlled by a 200-ml. mercury-toluene thermoregulator with Nichrome-mercury contacts in a 0.75-mm. bore capillary. The entire fluid in the bath is circulated at a rate of about seven times per minute. The extreme constancy of the temperature in the bath is principally attributable to the large volume, the thorough stirring, the good insulation, and the sensitivity of the mercury-toluene thermoregulator.

The choice of weight of the samples and the sensitivity of the springs depend primarily upon the amount of adsorption expected. Typical operating values for the adsorption of water vapor on various hydrous oxides often require a sample weight of about 100 to 150 mg. in platinum buckets of about 75 to 100 mg.; a larger sample weight may be used in aluminum buckets of about 10 to 25 mg. Aluminum buckets are fabricated from aluminum foil, and are pretreated with concentrated nitric acid to form a thin layer of alumina which renders the aluminum insensitive to the action of water vapor after accidental exposure to mercury vapor.

Aluminum buckets not pretreated with nitric acid have often reacted with water vapor at high relative humidities near saturation. It is believed that the amount of mercury vapor often present in laboratories (because of accidental spillage) is sufficient to catalyze the reaction with water vapor, especially in the absence of air at high relative humidities. Blank isotherms on treated and nontreated aluminum buckets have demonstrated that the amount of water adsorbed by the invisible film of aluminum oxide is entirely negligible.

Platinum buckets are fabricated from "dead soft" platinum foil, 0.0005 inch thick, which is easily obtainable from dental supply houses. The platinum buckets are preferred, except in instances where the weight of the bucket must be reduced to a minimum.

Considerable attention has been given to the question of the effect of traces of mercury vapor or grease on the samples. Shidei (*11*) observed that certain samples of alumina developed a slightly yellow color after exposure to a vacuum stopcock grease for many days in a high vacuum. In this laboratory, Simpson obtained six isotherms, simultaneously, which agreed closely for water vapor on a silica gel, using mercury cutoffs instead of grease-sealed stopcocks. The mercury cutoffs were then replaced with hand-ground stopcocks, lubricated with Apiezon-L grease, and the isotherms were repeated on the same samples. The resulting set of six isotherms agreed closely with the first set. The silica gels were then left in the apparatus for several weeks in high vacuum (about 10^{-6} mm. of mercury) and possible changes in weight were sought which might indicate a slow adsorption or deposition of grease from the stopcocks. No detectable change in weight was observed, but on removal of the samples from the apparatus, they were slightly yellow in color, in agreement with Shidei's results for alumina. These and other preliminary experiments suggest that the use of stopcocks and spherical joints is justified. The inherent ease and rapidity of operation permit many more isotherms to be obtained than would be possible if samples were sealed into the apparatus by a glass-blowing technique, and gases and vapors added and removed from the system by grease-free methods.

APPLICATIONS

The apparatus was primarily designed for the rapid determination of numerous water vapor isotherms on fifteen samples, simultaneously. In addition to sorption-desorption measurements, phase rule isotherms and isobars have been employed to detect definite hydrates or to follow changes in hydrates or definite hydroxides. This apparatus was originally constructed in 1942, has been modified in various ways, and has since that time been in almost constant use. At present over 500 samples have been examined, the resulting isotherms and isobars representing over 15,000 equilibrium point readings. Some of the results of this work have been previously reported (*1*, *4–8*).

ACKNOWLEDGMENTS

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Spectrographic Determination of Nitrogen in Some Organic Nitrogen Compounds

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A unique application of molecular emission spectra in the determination of organic nitrogen is described, in which a bothersome phenomenon has been utilized as the basis for a new method of analysis. The cyanogen band spectrum, normally in evidence when a carbon arc is burned in air, is produced "artificially" by introducing organic nitrogen compounds into the arc. Atmospheric nitrogen is removed by the use of a simple arc chamber through which an inert gas is passed. Densitometry of the band-head emissions produces working curves similar to those obtainable with atomic spectra. A range of concentrations has been studied for each of several nitrogen compounds of varying nitrogen content. An accuracy of ±2% was obtained with a visual-comparison densitometer. The method represents a departure from conventional emission spectroscopy in that the spectrum resulting from the excitation of a diatomic molecule is used successfully as though it were a line spectrum of a single atom. It should prove useful in determining the nitrogen content of gases, nitrogenous waste materials, etc.

THE cyanogen band spectrum is a familiar sight to spectroscopists, appearing at certain portions of the visible and ultraviolet region when a carbon arc is burned in air. Nitrogen of the air combines with carbon of the electrodes to form the CN molecule which manifests itself spectrally as a system of dense bands. These bands, in certain cases, completely obliterate some emission lines of certain elements which would otherwise be useful in analysis. This investigation is concerned with the utilization of the cyanogen spectra as a means of determining nitrogen in some organic nitrogen compounds.

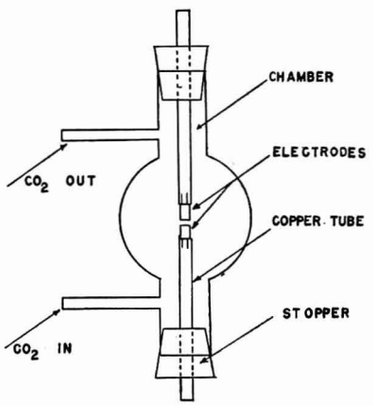

Figure 1. Arc Chamber

Because the cyanogen molecule is formed when a carbon arc is burned in air, it was necessary to remove air from the arc by employing a special arc chamber through which inert gas could be passed. This was accomplished by means of the apparatus shown in Figure 1. Carbon dioxide was found to be a convenient gas for this purpose. When certain organic nitrogen compounds were introduced into the carbon arc, the cyanogen spectrum was thereby obtained from the nitrogen of the particular compound used. The band head of the O—O (or principal) CN band at 3883.55 A. was used as the analysis line for nitrogen. Densitometric results indicated that band head intensity was proportional to concentration of organic nitrogen present.

APPARATUS

The arc chamber shown in Figure 1 was made from a 250-ml. distillation flask (borosilicate glass) selected for its clarity and freedom from flaws. The large tubes at top and bottom were made to hold 1-inch (2.5-cm.) cork stoppers through which the copper electrode holders were passed. These holders were of $^3/_8$-inch tubing, slotted and slightly enlarged at the ends to hold the graphite electrodes snugly. Rubber tubing was used to convey the carbon dioxide to and from the arc chamber. The outlet tube was carried to an exhaust fan or hood, because of the possible formation of toxic quantities of carbon monoxide at the hot graphite electrodes.

The spectrograph used was of the concave grating type, of 3-meter focal length and modified Eagle mounting (*4*). Excitation was provided by a General Electric Co. Spectrosource 1500-volt condenser discharge. This source is a thyratron-controlled type which utilizes a light leader-spark to trigger a 1500-volt heavy discharge from either a 0.5- or 2.0-microfarad capacitor sixty times a second (once for each positive half-cycle of the 60-cycle power supply). The 2.0-microfarad capacitor was employed for the work reported here.

Plates employed were Eastman Type 103-0 developed in an A.R.L.-Dietert developing machine.

Electrodes were regular grade graphite supplied by the National Carbon Co., Inc., and were in the form of $^5/_{16}$ × 12 inch rods. These were sawed flat to a length of 1.25 inches on a circular power saw used solely for that purpose.

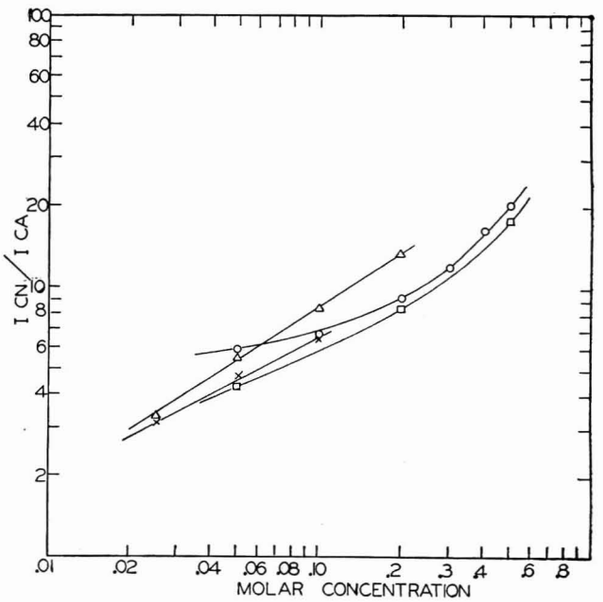

Figure 2. Working Curves

△ **Azobenzene**
× **Picric acid**
□ **Acetanilide**
○ ***p*-Phenylenediamine**

[1] Present address, Monsanto Chemical Co., St. Louis 4, Mo.

[2] Deceased.

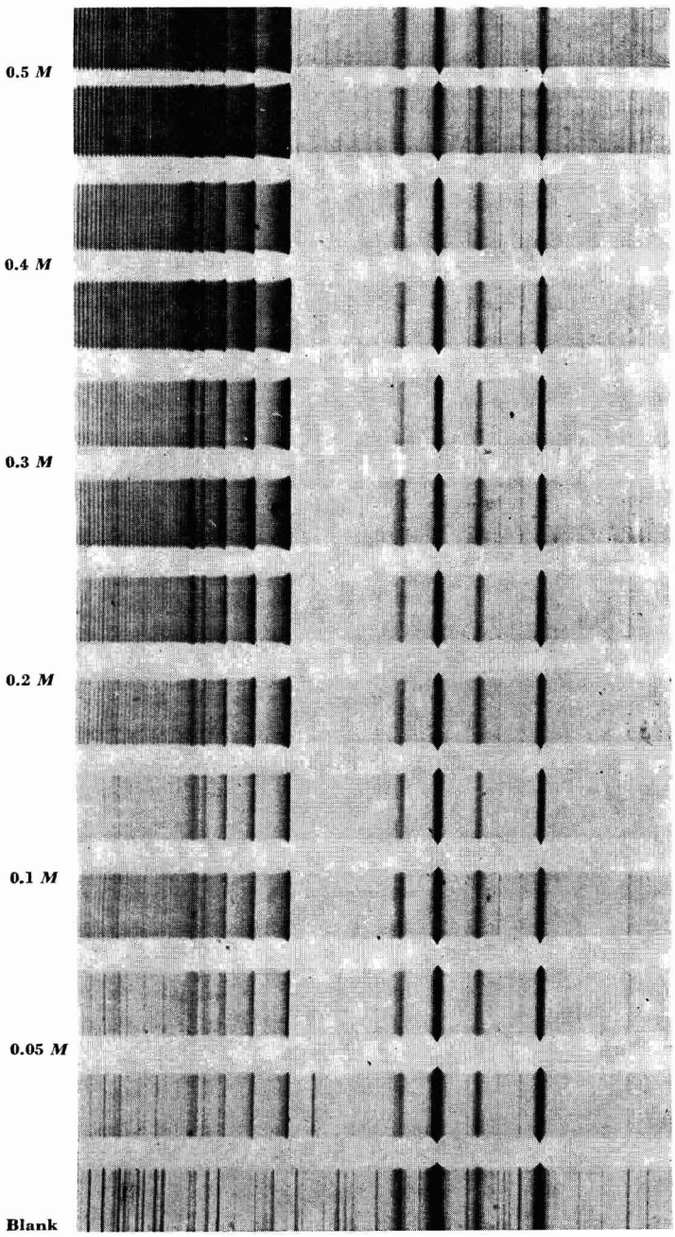

Figure 3. Spectra of *p*-Phenylenediamine

A Gaertner visual-matching densitometer was used to read all plates.

Exposures were timed with a Precision Scientific Co. electric timer.

EXPERIMENTAL

A series of electrodes was placed in a Transite block drilled to receive them; they were treated with redistilled kerosene to make them less porous and placed in an oven at 105° C. for 20 minutes. After being removed from the oven and allowed to cool, they were loaded with the compounds as follows: A molar solution of the organic nitrogen compound was prepared in ethyl alcohol and successive dilutions were made to achieve a suitable range of concentration. Using a 0.1-ml. serological pipet, 0.02 ml. of the solution was placed on the end of each electrode, or a total of 0.04 ml. per anode-cathode pair. The electrodes were then placed in an oven at 50° C. for 10 to 15 minutes to evaporate the alcohol, leaving the compound adhering to the electrode tips. Electrodes were placed in position by removing each stopper and copper holder as a unit from the arc chamber and inserting the electrode by means of a silver-plated forceps. When the stopper-tube-electrode units were returned to the chamber it was placed as a unit in the electrode stand. Positioning of the analytical gap was accomplished by the use of a light-lens-wall target system. Alignment completed, pressure tank carbon dioxide was allowed to flow through the chamber for 30 seconds prior to striking the arc and also during the exposure.

All exposures were of 10 seconds' duration. Slit width employed was 90 microns during most of the work, because it was necessary to produce a line wide enough to fill the density wedge gap in the Gaertner densitometer. Plates were developed (total darkness) in Eastman formula D-19b for 3 minutes and fixed in Eastman F-5 for 5 minutes. They were washed for 30 minutes and allowed to air-dry. Temperature during development and washing was 15° to 18° C. The internal standard line chosen was that of calcium at 4226.7 A., present as residuum in the graphite electrodes used. The log of the ratio of intensity of cyanogen (3883.5 A.) to intensity of calcium (4226.7 A.) was computed and plotted against log concentration of organic nitrogen present. Results obtained for these determinations are shown as working curves in Figure 2.

Figure 3 is a photograph of the spectra obtained by this procedure utilizing *p*-phenylenediamine in molar dilutions. Two spectra are included for each concentration level and the results were averaged for the pairs. This was the technique employed for all determinations. The bottom spectrum is a blank of the graphite electrodes arced in the carbon dioxide atmosphere, showing the absence of cyanogen.

A second type of data was obtained by preparing solutions of several compounds, varying in nitrogen content, 1 gram of each dissolved in 25 ml. of ethyl alcohol. These solutions were loaded in the manner previously described. Results of these determinations are shown in Figure 4. Log intensity ratios are plotted against log per cent nitrogen calculated for the compounds.

DISCUSSION

The calcium line at 4226.73 A. for the internal standard was selected after repeated determinations of its intensity showed it

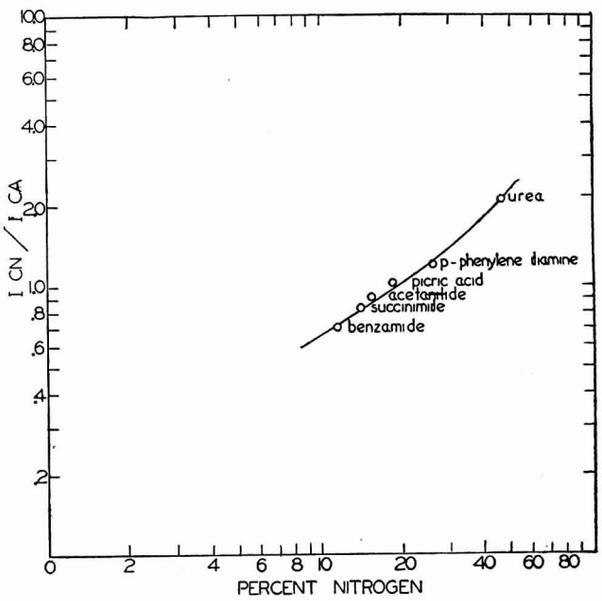

Figure 4. Spectrographic Determination of Nitrogen

One gram of compound in 25 ml. of ethyl alcohol

to have a fairly constant value in all the electrodes used, plus the fact that its intensity compared favorably with that of the cyanogen band head at the maximum nitrogen concentrations employed. The desirability of matching intensities between analysis and reference line has previously been established (*1*). All line intensities were corrected for background by the use of an accepted procedure (*3*). The photographic response charac-

teristics of the 103-0 plates used were measured by rotating a logarithmic step sector (ratio 1 to 1.5) at Sirks's focus (*5*) before the slit. An iron arc sector pattern was photographed on one of each new box of plates used and the H and D curve (*2*) thereby obtained.

Limitations imposed by the procedure, as herein outlined, are that the compound used should be (preferably) of a crystalline nature, and it should possess a suitably low volatility, such that oven or other drying will not volatilize the compound along with the alcohol carrier. Doubtless other modifications of the technique might be made toward investigating liquids and volatile compounds. The technique should prove of value in the study of nitrogenous wastes, etc., where a semiquantitative value would suffice.

Measurements of density with the visual-type densitometer used indicate that a precision of ±2.0% nitrogen may be obtained. This precision could conceivably be improved by the use of a photoelectric microphotometer wherein human error is eliminated or greatly reduced.

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Alphazurine G and Some *N,N'*-Substituted Benzidines as Redox Indicators

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The purpose of this study was to investigate the usefulness of a typical triarylmethane dyestuff as a redox indicator. The dye chosen for this purpose was Alphazurine G, C.I. No. 712, which had already proved to be an excellent and reliable indicator. The transition potentials of Alphazurine G in various concentrations of sulfuric, hydrochloric, and perchloric acids are reported for the titration of ferrous iron and ferrocyanide with cerate. Alphazurine G has also been found to give reliable results as a visual indicator for the titration of ferrous iron. Furthermore, two new oxidation indicators have been proposed, *N,N'*-tetramethylbenzidine and *N,N'*-tetramethylbenzidine-3-sulfonic acid. The former was found to be stable at temperatures up to 100° C. This information and these indicators may prove valuable in filling in the gaps in the existing knowledge concerning redox indicators.

THE triarylmethane dyes were introduced as reversible oxidation-reduction indicators in 1931 by Knop (*7*). Several investigators have applied a few of these indicators to specific titrations (*4, 5, 8, 9, 17*). Details as to the chemical constitution, trade names, Colour Index number, etc., of the dyes are given by Knop (*7*).

In contrast to the widely acclaimed *o*-phenanthroline or ferroin series of indicators also introduced in 1931 by Walden, Hammett, and Chapman (*16*) and exhaustively studied by Smith and coworkers (*2, 13*), the triarylmethane dyes have received only secondary interest in volumetric analysis. In view of their striking color changes, excellent reversibility, and extensive applicability to cerate oxidimetry, this is hardly justified. The limited use of these indicators is due in part to a lack of experimental evidence concerning their precise electrochemical and indicator behavior. Beyond the original approximate determination of the transition potentials of the dyes of Knop (*7*) and the re-evaluation of the transition potentials of Patent Blue V by Yoe and Boyd (*18*) and Setopalin C by Miller and Van Slyke (*11*), very little has been published concerning the electrochemical behavior of these indicators. No study has been made of the variation of transition potential with type and concentration of acid or in any titration other than the iron-permanganate reaction.

The purpose of this study was to investigate the redox indicator properties of a typical triarylmethane dyestuff with respect to the transition potential and its variation with type and concentration of acid, and the application of the dye to specific titrations. The dye chosen for this purpose was Alphazurine G, C.I. No. 712. It was introduced by Whitmoyer (*17*) for the titration of ferrous iron, ferrocyanide, and hydroquinone with cerate. The indicator properties of the triarylmethane dyes are similar, and though Eriogreen and Erioglaucine are perhaps the two best known dyes of the series, Alphazurine G was chosen as representative of the group because it had already proved to be an excellent and reliable indicator and should possibly receive more attention.

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REAGENTS

Alphazurine G, commercial sample, National Aniline Co., 74% active dye, was used without further purification.

Hexanitratoammonium cerate, C.P., G. F. Smith Chemical Co.

Ferrous sulfate, $FeSO_4.7H_2O$, C.P., Merck.

Stannous chloride, $SnCl_2.2H_2O$, C.P., Merck.

Potassium ferrocyanide, $K_4Fe(CN)_6.3H_2O$, C.P., Merck.

Sulfuric, hydrochloric, and perchloric acids of desired strength were prepared by proper dilution of the C.P. concentrated acids.

Cerium(IV) solutions in the above acids were prepared by dissolution of ceric hydroxide in the warm acid, using hexanitratoammonium cerate as a primary standard, according to the method of Smith and Fly (*13*).

APPARATUS

All potentiometric titrations and related e.m.f. measurements were made with a Leeds & Northrup Type Kl potentiometer and enclosed lamp and scale galvanometer. The calomel electrode was of the ground-glass cap salt-bridge type manufactured by Leeds & Northrup. Magnetic stirring and a titrating stand with fluorescent lamp were provided for all titrations.

EXPERIMENTAL

The transition potentials were determined by a modification of Knop's original procedure (*7*). Portions of approximately 0.1 *N* ferrous solutions in varying concentrations of sulfuric, hydro-

Table I. Transition Potentials of Alphazurine G in Sulfuric, Hydrochloric, and Perchloric Acids

Sulfuric Acid				
Molarity	1.0		2.0	4.0
E_t (v.), usual[a]	1.03		1.02	1.02
E_t (v.), Z-R[b]	0.92		0.93	0.93
Hydrochloric Acid				
Molarity	1.0		2.0	4.0
E_t (v.), usual	1.02		1.02	1.01
E_t (v.), Z-R	0.96		0.95	0.93
Perchloric Acid				
Molarity	1.0	2.0	4.0	6.0
E_t (v.), usual	1.07	1.06	1.07	1.06
E_t (v.), Z-R	1.02	1.02	1.03	1.03
Ferrocyanide Titrations				
Sulfuric acid	1.0 molar		0.99 v.	
Hydrochloric acid	1.0 molar		0.98 v.	

[a] Ferrous solutions titrated directly.
[b] Zimmermann-Reinhardt procedure.

chloric, and perchloric acids (prepared in all cases by dissolving the required weight of ferrous sulfate heptahydrate in the proper acid of desired molarity) were titrated under potentiometric control with 0.1 N cerate prepared in the same acid and concentration, using 2 drops of 0.01 M Alphazurine G as visual indicator. Potential readings were taken usually at 1-ml. intervals, except in the region of the mid-point of the titration where several readings were taken to interpolate for the formal potential of the ferric-ferrous system.

Within 0.1 to 0.2 ml. of the known equivalence point, 0.01 N, and finally 0.002 N, cerate was added dropwise to raise the potential slowly. The transition potential was recorded at the indicator color change as compared to a color standard containing the same quantities of acid, ferrous solution, and indicator and previously titrated to within 0.5 ml. of the end point. In all cases the titration was carried beyond the color change and then reversed by addition of 0.002 N ferrous solution. This was repeated several times, narrowing down the transition interval as much as possible with each reversal. At no time did a variation of transition potential occur with repeated reversal of the color. In some cases the reverse color change (addition of ferrous) was more distinct than the forward and was of greater value in evaluating the transition interval.

The saturated calomel electrode was used as the reference electrode. Hamer (*6*) has reviewed the e.m.f. values and liquid-junction potentials encountered with the various calomel electrodes and gives 0.246 volt as an appropriate value for the saturated calomel when working with solutions of strong acids. This value was used throughout as a standard e.m.f. for the reference electrode and all e.m.f. values were referred to the hydrogen electrode by adding the above value to the observed e.m.f., making no other corrections for liquid-junction potentials. However, in the measurements in perchloric acid media, a saturated sodium perchlorate salt bridge was interposed between the calomel electrode and the titration mixture, because perchloratocerate rapidly oxidizes chloride resulting from diffusion from the calomel salt bridge. The resulting unknown liquid-junction potential was evaluated by removing the perchlorate salt bridge midway through the titration and placing the reference electrode directly in the titration mixture just long enough to make a measurement. This was done again at the end of the titration and the two values were averaged to represent the mean correction. The magnitude of this correction varied from 70 to 90 mv. in the various perchloric acid concentrations studied.

In order to test the effect of mercuric chloride and other compounds on the transition potential, ferrous solutions, previous to titration, were treated as described by Rieman, Neuss, and Naiman (*12*) for the determination of iron by the Zimmerman-Reinhardt procedure. The comparison standard was treated in exactly the same manner and the titration made as previously described. The silky, white precipitate of mercurous chloride did not interfere in any way with the evaluation of the color change.

DISCUSSION

The formal potentials of the ferric-ferrous system were checked in all titrations to serve as references. In 1.0, 2.0, and 4.0 M sulfuric acid the values were 0.68, 0.68, and 0.69 volt, respectively. In 1.0, 2.0, 4.0, 6.0, and 8.0 M hydrochloric acid the values were 0.69, 0.68, 0.66, 0.61, and 0.56 volt. In similar concentrations of perchloric acid the values were 0.74, 0.74, 0.77, 0.78, and 0.87 volt. These results are in excellent agreement with similar data obtained by Smith and Richter (*14*).

The transition potentials of Alphazurine G under various conditions are summarized in Table I. All values are in volts referred to the hydrogen electrode as zero, and all values listed are the mean of three or more determinations. In concentrations greater than 4 M in sulfuric or hydrochloric acid media, the intense yellow color of the reduced form of Alphazurine G masked the transition interval to a point where it could not be determined with any accuracy. The transition potentials were also determined for the titration of ferrocyanide with cerate in 1 M sulfuric and hydrochloric acids.

An attempt was made to determine the transition potential by differential potentiometric titration of the dye and a reference redox system in the manner used by Smith and Richter (*14*) for *o*-phenanthroline and the substituted phenanthrolines. The stannic-stannous couple was chosen as the reference redox system because its fairly low formal potential could be expected to aid in obtaining a sharp differential oxidation. Dichromate in 1 M hydrochloric acid was used as the oxidant and a well-defined differential oxidation was obtained. However, the titration was not reproducible, the shape of the upper half of the titration curve and the volume increment between the oxidation of stannous ion and the dye being dependent upon rate of stirring, manner and rate of addition of oxidant, etc. In view of later studies, this is recognized as due to a lack of stability of the oxidized form of Alphazurine G.

Alphazurine G was tested as an indicator in the visual titrations of 0.1, 0.01, and 0.001 N ferrous solutions. Portions of these solutions were titrated (1) using 2 drops of approximately 0.007 M water solution of Alphazurine G, and (2) potentiometrically. All titrations were made in 1 M sulfuric acid. In the case of 0.1 N and 0.01 N solutions, the correction was negligible, amounting to only 0.03 ml. of 0.01 N cerate for the latter. For 0.001 N solutions the indicator correction was 0.27 ml. of 0.001 N cerate. The triphenylmethane dyes are subject to destructive side oxidation by the action of excess of strong oxidizing agents, which may result in variable indicator corrections if the oxidant is added in a variable or too rapid manner during the titration. A steady, dropwise addition of the oxidant is recommended for titrations using these indicators. Brennecke (*3*) has given a thorough review of this frequently overlooked problem.

TETRAMETHYLBENZIDINE AND TETRAMETHYLBENZIDINE-3-SULFONIC ACID

In connection with work on the mechanism of the oxidation of Alphazurine G it was desirable to prepare N,N'-tetramethylbenzidine and study its indicator properties.

The compound was prepared according to the method of Ullmann and Dieterle (*15*) and the purified product melted at 190° C. The 3-sulfonic acid was also prepared by the action of fuming sulfuric acid on the amine according to the directions in Beilstein (*1*). These compounds were made up as 0.2% solutions in 1 M hydrochloric acid for use as indicators. The amine is very soluble in dilute hydrochloric acid to give a clear, colorless solution. The ammonium salt of the sulfonic acid is dissolved in hydrochloric acid by the addition of a drop of concentrated sulfuric acid, giving a clear, pale yellow solution. The tetraethylbenzidine was also prepared, but was found to be unsatisfactory as a redox indicator.

The transition potentials of these compounds in molar sulfuric and perchloric acids were obtained by the same procedure used for Alphazurine G. These values for the tetramethylbenzidine are 0.86 volt in 1 M sulfuric and 0.90 volt in 1 M perchloric acid. For the 3-sulfonic acid derivative, the transition potentials are 0.88 in 1 M sulfuric and 0.91 volt in 1 M perchloric acid.

These compounds were then tested for their application to visual titrations, comparing visual and potentiometric titrations as before. For both indicators the corrections are negligible with 0.1 and 0.01 N solutions, while with 0.001 N solutions the corrections amount to +0.54 and +0.60 ml. of 0.001 N cerate

for tetramethylbenzidine and tetramethylbenzidine-3-sulfonic acid, respectively.

The use of these compounds as redox indicators has not been previously reported. This is due in all probability to the original observation by Michler and Pattinson (*10*) and Knop (*7*), that tetramethylbenzidine is only very slowly reversible in its color change—i.e., it does not immediately reverse its color when 1 drop of reducing agent such as ferrous ion is added to the oxidized form. However, it was found to be rapidly reversible when an excess of reducing agent is added, and with a countertitration the end point can be readily determined. The sulfonic acid derivative, on the other hand, is truly reversible. One drop of even 0.001 *N* ferrous or cerate solutions causes an immediate color shift in 1 *M* sulfuric acid.

The color change of both of these indicators is from colorless to a deep yellow. A faint pink develops within 0.5 to 1 ml. of the end point and serves as a "warning color" preceding the end point. In accord with their color change, they are not serviceable for titrations of ferrous iron in hydrochloric acid, because of the yellow color of ferric ion in this acid, which obscures the indicator color. They are likewise unsatisfactory for titrations of ferrocyanide, owing to the color of ferricyanide ion formed in the oxidation. Tetramethylbenzidine is more reliable than the sulfonic acid derivative in titrations of dilute solutions. The sulfonic acid was found superior to the tetramethylbenzidine in perchloric acid media by virtue of its more rapid color change.

As with the triphenylmethane dyes, the titration must be carried out slowly within 1 ml. of the end point, as the indicator response is sluggish in this region. This is more pronounced in very dilute solutions and at least 30 seconds should elapse between drops in titrations of 0.001 *N* solutions. However, in contrast to the triphenylmethane dyes, there is absolutely no fading of the end-point color.

Of special interest is the fact that the oxidized form of tetramethylbenzidine is stable at high temperatures. Two drops of the tetramethylbenzidine indicator solution and 1 drop of 0.1 *N* cerate in a volume of 100 ml. of 1 *M* sulfuric acid gave a deep yellow solution which showed no diminution in color after 10 minutes at the boiling point. This suggests that this indicator may be serviceable for titrations at elevated temperatures.

SUMMARY

The transition potentials of Alphazurine G have been studied in varying concentrations of sulfuric, hydrochloric, and perchloric acids for the titration of ferrous iron and ferrocyanide. The effect of mercuric chloride and other compounds present in the Zimmermann-Reinhardt iron titration on the transition potential of Alphazurine G has been determined. Alphazurine G has been tested as a visual indicator for the titration of ferrous iron and found to give reliable results when solutions from 0.1 to 0.001 *N* are used. No indicator corrections are involved with 0.1 *N* solutions.

N,N'-tetramethylbenzidine and *N,N'*-tetramethylbenzidine-3-sulfonic acid are proposed as two new oxidation-reduction indicators. These indicators have transition potentials in the 0.9-volt range and are satisfactory for titrations of ferrous iron in sulfuric and perchloric acid media, using solutions from 0.1 to 0.001 *N*. Tetramethylbenzidine may be used as a redox indicator at temperatures up to 100° C.

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Modified Colorimetric Assay of Pteroylglutamic Acid

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IN ADDITION to biological methods, various chemical and physical methods have been reported in the literature for the determination of pteroylglutamic acid, *N*-[4-{[(2-amino-4-hydroxy-6-pteridyl)methyl]amino}benzoyl]glutamic acid, either in purified preparations or in the presence of other substances. These include fluorometric (*1*, *14*), polarographic (*3*, *6*, *11*), and two colorimetric methods; one colorimetric method involves reduction with titanous chloride (*7*), and the other involves reduction with zinc in the presence of gelatin (*8*). In the colorimetric methods the amine formed by reduction is diazotized and coupled to the Bratton and Marshall (*2*) reagent, 1-naphthylethylenediamine, to produce a color which is measured spectrophotometrically. The color obtained by diazotizing *p*-aminobenzoic acid and coupling to the Bratton and Marshall reagent is used as the standard. The zinc reduction method is most generally used and is included in the "Pharmacopeia of the United States of America" (*13*).

The U.S.P. method has been of great value for the determination of pteroylglutamic acid in body fluids, natural products, capsules, elixirs, and other pharmaceutical preparations, but it is subject to several errors which limit its usefulness as a precise method of assay for the isolated material. These errors are caused by the use of zinc and acid for reduction, of *p*-aminoben-

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The method for the assay of pteroylglutamic acid (folic acid) in the "Pharmacopeia of the United States of America" (*13*) involves reduction with zinc and acid in the presence of gelatin, diazotization of the *p*-aminobenzoylglutamic acid thus produced, and coupling to 1-naphthylethylenediamine to form a color which is measured against that obtained from *p*-aminobenzoic acid as a standard. The assay values thus obtained are sometimes inaccurate because of an imperfect balance of positive and negative systematic errors. The method has been modified to eliminate these errors by using zinc amalgam for reduction and *p*-aminobenzoylglutamic acid as a standard, and by making some changes in technique. Values thus obtained are subject to no known systematic errors and have a standard deviation of ±0.8% (relative) for samples of assay values from 84 to 92%. The proposed method is recommended as more reliable than the U.S.P. method. Furthermore, the effect of zinc amalgam in avoiding some destructive action of zinc and acid and the necessity of using an appropriate standard are of more general interest.

zoic acid as a standard, and of methods of measurement which lack precision unless special techniques are employed. Reduction with zinc and acid leads to values which are low to varying degrees because of some overreduction. The use, as a standard, of *p*-aminobenzoic acid, which is not an actual product of the reduction of pteroylglutamic acid, causes high values under the conditions of the determination, with a resulting incomplete and erratic balance of errors in the assay value. A modified method of assay is described which employs zinc amalgam for reduction, *p*-aminobenzoylglutamic acid as the standard for color development, and altered measuring techniques for better precision. The investigations which led to this procedure are described and a discussion is given of the precision and accuracy of this method and of another method reported in the literature.

ZINC AMALGAM METHOD

Apparatus. The spectrophotometric measurements were made with either a modified General Electric (*10*) spectrophotometer or a Beckman DU spectrophotometer. Properly calibrated cells, usually 1 cm. in length, were used, but occasionally either longer or shorter cells were used when necessary to measure the color at a favorable transmittancy value.

Reagents. *p*-Aminobenzoylglutamic Acid. This material, used throughout this work as the colorimetric standard, was prepared by twice recrystallizing a technical grade of *p*-aminobenzoylglutamic acid from water. The substance was assayed both by a diazotization titration with standard 0.1 *N* sodium nitrite solution, using starch iodide paper as an external indicator, and by an alkalimetric titration with 0.1 *N* sodium hydroxide using bromothymol blue as an indicator. Assay values of 100.0 and 100.2%, respectively, were obtained by these methods. Another sample, similarly prepared, had a melting point of 183.4° C. on the Dennis-Shelton (*5*) bar. The assay values were 99.5 and 99.6%, respectively, by the above-mentioned methods. These two preparations were used interchangeably and their slightly different strengths were taken into account in the calculations.

p-Aminobenzoic Acid. Technical grade material, which assayed 97.5 and 97.1% by the diazotization and alkalimetric methods, respectively, was recrystallized once from water to yield a product which had an assay value of 99.9% as determined by both methods.

Zinc Amalgam. This was prepared by heating and stirring 10 grams of mossy zinc metal and 20 ml. of mercury at 150° C. until all the zinc dissolved. The amalgam should be liquid at room temperature. If it is not, a little more mercury should be added and the solid dissolved by further heating. The amalgam may be used until the zinc content falls to 0.2%, as calculated from its density at 25° C. by the following equation:

$$\% \text{ Zn} = \frac{13.534 - \text{density of amalgam}}{0.088} \quad (1)$$

The value 0.088 is the average slope of the curve obtained by plotting the values for the density of zinc amalgam against percentage of zinc as calculated from data in the "International Critical Tables" (*9*).

Sodium Nitrite. Reagent grade sodium nitrite (1 gram) was dissolved to make 1 liter of aqueous solution.

Ammonium Sulfamate. Reagent grade ammonium sulfamate (5 grams) was dissolved to make 1 liter of aqueous solution.

1-Naphthylethylenediamine Dihydrochloride. One hundred milligrams were dissolved to make 100 ml. of aqueous solution. This solution should be kept cold and in a dark bottle. It is suitable for use until it acquires a pink color.

Preparation of Standard Curve. A solution containing 100.0 mg. of *p*-aminobenzoylglutamic acid was diluted in distilled water to 1 liter in a volumetric flask. Twenty millimeters of this solution were further diluted to 100 ml. with distilled water to make a solution containing 20 mg. per liter of *p*-aminobenzoylglutamic acid. Then 5, 10, 15, 20, and 25 ml., respectively, of this dilute solution were transferred to 100-ml. volumetric flasks (preferably of red, low-actinic glass, although ordinary glass flasks may be used with little destruction of the diazonium compound formed in the subsequent reaction if the solutions are protected from direct sunlight). Each solution was further diluted to about 45 ml. with distilled water. To each solution were added 10 ml. of approximately normal hydrochloric acid and then 5 ml. of the sodium nitrite solution. The solutions were mixed well and allowed to stand for 2 minutes. Ammonium sulfamate solution (5 ml.) was added and the contents of the flasks were mixed well, and allowed to stand for 3 minutes. Then 5 ml. of the naphthylethylenediamine solution were added, and the solutions were mixed well, and allowed to stand for 10 minutes. The solutions were diluted to the mark with 0.15 *N* hydrochloric acid. Using a suitable spectrophotometer and a 1-cm. cell, the absorbancy (log $1/T$) (*12*) was measured at the absorption maximum which occurs in the 545 to 550 mμ region, with distilled water as a blank. A correction should be made for the color obtained when distilled water is treated in the same manner as the sample solution. The blank normally has a transmittancy of 98 to 99% relative to that of distilled water. If the transmittancy is significantly less than this, it is advisable to find out whether the reagents, particularly the water and the 1-naphthylethylenediamine, may be contaminated. The absorbancy of the blank was subtracted from the absorbancy of the standard. This gave the corrected absorbancy for the standard. The corrected absorbancy was plotted against the concentration of *p*-aminobenzoylglutamic acid. The plot, which will be called the standard curve, may show a slight deviation from Beer's law.

Analysis of Sample. Solution 1. A well-mixed sample (0.300 gram) was accurately weighed, dissolved in about 25 ml. of approximately 0.1 *N* sodium hydroxide, and diluted to 500 ml. in a volumetric flask with distilled water.

Solution 2. A 10-ml. aliquot of volumetric solution 1 was pipetted into a 100-ml. volumetric flask, 50 ml. of approximately 1 *N* hydrochloric acid were added, and the solution was made up to the mark with distilled water.

Solution *A*. About 60 ml. of volumetric solution 2 was transferred into a 4-ounce bottle having a paraffin-lined screw cap and containing about 5 ml. of zinc amalgam. The bottle was stoppered tightly and shaken for 30 minutes in a reciprocating-type shaking machine. Then 10 ml. of the solution were pipetted into a 100-ml. volumetric flask of low-actinic (red) glass, and 5 ml. of 1 *N* hydrochloric acid and 35 ml. of distilled water were added.

Solution *B*. To another 100-ml. low-actinic, volumetric flask 10 ml. of volumetric solution 1 were added, followed by 10 ml. of 1 *N* hydrochloric acid and 30 ml. of distilled water.

Solution *C*. To a third 100-ml. low-actinic flask, 10 ml. of an equal mixture of 1 *N* hydrochloric acid and distilled water, which had been treated with zinc amalgam in a manner similar to that used for the sample, were added. To this, 5 ml. of 1 *N* hydrochloric acid and 35 ml. of distilled water were added.

Solution *D*. To a fourth low-actinic flask 40 ml. of distilled water and 10 ml. of 1 *N* hydrochloric acid were added.

Each of the solutions, *A* to *D*, was treated in the manner described under preparation of the standard curve, starting with the addition of 5 ml. of sodium nitrite solution. The absorbancy of each of these solutions was measured at the wave length determined in setting up the standard curve (545 to 550 mμ), using distilled water as a blank. The concentration of *p*-aminobenzoylglutamic acid was read from the standard curve. The per cent pteroylglutamic (folic) acid was then calculated as follows:

Let A_1, B_1, C_1, and D_1 = mg./liter of *p*-aminobenzoylglutamic acid read from the standard curve, for solutions *A*, *B*, *C*, and *D*, respectively.

Let E = total (free plus combined) *p*-aminobenzoylglutamic acid (mg./liter) in the solution measured = $A_1 - C_1$.

Let mg./liter free *p*-aminobenzoylglutamic acid = $F = B_1 - D_1$.

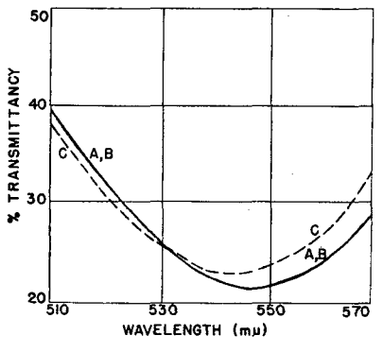

Figure 1. Effect of Amine on Curve Shape

Absorption data for compounds diazotized and coupled to naphthylethylenediamine
A. **Pteroylglutamic acid (5.4 mg./liter)**
B. ***p*-Aminobenzoylglutamic acid (3.26 mg./liter)**
C. ***p*-Aminobenzoic acid (1.68 mg./liter)**

Per cent free *p*-aminobenzoylglutamic acid = $\frac{F \times 100}{60.0}$ where 60.0 is the concentration (mg./liter) of the sample in the solution measured.

Per cent pteroylglutamic (folic) acid =

$$\frac{1.658(E - F/10) \times 100}{6.00} = 27.6\,(E - F/10)$$

In the above equation, 6.00 = mg./liter of sample in the solution measured, and 1.658 is the ratio of the molecular weight of pteroylglutamic acid (441.4) to that of *p*-aminobenzoylglutamic acid (266.3).

COLORIMETRIC STANDARD

Hutchings *et al.* (*8*) stated that pteroylglutamic acid, upon either chemical or catalytic reduction at an acid pH, is cleaved to yield a pteridine and *p*-aminobenzoylglutamic acid. Nevertheless, they recommended *p*-aminobenzoic acid as the standard in the belief that equimolecular quantities of *p*-aminobenzoylglutamic acid and *p*-aminobenzoic acid would give the same color when diazotized and coupled to 1-naphthylethylenediamine. It has been found in this study, however, that, as indicated by Figure 1, the absorption maximum obtained by diazotization and coupling of *p*-aminobenzoylglutamic acid in the concentration range useful for analysis occurs at a different wave length from that obtained by diazotizing and coupling an equivalent quantity of *p*-aminobenzoic acid, and coincides with that obtained by reducing, diazotizing, and coupling pteroylglutamic acid. In addition, as indicated by Figure 2, the absorption maximum of the color obtained from *p*-aminobenzoic acid shifts its position with changes in concentration, whereas the absorption maximum of the color obtained for *p*-aminobenzoylglutamic acid does not shift with concentration. It follows, therefore, that the choice of any particular wave length as a standard for the absorption maximum would be valid for only a narrow range of concentrations of samples. It is conceivable that a concentration was chosen in the work of Hutchings (*8*) at which the color for the two amines was equivalent, but at the concentrations chosen for this work it was found that *p*-aminobenzoylglutamic acid has about a 5% greater molar absorbancy index than *p*-aminobenzoic acid. These data are shown in Table I. The concentrations chosen are those to be expected for the pteroylglutamic acid samples being analyzed according to the described method. Furthermore, it is preferable to use as a standard a material which has an absorption curve which is not different from that produced by the treatment of the actual sample.

Table I. Comparison of Molar Absorbancy Indexes

(Measurements made with Beckman spectrophotometer at slit width of about 0.04 mm.)

Molarity of Solution $\times 10^5$	Molar Absorbancy Index at Absorption Maximum $\times 10^{-4}$
p-Aminobenzoylglutamic Acid	
1.12	5.40[a]
1.11	5.46
1.11	5.41
1.15	5.47
	Av. 5.44
p-Aminobenzoic Acid	
1.17	5.19[b]
1.10	5.21
1.18	5.25
1.08	5.14
1.13	5.11
	Av. 5.18

[a] Measured at 548 mμ, the absorption maximum.
[b] Measured at 543 mμ, the absorption maximum for this concentration.

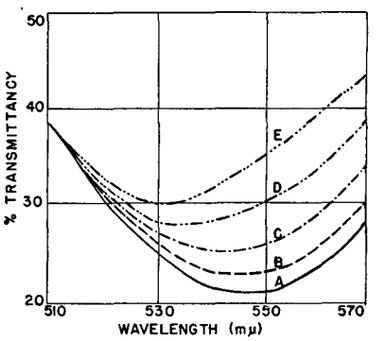

Figure 2. Effect of Concentration on Curve Shape

***p*-Aminobenzoic acid diazotized and coupled to naphthylethylenediamine**
Data calculated to a constant value for product of concentration and cell length
A. **0.77 mg./liter**
B. **1.54 mg./liter**
C. **3.08 mg./liter**
D. **7.0 mg./liter**
E. **14.0 mg./liter**

It is recommended that *p*-aminobenzoylglutamic acid be used as the standard for the analysis of pteroylglutamic acid samples. If for any reason it is desired to use *p*-aminobenzoic acid, a correction must be applied which takes into account the facts of the above discussion. Since the colored solution developed from *p*-aminobenzoic acid does not follow Beer's law, and indeed exhibits considerable shifts in the absorption maximum for small changes in concentration, this correction will necessarily be involved and cannot, therefore, be recommended.

METHOD OF REDUCTION

In the early stages of the analytical work the pteroylglutamic acid was reduced with zinc under the same conditions of acidity and concentration of sample as are recommended for the zinc amalgam. For the zinc reductions, two additions of zinc, each of approximately 0.5 gram, were made at 5-minute intervals, thus giving a total reduction time of 10 minutes. The solutions were swirled intermittently during this interval, after which the excess zinc was filtered off through a dry filter paper; the

Table II. Effect of Source of Zinc Used for Reduction

Zinc Used for Reduction	Pteroylglutamic Acid, %	
	Sample 1	Sample 2
Baker metal dust 1.2245	83.5, 83.7, 84.5, 84.6, 83.8, 83.3; av. 83.9	90.8, 90.5; av. 90.7
Baker metal powder dust 1.2844	82.9, 83.1, 79.4, 78.5, 83.3; av. 81.4	87.9
Commercial Zn (A)	84.9, 85.1, 87.6, 86.0; av. 85.9	91.0, 91.0; av. 91.0
Commercial Zn (B)	85.8	.....
Commercial Zn (C)	89.4, 86.5; av. 88.0	91.1, 91.4; av. 91.3
Commercial Zn (D)	88.4, 87.0; av. 87.7	.....
Commercial Zn (E)	86.4, 86.5; av. 86.5	.....
Amalgam method	88.4, 87.5, 88.6, 87.7; Av. 88.1	92.7, 92.5; av. 92.6

Table III. Effect of Concentration of Zinc in Zinc Amalgam and of Time of Reduction

Zinc, %	% Pteroylglutamic Acid	
	30-min. reduction	1-hour reduction
4.32	83.5, 83.9; av. 83.7	84.3, 84.4; av. 84.4
2.18	83.7, 84.0; av. 83.9	83.1, 84.0, 84.0, 84.3; av. 83.9
1.92	84.2, 83.8; av. 84.0	84.7, 84.5; av. 84.6
1.79	84.3, 83.8; av. 84.1	83.1, 84.1; av. 83.6
0.58	84.0, 84.5; av. 84.3	84.8, 84.3; av. 84.6
0.53	84.0, 83.8, 83.4, 84.3; av. 83.9	83.8, 84.0, 84.7, 84.8; av. 84.3
0.13	84.0, 84.0, 84.8, 84.4; av. 84.3	84.0, 83.8, 85.0, 84.8; av. 84.4

first portion of the filtrate was discarded. The color was developed on the remaining filtrate. The values obtained were somewhat erratic and depended upon the source of the zinc. Typical values are shown in Table II for two samples, together with comparable values by the zinc amalgam method. From this it can be seen that some of the specimens of zinc gave values closely approaching those obtained by the amalgam method, while others gave values which were considerably lower. In an attempt to see whether these difficulties were caused by the conditions of the reduction, the effects of time, acidity, and temperature were studied. It did not appear that any of these variations caused enough effect to account for the differences obtained with various specimens of zinc, although the effect of time of reduction suggested the possibility that the zinc reduction was too drastic. The values obtained on reduction for 1 hour were somewhat lower than those obtained after the usual 10-minute reduction, although the differences may not be significant in view of the precision involved. The values obtained were 85.9, 85.9, 86.4, and 83.3, average 84.7% for 1 hour; and 87.7, 86.1, 84.1, and 86.5, average 85.9% for 10 minutes. This suggested the use of zinc amalgam as the reductant because it should cause a milder reduction.

When zinc amalgam is used for the reduction, the value obtained is independent of the concentration of zinc in the amalgam over the range 0.13 to 4.3% zinc. These data, together with the effect of time of reduction, are shown in Table III. It had been found that 30 minutes was the minimum time required for complete reduction. Additional time of reduction up to 1 hour had no significant effect on the values obtained. (The samples used for the values in Tables II, III, and IV are all different; consequently, the values in different tables are not comparable. These data were obtained at lengthy time intervals and it was not convenient to repeat the work on the same sample.)

The values obtained with zinc amalgam as the reductant are not dependent upon the source of the zinc as is the case when zinc dust is used (Table IV). An attempt was made to prepare zinc amalgam from zinc dust to see if the dusts which had been unsatisfactory for the direct reduction might be satisfactory in an amalgam. It proved to be impossible, however, to prepare an amalgam that was usable for this determination from any available zinc dust. The difficulty was that not all the zinc dissolved in the amalgam; this was indicated by the liberation of considerable hydrogen during the reduction.

Simultaneously with the development of the zinc amalgam–reduction method described herein, Hutchings (*8*) reported the use of gelatin as a protective colloid in the reduction, using zinc metal as the reductant. On studying the zinc metal reduction procedure, both in the presence and in the absence of gelatin, it was found that the value obtained for pteroylglutamic acid depended not only on the particular zinc used but also, at least to some extent, on the gelatin used in carrying out the reduction. From Table V it can be seen that gelatin A apparently had no protective effect, while gelatin B may have had some such effect. In general, the values obtained using zinc and gelatin were somewhat lower than those obtained by reduction with zinc amalgam, although in some instances the differences were not significant. (Values by the colorimetric method using zinc amalgam had a standard deviation of ±0.8% relative, which will be discussed later in the paper.) To the authors' knowledge, there is no way of predicting whether a particular sample of zinc or gelatin is suitable for use without actual trial.

On treating *p*-aminobenzoylglutamic acid at low concentrations (40 to 60 mg. per liter) with either zinc or zinc amalgam, values are obtained which are rather erratic. When zinc is used as the reductant, the values are definitely low and there is a tendency at times to obtain low values also when zinc amalgam is used. At higher concentrations (3000 to 6000 mg. per liter) the values obtained using either reductant are no more erratic than one would expect from the reproducibility of the method and do not appear to be subject to any systematic error. These data are shown in Table VI. On reducing pteroylglutamic acid with zinc amalgam, the values obtained are independent of the concentration over a 100-fold concentration range. With zinc (no gelatin) there seems to be a definite trend toward lower values at the lower concentration. The values obtained at the higher concentration approach those obtained with the zinc amalgam (Table VI).

Table IV. Effect of Source of Zinc Used for Zinc Amalgam

Zinc Used	Pteroylglutamic Acid, %
Eimer & Amend mossy	83.2, 85.7, 83.5; av. 84.1
Baker sheet	84.9, 85.7, 84.5; av. 85.0
Baker sticks	83.5, 84.8, 84.8; av. 84.4
Baker mossy	84.9, 84.8, 84.8; av. 84.8

Table V. Effect of Zinc and Gelatin

Zinc Dust Used	Pteroylglutamic Acid, %		
	No gelatin	Gelatin *A*	Gelatin *B*
Commercial sample 1	83.1, 84.1; av. 83.6	84.6, 81.9; av. 83.3	84.2, 85.2, 85.2, 85.2; av. 85.0
Commercial sample 2	82.9, 83.3; av. 83.1	84.6, 83.6; av. 84.1	85.2, 87.9, 83.1; av. 85.4
Baker's C.P. 10845	82.4, 82.4; av. 82.4	82.7, 81.5; av. 82.1	.....
Merck reagent sample 1	82.6, 82.4; av. 82.5	83.0, 82.6; av. 82.8	.....
Merck reagent sample 2	82.1, 82.7; av. 82.4	83.5, 82.8; av. 83.2	.....
Merck reagent sample 3	82.6, 82.4; av. 82.5	82.7, 82.8; av. 82.8	.....
Zinc amalgam	85.4, 85.1, 85.5; av. 85.3		

DEVELOPMENT OF COLOR

A series of experiments was carried out in much the same manner as described for the preparation of the standard curve in order to study the effects of acidity, amounts of reagents, time of reaction and light on the diazonium compounds and the colors which are produced from them. There is no effect on the absorption curve if one half as much or twice as much hydrochloric acid as is recommended is used. Likewise, diazotization and coupling could be carried out without adding the 35 ml. of water with no significant effect on the final result. The amount of sodium nitrite can be one half of the standard amount or double that amount with no effect. It is necessary to add enough ammonium sulfamate to decompose the excess nitrous acid present, but adding a twofold excess over

Table VI. Effect of Concentration of *p*-Aminobenzoylglutamic Acid or Pteroylglutamic Acid on Reduction with Zinc or Zinc Amalgam

Concn. of Sample during Reduction, Mg./Liter	Per Cent Found	
	Zinc reduction	Zinc amalgam reduction
	p-Aminobenzoylglutamic Acid	
40 to 60	97.1, 96.4, 92.0, 95.2, 95.9, 95.3, 98.5, 96.3; av. 95.8	99.2, 98.4, 94.4, 95.0, 98.6, 98.4, 99.8, 98.6, 95.2, 97.4, 95.5, 99.6, 98.7; av. 97.6
3000 to 6000	99.8, 99.7; av. 99.8	100.2, 99.0, 101.0, 101.0, 99.7, 98.9, 99.8, 99.2, 99.8; av. 99.8
	Pteroylglutamic Acid	
A, 50	85.4, 86.0; av. 85.7	87.8, 87.6; av. 87.7
A, 5000	86.8, 86.0; av. 86.4	86.8, 87.1, 87.4; av. 87.1
B, 50	86.7, 86.8; av. 86.8	89.8, 89.2; av. 89.5
B, 5000	88.7, 87.5; av. 88.1	88.7, 90.8, 89.2; av. 89.6
C, 50	86.9, 87.8, 87.0, 87.0; av. 87.2	92.0, 90.0, 90.7, 91.2; av. 91.0
C, 5000	90.0, 88.7; av. 89.4	91.4, 91.4, 91.4; av. 91.4
D, 50	90.9, 88.8; av. 89.9	91.1, 90.9; av. 91.0
D, 5000	91.7, 91.3; av. 91.5	90.9, 91.1, 91.5; av. 91.2
E, 50	89.2, 88.9; av. 89.1	90.6, 92.0, 91.0; av. 91 2
E, 5000	90.5, 90.4; av. 90.5	90.9, 90.5; av. 90.7

Table VII. Aging of Original Acid and Alkaline Solutions

Sample	Aging in 0.1 *N* NaOH, Hours	Free *p*-Aminobenzoyl-glutamic Acid, %	Pteroylglutamic Acid, %
A	0	1.14	92.3
A	18	2.45	89.2
A	68	11.4	72.7
B	0	1.08	94.5
B	0.5	1.4	94.0
C	0	2.79	10.9
C	26	3.66	10.6
C	7[a]	4.10	9.9

[a] The aging in alkali for this experiment was followed by 19 hours' aging in 0.5 *N* hydrochloric acid.

the amount recommended has no effect. Also adding twice the recommended amount of 1-naphthylethylenediamine has no effect on the color produced provided that the solution of this reagent has not become discolored. The intervals between the additions of the several reagents can be increased to 5 minutes or decreased to one half the recommended time without significantly affecting the results. The 10-minute period suggested for the final color development appears to be adequate. It is recommended that the final solution be measured within 6 hours after the time of preparation. Up to about 2.5 hours there is no measurable change, but there is a loss of 6% of the original color strength observed at a 24-hour aging period. The aging of the original alkaline and acid solutions is of greater importance, as Table VII shows. In these experiments, the pteroylglutamic acid was dissolved in 0.1 *N* sodium hydroxide, and this solution was allowed to age for different lengths of time before the analysis. The aging of this alkaline solution is of significance if the free *p*-aminobenzoylglutamic acid is important, as is generally true for the purified pteroylglutamic acid samples. For such samples, the analysis should be made immediately after the dissolution of the sample. In any event, it is advisable to use the alkaline solution within 2 hours after its preparation. In the third experiment for sample *C*, the solution was allowed to age in 0.5 *N* hydrochloric acid after the initial exposure to alkali. On comparing the results of this determination with those of the second determination of this sample, it can be seen that aging in acid is more rapid than in alkali and it is, therefore, advisable to allow no undue lapse of time between acidification and the addition of the other reagents.

In some instances, a first analysis may result in the production of a color which is too strong for the use of the calibration curve. If this occurs, it is not possible to obtain a satisfactory value by diluting the final colored solution. From the approximate value obtained, a modification in the size of the initial sample or of the aliquots taken can be made, and a new set of solutions prepared. This will result in a considerably higher value than that which would have been obtained by mere dilution of the final colored solution.

The diazonium salt of *p*-aminobenzoylglutamic acid was unstable in sunlight. A series of experiments was carried out in which *p*-aminobenzoylglutamic acid was diazotized in red flasks, in ordinary glass in laboratory light, and in ordinary glass in direct sunlight. The values obtained are shown in Table VIII. From this it can be seen that the diazonium compound is unstable and should be protected, at least from direct sunlight. Laboratory light has little effect as Table VIII shows.

As mentioned previously, there are deviations from Beer's law, but in the working range normally used this is not serious, as shown by Table IX. Using the method described, most of the samples of pteroylglutamic acid for assay should yield final solutions containing between 2.5 and 3.5 mg. per liter of *p*-aminobenzoylglutamic acid. To illustrate the extent of the deviation from Beer's law, a fourfold change in concentration from 1 to 4 mg. per liter produces a change of 4% in the absorbancy index; thus, if the absorbancy index of 204, found for 3.97 mg. per liter of aminobenzoylglutamic acid, were to be applied to a sample containing 1 mg. per liter of aminobenzoylglutamic acid, the latter value would be high to the extent of 4% of the amount present. Over the narrow range of concentrations obtained on finished product samples, this deviation will have little effect provided that an approximately equivalent concentration of *p*-aminobenzoylglutamic acid is used in preparing the standard curve. Using a standard curve as described under the method, there is no appreciable error from this source.

PRECISION AND ACCURACY

The precision of the recommended method, expressed as the standard deviation of a single value from its mean, is ±0.8% (relative). This is the average value obtained for the standard deviation calculated from the analyses at different times of four samples ranging from 84 to 92%. From 6 to 18 replicates were obtained for each sample.

Although, as previously stated, it has not been proved that this method has no systematic error, it appears that the method

Table VIII. Effect of Light on Diazonium Compound

Conditions of Diazotization	*p*-Aminobenzoylglutamic Acid Found, %
Red flask, laboratory light	99.0, 100.2; av. 99.6
Ordinary glass, laboratory light	99.0, 98.7; av. 98.9
Ordinary glass, direct sunlight	56.2, 57.5; av. 56.9

Table IX. Deviation from Beer's Law

Concn. of *p*-Aminobenzoylglutamic Acid, Mg./Liter	Absorbancy (Cor. for Blank on Reagents)	Absorbancy Index
0.496	0.105	218
0.992	0.210	212
1.49	0.311	209
2.48	0.511	206
3.47	0.713	205
3.97	0.809	204
4.96	0.999	201

Table X. Analysis of Purified Samples

Sample	Pteroyl-glutamic Acid, %	*p*-Amino-benzoylglutamic Acid, %	H_2O, %[a]	Total %
A	91.8, 91.8, 92.2, 92.2; av. 92.0	.....	7.9	99.9
B	91.7, 92.1; av. 91.9	0.09, 0.10; av. 0.10	7.5, 7.6; av. 7.6	99.6
C	95.9, 96.2, 96.7, 96.7; av. 96.4	0.24, 0.32, 0.31; av. 0.29	3.5, 3.9; av. 3.7	100.4

[a] Determined by Karl Fischer method.

at least closely approximates this condition. Table X shows that for several samples of pteroylglutamic acid purified under different conditions, analyses for water (determined by the Karl Fischer method), *p*-aminobenzoylglutamic acid, and pteroylglutamic acid have been obtained which have added up to practically 100%. Although it is fully realized that this is not conclusive evidence, it is strongly indicative that the present method has no systematic errors. In addition, the values for the purified samples agree with those obtained by a method involving consumption of nitrous acid. This has been reported to form a nitrosamine in the 10 position (*4*). (The details of this method will be the subject of a later paper.) Both the colorimetric and the titrimetric methods depend upon the measurement of the secondary amine in the 10 position.

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RECEIVED November 15, 1950. The spectrophotometric terminology employed in this paper is that recommended in "Analytical Absorption Spectroscopy" (*12*).

Determination of Magnesium in Plant Tissue with Thiazole Yellow

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The object of this work was to find a precise and accurate direct method for the determination of magnesium in plant tissue which would eliminate time-consuming preliminary separations of cations. A reliable procedure was obtained by utilizing the color reaction of magnesium with thiazole yellow under alkaline conditions. The color was stabilized with polyvinyl alcohol and interferences were eliminated or compensated for by the addition of hydroxylamine, copper, aluminum, calcium, manganese, and phosphate. Results show increased sensitivity and reproducibility over other similar methods. Standard titrimetric or gravimetric procedures for the determination of magnesium are time-consuming and expensive. A dependable direct method using small amounts of sample containing semimicro amounts of magnesium has obvious advantages. Inasmuch as direct flame photometric procedures are now available for the determination of potassium and calcium, a direct method for magnesium will make possible the rapid estimation of the three major cation components in plant tissue.

THE direct colorimetric determination of magnesium with thiazole or titan yellow in plant or soil extracts has been modified in many ways in recent years (*2*, *6–8*). Essentially the modifications consist of the use of a stabilizing agent to prevent precipitation of the magnesium-dye lake, the addition of a reducing or complexing agent such as hydroxylamine, and the addition of various interfering ions at a concentration above which further moderate increments would not result in changes in color intensity.

In the authors' experience with stabilizing agents, starch and gum ghatti solutions have not proved satisfactory largely because colored solutions prepared with them lacked sensitivity and reproducibility. The use of polyvinyl alcohol as recommended by Ellis (*3*) was tried and the results confirmed the experience of Heagy (*4*) who found it greatly increased sensitivity on serum samples. Further gain in sensitivity was attained by increasing the alkali concentration; under this condition polyvinyl alcohol was stable and showed no off colors in contrast to starch and gum ghatti which darken to an orange-amber shade in concentrated alkali. The use of a high concentration of alkali has the additional advantage that variation in acidity of the ash solutions results in relatively slight variation in alkalinity of the final solutions for colorimetry.

The depressing effect of copper as noted by Willson and Wander (*9*) proved to be a necessary factor for increased sensitivity in the proposed method. Although the color intensities of blank and sample were less in the presence of 15 micrograms of copper, absorbance (optical density) (*5*) of the sample less that of the blank was two to three times greater than that obtained without copper.

The method described herein, therefore, proposes the use of polyvinyl alcohol as the stabilizing agent, an increased alkali concentration, and the incorporation of 15 micrograms of copper into the compensating solution prescribed by Peech and English (*7*) as modified by Drosdoff and Nearpass (*2*).

METHOD

Apparatus. Either the Evelyn photoelectric colorimeter with filter No. 515 or 540 or the Klett-Summerson photoelectric colorimeter with filter No. 54 has been employed satisfactorily for this determination. Carefully matched tubes were used.

Reagents. 1. Hydrochloric acid, 3 *N*
2. Sulfuric acid, concentrated
3. Hydrogen peroxide, 30%
4. Hydroxylamine hydrochloride, 1% (weight/volume)
5. Compensating solution, grams per liter:
Calcium chloride ($CaCl_2$), 1.40

Aluminum sulfate [$Al_2(SO_4)_3.18H_2O$], 0.37
Manganous sulfate ($MnSO_4.H_2O$), 0.16
Sodium phosphate ($Na_3PO_4.12H_2O$), 0.70
Copper sulfate ($CuSO_4.5H_2O$), 0.059
Hydrochloric acid, concentrated, 5 ml.

6. Polyvinyl alcohol, Du Pont, Elvanol, Grade 71-24, 2% (weight/volume). Mix 20 grams with about 400 ml. of water, heat to about 90° C., and stir until dissolved. Cool, dilute to 1 liter, and filter if not clear. Keep in refrigerator.

7. Mixed reagent. Mix equal parts of 4, 5, and 6 just before use.

8. Thiazole yellow (General Aniline Works, Inc., Albany, N. Y.), 0.02% (weight/volume). Prepare fresh weekly and keep in dark bottle. A concentrate of 0.5% in 50% ethyl alcohol in a dark bottle keeps indefinitely.

9. Sodium hydroxide, 10 *N*. Prepare with low carbonate alkali.

10. Magnesium standard. Dehydrate magnesium sulfate ($MgSO_4.7H_2O$) by heating at 300° C. for 7 hours or until constant weight is obtained. Prepare a standard stock solution containing 1 mg. of magnesium per ml. by dissolving 1.2375 grams of the anhydrous magnesium sulfate in water and diluting to 250 ml. Dilute to prepare a standard containing 10 micrograms of magnesium per ml.

Preparation of Sample. DRY ASH. Ash 10 grams of fresh or 1 gram of dry tissue in a 60-ml. porcelain crucible at 600° C. for 1 hour or until carbon-free. Dissolve with 15 ml. of 3 *N* hydrochloric acid, heating if necessary; transfer to a 100-ml. volumetric flask and dilute to volume. Filter, pipet 10 ml. to a 50-ml. volumetric flask and dilute to volume. This is designated "sample." The rest of the filtrate may be used for other determinations.

WET ASH. Transfer 2 grams of fresh or 0.2 gram of dry tissue to a 200-ml. Kjeldahl flask with about 20 ml. of water, add 4 ml. of sulfuric acid, and digest until fuming starts and the mixture is dark. Cool, add 11 drops of 30% hydrogen peroxide, and digest for a few minutes or until colorless. Cool and dilute to 100 ml. This is designated "sample."

Determination. Pipet into a colorimeter tube a 5-ml. aliquot of sample containing not more than 40 micrograms of magnesium when using filter No. 540 with the Evelyn colorimeter or No. 54 with the Klett instrument, or 25 micrograms when using filter No. 515 with the Evelyn instrument. Add 3 ml. of mixed reagent and mix by swirling (Evelyn tube) or stirring with a thin rod having a horizontally flattened end (Klett tube). Introduce 1 ml. of thiazole yellow reagent, mix well, and then add 2 ml. of 10 *N* sodium hydroxide. Mix thoroughly. After adding thiazole yellow, the sample should not be allowed to stand; the thiazole yellow should be followed immediately with the sodium hydroxide. Allow to stand 10 minutes, tap the tube gently if air bubbles adhere to the side of the tube, and read absorbance in a photoelectric colorimeter using a green filter. Set the instrument at zero absorbance with a blank prepared with an amount of acid approximately equivalent to that of the sample. Samples containing relatively high salt content, a condition seldom encountered, may develop slight turbidity after 10 minutes. In this case read the absorbance after 5 minutes, being careful to set the blank accurately at the same time interval.

Inasmuch as the addition of reagents requires less than 1 minute, a group of ten samples may be prepared in 10 minutes and transmission readings taken in another 10 minutes. Thus the method is extremely rapid.

Transfer aliquots of the 10 micrograms per ml. of standard to colorimeter tubes, dilute to 5 ml., and proceed with the determination. Prepare a standard curve by plotting the data obtained. A straight-line relationship exists between absorbance and concentration of magnesium.

RESULTS AND DISCUSSION

Effect of Polyvinyl Alcohol Concentration on Color Stabilization. Polyvinyl alcohol concentration of 1 ml. of 0.1% solution in a volume of 12.5 ml. as prescribed by Heagy (*4*) for plasma or serum extracts, was inadequate in preventing precipitation of the magnesium–thiazole yellow lake with plant ash solutions, owing probably to the comparatively higher salt content, particularly after addition of the compensating solution. Increasing polyvinyl alcohol concentration gave the results shown in Table I. One milliliter of 2% polyvinyl alcohol was taken as optimum for the prescribed procedure where a total volume of 11 ml. was finally obtained. The clarity of the solutions appeared to be further improved later when an increased sodium hydroxide concentration was used.

Table I. Effect of Polyvinyl Alcohol Concentration on Color Stabilization

(Solutions contain 5.0 ml. of compensating solution, 1 ml. of 5% hydroxylamine hydrochloride, 1 ml. of 0.1% thiazole yellow, and 5 ml. of 2.5 *N* sodium hydroxide in addition to polyvinyl alcohol and magnesium)

Polyvinyl Alcohol, Ml./50 Ml. of Mixture	Mg, γ/50 Ml. of Mixture	Result
0.25, 1%	125	Red ppt.
0.5, 1%	125	Red ppt.
1.0, 1%	125	Red ppt.
3.0, 1%	125	Turbid
5.0, 1%	125	Clear
5.0, 2%[a]	250	Clear
5.0, 4%	250	Clear

[a] Equivalent to 1 ml. of 2% per 10 ml.

Table II. Effect of Alkali Concentration on Color Intensity

(15 micrograms of Mg, 10 ml. of mixture, Evelyn photoelectric colorimeter, filter No. 515)

NaOH, me.	3	6	12	16	20
Absorbance	0.213	0.364	0.396	0.467	0.467

Sodium Hydroxide Concentration. Inasmuch as variable acidity of samples appeared to cause erratic results, the effect of alkali concentration was ascertained as shown in Table II. In the presence of polyvinyl alcohol, the magnesium–thiazole yellow color apparently increases markedly in intensity with increase in alkali content. Twenty milliequivalents of sodium hydroxide were taken as the optimum quantity for an 11-ml. volume; at this concentration slight variation in acidity of the sample causes negligible change in color intensity. This compares with 2.5 to 3.0 me. generally used. The high alkalinity proposed is apparently suitable only when polyvinyl alcohol is employed as the protective colloid. With starch or gum ghatti the color formed is of an orange-amber shade rather than red.

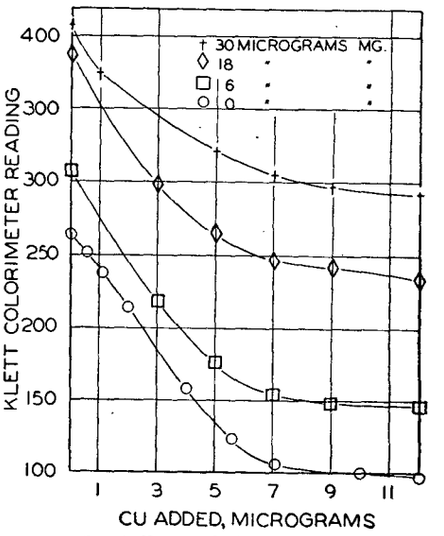

Figure 1. Effect of Added Copper on Color Intensity

Filter No. 54, 11-ml.

Effect of Copper. The depressing effect of copper on the intensity of the magnesium–thiazole yellow color as noted by Willson and Wander (*9*) is confirmed. However, under the conditions of the proposed method, the presence of copper is a unique advantage because apparently the color depression is in inverse proportion to the magnesium present. The net effect in the presence of 15 micrograms of copper is a depression of approximately 66% of the blank color and a depression of only 35% with 25 mi-

crograms of magnesium as shown in Table III. At least a two-fold increase in sensitivity is obtained.

The effect of adding cyanide as suggested by Willson and Wander is the complete elimination of the effect of copper and readings become high and sensitivity low as in the case where copper is absent (Table III). The addition of cyanide, therefore, is not prescribed.

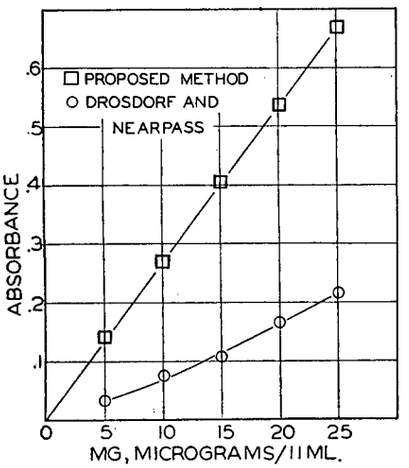

Figure 2. Comparison of Proposed Method and Drosdorf and Nearpass Method

Evelyn photoelectric colorimeter, filter No. 515

The increased sensitivity afforded by copper and polyvinyl alcohol with high alkali concentration is not additive but is dependent on both factors—that is, in the presence of copper, substituting starch together with low alkali concentration for polyvinyl alcohol with high alkali concentration does not increase sensitivity. Vice versa, without copper, polyvinyl alcohol with high alkali concentration also does not increase sensitivity.

Figure 1 shows the effect of adding copper to magnesium standards which contain approximately 3 micrograms of copper. Net absorbance reaches practically a constant level at 10 to 12 micrograms of added copper (total 13 to 15 micrograms). Above this point, an error within −3.1% will occur if the ratio of copper to magnesium is not greater than 1 to 2.5 as shown in Table IV

Table III. Effect of Copper on Absorbance and Sensitivity

Mg, γ	Cu, γ	Reading[a]	Net Reading	Sensitivity Gain[b]	Depression[c]	Depression, %[d]
0	0	445	...	...	...	...
25	0	525	80	...	...	...
0	15	150	...	...	295	66
25	15	343	193	2.4	182	35

[a] Klett-Summerson instrument, test tube, filter No. 54, 11-ml. mixture.
[b] $\frac{193}{80}$.
[c] 445 − 150; 525 − 343.
[d] $\frac{295}{445}$ (100); $\frac{182}{525}$ (100).

Table IV. Effect of Copper above 15 Micrograms

Mg, γ	Cu Added, γ	Total Cu, γ	Net Reading[a]	Decrease, %	Ratio Added Cu to Mg
25	0	15	191	...	...
25	5	20	188	1.6[b]	1 to 5
25	10	25	185	3.1	1 to 2.5
25	15	30	179	6.3	1 to 1.7

[a] Reading minus blank containing 15 micrograms copper, Klett-Summerson instrument, test tube, filter No. 54, 11-ml. mixture.
[b] For example: $\frac{191 - 188}{191}$ (100).

A correction based on Table IV may be applied to results obtained on relatively high copper samples. This has been unnecessary on pineapple tissue.

The proposed method eliminates interference from small amounts of copper when present in distilled water stored in partially corroded, tinned-copper tubing and tanks. Inasmuch as the same water is employed for standards and samples, any color-depression will be uniform throughout.

Comparison of Colorimetric Methods. The increased sensitivity of the proposed method is further shown in Figure 2 which represents a comparison of the method of Drosdoff and Nearpass and the proposed procedure on standard solutions of magnesium.

The Evelyn green filter No. 515 gives absorbance readings higher than No. 540 by the proposed method, whereas the difference is slight by the method of Drosdoff and Nearpass. Even with the Evelyn filter No. 540, absorbance by the proposed method is more than twice that of Drosdoff and Nearpass. With samples having a wide range of magnesium concentration, filter No. 540 is more practical than No. 515.

Effect of Manganese. Manganese develops a color with thiazole yellow similar to that of magnesium. The addition of hydroxylamine and the incorporation of 50 micrograms of manganese in the compensating solution as suggested by Drosdoff and Nearpass keep manganese interference to a minimum. However, an error within +5.5% will occur if the ratio of manganese to magnesium is not greater than 1 to 2.5 as shown in Table V. With samples containing a higher proportion of manganese a correction based on Table V may be applied to the magnesium values.

Table V. Effect of Manganese above 50 Micrograms

Mg, γ	Mn Added, γ	Total Mn, γ	Net Readings[a]	Increase, %	Ratio Added Mn to Mg
25	0	50	180	...	...
25	10	60	190	5.5	1 to 2.5
25	20	70	198	10.5	1 to 1.2
25	30	80	204	13.3	1 to 0.8

[a] Klett-Summerson instrument, test tube, filter No. 54, 11-ml. mixture, 24 micrograms of Cu.

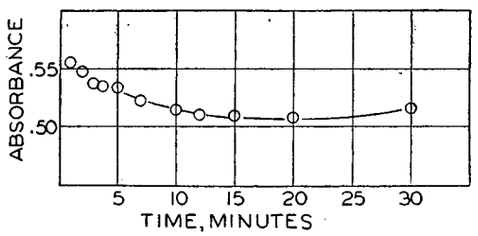

Figure 3. Stability of Magnesium–Thiazole Yellow Color

25 micrograms magnesium in 11-ml., Evelyn photoelectric colorimeter, filter No. 540

Stability of Color. Figure 3 shows the behavior of the magnesium–thiazole yellow color with time. Apparently at least 10 minutes are required for the color to become stable. Color intensity is practically constant from 10 to 20 minutes. Samples high in salt content may develop slight turbidity after 10 minutes, in which case readings at the three- to five-minute interval may be taken and compared with standards prepared similarly.

The initial high reading is due to the presence of small air bubbles which gradually rise to the surface within the first 3 minutes.

Order of Addition of Reagents and Other Effects. The order of addition of hydroxylamine, compensating solution, and stabilizer is not critical. This is followed by the dye and immediately the sodium hydroxide. With certain samples some

Table VI. Accuracy

Mg Present, γ	Mg Added, γ	Total	Total Found	Accuracy, %
20.0	12.5	32.5	33.0	101.5
20.0	12.5	32.5	33.0	101.5
18.5	12.5	31.0	32.4	104.5
19.6	12.5	32.1	32.8	102.1
24.5	12.5	37.0	37.3	100.8
12.7	12.5	25.2	25.6	101.6
			Mean	102

Table VII. Comparison of Colorimetric and Titrimetric Methods (*1*)

(With 50 samples, mean difference as % of titrimetric method = +1.7%' standard error = ±0.8%)

Mg in Fresh Tissue, %	
Colorimetric	Titrimetric
0.025	0.026
0.017	0.015
0.021	0.021
0.025	0.027
0.027	0.027
0.021	0.025
0.025	0.023
0.024	0.025
0.028	0.028
0.031	0.032
0.023	0.022
0.030	0.030
0.031	0.029
0.030	0.027
0.028	0.025
0.034	0.032
0.031	0.029
0.037	0.036
0.018	0.018
0.018	0.017

loss of color was noted if the sodium hydroxide was not added immediately. The sodium hydroxide should always be added last.

The first three reagents may be combined. This mixed reagent was found in practice to be stable for a month or more but this should be established for the prevailing conditions in the individual laboratory. The dilute dye was also stable for all practical purposes when protected from light and prepared with copper-free water. Thorough mixing is essential for reproducibility.

Contact with rubber tubing or stoppers must be avoided as higher and irregular values result. Contact with the mixed reagent or sample with reagents gave in some cases up to a third greater absorbance.

Although the prescribed compensating solution generally overcomes the effect of interfering ions, a further refinement in technique which contributes to greater accuracy involves incorporating into the standards average quantities of ions which are known from previous analyses of similar material to be present in the samples being analyzed. Thus the effect, particularly that of copper or manganese present in samples, may be equalized by adding appropriate quantities of these elements to the standards.

Accuracy. The accuracy of the method is shown in Table VI. Magnesium found averaged 102% of that present.

Comparison with Standard Method. Table VII presents typical comparative results on pineapple leaf tissue with the standard titrimetric procedure in which magnesium is precipitated as magnesium ammonium phosphate and its acid equivalent determined (*1*). A mean difference of 1.7% with a standard error of ±0.8% was obtained on 50 separate samples.

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Rapid 8-Quinolinol Procedures for Determination of Magnesium

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REDMOND and Bright (*5*) found that calcium precipitated as the oxalate does not retain any 8-quinolinol (8-hydroxyquinoline, oxine) when magnesium is later precipitated with 8-quinolinol in the same solution. They used an ammonia solution for the precipitation of magnesium with 8-quinolinol and had to make an R_2O_3 separation first because iron and aluminum also form insoluble oxinates under these conditions. Berg (*1*) used an alkaline tartrate solution for the precipitation of magnesium, which has been reported (*2*) to give a good separation from aluminum but not from iron. Other workers (*4*) have reported that the iron oxinates are very soluble in sodium hydroxide–tartrate solutions. It was apparent from this literature that it might be possible to determine magnesium directly with 8-quinolinol in an alkaline tartrate solution containing sodium oxalate. The conditions for such a procedure have been studied and methods for determining magnesium directly in leaf tissue solutions have been developed.

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REAGENTS

Reagents for Precipitating Magnesium. Oxalic–Tartaric Acid Solution. Dissolve 3 grams of oxalic acid and 25 grams of tartaric acid in 75 ml. of water. Adjust the final volume to 100 ml.

Sodium Hydroxide, 0.25 *N*. Dissolve 10.0 grams of sodium hydroxide in 1000 ml. of water.

Strong Sodium Hydroxide Solution, 10 *N*. Dissolve 40 grams of sodium hydroxide in 100 ml. of water.

Concentrated Hydrochloric Acid.

Potassium Cyanide Solution, 5%. Dissolve 5 grams of potassium cyanide in 100 ml. of water.

Bromothymol Blue, 0.05% in a 50% ethyl alcohol–water mixture.

Alcoholic 8-Quinolinol, 1%. Prepare a fresh 1% solution of 8-quinolinol in 95% ethyl alcohol as required.

Calcium Solution. Place 2.5 grams of calcium carbonate in a 1000-ml. volumetric flask. Add about 100 ml. of water and 5 ml. of concentrated hydrochloric acid. When the calcium carbonate has dissolved, dilute the solution to 1000 ml.

Reagents for Determining Magnesium in Precipitate. Wash Solution. Mix 500 ml. of 10% ammonium hydroxide with 500 ml. of 95% ethyl alcohol.

A rapid laboratory method has been developed for determining magnesium directly in aliquots of citrus leaf tissue solutions containing 0.1 to 0.6 mg. of magnesium. The presence of 4 mg. of calcium, 0.1 mg. of iron, 0.15 mg. of copper, and 0.1 mg. of aluminum did not interfere with the determination. Recovery of 0.1 mg. of magnesium added to leaf sample solutions ranged from 90 to 113%. The maximum deviation from an 8-quinolinol reference method which included preliminary separations of interfering cations was equivalent to 7.5% of the amount of magnesium determined. This method may be useful where the greater sensitivity of the thiazole yellow method is not required.

Ferric Chloride–Acetic Acid Reagent.

Ethyl Alcohol, 95%. Dissolve 20 grams of ferric chloride hexahydrate in 4 liters of water containing 20 ml. of glacial acetic acid.

Reagents for Determining Magnesium by Excess 8-Quinolinol. Sulfanilic Acid. Dissolve 1.72 grams of sulfanilic acid in 60 ml. of acetic acid. Adjust the solution to 200 ml. with water.

Sodium Nitrite. Dissolve 1.425 grams of sodium nitrite in 500 ml. of water.

Hydrochloric Acid, 1 *N*. Dilute 88.5 ml. of concentrated hydrochloric acid to 1000 ml.

Sodium Hydroxide, 2 *N*. Dissolve 80 grams of sodium hydroxide in approximately 900 ml. of water. When the solution cools adjust the volume to 1 liter.

Magnesium Standards. Dissolve 1.7638 grams of magnesium acetate tetrahydrate in 1 liter of distilled water. This solution contains 0.200 mg. of magnesium per ml.

PROCEDURES

Procedure for Precipitation of Magnesium. Pipet a suitable aliquot of the sample solution to contain between 0.05 and 0.55 mg. of magnesium into a 12-ml. calibrated centrifuge tube. Add 1.0 ml. of the oxalic–tartaric acid solution and one drop of bromothymol blue indicator. Adjust the total volume at this point to 6 ml. with water. Put a stirring rod in the tube and place the tube in a water bath at 95° C. Remove the tube after a minute or so and adjust the solution slightly acid to bromothymol blue with strong sodium hydroxide and concentrated hydrochloric acid, then add 0.25 *N* sodium hydroxide until the solution is just blue. While the solution is still hot add 5 drops of potassium cyanide and 1.0-ml. excess of 0.25 *N* sodium hydroxide. The well mixed solution should have a volume of less than 9 ml. at this point. Add 1.00 ml. of alcohol–8-quinolinol solution and stir until the magnesium precipitate is indicated by a further increase in the turbidity of the solution. This usually requires about 30 seconds' stirring with the stirring rod gently scraping the side of the tube to help start the precipitation. Let the solution stand at room temperature for 1 hour. Run a series of standards containing 0, 0.2, 0.4, and 0.6 mg. of magnesium along with the samples. Add 2 ml. of the calcium reagent to each standard and adjust the volume to 5 ml. before starting the procedure with the addition of 1 ml. of the oxalic-tartaric acid mix. Magnesium may be determined indirectly from the hydroxyquinolate in either the precipitate or the supernatant liquid.

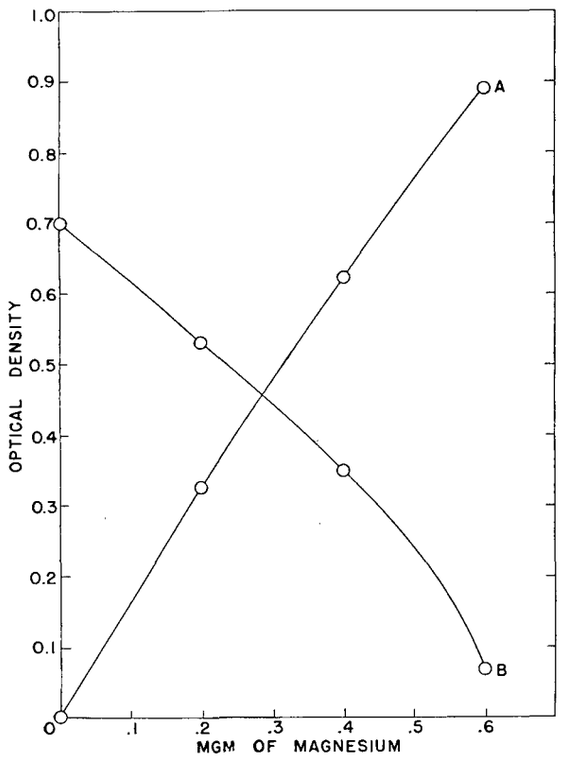

Figure 1. Calibration Curves for Determination of Magnesium with 8-Quinolinol

A. Optical curve at 650 mμ for ferric oxinate in dilute acetic acid

B. Optical density curve at 425 mμ for dye formed by coupling 8-quinolinol with diazotized sulfanilic acid

Determination of Magnesium in Precipitate (Precipitate Method). Wash down the stirring rod and sides of the tube with 1 ml. of 95% alcohol and centrifuge at 3000 r.p.m. for about 10 minutes. Pour off the supernatant liquid and invert the tube on a paper towel to drain for a few minutes. Add 10 ml. of the ammonia-alcohol wash solution and stir up the precipitate. Wash down the rod and sides of the tube with 1 ml. of 95% alcohol and centrifuge again at 3000 r.p.m. for 10 minutes. Pour off the liquid and drain the tubes as before. Wash the precipitate out of the tube into a 50-ml. graduate or volumetric flask with the ferric chloride–acetic acid solution. Adjust the volume to 50 ml. with the ferric chloride–acetic acid solution and allow about 30 minutes for complete solution of the precipitate. Mix and measure the absorbance at 650 microns in a photometer, using the zero standard for a reference cell at zero absorbance. An alternating current model Fisher electrophotometer has been used for this work.

Determination of Magnesium from Excess 8-Quinolinol (Excess Oxine Method). Remove the stirring rod from the solution containing the precipitate and allow it to drain for a few seconds. Adjust the volume to the 10-ml. mark with water and mix the solution thoroughly. Remove the stirring rod and centrifuge at 3000 r.p.m. for about 10 minutes. Draw off the film of precipitate remaining on the surface of the liquid with suction. Pipet a 1-ml. aliquot of the clear solution into a 100-ml. volumetric flask and add 5 ml. of 1 *N* hydrochloric acid and 55 ml. of water. (The small amount of hydrogen cyanide generated at this point does not diffuse from the flask and presents no hazard.) Then add 1 ml. of the sulfanilic acid and 1 ml. of the sodium nitrite solutions. Stopper the flask, mix well, and let this solution stand for about 15 minutes. Remove the stopper and add 20 ml. of 2 *N* sodium hydroxide. Mix the solution again and let it stand for another 10 minutes. Adjust to 100-ml. volume, mix, and measure the absorption at 425 microns in a photometer. In this case the zero magnesium standard has the greatest absorption. Adjust the photometer so that readings for both the zero and 0.6-mg. standards are on the scale. It has been found that when the optical density for the zero standard is set at 70 on the Fisher electrophotometer scale, a reading of about 8 is obtained from the 0.6-mg. standard. Using the zero standard for a reference cell, measure the absorbance of the standards and samples.

EXPERIMENTAL

Typical calibration curves for the two procedures are given in Figure 1. The slopes of these curves vary slightly with different sets of determinations and it is therefore necessary to run stand-

Table I. Effect of Time on Precipitation of 0.2 Mg. of Magnesium

(In presence of 2 mg. of Ca, 20 micrograms of Fe and Al, and 5 micrograms of Cu)

Time of Precipitation, Hours	Mg Found, Mg.
0.5	0.20
	0.20
1.0	0.19
	0.22
2	0.19
	0.20
4	0.20
	0.21

ards each time. Only slight changes were observed in the color of the solutions after 15 hours.

Effect of precipitation time on the determination of magnesium is shown in Table I. The precipitation time for the standards used for comparison was 2 hours.

The effect of possible interfering ions which are likely to be present in leaf samples is given in Table II. Data for determinations from the excess oxine procedure are not complete because only enough samples were run to confirm the results from the precipitation procedure in the range in which these elements were likely to be present in leaf samples.

The recovery of 0.1 mg. of magnesium added to citrus leaf sample solutions by the two procedures is shown in Table III.

A group of sample solutions which had previously been analyzed for magnesium by the regular oxine procedure (*3*, *6*) were analyzed again by the two procedures described in this paper. The reference procedure included an R_2O_3 separation and calcium oxalate separation before the precipitation of magnesium as the oxinate from an ammonia solution. Results for these analyses are presented in Table IV.

The new methods yielded values which were generally 0.01 to 0.02% higher than the reference method. However, this variation is well within the permissible limits of error for leaf tissue analysis.

Discussion of Precipitation Procedure. An accurate measure of the oxine is essential for the determination based on excess 8-quinolinol. Acid solutions of the reagent are generally stable and these were tried before using the comparatively volatile, unstable alcoholic solution. The 8-quinolinol is soluble in the oxalic-tartaric acid solution, but cannot be combined with this reagent because iron and probably the aluminum complexes with tartrate have to be formed first. It was impossible to prevent iron oxinate from precipitating with magnesium oxinate when oxalic acid, tartaric acid, and 8-quinolinol were used in a single solution. Erratic results were obtained with hydrochloric acid solutions of 8-quinolinol. The presence of acetic acid seemed to interfere with the formation of the iron tartrate complex. When 1 ml. of a 1% solution of 8-quinolinol in 0.5 *N* acetic acid was used for the precipitation of magnesium at a pH of 11.7, iron oxinate also precipitated. With a 1% alcoholic oxine solution, iron oxinate precipitated at pH 10.8 and under. At pH 11.1 and above there was no precipitation of iron. Low results were obtained which check samples when the pH for the precipitation was at 12 or over. The measured pH of a number of determinations indicated that satisfactory results could be obtained in the pH range of 11.1 to 11.7 for the precipitation.

Table II. Determination of 0.2 Mg. of Magnesium in Presence of Possible Interfering Ions

Ca, Mg.	Materials Added, γ			Magnesium Found, Mg.	
	Fe	Cu	Al	Precipitate method	Excess oxine method
0	...	...	...	0.18	0.19
1	...	...	...	0.20	0.19
3	...	...	...	0.19	0.20
5	...	...	...	0.20	0.20
4	10	...	...	0.20	...
4	50	...	...	0.20	...
4	100	...	...	0.20	...
2	50	...	...	0.20	0.19
4	50	50	...	0.20	...
4	50	100	...	0.20	...
4	50	150	...	0.19	...
2	50	50	...	0.21	0.23
4	50	100	50	0.20	...
4	50	100	100	0.20	...
2	50	50	50	0.19	0.21

Table III. Recovery of Magnesium Added to Citrus Leaf Sample Solutions

No.	Magnesium Added, Mg.	Magnesium Found, Mg.		% Recovery	
		Precipitate method	Excess oxine method	Precipitate method	Excess oxine method
1	0	0.19	...	...	...
1	0.1	0.29	...	100	...
2	0	0.40	...	...	...
2	0.1	0.50	...	100	...
3	0	0.33	0.31	...	...
3	0.1	0.43	0.41	100	100
4	0	0.38	0.37	...	...
4	0.1	0.49	0.46	110	90
5	0	0.39	0.40	...	...
5	0.1	0.50	0.50	110	100

Table IV. Magnesium in Citrus Leaf Samples

Reference Method, %	Precipitate Method, %	Excess Oxine Method, %
0.34	0.36	0.36
0.32	0.34	0.32
0.28	0.30	0.29
0.43	0.42	0.44
0.35	0.37	0.35
0.37	0.39	0.40
0.34	0.36	0.34
0.40	0.41	0.42
0.40	0.43	0.42
0.37	0.38	0.38
0.37	0.38	0.36

The presence of calcium in leaf sample solutions is advantageous. It adds bulk to the total precipitate and helps to collect the lighter, more flocculent magnesium oxinate. It is therefore convenient to add 2 mg. of calcium to each of the magnesium standards.

Because only 1 hour is allowed for the precipitation, it is important to start it properly by gently scraping the side of the centrifuge with a stirring rod for about 30 seconds, or more if necessary, after adding the alcoholic 8-quinolinol.

Maximum limits were not established for calcium, iron, copper, and aluminum, because such limits may be readily varied by changing the amounts of oxalic acid, tartaric acid, and potassium cyanide in solution. However, concentrations of interfering elements were studied which are well above any that are likely to occur in aliquots from plant tissue solutions. Magnesium may be precipitated with 8-quinolinol in the presence of ammonium oxalate and an excess of ammonium hydroxide. Such a procedure was used satisfactorily on most leaf sample solutions, but muddy precipitates were obtained with some samples, which resulted in higher values than were obtained by reference methods. The alkaline tartrate procedure described in this paper produced a clean magnesium oxinate precipitate with these samples and gave much closer agreement with reference methods.

DISCUSSION

Magnesium can be quantitatively precipitated in the presence of calcium, iron, aluminum, and copper from an alkaline tartrate solution containing sodium oxalate and potassium cyanide. The pH of the solution for the precipitation should be between 11.1 and 11.7. One hour has been found adequate for the precipitation, and the magnesium may be determined from the 8-quinolinol present in either the precipitate or supernatant liquid. Recovery of 0.1 mg. of magnesium added to leaf sample solutions ranged from 90 to 113%. In general, the results of analysis for a series of leaf sample solutions were slightly higher than those ob-

tained by a conventional oxine procedure. A maximum deviation from the reference method was equivalent to 7.5% of the amount determined.

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Determination of Scandium with 8-Quinolinol

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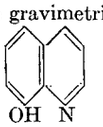
In order to standardize pure scandium-containing solutions, a method was sought which did not consume too much material, thereby conserving the standard solution for future use. 8-Quinolinol (oxine) was selected because it has a low gravimetric factor, and because it fulfills the requirements for a successful method. The precipitate formed between oxine and scandium ion has the formula $Sc(C_9H_6NO)_3.C_9H_7NO$, with the factor 7.24_3 for per cent scandium. The method is applicable to any solution containing scandium, provided other ions precipitated by oxine have been removed. It can be successfully employed for quantitative determination of 0.04 to 0.6 millimole of scandium.

IN THE course of an investigation of the chemistry of scandium, the need for a new method of standardizing solutions of pure scandium salts became apparent. Although methods for scandium are numerous, all have one or more disadvantages (Table I). In particular, those gravimetric procedures leading to scandium sesquioxide (65% scandium) are undesirable, inasmuch as they require that an excessively large portion of the available scandium be used for standardizations.

Because the gravimetric factor for metals determined by the 8-quinolinol, 

(oxine) method is generally low, and because it appeared that this reagent would otherwise fulfill the requirements for a successful method including ease of recovery of the rare and therefore valuable scandium, small sample size, simplicity, and accuracy, its use was indicated.

Berg (*1*) has applied the use of 8-quinolinol to the quantitative determination of almost 30 different elements. In most cases the compound formed between a transition element and oxine is one in which each oxine formally satisfies one covalent and one coordination bond—for example, $Al(C_9H_6NO)_3$. In a few cases (*2*), an additional molecule of oxine or water may enter the compound—for example, $Th(C_9H_6NO)_4.C_9H_7NO$.

REAGENTS

All materials used were C.P. grade, and unless otherwise stated were used as commercially available with no further purification.

Scandium perchlorate was prepared from purified scandium oxide as described elsewhere (*9*).

The 8-quinolinol reagent is 5% 8-quinolinol by weight in 2 *N* acetic acid. The 2 *N* ammonium hydroxide is adjusted to equal, as closely as practicable, the normality of the acetic acid in the 8-quinolinol reagent. The ammonium acetate solution is 2 *N*.

PROCEDURE

The sample to be analyzed should be separated by one of the standard methods (*3*, *8*) from aluminum, rare earths, and other materials giving a precipitate with oxine. The solution of pure scandium as the chloride, nitrate, or perchlorate is made up to volume in a volumetric flask. An aliquot containing approximately 0.03 millimole of scandium is diluted to a total volume of 110 ml., 5 drops of 0.005% aerosol in water solution are added and the solution is heated to 75° C. 8-Quinolinol reagent is added (10 ml.), followed by the addition with stirring of 45 ml. of a buffer made by mixing 30 ml. of ammonium acetate with 15 ml. of ammonium hydroxide. This buffer is designed so that the system after precipitation will have a pH of about 8.5. If more than 10 ml. of 8-quinolinol are used in the precipitation, the volume of ammonium hydroxide in the buffer must be increased by the same volume.

After standing, with occasional stirring, for 2 hours the lemon yellow precipitate is filtered through a weighed sintered-glass crucible, washed with a minimum quantity of distilled water (not more than 150 ml.) at room temperature, and heated to constant weight at 100° to 110° C. to remove free oxine which is always associated with the precipitate.

Inasmuch as the precipitate is $Sc(C_9H_6NO)_3.C_9H_7NO$, molecular weight, 622.70, the factor for per cent scandium is 7.24_3 and for per cent scandium sesquioxide, 11.10.

EXPERIMENTAL

Berg (*1*) has shown that successful use of 8-quinolinol for analytical purposes depends on many factors. With this in view, the effect of temperature of precipitation, quantities of reagent, quantities of buffer, pH of solution, temperature of ignition, use of Aerosol, and size of sample have been investigated. In all cases interfering ions must be absent.

Table II shows the results of varying the pH of the sample, keeping all other factors constant. All pH's were determined on a Beckman Model H-2 pH meter using a glass electrode standardized against a potassium acid phthalate buffer of known hydrogen ion concentration. The values shown are for the filtrate from

Table I. Gravimetric Methods for Determination of Scandium

Method	Reference	Objections
Hydroxide precipitation	(*10*)	Hard to filter and wash; must be ignited to oxide
Basic thiosulfate	(*7*)	Difficult to recover Sc and remove S
Fluosilicate	(*6*)	Difficult to recover Sc and remove SiF_6^{--}
Basic tartrate	(*3*)	Must be ignited to Sc_2O_3; high gravimetric factor
Oxalate precipitation	(*5*)	Not a quantitative method
Buffered pyridine	(*8*)	Must be ignited to Sc_2O_3

[1] Present address, Southwestern Louisiana Institute, Lafayette, La.

Table II. Effect of pH and Buffer

Run No.	Buffer Composition, Ml. NH_4OAc, 2 N	Buffer Composition, Ml. NH_4OH, 2 N	pH	Sc_2O_3 Taken, Gram	Sc_2O_3 Found, Gram
45	None	3.8	3.90	0.0240	0.0000
59	30	10	5.72	0.0240	0.0225
162	30	10	6.15	0.0231	0.0227
160	30	10	6.30	0.0231	0.0229
159	30	12	7.75	0.0231	0.0230
158	30	14	7.90	0.0231	0.0231
165	30	16	8.58	0.0231	0.0231

precipitation, but before the solutions were diluted by addition of wash water. The pH 3.9 is that pH at which the precipitate just dissolved. A pH range of 6.5 to 8.5 gives quantitative results. Mixing ammonium hydroxide and ammonium acetate prior to adding the buffer to the scandium solution was definitely superior to adding each of these solutions separately.

Aerosol in the precipitation was helpful because it made the precipitate easier to handle and prevented creeping of the precipitate. In addition, the compound did not stick to the glassware.

Another factor making the precipitate easier to handle was precipitation at 70° C., or thereabouts. Room temperature precipitation required digestion in order to form a precipitate which could be retained by the filter. Precipitation at various temperatures between room temperature and 70° C. was not found to offer any advantages over precipitation at 70° to 80° C.

The amount of reagent (oxine in 2 N acetic acid) used was critical. If insufficient oxine was added, the amount of precipitated material found was less than theoretical (Table III). An excess of oxine is therefore needed to ensure complete precipitation.

Table III. Effect of Quantities of Oxine

(Amount of buffer adjusted to achieve indicated pH)

Run No.	Ml. of 5% Soln. Oxine in 2 N HOAc	pH	Sc_2O_3 Taken, Gram	Sc_2O_3 Found, Gram
54	5	7.56	0.0239	0.0206
55	7	7.57	0.0239	0.0209
56	9	7.55	0.0239	0.0238
53	10	7.80	0.0239	0.0241

It was thought that, because the pH was critical, using oxine in methanol would avoid the addition of large amounts of acetic acid, thus eliminating the need for large amounts of buffer. Table IV shows that a large excess of oxine is needed. No advantages were found using methanol, ethyl alcohol, acetone, or dioxane, and the use of organic solvents was thus abandoned.

Table IV. Effect of Quantities of Oxine

(Organic solvent)

Run No.	Ml. of 5% Soln. Oxine in Methanol	pH	Sc_2O_3 Taken, Gram	Sc_2O_3 Found, Gram
83	5	9.82	0.0173	0.0165
84	7	9.78	0.0173	0.0166
85	9	9.75	0.0173	0.0169
68	10	9.90	0.0173	0.0173

Temperature of drying of the final precipitate was extremely important. At first, it was attempted to dry the compound to a constant weight corresponding to the formula $Sc(C_9H_6NO)_3$. However, 150 hours at 165° C. did not bring the compound to constant weight, so that plan was discarded. Subsequently, it was found that heating the compound at 100° to 110° C. for 2 to 5 hours brought it to constant weight at a composition corresponding to $Sc(C_9H_6NO)_3.C_9H_7NO$. Temperatures around 135° C. cause this compound to lose weight and approach, but not attain, the composition $Sc(C_9H_6NO)_3$. Table V shows the results of several different heating times and temperatures. Heating the precipitate at 100° to 110° C. to constant weight (usually 2 to 5 hours) is the desirable condition.

Analyses of solutions containing known amounts of scandium were carried out using the procedure described above. These determinations were made using sample sizes which would produce from 0.03 to 0.38 gram of precipitate (0.04 to 0.6 millimole). For all samples analyzed, the error was 0.7% or less (Table VI).

Table V. Effect of Heating Time and Temperature

Run No.	Theor. Wt., Gram	Temp., ° C.	Time of Heating	Wt. of Sample, Gram	Theor., %
172	0.2896	165	50 min.	0.2407	83.1
171	0.2896	165	1 hr. 37 min.	0.2446	84.5
170	0.2896	165	1 hr. 37 min.	0.2812	97.1
A[a]	0.2896	135	61 min.	0.2876	99.3
			2 hr. 5 min.	0.2869	99.1
			4 hr. 16 min.	0.2859	98.7
			6 hr. 53 min.	0.2843	98.2
			20 hr. 6 min.	0.2822	97.4
			90 hr. 52 min.	0.2488	85.9
B[b]	0.1539	110	3 hr. 18 min.	0.1545	100.4
			5 hr. 18 min.	0.1543	100.3
			7 hr. 18 min.	0.1538	99.9
167	0.2896	110	15 min.	0.2893	99.9
			66 min.	0.2890	99.8
168	0.2896	110	15 min.	0.2900	100.1
			66 min.	0.2899	100.1
169	0.2896	110	15 min.	0.2925	103.1
			66 min.	0.2901	100.2
			1 hr. 58 min.	0.2899	100.1

[a] Mass of sample is the average of masses of samples of runs 167, 168, and 169 after these had reached constant weight at 110° C.
[b] Mass of sample is the average of masses of samples in three concurrent runs.

For even smaller samples, a volumetric method is possible. The method is the bromometric one described in Kolthoff and Sandell (4). Typical results obtained are indicated in Table VII.

Because scandium oxinate is readily soluble in 2 N hydrochloric acid, the problem of recovery of the scandium is an easy one. Usually the material was dissolved in hydrochloric acid, brought practically to dryness in a mixture of nitric and hydrochloric acid

Table VI. Effect of Sample Size

Run No.	Theor., Gram	Actual, Gram	Yield, %
146	0.1512	0.1486	98.28
147	0.1512	0.1502	99.34
148	0.1512	0.1503	99.40
149	0.1512	0.1512	100.0
150	0.1512	0.1516	100.3
151	0.1512	0.1526	100.9
		Av.	99.70
152	0.0302	0.0299	99.0
153	0.0302	0.0300	99.3
154	0.0302	0.0301	99.7
		Av.	99.3
155	0.3780	0.3750	99.21
156	0.3780	0.3762	99.52
157	0.3780	0.3766	99.63
		Av.	99.45

Table VII. Determination of Scandium by Bromometric Method

Run No.[a]	Sc Taken, Mg.	Sc Found, Mg.
1	14.0	14.0
2	14.0	14.0
3	14.0	14.1
4	3.91	3.88
5	3.91	3.87
6	1.95	1.94
7	1.95	1.93
8	1.96	1.91
9	1.96	1.90
10	0.784	0.778
11	0.784	0.784

[a] Runs 1 to 7 made using 50-ml. burets and ca. 0.1 N solutions. Runs 8 to 11 made using 10-ml. burets and ca. 0.025 N solutions.

to destroy organic matter, and recovered by one of the standard procedures, the basic tartrate method (*3*) being preferred.

ACKNOWLEDGMENT

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Apparatus and Procedure for Rapid Automatic Adsorption, Surface Area, and Pore Volume Measurement

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Knowledge of surface area and pore structure is of primary importance in understanding the behavior of catalysts and other finely divided materials. The conventional Brunauer-Emmett-Teller method is laborious and time-consuming. Apparatus for continuous adsorption measurement has been developed. The method involves a constant slow flow of gas into the adsorbent section, so that equilibrium pressure is approximated and the amount adsorbed (uncorrected for dead space) is proportional to time. Surface area is calculated from the time required to reach a relative pressure end point of 0.2, using nitrogen as the adsorbate at liquid nitrogen temperature. This time is indicated automatically by a Bourdon gage with contact points or a vacuum recorder. The apparatus is very flexible and can also be used to equilibrate the sample at any desired pressure. This technique using a relative pressure of 0.97, followed by desorption through a wet-test meter, is chosen for pore volume measurement. The simplicity of procedure, short working time (15 minutes per test), ruggedness of apparatus, etc., place surface area determination in the same class as other routine physical tests. Surface area results can be expected to be within 5% of those of the Brunauer-Emmett-Teller method and to be reproducible within 3%.

IN CONTACT catalyst development work it is highly desirable to have rapid methods for the determination of surface area and pore volume. Catalytic activity defined by relative space velocity for given conversion is usually proportional to surface area when diffusion is not rate controlling, and the nature of the surface is constant. Porosity is important because of its effect on the diffusion of reactants and products to and from the internal surface and its effect on area stability. Higher pore volume for a given surface area would be expected to correlate with higher catalyst stability because of greater resistance to sintering. Porosity-area data may also be used in some cases to determine the cause of catalyst deactivation—for example, steam deactivation of silica-alumina cracking catalyst does not cause appreciable change in pore volume whereas thermal deactivation does. Therefore, pore volume data on deactivated cracking catalyst may give an idea as to the relative importance of these factors.

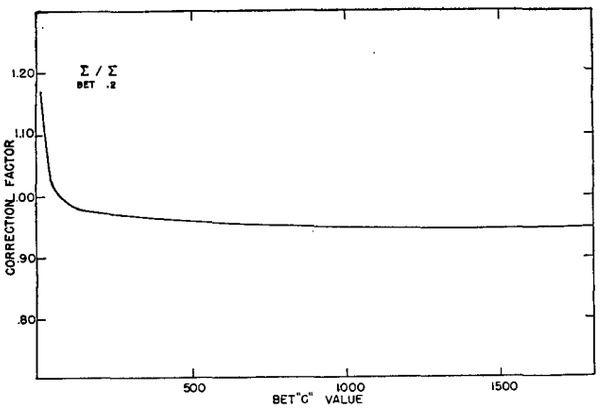

Figure 1. Correction Factor for Calculating BET Surface Area from Innes Surface Area[a]

[a] **Calculated from sorption at $P/P_o = 0.2$. $\left(\frac{\Sigma}{0.2} = 3.5V_{0.2}\right)$**

The work of Emmett and coworkers (*3,4*) has established that low temperature nitrogen-sorption measurements provide the best data for surface area determination. A number of methods have been proposed for the calculation of area from these data (*6*). Because of simplicity, the author prefers to use the relation, surface area = $3.5V_{0.2}$ where $V_{0.2}$ is the volume in milliliters at standard pressure and temperature sorbed at a relative pressure of 0.2. The theoretical agreement of this procedure with the Brunauer, Emmett, and Teller method (15.4 A.^{2} per molecule) as a function of the BET C value is shown in Figure 1 (obtained by substitution in the BET equation (*4*) of $P/P_o = 0.2$). The value, 15.4 A.^{2} per molecule, was recommended by Livingston for the nitrogen cross section (*5*); Emmett prefers the value of 16.2 A.^{2} per molecule. This is more generally used and would correspond to the relation, surface area = $3.68V_{0.2}$. Silica-alumina cracking catalysts ranging in area from 50 to 700 square meters per gram had on the average (25 samples) a C value of 70. The factor 3.5 was chosen so that perfect agreement would

be obtained on the average. The average deviation between individual determinations of BET area and $3.5V_{0.2}$ was 2%.

Conventional adsorption measurements are carried out by adding gas in increments and allowing it to equilibrate with the adsorbent after each addition. This requires considerable time if several points are determined and does not readily adapt itself to automatic operation. It appeared that more rapid results with automatic operation could be obtained by a continuous, constant, and slow introduction of adsorbate.

APPARATUS

The apparatus developed for continuous adsorption measurement is shown in Figure 2. The primary feature is that it is possible to obtain a constant, small rate of flow of gas into the adsorbent section so that the amount of gas introduced may be determined simply by a time measurement while the pressure in the adsorbent section approximates equilibrium pressure. This is accomplished by means of the Moore flow controller which operates to maintain a constant 3-pound-per-square-inch pressure drop across the capillary, or needle valve 2. A constant forepressure (6.0 pounds per square inch) is realized by the action of the pressure regulator and pressure relief valve. Hence, the flow into the adsorbent section should be constant despite variations of the pressure in the adsorbent section. To obtain the low flow rate desired, small diameter capillary tubing must be employed. Fever thermometer tubing with an internal diameter of about 0.001 inch served the purpose. Standard Hoke needle valves are used throughout and have proved satisfactory.

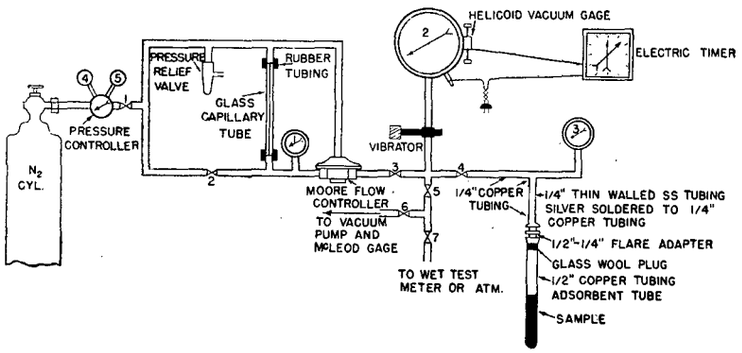

Figure 2. Apparatus for Continuous Adsorption, Surface Area, and Pore Volume Measurement

A Helicoid-type Bourdon gage (American Chain and Cable Co., Bridgeport, Conn.) with contact points was dependable for the vacuum measurements. Periodic checks against a mercury manometer showed no change in its calibration over several months' usage. Its good features include the following:

1. No volume change occurs with pressure change
2. Measurements can be made rapidly
3. The time to attain a given pressure can be automatically indicated by use of an electric timer

Bad features are a slight frictional effect which can be overcome by use of a vibrator or by tapping, and the necessity of calibration for precise work.

The apparatus is largely of metal construction. It is compact, inexpensive, and easy to construct and maintain.

Measurement and Constancy of Flow Rate. Flow rate can be readily and accurately determined by allowing the gas to flow into a given known volume and measuring the vacuum of this section as a function of time—for example, valves 4 and 5 may be closed with valve 3 (Figure 2) open and gage readings taken as a function of time. A typical plot of data obtained in this manner is given in Figure 3. Data on flow into a known larger volume where the pressure was measured with a mercury manometer are given in Figure 4. The constancy of flow rate despite change in pressure is indicated by the straight-line relationship. Flow rate can be determined from the slope of these plots by application of the perfect gas law. A measure of the flow rate may also be obtained from the reading of gage 1.

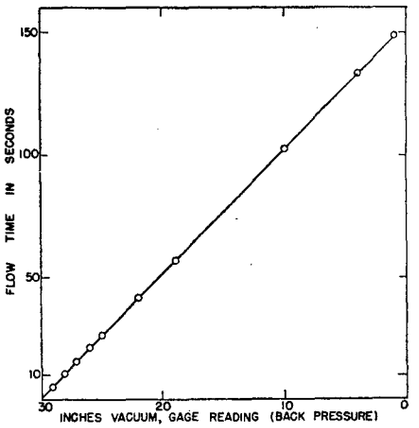

Figure 3. Determination of Constancy of Flow Rate into Small Volume with Varying Back Pressure

Volume, 28 ml.
Flow rate, 9.7 ml./minute, STP

At low back pressures in the range of primary interest (22 to 31 inches vacuum), no evidence of varying flow has ever been observed. However, with a forepressure less than 5 pounds per square inch, a back pressure greater than 20 inches vacuum, and flow rates less than 6 ml. per minute at standard temperature and pressure, the flow decreased slightly with increasing back pressure.

Approach to Equilibrium. This is naturally determined by the flow rate, size, packing, and nature of the sample as well as the relative pressure involved. If flow rate is to be constant over the whole range, the findings on one Moore flow controller and information from the Moore Products Co. of Philadelphia, Pa., suggest that flows in excess of 8 ml. per minute at standard temperature and pressure should be employed. (Also, the low flow require-

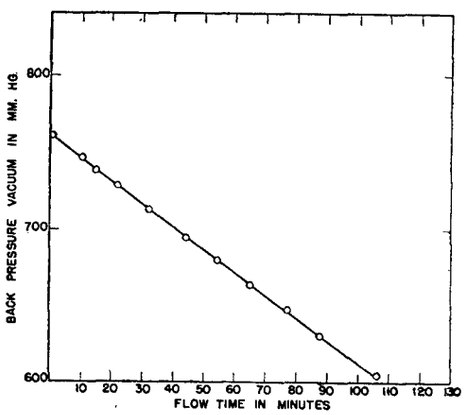

Figure 4. Determination of Constancy of Flow Rate into Large Volume

Volume, 3160 ml.
Flow rate, 5.7 ml./minute, STP

Table I. Water Pickup during Weighing

Time on Balance Pan[a], Min.	Sample Wt., Grams
0	3.34
5	3.36
12	3.40
25	3.45
120	3.55

[a] 60% relative humidity, 25° C., activated alumina sample.

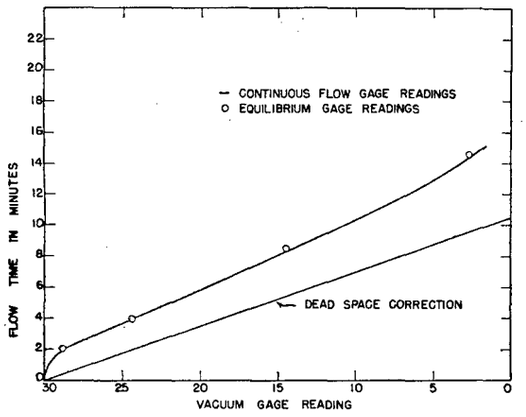

Figure 5. Comparison of Continuous Flow with Equilibrium Pressure Readings on Nonporous Iron Oxide

Sample, 9.7 grams
Flow rate, 10.2 ml./minute, STP

ments should be specified in ordering, as stock controllers, Type 63BD, are not normally designed for such a low flow.) However, it is possible to vary sample size over wide limits and, hence, approach equilibrium about as closely as desired.

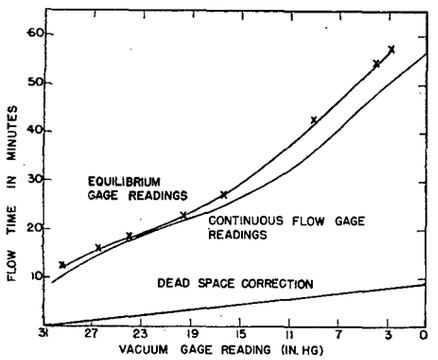

Figure 6. Comparison of Continuous Flow with Equilibrium Pressure Readings on Small Pore Size Alumina

Sample, 1.90 grams
Flow rate, 10.5 ml./minute, STP

Many observations on the nearness of approach to equilibrium with fairly small samples (5 to 10 grams) and a flow rate of 10 ml. per minute, showed that with nonporous or large pore (over 200 A.) materials, negligible pressure changes occurred on closing off, as shown in Figure 5. For small pore materials such as fresh silica gel–base catalyst using a 2-gram sample, equilibrium adsorption at a relative pressure of 0.2 was realized within 4% at a flow rate of 10 ml. per minute and within 2% at a flow rate of 7 ml. per minute (standard temperature and pressure). As the author was primarily interested in measuring area, the closeness of approach to equilibrium except near a relative pressure of 0.2 was not investigated thoroughly. A limited amount of work showed that equilibrium was most closely approached in the relative pressure region of 0.2, as illustrated in Figure 6.

Pretreatment of Sample. If a sample is not heated prior to or during outgassing, it may contain physically adsorbed water which might lower the surface area and pore volume results. High temperature heat treatment may of course cause sintering with decrease in surface area. However, most catalytic materials are used at fairly high temperatures and, if not, it is the area after reasonable heat treatment that is of importance anyway. Raising temperature is in general more effective in eliminating absorbed water than lowering pressure—that is, in general more water would be expected to be evolved in a given time on heating at 400° C. in a muffle open to the atmosphere (50% relative

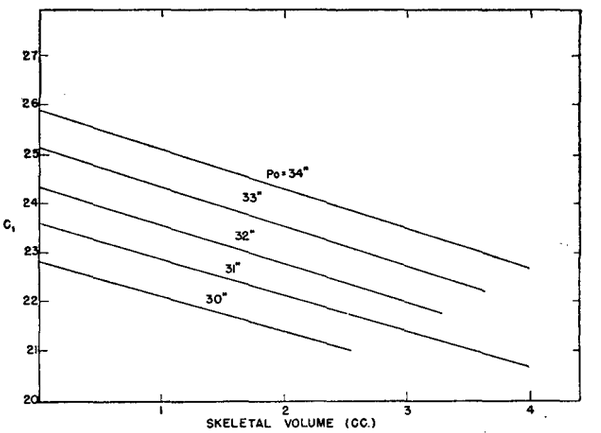

Figure 7. Chart Used for Dead Space Correction in Surface Area Determination

C_1, correction in milliliters, STP

humidity) than on evacuating at 200° C. and 0.01 mm. of mercury pressure because of the great dependence of adsorbate vapor pressure on temperature and the slowness of desorption at low adsorbate pressures. For these reasons as well as greater convenience, this laboratory pretreats the samples at an elevated temperature in a muffle to remove physically adsorbed water. The sample might be weighed before heat treatment but as specific area on a dry basis is usually more significant, it appears preferable to weigh the sample afterward. Weighing and transfer to the adsorbent tube must result in some water pickup unless it is carried out in a dry gas atmosphere. However, this precaution is not believed generally necessary if the weighing is carried out rapidly (less than 1 minute) with the possible exception of low area samples. An illustration of the negligible effect of brief atmospheric exposure is given in Table I; it can also be shown by calculating the possible water pickup for a 1-minute period if every molecule colliding with the external surface is adsorbed.

The surface area value obtained with 1-minute atmospheric exposure was 260 square meters per gram; after the 2-hour exposure it was 258 square meters per gram. Both measurements were made on the initial weight basis. Thus, in this case, negligible water adsorption occurred in 1 minute and the adsorption of large amounts of water did not effect the specific area results appreciably except for its effect on sample weight.

PROCEDURE

Surface Area Determination. Customary procedure is to place the samples in a temperature-controlled muffle (usually at 590° C.) and transfer to a desiccator after 1 hour until needed. A 2- to 20-gram sample is then rapidly weighed to ±1% and transferred to the adsorbent tube with the aid of a powder funnel and brush. It is then connected to the adsorbent tube and outgassed

for a few minutes to an absolute pressure of 0.3 mm. of mercury or less. The system is tested for tightness by measuring the leak rate on closing the valve to the pump and again measuring the pressure after a 30-second time interval. Valve 4 is then shut and the adsorbent tube is immersed in liquid nitrogen. Valve 3 is opened allowing nitrogen to flow through the controller section and into the vacuum pump for about 30 seconds until gage 1 shows that steady flow has been attained. Flow rate is then measured by determining with a stopwatch the time required for the vacuum to drop from 29 to 24 inches gage on closing valve 5.

Flow rate is then calculated from the relation,

$$r = \frac{\Delta P \times V_g \times 273}{t_1 \times 29.92 \times T}$$

where

r = flow rate, milliliters per minute, standard temperature and pressure
t_1 = time for indicated pressure change
ΔP = pressure change in inches of mercury
T = room temperature in degrees, absolute
V_g = volume of the gage section in milliliters

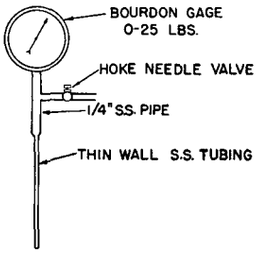

Figure 8. Nitrogen Vapor Pressure Thermometer for Measuring Saturation Pressure at Bath Temperature

This flow rate is normally constant within 1%, and only daily measurements are necessary if the room temperature is constant within ±2° C., and the barometric pressure constant within ±0.2 inch of mercury.

The contact points are then set to break at a relative pressure of 0.2. The gage reading at the end point ($p/p_o = 0.2$) is conveniently read off a graph as a function of barometric pressure. (In calculating p_o, it must be remembered, for accurate results, that commercial liquid nitrogen usually contains a per cent or more of oxygen, etc., and, hence, p_o is slightly greater than barometric pressure. With the author's source which is uniform and contains about 4% oxygen, the expression $p_o = p_b + 2.5$, where p_b = barometric pressure in inches of mercury, agrees with p_o values measured with the nitrogen–vapor pressure thermometer shown in Figure 8, except after long usage of the liquid nitrogen which results in higher p_o values, probably owing to selective vaporization of the nitrogen.)

The run is then started by opening valve 5 briefly before closing it, starting the timer and opening valve 4 to the adsorbent tube. The timer automatically shuts off when a relative pressure of 0.2 is reached.

The specific area is then calculated using the expression,

$$\underset{0.2}{\Sigma} = \frac{3.5\,(rt_{0.2} + C_1)}{W} \qquad (2)$$

where

$\underset{0.2}{\Sigma}$ = specific area in square meters per gram of heat-treated sample
$t_{0.2}$ = time in minutes for the relative pressure to reach a value of 0.2
W = sample weight in grams
C_1 = dead space correction in milliliters, standard temperature and pressure

The dead space correction is read off a graph such as the one shown in Figure 7 as a function of sample skeletal volume (W/d) and saturation pressure. The graph is based on the expression,

$$C_1 = -\frac{0.2p_oV_2}{\Delta p} + \frac{273}{78} \times \frac{0.2\,p_o}{29.92} \times \frac{W}{d} \qquad (3)$$

where

V_2 = a constant quantity = rt'
p_o = saturation pressure in inches of mercury
t' = time in minutes for the gage reading to change from 29- to 24-inch gage when a run is made with the adsorption tube empty and immersed in liquid nitrogen
r = flow rate in milliliters per minute, standard temperature and pressure
W = sample weight in grams
d = skeletal density of sample in grams per milliliter
Δp = pressure change in inches of mercury corresponding to a change of gage reading from 29 to 24 inches

The skeletal or material density can normally be estimated accurately enough from handbook values if the chemical nature of the adsorbent is known.

Pore Volume Determination. When pore volume is to be determined, nitrogen is rapidly introduced by partially opening valve 2 until the sample is fairly well saturated at a pressure approaching saturation. Valve 4 to the sample is then closed. If a noticeable pressure drop occurs in 1 minute, more nitrogen is added (approaching equilibrium by desorption was more rapid, and hence is the preferred procedure). Valve 7 is then opened to the atmosphere. Valve 5 is adjusted so that a reading corresponding to $0.97p_o$ is realized on the vacuum gage. The p_o value is determined with the nitrogen vapor pressure thermometer shown in Figure 8 together with the vacuum gage reading on evacuation. A relative pressure of 0.97 fills cylindrical pores smaller in diameter than 556 A. according to the Kelvin equation, or smaller than about 660 A. if multilayer adsorption is also considered (*2*). Use of a higher relative pressure might result in considerable interparticle condensation but would be necessary to get the total pore volume of large pore materials (*1*). Valve 4 is then opened which raises the gage reading until equilibrium is reached, at which point it will return to the initial setting of $0.97p_o$ because no gas will then be flowing in or out of the adsorbent section. After 30 minutes to 3 hours depending on the nature of the sample (large pore samples are slow in reaching equilibrium), valves 3 and 5 are simultaneously closed and if no measurable change in pressure occurs in a 1-minute period, valve 4 is closed, valves 5 and 7 are opened, and the wet test meter set at zero reading is connected. The adsorbent tube is then temporarily immersed in warm water in order to rapidly bring it to room temperature and valve 4 is partially opened.

From the wet test meter reading, R in cubic feet, after the adsorbent tube has come to room temperature, the specific pore volume, V_p, in milliliters per gram, is calculated as follows:

$$V_p = \frac{AFR + C_2}{W} \qquad (4)$$

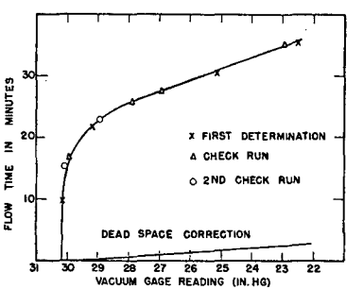

Figure 9. Typical Example of Reproducibility

Silica gel base catalyst
Sample, 2.0 grams
Flow rate, 9.7 ml./minute, STP

where

F = pressure and temperature factor to convert to 0° C., 760 mm of mercury, dry gas
W = sample weight in grams
A = conversion factor to convert cubic feet at standard temperature and pressure of gaseous nitrogen to milliliters of liquid nitrogen at −195° C., taken as

$$\frac{28 \times 28{,}316}{22{,}414 \times 0.808} = 43.8$$

The dead space correction, C_2 (in milliliters), is normally small and varies only slightly with skeletal volume and saturation pressure so that a correction (0.06 ml.) based on average conditions ordinarily gives results accurate within 1%. The dead space correction is calculated by considering both gas remaining after desorption and gas in the dead space during sorption. The full expression used is

$$C_2 = \frac{28}{22{,}414 \times 0.808}\left[\frac{0.97}{0.20}C_1 + V_g \times \frac{273}{T} \times \frac{0.97p_o}{29.92} + \frac{273}{T} \times \frac{p_b}{29.92}(V_a - W/d)\right] \qquad (5)$$

where

p_b = barometric pressure in inches of mercury
V_a = volume of the adsorbent section when empty (27.5 ml. for the author's apparatus)

DISCUSSION OF METHOD

The method has been applied to a variety of materials having surface areas ranging from 2 to 700 square meters per gram. This method is particularly advantageous for materials having specific areas in the range from 10 to 200 square meters per gram because of short time requirements. For accurate measurement of materials less than 10 square meters per gram, it would be advisable to reduce the dead space. BET C values (estimated from the pressure at $t = {}^1/_2 t_{0.2}$) were found in all cases to be greater than 30.

A check run was carried out on many samples immediately after the first run. Excellent reproducibility (within 2%) was invariably obtained, as illustrated in Figure 9. When check runs were carried out at a later date, reproducibility was within 4%. The average deviation from the mean of check determinations was 1.2%. The amount of error in area measurement resulting from various causes on a 200-square-meter sample is believed to be within the following limits:

Measurement of flow rate	1%
Hydration of sample from exposure to atmosphere during weighing	1%
Calibration of volumes	1%
Dead space correction	1%
Weighing and transfer of sample to adsorbent tube	2%
Measurement of flow time during adsorption to end point	1%
Temperature fluctuations	1%

Less than 15 minutes of working time is generally required for a surface area determination.

ALTERNATIVE PROCEDURES

Although this laboratory has adopted the procedures detailed herein, there are other alternative procedures and usages as follows:

1. The increment method of gas addition can be used if desired and the amount of gas introduced measured by the time of flow.
2. The gas can be allowed to equilibrate with the adsorbent at any desired pressure in the range from 0 to 33 inches of mercury by proper setting of valves 2, 5, 6, and 7; the desorption can be measured with a wet test meter or by the pressure developed on gage 3.
3. The continuous flow method can be used for pore volume determination as well as area measurement. The Helicoid gage has both high and low vacuum contact points. However, equilibrium cannot be approximated closely enough for accurate results (Figure 6).
4. Other more convenient gases such as n-butane at 0° C. might be employed for area and pore volume measurement. However, this has not been tried as yet.
5. A vacuum recorder with a short time cycle can be used in place of the Helicoid gage to record the adsorption isotherm directly (uncorrected for dead space).
6. A manifold with several adsorption tubes might be employed to increase the capacity of the apparatus, or the same 6-pound-per-square-inch nitrogen pressure sources may be used for several units of the type described.

ADDENDUM!

Improved operation has now been realized by use of a special low-flow Moore flow controller; by use of a low pressure, pressure regulator (Conoflow Corp., Philadelphia, Pa.) in series with the cylinder regulator, and replacement of the relief valve with a needle valve. A Precision Scientific Time It timer is now used. The new Moore controller gives a constant flow rate up to saturation pressure with flows less than 5 ml. (STP) per minute.

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Polarographic Investigations of Reactions in Aqueous Solutions Containing Copper and Cysteine (Cystine)

Amperometric Titration of Traces of Cysteine and Cystine with Cupric Copper as Reagent

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From a polarographic study of the reaction between cupric copper and cysteine in ammoniacal medium in the presence of sulfite, it was inferred that this reaction could be made the basis of a rapid amperometric titration of traces of cysteine and cystine, using the rotating platinum electrode as the indicator electrode. Procedures are given for the rapid and accurate determination of traces of cysteine and cystine at concentrations between 4×10^{-4} and 10^{-5} M. Cadmium, zinc, and iodide do not interfere. Cobalt interferes. An accurate and rapid determination of traces of cysteine and cystine is of great significance in the investigation of biological materials like normal and pathological blood sera. The new method is more specific and precise than methods that have been previously used.

AMPEROMETRIC titrations of sulfhydryl groups in amino acids and proteins with silver nitrate have been reported (*1*, *2*). No analytical use has been made of the reaction between cupric copper and cysteine in the presence of sulfite. From a polarographic study (*3*) it was inferred that it should be possible to make this reaction the basis of a rapid amperometric titration. It is shown in this paper that with the rotating platinum electrode as indicator electrode traces of cysteine can be titrated simply, rapidly, and accurately. The end point can be detected more sharply with cupric copper than with silver as a reagent. The diffusion current involved in the titrations with cupric copper corresponds to the reduction of cupric to cuprous copper, and not to a reduction to the metal. Copper salts catalyze air oxidation of cysteine (*5*), but it is possible to obtain accurate results with copper solutions containing air if titration mixtures of the proper composition are used.

Cupric copper reacts with cysteine and sulfite in ammoniacal medium to give cuprous copper and cysteine sulfonate (*3*) (designated as $RSSO_3^-$). As an intermediate, cuprous cysteinate (designated as RSCu) is formed from cysteine and cuprous copper, which under the proper conditions is oxidized further by cupric copper (see Equation 5). The partial reactions can be represented by the following equations; Equation 6a represents the over-all reaction, which is the basic reaction in the amperometric titration.

$$2Cu(II) + 2RS^- = RSSR + 2Cu(I) \quad (1)$$
$$2Cu(I) + 2RS^- = 2RSCu \quad (2)$$
$$RSSR + SO_3^{--} = RS^- + RSSO_3^- \quad (3)$$
$$2Cu(II) + 3RS^- + SO_3^{--} = 2RSCu + RSSO_3^- \quad (4)$$
$$2RSCu + 4Cu(II) + 2SO_3^{--} = 6Cu(I) + 2RSSO_3^- \quad (5)$$
$$6Cu(II) + 3RS^- + 3SO_3^{--} = 6Cu(I) + 3RSSO_3^- \quad (6)$$
$$\text{or } 2Cu(II) + RS^- + SO_3^{--} = 2Cu(I) + RSSO_3^- \quad (6a)$$

MATERIALS USED

Cysteine hydrochloride was a Paragon reagent grade product. Cystine was a Merck reagent grade product. Directions for weighing cysteine hydrochloride and the preparation of cysteine and cystine stock solutions were given in a recent paper (*2*). The copper metal used for the preparation of the standard cupric copper solutions was an Eimer and Amend, hydrogen-reduced C.P. product. Directions for the preparation of the copper solutions are given in the procedures.

EXPERIMENTAL METHODS

The equipment used for obtaining current voltage curves was a Heyrovský self-recording polarograph. The amperometric titrations were carried out with a manual apparatus and circuit described by Lingane and Kolthoff (*4*).

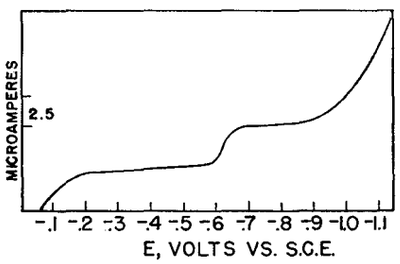

Figure 1. Current Voltage Curves

10^{-4} *M* Cupric copper in ammonia buffer (0.1 *M* NH_3, 0.1 *M* NH_4NO_3, 0.05 *M* Na_2SO_3) at rotating platinum electrode

A synchronous motor with gears provided rotation of the platinum wire electrode at 600 r.p.m. A constant speed of rotation of the platinum electrode is required only in the determination of current voltage curves. For routine titrations a synchronous motor is not necessary. All potentials are expressed versus the saturated calomel electrode (S.C.E.).

A 5-ml. semimicroburet divided into 0.01 ml. and a Gilmont ultramicroburet of 0.1-ml. capacity were used in the titrations.

Oxygen was removed from the solutions with a stream of nitrogen which was passed through two wash bottles containing ammonia buffers of the same composition as that in the titration mixture. This was necessary in order to maintain a constant ammonia concentration in the titration mixture during the analysis.

CURRENT VOLTAGE CURVES WITH ROTATING PLATINUM WIRE ELECTRODE

It has been shown (*2*) that cysteine, dissolved in an ammonia buffer, does not give an anodic diffusion current up to +1 volt if electrolyzed at the rotating platinum wire electrode. An ammoniacal cupric copper solution gives two well-defined cathodic waves at this electrode (see Figure 1). The first wave corresponds to the reduction of cupric to cuprous copper and starts at −0.1 volt, while the second wave is due to the reduction of cuprous copper to copper; it starts at about −0.6 volt (*vs.* S.C.E.). The diffusion currents of the two waves are practically equal. Figure 2 represents a polarogram at the rotating electrode of a reaction mixture containing cysteine and cupric copper in the mole ratio 1 to 2 and of an ammonia and sulfite concentration of 0.1 and 0.15 *M*, respectively. It is seen that this solution does not give an appreciable current between −0.3 and −0.6 volt.

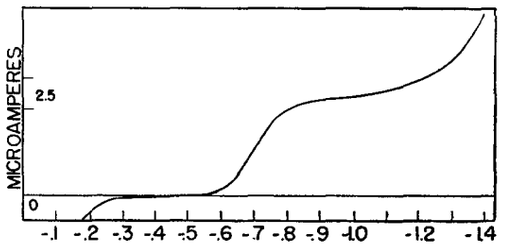

Figure 2. Current Voltage Curves

At rotated platinum electrode of 50 ml. of 10^{-4} *M* cysteine (0.1 *M* NH_3, 0.1 *M* NH_4Cl, 0.15 *M* Na_2SO_3) after addition of 2 ml. of 5×10^{-3} *M* cupric copper solution

The products of the reaction between cysteine and cupric copper are cysteine sulfonate and cuprous copper. Cysteine sulfonate does not give a wave, and cuprous copper under the experimental conditions is reduced at about −0.65 volt at the rotating platinum wire electrode. The addition of an excess of cupric copper to the above solution gives rise to the appearance of the wave of the cupric ammino ion, $Cu(II) + e \longrightarrow Cu(I)$. The height of this wave is proportional to the concentration of the cupric copper in the solution. Use is made of these observations in the amperometric titration of cysteine and cystine with cupric copper. The best results were obtained at an applied potential of −0.4 volt (*vs.* S.C.E.).

TITRATIONS WITH ROTATING PLATINUM WIRE ELECTRODE AS INDICATOR ELECTRODE

Titrations were carried out under varying conditions. The effects of the concentration of ammonia, ammonium nitrate, or chloride, of sodium sulfite, and of the cysteine concentration were studied. All titrations were carried out while nitrogen was bubbled through the titration mixture. Cysteine is air-oxidized relatively rapidly in alkaline solutions. In the presence of copper the system is extremely sensitive to air-oxidation. It is found that oxidation of cysteine by air dissolved in the cupric copper solution, used as titrating agent, is appreciable if the reaction between cupric copper and cysteine proceeds slowly.

The rate of this reaction is affected by ammonia and sulfite concentrations. The reaction rate increases with decreasing ammonia and increasing sulfite concentration (see *3*). If the reaction is slow, the current measured at −0.4 volt increases abruptly upon each addition of cupric copper and then decreases slowly until a constant value is attained (this is of the order of 0.05 to 0.1 μA. before the end point).

In titrations with mixtures of a comparatively small sulfite concentration the rate of the reaction is normal at the beginning of the titration but becomes markedly smaller as the reaction according to Equation 4 reaches completion. On further addition of cupric copper the reaction (oxidation of RSCu to Cu^+ and $RSSO_3^-$) proceeds faster again and soon reaches its initial rate, which persists throughout the remaining part of the titration. Mixtures of cysteine with zinc or cadmium ion react faster and at a uniform rate in titrations with cupric copper. This is found for mole ratios of initial cysteine to metal from 1:0.5 to 1:5.

The simplest way to obtain a uniform and great reaction rate is the proper adjustment of sulfite and ammonia concentration. Solutions containing 0.15 *M* sodium sulfite and 0.05 *M* ammonia are found to give the best results in titrations with air-containing

cupric copper solutions. Under these conditions the reaction is so fast that oxygen in the titrating agent does not interfere under the experimental conditions. With large ammonia or low sulfite concentrations the errors can amount to −25%. Titrations repeatedly done with 50 ml. of 3×10^{-4} to 8×10^{-5} M cysteine solutions which were 0.5 M in ammonia and 0.05 M in sulfite and with air-saturated 5×10^{-3} M cupric copper solution (total volume added, 4.7 to 1.6 ml.) gave end points at which the mole ratio RSH : Cu(II) was found to be about 1 to 1.5 instead of 1 to 2. This was found polarographically (see *3*) as well as in amperometric titrations with the rotating platinum wire electrode.

Because the amount of oxygen added to the titration mixture decreases with decreasing volume of the copper solution added, titration with relatively concentrated copper solutions is recommended. This is particularly important in titrations of very dilute cysteine solutions.

If an air-free cupric copper solution is used as titrating agent, ammonia and sulfite concentrations can be varied widely without affecting the results. For example, correct results were obtained with 0.5 to 0.05 M ammonia concentrations and with 0.05 to 0.2 M sulfite concentrations even though the reaction was very slow at high ammonia and low sulfite concentrations.

In the presence of ammonia, cupric copper solutions can be made air-free by the addition of sodium sulfite. Ammoniacal cupric copper solutions which were 5×10^{-3} to 10^{-3} M in copper, 1 to 0.5 M in ammonia, 0.1 to 0.05 M in ammonium chloride, and 0.05 to 0.025 M in sodium sulfite were found to be stable for about 2 hours after preparation. After this time a slow reduction of cupric copper to the cuprous state could be observed polarographically.

Experiments carried out under various conditions showed that the ammonium salt concentration of the titration mixture has hardly any effect on the result. Correct results are also obtained in the absence of ammonium salts.

PROCEDURES

1. Determination of Cysteine or Cystine. Introduce 25 to 100 ml. of a 0.05 to 0.025 M ammonia solution into a 150-ml. beaker which is provided with a rubber stopper with holes for electrode, buret, salt bridge, and inlet tube for nitrogen. Immerse a platinum wire electrode in the solution. Remove air with purified nitrogen, which is passed through during the entire titration. To the air-free ammonia solution add enough cysteine or cystine solution to make the mixture 4×10^{-4} to 10^{-5} M in RSH or RSSR. If the cysteine or cystine solution is acid, neutralize it with ammonia. Make the mixture 0.15 to 0.1 M in sulfite by adding the proper volume of 1 M sodium sulfite solution.

Immerse the salt bridge and tip of the buret in the solution. For titrations of 5 to 0.5 mg. of cystine (4×10^{-4} to 8×10^{-5} M) a semimicroburet with 0.01 to 0.002 M cupric copper solutions can be used, while quantities smaller than 0.5 mg. of RSSR (8×10^{-5} to 10^{-5} M) must be titrated with an ultramicroburet and more concentrated cupric copper solutions (0.05 M). Titrate at an applied potential of −0.4 volt *vs.* S.C.E. Allow the cupric copper solution to flow into the titration mixture at such a rate that the current remains practically constant (0 to −0.1 μA.) before the end point. After the end point the current increases rapidly. Measure the current after the end point after addition of three to four small increments of cupric copper solution. Plot the results and find the end point graphically. If carried out correctly, the time of titration proper is about 2 minutes.

NOTE. A new indicator electrode must be cleaned with concentrated nitric acid and rinsed with distilled water. It can be used immediately after cleaning. After each titration the electrode is rinsed with distilled water. If not in use, the electrode should be kept in distilled water. If kept in this way, a used electrode need not be cleaned with concentrated nitric acid.

According to Equation 6a, 1 ml. of 0.01 M copper solution corresponds to 0.788 mg. of cysteine hydrochloride. One mole of cystine in the presence of sulfite gives 1 mole of cysteine. One milliliter of 0.01 M copper solution corresponds to 1.20 mg. of cystine.

2. Modified Procedure for Cystine. Cystine can be reduced with sodium amalgam before the titration is carried out by the above method. After reduction with sodium amalgam 1 mole of cystine will use up 4 instead of 2 moles of cupric copper. Place about 5 ml. of a cystine solution, which should be at least 2×10^{-3} M in cystine and about 0.05 N in hydrochloric or sulfuric acid, in a test tube. Add a few drops of 1% sodium amalgam and let stand for 1.5 hours with occasional shaking. Treat an accurately measured portion of this solution further as in procedure 1.

3. Analysis of Mixtures of Cysteine and Cystine. For the analysis of mixtures of the two amino acids two amperometric titrations are required. One titration is carried out according to procedure 1. A second titration is done after previous treatment of the mixture with sodium amalgam, as in the modified procedure for cystine. If the number of milliliters of cupric copper solution consumed in the first and second titration are A and B, respectively, the difference $(B - A)$ corresponds to the molarity of cystine present in the mixture of the two amino acids. $(B - A)$ ml. of 0.01 M cupric copper is equivalent to 1.20 $(B - A)$ mg. of cystine.

NOTE. If the concentration of cysteine is small as compared to the cystine concentration, the cysteine is better determined by argentometric amperometric titration in the absence of sulfite (*2*), preferably adding the silver from an ultramicroburet.

PREPARATION OF STANDARD CUPRIC COPPER SOLUTION

Dissolve an accurately weighed amount of C.P. metallic copper in 6 N nitric acid. After complete dissolution of the copper, add a few drops of concentrated sulfuric acid and evaporate until the residue is nearly dry. Dissolve in distilled water, transfer into a volumetric flask, and make up to volume. The copper titer can be checked by iodometric titration or by electrolysis. Standardized cupric sulfate solutions may be used as well.

PREPARATION OF AIR-FREE AMMONIACAL CUPRIC COPPER SOLUTIONS

Place an accurately measured volume of a concentrated standard cupric copper solution (0.05 to 0.01 M in copper) in a volumetric flask. Add ammonia and sodium sulfite to make the solution 0.5 to 1 M and 0.05 to 0.025 M, respectively, in these constituents. Sulfite must be added after the ammonia. Make up the solution to volume with air-free distilled water. Copper solutions prepared in this way must be used within 2 hours after preparation.

RESULTS

Tables I, II, and III give the results of amperometric titrations of cysteine and cystine as obtained with air-containing cupric copper solutions as titrating agent.

Contrary to the argentometric titrations (*2*), it is seen that the cysteine concentration has hardly any effect on the results. This is found with RSH and RSSR at concentrations varying between 4×10^{-4} and 10^{-5} M. It is seen in Table I that at high ammonia and low sulfite concentration large negative errors are found, due to oxidation of cysteine by oxygen contained in the copper solution. The error is reduced to practically zero by using a more concentrated copper solution and adding it from an ultramicroburet (see Table III) or using air-free copper solutions (Table IV). Under the experimental conditions given in the procedure it is necessary to add the cupric copper from an ultramicroburet or to use air-free copper solutions when the cysteine or cystine concentration is less than 8×10^{-5} M. With an ultramicroburet cystine quantities as low as 60 micrograms in 25 ml. of titration mixture (10^{-5} M) can be determined with an accuracy of −1 to −2%. Using a semimicroburet 0.5 mg. of cystine (8×10^{-5} M) can be determined with an accuracy of −0.4%. The last experiment in Table II shows that the error becomes considerably greater if smaller quantities of cystine are titrated with a semimicroburet. From Tables I to III it is seen that best results with copper solutions containing air are obtained with ammonia and sulfite concentrations of 0.05 M and 0.15 to 0.1 M, respectively. If lower or higher ammonia concentrations are used, the sulfite concentration should be adjusted in approximately the same proportion. Thus it is also possible to obtain reasonably accurate results with solutions which are 0.1 or 0.025 M in ammonia if the corresponding sulfite concentrations are about 0.25 and 0.1 M, respectively. If the sulfite molarity is markedly lower than 0.1 M the reaction is slow, even if the

Table I. Amperometric Titration of 50-Ml. Cysteine Solution with 0.005 *M* Air-Containing Cupric Copper, Using 5-Ml. Semimicroburet

No. of Detns.	Init. RSH—Concn. of Soln. Titrated *M*	Cysteine Hydrochloride Taken *Mg.*	Cysteine Hydrochloride Found *Mg.*	Average Error %	Composition of Electrolyte
2	2×10^{-4}	1.58	1.58	+0.3	0.05 *M* NH_3, 0.05 *M* NH_4NO_3, 0.15 *M* Na_2SO_3
1	2×10^{-4}	1.58	1.51	−4.4	0.05 *M* NH_3, 0.05 *M* NH_4NO_3, 0.1 *M* Na_2SO_3
1	2×10^{-4}	1.58	1.58	−0.2	0.05 *M* NH_3, 0.05 *M* NH_4NO_3, 0.2 *M* Na_2SO_3
1	2×10^{-4}	1.58	1.63	+3.2	0.05 *M* NH_3, 0.05 *M* NH_4NO_3, 0.25 *M* Na_2SO_3
1	2×10^{-4}	1.58	1.54	−2.7	0.1 *M* NH_3, 0.1 *M* NH_4NO_3, 0.25 *M* Na_2SO_3
1	1.6×10^{-4}	1.26	1.26	0.0	0.05 *M* NH_3, 0.05 *M* NH_4NO_3, 0.15 *M* Na_2SO_3
1	1.2×10^{-4}	0.945	0.938	−0.7	0.05 *M* NH_3, 0.05 *M* NH_4NO_3, 0.15 *M* Na_2SO_3
1	1.6×10^{-4}[a]	0.630	0.630	0.0	0.05 *M* NH_3, 0.05 *M* NH_4NO_3, 0.15 *M* Na_2SO_3
2	2×10^{-4}	1.58	1.19	−25.0	0.5 *M* NH_3, 0.1 *M* NH_4NO_3, 0.05 *M* Na_2SO_3 (reaction slow)
1	2×10^{-4}	1.58	1.15	−27.0	1 *M* NH_3, 0.1 *M* NH_4NO_3, 0.05 *M* Na_2SO_3 (reaction very slow)
1	8×10^{-5}	0.630	0.488	−23.0	0.5 *M* NH_3, 0.1 *M* NH_4NO_3, 0.05 *M* Na_2SO_3 (reaction slow)

[a] Volume of titration mixture 25 ml.

ammonia concentration is low. The sharpness of the end point is illustrated in Figure 3, in which titration lines are given, measured in the titration of 0.96 and 0.12 mg. of cystine, respectively, in 25 ml. of solution (Figure 3). Table II shows that ammonium nitrate in concentrations of 0 to 0.3 *M* has no effect on the titration at an ammonia concentration of 0.05 *M*.

Experiments carried out in the absence of ammonia revealed that good results can be obtained if the solution is made 0.01 *M* in potassium hydroxide. Without alkali the results are not reproducible and depend greatly on the rate of addition of the cupric copper to the titration mixture. In the presence of alkali the reduction of the cupric copper to cuprous copper by sodium sulfite is delayed and the cupric copper is given enough time to react with cysteine.

Polarographically, as well as with the rotating platinum wire electrode as indicator electrode, it can be shown that cupric copper in an alkaline sulfite solution is stable for an appreciable period of time, even in the absence of ammonia. The difference in reactivity between copper and sulfite in the presence and absence of alkali is illustrated in Figure 4. A cupric copper solution was added at the same rate to 0.1 *M* sodium sulfite solutions in the absence and presence of 0.01 *M* potassium hydroxide. The line representing the diffusion current of the cupric copper flattens off in the absence of alkali, indicating the disappearance of the cupric copper due to reduction to cuprous copper. In the presence of alkali a straight line is obtained.

In an amperometric titration of cystine or cysteine in a solution which is 0.01 *M* in potassium hydroxide and 0.1 *M* in sodium sulfite, the slope of the excess reagent line is less than that obtained with an ammoniacal solution.

From a practical point of view, it is advisable to carry out the titrations in ammoniacal medium, whenever possible. The reaction is faster and the end point sharper in the presence of ammonia.

INTERFERENCES

Cadmium and zinc hardly interfere in cysteine titrations with cupric copper. In 2×10^{-4} *M* RSSR or RSH solutions cadmium and zinc in a concentration of 10^{-3} *M* do not interfere. In more dilute solutions (10^{-4} *M* or less in RSH) zinc in the mole ratio RSH : Zn = 1:10 affects the reproducibility of the results and may cause positive or negative errors of the order of 4%.

Table II. Amperometric Titration of Cystine with Air-Containing Cupric Solution, Using 5-Ml. Semimicroburet

No. of Detns.	Init. RSSR—Concn. of Soln. Titrated *M*	Cystine Taken *Mg.*	Cystine Found *Mg.*	Average Error %	Vol. of Titration Mixture *Ml.*	Concn. of Copper Solution *M*	Composition of Electrolyte
1	4×10^{-4}	4.80	4.80	0.0	50	10^{-2}	No NH_4^+, 0.1 *M* NH_3, 0.15 *M* Na_2SO_3
3	2×10^{-4}	4.80	4.79	−0.2	100	10^{-2}	No NH_4^+, 0.05 *M* NH_3, 0.15 *M* Na_2SO_3
1	2×10^{-4}	4.80	4.77	−0.7	100	10^{-2}	No NH_4^+, 0.05 *M* NH_3, 0.15 *M* Na_2SO_3, 10^{-4} *M* $CdCl_2$
1	2×10^{-4}	4.80	4.84	+0.7	100	10^{-2}	No NH_4^+, 0.05 *M* NH_3, 0.15 *M* Na_2SO_3, 2×10^{-4} *M* $CdCl_2$
1	2×10^{-4}	4.80	4.80	0.0	100	10^{-2}	No NH_4^+, 0.05 *M* NH_3, 0.15 *M* Na_2SO_3, 10^{-3} *M* $CdCl_2$
1	2×10^{-4}	4.80	4.80	0.0	100	10^{-2}	No NH_4^+, 0.05 *M* NH_3, 0.15 *M* Na_2SO_3, 2×10^{-4} *M* $ZnSO_4$
1	2×10^{-4}	4.80	4.80	0.0	100	10^{-2}	No NH_4^+, 0.05 *M* NH_3, 0.15 *M* Na_2SO_3, 10^{-3} *M* $ZnSO_4$
1	1.6×10^{-4}	3.84	3.88	+0.9	100	10^{-2}	0.05 *M* NH_3, 0.15 *M* Na_2SO_3
1	1.4×10^{-4}	3.36	3.36	0.0	100	10^{-2}	0.05 *M* NH_3, 0.15 *M* Na_2SO_3
1	1.2×10^{-4}	2.88	2.88	0.0	100	10^{-2}	0.05 *M* NH_3, 0.15 *M* Na_2SO_3
1	2×10^{-4}	2.40	2.40	0.0	50	10^{-2}	0.1 *M* NH_3, 0.15 *M* Na_2SO_3
1	2×10^{-4}	2.40	2.42	+0.85	50	5×10^{-3}	0.05 *M* NH_3, 0.05 *M* NH_4NO_3, 0.15 *M* Na_2SO_3
1	2×10^{-4}	2.40	2.40	0.0	50	5×10^{-3}	0.025 *M* NH_3, 0.025 *M* NH_4NO_3, 0.10 *M* Na_2SO_3
1	2×10^{-4}	2.40	2.40	0.0	50	5×10^{-3}	No NH_3, no NH_4^+, 0.10 *M* Na_2SO_3 (reaction slow, end point *not* sharp)
2	10^{-4}	2.40	2.41	+0.4	100	10^{-2}	0.05 *M* NH_3, 0.15 *M* Na_2SO_3
1	10^{-4}	2.40	2.40	0.0	100	10^{-2}	0.05 *M* NH_3, 0.3 *M* NH_4NO_3, 0.15 *M* Na_2SO_3
1	10^{-4}	2.40	2.47	+3.0	100	10^{-2}	0.05 *M* NH_3, 1.0 *M* NH_4NO_3, 0.15 *M* Na_2SO_3
1	10^{-4}	2.40	2.34	−2.5	100	10^{-2}	0.05 *M* NH_3, 0.15 *M* Na_2SO_3, 10^{-4} *M* $ZnSO_4$
2	10^{-4}	1.20	1.18	−1.7	50	2.5×10^{-3}	0.025 *M* NH_3, 0.025 *M* NH_4NO_3, 0.1 *M* Na_2SO_3
1	8×10^{-5}	0.961	0.950	−1.1	50	5×10^{-3}	0.025 *M* NH_3, 0.025 *M* NH_4NO_3, 0.1 *M* Na_2SO_3
3	1.6×10^{-4}	0.961	0.964	+0.3	25	2.5×10^{-3}	0.05 *M* NH_3, 0.05 *M* NH_4NO_3, 0.15 *M* Na_2SO_3
2	8×10^{-5}	0.961	0.959	−0.1	50	2.5×10^{-3}	0.05 *M* NH_3, 0.05 *M* NH_4NO_3, 0.15 *M* Na_2SO_3
2	1.2×10^{-4}	0.720	0.712	−1.1	25	2.5×10^{-3}	0.05 *M* NH_3, 0.05 *M* NH_4NO_3, 0.15 *M* Na_2SO_3
4	8×10^{-5}	0.480	0.497	+3.5	25	2.5×10^{-3}	0.05 *M* NH_3, 0.05 *M* NH_4NO_3, 0.15 *M* Na_2SO_3
1	8×10^{-5}	0.480	0.477	−0.6	25	2.5×10^{-3}	0.05 *M* NH_3, 0.05 *M* NH_4NO_3, 0.1 *M* Na_2SO_3
2	8.4×10^{-5}	0.480	0.478	−0.4	25–50	2.5×10^{-3}	Cystine reduced with sodium amalgam
1	4×10^{-5}	0.240	0.219	−9.0	25	2.5×10^{-3}	0.05 *M* NH_3, 0.05 *M* NH_4NO_3, 0.10 *M* Na_2SO_3

Table III. Amperometric Titration of Cystine with 0.05 *M* Air-Saturated Cupric Copper Solution, Using 0.1-Ml. Ultramicroburet

(Volume of titration mixture is 25 ml. in all titrations)

No. of Detns.	Init. RSSR—Concn. of Soln. Titrated *M*	Cystine Taken *Mg.*	Cystine Found *Mg.*	Average Error %	Composition of Electrolyte
2	6×10^{-5}	0.360	0.358	−0.6	0.05 *M* NH_3, 0.1 to 0.15 *M* Na_2SO_3
2	4×10^{-5}	0.240	0.240	0.0	0.05 *M* NH_3, 0.1 *M* Na_2SO_3
1	2×10^{-5}	0.120	0.116	−3.3	0.05 *M* NH_3, 0.05 *M* Na_2SO_3
1	2×10^{-5}	0.120	0.120	0.0	0.05 *M* NH_3, 0.1 *M* Na_2SO_3
1	10^{-5}	0.060	0.059	−1.7	0.05 *M* NH_3, 0.1 *M* Na_2SO_3
1	6×10^{-5}	0.360	0.351	−2.5	0.1 *M* NH_3, 0.1 *M* Na_2SO_3
1	4×10^{-5}	0.240	0.232	−3.3	0.1 *M* NH_3, 0.1 *M* Na_2SO_3
1	2×10^{-5}	0.120	0.118	−1.2	0.1 *M* NH_3, 0.1 *M* Na_2SO_3
1	10^{-5}	0.060	0.056	−6.0	0.1 *M* NH_3, 0.1 *M* Na_2SO_3
1	10^{-4}	0.600[a]	0.590	−1.7	0.5 *M* NH_3, 0.15 *M* Na_2SO_3 (reaction slow)
1	4×10^{-5}	0.240	0.272	+13.3	No NH_3, 0.1 *M* Na_2SO_3 (end point not sharp)
1	4×10^{-5}	0.240	0.238	−0.8	No NH_3, 0.01 *M* KOH, 0.1 *M* Na_2SO_3
1	4×10^{-5}	0.240	0.240	0.0	0.05 *M* NH_3, 0.1 *M* Na_2SO_3, 1.6×10^{-4} *M* KI
1	4×10^{-5}	0.240	0.242	+0.8	0.05 *M* NH_3, 0.1 *M* Na_2SO_3, 10^{-3} *M* KI

[a] Copper solution was 0.1 *M*.

Table IV. Amperometric Titration of Cysteine and Cystine with Air-Free Copper Solution, Using 5-Ml. Semimicroburet

No. of Detns.	Init. RSH— or RSSR—Concentration *M*	RSH.HCl or RSSR Taken *Mg.*	RSH.HCl or RSSR Found *Mg.*	Error %	Vol. of Titration Mixture *Ml.*	Composition of Cupric Copper Solution	Composition of Electrolyte
1	RSH.HCl 2×10^{-4}	RSH.HCl 1.577	1.55_3	−1.5	50	5×10^{-3} *M* Cu(II), 0.5 *M* NH_3	0.5 *M* NH_3, 0.1 *M* NH_4Cl, 0.05 *M* Na_2SO_3
1	RSH.HCl 1.6×10^{-4}	RSH.HCl 1.261	1.26_5	+0.3	50	0.1 *M* NH_4Cl, 0.05 *M* Na_2SO_3	0.5 *M* NH_3, 0.1 *M* NH_4Cl, 0.05 *M* Na_2SO_3
1	RSSR 2×10^{-4}	RSSR 2.403	2.40	0.0	50	5×10^{-3} *M* Cu(II), 0.5 *M* NH_3	0.5 *M* NH_3, 0.1 *M* NH_4Cl, 0.15 *M* Na_2SO_3
1	RSSR 1.2×10^{-4}	RSSR 1.442	1.44_2	0.0	50	0.05 *M* NH_4Cl, 0.05 *M* Na_2SO_3	0.5 *M* NH_3, 0.1 *M* NH_4Cl, 0.05 *M* Na_2SO_3
1	RSSR 8×10^{-5}	RSSR 0.481	0.481	0.0	25	10^{-3} *M* Cu(II), 1 *M* NH_3	0.5 *M* NH_3, 0.1 *M* NH_4Cl, 0.15 *M* Na_2SO_3
1	RSSR 8×10^{-5}	RSSR 0.481	0.480	−0.2	25	0.05 *M* NH_4Cl, 0.025 *M* Na_2SO_3	0.05 *M* NH_3, 0.05 *M* NH_4NO_3, 0.05 *M* Na_2SO_3
1	RSSR 4×10^{-5}	RSSR 0.240	0.234	−2.5	25	10^{-3} *M* Cu(II), 1 *M* NH_3	0.05 *M* NH_3, 0.05 *M* Na_2SO_3
1	RSSR 2×10^{-5}	RSSR 0.120	0.118	−1.6	25	0.05 *M* NH_4Cl, 0.025 *M* Na_2SO_3	0.05 *M* NH_3, 0.05 *M* Na_2SO_3

drop of air-containing cupric copper solution. The color became more intense with increasing concentration of cobalt. The rate of the reaction between cysteine and cupric copper is decreased exceedingly in the presence of cobalt and it is hardly possible to carry the titration to an end point. Apparently, the cobalt complex with cysteine or cystine is of an entirely different nature than that of zinc and cadmium. The three metals behave rather alike in their interference in argentometric cysteine titrations (*2*). Here the end point is found when RSH is transformed to RSAg, but in the titration with cupric copper under the authors' experimental conditions the RSCu formed is transformed quantitatively to cuprous copper at the end point.

Iodide ion does not interfere in titrations of cysteine with cupric copper. This was found in titrations of mixtures containing 4×10^{-5} *M* RSSR and 1.6×10^{-4} to 10^{-3} *M* potassium iodide (see Table III).

Cobalt behaves entirely differently from zinc and cadmium and interferes seriously. Titrations were carried out with 2×10^{-4} *M* RSSR solutions which were 0.05 *M* in ammonia and 0.15 *M* in sodium sulfite which contained cobalt in the following mole ratios RSSR : Co = 1:1, 1:0.5, 1:0.1, and 1:0.05. In all these experiments the color turned yellow on the addition of the first drop of air-containing cupric copper solution. The color became more intense with increasing concentration of cobalt. The rate of the reaction between cysteine and cupric copper is decreased exceedingly in the presence of cobalt and it is hardly possible to carry the titration to an end point. Apparently, the cobalt complex with cysteine or cystine is of an entirely different nature than that of zinc and cadmium. The three metals behave rather alike in their interference in argentometric cysteine titrations (*2*). Here the end point is found when RSH is transformed to RSAg, but in the titration with cupric copper under the authors' experimental conditions the RSCu formed is transformed quantitatively to cuprous copper at the end point.

Iodide ion does not interfere in titrations of cysteine with cupric copper. This was found in titrations of mixtures containing 4×10^{-5} *M* RSSR and 1.6×10^{-4} to 10^{-3} *M* potassium iodide (see Table III).

The titration with cupric copper has several advantages over that with silver. Especially with microquantities of RSH or RSSR, the copper titration is more precise and accurate. The surface conditions of the platinum wire electrode are not critical in titrations with cupric copper, because at the selected potential the cupric is reduced to cuprous copper and not the metal. Reproducible results are obtained when the electrode is cleaned in the way described in the procedure. Zinc and cadmium

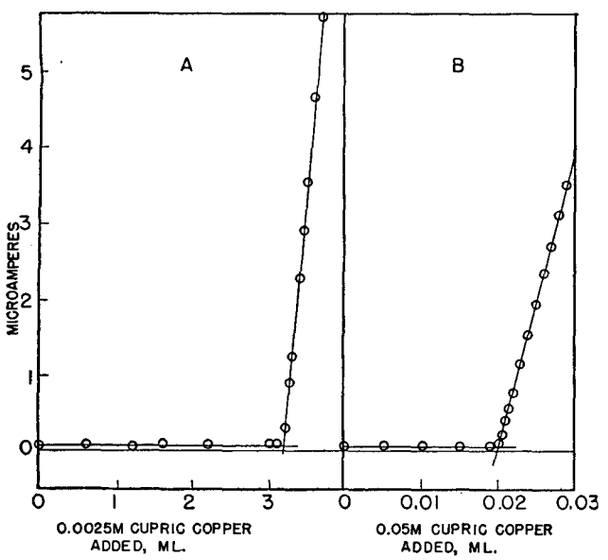

Figure 3. Amperometric Titrations

A. 0.96 mg. RSSR. Solution (25 ml.) 1.6×10^{-4} *M* in RSSR (semimicroburet)

B. 0.12 mg. RSSR. Solution (25 ml.) 2×10^{-5} *M* in RSSR (ultramicroburet)

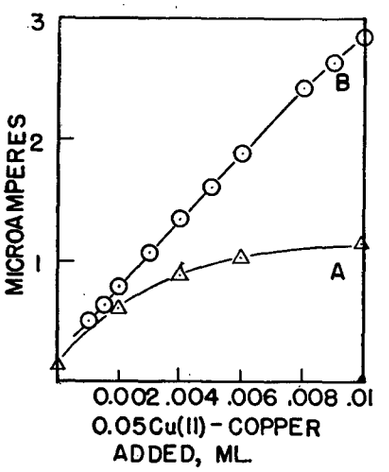

Figure 4. Diffusion Currents of Cupric Copper

Measured with rotated platinum wire electrode at −0.4 volt *vs.* S.C.E. upon addition of 0.05 *M* cupric copper to 25 ml. of 0.1 *M* sodium sulfite solution

***A.* In absence of KOH**

***B.* In presence of 0.01 *M* KOH**

interfere much more in the argentometric titration than in that with cupric copper. Iodide strongly interferes in the argentometric method but not in the copper method.

The accuracy and precision of the copper method are about $\pm 0.5\%$ with cysteine concentrations between 4×10^{-4} and 8×10^{-5} M, and about $\pm 1.5\%$ with cysteine concentrations between 6×10^{-5} and 10^{-5} M. With these dilute solutions the copper solution should be air-free or be added from an ultramicroburet.

ACKNOWLEDGMENT

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SUMMARY

The reaction between cupric copper and cysteine in a suitable ammonia buffer and in the presence of sulfite has been made the basis of a simple and accurate amperometric titration of cysteine and cystine, using the rotating platinum wire microelectrode as indicator electrode. Procedures are given for the rapid and accurate determination of traces of cysteine and cystine, which are more precise and accurate than the amperometric argentometric method.

The accuracy and precision are $\pm 0.5\%$ with cysteine concentrations between 4×10^{-4} and 8×10^{-5} M and about $\pm 1.5\%$ with cysteine concentrations between 6×10^{-5} and 10^{-5} M. With these dilute solutions the cupric copper solution should be air-free or added from an ultramicroburet. Cadmium does not interfere. Zinc does not interfere when its concentration does not exceed that of cysteine more than 10 times. Cobalt interferes. Iodide does not interfere.

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Determination of Small Quantities of Niacin in Presence of Niacinamide

Separation by Paper Partition Chromatography

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A NUMBER of investigators have developed methods for the determination of niacin and niacinamide in mixtures of these compounds. Lamb (*8*) and Melnick and Oser (*10*) determined both vitamins colorimetrically by means of the Koenig reaction (*6*), and by comparison of their respective reaction rates. First Scudi (*12*) and then Chaudhuri and Kodicek (*2, 5*) published fluorometric methods for the estimation of niacinamide in the presence of niacin. These authors determined total niacin colorimetrically after hydrolysis and subtracted the quantity of niacinamide found by their fluorometric procedure. Although adequate for the determination of niacinamide, these methods are apt to suffer from an appreciable inherent error if used for the determination of small quantities of niacin in presence of niacinamide.

Recently another method has been described by Ciuza (*3*). This author found that benzyl substitution on the pyridine nitrogen of nicotinamide prevented that compound from undergoing the Koenig reaction, whereas niacin is not easily benzylated under controlled conditions. By running total niacin and niacin after benzylation, both niacin and niacinamide may be determined by difference.

The microbiological method of Johnson (*4*) and its modification by Krehl (*7*) and associates, using *B. leuconostoc mesenteroides* has been successfully applied for the determination of niacin in presence of niacinamide.

It occurred to the authors that it might be possible to determine more accurately small quantities of niacin in the presence of large amounts of niacinamide, if those substances could first be separated. Paper partition chromatography appeared to present the most promising approach to making such separation.

PROCEDURE

Reagents. Cyanogen bromide reagent. A 4% aqueous solution, prepared by dissolving cyanogen bromide crystals, Eastman, in water. (It is stored in a refrigerator and used only if colorless.)

p-Aminoacetophenone reagent. *p*-Aminoacetophenone is recrystallized from a warm saturated solution in 95% ethyl alcohol by addition of about 3% distilled water. After cooling in the refrigerator, the almost colorless crystals are filtered off and dried in vacuo at room temperature. A 5% solution in 95% ethyl alcohol, when stored in the refrigerator, will keep for 1 week.

Ethyl acetate, C.P.

Ethyl acetate, C.P. Saturated with water.

Hydrochloric acid, 3 N.

Niacin standard. An aqueous solution containing 20 micrograms of United States Pharmacopoeia niacin per ml.

Apparatus. The apparatus used is similar to that described by

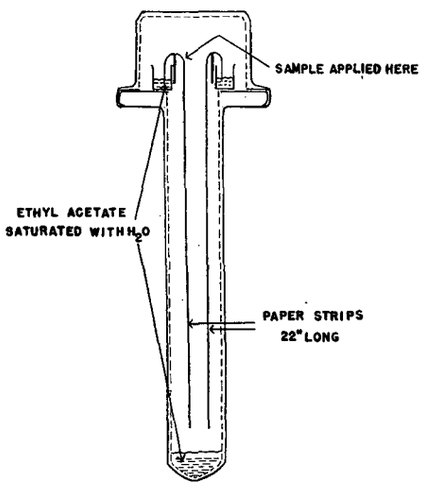

Figure 1. Apparatus for Chromatography

Niacinamide, a member of the vitamin B complex family, is a component of almost every multivitamin preparation on the market. Because niacin is apt to produce side reactions such as flushing, while niacinamide does not produce such reactions, it is of concern to the manufacturer to determine if and how much hydrolysis of niacinamide to niacin has taken place in his products. No simple method for the accurate determination of small quantities of niacin in the presence of large quantities of niacinamide has been available. In the present investigation niacin was separated from niacinamide by descending paper partition chromatography and both were assayed by colorimetric procedure. The stability of niacinamide in solutions of this vitamin alone and in multivitamin solutions at various pH levels and storage temperatures was investigated. Niacinamide was found to be stable in dry form as well as in solutions within the pH range 5.0 to 6.7. Less than 2% hydrolysis was observed at pH levels below 5.0 and storage at 45° C. for 6 weeks. By the technique described it was possible to separate niacin from niacinamide. The results indicate that niacinamide is a rather stable product within the pH levels indicated. A breakdown to niacin is likely to occur only at alkaline or very acid pH levels, which would not be encountered under normal manufacturing or storage conditions.

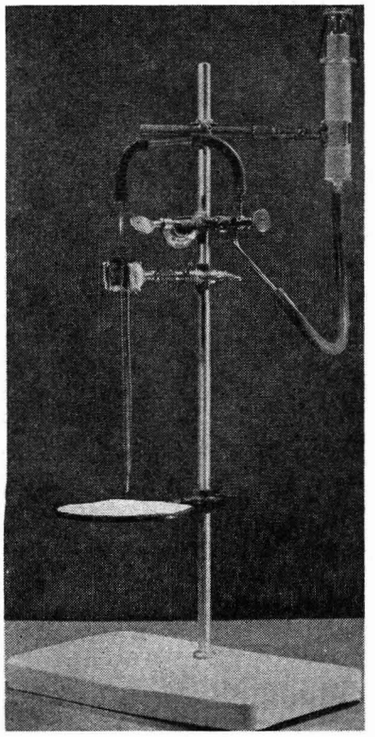

Figure 2. Apparatus for Delivery of Sample

Shepherd (*13*) for the quantitative separation of sulfonamides (Figure 1). The setup described by Winsten (*15*) might be used with equally good results.

The apparatus consists of a 3-inch borosilicate glass tube, about 22 inches (55 cm.) long, closed at one end. The other end is joined to a 6-inch borosilicate glass flange with ground surface. The solvent is contained in a 100-ml. beaker, with its rim removed and with one side flattened. A microscope slide is attached to the flattened side of the beaker by means of a wire. Two such beakers, each supporting a filter paper strip, can be used with one tube, with the beakers standing on the flange at opposite sides of the tube. About 50 ml. of ethyl acetate, saturated with water, are placed in the bottom of the tube. The same solvent is used in the beakers. The chromatogram is developed on Whatman filter paper No. 1, cut into strips of 1.25 × 22 inches. Exactly 0.1 or 0.2 ml. of sample solution is delivered along a horizontal line, marked with pencil on the paper at a point about 3 inches from one end, and this end of the paper strip is immersed in the beaker containing the solvent. The lower part of a 6-inch desiccator, inverted, is used as a cover to assure constant vapor saturation inside the apparatus.

In order to obtain a good chromatogram, the sample should be applied to the filter paper in a very narrow band. The apparatus shown in Figure 2 permits the accurate addition of as much as 0.2 ml. of sample. It consists of a 0.2-ml. micropipet, connected to an all-metal needle valve of the Hershberg-Southworth type (*14*), which in turn is attached to a 30-ml. all-glass hypodermic syringe. The piston of the syringe is attached to the cylinder by means of a coil spring in order to provide constant pressure. In order to fill the pipet, the valve is opened and the piston of the syringe withdrawn half way. The tip of the pipet is placed in the sample solution, which is then drawn up to a level above the zero mark. The valve is quickly closed and the solution is brought to the zero mark by opening the valve. One drop of the solution is placed on the paper; then the valve is closed. After the solution has evaporated on the paper, another drop is added until the required quantity of sample solution has been delivered. (When the solution was evaporated rapidly by application of heat to the paper, caking occurred and consequently a poor chromatogram was obtained.)

METHOD

The sample is applied to the strip and ethyl acetate saturated with water is used as the mobile phase. In approximately 3 hours at 25° C. the solvent front moves 16 inches. At the end of that time, the paper strip is carefully removed, air dried, and cut into 1-inch sections. Each of these sections is assayed by a modification of the colorimetric method of Arnold, Schreffler, and Lipsius (*1*) as follows:

A 1-inch section of the paper strip is placed in an amber 25-ml. glass-stoppered graduate. One milliliter of the phosphate buffer is added followed by 4 ml. of water. The graduate is placed in a water bath regulated at 80° C. for 10 minutes. Three milliliters of the cyanogen bromide reagent are added and the graduate is reheated in the 80° C. bath for exactly 5 minutes. The graduate is removed from the bath and cooled for 1 minute in a blast of air from a fan, then placed in an ice water bath for about 1 minute until the temperature of the solution is 20° to 22° C.

p-Aminoacetophenone reagent is added (0.5 ml.), the contents are well mixed, and 0.6 ml. of 3 *N* hydrochloric acid is added. After thorough shaking, the graduate is allowed to stand in the dark for 15 minutes.

Thirteen milliliters of ethyl acetate are added from a buret and the graduate is agitated in a mechanical shaker for 7 minutes. The contents of the graduate are transferred to a glass centrifuge tube equipped with a cork stopper. The tube is centrifuged at 2500 r.p.m. for 2 minutes. The ethyl acetate layer is carefully decanted into a suitable colorimeter tube and the absorbance is determined at 420 mμ in a photoelectric colorimeter.

Simultaneously a blank and a standard are run. The blank contains all the reagents. The standard consists of 3 ml. of niacin standard solution, 1 ml. of phosphate buffer, and 1 ml. of water, treated in the same manner as the sample.

Experimental. In order to determine the rate of flow (R_f) values of pure niacin and niacinamide, 60 micrograms of niacin were placed on the paper strip. All of the niacin was present on the first inch of the paper. The rate of flow was calculated as 0.10. When 450 micrograms of niacinamide were chromatographed on another strip, all of the niacinamide was present within the fifth to the seventh inch (Figure 3). Its rate of flow value was calculated as 0.37.

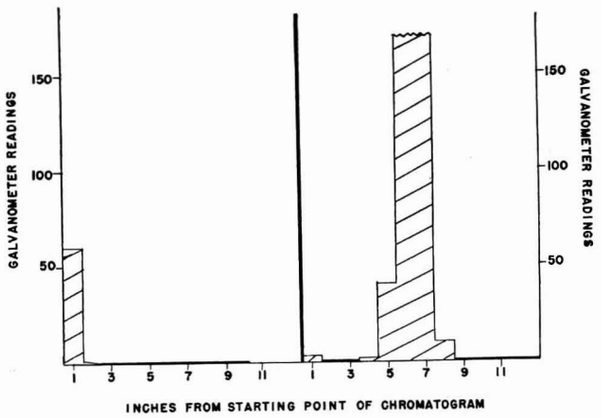

Figure 3. Chromatograms of Niacin (*left*) and Niacinamide (*right*)

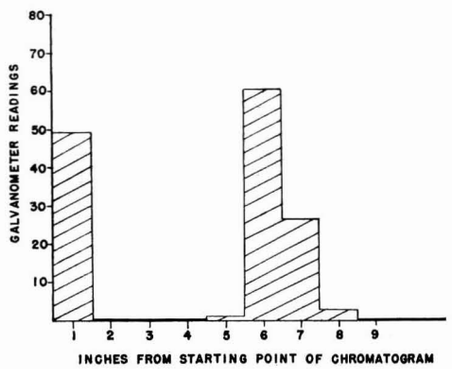

Figure 4. Separation of Niacin from Niacinamide from Solution of Equal Quantities of Each Vitamin

Experiments were conducted with solutions containing equal quantities of each vitamin (50 micrograms of each, Figure 4), and also 1 part of niacin in 100 parts of niacinamide (10 micrograms of niacin and 1 mg. of niacinamide, Figure 5). When these solutions were chromatographed, good separation and quantitative recovery of niacin were obtained. One division of the galvanometer reading shown in Figure 4 was equivalent to 1 microgram of niacin. When U.S.P. reference standard niacinamide was used as a standard, one division of the galvanometer reading was equivalent to approximately 2 micrograms of niacinamide.

pH EFFECT ON SEPARATION

Solutions containing 1 part of niacin to 10 parts of niacinamide were buffered at pH levels of 3.1, 4.1, 4.9, 5.8, and 6.6 with 0.1 *M* acetic acid–sodium acetate. Aliquots of these solutions equivalent to 50 micrograms of niacin were chromatographed. It can be seen from Figure 6 that good separation and recovery of niacin were obtained at all pH levels. However, the R_f of niacinamide was 0.43 at pH 3.1, and 0.40 at pH 4.1. At pH levels 4.9, 5.8, and 6.6, the R_f of the niacinamide appeared to be constant at 0.37. As added proof that the first inch of the strip contained all of the niacin added, a microbiological assay, using *B. leuconostoc mesenteroides*, was run on duplicate chromatograms. It was found that all of the niacin was present within the first inch of the paper.

Solutions were prepared containing 5 mg. of thiamin hydrochloride, 3 mg. of riboflavin, 3 mg. of pyridoxine hydrochloride, 3 mg. of panthenol, 50 mg. of ascorbic acid, 50 mg. of niacinamide and 5 mg. of niacin per ml. These solutions were buffered at various pH levels. When chromatographed in the manner described previously, only fair separation was obtained at a pH below 4, while at pH 5 separation was almost complete and at pH levels above 5 niacin could be completely separated from niacinamide (Figure 7). Therefore, in subsequent determinations a sodium acetate–acetic acid buffer of pH 6.0 was added to unknown multivitamin solutions before application to the chromatographic paper.

When determining the niacin content in multivitamin preparations, aliquots of the same sample solution should be applied to two separate strips and allowed to develop simultaneously. The same section used for the assay from one of the strips should be used as a blank for the second strip. This blank is run in the same manner as described for the sample except that the cyanogen bromide solution is omitted, and instead, 3 ml. of water are substituted.

STABILITY OF NIACINAMIDE

Mikkelsen (*11*), and later Meier (*9*), investigated the stability of solutions of pure niacinamide under various pH conditions. Using a direct titration with the addition of formaldehyde to bind any liberated ammonia, these authors found no hydrolysis in ampoules at pH 5 to 7 and only 0.3% at pH 8 after storage for 2 years.

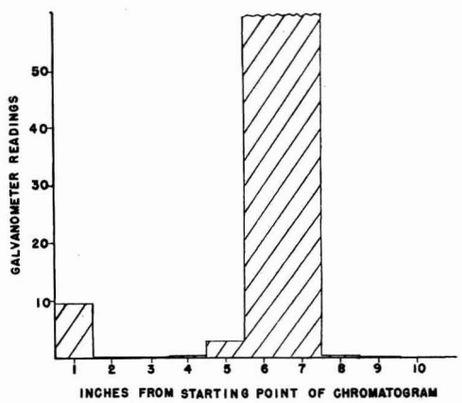

Figure 5. Separation of 1 Part of Niacin in 100 Parts of Niacinamide at pH 6.0

Using the previously described method of separations, the stability of niacinamide solutions at various conditions of pH and temperature was investigated. Five solutions, each containing 10 mg. of niacinamide per ml., were buffered to pH 3.1, 4.1, 5.0, 5.8, and 6.7, respectively, and sealed in ampoules. Four additional solutions containing, per milliliter of solution, 10 mg. of niacinamide, 5 mg. of thiamin hydrochloride, 3 mg. of riboflavin, 3 mg. of pyridoxine hydrochloride, 3 mg. of panthenol, and 50 mg. of ascorbic acid, and buffered to pH 3.0, 4.0, 4.9, and 5.6, respectively, were also sealed in ampoules. The ampoules were sterilized at 122° C. for 30 minutes. One half of these sterilized ampoules were placed in an oven at 45° C., whereas the remainder were kept at room temperature. Initially and after 6 weeks of aging, each of the solutions was chromatographed and the quan-

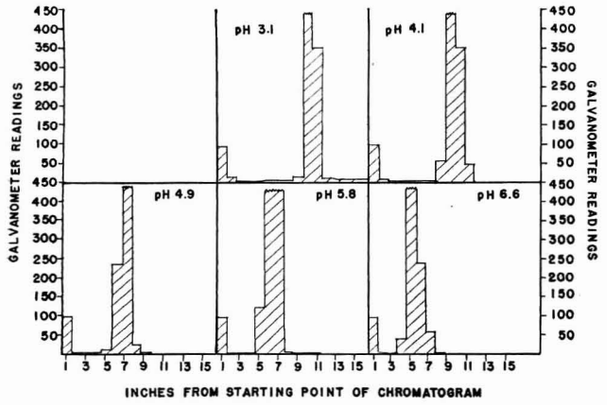

Figure 6. Separation of Niacin from Niacinamide in Buffered Solutions at Various pH Levels

tity of niacin determined. Table I indicates that niacinamide is rather stable at the pH levels investigated. Only after 6 weeks at 45° C. and at a pH below 5.0 did the niacinamide show any measurable sign of hydrolysis, not exceeding 2 per cent.

Samples of U.S.P. grade niacinamide from different manufacturers were chromatographed by the method described in order to determine the amount of niacin present. All of the batches tested contained less than 1% niacin. When these samples were placed in an oven at 45° C. for 6 weeks, no hydrolysis to niacin occurred.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Al Steyermark for his suggestions concerning use of the Hershberg-Southworth type needle valve, to Jack Scheiner for performing the microbiological determinations, and to Charles Pifer for his assistance in carrying out the experiments.

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Table I. Stability of Niacinamide Solutions

	pH	Niacinamide Hydrolyzed to Niacin, %: 6 weeks at room temp.	6 weeks at 45° C.
Solution containing 10 mg. of niacinamide per ml.	3.1	<1.0	1.2
	4.0	<1.0	1.0
	5.0	<1.0	<1.0
	5.9	<1.0	<1.0
	6.8	<1.0	<1.0
Solution containing 10 mg. of niacinamide per ml. plus ascorbic acid and B-complex vitamins	3.0	1.2	2.0
	4.0	<1.0	1.2
	4.9	<1.0	<1.0
	5.6	<1.0	<1.0

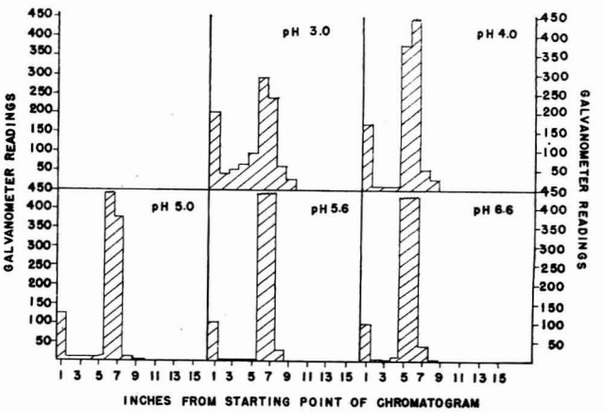

Figure 7. Separation of Niacin from Niacinamide in Buffered Multivitamin Solutions at Various pH Levels

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Rapid Micromethod for Alkaline Nitrobenzene Oxidation of Lignin and Determination of Aldehydes

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THE oxidation of lignin with alkaline nitrobenzene is usually carried out with about 20 grams of wood meal or other lignified material. This amount is necessary when using the methods of previous workers (*5*, *6*) for isolation of the aldehydes because a quantity of mixed aldehydes large enough for fractional sublimation must be obtained. Apart from being somewhat lengthy, the procedure is not readily adaptable to very small quantities of lignin. The following are examples of circumstances in which it is inconvenient to use more than a few milligrams of lignin: (*a*) during the examination of certain plant tissues; and (*b*) any study on the fractionation of lignin by chromatography, the Craig machine, or some other device.

Recently Bland (*1*) has shown that vanillin and syringaldehyde may be separated from one another by partition chromatography on a strip of paper, and it seemed likely that this method could be made quantitative. Moreover, the conditions used for separating the aldehydes might also separate them from the other components of the oxidation reaction mixture, and in this way

Table I. Relative Movement (R_f) Values of Aldehydes

Solvent	Vanillin	Syringaldehyde	*p*-Hydroxybenzaldehyde
n-Butyl ether–water	0.64	0.37	0.64
Petroleum ether (100° to 120° C.)–water	0.39	0.12	0.01

the lengthy purification procedure could be eliminated. The work described falls into two parts: (1) the separation of mixtures of vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde by chromatography and their quantitative evaluation; and (2) the alkaline nitrobenzene oxidation of wood on a micro scale and the subsequent determination of the aldehydes formed. *p*-Hydroxybenzaldehyde was included with the others because there is evidence that certain lignified materials give rise to this substance—for example, corn stalks, and rye straw (*3*, *4*).

EXPERIMENTAL

The apparatus used for chromatography was that of Consden, Gordon, and Martin (*2*). A strip of Whatman No. 1 filter paper 22 inches (55 cm.) long was used as the supporting medium and the aldehydes were applied to it from alcoholic solutions in 0.01-ml. spots 4.5 inches from one end. After running the chromatogram the aldehydes were detected by spraying the paper with a solution of 2,4-dinitrophenylhydrazine. A search for developing solvents gave only two that were suitable—petroleum ether (boiling range 100° to 120° C.) saturated with water as used by Bland, and *n*-butyl ether saturated with water. The former separated vanillin and syringaldehyde in 16 hours, the latter in 1.5 hours. Such chromatograms are shown in Figures 1 and 2. The R_f values are given in Table I. These R_f values illustrate the relative movements of the aldehydes; using *n*-butyl ether as solvent, vanillin and *p*-hydroxybenzaldehyde are not separated. Using petroleum ether, *p*-hydroxybenzaldehyde does not move at all; although this is satisfactory when using solutions of pure aldehydes, it is unsatisfactory when contaminants are present which themselves remain behind on the base line. Mixtures of the three aldehydes could be separated in a satisfactory way by using the upper layer of a 6 to 1 to 1 mixture of petroleum ether, *n*-butyl ether, and water. Such a chromatogram is shown in Figure 3. The time required for effective separation was 13 hours.

The quantitative determination of the aldehydes was carried out on a strip of paper 6 inches wide and 22 inches long. A line was drawn across the paper 4.5 inches from one end and another was drawn down the length of the paper 1 inch from one edge. A known volume of the mixture of aldehydes to be analyzed was spotted along the base line in the wide (5-inch) lane. Another spot was placed in the 1-inch wide lane. After developing the chromatogram, the narrow strip was cut off, sprayed to reveal the position

Table II. Determination of Vanillin–Syringaldehyde Mixtures[a] with *n*-Butyl Ether–Water as Solvent[b]

Present, Mg.		Found, Mg.[c]		Recovery, %[c]	
Vanillin	Syringaldehyde	Vanillin	Syringaldehyde	Vanillin	Syringaldehyde
0.025	0.025	0.024	0.024	97	95
0.050	0.050	0.048	0.046	96	93
0.100	0.100	0.095	0.095	95	95
0.200	0.200	0.190	0.180	95	90
0.500	0.500	0.440	0.450	88	90
1.000	1.000	0.870	0.850	87	85

[a] In alcoholic solution, 1% with respect to each.
[b] Chromatogram run for 1.75 hours.
[c] Average of three determinations.

Table III. Determination of Vanillin–Syringaldehyde–*p*-Hydroxybenzaldehyde Mixtures[a]

(6 to 1 to 1 mixture of petroleum ether, butyl ether, and water as solvent[b])

Present, Mg.			Found, Mg.[c]			Recovery, %		
Vanillin	Syringaldehyde	*p*-Hydroxybenzaldehyde	Vanillin	Syringaldehyde	*p*-Hydroxybenzaldehyde	Vanillin	Syringaldehyde	*p*-Hydroxybenzaldehyde
0.050	0.045	0.050	0.046	0.043	0.049	92	95	98
0.100	0.090	0.100	0.090	0.087	0.098	90	97	98
0.200	0.180	0.200	0.188	0.176	0.201	94	98	100
0.500	0.450	0.500	0.480	0.437	0.499	96	97	100

[a] In alcoholic solution, 1% with respect to each.
[b] Chromatogram run for 13 hours.
[c] Average of three determinations.

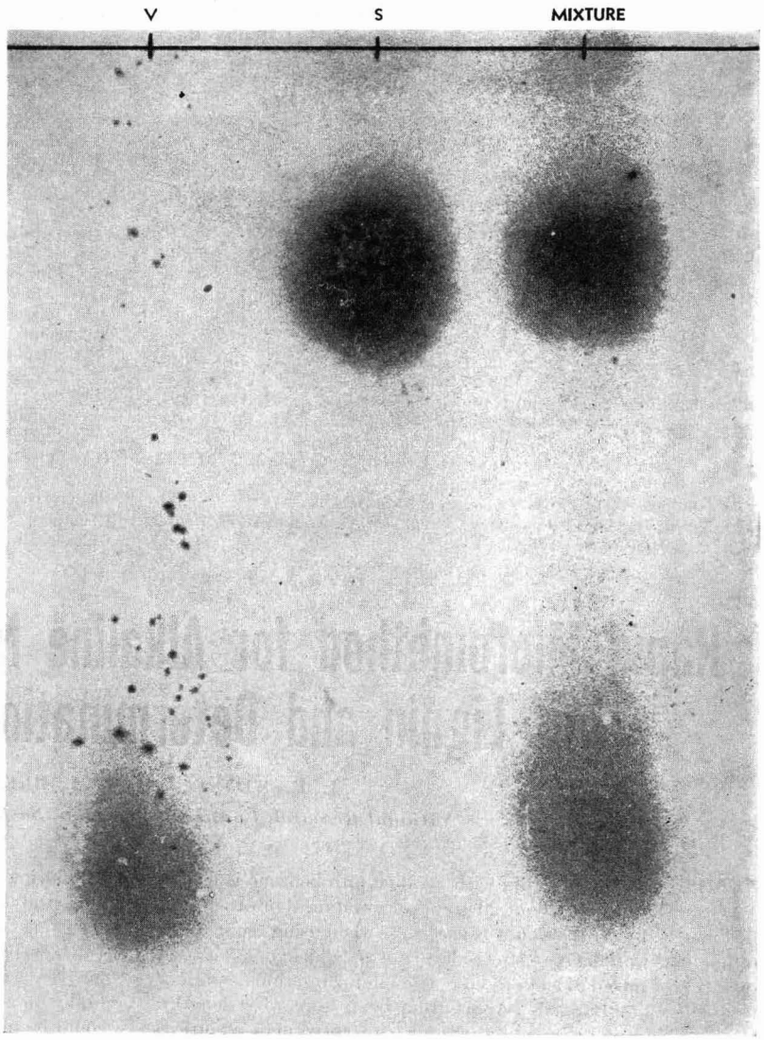

Figure 1. Separation of Vanillin and Syringaldehyde *n*-Butyl Ether
1.5 hours

During the analysis of plant tissue, a rapid quantitative micromethod was required for deciding unequivocally whether a particular fraction contained ligninlike substances. The production of vanillin on oxidation with alkaline nitrobenzene demonstrates the presence of the aromatic nucleus, and together with the formation of syringaldehyde and perhaps *p*-hydroxybenzaldehyde from certain plants, is probably a satisfactory criterion for lignin. The method involved separating the aldehydes chromatographically on a paper strip and determining the amount of each aldehyde spectrophotometrically after extraction from the paper. As little as 50 mg. of lignified material could be used; the oxidation was carried out with 0.06 ml. of nitrobenzene and 1.0 ml. of 8% sodium hydroxide at 160° C. for 2.5 hours. *n*-Butyl ether saturated with water separated vanillin and syringaldehyde from the reaction mixture and from each other in 1.5 hours; when *p*-hydroxybenzaldehyde was present a suitable solvent system was 6 parts of petroleum ether (boiling range 100° to 120° C.) and 1 part of *n*-butyl ether saturated with water. This method should be a valuable tool in differentiating between lignified and nonlignified material.

of the aldehydes, and these cut from the main chromatogram in 2- to 3-inch wide strips. A blank was also taken (Figure 4).

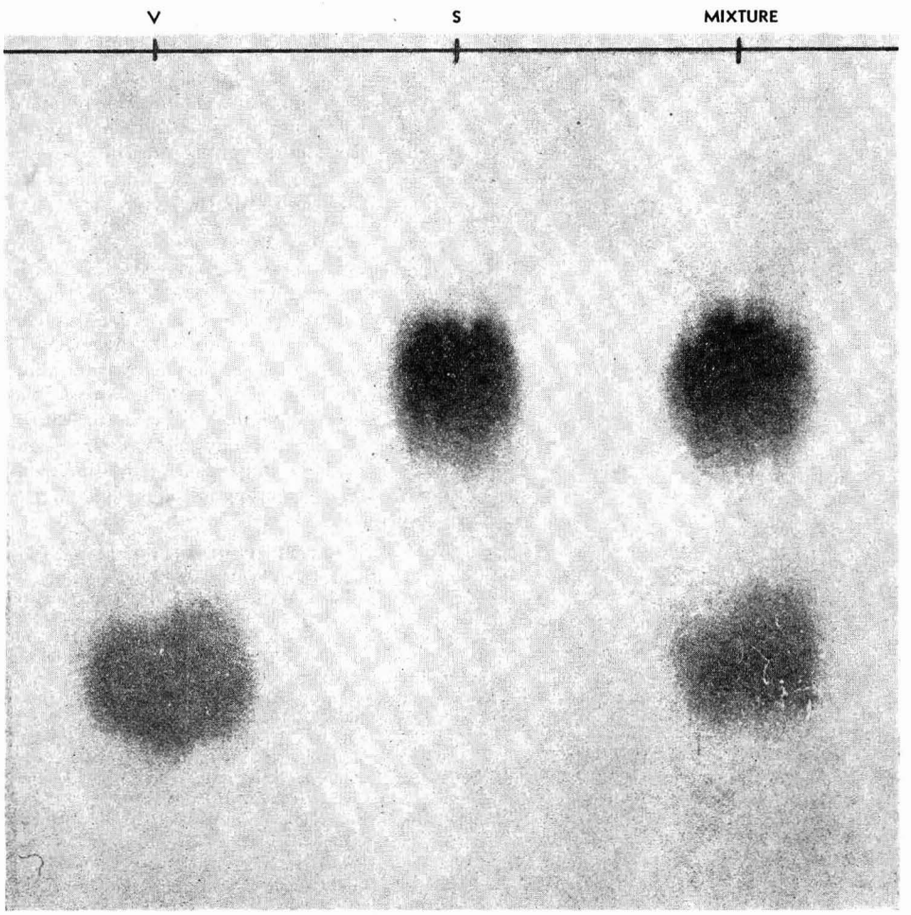

Figure 2. Separation of Vanillin and Syringaldehyde Petroleum Ether
16 hours

The strips of paper containing the pure aldehydes were rolled up and extracted in small Soxhlet extractors for 2 hours with about 25 ml. of ethyl alcohol. Following the procedure of Lemon (7), 4 ml. of 0.2% alcoholic potassium hydroxide were added to the extract which was made up to 50 ml.; the density of absorption was measured at the appropriate wave length with a Beckman spectrophotometer. These wave lengths are 352 mμ for vanillin, 368 mμ for syringaldehyde, and 335 mμ for *p*-hydroxybenzaldehyde. The amount of aldehyde present was then read directly from standard curves.

Results using *n*-butyl ether for vanillin and syringaldehyde are given in Table II. Results using the mixed solvents on mixtures of the three aldehydes are given in Table III. The recovery was usually less than 100%, probably because of the "tailing" which occurs; to reduce this effect it is advantageous to apply the aldehydes to the paper from dilute solution. One per cent should be considered a maximum concentration. Triplicate determinations using various amounts of the aldehydes showed a general deviation from the mean of somewhat less than ±3%.

This procedure has been found useful in determining the amount of vanillin or syringaldehyde in crude preparations of these compounds. It may also be used for the determination of vanillin in vanilla extract or any other natural source. The appropriate correction for chromatographic losses must be applied in every case.

MICROOXIDATION OF LIGNIFIED MATERIALS

The procedure used for the oxidation was identical with the macromethod but it was done on a greatly reduced scale. Stainless steel bombs of about 2.0-ml. capacity were used for the reaction; it was convenient to heat and shake them by placing them in a small hydrogenator bomb half-filled with water. In this way ten or more could be heated at once and the small pressure differential across the lead gaskets gave less chance of leakage. Glass reaction vessels were unsatisfactory owing to the neutralization of the alkali by the silica of the glass. Brass vessels were also unsatisfactory, but the reason is unknown. To each of these small reactors were added about 40 mg. of wood meal (10 mg. of lignin), 0.06 ml. of nitrobenzene, and 1.0 ml. of 2 *N* sodium hydroxide. After heating with shaking for 2 to 2.5 hours at 160° C., the bombs were cooled, opened, and centrifuged. An aliquot (0.2 ml. is convenient) was removed with a microburet and spotted along the base line of a paper chromatogram as previously described. The spots were acidified by passing the paper rapidly over a Petri dish containing boiling glacial acetic acid and the chromatogram was run in the normal way. The results for isoeugenol, spruce, and maple, corrected for chromato-

Table IV. Alkaline Nitrobenzene Microoxidation of Various Materials[a]

Materials	Amount, Mg.	Vanillin		Syringaldehyde	
		Mg.[b]	%[c]	Mg.[b]	%[c]
Isoeugenol	5.24	4.67	97.3	...	...
Isoeugenol	10.48	9.77	100.6	...	...
Isoeugenol	7.66	7.03	99.1	...	...
Spruce[d]	24.0	2.22	27.7	...	...
Spruce[d]	31.6	2.92	28.1	...	...
Spruce[d]	45.5	4.04	27.1	...	...
Maple[e]	51.8	1.50	13.2	3.3	28.9
Maple[e]	48.7	1.50	14.0	3.0	28.0

[a] 1.0 ml. of 2 *N* sodium hydroxide, 0.06 ml. of nitrobenzene, 160° C. for 2.5 hours.
[b] Corrected for chromatographic losses using Table II.
[c] Based on theory for isoeugenol and on lignin for woods.
[d] 32.8% Klason lignin.
[e] 22.0% Klason lignin.

graphic losses using Table II, are given in Table IV. Thus in one step is performed the whole of the purification and separation of the aldehydes. The nitrobenzene and azobenzene, being water insoluble, run at the liquid front. Other contaminants remain behind on the base line. Acetoguaiacone, when present, forms a distinct band of its own and does not interfere with the aldehydes. It may of course be estimated in the same way as described for the other components. The reasons for acidifying the oxidation liquor after placing it on the paper are to avoid dilution and to ensure that the phenolic substances are held in the aqueous phase while removing the aliquot. Theoretical

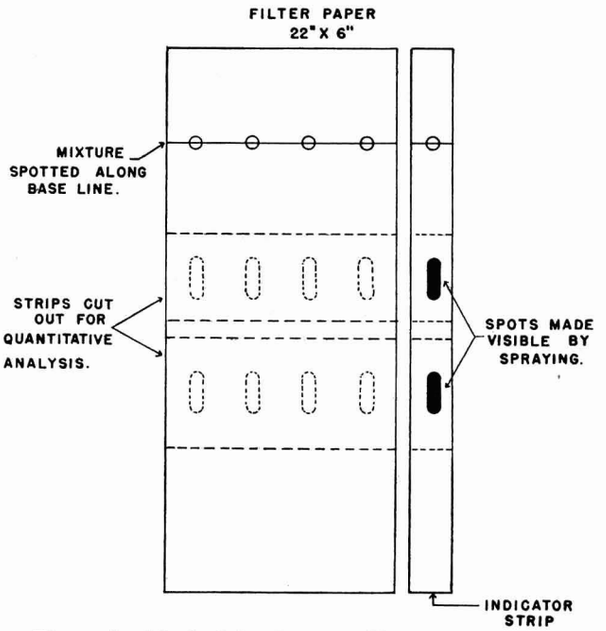

Figure 4. Method for Cutting Chromatogram for Quantitative Analysis

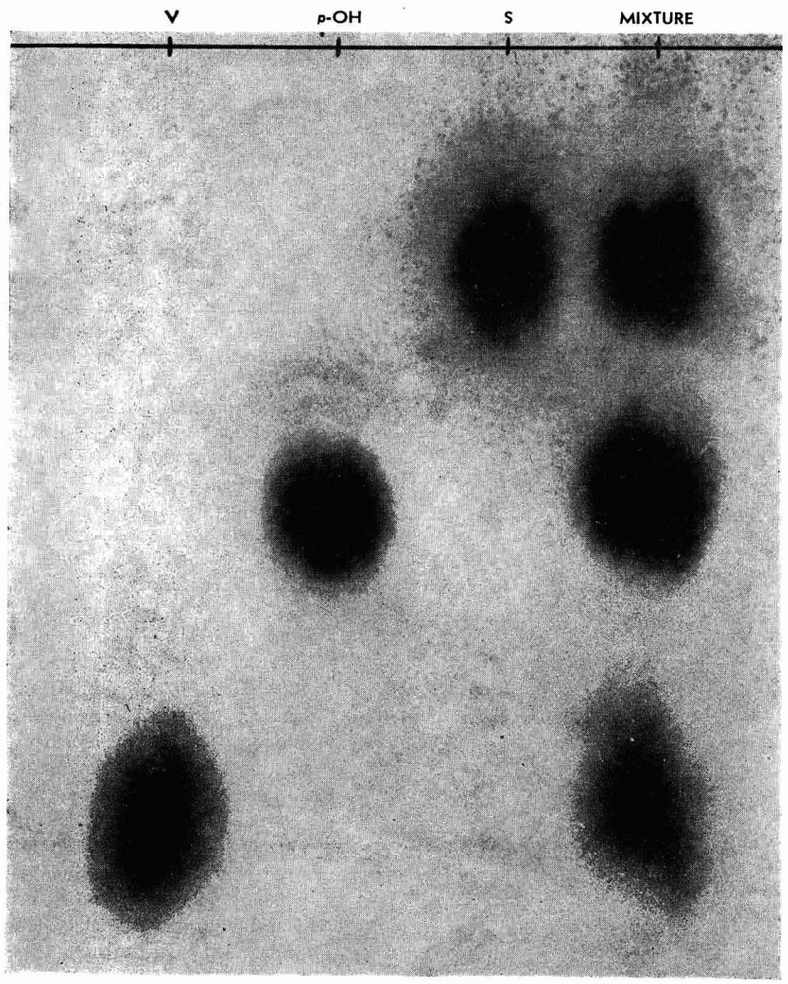

Figure 3. Separation and Vanillin, Syringaldehyde, and *p*-Hydroxybenzaldehyde Petroleum Ether–Butyl Ether

13 hours

yields of vanillin from isoeugenol are only possible if vanillin itself is stable under the conditions of the oxidation; to test this, vanillin was subjected to the standard conditions and the reaction liquor was chromatographed in the normal way. Yields of 99.3, 96.4, and 97.8% vanillin were obtained after correcting for chromatographic losses. The over-all error of the procedure, shown by a number of oxidations of isoeugenol to vanillin, is well within ±5%.

DISCUSSION

This method was developed as an aid in following the lignification of wheat plants during growth and has proved highly satisfactory for this purpose. Particularly valuable features are that many determinations can be carried out simultaneously, the whole procedure may be performed in a working day or a day and overnight, and during a large proportion of the time consumed no attention is required from the operator.

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Analysis of Fat Acid Oxidation Product by Countercurrent Distribution Methods

Model Compounds

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Countercurrent distribution techniques are currently providing valuable information in the study of fat acid oxidations. However, interpretation of results obtained by this new procedure of fractionation is contingent upon a knowledge of the influence of the functional groups upon the partition coefficient and the resulting weight distribution curve. Model compounds chosen for study and for distribution between hexane and 80% ethyl alcohol included methyl stearate, methyl hydroxy stearate, methyl dihydroxy stearate, methyl epoxy stearate, methyl keto stearate, methyl oleate, methyl ricinoleate, stearic acid, hydroxy stearic acid, azelaic acid, heptenal, nonenal, and mono- and diglycerides. The compounds investigated thus far behave nearly ideally in the concentration employed. Their weight distribution curves are predictable from their partition coefficient through use of the binomial theorem, and are little influenced by the presence of other dissolved solutes. The partition coefficients serve for qualitative identification and description of compounds; in addition, partition coefficients are useful because from them the degree of separation of compounds and the number of transfers that are required in the countercurrent distribution apparatus may be calculated.

THE statement "every scientific advance is an advance in method," finds support in numerous fields of research. One method recently introduced to the lipide field and applied with considerable success is Craig's countercurrent distribution procedure (*2*). However, interpretation of the results obtained on oxidized methyl linolenate requires a knowledge of the behavior of simpler systems.

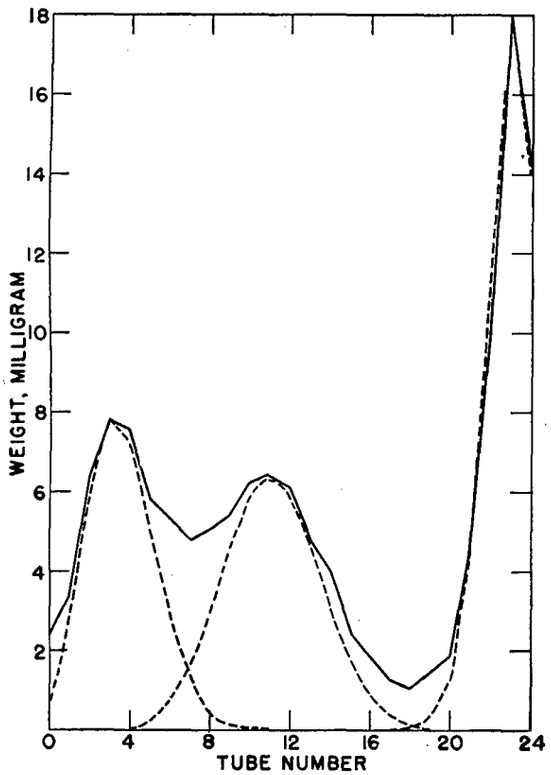

Figure 1. Countercurrent Distribution Curve of Dihydroxy Methyl Stearate, Hydroxy Methyl Stearate, and Methyl Stearate

Autoxidations of ethylenic compounds have been variously reported to introduce peroxidic, hydroxyl, keto, and oxirane groups as well as to promote polymerization. Further oxidation yields cleavage products including mono- and dicarboxylic acids, aldehydes, and various combinations of these functional groups. The behavior on countercurrent distribution of model compounds, some of which have been postulated as oxidation intermediates, containing these functional groups is the subject of this paper.

METHODS AND MATERIAL

A 25-tube Craig countercurrent distribution apparatus was used for fractionating the model compounds (*2*). This equipment consists, in principle, of a series of separatory funnels, each containing equal volumes of immiscible solvents. In the actual apparatus, the separatory funnels are formed by drilling a series of holes (called tubes) in a cylindrical stainless steel block. This block is cut near the middle, perpendicular to its axis. When the interface between the two immiscible solvents is adjusted to the level of the cut, the upper phase of each tube may readily be transferred to the adjacent tube by rotating the upper section, thus achieving countercurrent movement of solvents. Mixing the phases in all the tubes, simultaneously, is accomplished by rocking the whole apparatus to and fro a half turn. Fractionation of the material is effected by introducing the mixture into one of the tubes containing the immiscible solvents. Mixing and separation of the phases are alternated with transfer of the upper phase to next adjacent tubes until the upper section has made a complete revolution. A more complete description of the apparatus, the theory of operation, and some of its applications to lipides are included in the literature (*13, 14, 16, 17, 18*).

In the experiments described herein, 80% ethyl alcohol and hexane-pentane (35° to 60° C.) were used as the immiscible solvent pair. The weight of material fractionated ranged from 17 to 250 mg. Purification of the model compounds was generally necessary and was accomplished by recrystallization or countercurrent distribution procedure. The contents of each tube were withdrawn into weighed flasks after countercurrent distribution, numbered corresponding to the tubes, placed in a vacuum oven, taken to dryness, and again weighed. The weight of residue in each flask was then plotted against the tube number.

The partition coefficient, K, of each compound was calculated from the weights of each compound in pairs of tubes (T_r and T_{r-1}) chosen near the maximum in the weight curve by use of the equation (*20*)

$$K = \frac{T_r}{T_{r-1}} \times \frac{1}{F} \qquad (1)$$

Results of at least three calculations from three pairs of points were averaged to obtain the coefficient. The theoretical weight distribution curve can also be calculated by use of this equation and the experimentally determined partition coefficient. This calculated curve is designated by a broken line in the figures.

In a few instances, which will be discussed later, a 24-transfer distribution was insufficient to effect a satisfactory separation. However, the number of transfers necessary can be calculated by use of the equation

$$n = t^2 \left[\frac{K_a + K_b + 2K_aK_b}{K_b - K_a}\right]^2 \qquad (2)$$

where n = number of transfers; K_a = partition coefficient of compound a; K_b = partition coefficient of compound b; and t = a statistical coefficient for which the percentage impurity may be found in standard probability tables (*4, 14*).

RESULTS AND DISCUSSION

The results obtained by countercurrent fractionation of model compounds are shown in the following series of curves. Figure 1 gives the weight distribution curve of hydroxy methyl stearates. These compounds are of interest since the decomposition of peroxides leads to the formation of hydroxy acids and esters (*5–10, 19*). The data show that in fractionating an oxidation mixture, the maxima for the weight curves of monohydroxy esters and dihydroxy esters of C_{18} acids would appear in the vicinity of tube 12 and tube 4, respectively, for a 24-transfer distribution. The curve shows that the more polar the compound the lower the tube number in which the material will be found. To obtain dihydroxy and monohydroxy methyl stearate with 2% of material impurity, 44 transfers are calculated to be necessary.

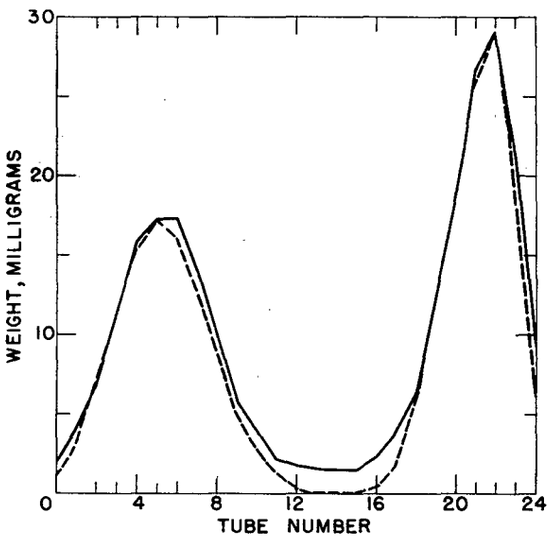

Figure 2. Countercurrent Distribution Curve of the Mono- and Diglycerides of Cottonseed Oil

The weight distribution curve for the mono- and diglycerides of cottonseed oil is shown in Figure 2. Polymer formation has been postulated (*12, 15*) and found to occur as a product in the oxidation of fat acids. If one considers the diglyceride as a model compound for a dimer with one hydroxyl group and the monoglyceride as a monomer with two hydroxyl groups, these data indicate that dimeric material containing one functional group would appear toward the higher number tubes where relatively nonpolar substances are found. The relative importance of polar and nonpolar groups in determining the partition ratio is illustrated by the monomer hydroxy ester of Figure 1 for which

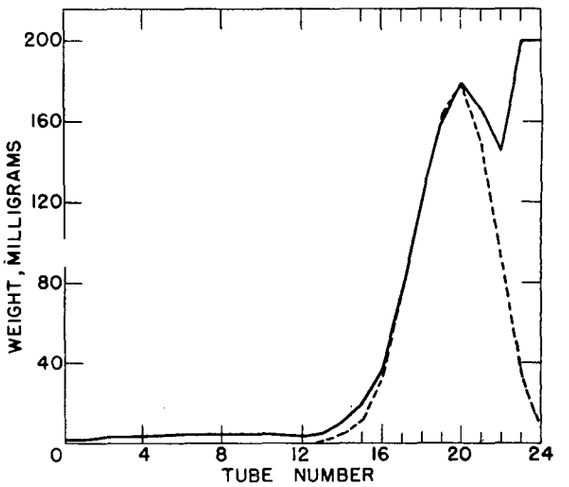

Figure 3. Countercurrent Distribution Curve of Epoxy Methyl Stearate and Methyl Stearate

the maximum is found in tube 12, whereas for the dimer hydroxy ester the maximum is found in tube 22. Therefore, the presence of an additional aliphatic group shifts the curve toward higher tube numbers.

Figure 3 gives the fractionation of epoxy methyl stearate and methyl stearate. A more complete (2% impurity) separation, by calculation, would require the application of 100 plates. Since an epoxy group is less polar than a hydroxyl group, the maximum for epoxy methyl stearate is expected and found in a higher tube number, 20. If additional functional groups, such as a hydroxy group, were present in the dimeric compounds described in the literature (*1, 19*), the maximum would occur toward tube numbers lower than 18.

The weight distribution curve for methyl oleate hydroperoxide, which is believed to be the first reaction product in the oxidation

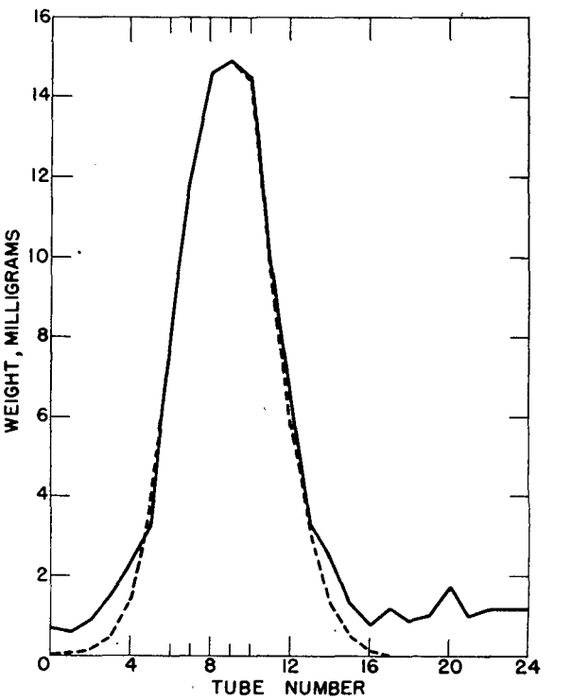

Figure 4. Countercurrent Distribution Curve of Methyl Oleate Hydroperoxide

of methyl oleate (*10*), is given in Figure 4. The hydroperoxide group, being more polar than the hydroxyl group, has shifted the curve toward lower tube numbers (maximum in tube 9).

Figure 5 shows the fractionation of 12-hydroxy stearic acid and methyl stearate. Replacement of the ester group in hydroxy methyl stearate by a carboxyl group has markedly shifted the curve toward a lower tube number.

The weight distribution curve of azelaic acid, a short chain dibasic acid postulated as one of the scission products in the oxidation of fat acids (*11*), is given in Figure 6. Owing to the presence of two carboxyl groups and a short carbon chain, this highly polar acid appears in the lower tube numbers.

The presence of a carbonyl group in methyl stearate shifts the curve toward lower tube numbers, as shown in Figure 7, for a mixture of 9 and 10 keto methyl stearate. Since experimental and theoretical curves agree closely, the position of the keto group on the carbon chain would appear to have little effect on the partition coefficient. Methyl keto stearate is not as polar as the monohydroxy stearate but is more polar than methyl epoxy stearate.

Figure 8 shows the effect on the weight distribution curve of the addition of a hydroxyl group to methyl oleate. The peaks for methyl ricinoleate and methyl oleate appear, in the same tubes, as those for hydroxy methyl stearate and methyl stearate, respectively. This indicates that the double bond has little effect upon the polarity of the compound.

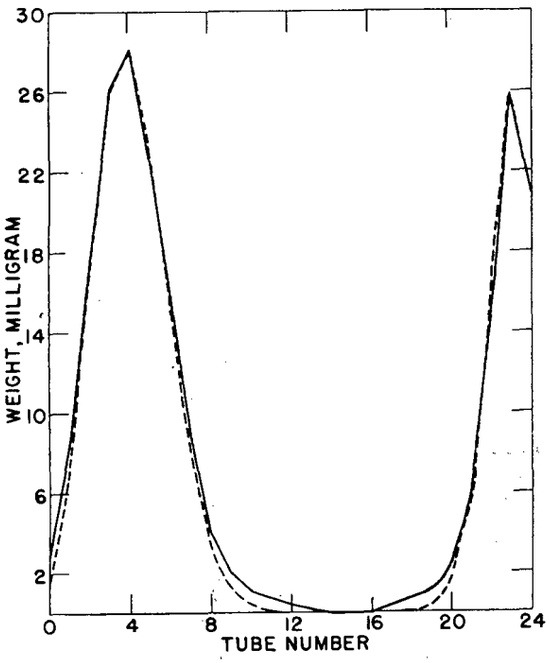

Figure 5. Countercurrent Distribution Curve of 12-Hydroxy Stearic Acid and Methyl Stearate

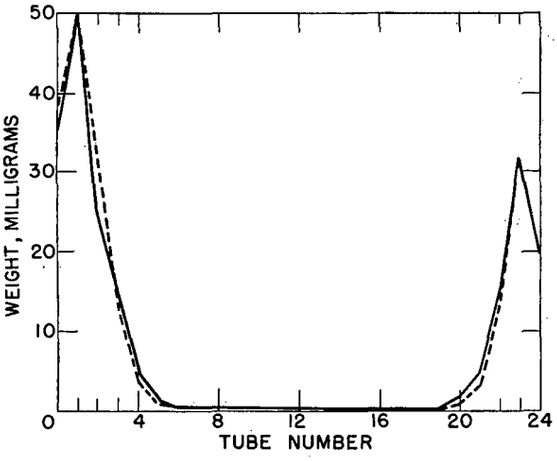

Figure 6. Countercurrent Distribution Curve of Azelaic Acid and Methyl Stearate

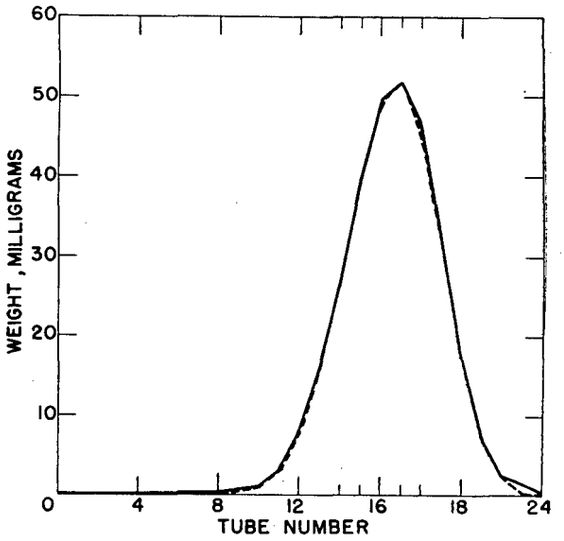

Figure 7. Countercurrent Distribution Curve of Keto Methyl Stearate

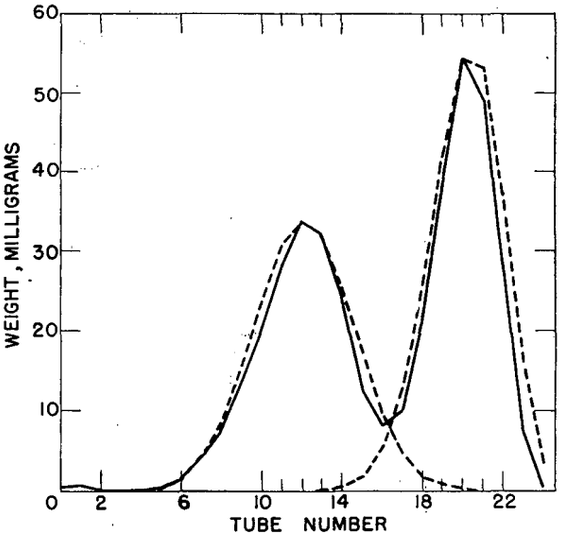

Figure 8. Countercurrent Distribution Curve of Methyl Ricinoleate and Methyl Stearate

The weight distribution curves for α-heptenal and α-nonenal, shown in Figures 9 and 10, respectively, are of particular interest because the former aldehyde has been isolated from reverted soybean oil (*3*). The maximum for the more polar α-heptenal occurred in tube 16 and the peak for α-nonenal was found in tube 18. An 85-transfer distribution would be necessary to separate a mixture of these two aldehydes (98% purity).

The weight in each tube was determined spectrophotometrically by measuring the absorption at a wave length of 2400 A. This wave length does not occur at the maximum but was selected in consideration of the solvent transparency and intensity of absorption. The absolute weight values given are relative since the distribution data show that impurities were present in the preparations at the time they were used for calibration and distribution.

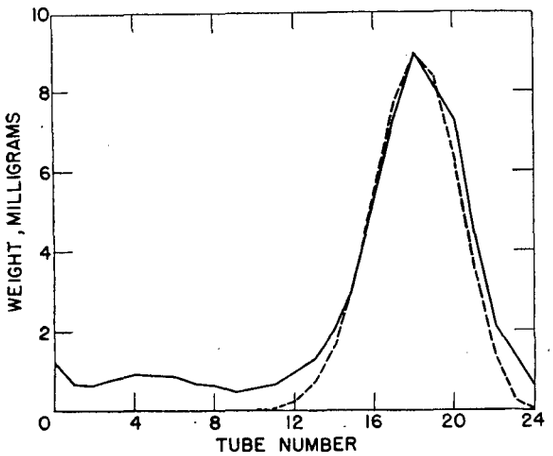

Figure 9. Countercurrent Distribution Curve of α-Heptenal

Table I gives a summary of the compounds fractionated, their partition coefficients, the tube number of the maximum in a 24-transfer distribution, and the important functional groups present. It becomes apparent that the partition coefficient may well assume the importance of other physical constants such as melting point, refractive index, and optical rotation. Not only do the coefficients serve for qualitative identification and description of compounds but, in addition, they have other useful features. If a pair of partition coefficients are given, the degree of separation of the compounds for any given number of transfers may be calculated or, conversely, the number of transfers necessary to achieve a desired purity or degree of separation may be calculated.

The compounds investigated thus far behave almost ideally in the concentration employed. Their weight distribution curves

Table I. Partition Coefficients and Positions of Maxima for Various Model Compounds

Compound	Partition Coefficient	Tube[a] Number	Functional Groups
Azelaic acid	0.05	1	di-COOH
Dihydroxy methyl stearate	0.17 to 0.21	3, 4	di-OH and COOR
12-Hydroxy stearic acid	0.20	4	OH and COOH
Monoglyceride of cottonseed oil	0.28	5	di-OH (monomer)
Methyl oleate hydroperoxide	0.57	9	OOH and COOR
Hydroxy methyl stearate	0.97 to 1.01	11, 12	OH and COOR
Methyl ricinoleate	1.01	12	OH, =, COOR
Heptenal	2.05	16	= and C=O
Keto methyl stearate	2.22	17	$-R_2C=O$ and COOR
Nonenal	2.96	18	= and C=O
Stearic acid	3.36	18	COOH
Epoxy methyl stearate	4.40	20	C—C and COOR (O)
Methyl oleate	5.15	20	= and COOR
Diglyceride of cottonseed oil	10.76	22	OH (dimer)
Methyl stearate	19.75 to 19.31	23	COOR

[a] Distribution (24-transfer) between hexane and 80% ethyl alcohol.

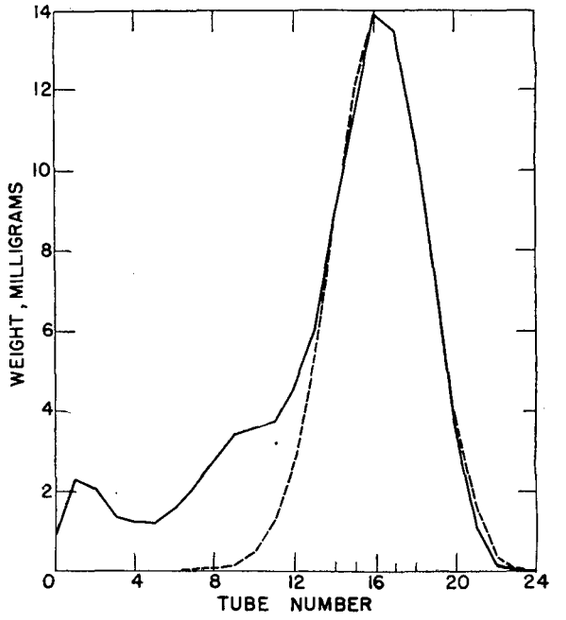

Figure 10. Countercurrent Distribution Curve of α-Nonenal

are predictable from their partition coefficient and the binomial theorem, and are little influenced by the presence of other dissolved solutes.

ACKNOWLEDGMENT

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Colorimetric Determination of Alkaloids in Tissues by Means of Methyl Orange

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A method was sought that would enable the toxicologist to estimate microgram quantities of alkaloids isolated from human tissues in a simple reliable routine analysis. Research has indicated that a relatively simple extraction method can isolate alkaloids from human tissues. The compound isolated is colored and lends itself to a colorimetric method for a quantitative determination. Identification can be made with existing physical methods. A group specific reaction for alkaloids requiring minimal amounts of original material gives a quantitative estimation of the drug concurrent with its detection.

IN 1940, Prudhomme (*7*) reported a colorimetric method for the determination of quinine based upon the reaction of that alkaloid with eosin to form a colored compound which is extractable with chloroform. Lehman and Aitken (*4*) in 1942 demonstrated the existence of a similar reaction between demerol and bromothymol blue which they used for the colorimetric determination of demerol in urine. In 1943, Oberst (*6*) slightly modified the method of Lehman and Aitken. Marshall and Rogers (*5*) in 1945 applied the bromothymol blue reaction to the determination of cinchona alkaloids. Brodie and Udenfriend (*1*) in 1945 introduced the sulfonic acid, methyl orange, for the colorimetric estimation of cinchona alkaloids and other organic bases in plasma and urine. The authors have modified the methyl orange reaction developed by Brodie and Udenfriend in order to apply it to the quantitative determination of alkaloids in human organs. The main steps in the method are as follows:

1. Extraction of alkaloids from tissue with boiling acidified water
2. Extraction of these alkaloids from the filtered aqueous solution by means of chloroform
3. Formation of a chloroform-soluble colored compound of the alkaloids with methyl orange

REAGENTS

Tartaric acid, saturated solution.

Sodium hydroxide solution, 4 *M*.

Chloroform, analytical grade essential.

Hydrion indicator paper B.

Methyl orange solution. Saturated solution essential. May be prepared in the following manner: Add 500 mg. of methyl orange to 100 ml. of water and maintain the mixture at a temperature of approximately 40° C. for about 20 minutes with occasional stirring. Allow the solution to cool to room temperature and filter.

Boric acid, saturated solution. To ensure a saturated solution, a small amount of solid boric acid should be maintained in contact with the solution.

Methyl orange reagent. Should be prepared just prior to use by mixing equal volumes of boric acid and methyl orange solutions.

Phosphate buffer, pH 8.0. Mix 25 ml. of 0.2 *M* potassium dihydrogen phosphate and 46.85 ml. of 0.1 *M* sodium hydroxide and dilute to 100 ml.

Ethyl alcohol, acidified by adding 2 ml. of concentrated sulfuric acid to 100 ml. of absolute ethyl alcohol.

PROCEDURE

Extraction of Alkaloid. To 500 grams of finely maccrated tissue, preferably brain or liver, contained in a 2-liter Florence flask, are added 500 ml. of water and 2 ml. of the tartaric acid solution. The mixture is steam distilled until about 150 ml. of distillate have been collected. During the steam distillation process, the flask and its contents are immersed in a boiling water bath. This operation serves to coagulate proteins and to facilitate the extraction of alkaloids from the tissue. If desired, the distillate may be examined for volatile poisons, but in the present investigation, inasmuch as these substances were not pertinent to an evaluation of the colorimetric method for alkaloids, the distillate was discarded. The total volume of material in the flask is measured and then filtered while still hot. The volume of insoluble matter is estimated as 30% of the original brain or liver tissue, in the present case 150 ml. (0.30 × 500). If other tissues are used, a correction should be applied depending on the per cent of solids present in that tissue. This amount is subtracted from the total volume in order to estimate the volume of the aqueous extract. An aliquot of the aqueous extract is employed for subsequent steps of the analysis.

Quantitative Determination. Transfer 25 ml. of aqueous extract into a 60-ml. glass-stoppered bottle and add 4 *M* sodium hydroxide solution until the pH of this aliquot attains a value of 7.5 to 8.2 as indicated by the use of Hydrion indicator paper B. Two to four drops of the sodium hydroxide solution will usually be sufficient to attain the proper pH value as indicated by the development of a green color on the indicator paper. Other experiments performed in this research project indicate that, at this pH, alkaloids are completely extracted by the chloroform and at the same time the extraction of organic bases normally present in tissues is kept at a minimum.

After the adjustment of the pH, 25 ml. of chloroform are added and the mixture is shaken vigorously for 20 minutes, preferably on a mechanical shaking machine. The bottle and contents are then centrifuged at high speed for 3 to 4 minutes. If an emulsion is evident at the interface, the mixture should be stirred vigorously with a glass rod and again centrifuged. The aqueous layer and any remaining emulsion at the interface are then removed by aspiration. The remaining chloroform phase is transferred to a test tube and again centrifuged. Any remaining aqueous layer and residual emulsion are removed by aspiration.

The chloroform layer is transferred to a glass-stoppered bottle and mixed with 0.7 ml. of the methyl orange reagent, and the resultant mixture is shaken mechanically for about 10 minutes. After shaking, the mixture is transferred to a test tube and the aqueous methyl orange layer is removed by aspiration. The content of the test tube is centrifuged, and all remaining aqueous methyl orange layer is removed by aspiration. It is imperative to remove all of the aqueous methyl orange solution to ensure that subsequent readings will be valid. Ten milliliters of the chloroform solution are pipetted into a cuvette and 1.0 ml. of the acidified alcohol reagent is added. In the present investigation a Coleman Junior spectrophotometer, Model 6 A.S., was employed to determine the optical densities of the solutions at 520 mμ using reagent blank to set the instrument at 100% transmission.

In cases where the alkaloid content is extremely small, the sensitivity may be increased twenty times by extracting the 10

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ml. of chloroform solution containing the alkaloid–methyl orange compound with 0.5 ml. of 1 *M* hydrochloric acid and reading this aqueous layer in a suitable cuvette at the same wave length. Thus the colored compound has been concentrated from a volume of 10 ml. to 0.5 ml.

The analysis of plasma for alkaloids may be conducted by the original method of Brodie and Udenfriend (*1*).

Table I. Recovery of Alkaloids and Other Organic Bases from Tissue Using Methyl Orange Method

(Recovery, mg.)

	Alkaloid Added to 500 Grams of Tissue, Mg.			
	1.00	2.00	3.00	4.00
Atropine	1.18	2.16	3.13	4.20
	...	2.08	3.00	4.12
Benzedrine	1.12	2.02	3.10	3.96
	0.91	2.07	3.06	4.04
Cocaine	0.92	1.34	2.63	3.12
	...	1.42	2.36	2.93
Codeine	0.97	2.06	2.71	3.95
	0.91	1.85	2.88	3.70
Demerol	1.05	1.84	3.06	4.01
	1.08	1.93	2.96	...
Dilaudid	1.10	2.03	3.10	3.97
	1.09	2.06	3.00	3.70
Heroin	1.05	2.09	3.05	4.10
	1.03	2.00	2.98	4.15
Neohetramine	0.99	1.92	2.74	3.82
Nicotine	0.97	2.13	2.95	3.96
	0.96	1.98	3.03	3.90
Nupercaine	0.93	0.93	1.18	1.58
Pontocaine	0.76	1.00	1.22	1.84
	1.02	1.91	1.45	2.38
Quinine	0.93	1.91	2.94	3.96
	0.99	1.91	2.98	...
Strychnine	0.93	2.10	2.85	3.61
	0.96	1.88	2.74	...

Isolation and Identification. The aqueous extract of the tissue (100 ml.) is made alkaline and shaken with 100 ml. of chloroform in a 250-ml. bottle by means of a mechanical shaker. The mixture is then centrifuged to break any emulsion and the aqueous phase is removed by aspiration. The chloroform layer is evaporated to about 5 ml. and transferred to a sublimation tube, and all the remaining chloroform is then evaporated. The residue is subjected to a vacuum sublimation procedure as described by Gettler, Umberger, and Goldbaum (*2*). The resultant sublimate will contain the pure alkaloid which may then be identified by crystalline structure, micro melting point, and eutectic point using Kofler's technique (*3*), and also by color reactions. An excellent English presentation of Kofler's technique is given by Reimers (*8*).

EXPERIMENTAL WORK

Standard Optical Density Curves. A sample of the pure alkaloidal salt, equivalent to 50 mg. of the free base, is weighed and dissolved in 250 ml. of the phosphate buffer solution. Five milliliters of this solution are diluted to 100 ml. with distilled water to yield a working standard alkaloid solution equivalent to 10 micrograms per ml. of solution. Into a series of 60-ml. glass-stoppered bottles there are pipetted 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 ml. of the working standard alkaloid solution, containing 25, 50, 75, 100, 125, and 150 micrograms of the alkaloid, respectively. Volumes of the buffer solution are added to each of these standard samples in order to bring the volume to a total of 25 ml. These solutions were processed in the manner described above for the aqueous extract. The samples prepared represented an alkaloid content of 1, 2, 3, 4, 5, and 6 micrograms per ml. of chloroform solvent. These alkaloidal concentrations were plotted against their corresponding optical densities thus obtaining the standard optical density curve shown in Figure 1. The rectilinear nature of the curve indicates that the compounds obey Beer's law within the range from 1 to 6 micrograms per ml.

ANALYSIS OF TISSUES CONTAINING NO ALKALOIDS

Upon application of the above method to 70 human brains containing no alkaloids, the optical density of the chloroform solution of methyl orange, the tissue blank, was 0.013 ± 0.003. Inasmuch as the reagent blank gives an optical density reading of 0.008, it is evident that a correction for the normal brain tissue blank is not necessary. The liver tissue blanks were somewhat higher with an average optical density of 0.028. With liver this value should be subtracted from the optical density of solutions containing alkaloids, especially if the alkaloidal content is very small. It was also found that the tissue blank does not increase if the aqueous extract of the tissues is kept in the refrigerator from 4 to 6 days. Putrefied tissue blanks, however, are so high as to invalidate the method.

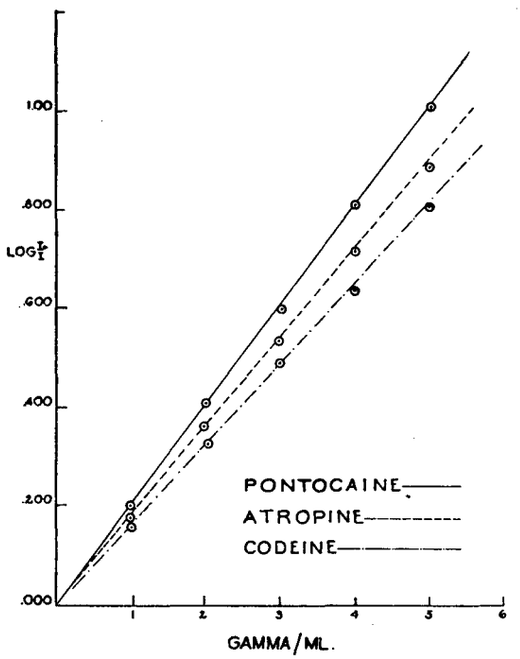

Figure 1. Effect of Concentration on Optical Density

Because the reaction involved is a general one for alkaloids and many synthetic organic bases, a reading equivalent to the tissue blank (0.013) indicates that none of these substances is present. The method, therefore, also serves as a general qualitative test. If the optical density of the processed aqueous extract is definitely higher than the optical density of the tissue blank, some basic organic substance is present. The next step is the isolation and identification of the substance, followed by the preparation of a standard optical density curve for that substance.

ANALYSIS OF TISSUES CONTAINING ALKALOIDS

Known quantities of alkaloids were added to tissues. These were then processed in the manner described. From the optical density of the colored chloroform extract, the micrograms of alkaloid present per milliliter of chloroform solvent are obtained from the standard curve. This value multiplied by the corrected

volume of the original filtrate will give the quantity of alkaloid present in the 500 grams of tissue used for the analysis. The results are given in Table I. Each value is given as the average of two concurrent analyses of the same sample. Duplicate values checked within an average of 3%.

Should the chloroform solvent contain much more than 6 micrograms of the alkaloid per ml., the analysis should be repeated, using a smaller volume of the aqueous extract. Similarly, should the solvent contain less than 1 microgram of alkaloid, a larger volume of the aqueous extract should be processed.

A direct comparison of the color of the final chloroform extract of tissue filtrate with that of a standard alkaloid solution processed in the same manner, may also be used.

An analysis of the data in Table I leads to the following conclusions:

1. The recoveries of most of the alkaloids and other organic compounds are good.
2. Some antihistamines—for example, Neohetramine—can also be determined by this method with good results.
3. The low recoveries in the case of cocaine are probably due to decomposition during the steam distillation, because aqueous solutions of cocaine that were not heated gave good recoveries.
4. The *p*-aminobenzoic acid derivative pontocaine, and nupercaine, gave good recoveries from aqueous solutions under the identical conditions of the method. When added to tissues, however, the recoveries were low. This was probably due to decomposition by enzymatic action. *p*-Aminobenzoic acid is not determinable by this method and the aliphatic base obtained from the decomposition is extractable only at high alkalinity and with another solvent.
5. Morphine, not included in Table I, gave poor results under the conditions of this method. Further studies involving the recovery of morphine by altering experimental conditions are contemplated. The poor results are probably attributable to the amphoteric nature of morphine.

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Colorimetric Determination of Tungsten

Study of Variables Involved in Stannous Chloride–Thiocyanate Method

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AND
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Erratic results in the determination of tungsten led to a study of the variables influencing the stannous chloride thiocyanate method. Free acid and chloride concentrations determine the degree of reduction of the tungsten. With 7.00, 5.95, and 3.63 moles of chloride per liter, the lower limits of free acid to achieve complete reduction are 9.5, 11.2, and 13.9 moles per liter, respectively. The thiocyanate concentration should be maintained about 0.2 molar for best results. The reliability of the method is thereby improved by selection of optimum operating conditions.

THERE are only a few chemical methods for determining trace amounts of tungsten. Current interest is directed primarily toward the colorimetric method, based on the reduction of tungstate and subsequent formation of the yellow tungsten thiocyanate complex. A bibliography relating to the development of the method is given by Geld and Carroll (*2*). The changes in procedure and reagents suggested in the literature stress the need for studying the reactions involved.

This paper discusses some of the variables involved in the stannous chloride thiocyanate method, and makes possible selection of optimum operating conditions, thereby improving the reliability of the method. Experiments on the nature and rate of formation of the color when the reduction and color development are carried out simultaneously indicate the limitations of this method. A modified procedure, separating the reduction and complexation steps, is considered in detail. The influence of free acid, chloride, and stannous ion concentrations on the reduction and the choice of proper conditions for color development comprise the major parts of the investigation.

The addition of stannous chloride and then potassium thiocyanate to an acid tungstate solution results in a greenish color, whereas the same additions to an initially alkaline solution yield a yellow color. Two tungstate samples treated according to the general procedure of Sandell (*4*) were identical in all respects, except that one was initially acid and the other initially alkaline. The two absorption curves, plotted in Figure 1, show a maximum

of 400 mμ, characteristic of the tungsten thiocyanate complex. The suggestion of Gentry and Sherrington (*3*) that the color of the green solution is due to the additive effect of blue quinquevalent compounds of tungsten is supported by the lower absorption of the thiocyanate complex and the somewhat greater absorption in the red region.

The slow development of the color at room temperature is another disadvantage. Two to 5 hours are required to achieve complete reduction of the tungsten and stabilization of the color in solutions containing 0.2 to 6 p.p.m. of tungsten. Raising the temperature leads to extensive decomposition of the thiocyanate into hydrogen sulfide.

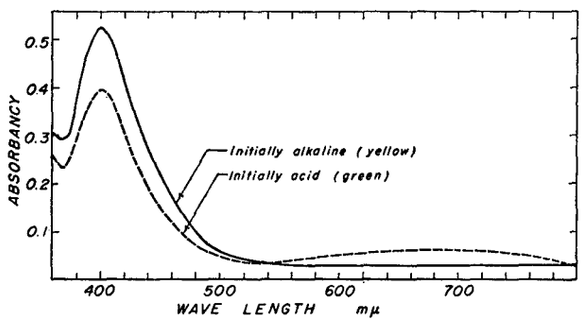

Figure 1. Absorption Curves of Tungsten Thiocyanate Complexes

The use of other reducing agents such as amalgams (*3*) and titanous chloride (*1*) has been suggested for more rapid and complete reduction. However, stannous chloride is desirable as it is colorless, readily available, and capable of yielding reliable results. Geld and Carroll (*2*), by first reducing with stannous chloride at the boiling point of the solution, cooling, and then adding the thiocyanate, achieve complete reduction in a matter of minutes. Under these drastic conditions the yellow complex is formed, even though the reduction is made in an initially acid solution. They applied the method to the determination of tungsten in high-temperature alloys.

Chloride and free acid concentrations influence the electrode potentials of the redox system and may determine the extent and rate of reduction. The next series of experiments relate the formation of the complex tungsten thiocyanate to these solution conditions, when the reduction and complexation steps are performed separately.

GENERAL EXPERIMENTAL PROCEDURE

Solutions. Sodium tungstate (0.276 gram, 72.5% tungsten) is dissolved in water containing a few tenths of a gram of sodium hydroxide and then diluted to 2 liters. A tungsten concentration of 0.10 mg. by weight per ml. is obtained.

Stannous chloride, 2 *M*, is prepared by dissolving 112.9 grams of stannous chloride dihydrate in concentrated hydrochloric acid and making up to 250 ml. with concentrated hydrochloric acid.

Potassium thiocyanate, 20%, is made by dissolving 20 grams of potassium thiocyanate in 80 ml. of water.

Magnesium chloride, 8 *M*, is made by dissolving 250 grams of magnesium chloride hexahydrate in 100 ml. of water. The solution is assayed by means of a chloride determination.

The system to be studied is placed in a 50-ml. borosilicate glass volumetric flask and adjusted to the mark, and a 5.00-ml. aliquot for analysis is transferred to a 100-ml. volumetric flask. The reaction flask is immersed in a boiling water bath for 5 minutes and cooled in running water at 13° to 15° C. for 2 minutes. The reduction mixture is poured into 10.00 ml. of potassium thiocyanate and 25.00 ml. of water in a 100-ml. volumetric flask. Water is added approximately to the mark and the samples are thermostated at 25.0° C. Extinction readings at a wave length of 400 mμ are taken after 1 hour, using a Beckman DU spectrophotometer. A blank sample containing no tungsten or magnesium chloride, but otherwise identical, is carried through the procedure and used to obtain 100% transmittance. The aliquots are analyzed for stannous ion, free acid, and total chloride.

Methods of Analysis. The 5.00-ml. aliquot is diluted to 100 ml. and suitable fractions are removed for the various analyses.

Tin Determination. The stannous ion concentration is determined in the conventional manner by titrating with standard iodine solution, which has been standardized previously against arsenious oxide.

Free Acid Determination. The method for the total free acid is based on the procedure recommended for the determination of free acid in the presence of a hydrolyzable salt. Best results are obtained by a preliminary oxidation of tin with hydrogen peroxide followed by complexation with tartrate. Five grams of disodium tartrate are dissolved in 100 ml. of water, 2 ml. of 30% hydrogen peroxide are added, and the pH of the solution is adjusted to a phenolphthalein end point with standard sodium hydroxide. An aliquot of the sample to be analyzed is added and the solution is again brought to the phenolphthalein end point, with standard sodium hydroxide.

Total Chloride Determination. Following oxidation of the stannous ion with hydrogen peroxide and neutralization of most of the free acid with sodium hydroxide, the total chloride is determined by titrating with standard silver nitrate, using dichlorofluorescein indicator. Two milliliters of 30% hydrogen peroxide and about 0.1 gram of dextrin are added to 100 ml. of water. A suitable aliquot of the sample is added together with sufficient chloride-free sodium hydroxide to neutralize 99% of the free acid. Standard 0.1 *N* silver nitrate, prepared by the direct solution of the salt in water, is used to titrate the chloride. Despite precipitation of the tin, the results on synthetic samples and further checks with the Volhard method have shown the adsorption indicator method to be accurate.

Effect of Chloride Concentration on Reduction of Tungstate with Stannous Chloride. Those factors influencing the strength of the stannous chloride as a reducing agent should exert the most profound influence on the reduction step. The significance of the ionic activities is evident from the equation for the reduction potential of the stannous-stannic system.

$$E = E^0 - \frac{RT}{nF} \ln (a_{Sn^{++}}/a_{Sn^{++++}})$$

The standard electrode potential, E^0, is equal to +0.13 volt, and a represents the activities of the ions. R, T, n, and F have their conventional meanings. The more negative the value of E, the stronger is the reducing agent. Hence, increasing the ratio of stannous to stannic ion activities will result in a more negative reduction potential.

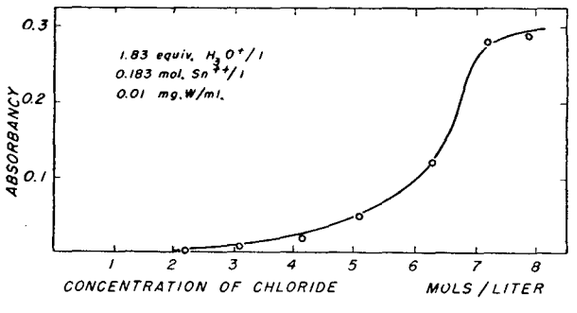

Figure 2. Effect of Chloride Concentration on Reduction of Tungsten

Bivalent and tetravalent tin in hydrochloric acid solutions are known to exist primarily as complex ions, $SnCl_4^{--}$ and $SnCl_6^{--}$, and the activities of stannous and stannic ions will depend upon the dissociation of these complexes. If the activity of the $SnCl_4^{--}$ ion is assumed to be large and relatively constant (as it would be in these experiments), and the dissociation of $SnCl_6^{--}$ is assumed to be small, an increase in the chloride activity will cause a relatively greater decrease in the stannic activity than in the stannous activity. Thus the strength of stannous chloride as a reducing agent may be increased by increasing the chloride activity.

Experimental confirmation is obtained by selecting concentrations of free acid and stannous chloride known to yield only a partial reduction of tungsten. Then the effect of increasing the chloride ion concentration is studied by adding varying amounts of strong magnesium chloride solution to a series of samples.

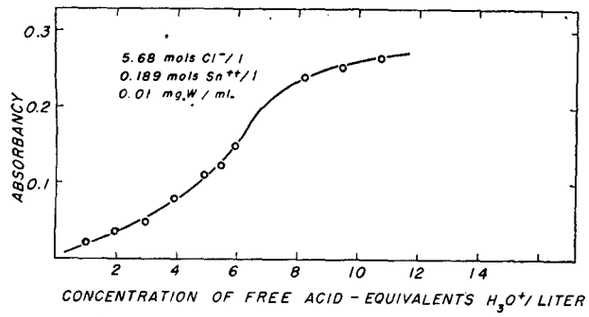

Figure 3. Effect of Free Acid on Reduction of Tungsten

The procedure consists of placing 5.00 ml. of concentrated hydrochloric acid, the desired amount of magnesium chloride solution, and 5.00 ml. of sodium tungstate solution in a 50-ml. volumetric flask. Five milliliters of stannous chloride are added, the sample is diluted to the mark with water, and a 5.00-ml. aliquot is removed for analysis. The reduction, color development, and analyses are carried out by the methods already described.

The results of these experiments, plotted in Figure 2, show the pronounced effect of the chloride concentration in increasing the reducing power of the stannous-stannic system. At the high concentration levels used, little is known about the activity coefficients except that they are considerably greater than 1. Thus the effective concentration is even greater than the analytically determined values. Unfortunately, the solubility of magnesium chloride does not permit chloride concentrations above those used, but it appears that essentially complete reduction is attained above 7 moles per liter of chloride ion.

Attempts to use stannous sulfate as the reducing agent gave results that were qualitatively similar to the above runs. Because of the low solubility of stannous sulfate, a weak reducing system resulted, even with the addition of chloride; consequently, only a small percentage increase in the extent of tungsten reduction was noted.

Effect of Free Acid on Reduction of Tungstate with Stannous Chloride. The dependence of the rate of reduction on acidity was suggested by Sandell (*5*), although as hydrochloric acid was used the combined effects of both acid and chloride were observed. Increased acidity will decrease the dissociation of both H_2SnCl_4 and H_2SnCl_6, but should not markedly influence the ratio of stannous to stannic activities.

The exact nature of the tungsten thiocyanate complex is not known. Work in progress at Oregon State College indicates that tungsten has a valence state of 5. A possible half-cell reaction might be:

$$WO_4^{--} + 8H_3O^+ + 1(e) = W^{5+} + 12H_2O$$

The corresponding electrode potential equation indicates the role of the free acid. As the acidity is increased, the electrode potential is made more positive, thus increasing the oxidizing power of the tungsten system. Consequently, its reduction with stannous chloride is facilitated.

Experimental verification is obtained in a manner analogous to the chloride experiment. Samples are prepared in which the stannous chloride and magnesium chloride concentrations are known to produce only slight reduction of the tungsten. The total free acid is then varied by means of additions of sulfuric acid. The experimental technique and methods of analysis are the same as described before. The results are plotted in Figure 3 and show the desirable effect of operating at high acidities.

Combined Effect of Chloride and Free Acid on Reduction of Tungstate with Stannous Chloride. The experiments thus far were not conducted on practical systems, owing to the presence of large amounts of magnesium chloride and the independent variation of the chloride and free acid. In actual practice, varying amounts of sulfuric acid may be present, while the addition of hydrochloric acid will simultaneously increase both the free acid and chloride concentrations. With this in mind, a family of curves is prepared using a series of fixed total chloride concentrations as parameters and varying the free acid by means of sulfuric acid additions. Only stannous chloride and hydrochloric and sulfuric acids are used to control the concentrations of chloride and free acid.

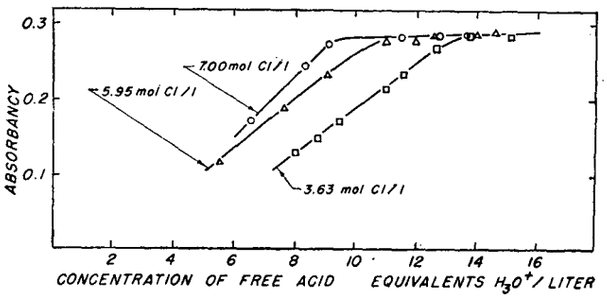

Figure 4. Combined Effects of Chloride and Free Acid on Reduction of Tungsten

The results of these runs, plotted in Figure 4, supply the essential data required for the selection of suitable reduction conditions. From the three curves the limiting free acid concentrations were found to be 9.5, 11.2, and 13.9 for curves with 7.00, 5.95, and 3.63 moles of chloride per liter, respectively. Free acid concentrations above these values produce no increase in extinction; hence, it may be assumed that complete reduction of the tungsten has taken place. The decrease in absorbancy, below the critical concentrations of chloride and free acid, shows clearly why erratic results would be obtained with the stannous chloride method of reduction, if adequate control were not maintained.

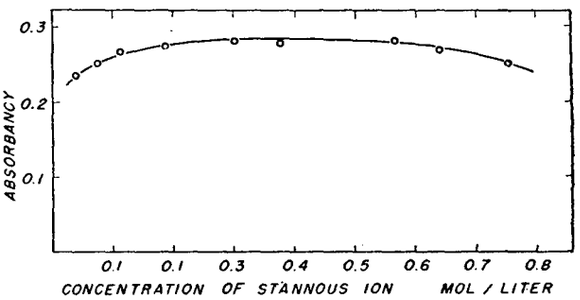

Figure 5. Effect of Stannous Chloride Concentration on Reduction of Tungsten

Effect of Stannous Chloride on Reduction. Under conditions such that stannous chloride is a sufficiently strong reducing agent, and with a preponderance of tin over tungsten, a variation in the tin concentration would not be expected to result in a significant change in the amount of tungsten reduced. This is borne out in Figure 5, which shows a wide variation in the permissible stannous chloride concentration.

Table I. Effect of Time on Color Development

(0.45 mg. of tungsten reduced with 10 ml. of concentrated H_2SO_4, 20 ml. of concentrated HCl, 5 ml. of $SnCl_2$ per 50 ml.; color developed with 10 ml. of 2 *M* KCNS per 100 ml.)

Null Cell	Sample Cell	Absorbancy after 0.5 hour	1 hour	2 hours	3 hours	4 hours
Water	Blank	0.002	0.005	0.009	0.014	0.018
Water	Tungsten	0.278	0.282	0.287	0.293	0.299
Blank	Tungsten	0.277	0.278	0.279	0.279	0.280

Table II. Effect of Potassium Thiocyanate Concentration on Color Development

(0.45 mg. of tungsten reduced as in Table I)

Volume of 2 *M* KCNS, Ml.	Absorbancy after 0.5 hour	1 hour
3.00	0.255	0.247
5.00	0.272	0.268
8.00	0.272	0.268
10.00	0.277	0.277
20.00	0.281	0.280

Color Development with Potassium Thiocyanate. Some difficulty is encountered in obtaining a stable color system. When small amounts of potassium thiocyanate are used the color fades on standing; with increasing amounts the color intensifies. Presumably the fading is due to reoxidation of the tungsten and occurs when insufficient thiocyanate is available to repress the dissociation of the complex. The intensification of the color is due to the formation of colored decomposition products of the thiocyanate. The data in Table I confirm the stability of the tungsten complex and show clearly the role of the reagent blank in increasing the absorbancy of the system. Table II emphasizes the need for an adequate concentration of thiocyanate both to develop the color fully and to prevent fading.

In the previous work the samples were thermostated at 25.0° C. to minimize any error due to temperature variation. This is an undesirable requirement for an analytical method, and several runs at 12.4°, 25.0°, and 34.6° C. were compared to ascertain the necessity for maintaining a constant temperature. No significant difference in extinction or stability could be detected over a period of 4 hours. A large blank due to a greater rate of decomposition of the thiocyanate at high temperatures makes it desirable to maintain the temperature around 25° C. or less.

RECOMMENDED PROCEDURE FOR REDUCTION AND COMPLEXATION OF TUNGSTEN

The sample containing from 0.1 to 1.5 mg. of tungsten is placed in a 100-ml. borosilicate volumetric flask, water is added to adjust the volume to 15 ml. and 10 ml. of concentrated sulfuric acid are added, mixed, and cooled. This is followed by 20 ml. of concentrated hydrochloric acid and 5 ml. of 2 *M* stannous chloride. The solution is then placed in a boiling water bath for 5 minutes. After cooling for 3 minutes in running cold water (10° to 15° C.), 10 ml. of 2 *M* potassium thiocyanate are added, and the volume is adjusted to the mark with distilled water. The extinction is determined after 15 minutes, using a wave length of 400 mμ. A reagent blank carried through the same procedure may be used in the null position. A standardization curve prepared according to this procedure shows strict adherence to Beer's law. The absorbancy index, *a*, in the equation

$$A = \log (I_0/I) = a \times c \times l$$

is 62.5 when the concentration is expressed in milligrams per milliliter and the length of the optical path in centimeters.

ACKNOWLEDGMENT

Special acknowledgment is made to E. C. Gilbert for critical review of the manuscript.

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Instrument for Internal Standard Flame Photometry

Application to Determination of Calcium in Rare Earths

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IN THE application of direct photoelectric methods to the measurement of intensities of flame spectra, a new analytical method known as flame photometry has been developed (*2*). Flame photometric techniques using a monochromator in place of glass and interference filters for spectral isolation have become the preferred method because of the reduction of spectral interference and over-all background radiation. The practice with several of the flame photometers of the monochromator type commercially available (*1, 9*) is to measure the spectral line intensity of an element of interest and to compare this with measurements made on standard samples. This technique of measurement does not take advantage of the increased precision and greater freedom from extraneous influences afforded by application of the well-known internal standard principle (*8*). A ratio flame photometer using this principle and its commercial counterpart have been described (*3, 15*), but this instrument is restricted to a single element, lithium, as an internal standard.

With a few relatively simple modifications of a laboratory monochromator, it is possible to have an instrument offering the advantages of internal standardization, and in addition, greater flexibility in choosing internal standard lines best suited for various analyses. Furthermore, the need for the more elaborate amplification circuits of the commercial flame photometers is eliminated by the use of multiplier phototubes for measurement of spectral line–intensity ratios. The fact that line-intensity ratios in flame excitation under controlled conditions do not undergo the continuous changes characteristic of electrical excitation allows the use of simple electrical circuits with suitable band pass to eliminate small, short-period fluctuations. Although this instrument is constructed around a commonly available monochromator, the design can readily be extended to any spectrometer or spectrograph.

INTERNAL STANDARD FLAME PHOTOMETER

Monochromator. The schematic diagrams in Figures 1 and 2 show the instrument as built around a Gaertner constant-deviation monochromator. The burner was positioned to place the

Microquantities of many elements not readily determined by usual methods of analytical chemistry can be conveniently measured by flame photometric methods. Intensity comparison of the unknown with a series of standards is a common technique in flame photometry. This procedure does not take advantage of the usually greater precision and freedom from extraneous influences afforded by use of the internal standard principle. Although flame photometers utilizing this principle have been described, these instruments have been restricted to lithium as an internal standard. In this paper details are given for simple modifications of a laboratory monochromator to provide both internal standardization and greater flexibility in choosing line pairs. Need for auxiliary amplification is eliminated by using multiplier phototubes in a differential photometer circuit. A minimum sample is consumed through the use of an atomizing system with provisions for recycling and sa mple recovery. In the determination of calcium in the rare earths in a concentration range from 0.025 to 2.5%, the average deviation was about ±1.5%. The basic design is applicable to most spectrometers or spectrographs. The method should be useful not only for calcium determinations in other matrices but with proper modifications of experimental setup, for determination of other elements susceptible to flame excitation.

tip of the blue cone of the flame about 15 mm. below the optical axis of the spectrometer. Between the burner and the entrance slit of the monochromator, but not shown in the diagram, is a sliding cover serving a dual purpose as a shutter and as a protection for the slit jaws from soot from the flame when igniting the acetylene.

In place of the eyepiece or exit slit mechanism normally located at or near the focal plane of the spectrometer, a brass-faceplate adapter for mounting the exit slits, reflecting mirrors, and photomultiplier tube–housing assembly were fitted into the telescope tube. Provisions were also made for rotating the entire assembly to obtain parallel orientation of the slits with the spectral lines.

Flame excitation gives rise to only low temperature lines typical of atoms or molecules in the lowest state of excitation. Consequently, the spectra are very simple. This factor permits a great simplification in the design of the exit slits for measuring line pairs for a variety of analyses. The exit slits were made by first photographing the spectrum of the lines under consideration (calcium, 4227 A., and manganese, 4031-3-4 A. in the example described later) on 2 × 2 inch squares of Eastman Spectrum Analysis No. 1 plates which were placed on the telescope tube adapter. These were exposed for 30 seconds with an entrance slit width of 0.1 mm. A positive image was then printed on a square cut from an Eastman 548-G plate. These plates were mounted on the brass faceplate as previously indicated.

In attempting to print a positive which had sufficient blackening to exclude all light adjacent to the spectral line, the lines themselves were exposed slightly with a consequent loss in transmission in the developed image. Therefore the positive print exposures were made to obtain images with as much blackening as possible without definition or transmission losses of the lines; the adjacent portions of the plate were then painted to eliminate the remainder of the over-all radiation coming through. For the calcium and manganese lines the linear separation was approximately 3.6 mm. The entire exit slit length of 14.7 mm. was used.

After passage through their respective exit slits the spectral lines were reflected by first-surface mirrors (supplied by Evaporated Metal Films Corp., Ithaca, N. Y.) to the photocathodes of the high sensitivity 1P21 multiplier phototubes. Inasmuch as exit slits of this type can be readily made for other spectrometers and spectrographs that may be available, the design is readily adaptable to these instruments.

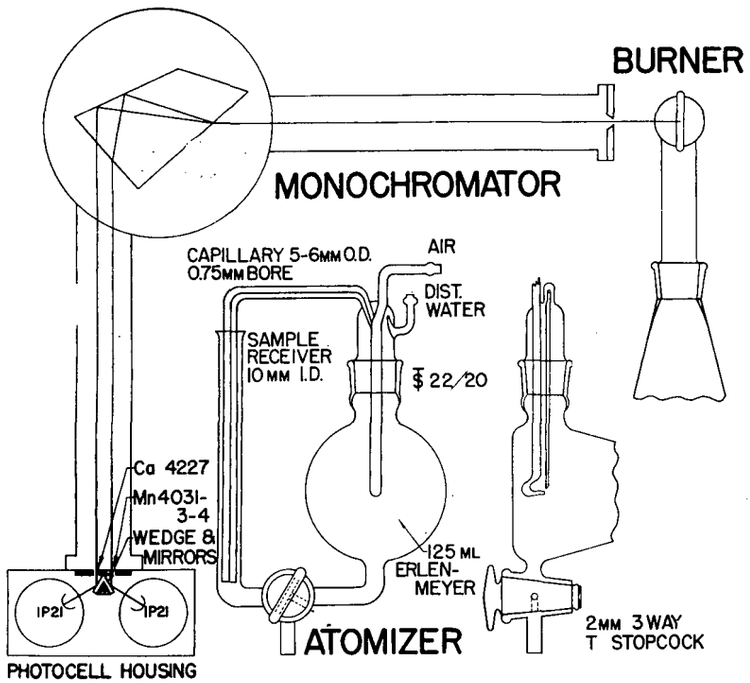

Figure 1. Monochromator Adaptations and Recycling Atomizer Assembly

Atomizer and Burner. The air-acetylene flame-excitation equipment used follows, in principle, Lundegårdh's (*12*) design, details of which have been reported in the literature (*5*, *13*). A few of the modifications which appeared desirable are indicated in the following discussion.

The burner and tip were fabricated of stainless steel. Although the acetylene pressure could be adjusted to the same gage reading, there were instances in which there was a change in the quantity of acetylene flowing to the burner owing to a clogging of the orifice. In addition to the usual manometer for measuring acetylene pressure, a capillary flow-rate meter was inserted in the line from the acetylene tank to the burner. An appreciable change in the gage pressure for the same flow rate was thus indicative of a partially clogged acetylene orifice.

The all-glass atomizer and solution recirculation system are shown in the lower right-hand portion of Figure 1. One advantage of such a system is that the burner can be kept in continuous operation without the need for turning off the burner, introducing a new sample into the chamber, and relighting the burner, as is done in the usual Lundegårdh technique. It is also pos-

sible to recycle the solution for readings requiring several minutes or more to complete. Most of the sample can also be recovered with this system. Considerably greater air pressure is required to start the atomizing process when the receiver tube is only partially filled. In manipulating the equipment, the three-way stopcock is turned so the receiver surrounding the capillary can first be completely filled with sample. The solution is then easily drawn up into the capillary tube, and when the flow has been started, the stopcock is turned to allow the solution to recycle. After completion of a determination, the sample is drained out of the chamber. The stopcock is then turned so that the receiver tube can be filled with distilled water while at the same time the chamber is being flushed with a fine spray of distilled water from an external line. The washing and draining of the atomizing chamber are repeated three or four times, after which the equipment is ready for another determination.

The performance and operation of any given setup of flame excitation equipment is unique in that it is dependent on the particular dimensions of the orifices and other geometry of the apparatus. On the basis of the behavior of the flame at various air-acetylene combinations, an air pressure of 75 cm. of mercury, a capillary flow-rate meter reading of 17.5 cm. of water, and an acetylene gage pressure of 4 cm. of water gave the most reproducible bridge readings or intensity ratios.

Photomultiplier Detector and Bridge Circuits. Multiplier phototubes are now an accepted measurement device for arc and spark emission analyses (*4, 10, 16*) but their use in flame photometry has been limited (*11, 14, 17*). While this manuscript was in preparation, a commercial flame photometer using photomultiplier tubes was announced (*1*). The slow acceptance of multiplier phototubes has apparently been caused by several inherent limitations of these tubes—namely, fatigue, and large changes in sensitivity with voltage applied to the dynodes (*7*). These limitations are readily overcome by use of a bridge circuit and proper experimental conditions. Compared to conventional phototubes, photomultipliers afford high enough sensitivity to eliminate the

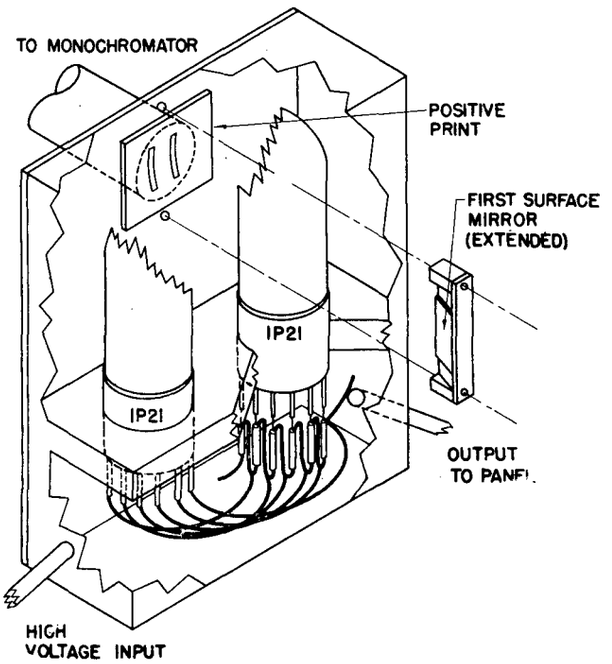

Figure 2. Cutaway View of Slits and Reflecting Mirrors in Photocell Housing

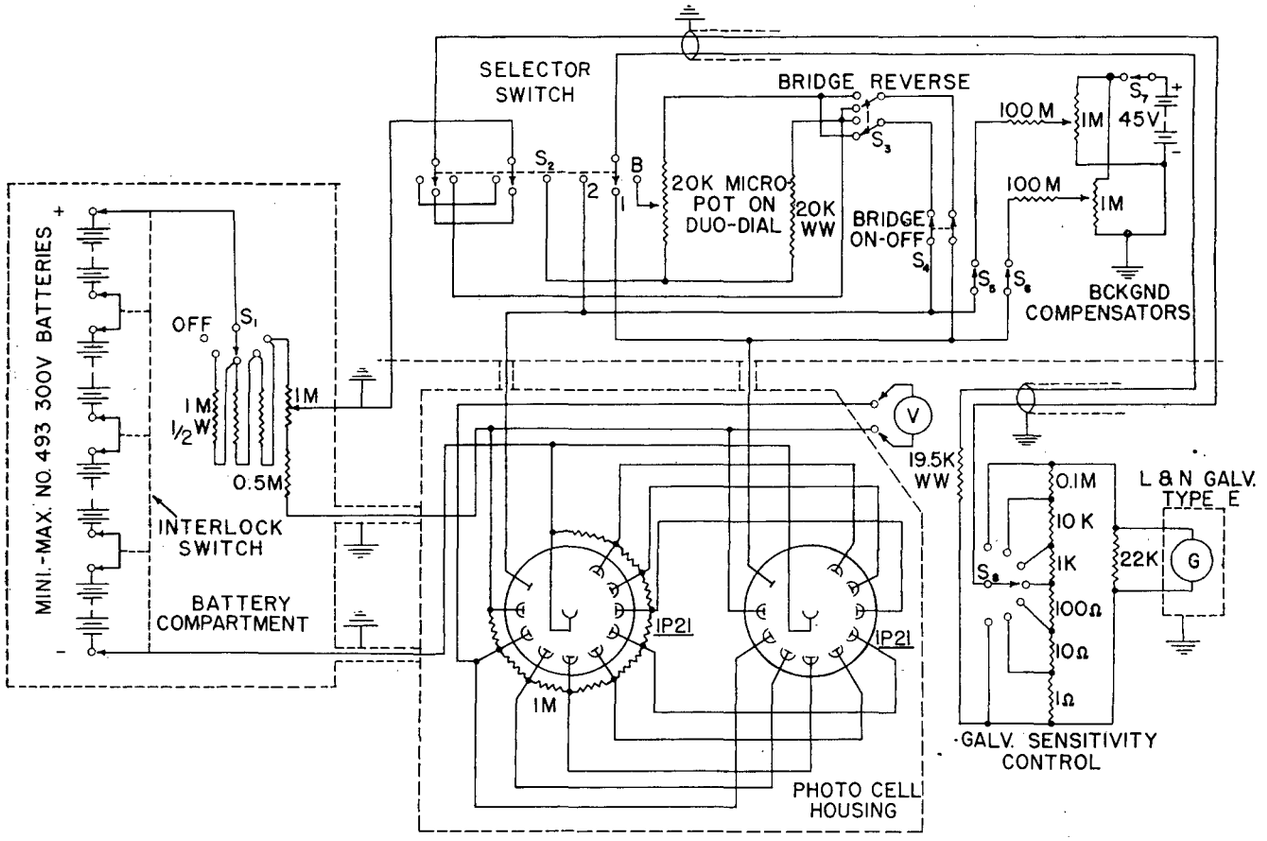

Figure 3. Circuit Diagram of Internal Standard Flame Photometer

need for additional amplification. Thus a photometer can be obtained with a simple circuit involving no more than a battery source, photomultipliers, resistances, and a sensitive null-reading device.

The circuit diagram for the photometer is shown in Figure 3. A dynode potential of approximately 100 volts per stage was obtained by setting S_1 to proper voltage as measured with an external voltmeter connected to pinjacks wired from the 8th and 9th dynodes. A Leeds and Northrup Type E galvanometer, Catalog No. 2430-D, having a maximum sensitivity of 4×10^{-10} ampere, and a period of 3.2 seconds was used both as the null-indicating device and, with the shunt control, as a current-measuring device. Effects of changes in sensitivity owing to fatigue could be avoided, ideally, by having two phototubes perfectly matched with respect to this characteristic, but this is hardly practicable inasmuch as a large number of tubes are generally not available for matching purposes. Fatigue is dependent on the amount of current drawn (*6*). Therefore, to minimize this effect, it is advisable to adjust concentrations in order that currents of 10^{-6} ampere are never exceeded. In cases where tubes having the same fatigue rate are not available, it would be possible further to compensate for differences by subjecting the tube having the lower fatigue rate to a greater current flow—for example, as could be done by increasing the concentration of the manganese standard. It would also be feasible to substitute a less expensive 931-A tube for the 1P21 used to measure the manganese photocurrent since sensitivity limitations do not enter in for the internal standard element. Changes in phototube sensitivity resulting from voltage changes have been adequately compensated by use of two tubes in the bridge arrangement as shown. Restandardization can be done simply by running a reference sample of known concentration at regular intervals as is done in the case of direct photoelectric measurements with arc or spark emission sources.

A series of calcium solutions of known concentration was run to determine the lower limit of detectability with the particular excitation source and photomeasuring device. Concentrations as low as 1.0 p.p.m. calcium in solution were measurable although the magnitude of the random variations made readings somewhat unreliable. The random variation of the dark current of the particular 1P21 photomultiplier used was less than 1 scale division at maximum galvanometer sensitivity whereas that of the flame background was 3 to 4 times this deviation. Thus the limiting factor in this instance could be attributed to the latter cause. Much of the unsteadiness in the flame was due to air currents. Under internal standard conditions these fluctuations are smoothed out by the bridge circuit. The upper limit of the concentration range is governed by spectral excitation effects and phototube considerations. The 4227 A. line of calcium is susceptible to reversal and self-absorption with increasing concentration. For a working concentration range of 5 to 500 p.p.m. calcium in solution, currents from 10^{-9} ampere to a maximum of the order of 10^{-7} ampere were drawn through the phototubes.

Bridge Manipulations. If a true difference between the IR (current times resistance) drop from the current of the test element and internal standard radiations is to be obtained on the bridge, the voltage drop resulting from the residual current from flame background, general spectral radiation, and random thermionic emission of the tubes must be compensated for by putting a counter electromotive force across each arm of the bridge as follows:

A solution of the matrix, free of the element to be determined, is introduced into the atomizing system. Selector switch S_2 (Figure 3) is set to position 1 with the bridge on-off switch, S_4, in the off position. Then the appropriate compensator dial is adjusted to give a zero current reading on the galvanometer at maximum sensitivity. This same procedure is repeated when S_2 is in position 2, after which the selector switch is set to the B position and S_4 is turned to the on position. If a null reading is obtained on the galvanometer irrespective of the position of the helical $20K$ precision-slide wire resistance (Micropot manufactured by Gibbs Division, Borg Corp., Delevan, Wis.), the IR drop across each arm of the bridge is zero and the compensators have been properly adjusted.

The Micropot was wired so that readings on the Duodial scale (made by Beckman Instruments, Inc., South Pasadena, Calif.) go from 1000 to 0 for the condition $I_{PA} > I_{PB}$ to $I_{PA} = I_{PB}$, where I_{PA} and I_{PB} are, respectively, the net photocurrents on the multipliers picking up radiations from elements A and B. Thus, for this condition the bridge reversing switch must be in such a position that the photocurrent of A goes through the Micropot arm of the bridge if a null reading is to be obtained. When $I_{PB} > I_{PA}$, S_3 must be reversed and dial readings go from 0 for $I_{PB} = I_{PA}$, toward 1000 as I_{PB} becomes increasingly greater than I_{PA}.

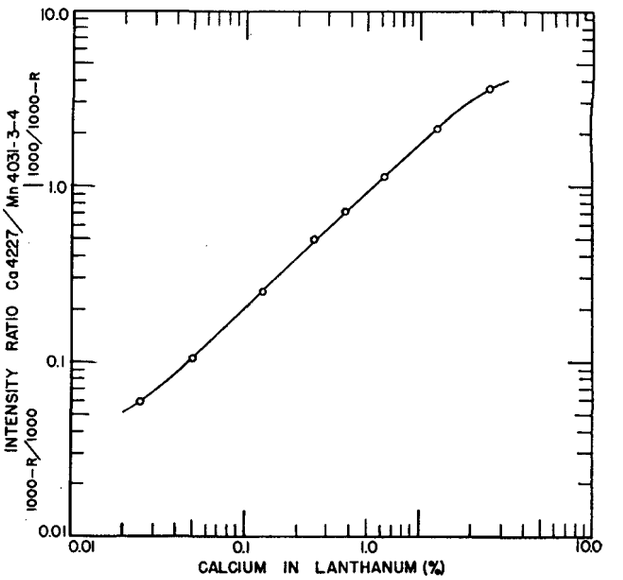

Figure 4. Calibration Curve for Determination of Calcium in Lanthanum

Calculations. Bridge readings with the arrangement of the circuit and Duodial as described do not give photocurrent ratios directly. To obtain a relationship between these readings and photocurrent ratios, the factors $(1000-R)/(1000)$ for the case where $I_{PA} > I_{PB}$, and $(1000)/(1000-R)$ for $I_{PB} > I_{PA}$ were applied. Inasmuch as photomultipliers respond linearly to changes in light, intensity ratios are equivalent to current ratios. Following the usual practice in spectrographic analysis, the log of intensity ratio as represented by $(1000-R)/(1000)$ or $(1000)/(1000-R)$ can then be plotted as a function of the log of the concentration.

DETERMINATION OF CALCIUM IN RARE EARTHS

Preparation of Standards. Solutions of the rare earths were prepared by dissolving the oxides equivalent to 0.500 gram of the metal in a minimum of calcium-free hydrochloric acid, evaporating to crystals of the chloride, and redissolving the crystals in a few milliliters of redistilled water. Standard solutions of calcium were then added to provide calcium to rare earth percentages ranging from 0.025 to 2.5. Constant amounts of a standard solution of manganese were added so that upon dilution of the combined rare earth, calcium, and manganese solutions to 25 ml., the manganese concentration was 0.03%. This provided a 2% solution of the matrix rare earth. The standard calcium solution (2 mg. per ml.) was made by dissolving double-distilled calcium metal in dilute calcium-free hydrochloric acid. The standard manganese solution (1 mg. per ml.) was similarly prepared from electrolytic metal. The calcium-free hydrochloric acid was pre-

Table I. Reproducibility Data

Concn. Calcium in Lanthanum, %	Bridge Readings[a] 3/28/50	3/30/50	4/5/50	Mean
0.025	0.066	0.054	0.060	0.060
0.050	0.106	0.102	0.109	0.104
0.125	0.259	0.260	0.256	0.258
0.250	0.495	0.500	0.504	0.500
0.375	0.727	0.708	0.734	0.723
0.625	1.16	1.14	1.15	1.15
1.25	2.18	2.17	2.17	2.17
2.50	3.73	3.68	3.67	3.69

[a] From the factor $(1000-R)/(1000)$ or $(1000)/(1000-R)$.

pared by passing hydrogen chloride gas through double-distilled water in a quartz container.

RESULTS

The calibration curve for the determination of calcium in lanthanum is shown in Figure 4. Similar calibration curves for determining calcium in samarium, neodymium, cerium, and praseodymium showed slight but significant shifts in the intercepts as compared to lanthanum. By measuring this difference, a single curve could be used for measuring calcium in the rare earths mentioned.

Although no extensive studies have been made on the precision of measurements, preliminary results indicate a mean deviation of about ±1.5%. Much of this variation may be attributed to results obtained at the lowest concentration. The results in Table I summarize calibration data obtained on three different runs.

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Determination of Molybenum in Soils and Rocks

A Geochemical Semimicro Field Method

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Reconnaissance work in geochemical prospecting requires a simple, rapid, and moderately accurate method for the determination of small amounts of molybdenum in soils and rocks. The useful range of the suggested procedure is from 1 to 32 p.p.m. of molybdenum, but the upper limit can be extended. Duplicate determinations on eight soil samples containing less than 10 p.p.m. of molybdenum agree within 1 p.p.m., and a comparison of field results with those obtained by a conventional laboratory procedure shows that the method is sufficiently accurate for use in geochemical prospecting. The time required for analysis and the quantities of reagents needed have been decreased to provide essentially a "test tube" method for the determination of molybdenum in soils and rocks. With a minimum amount of skill, one analyst can make 30 molybdenum determinations in an 8-hour day.

A SIMPLE, rapid quantitative method for determining small amounts of molybdenum in soils and rocks was needed in connection with the field work of the Geochemical Prospecting Group of the U. S. Geological Survey.

Geochemical prospecting is comparatively new in the United States. Briefly, one may expect that a soil developed over an ore body will contain more of the elements of the ore than one developed elsewhere. The presence of large amounts of zinc, copper, cobalt, nickel, tin, tungsten, and other elements in soil samples may indicate the presence of mineralization below the surface. The suggestion that the molybdenum content of soils be used as a guide in this practical problem led to the development of the following procedure.

A suitable method for the determination of molybdenum must take into account the ranges in which the element occurs in soils and rocks.

Early reports from the Jealott's Hill Research Station (*7*) gave values of 10 to 100 parts per million (p.p.m.), and the vegetation growing on the sampled soils after heavy liming contained enough molybdenum to be toxic to grazing cattle. Later, Perrin (*16*) analyzed 8 soils in New Zealand, where symptoms due to molybdenum deficiency were prevalent, and found quantities ranging from 0.28 to 1.28 p.p.m. Bertrand (*4*) found a range of 4.3 to 69 p.p.m. in 20 soils in France. The amounts of molybdenum in 275 soils from various parts of the United States ranged from 0.6 to 31.6 p.p.m., with 85% of those analyzed falling in the range of 1 to 4 p.p.m. (*19*). In California, 10 of 20 soils analyzed by Barshad (*3*) contained less than 1 p.p.m. of molybdenum in the

surface 12 inches, and 7 of the remaining 10 samples contained from 1 to 4 p.p.m. in the surface layer.

The data in the literature on molybdenum in rocks are extensive. Shortly before 1900, Hillebrand (*11*) found traces of molybdenum in diorites from California and rhyolites from Utah. He concluded that the element tends to concentrate in the more siliceous rocks. Later, Ferguson (*6*) analyzed a basalt from Hawaii and found 0.01% molybdenum trioxide. The presence of a detectable amount of molybdenum in a less siliceous rock was surprising. The highest molybdenum content in igneous rocks in the United States reported by Sandell and Goldich (*22*) was 7 p.p.m., but a magnetite-ilmenite sand derived from basic rocks in Minnesota contained 13 p.p.m. More recently, Földvari (*8*) found from 1 to 80 p.p.m. in rocks related to andesitic eruptives and their postmagmatic derivatives. He also found high amounts of molybdenum in iron-bearing quartzites, but neither he nor Rankama (*17*) found detectable quantities in granitic rocks. However, a composite of 67 gabbros and norites from central Europe contained 3 p.p.m., and a composite of 282 volcanic rocks from the same area contained 15 p.p.m. (*10*). Landergren (*14*) in a study of the geochemistry of Swedish iron ores found three different ores in which the molybdenum contents were 30 to 80 p.p.m., 30 to 200 p.p.m., and 30 to 500 p.p.m.

The available data indicate that bituminous schists as well as some shales contain considerably more molybdenum than the rocks mentioned above. Brockamp (*5*) reported molybdenum contents as high as 230 p.p.m. in bituminous schists; Schneiderhöhn *et al.* (*23*) reported values as high as 1500 p.p.m. in similar materials. In a study of Swedish oil shales, Assarsson (*1*) found values ranging from 80 to 300 p.p.m.; Westergard (*24*) reported contents of 110 to 160 p.p.m. of molybdenum in alum shales.

Small amounts of molybdenum can be determined with potassium ethyl xanthate, phenylhydrazine, sodium thiosulfate, and thiocyanate in the presence of stannous chloride as a reducing agent. The thiocyanate is the most sensitive, and, at the same time, can be made almost specific. As little as 0.01 microgram of molybdenum per ml. is easily detectable. In two separate reviews of colorimetric methods for the estimation of molybdenum in soils and plants (*12*, *15*) the thiocyanate method is preferred.

REAGENTS AND APPARATUS

Flux, a mixture of equal parts of sodium carbonate and potassium nitrate, ground to pass an 80-mesh silk bolting-cloth sieve, thoroughly mixed, and passed through the sieve again.

Sodium tartrate, reagent quality.

Potassium thiocyanate, 5 grams of potassium thiocyanate dissolved in 95 grams of water.

Stannous chloride, 10 grams of $SnCl_2.2H_2O$ dissolved in 100 ml. of 2 *M* hydrochloric acid. The addition of tin promotes the stability, but fresh solutions should be prepared at weekly intervals.

Standard molybdenum solution, 0.01% molybdenum, solution A. Dissolve 0.075 gram of pure molybdenum trioxide in dilute sodium hydroxide, dilute with water, add hydrochloric acid until solution is just acid, and make up to 500 ml. with water. This solution contains 100 micrograms of molybdenum per ml.

Standard molybdenum solution, 0.0001% molybdenum, solution B. Prepared daily at least 1 hour before use by diluting 1 ml. of the 0.01% solution with water to 100 ml.

Hydrochloric acid, concentrated.

Hydrochloric acid, 1 *M*.

Potassium nitrate, 10 grams of potassium nitrate dissolved in 90 grams of water.

Isopropyl ether. Practical grade is suitable, if it is absolutely free from peroxides. On the day during which it is to be used, saturate the ether with a mixture of equal amounts of stannous chloride and potassium thiocyanate solutions.

Phenolphthalein indicator, 1% in alcohol.

Lucite spoon, a lucite bar with cavity of 0.25 ml. drilled near end.

Agate or mullite mortar and pestle, outside diameter of mortar, 75 mm.

One sieve, 80 mesh. The sieve consists of a piece of silk bolting cloth in an aluminum holder having an outside diameter of 100 mm. and an aluminum receiver.

One small camel's-hair brush.

One small spatula (3 mm. in diameter), made by hammering one end of a Nichrome wire.

One 100-ml. borosilicate glass volumetric flask with stopper.

Twenty borosilicate glass culture tubes, 16 × 150 mm., marked at 5 ml.

Borosilicate glass culture tubes, 16 × 150 mm., unmarked. Discard after each test.

Two 1-ml. pipets calibrated in hundredths of a ml.

One 2-ml. pipet calibrated in tenths of a ml.

One 5-ml. pipet calibrated in tenths of a ml.

One 10-ml. pipet.

One test tube rack holding at least 20 tubes.

Balance, torsion, with sensitivity of 0.002 gram.

Filters, made by fusing a disk of sintered glass in the end of a glass tube 7.5 mm. in inside diameter.

One portable gasoline stove. A Coleman pocket stove used by Lakin *et al.* (*13*) was found to be satisfactory.

Water, purified by passing tap water through one of the several types of demineralizers now commercially available. The Bantam Demineralizer manufactured by Barnstead Still and Sterilizer Co. was used in this work.

Cork stoppers, for the culture tubes.

PROCEDURE

In the mullite mortar 0.1 gram of the finely ground sample is mixed intimately with 0.5 gram of the flux. The mixture is transferred to an unmarked culture tube (16 × 150 mm.) and tapped gently to dislodge the sample from the side of the tube. The tube is then heated and rotated over the Coleman stove to effect fusion. Usually 4 to 5 minutes is required; by the time all the sample has been attacked the tube is filled with brown fumes from the nitrate decomposition. With a suitable rack, 3 or 4 samples can be fused simultaneously. After the fusion is completed, the tubes are removed from the flame, placed in a rack, and allowed to cool. A white or light gray mass indicates that practically all of the organic matter has been destroyed. While the tubes are cooling, water is poured into the top of the metal carrier of the Coleman stove until it is about half full. This is then placed on the stove and the water brought to a boil. Four milliliters of purified water are pipetted into each tube and the tube is placed in the boiling water for 3 to 5 minutes. The tubes are then removed from the water bath and placed in a rack. A filter is inserted into each tube and an ear syringe placed in the top of the filter in order to produce a vacuum in the filter tube. In this manner a rapid filtration under field conditions can be made. After the analysis is completed, the tube used for the fusion is destroyed, as the action of the flux on the tube makes further use impracticable. The filter tube is washed and retained for future use.

To make the estimation, a suitable aliquot of the filtrate should be transferred to a marked culture tube (16 × 150 mm.). For exploratory work, a 1-ml. aliquot, containing 0.025 gram of sample, is convenient. One drop of 1% phenolphthalein indicator and 1 *M* hydrochloric acid is added drop by drop until the red color of the solution disappears. An excess should be avoided. One large spoonful (0.2 gram) of sodium tartrate is dissolved in the colorless solution and water is added to bring the volume up to the mark (5 ml.). Then 0.5 ml. of concentrated hydrochloric acid is added and the solution shaken so as to liberate carbon dioxide. Add 0.3 ml. of 5% potassium thiocyanate and 0.5 ml. of stannous chloride reagent, shaking after each addition. The solution is allowed to stand for 0.5 to 1 minute, then 0.3 ml. of isopropyl ether is added and the solution is shaken thoroughly. A cork is placed in each tube and within about 10 minutes the amber-colored organic layer can be viewed against a white background and compared with standard solutions. For material containing from 1 to 32 p.p.m. of molybdenum, the quantity of the element in the lowest standard solution should be 0.03 microgram, and the quantity in the highest standard solution should be 0.8 microgram.

The standard solutions are prepared by: pipetting the appropriate volume of the standard molybdenum solution B into a culture tube (16 × 150 mm.), then adding 0.5 ml. of potassium nitrate solution and 1 spoonful of sodium tartrate. Approximately 2 ml. of water are added and shaken to effect a clear solution. The volume is made up to 5 ml. with water and the adjustment of the acidity, the addition of the thiocyanate and stannous chloride reagents, and, finally, the extraction with isopropyl ether are the same as mentioned above.

For an aliquot containing 0.025 gram, the number of micrograms of molybdenum is multiplied by 40 to convert the results to parts per million.

DISCUSSION

The thiocyanate–stannous chloride method with an extraction by isopropyl ether is practically free from interferences. The

Table I. Precision of Field Determinations of Molybdenum in Soils

Sample No.	Source of Soil Sample	Laboratory Detn. of Mo, P.P.M.	Field Method, P.P.M. of Mo	
			Detn. 1	Detn. 2
2	Winthrop, Iowa	1	1	1
3	Southeast Utah	1	1	2
6	Carrizo Mts., Ariz.	1	2	2
7	Southeast Utah	2	2	2
9	Carrizo Mts., Ariz.	2	3	3
10	Carrizo Mts., Ariz.	2	2	3
11	Carrizo Mts., Ariz.	2	1	2
16	San Manuel, Ariz.	3	2	2
24	Raleigh, N. C.	12	10	10
31	San Manuel, Ariz.	20	24	16
34	San Manuel, Ariz.	28	16	24
39	San Manuel, Ariz.	43	48	36
42	San Manuel, Ariz.	62	68	72
45	San Manuel, Ariz.	116	104	130

elements possibly occurring in soils and rocks—iron, aluminum, titanium, manganese, phosphorus, chromium, vanadium, tantalum, and fluorine—do not interfere to any appreciable extent (*21*). The addition of tartrate or citrate prevents a reaction between tungsten and thiocyanate; otherwise, the complex formed would be extracted and would interfere by altering the color of the ether solution. Moderate amounts of fluoride and phosphate are without effect on the determination. Similarly, small amounts of gold, selenium, and tellurium do not interfere. Although the white, insoluble cuprous thiocyanate is formed with large amounts of copper, thiocyanate is always present in excess and, apparently, the reaction does not prevent a satisfactory determination of molybdenum. According to Goldschmidt (*9*), molybdenum is 10,000 times as abundant as rhenium in the earth's crust; therefore, as an interference, rhenium may be ignored in the suggested method.

To test the reproducibility of the proposed procedure, duplicate determinations were made at random on 14 soil samples. The order of agreement is shown by the data in Table I.

The differences are not outside the allowable range for a satisfactory field test.

The suggested procedure without any changes is applicable to samples containing from 1 to 32 p.p.m. of molybdenum. The upper limit results from the small volume of isopropyl ether used to extract the thiocyanate complex. To determine the maximum amount of molybdenum extractable in the field procedure, solutions containing increasing quantities of molybdenum were extracted with 0.3-ml. portions of the ether under the conditions of the test. Differences in the intensity of the amber color of the organic layer could be seen in samples containing 0.1 to 0.8 microgram, which corresponds to 4 to 32 p.p.m. molybdenum in the samples as taken for the field test. Solutions containing 0.9 to 2.0 micrograms of molybdenum gave colors whose intensity could not be distinguished from that given by 0.8 microgram of molybdenum. By an increase in the volume of extractant to 0.6 ml., the tubes containing 0.8 and 0.9 microgram, respectively, of molybdenums are distinguishable. Obviously, this makes possible an extension of the upper limits of the field method. One method calls for doubling or trebling the volume of ether, adding more molybdenum to the standard solution, and shaking until the color of the extracted complex matches that of the samples. However, all of the values in this paper greater than 32 p.p.m. were determined by the following procedure:

The volume of the amber-colored ether layer over the sample was increased by adding more of the organic extractant in 0.3-ml. portions and shaking thoroughly after each addition until the hue is approximately that of the median standard previously prepared. An exact comparison was made by transferring a 0.3-ml. aliquot of the large ether layer over the samples to a clean tube containing a volume of water equal to that of the aqueous phase in the sample tube.

This procedure does not involve any changes in the original standards, and is, therefore, more suitable for routine determinations.

As a test of the accuracy of the field method, comparisons were made between the values obtained for molybdenum by the method here proposed and the laboratory method of Sandell (*20*), as modified by Robinson (*18*) and Barshad (*2*). Barshad observed that the nitrate ion enhances the color of the thiocyanate complex. The results on 45 soil and rock samples are shown in Table II, in order of increasing molybdenum content.

The agreement between the two methods is good. In fact, the data indicate that when the highest degree of accuracy is less important than a rapid accumulation of quantitative data, the field method is a valuable supplement in the laboratory as well as in the field.

The smaller sample requires correspondingly smaller amounts of chemicals. Culture tubes are used in both the sample preparation and in the estimation. With the exception of the filter tubes, which are used repeatedly, the apparatus is simple, in expensive, and readily available. Furthermore, all the equipment can be assembled in a small case. Thus, satisfactory molybdenum determinations can be made in temporary quarters under field conditions, and one analyst, with a little experience, can make 30 or more determinations in the field during a single working day.

Table II. Determination of Molybdenum in Soils and Rocks by Laboratory and Field Methods

Sample No.	Source of Sample[a]	Laboratory Detn. of No. P.P.M.	Mo Found by Field Method, P.P.M.
1	Raleigh, N. C.	1	1
2	Winthrop, Iowa	1	1
3	Southeast Utah	1	2
4	Southeast Utah	1	2
5	Carrizo Mts., Ariz.	1	4
6	Carrizo Mts., Ariz.	1	2
7	Southeast Utah	2	2
8	Southeast Utah	2	3
9	Carrizo Mts., Ariz.	2	3
10	Carrizo Mts., Ariz.	2	3
11	Carrizo Mts., Ariz.	2	2
12	San Manuel, Ariz.	2	6
13	San Manuel, Ariz.	2	6
14	San Manuel, Ariz.	2	3
15	San Manuel, Ariz.	3	6
16	San Manuel, Ariz.	3	6
17	San Manuel, Ariz.	3	3
18	San Manuel, Ariz.	3	3
19	Utah	4	3
20	San Manuel, Ariz.	4	5
21	San Manuel, Ariz.	5	10
22	San Manuel, Ariz.	9	8
23	San Manuel, Ariz.	9	8
24	Raleigh, N. C.	12	10
25	San Manuel, Ariz.	12	12
26	San Manuel, Ariz.	13	7
27	San Manuel, Ariz.	13	12
28	San Manuel, Ariz.	14	12
29	San Manuel, Ariz.	15	10
30	San Manuel, Ariz.	15	12
31	San Manuel, Ariz.	20	24
32	San Manuel, Ariz.	26	24
33	San Manuel, Ariz.	27	24
34	San Manuel, Ariz.	28	24
35	San Manuel, Ariz.	29	24
36	San Manuel, Ariz.	29	32
37	Near Hayfield, Va.	32	24
38	San Manuel, Ariz.	32	32
39	San Manuel, Ariz.	43	48
40	San Manuel, Ariz.	50	40
41	San Manuel, Ariz.	58	40
42	San Manuel, Ariz.	62	68
43	San Manuel, Ariz.	70	56
44	San Manuel, Ariz.	78	40
45	San Manuel, Ariz.	116	128

[a] All the samples were obtained from soil except Sample 19 which was from vanadium ore.

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NOTES ON ANALYTICAL PROCEDURES . . .

Determination of Nitrogen Adsorption-Desorption Isotherms

Estimation of Total Pore Volumes of Porous Solids

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IT IS the purpose of this note to call attention to errors which may inadvertently be made in determining gas adsorption-desorption isotherms, and, particularly, in their use for the estimation of total pore volume of the adsorbent. The nature of these errors is illustrated in Figure 1, which shows an adsorption isotherm and two desorption isotherms determined on a single adsorbent which exhibits a type II isotherm according to Brunauer's classification (*1*).

An adsorption isotherm for nitrogen at −195° C. was determined using an oxygen thermometer to measure P_0, the saturation pressure of nitrogen. This isotherm indicated the adsorption, at saturation, of 153 ml. per gram, standard temperature and pressure, of nitrogen, corresponding to a total pore volume of 0.238 ml. per gram. The oxygen thermometer was replaced with a nitrogen thermometer, and the determination was repeated. The desorption branch of the isotherm obtained is designated in the figure as "erroneous desorption isotherm." This isotherm indicated adsorption of 170 ml. per gram of nitrogen at saturation, or a total pore volume of 0.264 ml. per gram.

The density of the material was then measured both by displacement of mercury and by displacement of helium. From the difference in densities, a total pore volume of 0.298 ml. per gram was computed which corresponds to 192 ml. of nitrogen at saturation. Therefore, the desorption branch of the isotherm was redetermined using an initial volume of nitrogen well in excess of 192 ml. The result is shown in the figure as the "correct desorption isotherm."

From these observations it is apparent that it is unwise to attempt to determine total pore volumes of adsorbents which possess a considerable volume in large pores by means of adsorption-desorption isotherms. It also appears that the use of an oxygen thermometer to measure the saturation pressure of nitrogen may result in errors of greater magnitude than does the use of a nitrogen thermometer.

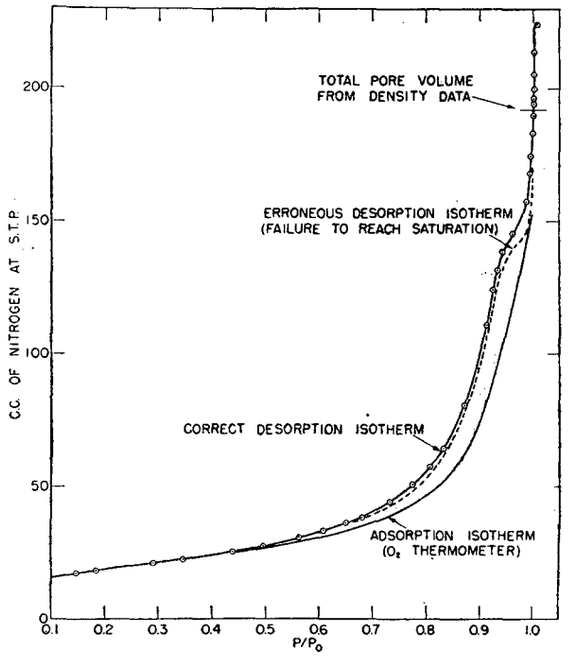

Figure 1. Adsorption-Desorption Isotherm for Nitrogen
Sample 452 at −195° C.

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To avoid the uncertainty resulting from the attempt to estimate total pore volumes from adsorption isotherms, Innes suggests that the volume adsorbed at $P/P_0 = 0.97$ be taken as the measure of total pore volume (*3*). This appears to be an appropriate convention for isotherms of types I, IV, or V, but it is unsuitable for isotherms of types II or III. For the example shown in Figure 1, the volume of nitrogen adsorbed at a relative pressure of 0.97 is 147.5 ml. per gram or 0.229 ml. per gram of liquid nitrogen at its boiling point. This differs from the total pore volume determined from the density data by 0.069 ml. per gram of liquid or 23.2%.

The data also indicate that failure to achieve saturation not only leads to erroneous results at high relative pressures, but also yields an erroneous desorption isotherm at all relative pressures above that at which the desorption branch rejoins the adsorption branch. According to Brunauer (*2*), desorption from a relative pressure less than unity results in a scanning of the hysteresis loop. The data of Figure 1 appear to indicate that this is only approximately true.

The observations suggest not only that accurate estimations of pore volume cannot be made by means of gas adsorption measurements in the case of type II and III isotherms, but also that to be certain of obtaining reliable desorption isotherms of these types, a measurement of total pore volume by means of density determinations should be made to provide assurance that saturation of the sample has actually been accomplished prior to desorption.

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Evaporation Errors in Determination of Trace Concentrations of Low Molecular Weight Solutes in Carbon Tetrachloride

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IN THE quantitative analysis of very low concentrations of polar solutes in nonpolar solvents, the role of evaporation may be seriously underestimated. Loss of solute at an unexpectedly rapid rate under conditions of sample handling normally considered proper can prove to be a major source of analytical error. An experience of this sort was encountered by the authors in the course of applying infrared methods for the determination of trace amounts of water and ethyl alcohol in carbon tetrachloride and of water in liquid bromine. In determinations of water in the two solvents a rapid loss or gain occurred at exposure, depending on the atmospheric humidity.

In order to understand these effects and their orders of magnitude, some measurements were carried out on the differential evaporation rates of carbon disulfide, ethyl alcohol, *n*-butyl alcohol, and acetic acid from solutions of each in carbon tetrachloride. These compounds were chosen to illustrate the effects of boiling point, molecular weight, and polar or nonpolar nature of the solute. Equivalent data on water were not obtained owing to the complication of atmospheric humidity. The significance of these measurements in the field of trace analysis made it seem worth while to present them here.

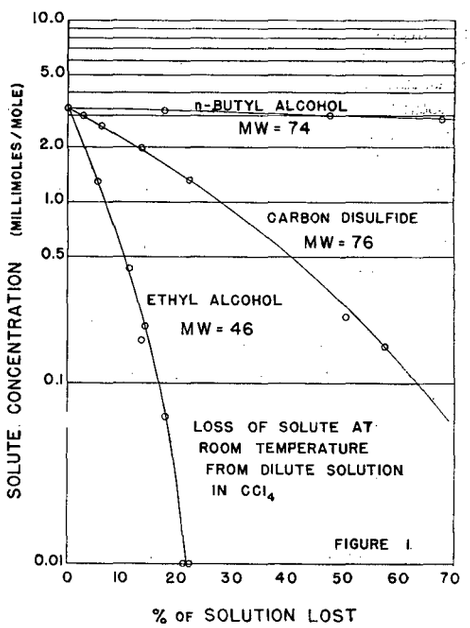
EXPERIMENTAL

Solutions in carbon tetrachloride of ethyl alcohol, carbon disulfide, and *n*-butyl alcohol were made up at concentrations of 3.3 millimoles per mole. Approximately 40 ml. of alcohol solutions were placed in Petri dishes (90 × 20 mm.) and allowed to evaporate from the open dishes at room temperature. Samples were analyzed for alcohol after various amounts of solution had evaporated. A similar experiment was performed for carbon disulfide in carbon tetrachloride except that, for sampling purposes, 90 ml. of solution were placed in the Petri dishes. The results of the experiments, summarized in Figure 1, show the concentration of solute against per cent loss in weight of solution.

The analyses were carried out with a lithium fluoride prism spectrometer. The alcohols were measured at the 2.7μ hydroxyl band, and the carbon disulfide at its 4.7μ band, using the well-known base-line method (*1*). A cell 3.5 mm. thick was used for the alcohols. For acetic acid, which was investigated less thoroughly, the carbonyl band at 5.84μ was measured using a cell length of 3.5 mm. The determination of carbon disulfide required a 5.0-cm. cell.

DISCUSSION

In order to understand the significance of the results indicated by Figure 1, it is well to recall some simple considerations of vapor pressure diagrams. For the binary systems studied, at the pure carbon tetrachloride end of a vapor pressure diagram qualitatively we always have the situation as illustrated by Figure 2. From the usual arguments, it is seen that if a portion of the solution is volatilized, the vapor will be richer in the minor constituent and the liquid remaining behind will approach pure carbon tetrachloride in composition.



Qualitatively the results of the experiments come out as expected. However, Figure 1 shows that after only 12% of the carbon tetrachloride solution has evaporated, the concentration of ethyl alcohol falls to 10% of the starting concentration. This rapid loss of solute is at first glance surprising, perhaps because of a tendency to think of the volatility of ethyl alcohol in terms of the properties of the pure liquid.

In these very dilute solutions, the solute behavior is expressed by Henry's law:

$$P_x = N_x k$$

where k is a constant characteristic of the solvent-solute system. The Henry's law constant, in thermodynamic terms, is an expression of the escaping tendency, or fugacity, of the solute. This may be abnormally high for a substance like ethyl alcohol, because the association effect from hydroxyl bonding is eliminated in very dilute solution. We are then dealing with a low molecular weight species, and the solvent-solute interaction becomes one common to polar-nonpolar systems generally.

Even a substance like *n*-butyl alcohol tends to escape from carbon tetrachloride, although the effect is rather slight. It is interesting to note the difference of behavior between carbon disulfide and *n*-butyl alcohol, which have very nearly equal molecular weights, but are very different with respect to polar character.

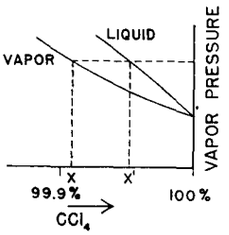


Figure 2

Acetic acid was also investigated to some extent. At 0.1% concentration very little change in concentration is noted upon partially evaporating the solution. But at lower concentrations, about 0.01%, there is a marked lowering of acetic acid content with evaporation. This is probably explained by the fact that at the higher concentration the acid is present to a high degree as the dimer. However, at 0.01% and lower, there is a noteworthy increase of monomer to dimer ratio as shown by the infrared spectrum, so that at very low concentrations a low molecular weight species is present (60 for the monomer as against 120 for the dimer).

As a rule, the authors concluded that the loss of solute is proportionately greater the lower the concentration for the compounds investigated. If this latter observation is correct, the necessity for emphasis on extreme care in sample handling is increased at minute concentrations for solutions such as those considered here.

The determination of water in nonpolar solvents has been found to be subject to all the effects mentioned, to an extreme degree. It might have been more to the point to illustrate this discussion with a water-containing system. But the kind of experiment performed for the systems chosen would offer formidable difficulties when water is involved. Under ordinary conditions the experimenter would be operating in an atmosphere containing amounts of solute (water) significant with respect to the solution concentrations of interest. Evaporating carbon tetrachloride containing small amounts of water leads to either a decrease or an increase in water content. An equilibrium is reached with atmospheric water vapor concentration depending on the humidity.

If these observations are accepted, several rather obvious precautions must be taken in handling solutions of this kind for analysis. Determinations on hundreds of samples analyzed independently by both chemical and spectroscopic means gave very erratic results until the sources of error were successfully traced back to handling procedures ordinarily considered entirely adequate. Samples must be kept hermetically sealed. Transferring a sample from one vessel to another must be done with considerable care. Pouring a carbon tetrachloride solution containing a few parts per million of water, in such a way as to expose a considerable surface of solution to the atmosphere, can greatly affect the concentration of water. Simple calculation may show that the amount of vapor space above the solution in a container may be significant with respect to the solution volume itself.

The investigator must be prepared to ask himself if the determination of water content has any meaning. According to the history of the sample, water content may be more a function of atmospheric conditions coupled with sample handling than anything else. Only when the sample container is handled with very special care will water concentration be anything more than an expression of solvent–atmospheric water vapor equilibrium.

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Spectrographic Determination of Silicon in Uranyl Nitrate Solutions

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SILICON has been determined in uranous uranic oxide (U_3O_8) and in uranium metal and compounds after conversion to U_3O_8, according to the direct current arc carrier distillation method (*1*), and in uranium metal by Walsh (*2*), who used graphite counter electrodes in the controlled alternating current arc.

A number of uranyl nitrate solutions, each containing about 300 mg. of uranium per ml. of dilute nitric acid solution, were to be examined for silicon. The carrier distillation method was considered too slow for this purpose, as it would involve the conversion of the whole or most of the sample to U_3O_8 and the subsequent operations of mixing with the carrier are time-consuming. Because the only impurity element sought was silicon and there were sensitive silicon lines located in a region free from an excessive number of uranium lines, it was decided to apply a direct burn procedure and to use uranium itself as an internal standard.

PREPARATION OF STANDARDS

A pure sample of U_3O_8 was prepared by extraction of strong uranyl nitrate solution with ether, removal of the ether under reduced pressure, and final ignition of the residue to U_3O_8. Pure precipitated silica was diluted with this base by thorough grinding in an agate mortar to obtain the standards: 5000, 1000, 500, 100, 50, and 10 p.p.m. of silicon relative to U_3O_8. A blank estimation of silicon was carried out on the pure U_3O_8 used for the dilution.

PRELIMINARY INVESTIGATION

The electrodes were shaped from National Carbon Co. pure graphite rod 0.5 inch (1.25 cm.) in diameter to fit on a graphite support 0.125 inch in diameter, and had a crater $^5/_{32}$ inch in diameter × $^1/_{20}$ inch deep. They were preburned for 30 seconds at 10 amperes before loading, to remove surface contamination and reduce the silicon in the electrode material to a uniform low level.

A moving plate exposure was first made on a 6-mg. charge in a 10-ampere direct current arc to study the relative emission rates of silicon and uranium. It was found that the silicon intensities followed the uranium fairly closely and fell off rapidly after 20 seconds, becoming zero at 30 seconds. The exposure conditions adopted were 30 seconds at 10 amperes with the sample as anode.

To investigate the effect of charge weight on the result, weights of 4, 5, 6, and 7 mg. of the 500 p.p.m. standard were exposed; the intensity ratio was found to remain reasonably constant.

It had been shown in connection with other work that in general the sensitivity of trace elements added in the form of an aqueous solution was higher if the solution were prevented from soaking into the porous graphite electrode by the presence of a thin grease film. Furthermore, nonpenetration of the electrode

by the samples was desirable, as it was intended to add the standards in the form of solid U_3O_8. The grease film was prepared by evaporating one drop of a solution of Apiezon M grease in analytical reagent 60° to 80° C. petroleum ether (0.5 gram in 10 ml.) on the surface. The film was, however, attacked and penetrated with consequent loss in sensitivity for silicon, when solutions containing much nitric acid were evaporated on it. This difficulty was overcome by allowing the electrode with its drop of solution to stand for a few minutes in an atmosphere of ammonia until the yellow ammonium diuranate commenced to form. The ammonia atmosphere was simply obtained by placing a small beaker of 0.880 specific gravity ammonia solution under the crystallizing dish which was used as a dust cover for the electrodes.

To check the sample preparation, several milliliters of a sample were evaporated in a platinum crucible and ignited to U_3O_8. Quadruplicate exposures were made on 6-mg. aliquots of this residue and compared with the results obtained by treatment of the solution on the electrodes as described above with the following results:

1. Ignition in platinum, 93, 100, 98, 100; mean 98 p.p.m. silicon
2. Ignition on the electrodes, 115, 107, 114, 105; mean 110 p.p.m. silicon

The somewhat higher figure arrived at by method 2 was not due to the blank on the grease, as this was negligible, but might have been due to slight penetration of the graphite by the nitric acid resulting in the introduction into the arc stream of a small amount of silicate from the electrode. However, a blank run on nitric acid alone showed that no silicon was introduced in this way. The difference is probably due to small losses of silicon on the walls of the platinum crucible in method 1; no such loss can occur when the solution is processed directly on the electrode.

PROCEDURE

A suitable volume of the solution for analysis, in this case 0.02 ml. which contained 6 mg. of U_3O_8, was transferred by means of a graduated pipet with plunger control to the prepared electrode, the free acidity was neutralized as described above, and the solution was evaporated to dryness under radiant heat lamps. The samples could not be arced in this condition, because copious vapor evolution would have expelled the charge bodily from the electrode cup. They were therefore first ignited for a short time in a Bunsen flame to convert the uranium into U_3O_8, the base of the electrode being held in platinum-tipped tongs. A little care was necessary in the first stage of the ignition; the surface of the charge had to be heated just outside the flame until the oxide crust became sufficiently porous to allow the passage of vapor from within the bulk of the charge. After the first two or three ignitions this procedure gave no difficulty.

Table I. Reproducibility of Analysis

Sample	Silicon P.P.M. 1	2	3	4	Mean
1	96	102	109	103	102.5
2	71	63	65	69	67.0
3	85	77	76	87	81.2
4	66	73	73	79	72.8

At least two exposures were made on each solution together with the synthetic standards (6-mg. charge) on the same photographic plate under the following conditions.

Baird, grating spectrograph (3-meter), 15,000 lines per inch. First order. Range 2.5 (2165 to 3580 A.). Slit 10 microns. Grating 3-cm. aperture, image of source focused on grating. Plate Ilford Ordinary. Exposure 30 seconds at 10 amperes, sample as anode. Plate calibration 1.5-step sector at secondary focus, range 8-second order. Hilger Littrow spectrograph. Range 2220 to 2900 A. Slit 10 microns. Plate Ilford Ordinary. Exposure 30 seconds at 10 amperes, sample as anode.

A suitable line pair for use with both spectrographs was Si 2516.1 A. and U 2519.0 A., measurement being made with the Hilger nonrecording microphotometer. A calibration curve was drawn up of log intensity ratio against log of parts per million of silicon in U_3O_8.

RESULTS

Results were expressed as parts per million of silicon in U_3O_8 and, assuming that the concentration of uranium in the original solution was known, could be converted into micrograms per milliliter of solution. Typical results on samples run in quadruplicate are shown in Table I. The standard deviation calculated on sixty duplicate sample results was 9.42%.

ACKNOWLEDGMENT

Acknowledgment is made to the Director, Atomic Energy Research Establishment, Harwell, England, for permission to publish this paper.

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Tiselius-Claesson Interferometric Adsorption Analysis Apparatus

Improvements in Design and Use

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THE adsorption analysis apparatus developed by Tiselius and Claesson (*1*, *11*) has proved to be a valuable tool in the chromatographic separation of sugars (*9*, *12*), amino acids and peptides (*10*), fatty acids (*4*, *6*, *7*), and macromolecules (*2*, *8*). Experience gained through building two instruments for this laboratory has led to some modifications in design that have increased the versatility of the apparatus and altered and simplified its manipulation.

The Tiselius-Claesson apparatus consists of two main parts, the vertical chromatographic column and the horizontal interferometer by means of which observations are made upon the effluent. The model described is shown in Figure 1.

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COUPLED FILTER SYSTEM

The chromatographic column, *A*, is segmented, consecutive segments decreasing in internal diameter from the top to the bottom of the column. The filters (segments) are joined by couplings having capillary bores through which the effluent passes. This arrangement, first used by Hagdahl (*3*), thoroughly mixes the effluent from one filter and passes it on to a fresh filter of smaller diameter. The inevitable irregular fronts are thus "ironed out," and, as the fronts pass to successively smaller filters, these irregularities are expressed in smaller and smaller volumes of effluent. Frequently fronts are observed encompassing only 1 or 2 ml. Without this coupled filter system and the mixer, observation of the separated zones is difficult.

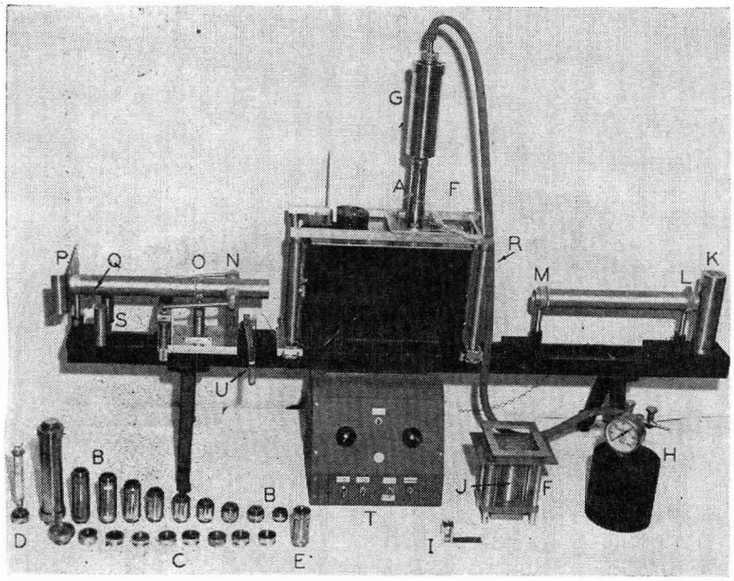

Figure 1. Modified Tiselius-Claesson Adsorption Analysis Apparatus

A.	Chromatographic column	*L.*	Adjustable slit
B.	Coupled filters (segments)	*M.*	Plano-convex lens
C.	Filter couplings	*N.*	Compensator
D.	Column head with syringe in adapter	*O.*	Plano-convex lens
E.	Mixer	*P.*	Eyepiece
F.	Cuvette	*Q.*	Adjustable hair line
G.	Driving syringe	*R.*	Double thermostated bath
H.	Pressure chamber	*S.*	Micrometer lamps
I.	Ultraviolet absorption cuvette	*T.*	Control box
J.	Spacer on spool for "crossing over"	*U.*	Receiver
K.	Lamp housing for band lamp		

The set of coupled filters pictured in Figure 1 is constructed of No. 303 stainless steel and is resistant to corrosion. The outer diameter of the filters has been increased to 1.25 inches (3.1 cm.) to allow larger internal diameters for greater capacity, to permit stronger threads, and to lend sturdiness to the system. Nine standard filters of capacities varying from 0.5 to 20 cc. are shown in Figure 1, *B*. The ratios of length to diameter are approximately 4. To their left is an oversize filter of 100-cc. capacity used to build columns of high capacity. In front of the filters is a row of identical and interchangeable filter couplings, *C*. These carry polyethylene gaskets in both faces.

The column head is fitted with Luer-Lok syringe adapter, *D*, making possible convenient and quantitative charging of the column with the sample for analysis. The bottom of the column carries a mixer, *E* (*3*), the function of which is to homogenize the effluent and prevent any layering of the solution in the observation cuvette, *F*.

The syringe, *G*, which drives the column is a stainless steel hollow-honed cylinder which contains a close-fitting piston. The cap of the syringe is provided with a detachable fitting connected to a source of compressed gas, *H*, by pressure tubing. The nose of the syringe is threaded to fit the column head, *D*. Recently it has been found that for use with solutions that do not attack rubber, a piston carrying two rubber O-rings makes a more leakproof fit and no special care is required to prevent scoring of the honed surface. With the steel piston removed, small syringes can conveniently be placed within the cylinder for delivery of small volumes of liquid into the column.

THE CUVETTE

For use with fatty acid separations it was necessary to abandon the conventional gold-plated brass cuvette to prevent leaching of copper by the fatty acid solutions.

The cuvette, shown in Figure 2, is made of stainless steel and represents a new type of construction. It consists of two blocks of stainless steel with channels milled into their surfaces. The observation channels were milled into the upper surface of the lower block, *B*, and channels to conduct the liquid to the needle tubing outlets, *G*, were milled into the lower surface of the upper block, *A*. The two blocks are bolted together with a polyethylene gasket, *C*, separating them. Glass faceplates, *E*, over polyethylene gaskets, *D*, are held in place on the ends of the cuvette by brass end plates, *F*. The outlet tubes, *G*, made of stainless steel needle tubing can be connected conveniently to capillary plastic tubing to conduct the effluent wherever desired. An extra opening to the flowing channel is provided to allow the flushing out of any interfering bubbles which may collect in the cuvette and prevent observation. The needle tubings are protected by larger jacket tubings, *H*, which terminate in the cuvette cover, *I*. By providing the cuvette with its own cover the instrument can be used in a windy place without any noticeable disturbance of the interference fringes. By using interchangeable cuvettes of differing lengths, one can quickly select different ranges of refractive index without making adjustments of the instrument itself.

When the "crossing-over" technique is used (*5*), a spool, *J*, carrying a few meters of capillary plastic tubing, is placed around the foot of the column on the cuvette, and the ends of the spool are connected to the flowing tube and one inlet of the comparison tube. Effluent is then collected from the other comparison tube inlet.

An auxiliary cuvette (Figure 1, *I*) has been constructed to allow simultaneous observations to be made in the Beckman spectrophotometer. This cuvette consists of a quartz cell having a 0.2-mm. light path and is provided with an inlet at the bottom and an outlet at the top. The entire unit replaces the cuvette carrier of the Beckman spectrophotometer. The interferometer cuvette is connected with the Beckman cuvette by the capillary plastic tubing.

OPTICAL SYSTEM

The optical system, similar to that of Claesson (*1*), is composed of a horizontal band lamp, *K*, a vertical slit, *L*, a plano-convex lens, *M*, providing parallel rays to the cuvette, *F*, two compensator plates, *N*, and a second plano-convex lens, *O*, which brings the two rays to cross at the focal point of the cylindrical lens in the eyepiece, *P*.

The compensator, *N*, is the only part of the optical system that has been significantly modified. A view of the compensator system with shade tube removed is shown in Figure 3. In the compensator, the glass plates, *A*, are mounted in rods, *B*, which rotate about axes which intercept the light beams. With this arrangement, the same spot of the compensator glass intercepts the light ray in all angular positions of the plate, and the glass does not move out of the light path. The arms of the compensator, *C*, which are activated by the micrometers, *D*, are not permanently fixed to the rods bearing the compensator glasses, but are fixed to sleeves, *E*, which can be adjusted to positions selected to provide for any desired angular range of the compensator glasses for the same fixed 25-mm. range on the micrometer. Thus the sensitivity of the micrometers can be altered to suit the general needs of the investigator. By mounting the micrometers below the optical path and by shortening the focal length of the plano-convex lens, *O*, the operator can more comfortably reach and read the micrometers.

The eyepiece, *P*, is provided with vertical and horizontal slits which allow the operator to exclude stray or reflected light and to select only the rays involved in the interference pattern. The eyepiece is also provided with a curved eye shade, excluding side light from the operator's eye. An adjustable fine glass hairline, *Q*, is mounted at the focal point of the cylindrical lens of the eyepiece.

THERMOSTATED BATH

The double-walled bath of the original instruments (*1, 11*) has been retained. An attempt was made to use a simple bath, but the disturbances caused by stirring and heating were too great.

The outer bath, *R*, containing water is provided with a level indicator, and the cover of the bath carries a motor stirrer, a thermoregulator (37° ± 0.2°), two electric heaters, and a thermometer. When the cuvette is in place, its cover completely covers the opening of the inner bath, minimizing cooling by the atmosphere. The heaters are connected in such a manner that they can be used either in parallel for rapid heating, or in series for

sustained heating free from surges in temperature. Controls for motor, heaters, micrometer lamps, *S*, and band lamp are grouped in a control box, *T*.

USE OF THE APPARATUS

A unified description of the manipulations of the adsorption analysis apparatus and the coupled filters is not available in the literature. To give a clear picture of how the apparatus is used, the manipulations involved in a simple displacement experiment are described here.

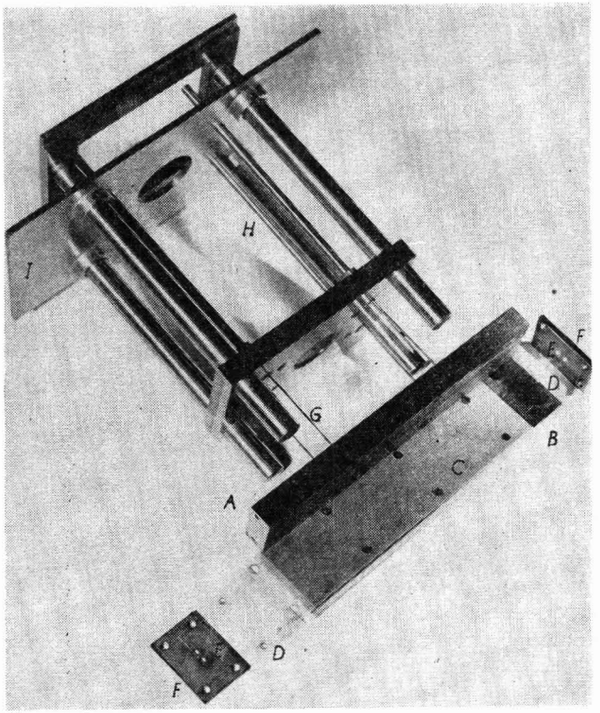

Figure 2. Exploded View of Cuvette

A.	Upper cuvette block	*F.*	End plates
B.	Lower cuvette block	*G.*	Needle tubing outlet tubes
C.	Polyethylene gasket	*H.*	Jackets for needle tubing
D.	Gaskets for windows	*I.*	Cuvette cover
E.	Windows		

The number and sizes of filters chosen for a desired separation depend upon the quantity of the sample, retention of displacer, and difficulty of separation of components. Columns should always be built with lowest filter the smallest (0.5 cc.), increasing the capacity of the column by adding larger filters at the top. Neighboring filters should preferably not differ in capacity in steps greater than a factor of 2.5.

The filters, *B*, are provided with perforated filter bottoms over which close-fitting circles of filter paper are placed. A slurry of adsorbent in the solvent to be used in the experiment is poured into the largest filter of the column, and the solvent is drained from below by slight suction. When the filter is full of packed, yet moist, adsorbent, the absorbent is pressed down with a spatula, and the excess is removed. A filter paper circle is laid over the adsorbent. The filter is now inverted and screwed into the column head, *D*, which is previously flooded with solvent. The column head is then screwed into the syringe, *G*, previously filled with solvent, care being taken that no bubbles are trapped within the system. The syringe with the one filter is connected to the compressed gas, and solvent is forced through the filter to wash it. The next filter is packed and inverted, and its top is joined to a coupling previously flooded with solvent. The other side of the coupling is then filled with solvent and joined to the bottom of the first filter. The remaining filters are packed and assembled in the same way, and the mixer, *E*, is joined to the bottom filter. This method of building and washing a column saves considerable time, for when the column is built, it is already nearly washed.

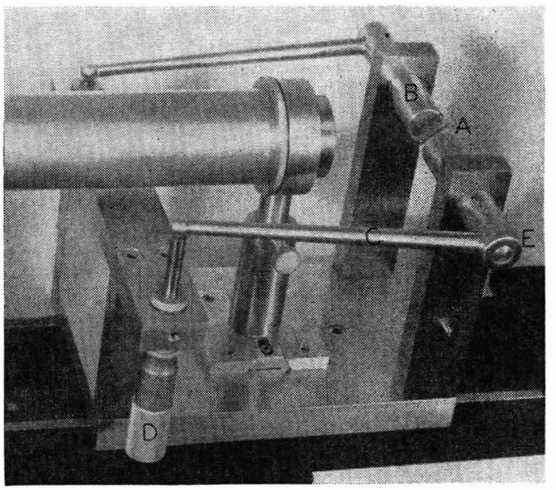

Figure 3. Compensator with Shade Tube Removed

A.	Compensator glasses	*C.*	Compensator arm
B.	Rod	*D.*	Micrometer

The sample to be separated is dissolved in a convenient volume (5 to 10 ml.) of the solvent, and placed in a Luer-Lok syringe. The stainless steel syringe is removed from the column and the glass syringe is fixed in its adapter. The contents of the syringe are discharged into the filter column by hand. It is often convenient to loosen a joint in the center of the column before pressing in the sample, thereby reducing the back pressure. The column is then retightened, the syringe removed from its socket, and the column replaced on the large syringe which has been filled with displacer solution. The column with syringe is then screwed into its place in the cuvette and the cuvette is placed in the bath.

The contents of the comparison tube are next washed out with solvent from a glass syringe and the needle tubings are joined by a short length of plastic capillary tubing. The flowing channel is also flushed with solvent and the wash outlet plugged. The driving syringe is connected to the source of gas pressure and the development of the column is begun.

When the apparatus comes to bath temperature, the fringes are located at or near the zero (base line) value on the micrometer. If fringes cannot be found, the cuvette channels are inspected by means of a mirror. Should the light be obscured, the channels are washed again until the light emerges from both channels. The usual source of trouble is the lodging of bubbles in the channels. If bubbles appear in the flowing channel during an experiment, they usually can be dislodged by pinching the plastic outflow tube. This develops internal pressure enough to dissolve the bubble partially, and when the pressure is released the bubble is flushed out. Pinching the tube a few times usually removes the bubble from the channel; if not, it can always be washed away by a stream of solvent injected into the wash tube.

Observations are made at convenient intervals of volume during the course of the experiment by turning the micrometer screws to bring the zone of interference fringes across the hairline, approximately the same number of fringes lying on either side. A convenient flow rate is 20 to 30 ml. per hour. When the micrometer reading reaches the value previously determined for the displacer solution, the experiment is terminated, for at that time the column is saturated with displacer and the sample has been displaced.

When high concentrations of displacer are to be used, or when the components have high refractive indexes, so that measurements would normally exceed the range of the two micrometer screws, the crossing-over technique (*5*) is of value. The effluent is conducted from the outflow tube. through a plastic capillary

tube spacer wound on a spool, to the inlet of the comparison tube, and from thence to the receiver, *U*. Thus, refractive index gradients across a volume interval equal to the spacer are measured. As long as the concentration of substance in the effluent is constant, the refractive index gradient is zero. When a zone of substance arrives in the cuvette, the refractive index rises to a new level, and this is then observed as a spike. Thus, rather than a stepwise rise in refractive index, one obtains a series of maxima rising from the base line, corresponding to the steps of the ordinary displacement diagram.

In the discussion here, displacement separation has been used as an example. The apparatus is well adapted also to elution, frontal, and carrier displacement separation.

ACKNOWLEDGMENT

Opportunity is taken to commend the workmanship of D. Milton Kvanbeck of Minneapolis, Minn., who constructed the custom-built model discussed in this paper. This work was supported by a grant-in-aid from the Williams-Waterman Fund of the Research Corp.

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Microdetermination of Chromium in Catgut Sutures

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A TITRIMETRIC method for the determination of chromium in catgut sutures, using 1- to 2-gram samples, has been reported (*5*). However, studies in progress at this laboratory involve the determination of chromium in small strips of catgut suture, weighing 3 to 4 mg. As the chromium content of these sutures ranges between 0.15 and 0.25%, a more sensitive method of analysis is required.

The high sensitivity of the diphenylcarbazide method for determining chromium (*3*) has been adapted to the determination of chromium in leather (*2*). The procedure described is not within the working range for the minute quantities of chromium to be determined in catgut sutures. Modifications of this procedure have, however, afforded the accurate determination of chromium in 3- to 4-mg. samples of catgut suture. The method is simple, rapid, and should be generally applicable to the determination of minute amounts of chromium in biological materials—e.g., in the stratoassessographic analysis of chrome-tanned leather (*1*).

Iron, vanadium, molybdenum, and mercury interfere with color development in the diphenylcarbazide method. Of these, iron is the only interfering metal that may possibly be encountered in catgut sutures. (Phenyl mercuric benzoate is added to certain tubing fluids. However, the amount employed is insufficient to produce interference in the diphenylcarbazide reaction.) The addition of phosphoric acid, to complex the iron possibly present, effectively minimizes this interference (*2*).

A loss of chromium in the perchloric acid oxidation, resulting from the formation of chromyl chloride, may occur (*4*). This is obviated by adjusting the temperature below the boiling point of constant boiling perchloric acid, and yet maintaining a sufficiently high temperature to permit complete conversion of chromium to the hexavalent state.

PREPARATION OF SAMPLE

The sutures, following removal from the tubes, were cut into 1-inch (2.5-cm.) strips, allowed to come to equilibrium in a room which remained relatively constant with regard to humidity and temperature, and then weighed. For determining absolute values of chromium content, 1-inch strips of suture were transferred to micro weighing bottles, oven-dried under vacuum at 70° C. for 18 hours, and weighed.

REAGENTS

Buffer solution, pH 1.6. Take 250 ml. of 0.2 *N* potassium chloride (14.9 grams per liter), mix with 150 ml. of 0.2 *N* hydrochloric acid (17.1 grams of concentrated hydrochloric acid per liter), and dilute to 1 liter.

Diphenylcarbazide, 0.25% in 1 to 1 acetone-water. This solution should be prepared daily and kept in the dark while not in use.

EXPERIMENTAL PROCEDURE

The sample is transferred to a 12 × 75 mm. test tube. Two drops of concentrated sulfuric acid and 4 drops of concentrated nitric acid are added, and the mixture is boiled gently on a hot plate until carbonization occurs. The sample is cooled, and one drop of hydrogen peroxide (30%) is added.

The mixture is heated again to charring, removed from the hot plate, and cooled, and the treatment with a drop of hydrogen peroxide is repeated. Usually 3 drops of hydrogen peroxide are required to effect complete digestion of the sample in this manner. Following clearing, the sample is allowed to remain on the hot plate for 0.5 hour to remove traces of unreacted hydrogen peroxide.

After cooling, 2 drops of constant boiling perchloric acid (70 to 72%) are added to the digestion tube. The test tube is inserted about 15 mm. below the surface of a constant temperature bath adjusted at 200° ± 2° C. and oxidized for 15 minutes. After oxidation, the tube is immediately inserted into a cold water bath and diluted with distilled water to a volume of approximately 3 ml. The solution is transferred into a 25-ml. volumetric flask. About 10 ml. of water are used for the transfer. The volumetric flask is placed on a hot plate and the solution is boiled for several minutes to remove liberated chlorine. After cooling, one drop of concentrated phosphoric acid and 10 ml. of the buffer solution are added. One milliliter of diphenylcarbazide is transferred to the flask, water is added to the mark, and the solution is shaken. The color is allowed to develop for 15 minutes. Readings are made in an Evelyn colorimeter using a No. 540 Evelyn filter.

PREPARATION OF STANDARD GRAPH

Known samples of hexavalent chromium at five concentrations, ranging from 3.6 to 10.8 micrograms, were transferred to the digestion tubes, reduced with an excess of sodium thiosulfate, digested, and oxidized in the manner described. Six samples at each concentration were determined. The color developed was read against a blank solution treated similarly. Per cent transmittance for the concentrations indicated ranged from 25 to 65. The optical density of the mean reading at each concentration was plotted; Beer's law was followed. The average deviation from the mean values of optical density at the particular concentrations was 1.7% and the maximum deviation from the mean (at 3.6 micrograms) was 6.2%.

RECOVERY EXPERIMENTS

Solutions of potassium dichromate were added to 1-inch (2.5-cm.) strips of ordinary nonchromicized catgut. Four samples at

Table I. Determination of Chromium in Catgut Sutures

Catgut Sutures	No. of Detns.	Average Weight	Average Chromium	Average Deviation	Maximum Deviation
		Mg.	%	%	%
I (Air-dried)	6	3.85	0.179	2.7	7.2
II (Air-dried)	19	3.75	0.177	3.4	9.0
III (Oven-dried)	11	3.27	0.166	3.0	6.0

each of three concentrations (5.4, 7.2, and 9.0 micrograms) were determined. Two samples at each concentration were reduced with sodium thiosulfate prior to analysis. No differences could be found in recoveries between reduced and unreduced samples. The average deviation from the amounts added was 1.7%, and the maximum deviation was 6.9%. Four samples of catgut suture, to which no chromium was added, gave the same reading as the blank.

RESULTS AND DISCUSSION

The chromium content of 1-inch strips of three sutures, prepared by the air-dried (samples I and II) and dry-weight methods (sample III), was determined in several separate runs. The results are indicated in Table I.

Evaluation of the results indicates that the procedure is adaptable to the measurement of minute quantities of chromium in catgut sutures. The average deviations from the mean are 2.7, 3.4, and 3.0% and the maximum deviations are 7.2, 9.0, and 6.0% for strips from samples I, II, and III, respectively.

The increase of average deviation for the samples of catgut suture over that of standard chromium solutions and recoveries of chromium added to sutures, may result from small variations in chromicization along the length of the suture, rather than errors inherent in the procedure.

ACKNOWLEDGMENT

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Determination of Serum Iodine

Evaluation by Radioactive Tracer Technique of the Alkaline Fusion Method

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THE literature reports many methods for the determination of micro amounts of iodine in organic material. All procedures follow three basic steps: separation of the bound iodine from the free iodide, digestion of the bound iodine and other organic constituents of the serum, and quantitative determination of the iodine either colorimetrically or titrimetrically.

However, two basically different principles underlie all these procedures: digestion in an acid medium followed by distillation or other complicated separations and then a quantitative determination of the iodine, and alkaline digestion followed by the quantitative determination of iodine in the presence of all the digestion products.

Barker (*1*) used a chromic acid digestion procedure, a modification of the Chaney (*2*) procedure, distilling the iodine into an arsenite solution, to which was added a known amount of ceric ammonium sulfate. This oxidation-reduction reaction

$$(2Ce^{IV} + As^{III} \xrightarrow[\text{yellow}]{I^-} \underset{\text{colorless}}{2Ce^{III} + As^{V}})$$

is catalyzed by iodine as iodide. The iodine may be quantitatively estimated by measuring the time rate of change of the reaction. This relationship was first studied by Sandell and Kolthoff (*7*).

Salter (*4–6*) used an alkaline digestion followed by the quantitative determination of the iodine in the presence of the fusion products, using the same ceric sulfate reaction.

Salter's (*3*) method for the determination of iodine in blood serum was chosen in this laboratory in preference to other procedures. This procedure used an alkaline digestion, preventing the loss of iodine by volatilization even in the presence of strong oxidizing agents. The procedure was relatively rapid, economical as to both chemicals and apparatus, and comparatively simple—i.e., free from complicated separations by distillation or preferential solvent action.

At the time this procedure was adopted, work was initiated immediately to check the accuracy of the method. Radioactive iodine (I^{131}) was used as a tracer element to determine quantitatively the accuracy of the procedure and also to check the reactions step by step to determine if and where the losses might occur. In order to do this the procedure was divided into four major steps and recovery was checked at each step.

1. Separation of the bound iodine from the free iodide
2. Digestion of the organic constituents in the strongly alkaline medium
3. Acidification of the alkaline fusion material before the colorimetric determination of iodine
4. Comparison of the amount of total iodine determined as such and determined as the sum of protein-bound and free iodine

REAGENTS AND APPARATUS

All reagents for serum iodine determination were prepared as directed by Salter and Johnston (*3*). Sodium hydroxide, 4 *N* 1% and 10% potassium nitrate, and 1.4 *N* sulfuric acid were used. The Geiger-Müller counter, scale of 64, self-registering type, was Model 163 manufactured by Nuclear Instrument Co., Chicago, Ill.

A counting chamber holding the counter head at a fixed distance from the sample cups was used. The cups were placed on a sliding tray which directed the cups below the counter head. The counting chamber was built by this laboratory.

Stainless steel cups 1 inch (2.5 cm.) in diameter and 0.25 inch in depth were used as radioactive sample containers.

Serum from either euthyroid or hyperthyroid patients was used. The majority of these patients had previously received 50 microcuries of radioactive iodine (I^{131}) used in a diagnostic procedure to determine the relative metabolism of the thyroid gland. In a few instances, the hyperthyroid patients had received from 4 to 6 millicuries of radioactive iodine as a therapeutic dose. In either case blood was drawn 26 hours after radioactive iodine was given to the tracer patients and 2 to 3 hours following dosage of 4 to 6 millicuries.

A saturated solution of sodium hydroxide (75 grams per 100 ml. of solution) was used to ensure an alkaline medium throughout the counting.

PROCEDURE

Testing Fusion Ash for Loss after Digestion. To 1 ml. of radioactive serum were added 0.5 ml. of 4 *N* sodium hydroxide and

Table I. Recovery after Ashing

Sample No.	Standard, 0.2 Ml. of Serum, Counts/Min.	After Ashing, 0.2 Ml., Counts/Min.	% of Standard	% Error
1	461	444	96.3	−3.7
2	437	427	97.7	−2.3
3	3647	3807	104.4	+4.4
4	319	326	102.1	+2.1
5	8295	7942	95.7	−4.3
6	247	249	100.9	+0.9
7	9538	9521	99.8	−0.2
8	188	190	101.1	+1.1
9	437	437	100.0	0.0
		Av. recovery	99.8	

0.1 ml. of 10% potassium nitrate in a combustion tube. The combustion tube (10 × 1 cm. borosilicate glass) was etched with saturated sodium hydroxide previous to use. This solution was mixed and evaporated to dryness over a microburner, with care to ensure the wide distribution of solid matter in a thin film.

This dark brown ash was then heated to 500° C. in a muffle furnace. The furnace was shut off and the ash allowed to cool for 0.5 hour. To the ash was then added 0.5 ml. of 1.0% potassium nitrate solution, with slow washing down of the sides of the combustion tube. This dark turbid liquid was then evaporated again over a microburner to an expansive dry film and returned to the muffle furnace to be reheated to 500° C. The furnace was allowed to cool again for 0.5 hour and the combustion tube was removed.

The ash (white) was diluted to 5.00 ml. with distilled water. Three 1.0-ml. aliquots were removed and introduced into the steel cups. One drop of saturated sodium hydroxide was added to each sample and the cups were placed beneath an infrared lamp to dry. Each cup contained 0.2 ml. of the original/ml. of serum.

A 0.2-ml. sample of unashed radioactive serum was introduced into each of three more steel cups plus the usual drop of saturated sodium hydroxide. These three cups contained the reference standards of original serum to which the 1.0-ml. aliquots from the ashed serum were compared. These cups too were dried beneath the infrared lamp. The results are noted in Table I.

The average recovery after ashing was 99.8%. Each of the counts recorded in all tables was the average count of three or more like samples, with the background count subtracted in each case. Average background for this geographic location and with the equipment employed was 16 counts per minute.

Standard Geiger-counting procedure was used. Contamination of the counting chamber was checked first, a 3-minute background was taken (a background count is a count of cosmic radiation for a geographical area plus the small amount of contamination near the counter), and the samples were counted from 3 to 5 minutes, depending on the concentration of the radioactive iodine. Each count was taken for 3 to 5 minutes in order to make a minimal count of approximately 1000. The results from the three aliquots were averaged after correction for background. There was no need for a self-absorption correction in any of the procedures, because every sample weighed 10 mg. per sq. cm. or less (*8*).

The next step of the procedure to be analyzed was the separation of protein-bound iodine from the inorganic iodine.

Testing Inorganic Iodine Fusion Ash for Loss during Separation. To 1.0 ml. of nonradioactive serum was added a known amount of radioactive iodine; to this solution were then added 4.0 ml. of distilled water and 0.27 ml. of 0.2 *N* acetic acid. The solution was adjusted to pH 6 with either dilute alkali (sodium hydroxide) or acid (sulfuric acid), thoroughly mixed by agitation, and placed in a water bath at room temperature. The bath was brought to a boil, causing coagulation and flocculation and completion of precipitation. The tube and contents were allowed to cool for 20 minutes at 76° C. and were then centrifuged for 10 minutes at 2000 r.p.m. The supernatant solution was poured into another combustion tube. The precipitate was washed three times, centrifuged with each washing, and poured into the combustion tube containing the first washing. The washings were made alkaline with 4 drops of ammonium hydroxide (specific gravity 0.90) and slowly dried in a hot air oven at 37° C. When dry, this tube was treated as when testing fusion ash for loss after digestion, except that the final ash was dissolved with 1 ml. of distilled water, and introduced into a steel sample cup. All radioiodine added should be found in the inorganic fraction. The usual drop of saturated sodium hydroxide was added. In the analysis of this step triplicate samples were prepared, dried, and then compared with three standard samples of radioiodine (which are equal in amount to that which was added to the nonradioactive serum) heated to dryness only before counting.

The results of this procedure are shown in Table II.

Table II. Recovery of Radioactive Iodine Added to Serum

Sample No.	Standard (I^{131}), Counts/Min.	After Separation, Inorganic Iodine Fraction (I^{131}), Counts/Min.	% Reclaimed	% Error
1	388	394	101.5	+1.5
2	463	465	100.4	+0.4
3	335	329	101.8	+1.8
4	340	330	97.1	−2.9
5	478	476	99.6	−0.4
		Av. reclaimed	100.1	

An average of 100.1% was reclaimed; the separation of inorganic iodine was adequate and there seemed to be no absorption to or combination with protein (in vitro) before or after precipitation. The protein precipitate was checked for radioactivity and the count was the same as background, showing that no iodine (I^{131}) had been held by the solid protein.

Loss of Iodine. Comparison of the loss of iodine in 1 ml. of radioactive serum measured as total iodine against the loss in 1 ml. of radioactive serum calculated as the sum of the bound and free iodine entailed ashing 1-ml. samples of serum as described above. The ash was then treated with 3 ml. of distilled water and 2 ml. of 1.4 *N* sulfuric acid. The sulfuric acid reacts with the ash, liberating iodide which was to catalyze the arsenious acid–ceric ammonium sulfate reaction. The ash dissolved in the dilute solution of iodine-free sulfuric acid with some effervescence. The effervescence of reaction is kept well under control by gently agitating the combustion tube until the solution is complete. Three 1-ml. aliquots were transferred to steel cups, made alkaline, and dried as previously noted. Three 0.2-ml. samples of the same untreated radioactive serum were prepared and dried as previously explained. The two sets of samples were compared for loss during acidification of the iodine.

The results are shown in Table III.

Table III. Recovery of Total Iodine after Acidification

Sample No.	Standard, 0.2 Ml., Counts/Min.	After Acidification, 0.2 Ml., Counts/Min.	% Recovery	% Error
1	491	450	91.7	8.3
2	247	216	87.4	12.6
3	188	145	77.1	22.9
4	9538	6979	73.1	26.9
5	3647	3357	92.0	8.0
6	319	262	82.1	17.9
7	437	344	78.7	21.3
8	437	365	83.5	16.5
		Av. recovery	83.2	

For checking the loss during acidification of the protein-bound iodine and iodide fraction, the combined counts from the two fractions separated before fusion were compared to 0.2 ml. of radioactive serum. The 1.0-ml. sample of radioactive serum was separated as in testing inorganic fusion ash for loss during separation and the organic curd and washings (iodide) were treated as in testing fusion ash for loss after digestion. The resulting fusion ash was dissolved with 3.0 ml. of distilled water and 2.0 ml. of 1.4 *N* sulfuric acid and aliquots were taken from each tube and treated for the counting procedure. Therefore, the count from 0.2 ml. of radioactive serum was the standard and the count from 1.0 ml. of protein-bound iodine plus 1.0 ml. of the iodide fraction was compared to the standard. The 1.0 ml. of protein-bound iodine plus the 1.0 ml. of iodide was equal to 0.2 ml. of the original 1.0 ml. of serum.

The results from this analysis are presented in Table IV.

Comparison of the results recorded in Table III (average recovery of total iodine = 83.2%) with those in Table IV (average recovery of total iodine, protein-bound iodine plus iodide =

99.3%) shows that a more complete recovery of total iodine is obtained by summation of the bound and free iodine determined separately.

DISCUSSION

The experimental evidence found by the procedures described in this paper indicate that losses occurring during the alkaline fusion procedure were negligible. The experimental evidence indicates that acidification of smaller amounts of ashed serum per unit volume of solution resulted in a smaller loss. This was proved by the fact that the total iodine determined in 1 ml. of serum was of the order of 82.3% of the original serum, or of the total iodine calculated as the sum of the protein-bound iodine contained in 1 ml. of serum and the inorganic iodine in the same 1 ml. of serum. Hence, a dilution of 100% gave a 99.3% recovery. Thus it is necessary only to determine protein-bound and free iodine, omitting the step of determining total iodine as such. From 100 cases the total iodine determined on 0.5 ml. of serum chemically varied in the order of ±0.2 microgram from the total iodine calculated by the two separate fractions.

CONCLUSIONS

Salter's method for blood serum iodine gives both precision and accuracy, if total iodine is calculated as the sum of two fractions, inorganic and organic. The biologically important protein-bound iodine is shown to be determined accurately.

The precipitation of protein-bound iodine is adequate as noted in Table II (average recovery 100.1%).

The alkaline digestion procedure gives an average recovery of 99.8% (see Table I).

An adequate recovery of total iodine present is obtained by combining the results of the protein-bound fraction and iodide fraction by separation before digestion (Table IV average recovery, 99.3%).

Table IV. Recovery of Total Iodine after Separation and Fusion

Sample No.	Standard Counts	Protein-Bound Iodine and Iodide Counts	% Recovery	% Error
1	405	400	98.8	−1.2
2	330	348	105.4	+5.4
3	404	375	92.8	−7.2
4	351	348	99.1	−0.9
5	465	469	100.9	+0.9
6	8041	7952	98.9	−1.1
		Av. recovery	99.3	

There is a large and variable loss upon acidification of the total iodine sample following digestion (Table III average recovery, 83.2%). This may be avoided by the modification of the method presented in this paper.

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Conversion of Pfund Gage Reading to Dry Film Thickness

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THE Pfund gage (*2*) has for many years been employed for measuring the wet thickness of applied organic coating films. Briefly, this instrument is a plano-convex lens of known radius of curvature, mounted so that its convex surface can be pressed reproducibly into the wet coating film down to the substrate; the diameter of the paint spot thus produced on the convex surface of the lens is employed as a measure of the thickness of the film.

The author has frequently needed to determine the thickness of dried coating films where direct measurement is inconvenient. In many instances, direct measurement of the thickness of the dried film is either very difficult or impossible, because of the nature of the article to which the coating is applied. On the other hand, very few instances have been found in which the Pfund gage could not be employed to obtain an estimate of wet film thickness; later touching up of the small marks left in the finish is ordinarily a simple procedure. Accordingly, an equation has been developed relating the Pfund gage reading to the resulting dry film thickness and a nomogram constructed for the solution of this equation throughout the useful arguments of its factors.

The equation is developed as follows:

Let T = thickness of wet film, millimeters
L = diameter of spot on Pfund film thickness gage, millimeters
r = radius of curvature of lens of Pfund gage = 250 mm.

Then

$$T = \frac{L^2}{16r} \qquad (1)$$

Let W = weight in milligrams of wet coating film on any selected area, A
A = selected area, square inches
G = weight per gallon of coating material, pounds as prepared for application
m = mm. per inch = 25.40005
n = cubic inches per gallon = 231
g = mm. per pound = 453592.4277

Then

(Thickness of wet paint in inches) = $\frac{\text{(weight of wet film per sq. inch)}}{\text{(weight of 1 cu. inch of coating)}}$

Hence,

$$T = \frac{\frac{W}{A}m}{\frac{G}{n}g} = \frac{W}{AG} \times \frac{mn}{g} \qquad (2)$$

Combining Equations 1 and 2,

$$\frac{L^2}{16r} = \frac{W}{AG} \times \frac{mn}{g}$$

and

$$W = L^2AG \times \frac{g}{16rmn} \qquad (3)$$

Let S = per cent solids of the coating material divided by 100—i.e., weight of a sample of the coating material after drying in accordance with a schedule

ordinarily employed to reduce a film of the coating to a state suitable for its ultimate intended use divided by the weight of the corresponding wet sample of the coating material as prepared for application

W_d = weight in milligrams of the dried coating film on the selected area, A—i.e., dried in accordance with a schedule that will reduce the coating film to a state suitable for its ultimate intended use

Then

$$W_d = WS \tag{4}$$

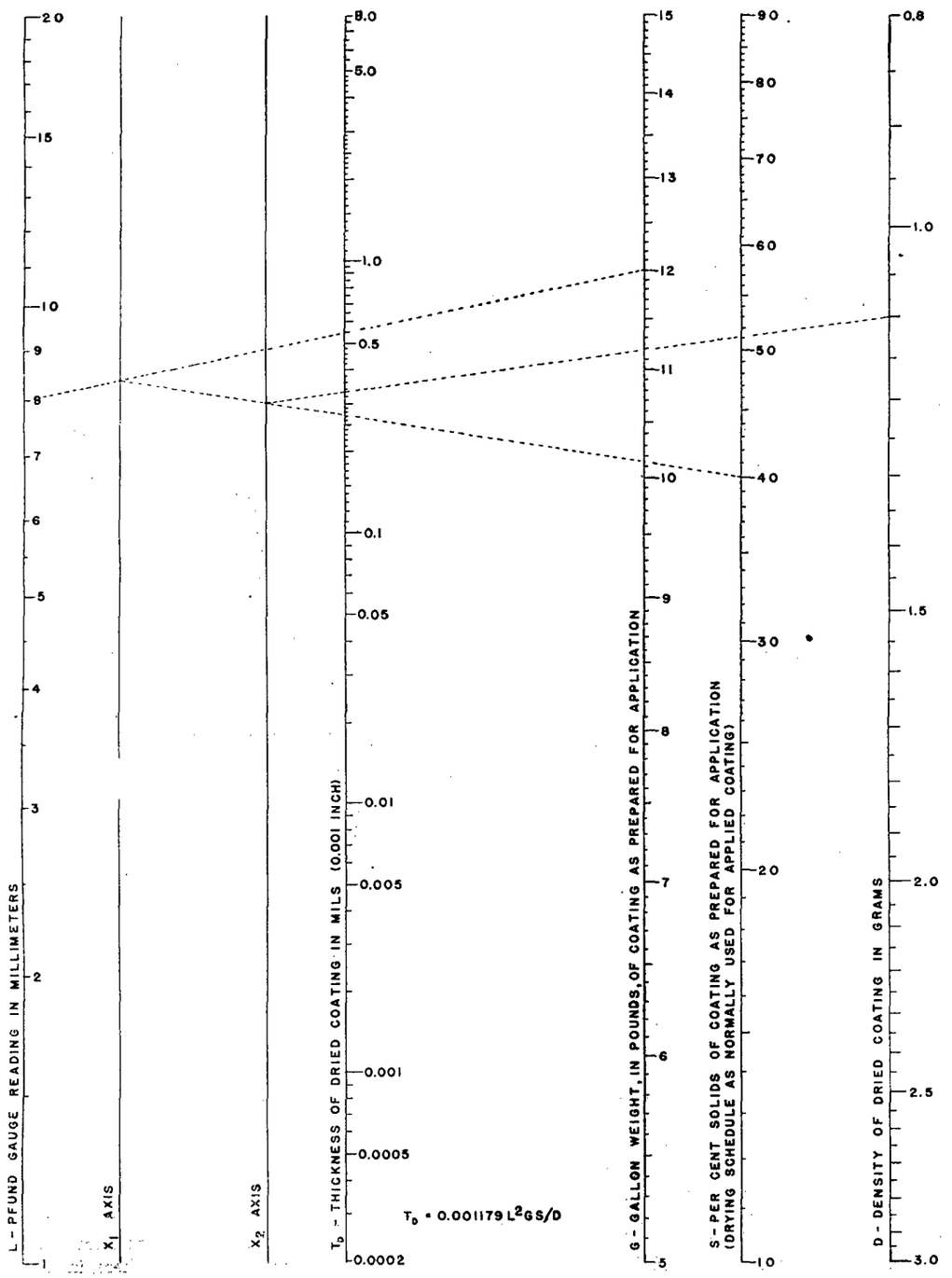
and substituting Equation 3 into Equation 4,

$$W_d = L^2 AGS \times \frac{g}{16rmn} \tag{5}$$

Let D = density (or specific gravity referred to water at 4° C.) of the dried coating film. The density of the film may be determined by flotation of a detached specimen of the dried film in a salt solution, the specific gravity of which is adjusted so that the film specimen neither rises nor falls in the liquid, and subsequent measurement of the specific gravity of the salt solution (*1*)

v = cubic centimeters per cubic inch = 16.38716

T_d = thickness in mils (0.001 inch) of dried film



Then

$$T_d = \frac{W_d}{DvA} \tag{6}$$

(The dimensions of Equation 6 might appear to be in terms of inches; multiplication of both sides of the equation by 1000 might seem to be required in order to express T_d in mils. However, because W_d is expressed in milligrams and D is expressed in grams, a factor of 1000 must appear in the denominator of the right-hand side of Equation 6. Hence the factors of 1000 on the right-hand side cancel and the 1000 times inches on the left-hand side become mils.)

Substituting Equation 5 in Equation 6

$$T_d = \frac{L^2GS}{Dv} \times \frac{A}{A} \times \frac{g}{16rmn}$$

and

$$T_d = \frac{L^2GS}{D} \times \frac{g}{16rmnv} \tag{7}$$

Finally, substituting the values of the constants in Equation 7,

$$T_d = \frac{L^2GS}{D} \times 0.001179 \tag{8}$$

The practical validity of Equation 8 depends upon the physical and chemical properties of the coating material associated with two of the equation factors, D and G. Considering the first of these two, the method for determination of the dried film density will provide accurate results, if proper attention is accorded the details of the experimental procedure. However, the method is tedious to perform. For practical purposes, therefore, it is satisfactory to determine the density of a particular coating material on a specimen of its dried film which is known to have been cured carefully in accordance with the recommended practice. This density value is thereafter employed as if it were a constant associated with the coating material, including subsequent batches of the same material. In using this practical expedient, it must be recognized that in so far as the density of the dried film is sensitive to curing schedule variations, the use of the constant value of D will tend to invalidate Equation 8. It is assumed that the curing practice in subsequent coating application schedules will be substantially in accord with that used for establishing the constant. Physical and chemical tests on the successive batches of the coating material minimize the chance that changes in formulation have occurred which would significantly alter the dried film density.

Equation 8 has so far been applied mainly to industrial organic coatings, for which the cure is effected by forced drying at controlled elevated temperatures. These precautionary measures, the materials employed, and the conditions under which they are employed are conducive to satisfactory stability of the value of D.

With regard to the value of factor G, it is assumed that the gallon weight of the coating material as prepared for application (at which time the value of G may be determined quickly and conveniently by means of a gallon weight cup or a hydrometer) is the same as that value which it will be supposed might be determined for the applied coating at the time of measurement with the Pfund gage. Theoretically, evaporation of solvent from the coating material during the application process and subsequently during any delay time prior to obtaining the Pfund gage reading will generally tend to result in a gallon weight effectively higher at the time of the reading than prior to coating application. The more highly volatile the solvent with which the coating material is reduced for application, and the longer the delay between the time of coating application and the time of making a Pfund gage reading, the more important in respect to invalidating Equation 8 does this theoretical consideration become.

An example of one of the most severe conditions in this regard would be encountered in spray finishing with a nitrocellulose lacquer; the solvent has high volatility, the volatility is assisted by the spraying operation, and the reading with the Pfund gage must be delayed until the end of the spraying operation. Spray application methods, in general, have been found to invalidate Equation 8 even when solvents of low volatility have been employed. On the other hand, results with roller-coated industrial finishes subsequently cured at elevated temperatures have been highly satisfactory. Here, the cheaper solvents of low volatility are ordinarily employed and a Pfund gage reading may be made in a matter of seconds after the coated article leaves the coating machine. Under these conditions, the errors associated with the value of G are well within the uncertainty of the Pfund gage reading itself.

The nomogram is constructed to provide a rapid solution to Equation 8.

Assume that an organic coating as prepared for application has a weight of 12 pounds per gallon and 40% solids, and that the density of the dried coating film is 1.1 grams per cubic centimeter. This coating is to be applied at a wet thickness which will provide a Pfund gage spot diameter of 8 mm. What will be the resulting dry film thickness in mils? The solution to this problem is drawn as a key on the nomogram. Draw a straight line connecting 8 and 12 on the L and G axes, respectively. With the intersection of the L to G line and the x_1 axis as a starting point, draw a straight line to 40 on the S axis. With the intersection of the x_1 to S line and the x_2 axis as a starting point, draw a straight line to 1.1 on the D axis. The intersection of the x_2 to D line with the T_d axis indicates a dry film thickness of 0.33 mil.

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RECEIVED May 29, 1950.

Oxygen Removal in the Polarography of Biological Solutions

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IN CONNECTION with work in this laboratory on the polarography of metal solutions in the presence of proteins, it was necessary to develop a new method for the removal of oxygen, which should find application in any polarographic analysis of solutions containing proteins or other large molecules of biological origin—for example, analyses based on the catalytic sulfhydryl wave (*3*). Gases cannot be bubbled through such solutions because of the formation of very stable foams, and the usual method of oxygen removal is therefore not possible.

This same problem is encountered in pH measurement by means of the hydrogen electrode: Solutions are customarily saturated with hydrogen by bubbling the gas through the solution, but this cannot be done for biological solutions. A special cell has therefore been devised for such solutions by Clark (*1*), in which continuous rocking causes continuous breaking and renewal of the solution surface, and, therefore, fairly rapid equilibration with the surrounding atmosphere, in this case hydrogen.

The authors have applied Clark's technique to the removal of

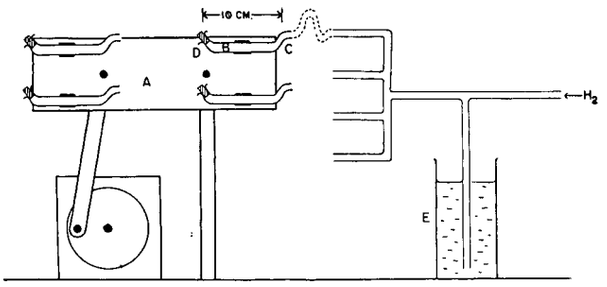

Figure 1. Diagram of Apparatus

oxygen from biological solutions. The apparatus is shown in Figure 1.

The board, *A*, is pivoted at its right end and is caused to rock through an angle of about 20° by the motor-driven speed reducer, which rotates at a speed of 30 r.p.m. Four clamps or fuse clips on the board hold four glass vessels, *B*, in which solutions can be placed. Each vessel is a little more than 10 cm. in overall length, and 1 cm. in external diameter. It is designed to hold about 3 to 4 ml. of solution. The openings, *C*, of the vessels are connected by rubber tubing to a tank of pure hydrogen or nitrogen (only one connection is shown in the figure), and the vessels are flushed out with hydrogen for a few minutes before the solutions are introduced through *D*. The board should be horizontal when the solutions are introduced, and the vessels should not be more than half filled. Openings *D* are then tightly stoppered, and the solutions are rocked for about 1 hour.

A beaker, *E*, filled with a salt solution with about the same vapor pressure as the solutions in the vessels, acts as a safety valve to prevent build-up of gas pressure. The gas flow during the rocking operation is so adjusted that very slow bubbling occurs at this safety valve. The rocking may be stopped after 30 minutes and the system again flushed out to remove the small amount of oxygen accumulated in the atmosphere at that time. In any event equilibrium is established after about 1 hour, and only a negligibly small fraction of the oxygen originally present in the solution remains.

Polarograms obtained with a 1% solution of bovine serum albumin in tartrate medium before and after treatment are shown in Figure 2.

For solutions containing no substances which are reducible by hydrogen, an additional refinement is possible. A platinized platinum disk, sealed into glass tubing, is wedged tightly into opening *D* in place of the stopper described above, and hydrogen is used as the inert gas. Catalytic hydrogen–oxygen combination should take place during the shaking process, and should cause complete oxygen removal to be accomplished somewhat sooner. This procedure is not necessary for ordinary polarographic work, which requires only that the oxygen content shall be reduced to a small value.

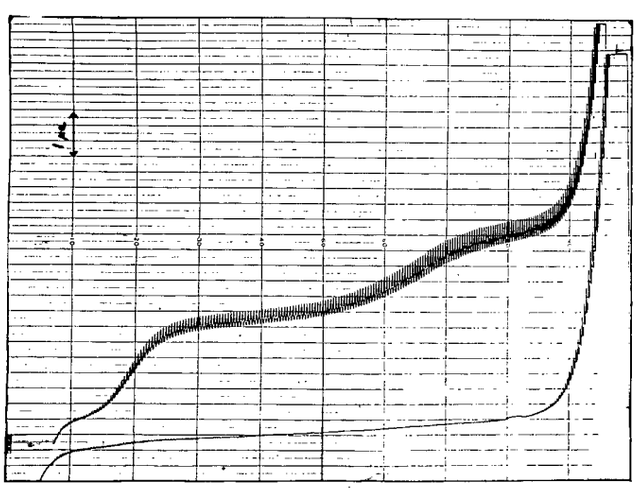

Figure 2. Polarograms of 1% Solution of Bovine Serum Albumin

Before (*upper*) and after (*lower*) oxygen removal

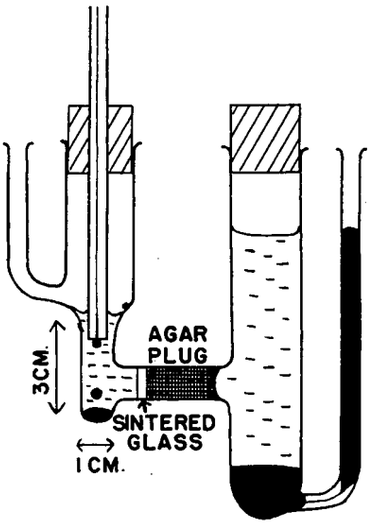

Figure 3. Diagram of Polarographic Cell

Because it is generally not desirable to use a large volume of solution where biological materials are involved, the familiar H-type polarographic cell developed by Lingane and Laitinen (*2*), which contains an internal reference electrode, has been modified for use with 2 to 3 ml. of solution, and to take account of the fact that oxygen need not be removed in the cell. Provision has been made for the passage of a stream of inert gas over the solution during analysis, but none for passage of gas through the solution. A diagram of the cell is shown in Figure 3. Deoxygenated solutions can be poured directly into these cells for immediate analysis. No appreciable reoxygenation ordinarily occurs.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Homer Hall for designing and constructing the shaking apparatus.

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(3) *Ibid.*, p. 411.

RECEIVED August 14, 1950.

Converting Platinum Resistance to Temperature—Correction

In the article on "Converting Platinum Resistance to Temperature" [Eggenberger, ANAL. CHEM., 22, 1335 (1950)] an error occurs in one of the formulas.

$$t_a = A - B\sqrt{S + 10\Delta R}$$

should read

$$t_a = A - B\sqrt{S} + 10\Delta R$$

A Simple Surface Tensiometer

N. B. COLTHUP AND R. E. TORLEY

Stamford Research Laboratories, American Cyanamid Co., Stamford, Conn.

A SIMPLE and direct method has been developed for the rapid measurement of surface tension. The equipment required is generally available and the construction is relatively easy. The method has been found to be sufficiently accurate for general usage and it has proved useful as a rapid means of analysis where surface tension values are functions of the concentrations of the component to be determined.

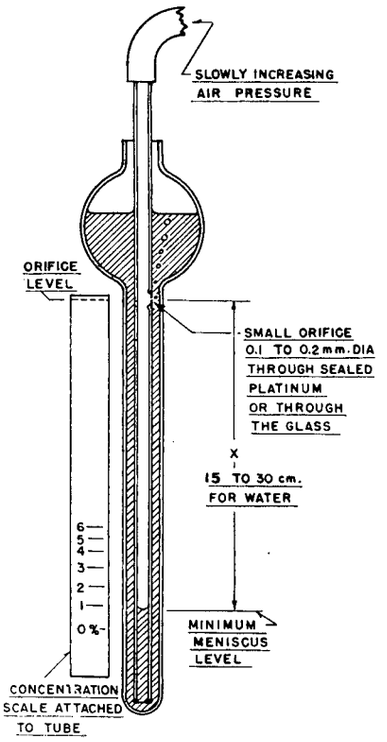

Figure 1

Essentially, the method is an adaptation of the maximum bubble-pressure method, in that the pressure required to force air through a submerged orifice is related to the surface tension of the liquid. However, the experimental details are simpler and the effective speed of a determination is somewhat greater.

A schematic drawing of the apparatus is shown in Figure 1.

It consists of a reservoir for the liquid to be measured, in which the measuring tube is suspended. The latter has a small orifice in the side and is adjusted so that this orifice is under the surface of the liquid. The upper end of the tube is attached to a supply of air or any other inert gas such as nitrogen which is available under pressure. The air is admitted to the tube in a manner such as slowly to increase the pressure until a bubble is forced through the orifice. At the same time the liquid in the lower portion of the measuring tube will be forced out through the bottom and the displacement of the meniscus level below the orifice is read at the moment that the bubble appears. The surface tension can then be computed from the displacement of the meniscus, the diameter of the orifice, and the density of the liquid.

The equation relating the conditions in this system is $xg\rho = 4\gamma/d$ where x is the displacement of the meniscus below the orifice at the moment bubbling occurs, g is the gravity constant, ρ is the density of the liquid, d is the diameter of the orifice, and γ is the surface tension. For all practical purposes, the head of the liquid above the orifice may be neglected, as its effect on the bubble diameter is negligible for heads up to a decimeter or more of water.

CONSTRUCTION AND OPERATION

The reservoir and tube may be made of any transparent material of suitable depth, their relative dimensions being dictated only by the volume of liquid available. The choice of the bubble orifice diameter, on the other hand, depends on the accuracy desired, inasmuch as a smaller diameter will lead to a larger meniscus displacement with a subsequently greater accuracy in its measurement.

In this case the tube was of soft glass having a 10-mm. bore and 1-mm. wall thickness, and was 35 cm. long. The reservoir was a borosilicate glass tube of 15-mm. bore and was 30 mm. long. The orifice diameter was on the order of 0.16 mm., giving a meniscus displacement of about 18 cm. for distilled water. One of the most suitable materials for constructing the orifice is sheet platinum because of the readiness with which it may be wet. A needle was used to punch the hole in the metal and it was then sealed to the soft glass tube at a point about 20 cm. from the lower end. If sheet platinum is not available, the orifice may be constructed by thinning a section of the wall of the glass tube and punching a hole with a hot pointed tungsten rod.

Because the meniscus displacement may be read best by slowly and steadily increasing the pressure on the system, it is convenient to use a supply such as the laboratory air line or tank gas. If the pressure is properly applied, the meniscus will lower slowly until bubbling commences, then rise until it ceases. This cycle will be repeated, readings being taken at each minimum point. In this manner a series of readings may be averaged for optimum precision. In practice it was found that the most suitable rate of depression of the meniscus level for this particular orifice was about 2 or 3 mm. per second.

It is necessary to exercise considerable care to keep the orifice clean. A hot chromic acid bath was found advisable immediately prior to the measurements.

A linear scale may be attached to the reservoir or etched on the glass of the tube for measuring the depression of the meniscus level; the latter is preferable from consideration of possible errors resulting from parallax. If the densities of the liquids to be measured are the same or very nearly so, the scale may be constructed to read directly in terms of dynes per centimeter. Similarly, if the surface tension of a solution is essentially a function of the concentration of a given solute, the scale may be calibrated in terms of its percentage.

Table I. Surface Tension Values

Substance	x, Cm.	Temp., ° C.	Measured, Dynes/Cm.	Reported, Dynes/Cm.	References
Carbon tetrachloride	4.19	25.0	25.9	26.0	(1)
				26.16	(3)
Acetone	7.43	25.0	22.7	23.0	(2)
				22.68	(3)
Benzene	8.27	25.0	28.2	28.1	(1)
				28.22	(3)

From several series of twenty measurements each it was found that the standard deviation of the displacement readings was ±0.01 cm. The agreement between the theoretical value for the orifice diameter as determined using fresh redistilled water and the value obtained with a microscope was good within the accuracy of the optical method, the two figures being 0.01590 ± 0.00002 and 0.0159 ± 0.0002 cm., respectively. Using the orifice

diameter obtained with water as the standard, the surface tensions of freshly distilled and dried carbon tetrachloride, acetone, and benzene were obtained. The agreements of these with values given in the literature are shown in Table I. Of the literature values listed, those of Andreas, Hauser, and Tucker (*1*) were obtained by the pendant drop method, while those reported by Hennaut-Roland and Lek (*2*) were obtained by the capillary rise method of Richards and Coombs (*4*). No reference to the method used to determine the surface tension of acetone was given in the handbook (*3*).

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RECEIVED August 16, 1950.

Density-Composition Relation of Mixtures of Trichlorosilane and Tetrachlorosilane

ROY I. GRADY, JOHN W. CHITTUM, AND C. K. LYON[1]

The College of Wooster, Wooster, Ohio

WHEN trichlorosilane is prepared by passing anhydrous hydrogen chloride over silicon, the product is largely (99% or more) a mixture of trichlorosilane and tetrachlorosilane. Depending upon conditions, such as the rate of flow of the gas and the temperature at which the reaction takes place, the composition of the product may vary greatly. In this laboratory yields ranged from 24 to 93% of trichlorosilane, in agreement with results obtained by Booth and Stillwell (*2*).

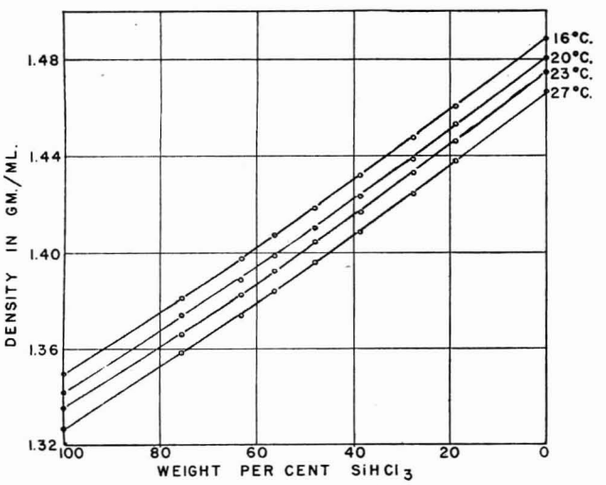

Figure 1. Densities in the System Trichlorosilane-Tetrachlorosilane

A simple relation should exist between the density and the composition of this product. It seemed desirable to prepare a density-composition table for different mixtures of trichlorosilane and tetrachlorosilane at various temperatures, so that the composition of a given mixture could be quickly estimated. In Table I the densities of pure trichlorosilane, pure tetrachlorosilane, and seven weight-per cent mixtures of pure tetrachlorosilane and trichlorosilane are shown at four different temperatures: 16°, 20°, 23°, and 27° C. All densities were determined by means of an Ostwald pycnometer with ground-glass caps, and a volume of approximately 22 ml.

The trichlorosilane used was prepared in this laboratory by passing anhydrous hydrogen chloride over ferrosilicon at elevated temperatures. The trichlorosilane was purified in three stages: by ordinary distillation; through an insulated 17-inch (42.5-cm.) Vigreux column; and finally through a carefully insulated 44-inch Vigreux column. After purification, the trichlorosilane showed a constant boiling point, corrected to 760 mm., of 31.7–31.9° C. and had a density of 1.3415 grams per ml. at 20° C. Booth and Stillwell (*2*) found the boiling point of trichlorosilane to be 31.5° ± 0.1° C. at 760 mm. Stock and Zeidler (*4*) gave the boiling point as 31.8° C. at 760 mm.

The tetrachlorosilane was obtained from The Niagara Smelting Corp., Niagara Falls, N. Y. It was redistilled, and the portion used had a density of 1.4807 grams per ml. at 20° C. It boiled constantly at 56.0° C. at 735.2 mm. Among the recorded values for the boiling point of tetrachlorosilane is 57.57° C. at 1 atmosphere (*3*).

By plotting the data in Table I it becomes possible to interpolate for the composition of any per cent mixture in the system at any temperature between 16° and 27° C. (see Figure 1). Moderate extrapolations to other temperatures beyond this range is possible. The lines are almost linear, indicating a high degree of ideality in the solutions, but not to the extent shown by mixtures of dimethyldichlorosilane and methyltrichlorosilane (*1*).

Table I. Densities in the System Trichlorosilane–Tetrachlorosilane

$SiHCl_3$, Wt. %	Density at 16° C.	Density at 20° C.	Density at 23° C.	Density at 27° C.
100	1.3497	1.3415	1.3350	1.3264
75.86	1.3805	1.3733	1.3659	1.3583
63.28	1.3968	1.3886	1.3825	1.3740
56.48	1.4070	1.3984	1.3921	1.3836
47.71	1.4186	1.4104	1.4040	1.3951
38.68	1.4315	1.4233	1.4169	1.4084
27.79	1.4471	1.4386	1.4328	1.4241
19.02	1.4599	1.4519	1.4459	1.4374
0.0	1.4887	1.4807	1.4746	1.4662

To illustrate the use of this graph: A certain mixture having a density of 1.440 grams per ml. at 18° C. would contain 30% by weight of trichlorosilane.

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(4) Stock, A., and Zeidler, F., *Ber.*, **56B**, 986 (1923).

RECEIVED July 31, 1950.

[1] Present address, Department of Chemistry, Northwestern University, Evanston, Ill.

CRYSTALLOGRAPHIC DATA

44. Sodium Metaborate Dihydrate, $NaBO_2.2H_2O$ (2)

Contributed by JOHN KRC, JR., Armour Research Foundation, Illinois Institute of Technology, Chicago 16, Ill.

THE dihydrate of sodium metaborate is the stable hydrate at room temperature (*1*). It can be recrystallized on a microscope slide from strong aqueous sodium hydroxide solutions as needles and tablets (Figure 1). Sodium metaborate dihydrate is almost monoclinic, with both α and γ almost exactly 90°. The orthographic projection (Figure 2) is drawn to emphasize the relationship to the corresponding monoclinic form.

CRYSTAL MORPHOLOGY

Crystal System. Triclinic.

Form and Habit. Needles and tablets (lying on 010) showing forms {011} and {100} and occasionally {001} and {111}.

Axial Ratio. $a:b:c$ = 0.641:1:0.556. $a:b:c$ = 0.5573:1:0.6421 (*1*).

Interfacial Angles (Polar). $011 \wedge 0\bar{1}1$ = 50°; $010 \wedge 011$ = 65°.

Crystal Angles. α = 91.5°; β = 122.5°; γ = 89°. α = 91°; β = 57°; γ = 91.5° (*1*).

Cleavage. Excellent; parallel to 010 and 100.

X-RAY DIFFRACTION DATA

Cell Dimensions. a = 6.78 A.; b = 10.58 A.; c = 5.88 A. a = 5.86 A.; b = 10.51 A.; c = 6.75 A. (*1*).

Formula Weights per Cell. 4.

Principal Lines

d	I/I_1	d	I/I_1
5.71	0.04	2.008	0.15
5.29	1.00	1.980	0.16
5.03	0.60	1.935	0.18
4.48	0.12	1.901	0.20
3.80	0.61	1.872	Very weak
3.63	Very weak	1.849	0.07
2.91	0.24	1.805	0.10
2.87	0.60	1.787	0.04
2.75	0.34	1.767	0.06
2.64	0.30	1.732	Very weak
2.51	0.51	1.707	0.09
2.42	0.42	1.686	0.17
2.33	0.25	1.655	Very weak
2.29	Very weak	1.634	0.13
2.24	0.23	1.608	0.14
2.21	0.16	1.579	Very weak
2.16	0.13	1.543	0.06
2.13	0.06	1.509	0.05

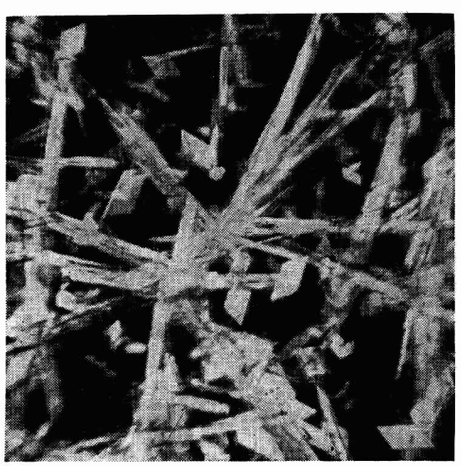

Figure 1. Crystals of Sodium Metaborate Dihydrate

Tablet and needles from aqueous sodium hydroxide, crossed Nicols

Formula Weight. 101.9.

Density. 1.905 (flotation and density balance); 1.89 (x-ray)., 1.909 ± 0.006 (*1*).

OPTICAL PROPERTIES

Refractive Indexes (5893 A.; 25° C.). α = 1.439 ± 0.002 ≃ α'. β = 1.473 ± 0.002 ≃ β'. γ = 1.484 ± 0.002 ≃ γ'. β = 1.469 (*1*).

Optic Axial Angles (5893 A.; 25° C.). $2V$ = 58° (calculated from α, β, and γ). $2V$ = 58°, 62° (*1*). $2E$ = 90° (calculated from $2V$ and β).

Dispersion. $r > v$, strong.

Optic Axial Plane. Approximately ⊥ 010.

Sign of Double Refraction. Negative.

Acute Bisectrix. α.

Extinction. $\alpha' \wedge c$ = 43° in obtuse β; $\alpha'' \wedge c$ = 4° in acute α (Figure 2).

Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma}$ = 1.465 ± 0.002. $n_{average}$ = 1.477 (calculated from Gladstone and Dale formula). R (calcd.) = 30.2. R (obsvd.) = 29.6.

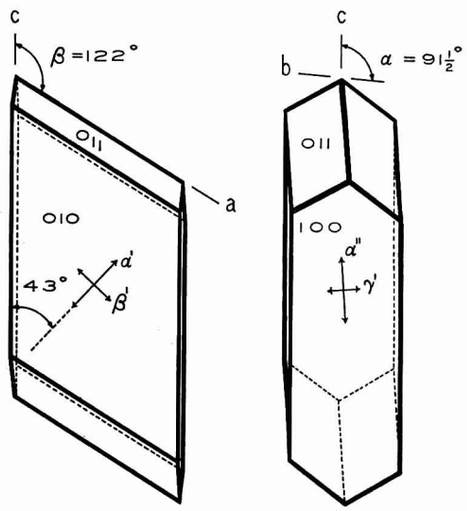

Figure 2. Orthographic Projection of Typical Tablet of Sodium Metaborate Dihydrate from Aqueous Sodium Hydroxide

FUSION DATA. Sodium metaborate dihydrate has an equilibrium transition temperature of 54° C. with $NaBO_2.0.5H_2O$ (*1*). When heated on a hot stage, however, no transition is observed until a temperature of 106° to 110° C. is reached, at which temperature dehydration is observed to proceed slowly from the outer portions of the crystals toward the inner portions of the crystals. The crystals remaining after dehydration are isotropic and transparent and retain the form of the original sodium metaborate dihydrate crystals. On further heating, the isotropic crystals melt with the evolution of gases at about 130° C. Eventually an amorphous and extremely viscous mass is obtained which soon sets to an amorphous sodium borate glass.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the assistance of Irene Corvin in the determination of the powder x-ray spacings and intensities.

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CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Section, Armour Research Foundation, Illinois Institute of Technology, Chicago 16, Ill.

Book Reviews

Methods in Food Analysis Applied to Plant Products. *Maynard A. Joslyn.* 525 pages. Academic Press, Inc., 125 East 23rd St., New York 10, N. Y. Price, $8.50.

Twenty-odd years of teaching by the author have convinced him that students engaged in the laboratory examination of fruit and vegetable products fail to understand the principles of the current methods of analysis, even though these students had prior training in quantitative analyses, organic and inorganic chemistry, and bacteriology. The present book is an attempt to teach such students to apply their training in the fundamentals to food analysis. The result is not just another book on food analysis. The text stresses the principles and limitations of analytical procedures used in food analysis, rather than practices. The discussion is up to date and wide in scope. At the end of each chapter are selected references, so that the reader may go further into the subject.

There may be a few minor criticisms that do not detract from the value of the book. For instance, some food analysts may question the wisdom of allowing fruit juices to ferment prior to drying for ashing (page 89). The reviewer believes that potential dangers arising from the use of perchloric acid have not been emphasized enough to impress the student (page 108). The primary products of oxidation of fats and oils are not in every case peroxides (page 263). Initial oxidation products of unconjugated fatty acids, for instance, are considered to be hydroperoxides. Hazes and turbidities in bottle beer may also be due to proteins, an important cause, and not mentioned (page 265). That part of Table 17 on page 195 should be on page 194, and part of the table on page 194 would then be shifted to page 195.

This book should be a required text for every student taking a comprehensive course in food technology. Food technologists, rusty in their fundamentals, will also find the book useful in refreshing their memories.

HARRY VON LOESECKE

Organic Reagents for Organic Analysis. *Hopkin and Williams Research Laboratory Staff.* 2nd ed. 263 pages. Chemical Publishing Co., 26 Court St., Brooklyn 2, N. Y., 1950. Price, $5.

Although the title of this book does not imply such a narrow limitation, the stated objective was to include "the use of organic reagents in preparing derivatives of organic substances for purposes of identification by melting points."

In a preliminary general survey (32 pages) some fifteen kinds of chemical compounds are considered in terms of useful derivatives. Examples are carboxylic acids, halides, and sulfonamides. In general, selected reagents are recommended for identifying each kind of compound, and often mention is made of various other reagents which have been used (with appropriate references).

The next 100 pages are devoted to instructions for using 48 of these selected reagents, the selections ranging from acetic anhydride to *p*-xenylisocyanate. Under each reagent there are considered the kinds of compounds for which it is applicable. Thus, acetic anhydride is applied to alcohols and phenols and to amines, with citations to various references.

Then follow 106 pages of tabulated melting points of derivatives of specific examples of the fifteen different kinds of compounds with one or more of the selected reagents. Names of classes, specific compounds, and reagents are arranged alphabetically. Many of the constants included were taken from the literature. Those determined in the Hopkin and Williams laboratory are indicated, and the simple apparatus used is described. The subject index (12 pages) does not include the substances listed in the tables.

Altogether, this compilation seems a useful source for the information included. The reviewer missed some comment on the reliability of the constants.

M. G. MELLON

The Polarographic Method of Analysis. *O. H. Müller.* Contributions to Chemical Education No. 2. 2nd ed. xii + 209 pages. Chemical Education Publishing Co., Easton, Pa., 1951. Price, $3.50.

In this second revised and augmented edition, the author purposes "to present a simple account of polarography in a form which can be used by teachers and students in physical chemistry as well as in advanced courses in analytical chemistry." The emphasis is on principles and the scope is descriptive rather than mathematical. It begins with an excellent review of electroanalysis, showing the relation of polarography to other electro methods. A brief description of apparatus includes equipment constructable from parts readily available in many laboratories. Fundamentals of quantitative and qualitative analysis and recent developments are discussed in some detail. Chapters on applications and suggestions for practical polarography complete the book.

A particularly good feature is the inclusion of 26 experiments, and the graphs and tables of data obtained from them constitute the illustrative material of the text. These experiments could be undertaken with profit by anyone desiring an experimental indoctrination in polarography.

The book is especially recommended to students, beginners in the field, and anyone desiring a brief but comprehensive introduction of the fundamentals of polarographic measurements.

JOHN K. TAYLOR

Colorimetric Determination of Traces of Metals. *E. B. Sandell.* Volume III. 2nd edition, revised. xix + 673 pages. Interscience Publishers, Inc., 250 Fifth Ave., New York 1, N. Y., 1950. Price, $9.

The present volume will come as the welcome renewal of an old friend of the analytical chemist, needing but little introduction to those in the field. The volume consists of two main parts, the first dealing with the general requirements of colorimetric and photometric trace analysis and the second part dealing in some detail with the procedures recommended for the separation and determination of 45 elements and the rare earths.

The first section of this book is divided into four parts covering in a general manner (1) trace analysis, (2) methods for the separation and isolation of traces, (3) colorimetry and spectrophotometry, and (4) general colorimetric reagents. The material has been extensively revised and enlarged by about 25%.

The plan of the second section is to present for the element concerned, first, the methods of separation of the element from its likely associates, secondly, the methods available for the colorimetric determination, and finally, detailed procedures for a number of classes of material. This section has been extensively rewritten and enlarged by about 40%. The revision appears to be complete through 1948.

The methods in this book are critically selected and the selection is based in no small measure on the personal experience of the author and his students. The result is a volume which is a laboratory manual of value to the practical analyst as well as a textbook for the advanced student. The fact that an extensive revision is both necessary and practical in a short period of six years is a tribute to both the author's energy and the large advances made in photometric analyses in the past decade. While

a large part of this progress has been in the practical application of known processes to newer materials, progress has also been made in the discovery of new reagents for separations and determinations, and these advances are reflected in the present volume.

A number of small errors of fact in the first edition have been corrected. The printing and binding are good. A third edition could profitably include chapters on important nonmetallics such as phosphorus and boron. The inclusion of more transmittancy–wave-length curves showing the absorbing characteristics of the various compounds would aid considerably in adapting the methods to materials not covered in the volume.

J. L. HAGUE

Practical Microscopy. *L. C. Martin* and *B. K. Johnson.* 124 pages. Chemical Publishing Co., 26 Court St., Brooklyn 2, N. Y., 1951. Price, $2.50.

Most of the small books on microscopy for the beginner or hobbyist deal primarily with sample preparation and a description of objects as seen in the microscope, principally biological. However, in this book, the major emphasis is placed on the optics and a description of the various kinds of microscopes and their proper use. To cover such a wide field, the treatment must of necessity be very brief in such a small book. The English authors, not microscopists, attempt to cover all phases of microscopy, but unfortunately some material, as might be expected, is not up to date even though the book was published in 1951. In the preface the authors state that the book is brought up to date simply by the addition of a chapter (six pages) on the electron microscope.

The book is kept strictly British in language, terms, references, instruments, lens designations, and spelling, although there are a few references and pictures of old German microscopes. To introduce such a publication to this country would only create confusion and frustration, particularly to the inexperienced microscopist for whom the book is intended. No mention is made of any American microscopes or standards, nor is there any attempt to relate the English trade names of materials mentioned to their American counterpart. Only a few ancient English literature references are given covering specific points with, of course, some books (no dates) of a general nature to expand the material.

The book has an excellent, but brief, treatment of light microscope optics and the English microscopes, certainly an important part of practical microscopy in any country; however, the publication should remain in England and not create conflict with different standards and nomenclature outside.

ERNEST F. FULLAM

Einführung in die quantitative chemische Analyse. *A. Gehring* and *R. Fresenius.* viii + 220 pages. Friedr. Vieweg & Sohn, Braunschweig, Germany, 1949. Price, DM 9.50 ($2.26).

This introductory text is designed for a two-semester course in quantitative analysis. The first 17 pages present a concise description of the basic operations of gravimetric analysis. In the next 93 pages attention is directed to the more important cations and anions, which are taken up individually in successive sections of the book. After presenting a summary statement on the various methods—gravimetric, volumetric, colorimetric, etc.—for determining the ion in question, each section describes in concise fashion the details of at least one gravimetric method. Methods for quantitative separation of the various cations and anions are described in the next 47 pages. Eighteen pages are then devoted to the fundamentals of electrochemical analysis and to descriptions of typical methods. The quantitative analysis of various technically important materials, such as gypsum, limestone, dolomite, wolframite, fertilizers, and the like, is described in detail. Possibility of difficulties and corresponding precautions are mentioned at the proper places throughout the book. This well-organized, clearly written text not only provides the beginner with practical laboratory instruction but also gives him the background needed to understand the basic principles of gravimetric and electrochemical analysis and the practical application of these principles.

J. W. PERRY

Einführung in die qualitative chemische Analyse. *R. Fresenius* and *A. Gehring.* viii + 263 pages. Friedr. Vieweg & Sohn, Braunschweig, Germany, 1949. Price, DM 12 ($2.85).

The introductory section (15 pages) of the book discusses basic physical chemistry, with emphasis on the mass action law, and also describes simple typical test procedures. The reactions of importance in qualitative analysis are then described (100 pages) by separate individual consideration of the more important cations and anions. The separation schemes discussed in the next 101 pages might be described as (1) conventional schemes for the more important cations and anions and (2) somewhat more elaborate schemes needed to cope either with less common elements, such as Au, Be, W, etc., or with interfering ions of various kinds. The final section of the book (18 pages) first presents a brief summary of the theoretical basis of spectrographic qualitative analysis and then reviews a fairly large number of spot tests in some detail. A supplement is devoted to the preparation of reagents. Throughout the book, attention is directed to possible sources of difficulty and to corresponding precautions. This is a well-written text which should prove well suited both for teaching beginners and as a reference work for a wide range of problems.

J. W. PERRY

The Analytical Balance. Its Care and Use. *W. M. MacNevin.* xiii + 60 pages. Handbook Publishers, Inc., Sandusky, Ohio. Price, $1.50.

As the title implies, this is a practical monograph on the care and use of the analytical balance. It might be particularly useful to instructors, graduate students, research workers, and others who are responsible for maintenance of balances, as it deals with selecting, mounting, cleaning, adjusting, testing, and repairing. A very specific distinction is drawn between those repairs which can be made by the average scientific worker and those which cannot. More repairs can be made by the average worker than is commonly believed. By showing how, the price of this book may be regained many times over. Of particular use are lists of names and addresses of balance manufacturers and repair specialists. While there are available in the literature articles which deal with balance maintenance, in the opinion of the reviewer, this little book seems superior to most of them with respect to understandability and timeliness.

W. J. BLAEDEL

Qualitative Analysis. Analytical Procedures. *J. C. Hackney.* viii + 144 pages. J. C. Hackney, Indiana University, Calumet Center, East Chicago, Ind., 1950. Price, $1.75.

This is Part III of a book on qualitative analysis and is not intended as an independent textbook. The user must rely upon other texts for the remainder of the course, as Part I, Principles and Calculations, and Part II, Properties and Descriptive Chemistry of the Elements, are in preparation.

The present volume is intended to supply specific instructions for the systematic analysis of the common inorganic cations and anions, and to discuss the chemical reactions involved. The

directions for the laboratory procedures are unusually thorough and detailed. Likewise, the notes on the procedures are comprehensive and well explain the reasons for the various steps. Sufficient tests are given to make possible the determination of the original state of the element in the sample. The remarks on possible dangerous chemicals are more complete than usual and are well taken.

The book is the second printing of a mimeographed edition and the binding is not very sturdy. There are a number of mistakes in spelling and some in grammar, which presumably would be eliminated in a printed edition. There are no literature references at all and preliminary experiments are yet to be added. It would seem premature to place this book on the market in its present state.

ELLWOOD M. HAMMAKER

Official Methods of Analysis of the Association of Official Agricultural Chemists. *H. A. Lepper*, Chairman. 7th edition. xv + 910 pages. Association of Official Agricultural Chemists, P.O. Box 540, Benjamin Franklin Station, Washington 4, D. C., 1950. Price, domestic $10, foreign $10.50.

Quinquennially, food chemists throughout the world await the appearance of a new edition of the AOAC book of methods. This new edition contains much new material without increasing the number of pages. As stated in its preface, "The title of the present edition, 'Official Methods of Analysis,' reflects the recent action of the Association in deleting the 'tentative' classification. Methods now are designated 'first action' upon first adoption and 'official' upon subsequent final adoption. Thus is continued the long-established custom of providing a year's notice to all who may be interested (by publication of a method upon first adoption) that final adoption is pending, thereby permitting opportunity for such further critical study as may be desirable before final approval of the official status is granted."

In comparing this new edition with the sixth edition, 1945, one notes that the chapters on naval stores, leathers, and tanning materials have been deleted, as has the section in the appendix dealing with definitions of terms and interpretations of results on fertilizers and liming materials. A new chapter entitled "Economic Poisons" includes insecticides, fungicides, herbicides, rodenticides, and disinfectants. The space given to preservatives, cosmetics, and economic poisons has been substantially enlarged. The chapter on preservatives includes both preservatives and artificial sweeteners. The text of the various methods is written in a lucid style which enhances the value of the publication. It is singularly free from typographical errors and the cross referencing is simple and effective. Ever since the appearance of the first edition of this publication, each edition has been characterized by the utmost care in the preparation of the manuscript. All of the methods published, particularly those designated as "official," have been adopted only after careful and painstaking collaborative studies to determine their reproducibility in the hands of different analysts. This book is truly the food chemist's *vade mecum* and reflects the greatest professional credit on the officials and workers in the Association of Official Agricultural Chemists who, by their untiring efforts, have produced this monumental work on food, drug, and cosmetic analysis.

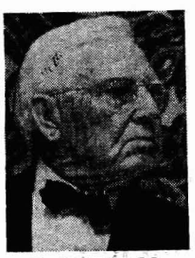
F. C. BLANCK

NEW BOOK

Les Méthodes d'Analyse des Réactions en Solution. *G. Charlot* and *R. Gauguin*. viii + 328 pages. Masson et Cie, 120 Boul. Saint-Germain, Paris VI^e, France. $6.50.

Scientific Apparatus Makers Meeting

THE climax of the 33rd annual meeting of the Scientific Apparatus Makers Association, White Sulphur Springs, W. Va., April 15 to 18, was the presentation of the SAMA Award for outstanding service to Col. Evan E. Kimble, pioneer leader in the field of machine- and handmade glassware and founder of the Kimble Glass Co., now a division of Owens-Illinois Glass Co.



E. E. Kimble

The SAMA Award has been presented only twice since the organization was founded—to Morris Leeds, founder and chairman of the board, Leeds & Northrup Co., who was honored in 1949, and to Harvey N. Ott, a founder and former president of the Spencer Lens Co., now a division of the American Optical Co., who received the award in 1950. Colonel Kimble, who is 83, flew from his Florida home to accept the award at the annual SAMA dinner. Presentation of the engraved, leather-bound award certificate was made by H. B. Richmond, chairman of the board, General Radio Corp., and a past president of SAMA.

In his presentation, Mr. Richmond called Colonel Kimble "the person, more than any other individual, who made America free of European influence in the availability of chemical glassware, especially in accurate tubing." Colonel Kimble led the fight at the end of World War I "to protect American-made chemical ware through tariff protection, so that the United States could stand free and independent from its former position of complete dependence upon European sources," Richmond said.

Three scientific instrument and laboratory apparatus companies—Corning Glass Works, Eimer and Amend Division of Fisher Scientific Co., and Taylor Instrument Companies—were honored at the SAMA dinner, when their representatives accepted membership certificates for their companies in the SAMA Century Club. The club, now numbering six members, was founded in 1943 to recognize and honor 100 years of service to American science by instrument and apparatus makers and distributors. It is sponsored by the Scientific Apparatus Makers Association, the industry's national organization.

Left to right. W. J. Murphy, Paul Block, Harlan Hobbs, Gerard Piel, and R. J. Painter

A feature of the meeting was a forum on the topic "What Does America Expect from the American Scientific Instrument Industry?" Harlan Hobbs, Kimble Glass Co., and chairman of the SAMA Public Information Committee, presided. The participants were: Walter J. Murphy, editor, ANALYTICAL CHEMISTRY, *Industrial and Engineering Chemistry*, and *Chemical and Engineering News*, and director of the ACS News Service; Gerard

Piel, editor and publisher, *Scientific American;* Paul Block, publisher, *Toledo Blade* and *Pittsburgh Post Gazette;* and Robert J. Painter, ASTM assistant secretary and editor of the *ASTM Bulletin* who substituted for Howard W. Blakeslee, science editor of the Associated Press.

Murphy discussed the question "What Does the American Chemist Expect of the Industry?"; Gerard Piel spoke on the topic "Can We Attain and Maintain World Leadership?"; Paul Block replied to the question "What Is the Lay Public's Interest in the Scientific Instrument Industry?"; while Mr. Painter attempted to answer the question "What Is Expected of the Industry in Case of Another Major National Emergency?"

Complimenting the scientific apparatus and instrument industry for its outstanding accomplishments, Murphy warned that most of the training emphasis may be pitched at the technician level, and that too little emphasis will be placed on the teaching of the fundamentals upon which the field of instrumentation has been created. He urged instrument makers and large employers of analytical chemists to provide more graduate and postgraduate fellowships so as to create an adequate number of highly trained personnel for the future.

Piel reported that among the more theoretical researchers in this country, there is a definite suspicion that the scientific apparatus industry in America has attained its present pre-eminence largely through default. "His contacts," he said, "seemed to feel that a very large proportion of the fundamental research that has made possible the advances in instrumentation have been made abroad." He urged American manufacturers to increase heavily the amount of fundamental research now being conducted.

Block, in a very interesting and illuminating discussion of the point of view of the public, reported that the accomplishments of the industry are practically unknown to the man on the street. This statement led to considerable discussion as to ways and means of better informing the public of what the industry is doing to help to improve the standard of living and to provide the sinews of war.

Painter reviewed briefly the volume of instruments and apparatus required in World War II and pointed out significantly that even greater production demands will be made upon instrument makers if we are forced to fight a third world war.

Heavy emphasis was placed at the meeting on relationships with government in an emergency. "The Controlled Materials Plan" was discussed at length by Walter C. Skuce, staff assistant to the administrator, National Production Authority, while "The Military Production Program" was analyzed by Captain Rawson Bennett, director, Electronic Production Resources Agency, Munitions Board. "Priority Controls Affecting Scientific Instrument Manufacturers and Distributors" was discussed by J. H. Kincaid, chief of the Scientific Instruments Section, Scientific and Technical Equipment Division, National Production Authority.

The principal address on April 17 was given by Henry F. Dever, president, Minneapolis-Honeywell Regulator Co., Brown Instruments Division. In this talk "Living in an Emergency Economy," he pointed out that today's growing manpower shortage will force the management of American industry to put an entirely new emphasis on manpower-strengthening programs. "From now on," said Dever, "this subject will compete actively with new machine and tooling programs for the attention of progressive industry executives."

According to Dever, special emphasis must be placed on engineering manpower, which is highly important to the scientific instruments field. He pointed out the present scarcity of engineers, young or old, and prophesied that the end of this shortage is not yet in sight.

Dever stated his belief that industry has the power to increase productivity in various ways. He outlined methods that could be followed to increase worker effectiveness and maximum coordination within each organization. He particularly advised his audience to watch wage and salary relationships between new and old employees and between rank and file workers at supervisory levels. "The supervisors," he said "are your key people—the backbone of your organization. Obviously to such people salary is not everything, but it ranks high in the morale factor. I think you cannot afford not to be right in wage and salary levels in these echelons."

In closing, the speaker stated, "the key to the progress of America's engineering and manufacturing organizations has long been their aggressive interest in adopting improved manufacturing methods, based upon exhaustive analysis of new machines and new techniques as they are developed. Even though the sums of money involved may be large, approval is given because confidence has been built up that the expenditure of today makes possible the profit of tomorrow."

At the closing session J. Clair Evans of Denver Fire Clay was elected president.

THE ANALYST'S CALENDAR....

Standards and Standard Methods to Be Discussed at Summer Symposium in Washington

A RECORD attendance is expected for the Fourth Annual Summer Symposium, cosponsored by the Division of Analytical Chemistry of the ACS and ANALYTICAL CHEMISTRY. To be held at the Shoreham Hotel, Washington, D. C., on June 14, 15, and 16, the subject of the symposium will be "Standards and Standard Methods." The cosponsoring organizations have arranged the program in Washington in honor of the National Bureau of Standards, which is commemorating its fiftieth anniversary this year.

R. M. Fowler, Union Carbide and Carbon Research Laboratories, Inc., Niagara Falls, N. Y., is general chairman of the symposium and H. A. Bright, National Bureau of Standards, is chairman of local arrangements.

CHEMISTRY IN CRIME DETECTION

On Friday at 6:30 P.M. there will be a dinner meeting, at which Wallace R. Brode, associate director of the National Bureau of Standards, will act as toastmaster. Donald J. Parsons, scientific

chief of the laboratory of the Federal Bureau of Investigation, will speak on "Chemistry in Crime Detection." He will point out some of the scientific and analytical problems inherent in the work of the FBI and explain the special methods and techniques developed. Parsons will also discuss recent interesting cases from the files of the FBI to illustrate the application of chemistry in crime detection.

Those planning to attend the dinner should send advance notice to T. P. Sager, Division of Chemistry, National Bureau of Standards, Washington 25, D. C.

D. J. Parsons

HOTEL RESERVATIONS

The Shoreham Hotel has set aside a block of rooms for guests attending the symposium. It is important that individuals make their own reservations with the hotel and that in so doing they tell the hotel that they are attending the Analytical Symposium. Reservations must be made promptly, as the hotel will not guarantee reservations after June 1.

Rates at the Shoreham are $6 to $8 for single occupancy and $9 to $10 for double occupancy.

Rates at the Wardman Park Hotel, which is one block from the Shoreham, are $5 to $8 for single occupancy and $8 to $11 for double occupancy.

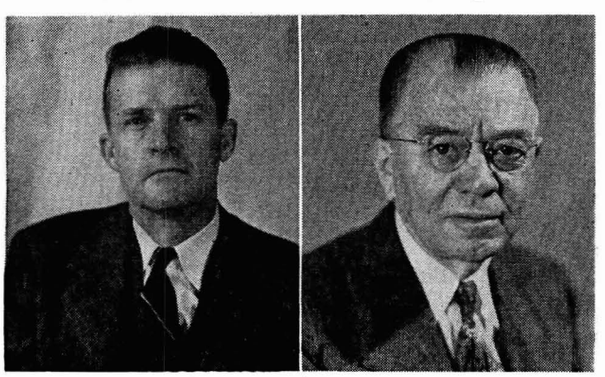
REGISTRATION

Registration will be at the Shoreham Hotel on Thursday, June 14, from 1 to 6 P.M.; on Friday from 9 A.M. to 6 P.M.; and on Saturday from 9 A.M. to 12 noon.

Registration fees will be $3 for individual members of the Society, designated representatives of corporation members (only one for each corporation membership), and visitors other than chemists or chemical engineers residing in the United States; $6 for nonmember chemists or chemical engineers residing in the United States, regardless of nationality. Associates of divisions of local sections are not members of the ACS, and if they are chemists or chemical engineers, are subject to the $6 fee. Full-time students of chemistry, both graduate and undergraduate, are given the courtesy of registration on the same basis as members of the Society.

The final program is as follows:

R. M. Fowler **H. A. Bright**



National Bureau of Standards

Thursday, June 14

1:00 P.M. Registration
1:30 P.M. Visit to National Bureau of Standards
5:00 P.M. Tea at residence of director of bureau

Friday, June 15

9:00 A.M. Registration
10:00 A.M. Welcome by chairman of division, by chairman of symposium, and by director of National Bureau of Standards
10:10 A.M. Basic Philosophy of Standards. GEORGE D. BEAL, Mellon Institute
11:00 A.M. Study of a Fundamental Standard—Hydrochloric Acid. H. A. LIEBHAFKSY, General Electric Co.
2:00 P.M. Preparation of Pure Chemicals for Standards. H. V. FARR, A. Q. BUTLER, AND S. M. TUTHILL, Mallinckrodt Chemical Works
2:30 P.M. Standards for Reagent Chemicals. EDWARD WICHERS, National Bureau of Standards
3:00 to 3:15 P.M. Recess
3:15 P.M. Requirements for Primary Redox Standards. V. A. STENGER, Dow Chemical Co.
4:00 P.M. Standard Sample Program of the National Bureau of Standards. H. A. BRIGHT, National Bureau of Standards
6:30 P.M. Dinner at Shoreham Hotel (advance reservation) Toastmaster, WALLACE R. BRODE, associate director, National Bureau of Standards. Speaker, D. J. PARSONS, Federal Bureau of Investigation, "Chemistry in Crime Detection"

Saturday, June 16

9:30 A.M. Emission Spectrographic Standards. B. F. SCRIBNER, National Bureau of Standards
10:00 A.M. Intercompany Standards in the Steel Industry. ARBA THOMAS, Armco Steel Co.
10:20 to 10:45 A.M. Recess
10:45 A.M. Standardization in the Chemical Industry. W. A. KIRKLIN AND W. W. BECKER, Hercules Powder Co.
11:15 A.M. Intercompany Standards and Standardization in the Petroleum Industry. F. D. TUEMMLER, Shell Development Co.
2:00 P.M. Standards of Unstable Materials. L. E. WEST, Eastman Kodak Co.
2:30 P.M. Standardization of Microchemical Methods and Apparatus. C. O. WILLITS, Eastern Regional Research Laboratory
3:00 to 3:15 P.M. Recess
3:15 P.M. An Investigation of Precision and Accuracy in Chemical Analysis. W. G. SCHLECHT, U. S. Geological Survey
3:45 P.M. Design and Interpretation of Interlaboratory Studies of Test Methods. GRANT WERNIMONT, Eastman Kodak Co.

TOUR OF LABORATORIES AT NATIONAL BUREAU OF STANDARDS

A guided tour of selected laboratories is planned for members of the Society and their guests on Thursday, June 14, to begin at 2 P.M. Those members who wish to visit laboratories that are not on the guided tour can arrange for this when they arrive at the bureau. Headquarters for all ACS visitors will be Room 214 Chemistry Building (Chemistry Lecture Room).

The guided tour will include such activities as standards of length and mass, the production and analysis of standard samples, microwave spectroscopy, the omegatron, and the determination of the gyromagnetic ratio of the proton. The tour will end at 5 P.M. at a tea to be held at the residence of the director, Edward U. Condon, which is located on the bureau's grounds.

The bureau is located about 5 minutes' distance by taxi from the Shoreham Hotel. A limited number of parking spaces will be reserved on the bureau's grounds for private cars.

Gordon Research Conferences

THE 1951 Gordon Research Conferences, sponsored by the American Association for the Advancement of Science, are to be held from June 18 to August 31 at Colby Junior College, New London, N. H., and New Hampton School, New Hampton, N. H. Those interested in attending the conferences should send their names immediately to W. George Parks, Department of Chemistry, University of Rhode Island, Kingston, R. I. The program includes sessions of especial interest to analytical chemists.

At Colby Junior College

INSTRUMENTATION

H. W. WASHBURN, Chairman
HOWARD CARY, Vice Chairman

July 30
- Recent Advances in Infrared Instrumentation. VAN ZANDT WILLIAMS.
- Microspectrophotometry. R. C. MELLORS.
- Interference Filter Spectroscopy. BRUCE BILLINGS.

July 31
- Refractometry. J. W. FORREST.
- Instrument Art and Experimental Science. JOHN STRONG.
- Magnetic Amplifiers. G. W. DOWNS.

August 1
- Magnetic Measurements in Physical Chemistry. P. W. SELWOOD.
- Instrumentation Research at the Bureau of Standards. W. A. WILDHACK.
- Process Control Instrumentation. E. C. MILLER.

August 2
- Mass Spectrometry of Solids. JOHN HIPPLE.
- Automatic Mass Spectrometer Process Control.
- Mass Spectrometer for Aqueous Samples.

August 3
- Microwave Refractometry.
- Neutron Spectroscopy.

At New Hampton School

STATISTICS IN CHEMISTRY

H. M. SMALLWOOD, Chairman
J. W. Tukey, Vice Chairman

July 23. **Statistics in Analytical Chemistry**
- Discussion. GRANT WERNIMONT.
- Control of Accuracy and Precision of Industrial Tests and Analyses. J. A. MITCHELL.
- Design of Experimental Work to Develop Analytical Methods. JOHN MANDEL.
- Evaluation of Elementary Causes of Deviations in Spectrochemical Analyses. JOSEPH GEFFNER.
- Standardization of Test Methods through Interlaboratory Tests. M. M. SANDOMIRE.

July 24. **Role of Statistics in Chemistry**
- Discussion. J. W. TUKEY.
- What Kind of Statistics Is Important in Chemistry? GEORGE KIMBALL.
- Comparative Experimentation. W. G. COCHRAN.
- Empirical Knowledge *vs.* Insight. J. W. TUKEY.
- **Training in Statistics for Chemists.** Round Table Discussion.

July 25
- Clinic. Discussion of concrete problems by members of the conference.

Statistics in Laboratory Experimention
- Discussion. W. J. YOUDEN.
- Problems in Spectrochemical Analysis. B. F. SCRIBNER.
- Use of Ranks in Latin Square Designs. FRANK WILCOXON.

July 26. **Statistics in Laboratory Experimentation** (*continued*)
- Precision Reference Temperatures. J. I. MINOR.
- Selection of Economic Precision Limits for Laboratory Tests. LOUIS TANNER.

Statistics in Industrial Research
- Discussion. R. H. NOEL.
- Resolution of Interactions in Chemical Experimentation. W. L. GORE.
- Utility of Variance Analysis. A. W. KIMBALL, JR.

July 27. **Statistics in Industrial Research** (*continued*)
- Role of Statistics in Increasing Productivity of Industrial Research. C. A. Bicking.
- Subject to be announced. K. A. Brownlee.
- Linear Extrapolation. C. DANIEL.

CURRENT TRENDS IN ANALYTICAL CHEMISTRY

W. E. CAMPBELL, Chairman
J. J. LINGANE, Vice Chairman

Trace Analysis

August 6
- Philosophy of Trace Analysis. S. E. Q. ASHLEY.
- Fluorescence Analysis. C. E. WHITE.

August 7
- Colorimeter Trace Analysis. J. YOE.
- Trace Analysis of Petroleum Products. H. LEVIN.

August 8
- Colorimetric Trace Analysis. E. H. SWIFT.
- Colorimetric Trace Analysis. W. D. COOKE.
- Electrolytic Trace Analysis Involving Radioactive Indicators. L. B. Rogers.
- Trace Analysis Involving Electrolytic Reactions at a Rotated Mercury Electrode. T. S. LEE.

August 9
- Trace Gas Analysis. L. K. NASH.
- Radioactive Tracer Analysis.

August 10
- Isotopic Dilution Analysis.

American Society for Quality Control. Annual Convention, Cleveland, Ohio, May 23 and 24

Society for Applied Spectroscopy. Sixth Annual Meeting, New York, N. Y., May 25 and 26

Symposium on Molecular Structure and Spectroscopy. Ohio State University, Columbus, Ohio, June 11 to 16

Fourth Annual Summer Symposium. Washington, D. C., June 14 to 15

American Council of Commercial Laboratories. Los Angeles, Calif., June 14 and 15

American Society for Testing Materials. Atlantic City, June 18 to 22

Gordon Research Conference. Colby Junior College, New London, N. H., and New Hampton School, New Hampton, N. H., June 18 to August 31

International Union of Crystallography. Second General Assembly, Stockholm, Sweden, June 27 to July 3

International Congress on Analytical Chemistry. United Kingdom, August 8 to 13

3.622. Mold appeared in five other saturated solutions at ages of 15, 8, 30, 30, and 8 days. After 17 days the pH of these solutions had increased by 0.000, 0.040, 0.008, 0.001, and 0.028 unit, respectively.

All pH values were computed from the e.m.f. at 25° of a double hydrogen-electrode cell in which a saturated solution of potassium chloride was interposed between the two electrode compartments. A phosphate buffer mixture 0.025 *M* with respect to both potassium dihydrogen phosphate and disodium hydrogen phosphate, pH 6.860 at 25° (*2*, *3*), served as a reference standard.

The author's experience fails to confirm Lingane's statement (*4*) that solutions of potassium hydrogen tartrate appear more stable than those of potassium hydrogen phthalate. The pH of a 0.05 *M* phthalate buffer solution, prepared and preserved in the same manner as the tartrate solutions, was found to have increased 0.005 unit in 130 days. The stability of Lingane's tartrate solution over an extended period illustrates the differences that may be encountered as the result of chance contamination by molds and other microorganisms. Inasmuch as sterile conditions are not conveniently maintained in the preparation and use of tartrate pH standards, it is important that these solutions be made as needed.

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(2) Bates, R. G., and Acree, S. F., *J. Research Natl. Bur. Standards*, **34**, 373 (1945).
(3) Hitchcock, D. I., and Taylor, A. C., *J. Am. Chem. Soc.*, **59**, 1812 (1937).
(4) Lingane, J. J., ANAL. CHEM., **19**, 810 (1947).

Electronic Timer-Controller. Donald M. Peppard, Wyandotte Chemicals Corp., Wyandotte, Mich.

DURING the past few years magnetically operated still heads for distilling columns (Oldershaw, Piros-Glover, Du Pont, and others) have become increasingly popular. A necessary auxiliary instrument with such a still head is a timer-controller to operate the valve and control the reflux ratio. The timer-controller described is simple, inexpensive, accurate, and extremely easy to use, and its reliability has been demonstrated by steady use for 3 years in this and other laboratories. It has all the flexibility associated with the electronic timers previously described (*3*) plus certain added features. Thus, the present instrument has a circuit compensated for line voltage variations, and the time intervals are independent of the relay adjustment and remain constant for the useful life of the tube. Furthermore, it is usually possible to replace the tubes without having to make compensating adjustments.

The circuit uses a condenser-resistor combination as the basic timing element (*2*). It consists of a condenser whose charge and discharge times determine the off and on periods of the controlled circuit. The desired periods may be varied over a wide range by the choice of resistance and capacitance values. Those given are for a particular model designed for distillation purposes and will serve as guides to the constructor building a unit for other purposes.

Figure 1 gives a diagram of the timer.

The transformer, T, supplies the filament voltages for tubes V_1 and V_2, and also the high voltage for V_1. V_1 rectifies the high voltage supplying about 250 volts across R_1R_2. At the start of a cycle condenser C_2 is charged through the resistors of switch S_2 and relay L. C_2 is connected to the grid of V_2 through R_7 and R_8. The grid potential of V_2 therefore rises as C_2 is charged. Superimposed on this direct current voltage is an alternating current voltage, from C_5, R_9 through C_4, which is 90 electrical degrees ahead of the voltage applied to the plate of V_2 (*1*). This leading voltage does several things—it ensures that V_2 will start conducting and stop conducting instantaneously, thus making the timing interval independent of the relay type or adjustments; it minimizes the effects of individual tube characteristics; and it provides compensation for line voltage fluctuations. The grid of V_2 reaching a potential P_1 will fire the tube, thus operating the relay. C_2 will now discharge through L and R_5. When the voltage of C_2 has dropped sufficiently to reduce the potential at the grid of V_2 to P_2 the tube will suddenly stop conducting, allowing the relay to drop out and thus initiating another cycle.

The other set of contacts on L supply power to the outlet receptacles. The pilot lights indicate the duration of the timed intervals, permitting easy calibration. Switch S_2 will turn off both outlets without shutting off the timer. S_4 will turn on outlet *NO* without affecting the timer. This switch could be a double-pole switch and turn on both outlets.

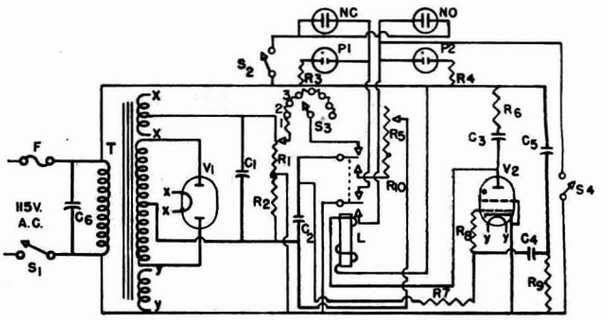


Figure 1. Diagram of Timer-Controller

C_1. 1 mfd., 600 volts
C_2. 5 to 10 mfd. Hermetically sealed with high internal resistance. Ceresin wax or silicone used to stop surface leakage at all high impedance points
C_3. 4 mfd., 450 volts electrolytic
C_4. 0.02 mfd., 600 volts. Hermetically sealed with high internal resistance
C_5. 0.05 mfd., 600 volts
L. D.P.D.T. relay, 1–10 kilohms d.c. Ceresin wax or silicone used to stop surface leakage at all high impedance points
P_1, P_2. NE51 neon
R_1. 10,000 ohms, 10 watts, adjustable
R_2. 10,000 ohms, 10 watts
R_3, R_4. 200,000 ohms, 0.5 watt
R_5. 0.5 megohm
R_6. 500 ohms, 5 watts
R_7. 5 megohms, 1 watt
R_8. 1 megohm, 1 watt
R_9. 4500 ohms, 1 watt
R_{10}. 50,000 ohms, 0.5 watt
S_1, S_2, S_4. S.P.S.T. toggle switch
S_3. Ceramic selector switch (Mallory 172C). Resistors chosen to suit time intervals desired (0.22, 0.22, 0.22, 1.2 megohms, etc.)
T. 240–0–240, 6.3, 5 volts
V_1. 5Y3
V_2. 2050
F. Fuse, 1 amp.
NC. Outlet
NO. Outlet

Four timers have been built and used to operate magnetic still heads, reciprocating stirrers, and magnetic pumps (*4*). Like all devices using relays, the contacts may require occasional cleaning. The frequency of servicing is determined to a large extent by the amount of dust and fumes to which a given relay design is subjected. Sealed relay contacts, if available, will, in most instances, eliminate any trouble due to this cause. Relay coil resistances are not critical within the maximum current rating of the tube, 1000 ohms being a safe lower limit. Above 3000 ohms it is suggested that the value of R_6 be increased or the value of C_3 decreased, so that the time constant of the relay circuit will not be made too long, particularly when time intervals of less than 1 second are desired. With reference to the voltage divider, S_3, heating the resistors while soldering them in place may alter their values. If this happens, this resistor is discarded and replaced with one with longer leads. It is better and easier to use two resistors in series or parallel to get the exact value desired, rather than to buy expensive precision resistors or try to select a particular value from an assortment.

If a suitable transformer is not available, a high voltage supply may be made by substituting a selenium rectifier voltage-doubler for the transformer-rectifier tube combination shown. The heater of the 2050 could be operated by a small transformer or in series with a resistor across the line.

LITERATURE CITED

(1) Cockrell, W. D., "Industrial Electronic Control," p. 186, New York, McGraw-Hill Book Co., 1944.
(2) *Electronics*, **14**, Reference charts, 33–64 (June 1941).
(3) Harvey, R. B., IND. ENG. CHEM., ANAL. ED., **18**, 331 (1946).
(4) Oliver, G. D., Bickford, W. G., Todd, S. S., and Fynn, P. J., *Ibid.*, **17**, 158 (1945).

AIDS FOR THE ANALYST.....

Distillation of Rubber Cement. Jerome L. Been, Martin M. Grover, and Kenneth J. Ewing, Rubber & Asbestos Corp., Bloomfield, N. J.

THE following method has been found useful in application of Engler distillation (*1*) to rubber cement, to determine whether two cements have the same solvent combination or to determine the amount and kind of solvent present in a cement of unknown composition.

The equipment is set up as directed by the American Society for Testing Materials (*1*), and 100 grams of rubber cement are charged into the flask. An oil bath is used to prevent local overheating and subsequent decomposition of the cement. It is convenient to collect the solvent on a tared balance, recording temperatures at every 2 grams of solvent recovered.

In order to carry the distillation to completion, 0.5 gram of high-boiling silicone oil is added. Without it, the cement will foam and slug over and the rubber film will not break to allow the solvent to escape at the required rate. The silicone oil must be sufficiently high boiling so that it will not distill over (the oil particularly recommended is No. 9981LTNV70, made by the General Electric Co., Schenectady, N. Y.). Such an oil exerts a negligible effect on the vapor pressure of the solvent.

It is probable that other high-boiling surface active agents may be used in place of silicone oil. The procedure is also suggested for use with other high polymer solutions of high viscosity.

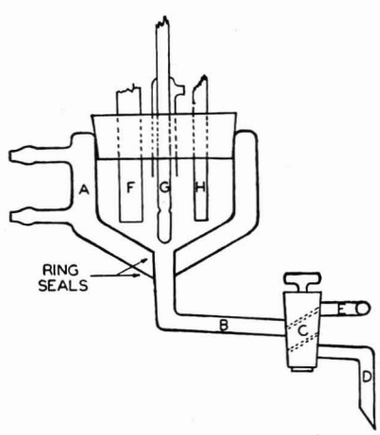
LITERATURE CITED

(1) American Society for Testing Materials, Philadelphia, Pa. "Standard Method of Test for Distillation of Gasoline, Naphtha, Kerosene, and Similar Petroleum Products," A.S.T.M. Designation D 86–46.

Versatile Cell for Routine Polarographic Analysis. Leo D. Frederickson, Jr.[1], Monsanto Chemical Co., Monsanto, Ill.

MANY types of polarographic cells have been described in the literature, to meet general needs and for specific applications. Lingane (*3*) designed a cell for rapid analysis, while Langer (*1*) describes a unique cell incorporating an external reference anode. Other general types are discussed in the review of polarographic instrumentation by Lingane (*2*).

[1] Present address, Monsanto Chemical Co., St. Louis, Mo.



Frederickson Cell

In routine polarographic analysis, most of the previous designs suffer from the disadvantage that they are of delicate construction, elaborate in design, and because of the necessity of removal to clean and recharge them, are subject to breakage. Time is also consumed in the removing and cleaning operation.

Specifications. Outside diameter of the jacket is 80 mm. Inside diameter of the solution chamber is 55 mm. Depth of solution chamber, to cone, is 38 mm. A stopcock, size 2, narrow bore is used. Tubing is 8 mm. in outside diameter. Borosilicate glass, 1.5 mm. thick, is used in the body of the cell.

The present cell (Figure 1) was designed to provide a unit for routine use. It is permanently mountable, by the use of a finger-type clamp, and incorporates the following features: construction of heavy borosilicate glass; a thermal jacket, *A*, through which water from a constant-temperature bath is circulated, a combination gas and drain tube, *B*, to provide rapid draining and cleaning (through *D*) as well as to allow the passage of inert gas (through *E*) to the solution to be analyzed, depending upon the position of the stopcock, *C*.

The cell as normally used in this laboratory is fitted with a large (size 12) rubber stopper through which are passed a gas-exit tube built to contain a thermometer, *G*, a reference anode, *F*, and the dropping mercury electrode, *H*. The silver–silver chloride combination using 1 *N* potassium chloride as electrolyte is employed as the external anode.

The cell is capable of analyzing a minimum of 10 and a maximum of 50 ml. of solution.

ACKNOWLEDGMENT

The author wishes to acknowledge the assistance of Ray Rose, who performed the glass blowing.

LITERATURE CITED

(1) Langer, Alois, IND. ENG. CHEM., ANAL. ED., **17**, 454 (1945).
(2) Lingane, J. J., ANAL. CHEM., **21**, 47 (1949).
(3) Lingane, J. J., IND. ENG. CHEM., ANAL. ED., **16**, 329 (1944).

Mold Growth and the pH of Tartrate Buffer Solutions. Roger G. Bates, National Bureau of Standards, Washington 25, D. C.

ALTHOUGH solutions of potassium hydrogen tartrate are convenient standards for the calibration of pH equipment, many users may not be aware that the pH may be changed in a relatively short time by molding. Hitchcock and Taylor (*3*) studied a 0.03 *M* solution and noted a susceptibility to mold growth, but did not estimate the resulting change of pH. Lingane (*4*) recommended a saturated solution of potassium hydrogen tartrate because of its ease of preparation, reproducibility, and adequate buffer capacity, but made no mention of the tendency to mold. Although he observed an increase of only 0.03 pH unit after a year, Lingane suggested that the solution be freshly prepared as needed.

At the time of preparation, the pH of the 0.03 *M* solution was found to be 3.569 at 25° C., as compared with 3.567 given by Hitchcock and Taylor (*3*) and 3.569 to 3.575 computed by the writer (*1*) from the e.m.f. of cells without liquid junction. A fluff of mold appeared in about 14 days. At 54 days the pH was found to have risen to 3.591 and, after 93 days, to 3.609.

The initial pH of three saturated solutions was found to be 3.561 ± 0.003 at 25°. One of these solutions molded in 7 days and was found 18 days later to have a pH of 3.660, or 0.1 unit higher than its original value. The pH of another remained unchanged for 13 days and there was no noticeable molding. Three weeks later mold had formed and the pH had risen 0.06 unit to

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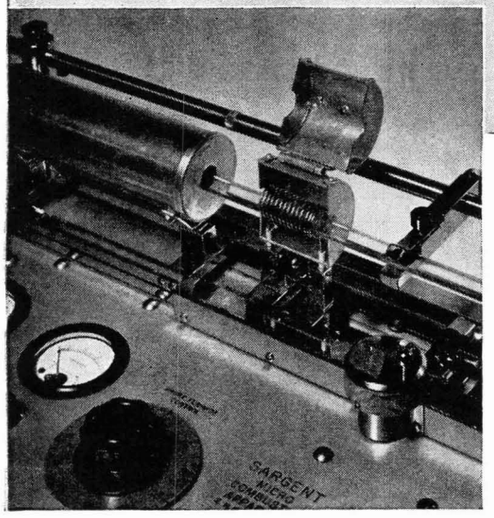

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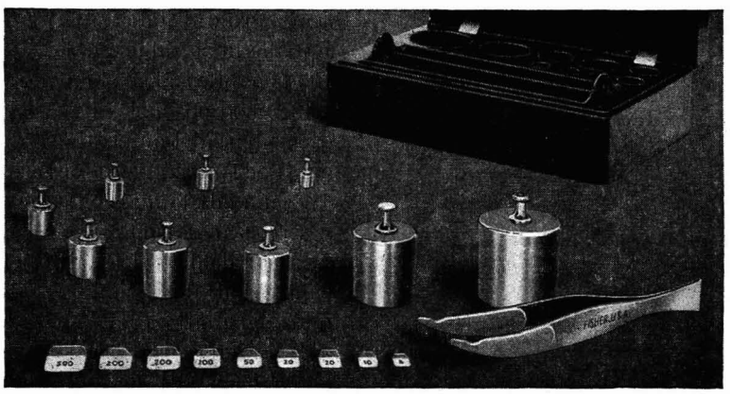

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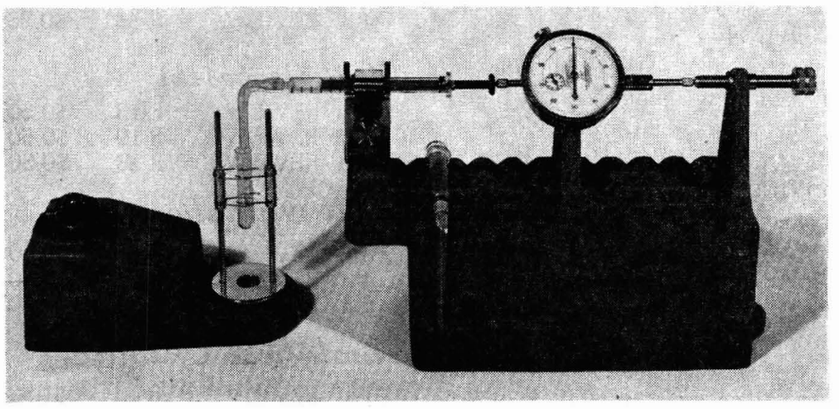
INSTRUMENTATION

A recently developed syringe microburet, a substitute for the analyst's conventional buret, is of good mechanical design and operational simplicity

by R. H. Müller

ANALYTICAL chemistry has borrowed techniques from almost every branch of science. Although the instrumental approach has profited most from electrical, electronic, optical, and mechanical advances, one cannot afford to overlook the impressive array of techniques which have become available as the result of the labors of the biochemist, the biologist, and physiologist.



COURTESY E. MACHLETT & SON

Figure 1. Syringe Microburet

Microchemical analysis, whether organic or inorganic, is in many respects a scaling down of classical procedures. However, anyone who has ever performed a creditable microdetermination will recognize that this is a slightly scandalous oversimplification. The fact remains that, aside from the skill, care, and meticulous attention to detail which one must observe, the operations are essentially classical.

On the other hand, if one is required to titrate a single drop of solution or perform a surgical operation on a single-cell organism in order to determine composition, it is obvious that some unconventional tools and instruments will be required. Micrurgical techniques of astounding precision and delicacy have been devised and, although the interest may be primarily in some morphological aspect of the specimen, more often than not some chemical information is also sought and obtained. Modern micrurgy has even created the need for the micro tool maker and the outstanding expert is Fonbrune of the Pasteur Institute. His microforge enables an expert to fashion tools and implements invisible to the unaided eye. We may well expect to see the full resources of modern instrumentation ultimately brought to bear on ultramicroscopic specimens. The microbiologist is showing us the way, outlining the problems and defining the objectives.

Syringe Microburet

The syringe buret has long served as a substitute for the conventional buret of the analyst. In many respects it is superior. Its precision can be very high, it is readily adapted to motor drive, and it is produced by semiautomatic methods at very low cost. Microchemists have devised many microburets depending upon displacement effects, but a recent version strikes us as an example of extremely good mechanical design and operational simplicity.

The Model SB-1 syringe microburet shown in Figure 1 is manufactured by the Micro-Metric Instrument Co., 2891 East 79th St., Cleveland 4, Ohio. Among its advantages are the fact that it requires no mercury, and has a direct-reading dial readable in increments as low as 0.0002 cc. and a rotatable rim for quick zero setting. No calibrations are required and various syringes can be interchanged rapidly, either for range selection or for use with an unlimited number of reagents. The microburet is manually actuated and the rugged components are mounted on a neat hollow cast aluminum base finished in gray crackle lacquer.

The principle of operation may be seen in Figure 1. The syringe of desired capacity is very readily mounted in a V-block where it is held by a phosphor-bronze strap. The V-block assembly is pivoted at the bottom and can be rotated about this pivot in a plane perpendicular to the drive axis and brought into line with the drive axis. The drive element is the stem of the micrometer dial gage. The stem carries a plate at the left which thrusts against the plunger of the syringe and the right end of the gage stem is driven by the feed screw on the right. Motion of the gage stem, and of the syringe plunger which it drives, is indicated directly on the dial in thousandths of an inch. The total possible excursion is slightly over 1 inch. A smaller dial reads tenths of an inch directly.

INSTRUMENTATION

Loading is a very simple and rapid operation. With the desired syringe in position and its microtip immersed in the reagent, the plunger is drawn from left to right until it bears against the dial gage plate. The microburet feed screw at the extreme right can be advanced to expel a little reagent and, after the tip is wiped, loading is completed. The lock nut on the dial gage is released and the knurled rim is now rotated to bring the scale to zero, at which point it is locked. Subsequent motion of the feed screw will now expel reagent, and the amount in microliters is indicated directly by the dial. For syringes ranging from 0.200 cc. per inch to 5.00 cc. per inch, the dial readings will correspond to 0.200 microliter per division to 5.00 microliters per division, respectively.

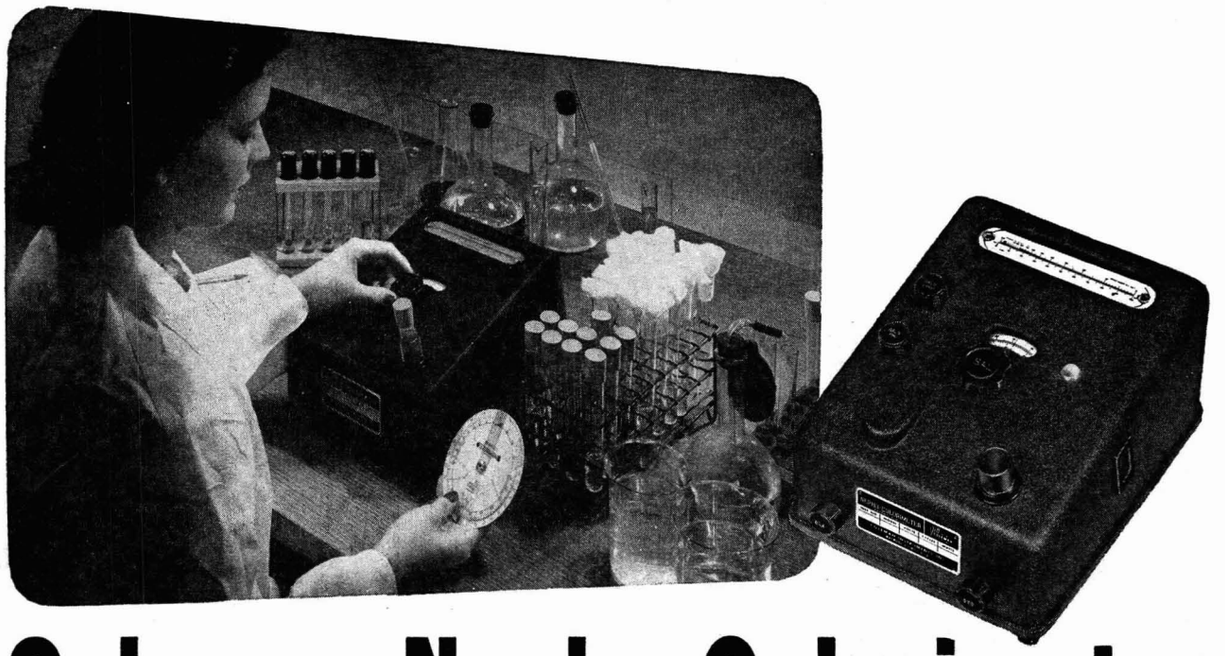
Reference to this principle of microburet techniques may be found in the work of Lazarow [Lazarow, A., *J. Lab. Clin. Med.*, **35**, 810 (1950)]. The SB-1 instrument can also be used in conjunction with the Beckman spectrophotometer microcells as described by Lowry and Bessey [*J. Biol. Chem.*, **163**, 633 (1946)].

Also shown in conjunction with the syringe microburet in Figure 1 is the Model MTS-1 micro test tube stirrer, the base of which can be driven at variable speeds. Vertical rods attached to this base carry coiled springs into which the sample tube can be inserted. The delivery tip is used as a stirrer. A magnetic mixer is also available, the Model MM-1.

Manometer Calibrator

Another useful device manufactured by the same company is the Warburg manometer calibrator, Model WMC-1. In addition to being the fastest known method for calibrating a Warburg manometer, it eliminates the toilsome use of mercury or gravimetric procedures, and is direct reading to 0.001 cc. and with an accuracy of 0.2%. It

(*Continued on page 26A*)



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INSTRUMENTATION

is available in all standard tapers. As shown in Figure 2, the calibrator is fitted to the ground joint of the Warburg and fluid displacement is effected by means of a piston of exactly 1 sq. cm. area driven by a high quality metric micrometer. As a consequence of this construction, each small division of the micrometer is equivalent to 0.001 cc. The plastic top forms a cavity for the measuring liquid (water). The plastic tops are available in different tapers and are all interchangeable. Aside from the micrometer and plastic adapter, the parts are made of stainless steel and one neoprene rubber gasket.

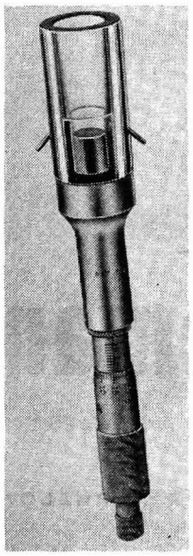

Figure 2. Manometer Calibrator

Beyond the intended purpose of this calibrator, it is evident that it would be extremely useful for accurate fluid or gas volume displacement measurements.

Information on these and related devices can be obtained from the manufacturer or from Ross G. Harrison, Jr., vice president in charge of sales, who is available through the Eastern Office, Box 255, Princeton, N. J.

These developments indicate the growing extent to which standard precision mechanical components are being adapted to the measurement problems of the chemist. This is particularly true with the increasing use of the machinist's precision micrometer and dial gage. All too frequently chemists find themselves devising feed screw mechanisms for a particular purpose and are later surprised to find that this simple machine tool does a better job at 10% of the cost.

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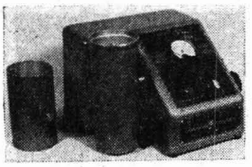
Moisture Meter

A new instrument in the field of moisture measurement has been reported by the Halross Instruments Corp. Known as Model 919, it is a portable electronic device, designed to determine the moisture in cereal crops and a wide variety of other materials. The meter consists of a die-cast aluminum case, containing the batteries and measuring circuit, together with a detachable cell. The circuit is composed of two tubes and their associated components, a standard for calibration purposes, and a variable condenser on which is mounted the scale drum of the instrument. The complete circuit is compensated for temperature variations. For alternating current operation, a small power supply is available which fits into the battery compartment. The measuring cell incorporates a novel feature that compensates for various material densities. The meter will operate within a temperature range from −20° to 110° F. and at relative humidities up to 95%. **1**



Monochromator

The new grating monochromator offered by Baird Associates may be used over a spectral range extending from the ultraviolet (2000 A.) into the infrared to 6 microns without interchange of optical components. This results from the exclusive use of mirror optics. The grating monochromator is said to provide spectral purity equal to or better than a double monochromator of the prism type. As is true of all grating devices, this instrument exhibits the phenomenon of overlapping orders. For example, if the monochromator is set for 2 microns, it will also pass light of wave lengths 1, 0.66, 0.5, 0.4, 0.33 micron, and others—if the source emits radiations at these other wave lengths. These overlapping orders may be eliminated by the use of appropriate filters or the aid of a fore-prism of low dispersion.

The amplifier is designed to operate in conjunction with a light chopper synchronous generator combination. The input impedance is variable over a range sufficient to match either a photomultiplier or a photoconductive cell (sulfide or telluride). The use of a synchronous rectification system provides accurate linearity of output d.c. voltage with respect to light intensity incident on the receiver and permits attainment of an effective over-all band width as small as 0.05 cycle per second.

The sensitive elements are prepared on small rectangular pieces of glass provided with contact electrodes. They are available in a variety of configurations, from one with a sensitive area of 0.031 × 0.031 inch to others that are 1 inch deep and have almost any desired width for use behind an exit slit. The cells may be operated with the sensitive surface exposed directly to the incoming radiation (no window). Peak response is at about 2.5 microns. Response drops to 1% of the peak at about 3.5 microns at room temperature, although with dry ice cooling the response may be extended beyond 4 microns. **2**

Umbrella Stirrer

The problem of inserting 4-inch stirrer-propellers through narrow-necked flasks and similar vessels has been solved by Fisher Scientific Co. The latest device, called an umbrella stirrer, consists of four blades that can be closed to pass through the neck of a flask, then opened for maximum stirring effectiveness. The stirrer is particularly valuable when used in connection with 3-necked flasks in cases where a minimum exposure to air is desirable. The 12-inch stirrer shaft fits into chucks of any motor which can accommodate 0.25-inch rods. All parts of the stirrer are made of corrosion-resistant stainless steel. Its blades can be twisted within the flask, so that a variety of shearing actions can be achieved. **3**

Data Recorder

A new electronic device designed to read test instruments at speeds of up to 50,000 readings per second and record the readings on tape or punched cards ready for computation has been announced by Arthur D. Little, Inc. Called the digital reader, the instrument can eliminate the cumbersome effort involved in recording and computing data from a great variety of instruments used in analytical and research work. At present, it is often necessary for research workers to photograph instrument dials at stated intervals, then laboriously

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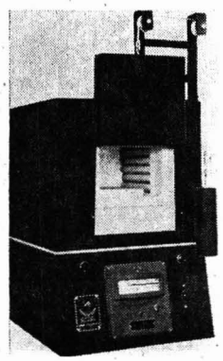
jot down the readings in numerical form and arrange them for computation. Some recording instruments trace out a chart which must be similarly examined and translated into numerical form. When the digital reader is linked to the instruments to be recorded, the device rapidly converts the electrical signals from the instruments directly to convenient binary-digital form for immediate analysis, computation, smoothing, or storage on recording media.

Arthur D. Little, Inc., built its first digital reader to record data on infrared spectra. The original models proved so effective that the company is now building units for other applications. The instrument is about 20 × 30 × 5 inches. It operates on 110 to 125 volts and can be incorporated into multiple-instrument reading and recording systems. **4**

Electric Furnace

The new electric furnace offered by K. H. Huppert Co. gives the operator automatic control of temperatures ranging from 300° to 2200° F. Heretofore in the case of electric furnaces of this type, the lower temperature could not ordinarily be controlled without additional equipment. Model 869 eliminates the need for such auxiliary apparatus. The furnace is 8 × 6 × 9 inches in size, having a maximum power consumption of 4 kw. It is wired for 220-volt single-phase operation. The furnace has been equipped with a counterweighted door which rides in a wedge slide, to seal the door in the closed position. The heating elements are made of nonscaling, nonflaking high-temperature coiled alloy wire. All contacts are fully enclosed. Floor and bench models are available. **5**



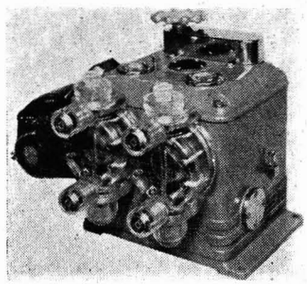
Radiation Counter

Radiation Counter Laboratories has come forth with a new fusion seal mica window counter, which may be used in any installation in which the Tracerlab TGC-1 or TGC-2 is employed. In this new counter, the thin mica window is held in place by a low-temperature polymerizing fusion seal. A fitting provides against leakage. Counters are available with mica window thicknesses of between 3 and 4 mg. per square cm. For experimenters working with less energetic emitters, such as C^{14} and C^{35}, mica window thicknesses of less than 2 mg. per cm. are available. The counter has a useful life of approximately 10^8 counts.

The tube, 3.625 inches high, may be firmly supported on a standard RCL Lucite mount by a metal attachment, which also permits an accurate adjustment of the tube position. The mica window is 1.125 inches in diameter, while the body of the counter is 1.5 inches in diameter. The sample may be held within 0.03 inch of the window. **6**

Diaphragm Pump

%Proportioneers, Inc.%, has developed a new Model 2-47CF chemical feeder having a recommended delivery range of from 1 to 15 gallons per hour. This positive displacement, duplex diaphragm pump is equipped with two plastic heads and is driven by a 0.25-hp. motor. The pump will draw solutions or slurries from a container and force them into a line or vessel up to pressures of 100 pounds per square inch gage. By means of special operating cams and rocker bars, the acceleration curve of one feeding head is exactly balanced by the deceleration curve of the second feeding head, so that the resulting delivery from the two discharge ports manifolded into a common line is smooth and pulsation-free. While the unit is in motion, the feed rate is manually adjustable by the setting of a knob which controls the amount delivered from each head. The feed rate is also manually adjustable by a change in the position of a V-belt on three-step cone pulleys. All moving parts are enclosed in an oil bath. The unit may be used at temperatures not in excess of 100° F. **7**



Direct Current Amplifier

In many fields, the limits of research are defined by the low levels of the phenomena to be studied. A new instrument that is extending these limits below their recent boundaries is the ultrasensitive General Motors synchronous breaker-type d.c. amplifier whose sensitivity extends down into the range immediately adjacent to the Johnson noise level—the noise resulting from the random motion of electrons in the circuit. Originally developed in the laboratories of General Motors Corp. during World War II, the present advanced models are being produced by the Liston-Folb Instrument Corp. In addition to its exceptional sensitivity, the Liston-Folb d.c. amplifier exhibits stability in both zero reading and gain in the order of 0.005 microvolt and 0.3%, respectively, over periods of 8 hours or more.

The breaker-type amplifier employs two synchronously driven breakers, which convert d.c. inputs to a.c. for ampli-

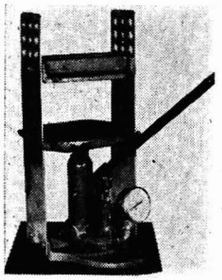
fication and then back to d.c. for output. This system discriminates against interference from a.c. signals originating in neighboring electrical equipment and produces an output of the same polarity as the input. In Model 10, the response is flat to 1 cycle per second, while Model 10-A is linear to 15 cycles per second. Both models are available for 110-volt 60-cycle, 50 volt, or 6-volt power supplies. The expected input impedance should be specified when ordering. **8**

Illuminator

The new Daylite illuminator, manufactured by Hellige, Inc., provides suitable light for colorimetric work and ensures reproducible results independent of time or weather. With this accessory, the company's nonfading glass color standards of the daylight series can also be used in artificial light. The illuminator is equipped with a specially coated bulb and Corning Daylite glass filter and should not be confused with lamps using an ordinary bulb or blue glass plate. The ventilated metal housing measures 3.75 × 4.75 × 3.75 inches and has a sloping front to hold the comparator at an angle of 45° for increased ease of operation. It is furnished for 110 to 125 or 220 to 230 volts a.c. or d.c. **9**

Laboratory Press

Just announced by the Knuth Engineering Co. is the standardization and factory production of the K&K press, designed for laboratories in need of a portable, low-pressure unit. Model 100 is a simplification of a press previously restricted to custom designs by special order. It is designed for pressures up to 83 pounds per square inch. The unit employs a standard dial gage which indicates the number of pounds of direct hydraulic pressure, with maximum loads up to 3000 pounds. New accessories are being planned and developed. The press may be used in crushing rock, extracting liquor from pulp, pressing samples, and in many other ways. **10**

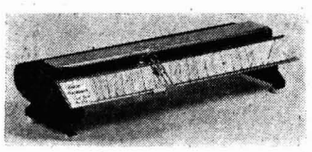


Controlling Pyrometer

The automatic pyrometer manufactured by Assembly Products, Inc., may be used to maintain a preset temperature by automatic on-off control. A switch permits the device to be changed from automatic to limit control. As a limit control, it operates as a shutoff device or safety warning unit which will make contact, when a preset temperature is reached, to sound an alarm or shut off a furnace. The contact holds until reset. No special calibration equipment is required for this instrument. Furnished with a thermocouple, the meter comes in various models covering such ranges as 0° to 260°, 400°, 500°, 800°, 1100°, 1370°, and 1650° C. A 4.5-inch meter dial indicates the temperature. **11**

Hot Bench

The Kofler hot bench, distributed by William J. Hacker & Co., is used in determining melting points. It consists mainly of a metal body, 38 mm. wide by 370 mm. long, electrically heated at one end to provide an almost linear temperature drop. The temperature varies from approximately 50° to 260° C. A scale with temperature lines marked at every 2° and a special reading device are provided. In the determination of a melting point, the substance is laid directly upon the chromium-plated surface of the hot bench. In the case of pure substances, a sharp dividing line will develop between the fluid and solid phases within a few seconds. The determination of a melting point, including the prior adjustment of the reading device, takes approximately 1 minute. The margin of error in careful work is usually not more than ±1° C. **12**



Bromo Fatty Acids

Alpha-bromo derivatives of high-purity lauric, myristic, and stearic acids are now available from Sapon Laboratories in research quantities. The materials are white to pale yellow waxes having low melting points. The manufacturer states that the potentially low cost of the bromo acids makes these compounds of interest in the preparation of specialty surface active materials for the petroleum, rubber, textile, and metal working industries. The present materials replace previously offered derivatives of 90%-grade fatty acids. **13**

Microparticle Classifier

Particles below 60 microns may be accurately separated with the microparticle classifier offered by Harry W. Dietert Co. The classifer will separate mixed particles into 8 fractions from 0 to 60 microns plus oversize residue within 2 hours. It may be applied to any type of material, such as drugs, chemicals, dusts, and powders. The sample may be as small as 10 grams. The gentle air sorting process does not alter the sizes of the particles. All parts of the classifier in contact with the sample are made of stainless steel and may be kept clean and sterile. **14**

pH Meter

Manufactured by Analytical Measurements, Inc., a new pocket-size pH meter and companion probe unit is now available for on-the-spot pH determinations. Completely self-contained with batteries, in a 3 × 5.88 × 2.5 inch Bakelite case, this instrument is furnished, camera fashion, in an ever-ready case with plastic tubes of buffer and potassium chloride solutions. The total weight is 3 pounds. Both waterproof and fungusproof, the case comes with a combination hand-and-shoulder strap, allowing the instrument to be slung over the shoulder or hung around the neck, leaving both hands free. Supports and beakers are eliminated by combining the calomel and glass electrodes with the sample holder in a single polyethylene probe unit. A sample volume of only 0.5 ml. is required.

The meter is scaled from pH 2 to 12 for easy reading. A simple adjustment permits readings from 0 to 14. Accuracy of 0.1 pH is obtainable. Hearing aid–type batteries provide up to 1300 hours of operation. The electrometer tube, switch, and input connector are sealed in a single unit. The one-knob-control and continuous-reading features of this instrument simplify operation for untrained personnel. **15**

Induction Heater

An improved electronic-type 20-kw. induction heater, featuring a nonventilated, dustproof NEMA Type 12 enclosure, has been announced by General Electric Co. The new heater has been designed so that only the control and accessories required for a particular heating application need be purchased. The totally enclosed steel cabinet is equipped from felt-gasketed and bolted doors to protect the components from dirt, grit, or oily vapors, thus reducing the need

for maintenance and providing a minimum of "downtime." The heater is certified to meet FCC regulations.

In addition to water cooling, blowers recirculate air within the enclosure to cool the seals of the oscillator tube and the bases of the rectifier tubes. Oscillator, control, and rectifier panel, as well as other components, are located within the cabinet in order to be easily accessible for maintenance. Long-scale, switchboard-type instruments provide improved readability, enabling the operator to determine operating characteristics quickly and accurately. The heater can be used for long- or short-run production, and is available in two models—with or without variable power adjustment.

For the short-run production of a wide variety of parts, GE recommends the Type HM-20L1 heater. This model has a variable power adjustment from 0 to 100% by means of a rheostat, either mounted in the work table or purchased separately for mounting elsewhere. The versatility of this unit enables the operator to perform rapid on-off heater operation to harden selected areas accurately in less time. For long-run, higher-production applications which do not have rapid cycling, the Type HM-20L2 heater (without variable power adjustment) is recommended. On such applications, the heater is initially set to heat a specific part and no further adjustment is necessary.

The complete heater, in either model, weighs approximately 3600 pounds. Units are available for operation on 230-, 460-, or 550-volt, three-phase, 60-cycle power supplies. **16**

Manometer Calibrator

E. Machlett & Son offers a new instrument for the calibration of Warburg constant-volume manometers. The device is said to simplify and shorten the calibration procedure by eliminating the handling and weighing of mercury. Each division of the micrometer scale of the calibrator represents a volume displacement of 0.001 ml. The calibrator can be used wherever extreme accuracy is required to determine fluid or gas volume displacement, as in the case of pipets, microburets, and similar apparatus. **17**

MANUFACTURERS' LITERATURE

Dynel. An 8-page pamphlet describes properties of dynel: tenacity, elongation, stiffness, toughness, flammability, moisture regain, light stability, and resistance to water, chemicals, insects, and mildew. Information on price and standard sizes and lengths of dynel staple is also included. Carbide and Carbon Chemicals Division. **18**

Silica Aerogel. Applications of Santocel C, a finely divided, pure white silica aerogel, are described in new 16-page booklet. Typical grinding and reducing procedures for oleoresinous and alkyd flat coatings are explained. Product is used in flat and semigloss varnishes, as well as in lacquers, plastisols, organosols, and free films. Merrimac Division. **19**

Apparatus. Current issue of *Better Analysis* presents articles on flame photometer, grating monochromator, and micromulls. Baird Associates, Inc. **20**

o-Nitrobiphenyl. Technical Bulletin O-D-102 on *o*-nitrobiphenyl gives physical and chemical properties, plasticizing and fungicidal characteristics, toxicity, and suggested uses. Monsanto Chemical Co. **21**

Humidity Control. A 4-page bulletin describes the Kathabar system of humidity control, which combines chemical dehumidification with mechanical refrigeration to obtain air of low dew point. Air at subzero temperatures can be produced without formation of frost on the cooling coils. Surface Combustion Corp. **22**

Potentiometers. Catalog 15-15, well illustrated with photographs and drawings, gives information on potentiometers, strip chart controllers and recorders, circular chart controllers and recorders, switches, thermocouples, motors, and motorized valves. Minneapolis-Honeywell Regulator Co. **23**

Bismuth Wire. New 20-page booklet discusses ductile bismuth wire and ribbon. Description of electrical properties and list of references are included. Fitzpatrick Electric Supply Co. **24**

Peracetic Acid. "Peracetic Acid, A New Bactericide-Fungicide" is title of 21-page bulletin which covers general properties of product, bactericidal properties, equipment sanitization, enzyme inactivation, toxicity, analysis, and suggested applications in the food industry. Buffalo Electro-Chemical Co. **25**

Organic Chemicals. Attractive, well illustrated, 32-page catalog gives physical properties, uses, and container specifications for aliphatic chemicals (acids, anhydrides, alcohols, aldehydes, plasticizers, and solvents), aromatic chemicals (amines, aminophenols, phenols, and phenol ethers), cellulose products, and inorganic chemicals. Tennessee Eastman Co. **26**

Nichrome Wire. Information on Nichrome and other high-nickel heat- and corrosion-resistant alloys is given in 75-page publication. Creep data, heating element data, life expectancy, temperature resistance, weights, wire data, strip data, and other details are included. Driver-Harris Co. **27**

Water Softeners. A 115-page book describes polyamino acids and salts (Versenes) employed as water softeners. Sections cover general information, applications, toxicity, and analytical methods (microanalysis, water hardness determination by Versenate method, and analysis of Versene in soap). Bersworth Chemical Co. **28**

Wave Analyzer. A 4-page bulletin describes analyzer that has been designed primarily for analysis of complex vibration wave forms, but is equally applicable to the measurement of audio and power frequency wave forms up to 21 kilocycles per second. Muirhead & Co., Ltd. **29**

Time Interval Meter. Pamphlet outlines basic design, operation, specifications, and applications of time interval meter which provides a direct reading of elapsed time between any two events in the range of 10 microseconds to 1 second. Accuracy of measurement is ±10 microseconds. Berkeley Scientific Corp. **30**

Methoxychlor Determinations. Laboratory procedures for methoxychlor determinations are described in technical bulletin. Procedure can be used to distinguish amount of compound in spray residues on crops, as well as deposits in milk or in the tissues of meat. E. I. du Pont de Nemours & Co. **31**

Thermoregulators and Thermostats. Well illustrated with photographs and cutaway drawings, 12-page catalog discusses mercury-activated thermoregulators and thermostats, which may be selected according to accuracy requirements from ±0.005° to ±0.10° C. H-B Instrument Co. **32**

Spectrographic Outfits. A 12-page illustrated brochure describes automatic large (F_D 170 cm.), medium (F_D 60 cm.), and intermediate and small quartz spectrographs (F_D 38 and 20 cm.). Any one or more of these instruments may be used in analysis of steels, heavy metals, aluminum and other nonferrous alloys, alkalies, or biological samples. Jarrell-Ash Co. **33**

Laboratory Equipment. A 28-page booklet entitled "What's New for the Laboratory?" describes tumbler-mixer, glass solvent trough for use in paper chromatography, viscometer, pyrometer, mercury filter, Geiger counters, Van Slyke blood gas apparatus, and other laboratory equipment. Scientific Glass Apparatus Co. **34**

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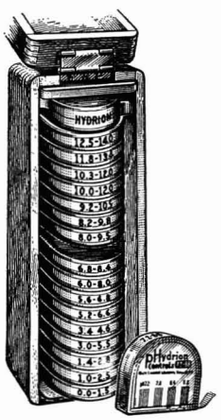

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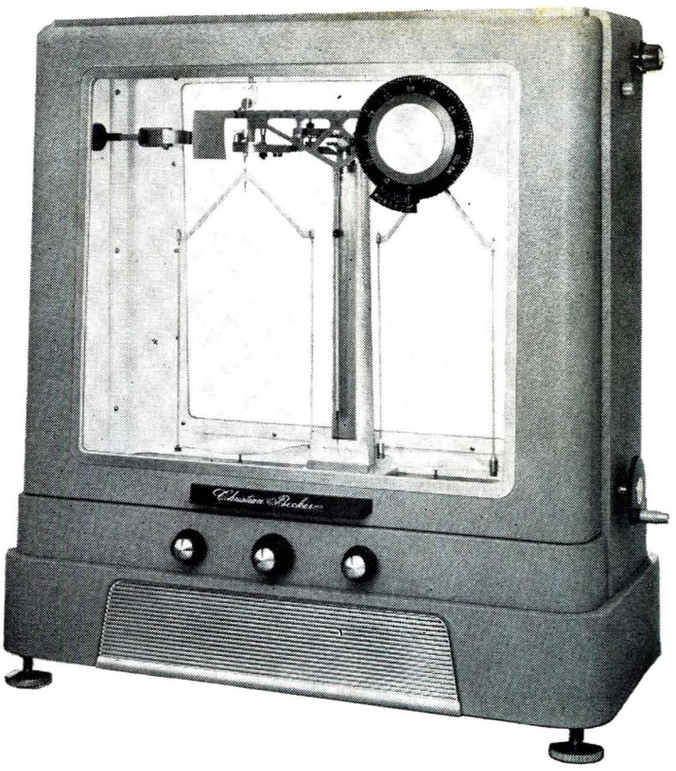

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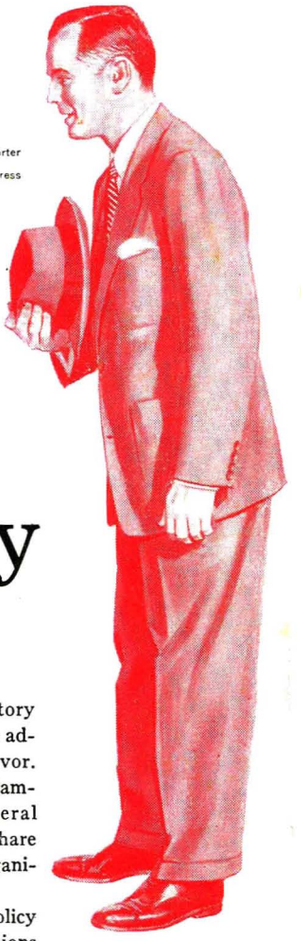
1917 Committee on Analytical Reagents established

1937 National charter granted by 75th Congress

Hats Off to the A.C.S. on its 75th Anniversary



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Starting with a group of thirty-five chemists in 1876, and building up to a current membership of nearly sixty-five thousand, the A.C.S. has served chemists and the chemical industry well in countless ways!

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