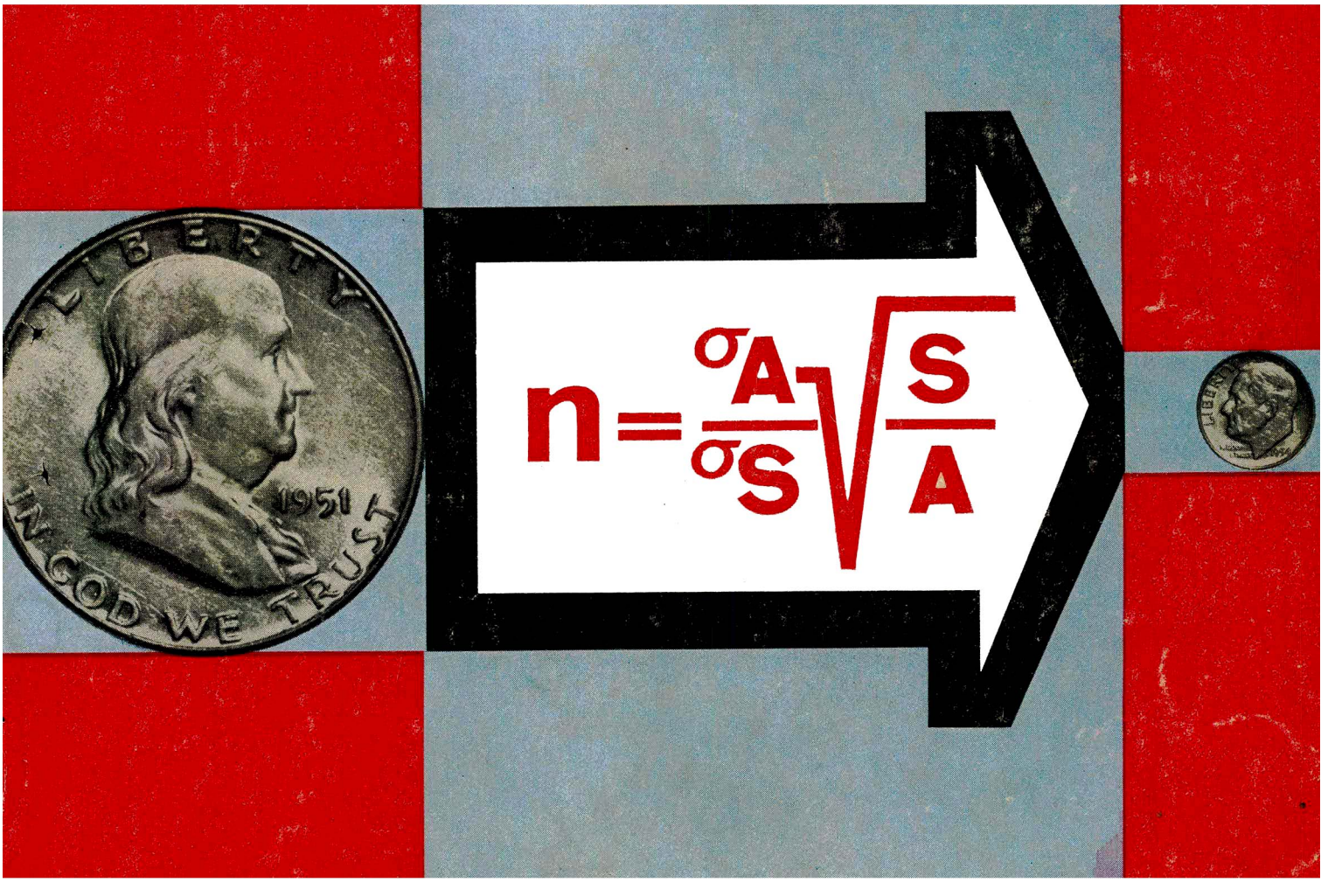
MAY 1959

ANALYTICAL CHEMISTRY



STATISTICS POINT WAY TO SAVINGS IN ANALYSIS ..... 21 A

▶ Progress In Radiochemical and Differential Migration Analyses 792, 818

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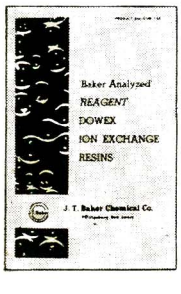
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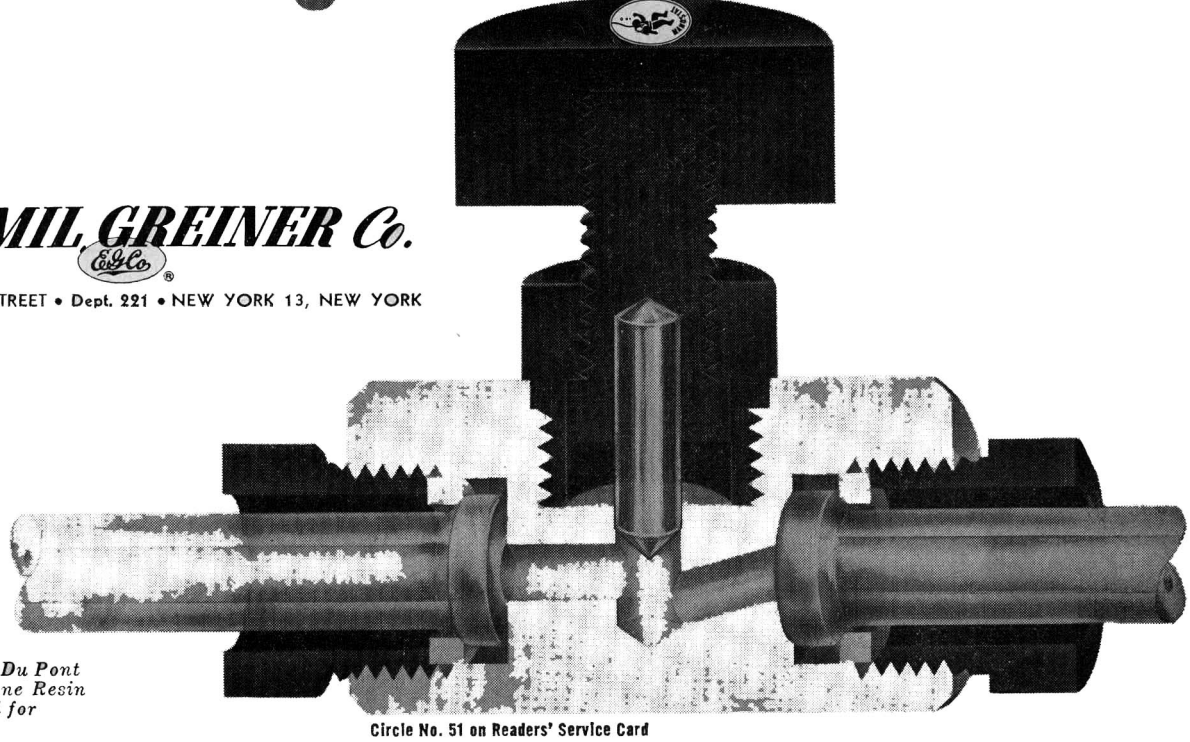
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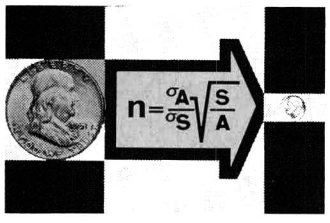
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ANALYTICAL CHEMISTRY

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THIS MONTH'S COVER

Application of statistics to analytical problems can indicate ways to cut costs of analyses. Analytical chemists, who examine all advances in analytical methods, reagents, and instrumentation as potential new tools, seldom consider statistics as a valuable adjunct. Although the language and mathematics of the statistician appear forbidding, the principles are relatively simple. Typical examples in this month's Report for Analytical Chemists illustrate the principles without the mathematics. See page 21 A.

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Issued April 24, 1959

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GUIDE FOR AUTHORS, published in December 1958 issue, page 2104, outlines scope and copy requirements to be observed in preparing manuscripts for consideration. Manuscript (2 copies) should be submitted to the Editor, 1155 Sixteenth St., N. W., Washington 6, D. C.

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Members, foreign and domestic	$ 4.00	$ 7.00	$10.00
Nonmembers, domestic and Canada	5.00	9.00	12.00
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Published monthly by the American Chemical Society, from 20th and Northampton Sts., Easton, Pa. Executive Offices, Editorial Headquarters, and Circulation Department, 1155 Sixteenth St., N. W., Washington 6, D. C. Advertising Office: 430 Park Ave., New York 22, N. Y. Branch Editorial Offices: 36 So. Wabash Ave., Chicago 3, Ill.; 718 Melrose Bldg., Houston 2, Tex.; 2 Park Ave., New York 16, N. Y.; 703 Mechanics' Institute Bldg., 57 Post St., San Francisco 4, Calif.; Bush House, Aldwych, London. Second class mail privileges authorized at Easton, Pa.

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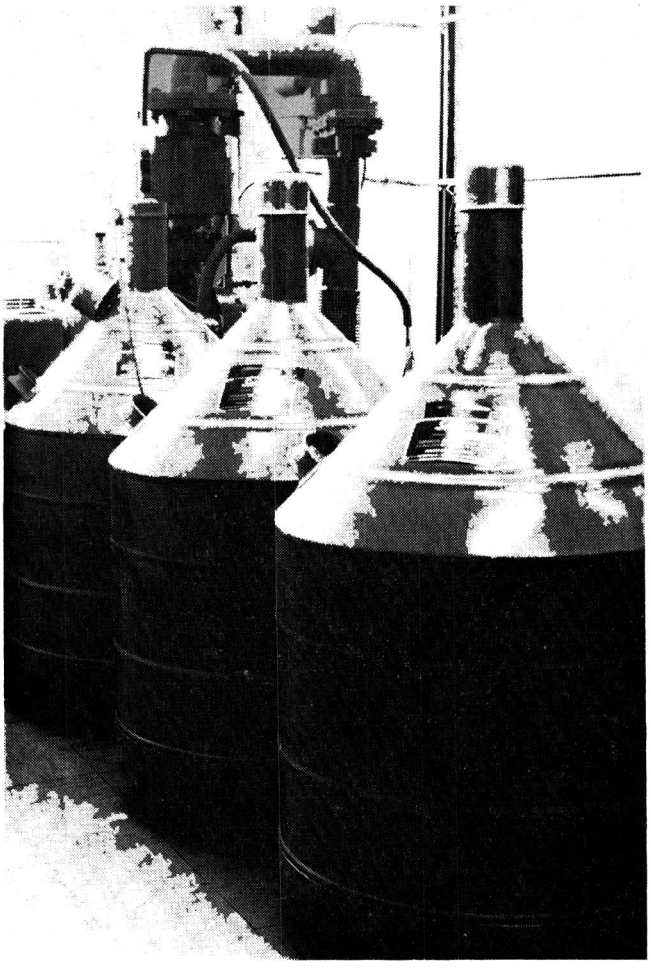
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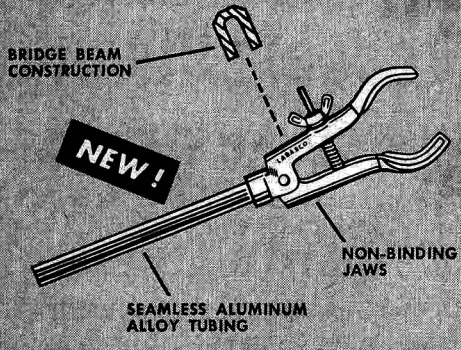
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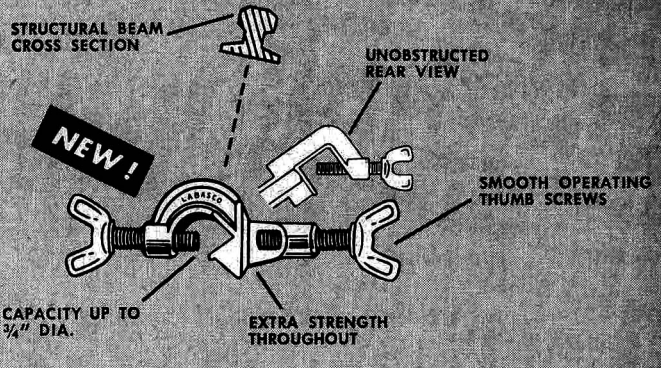
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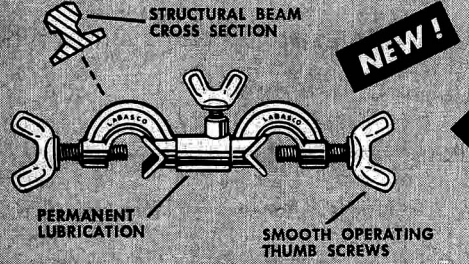
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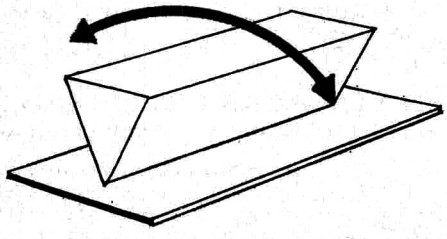
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AC BRIEFS

Highlights of the scientific and technical articles in this issue

Sensitivity Charts for Neutron Activation Analysis

Sensitivity charts for irradiation periods of 6 minutes, 10 hours, and 1000 hours are presented for certain elements activated by the thermal neutron procedure. These values are useful in comparing sensitivities of activation analysis with other analytical methods.

W. W. MEINKE, University of Michigan, Ann Arbor, Mich.

Anal. Chem. 31, 792 (1959)

Absolute Beta Standardization Using 4 Pi Beta-Gamma Coincidence Techniques

The use of the $4\pi\beta$-γ coincidence counter is recommended as a primary beta-standardizing method. It is free of many disadvantages but requires a true beta-gamma coincidence. The 4π method can be tested and evaluated against this procedure.

RAY GUNNINK, L. J. COLBY, JR., and J. W. COBBLE, Purdue University, Lafayette, Ind.

Anal. Chem. 31, 796 (1959)

Precision Calibration of Large 4 Pi Scintillation Crystals for Routine Gamma Counting

A large well-type scintillation crystal counter is calibrated by use of the $4\pi\beta$-γ coincidence method. The system is applicable to analyses of mixed isotopes, decay schemes, and counting of electron capture isotopes.

L. J. COLBY, JR., and J. W. COBBLE, Purdue University, Lafayette, Ind.

Anal. Chem. 31, 798 (1959)

Direct Estimation of Gamma-Ray Abundances in Radionuclide Mixtures. Complement Subtraction Method

A direct, rapid technique is devised for the estimation of gamma-emitting radionuclides in mixtures. The method combines an Argonne-type 256-channel analyzer and a dead-time correcting device. Pure calibrated standards for each radionuclide are required. Four component mixtures are quantitatively estimated.

WILLIAM LEE, U. S. Naval Radiological Defense Laboratory, San Francisco 24, Calif.

Anal. Chem. 31, 800 (1959)

Analysis of Fission Product Mixtures

A quantitative procedure is devised for the rapid and specific separation of radioactive fission product mixtures. Analyses are made in hydrochloric acid–hydrofluoric acid solution, rendering recoveries greater than 97% and high decontamination factors. The method is applicable to air-filter, soil, sediment, vegetable, and aqueous samples.

E. L. GEIGER, E. I. du Pont de Nemours & Co., Inc., Aiken, S. C.

Anal. Chem. 31, 806 (1959)

X-Ray Emission Spectrographic Analysis of High-Purity Rare Earth Oxides

A fluorescent x-ray procedure is devised for determining rare earth impurities in highly purified rare earth oxide samples. The accuracy is dependent upon the intensity of the spectral line above the background interference. Impurity concentrations between 0.01 to 0.1% are determined with an average error of 10%.

F. W. LYTLE and H. H. HEADY, U. S. Bureau of Mines, Reno, Nev.

Anal. Chem. 31, 809 (1959)

Radiometric Analysis of Leached Uranium-Thorium Ore Samples with the Beta-Gamma-Gamma Method

A radiometric method is developed for the quantitative analysis of leached uranium-thorium ore. Results indicate favorable agreement with chemical analysis for uranium oxide and thorium oxide. Minimum concentration determined is 0.1% in 18 grams of sample ore.

P. W. DE LANGE, National Physical Research Laboratory, Pretoria, South Africa

Anal. Chem. 31, 812 (1959)

Study of the Chemistry of Scandium on a Tracer Scale with Scandium-46

A study is made of mixed crystal formation of ferric sulfate containing tracer quantities of scandium-46, yttrium-91, and europium-152 and -154. Indications are that scandium is more similar to the iron family than to the rare earth elements.

B. C. PURKAYASTHA and V. R. PAI VERNEKER, Institute of Nuclear Physics, Calcutta, India

Anal. Chem. 31, 814 (1959)

Properties of Ethyl Esters of Certain Amino Acids as Related to Artifacts in Paper Chromatography

The formation of unknown substances formed during the alcoholic extraction of amino acids from plant materials or protein hydrolyzates is studied. The nature of the change, the identity of the unknown, and the conditions of the reaction are the points of investigation.

J. E. DeVAY, A. R. WEINHOLD, and GUNTER ZWEIG, University of California, Davis, Calif.

Anal. Chem. 31, 815 (1959)

Requirements for Analysis by Differential Migration

The important features common to all differential migration methods of analysis are reviewed. These methods serve primarily for the resolution of mixtures of all kinds of chemical substances. They allow comparison, description, and isolation of constituents of component mixtures. The methods present a wide variety of modification and application.

H. H. STRAIN, Argonne National Laboratory, Lemont, Ill.

Anal. Chem. 31, 818 (1959)

Some Artifacts in Paper Chromatography

An analytical investigation is made for the identification of artifacts in paper chromatography of amino acids, organic acids–bases, and inorganic ions. Certain isolated artifacts are identified by using infrared spectrographic techniques. Recommendations are made to minimize artifact occurrence.

GUNTER ZWEIG, University of California, Davis, Calif.

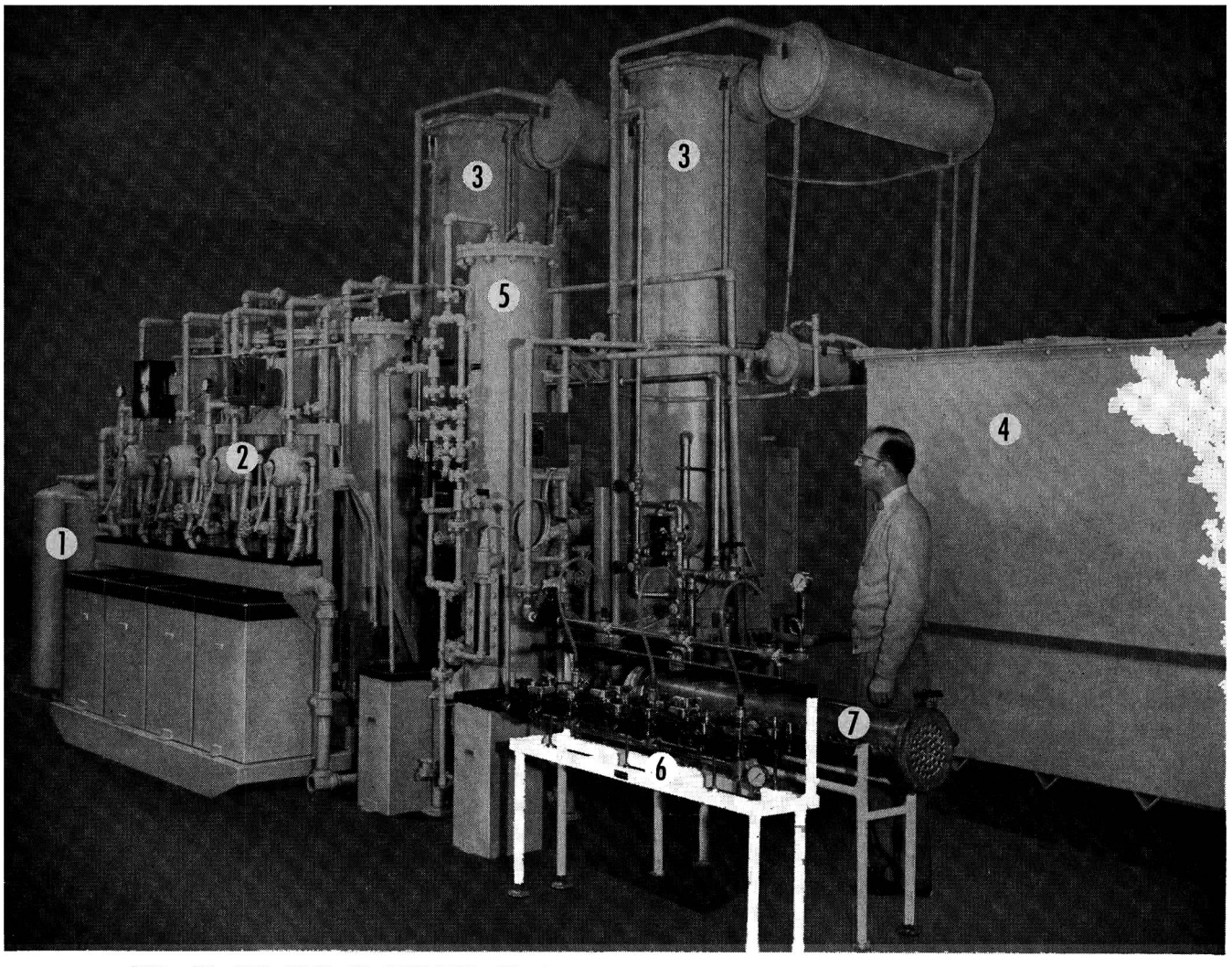
Anal. Chem. 31, 821 (1959)

Centrifugal Force in Paper Chromatography and Electrophoresis

A simple and effective apparatus is described for the application of centrifugal force to paper chromatography and ionography. Chromatogram patterns are similar to those obtained under usual conditions but the development time is markedly reduced. Centrifugal force is combined with high voltage direct current electrical field to produce a rapid planar electrophoretic technique.

H. J. McDONALD, L. P. RIBEIRO, and L. J. BANASZAK, Loyola University, Chicago 12, Ill.

Anal. Chem. 31, 825 (1959)



NEW BARNSTEAD WATER PURIFICATION EQUIPMENT PRODUCES 18,000,000 OHM WATER IN PRODUCTION QUANTITIES

PROBLEM: A large electronics firm needed 18,000,000 ohm* water free of bacteria, organics, silica, and submicroscopic particles. Existing purification methods could not produce it in either the purity required nor in the quantities needed. Further, the slightest amount of impurities in the firm's rinsing and washing operations would result in a high rate of rejects, raised manufacturing costs, and in some cases a permanently damaged product, noticeable when in use by the ultimate consumer.

SOLUTION: Since demineralizers would provide high ionic purity but would not solve the problem of bacteria, colloidal impurities, dissolved gases, organic matter etc., the use of distillation combined with other purification methods became necessary. Barnstead recommended the following equipment operating in series. 1. Sand and carbon filter for preliminary treatment of raw water. 2. A four-bed, extra-high capacity demineralizer for removal of most ionizable impurities. 3. Two Barnstead new high-purity Stills to remove the remaining non-ionizable, suspended, and gaseous impurities. 4. Box-type tin-lined tank for intermediate storage. 5. Mixed-bed demineralizer for removal of final traces of ionizable impurities. 6. Barnstead MF® Submicron Filter for removal of submicroscopic particles down to 0.45 micron. 7. Tin-lined heater for final heating of water to desired temperature for manufacturing operation.

RESULT: This Barnstead Pure Water Equipment, especially designed for this particular manufacturing operation, produces 18,000,000 ohm water completely free of bacteria, organics, silica, and submicroscopic particles. Thus a new manufacturing procedure becomes a reality through Barnstead Pure Water Engineering.

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Diffusion Analysis of Ions in Gels

The partial resolution and detection of some common cations and anions by radial differential diffusion in concentrated gels is described. The factors of concentration and time and distance traveled in the medium are essentials in the determination of cationic or anionic strength.

MARVIN ANTELMAN, Hampshire Chemical Corp., Nashua, N. H.
SISTER DENISE EBY, St. Joseph College, Emmitsburg, Md.
G. B. KAUFFMAN, Fresno State College, Fresno, Calif.

Anal. Chem. 31, 829 (1959)

Carrier Displacement Chromatography on Ion Exchange Resins

The separation of amino acids by chromatographic ion exchange techniques is enhanced by using carrier displacement. Volatile bases are used for cation resin columns and volatile acids for anion resin columns. Carrier substances are also used to saturate resins prior to application of anion acid solution.

D. L. BUCHANAN, Yale University, New Haven, Conn.

Anal. Chem. 31, 833 (1959)

Isolation of Cytoplasmic Particulates from Animal Tissues by Density-Gradient Centrifugation

Cytoplasmic particulates from animal tissue are isolated by means of a density-gradient centrifugation technique. Principal use of the method is in comparing intercellular material of normal and abnormal tissues.

J. F. THOMSON, Argonne National Laboratory, Lemont, Ill.

Anal. Chem. 31, 836 (1959)

Isolation of Carotenoids, Coumestrol, Chlorogenic Acid, and Antibiotics. Application of Countercurrent Distribution

Various biologically active materials are isolated from their natural sources by countercurrent distribution technique. The isolated compounds include carotenoids, coumestrol, chlorogenic acid, and antibiotics.

C. R. THOMPSON, A. L. CURL, and E. M. BICKOFF, Western Regional Research Laboratory, Albany 10, Calif.

Anal. Chem. 31, 838 (1959)

Examination of Oxyacids of Phosphorus by Electrochromatography

Mixtures of the oxyacids of phosphorus are resolved and estimated by means of a differential electrical migration in moist paper. Mobility of the oxyacids is related to the solvent employed. Location and estimation of the various acids is facilitated by the use of radioactive tracers and neutron activation techniques.

T. R. SATO, Argonne National Laboratory, Lemont, Ill.

Anal. Chem. 31, 841 (1959)

Separation of Antibodies by Starch Zone Electrophoresis

Zone electrophoresis in starch medium is used to characterize rabbit antibodies effective against sheep red blood cells. Two types of hemolysins appeared, one of high molecular weight and one of ordinary globulin size. Their appearance is a function of the immunization schedule.

PETER STELOS and W. H. TALIAFERRO, University of Chicago, Chicago 37, Ill.

Anal. Chem. 31, 845 (1959)

Separations by Continuous Electrochromatography

The operating principles and the typical experimental techniques involved in continuous electrochromatography are discussed in relation to the separation and analysis of complex mixtures of labile biological materials. Correlation is made between the individuality of sample patterns and special biological activities.

ARTHUR KARLER, Karler Laboratories, Berkeley, Calif.

Anal. Chem. 31, 848 (1959

Separation of Lipides by Gas-Liquid Chromatography

A modified ionization detection system and high efficiency capillary columns are employed in a gas-liquid chromatographic method. It is applicable to the analysis of mixtures of long-chain saturated and unsaturated acid esters The ionization detection system is not sensitive to changes in pressure and temperature. The optimum sample load is in the region of 1 gamma or less.

S. R. LIPSKY, R. A. LANDOWNE, and J. E. LOVELOCK, Yale University School of Medicine, New Haven, Conn.

Anal. Chem. 31, 852 (1959)

Variable Gradient Device for Chromatography

The construction details and quantitative relationship are given for variable gradient for chromatography. Small changes in specific portions of the elution gradient are possible without physical change in the apparatus. Gradient data obtained permit exact duplication.

E. A. PETERSON and H. A. SOBER, U. S. Department of Health, Education, and Welfare, Bethesda, Md.

Anal. Chem. 31, 857 (1959)

Automatic Derivative Potentiometric and Spectrophotometric Titrations of Organic Acids

An automatic derivative potentiometric and spectrophotometric titration method is developed for determining carboxylic acids, sulfonamides, imides, mercaptans, phenols, and enols. The acids are titrated with tri-*n*-butylmethylammonium hydroxide in acetone using an electrode pair or indicator most applicable to the acid type.

H. V. MALMSTADT and D. A. VASSALLO, University of Illinois, Urbana, Ill.

Anal. Chem. 31, 862 (1959)

Gas Chromatographic Determination of Fuel Dilution in Lubricating Oils

An accurate and sensitive gas chromatographic method is developed for measuring fuel dilution in crankcase oil. A preheater block flashes the diluent from the oil into a helium stream which carries it into a gas chromatographic column. Each analysis requires an internal standard of known concentration.

R. S. PORTER and J. F. JOHNSON, California Research Corp., Richmond, Calif.

Anal. Chem. 31, 866 (1959)

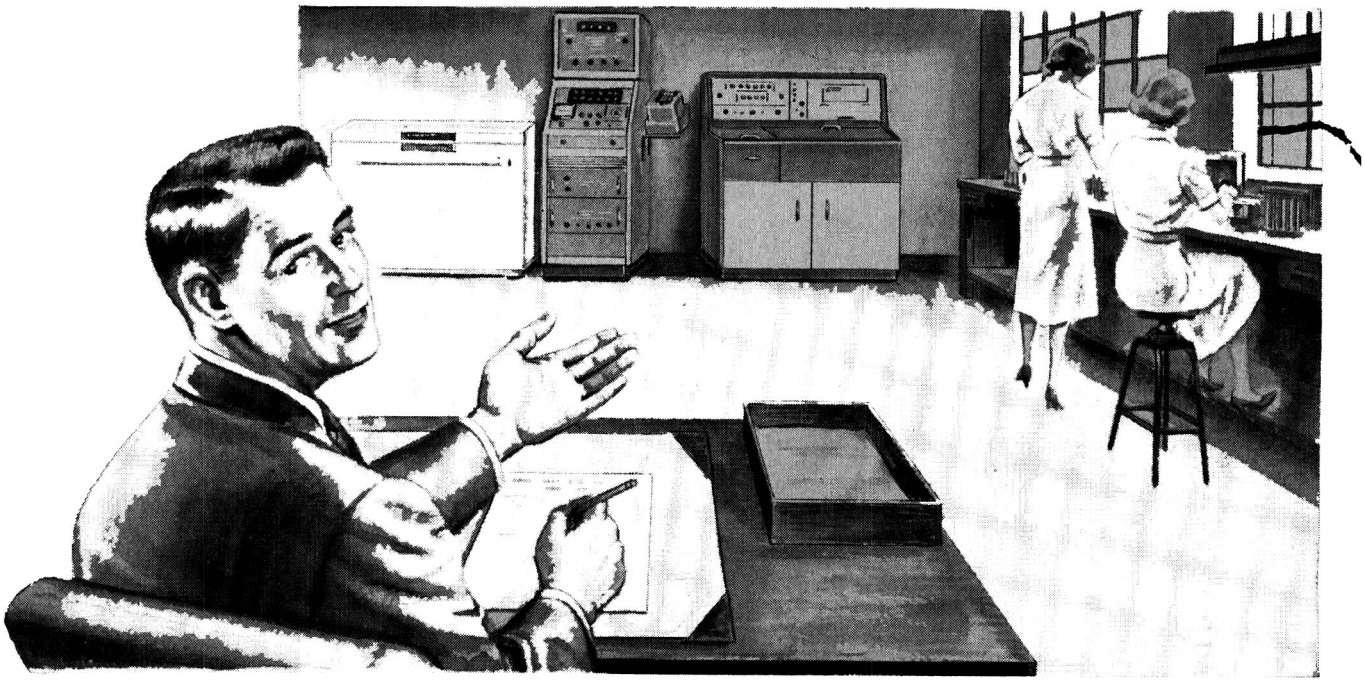
Gas Chromatographic Determination of Dissolved Oxygen in Lubricating Oil

A chromatographic technique is developed for determining oxygen in lubricating oil. The sample is passed over a Molecular Sieve column and oxygen is separated and determined. Method is applicable for the determination of other gases.

P. G. ELSEY, Ethyl Corp., Detroit, Mich.

Anal. Chem. 31, 869 (1959)

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Paper Chromatography of 2,4-Dinitrophenylhydrazones. Resolution of 2-Alkanone, *n*-Alkanal, Alk-2-enal, and Alk-2,4-dienal Derivatives

A rapid ascending paper chromatographic method is developed for the separation and identification of 2-alkanone, *n*-alkanal, alk-2-enal, and alk-2,4-dienal derivatives in mixtures. The sample is reacted to form the 2,4-dinitrophenylhydrazones which are resolved with petroleum ether on untreated filter paper.

A. M. GADDIS and REX ELLIS, U. S. Dept. of Agriculture, Beltsville, Md.

Anal. Chem. 31, 870 (1959)

Separation and Identification of *n*-Alkanals, 2-Alkanones, 2-Alkenals, and 2,4-Alkadienals by Paper Chromatography and Direct Spectrophotometric Examination

A paper chromatographic method is developed for the separation and determination of *n*-alkanals, 2-alkanones, 2-alkenals, and 2,4-alkadienals. After resolution the spots are identified as the 2-4-dinitrophenylhydrazone complex and spectrographically analyzed. The method allows as little as 5 gamma of material per spot.

MASAHIDE NONAKA, E. L. PIPPEN, and G. F. BAILEY, U. S. Dept. of Agriculture, Albany 10, Calif.

Anal. Chem. 31, 875 (1959)

Photoelectric Technique for Spectrophotometry between 194 and 225 Millimicrons

A spectrophotometer capable of accurate measurements of the absorption of ultraviolet light of wave length between 194 and 225 mμ is described. The technique allows far-infrared analysis. The absorption spectra of certain biologically active compounds are presented.

R. E. HANSEN and M. V. BUELL, University of Chicago, Chicago 37, Ill.

Anal. Chem. 31, 878 (1959)

Spectrophotometric Determination of Palladium with Phenyl-alpha-pyridyl Ketoxime

A spectrophotometric method is devised for determining palladium by complexing with phenyl-α-pyridyl ketoxime. The complex exhibits maximum absorption at 340 and 410 mμ. Interfering substances, except gold and cyanide, are eliminated by masking with EDTA. Concentration range at 410 mμ is 2 to 10 p.p.m. and at 340 mμ is 1.5 to 8 p.p.m.

BUDDHADEV SEN, Louisiana State University, Baton Rouge, La.

Anal. Chem. 31, 881 (1959)

Tracer Investigation of Iridium-Platinum Separation by Anion Exchange

An anion exchange procedure is devised for the separation of iridium and platinum. Sample is dissolved in hydrochloric acid, treated, and applied to a Dowex A-1 column. Iridium is eluted with 9*N* hydrochloric acid and platinum with 50% perchloric acid. The procedure is verified by radiotracer techniques.

D. D. BUSCH, J. M. PROSPERO, and R. A. NAUMANN, Princeton University, Princeton, N. J.

Anal. Chem. 31, 884 (1959)

Principles of End Point Detection in Chelometric Titrations Using Metallochromic Indicators. Characterization of End Point Sharpness

In chelometric titrations using metallochromic indicators, a variety of equilibria are involved. The sharpness of the end point is quantitatively established through use of pM-pH or pY-pH diagrams. The diagrams allow quantitative characterization of the color change in the titration independent of the system being considered. Other advantages are realized.

C. N. REILLEY and R. W. SCHMIDT, University of North Carolina, Chapel Hill, N. C.

Anal. Chem. 31, 887 (1959)

Trace Determination of Copper and Cobalt by Chelate Chromatography and Dithizone Photometry

A photometric method is developed for determining microquantities of copper and cobalt. The metals are chelated with sodium diethyldithiocarbamate and adsorbed on activated silica. After separation from the column the chelate is converted to the dithizone complex and photometrically measured. The method is applied to analysis of plant materials.

D. M. SMITH and J. R. HAYES, The Pennsylvania State University, University Park, Pa.

Anal. Chem. 31, 898 (1959)

Rapid Method for Determination of Small Amounts of Carbon Monoxide in Gas Mixtures

A gravimetric method is developed for determining carbon monoxide in gas mixtures. The carbon monoxide is converted to carbon dioxide and absorbed in an Ascarite tube. The method is sensitive to 0.1 to 1.5% carbon monoxide in 500 milliliters of gas. For smaller concentrations larger sample volumes are required.

IHOR LYSYJ, J. E. ZAREMBO, and ARNOLD HANLEY, Food Machinery and Chemical Corp., Princeton, N. J.

Anal. Chem. 31, 902 (1959)

Photometric Determination of Tantalum with Phenylfluorone

A colorimetric method is developed for determining trace quantities of tantalum using phenylfluorone. Tantalum is extracted from acid solution with methyl isobutyl ketone. The metal-phenylfluorone complex absorbance is measured at 530 mμ. Interfering substances are masked by EDTA.

C. L. LUKE, Bell Telephone Laboratories, Inc., Murray Hill, N. J.

Anal. Chem. 31, 904 (1959)

Hydrocarbon Compound–Type Analysis of Automobile Exhaust Gases by Mass Spectrometry

A mass spectrometric method is devised for the determination of hydrocarbon-type compounds in gaseous mixtures. The method permits the differential analysis of olefinic and cycloparaffinic compounds which exhibit similar mass spectral patterns. The method is applied to analysis of automobile exhaust gas.

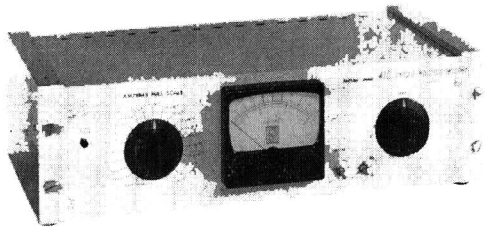
D. M. COULSON, Stanford Research Institute, Menlo Park, Calif.

Anal. Chem. 31, 906 (1959)

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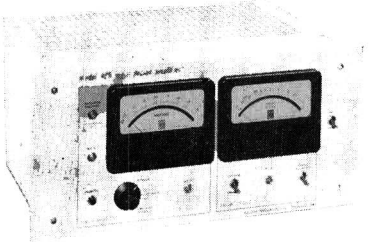
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linear micro-microammeters



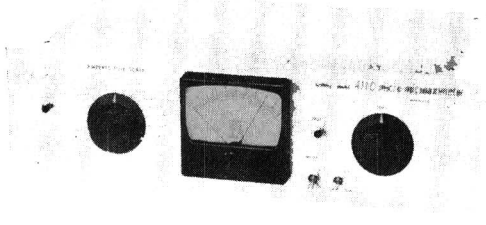
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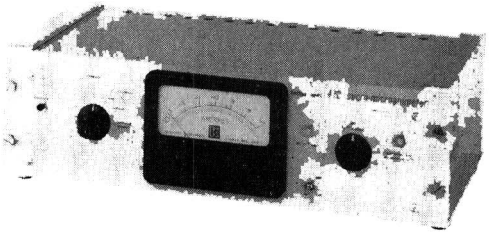
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Identification of Position Isomers of Some Stearic Acid Derivatives by Infrared Spectroscopy. Study of *trans* 6- through 11-Octadecenoic Acids and Corresponding Contiguously Substituted Dihydroxystearic Acids in the Crystalline State

Five position isomers of *trans*-octadecenoic acid are studied for correlation between the band progression in the 1180 to 1350 cm.$^{-1}$ region and the position of the double bond. No distinct carboxyl band is found in this region. Dihydroxy acids are studied in the 800- to 1200- cm.$^{-1}$ region.

HEINO SUSI, U. S. Department of Agriculture, Philadelphia 18, Pa.

Anal. Chem. 31, 910 (1959)

Spectrophotometric Determination of Residual Δ^4-3-Ketosteroids in Bulk $\Delta^{1,4}$-3-Ketosteroids. Differential Rates of Thiosemicarbazone Formation

A spectrophotometric method is devised for determination of Δ^1-3-ketosteroids in presence of $\Delta^{1,4}$-3-ketosteroids. The method is based on the reaction rate differential in the formation of the thiosemicarbazones. The Δ^1-3-ketosteroids–thiosemicarbazone complex is measured at 302 mμ.

A. A. FORIST, The Upjohn Co., Kalamazoo, Mich.

Anal. Chem. 31, 913 (1959)

Determination of Bound Styrene in Raw and Cured Polymers by Nitration

A spectrophotometric method is devised for determining the styrene content of polymers of raw and cured stocks. After proper extraction the polymer is nitrated yielding *p*-nitrobenzoic acid. The absorbance is measured at 265, 273.75, and 285 mμ. Precision and accuracy are in the order of 0.2%.

C. L. HILTON, J. E. NEWELL, and JACOB TOLSMA, U. S. Rubber Co., Wayne, N. J.

Anal. Chem. 31, 915 (1959)

Sodium-p-(mercaptoacetamido)benzene Sulfonate as Colorimetric Reagent for Cobalt

A sensitive method for determining cobalt(II) is developed using sodium-*p*-(mercaptoacetamido)benzene sulfonate as a specific reagent. A brown-red complex and a brown complex are formed with absorbance peaks at 475 mμ and 390 mμ, respectively, depending upon the pH. Iron and nickel produce slight interference.

H. K. L. GUPTA and N. C. SOGANI, Government College, Ajmer, India

Anal. Chem. 31, 918 (1959)

Ion Exchange Separations. Chromatographic Separations Based on Ion Charge

An ion exchange chromatographic separation procedure is developed for the analysis of trivalent metal ions. A cation exchange resin is used in the column and a solution of ethylenediammonium chloride as the elutant. Variables in the separation of zinc and lanthanum are studied.

J. S. FRITZ and S. K. KARRAKER, Iowa State College, Ames, Iowa.

Anal. Chem. 31, 921 (1959)

Acetylacetone as Analytical Extraction Reagent. Calculation of Successive Formation Constants from Extraction Data

A study is made of the formation and extraction of certain metallic acetylacetonates with acetylacetone. From extraction data successive formation constants for nine acetylacetonates are calculated. These values are in close agreement with those obtained potentiometrically.

ANOOP KRISHEN and HENRY FREISER, University of Pittsburgh, Pittsburgh 13, Pa.

Anal. Chem. 31, 923 (1959)

Polychromatic Technique for the Identification of Amino Acids on Paper Chromatograms

A simple, rapid technique for identification of amino acids in protein hydrolyzates is described. The amino acid mixture is resolved on a paper chromatogram using *n*-butyl alcohol–acetic acid–water solvent system. The paper is sprayed with a ninhydrin–cupric nitrate solution producing a polychromatic reaction, from which the amino acids are identified.

E. D. MOFFAT and R. I. LYTLE, Naval Medical Research Unit No. 4, USNTC, Great Lakes, Ill.

Anal. Chem. 31, 926 (1959)

Titrimetric Determination of Alkyl Mercaptan–Dialkyl Sulfide and Alkyl Mercaptan–Alkyl Disulfide Mixtures

Accurate titrimetric methods are developed for the determination of mixtures of alkyl mercaptan–dialkyl sulfide and alkyl mercaptan–alkyl disulfide. The titrants used are standard iodine and bromide-bromate solutions. Direct titration does not proceed for these substances in the presence of alcohol.

BRUNO JASELSKIS, University of Michigan, Ann Arbor, Mich.

Anal. Chem. 31, 928 (1959)

Spectrophotometric Determination of Traces of Nickel with 4-Isopropyl-1,2-cyclohexanedionedioxime

A spectrophotometric method is developed for determining micro quantities of nickel. The metal is reacted with 4-isopropyl-1,2-cyclohexanedionedioxime and the complex extracted with xylene and the absorbance measured at 383 mμ. Masking agents are used to control the interference of iron, copper, and cobalt ions.

B. L. McDOWELL, A. S. MEYER, Jr., R. E. FEATHERS, Jr., and J. C. WHITE, Oak Ridge National Laboratory, Oak Ridge, Tenn.

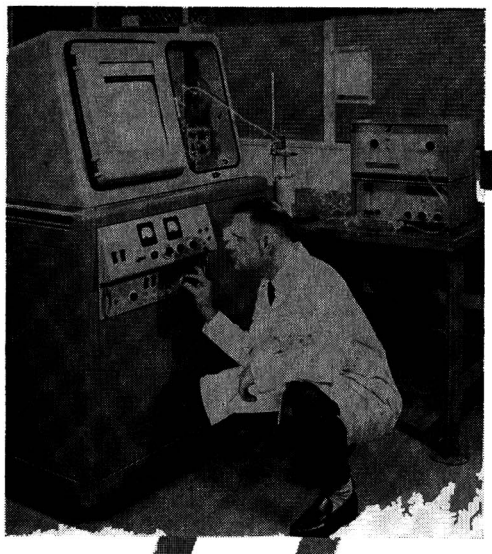
Anal. Chem. 31, 931 (1959)

Mass Spectrometric Analysis of Aliphatic Amides

The mass spectra of 35 aliphatic amides, comprising primary, secondary, and tertiary forms, are tabulated and correlated. Special note is given to the fragmentation and rearrangement ions and to the common spectral characteristics. The data aid in the determination of molecular structure of aliphatic amides.

J. A. GILPIN, The Dow Chemical Co., Midland, Mich.

Anal. Chem. 31, 935 (1959)



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BRIEFS

Determination of Traces of Mercury in Biological Material by Catalytic Action of Mercuric Ions. Microelectrolysis of Mercury

A sensitive method is described for determining the mercury content of biological materials. The method is based on the catalytic action of mercuric ion in the reaction between potassium ferrocyanide and nitrosobenzene which produces a violet color. Mercury is liberated by acid hydrolysis and bromine oxidation and extracted by electrolysis.

DUŠANKA PAVLOVIĆ and SMILJKO AŠPERGER, Institute "Rudjer Bošković," Zagreb, Croatia, Yugoslavia, University of Zagreb, Jukoviceva 1, Zagreb, Croatia, Yugoslavia

Anal. Chem. 31, 939 (1959)

Accurate Determination of Uranium in the Presence of Small Amounts of Molybdenum. Lead Reductor and Stannous Chloride Methods

A titrimetric method is devised for the accurate determination of uranium in the presence of molybdenum. Samples are dissolved in nitric acid and fumed to dryness with sulfuric acid. The residue is taken up in an acid solution and passed through a lead reductor. The effluent is collected under ferric sulfate and titrated with potassium dichromate. The stannous chloride method is investigated.

J. T. BYRNE, M. K. LARSEN, and J. L. PFLUG, The Dow Chemical Co., Denver, Colo.

Anal. Chem. 31, 942 (1959)

Resolution of Isomeric Xylenes by Gas-Liquid Chromatography

A gas-liquid chromatographic procedure is developed for separating isomeric xylenes. Various polar stationary liquid substrates are examined in conjunction with an aqua regia–treated C-22 firebrick column.

ALBERT ZLATKIS, S.-Y. LING, and H. R. KAUFMAN, University of Houston, Houston, Tex.

Anal. Chem. 31, 945 (1959)

Separation and Microidentification of Metallic Ions by Solvent Extraction and Ring Oven Techniques

A micromethod for separation and identification of 35 metallic ions in a small amount of sample is developed using solvent extraction and ring oven techniques. Success of the ring oven technique depends almost entirely on the specificity and sensitivity of the reagents.

P. W. WEST and A. K. MUKHERJI, Louisiana State University, Baton Rouge, La.

Anal. Chem. 31, 947 (1959)

Rapid Paper Chromatographic Microassay of Free and Ester Cholesterol of Blood

A simple and rapid quantitative paper chromatographic method is devised for determining free and ester cholesterol in blood serum. The sample is applied to Whatman No. 1 filter paper impregnated with zinc carbonate, and chromatographed with 1.5% ethyl ether in cyclohexane.

M. L. QUAIFE and R. P. GEYER, Harvard School of Public Health, Boston, Mass., H. R. BOLLIGER, F. Hoffmann-La Roche, Basel, Switzerland

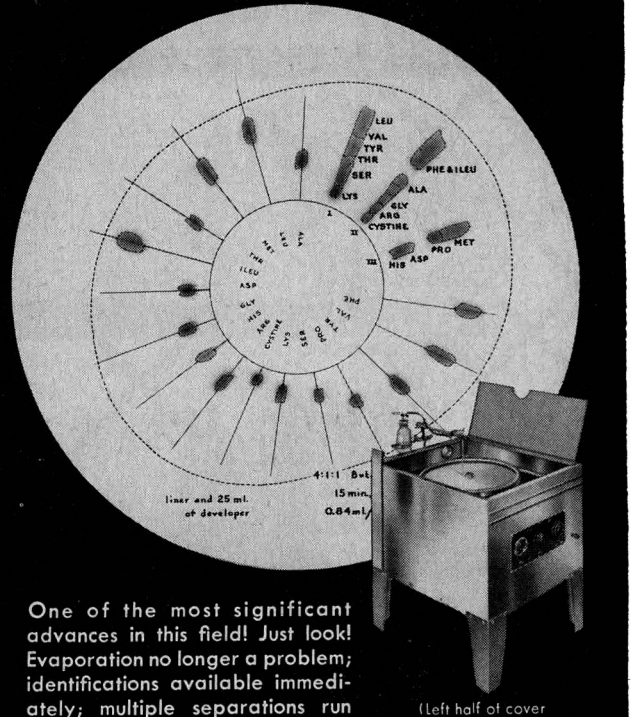
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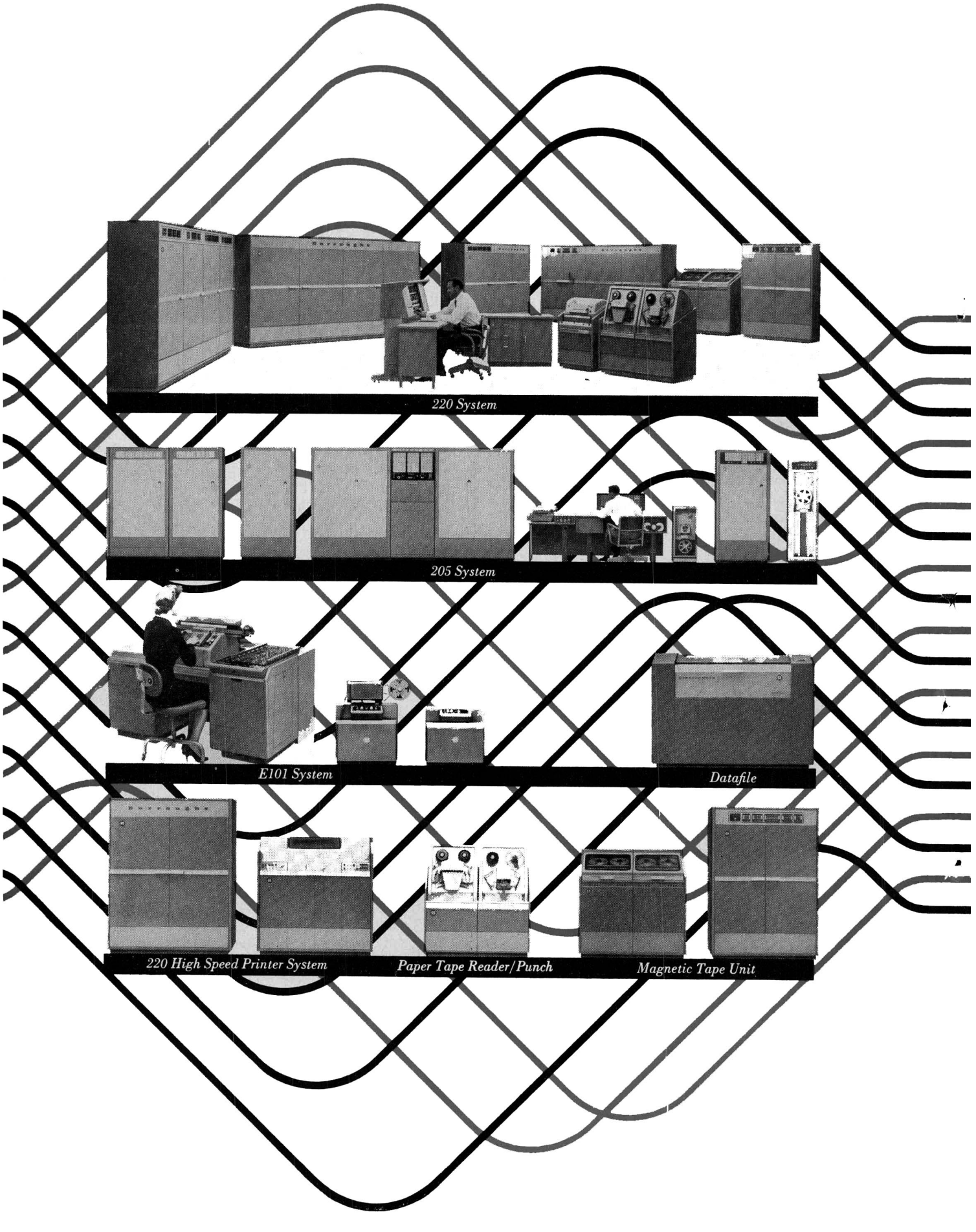
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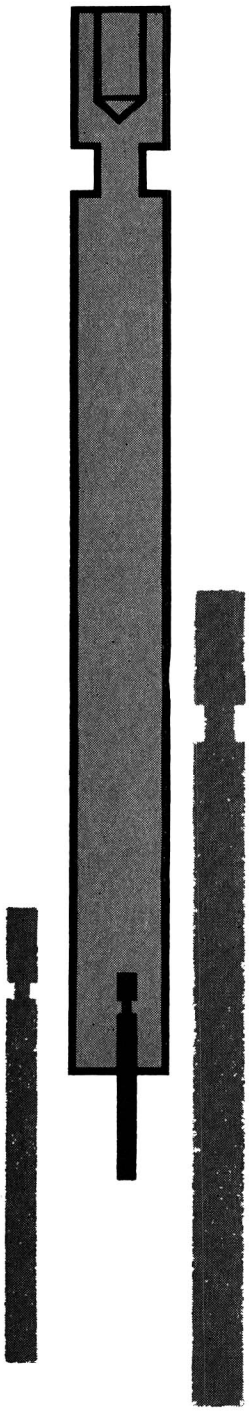
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*6. **Man-hours.*** Time needed for machining is expensive . . . particularly when it's time taken from important lab work.

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Statistics—1959 Tool for 1959 Analytical Problems

The analytical chemist, who is quick to visualize the potential of chromatography, automatic emission spectrography, nuclear magnetic resonance, radioisotopes, and organic reagents for inorganic analyses, should view statistics in the same light. Trying to solve today's analytical problems without a knowledge of statistical techniques is like trying to do a job with 1950 tools.

This month's Report for Analytical Chemists is intended to present practical examples of applications of statistics to everyday problems encountered in the analytical laboratory without going into details of the statistics themselves.

THE analytical chemist sits on a hot spot in today's highly technological society. Millions of dollars are being spent annually on research directed toward discovering new products, and the analytical chemist must assist this research by having available, when needed, methods which will tell the research man how much product he's got (if he's got any) and what the impurities are in his new composition of matter, or from his improved process for producing an old product. The solution of this type problem often involves the working out of an entirely new method of analysis to cope with possible interfering substances or to detect, and measure, hitherto unknown compounds.

Modern industry is leaning more and more toward catalyzed reactions, and often these catalysts are expensive; an example is the alumina-supported platinum catalyst so widely used for aromatization. It is not unusual for a million dollars' worth of this catalyst to be ordered, and when it reaches your plant, who decides the billed amount of platinum is in the pilled catalyst? The analytical chemist. If this catalyst becomes spent through use and is shipped back for reworking, how do you know how much platinum (part of your company's capital) you are sending out of your control? The analytical chemist has to tell you.

A heat exchanger in the plant fouls up with scale and sludge; a line, pump or vessel corrodes out; product quality goes to pot and shipments are returned; who gets the ox out of the ditch so that effective corrective measures can be taken quickly to minimize financial losses? The analytical chemist.

A customer who has been satisfied with your product if the sulfur content is below 0.01% suddenly wants not more than 5 p.p.m. of sulfur. Will your old method of analysis still be good enough? How do you know? Will a new me you found in the literature be cise enough? How do you out? Can you alter some step steps in your old procedure thereby gain enough precision analyze for this low sulfur con consistently, correctly? Wh steps should you try to change

Everybody admits that you analytical chemist) have a so technical background, are fa with the literature in your and by attending ACS or o professional society meetings abreast of the new developm But have you? Things have happening in the field of statis that will help you if you will use them in your daily work, since you don't normally read *metrics, Biometrika, Journal of American Statistical Associa* or other allied journals, these t are not found in your kit. As result you're trying to do a 1 job with 1950 tools. If you're ing chromatography, aut emission spectrography (such the Quantometer), organic re for inorganic analyses, nucl magnetic resonance, and radio topes, why not also use statistics

In 1951 Dr. W. J. Youd frequent contributor to this other magazines, wrote a bo "Statistical Methods for Chem In 1947 (revised 1957) a working with Imperial Ch Industries Ltd. issued a book, the editorship of O. L. Da "Statistical Methods in Resea and Production." A compa work, also edited by Davies, " Design and Analysis of Ind Experiments," first appeared 1954. Do you have any or all these books on your desk? they in your company libra Have you read them? The Che cal Division of the American ciety for Quality Control and section of Physical and Engine ing Sciences of the American tistical Association are jointly spo soring a new magazine, *Tech metrics,* which is appearing



C. T. Shewell was born in St. Louis, Mo., in 1902, graduated from high school there, attended the United States Naval Academy and the Massachusetts Institute of Technology. He went to work for the Humble Oil & Refining Co. in 1925, and has held a series of jobs ranging from chemical engineer to senior research specialist, the title of his present job. He was active in ASTM Committee D-2 and the Committee on Analytical Research of API during the war years, and is a member of American Statistical Association and Fellow and National Director for District 14 of American Society for Quality Control. He is author of several papers on statistics.

He has discussed statistics at local meetings of the American Chemical Society, American Society for Quality Control, and the American Institute of Industrial Engineers.

spring under the editorship of Dr. J. S. Hunter of Princeton. It will also be directed towards your area of interest.

Application of Statistics to Analysis

Having now gotten over the build-up, just what am I trying to sell you? A logical method of planning your work to ensure that you get the relevant data, that you get enough data (but not an unnecessarily large quantity of it) to have confidence in your conclusions, and a method for condensing blocks of data into a manageable, understandable, easily explainable form. Let us examine a few typical cases to see where statistics can be of daily help in your own job.

In Figure 1 there is a schematic diagram of the necessary steps in the analysis of a nonhomogeneous solid for a metallic element. The first step, quartering or sample size reduction, has sampling errors associated with it, since some of the lumps or pills may not be of the same composition as others due to contamination by corrosion products or scale, or migration of the catalyst. These errors in sampling may or may not be serious, but a set of statistically designed experiments will allow the estimation of the size of this error, and if serious, proper steps may then be taken to minimize its over-all effect.

What is this statistically designed set of experiments which will minimize the effect of the sampling error? Let it be assumed that only 12 analyses can be "wasted" in this study of a lot of catalyst. We can

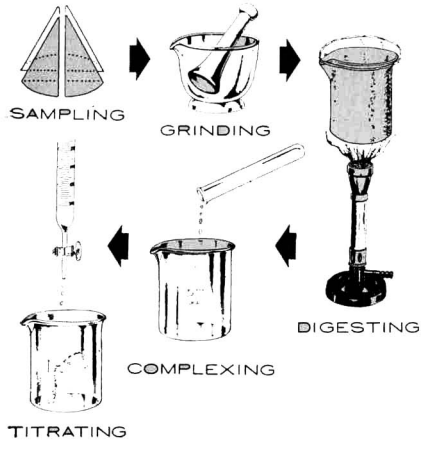


Figure 1. Typical steps in an analysis

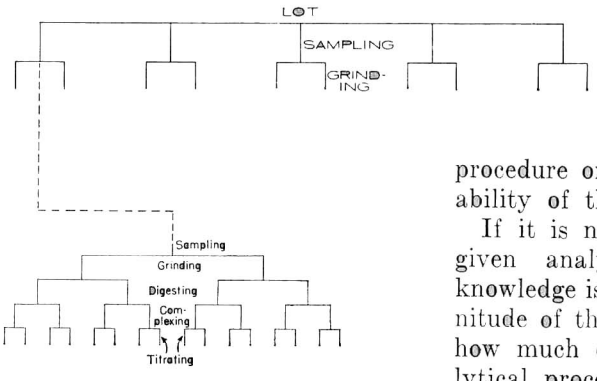


Figure 2. Breakdown of analytical procedure to allow error evaluation

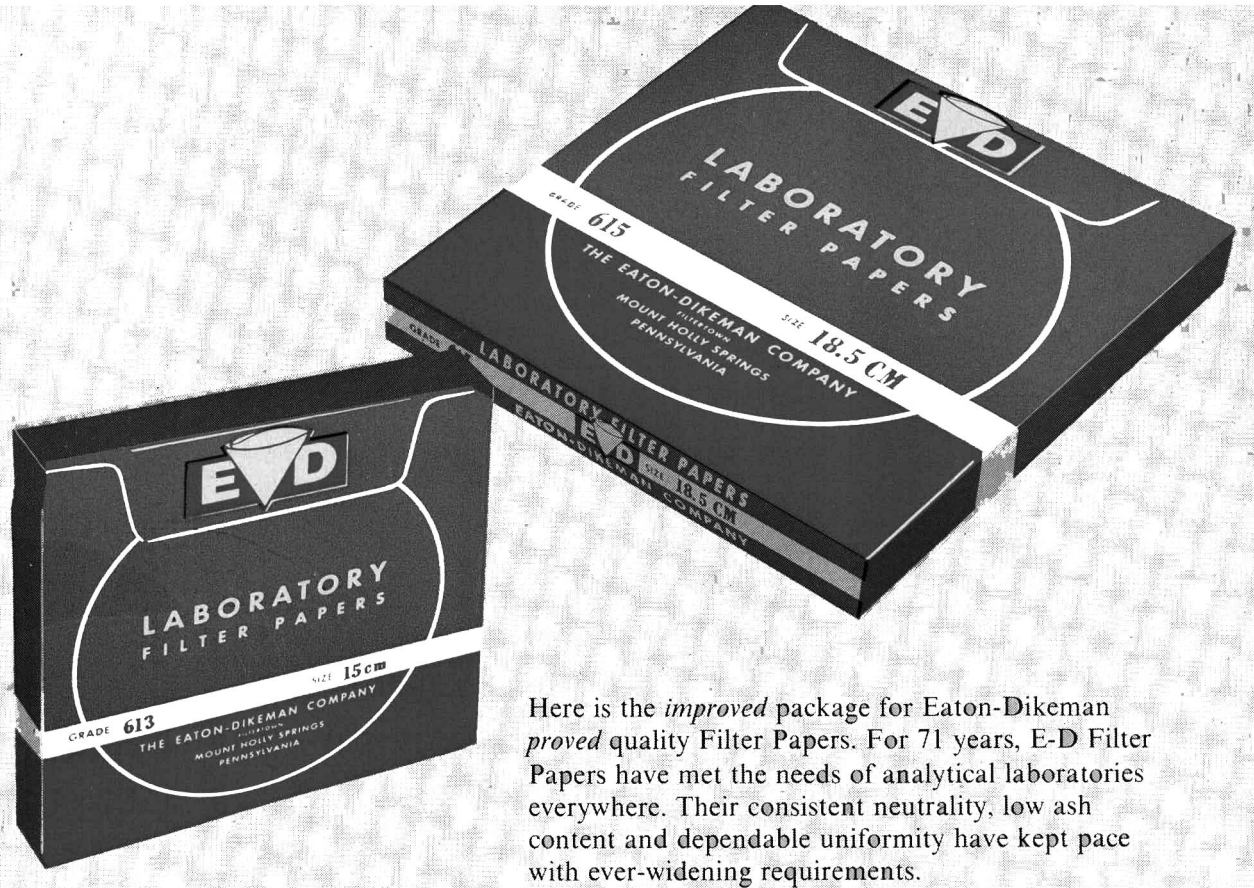
take one sample and analyze it 12 times, or take two samples and analyze each six times, or any other set of factors which result in 12 analyses. The problem is to choose that set of samples and analyses per sample which will give maximum precision to the over-all result. Strangely enough, and probably contrary to most chemists' intuitive feelings even if not contrary to their formal instruction, the correct design is to make only one analysis per sample. While this procedure leads to maximum precision in every case where sampling error is large compared to analytical error, it does not allow quantitative estimation of the sampling and analytical errors separately.

A quite different design is needed for these estimates. The sampling error cannot be measured separately from the analytical error, but the over-all error and the analytical error can be so estimated, and the sampling error computed from these two which can be estimated. The design which accomplishes this is one in which at least two samples are each analyzed at least in duplicate, but preferably something like three to four samples each analyzed three or four times.

Perhaps at this point an explanation of what is meant by "analyzed three times" should be made. Again referring to Figure 1, a portion of the ground material carried through the titration step represents one analysis; three such portions from the same grinding step independently carried through the titration are what is meant by "analyzed three times." It does not mean three titrations on aliquots from one flask of solution. This latter procedure only measures the variability of the titration step.

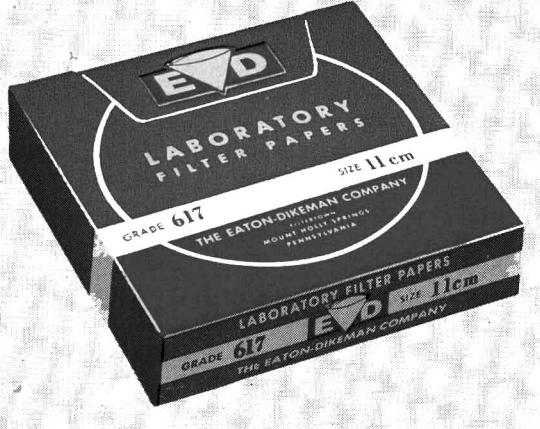
If it is necessary to improve a given analytical method, some knowledge is needed as to the magnitude of the total variability and how much each step in the analytical process contributes to this over-all error, so that effort may be concentrated on the step or steps where the greatest possible improvement may be obtained. How does one obtain this information in the best manner? Figure 2 shows the process of Figure 1 broken down into a pattern which would allow statistical evaluation of the error associated with each step in the analysis. The differences observed between aliquots at the titration step are due solely to such things as slight (0.005 ml.) errors in buret readings, difference in reagent addition time, slight over- or under-titration, temperature changes, etc. The observed differences at the complexing step are caused by incomplete complexing as well as titration errors. Statistical methods of computation will allow the calculation of the complexing error alone from the total error at this stage and the titration error. The same is true at each stage of the analytical process, the computations being performed in the reverse order to the actual operations.

As a result of these designed experiments and analytical results one now knows, for example, that the major source of variability is the grinding step, the next largest being the digestion step due to occlusion within insoluble residues, perhaps. You are the expert, however, and once statistics has pointed the finger of knowledge at the sources of your trouble, it is up to you to devise ways of eliminating these troublesome areas. You put on your thinking cap and finally come up with what looks like a good revision to the method which either eliminates the trouble spot completely, or drastically reduces its effect. Now, however, you are called upon to compare the two methods of analysis to demonstrate



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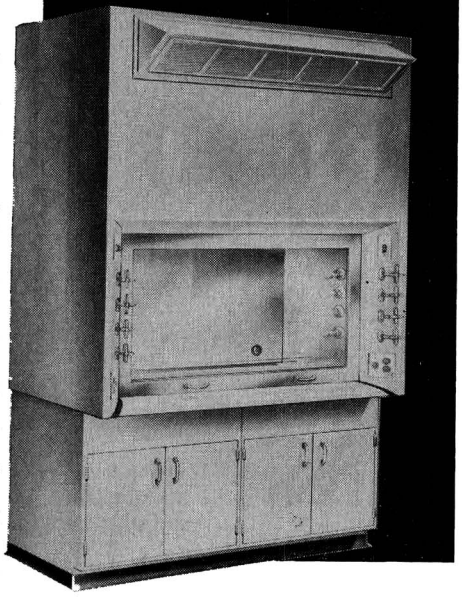
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that you have improved the analysis, without introducing some new and troublesome aspect. Again a designed experiment, utilizing both methods on several different lots, is called for. This will afford a statistically sound test of significance of any observed differences in the analyses by the two methods. Note carefully that I did not specify several different concentrations of a synthetic sample of known composition and no impurities. It is very important to evaluate methods on actual production samples in order that the method may be exposed to all the possible interferences it will be confronted with in actual use. It is also important to have the production samples used cover the widest expected concentration range, and at least three, preferably more, concentrations should be employed.

A favorite trick of the statistician is played on the analyst who boasts that his analyses are "right." The statistician finds out what the er-

Interferer 1		L								H					
Interferer 2		L				H				L				H	
Interferer 3		L		H		L		H		L		H		L		H	
pH		L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Concentration	L	3	26	20	15	25	17	32	22	9	14	7	19	27	18	8	31
	H	13	2	30	10	23	11	5	1	12	28	29	21	4	16	24	6

Figure 3. Factorial design to estimate effects of variables

Interferer 1		H								L					
Interferer 2		H				L				H				L	
Interferer 3		H		L		H		L		H		L		H		L	
pH		H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L
Concentration	L	1 4			24 31		8 19	28 30			7 13	14 17		3 22			9 27
	H		2 25	5 26		18 23			16 21	29 32			15 20		6 12	10 11	

Figure 4. Factorial design which allows estimation of errors

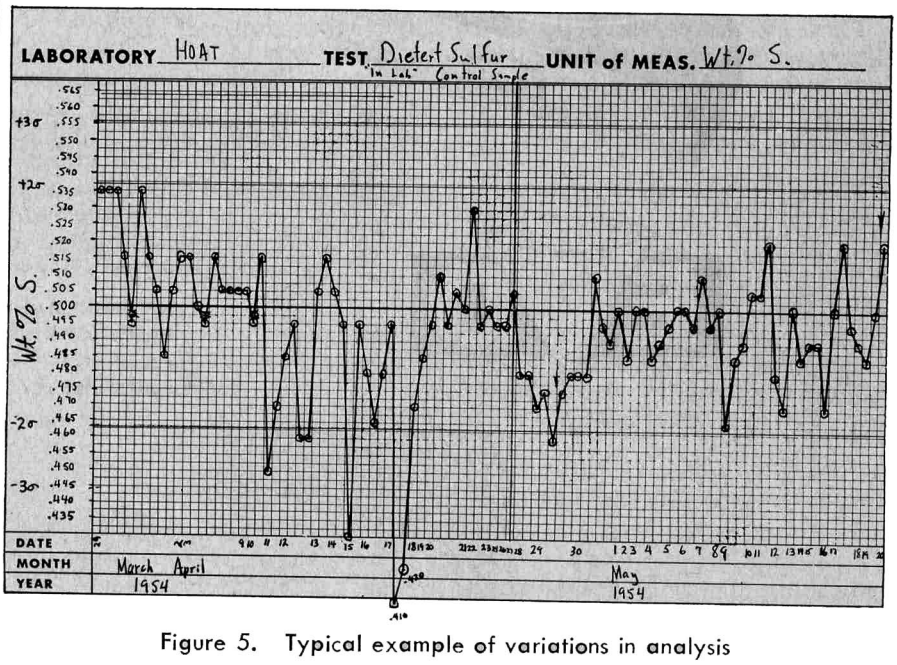
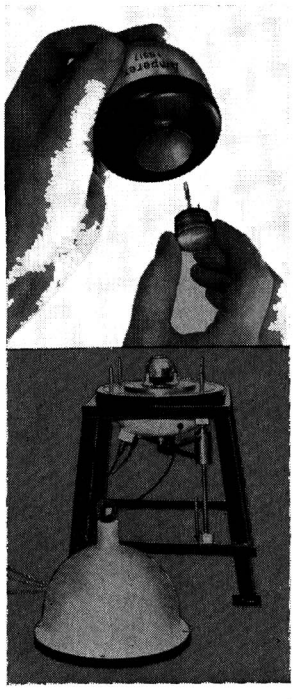
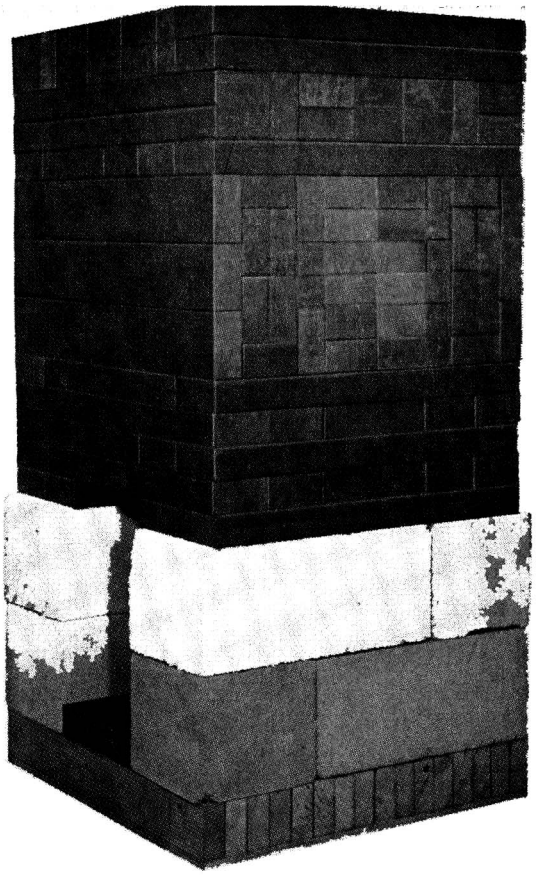


Figure 5. Typical example of variations in analysis

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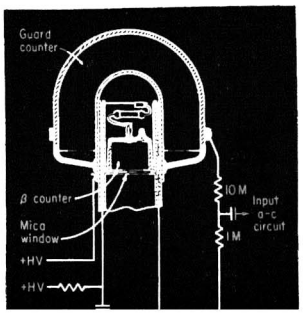
Because of its unique construction, processing, and gas mixture, the cosmic ray efficiency of the guard counter is virtually 100%. Thus, NO cosmic ray can actuate the inner tube without simultaneously actuating the guard counter.

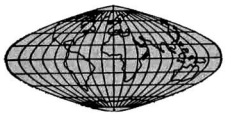
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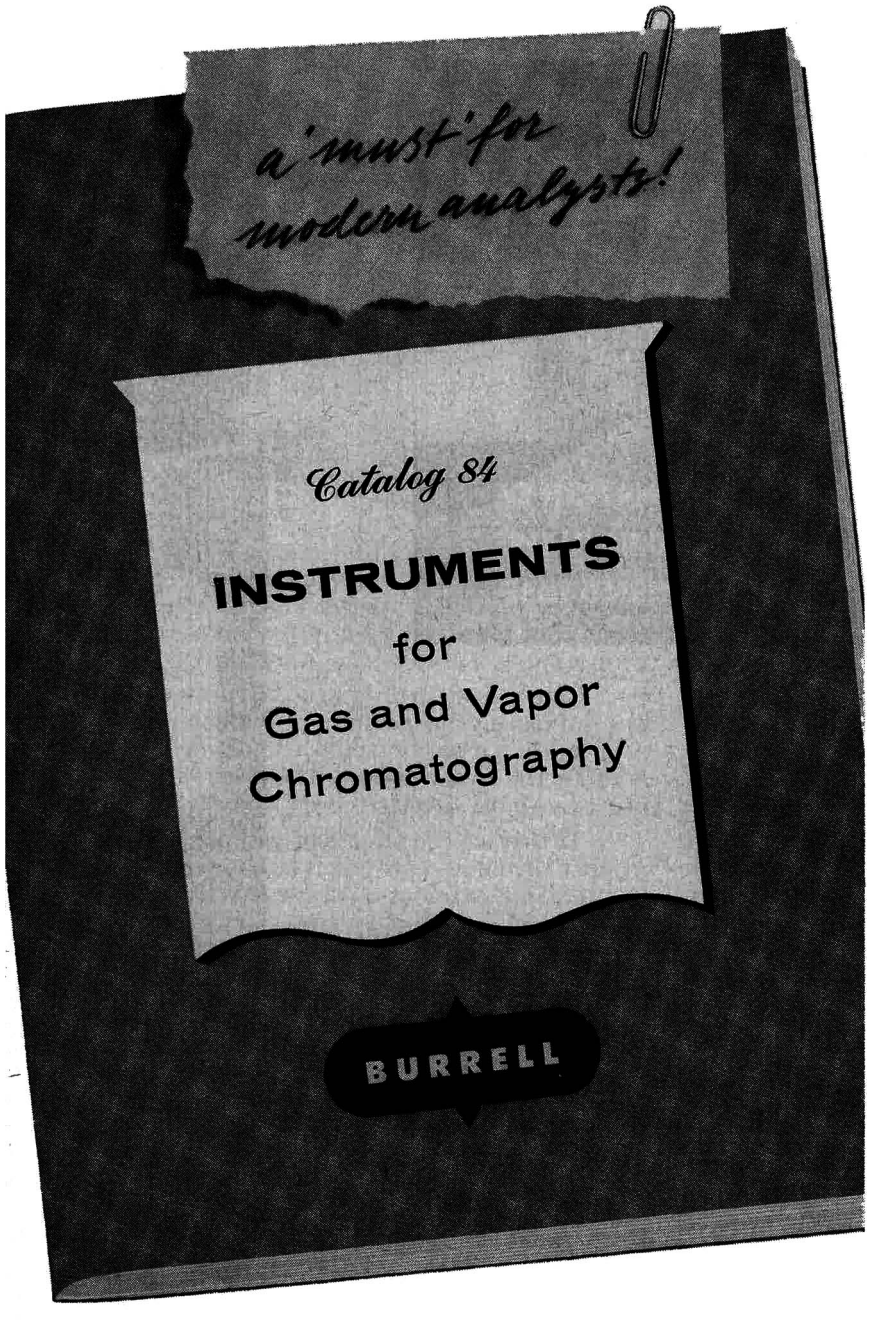
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rors of the analysis are, and then submits four samples, three of which are identical and the fourth differs from them by about one standard deviation, and asks the analyst to pick out the maverick on the basis of one analysis per sample. ASTM cooperative samples are usually "loaded" in this fashion.

Minimizing Laboratory Operating Costs

If you are worried about the costs in your laboratory operation, a statistically determined formula shows how to minimize sampling and analytical costs for a specified over-all variability. Although this article is supposed to be nontechnical in the sense of trying to teach statistics, this one formula is given. It is $n = \frac{\sigma_A}{\sigma_S}\sqrt{\frac{S}{A}}$ where n is the number of times each sample is analyzed, σ_A is the standard deviation of the analysis, σ_S is the standard deviation of sampling, S is the cost of obtaining a sample, and A is the cost of one analysis. If samples cannot be bulked, the actual n is chosen as the nearest integral value to the computed value, since application of the formula rarely results in an integral value (see pp. 111–112 of "Design and Analysis of Industrial Experiments").

If a new method of analysis must be devised to meet a changing or new plant operation or area of research, statistics can really help you reduce the volume of your work necessary to prove out your method. Assume that there are three compounds or elements, which might be present in a real sample, which you suspect might interfere in the analytical scheme; also assume that pH is felt to be an important variable; of course, concentration of the principal product or impurity is an important variable as you are trying to determine the "accuracy" of the method for this product or attempting to find the smallest detectable quantity if it is an analysis for an impurity. This gives five variables whose effects on the analysis must be investigated: interferer 1, interferer 2, interferer 3, pH, and concentration. A complete factorial design, which will allow estimation of the main

effects of each of the five variables as well as their two factor interactions is shown in Figure 3, in which the numbers indicate the order in which the analyses are to be made. This is a completely randomized set and does not give any information as to over-all error, unless one is willing to accept the higher order interactions as equivalent to error. The letters H and L merely indicate a high (H) or low (L) level of the corresponding factor, the actual values being chosen to lie within the region of factor space of interest.

The same analytical effort can be expended in another way, as shown in Figure 4, where a half replicate of the design of Figure 3 has been employed, each chosen run being duplicated in a random manner. This design will give the same information as that of Figure 3, and in addition will give a valid measure of error without any assumptions, against which the main effects and interactions may be judged for significance.

Perhaps all your methods work beautifully when in the hands of an experienced graduate analytical chemist, but what happens when you turn them over to routine analysts, who may not be graduates? Can they misinterpret the instructions in such a manner that a bias is introduced, or perform some step a little sloppily so that the variability is larger than it should be? What's the easy way to determine if either of these things is happening in your routine analytical laboratory? One way is to provide control charts on at least the critical analyses they perform on which are plotted their results of "blind" samples you have selected and perhaps standardized yourself. These charts will quickly reflect a level shift (bias) or variability change. Figure 5 shows one of our charts on Dietert sulfur. It is obvious that the same result was not obtained every time, and this is typical of every analytical method, from the most precise work at universities to determine atomic weight to routine analyses in anybody's plant. The minute there is variability in a measurement, then statistics should be used to measure and interpret it.

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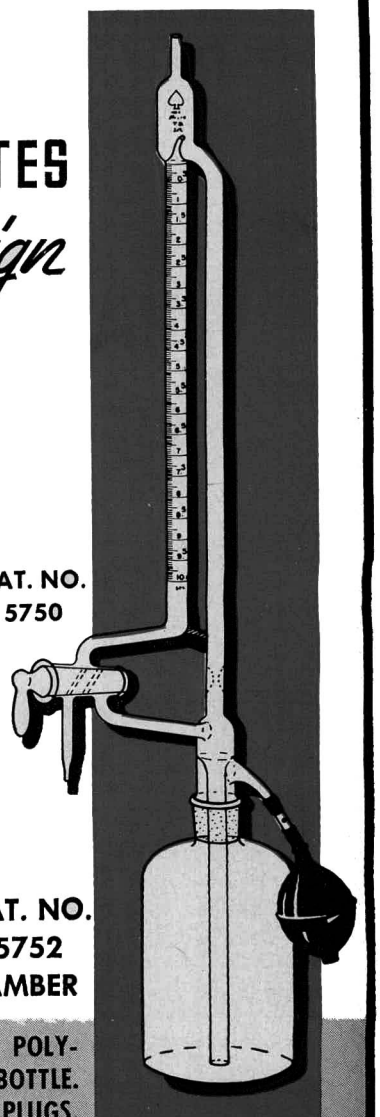
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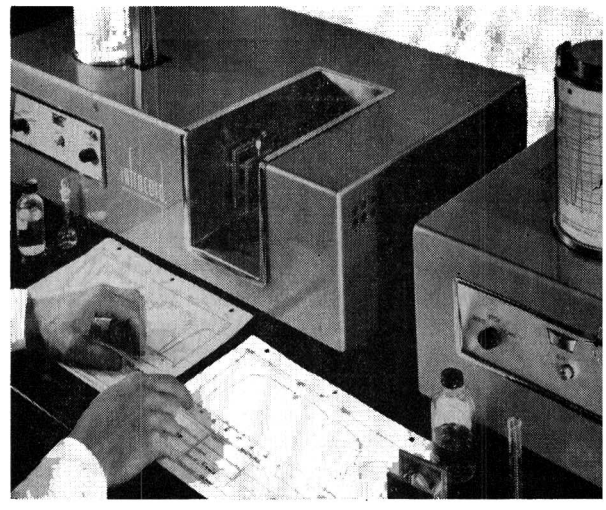
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WHAT THE INFRACORD® DOES FOR THE ORGANIC CHEMIST

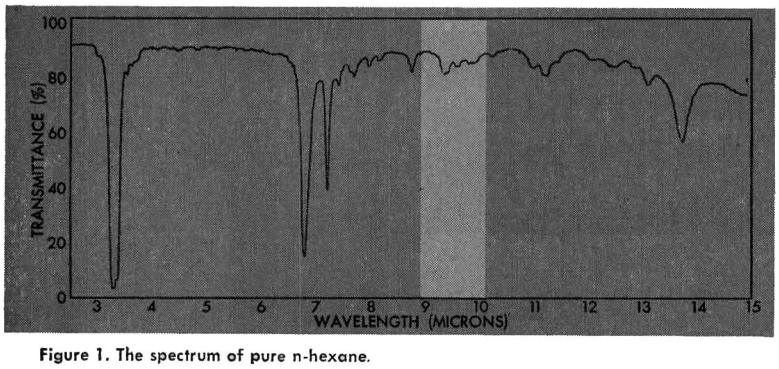


Figure 1. The spectrum of pure n-hexane.

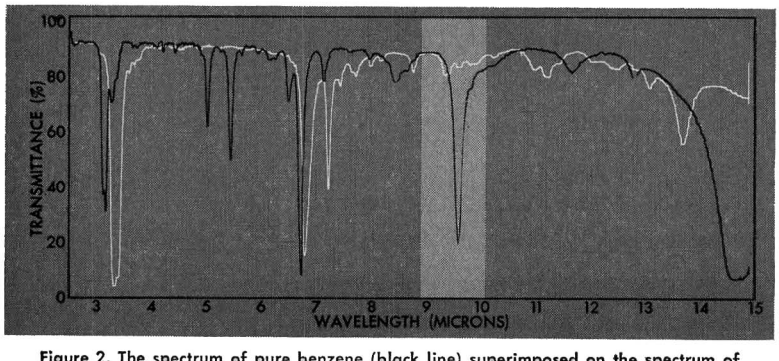


Figure 2. The spectrum of pure benzene (black line) superimposed on the spectrum of pure n-hexane (white line). Since n-hexane has no absorbance band in the 9.6 micron region, the 9.6 micron band of benzene (paler blue area) is selected as the band of interest.

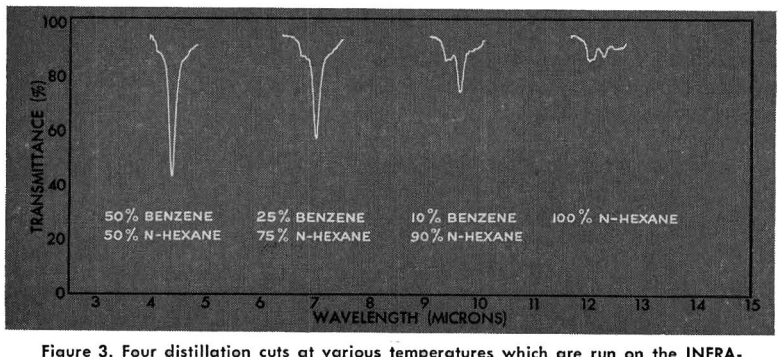


Figure 3. Four distillation cuts at various temperatures which are run on the INFRACORD in the 9 to 10 micron region. The chemist can watch the progress of his distillation until the benzene band disappears and he knows the process is complete.

A Perkin-Elmer® INFRACORD identifies unknowns; performs qualitative and quantitative analyses; controls the purity of products with speed, accuracy and reproducibility.

The introduction of Perkin-Elmer's NaCl INFRACORD Spectrophotometer two years ago placed infrared analysis for the first time within the reach of every chemist and spectroscopist.

Hundreds of INFRACORDS are now in use—in large and small laboratories—where they are helping the organic chemist with immediate and continuous control of his analyses. The INFRACORD instrument has proved a sound investment because it provides a wealth of information in minutes which would take hours or be impossible by classical methods.

How good is his distillation? How complete is his refluxing? How far has his reaction gone? The chemist with an INFRACORD Spectrophotometer at his elbow can get fast, accurate answers to these and many other of his problems.

Take the example of the chemist who wants to distill n-hexane containing a benzene impurity (Figures 1-3). From his spectra references, he first compares the spectra of pure hexane with pure benzene and selects the 9.6 micron band of benzene, since it has no interfering band from hexane in the same region. Then, as his distillation proceeds, he takes cuts at various temperatures and records the intensity of the selected band. When the 9.6 micron band disappears, his distillation is complete. The procedure is simple, efficient, accurate. There is no waiting or waste.

Qualitative Analysis with the INFRACORD

A big advantage of infrared to the chemist is that the spectrum allows him to observe the effects of his process on sensitive chemical groups in the molecule.

Each functional group has a characteristic absorption band, as distinctive from the bands of other groups as one fingerprint is from another. Standard references give band assignments for thousands of organic compounds. With these, plus his knowledge of the bond concept, the chemist quickly identifies the sample. The presence and location of the bands also tell him how the groups are assembled in the molecule.

The INFRACORD provides, on notebook-size paper for easy reference and filing, an accurate "fingerprint" of the unknown compound for correlation with known spectra. Identification then becomes a simple sorting procedure, requiring no special training in spectroscopy.

Quantitative Analysis with the INFRACORD

Since the depth of an absorption band is related to the concentration of the component, the amount of any material present in a sample is determined by comparing the depth of its band with the depth of the same band in a known concentration of the material. Usually, a few known concentrations are made up and run to provide a working curve of absorbance vs. concentration as a reference.

The INFRACORD can detect changes in concentration of ±1%. This sensitivity is another advantage of the instrument's double beam system, which permits differential analysis of two similar mixtures, expanding small differences in two compounds for accurate quantitative measurement.

Quality Control with the INFRACORD

The INFRACORD plays an important role through all processing steps, from raw material checks to following the progress of a reaction, determining yields and, finally, analysis of the finished product. Many companies use it to establish limits of material acceptance and as a specification of product purity.

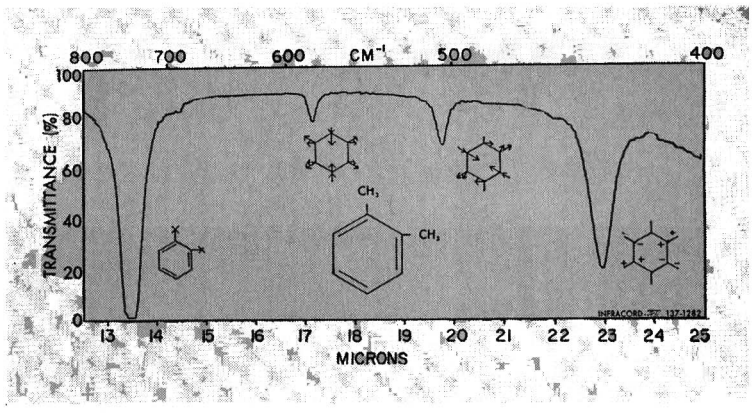
When the analyst knows what impurities he is looking for, he doesn't even have to run through an entire analysis—although that takes only 12 minutes. Using manual scan, he simply turns to the wavelength of interest and, within a minute or less, determines the presence or absence of the impurity.

Easy to Operate, Moderately Priced

With all its precision performance, the INFRACORD can be operated by untrained personnel with a minimum of instruction. Equally important, results of routine analyses can be interpreted accurately by the bench chemist without reference to the central laboratory. Thus, both the spectroscopist and the ultra-precision instruments of the central lab are freed for R & D work.

THE NEW KBr MODEL 137 INFRACORD

Complementing the NaCl Model 137, which operates from 2.5 to 15 microns, Perkin-Elmer now makes available for studies at longer wavelengths the KBr Model 137 INFRACORD, covering the region from 12.5 to 25 microns, with potassium bromide prism and optics. The KBr INFRACORD incorporates all the design and performance features of its companion NaCl instrument.



Shown above is a KBr INFRACORD spectrum of o-xylene; it illustrates the specificity of the bands associated with a change in the nature of a ring substituent, demonstrates one of the analytical advantages of working at the longer IR wavelengths.

The NaCl INFRACORD is priced at $4,050.00, f.o.b. Norwalk, Conn. The KBr instrument is $4,250.00. A full line of accessories for special types of analyses is also available, for example:

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- Microcells for small quantities of sample in solution, in various thicknesses
- Variable space cell with windows for compensation of solvents
- 5cm. and 10cm. gas cells for gas mixtures
- Minimum volume gas cell with windows (25cc. vol., 7.5cm. path length) for gas analysis, including chromatographic fractions
- 1-meter gas cell with windows and adaptor for mounting, for impurity studies
- Evacuable KBr dies with removable cone for solid samples
- KBr disc holder for solid samples
- Vial vibrator for KBr solid sample preparation
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- Specular reflectance attachment for measuring films deposited on IR-reflecting surfaces
- Polarizer accessory for analysis of substances in polarized IR beam
- Chart paper for linear transmittance and non-linear absorbance, as well as unmarked and gridless paper for special recording.

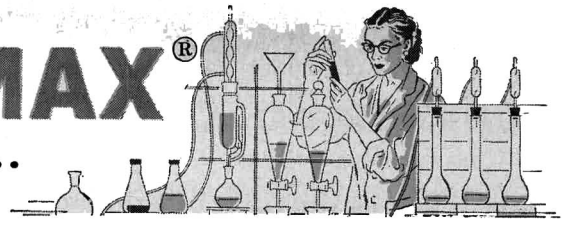
For further information on either the NaCl or KBr INFRACORD, write to us at 750 Main Avenue, Norwalk, Conn.

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Twelfth Summer Symposium to Feature Complex Reactions

Annual symposium of Division of Analytical Chemistry and ANALYTICAL CHEMISTRY to be held at University of Illinois June 10 to 12

Complex reactions in analytical chemistry will be the subject under consideration at the 12th annual Summer Symposium on Analytical Chemistry to be held at the University of Illinois, June 10 to 12.

Conducted under sponsorship of the American Chemical Society's Division of Analytical Chemistry and ANALYTICAL CHEMISTRY, the symposium will present 14 papers in a series of six sessions. In addition William L. Everitt, Georgetown University, will talk at the symposium banquet on the subject, "Science and Engineering in Russia—Personal Observation."

The formal technical sessions are to be interspersed with a series of social activities. These include a luncheon, followed by a tour at the Allerton Park estate in Monticello, about 20 miles from the University, an informal smoker and reception, and a banquet with entertainment and a featured speaker. Also available for use are the two University golf courses, swimming pools, tennis courts, and other athletic facilities. There are several small rooms available for those who wish to engage in informal discussions.

There are several types of housing arrangements to be used at the option of individuals attending the symposium. In addition to four hotels located in Urbana and Champaign and the various motels in the area, rooms in the brand new Men's Residence Halls have been listed for use at nominal rates. These are available for both single persons and married couples. It is suggested that best arrangements for families with children can be made at the local hostelries.

Charles N. Reilley, University of North Carolina, is the program chairman for the symposium, while G. L. Clark, University of Illinois, is chairman in charge of local arrangements.

Registration for the meeting will begin on Tuesday evening, June 9, in the lounge of the Men's Residence Halls. The technical sessions start on Wednesday morning at 9 A.M., Wednesday evening at 8 P.M., Thursdays at 9 A.M., and 2 P.M., and Friday at 9 A.M. and 2 P.M.

On Wednesday at 12:30 P.M. an informal luncheon will be held in the residence hall dining room, followed by the tour of Allerton Park, starting at 1:30 P.M. Informal dinner will be again at the residence hall at 6 P.M., and at 9:30, following the evening symposium session, an informal smoker and reception will be held in the lounge of the residence halls.

The symposium banquet is scheduled for Thursday evening at 7 o'clock, with William L. Everitt speaking as noted above.

While no events are scheduled for Friday evening, those wishing to do so may remain overnight at the residence halls.

Technical Program

Increasing Selectivity by Masking. K. L. Cheng, Kelsey & Hayes Co.

Complex Titrations, Principles and Methodology. Herman Flaschka, Georgia Institute of Technology.

Application of Chelating Agents to Analytical Separation Processes. Henry Freiser, University of Arizona.

Complexes in Cation Exchange Separations. J. S. Fritz, Iowa State College.

Current Developments in the Solution Chemistry of Complexes. D. N. Hume, Massachusetts Institute of Technology.

Anion Exchange Separation of Metal Ions. Kurt Kraus, Oak Ridge National Laboratory.

Analytical Applications of EDTA in the Paint Industry. C. A. Lucchesi, Sherwin-Williams Co.

Coordination Kinetics Applied in the Separation of Metal Ions. D. W. Margerum, Purdue University.

Reactions and Potentiometric Titrations of Highly Charged Metal Ions with Multidentate Ligands. A. E. Martell, Clark University.

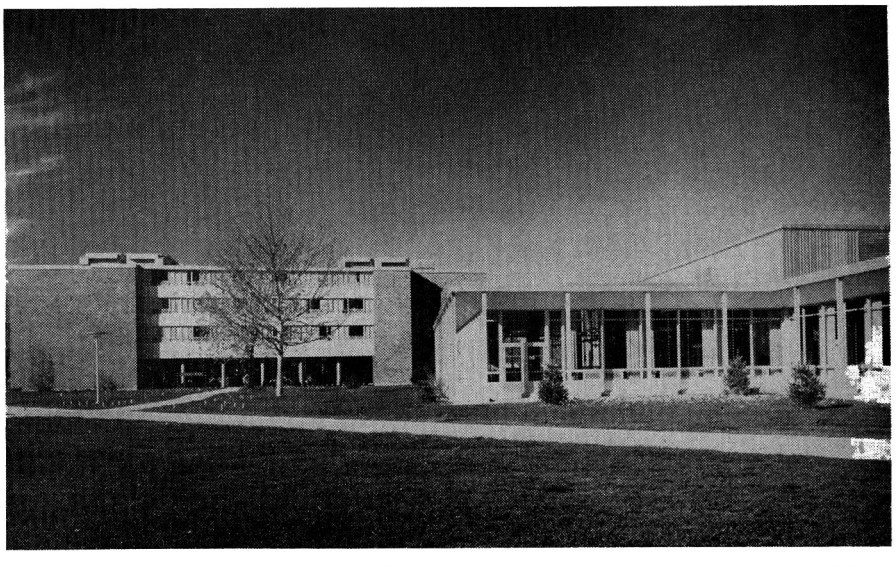
Reaction Rates and Stability of Metal Complexes. R. G. Pearson, Northwestern University.

Polarography of Metal Complexes. R. L. Pecsok, University of California at Los Angeles.

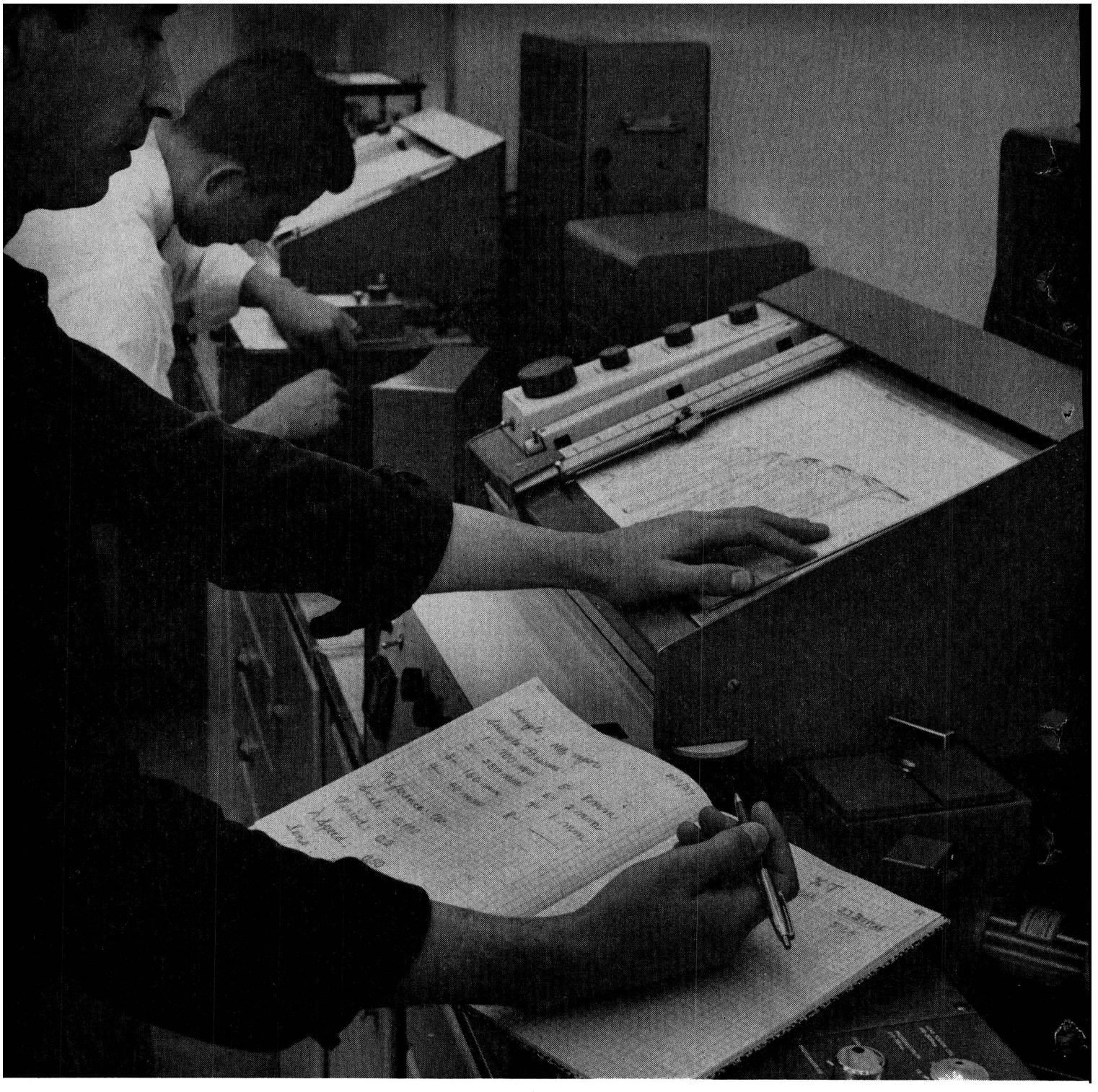
End Point Detection in Complex Titrations. C. N. Reilley, University of North Carolina.

Relationships between Metal Complex Stability and the Structure of the Complexing Agents. Gerald Schwarzenbach, Eidg. Technische Hochschule, Zürich, Switzerland.

Metalfluorechromic Indicators: Theoretical Considerations and Applications. D. H. Wilkins, General Electric Research Laboratory.



New University of Illinois Men's Residence Halls, scene of much of the 12th annual Summer Symposium's activities



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ANALYTICAL CHEMISTRY NEWS

MAY 1959

Microscopy Advances Highlighted at ACS Meeting

Inorganic analysis and instrumentation developments on Analytical Division program

BOSTON.—All records for a spring meeting were broken when approximately 8500 chemists gathered here for the week-long 135th National Meeting of the ACS. The analytical chemists were present in large numbers for their five-day program.

Three symposia highlighted the Analytical Division program: microscopy, inorganic analytical chemistry (Fisher Award), and instrumentation (Beckman Award). A special feature was an informal discussion of electroanalytical chemistry. Trends in clinical chemistry, subject of the dinner speaker, highlighted one field of analytical chemistry often overlooked by the analytical chemist.

Renewed Interest in Microscopy

Microscopy has undergone many changes since the last symposium on this topic was held by the Analytical Division a decade ago. The present symposium presented a well rounded discussion of the education and employment of microscopists and their tools, and applications. It was brought out by several speakers that microscopy, although recognized as a valuable tool, is often overlooked by the chemist. In many cases, even use of simple techniques and tools will solve what appear to be difficult problems.

The symposium, divided into four parts, dealt with microscopical specializations, professional activities, education, and management.

Specializations. The 19th century saw a rapid development of microscopy and its eventual decline, said Harold F. Schaeffer, College of Emporia. Early in the 19th century, microscopists were hindered by the lack of achromatic objectives. As this deficiency was overcome, microscopy fluorished. In the latter part of the century interest declined. Foundations had been laid, however, for important advances in the 20th century.

The past 50 years has seen a remarkable development in x-ray microscopy, George L. Clark, University of Illinois, stated. Areas of improvement include progress toward grainless photographic emulsions, reflecting lens microscopes, projection microradiography apparatus, use of soft x-rays, and a host of others.

A method and equipment developed by E. Cameron, University of Wisconsin, have made possible the determination of optical properties with reflected light rather than with transmitted light, said H. W. Zieler, Zieler Instrument Co. This makes possible studies of anisotropic opaque objects with a polarizing microscope.

A technique and equipment used by mineralogists and petrographers in crystal studies have generally been overlooked by the chemist according to Charles D. Jeffries, Pennsylvania State University. These are the universal stage procedures, including Emmons' five-axis stage. Such a method helps in the determination of such optical data as refractive indices, dispersion birefringence, and optic angle.

The electron probe instrument may prove to be a rival for the electron microscope, reported R. M. Fisher, U. S. Steel Corp. In this device, the electron optical system produces a narrow beam of electrons ranging from 100 A. to several microns in diameter. With this device, it is possible to examine the topography of a specimen without replica techniques.

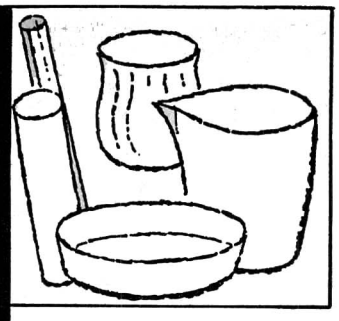
The field emission microscope in the hands of a skillful and imaginative operator is a promising research tool according to Erwin W. Müller, Pennsylvania State University. Although it is a specialized instrument with many limitations, it is useful in the form of a field electron microscope in studying adsorption layers on metal crystals at temperatures ranging from 0° to 2000° K. As a low temperature field ion microscope, it is very powerful with a resolution up to 2.3 A. It can show individual atoms in a crystal lattice.

Electron diffraction and microscopical studies of surfaces of anodically polarized stainless steels have been helpful in trying to determine reasons for passivation of such materials, said F. G. Rowe, Continental Oil Co. He



William B. Mason, University of Rochester (2nd from left) was dinner speaker at the Union Oyster House. Others at head table (left to right) are Warren W. Brandt, Purdue University, chairman; Mrs. Brandt, Herman Liebhafsky, General Electric Co., chairman-elect, and James I. Hoffman, National Bureau of Standards, 1959 Fisher award recipient

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Analysts Honored at Boston Meeting



James I. Hoffman, National Bureau of Standards (left) received the Fisher Award in Analytical Chemistry from Claude A. Pamplin, Fisher Scientific Co. He was given this award "in recognition of his many distinguished contributions to the advancement of analytical chemistry, and for noteworthy authorship."

Hoffman, a veteran of 39 years at the National Bureau of Standards, is now head of the metallurgy division after having worked for many years in the chemistry division.

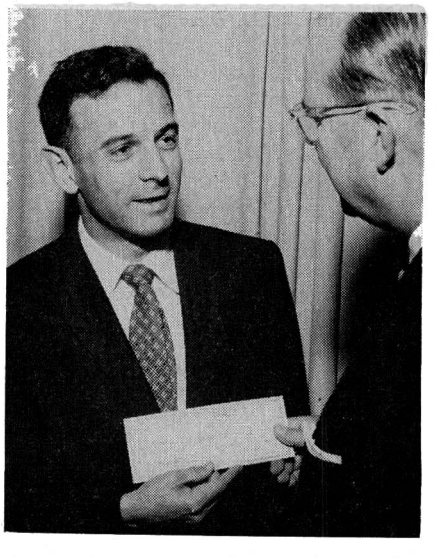
His work includes development of phosphate rock standards, alumina recovery and analysis, purification of uranium, and authorship of several books on analysis.



Howard Cary, president of Applied Physics Corp. (left) received the Beckman Award in Chemical Instrumentation from Henry J. Noebels, Beckman Instruments, Inc.

The award citation describes Cary as one "whose insight, ingenuity, and urge to achieve perfection in instrument design have aided in the further development of higher standards of performance for instruments used for chemical analyses."

Cary was instrumental in developing pH meters, glass pH electrodes, quartz spectrophotometers, commercial infrared spectrophotometers, photoelectric Raman spectrographs, vibrating reed amplifiers and electrometers, and spectrophotometers.



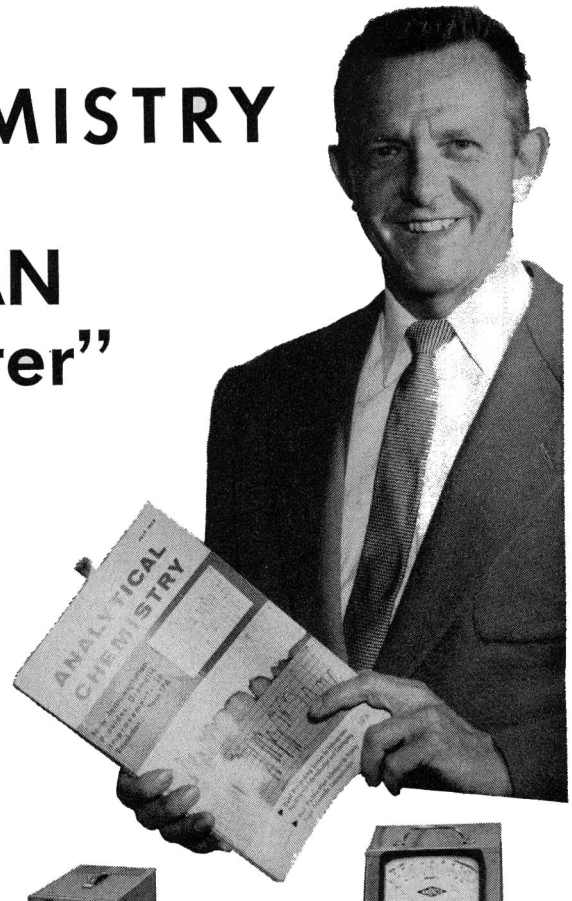
George C. Pimentel (left) received the American Chemical Society Award in Petroleum Chemistry from Chester A. Warner, Precision Scientific Co.

The award, given by the Precision Scientific Co., was presented to Pimentel, associate professor, University of California, "for outstanding discoveries involving the application of infrared spectroscopy to the study of reactive chemical species."

More specifically, he developed the matrix isolation technique for studying free radicals. By this technique, he can observe infrared spectra of radicals at temperatures down to absolute zero.

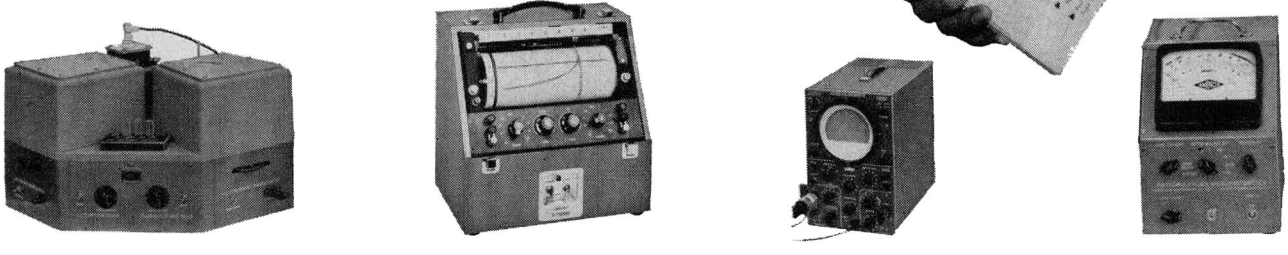
He has also worked on infrared spectra of molecular crystals, the mixed crystal technique, infrared spectra of water adsorbed on silica gel, and proton magnetic resonance research.

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The American Instrument Co., Inc., 8030 Georgia Avenue, Silver Spring, Maryland, has compiled a list of more than 200 compounds analyzed by the SPF which shows activation and fluorescence maximum wavelengths, pH, and ultimate sensitivity. The list includes a newly-compiled bibliography of papers that describe new techniques and applications, a full description of the Aminco Spectrophotofluorometer, and selected reprints of technical papers.

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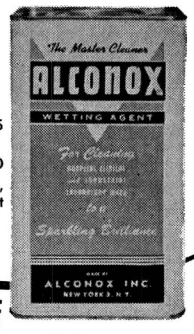
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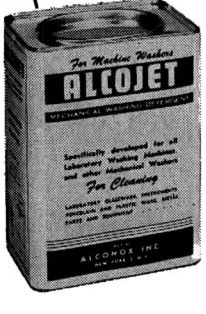
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concludes that the passive layer formed on stainless steel by the atmosphere is a crystalline oxide of chromium, iron, and nickel, depending on the alloy involved. Anodic passivation destroys this layer. The new passive layer, he believes, is something beyond a material barrier, such as an energy barrier.

Professional Activities. The electron microscope has several limitations, said F. A. Hamm, Minnesota Mining and Manufacturing Co. The microscopist must, therefore, educate management as to the limitations and deficiencies of this tool. This is particularly true with respect to particle size distribution in photosensitive systems. Some limitations, however, are those resulting from lack of ingenuity on the part of the electron microscopist.

The microscopist in the chemical laboratory who works with transparent hard materials often overlooks techniques used in other fields, said R. E. Wright, Shell Chemical Corp. In the case of materials not adapted to microtomy, petrographic grinding techniques may be used. Sources of information for such techniques are often found in the literature of the petrographer and ceramicist rather than the chemical journals.

To learn about living cells, it is necessary to study living cells and not static ones, said George O. Gey, Johns Hopkins University. He has developed original instrumentation and techniques which permit him to observe growing cells and photograph them on motion picture film. With such photomicrographs, he has been able to observe mutations of living cells.

Microscopy can be very helpful in solving diverse problems encountered in industrial applied research, said Charles M. Schwartz, Battelle Memorial Institute. To be fully effective, however, conventional microscopy must often be supplemented by specialized microscopical techniques. Lack of such equipment and skills can hamper the solution of some product improvement or development projects.

The chemical microscopist in government is apt to encounter a wide variety of problems, reported Charles P. Saylor, National Bureau of Standards. NBS, in addition to establishing standards of particle size, shape, and distribution, is called on to do such specialized jobs as examining works of art for fraud or alteration, examining evidence in criminal cases, and the like.

Education. There are many advantages to getting students used to working with microscopes by having them use them in courses in qualitative and quantitative analysis and physical chemistry. This is a particularly useful way to teach the use of microscopes to undergraduates, said Warren W. Brandt, Purdue University.

Even if a chemical engineer never uses a microscope after he graduates, training in its use sharpens his powers of observation and interpretation, said C. W. Mason, Cornell University. In solving chemical engineering problems, very often a careful microscopical examination of the materials involved will give many clues as to solution to the problem at hand, particularly where physical structure may be as important as chemical properties.

A pioneer in teaching chemical microscopy, Mary L. Willard, Pennsylvania State University, has developed several courses in the field for both beginners and advanced students. She raised questions as to whether such courses should concentrate on a few fundamental areas or develop skills in many specific areas.

Requirements for an electron microscopist, said Cecil E. Hall, Massachusetts Institute of Technology, include a background in physics and mathematics and generally advanced training in such fields as biology, metallurgy, or medicine. With such a background, a one-term lecture and laboratory course, followed by a year's experience, should qualify one to be an electron microscopist. The principles and uses of the electron microscope could be included in many science courses to give students enough knowledge of this tool to collaborate intelligently with specialists.

In many cases, skill in the use of a polarizing microscope would save a research chemist much time, reported E. G. Rochow, Harvard University. In some inorganic preparations, for example, the product may appear in two or more phases whose physical properties differ sufficiently to cast doubt on the identity of the product. A few simple observations under a microscope would indicate such conditions.

Management. The electron microscopist at the management level must be able to handle both administrative and technical aspects of the work, said Max Teague, Chrysler Corp.

Electron microscopy is still less of a science than an art, said J. H. L. Watson, Ford Institute for Medical Research. Based on a survey, he feels that more trained men are needed in this field, that management should specify more clearly the nature of the duties and responsibilities of those connected with this work, and that a distinction should be made in responsibilities of technicians, research men, and managers.

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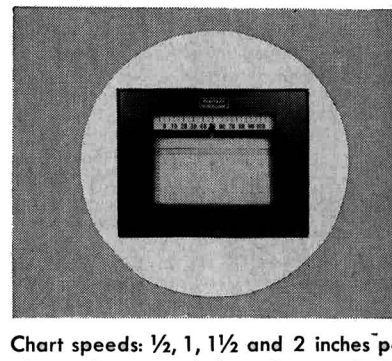


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The microscopist in solid state work has the opportunity, not only to deliver highly skilled analyses of constitution and structure but to study the basic aspects of atomic and molecular phenomena.

The professional microscopist in industry, said James Hillier, RCA Laboratories, must not only be a skilled operator of his instruments but must be given a broad understanding of the company's research problems. Responsibility for securing skilled, creative personnel and keeping them informed falls to the supervisor.

George L. Royer, American Cyanamid Co., who also discussed management's views, said that in some companies, advancement in industrial research groups is not limited to the administrative ladder but that professional ladders have been established. Thus, a man can progress in his field without leaving his work to go into purely administrative work.

Symposium Honors J. I. Hoffman

Development of standard samples is an essential element in the development of analytical chemistry, said Fisher Award winner James I. Hoffman, National Bureau of Standards.

Almost all analytical methods depend on reference to some material whose composition or behavior is known. Without reference standards, the analytical chemist would have no way of determining the validity of his results.

Development of reference standard goes hand in hand with development of chemical methods of separation and analysis. One must have materials of known purity to mix blend or alloy or methods of proved reliability for analysis of existing materials.

Certified standards are even more essential in the field of industrial analysis, which uses instrumentation and automation to a considerable extent.

The National Bureau of Standards has a very active standard sample program, Hoffman said.

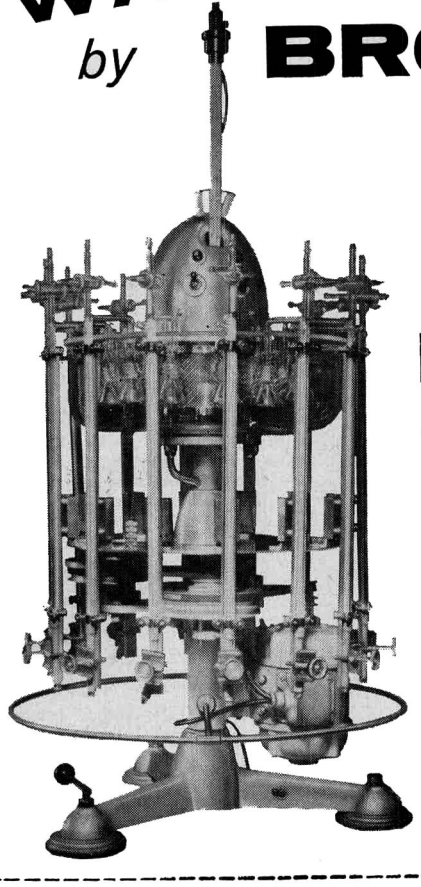
The symposium was devoted to discussion of recent developments and current problems in inorganic analytical chemistry. Specific subjects covered were nuclear materials, fertilizers, and metals.

The nuclear field was described by C. J. Rodden, Atomic Energy Commission. He said that analysis of these materials is arbitrarily divided into major and minor constituents. Major ones are fuel materials and moderators. Fuels consist of uranium, thorium, plutonium, and alloys; moderators include heavy water, graphite, and beryllium. Minor constituents fall in two categories: those which absorb neutrons and those which adversely effect the metallurgy of the fuel or moderator.

In the fertilizer area, K. D. Jacob, U. S. Department of Agriculture, stated that 16 or more elements are recognized as essential to growth of higher forms of plant life. These are carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, chlorine, copper, iron, manganese, molybdenum, and zinc. While official analytical methods have existed for many decades for major fertilizer materials (nitrogen, phosphorus, and potassium), official methods for others have been of more recent origin. Methods used include improved old ones and new ones. Instrumentation and organic reagents are being used widely. An insistent need is for better laboratory techniques for rapid estimation of nutritive potential of fertilizers.

Standards have become of increasing importance in the development of methods of analysis, said Robert M. Fowler, Electro Metallurgical Co. This is particularly true as the era of purely

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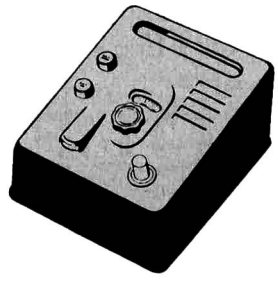
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classical methods has given way to instrumental methods. Analytical chemists in this field face several challenging problems, including determination of gases in metals, estimation of metallic compounds, and associations existing in metals in both liquid and solid states.

Symposium Honors Howard Cary

In developing complex analytical instrumentation, two laws hold, said Howard Cary, Beckman Award recipient. These are: law of conservation of difficulties which says that no matter how many difficulties are overcome, new ones develop so that the total number remains constant; and the second law, which says that if anything can go wrong, it will.

The analytical instrument industry, he said, consists of approximately 50 companies in the United States, of which about a dozen make complex instruments. Annual output of this industry totals $200 to $300 million.

This phase of the instrumentation industry has several characteristics and methods of operation. Success depends in part, he said, on teamwork by skilled men who have worked together for a long time.

Decisions facing the instrument maker fall in four major categories: what to make; how to make it; construction and testing of prototypes; and production engineering.

Estimated costs and sales analyses are major items which are difficult to determine accurately.

One major difficulty facing instrument makers is the lack of basic scientific knowledge. Most of the basic data come from academic sources.

Suggestions for new instruments often come from outside, from those who need an instrument to do a job, from those who have developed a research instrument for laboratory use, from the armed services, and from other manufacturers.

Before development is undertaken, an effort is made to obtain costs and sales data. Development costs are difficult to determine and market surveys for new instruments are unreliable, he said.

In developing an instrument, it is necessary to determine performance, cost, reliability, and service needs; to determine where components can be obtained with particular attention to items which can be supplied by only one source. Project engineering refinement is particularly important.

After the prototype is made and tested, it is difficult to decide when to stop redesigning and improving and go into production. Generally it is

better to produce an instrument of moderate performance soon than to hold off a long time until the "ultimate" model can be produced.

The production phase presents problems of whether to build or buy components, tool design, development of parts lists with drawings and specifications, and assembly and test procedures.

In determining costs, it is necessary to include costs of unsuccessful projects, and to consider the cost of developing one-of-a-kind or limited production items. Cary estimates development costs of a successful instrument at 10 to 15% of the sales price of the first five years' sales. Changes in design increase costs 2 to 5%.

Continued growth in the numbers and types of instruments and the ever-increasing variety of uses to which they are being put in analysis was emphasized in the papers presented at the Beckman symposium.

Typical examples include a high resolution spectrofluorophotometer which is made from commercially available components. It was described by R. E. Rehwoldt, Lehigh University.

Scale expansion of the intensity ordinate of infrared spectrophotometers increases sensitivity and allows discernment of very weak absorption bands, said D. F. Westneat of Du Pont. The system involves use of high length to volume gas cells.

Other instruments include an automatic recording velocity-servo potentiometric titrator described by Myron T. Kelley, Oak Ridge National Laboratory and a multifunction automatic recording phototitrator by Paul W. Mullen of Du Pont.

Saul Gordon, of Gordon and Campbell, stated that instrumentation developments allow applications of thermogravimetry under conditions of controlled atmospheres as well as at ambient and reduced pressures.

Continuous titrations in fused salts can now be carried out, said Joseph Jordan, Pennsylvania State University. Temperatures varying from 150° to 500° C. are used.

William S. Pappas, Union Carbide Nuclear Corp., described a low cost continuous analyzer which is made to determine moisture in air.

Richard D. Dierks, Hanford Atomic Products Operations, described a modified single-beam filter photometer used to analyze radioactive process streams. This instrument avoids radiation exposure of operating personnel.

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(Continued on page 46 A)

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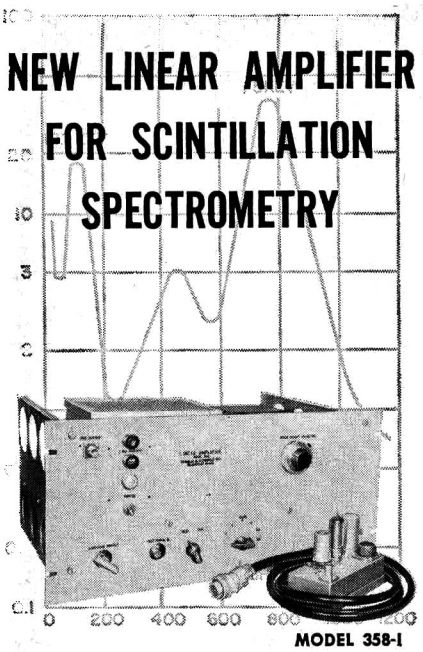
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ANALYST'S CALENDAR

May 2 — **Analytical Group, New York Section, ACS,** College of the City of New York. Weighs and Means in Analytical Chemistry. *Contact:* E. A. Wynne, Fisher Scientific Co., 1 Reagent Lane, Fair Lawn, N. J. Page 42 A, April I.

May 3 to 7 — **Electrochemical Society 115th National Meeting,** Sheraton Hotel, Philadelphia, Pa. *Contact:* H. C. Mandell, Jr., Pennsalt Chemicals Corp., P. O. Box 4388, Philadelphia, Pa. See page 59 A.

May 5 — **Society for Applied Spectroscopy,** Hotel New Yorker, New York. Topic: Determination of the Ratio of Phenol-Hydroxy & Methyl Groups in Silicones by Infrared Spectroscopy. Speaker: E. R. Shull, Linde Air Products, Inc. *Contact:* Paul Lublin, Sylvania Research Laboratories, Bayside, N. Y.

May 5 to 7 — **14th Purdue Industrial Waste Conference,** Purdue University. *Contact:* D. E. Bloodgood, Purdue University, Lafayette, Ind.

May 8 — **American Chemical Society, Analytical & Microchemical Group. Philadelphia Section.** Socony Mobil Oil Co., Inc., Paulsboro, N. J. Open house. *Contact:* Sidney Alpert, National Drug Company, Haines & McCallum Streets, Philadelphia 44, Pa.

May 11 to 13 — **Symposium on Instrumentation and Computation in Process Development and Plant Design,** Westminster, England. Sponsored jointly by the Institution of Chemical Engineers, Society of Instrumental Technology, British Computer Society. *Contact:* Institute of Chemical Engineers, 16 Belgrave Sq., London, S.W. 1, England.

May 12 — **American Association of Spectrographers.** Western Society of Engineers Building, Chicago, Ill. Topic: Use of Organic Solvents in Flame Photometry. Speaker: J. A. Dean, University of Tennessee. *Contact:* C. A. Lucchesi, Sherwin-Williams Co., Chicago 28, Ill.

May 17 — **Seventh Meeting on Mass Spectrometry,** ASTM Committee E-14, Hotel Statler, Los Angeles. *Contact:* A. G. Sharkey, U. S. Bureau of Mines, 1200 Forbes Ave., Pittsburgh 13, Pa. Page 51 A, Nov.

May 18 to 20 — **5th National Symposium on Instrumental Methods of Analysis,** Houston, Tex. Instrument Society of America. *Contact:* M. D. Weiss, program chairman, Union Carbide Olefins Co., South Charleston, W. Va. Page 54 A, January.

May 18 to 22 — **ASTM Committee E-14 on Mass Spectrometry,** Statler Hotel, Los Angeles, Calif. *Contact:* American Society for Testing Materials, 1916 Race St., Philadelphia 3, Pa.

May 20 — **Spectroscopy Society of Pittsburgh.** Mellon Institute. Topic: Our Challenge in Technical Services. Speaker: Neil E. Gordon, Jr., Westinghouse Electric Corp. *Contact:* A. G. Sharkey, Jr., U. S. Bureau of Mines, 1200 Forbes Ave., Pittsburgh 13, Pa.

May 20 — **Cleveland Society of Spectroscopy & Western Reserve University. 4th Annual Conference.** Case Institute of Technology, Cleveland. *Contact:* Mildred S. Vigler, Standard Oil Company, Cleveland 28, Ohio.

May 21 — **American Chemical Society, Analytical & Microchemical Group. Philadelphia Section.** University of Pennsylvania Museum. Topic: Titration in Nonaqueous Solutions. Speaker: E. G. Wollish, Hoffmann-LaRoche, Inc. *Contact:* Sidney Alpert, National Drug Co., Haines & McCallum Sts., Philadelphia 44, Pa.

May 27 to 28 — **24th Midyear Meeting, Division of Refining,** American Petroleum Institute, Statler Hotel, New York, N. Y. *Contact:* API, 50 W. 50th St., New York 20, N. Y. Page 50 A, April I.

May 28 — **Metropolitan Microchemical Society.** American Museum of Natural History, New York, N. Y. Subject: The RCA Electronic Music Synthesizer. Speaker: H. Donley, RCA Research Laboratories. *Contact:* Erik R. Hoffmann, Ethicon, Inc., P. O. Box 151, Somerville, N. J.

May 30 to June 5 — **Fifth World Petroleum Congress.** Coliseum, New York. *Contact:* Fifth World Petroleum Congress Inc., 527 Madison Ave., New York 27, N. Y. Page 36 A, January.

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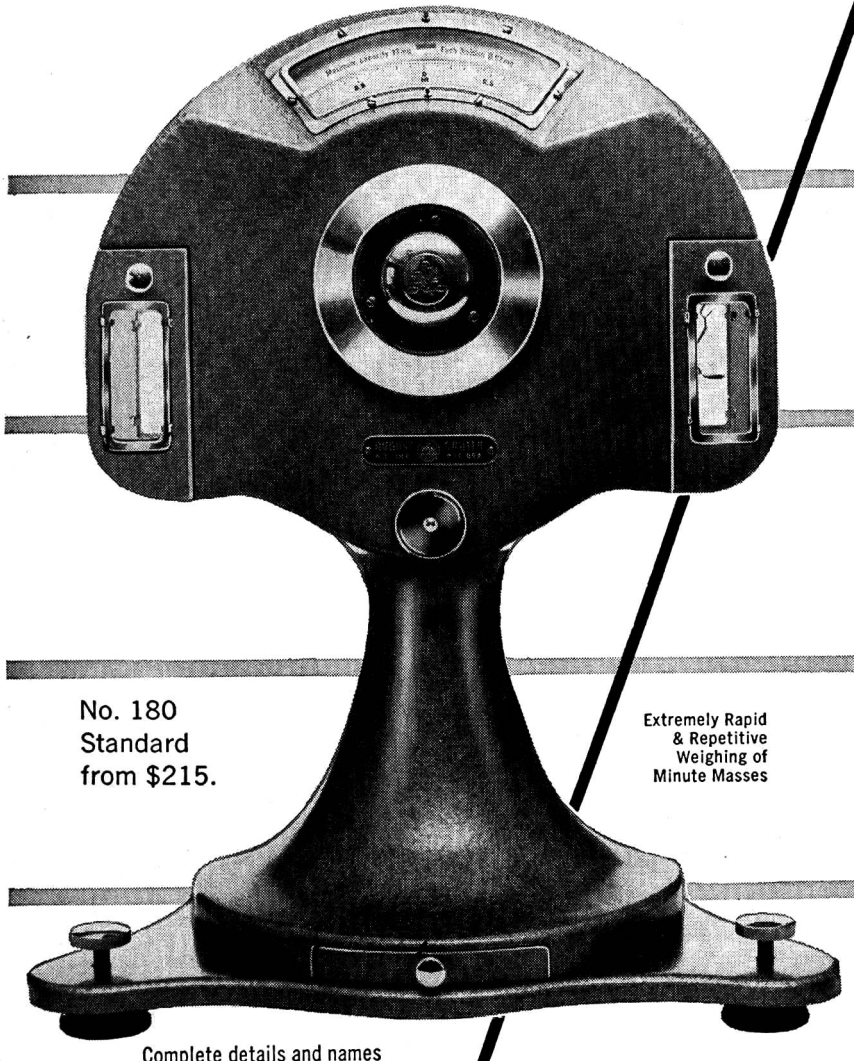
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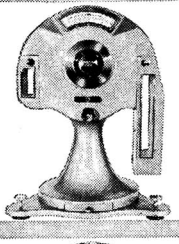
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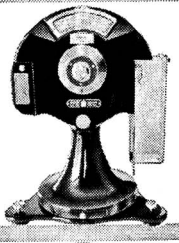
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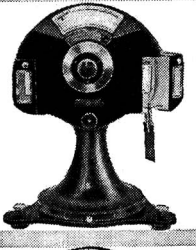
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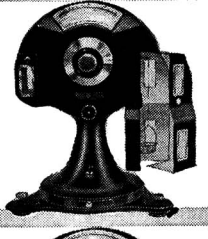
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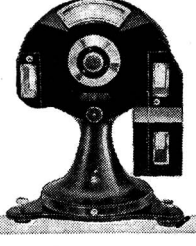
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ANALYST'S CALENDAR *(Continued)*

Coming Events

June—date undetermined—European Molecular Spectroscopy Group, 4th biennial meeting. Bologna, Italy. *Contact:* Reinhard Mecke, University of Freiburg, Freiburg, Germany.

June 1 to 4—Tenth Annual Symposium on Spectroscopy, American Association of Spectrographers, Conrad Hilton Hotel, Chicago. *Contact:* H. M. Wilson, Continental Can Co., 7622 S. Racine Ave., Chicago 20, Ill. Page 50 A.

June 4 to 8—International Symposium on Electrolytes. Italian Society for the Progress of Science, Trieste, Italy. *Contact:* Societa Italiana per el Progresso delle Scienze, Piazzale delle Scienze 7, Rome, Italy.

June 9 to 11—International Symposium on Interferometry, Teddington, England. *Contact:* International Symposium on Interferometry, National Physical Laboratory, Teddington, England.

June 10 to 12—Division of Analytical Chemistry and *Analytical Chemistry,* University of Illinois. 1959 Summer Symposium. Topic: Complex Reactions in Analytical Chemistry. *Contact:* G. L. Clark, University of Illinois, Urbana, Ill.

June 10 to 12—Second International Symposium on Gas Chromatography, East Lansing, Mich. Instrument Society of America. *Contact:* H. S. Kindler, ISA, 313 Sixth Ave., Pittsburgh 22, Pa.

June 10 to 12—International Union of Crystallography, Karolinska Institutet, Stockholm, Sweden, Conferences on Precision Lattice Parameter Determination and Counter Methods for Crystal Structure Analysis. *Contact:* William Parrish, Philips Laboratories, Irvington-on-Hudson, N. Y. Page 50 A, Nov.

June 15 to 17—Second International Symposium on X-Ray Microscopy and X-Ray Microanalysis, Stockholm. *Contact:* G. Hoglund, Institutionen for Medicinsk Tysik, Karolinska Institutet, Stockholm 60, Sweden. Page 50 A, Nov.

June 15 to 19—Ohio State University. Symposium on Molecular Structure and Spectroscopy. *Contact:* R. A. Oetjen, The Ohio State University, Columbus 10, Ohio. Page 56 A, March.

June 15 to Sept. 4—Gordon Research Conferences. Colby Junior College, New London, N. H.; New Hampton School, New Hampton, N. H.; and Kimball Union Academy, Meriden, N. H. *Contact:* W. Alec Jordan, 52 Park Ave., New York 16, N. Y. Page 58 A.

June 21 to 26—American Society for Testing Materials, Annual Meeting. Atlantic City, N. J. *Contact:* ASTM, 1916 Race St., Philadelphia 3, Pa.

June 24 to 26—Second Nuclear Instrumentation Symposium, Idaho Falls, Idaho. Instrument Society of America. *Contact:* H. S. Kindler, ISA, 313 Sixth Ave., Pittsburgh 22, Pa.

August 10 to 11—Rocky Mountain Spectroscopy Conference. Rocky Mountain Spectroscopy Society. Denver, Colo. *Contact:* Rocky Mountain Spectroscopy Conference, 718 Sherman St., Denver 3, Colo.

August 12 to 14—8th Annual Conference on Applications of X-Ray Analysis, Stanley Hotel, Estes Park, Colo. Sponsor: Denver Research Institute. *Contact:* W. R. Mueller, Denver Research Institute, University of Denver, Denver 10, Colo. Page 47 A, March.

August 24 to 29—Second International Congress of Polarography. Polarographic Society. University of Cambridge, England. *Contact:* G. F. Reynolds, Ministry of Supply, C. 36, Royal Arsenal, Woolwich, London S.E. 18, England. Page 41 A, January.

August 30 to Sept. 4—Chemical Institute of Canada, Physical Chemistry Division Symposium on Mass Spectrometry in Chemistry. McMaster University, Hamilton, Ont. *Contact:* Chemical Institute of Canada, 18 Rideau St., Ottawa 2, Can.

Sept. 1 to 8—International Conference on Analytical Chemistry, Czechoslovak Academy of Science, Prague, Czechoslovakia. *Contact:* Prof. Dr. Ing. Vaclav Hovorka, Praha-Dejvice, Technicka ul. 1905, Czechoslovakia. Page 46 A, March.

Sept. 9 to 12—Electron Microscope Society. 17th Annual Meeting. Ohio State University. *Contact:* S. S. Breese, Jr., Plum Island Animal Disease Laboratory, Greenpoint, L. I., N. Y. (re papers); W. J. Frajola, Ohio State University, Columbus, Ohio (re exhibits).

Sept. 13 to 18—American Chemical Society, 136th National Meeting, Atlantic City, N. J.

Sept. 14 to 16—Sixth Ottawa Symposium on Applied Spectroscopy. Canadian Association for Applied Spectroscopy. Ottawa, Canada. *Contact:* W. O. Taylor, Ontario Department of Mines, Parliament Buildings, Toronto, Ont., Page 42 A, April I.

Sept. 14 to 19—VIII Colloquium Spectroscopicum Internationale, Lucerne, Switzerland. *Contact:* H. Guyer, VIIIth Colloquium Spectroscopicum Internationale, P. O. Box, Schaff Hausen, Switzerland.

Sept. 21 to 25—14th Annual Instrument-Automation Conference and Exhibit, International Amphitheater, Chicago, Ill. Instrument Society of America. *Contact:* H. S. Kindler, ISA, 313 Sixth Ave., Pittsburgh 22, Pa., re program and F. J. Tabery, 3443 South Hill St., Los Angeles 7, Calif., re exhibits.

Sept. 28 to Oct. 1—Ninth Annual Instrument Symposium & Research Equipment Exhibit, National Institutes of Health. *Contact:* J. B. Davis National Institutes of Health, Bethesda 14, Md. Page 59 A.

October 1—American Chemical Society. Analytical and Microchemical Group. Philadelphia Section. Philadelphia Textile Institute. Topic: Determination of Molecular Weights. Speakers: Harry Mason, Rohm & Haas and C. L. Ogg, U. S. Dept. of Agriculture.

October 11 to 16—ASTM Spectroscopy Symposium, ASTM Committees E-2 and E-13, San Francisco. *Contact:* R. J. Wirshing, General Motors Corp., Box 188, North End Station, Detroit 2, Mich.

Oct. 26 to 28—Anachem Conference. Assn of Analytical Chemists. Wayne State University, Detroit. *Contact:* S. O. Roe, Ethyl Corporation, 1600 W. Eight Mile Rd., Ferndale 20, Detroit, Mich. See page 46 A, March.

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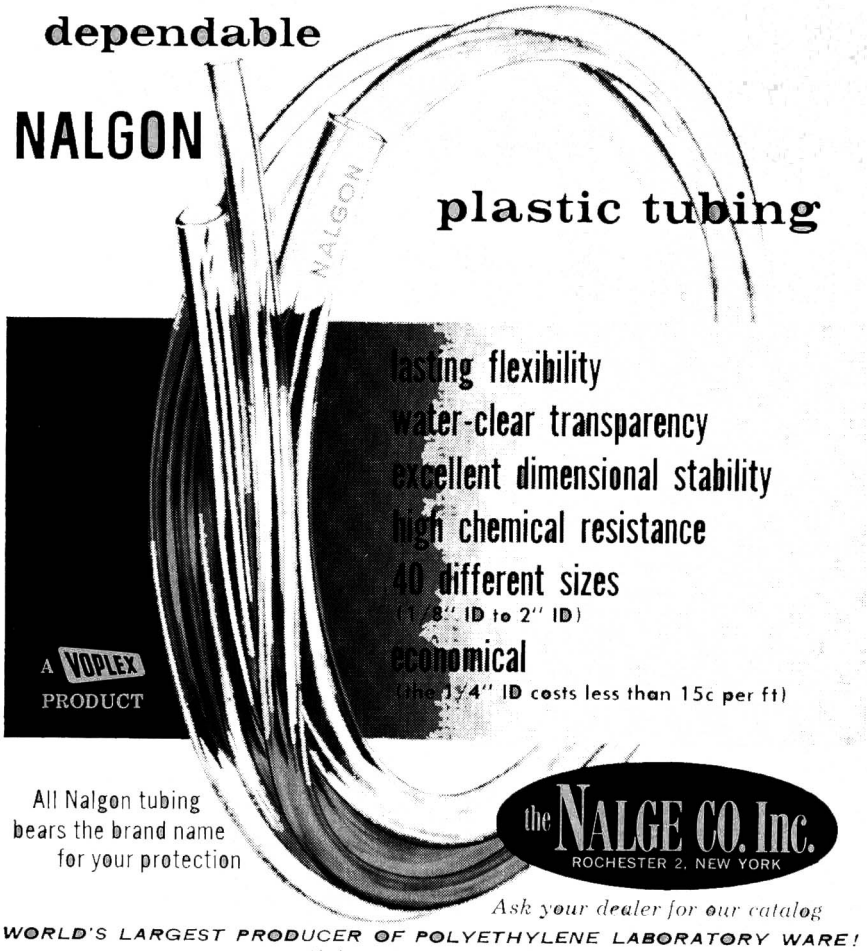
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industrial application, reported Robert T. Sheen, Milton Roy Co.

Applications described include the use of ultraviolet absorption spectroscopy in forensic chemistry. Henry J. Noebels, Beckman Instruments, said that this approach is desirable, since samples available in crime cases are usually very small. Application involves preparation of reference curves of compounds of toxicological interest.

Other instrumental applications described include the investigation of reactions of ozone with hydrocarbons by use of infrared absorption techniques. This was presented by Raymond R. Sawyer, Perkin-Elmer Corp. Cyanogen-oxygen flames have thermal characteristics which make them suitable for spectrochemical analysis, Bert L. Vallee, Peter Bent Brigham Hospital, reported. By combining a burner with a direct-reading spectrometer, very small samples may be analyzed.

A new method of electrochemical analysis involves use of a cell with a working electrode of large area and controlled potential. Edgar L. Eckfeldt, Leeds & Northrup, says the system offers possibilities for continuous measurement of low concentrations of electro-oxidizable or reducible substances.

A micro oven and an electrolytic hygrometer are essential elements in the determination of microgram quantities of water in paper, said Robert G. Armstrong, Continental Can Co.

Determination of surface areas of solids may be accomplished by allowing a condensable gas to be adsorbed on the solid, heating the solid, causing desorption, and measuring the gas by means of a thermal conductivity cell and recorder. This development was described by C. F. Lee, Shell Development Co.

Primary justification for use of gas chromatography in process monitoring and control is found in the degree of improvement in control of the process, said H. N. Claudy, Perkin-Elmer Corp. This results from increased frequency with which operators are supplied with process stream composition information or from direct incorporation of the analyzer in a control system. Experience indicates that the laboratory work load is generally not reduced.

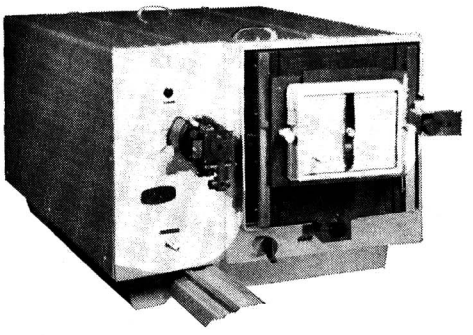
Other gas chromatography applications described include the analysis of mixed utility gases, by J. H. Leggoe, Philadelphia Electric Co., analysis of trace hydrocarbon impurities in petroleum benzene and toluene by F. A. Fabrizio, Sun Oil Co., and use of Golay capillary columns for many applications including multicomponent analysis of catalytic reformates, by R. D. Condon, Perkin-Elmer Corp.

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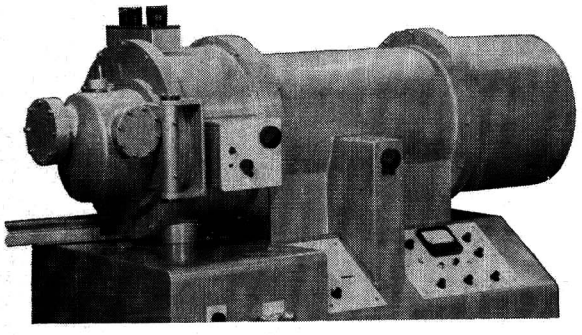
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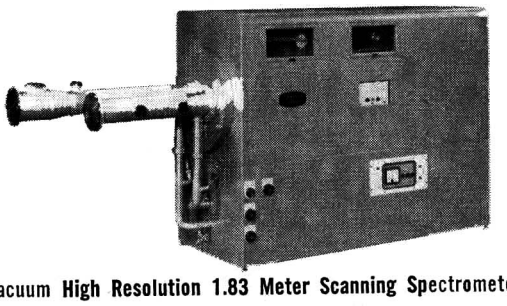
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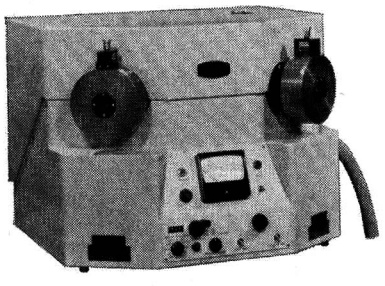
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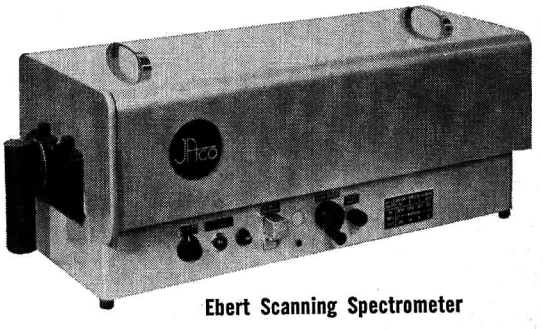
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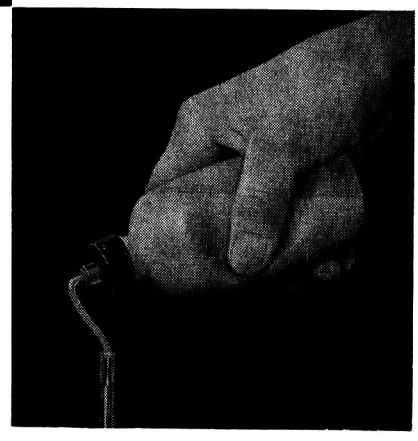
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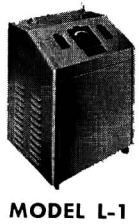
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Electroanalytical Chemistry

Scheduling an informal session to discuss electroanalytical chemistry was an innovation for the division.

It was felt that such a program would encourage informal discussion of pending work not ready for publication, unsolved problems, and in general the type of exchange of ideas which often takes place in the corridors at meetings.

Those responsible for the program, including L. B. Rogers of Massachusetts Institute of Technology and William N. Reinmuth of Columbia University, did not set any time limit to the discussion. The result: discussions were still in progress three hours after starting. The session ended only because the hotel management needed the room for other scheduled events.

Success of this program is expected to lead to such discussions at future meetings.

Trends in Clinical Chemistry

There are seven distinct trends in clinical chemistry, stated William B. Mason, divisional dinner speaker. Mason, a Ph.D. analytical chemist and holder of an M.D. degree, divides his time between the schools of medicine and biochemistry at the University of Rochester.

Mason noted that analytical chemists show little interest in clinical chemistry, although this is a field which is essentially analytical in nature.

The seven major trends Mason noted in clinical chemistry are:

1. More determinations. Results of a wide variety of clinical tests are of great assistance to doctors in diagnosing and treating a host of diseases, thus leading doctors to request more and more tests.

2. Faster determinations. Determinations are made much more rapidly than in the past and thus are of greater value to the doctors.

3. Automation. The demand for more clinical tests and rapid results has encouraged development of automatic procedures. Streamlined production-line determinations are becoming more commonplace.

4. Greater reliability. Progress is being made in standardizing existing procedures and developing good standards. New instrumentation is helpful in this regard. Agreement on results and quality control, at least on an interlaboratory basis, has been miserable, he said.

5. Low concentration work. The most exciting trend and one of the most challenging in this field is the development of methods for determining materials present in body fluids in extremely minute amounts—for ex-



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ample, 1 in 20 ml. of blood. Protein-bound iodine, steroids in blood, catechol amines in blood, and enzymes in blood are examples.

6. Changing status of clinical chemists. Another significant trend is the development of a better understanding of the respective roles of the clinical chemist and the medical doctor. Today there is a greater understanding between the two groups. One development in this regard, and one which Mason believes will help enhance the professional status of clinical chemistry, is the establishment of legislation with licensing procedures for clinical chemists. This step, he feels, will help eliminate the unscrupulous and unskilled "fast-buck boys" who see clinical chemistry as a lucrative field.

7. Disease type changes. Advances in the medical field are changing the work of the clinical chemist. As certain types of diseases are brought under better control, the clinical work load shifts. At present much work is being conducted on diseases which are considered biochemical in nature. This, in turn, is leading to a need for better trained clinical chemists.

Spectrographers Meet in Chicago

The field of far infrared spectroscopy will be highlighted at the 10th annual Symposium on Spectroscopy to be held at the Conrad Hilton Hotel in Chicago, June 1 to 4. The meeting, sponsored by the American Association of Spectrographers and its parent society, the Society for Applied Spectroscopy, will feature 44 foreign and domestic papers in all fields of spectroscopy. There will also be panel discussions on direct readers in optical emission spectroscopy and x-ray emission spectroscopy. Seminars in x-ray, far infrared, infrared, emission, and flame spectroscopy will be presented, and problem clinics will be conducted as in previous meetings.

A concurrent exhibition of recent instrumentation in spectrochemistry will feature the offerings of various manufacturers. J. R. Churchill, Alcoa Research Laboratories, will be the speaker at the symposium banquet on June 2.

Registration is open to all interested persons at a fee of $14.50, with the exception of students for whom the charge is $1.00. Further information may be obtained from Harry M. Wilson, Continental Can Company, C. R. & E.



R. C. Chirnside, chief chemist of General Electric Company's Research Laboratories at Wembley, England, has been nominated by the Council of the Society for Analytical Chemistry as president-designate, to take office at the annual general meeting in March 1959. He has taken a prominent part in the activities of IUPAC, and was honorary secretary of the International Congress at Oxford in 1952.

Millipore BRIEF #217

Critical Comparison of Collection Efficiencies of Commonly Used Aerosol Sampling Devices.

The extent to which the theory of collection techniques could be applied to commonly-used field instruments has been determined. Instruments included sedimentation chambers, MSA electric precipitator, Greenberg-Smith impinger, Millipore Filters, Cassella thermal precipitator and an impactor. Collection efficiencies for MF's were greater than 99% for all aerosols. Glycerol aerosols were collected at greater than 99.995% with the MF — the limit of measurement.

Schadt, C., Cadle, R. D. Analytical Chemistry, 29:6:864-68, June, 1957

Millipore BRIEF #166

Use of Membrane Filters in the Measurement of Biological Incorporation of Radioactive Isotopes.

A technique is presented for accurately estimating by direct radiation counting the total isotope incorporation into metabolizing cells. After exposure to the labeled substrate ($C^{14}O_2$) the cells are killed, transferred to 10-20 ml. H_2O, and filtered through a 1" HA Millipore filter. After flushing and drying, the MF is introduced into a gas-flow chamber for direct counting of B radiation from the dry cells.

Atkinson, D. E., McFadden, B. A. Journal Bacteriology, 71:1:123-24, 1956

Millipore BRIEF #212

A New Non-destructive Method for the Bacteriological Sampling of Meats.

A non-destructive MF method for the bacteriological sampling of meat is described which gives best results as reliable as those obtained using destructive sampling. A water-saturated sterile type HA Millipore filter is pressed with a sterile spatula against the meat to be sampled for one minute. The MF is then shredded and an aliquot plated on TGE agar. Incubation for 3 days at 20°C follows.

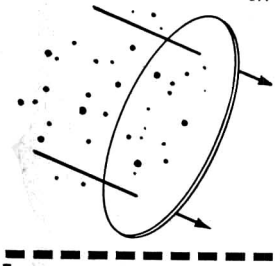
Silliker, J. H., Andrews, H. P., Murphy, J. F. Food Technology, XI:6:317-18, June, 1957

Millipore BRIEF #233

Modified Determination of Radium in Water.

Radium is a source of radioactivity in water and its detection is important as a possible health hazard, industrial product variant, clue to subsurface conditions and means of studying water movement. The U. S. Geological Survey uses a modification of the Harley and Foti method embodying a barium sulfate-carrier precipitation followed by filtration through a black 47mm diameter type HA Millipore Filter, and counting of activity after a prescribed aging period.

Barker, F. B., Thatcher, L. L. Analytical Chemistry, 29:11:1573-75, November, 1957



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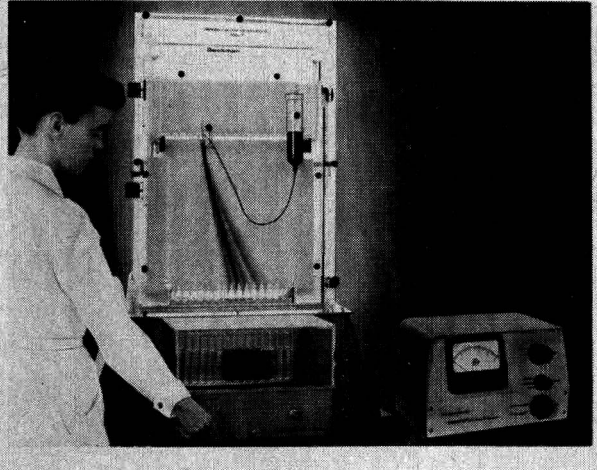
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The program of papers follows:

June 1

X-ray Spectroscopy in Storage Battery Research. G. L. Clark, R. H. Hunt, and D. J. Manus, University of Illinois.

Glass Defect Analysis by X-ray Spectroscopy. P. R. Eggers, Ford Motor Co.

100 Kilovolt Fluorescent X-ray Spectrography of the Rare Earths and Rare Metals. M. Salmon, Fluo-X-Spec Laboratories.

Application of the Multicomponent X-ray Spectrograph to the Analysis of Complex Systems. B. J. Mitchell, Electro Metallurgical Co.

Determination of TEL in Gasoline by X-ray Fluorescence. J. Hansen, Esso Research and Engineering Co.

Chemical Applications of Infrared Spectroscopy beyond 50 Microns. R. C. Lord, Massachusetts Institute of Technology.

Measurement of Far Infrared Spectra. E. K. Plyler, National Bureau of Standards.

Far Infrared Instrumentation. H. W. Marshall, Perkin-Elmer Corp.

Studies of Far Infrared Spectra of Benzene Derivatives: Effects of the Properties of Substituents on the Frequencies of Certain Absorption Bands. W. C. Corburn, Jr., C. V. Stephenson, and W. S. Wilcox, Southern Research Institute.

Internal Rotational Effects in the Far Infrared. L. A. Harrah and D. W. Mayo, Wright Air Development Center.

Infrared Spectra of Fatty Acids, Soaps, and Triglycerides from 14–30 Microns. R. O. Crisler and A. J. Fenton, Procter & Gamble Co.

Analysis of Phenylchlorosilanes by Far Infrared Absorption. M. E. Grenoble and P. J. Launer, General Electric Co.

Infrared Spectra of Mono-Substituted Pyridines from 15 to 35 Microns. R. Isaac, The Anderson Physical Laboratory, and Freeman Bentley, Wright Air Development Center.

Techniques and Applications in the CsBr Region. J. E. Stewart, Beckman Instrument.

June 3

New X-ray Equipment. V. E. Buhrke, Radio Corp. of America.

Precision in X-ray Emission Spectrography. P. D. Zemany, H. G. Pfeiffer, and H. A. Liebhafsky, General Electric Co.

Current Applications of the Electron Probe Microanalyzer. L. S. Birks and R. E. Seebold, U. S. Naval Research Laboratory.

Matrix Compensation in X-ray Fluorescence Analysis. Jacob Sherman, Philadelphia Naval Shipyard.

Comparative Study of X-ray and Optical Spectrochemistry. Frederick Brech, Jarrell-Ash Co.

Analysis Lines for Visual Analysis of Trace Elements in Steels. W. Kandlbauer, Gebr. Bohler and Co.

Physical Phenomena in Electric Sparks and Arcs and in Flames and Their Significance to the Spectral Emission from These Light Sources. Jan van Calker, University of Münster.

Accuracy in Spectrochemical Analysis. A. J. Mitteldorf, Spex Industries, Inc., and Arno Arrak, Belman Smelting and Refining Co.

Spectroscopy without Spectrographs. Mark Fred, Argonne National Laboratory.

Spectrochemical Analysis of Anions. C. E. Harvey, C. E. Harvey Associates.

Steps in the Manufacture of Diffraction Gratings. E. Leibhardt, Diffraction Products, Inc.

Spectrographic Analysis of Tantalum Metal Powder by the Carrier Distillation Method. Dimetro Andrychuk and J. Massengale, Texas Instruments, Inc.

June 4

Spectrographic Analysis of Hafnium for Al, Cu, Fe, Ti, W, Zr by a Point to Plane Technique. R. C. Brayer, R. F. O'Connel, A. S. Powell, and R. H. Gale, Combustion Engineering, Inc.

Application of Flame Spectra to the Analysis of Rare Earths. V. A. Fassel, R. N. Kniseley, R. H. Curry, R. H. Heidel, and C. G. Tremmel, Iowa State College.

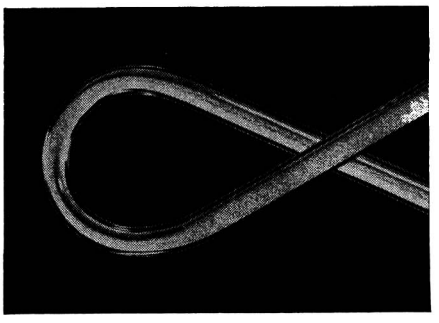
Comprehensive Review of Spectrochemical Arc-Spark Solution Methods. T. H. Zink, Armour Research Foundation.

Preliminary Report on the Application of Triggered Discharges to Spectrochemical Analysis. E. B. Owens, M. I. T. Lincoln Laboratories, Frederick Brech and R. W. Tabeling, Jarrell-Ash Co.

Spectrochemical Analysis of High Purity Silicon Carbide. E. S. Hodge and W. K. Baer, Mellon Institute.

Spectroscopy of Reaction Intermediates in Condensed Systems. E. W. Abrahamson, Syracuse University.

New Instruments and Spectroscopy in the Vacuum Ultraviolet. R. K. Brehm, Jarrell-Ash Co.



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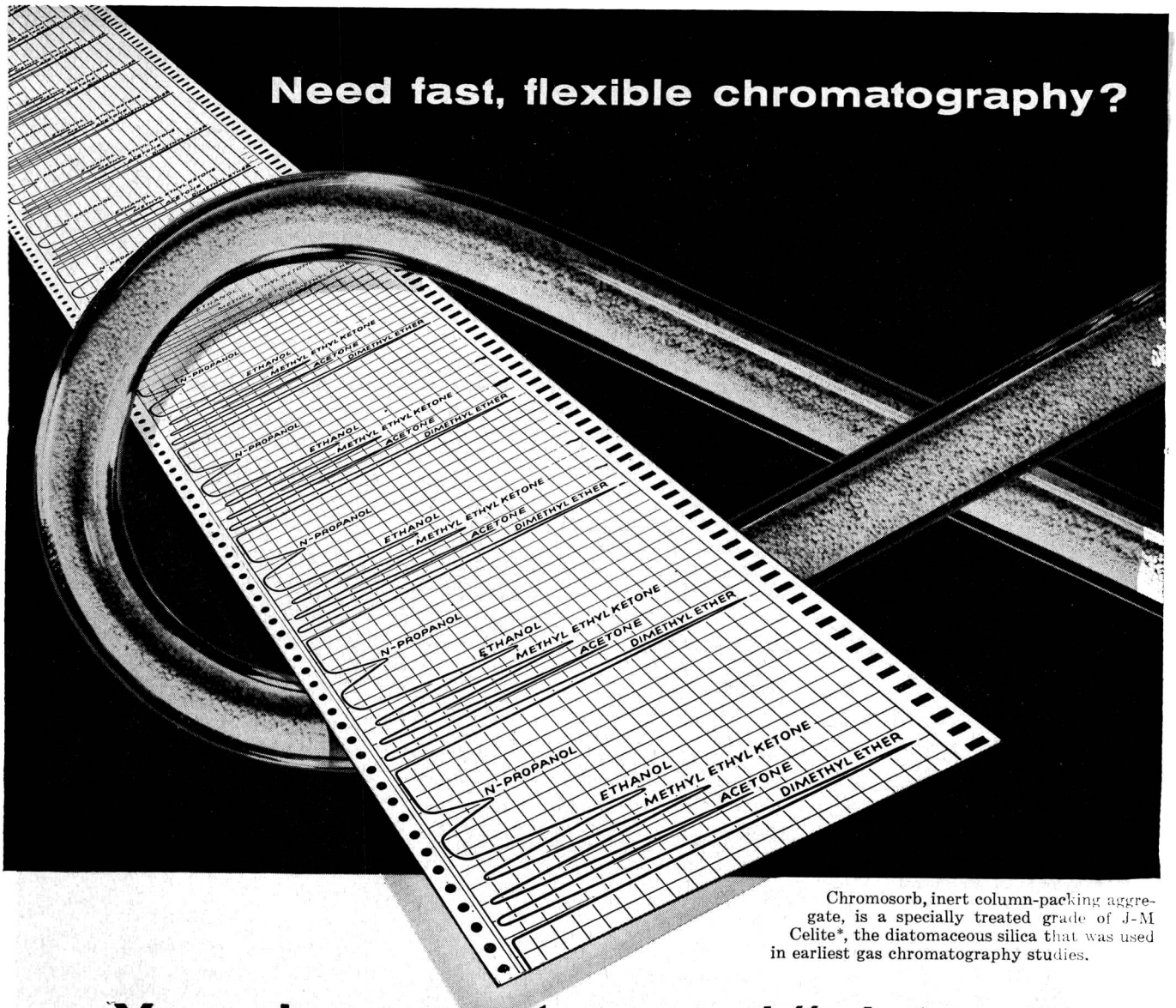
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pH (avg.)	6-7
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Typical Properties

Flux calcined diatomaceous earth aggregate.

Color	white
Free Fall Density—lbs./cu. ft. (avg.)	15-16
Specific Gravity—true	2.30
Water Absorption—cc./gr. (avg.)	4.0-5.0
Moisture—% by weight, maximum	1.0
pH (avg.)	8-10
Surface Area (BET Method)—sq. m./gr. (avg.)	3-4

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Line-Width Measurements for Spectrographic Photometry. J. A. Perry, Sinclair Research Laboratories, Inc.

Emission Spectrochemical Analysis by the Spark and Spray Solution Technique. A. L. Schalge and H. V. Malmstadt, University of Illinois.

Change in Nitrate Symmetry in Metallic Nitrates and in Various Nitrate-Organophosphorus Complexes. J. R. Ferraro, Argonne National Laboratory.

Fundamental Absorption Bands of Substituted Pyridines. R. P. Mariella and E. M. Godar, Loyola University.

Some Results of Raman Resonance Spectroscopy. F. Dörr and W. Maier, Technical University, Munich.

Simple Relations between Spectroscopic and Thermal Energies. F. Dörr, Technical University, Munich.

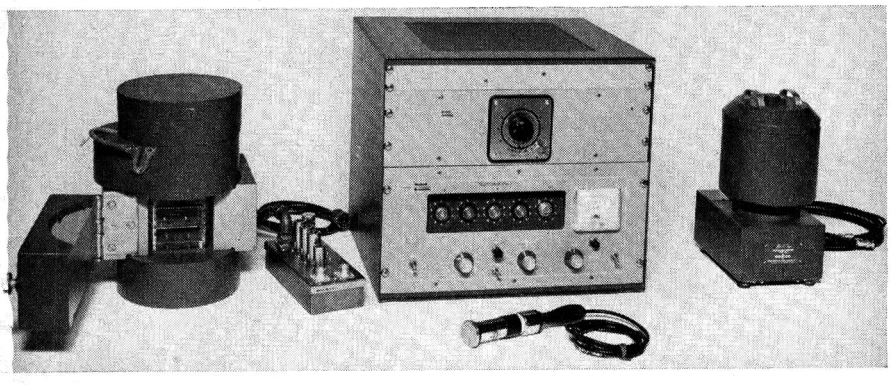
Infrared Absorption Spectra of Some Inorganic Sulfates. Hidalgo, Institute de Optica "Daza de Valdes," Madrid.

Error Prediction in Multicomponent Analysis. J. A. Perry, Sinclair Research Laboratories, Inc.

Determination of 1,10-Phenanthroline by Ultraviolet Spectroscopy. P. J. Secrest, J. A. Pawley, and C. A. Lucchesi, Sherwin-Williams Co.

Interdependence of the Intensity of Raman Lines of Carbon Tetrachloride on the Frequency of the Exciting Line. W. Hoffman and H. Moser, University of Munich.

Infrared Spectroscopy. S. E. Wiberly, Rensselaer Polytechnic Institute.



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ISA Schedules Instrumentation Advances Program

Advances in analysis instrumentation applying to several branches of science and at least six industries will be described at the Fifth National Symposium on Instrumental Methods of Analysis sponsored by the Instrument Society of America. The meeting will be held at the Shamrock Hotel, Houston, May 18 to 20. There will also be an exhibit of analytical instruments.

The program appears below:

May 18

General

Keynote address: J. D. Lindsay, A. & M. College of Texas.

Economic Evaluation—A Challenge to the Instrument Engineer. K. V. Kratochvil, Phillips Petroleum Co.

New Developments in Instrumental Analysis. D. D. De Ford, Northwestern University.

Instrumentation in Chemical Oceanography. D. W. Hood and E. R. Ibert, A. & M. College of Texas.

Analysis Instrumentation and Computer Control. T. J. Williams and H. S. Min, Monsanto Chemical Co.

Advances in Process Chromatography. P. H. Stirling, Canadian Industries, Ltd.

May 19

Chemical Methods of Analysis

Process Control Mercaptan Titrator. F. A. Leisey, Standard Oil Co. of Indiana.

Precise Method for Measurement of Boiling Point Rise. F. E. Moore, The Foxboro Co.

Automatic Potentiometric Titrator for Available Chlorine in Sodium Hypochlorite. J. B. Beaugh and R. C. Halter, Humble Oil Co.

Continuous Liquid Stream Boron Analyzer. A. Martin, Callery Chemical Co.

Recent Applications in Automatic Colorimetric Analyzers and Titrators. R. T. Sheen and Hal Freedman, Milton Roy Co.

Physical Methods of Analysis

Simple Recording Spectrophotometer for Dense Light-Scattering Materials. K. H. Norris and W. L. Butler, U. S. Department of Agriculture.

Selective Indication of One Component of a Multicomponent Gas Stream by Thermal Analysis. G. C. Flumerfelt, Mine Safety Appliances Co.

Nondispersive Infrared Studies of Automobile Exhaust Gases. R. L. Chapman, Beckman Instrument Co.

New Design Techniques in Process Instrumentation

Designing a Katharometer for Gas Chromatography. P. H. Stirling and D. McSloan, Canadian Industries, Ltd.

Developing Process Instrumentation. F. W. Karasek, Phillips Petroleum Co.

An Overlooked Temperature Effect on Ultraviolet Gas Analyzers. Lewis Fowler, Monsanto Chemical Co.

Explosion-proof Beta Ray Gravitometer for Refinery Service. M. B. Fallgatter, Shell Development Co.

May 20

Electrochemical Methods of Analysis

Radio Frequency Conductivity Meter. George Huebner, A. & M. College of Texas.

Applications of Nonspecific Electrochemical Analyzers to Complex Streams. R. M. Green, The Foxboro Co.

Glass Electrodes for the Direct Measurement of Sodium Ion Concentration in Aqueous Solutions. John Leonard, Beckman Instrument Co.

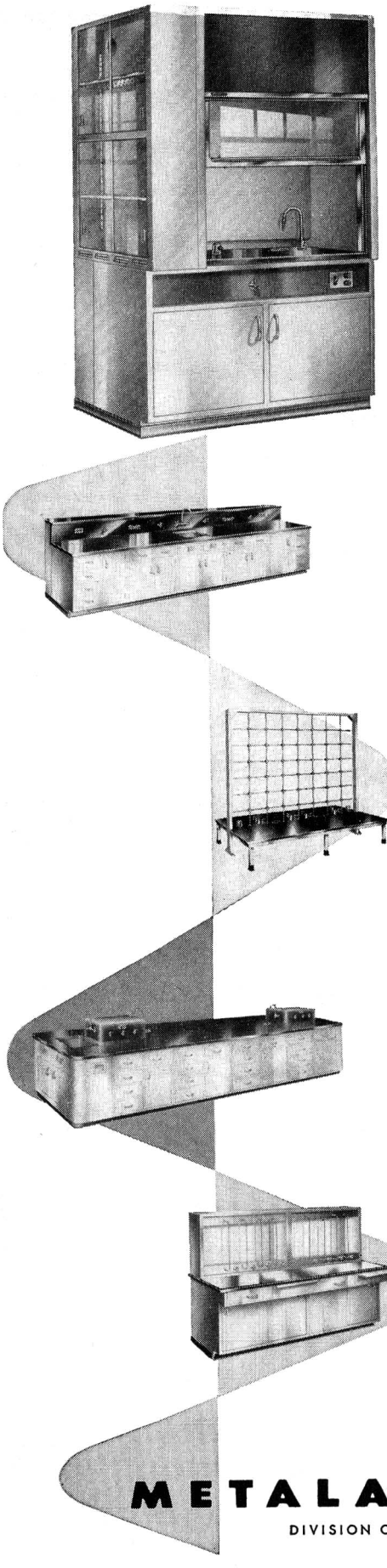
Sampling Systems for Process Analyzers

Sampling Systems for Chromatographs. D. C. Field, Esso Standard Oil Co.

Multiple Stream Chromatographs. D. M. Wreyford, Texas Butadiene & Chemical Co.

Sampling for Process Analysis. H. J. Cannon, Shell Oil Co.

Sample Handling Problems for Continuous High Sensitivity Analyzers. F. R. Gilmer, E. I. du Pont de Nemours & Co., Inc.



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Process Chromatography

Chromatographic Back Flushing for Heavy End Analysis. H. N. Claudy, Perkin-Elmer Corp.

Design and Performance of a New Ionization Detection System for Gas-Liquid Chromatography. F. E. Wing, Jr., Jarrell-Ash Corp.

Successful Applications of Process Chromatography to Plant Process Streams. M. V. Perry, Consolidated Electrodynamics Corp.

Process Chromatography Utilizing Catalytic Combustion Principles. F. Taylor, Jr., Davis Instrument Co., W. Herkness and D. N. Campbell, Greenbrier Instrument Co.

Ohio State Holds Spectroscopy Symposium

The annual Symposium on Molecular Structure and Spectroscopy will be held at The Ohio State University, June 15 to 19, under sponsorship of the Department of Physics and Astronomy. As in previous years, electronic band spectroscopic problems will be discussed early in the week, and infrared and radiofrequency spectroscopy will be discussed later. Also, morning sessions will be devoted to invited papers and the afternoons to contributed papers. Information on all phases of the meeting may be obtained from Robert A. Oetjen, Department of Physics and Astronomy, The Ohio State University, Columbus 10, Ohio.

The program of papers follows:

June 15

Vacuum Ultraviolet Spectroscopy. P. G. Wilkinson, University of Chicago.

Electronic Spectra of Transition Metal Ions in Crystals. D. S. McClure, Radio Corp. of America.

Some Problems in the Electronic Structure of Conjugated Chains. Yuzuru Ooshika, Polytechnic Institute of Brooklyn.

Walsh's Rules. C. E. Wulfman, Defiance College.

Survey of Wave Functions for Two-Electron Systems. C. C. J. Roothaan, University of Chicago.

June 16

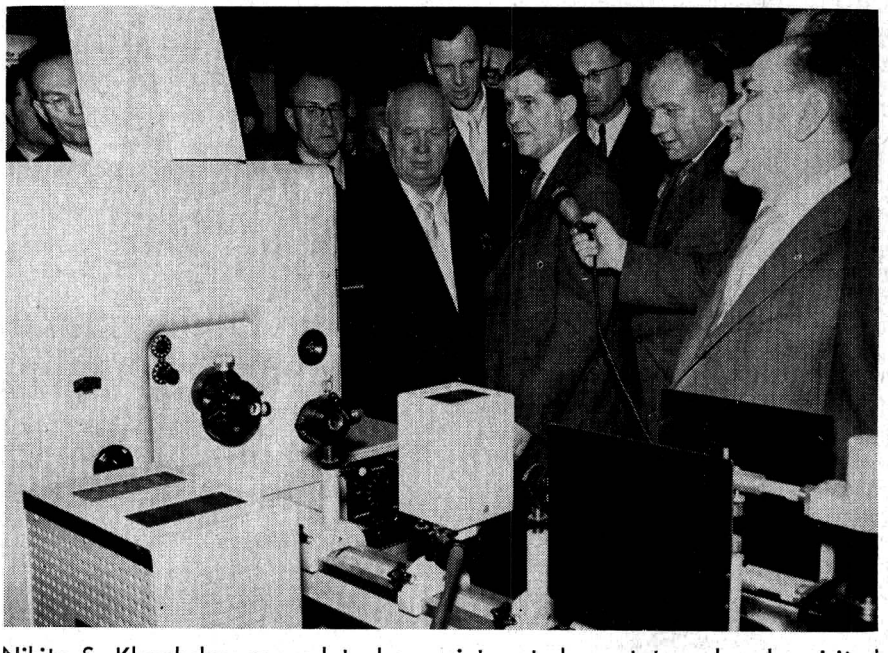
Electron Spin Resonance in Biochemical and Biological Systems. Barry Commoner, Washington University.

Emission Spectra of Trapped Radicals. H. P. Broida, National Bureau of Standards.

Electronic Field Effect in Vibrational Spectra. C. W. Peters, University of Michigan.

June 17

Generalized Orbital Angular Momentum and the N-Fold Degenerate Quantum-Mechanical Oscillator. J. D. Louck, Los Alamos Scientific Laboratory.



Nikita S. Khrushchev proved to be an interested spectator when he visited the Spring Fair at Leipzig in East Germany this year. He is shown in the Hall of Precision Tools and Optics of East Germany, inspecting a 2-meter plane grating spectrograph produced by VEB Zeiss of Jena.

Computation of Asymmetric Rotator Constants from Energy Moments. P. M. Parker, Michigan State University, and L. C. Brown, Ohio State University.

Dipole Moment Function of Diatomic Molecules. Harold Salwen, Harvard University, and John Trischka, Syracuse University.

June 18

Current Status of Some Spectroscopic Problems of Astrophysical Interest. Andrew McKellar, Canadian Department of Mines and Technical Surveys.

Some Infrared Spectra of Hydrocarbons Chemisorbed on Silica-Supported Metals. N. Sheppard, Cambridge University.

Reflection Measurements in the Infrared Spectrum. J. Lecomte, French National Research Center.

June 19

Recent Studies in High Resolution Raman Spectroscopy. H. L. Welsh, University of Toronto.

Present Status of IR Photoconductive Detectors. Henry Levinstein, Syracuse University.

Far Infrared Interferometric Modulation at The Johns Hopkins University. G. A. Vanasse, Johns Hopkins University.

Third Gatlinburg Conference in Planning Stage

The Oak Ridge National Laboratory has announced that the Third Conference on Analytical Chemistry in Nuclear Reactor Technology will be held at Gatlinburg, Tenn., on Oct. 26 to 28, 1959.

The general theme of the conference, analysis of reactor materials following operation of nuclear reactors, complements those of previous meetings. These were advances in the chemical analysis of important reactor materials, and the role of analytical chemistry in the startup and operation of nuclear reactors.

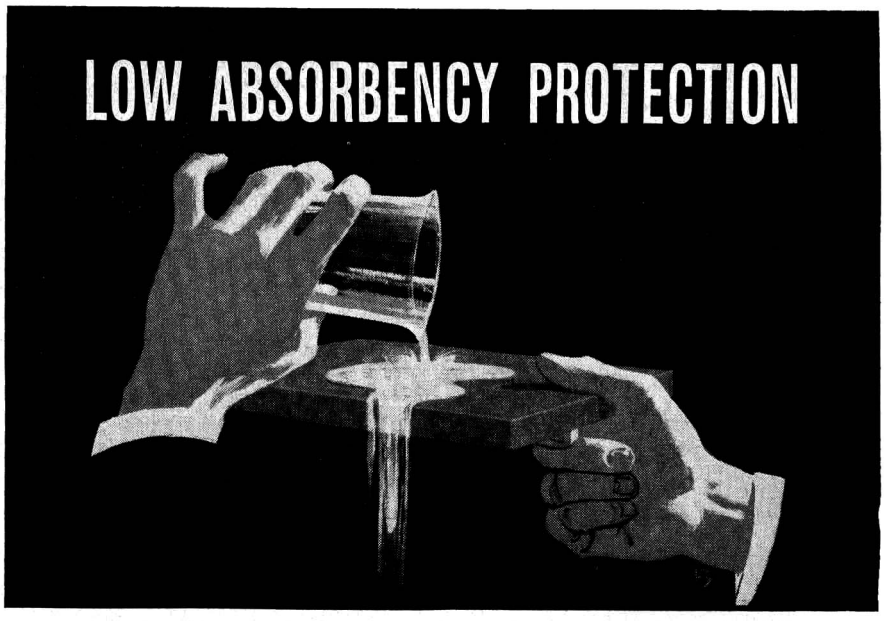
The contribution of papers pertinent to the subjects to be emphasized is solicited. Tentatively, six sessions are planned, with general topics as follows:

October 26

Analysis of Spent Reactor Fuels

Analytical Chemistry of Exposed Blanket Materials.

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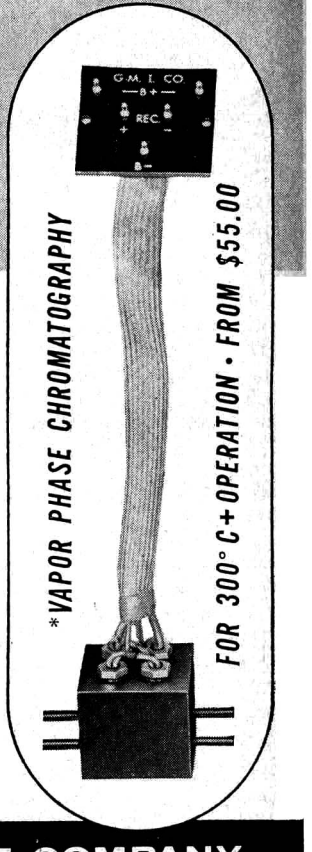


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October 27

Analytical Chemistry in the Reprocessing of Fuels and Blanket Materials.

The Analysis of Corrosion and Erosion Products.

October 28

Analysis of Fission Products Mixtures.

Analytical Chemistry of Plutonium and Transplutonic Elements.

Those desiring to present papers are requested to submit abstracts of about 500 words not later than Aug. 1, 1959, and to indicate the time required for presentation. The time should not exceed 30 minutes.

In addition to the technical program, an informal dinner and dance will be held on Oct. 27. The meeting is unclassified, and all interested persons are invited to attend. There is no registration fee. However, for those attending the dinner a nominal charge will be made.

All communications concerning the conference, including submission of abstracts, should be sent to C. D. Susano, Oak Ridge National Laboratory, P. O. Box Y, Oak Ridge, Tenn.

Information about accommodations and reservations may be obtained from Tom Woods, Manager, Mountain View Hotel, Gatlinburg, Tenn.

Gordon Research Conferences Schedule

Several sessions of interest to analytical chemists are included in the Gordon Research Conferences program to be held June 15 to September 4. Meetings will be held at Colby Junior College, New London, N. H., New Hampton School, New Hampton, N. H., and Kimball Union Academy, Meriden, N. H.

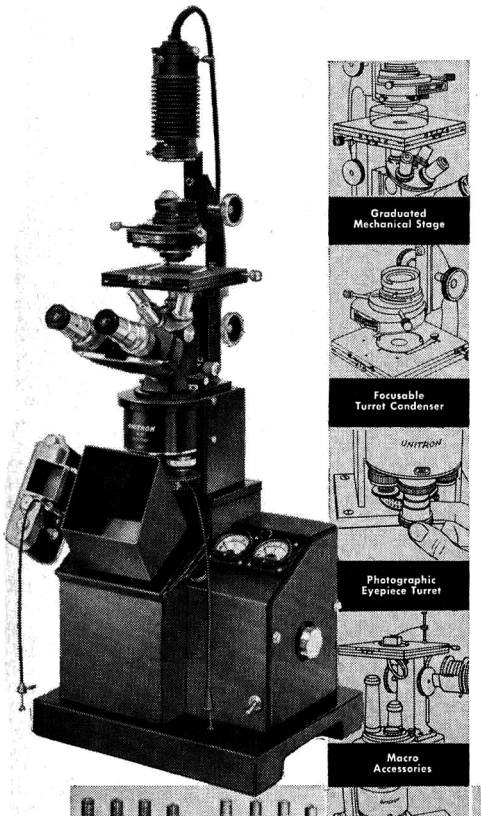
Among the 36 conferences scheduled over a 12-week period are several of interest to analytical chemists: analytical chemistry, chairmaned by John Mitchell, Jr., Du Pont, New Hampton School, August 17 to 21; instrumentation, chairmaned by C. E. Berry, Consolidated Electrodynamics Corp., Colby Junior College, August 24 to 28; magnetic resonance, C. A. Hutchinson, Jr., Enrico Fermi Institute for Nuclear Studies, New Hampton School, July 6 to 10; separation and purification, E. S. Perry, Eastman Kodak Co., Colby Junior College, June 15 to 19; molten salts, B. R. Sundheim, Washington State College, Kimball Union Academy, August 21 to Sept. 4; and radiation chemistry, W. M. Garrison, University of California, New Hampton School, July 27 to 31.

Other topics scheduled are: petroleum; catalysis; polymers; textiles; corrosion; organic coatings; medicinal chemistry; elastomers; food and nutrition; cancer; chemistry and physics of liquids; chemistry of coal; proteins and nucleic acids; organic reactions and processes; microbiological deterioration; steroids and related natural products; inorganic chemistry; statistics in chemistry and chemical engineering; chemistry of adhesion; lipide metabolism; physical metallurgy; nuclear chemistry; solid state studies in ceramics; chemistry, physiology, and structure of bones and teeth; cell structure and metabolism; chemistry at interfaces; biochemistry and agriculture; toxicology and safety evaluations; chemistry and physics of metals; photonuclear reactions.

Details concerning the program, registration fees, and other details are available from W. G. Parks, Director, Gordon Research Conferences, University of Rhode Island, Kingston, R. I.

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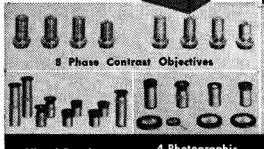
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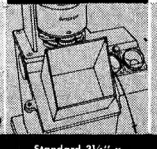
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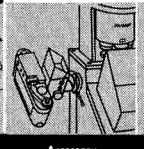
UNITRON'S unique Phase-Camera-Microscope will assure you a **CONVENIENCE** of operation impossible to duplicate with a conventional microscope stand. For example, a specimen consisting of a tissue culture or aqueous solution may be examined in the very petri dish in which it has been prepared. Merely place the dish on the microscope stage; examine the contents visually, project the image on the viewing screen, or photograph — all in rapid sequence and at magnifications up to 2000X. With the inverted design, there is no need to contend with slipping cover glasses or evaporating fluids. Using the phase optics, you dispense with messy chemical staining. Aided by the self-contained illumination system and built-in cameras, you eliminate troublesome problems of alignment and vibration.

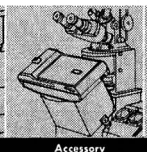
UNITRON'S Phase-Camera-Microscope, optically equipped for Bright and Dark Phase Contrast, bright and dark field, and polarized light, offers the microscopist a working tool of unmatched **VERSATILITY.** The overhead turret phase condenser is centerable, divisible, and provides long working distances sufficient even for micro-manipulation. Provision is included for four different cameras — 3¼" x 4¼", 35mm., Polaroid, and motion picture. Available at extra cost are macro accessories for magnifications as low as 5X and accessories for opaque specimens. This UNITRON model meets not only your urgent needs of today — its versatility is your assurance of continued usefulness in the future.

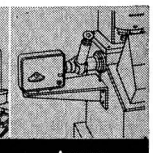
UNITRON'S Phase-Camera-Microscope is the ideal all-purpose instrument for visual examination, viewing, and photo-recording. Completely equipped with an impressive array of optics, its cost is actually lower than that of a conventional phase stand, camera and illuminator — true **ECONOMY** for any laboratory.


8 Phase Contrast Objectives









Visual Eyepieces | 4 Photographic Eyepieces, Filters. | Standard 3¼" x 4¼" Camera | Accessory 35mm. Camera | Accessory 3¼" x 4¼" Polaroid Land Camera | Accessory Motion Picture Camera

UNITRON'S Phase-Camera-Microscope Model BU-13 as described including binocular body; 8 phase objectives 10X, 20X, 40X, 100X oil, in both bright and dark contrast; paired visual eyepieces R5X, P10X, Ke15X; focusing telescope; four photographic eyepieces 10X, 15X, 20X, Micrometer; phase condenser; filters; polarizing accessories; special glassware, etc.

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NIH Conference-Exhibit

The Ninth Annual Instrument Symposium and Research Equipment Exhibit will be held September 28 through October 1, 1959, at the National Institutes of Health, Bethesda, Maryland.

Sponsors of the exhibit are the nation's leading instrument manufacturers, who will display the newest developments in laboratory glassware and electronic, surgical, radiation, optical, gas-sampling, and other research equipment.

Sponsors of the symposium are the Washington, D. C., sections of the American Association of Clinical Chemists, American Chemical Society, Instrument Society of America, Professional Group on Medical Electronics of the Institute of Radio Engineers, Society of American Bacteriologists, and the Society of Experimental Biology and Medicine.

For additional information about the symposium and exhibit, contact James B. Davis, National Institutes of Health, Public Health Service, Bethesda 14, Md.

Electrochemical Society Meeting

The 115th National Meeting of the Electrochemical Society will be held at the Sheraton Hotel, Philadelphia, May 3 to 7.

Divisions which will meet include: theoretical, industrial, electrolytic, electrothermic and metallurgy, electronics, and electric insulation.

Special symposia on liquid dielectrics and electrode processes are also scheduled. Several foreign speakers will take part.

Details are available from H. C. Mandell, Jr., Pennsalt Chemicals Corp., P. O. Box 4388, Philadelphia, Pa.

New Nickel Oxide Standard

A new standard sample of nickel oxide powder is now available from the National Bureau of Standards. Analyzed and certified for nine minor and trace elements, the standard is intended for checking and calibrating spectrochemical and chemical methods employed in the analysis of high-purity nickel, particularly electronic-grade and electrolytic nickel.

The new standard NBS 673 supplements the nickel oxide standard samples NBS 671 and 672, issued in 1957. All three samples have been prepared by a cooperative program between the Bureau and a task group of Committee

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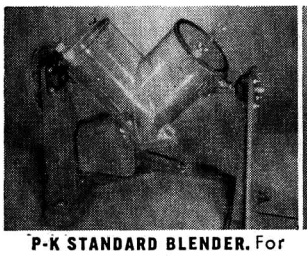
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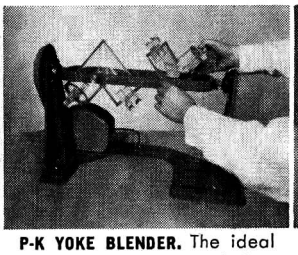
Performance of these P-K laboratory blenders is duplicated regularly in production models in capacities up to 500 cubic feet. P-K "Twin-Shell" lab blenders are available with either heavy transparent Lucite or stainless steel shells.

Order from description below, or send coupon for Bulletin 15A-1.

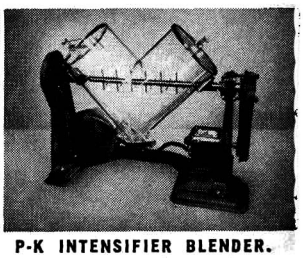
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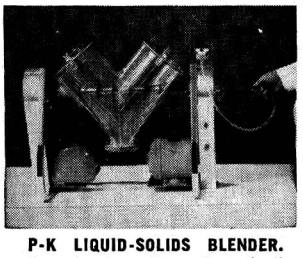
P-K STANDARD BLENDER. For plain tumble-blending of dry materials. No attrition or crystal fracture. Easy to clean. Average blending time 3-5 minutes. 4- and 8-quart interchangeable shells.
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8-qt. size $225



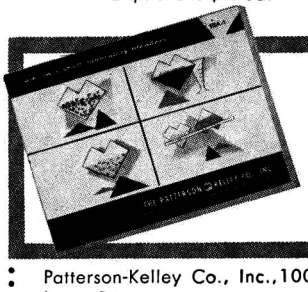
P-K YOKE BLENDER. The ideal blender for small-scale lab work. Yoke frame houses one 2-quart shell, two 1-quart shells, two 1-pint shells or combinations.
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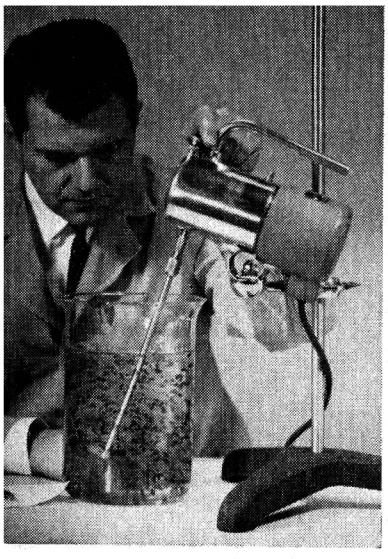
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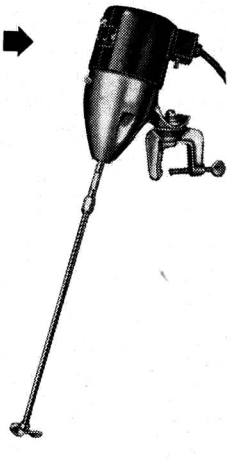
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F-1 of the American Society for Testing Materials. Similar to the other two, the new standard is designed primarily for application in the spectrographic analysis of nickel and nickel alloys by ASTM Tentative Method E 129-57T, *Methods for Emission Spectrochemical Analysis*. The three standards are also equally suited for chemical analysis.

The nickel oxide standard is packaged in bottles containing 25 grams and is available from the Standard Sample Clerk, National Bureau of Standards, Washington 25, D. C. The fee is $8.00 per sample. A provisional certificate of analyses accompanying the standard sample lists the analytical results of the cooperating laboratories.

Norelco School Scheduled for Chicago

The 33rd session of the Norelco X-ray Diffraction and Spectrographic School for those interested in the latest x-ray methods of analysis will be held at the Edgewater Beach Hotel, Chicago, June 1 to 5. Sponsored by the Instruments Division, Philips Electronics, Inc., the session is subject to limited registration—125 persons for the first four days and 150 persons on the final day, Friday.

Monday through Thursday will be devoted to classroom and laboratory work, wherein participants discuss their own problems and become familiar with use of all types of equipment. Friday is to be devoted to actual application problems, when guest speakers discuss methods currently in use by researchers and industrial plants.

Basic subjects to be covered include x-ray diffraction, diffractometry, and spectrography. There will also be discussions on new camera techniques, electron microscopy, and electron diffraction. There is no registration fee for the sessions.

Short Gas Chromatography Course Offered

An intensive three-day course in gas chromatography for chemists, industrial supervisors, engineers, physicists, and doctors will be given June 15 to 17 at UCLA. Offered by University Physical Sciences and Engineering Extension, the basic course will be divided between lectures and small group laboratory demonstrations. Lectures will be given by a group of leading scientists, coordinated by Robert L. Pecsok, UCLA Department of Chemistry. Laboratory demonstrations will be conducted in 10 laboratories, with instructors and equipment furnished by various manufacturing companies. Enrollment will be limited, with a bachelor's degree in science or engineering, or equivalent, required as a prerequisite. Information and applications may be obtained through Physical Sciences Extension, Mathematical Sciences Building 6115, University of California, Los Angeles 24, Calif.

Rensselaer Instrumental Analysis Course

Rensselaer Polytechnic Institute will offer its fourth special comprehensive course in instrumental analysis, June 15 to June 20. This course, which covers both theoretical concepts and practical applications of instrumental analysis, is intended to broaden the training of chemists and chemical engineers in industry and in academic and governmental work.

The course will cover ultraviolet, infrared, and Raman spectroscopy, emission spectroscopy, mass spectrometry, coulometry, polarography, and gas chromatography. Lectures will be given by outstanding authorities in the various fields.

Closing date for registration is June 1. Complete details may be obtained from S. E. Wiberley, Rensselaer Polytechnic Institute, Troy, N. Y.

Russian Courses for Scientists

Russian for research, two intensive, six-week summer courses designed for scientists and engineers who must keep abreast of current Russian publications, will be offered in June and July by the Polytechnic Institute of Brooklyn.

The first course, Russian for Research I, will be offered on Tuesday and Thursday evenings, 6:30 to 9:30 P.M., from June 1 to July 18. Designed for graduate or undergraduate students, it will present an introduction to grammar and problems of syntax and idioms with emphasis on scientific technology. There are no prerequisites. The second course, Russian for Research II, will be offered at the same hours from July 27 to September 21 and will include readings from periodicals and journals covering several fields of science and engineering. Prerequisite for this course is Russian for Research I or the equivalent.

Information on the two offerings can be obtained from the Department of Modern Languages, Polytechnic Institute of Brooklyn, 333 Jay St., Brooklyn 1, N. Y.

MIT Spectroscopy Course

Techniques and applications of infrared spectroscopy will be covered in two one-week courses at the Massachusetts Institute of Technology. The techniques course, June 22 through 26, will consist of lectures and laboratory work. It is aimed at those with a bachelor's degree and some background in research but without the previous infrared experience.

The one-week course on applications, June 29 through July 3, will also consist of lecture and laboratory sessions. Applications to be covered include theory of absorption, use of infrared methods in chemical analysis, and use of catalogs and punched cards for spectra identification.

Details are available from J. M. Austin, Massachusetts Institute of Technology, Cambridge 39, Mass.

Chemical Microscopy Course at Cornell

A 4-week course in chemical microscopy will be given at Cornell University, beginning July 6. It will include measurements and particle size determination, quantitative analyses, double refraction of crystals and polymeric materials, physical chemistry of crystallization, optics and photomicrography, and an introduction to qualitative analysis.

Enrollment is limited, and should be firm by June 1.

Further details are available from C. W. Mason, Cornell University, Ithaca, N. Y.

BC Spectrography Course

Boston College will give an intensive two-week course in modern industrial spectrography July 20 to 31. The course is designed for industrial chemists and physicists interested in the techniques of emission spectroscopy as an analytical tool.

Details are available from J. J. Devlin, S.J., Physics Department, Boston College, Chestnut Hill 67, Mass.

Arizona State Spectroscopy Course

Arizona State University will offer its 4th annual course in modern industrial spectroscopy August 17 through 28. The course is designed particularly to train chemists and physicists from industrial laboratories which use spectrographic and spectrophotometric equipment.

The program includes basic theoretical considerations and practical instrumental training. The instructional staff will include members of the Departments of Chemistry and Physics augmented by invited guest speakers from industrial laboratories and technical personnel from manufacturers of analytical instruments.

Complete information is available from Jacob Fuchs, Arizona State University, Tempe, Ariz.

Fisk Infrared Spectroscopy Institute

The Tenth Annual Fisk University Infrared Spectroscopy Institute will be held at Fisk University, Nashville, Tenn., August 24 to 29.

The institute is designed to provide chemists, physicists, engineers, and medical scientists with sufficient background training to make effective use of infrared spectroscopy in their laboratories.

The program provides general lectures and discussions on theory, techniques, and application, laboratory work, and a workshop devoted to the interpretation of infrared spectra. The institute has two laboratory programs. The first is for participants with no previous experience and the second, more flexible program, is for experienced participants.

A special session devoted to gas chromatography has been scheduled for a four-day period preceding the infrared institute. This session, extending from August 19 to 22, will provide instruction in the theory, applications, and techniques of gas chromatography.

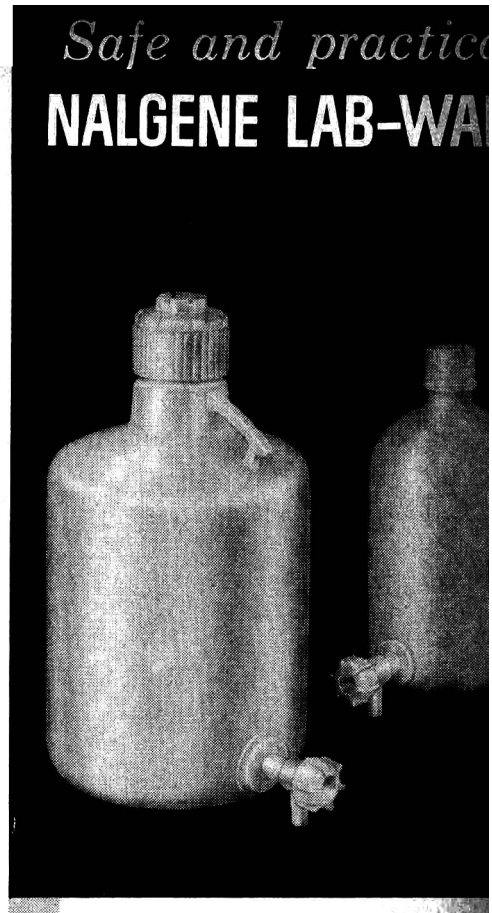
Further information may be obtained by writing Nelson Fuson, Fisk University, Nashville 8, Tenn.

Canisius Infrared Spectroscopy Institute

Canisius College will offer its third annual week-long course in infrared spectroscopy August 24 through 28. The course is designed for those in industry with little or no experience in the field. Sessions will cover elementary and advanced theory, industrial applications, and future in research and industry. Both lectures and laboratory training are included. Lecturers will include faculty members, visiting professors, and invited outside experts.

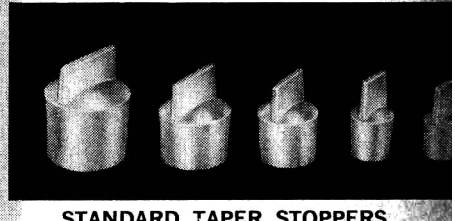
Details are available from H. A. Szymanski, Infrared Spectroscopy Institute, Canisius College, Buffalo 8, N. Y.

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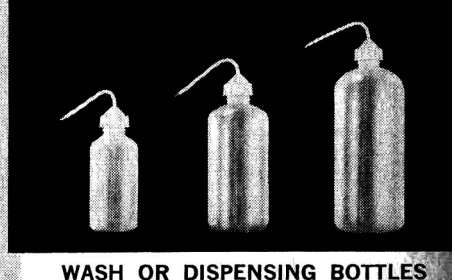


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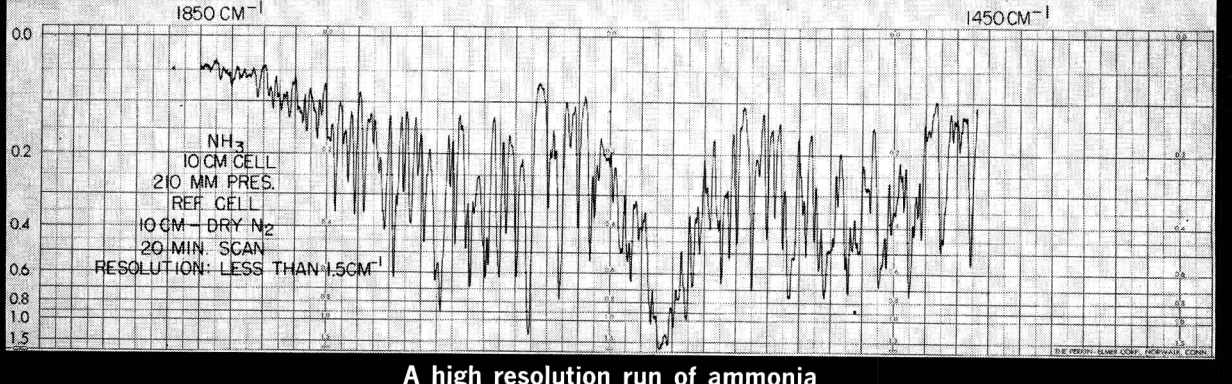
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the EDITOR'S column

L.T. Hallett, Editor

A GRATING spectrometer which gives total resolution of 0.05 cm.$^{-1}$ and partial resolution of 0.025 cm.$^{-1}$ in the region of 3 to 6 microns has been designed and constructed by the National Bureau of Standards. The new instrument, which has three times better resolution than NBS's previous instrument, was developed by E. K. Plyer and L. R. Blaine.

This spectrometer can be used as a single-pass or double-pass instrument. It employs a special drive mechanism which allows fast or slow scanning of small portions of a rotational-vibrational band. This allows not only resolution of rotational lines but also at slow speeds it permits detection of fine-structure splitting of individual lines.

This high resolution provides a means for investigating larger molecules in the infrared and for determining molecular constants. It is also used to separate components of overlapping bands, such as atmospheric water vapor and ammonia, thus making classification of spectra more accurate.

Key to high resolution lies in improved detectors and gratings used in conjunction with special optical systems for double-passing the radiation to the grating. The spectrometer arrangement generally follows the accepted pattern for absorption spectrum measurements. The substance under study is in an absorption cell through which radiation is passed before entering one of the optical systems used.

An arc source, filtered to eliminate radiation from higher-order spectra, provides continuous radiant energy. The filtered radiant energy, which is approximately 90% of initial radiation, is sent through a 1-meter long absorption cell. Through use of mirrors, the effective path is 6 meters. Transmitted radiation is resolved in the spectrometer. The radiation, modulated at 90 cps, is detected by a photodetecting cell, amplified, and recorded.

Two equally successful optical systems have been devised. In one, radiation passes through a slit, and is ren-

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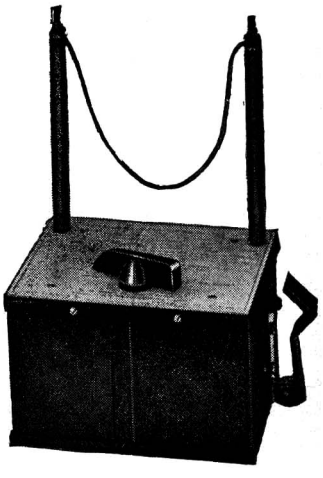
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Glass tubing, bottles, or jars up to 3 inches in diameter can be cut neatly and quickly with this cutter. Soft glass, Pyrex-Brand Glass, or other hard-glass tubes can be cut with equal ease. The article is first encircled with a scratch made by a cutter wheel conveniently mounted on the side of the transformer, the scratch is heated by contact with the hot wire, and then cooled quickly by applying water or by blowing on it. No other equipment is required.

The cutting wire is supported on two insulated posts and is heated by current from a 12-volt transformer serving as the base. No. 24 (B & S gauge) nichrome wire is used and is easily replaced. Three extra wires are included. By means of an adjustment on the transformer, the current can be controlled to give the optimum heat for whatever type of glass may be used. An instruction plate is mounted near this control. The unit operates on 115 volts, 50 or 60 cycle A.C. Over-all dimensions are 6 x 4½ x 9 inches high.

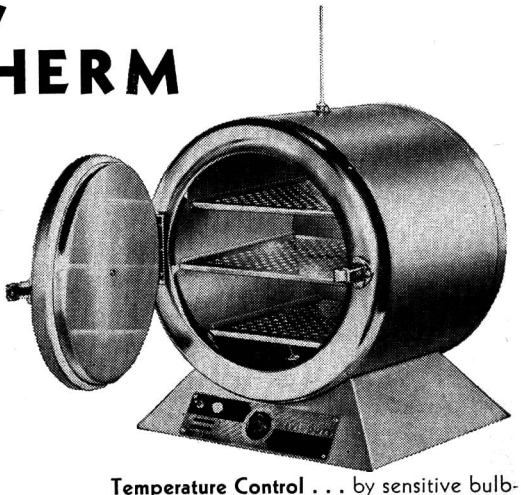


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dered parallel by a paraboloidal mirror. The parallel beam is reflected from the grating, intercepted by an optical flat, and reflected back to the grating. The beam returns to the mirror almost along its original path and is reflected through a second slit to the detector. The resulting spectrum partially overlaps the spectrum provided by the beam which has been reflected only once from the grating. Dispersions are almost identical and the wave lengths are so close that they cannot be separated by transmission filters. The two images on the exit slit are thus arranged so that one image lies slightly above the other. By screening part of the exit slit, the instrument can be made double-pass only. By screening the optical flat so that no second reflection to the grating is possible, the instrument is single-pass.

The second optical system for double-passing consists of two small plane mirrors placed behind the entrance slit to send energy back to the grating a second time. The dispersions resulting from double- and single-passing are practically identical. Spectra are distinguishable from one another as the single-pass image is curved while the double-pass one is approximately straight. Separation of the two spectra is effected by chopping either the single-beam or double-beam radiation. The detection system, a narrow-band pass filter, allows the 90-cycle chopped pulses to be amplified.

To assure accuracy in measuring absorption lines, the calibrated fringe system of a Fabry-Perot interferometer is recorded simultaneously on the same chart by one pen of a two-pen recorder. The calibrated fringes allow very precise determination of position of absorption lines. Wave lengths can be measured to one part in 500,000.

Rotating the grating allows scanning of portions of the spectrum. Slow rotation speeds are required to obtain highest resolution, for example, one wave number in 30 minutes in the 3020 cm.$^{-1}$ (3.3-micron) region.

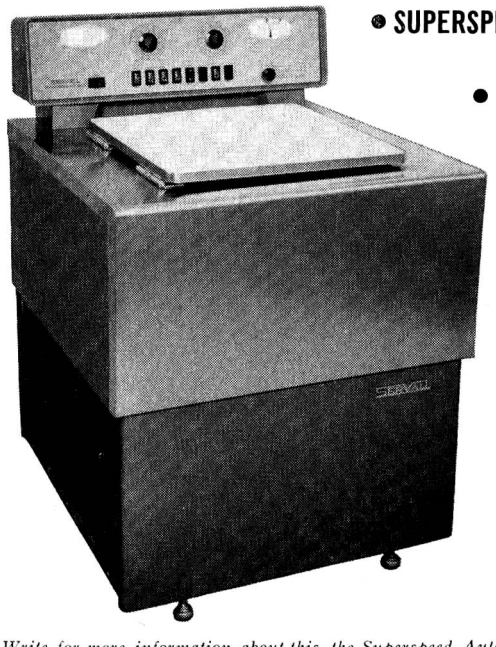
Larger regions can be scanned more quickly because of the versatile drive system. The gear arrangement allows the grating mount to be rotated at 32 speeds varying from 2.5 to 800 minutes per degree of rotation. Drive irregularity errors are less than 0.006 cm.$^{-1}$.

Further details will be found in an article "Infrared High Resolution Grating Spectrometer" by E. K. Plyler and L. R. Blaine [*J. Research Natl. Bur. Standards* **62**, 7 (1959)].

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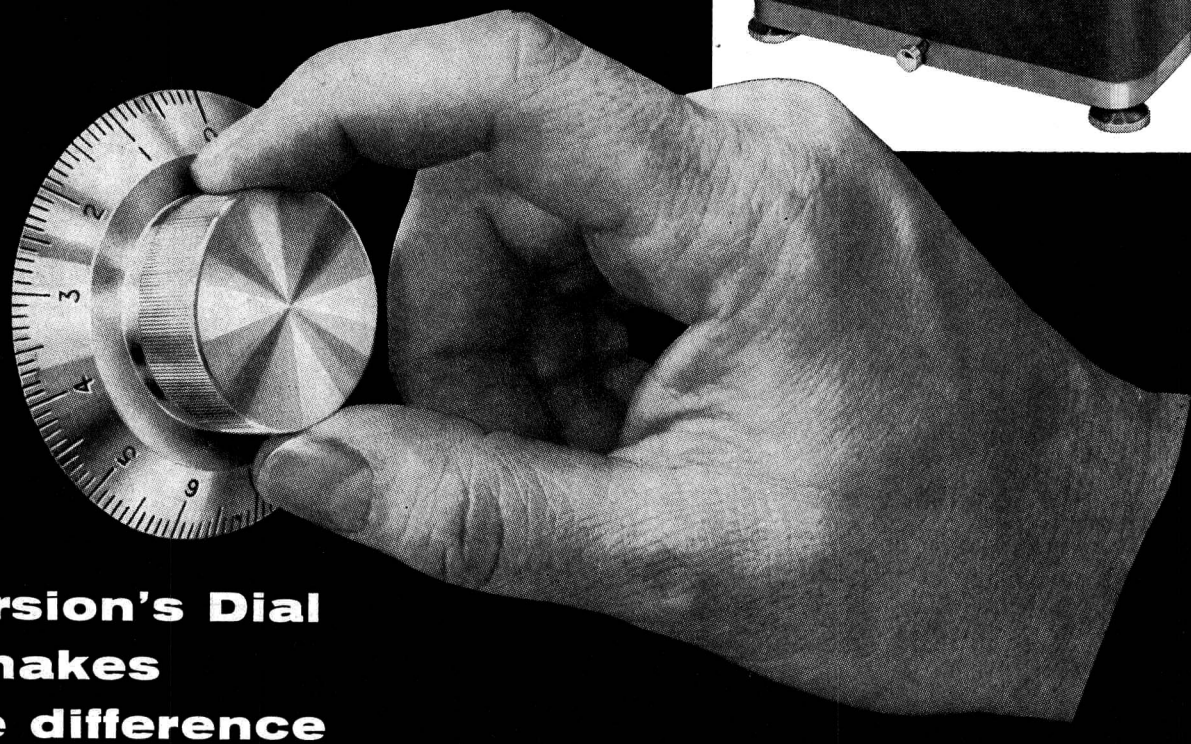
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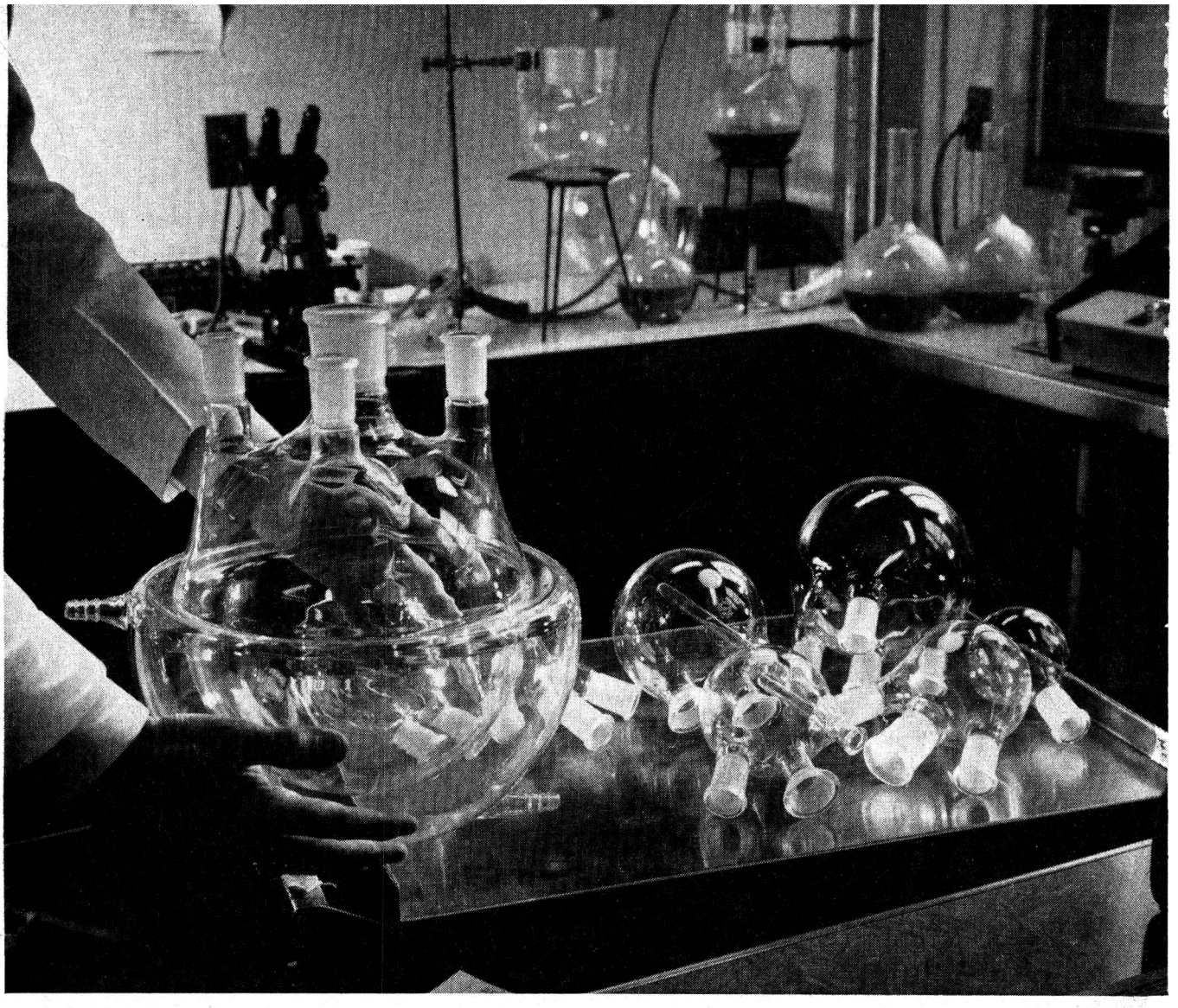
DLT2, shown above, has a capacity of 120 grams, and a sensibility reciprocal of 10 mgs. (10 mgs. moves the rest point one division).

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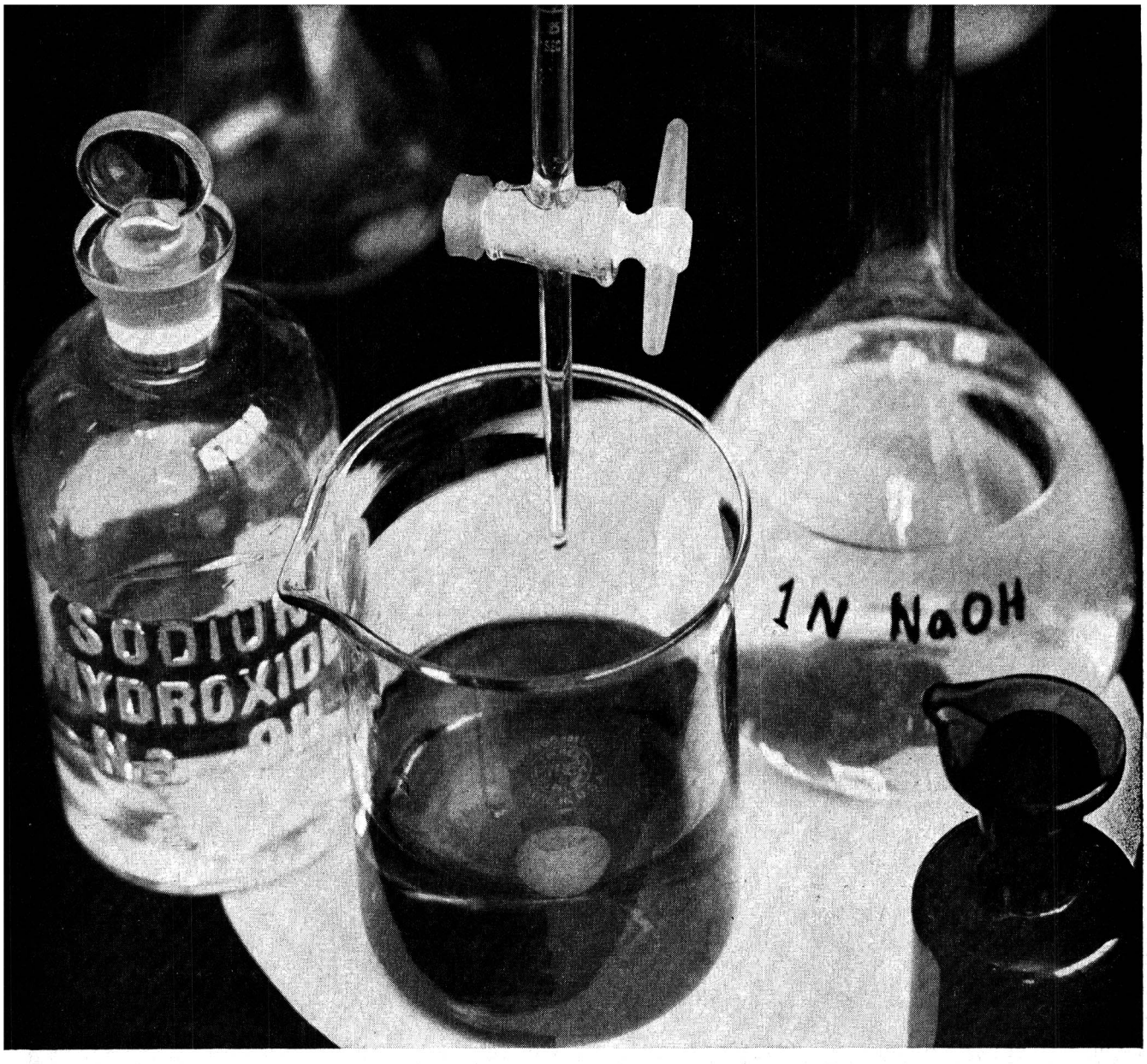
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The new Research Center is trimmed with fluted asbestos in an attractive windowless design. Separate entrances are provided for the main office wing, laboratory sections, and pilot plant

LABORATORY OF THE MONTH

Bioferm Enters Second Decade with New Research Center

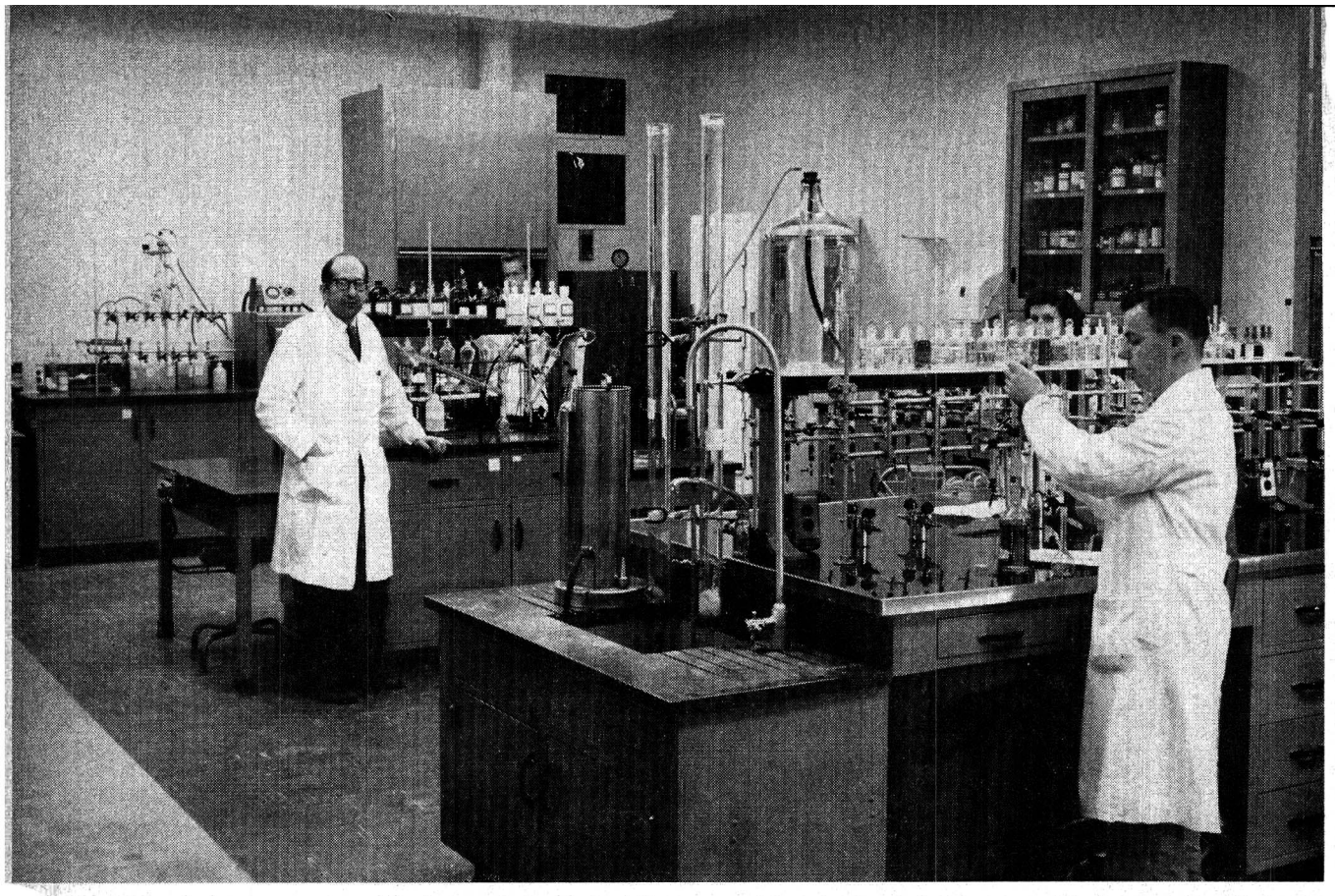
DEDICATION of a $500,000 Research Center in Wasco, Calif., near Bakersfield, was a fitting start for Bioferm Corporation's second decade of research and development. The manufacture of organic chemicals by means of fermentation for the pharmaceutical, feed, and insecticide industries requires continuous new product and process development. Bioferm Corporation (formerly Pacific Yeast Products, Inc.) is initiating its next ten year program with a staff of more than half a hundred scientists and technicians in the new air-conditioned 25,000 square foot Research Center. Plans call for a research staff of 200. A 7 acre plot provides ample room for expansion.

The analytical laboratory group is regarded as a key group and is responsible for quality control of products and raw materials, methods research, and the training of technicians to staff pilot plant and manufacturing control laboratories. Analytical work is concentrated here for manufacturing, development pilot plant and research functions (biochemical, microbiological, entomological, and organic). To carry out this extensive program, the analytical group is divided into a central laboratory and an instrument laboratory. Each is individually air-conditioned to prevent any cross contamination since the group is charged with the determination of vitamins and antibiotics which may be present in crude materials only in parts per billion. Analytical work is carried out in large stainless steel hoods or in center island sections equipped with usual services. One section is set aside for isotope dilution assay work.

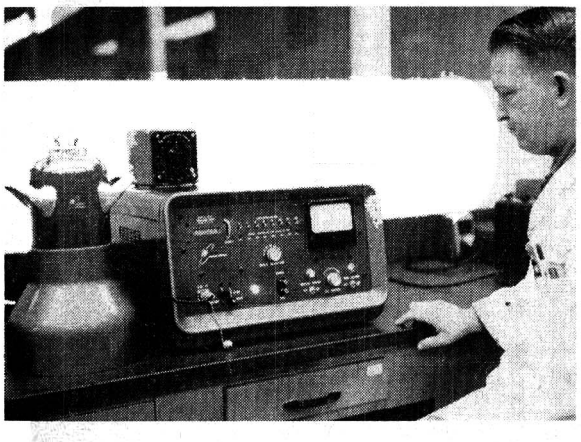
The instrument laboratory houses automatic balance equipment (mounted on a vibration-free table), vapor phase chromatographic equipment, a scintillation well counting instrument for isotope work, and two spectrophotometers fitted for flame photometry and ultraviolet work.

These facilities are housed in a large, glass partitioned, well lighted section which is painted with bright color and sound-conditioned with acoustical tile. Serving this section is a lower floor bank of 4 air-conditioned walk-in incubators each supplemented with a walk-in refrigerator. These can be used for stability studies, microorganism development in rotary or reciprocating shaker equipment, storage, and for much of the paper chromatographic and paper electrophoresis work. This area also houses the vacuum and compressed air system and an automatic still which maintains a 100 gallon supply of distilled water piped to each bench through aluminum tubing.

Other facilities at the Research Center include a library-conference room which is very essential due to the center's isolated location, a cafeteria–coffee room which is open 24 hours a day, first aid and smoking-rest rooms, plant-wide paging system, central stock room, storage area, freight elevator, and truck-loading dock.



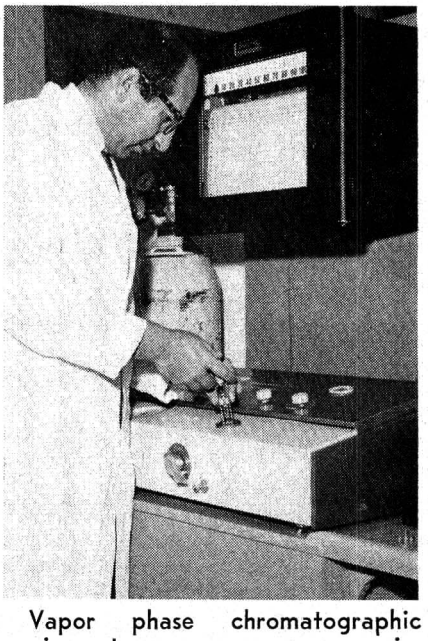
This corner of the large, centrally-located Analytical Laboratory is equipped for isotope dilution assay. Stainless steel hoods house solvent extraction equipment. A formica topped center table supports purification columns. Refrigerated storage of samples awaiting assay (background) is supplemented by walk-in refrigeration and deep freeze equipment on the lower floor. This section of the laboratory contains the clerical and file sections for recording, expediting, and reporting several thousand assays each month. 1200 isotope dilution determinations of vitamin B_{12} alone are carried out monthly. Provision was made for future addition of distillation and extraction equipment



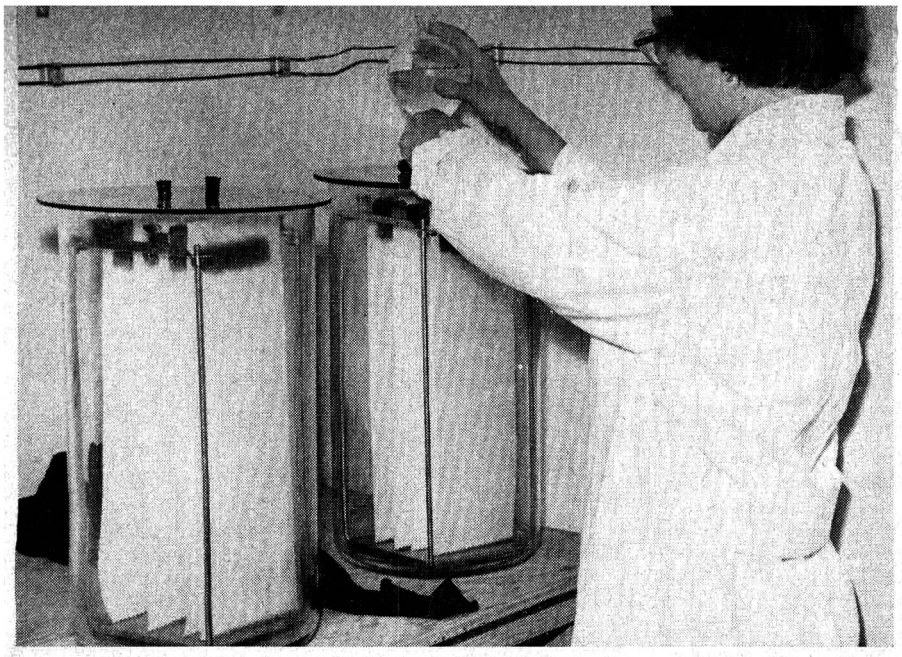
The scintillation well counter is a preferred instrument for determination of vitamin B_{12} by the isotope dilution method. This equipment was used for the collaborative work carried out in this laboratory during establishment of the official USP vitamin B_{12} assay method. The method uses vitamin B_{12} containing a labelled cobalt-60 atom.



One essential piece of equipment is the semiautomatic balance. This is mounted on a specially constructed vibration-free table in the separate instrument laboratory and is thus protected from vibrations or drafts. The instrument laboratory is separately air-conditioned to hold the temperature within 1 or 2° F. 24 hours a day

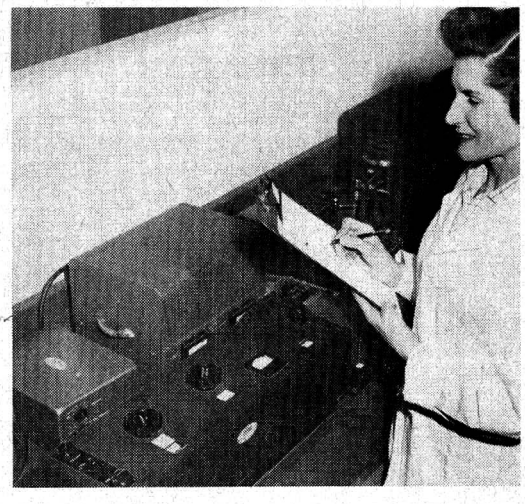


Vapor phase chromatographic equipment serves many purposes including simultaneous determination of phenol and chloroform, a wide variety of research and control functions for qualitative screening of fermentation broths, and quantitative estimation of organic acids and solvents. It is housed in the air-conditioned instrument laboratory

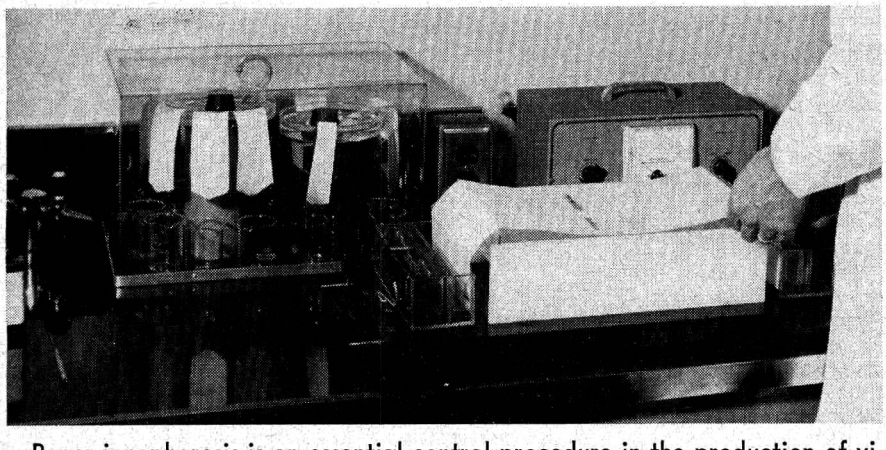


Paper chromatography is an analytical tool used in quality control of production of antibiotics and vitamins. Chromatography equipment shown is housed in a special high temperature walk-in incubator. The equipment is normally covered by a black cloth shown to protect from any influence of light

LAB OF THE MONTH



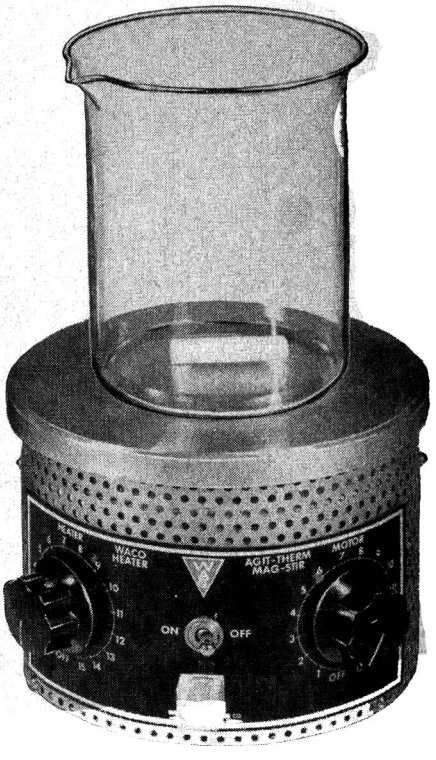
The instrument laboratory houses two spectrophotometers, key instruments for determination of vitamin B_{12}. This vitamin has distinct absorption peaks in the ultraviolet and visible light ranges which serve for quantitative estimation. These instruments are also used for flame photometric determination of metallic ions and for a wide variety of colorimetric assays for organic chemicals in fermentation broths



Paper ionophoresis is an essential control procedure in the production of vitamin B_{12}. The apparatus shown, housed in the instrument laboratory to protect it from drafts or undue changes in temperature, is normally covered by a black cloth to eliminate any influence of light on the ionogram. Pigments separated by this instrument are being eluted for analysis in the apparatus (left)

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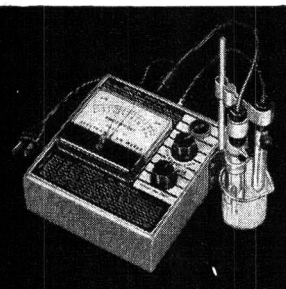
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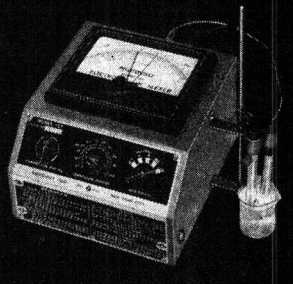
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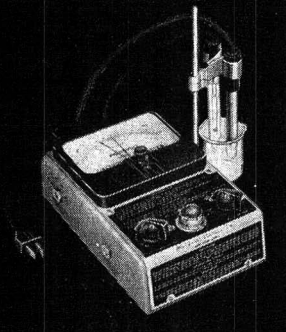
All prices include set of glass electrode and calomel electrode, or combination glass-and-calomel electrode (probe).



MODEL 110



MODEL 125



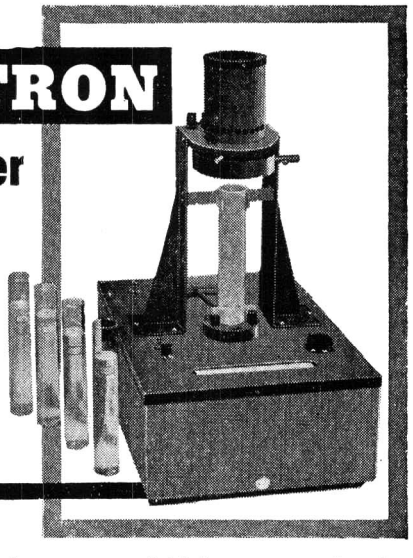
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See also Bulletin #138 on pH Meter Tester Mod. 25 for checking the performance of pH meters without electrodes or buffers, applicable to pH meters of any make. **$58.—**

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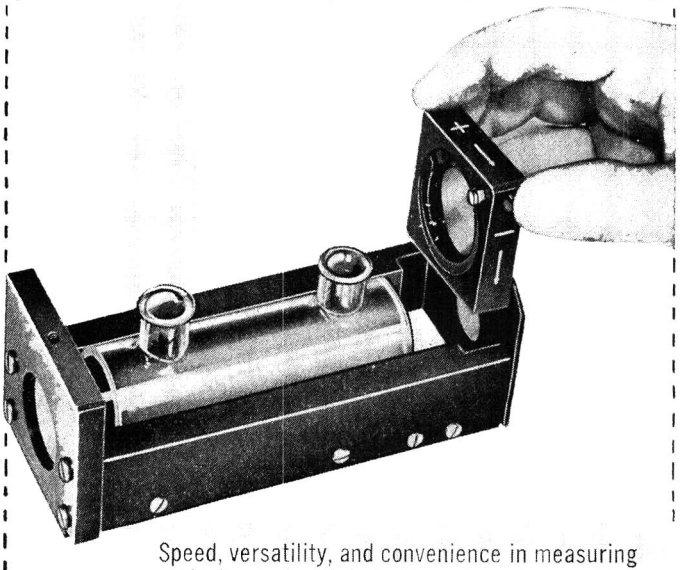
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LUMETRON Model 402-EP



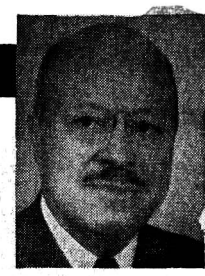
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by Ralph H. Müller

Use of instrumentation and electronics in teaching considered essential notwithstanding vigorous opposition by some educators and foundations

THE editorial in the current issue of *Instruments and Control Systems* [32, No. 3, 375 (1959)] has to be read to be believed. It is entitled "Anti-Hardwarism, a Threat to American Education and the Instrument Industry." For some three years a Pittsburgh group has worked with a public high school system, attempting to upgrade a physics curriculum by introducing instrumentation and electronics. This program has been relentlessly and vigorously opposed by university groups and the national foundations. Despite this, of 56 students who took this program in its first year, 41 are now in college.

We quote from the editorial by Dean J. D. Ryder of Michigan State University, who says, "To join science with hardware results in the training of technicians, which is not the objective of the university." The mental roadblock is obvious. The anti-hardware philosophy has blinded this man to the obvious fact that modern scientific apparatus is scientific, and that it can be used effectively to teach science.

Dean Ryder also states in one of his editorials in the *Proceedings of the Institute of Radio Engineers*—"many of our departments have made advances in removing the hardware and are attacking the subject at a high science level."

When the foundations get into the act, Editor Aronsen of *Instruments and Control Systems* tells us:

One of the things we get is a Visiting Scientist Program in Physics, sponsored by the National Science Foundation, in which 100 prominent physicists are paid to visit high schools and colleges and chat with physics teachers. (Cost: Hundreds of thousands of dollars to date. Results: Some nice chats.)

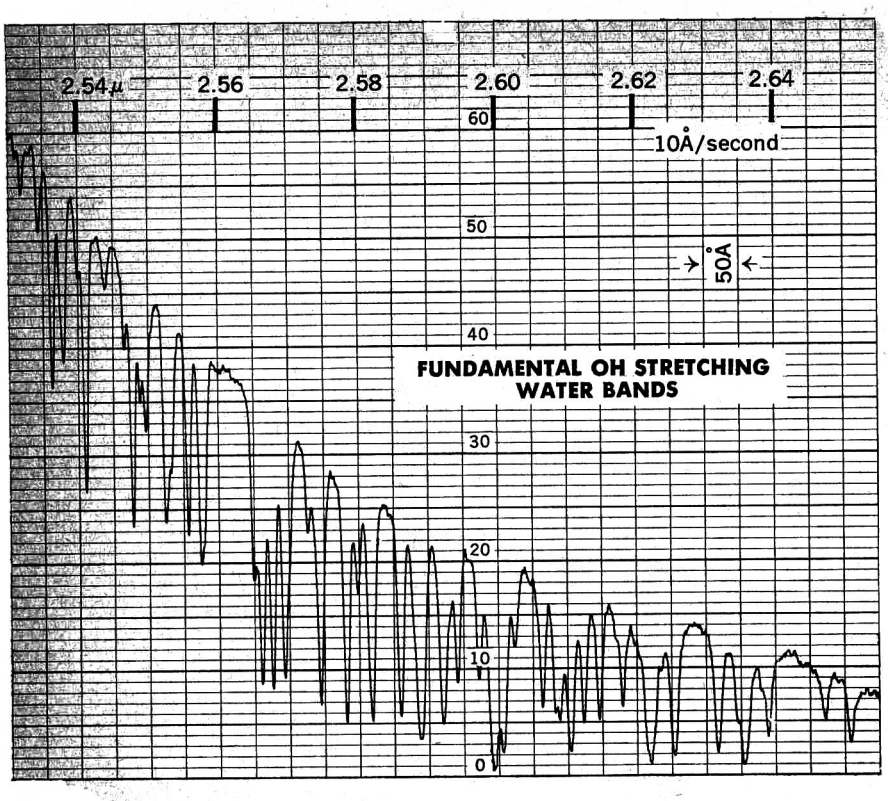
Another thing you get is a high-school physics curriculum designed by the Massachusetts Institute of Technology (and supported by *several* national foundations) wherein the high-school professor is urged to use toothpicks, bamboo rods, and wood blocks to illustrate the principles of physics. Toothpicks are "academic"; an actual instrument is "vocational." (Cost of this course has been several million dollars to date. Results: Lots of wood blocks.)

At our age, we hesitate to talk of the "good old days" (daze) but when our well heeled country is reduced to the penury of teaching its youth with "kiddy-blocks," we wax nostalgic. It is still possible to flabbergast our friends by stating that we attended a Manual Training High School. When we add that one third of the teachers were Ph.D.'s, we are suspected of downright lying. To support the facts and to make them understandable, we hasten to add that this period was on the eve of World War I, long before John Dewey started to collect powdered unicorn's hoofs, laudanum, condensed moonbeams, and other ingredients for the witches' brew which became Progressive Education.

The physics laboratory was a thing of beauty. It was headed by a Ph.D. from the University of Strasbourg. He was also secretary of the Franklin Institute and his assistant held a master's degree and was the nephew of Henry Augustus Rowland. The laboratory was a real physics laboratory, presided over by physicists—and incidentally—excellent teachers. The elegant apparatus was all imported from Leybold u. Nachfolger in Cologne, because the American instrument industry was almost nonexistent at that time, and the use of toothpicks was eschewed by all except the lower strata of society. One gained immediate respect for such equipment and promptly inferred that physics was a precise, orderly, and respectable profession. It was years later at the university that we witnessed a lecture demonstration made with very poor equipment at which the professor said, "Gentlemen, the experiment has failed, but the principle remains the same." Humorous—but not convincing.

The cultural features of that high school would be completely incomprehensible to a modern educator. If we were simultaneously studying Greek civilization in one classroom, making an original design based upon a Greek motif in the art department, and later hammering it out in wrought iron at a flaming forge, we, in youthful excitement, believed we were beginning to understand the whole story of artistic creation. Let any dyspeptic votary of the arts stand in the Loggia at Florence and gaze at Benvenuto Cellini's "Perseus with the Head of Medusa" and think he understands this jovial Renaissance rogue's art. With that work, and his enchanting chalices, Cellini was master of every detail. He was a gifted artist and a superb technician. He might curse and abuse his technicians and laborers, but when the casting was to be done he did it and no one danced more nimbly than he to avoid the bouncing globules of molten bronze as they spilled over the foundry floor. Technicians indeed! Would our deans and foundation executives be content to have the surgeon spout Latin and let the hospital orderly remove their appendixes? A truly great artist, musician, surgeon, scientist, or engineer is master of every detail of his profession and can do any part of it as well as or better than a technician. Whenever he loses that competence, becomes wholly dependent upon his technicians, and what is worse, begins to despise technicians, it is high time for him to get behind a desk, write memoranda and edicts, and begin to order the work and affairs of better men. Moreover, he will be better paid for his services.

This is no catastrophe threatening the instrument industry nor will it in any way diminish the use of instruments in research and technology. It is just another example of muddy thinking in some low and high places. Nor will this disdain for craftsmanship and skill have too serious effect upon our youth. Although most students get



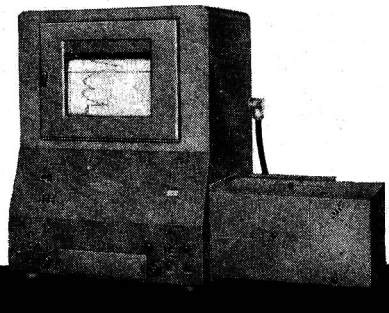
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poor advice, they manage to struggle along. One or two inspiring and competent teachers make up for all the martinets and light-weights.

We all do well to recall the history of the American apparatus and instrument industry and that it is less than a half century old. That it is largely responsible for our prosperity and technical supremacy, is easily demonstrated. That any person in his right mind can imagine that a scientist or engineer can do without the best instrumental resources available is incomprehensible. Nor can such devices be relegated to the exclusive use of so-called technicians. Suppose we consider the scientist ready to make some precise measurements. He turns on switches and nothing happens. More twiddling of dials and more snapping of switches. He calls the technician, who crawls under the chassis, changes a few tubes, checks resistors, capacitors and wiring, connects an oscilloscope, and checks a dozen wave forms. In a little while, he emerges, flips a few more switches, reads a few meters, and then announces that the instrument is in perfect order and on calibration. The scientist introduces the specimen, pushes the correct button (after a second attempt). The recorded data are sent to the computing center, encoded by a lady technician, and the collated data are returned to the scientist who finds that the results fit an equation derived many years ago (by someone else). Question: Which is the scientist and which is the technician?

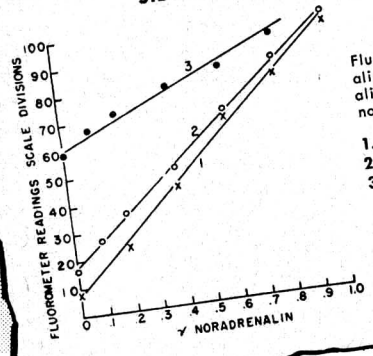
There are several fields of research in which it can be stated that the technical and instrumental aspects are far more complicated than the scientific problem on which they are brought to bear. If the performance, the precision, and reliability of the results depend very largely upon the technician and are but poorly or vaguely understood by the scientist, one must ask again, who is the technician? Let the scientist who disdains instruments, gadgets, and electronic nuisances and values only the results which they furnish him in some ill-understood way take heed. He may come to grief or have to change his attitude. It happened a thousand years ago!

In the ancient universities of Padua, Bologna, and Paris, the professors of medicine lectured from a pulpit. Clad in semi-ecclesiastical robes and bearing princely decorations they proclaimed the infallible doctrines of Aristotle, Hippocrates, and Galen. Students were bored, then as now, by pontifical utterances and they didn't perk up until a group of technicians entered the scene. These venturesome and courageous

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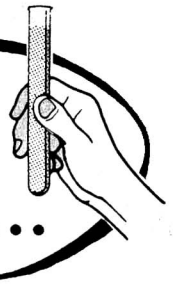
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SIDNEY ROSTON, Anal. Chem. Vol. 30, Pg. 1363



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2. Adrenalin, 1 γ; water reading, 6
3. Adrenalin, 5 γ; water reading, 6



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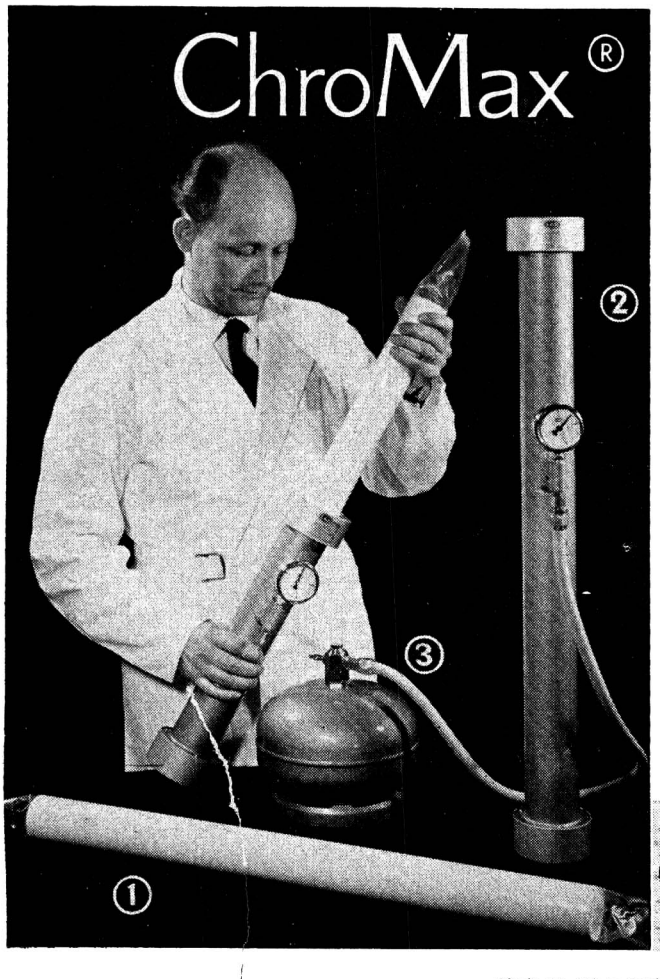
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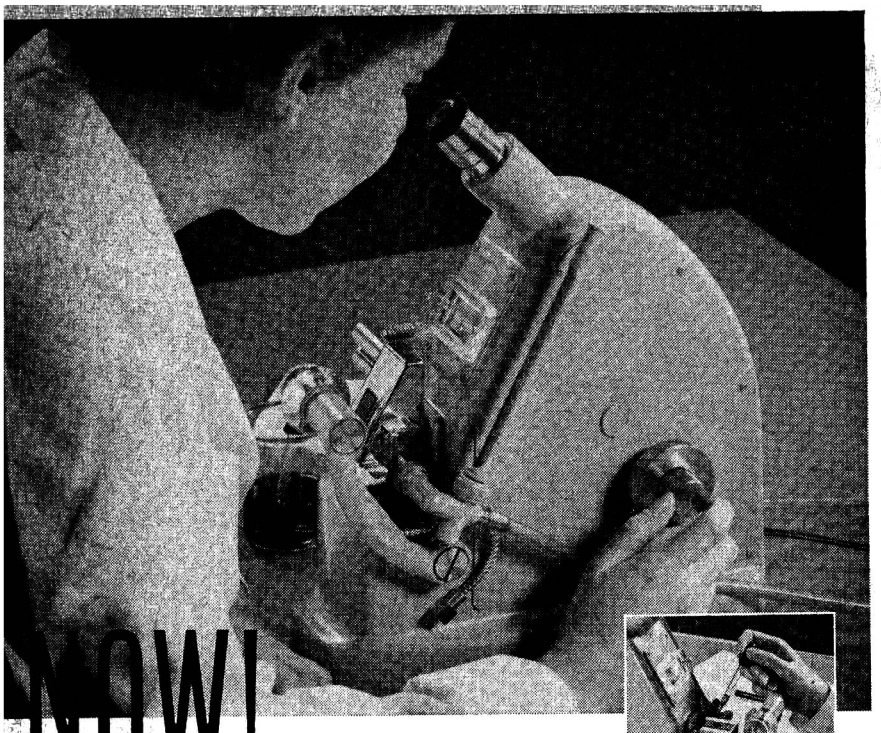
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technicians were beginning to disect cadavers in the lecture hall and, to use a modern and inelegant expression, "making a monkey of the learned professor." Students are not nearly so stupid as their mentors believe, and what was exposed before their eyes became a realistic approach to the practice of medicine and surgery. The professors droned on, quoting the alleged location of the heart, liver, spleen, and the spirit, but thanks to the technicians, their true location and function were apparent to anyone who could see. The lectures continued unabated, as mellifluous as a sonorous Gregorian chant, but the students sought out the technicians and learned as much as they could from them.

In the contemporary traditions of the union hierarchy, this could have led to a serious jurisdictional dispute, but all honor to the medieval professors of medicine (or was it good business sense?); the professors put their robes in moth balls and joined the technicians, and medicine became an experimental factual science, leaving speculation, theory, and divination to the philosophers and the theologians.

Let us make no mistake! The recent emphasis on mathematics and the sound grounding on fundamental principles has paid off handsomely. No intelligent person would insist upon a closed loop of string bearing three, four, and five knots, equally spaced, in order to prove Pythagoras' principle, even if the practical Egyptians did use the scheme to lay out precise right angles for their pyramids. What we may rightly object to, is the inference, or the downright claim, that all the laws of physical science came as a revelation to the human mind—as if they were handed down by Moses along with the sacred tablets. Our science has been a magnificent interplay between sound measurement and inspired dreams. To the scientist who, in contemporary style, likes to dress up lousy measurements (we use the term advisedly) with second-order differential equations and abundant statistical analysis, let us point out gently that there are never more than three or four outstanding theoretical giants in a century. The rest of us must measure, count, and compare, with patience and diligence—and never more than 3 feet away from our technicians.

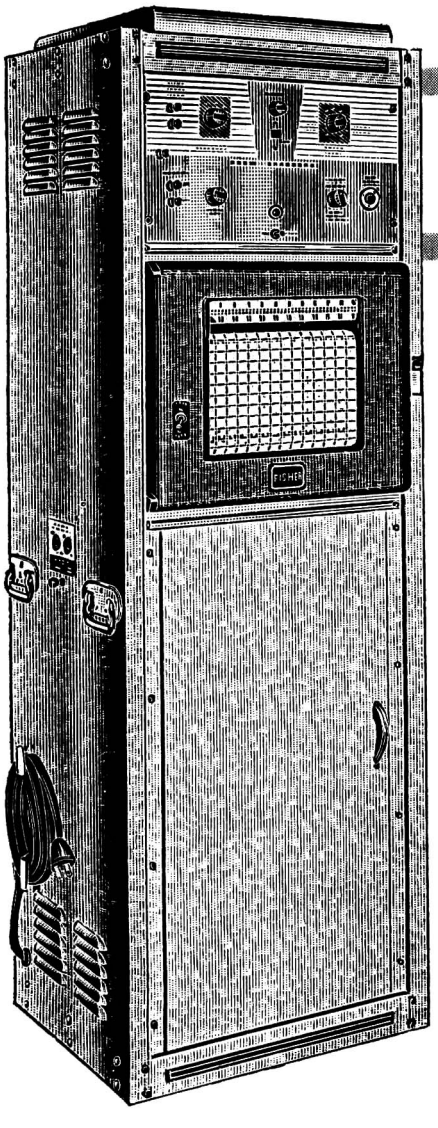
When we continue to hear such pontifical statements from high places, statements which are in flat contradiction to the most elegant practices in physical science, we can begin to understand the nervous freshman's Spoonerism as he sought an interview—"Is the Bean dizzy?"

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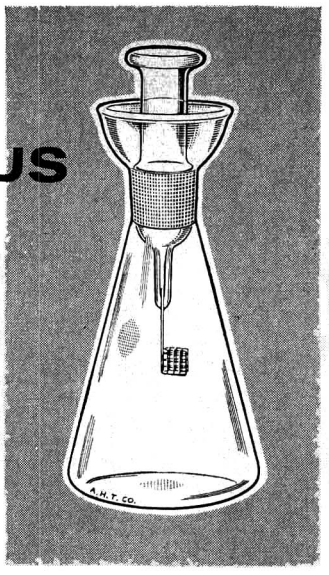
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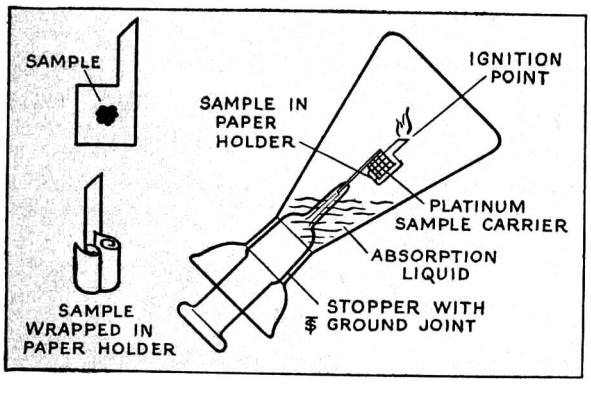
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See Wolfgang Schöniger, *Mikrochimica Acta, 1955, Heft 1, pp. 123-129;* and ibid. *1956, Heft 1-6, pp. 869-876.* See also Kenneth D. Fleischer et al, *Analytical Chemistry, Vol. 30, No. 1 (Jan., 1958), pp. 152-153,* and Ihor Lysyj and John E. Zarembo, *Analytical Chemistry, Vol. 30, No. 3 (Mar., 1958), pp. 428-430.*



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Instrumentation—New Frontier in Industrial Plant Management

Modern instrumentation is the essential element in future growth of our economy

In modern instrumentation lies our hope for the extra hands to do the work, the extra effort to make our work effective, the extra energy that will be needed to power our entire economy in an upward climb." So stated Henry B. du Pont, vice president and director, E. I. du Pont de Nemours and Co., at the Executives' Day Program of the Instrument Society of America. Topic of the program was Management's Status in Instrumentation.

The major significance of instrumentation development is not scientific or economic, but social, he said. The kind of a world we will live in 20 years from now will depend on the degree of expansion of instrumentation. Today, with a working force of 65 million out of a population of 180 million, each employed person is supplying goods and services for himself plus two other persons.

If our living standard continues its present rate of increase, in 20 years each person will require double the present day amount of goods and services. To attain this goal, productivity must increase proportionately. Since the labor force in manufacturing and agriculture is expected to increase by no more than 20% over 20 years, the increase in productivity can come only through longer working hours, fewer persons in service industries, advances in technology, or advances in instrumentation. The only alternative is a drop in living standards. Since advances in technology can not be expected to produce the required rate of growth, only through modern instrumentation can the goal be reached.

To be most effective, instrumentation must be applied at the four major levels of business. These are the control of: process variables, production units, plants, and business. Considerable progress has been made already in effecting control of process variables and production units. Over-all plant control and control of a business are exceedingly complex and still present a major challenge to instrumentation experts.

Even though the scientists and engineers develop needed instrumentation, it is essential that management recognize the necessity of adopting it. This will require integration of the skills and abilities of those developing these tools with those on the management team.

The analytical chemist is playing a major role in this fundamental change in our way of life. Even today, the chemical industry is in the forefront in instrumentation applications. Whole processes in some chemical plants are now wholly continuous and fully automatic. Many of the contributions to such automation are the product of the analytical chemist. These efforts, generally aimed at getting results faster or more economically or improving quality, take on a greater significance when it is realized that our future is linked directly to the success or failure of those who are advancing the field of instrumentation.

Sensitivity Charts for Neutron Activation Analysis

W. WAYNE MEINKE

Department of Chemistry, University of Michigan, Ann Arbor, Mich.

▶ **Sensitivity values for elements activated by thermal neutrons have been plotted in three charts for irradiation periods of 6 minutes, 10 hours, and 1000 hours. These calculated values are based on a neutron flux of 10^{12} neutrons cm.$^{-2}$ sec.$^{-1}$ Counting efficiencies (mostly for Geiger counting) were considered but decay time required for radiochemical separations was not included.**

SEVERAL concise summaries of sensitivity of the elements for thermal neutron activation analysis have been published (*1–3, 5*).

Leddicotte and Reynolds (*2*) computed the sensitivity of activation analysis for each element, assuming a neutron flux of 5×10^{11} neutrons cm.$^{-2}$ second^{-1} and an irradiation period of 10 half lives (saturation) or 30 days, whichever was shorter, but did not take into account the counting efficiency of the products. These sensitivity values have been used (*3*) in comparing the sensitivities of activation analysis with other conventional analytical methods.

Jenkins and Smales (*1*) assumed a similar irradiation period in their computations, but they assumed a neutron flux of 10^{12} neutrons cm.$^{-2}$ second^{-1}, introduced a time of 2 hours for radiochemical separation, and also considered the detector counting efficiencies for the beta- or gamma-emitting isotopes.

Recently in this laboratory Schindewolf (*5*) computed sensitivities based on a neutron flux of 10^{12} neutrons cm.$^{-2}$ second^{-1} and irradiation times of 6 and 600 minutes (10 hours). He considered the counting efficiency (using a Geiger counter in most cases) but did not include the time required for radiochemical separations. These very useful comparisons have been transposed into the graphical form used in a previous publication (*4*).

The values given by Schindewolf for activations of 6 minutes and 10 hours have been extended to 1000 hours and plotted to give three graphs, showing how the sensitivity for activation analysis can be varied, and discrimination obtained, by using different times of irradiation. If the graph for 1000-hour activation is compared to a plot of the cross sections (*4*), it is apparent that a number of the isotopes in the lower left-hand corner with low cross section and long half life have been omitted. This is the result of Schindewolf's original interest in short-lived activities. All these isotopes fall below the detection limit for a 6-minute irradiation and no attempt has been made to include them.

In the graphs the negative logarithm of the sensitivity, pS, has been plotted. This is defined as the number of grams of an element required to give about 60 counts a minute on a Geiger counter. This pS is similar to the pH used in acid-base work and is used as a convenience in plotting these sensitivities. For example, the sensitivity for vanadium-51 for a 6-minute irradiation is 1.7×10^{-10} gram, while that for cobalt-59 is 4.5×10^{-8} gram.

It is possible to change the sensitivities by using more sensitive detectors, etc., but these plots are intended to give a general impression of the relative sensitivities of these isotopes.

An isotope that is underlined on the graph indicates (*4*) that neutron activation produces a metastable daughter activity. A wavy line, on the other hand, indicates activation to the ground state of an isomeric pair. In most cases the metastable state decays independently by beta emission or contributes only a small amount to the activity of the ground state. In some cases, however, the metastable state decays completely into the ground state both directly and by decay of the short-lived metastable state.

Further information on these sensitivities is given in the original article by Schindewolf (*5*). These sensitivity values have been based primarily on Geiger counter detection. Scintillation counters were considered only when no beta emission was reported for the isotope. Similar graphs of this sort could be made for a scintillation well counter utilizing the gamma radiations given off by the isotope. In some cases these would juxtapose the sensitivities for a particular isotope.

ACKNOWLEDGMENT

This work has received the combined support of the U. S. Atomic Energy Commission and the Michigan Memorial Phoenix Project. Thanks are due U. L. Schindewolf for extending his published sensitivity values to 1000 hours. The help of M. L. Sargent and R. S. Maddock in preparing the graphs is also greatly appreciated.

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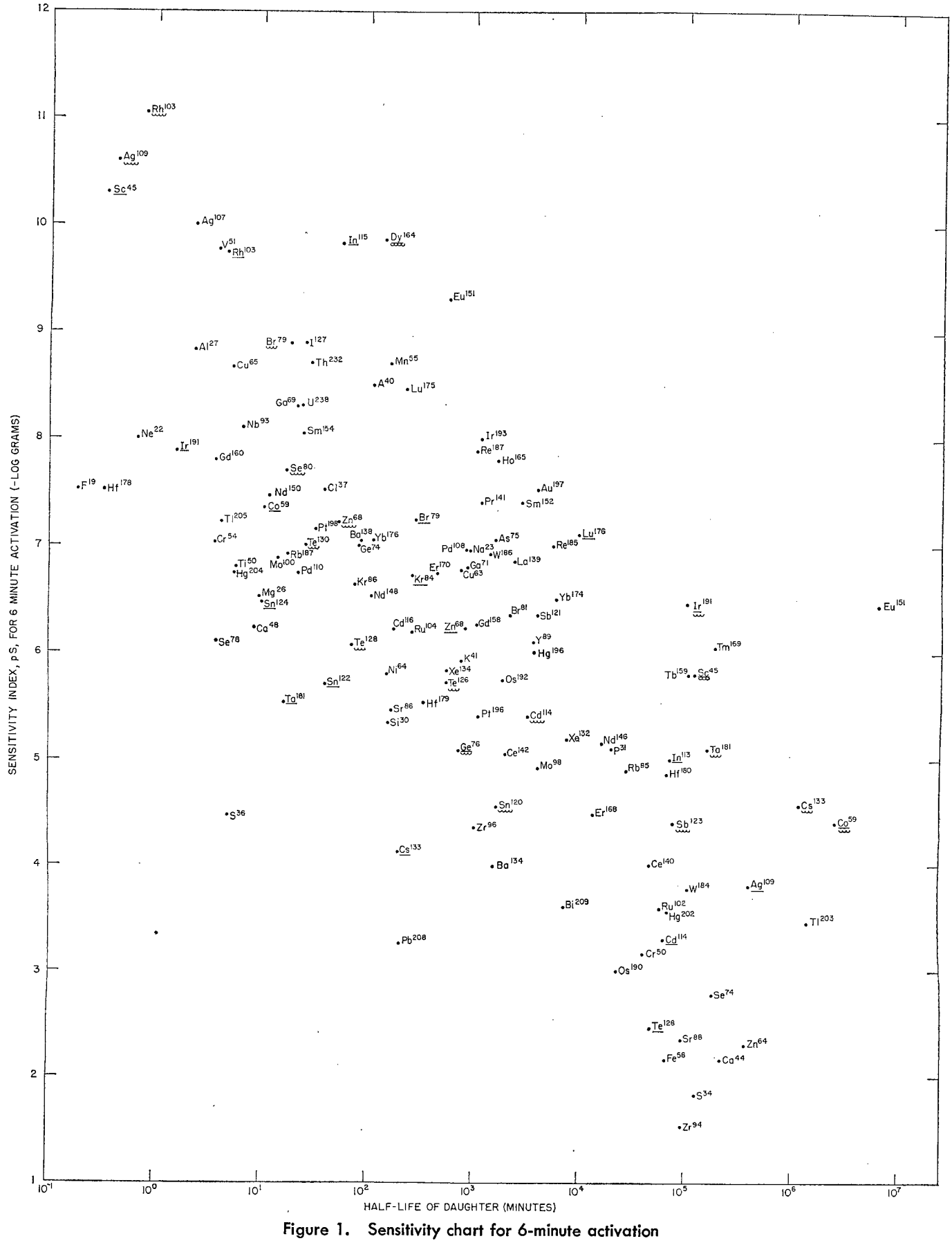


Figure 1. Sensitivity chart for 6-minute activation

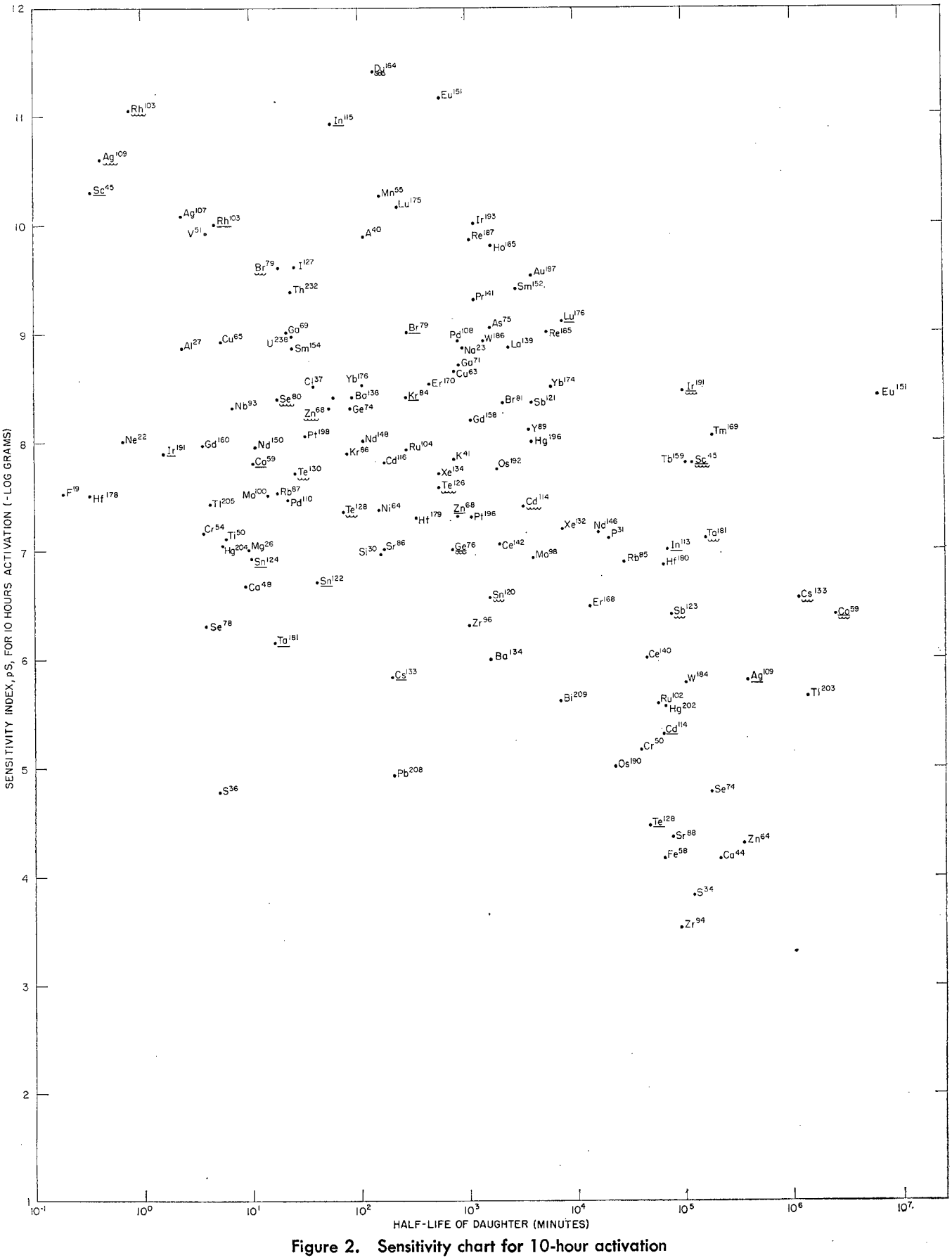


Figure 2. Sensitivity chart for 10-hour activation

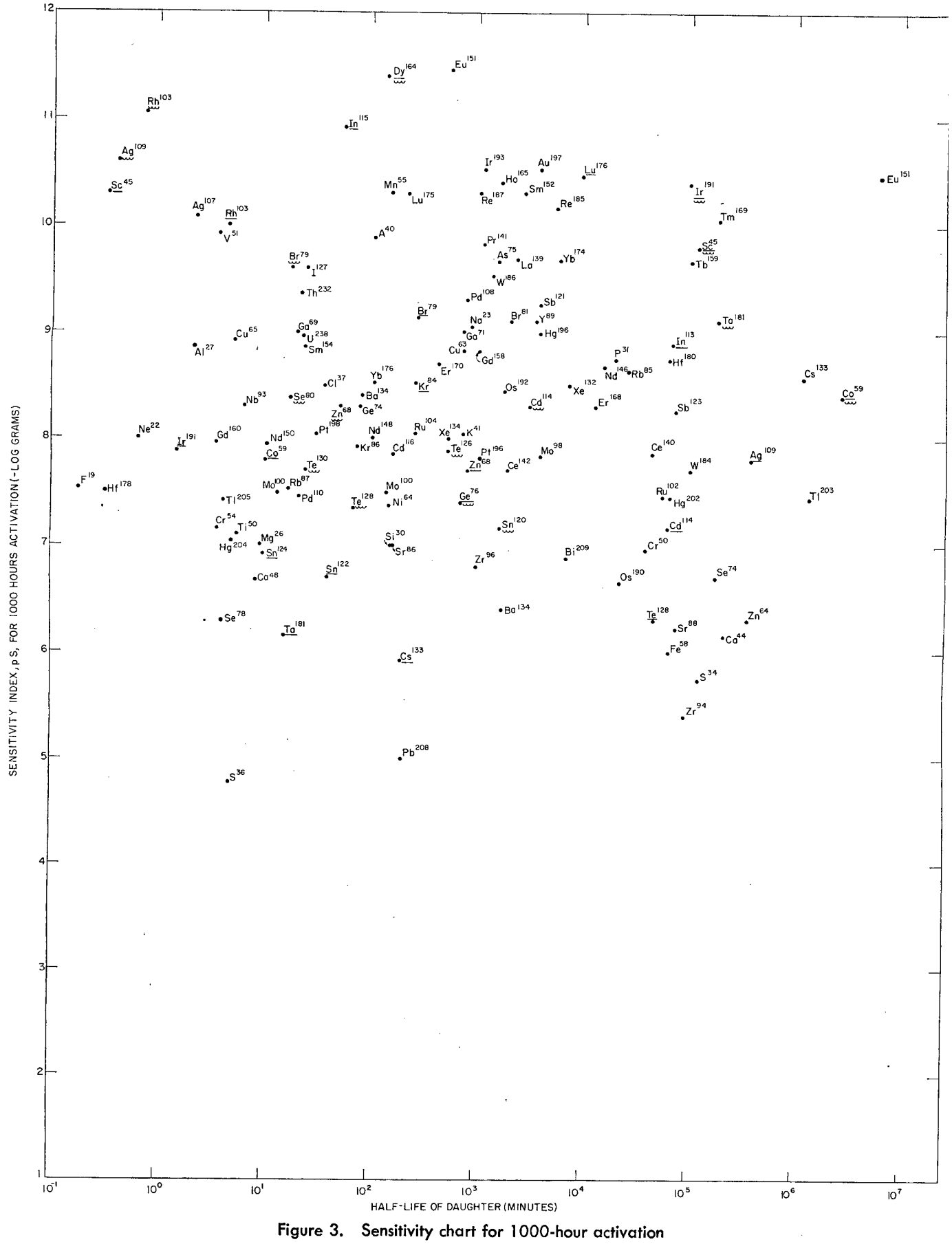


Figure 3. Sensitivity chart for 1000-hour activation

Absolute Beta Standardization Using 4 Pi Beta-Gamma Coincidence Techniques

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▶ The $4\pi\beta$-γ coincidence technique has been used to study the efficiency of the so-called 4π proportional counter. In most cases the counting efficiency varies approximately exponentially with the energy, dropping in the case of cobalt-60 to ~90%. The advantages of the technique are discussed and recommendations made as to a primary standardization method.

THE accurate determination of the absolute disintegration rate of beta-emitting isotopes continues to be important. Although several methods for standardization are widely used, each has certain limitations. These methods include coincidence techniques, the 4π geometry counter, the internal gas counter, absolute assay of a daughter or parent alpha-emitting isotope, and total absorption scintillation counting of coincident gamma-radiation. The most generally applicable methods have been the coincidence and 4π geometry techniques.

The coincidence method requires a β- and γ-ray in coincidence (or sometimes, two γ-rays). In the ordinary coincidence method, if branching occurs, or there is any angular correlation between the gammas or gamma and beta, further (and sometimes unknown) corrections must be made.

The 4π proportional counter is theoretically an excellent device for absolute beta counting and has been investigated extensively by Pate and Yaffe (*2–5*). However, these authors admit that the method is frequently unreliable, as not only as high a specific activity as possible is needed to eliminate inert solids that could cause self-absorption by the sample, but (*2*) also too high specific activity is to be avoided, to prevent accidental absorption of the carrier-free isotope on pipets, bottles, and other glassware. For the weaker beta emitters the slightest contamination with dust or silica (a 1-micron particle ≈200 γ per sq. cm.) will completely invalidate the results and in many cases it is not possible to prepare high specific activities. In other cases it may be necessary to assay accurately a sample known to contain dissolved inert solids.

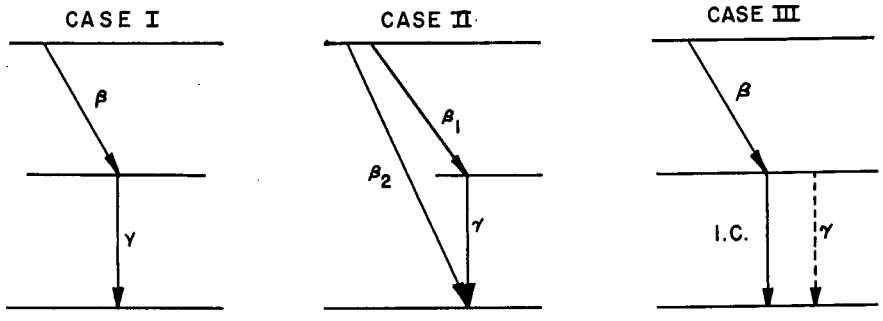


Figure 1. Decay schemes

$N_\beta = Nf_\beta$	$N_\beta = NP_1f_{\beta_1} + NP_2f_{\beta_2}$	$N_\beta = Nf_\beta + NP_ef_e - NP_ef_\beta f_e$
$N_\gamma = Nf_\gamma$	$N_\gamma = NP_1f_\gamma$	$N_\gamma = NP_\gamma f_\gamma$
$N_{\beta-\gamma} = Nf_\beta f_\gamma$	$N_{\beta-\gamma} = NP_1f_{\beta_1}f_\gamma$	$N_{\beta-\gamma} = NP_\gamma f_\beta f_\gamma$
$N = \frac{N_\beta N_\gamma}{N_{\beta-\gamma}}$	$\frac{N_\beta N_\gamma}{N_{\beta-\gamma}} =$	$\frac{N_\beta N_\gamma}{N_{\beta-\gamma}} =$
	$N\frac{(P_1f_{\beta_1} + P_2f_{\beta_2})}{f_{\beta_2}} =$	$N\frac{[f_\beta + P_ef_e(1-f_\beta)]}{f_\beta}$
	$N\left(P_1 + \frac{f_{\beta_2}}{f_{\beta_1}}P_2\right)$	

$N_{\beta-\gamma}/N_\gamma$ is equal to f_β

where

N_β, N_γ, $N_{\beta-\gamma}$ are the observed β, γ, and β-γ coincidence counting rates, respectively.

N is the absolute disintegration rate.

f_β, f_γ, and f_e are the counting efficiencies for the betas, gammas, and conversion electrons, respectively.

P_1, P_2, P_e, and $P\gamma$ are the decay probabilities: $P_1 + P_2 = 1$ and $P_e + P\gamma = 1$.

In the last analysis the accuracy of the 4π counter depends largely upon using as the assay the highest reproducible possible counting rate that has been obtained after many trials. This is a highly unsatisfactory basis of standardization. A partial and more accurate solution is the use of the $4\pi\beta$-γ coincidence counter, which combines many advantages of 4π and coincidence counting but is free of many disadvantages [except the requirement of a true (undelayed) beta-gamma coincidence]. This communication indicates the desirability of adopting this counter as a primary beta-standardizing method, against which the 4π method can be tested and evaluated.

THE GENERAL METHOD

Consider some general, but simplified, decay schemes (Figure 1).

In Case I, ordinary β-γ coincidence will work about as well as the $4\pi\beta$-γ coincidence method, if there is no angular correlation between the beta-particle and γ-ray. However, in the very common Case II, the beta-counting efficiencies (or their ratios) are not generally known. In the ordinary coincidence method this case cannot be handled accurately, but in the $4\pi\beta$-γ coincidence method it becomes much less serious, because practically all (85 to 100%) of the betas are being counted. For example, in Case II, β_2 will have a higher energy than β_1 and thus will be counted with a higher efficiency. As a first approximation, f_{β_2} can be set equal to unity, especially if $\beta_2 \geq 1$ m.e.v. The equation for Case II now becomes:

$$\frac{N_\beta N_{1\gamma}}{N_{\beta-\gamma}} = N\left(P_1 + \frac{P_2}{f_{\beta_1}}\right) = N\left(P_1 + \frac{P_2N_\gamma}{N_{\beta\gamma}}\right) \quad (1)$$

since

$$f_{\beta_1} = \frac{N_{\beta\gamma}}{N_\gamma} \quad (2)$$

This equation is now not sensitive to errors in P_1 and P_2.

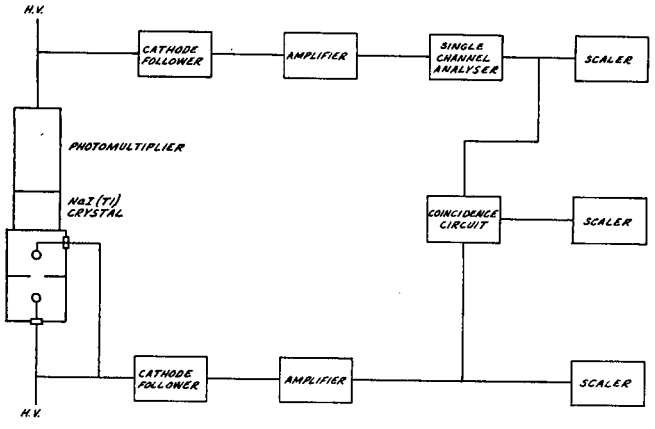


Figure 2. Block diagram of the $4\pi\beta$-γ coincidence counting assembly

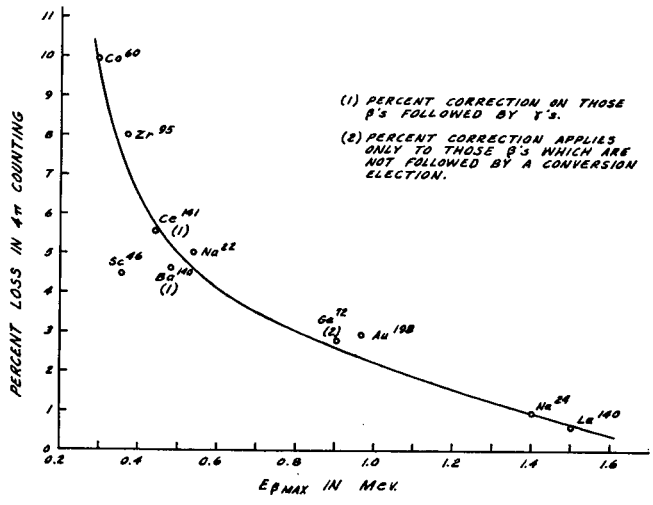


Figure 3. Approximate 4π counting efficiencies as determined by $4\pi\beta$-γ coincidence counting

Consider a decay scheme in which the real values of $P_1 = P_2 = 0.5$, $f_{\beta_2} = 1$ and $f_{\beta_1} = 0.90$ (very reasonable numbers). If an error were made in the decay scheme and P_1 and P_2 were reported as 0.3 and 0.7, the ratio of the true absolute disintegration rate to the one based on a poor choice of P_1 and P_2 would be 1.00/0.982, or an error of about 2%. The values for some representative counting efficiencies are given in the discussion. A similar situation holds for even more complicated decay schemes, if reasonable estimates can be made for the branching ratios. This method is applicable even if weak betas are involved, and, as f_{β_1} approaches unity the standardization becomes independent of any branching ratios. In the ordinary or $2\pi\beta$-γ coincidence technique the situation is different and the errors involved, especially those due to unequal scattering of the various beta groups, can be serious.

Case III involves conversion of a γ-ray; even for rather high degrees of conversion, the method is insensitive to errors in P_e and f_e.

One important aspect of such studies is that it provides a direct test of the counting efficiency of the $4\pi\beta$ counting method itself, because of the relation given in Equation 2; in effect this coincidence method can be used to standardize the 4π counter. The method is not new and the authors are indebted to Ralph Brosi of ORNL for pointing it out.

EXPERIMENTAL

The 4π counter used in this research is of the type that accepts circular mounting rings, placed between the two halves of the assembly. On this ring a thin sheet of aluminum foil is placed and a small (*6*) circular aperture is cut. The authors are in agreement with others on the size of this hole and the need for sputtering the sample with gold or aluminum to make it conducting (*3*, *7*). Both Zapon and VYNS films have been used, ranging from 1 to 10 γ per sq. cm. The thickness was negligible (*4*) and was estimated by the color fringes (*2*) and/or weighing on a microbalance.

The radioactive solutions were pipetted directly onto the film. If extremely high specific activity samples are used care must be taken to prove that the activity is not adsorbed in the glassware. If the amount of solids present in the sample is >1 γ, insulin or aerosol O.T may be used to ensure spreading of the sample (*8*). After drying, an aluminum conducting surface of $\sim$3 to 5 γ per sq. cm. was deposited for these studies.

In experiments with cobalt-60 of very high specific activity, extreme care was taken to ensure the reduction of inactive solids to as low a value as possible. In one case the cobalt-60 solution was allowed to absorb directly on the film, which was then washed with distilled water. Even in this extreme case, the highest 4π counter efficiency that could be obtained was 0.95. The authors believe that such a procedure is not adapted to precise standardization and is needlessly time-consuming and unreliable. (This is not to say that very nearly 100% counting efficiencies for weak betas cannot be obtained: merely that none have been observed in this laboratory.)

The counting arrangement is schematically illustrated in Figure 2.

The 4π counter was modified from a commercial model (Radiation Counter Laboratories, Skokie, Ill., Mark 12, Model 4) by constructing a new upper half which had the electrode connection placed in the side, with a thin top wall (approximately $^1/_{16}$ inch). The base of a $1^3/_4 \times 2$ inch sodium iodide (Tl) scintillation counter assembly was rigidly mounted and placed against the top of the proportional counter. C.P. methane was used in the proportional counter at a plateau voltage of approximately 4000 volts. The scintillation counter was used in conjunction with a single-channel analyzer, so that various β-γ-ray coincidences could be separately studied. An amplifier gain of approximately 1000 to 2000 was used; examination of the pulses indicated that no true pulses from the counter were being lost.

DISCUSSION

The results obtained for some ten different nuclides are summarized in Figure 3, which indicates the per cent loss of true 4π counting as obtained from the $N_{\beta-\gamma}/N_\beta$ ratios. The curve is approximately exponential over the range of beta-energies studied and has an equation of the form:

$$\text{Log}\,(1-f_\beta) = -0.80 - E_\beta\,(\text{m.e.v.}) \quad (3)$$

$$E_\beta \geq 0.3 \text{ m.e.v.}$$

Undoubtedly the curve will change slope and eventually approach 100% loss at the lowest energies.

As might be expected, some of the low energy values are not entirely reproducible. For example, with a great deal of care a few f_β values for cobalt-60 as high as 0.95 have been obtained; with very low specific activity and poor technique, values as low as 0.85 are obtained. The value on the curve of 0.90 merely represents a "best" average. At the higher energies the data are more reproducible and the curve in Figure 3 has been used in this laboratory to calibrate the 4π counter for isotopes having no β-γ coincidences. It is believed such a procedure is far more satisfactory than assuming 100% efficiency, especially at low energies and when extremely high specific activities are not always available or desirable. The data in Figure 3 can also be used to estimate certain relative beta-count-

ing efficiencies for more complicated decay schemes. Microscopic observation indicates that much of the loss is still caused by absorption from infinitesimal amounts of inert solids which aggregate into particles of 0.01 to 0.1 micron on evaporation. Similar conclusions have been obtained in other laboratories (*1*).

ADVANTAGES OF $4\pi\beta$-γ COINCIDENCE STANDARDIZATION METHOD

Only approximate branching ratios need be known in the more complicated decay schemes. No corrections are required for "chance" coincidences or angular correlation, because practically all of the betas are being counted. Almost any specific activity sample can be assayed. The 4π counting technique can be "standardized" for beta-emitters having no coincidences. Theoretically, statistical variations in the true N_γ and $N_{\beta\text{-}\gamma}$ counting rates should cancel each other almost exactly in the $4\pi\beta$-γ method but not in the ordinary β-γ technique. Different back scattering and counting efficiencies for beta activity in decays involving more than one beta group have no effect on the method.

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Precision Calibration of Large 4 Pi Scintillation Crystals for Routine Gamma Counting

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▶ By use of new techniques in isotope calibration, and scintillation spectrometry, a large well-type scintillation crystal counter has been calibrated very accurately. This now readily available counting system can be used routinely for mixed isotope analysis, for absolute counting of isotopes, and for decay scheme analysis. Accurate absolute counting of electron-capture isotopes is a further possible application.

THE recent use of large crystal scintillation counters (*1*, *5*) has markedly improved both the sensitivity and accuracy of γ-ray counting. The efficiencies of these instruments now approach that of high pressure ionization chambers (*2*, *4*) and they are much more sensitive and readily obtainable. Scintillation techniques are highly developed (*7*) and the instrumentation appears to be reasonably stable with time.

In this laboratory a large crystal, 4π, scintillation counter is being used for routine, absolute analysis of tracer level fission product activities and for nuclear decay scheme studies. It can also be used with isotopic mixtures. Several curves have been published (*8*, *9*) on the photo efficiencies of crystals based largely on calibration with isotopes standardized by $4\pi\beta$ proportional counting and the method of intrinsic photo efficiencies of P. R. Bell using mass absorption coefficients. Because of the possibility of errors in standardization techniques (*6*), the calibration curves so obtained do not demonstrate the inherent accuracy obtainable with the scintillation counting method. In the present research, calibration was achieved by use of the $4\pi\beta$-γ coincidence method (*3*, *6*), which is superior to normal $4\pi\beta$ standardization. The calibration curve of γ-ray photopeak efficiencies so obtained is believed to be accurate to $\pm 3\%$.

EXPERIMENTAL

The crystal used in this research was a cylinder 5 inches in diameter and 5 inches high of sodium iodide (Tl) with a $^5/_8$-inch diameter and $2^1/_2$ inch deep well. This crystal was obtained commercially from Harshaw Chemical and was optically coupled, by using silicone oil, to a Dumont 6364 5-inch multiplier phototube. The instrumentation varied with the need, but one experimental setup is indicated in Figure 1, which facilitates rapid multicomponent analysis and determination of photopeak efficiencies. The system has a resolution of 11.4% for the cesium-137 gamma activity with the source in the well—i.e., at the center of the crystal.

For high sensitivity the background can be reduced by proper shielding; in this laboratory 4 inches of lead is commonly used, resulting in a background of 600 counts per minute under the cesium-137 photopeak. For "low-level" counting an anticoincidence arrangement would be required.

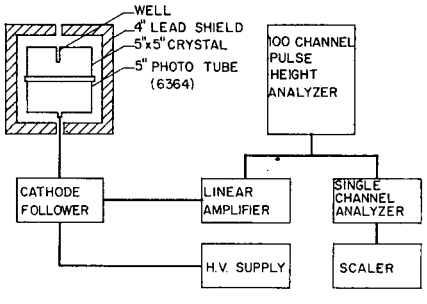


Figure 1. Experimental arrangement of instruments

Efficiencies were obtained by first standardizing the isotopes by the $4\pi\beta$-γ coincidence method (*6*) or in some cases in a previously calibrated $4\pi\beta$ counter (*6*, *11*, *12*). Appropriate aliquots (10 or 25 μl.) of the standardized solution were then transferred into a 3-inch borosilicate glass test tube which fitted into the well. The photopeak count rate for monoenergetic γ-rays was obtained by summing the appropriate channels in a 100-channel energy analysis of the sample.

In the case of sodium-22, scandium-46, and cobalt-60 the sum peak for the coincident gamma activities was counted, resulting in the product of photopeak efficiencies for the two γ-rays involved (*13*). Such a procedure contributes appreciably to the accuracy of the determination, because this photo-

peak (sum peak) does not require the somewhat arbitrary subtraction of a Compton distribution due to a gamma of higher energy. Further, the sum peak is usually well separated from the rest of the spectra, shows almost perfect Gaussian shape, and has little Compton distribution. The data obtained are summarized in Table I.

Through judicious choice of isotopes having overlapping γ-ray energies, it is possible by successive approximations on the data in Table I to construct a curve of single photopeak efficiencies as given in Figure 2. Although a 100-channel analyzer facilitated collecting the data, a single-channel analyzer will work almost as well, the largest disadvantage being the electronic drift over the time required to collect all the necessary information.

DISCUSSION

The very high efficiencies, especially at the lower energies, are apparent from Figure 2. However, for photons below 0.2-m.e.v. absorption in the glass sample holder and aluminum in the walls of the crystal well (~300 of mg. per sq. cm. absorber) becomes appreciable (solid line). (By use of mass absorption coefficient data the solid line below 0.279 m.e.v. was calculated.) The dotted line would be the theoretically expected behavior, which in this particular experimental apparatus approached 99.5% of 4π.

In the resolution of one isotope from another, or in decay scheme work, it is necessary to subtract or "peel off" the Compton distribution of the higher energy γ-ray from the photopeak of the less energetic γ-ray. The errors as introduced with large crystals are appreciably less, because the Compton contribution is very much reduced. This has been particularly useful in this laboratory for mixed fission product analyses.

By using this calibrated crystal as a secondary standard, electron capture isotopes (which are used as standards in calibrating crystals) can be calibrated to a much higher accuracy ($\pm 3\%$) than previously possible. If only x-rays are emitted, the accuracy would be $\sim \pm 10\%$.

Table I. Photopeak Calibration and Efficiencies (Primary Standards)[a]

Isotope	E, M.E.V.	Calibration Method	ϵ_1 ϵ_2	Efficiency, %
Hg^{203}[b]	0.279	$4\pi\beta\gamma$ coinc.	...	89.6
Au^{198}	0.412	$4\pi\beta\gamma + 4\pi\beta$[c]	...	74.9
Na^{22}	0.511	$4\pi\beta\gamma$ coinc.[d]	0.1716	69.0
Cs^{137}	0.6616	$4\pi\beta$	...	61.4
Sc^{46}	0.885	$4\pi\beta\gamma$ coinc.	0.2075	50.6
Sc^{46}	1.119	$4\pi\beta\gamma$ coinc.	0.2075	41.0
Co^{60}	1.17	$4\pi\beta\gamma$ coinc.	0.1353	38.6
Na^{22}	1.27	$4\pi\beta\gamma$ coinc.[d]	0.1716	36.0
Co^{60}	1.33	$4\pi\beta\gamma$ coinc.	0.1353	35.0

[a] Two other secondary standards, Ce^{143} (0.294 m.e.v.) and I^{131} (0.364 m.e.v.) gave efficiencies of 87.5 and 78.3%, respectively, which agrees well with other data.
[b] Using recent data of Nordling (*10*).
[c] Using recent data of Wapstra (*14*).
[d] Data from coincidence of 0.51 m.e.v. gamma.

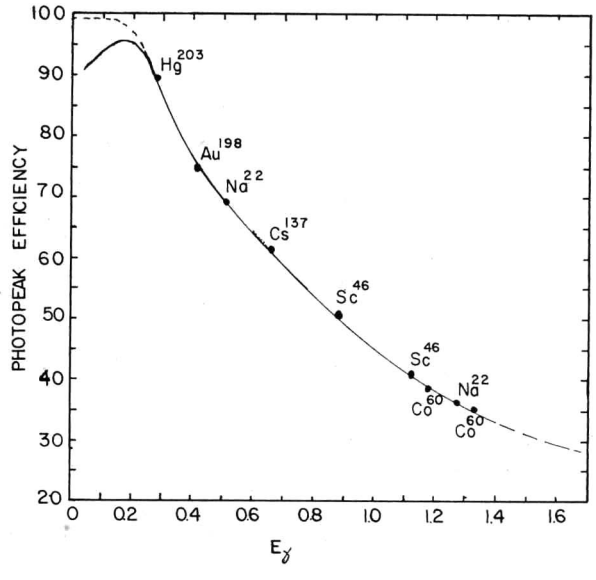


Figure 2. Photopeak efficiencies in 5-inch well crystal

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Direct Estimation of Gamma-Ray Abundances in Radionuclide Mixtures

Complement Subtraction Method

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▶ A method is described for directly determining the absolute abundances of gamma-emitting radionuclides in complex mixtures without manipulation of data. A pure standard of known disintegration rate is subtracted directly from pulse height data stored in the Argonne-type 256-channel analyzer. Abundance is determined by comparing counting times read directly from a timing scaler. The technique can be applied to any mixture in which the photopeak of interest can be definitely identified. As the correct end point is determined by visual inspection of the analyzer display screen, other constituents of the mixture need not be known. The method is adequately demonstrated by quantitative estimation of each component in four-component mixtures.

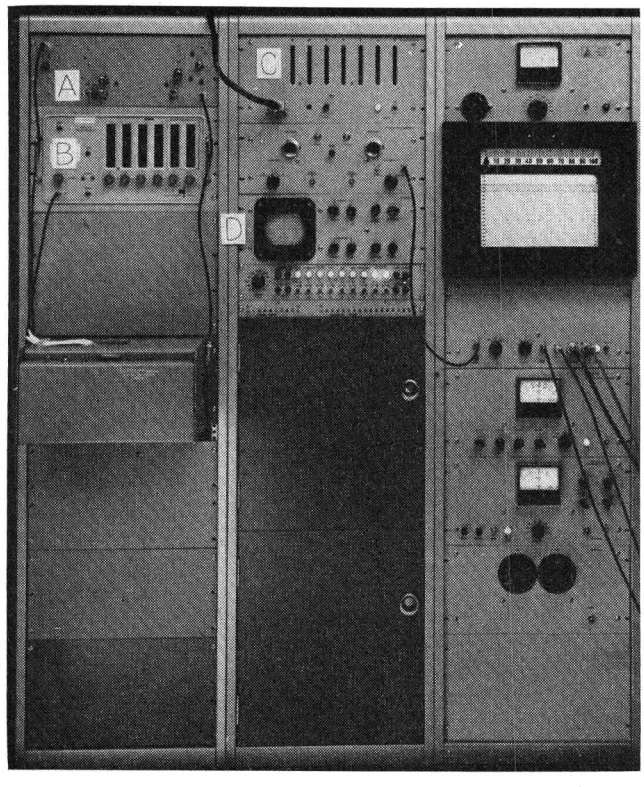


Figure 1. 256-Channel pulse height analyzer

A. Precision time base
B. Timing scaler
C. Gross count scaler
D. Viewing screen and control panel

ALTHOUGH the γ-ray scintillation pulse height analyzer is finding increasing acceptance as a standard piece of equipment in the nuclear research laboratory (*2, 14*), its more general application to the routine quantitative analysis of gamma-emitting radionuclide mixtures has been hindered by the lack of a convenient method for estimating abundances from the pulse height distribution data. Methods which have been used require that operating conditions of the equipment, such as voltage gain, linearity, channel width, sample geometry, and detector characteristics be accurately standardized as a prerequisite for precision (*10*). Pulse height distribution data once obtained must be subjected to some type of graphical or numerical analysis for interpretation (*1, 3, 6, 8*).

The present paper describes a technique which permits rapid, direct estimation of gamma-emitting radionuclides in mixtures relatively independent of the requirement for maintaining standard measuring conditions. A unique "complement-subtraction" feature of the Argonne-type 256-channel analyzer combined with a specially designed dead-time correcting device makes possible a direct nonmathematical procedure. In using this procedure, a pure calibrated standard sample of each radionuclide sought must be available in a form similar to the sample being analyzed.

EQUIPMENT AND APPARATUS

The "complement" function of the Argonne-type 256-channel analyzer converts the information which is stored in each channel of the memory to the full capacity (65,535 counts) minus the number of counts accumulated in the channel (*13*). The result of this mode of operation as viewed on the monitoring screen is a complete inversion of the pulse height distribution curve. If subsequently more counts from the same source are added to the complemented memory, these additional counts will, in effect, be subtracted from the original data stored in the memory—hence the term "complement subtraction." When a number of counts exactly equal to that which was stored in the memory before complementing has been added, the result as viewed on the monitoring screen is a complete erasure of the distribution curve. This technique has been suggested as a means of subtracting background from an accumulated spectrum (*13*).

It was felt that the same procedure could be used to subtract a single photopeak from a complex spectrum containing several such component peaks. In this case the result of subtraction would not be complete erasure of the spectrum but merely the removal of the one peak. The end point for complete removal could be determined by close observation of the expanded photopeak trace on the monitoring screen during the process of subtraction. If the source of the subtracted counts were an accurately calibrated standard of the radionuclide in question, the amount subtracted would be a direct measure of abundance.

Before any such subtraction method could be applied quantitatively, however, it was necessary to provide means for correcting the inevitable discrepancy in instrumental dead time due to differences in counting rate between samples and standards. This was further complicated by the fact that in the Argonne-

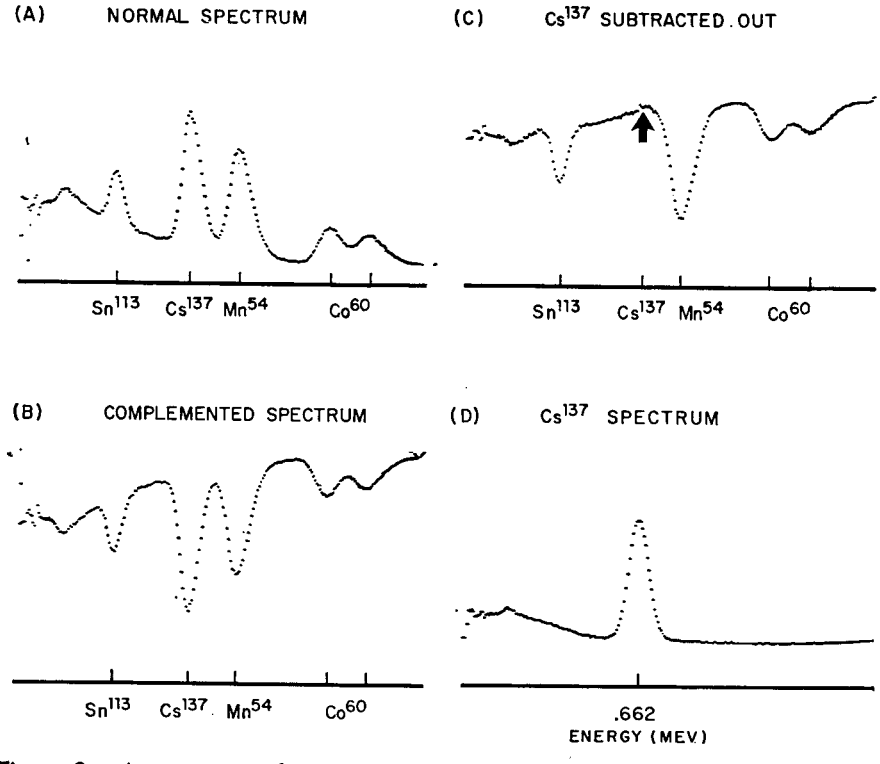


Figure 2. Appearance of pulse spectrum on viewing screen at various stages of subtraction process

type analyzer the dead time increases not only with count rate but also with the energy of the γ-ray being analyzed. A convenient solution to this problem is a timing device which, in effect, automatically corrects for dead time. The theoretical and technical aspects relating to the design of such dead-time correcting devices have been adequately described (*4, 7, 15*).

Figure 1 is a photograph of the 256-channel analyzer with the dead-time correcting unit installed. A precision 1-kc. pulse train, generated in unit *A* is admitted to a presettable timing scaler, *B*, through a normally open gate which is coupled to the control panel, *D*, of the analyzer. Whenever an event occurs which produces dead time in the analyzer, a closure pulse of duration equal to the dead-time interval is applied to the gate. Timing pulses which arrive at the gate during the dead-time interval are thus prevented from entering the timing scaler, so that the timing scaler and the analyzer both have the same dead time. The accumulated action of the analyzer is automatically terminated when the preset number of timing pulses is reached. A separate scaler, *C*, registers the total sample counts accumulated during this interval. Analyzer and timing circuits can be simultaneously interrupted by means of the operate switch on the analyzer control panel, permitting the operator to meter in very small increments of sampling time while observing the effect on the monitoring screen. This is an important feature as the end point of the subtraction process is approached.

The design of the timing device is such that it does not interfere with normal operation of the analyzer. If desired, the control action of the timer can be deleted and the analyzer operated in conventional fashion. Correction for dead time with this device is satisfactory for gross input rates in excess of 1,000,000 counts per minute (*4*).

EXPERIMENTAL PROCEDURE

A 20-ml. celluloid test tube containing the sample being analyzed is placed in a 3-inch well-type sodium iodide (Tl) crystal (Harshaw No. 12AW12S6) mounted on a Dumont No. 6364 5-inch multiplier phototube. The output of the detector is connected to the amplifier of the 256-channel analyzer. An appropriate portion of the pulse spectrum of the sample is collected for an accurately measured length of time, the timing device described being used. The memory is then complemented. A test tube containing an accurately calibrated standard of the radionuclide to be determined is then substituted for the sample. Counts from the standard are now entered into the memory and subtracted from the data previously entered. The photopeak of the radionuclide being determined is monitored continuously on the viewing screen to follow the progress of the subtraction. As the end point is approached, the display scale switch may be advanced, thus enlarging the image and permitting greater precision in determining the end point. When the end point has been reached, the accumulate action of the analyzer is manually switched off. This automatically stops both the timing device and the scaler registering total counts. Abundance of the radionuclide present in the sample is readily computed from the observed counting time and the known disintegration rate of the standard.

Figure 2 shows the actual steps in subtracting cesium-137 from a mixture as viewed on the monitoring screen.

EXPERIMENTAL RESULTS AND DISCUSSION

Studies on Single Radionuclides. To obtain a reasonable estimate of the maximum precision and accuracy which could be achieved under nearly ideal conditions, the method was first tested with samples of single, pure radionuclides. It was also desired to measure the effect of varying conditions of voltage gain (in this equipment the same as effective channel width) and count rate on the accuracy and precision of the method. Radionuclides used for this phase included cesium-137 as an example of a monoenergetic emitter and cobalt-60 as an example of a more complex type.

In each case a known quantity of the radionuclide in terms of rate and time was first stored in the memory. The same radionuclide was then subtracted either at the same or a different rate. As only one radionuclide was counted, the representation on the monitoring screen for each channel diminished and eventually dropped from view as the particular channel became filled. As the contents of each channel were subtracted to zero, the representation on the viewing screen disappeared at the top and reappeared at the bottom of the screen.

In the photographs of Figure 3 the zero level has been artificially depressed in order to retain these overflow points. The normal overflow level is indicated by the dashed lines.

Because the subtraction is a random process over the entire distribution, the peaks seldom subtracted in a completely uniform manner. Therefore, three end points were arbitrarily selected for these tests (Figure 3): (1) the point at which the representation of any channel in the region of the peak drops from view; (2) the point at which as many channels remain as those that have dropped out; and (3) the point when all representation in the region of the peak has disappeared and the identity of the peak is completely lost. End points 1 and 3 thus represent extremes on either side of an idealized end point 2. The spread is indicative of the amount of uncertainty in determining the correct end point. In actual practice it is often possible to determine end point 2 within much closer limits than end points 1 or 3.

The effects of varying counting intervals and count rate were studied by subtracting cobalt-60 with the channel

Table I. Recovery of Cobalt-60 at Different Counting Rates

Photopeak Energy, M.E.V.	Rate, D.P.M.	Counting Interval, Sec.	Average Recovery, % (Std. Dev.) End point 1	End point 2	End point 3
1.17	1.06×10^5	60	94.88 (1.85)	99.71 (0.91)	106.45 (2.37)
	1.06×10^5	300	96.15 (1.37)	100.23 (0.25)	104.83 (1.55)
	5.28×10^5	60	96.70 (0.82)	99.87 (0.33)	103.50 (0.80)
	2.74×10^4	600	91.26 (1.28)	99.46 (0.92)	107.76 (0.91)

Table II. Recovery of Cesium-137 with Different Channel Widths

Photopeak Energy, M.E.V.	Rate, D.P.M.	Counting Interval, Sec.	Channel Width, K.E.V.	Average Recovery, % (Std. Dev.) End point 1	End point 2	End point 3
0.662	8.60×10^5	60	5	97.40 (0.76)	99.92 (0.252)	103.23 (0.80)
			10	98.35 (1.10)	100.05 (0.588)	102.43 (1.46)
			20	97.25 (1.48)	99.70 (0.452)	103.65 (2.27)

Table III. Recovery of Individual Radionuclides in Virtual Mixtures

Sample	Photopeak Energy, M.E.V.	Rate, D.P.M.	Counting Interval, Sec.	Channel Width, K.E.V.	Average Recovery, %	Standard Deviation, %
Ce^{144}	0.134	2.99×10^6	120	5	99.65	1.03
Cs^{137}	0.662	8.60×10^5	60	10	99.39	0.656
Co^{60}	1.17	5.28×10^5	120	10	100.75	1.40

Table IV. Recovery of Individual Radionuclides in Actual Mixtures

Isotope	D.P.M. Added	D.P.M. Found	Approx. Compn., % of Counts	Av. Recovery, %	Standard Deviation, %
		Sample Mixture A(1)			
Sn^{113}	1.96×10^4	1.486×10^4	1	75.8	8.47
Cs^{137}	1.72×10^5	1.809×10^5	9	105.2	2.06
Mn^{54}	1.67×10^5	1.668×10^5	9	99.9	2.25
Co^{60}	1.06×10^6	1.073×10^6	81	101.0	0.962
		Sample Mixture A(2)			
Sn^{113}		1.332×10^4		68.0	5.36
Cs^{137}		1.724×10^5		100.2	1.25
Mn^{54}		1.655×10^5		99.1	3.16
Co^{60}		1.074×10^6		101.0	0.915
		Sample Mixture B(1)			
Sn^{113}	1.96×10^5	1.731×10^5	9	88.3	0.679
Cs^{137}	1.72×10^5	1.686×10^5	6	98.0	1.41
Mn^{54}	1.34×10^6	1.293×10^6	44	96.5	1.61
Co^{60}	7.95×10^5	7.94×10^5	41	99.9	0.123
		Sample Mixture B(2)			
Sn^{113}		1.829×10^5		93.3	1.37
Cs^{137}		1.613×10^5		93.8	2.09
Mn^{54}		1.240×10^6		92.5	1.44
Co^{60}		7.85×10^5		98.7	0.088
		Sample Mixture C(1)			
Sn^{113}	3.93×10^5	3.569×10^5	12	90.8	2.31
Cs^{137}	8.60×10^5	9.08×10^5	19	105.6	2.93
Mn^{54}	1.34×10^6	1.227×10^6	31	91.6	0.828
Co^{60}	1.06×10^6	9.98×10^5	38	94.2	0.849
		Sample Mixture C(2)			
Sn^{113}		3.619×10^5		92.1	1.71
Cs^{137}		8.72×10^5		101.4	1.58
Mn^{54}		1.294×10^6		96.6	0.590
Co^{60}		9.97×10^5		94.1	0.264
		Sample Mixture D(1)			
Sn^{113}	2.45×10^4	2.302×10^4	7	94.0	1.35
Cs^{137}	1.72×10^5	1.748×10^5	33	101.6	1.51
Mn^{54}	1.34×10^5	1.361×10^5	27	101.6	2.15
Co^{60}	1.06×10^5	1.062×10^5	33	100.2	1.44
		Sample Mixture D(2)			
Sn^{113}		2.230×10^4		91.0	2.67
Cs^{137}		1.724×10^5		100.2	2.52
Mn^{54}		1.326×10^5		99.0	1.81
Co^{60}		1.031×10^5		97.3	1.37

width set at 20 k.e.v. (Table I). Recovery values are averages of 10 separate estimations. The standard deviation has been calculated as a measure of the precision, and the range between end points 1 and 3 as an index of the sensitivity.

Precision and sensitivity are both improved when more counts are accumulated. However, there seems to be little difference in actual recovery over the range of input rates which were evaluated (accuracy of end point 2 in Table I). The total storage capacity of the analyzer naturally limits the maximum number of counts that can be accumulated. There are also certain practical considerations in determining the best input rate to use.

Limitations on Maximum Input Rate of Samples or Standards. The analyzer fails to store pulses correctly.

Dead-time compensation becomes incomplete.

Coincidence summations take place with resultant spectral distortions.

Difficulty occurs in metering in small increments as the end point is approached.

Factors Influencing Selection of Lowest Rate. Count Duration. Slow input rates require more time for each determination, which is undesirable where samples are many in number or of short half life.

Instrumental Stability. No correction is needed for background even for the lowest activity samples; by means of the dead-time correcting device the proper sampling interval is always assured. However, the possibility of instrumental drift over an extended counting period is increased, resulting in impairment of the precision.

In general, the best compromise was a rate chosen for utilizing the full storage capacity of the instrument, keeping the time consumed for an analysis on the order of a few minutes. These requirements were usually met by accumulating for 1 or 2 minutes at a rate of 100,000 to 500,000 counts per minute.

The effect of varying channel width on the end point determination in a similar experiment in which cesium-137 was used as the gamma source is shown in Table II. Gain settings which gave effective channel widths of 5, 10, and 20 k.e.v. were used. Recovery figures are the average of five separate estimations.

Varying the channel width seems to have little effect on the actual recovery. Precision seems, however, to be slightly greater at the higher gain settings (narrower channel widths). Once again important practical considerations influence the choice of channel widths.

Effect of Wide Channels. Fine detail in complex mixtures is lost.

Effects of voltage drifts become more pronounced.

The end point is passed too abruptly.

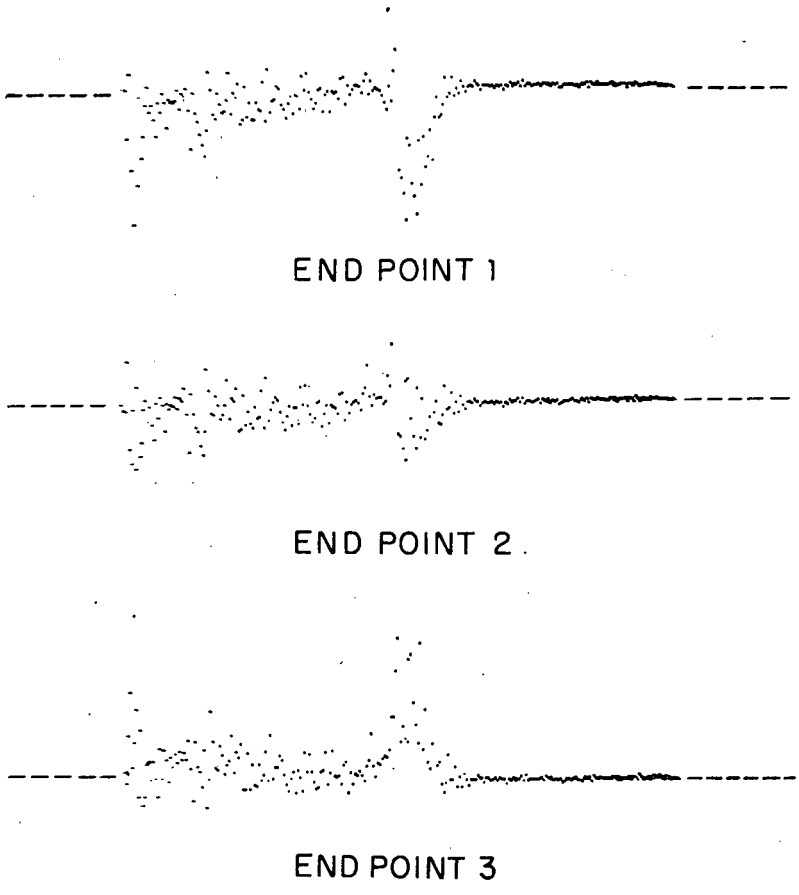


Figure 3. End points used in evaluating precision of subtraction process

Zero level has been depressed to show overflow channels (above dashed line) which are normally off screen

Storage capacity is limited, because more counts are stored in each channel.

Effect of Narrow Channels. The upper energy level is limited because of the fixed number of channels available.

Peaks are excessively broadened, increasing the scattering around the end point.

The time consumed by each analysis is lengthened because of the lower effective counting rates in each channel.

The best compromise seems to be a setting which spreads the peak being observed over approximately 10 to 20 channels. In actual use of the method with mixtures for which the exact nature of the residual spectrum under the subtracted peak is unknown, the peak is first deliberately oversubtracted at a wide channel width. This enables a quick estimate to be made, because overshoot is very easy to detect when the peak is compressed into a small number of channels. This procedure is repeated at the narrower channel width and the excess of counts removed is carefully restored. By alternately adding and removing counts, the end point may be crossed over several times, until the operator is thoroughly familiar with the appearance of the residual spectrum.

Studies of Virtual Mixtures. It was next desired to estimate the order of precision and accuracy which might be expected in estimating one component of a mixture of gamma-emit-

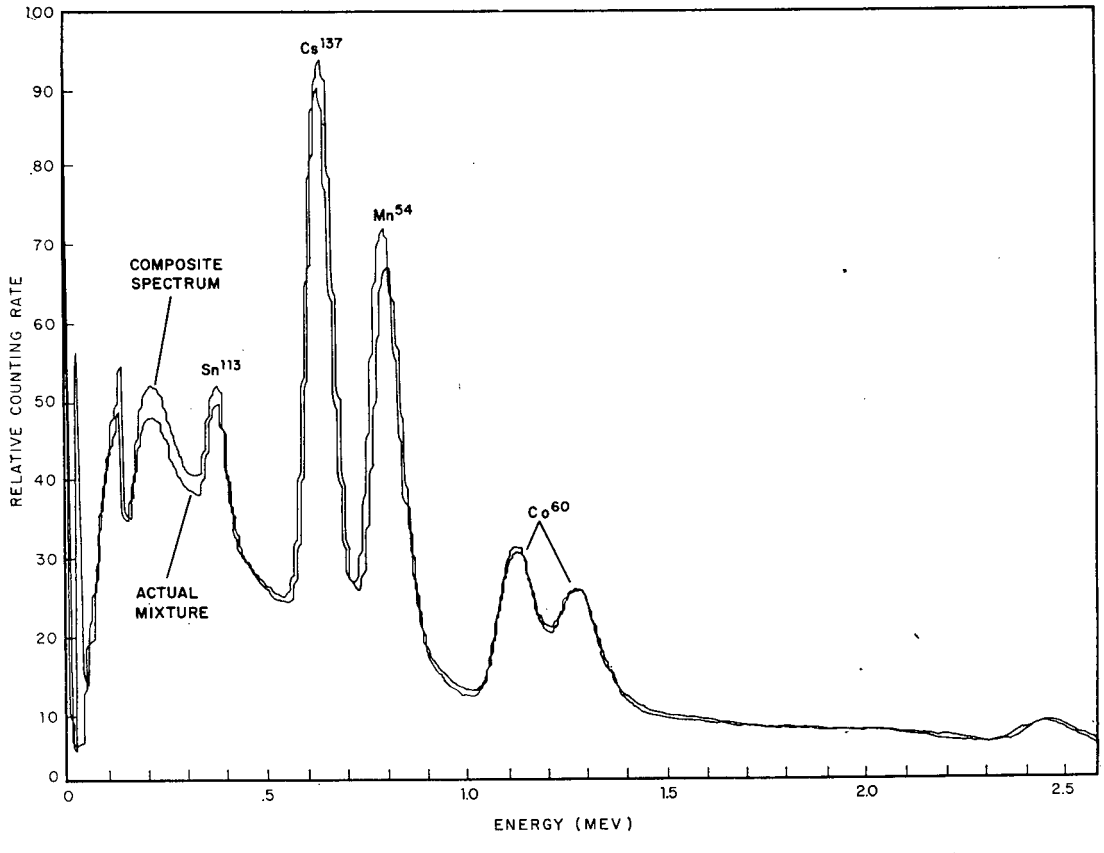


Figure 4. Distortion of gamma pulse spectrum by coincidence summation in a 3 × 3 inch sodium iodide (Tl) well crystal

ting radionuclides. In this experiment a virtual mixture was synthesized by summing the individual pulse spectra from known quantities of cerium-144, cesium-137, and cobalt-60 in the memory of the analyzer. An attempt was then made to recover each of the three radionuclides quantitatively in turn. The same mixture ratios were used in every case (Table III). Recovery values represent an average of 10 separate estimations. From these results it appeared that the technique could be successfully applied to mixtures of gamma emitters with excellent precision and accuracy.

The question may arise as to whether "mixtures" made in this fashion—that is, by summing separate spectra in the memory of the analyzer—may be different from that obtained from an actual sample mixture. In very active samples sufficient accidental coincidences may occur within the detector to cause significant distortion of the resultant pulse distribution. Some distortion of this type is certain to be encountered in every case where a sample consists of a mixture of radionuclides. To test the possibility of excessive spectral distortion in the samples, one of the mixtures used in subsequent studies on actual mixtures was synthesized by summing the appropriate quantities of each component. Mixture C was selected because it had the highest counting rate of the samples tested, being in excess of 1,000,000 cpm. The resulting composite spectrum was then compared with that of the actual mixture. Figure 4 shows the actual strip recorder tracings of the two spectra superimposed for purposes of comparison. As can be seen from inspection of the figure, the losses are relatively slight and occur mostly in the region of the backscatter peak and the tin-113 peak. This is compensated by a slight increase in the background at higher energies—for example, in the valley bridging the manganese-54 and the 1.17-m.e.v. cobalt-60 peaks. Because accidental coincidences are rate-dependent, distortions of this type can be minimized by keeping the input rate sufficiently low.

At first glance it may seem that results would be better if the contribution due to neighboring peaks which might "interfere" with the correct estimation of the end point were first removed and the estimation made on the remaining spectrum. For instance, in the synthetic mixture the peaks due to cobalt-60 could be subtracted first and subsequently cesium-137 estimated on the remaining "cleaned-up" spectrum. This is the stripping technique so often used in graphical and numerical analysis. In actual practice this does not appear to be of advantage, because the extra step introduces additional error. As the entire process of accumulating and subtracting takes but a few minutes, it is just as easy to use the original spectrum for each element to be estimated. A comparison of the results obtained from these mixtures with those obtained in estimating radionuclides singly (see Tables I, II, and III) indicates that the presence of the other constituents has no influence on accuracy or precision.

In general, this method would appear to be less sensitive to the influence of other components in a mixture than other methods which have been used. Minor peaks which may otherwise be overlooked in the spectrum of unknown mixtures are often revealed in the residual spectrum resulting from the use of the complement-subtraction technique.

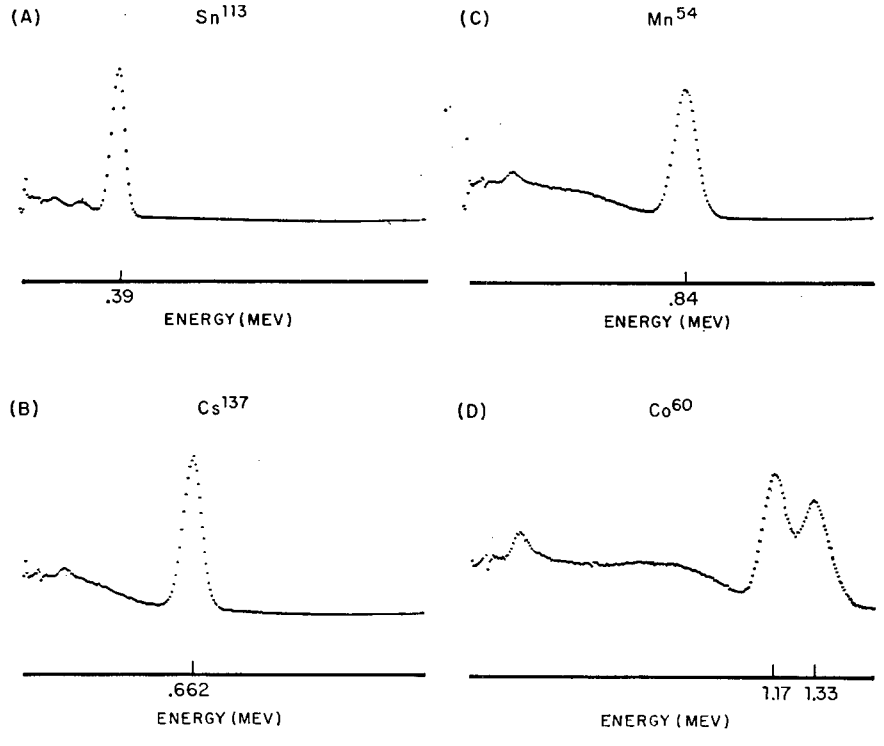


Figure 5. γ-Ray pulse height distributions of individual radionuclides used in preparing mixtures

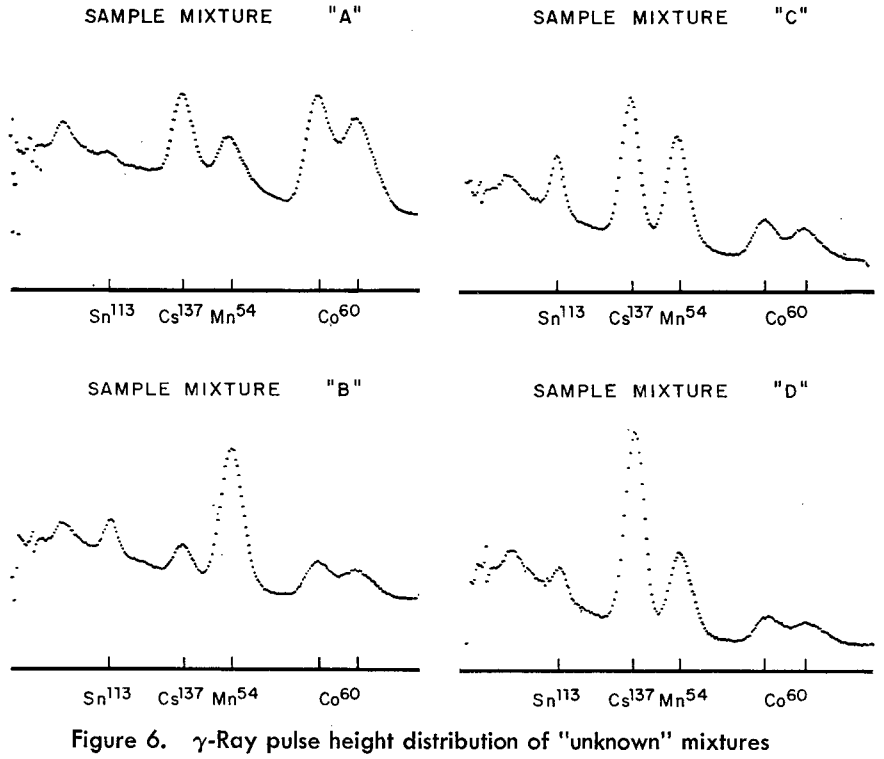


Figure 6. γ-Ray pulse height distribution of "unknown" mixtures

Studies on Actual Sample Mixtures. For an evaluation of the technique under simulated working conditions four sample mixtures were prepared in duplicate. These mixtures contained tin-113, cesium-137, manganese-54, and cobalt-60 in varying amounts. Figure 5 shows the individual γ-ray pulse height distribution of each radionuclide. The exact composition of each mixture was withheld from the investigator until the analyses were completed. Mixtures were identified only as A, B, C, and D (Figure 6). The sample mixtures were prepared by pipetting 5- to 50-μl. aliquots of the solutions used in making up the standards. As the radioactive decay of the sample mixtures and the standards was thus identical, no correction was necessary. Standards used were calibrated in disintegrations per minute.

In analyzing the mixtures, losses due to accidental coincidences have been disregarded, as it was estimated that these would be very small at the counting rates encountered.

Results of actual analyses of these mixtures by the complement subtraction technique are compared to the known composition in Table IV. Recovery values represent an average of at least five separate estimations. In general, the recovery is excellent. A comparison of the results for duplicate mixtures showed differences in composition somewhat greater than would be expected from the associated errors. For this reason they have been listed separately.

The recovery of tin-113 tends to be lower than the other radionuclides, especially in mixture A. Because the actual quantity of tin-113 in this mixture represents such a small percentage of the total activity (about 1% of the total counts), it may be assumed that the low recovery is in large part due to poor sampling statistics. However, the activity of the available tin-113 standards was considerably lower than of any of the other radionuclides, necessitating a much longer time to carry out the subtraction. This increased the amount of scatter due to background, especially as the peak of tin-113 is in the energy region most affected by background. The longer time used in subtracting increases the possibility of instrumental drift during the operation. To evaluate the effect of drift, a virtual mixture of A was synthesized and a separate series of tin-113 recovery experiments performed. In 4 out of 10 estimations performed no drift effects in the end point were perceptible. The average recovery was 100.27%. Recovery in the six determinations in which drift was evident ran from 80.80 to 96.66%, averaging 89.47%. In practice these difficulties are best circumvented when possible by the use of suitably high counting rates in samples and standards.

INSTRUMENTAL DRIFT

Requirements for short-term instrument stability during the actual period of accumulating and subtracting are stringent. Very small changes in voltage gain during this interval had a pronounced effect on the appearance of the end point. The over-all effect was a shift in the location of the peaks, resulting in a jagged appearance in the residual spectrum. Although the correct end point still can be estimated by a slightly different procedure (Figure 7, *A,B,C*), the precision is naturally inferior to that obtainable with the normal end point (Figure 7, *D, E, F*). A study of the effects of instrument drift indicates that distortion of the end point in this manner invariably results in impaired recovery. By spreading the peak over a larger number of channels, the effect of a minor shift in gain of this type is minimized at the expense of a slight loss in resolution of the end point. Using a higher count rate and subtracting immediately after accumulation also reduces the possibility of instrumental drift during the operation.

An investigation of the sources of gain shift revealed that the pulse analyzer itself was stable in this respect. The major sources of drift were variations of line voltage and photomultiplier gain. Monitoring of the line voltage showed many wide fluctuations during the working day. Less than 5% drift in line voltage caused a variation of several channels. A survey of the multiplier phototubes on hand showed that some tubes exhibited a much greater tendency to vary in gain characteristics than others. Subsequent to the taking of the data presented in this paper, a heavy-duty line-voltage regulating system was installed which has eliminated most of the trouble arising from voltage drifts. Drift arising in the detector has been minimized by use of an especially selected multiplier phototube. It is also very important that there be no shift in spectral distribution due to varying input rates. In other words, at the highest rates encountered a pulse of a given amplitude would be stored in the same channel as at the lower rates. The equipment was tested over extremes of the range of count rate covered in the present work by alternately accumulating and subtracting with widely varying input count rates. No detectable shift in the location of peaks from this cause was encountered.

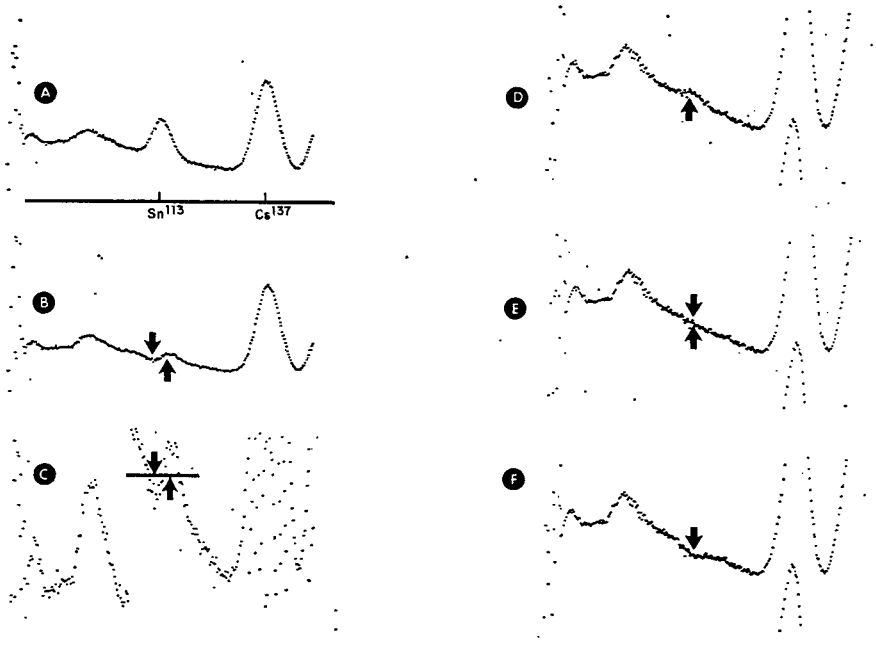


Figure 7. Effect of instrumental drift on end point in determination of tin-113

A. Sn^{113} peak before subtraction
B. Subtracted spectrum showing shifting of Sn^{113} peak during subtraction
C. Expanded view of *B* showing method of estimating correct end point
D. Sn^{113} peak 0.1% undersubtracted
E. Normal end point
F. Sn^{113} peak 0.1% oversubtracted

CONCLUSION

The complement-subtraction method permits rapid, direct estimation of γ-ray abundances in a mixture. Analyses can be performed by relatively unskilled personnel, as the results are read directly from scalers and further graphical or numerical analysis is not required. Accuracy of the technique for absolute quantitation appears to be limited at

present largely by the availability of suitable calibrated standards of the isotopes sought. Where the results are expressed relatively in per cent of some known level of original activity, such as in isotope dilution or activation analysis, this method seems especially suitable. Applications of this type are numerous in industry and medicine (*5, 9, 11, 12*).

ACKNOWLEDGMENT

The author thanks Daniel L. Love for furnishing the gamma standards and sample mixtures used in this work.

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RECEIVED for review September 19, 1958. Accepted December 24, 1958. Presented in part before Division of Analytical Chemistry, Symposium on Radiochemical Analysis, 133rd Meeting, ACS, San Francisco, Calif., April 1958.

Analysis of Fission Product Mixtures

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▶ A procedure has been developed for the rapid, sequential separation of radioactive ruthenium, strontium, rare earths, zirconium-niobium, and cesium with recoveries greater than 97%, and individual decontamination factors greater than 1000. High decontamination factors are also obtained with radioactive iodine, cobalt, iron, chromium, and zinc. The precision of each analysis is within ±4% at the 90% confidence limit. Designed for the examination of air-filter, soil, sediment, vegetation, and aqueous samples, the analyses are made on 3*N* hydrochloric acid–0.1*N* hydrofluoric acid solution. With this solution, fission products are quantitatively leached from samples which cannot be easily dissolved. A complete analysis can be made in 16 hours, and a trained technician can analyze 40 samples per 40-hour week. By providing nearly quantitative recoveries and high precision, these procedures eliminate the necessity for gravimetric yield determinations without sacrificing specificity. Considerable application to other areas of radiochemistry is anticipated.

DEVELOPMENT and evaluation of fission product procedures began with the Manhattan Project (*1*). Some of these procedures have been modified and new ones have been developed. Radioisotopes of almost any element can now be separated from those of almost any other element (*3*); however, very few procedures are quantitative or simple. These attributes have been sacrificed to obtain the desired specificity. Being nonquantitative, such procedures require that the carriers be standardized and gravimetric yield be determined for each sample. This investigation was made to develop quantitative procedures that are specific and relatively simple for the analysis of aged fission product mixtures.

In health physics environmental monitoring programs, air-filter, soil, sediment, vegetation, and water samples are analyzed for fission products. Fission products of interest include radioisotopes of ruthenium, strontium, yttrium, rare earths, cesium, zirconium, and niobium. These fission products and their daughter isobars represent the major components of a fission product mixture that has aged 2 months or more.

Fission product mixtures that have aged less than 2 months contain radionuclides other than isotopes of the elements listed above. Such radionuclides may or may not interfere with the procedure that was developed. However, any interference will be eliminated through radioactive decay within 2 months.

EXPERIMENTAL

Filters used to monitor air for radioactive particulate matter include Hollingsworth Vose No. 70 (HV-70) filters and Mine Safety Appliances No. 1106B (MSA-1106B) filters (*4*). The HV-70 filter is impregnated with silver nitrate to facilitate the collection of gaseous radioiodine. Because this filter is difficult to dissolve, a leaching procedure was developed for the recovery of fission products from the insoluble residue. A 3*N* hydrochloric acid–0.1*N* hydrofluoric acid solution leaches more than 99.9% of the ruthenium, strontium, yttrium, rare earths, cesium, zirconium, and niobium from the insoluble residue. Solutions of nitric, hydrochloric, and nitric-hydrofluoric acids do not leach zirconium and niobium quantitatively.

The MSA-1106B filter is used when collection of iodine-131 is not necessary. This filter dissolves completely in 3*N* hydrochloric acid–0.1*N* hydrofluoric acid solution after first being treated with a nitric acid–hydrofluoric acid solution. The 3*N* hydrochloric acid–0.1*N* hydrofluoric acid solution is also used to leach fission products from soil and ashed vegetation. This technique was quantitative for soil samples contaminated with fission products and for vegetation grown in a contaminated environment.

For the evaluation, the amount of radioactive tracer added and the amount recovered were compared using a sodium iodide (thallium) detector $1^3/_4$ (diameter) × 2 inches with a central well $^5/_8$ (diameter) × $1^1/_2$ inches. Tracer solutions were counted in polystyrene vials $^1/_2$ (inside diameter) × 2 inches. Tracer concentrations that provide 10^4 to 10^6 counts per minute (c/m) were used to obtain recovery and decontamination factor (DF) data, the higher concentrations being used to determine the decontamination factor. No at-

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tempt was made to determine the decontamination factor exactly if it exceeded 10^4.

Reagents. CARRIER SOLUTIONS. These are not standardized, because recoveries are nearly quantitative and gravimetric yield determinations are not required. Chemically pure reagents were used to prepare the following

Element	Solution Concn., Mg./Ml.	Solute and Solvent
Ru	25	$RuCl_4.5H_2O$ in H_2O
Cr	10	$CrCl_3.6H_2O$ in 1*N* HCl
Zr	20	$ZrOCl_2.8H_2O$ in H_2O
Ce	15	$Ce(NO_3)_3.6H_2O$ in H_2O
Sr	20	$Sr(NO_3)_2$ in H_2O
Ce	5	$Ce(NO_3)_3.6H_2O$ in 1*N* HNO_3
Cs	1	$CsNO_3$ in H_2O
Zr	100	$ZrO(NO_3)_2.2H_2O$ in 1*N* HNO_3

SPECIAL SOLUTIONS. Thioacetamide, 0.1 gram per ml. Dissolve 10 grams of thioacetamide in slightly warm distilled water. Mix thoroughly, allow to cool, and dilute to 100 ml.

Nitric Acid–Boric Acid Solution. Transfer 50 ml. of a saturated boric acid solution, 50 ml. of distilled water, and 100 ml. of concentrated nitric acid to a labeled reagent bottle. Mix thoroughly by swirling gently with the glass stopper removed. Allow to cool and replace the glass stopper.

Phosphotungstic Acid, 0.05*M* solution. Transfer the contents of a freshly opened, 100-gram bottle of Eastman Kodak Co. phosphotungstic acid, $P_2O_5.24WO_3.44H_2O$ (molecular weight = 6500), to a 500-ml. graduated cylinder. Dissolve in distilled water and dilute to 300 ml. Mix thoroughly and transfer to a labeled reagent bottle.

Procedure. FIRST PRECIPITATION OF RUTHENIUM. Transfer 25 ml. of the 3*N* hydrochloric acid–0.1*N* hydrofluoric acid sample solution to a 50-ml. Erlenmeyer flask. Add 1 ml. of ruthenium carrier and 1 ml. of chromium carrier. Adjust to 35 ml. with distilled water. Mix by swirling gently. Add 1 ml. of 0.1 gram per ml. of thioacetamide. Place the flask on a hot plate adjusted to maintain the temperature of the solution between 90° and 100° C. Do not boil.

Allow to remain on the hot plate until the precipitate settles to the bottom of the flask and the supernate clears (approximately 45 minutes). Filter with HA Millipore paper, collecting the filtrate in a clean 250-ml. suction flask. Use 2*N* hydrochloric acid–0.08*N* hydrofluoric acid to transfer the precipitate quantitatively to the filter and wash the precipitate and filter. Wash finally with a small amount of ethyl alcohol.

Transfer the filtrate from the suction flask to a 150-ml. beaker. Wash the suction flask with small portions of 2*N* hydrochloric acid–0.08*N* hydrofluoric acid solution. Add the wash solution to the 150-ml. beaker. Place the beaker on a hot plate and carefully evaporate the solution to dryness. Allow the residue to char. Retain this portion for subsequent determination of strontium, zirconium-niobium, cesium, and rare earths.

SECOND PRECIPITATION OF RUTHENIUM. Place the filter containing the ruthenium sulfide inside a 50-ml. Erlenmeyer flask. Insert filter in such a way as to have the precipitate side down and flat on the bottom of the flask. Add 2 or 3 ml. of 8*N* nitric acid; this reacts vigorously, dissolving the precipitate. The reaction should be completed before the flask is placed on a hot plate. Boil to dryness and leave on the hot plate until fuming ceases. Add 3 or 4 ml. of 1.5*N* hydrochloric acid and evaporate to dryness on the hot plate. When the fuming ceases, remove from the hot plate and allow to cool.

Dissolve the residue in 25 ml. of 3*N* hydrochloric acid–0.1*N* hydrofluoric acid solution. Add 1 ml. of chromium carrier, 1 ml. of ruthenium carrier, and 1 ml. of 20 mg. per ml. zirconium carrier, and repeat the thioacetamide precipitation.

Mount the ruthenium precipitate for beta counting. Discard the filtrate.

PRECIPITATION OF STRONTIUM AND RARE EARTHS. To the charred filtrate from the first thioacetamide precipitation, add 2 to 3 ml. of concentrated nitric acid and again evaporate to dryness. Do not char. Dissolve the residue in 3 to 4 ml. of 3*N* hydrochloric acid–0.1*N* hydrofluoric acid solution and again evaporate to dryness. Remove the beaker from the hot plate and allow to cool 3 to 4 minutes. Add 10 ml. of 0.1*N* sulfuric acid–0.3*N* hydrofluoric acid solution and 1 ml. of 20 mg. per ml. zirconium carrier. Mix thoroughly, then add 1 ml. of 15 mg. per ml. cerium(III) carrier. Cover with a watch glass and allow the sample to digest 30 minutes on a hot plate adjusted to maintain the temperature of the solution between 90° and 100° C.

Transfer to a 50-ml. round-bottomed glass centrifuge tube. Wash with small aliquots of 0.1*N* sulfuric acid–0.3*N* hydrofluoric acid solution until the total volume of the sample is 20 ml.

Centrifuge and decant the supernate into a Dowex-2 anion exchange column 0.64 cm. in inside diameter × 12.7 cm., previously prepared by washing with 10 ml. of 0.1*N* sulfuric acid–0.3*N* hydrofluoric acid solution. Wash the precipitate with 5 ml. of 0.1*N* sulfuric acid–0.3*N* hydrofluoric acid solution, centrifuge, and decant the wash solution to the reservoir of the anion exchange column. Collect elutriant from the column in a 100-ml. beaker.

SEPARATION OF STRONTIUM AND RARE EARTHS. Place the centrifuge tube in a hot water bath and dissolve the fluoride precipitate in 3 ml. of the nitric acid–boric acid solution. Add 0.5 ml. of 20 mg. per ml. strontium carrier and mix thoroughly. Add 20 ml. of fuming nitric acid in 5-ml. increments. Let stand for 10 minutes in an ice bath. Centrifuge and decant the supernate into a 100-ml. Lusteroid tube containing 65 ml. of distilled water. Retain this portion for rare earth determination.

PURIFICATION OF STRONTIUM. Dissolve the nitrate precipitate in 15 ml. of distilled water and add 1 ml. of 5 mg. per ml. cerium(III) carrier. Add 1.5 ml. of concentrated ammonium hydroxide. Centrifuge and transfer supernate to a conical, 50-ml. centrifuge tube. Wash the precipitate with 5 ml. of dilute (1 to 100) ammonium hydroxide solution. Centrifuge and add the supernate to the conical centrifuge tube. Save the precipitate for rare earth determination.

To the solution in the conical tube add 1 ml. of ethyl alcohol, then 5 ml. of saturated ammonium oxalate. Place in an ice bath for 10 minutes. Centrifuge and discard the supernate.

Mount the strontium precipitate for beta counting and count immediately.

PURIFICATION OF RARE EARTHS. From the 100-ml. Lusteroid tube containing the supernate from the strontium nitrate precipitation, pour 25 to 30 ml. into the tube containing the hydroxide precipitate obtained in the purification of the strontium portion. Pour this back into the Lusteroid tube and wash the glass tube with 5 ml. of distilled water to complete the transfer.

To the Lusteroid tube add 1 ml. of 100 mg. per ml. zirconium carrier. Add 15 ml. of concentrated hydrofluoric acid. Stir with a polyethylene-encased stirring rod. Let stand 15 minutes. Centrifuge and discard the supernate, allowing the tube to drain thoroughly. Dissolve the precipitate in 5 ml. of the nitric acid–boric acid solution with the aid of a hot water bath. Add 50 ml. of distilled water and 1 ml. of saturated strontium nitrate solution. Add 3 ml. of concentrated ammonium hydroxide. Centrifuge and discard supernate.

Dissolve the precipitate in 5 ml. of nitric acid–boric acid solution and repeat the precipitation.

Mount the rare earth precipitate for beta counting and count immediately.

ANION EXCHANGE REMOVAL OF ZIRCONIUM-NIOBIUM. When the supernatant solution from the strontium and rare earths precipitation has passed through the anion exchange column, wash the column with three 10-ml. aliquots of 0.1*N* sulfuric acid–0.3*N* hydrofluoric acid solution, allowing each portion to drain through the column before addition of the next aliquot. Set aside the combined column elutriant and washes for cesium determination. Add 25 ml. of 6*N* hydrochloric acid–0.5*N* hydrofluoric acid solution to the column reservoir and collect the elutriant in a 50-ml. beaker. Add 10 ml. of 6*N* hydrochloric acid–0.5*N* hydrofluoric acid solution to complete the elution of zirconium-niobium from the column. Place the 50-ml. beaker on a hot plate and carefully evaporate to 1 to 2 ml. Do not allow the sample to evaporate to dryness.

Transfer the sample quantitatively to a labeled 5-ml. polystyrene vial, using small aliquots of 2*N* hydrochloric acid–0.08*N* hydrofluoric acid solution to complete the transfer. Adjust the volume

Table I. Recovery and Precision

Element	Recovery, %	Precision, %[a]
Ruthenium	98.8	±3.4
Strontium	97.0	3.0
Rare earths	99.3	1.9
Zirconium-niobium	99.0	2.3
Cesium	99.2	3.8

[a] 90% confidence level.

0.08*N* hydrofluoric acid solution, ruthenium is quantitatively separated from 99.9% of all other contaminants normally encountered in fission product mixtures that have aged 2 months or more.

The supernate from the first ruthenium sulfide precipitation is evaporated to dryness and then carried through a series of acid evaporations to eliminate radioiodine. This treatment also prepares the sample for removal of rare earths and strontium on cerium fluoride precipitation, which is made from a solution that contains no strontium or barium carriers.

Table II. Decontamination Factors

Tracer	Ru	Sr	RE[a]	Zr-Nb	Cs
Ru^{103}	...	$>10^4$	$>10^4$	$>10^4$	$>10^4$
Sr^{85}	$>10^4$	...	4×10^3	1.4×10^3	7×10^3
$Ce\text{-}Pr^{144}$	$>10^4$	2×10^3	...	1.1×10^3	9×10^3
$Zr\text{-}Nb^{95}$	2×10^3	4×10^3	2×10^3	...	$>10^4$
Cs^{137}	$>10^4$	$>10^4$	6×10^3	2×10^3	...
Fe^{59}	$>10^4$	$>10^4$	$>10^4$	1.5×10^3	1×10^3
Co^{60}	2×10^3	$>10^4$	$>10^4$	$>10^4$	$>10^4$
Cr^{51}	1×10^3	$>10^4$	4×10^3	$>10^4$[b]	2×10^3
Zn^{65}	$>10^4$	$>10^4$	$>10^4$	$>10^4$	1.2×10^3
I^{131}	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$

[a] Rare earths.
[b] Sample did not contain Ag^+.

to exactly 4 ml. Place the cap on the vial and mix thoroughly. Count the sample in a well-type, sodium iodide (thallium) crystal, gamma counter. This portion contains zirconium-95 and niobium-95.

PRECIPITATION OF CESIUM. Add 16 ml. of concentrated nitric acid and 1 ml. of cesium carrier to the 0.1*N* sulfuric acid–0.3*N* hydrofluoric acid column elutriant. Add 1 ml. of 0.05*M* phosphotungstic acid. Let stand for 10 minutes. Filter with HA Millipore paper. Wash with 4*N* nitric acid and dry the precipitate with suction continued for 3 or 4 minutes.

Mount the sample for beta or gamma counting. For gamma counting, allow 30 minutes for barium-137 to establish equilibrium with cesium-137.

RESULTS AND DISCUSSION

Ruthenium sulfide is homogeneously precipitated from the hydrochloric acid–hydrofluoric acid solution by thermal decomposition of thioacetamide. From a hydrochloric acid–hydrofluoric acid mixture the precipitation is very specific for ruthenium. From 2*N* hydrochloric acid–0.08*N* hydrofluoric acid solution, 0.4% of the zirconium–niobium-95 is carried on the sulfide precipitate; however, this interference is easily eliminated by a second sulfide precipitation and addition of holdback carriers. The specificity increases with increasing hydrochloric acid concentration. However, at acid concentrations much above 2*N*, ruthenium sulfide has a tendency to decompose with heating. Therefore, a concentration of 2*N* hydrochloric acid–0.08*N* hydrofluoric acid solution is considered optimum. The presence of a small amount of hydrofluoric acid enhances the specificity of the precipitation for ruthenium. With two precipitations from 2*N* hydrochloric acid–

The precipitation of strontium nitrate from concentrated nitric acid solutions was first described by Willard and Goodspeed in 1936 (*7*). This strontium nitrate precipitation is followed by a hydroxide scavenge precipitation from the strontium portion to complete the separation of strontium and rare earths. Strontium is then precipitated as strontium oxalate (*2*, *6*).

Because barium-140 is reduced by radioactive decays to a negligible amount in aged fission product mixtures, the necessary steps for the separation of barium-140 from the strontium portion were omitted. Should barium-140 be present, the strontium portion should be purified by barium chromate scavenge or repeated oxalate precipitations.

Lanthanide rare earths are determined as a group that includes yttrium. They are purified with a fluoride precipitation containing zirconium holdback carrier and two hydroxide precipitations with strontium holdback carrier.

The zirconium-95 and niobium-95 isobars are determined together; however, they are easily separated by Dowex-2 anion exchange using hydrochloric acid–hydrofluoric acid solutions, if desired.

The absorption of zirconium and niobium from 0.1*N* sulfuric acid–0.3*N* hydrofluoric acid solution by Dowex-2 anion exchange resin was reported by Wish and Rowell (*8*) in 1956. After extensive evaluation, this technique was incorporated in this procedure. The solution was allowed to pass through the column under gravity flow conditions. A 200- to 400-mesh resin was used. Zirconium and niobium were eluted quantitatively from the column with 6*N* hydrochloric acid–0.5*N* hydrofluoric acid solution. No additional purification was necessary.

Phosphotungstate precipitation is a modification of a procedure reported by Mizzan (*5*). For samples containing fission products as the only beta-gamma emitters, a more specific precipitation is obtained in hydrochloric acid–hydrofluoric acid solution than in nitric acid–hydrofluoric acid solution. However, because most of the fission products have been removed prior to the cesium precipitation, a higher degree of specificity is not normally required. If the sample contains chromium-51, which is produced by neutron irradiation of stainless steel, the nitric acid–sulfuric acid–hydrofluoric acid solution is better than a hydrochloric acid–sulfuric acid–hydrofluoric acid solution.

To evaluate the procedure, more than a hundred silver nitrate–impregnated HV-70 filters were spiked with radioactive tracers, leached with 3*N* hydrochloric acid–0.1*N* hydrofluoric acid solution, and analyzed for ruthenium, strontium, rare earths, zirconium-niobium, and cesium. Gamma emitters, ruthenium-103, strontium-85, cerium–praseodymium-144, zirconium–niobium-95, and cesium–barium-137 were used to determine chemical recoveries and decontamination factors.

Recovery and precision data are summarized in Table I. The decontamination factor data are summarized in Table II. Individual decontamination factors greater than 10^3 were considered adequate, except for iodine-131. Being volatile, iodine-131 is sometimes released to the atmosphere in much greater quantities than other fission products. For this reason, a decontamination factor greater than 10^4 is considered desirable for iodine. The procedure provides adequate decontamination from iodine-131 in every case.

Occasionally, samples are analyzed that contain, in addition to fission products, radionuclides which are produced by neutron irradiation of structural components of a reactor—e.g., stainless steel. Radioactive isotopes of iron, cobalt, chromium, and zinc were considered as possible contaminants; therefore, decontamination factors were determined for iron-59, cobalt-60, chromium-51, and zinc-65. These data are included in Table II.

For samples containing silver ion in large amounts—e.g., silver nitrate–impregnated filter samples—0.1 to 0.2% of any chromium-51 that may be present contaminates the zirconium-niobium portion. For samples that do not contain silver, decontamination factors greater than 10^4 are obtained

for chromium-51. In every case, adequate decontamination factors are obtained for iron, cobalt, chromium, and zinc.

A complete analysis requires approximately 16 hours; however, approximately 50% of this time, the analyst is waiting for digestion or ion exchange steps. A trained technician can analyze 40 samples in a 40-hour work week.

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RECEIVED for review October 17, 1958. Accepted February 11, 1959. Division of Analytical Chemistry, Symposium on Radiochemical Analysis, 133rd Meeting, ACS, San Francisco, Calif., April 1958. Information developed during work under Contract AT(07-2)-1 with the Atomic Energy Commission, whose permission to publish is gratefully acknowledged.

X-Ray Emission Spectrographic Analysis of High-Purity Rare Earth Oxides

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▶ Minor amounts of rare earth elements (0.01 to 1.0%) in high-purity rare earth oxides may be determined by a fluorescent x-ray procedure. Basic to the method is accurate determination of spectral line intensity above background. Sample and standard preparation, choice of analytical lines, and utilization of a helium path to increase x-ray intensities are discussed. Precision and accuracy were evaluated by analyzing samples of known composition. The average of five separate determinations showed an average error of about 10%.

INSTRUMENTAL analysis has become an indispensable tool in research and production of the rare earth elements. This development can be attributed mainly to a combination of two factors—the marked chemical similarity of the rare earth group of elements, which excludes classical analysis methods, and the speed and accuracy of instrumental analysis techniques. With continued improvements in methods of producing high-purity rare earth elements, keeping pace instrumentally is becoming exceedingly more difficult. Accordingly, more and more emphasis is being placed on improving instrumental limits of detection and accuracy.

The methods employed most frequently for determining impurities in rare earth elements involve use of the optical emission spectrograph (*3, 4*) and the spectrophotometer (*9*). Although numerous x-ray emission spectrographic methods (*6–8*) have been developed for analyzing rare earths, these techniques have not included detection of low-level impurities. This investigation was conducted to find the precision and accuracy obtainable by x-ray fluorescence in determining impurities in rare earth oxides in the 0.01 to 1.0% concentration range.

INSTRUMENTATION

A General Electric XRD-5 spectrograph, equipped with a tungsten target x-ray tube, was operated at 50-kv. peak and 40 ma. Lithium fluoride was used as the diffracting crystal, and collimation was provided by an 0.005-inch Soller slit. An argon-filled Geiger proportional counter was operated in the proportional region in conjunction with a decade-type scaler. The analytical lines were chosen from the rare earth *L* spectra.

Table I shows the tabulations of the analytical lines for each matrix.

Calibration was accomplished by counting each standard twice for 4000 counts (probable error equals 1%), averaging, and computing intensity in counts per second (c.p.s.). Usually, one calibration sufficed for a few months' constant instrumental operation, if no changes were made in the instrument. Periodically, standard samples were rerun to check the calibration.

Some of the rare earth lines used were of long wave length. To decrease absorption effects and increase intensity, a helium atmosphere was introduced by enclosing the optical path in a plastic bag and maintaining a positive pressure at low flow rates. Each element analyzed indicated an appreciable gain in net intensity over the air path, varying for lanthanum, praseodymium, neodymium, and samarium by a factor of 3.6, 2.6, 2.1, and 1.8, respectively. This would appear to increase sensitivity levels by a like factor. However, only a slight increase in peak-to-background ratios was obtained; accordingly, no significant increase in sensitivity or accuracy was noted. The net intensities above background and peak-background ratios for several elements in ceric oxide, with air and helium atmospheres, are summarized in Table I. Net intensities and peak-background ratios in air for elements in other rare earth matrices are also included.

PREPARATION OF STANDARDS

Standards were prepared by precipitation to simulate the type of samples submitted for analysis. Each set of standards was precipitated from acid solution as the oxalate and converted to the oxide by ignition for 1 hour at 1000° C. Some rare earth oxalates were slightly soluble and required evaporation and reprecipitation. Concentrations of the impurity elements were varied in convenient steps from 0.005 to 1.0%.

Rare earth oxides to be analyzed were ground under alcohol in a mechanical mullite mortar for 20 minutes to achieve uniformly fine particle size. Because of the abrasive quality of the oxides, dilution with an abrasive as an aid in grinding was not necessary. After evaporation of the alcohol, each sample was ignited at 1000° C. for a short time and kept in a tightly capped bottle in a desiccator until packed into

Table I. Analytical Angles for Each Matrix, Net Intensity, and Peak-Background Ratios[a] at 1% Concentration Level

	Element					
	La	Ce	Pr	Nd	Sm	Y
	Analytical Angle					
	$L\alpha_1$, 82.85	$L\alpha_1$, 78.96	$L\beta_1$, 68.23	$L\beta_1$, 65.06	$L\beta_1$, 59.48	$K\alpha_1$, 23.75
	CeO_2 Matrix					
Air						
Net intensity, c.p.s.	6.74	...	15.43	20.80	20.29	...
Ratio	3.29	...	1.46	1.22	3.20	...
Helium						
Net intensity, c.p.s.	24.00	...	39.90	42.96	37.34	...
Ratio	5.10	...	1.80	1.23	3.81	...
	Pr_6O_{11} Matrix					
Net intensity, c.p.s.	4.55	6.94	...	20.33	20.55	55.85
Ratio	3.03	1.81	...	4.52	3.14	5.82
						Eu
	Nd_2O_3 Matrix					$L\beta_{1-2}$, 144.85
Net intensity, c.p.s.	5.03	8.00	14.00	...	30.10	4.65
Ratio	0.59	2.32	2.33	...	0.96	1.21
	Element					
	Ce	Pr	Nd	Eu	Gd	Y
	Analytical Angle					
	$L\alpha_1$, 78.96	$L\beta_1$, 68.23	$L\alpha_1$, 72.08	$L\beta_{1-2}$, 144.85	$L\alpha_1$, 61.05	$K\alpha$, 23.75
	Sm_2O_3 Matrix					
Net intensity, c.p.s.	6.86	14.46	18.51	6.45	63.96	32.24
Ratio	2.25	1.50	6.11	1.29	3.85	5.89
	Element					
	Eu	Gd	Tb	Dy	Er	Yb
	Analytical Angle					
	$L\alpha_1$, 63.51	$L\alpha_1$, 61.05	$L\alpha_1$, 58.76	$L\beta_1$, 50.24	$L\beta_1$, 46.41	$L\alpha_{1-2}$, 112.25
	Y_2O_3 Matrix					
Net intensity, c.p.s.	55.49	73.17	62.74	48.27	81.77	12.35
Ratio	6.80	8.88	7.20	2.19	3.33	1.08

[a] Peak/bg. = $\frac{\text{gross c.p.s.—bg. c.p.s.}}{\text{bg. c.p.s.}}$

a sample holder and analyzed. This was done to minimize hydration and carbonation of the oxide material.

BACKGROUND DETERMINATION

In any trace-analysis technique, determination of spectral line intensity above background is very important (*1*, *2*). The usual method of determining background consists of counting in an interference-free area near, and on both sides of, the analytical line. An average value is used as the background intensity. However, this approximation method was insufficiently accurate for determining minor amounts of rare earths in a rare earth oxide.

The absolute background intensity of each analytical line in a particular matrix was evaluated by using an extremely high-purity sample of the type to be analyzed as a blank. High-purity samples were obtained from commercial sources or prepared by ion exchange. Purity was checked by spectrographic and spectrophotometric methods. However, it was sometimes impossible to obtain a standard pure enough for use as a blank with respect to all of the impurity elements. The procedure followed was to determine background intensity for the desired analytical line in several of the highest purity samples available. The lowest intensity obtained was used as the background value for that analytical line in that particular matrix.

Interelement effects due to the presence of impurities in the 0.01 to 1.0% concentration range were negligible. The worst interference possibility, for example, was in the determination of cerium in samarium oxide. Enhancement of the Ce$L\alpha$ line by the Pr$L\gamma$, Nd$L\gamma$, Sm$L\beta$, Sm$L\gamma$, Eu$L\alpha$, Eu$L\beta$, Eu$L\gamma$, Gd$L\alpha$, Gd$L\beta$, Gd$L\gamma$, and Y$K\alpha$-2 and $K\beta$-2 lines was possible. However, a samarium oxide containing 1% of each of these elements caused an increase of only 0.5 c.p.s. in cerium intensity, representing about 0.02% cerium. Obviously, in high-purity samarium these impurities would be present at much lower levels and would consequently make an insignificant contribution to the cerium intensity.

Because the availability of high-purity oxide was limited, standard series were prepared from rare earth oxides of 99.9+% purity. After x-ray intensities were obtained, the background intensity of each analytical line for that particular lot was determined, and was then subtracted from each standard. The net intensity thus obtained for each standard shown in Table II was due only to the added amount of rare earth impurity. Per cent oxide was plotted against net intensity for each impurity to give a standard curve through zero. Although this curve was completely valid, the background intensity due only to the major element was not known. To analyze unknown samples, the background, as determined from the high-purity sample, was subtracted from the scaled intensity of each unknown. In Table II the background intensity determined from a high-purity sample, Ce-JM, is equal to 11.14 c.p.s. By subtracting this value from the background intensity of the standard material, 11.79–11.14 = 0.65, the residual per cent La_2O_3 = 0.01 may be read directly from the standard working curve. Although the data were accurate to only 1%, one more figure than significant was carried through the calculations. Determined percentages were rounded off to the number of figures significant.

RARE-EARTH ANALYSIS

In developing a routine analytical method, the degree of accuracy desired and the counting time necessary to attain it must often be compromised to make the method practical. It was possible with the instrumental arrangement used to operate at 1% probable error in determining all intensities. The counting time necessary for accumulating the required number of counts (4000) varied from 1 to 10 minutes, averaging about 3. The error inherent in each analysis depended on the background and net intensity of that particular case.

Statistical methods were invaluable in predicting errors at a given concentration level for a particular count situation. The inherent error was conveniently estimated by extrapolating data more easily determined at a higher concentration. For example, the net intensity and background at the 1.0% level for La$L\alpha_1$ line in cerium oxide were, respectively, 6.74 and 2.05 c.p.s. (see Table I). An estimate of the error at 0.01% was calculated thus:

The net count at 0.01% would be 6.74/100 = 0.07 count per second, or a total count of 2.12. All data are good to 1.0% or 2.12 ± 0.02 and 2.05 ± 0.02. A statistical consideration (*5*) of adding and subtracting intensities leads to:

$$\text{Probable error} = 2/3(0.02^2 + 0.02^2)^{1/2} = 0.019 \text{ c.p.s.}$$

Table II. Background Determination by High-Purity Sample, Lanthanum Oxide in Cerium Oxide

Standard No.	La_2O_3 Added, %	Intensity[a] $La L\alpha_1$, c.p.s.	Less Matrix Background, 11.79 c.p.s.
Ce-1	1.0	70.67	58.88
Ce-2	0.5	41.02	29.23
Ce-3	0.2	23.05	11.26
Ce-4	0.1	17.05	5.26
Ce-5	0.05	14.11	2.32
Ce-6	0.01	12.02	0.23
Ce-7	0.005	12.00	0.21
Ce-8 (blank)	0.0	11.79	...
Ce-JM[b]	...	11.14	...

[a] Values taken in helium atmosphere with 0.010-inch Soller slit.
[b] Johnson-Matthey Catalog No. 304, 99.99% pure CeO_2, relative to rare earths present; analysis by supplier indicated no lanthanum.

Finally, considering only the error involved in counting,

$$2.12 \pm 0.02 - 2.05 \pm 0.02 = 0.07 \pm 0.019 = \pm 27\%$$

This analysis is applicable to all data in Table I and may be used to estimate the inherent error in a given analytical situation. Table III shows the predicted probable error at 0.01% concentration for elements in ceric oxide in air and helium.

Synthetic samples of known composition were prepared and analyzed to determine the actual precision and accuracy of the method. The precision was determined by analyzing five individual samples from each synthetic mixture. The accuracy of individual and replicate analyses was calculated, using statistical methods (*5*), from the five individual analyses of each known sample.

The significance of statistical evaluations based on only five determinations might be questioned: however, accumulation of many similar sets of data tends to validate the precision and accuracy thus determined. The composite gives a rather accurate representation of the precision and accuracy attainable by this x-ray technique for various concentration levels of impurities. These data for a samarium oxide matrix are presented in Table IV.

In all instances the impurity elements are those expected when the element was purified by ion exchange. The known percentage was obtained by adding various amounts to a high-purity material and determining the residual per cent by the method described. Each per cent found is the average of five individual determinations. The accuracy and the precision were evaluated at ±2 sigma, or the 95% confidence level; errors of individual analyses lie within the limits listed 95% of the time. Where precision and accuracy are about the same, the maximum accuracy has apparently been attained. However, in some instances precision is good, but there is a fairly large error.

An analysis summary in Table V shows the average error of analysis for several rare earth elements at different concentration levels. For samples analyzed in quintuplicate, the average error varies from 0 to 30% of the amount present, depending on the element and concentration. The overall average error is about 10%.

The fluorescent x-ray analysis technique is applicable to the determination of rare earth impurities in any single rare earth oxide. Individual sensitivities of about 0.01% oxide were attained, but may vary depending on the element and matrix. Although fluorescent x-ray analysis of high-purity rare earth oxides is rapid and reasonably accurate, the best analytical situation is realized when a combination of methods (emission spectrography, x-ray spectroscopy, and spectrophotometry) is used conjointly to complement each other.

Table III. Predicted Probable Error at 0.01% Concentration Level in Ceric Oxide Matrix

	Element							
	La	Pr	Nd	Sm	La	Pr	Nd	Sm
	In Air				In Helium			
Gross intensity, c.p.s.	2.12	10.73	17.27	6.55	4.95	22.61	35.39	10.17
Background intensity, c.p.s.	2.05	10.58	17.06	6.35	4.71	22.21	34.96	9.80
Net intensity, c.p.s.	0.07	0.15	0.21	0.20	0.24	0.40	0.43	0.37
Probable error, %	±27.0	±69.0	±76.0	±30.0	±20.0	±53.0	±76.0	±25.0

Table IV. Precision and Accuracy in Samarium Oxide Matrix[a]

	Element					
	Ce	Pr	Nd	Eu	Gd	Y
Known	0.418	0.617	0.500	0.822	0.307	0.204
Found[b]	0.404	0.560	0.491	0.738	0.302	0.204
Error	3.3	9.2	1.8	10.2	1.6	0
Accuracy[c]	±13.0	±21.0	±8.8	±25.0	±12.0	±2.0
Precision[c]	±11.0	±7.4	±7.8	±7.4	±11.0	±2.0
Known	0.098	0.137	0.100	0.182	0.067	0.044
Found[b]	0.097	0.138	0.093	0.179	0.067	0.047
Error	1.0	0.7	7.0	1.6	0	6.8
Accuracy[c]	±9.8	±26.0	±17.0	±21.0	±14.0	±26.0
Precision[c]	±9.0	±25.0	±7.8	±21.0	±14.0	±21.0
Known	0.034	0.041	0.020	0.054	0.019	0.012
Found[b]	0.037	0.046	0.022	0.058	0.025	0.012
Error	8.1	12.0	10.0	7.4	31.6	0
Accuracy[c]	±127.0	±113.0	±56.0	±56.0	±127.0	±140.0
Precision[c]	±114.0	±95.0	±46.0	±50.0	±69.0	±140.0

[a] All figures as % oxide.
[b] An average of 5 determinations.
[c] 95% confidence level; precision and accuracy of individual analyses lie within limits listed 95% of time.

Table V. Accuracy of Analysis Summary[a]

Concn. Range	Element, Average %[b] Error							
	La	Ce	Pr	Nd	Sm	Eu	Gd	Y
0.3–0.8	15	2	4	4	1	6	2	0
0.06–0.2	21	9	7	4	5	6	0	7
0.01–0.05	29	5	14	19	15	15	32	0

[a] All figures as % oxide.
[b] Based on comparison of average of 5 determinations to known value.

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RECEIVED for review September 8, 1958. Accepted January 15, 1959.

Radiometric Analysis of Leached Uranium-Thorium Ore Samples with the Beta-Gamma-Gamma Method

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▶ **A radiometric method for the quantitative analysis of naturally leached uranium-thorium ore samples is presented. Ordinary β-γ method calculations are done after the thorium oxide, ThO_2, concentraton has been determined, utilizing the 2.6-m.e.v. γ-photopeak from ThC″ (Tl^{208}). Radiometric results of 24 samples are tabulated and agree well with chemical values for the uranium oxide, U_3O_8, and ThO_2 contents. When 18-gram samples are used, drift of the photopeak of the 2.6-m.e.v. γ has been less than 0.5% per week. Variations of 5° C. in a room temperature of 21° C. had no effect on the stability of the apparatus.**

ALTHOUGH the β-γ equilibrium method (*4*, *7*) as used in practice (*1–3*) has given excellent results for leached uranium ores and unleached uranium-thorium ores, the results for naturally leached uranium-thorium ores have not been satisfactory. Reports have appeared on the rather artificial extension of the β-γ method to include such cases and, without doubt, produced excellent results. However, similar conditions could not be reproduced in this laboratory (*1*).

This paper presents an extension of the equilibrium method that can be reproduced in any laboratory where reliable drift-free electronic equipment is available.

EXTENSION OF THEORY INVOLVED IN EQUILIBRIUM METHOD TO β-γ'-γ''-METHOD

Consider the case where the uranium series has been naturally leached or redeposited in the presence of thorium minerals (*1*). No current evidence exists that natural leaching of thorium-minerals takes place. By grouping the uranium series into a uranium and a radium group, the following expression can be obtained for the beta counting rate:

$$C_\beta = U\,(\beta_u + p\beta_r) + T\beta_t \qquad (1)$$

Similarly, for the gamma counting rate, C'_γ, obtained at the lowest possible discrimination level—100 k.e.v.– integral counted—there is

$$C'_\gamma = U(\gamma'_u + p\gamma_r') + T\gamma_t' \qquad (2)$$

A third equation might be obtained by screening some of the β-rays. It will involve weight corrections, because a large amount of γ-rays will be slowed down and counted by the Geiger tube.

A more straightforward possibility is to utilize the 2.6-m.e.v. γ-photopeak from ThC″ in the thorium series. If the window of a differential discriminator is set over this photopeak, a small amount of the low intensity 2.42-m.e.v. γ-photopeak from RaC(Bi^{214}) will also be registered. The following expression for this gamma counting rate will be obtained:

$$C''_\gamma = Up\gamma''_r + T\gamma''_t \qquad (3)$$

The solving of Equations 1, 2, and 3 for T gives the expression:

$$T = \frac{C_\beta + C''_r\left(\frac{\gamma'_r}{\gamma''_r}\cdot\frac{\beta_u}{\gamma'_u} - \frac{\beta_r}{\gamma''_r}\right) - \frac{C'_\gamma\cdot\beta_u}{\gamma'_u}}{\left(\beta'_t - \gamma''_t\frac{\beta_r}{\gamma''_r}\right) - \frac{\beta_u}{\gamma'_u}\left(\gamma'_t - \gamma''_t\frac{\gamma'_r}{\gamma''_r}\right)} \qquad (4)$$

To obtain the U value, a similar formula can be deduced or the % ThO_2 value obtained can be utilized to calculate the number of betas and gammas resulting from elements in the thorium-232 series. The amount of betas and gammas due to elements in the uranium-238 series can then be obtained by subtraction. The nominal formulas are used, for the determination of the % U_3O_8 and the p-value (*1*):

$$U = U'_\beta(1 + A) - U'_\gamma A \qquad (5)$$

and

$$p = \frac{U'_\gamma A/_a - U'_\beta A + 1/x}{U} \qquad (6)$$

This procedure proved the most easily applicable in an assay laboratory.

APPARATUS

For the routine application of gamma-discrimination it is necessary to have well stabilized counting conditions. Of the locally available equipment, the following arrangement was selected.

Gamma-Counting Unit. High voltage supply. Modified Tracerlab RLI-7 (Tracerlab, Inc.) with a measured drift of 0.004% per 9 hours.

Linear amplifier. Tracerlab RLA-1 (improved) with the proper 6BN6 discriminator. A window widener was available but was not used because its response was frequency-dependent above 1000 counts per second.

Multiplier phototube, Radio Corporation of America 6655 (unselected) with a 2 × 1¾ inch ϕ sodium iodide Harshaw crystal (Harshaw Scientific).

Scaler. Locally designed, using Philips EIT tubes. With this arrangement, the drift of the 2.6-m.e.v. γ-photopeak of the ThC″ has been less than 0.5% per week and could be daily compensated for with a 11-step attenuator ranging from 1.00 to 1.125. A drift of ± 5° C. at a room temperature of 21° C. had no effect on the peak position.

Beta-Counting Unit. High voltage supply. Dynatron Type 1082 A.

Preamplifier. Dynatron 1014 A set at a quenching time of 300 μsec.

Geiger tube. Tracerlab TGC-2 with window thickness of 2.1 mg. per sq. cm.

Scaler. Dynatron Type 200.

The arrangement of the apparatus is similar to that described (*1*). The sample pan containing the mineral sample to be analyzed is placed between the window of the Geiger tube and the sodium iodide crystal, all mounted inside a lead castle.

TECHNIQUE

Using about 18-gram samples, the three counting rates C_β, C'_γ, and C''_γ were obtained.

Only background correction was made on the β-count after the dead time correction was carried out. (The γ-sensitivity of the Geiger tube is negligible.)

For the two γ-counts, background and sample-weight corrections were necessary and were made (*1*).

No daily corrections were made on the results of the γ-counter, but an enclosed 10-gram 42% U_3O_8 sample served as a reference source.

Standardization was done with a 0.512% U_3O_8 standard obtained from the Canadian Department of Mines and Technical Surveys. The U_3O_8 concentration in this sample as well as that in a 5.4% U_3O_8 (pure uranium separated from daughter products) sample was obtained on a mass spectrometer at the National Physical Research Laboratory. The pure uranium sample is more than 3 years old and therefore contains its immediate daughter elements.

A uranothorianite sample, T249/11, which gave the best checks with chemical values on the β-γ apparatus was used to obtain the constants for thorium. The chemical values are 0.453% ThO_2 and 0.142% U_3O_8.

The necessary sample preparations and the influence of nonuniformity have been described (*1*). The same sample treatment used before—methanol mixing of samples—gave good results.

EXPERIMENTAL RESULTS

The total counting times involved in obtaining the results tabulated in Table I amounted to 80, 20, and 60 minutes, for C_β, C'_γ, and C''_γ, respectively.

The first four samples are Dominion reef-outcrop samples and are probably not in equilibrium. From the p-values listed it seems that uranium redeposit (or radium group loss) took place—more in one case than in the other. More important, however, are the good checks obtained for the ThO_2 values with the listed chemical values. To a lesser extent it is also the case for the U_3O_8 values, although slightly high. The two sets of chemical values were obtained by two different analytical laboratories.

Applying the ordinary β-γ method (*1, 3*) calculations (where p is assumed to be 1) gave negative results for the ThO_2 contents. Applying the ordinary γ'-γ'' method (*8*) gave results for ThO_2 not much different from those obtained with the proposed β-γ'-γ'' method, but the U_3O_8 values were below the chemical values.

Samples 5 and 6 are composite samples and also showed a slight difference in the equilibrium condition of the uranium series.

Samples 7 to 15 and 16 to 24 were obtained by sampling successively across the Dominion reef in two different cases. The β-γ method gave confusing results, but with the β-γ'-γ'' method, there is only one serious anomaly in the U_3O_8 values (assuming that the chemical figures are all in order)—sample 11—and one anomaly in the ThO_2 values—sample 18.

No explanation can be given for these differences other than that it is perhaps failure of initial assumptions. Radiochemical analysis of disequilibrium ores by Rosholt seems to prove that the generalized grouping of uranium and radium, is not specific enough (*5, 6*).

The errors involved in these tabulated radiometric values are best illustrated with a specific example—for sample 7 the results are: $\%ThO_2 = 0.039 \pm 0.003\%$, $\%U_3O_8 = 0.085 \pm 0.002\%$, and $p = 0.81 \pm 0.05$. The error to sample value ratio will naturally become smaller for higher concentration material.

When only 5-minute counting is carried out on two repeat samples, the minimum concentrations of U_3O_8 and ThO_2 that can be determined with the β-γ'-γ'' method are 0.1% for both unknowns when an 18-gram material is used. However, the grades of some samples were rather low and the amounts were less than 35 grams, so that a 90-gram sample container which is normally used for low grade ore, could not be utilized.

Table I. Radiometric and Chemical Values for Leached Mixed Ore Samples

NPRL Sample No.	Original No.	Radiometric Values			Chemical Values, %	
		ThO_2, %	U_3O_8, %	p-value	ThO_2	U_3O_8
1	A418	0.15	0.16	0.53	0.15, 0.15	0.11, 0.10
2	A419	0.14	0.35	0.37	0.12, 0.13	0.22, 0.19
3	A426	0.25	0.70	0.61	0.27, 0.29	0.59, 0.53
4	A432	0.024	0.028	0.55	0.022, 0.025	0.022, 0.037
5	KCGI	0.093	0.32	0.66	0.008, 0.22	..., 0.23
6	KCGIV	0.23	0.62	0.77	0.20, 0.20	..., 0.53
7	D622A	0.039 ±.003	0.085 ±.002	0.81 ±.05	0.050	0.080
8	B	0.046	0.14	0.76	0.055	0.13
9	C	0.093	0.51	0.85	0.098	0.54
10	D	0.057	0.19	0.92	0.065	0.21
11	E	0.064	0.17	1.00	0.055	0.066
12	F	0.19	0.67	1.06	0.18	0.75
13	G	0.12	0.22	1.28	0.13	0.27
14	H	0.18	0.89	0.92	0.18	0.89
15	I	0.20	0.29	1.04	0.22	0.33
16	D540A	0.16	0.54	0.70	0.19	0.46
17	B	0.072	0.33	0.62	0.085	0.30
18	C	0.11	0.61	0.87	0.25	0.47
19	D	0.12	0.41	1.26	0.14	0.48
20	E	0.19	1.11	1.09	0.17	1.12
21	F	0.18	0.65	0.97	0.16	0.62
22	G	0.22	0.55	0.87	0.25	0.47
23	H	0.021	0.069	1.00	0.020	0.074
24	I	0.12	0.25	1.12	0.13	0.26

NOMENCLATURE

U = concentration of uranium in % U_3O_8

T = concentration of thorium in % ThO_2

p = equilibrium value of uranium series

$$= \frac{\text{uranium-radium ratio in leached or enriched ore}}{\text{uranium-radium ratio in equilibrium ore}}$$

β_u = counting rate of β-particles originating in the uranium group per 1% U_3O_8

γ'_u = counting rate of γ-rays originating in the uranium group per 1% U_3O_8 per 18 grams with integral discriminator on 100 k.e.v.

β_r, β_t = counting rate of β-particles originating in the radium group per 1% U_3O_8 (equivalent) and for 1% ThO_2, respectively

γ'_r, γ'_t = similar to γ'_u but for radium group and thorium series, respectively

γ''_r, γ''_t = counting rate of γ-rays originating in radium group and thorium series per 1% U_3O_8 (equivalent) and 1% ThO_2, respectively, per 18 grams, falling in the 2.6-m.e.v. γ-photopeak of ThC''

U'_β, U'_γ = apparent uranium concentrations derived from the thorium corrected β and γ measurements—

$$U'_\beta = \frac{(C_\beta - T.\beta_t)}{\beta_{u+r}}, \quad U'_\gamma = \frac{(C_\gamma - T.\gamma'_t)}{\gamma'_{u+r}}$$

$$A = \frac{a(1+x)}{x-a}, \quad a = \frac{\beta_r}{\beta_u}, \quad x = \frac{\gamma'_r}{\gamma'_u}$$

ACKNOWLEDGMENT

The assistance given by Douglas Simpson in obtaining some of the samples is acknowledged. The author is also indebted to Peter Laxen and Trevor Steele of the Government Metallurgical Laboratory for supplying the two series of reef section samples, as well as their chemical values. Franz Strelow of the National Chemical Research Laboratory is thanked for some duplicate chemical values.

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RECEIVED for review June 9, 1958. Accepted December 11, 1958. Work completed as part of a project undertaken by the National Physical Research Laboratory on behalf of the South African Atomic Energy Board, and published with the permission of these two organizations.

Study of the Chemistry of Scandium on a Tracer Scale with Scandium-46

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▶The coprecipitation of scandium, yttrium, and europium with ferric sulfate was studied with scandium-46, yttrium-91, and europium-152 and -154 as tracer ions. Scandium is carried by ferric sulfate through mixed crystal formation. Europium and yttrium are also carried and the data are in close agreement with the Freundlich adsorption isotherm. The study indicates that scandium is more akin to the iron family than to the rare earths.

BY SPECTRAL ANALYSIS scandium has been detected in almost all the rocks which form the earth's crust (*1*). It is therefore of interest to apply scandium-46 to the study of the chemistry of scandium. The study of its chemical properties reveals that scandium is more akin to the transitional elements than to the rare earths, though its existence in the rare earth minerals is also always observed (*2*). The study on a tracer scale may reveal interesting aspects of its analogy and give as much information as a thorough study without using large quantities of scandium. With this in view the chemistry of scandium was studied. This work, however, refers to the study of mixed crystal formation of ferric sulfate with tracer quantities of scandium; yttrium and europium have also been included.

EXPERIMENTAL

Scandium-46 (86 days) and yttrium-91 (57 days) of high specific activity were supplied by the Atomic Energy Research Establishment, Harwell, England. Europium tracer was prepared by the same concern by $(n\gamma)$ reaction on natural europium. Natural scandium used in the experiment was Specpure, purchased from Johnson & Mathey Co., Ltd. Ferric sulfate was of analytical reagent quality. It was dissolved in sulfuric acid and filtered through sintered glass and a 2*N* stock solution was made.

In a typical experiment an aliquot portion of the ferric sulfate stock solution was taken. Scandium-46, yttrium-91, or europium-152 and -154 was added; it was kept in a thermostat (sensitivity ±0.05° C.). Glacial acetic acid of analytical reagent quality in another container was kept in the same thermostat. When the solution attained the temperature of the thermostat, glacial acetic acid was added drop by drop with constant stirring (electrical stirrer) until a fraction of the iron was precipitated as sulfate; it was then stirred for a few minutes and filtered through sintered glass. The precipitate was washed with a mixture of water and glacial acetic acid having the same composition as the mother liquor. The solid was taken up with hydrochloric acid.

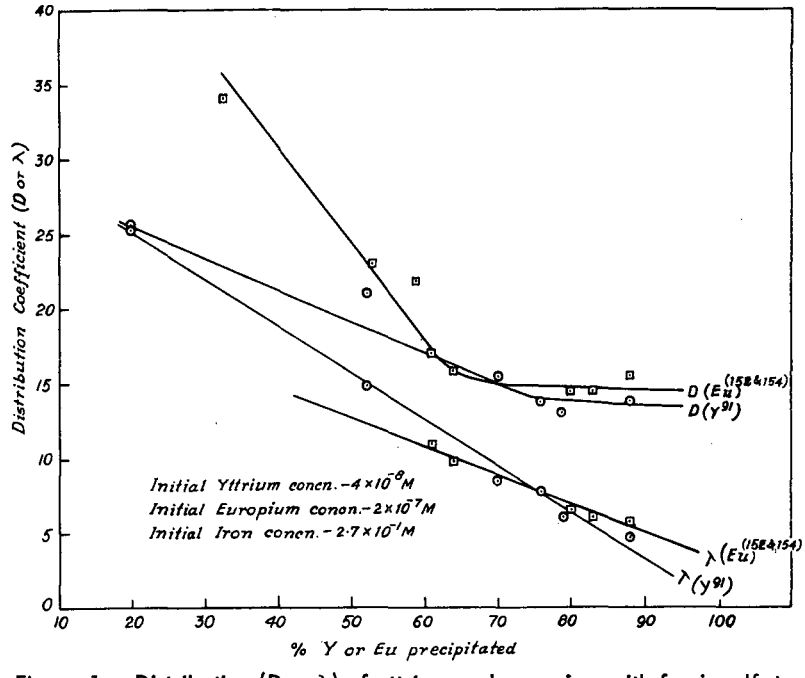


Figure 1. Distribution (D or λ) of yttrium and europium with ferric sulfate at 36° C.

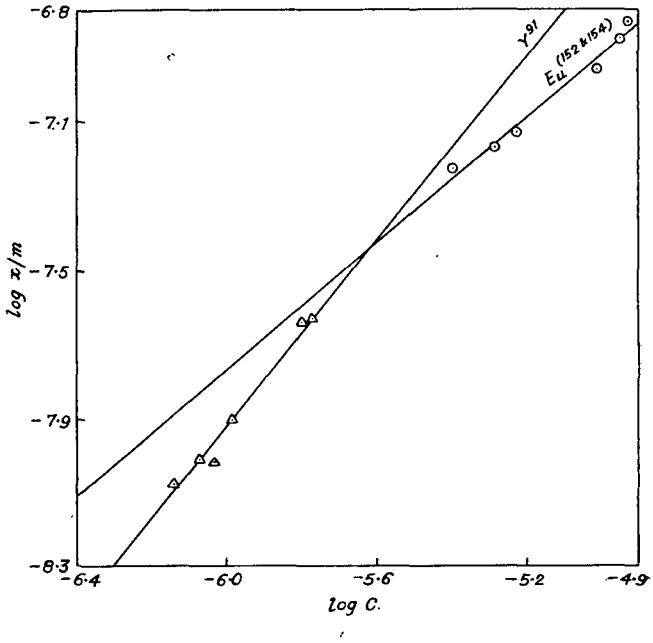


Figure 2. Freundlich adsorption isotherm on carrying of europium and yttrium by ferric sulfate

x. Amount of europium or yttrium carried, grams per liter
C. Concentration of europium or yttrium remaining in solution, grams per liter
m. Amount of iron in precipitate, grams per liter

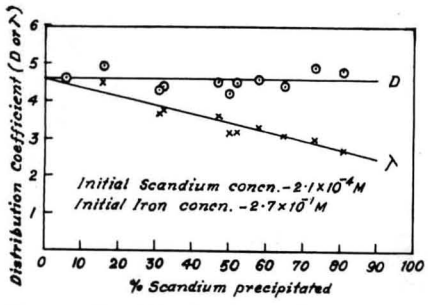


Figure 3. Distribution of scandium with ferric sulfate at 36° C.

and the iron was estimated volumetrically by dichromate and scandium-46 was estimated by a liquid counter. That the precipitate was ferric sulfate was ascertained by determining the iron sulfate ratio in the precipitate.

Homogeneous and heterogeneous distribution laws were calculated from the following expressions:

$$\left(\frac{\text{tracer}}{\text{carrier}}\right)_{\text{solid}} = D\left(\frac{\text{tracer}}{\text{carrier}}\right)_{\text{solution}} \quad (1)$$

$$\ln \frac{\text{initial tracer}}{\text{tracer in solution}} = \lambda \ln \frac{\text{initial carrier}}{\text{carrier in solution}} \quad (2)$$

In the calculations concentrations of tracer and carrier were taken as moles per liter.

The carrying of scandium by ferric sulfate has been studied in three different concentrations. The constancy of the Berthelot-Nernst distribution factor has been observed. With europium and yttrium under identical conditions, neither D nor λ is constant but the uptake is in close agreement with the Freundlich adsorption isotherm (Figures 1 and 2). The constant value of D observed in the case of iron is evidence that iron sulfate and scandium sulfate in the range of concentrations studied form mixed crystals. This study leads indirectly to the inference that scandium is more akin to the iron family than to the rare earths, although mixed crystal formation is not always a criterion for chemical resemblance. In the case of mixed crystal formation the conditions under which ferric sulfate was precipitated should give a constant value of λ; but probably because of the fineness of the precipitate, the diffusion rate through the crystal lattice was so rapid that a uniform distribution was found, as evidenced by the constancy of D (Figure 3 and Table I).

Table I. Coprecipitation of Scandium with Ferric Sulfate at 36° C.

(Iron 2.7 × $10^{-1}M$)

Precipitated, % Sc	Precipitated, % Fe	D	λ
Scandium 2.1 × $10^{-4}M$			
7.36	1.73	4.51	4.37
16.48	3.84	4.94	4.60
18.78	5.62	3.88	3.59
30.66	9.37	4.28	3.72
31.62	9.78	4.27	3.69
47.00	16.59	4.46	3.50
49.82	19.21	4.18	3.23
51.74	19.21	4.51	3.42
52.12	20.33	4.28	3.24
58.25	23.15	4.63	3.31
59.88	25.29	4.41	3.13
65.51	31.14	4.20	2.85
72.83	35.36	4.90	2.99
81.36	47.41	4.84	2.61
	Mean	4.45	
Scandium 2.1 × $10^{-5}M$			
15.87	4.12	4.39	4.12
28.31	8.47	4.27	3.76
41.49	13.87	4.40	3.55
43.61	15.59	4.19	3.38
Scandium 1 × $10^{-3}M$			
16.20	3.98	4.67	...
39.60	12.45	4.61	...
60.01	24.29	4.68	...

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RECEIVED for review May 14, 1958. Accepted December 17, 1958.

Properties of Ethyl Esters of Certain Amino Acids as Related to Artifacts in Paper Chromatography

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▶ Solutions of single amino acids in 70% ethanol or 10% 2-propanol at different pH values were chromatographed at various intervals after their preparation. In ethanol solutions acidified with hydrochloric acid to pH 1.5 to 3.0, newly formed ninhydrin-reactive substances were easily detected within 36 hours after solutions of L-glutamic acid, L-aspartic acid, β-alanine, and γ-aminobutyric acid were prepared; these new substances were identified as the monoethyl esters of the respective amino acids. Esters of these amino acids were not found in 2-propanol solutions or in ethanol solutions above pH 3.0. None of the other commonly occurring α-amino acids formed esters in the alcoholic solutions, even after 5 months at 24° C. The importance of these esters as artifacts in the chromatographic analysis of amino acid solutions and procedures to reduce or prevent their formation are discussed.

AMINO ACID analyses by paper partition chromatography have often revealed the presence in biological materials of unknown ninhydrin-reacting substances. Subsequent identification of these unknown compounds has, in several instances, increased our knowledge of their natural occurrence (*8*). Frequently, however, these unknowns are artifacts that may be formed during the preparation and storage of amino acid extracts or during the development of the chromatograms (*1*, *4*, *7*, *8*, *11*, *12*). Block and Bolling (*2*) warned that using ethanol as a solvent for amino acid residues in concentrated extracts might result in the formation of esters, and recommended using 10% 2-propanol instead.

In the authors experience, when amino acid residues in plant materials or protein hydrolyzates are dissolved in 70 to 80% ethanol, changes in the ninhydrin-reactive components of the preparations often follow within short periods. In preliminary studies on the efficiency of various alcohol solutions in extracting plant materials, the highest concentration of ninhydrin-reacting substances was obtained with cold 70% ethanol at pH 2.0 to 3.0. However, the formation of unknown substances was most apparent in the acidified extracts. It was further demonstrated that these changes were reduced to a minimum or prevented by adjusting the extracts to pH 5.0 after extraction. The present study was conducted to determine the nature of these

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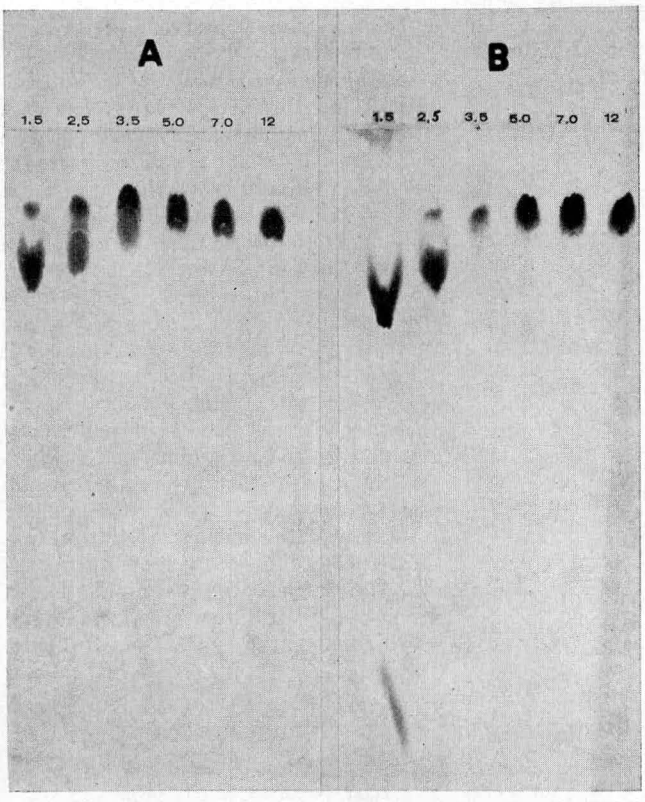


Figure 1. Chromatograms of L-aspartic acid in 70% ethanol solutions at different pH values

A. Solutions chromatographed immediately after preparation with phenol saturated with water

B. Same solutions chromatographed 3 weeks after preparation

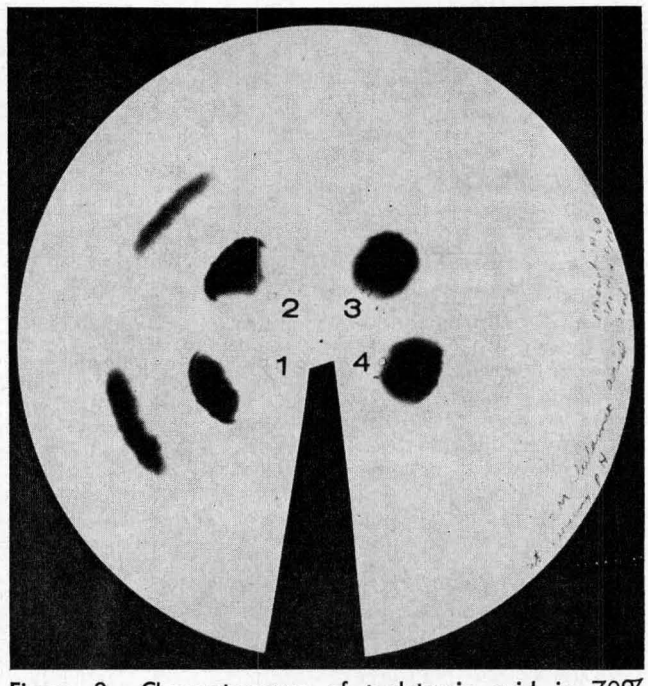


Figure 2. Chromatograms of L-glutamic acid in 70% ethanol solutions at different pH values

Solutions chromatographed 1 week after preparation with phenol saturated with water

1. pH 1.5
2. pH 2.5
3. pH 4.0
4. pH 6.0

changes, the identity of the resulting unknown compounds, and the conditions that favor these reactions.

EXPERIMENTAL PROCEDURE

Solutions of single amino acids (0.02 *M*) were prepared by dissolving the crystals in 70% (v./v.) ethanol or 10% (v./v.) 2-propanol. The solutions were adjusted to various pH levels with hydrochloric acid or sodium hydroxide and a Beckman potentiometer, Model G, and stored at 24° C. Paper chromatograms (Whatman No. 1) spotted by micropipet with 20 μl. of the amino acid solutions were developed with phenol saturated with water or with 1-butanol–acetic acid–water (4:1:5). Locations of the ninhydrin-reactive substances were visualized by dipping the chromatograms in an acetone solution containing 0.2% ninhydrin. After drying, the chromatograms were placed in an oven at 70° C. for 5 minutes.

RESULTS AND DISCUSSION

When the solutions of amino acids were spotted on chromatograms immediately after preparation, development with phenol caused the following amino acids to move as single spots: glycine, L-serine, L-α-alanine, L-arginine, L-lysine, L-proline, L-valine, L-leucine, L-tryptophan, L-tyrosine, L-phenylalanine, L-methionine, L-cystine, L-threonine, L-histidine, DL-pipecolic acid, γ-aminobutyric acid, and β-alanine. The R_F values of the spots for the different amino acids were similar to those reported by other workers (*3*, *9*). In contrast, when alcohol solutions of L-aspartic acid and L-glutamic acid were spotted on chromatograms immediately after preparation, two spots were evident for each of the two amino acids. The R_F values were 0.29 and 0.35 for glutamic acid, and 0.13 and 0.18 for aspartic acid.

Double spots on chromatograms developed with phenol have been reported for these amino acids (*4*, *5*, *12*). Weinhold, DeVay, and Zweig (*12*) indicated that for glutamic acid and aspartic acid, the slower moving substances were the partially dissociated anions, and the faster moving substances were the cations. The primary evidence was the relative concentration of these ions in alcohol solutions adjusted to different pH levels. When glutamic or aspartic acid is spotted on chromatograms, the concentration of the ions is apparently little influenced by the hydrogen-ion activity of the phenol solvent (pH 4.1) used to irrigate the papers (Figures 1, *A*, and 2); however, the R_F values of these substances are markedly influenced by the pH of both the amino acid solutions (Figure 1) and the chromatographic solvent (*12*). The importance to R_F values of the pH of amino acid solutions before chromatographic development has been emphasized by Landua, Fuerst, and Awapara (*8*) and McFarren (*10*).

Paper electrophoresis produced additional evidence of the ionic nature of the double spots for the dicarboxylic amino acids. Ethanol solutions at pH 1.5 to 3.0 containing relatively high concentrations of the cation, and solutions at pH 3.5 to 5.0 containing high concentrations of the anion, were spotted on strips of Whatman No. 1 paper, 3 inches wide, moistened with solutions of the electrolyte (0.05*M* potassium chloride adjusted to the same pH with hydrochloric acid as the alcohol solutions of the amino acids), and developed in a closed system for 3 hours at 300 volts.

The locations of the ions were visualized by dipping the strips in a solution of acetone and 0.2% ninhydrin. Glucose was used as an indicator of electroendosmosis and visualized with ammoniacal silver nitrate. The movement and concentration of the various forms of glutamic acid and aspartic acid, as compared in paper chromatography and electrophoresis, further indicated that the faster moving substances on paper partition chromatograms were cations and the slower moving substances were anions. From the size and color intensity of the different spots on chromatograms of these amino acids, it was also evident that solutions between pH 3.0 and 3.5 contained similar concentrations of both the anion and cation forms, that the concentrations of the cation increased markedly at lower pH values, with a corresponding decrease in the anion, and that the anion increased at higher pH values with a decrease in the cationic form. These relationships are shown by a series

of chromatograms of aspartic acid in Figure 1, *A*.

At various intervals from 36 hours to 5 months after the original solutions of amino acids were prepared and analyzed, they were chromatographed as previously described, with phenol as the irrigating solvent. None of the alcohol solutions of the α-amino acids, except those of glutamic acid and aspartic acid, gave multiple spots. However, ethanol solutions of β-alanine below pH 3 yielded two spots (R_F 0.57, 0.83), whereas β-alanine above pH 3 dissolved in ethanol or 2-propanol gave a single spot (R_F 0.57). Ethanol solutions of γ-aminobutyric acid at different pH levels gave two spots (R_F 0.0, 0.77), while solutions below pH 3 gave an additional spot (0.80). However, when γ-aminobutyric acid was dissolved in 2-propanol and adjusted to different pH values, only one spot (R_F 0.77) was found on the chromatograms. In the 2-propanol solutions at different pH values and the ethanol solutions above pH 3.0, glutamic acid and aspartic acid gave the two spots previously described as the ionic forms of these amino acids. In contrast, the ethanol solutions of the dicarboxylic amino acids below pH 3 gave four spots for glutamic acid and three spots for aspartic acid. Table I gives the colors and R_F values of the multiple spots for solutions at pH 1.5 of glutamic acid, aspartic acid, γ-aminobutyric, and β-alanine.

Earlier reports on the occurrence of ethyl esters in ethanol solutions of aspartic acid (*7*) and glutamic acid (*7*, *11*) indicated that the substances observed at R_F values 0.88, 0.80, 0.83, and 0.80 for glutamic acid, aspartic acid, β-alanine, and γ-aminobutyric acid, respectively, might also be the ethyl esters of these amino acids. In order to characterize them, these unknowns were isolated by chromatographing large amounts of the ethanol solutions (pH 1.5) of glutamic acid, aspartic acid, γ-aminobutyric acid, and β-alanine. The unknowns were then eluted from the chromatograms with 0.01*N* hydrochloric acid and crystallized as their hydrochlorides. The following evidence established the structure of these compounds as the monoethyl esters of the amino acids:

1. Hydrolysis of the unknowns with hydrochloric acid or sodium hydroxide yielded the parent amino acids.
2. Carbon, hydrogen, and ethoxy analyses of the unknowns indicated that the compounds were the monoethyl esters of the dicarboxylic acids and the ethyl esters of γ-aminobutyric acid and β-alanine.
3. A pure sample of the γ-ethyl ester of glutamic acid and the unknown gave identical R_F values on chromatograms developed with phenol-water or 1-butanol–acetic acid–water. Moreover, the infrared spectra for the pure sample and the unknown were identical. It was thus concluded that the unknown from solutions of glutamic acid was the γ-ethyl ester.
4. None of the α-amino acids studied, other than the dicarboxylic acids, or γ-aminobutyric, and β-alanine, formed ethyl esters under the conditions of this experiment. Because amino acids with the amino group in the beta and gamma positions formed the esters readily, it was apparent that an α-amino group hindered the esterification of the C-1 carboxyl group. This hindrance was easily overcome by increasing the hydrochloric acid concentration in the solutions. The R_F value and color of the monoethyl ester of aspartic acid were similar to those reported by Koch and Hanson (*7*) for the β-ethyl ester of aspartic acid. It was thus concluded that the ester of aspartic acid formed in 70% ethanol solutions (pH 1.5 to 3.0) was the β-ethyl ester.

The unknown ninhydrin-reactive substances (R_FO, Table I) that formed in the ethanol solutions of glutamic acid and γ-aminobutyric acid have not yet been identified. Heyns, Koch, and Königsdorf (*6*) found that solutions of glutamic acid acidified with hydrochloric acid and subsequently chromatographed with 1-butanol–acetic acid–water formed a ninhydrin-reactive substance that remained at the origin. They concluded that this spot resulted from interaction of glutamic acid with the irrigating solvent, giving rise to the γ-butyl ester of glutamic acid. Evidence in the present study did not support this view, for the unknown was not present when freshly prepared solutions were chromatographed with 1-butanol–acetic acid–water, though it was found in older solutions.

It is of interest to note in Figure 1,*B*, that the formation of the β-ethyl ester of aspartic acid occurred only in solutions at pH 1.5 and 2.5, with a corresponding decrease in the concentration of the slower moving (anionic) form of aspartic acid. A similar observation was made for solutions of glutamic acid (Figure 2). The weak ninhydrin reaction given by the chromatogram of the solution at pH 3.5 resulted from the crystallization of aspartic acid at this pH.

The observed occurrence of ethyl esters of amino acids as artifacts in ethanol extracts of the fungus *Fusarium moniliforme* will be reported elsewhere. However, the formation of esters in amino acid solutions may be minimized by adjusting ethanol extracts to a pH above 3.0 and redissolving the amino acids from concentrated residues with 10% 2-propanol. Further, the tendency of either glutamic acid or aspartic acid to split into two distinct spots may be reduced by adjusting the pH of the preparations to about pH 5.0.

Table I. R_F Values and Colors of Ninhydrin-Reactive Substances Formed in 70% Ethanol Solutions (pH 1.5) of Certain Amino Acids

(Irrigating solvent; phenol saturated with water, 24° C.)

Ninhydrin-Reactive Substance	R_F	Color Reaction with Ninhydrin
L-Glutamic acid		
1	0	Purple
2	0.29	Bluish purple
3	0.35	Purple
4	0.88	Purple
L-Aspartic acid		
1	0.13	Blue
2	0.18	Blue
3	0.80	Orange
γ-Aminobutyric acid		
1	0	Purple
2	0.77	Purple
3	0.80	Purple
β-Alanine		
1	0.57	Bluish purple
2	0.83	Orange

ACKNOWLEDGMENT

The authors are indebted to Felix Lukezic, Jack Paxton, and Gladys Cossens for assistance during this study; to the Perkin-Elmer Corp. and the Scientific Instruments Division, Beckman Instruments, Inc., for determining the infrared absorption spectra of certain compounds; and to General Mills Research Laboratories for a sample of the γ-ethyl ester of glutamic acid. Thanks are given the Microanalytical Laboratory, University of California, for certain carbon, hydrogen, and ethoxy analyses.

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RECEIVED for review November 28, 1958. Accepted January 28, 1959.

Separation through Differential Migration Analysis

A group of papers presented before the Division of Analytical Chemistry at the 134th Meeting of the American Chemical Society, Chicago, Ill., September 1958

Requirements for Analysis by Differential Migration

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▶ Differential migration is the basis of several widely applicable techniques for the resolution of mixtures of chemical substances. It also serves for the description and comparison of the substances themselves. For examination by differential migration, substances must be molecularly dispersed as in a gas, vapor, or solution. Permeable migration media, ranging from a vacuum to stabilized solutions, are required. For the most extensive separations, the mixture must be added to the migration medium as a narrow zone. To promote migration, a driving force is required, and the resultant migration is usually opposed by a resistive action. The driving force, the resistive action, or the resultant of these effects must produce selective or differential migration of the components. Two driving forces may be applied transversely, providing two-way, discontinuous separations from spots of the mixture or continuous separations from a narrow stream. Substances separated by differential migration may be located and estimated by methods that vary with the substances and the properties of the medium. Substances may be described, compared, and identified by their rates of migration or migration relative to other substances. Migration methods may be varied in so many ways that there is no systematic basis for classification of all variations.

THE Symposium on Differential Migration Methods of Analysis was designed to illustrate the importance of differential migration effects in analytical chemistry. Although these techniques are of great usefulness to analytical chemists, many of the methods were developed in other fields. For example, chromatography was devised and refined in botany and plant physiology. Two of its important modifications, paper chromatography and gas partition chromatography, were first demonstrated in organic chemistry and biochemistry. Electrochromatography (zone electrophoresis) was worked out in biochemistry and inorganic and clinical chemistry. Continuous electrochromatography was a product of biochemical and inorganic investigations. Liquid-liquid partition, partition chromatography, countercurrent distribution, and density-gradient centrifugation were developed in biochemical research and mass spectrometry was developed in physics, particularly nuclear physics. Many of these techniques have only recently been adopted in conventional analytical chemistry, and few have been included in formal courses of instruction in analytical chemistry.

Differential migration methods of analysis serve primarily for the resolution of mixtures of chemical substances. They permit comparison, description, and isolation of the constituents of multicomponent mixtures. They are applicable to the investigation of virtually all kinds of chemical substances, including unknown or undescribed compounds. They are useful in all branches of science concerned with chemical compounds and their reactions. Because of their usefulness as exploratory or research tools, they have found their greatest application in the hands of specialists in various areas of research concerning chemical substances and their reactions.

These differential migration methods are very simple in principle, but they are endlessly variable with respect to modification and application. Consequently there have been few attempts to systematize the basic principles and the requisite conditions (*1–8*). This paper describes the important features that are common to all differential migration methods of analysis. Special modifications and applications of the technique are illustrated by the other papers of the symposium.

REQUISITES FOR DIFFERENTIAL MIGRATION

Differential migration methods of analysis and some of their principal requirements are summarized in Table I. All these methods require the use of molecularly dispersed mixtures, in the form of a gas, a vapor, or a solution. Mixtures of solids or of liquids should be vaporizable or soluble without chemical change or alteration. As most substances may be dissolved, whereas fewer substances may be vaporized, migration methods that utilize solutions (solution chromatography, electrochromatography, batchwise countercurrent partition, sedimentation, and diffusion) are more widely applicable than those that utilize gases and vapors (gas chromatography, mass spectrometry, and thermal gravitational diffusion) (*2*).

All differential migration methods of analysis require the use of special, permeable, migration media. These media include a vacuum, as in mass spectrometry, gases, as in gas chromatography, and solutions, as in solution chromatography, electrochromatography countercurrent partition, differential sedimentation, and diffusion.

With many differential migration methods of analysis, the migration media must be stabilized to prevent mixing of the migrating substances. In electrochromatography and diffusion, for example, paper and gels serve as stabilization agents. In electrochromatography and differential sedimentation, density gradients also serve as stabilization agents. With many of these methods, the stabilization agent has little or no effect upon the migrating substances. With chromatographic methods, how-

ever, the powdered or fibrous sorptive media not only stabilize the flow of gas or solution but also effect the separations through their selective sorption of the migrating substances.

For the migration of the constituents of mixtures, a driving force is required. It may be nonselective, as flow of gas in gas chromatography and as flow of solution in solution chromatography (produced by gravity, suction, pressure, or centrifugal force). It may be selective, as direct current electrical potential in mass spectrometry and in electrochromatography, as centrifugal force in differential sedimentation, and as thermal diffusion in thermal gravitational diffusion.

The migration of the molecules usually encounters a resistive action in the migration medium. This resistive effect may be negligible in a vacuum, as in mass spectrometry. But under other conditions, it may be very great (nearly large enough to stop the migration), as in some modifications of chromatography. It is nonselective in some circumstances, as in certain modifications of electrochromatography, yet in other circumstances it is the basis of the selective migrations, as in chromatography and some modifications of electrochromatography.

The driving force, the resistive action, or the resultant of these two effects must produce differential migration of the components of mixtures. Driving forces may be utilized in various geometric arrangements to improve the separations. For example, two different driving forces may be applied transversely and in succession, as in mass spectrometry, or two resistive forces may be utilized transversely and in succession, as in two-way paper chromatography. Two different forces may also be applied transversely and simultaneously as in batchwise separations by chromatography plus electrochromatography and in continuous separations by continuous electrochromatography.

In all differential migration methods of analysis, the dimensions of the initial zone of the mixture determine, to a large degree, the extent of the separations. The over-all separation depends upon the relative rates of migration, the dimensions of the initial zone of the mixture, and the distance of migration.

If the initial zone of the mixture is very wide or continuous, the components form overlapping zones with only partial separations at the leading regions of the zones. Examples of these partial separations are found in capillary analysis, break-through or frontal analysis, the moving boundary method of electrical migration or electrophoresis, and most diffusion methods. But if the initial zone of the mixture is narrow, the components will yield separate zones each

Table I. Differential Migration Methods of Analysis and Some of Their Requirements

Methods	State of Mixture	Migration Medium	Driving Force	Resistive Action	Detection Techniques	Substances Separated	Operation	Zones
Chromatography								
Gas								
Adsorption (columns)	Gas, vapor	Porous sorbent	Gas flow (nonselective)	Adsorption (selective)	One to few	All kinds	Batchwise	Separate
Partition (columns)	Gas, vapor	Porous sorbent	Gas flow (nonselective)	Solution (selective)	One to few	All kinds	Batchwise	Separate
Solution								
Adsorption (columns, paper)	Solution	Porous sorbent	Solvent flow (nonselective)	Adsorption (selective)	Many	All kinds	Batchwise	Separate
Partition (columns, paper)	Solution	Porous sorbent	Solvent flow (nonselective)	Solution (selective)	Many	All kinds	Batchwise	Separate
Ion exchange (columns)	Solution	Porous sorbent	Solvent flow (nonselective)	Ion exchange (selective)	Many	Ions	Batchwise	Separate
Electrochromatography (zone electrophoresis)	Solution	Electrolytic solutions in gels or porous solids	D.C. potential (selective)	Viscosity ± sorption (selective)	Many	Ions	Batchwise	Separate
Continuous electrochromatography	Solution	Electrolytic solutions in porous solids	D.C. potential (selective) plus transverse flow of solution (nonselective)	Viscosity ± sorption (selective)	Many	Ions	Continuous	Separate
Multiple partition (Craig)	Solution	Solvents	Chemical potential + mechanical transfer	Partition (solubility) (selective)	Many	All kinds	Batchwise	Separate
Mass spectrometry								
Magnetic	Gas, vapor	Vacuum	D.C. potential + transverse magnetic field (selective)	None	One	All kinds	Continuous	Separate
Time-of-flight	Gas, vapor	Vacuum	D.C. potential (selective)	None	One	All kinds	Intermittent	One only
Thermal gravitational diffusion	Gas, vapor, solution	Gas, solution	Thermal gradient (selective) + gravity	Viscosity	Many	All kinds	Batchwise	Adjacent
Diffusion	Solution	Solution	Concentration gradient	Viscosity	Many	All kinds	Batchwise	Overlapping
Differential sedimentation								
Solution (only)	Solution	Solution	Centrifugal force (selective)	Viscosity, concentration gradient	One to few	All kinds	Batchwise	Overlapping
Density gradient	Solution	Solution	Centrifugal force (selective)	Viscosity, concentration gradient	One to few	All kinds	Batchwise	Separate

containing a single constituent of the mixture, as in chromatography, electrochromatography, etc. (see Table I). An exception is diffusion.

The definition of the zones of migrating substances varies with the migration systems. With some systems, as in partition and ion exchange chromatography, the zones are usually diffuse at both the leading and trailing boundaries. With other systems, as in adsorption chromatography, the zones are very diffuse at the trailing boundaries but very sharp at the leading boundaries.

The rate of migration of a substance serves as a basis for its description and its comparison with other substances. In many systems, the rate of migration varies with many different conditions, such as the intensity of the driving force and the resistive action, the concentration of the migrating substance, the temperature, and the permeability of the migration medium. All these conditions must be carefully standardized and specified, if the migration rate is to represent reproducible characteristics of the migrating substance. In solution chromatography, the rate of migration of the solute relative to the migration of the wash liquid, the R or R_F value, is widely employed for the description of all kinds of soluble substances. The rate of migration relative to an authentic reference substance and the migration sequences of various substances (which may vary with the migration conditions) (*3*) are widely employed for the description of unknown substances separated by chromatography.

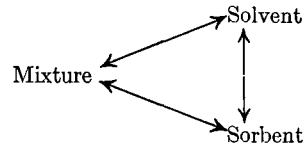
The quantity and the concentration of the mixtures that may be separated by differential migration methods are limited by the migration systems themselves. As a rule, the upper limits of quantity and concentration are small compared to chemical methods of separation. But the lower limits are usually much lower than those encountered in chemical separations. In fact, most of the differential migration methods permit separations of the smallest quantities that can be detected by the most sensitive tests. For this reason, differential migration methods are remarkably effective with substances that are found in minute quantities—for example, the synthetic transuranium elements, fission products, neutron activation products, vitamins, hormones, and enzymes.

Most differential migration methods must be supplemented with detection techniques for the location, detection, and estimation of the separated substances. With separatory methods based upon the use of solutions, wherein the substances are diluted with many molecules of the solvent, these detection techniques may depend upon various characteristics of the separated substances, such as their nuclear, physical, chemical, or biological properties (*2*). With gas chromatography, wherein the substances are diluted with relatively few molecules of the wash gas, several detection techniques, such as the gas density balance and the ionization of the gas fractions, serve as nigh universal procedures for the detection of all kinds of gaseous substances. With mass spectrometry, the detection of the charge carried by the separated substances is an extremely sensitive and universally applicable technique for the detection of the separated substances. For this reason, mass spectrometry itself is employed for the detection and estimation of volatile substances separated by other differential migration techniques.

VARIABILITY OF DIFFERENTIAL MIGRATION METHODS

Differential migration methods are variable in so many ways that it is impossible to consider all the ramifications in a brief report. Chromatography is the most variable, and in many respects, the most adaptable, because it may be used for the examination of both vapors and solutions of all kinds of chemical substances (*2*).

Some illustrations of the complexity of differential migration may be gained by a brief consideration of chromatographic methods. In all chromatographic techniques, there is a dynamic interaction among the solvent, the sorbent, and the components of the mixture—e.g.,

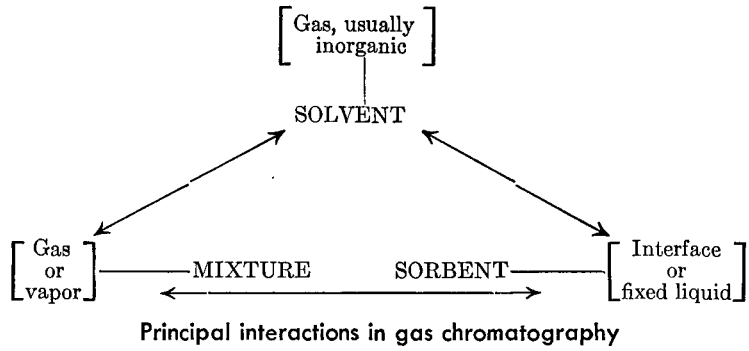


This dynamic reversible system is requisite for the migrations in all chromatographic separations. But for effective separations, an additional condition must be operative. The distribution of the components of the mixture between the solvent and the sorbent must be different. Except in homologous series, this preferential distribution, or selective sorption as it is commonly called, varies seemingly unpredictably with variation of the sorbed substances, solvent, and sorbent (*3*, *4*).

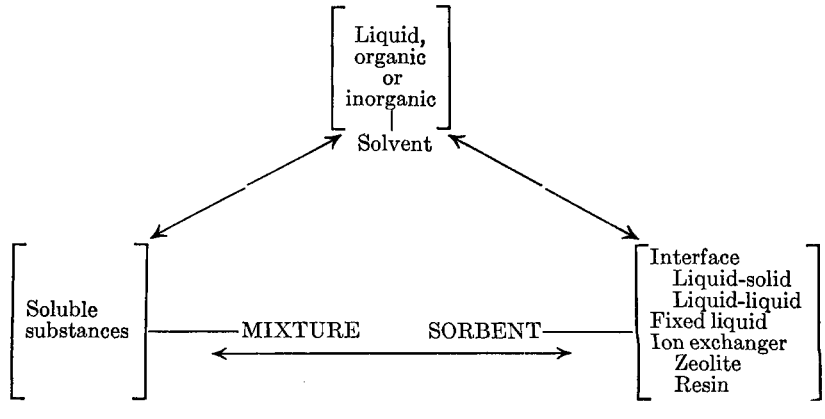
Because chromatographic separations may be made with mixtures of gases, vapors, and solutes, chromatographic methods fall into two principal categories: gas chromatography, for use with gases and vapors, and solution chromatography, for use with solutions. The principal interactions in gas chromatography are shown below.

The principal interactions in solution chromatography (*3*) are shown below.

These sorptive systems may be modified in many ways. Both the solvent and the sorbent may be varied with respect to their chemical and physical properties. Solvents ranging from the nonpolar hydrocarbons to the polar acids, bases, and aqueous solutions have been employed. Sorbents have ranged from the surface active powders (ad-



Principal interactions in gas chromatography



Principal interactions in solution chromatography

sorption chromatography) through the fixed liquids immiscible in the wash liquid (partition chromatography) to the ion exchange resins and zeolites (ion exchange chromatography). As yet there is no precise, comprehensive basis for the correlation of the numerous combinations of solutes, solvents, and sorbents that provide the dynamic and selective interactions essential to all chromatographic separations.

The geometry of the sorbent may also be varied. Examples of this kind of variation are the dimensions of the tubes and columns in gas and solution chromatography, and the variation of paper from strips to sheets in paper chromatography, two-way paper chromatography, and radial paper chromatography (*1*, *4–7*).

No simple relationship between the chemical composition or molecular structure of unrelated substances and their sorbability in the chromatographic columns has been established (*3*). For this reason, it is impossible to deduce composition or structure from chromatographic behavior alone. From this standpoint, chromatographic methods (and virtually all differential migration methods) cannot replace the conventional chemical methods for the determination of composition and structure. These differential migration methods do not even serve for the classification and identification of substances unless authentic preparations are available for comparison.

Differential migration methods are most effective for the resolution of mixtures, the partial description of various substances, and the identification of substances by comparison with authentic preparations. In all these respects, these methods augment and improve the armamentarium of the analyst.

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RECEIVED for review December 16, 1958. Accepted February 27, 1959. Work performed under the auspices of the U. S. Atomic Energy Commission.

Some Artifacts in Paper Chromatography

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▶ **Artifact spots have been observed on paper chromatograms of amino acids, organic acids–bases, and inorganic ions. Some of these artifacts have now been identified as the ethyl esters of amino acids and separate ionic species of dicarboxylic amino acids, by study of infrared spectra of isolated artifacts. Other artifacts discussed include "comet" and "masking" effects of the organic acids, geometric isomers of keto-acid derivatives, and multiple ionic species of inorganic anions. Caution must be exercised in interpreting "unknown" chromatographic spots. Corrective measures are recommended to minimize the occurrence of artifacts.**

THE technique of paper chromatography has become an almost indispensable tool to the researcher who is dealing with micro amounts of a multicomponent mixture. Were it not for paper chromatography, the separation of the amino acids from protein hydrolyzates would be a time-consuming task. The metabolic fate of biological intermediates has been frequently elucidated by the combination of paper chromatography and autoradiography. The observation of an "unknown spot" on a paper chromatogram has led to the eventual discovery of hitherto unknown compounds. These "unknown chromatographic spots" must be scrutinized very carefully, however, before deciding that they represent undiscovered compounds. Some spots may represent "multiple spots" or artifacts due to different ionic species of polar compounds or experimental manipulations.

ARTIFACTS OF AMINO ACIDS

Koch and Hanson (*13*) observed the formation of the monoethyl esters of aspartic and glutamic acids by storing these amino acids in ethanol–*N* hydrochloric acid (75:25 v./v.) for several days. Plaisted (*18*) has also reported that glutamic acid in the presence of 80% ethanol and a strong cationic ion exchange resin (Dowex 50 H^+) was converted to the γ-ethyl ester of glutamic acid. This compound gives a ninhydrin-positive reaction and occupies a position near the leucines on a two-dimensional chromatogram, when phenol and butanol–acetic acid–water are used as solvents. This ninhydrin spot may be mistaken for an unknown amino acid when plant extracts are chromatographed. Furthermore, for quantitative analysis of the amino acids by paper chromatography, the value for glutamic acid would be low if the γ-ethyl ester were ignored.

DeVay, Weinhold, and Zweig (*7*) have studied the reaction between ethanol and the commonly occurring amino acids in the presence of hydrochloric acid, and found that the following amino acids formed ethyl esters with ease: glutamic acid, aspartic acid, γ-aminobutyric acid, and β-alanine. Glutamic and aspartic acids chromatographed in phenol produced multiple spots due to two ionic species of the dicarboxylic amino acids. With butanol–acetic acid–water as solvent only one additional artifact was observed, because the ionization was repressed (see Table I). The R_f value for the fast moving artifact of "old" glutamic acid was the same as that for authentic γ-ethyl ester glutamic acid. (The authentic sample of the ester contained a trace of glutamic acid.) The artifact spot of the four amino acids under investigation was faster moving in the two solvent systems than the parent amino acids. In this respect the chromatographic behavior was the same as that of γ-ethyl ester glutamic acid.

The compounds, formed by the action of ethanol and hydrochloric acid with the amino acids, were isolated in milligram quantities. Thirty-six-hour-old solutions of each amino acid were streaked on Whatman 3MM paper (18 × 22 inches) and chromatographed with phenol-water or butanol–acetic acid–water as solvents by the descending technique. Guide strips were developed with ninhydrin, and the faster moving, major band was eluted with dilute hydrochloric acid. The concentrated elu-

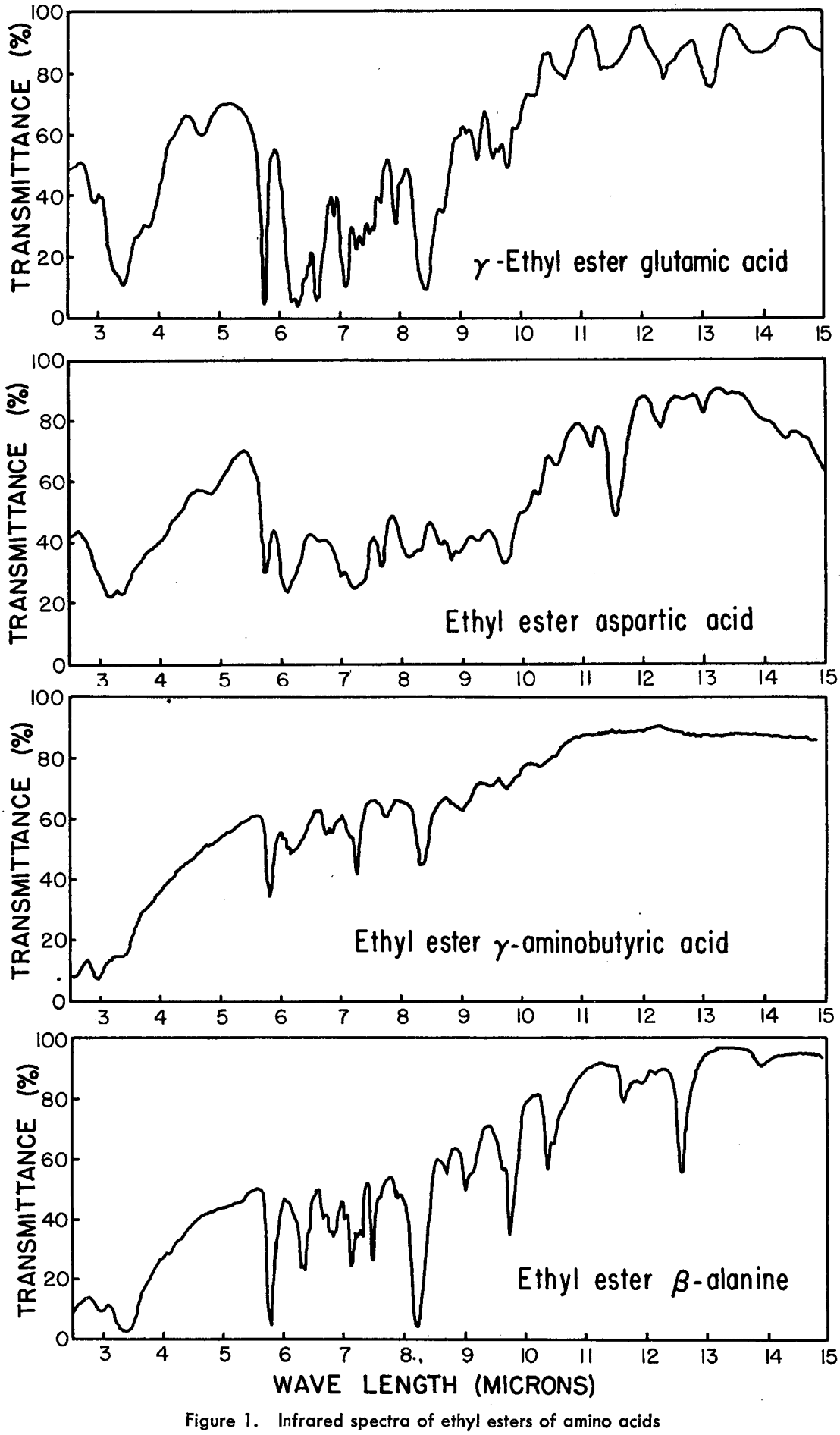


Figure 1. Infrared spectra of ethyl esters of amino acids

γ-Ethyl ester of glutamic acid, authentic sample; all others synthesized and purified as described in text

ates were examined by infrared spectroscopy, ethoxy-group determination, and elemental analysis.

Figure 1 shows the infrared spectra of the four amino acid derivatives isolated by the above procedure. These spectra were obtained from about 1 to 2 mg. of the concentrated eluate by the potassium bromide–pellet technique. The spectrum of an authentic sample of γ-ethyl ester of glutamic acid was identical with that of the unknown concentrate, derived from glutamic acid, giving further evidence about the identity of the derived compound. No authentic samples of the ethyl esters of the other amino acids were available, and their infrared spectra could thus not be compared. However, two strong bands in the regions of 5.7 and 8.2 microns indicated the presence of an ester linkage; these two bands were absent from the parent amino acids (*3*).

Ethoxy-group determination by the method of Pregl (*19*) was positive, but not quantitative. Carbon-hydrogen determinations, likewise, were not quantitative, indicating a mixture of amino acids and their ethyl esters. This latter point was demonstrated by paper chromatography of the purified esters; the concentrates were shown to be a mixture of the parent amino acids and their derivatives. It seemed difficult to prevent slight hydrolysis of the esters during chromatographic development.

Heyns has also observed artifact spots when chromatographing glutamic acid with butanol–acetic acid (*10*). Although he did not isolate these artifacts, he reasoned that one of them probably was identical with the γ-butyl ester of glutamic acid. Other artifacts, thought to be due to the interaction of glutamic acid and glycine, have been shown actually to be due to the interaction of glutamic acid and phenol (*2, 9*).

To minimize the formation of the ethyl esters of these four amino acids, the samples should not be stored over ethanol for any length of time. This is especially applicable to plant extracts having a high acid content. 2-Propanol is the recommended solvent for the amino acids from protein hydrolyzates, because, according to Block, this solvent is an effective preservative and yet does not cause esterification (*5*). A sample of glutamic acid may be kept indefinitely in a solution of 10% aqueous 2-propanol, acidified with 0.1*N* hydrochloric acid. Figure 2 shows a chromatogram of glutamic acid dissolved in acidified 2-propanol and ethanol. No 2-propanol ester is formed, whereas the ethyl ester may be clearly seen.

Figure 2 also illustrates another phenomenon of the dicarboxylic amino acids, the formation of "multiple spots" with an unbuffered solvent like phenol-water (spots 5 and 6). When Dent published his map of amino acids in 1947 (*6*), he correctly showed that aspartic and glutamic acids split into two spots each with phenol as solvent. How these multiple spots may lead to a false interpretation of an experiment is illustrated in Figure 3. This is an autoradiogram of an alcohol extract of an excised bean leaf, treated with the herbicide Simazine (Geigy Product) and exposed to $C^{14}O_2$ in the light. The effect of the herbicide seemed to lie in the formation of compounds A and B (see Figure 3). Cochromatography with aspartic acid showed that both spots were due to aspartic acid, and that no new compound has been formed (*20*).

DeVay, Weinhold, and Zweig (*7*) have studied the chromatography of aspartic and glutamic acids in phenol, saturated with buffers at pH 2.5 (below the isoelectric point) and pH 7.2 (above the isoelectric point). Figure 4 shows that each amino acid produced single spots at each pH, but that the R_f values were lower at the higher pH. This finding seemed to indicate that the anion of each amino acid gave a lower R_f value than the corresponding cation. At an intermediate pH, as may be expected from unbuffered phenol, both ionic species exist in equilibrium, thus producing two spots or a streak, (Figures 2 and 3).

The formation of multiple spots of the dicarboxylic amino acids may be prevented by the use of buffered phenol or buffered paper (*4, 14, 16*). Because commercially available phenol has a high acid content, it is usually distilled. A recent innovation is the availability of neutral "liquid phenol–without preservative" which bears on its label the inscription "suitability for amino acid paper chromatography—to pass test."

Similar difficulties, like streaking or multiple spots, have been observed with the basic amino acids. Aronoff (*1*) has attributed multiple spots of lysine, when phenol-water was used as solvent, to the formation of association complexes with the solvent.

Table I. R_f Values of Amino Acids, Dissolved in Ethanol–Hydrochloric Acid

[Solvent. 1-Butanol–acetic acid–water (4:1:5, v./v.)]

Amino Acids	Fresh Solution	36-Hour-Old Solution	Color Ninhydrin
Glutamic acid	0.35	0.35	Purple
		0.60	Purple
γ-Ethyl ester glutamic acid	0.35 (minor)	0.35 (minor)	Purple
	0.60	0.60	Purple
Aspartic acid	0.28	0.28	Blue
		0.50	Yellow-brown
γ-Aminobutyric acid	0.45	0.45	Purple
		0.73	Purple
β-Alanine	0.37	0.37	Blue
		0.68	Green-blue

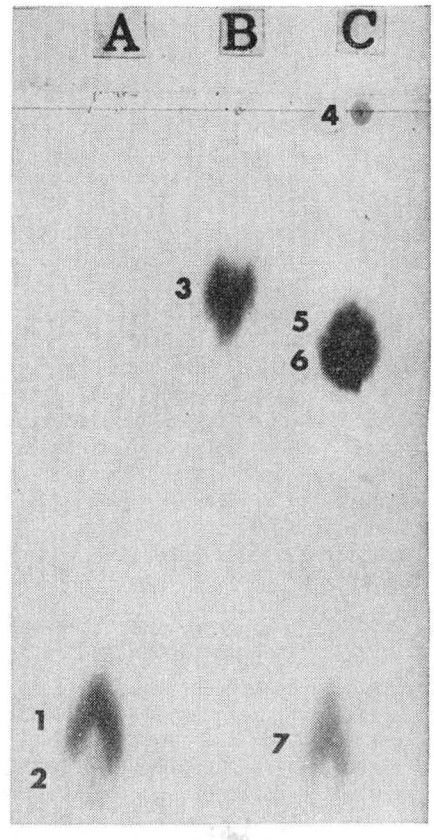


Figure 2. One-dimensional paper chromatograms

A. γ-Ethyl ester glutamic acid
B. Glutamic acid in 10% 2-propanol
C. Glutamic acid in 80% ethanol, trace HCl
Solvent, phenol-water

ARTIFACTS OF ORGANIC ACIDS AND BASES

Multiple spots or long trails, known as "comets," have been observed frequently during the chromatography of organic acids and bases. These artifacts are again due to the ionization of these compounds into more than one form. In the case of the acids, this may be overcome by the addition of a "swamp" acid to the solvent, like formic acid, in order to repress the ionization of the organic acid to be chromatographed (*15*). For weak bases, like alkaloids, a solvent should contain a mineral acid with a common anion (*17*). Thus, a single species, the salt, results in a single, well-defined spot.

Another phenomenon, that of "masking," has been observed when chromatographing organic acids. This artifact

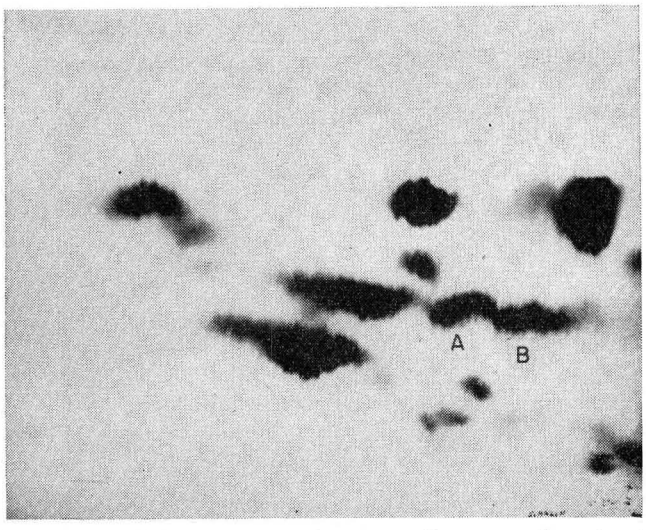


Figure 3. Two-dimensional autoradiogram of extract from $C^{14}O_2$-exposed bean leaf

1. First solvent, phenol-water (right to left)
2. Second solvent, butanol–propionic acid–water (down-up)

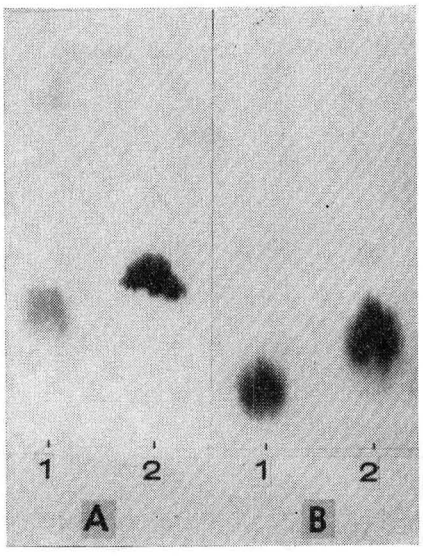


Figure 4. One-dimensional chromatograms

1. Aspartic acid
2. Glutamic acid

Solvents.

A. Phenol-buffered pH 2.5
B. Phenol-buffered pH 7.2

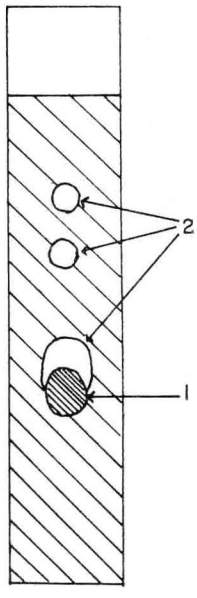


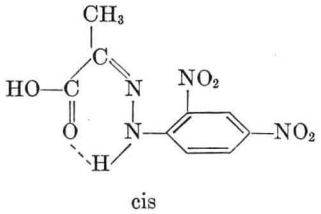
Figure 5. One-dimensional paper chromatogram of alcohol extract of excised bean leaf

1. Dark-blue spot due to Na^+ ions
2. Unidentified organic acids-yellow spots. Solvent, ether–formic acid–water, 5:2:1, v./v.

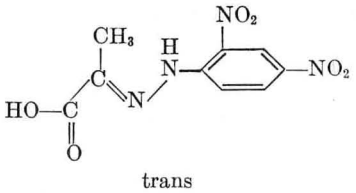
occurs when the developed chromatogram is sprayed with an acid-base indicator, like bromophenol-blue, and when sodium or similar ions are present in the original sample. The spot due to the interfering cation turns dark-blue and may thus mask an acid (yellow) (present as the anion) which may have the same R_f value. When the R_f values are similar, a crescent-shaped spot results, as illustrated in Figure 5. This artifact may be prevented by first purifying the sample by ion exchange or ether extraction.

ARTIFACTS OF 2,4-DINITROPHENYLHYDRAZONES

Multiple spots occur when the 2,4-dinitrophenylhydrazones of pyruvic and oxalacetic acids are chromatographed. This has been shown to be due to the differential migration of the cis and trans isomers of the phenylhydrazones (*12*). The two structures are:



cis



trans

ARTIFACTS DUE TO PURIFICATION

In the purification of sugars from plant extracts by passage through a strong alkaline ion exchange resin (Dowex 2 OH^-), lactic acid is formed. On spraying the chromatogram with silver nitrate, a false spot, due to lactic acid, may appear. The remedy is purification of the sugar solution by a basic ion exchange resin in the carbonate cycle (*11*).

ARTIFACTS OF INORGANIC IONS

Multiple spots in the paper chromatography of inorganic ions have been reported by Erdem (*8*). He found that cadmium (II) acetate resulted in three spots when chromatographed with a solvent containing ammonium hydroxide. Sprayed with quercetin, these three spots have been tentatively identified as cadmium-ammonia complexes. Erdem showed this by comparing their color with that of synthetic cadmium-ammonia mixtures on spot tests. He observed similar colors ranging from yellow, to brown, to purple.

Erdem (*8*) observed that disodium hydrogen phosphate and disodium hydrogen arsenate gave two spots with benzene-ethanol-water solvent, presumably due to the mono- and dibasic ions. When phosphorus and arsenic acids were used, only single spots resulted.

ACKNOWLEDGMENT

The author is indebted to Paul O. Amrhein, Perkin-Elmer Corp., for obtaining the infrared spectra.

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RECEIVED for review November 26, 1958. Accepted January 30, 1959.

Centrifugal Force in Paper Chromatography and Electrophoresis

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▶ A simple apparatus and techniques have been developed for centrifugally accelerated chromatographic separations, one- and two-dimensional, in circular, rotating paper sheets. The developed chromatograms resemble the classical radial patterns but development time is markedly reduced. For 16 amino acids, R_f values, using two developing solvents, were obtained by the technique described and the conventional descending technique. Utilizing centrifugal force in combination with a high voltage direct current electrical field has made possible electrochromatographic separations, in horizontal rectangular paper sheets. Electrophoresis may be discontinuous or continuous flow. Centrifugal and electrical forces may be applied independently, in sequence, or simultaneously.

THE application of centrifugal force to paper chromatography and ionography provides a new dimension in these widely used techniques. A simple and effective apparatus is described for carrying out centrifugally accelerated separations in paper disks rotating in a horizontal plane. Use of centrifugal force in combination with a high voltage direct current electrical field has made possible relatively rapid electrochromatographic separations or planar electrophoresis, somewhat analogous to the type formerly achieved by the time-consuming technique of hanging curtain electrophoresis.

CENTRIFUGALLY ACCELERATED PAPER CHROMATOGRAPHY

Some details of the technique, and earlier modifications of the apparatus, for carrying out centrifugally accelerated chromatographic separations in rotating circular paper sheets, have been described (*2, 3, 6*). A technique for conducting two-dimensional separations on a rotating paper disk has been published (*5*). The developed chromatograms resemble those resulting from the classical stationary circular paper method, but the development time for an acceptable pattern 35 cm. in diameter is reduced from several hours to 5 to 20 minutes.

The apparatus consists essentially of a carefully balanced motor-driven disk-shaped head, rotating in a horizontal plane, and completely enclosing a circular sheet of filter paper held in position at the center. While rotating, the paper sheet assumes a taut horizontal position. The solution to be fractionated is applied, by micropipet, to the paper surface in the form of dots, or whole or partial rings. While the paper disk, 18 inches in diameter, is rotated at from 300 to 1000 r.p.m., the developing solution is added at a point away from its center as a fine, continuous stream.

A schematic diagram of the current modification of the apparatus is shown in Figure 1.

A constant-speed, 1/4-hp. motor is coupled with a variable-speed drive, completely mechanical in operation. This arrangement was designed to permit reproducible, constant speeds of the rotor from 250 to 1000 r.p.m. The unit is equipped with a brake to stop the rotor rapidly at the end of a run, a desirable feature if diffusion and consequent enlargement of the colored zones occupied by individual components of a mixture are to be avoided. A tachometer and automatic timer are also provided. The rotor, made of Monel alloy, has an outside diameter of 50 cm., with a spacing of 2.5 cm. between the lid and base.

The device for feeding the developing liquid to the paper during a run consists of a screw-top glass pressure vessel containing the solution, which is fed through the capillary tip under 8 to 11 pounds nitrogen pressure. Rapid interchange of the capillary tips is provided for through use of a ball and socket joint between the tip and the main glass tubing. For proper development of the chromatogram, the liquid must be added as a fine continuous stream, at about 0.8 to 1.5 ml. per minute, calibrated as water at 25° C., depending on the particular paper, rotor speed, etc.

The rate of flow of liquid through the capillary tip is usually calibrated with water at 25° C. This makes it unnecessary to restandardize the capillary for each solvent used. To initiate a sufficiently fine stream, rather than a series of drops, is sometimes difficult if a drop once forms on the tip of the capillary. This problem can be solved by touching the drop on the tip of the capillary with a clean dry paper tissue, while the liquid in the capillary is under gas pressure. The drop will be absorbed in the paper and a continuous stream of liquid will begin to flow. It was found advisable to filter all developing solvents through Whatman No. 1 paper to prevent clogging of the capillary tips during a run.

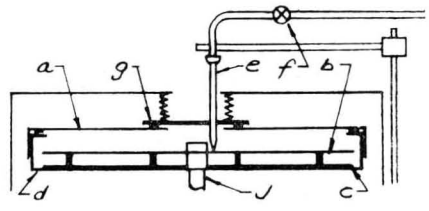


Figure 1. Cross section of main features of apparatus for centrifugally accelerated chromatographic separations

- *a.* Lid of rotor
- *b.* Circular sheet of paper, 18 inches in diameter, on which chromatographic separations take place—e.g., Whatman 3MM: it rests lightly on numerous support points
- *c.* Liner, of heavy-stock paper—e.g., Eaton-Dikeman No. 320, in close contact with bottom of rotor, *d*, so support points protrude through
- *e.* Removable glass capillary delivery tube
- *f.* Stopcock to regulate liquid flow to rotating paper disk
- *g.* Spring-loaded closure device, for opening in lid of rotor, equipped with Teflon bearing ring
- *j.* Drive shaft of rotor

To have the rotating paper sheet completely enclosed and yet at the same time add liquid from the capillary at a point away from the center, a specially designed closure device is provided for the opening, 8 cm. in diameter, in the center of the rotor lid. The closure device, shown in Figure 1, is essentially a spring-loaded flat cap, *g*, equipped with a thin circular Teflon bearing ring, 9.5 cm. in diameter, partially embedded in its surface, and held in position by snapping it just over center into a prepared groove. While the rotor is spinning, the cap remains stationary, the Teflon ring bearing down gently on the lid of the rotor to seal it completely. Although the Teflon ring may be used without any lubricant, a thin film of Dow-Corning stopcock grease, a silicone lubricant, decreases wear and improves the seal. The cap has a small

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hole through which the capillary feed tip, *e*, can be inserted during a run.

In the early phases of the development of the apparatus and technique, dye mixtures were often used because they yielded colored chromatographic patterns without further development, and required simple water solutions for their development, thus avoiding the problems involved in differential evaporation rates of individual solvents from multisolvent solutions. For the separation of such mixtures as bromophenol blue, methyl orange, and methyl red, Whatman No. 1 filter paper was used with an aqueous Veronal buffer solution of ionic strength 0.05 and a pH of 8.7. For this system the R_f values, 0.86, 0.22, and 0.64, respectively, of the dyes were essentially unaffected by changes in rotational speed of the rotor from 300 to 925 r.p.m. (*6*). The flow of solvent was important in developing satisfactory chromatograms; 1.2 ml. per minute was satisfactory at all rotational speeds above 250 r.p.m. The shape of the developed chromatogram was elliptical rather than circular in all types of paper utilized. For Whatman No. 1 and 3MM, the major axis of the ellipse was parallel to the machine direction of the paper. The R_f values for the dye components were essentially the same for Whatman No. 1, Eaton-Dikeman No. 613, and Cremer-Tiselius Munktells (*6*), and within the limits of experimental error, were the same whether the separation was carried out in a direction parallel to the long axis of the ellipse or at right angles to it.

Amino acid mixtures were fractionated using Whatman 3MM paper and a solvent system of butanol–glacial acetic acid–water, in the volume ratio of 4:1:1, respectively, or methyl ethyl ketone–propionic acid–water, in the volume ratio of 15:5:6, respectively (*1*). Solutions containing 10 mmoles per liter of each amino acid were prepared, using as solvent an aqueous solution containing 10% isopropyl alcohol. In the case of tyrosine and cystine, and in the mixtures containing these amino acids, 6*N* hydrochloric acid was added, drop by drop, with stirring, until solution was complete. In almost all experiments, 2-μl. aliquots of the amino acid solutions were spotted on the paper sheet 7 cm. from the center. When the solution was applied to the paper in the form of an arc, 10- to 20-μl. samples were used. After development, all chromatograms were dried in a current of warm air, sprayed with a solution containing 0.2% ninhydrin in 95% butanol–5% acetic acid (2*N*). Color development of the chromatograms was carried out by placing the paper sheets in a current of warm air.

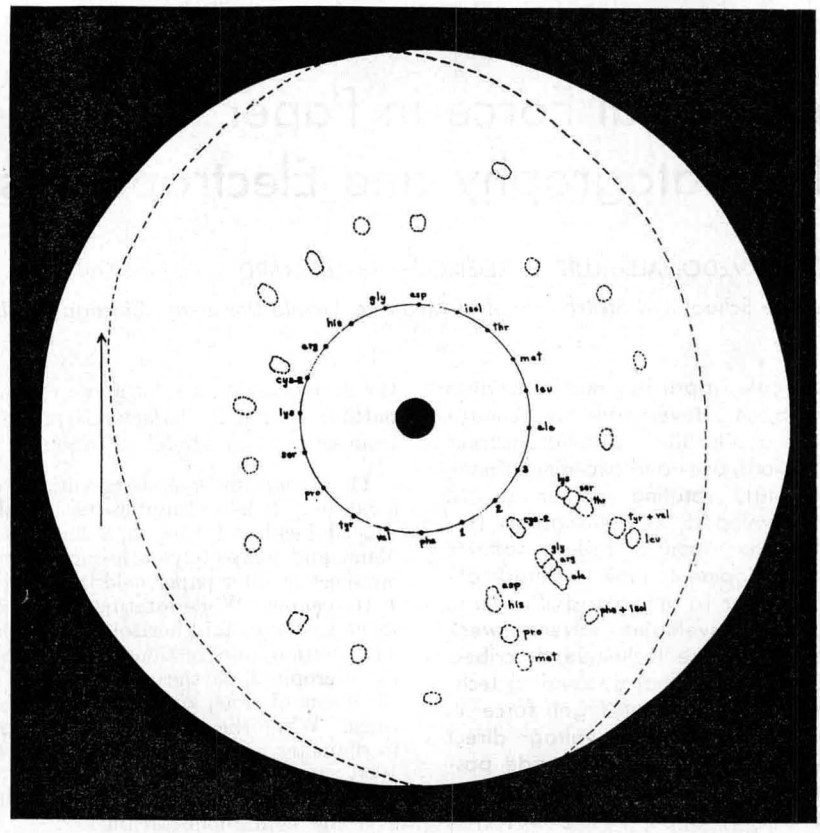


Figure 2. Relative positions and migration distances of 16 amino acids, and degree of separation of three mixtures of amino acids

Elliptical broken line indicates perimeter of developing solvent at end of run. Arrow indicates long direction of Whatman 3MM sheets, $18^1/_4 \times 22^1/_2$ inches, from which circular sheet was cut. Dots on circular line, 7 cm. from center of disk, represent points of application of amino acids

Table I. R_f Values of Amino Acids

(Whatman 3MM paper, 25° C.)

Amino Acid	Butanol–Glacial Acetic Acid–H_2O, Vol. Ratio, 4:1:1		Methyl Ethyl Ketone–Propionic Acid–H_2O, Vol. Ratio, 15:5:6	
	Conventional, descending	Centrifugally accelerated	Conventional, descending	Centrifugally accelerated
Cystine	0.06	0.08	0.04	0.15
Lysine	0.12	0.17	0.11	0.32
Histidine	0.14	0.18	0.13	0.35
Arginine	0.16	0.22	0.16	0.40
Aspartic	0.18	0.23	0.13	0.32
Serine	0.19	0.24	0.14	0.33
Glycine	0.20	0.26	0.16	0.35
Threonine	0.24	0.31	0.18	0.40
Alanine	0.31	0.38	0.23	0.44
Proline	0.34	0.41	0.27	0.48
Tyrosine	0.39	0.45	0.41	0.57
Methionine	0.48	0.55	0.43	0.57
Valine	0.53	0.59	0.41	0.56
Phenylalanine	0.55	0.63	0.54	0.63
Isoleucine	0.62	0.68	0.52	0.63
Leucine	0.65	0.70	0.55	0.65
Development time	7 hr.	15 min.	4 hr.	17 min.

Table I shows R_f values for 16 individual amino acids, as obtained by centrifugally accelerated paper chromatography and by the conventional descending technique, under similar conditions (paper, migration distance, developing solvent, temperature, etc.). The R_f values represent the average of at least three separate determinations. In the centrifugally accelerated experiments, the average calculated standard deviation for the butanol–acetic acid–water solvent was 0.011, and for the methyl ethyl ketone–propionic acid–water solvent was 0.021. In the experiments using the descending technique, the average calculated standard deviation for both developing solvents was 0.006. The R_f values of the amino acids

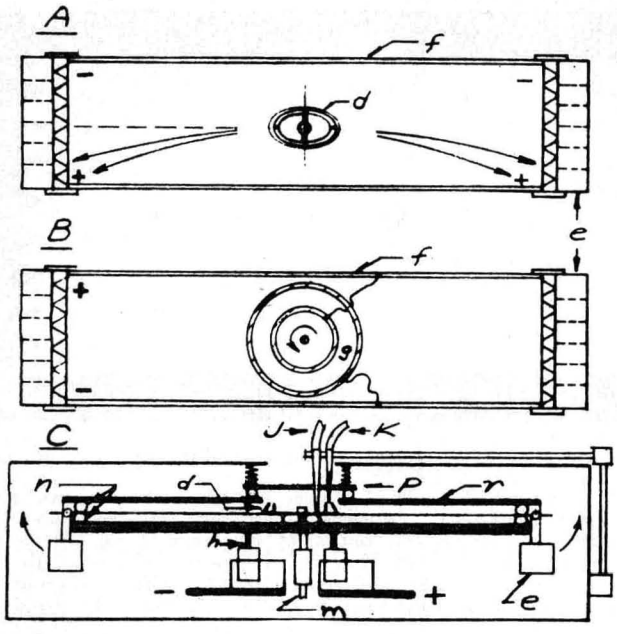


Figure 3. Apparatus for centrifugally accelerated electrochromatography

A. Top view of rotor
B. Underneath side of rotor
C. Side view of apparatus, with drive mechanism, brake, tachometer, automatic timer, deleted
d. Oval feeder cup
e. Collector troughs, swing out when heat rotates
f. Platinum-plated electrodes
g. Brass collector rings
h. Spring-loaded graphite brushes
j. Capillary feed for developing liquid
k. Capillary feed for solution to be fractionated
m. Drive shaft of rotor
n. Silicone gasket, above and below paper sheet
p. Spring-loaded closure device, for opening in top section, r, of rotor

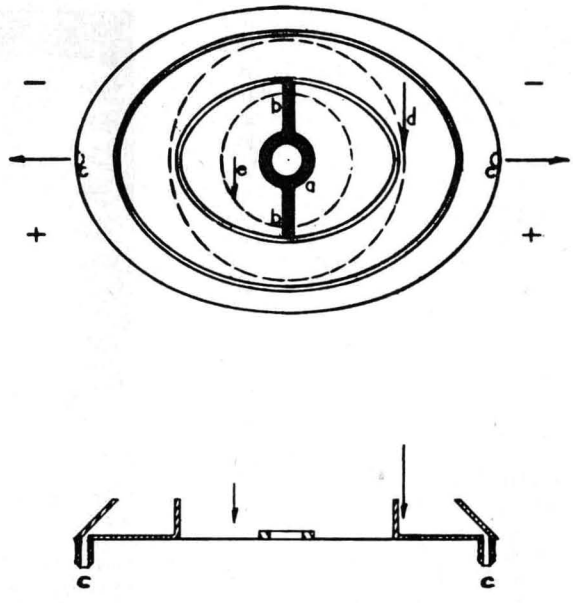


Figure 4. Elliptical feeder cup attached to drive shaft of the rotor by circular hub *a* and two spokes, *b*

Inner circular broken line represents path traced on paper sheet by stream of liquid from a glass capillary tip, represented by arrow e, used to develop the electrochromatogram. Outer circular broken line indicates path of stream of liquid containing solution to be fractionated as it is fed into the feeder cup, from a second capillary tip, *d*, for continuous operation of unit. The two stainless steel capillaries, c, one at each end of bottom of oval cup, allow solution to be fractionated to leak into paper sheet at two points symmetrically positioned away from center of sheet, and along median line parallel to its longer sides

studied, obtained on the rotating paper disk, were in all cases slightly higher than those obtained by the conventional descending technique.

A liner of Eaton-Dikeman, No. 320, a heavy stock paper approximately 2.5 mm. thick and 18 inches in diameter, was laid down in close contact with the bottom of the lower section of the rotor. Holes were cut in the paper sheet, so that the stainless steel support points protruded through. One hundred milliliters of developing solvent was spread over its surface, from a pipet, and a second sheet of Whatman 3MM paper was mounted above it, resting lightly over the stainless steel support points. The lid was placed on the rotor, the Teflon-ringed closer device lowered over the opening in the center of the lid, and the rotor run at 900 r.p.m. for 5 minutes. In this manner, the atmosphere within the rotor was equilibrated with respect to solvent vapor. After 5 minutes, the flow of developing solvent was started. In the case of the butanol–acetic acid–water solvent, the true flow rate of solvent was 1.0 ml. per minute, and it was continued for 15 minutes. For the methyl ethyl ketone–propionic acid–water solvent, the true flow rate was 1.1 ml. per minute, and it was run for 17 minutes. Whatman 3MM paper has also been used as the liner in the rotor but does not appear to be as satisfactory as the much heavier stock paper, Eaton-Dikeman No. 320. With a liner of Whatman 3MM, 25 ml. of solvent was used to wet it initially, and before each subsequent run. The former paper stays flat in the bottom of the rotor and has less tendency to lose the solvent from its perimeter when rotated at high speed. The same liner may be utilized for several runs. Each time a new sheet of Whatman 3MM paper is placed in the rotor 25 ml. of developing solvent is added to the Eaton-Dikeman No. 320 liner.

The R_f values appeared, within the limits of experimental error, to be the same regardless of the angle of their path of migration to the major axis of the ellipse representing the perimeter of the solvent front.

A chromatogram, developed with methyl ethyl ketone–propionic acid–water, showing the relative positions reached by 16 individual amino acids and the degree of separation of three mixtures of amino acids, is shown schematically in Figure 2. The arrow was drawn parallel to the long direction of the Whatman 3MM sheets, $18^1/_4 \times 22^1/_2$ inches, from which the circular sheet was cut. The arrow is parallel to the major axis of the ellipse marking the perimeter of the solvent front. The experimental conditions were as described above for this solvent.

CENTRIFUGALLY ACCELERATED ELECTROCHROMATOGRAPHY

In the usual curtain electrophoresis, the solution to be fractionated is applied to the top of a vertical sheet of paper, the edges of which are fitted with electrodes. In the newer planar electrophoresis described here, the paper is horizontal and is rotated so that the material undergoing fractionation moves across the paper under the stimulus of both electrical and centrifugal, instead of electrical and gravitational, forces.

Some details of the technique and early modifications of the apparatus for carrying out the centrifugally accelerated electrochromatographic separations have been published (*2*, *4*, *7*). A schematic diagram of the current apparatus is illustrated in Figure 3. The drive mechanism and auxiliary components, such as brake, tachometer, and automatic timer, are similar to those of the centrifugally accelerated paper chromatography apparatus.

The rotatable head consists of a lower (Bakelite) and an upper (Lucite) shallow tray (each section measuring 56 × 23 cm.) which can be clamped together to yield a chamber. The rectangular sheet of filter paper is moistened, blotted with Whatman 3MM paper,

then clamped in position. Attached along the full length of each side of the bottom tray are platinum-plated electrodes, *f*, connected to a high voltage direct current source, through two brass collector rings, *g*, and spring-loaded graphite brushes, *h* (Figure 3, *C*). The upper tray, with the paper sheet held in position, is placed over the lower tray, so that the platinum electrodes on both trays make good contact with the long sides of the paper sheet, and is then clamped in position. The paper sheet when positioned properly does not touch either the upper or lower plate but is suspended in a horizontal plane approximately midway between them. A collection trough, *e*, divided into nine individual compartments, is attached by a hinge arrangement to each end of the bottom tray in such a way that the troughs swing out into the same horizontal plane as the plane of rotation of the rotor, when the latter is set in motion. A series of V-shaped drip points is cut into each end of the rectangular paper sheet, to correspond with the number of drip collector compartments or cups in each of the two collector troughs.

For continuous operation, the solution to be fractionated is added continuously from a glass capillary tip to the oval, shallow, stainless steel cup, *d* (Figure 3). It is designed as an annular trough (outside dimensions, major axis, 9.5 cm.; minor axis, 9 cm.) attached to the drive shaft of the rotor by two slender spokes, *b* (Figure 4). The cup rotates with the paper sheet. The major axis of the oval or elliptical cup is arranged parallel to the long dimension of the rotor and the spokes holding the cup to the axle are at right angles to this, or congruent with the minor axis of the ellipse. Two stainless steel capillary tips, one in each end of the bottom of the oval cup, are provided so that the solution to be fractionated can leak into the paper sheet at two points symmetrically positioned away from the central point of the rectangular sheet and along the median line parallel to the long sides of the paper sheet. The centrifugal force tends to propel the solution in a thin straight line from the center, while the electrical field, acting at an angle to the centrifugal field, causes a drift of positively or negatively charged components to the electrode of opposite electrical sign. The result is that the components of the migrant solution assume distinct paths of migration (Figure 3, *A*).

The design of the oval feeder cup (Figure 4) is essential to the proper operation of the unit as a whole.

As the liquid to be fractionated is added to the cup from the glass capillary tip, represented by the arrow, *d*, held firmly in place above its annular opening under continuous-operation conditions, it describes a circle within the annular opening of the cup as the paper sheet turns through 360 degrees. The placement of the capillary tip must be adjusted closely so that the circle remains within the annular opening of the cup through a full turn of the paper sheet; otherwise the liquid to be fractionated would spill over the edge of the cup and onto the paper sheet. For batch-type operation, a small sample of the mixture can be added to the cup, and no further additions made. For micro quantities of sample, the paper sheet may simply be "spotted" with the liquid to be analyzed, at two points away from the center, as shown by the arrows in Figure 5, and the development carried through in the usual way.

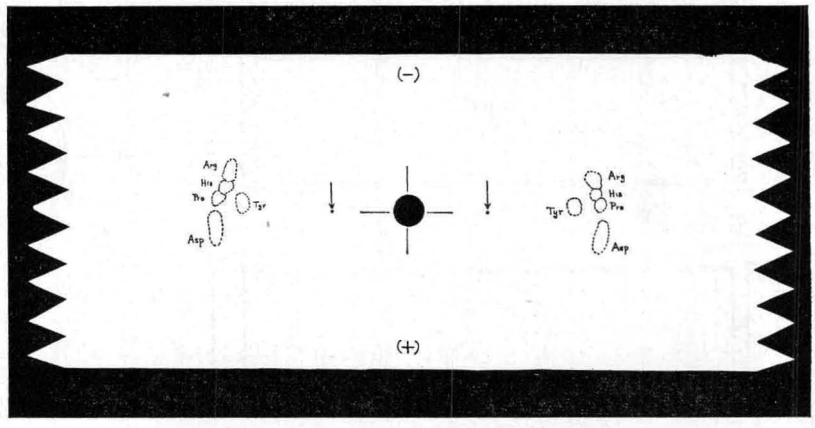


Figure 5. Electrochromatogram from spot application of 5-μl. aliquot

Solution containing 0.01 mole per liter, each, of arginine, histidine, proline, tyrosine, and aspartic acid. Experimental conditions. Phosphate buffer; pH, 6.4; ionic strength, 0.008; potential gradient, 17 volts per cm.; current, 16 mm.; rotor speed, 316 r.p.m.; temperature, 25° C.; paper, Whatman 3MM, 23 × 56 cm.; flow rate of developing solution, 0.8 ml. per minute. Time, 15 minutes. Displacement of amino acids, cm., from point of origin to approximate center of spot: arginine, 8.2; histidine, 8.1; proline, 8.6; tyrosine, 6.6; aspartic acid, 9.0

As the paper sheet rotates, provision must also be made for introduction of more liquid near its center, to prevent drying out. The liquid for this purpose is added to the rotating paper sheet in a thin, continuous jetlike stream from a second glass capillary tip, represented in Figure 4 by the arrow, *e*. The developing liquid in this way is added directly to the paper as a ring (rather than at two point sources, *c*, as is the solution to be fractionated), which, under the influence of the centrifugal field, spreads out uniformly in a widening circular path so as to saturate the paper sheet thoroughly from the center on out to its perimeter. The capillary tip is positioned carefully over the paper, so that the diameter of the initial ring of developing liquid is somewhat less than the shorter diameter of the inside wall of the elliptical cup.

To cause the electrophoretic separations, a direct current potential is applied to the strip electrodes, to yield a potential gradient of from 8 to 40 volts per cm. across the dampened paper sheet. Two Heathkit units, Model PS-3 (Heath Co., Benton Harbor, Mich.), connected in series may be used as the source of electrical power, and the ionic strength of the buffer solution saturating the paper can be adjusted to maintain the current carried by the sheet within the rated capacity of the power supply.

No great problem is introduced by the fact that the electrodes run along the full length of each side of the paper sheet. As the developing liquid is introduced near the center of the sheet, it flows out at a uniform rate to form a circle of steadily increasing diameter. As the front of this circular wave reaches the sides of the sheet where the electrodes are attached, it divides evenly and flows along the electrodes to the four outside corner collection cups, impelled by centrifugal force. In this way, any electrode products which collect in the edges of the paper sheet in contact with the electrodes are continually washed away.

The hole in the center of the top plate of the rotor, which accommodates the elliptical cup, and through which both the solution to be fractionated and the developing liquid are added, is sealed as in the simpler chromatographic unit. The spring-loaded closure device and Teflon bearing ring are represented in Figure 3, *C*, by *p*. As an additional measure, to reduce evaporation of solvent from the paper and maintain equilibrium conditions as regards solvent vapor within the rotating head, soft silicone rubber gaskets, 1 × 20 × 0.3 cm., make intimate contact with the paper sheet at each end of the rectangular head. The gaskets (*n*, Figure 3, *C*), one above and one below the paper sheet at each end of the rotor, are positioned adjacent to the serrated ends of the paper sheet, but allow the nine paper points to be exposed within the compartments of the collection troughs.

In general, heavier stock papers—e.g., Whatman 3MM—were preferable for planar electrophoresis. For separations of dye mixtures, such as aniline red, bromophenol blue, and methyl orange, generally satisfactory conditions were: Veronal buffer, pH 8.6, ionic strength 0.01; rotor speed, 300 to 400 r.p.m.; flow rate of developing solvent, 0.6 to 0.8 ml. per minute. The paper was prewetted, mounted in the

head of the instrument, and rotated for 5 minutes to achieve an even wetness. It was then stopped and 5-μl. aliquots of the mixture to be separated were applied. The rotor was now set at 400 r.p.m., the developing solution added as a fine stream, and a direct current potential of 18 volts per cm. applied. After 20 minutes, a bromophenol blue spot had traveled outward 7.5 cm. (chromatographic displacement); and 2 cm. in the direction of the anode [ionographic migration (*7*)]. For separation of amino acids, representative experimental conditions might be illustrated by the fractionation of a solution containing glycine, lysine, and glutamic acid. The conditions were: sample, 0.01*M* solution of glycine, lysine, and glutamic acid; rotor speed, 400 r.p.m.; flow of rate of developing solvent, 0.6 ml. per minute; buffer and other conditions as above. After 15 minutes, the amino acids had all moved 7.5 cm. from the point of application and were 1.9 cm. apart (*7*).

The electrochromatogram shown in Figure 5 is a further illustration of the type of separations achieved. It was obtained from a spot application at the two points indicated by the arrows.

A 5-μl. aliquot of a solution containing 0.01 mole per liter each of arginine, histidine, proline, tyrosine, and aspartic acid was applied. The experimental conditions were: phosphate buffer, pH 6.4; ionic strength, 0.008; potential gradient, 17 volts per cm.; current, 16 ma.; rotor speed, 316 r.p.m.; temperature, 25° C.; paper, Whatman 3MM, 23 × 46 cm.; flow rate of developing solution, 0.8 ml. per minute; time, 15 minutes. The displacement of the amino acids, in centimeters, from the point of origin to the approximate center of each zone, was: arginine, 8.2; histidine, 8.1; proline, 8.6; tyrosine, 6.6; aspartic acid, 9.0. The distance of separation between the arginine and aspartic acid zones was 4.5 cm.

As the pattern on one half of the electrochromatogram is practically a mirror image of that on the other, an authentic sample of one amino acid can be applied to the paper on one side and a mixture on the other. When development has been completed, the paper sheet can be folded in half, and the presence or absence of the known amino acid in the mixture determined by observing congruence of developed zones.

The centrifugal and electrical forces may be applied independently, in sequence, or simultaneously. It is thus possible to carry out simple chromatographic or ionographic as well as electrochromatographic separations.

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RECEIVED for review November 17, 1958. Accepted March 4, 1959. Project supported in part by a grant-in-aid from the Chicago Heart Association. Leonard J. Banaszak is holder of the New Horizons Fellowship, for 1958–59, supported by Labline, Inc., Chicago. Luiz P. Ribeiro, postgraduate research fellow 1957–58, acknowledges receipt of a travel grant from the National Research Council of Brazil. The apparatus described in this article is manufactured by and available from Labline, Inc., Chicago 22, Ill.

Diffusion Analysis of Ions in Gels

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▶ **Methods were needed for qualitative separation of ions and their quantitative estimation by radial diffusion in gels. Mixtures of two and three cations were easily identified after resolution in gelatin and poly(vinyl alcohol) gels. Diffusion distances for cations and anions were noted and compared in gelatin and sodium alginate gels. Individual cations and anions diffuse in accordance with Fick's law, and can be determined by plotting the variation of radial distance against ionic concentration. Common cations and anions may be quantitatively determined in concentrations between 0.1N and 10.0N, with an accuracy to approximately 1%. This technique is valid for both the cationic and anionic species in a given compound, as each constituent diffuses independently.**

THE objective of this work was to develop further qualitative and quantitative tests for ions by utilizing the property of diffusion in gelatinous media in contradistinction to solvent flow capillary action and absorption *vs.* gravity, the properties utilized in paper chromatographic separations. Whereas analytical separations have been accomplished by diffusion alone in gels (*1–8*), no method has been previously developed for utilizing the property of diffusion (independent of current or other influence) for determining ions quantitatively.

This paper describes the partial resolution and detection of some common cations and anions by radial differential diffusion taking place in concentrated gels under the influence of chemical potential gradients, and the behavior of individual ions with respect to the variables of time, concentration, and distance when undergoing diffusion, which enables their determination.

GEL PREPARATION

Thick concentrated gels of gelatin, poly(vinyl alcohol), and sodium alginate were prepared. Gelatin plates were prepared by softening 16 grams of food grade gelatin in 50 ml. of cold distilled water, then adding 150 ml. of boiling distilled water instantly to the mass. The hot solution was poured into Petri dishes to a depth of 1.5 cm. (watch glasses may also be used). Sodium alginate plates were prepared from Kelcosol (Kelco Co.) in the same manner by dissolving 3 grams of alginate in 100 ml. of hot distilled water. Poly(vinyl alcohol) plates were prepared from Elvanol 72-60 (Du Pont). In this case it was necessary to heat the alcohol (40 grams per 200 ml. of distilled

water)–water solution for 30 minutes to ensure uniform gelation. Refrigeration hastened gelling.

PROCEDURE

Qualitative. The mixtures to be resolved (0.1*N* solutions of cations) were added 0.2 to 0.4 ml. at a time from a pipet to the approximate center of the plates. After a few minutes had elapsed, noticeable diffusion had taken place. Some gel surfaces were completely dry, while others were wet with excess liquid. Wet surfaces were carefully dried by gently dipping the edge of a filter paper into the liquid. Developing reagents were then added in a sufficient amount to cover the surfaces and develop distinct concentric rings. The developing may take from less than a minute to an hour, depending on the reagents concerned. Some combinations of cations and developers failed to give good results.

Quantitative. Gel plates were placed on a polar coordinate paper so that the periphery of the Petri dishes coincided concentrically with the circles on the paper. Carefully measured drops (0.05 ml.) of individual ions of known concentration were placed at the center of the gel plates over the central circle of the polar coordinate paper. This radius, which was constant in all cases, is termed the initial radius, $r°$. Care had to be taken that the drops delivered were perfectly round with no distortion, that the plates were not jarred, and that the temperature remained constant within 1° or 2°.

The radii traveled by the ions after specific time intervals from $r°$ were measured for various concentrations. Measurement was facilitated by rendering invisible ions visible with a suitable developer. This arrested the diffusion at the time interval, necessitating separate ion development for each time interval.

Two techniques were used to measure the diffusion radius, r, of an ion. One technique consisted in projecting the image of the ionic zone with an opaque projector onto a screen, and measuring distance r, on the screen with a millimeter ruler. Another method was to span r with a fine bow divider from a drawing set, then lay the bow divider against the millimeter scale of a Vernier caliper which could be accurately read to millimeters and estimated to tenths of millimeters.

The first technique was useful for relative readings and comparison, while the second gave the actual distance traveled by the ion in the gel system. The actual readings were recorded and calibration curves were made for various ions by plotting the distances traveled as ordinates against the logs of the concentrations of the diffused ions for constant time intervals. Time was plotted as abscissa, *vs.* distance traveled for ions, holding concentration constant.

Students were then asked to prepare standard series of solutions of various ions, duplicate the above procedure in the same medium for the same time interval, and then construct calibration curves of log concentration *vs.* distance for the time interval. The students were then given unknowns of the ions which they diffused for the same time interval, and asked to evaluate the concentrations of their unknowns by measurement of r and comparison with their calibration curves. Compounds were diffused and r of both the cation constituent and the anion was measured and compared for a single compound.

Table I. Separation of Cation Pairs in Gelatin by Selective Diffusion

Compounds Diffused	Developer	Colors and Ions: Inner	Colors and Ions: Outer
$HAuCl_4$, $HgCl_2$	Oxine	Purple, Au^{+++} Hg^{++},	Yellow, Hg^{++}
$Fe(NO_3)_3$, $Cu(NO_3)_2$	Oxine	Orange brown, Fe^{+++}, Cu^{++}	Yellow-green, Cu^{++}
$Cu(NO_3)_2$, $HgCl_2$	Oxine	Yellow-green, Cu^{++}, Hg^{++}	Yellow, Hg^{++}
$HAuCl_4$, $Cu(NO_3)_2$	Anthranilic acid	Purple brown, Au^{+++}, Cu^{++}	Violet, Cu^{++}
$Cu(NO_3)_2$, $Bi(NO_3)_3$	Oxine	Whitish yellow, Cu^{++}, Bi^{+++}	Yellow-green, Cu^{++}
$Hg(NO_3)_2$, $Cu(NO_3)_2$	Cyanuric acid	Orange, Hg^{++}, Cu^{++}	Violet, Cu^{++}
$Bi(NO_3)_3$, $Cu(NO_3)_2$	Oxine	Gray, Bi^{+++}, Cu^{++}	Green, Cu^{++}
$Hg(NO_3)_2$, $Pb(NO_3)_2$	KI	Brown, Hg^{++}, Pb^{++}	Yellow, Pb^{++}
$FeCl_3$, $Ni(NO_3)_2$	Oxine	Brown, Fe^{+++}, Ni^{++}	Green, Ni^{++}
$Bi(NO_3)_2$, $Hg(NO_3)_2$	K_2CrO_4	Yellow, orange, Bi^{+++}, Hg^{++}	Yellow, Bi^{+++}
$Zn(NO_3)_2$, $FeCl_3$	$K_3Fe(CN)_4$	Brown, Zn^{++}, Fe^{+++}	Yellow, Zn^{++}
$FeCl_3$, $Hg(NO_3)_2$	Cyanuric acid	Yellow-brown, Fe^{+++}, Hg^{++}	Green, Fe^{+++}
$Ni(NO_3)_2$, $Hg(NO_3)_2$	Cyanuric acid + rubeanic acid	Yellow, Ni^{++}, Hg^{++}	Violet, Ni^{++}
$Mn(NO_3)_2$, $Hg(NO_3)_2$	$K_4Fe(CN)_6$	Yellow-green, Mn^{++}, Hg^{++}	White, Mn^{++}
$Ni(NO_3)_2$, $Co(NO_3)_2$	Na_3PO_4 + dimethyl-glyoxime	Rust, Co^{++}, Ni^{++}	Pink, Ni^{++}
$SnCl_2$, $Ni(NO_3)_2$	Rubeanic acid	Brown, Ni^{++}, Sn^{++}	Violet, Ni^{++}

Table II. Separations of Three Cations in Gelatin by Selective Diffusion

Compounds Diffused	Developers	Colors and Ions: Inner zone	Middle zone	Outer zone
$FeCl_3$, $Hg(NO_3)_2$, $Ni(NO_3)_2$	RA&KF	Brown, Fe^{+++}, Hg^{++}, Ni^{++}	Blue, Fe^{+++}, Ni^{++}	Violet, Ni^{++}
$Mn(NO_3)_2$, $Ni(NO_3)_2$, $Cu(NO_3)_2$	RA	Red, Mn^{++}, Cu^{++}, Ni^{++}	Purple, Ni^{++}, Mn^{++}	White, Mn^{++}
$Ni(NO_3)_2$, $Cu(NO_3)_2$, $FeCl_3$	RA	Brown-green, Ni^{++}, Cu^{++}, Fe^{+++}	Blue-green, Ni^{++}, Cu^{++}	Violet, Ni^{++}
$Mn(NO_3)_2$, $Ni(NO_3)_2$, $FeCl_3$	RA&KF	Blue, Mn^{++}, Ni^{++}, Fe^{+++}	Violet, Ni^{++}, Mn^{++}	White, Mn^{++}
$Mn(NO_3)_2$, $Hg(NO_3)_2$, $Cu(NO_3)_2$	RA&KF	Brown-green, Mn^{++}, Cu^{++}, Hg^{++}	Violet, Cu^{++}, Mn^{++}	White, Mn^{++}
$Mn(NO_3)_2$, $Hg(NO_3)_2$, $Cu(NO_3)_2$	KF	Brown, Mn^{++}, Hg^{++}, Cu^{++}	Maroon, Mn^{++}, Cu^{++}	White, Mn^{++}
$Mn(NO_3)_2$, $Hg(NO_3)_2$, $FeCl_3$	KF	Green, Mn^{++}, Hg^{++}, Fe^{+++}	Blue, Fe^{++}, Mn^{++}	White, Mn^{++}

KF. Potassium ferrocyanide
RA. Rubeanic acid

RESULTS

Qualitative. Many cation combinations were qualitatively resolved in gelatin (Tables I and II) and Elvanol (Table III). Considering the results obtained here in conjunction with previous results (*1*), 0.1*N* solutions of cations when mutually diffused in twos or threes will fall into the following category, in order of greater diffusion distance:

Mn(II) > Ni(II) > Co(II) > Ag(I) > Cu(II) > Fe(III) > Pb(II) > Bi(III) > Hg(II) > Au(III) > Hg(I)

Quantitative. Figure 1 compares curves obtained by plotting time *vs.*

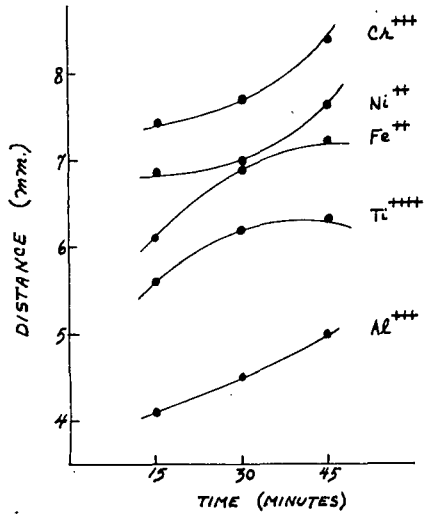


Figure 1. Variation of distance, r, with time, t, for 0.1N solutions of various cations in gelatin

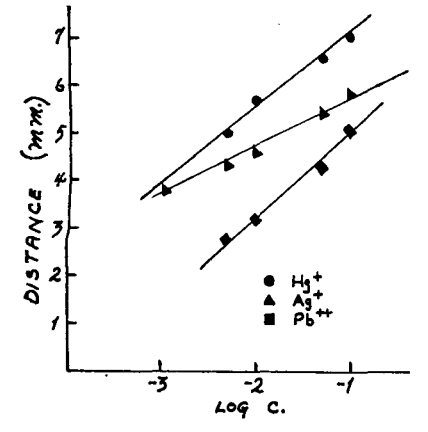


Figure 2. Log concentration vs. r for group I cations, after 15 minutes' diffusion in gelatin

distance for 0.1N solutions of various cation nitrates diffusing in gelatin. Figures 2 and 3 illustrate how log concentration varies with distance, enabling determination of ions diffusing in a fixed period of time (15 minutes in this case). Constructing such curves for knowns enabled students to determine the concentration of unknown solutions of lead nitrate. By diffusing the solutions in gelatin for 15 minutes, and then arresting the diffusion with saturated potassium iodide, measurement of the yellow zone radii, 0.1000, 0.5000, 1.0000, 5.0000, and 10.0000N solutions could be identified with reasonable accuracy. Out of 28 trials, the results were accurate to ±0.68%.

A plot of time *vs.* distance at various concentrations is illustrated in Figure 4, from which the effect of concentration on the slope of an r *vs.* t plot may be noted.

Halide anion radii were developed with silver nitrate and calcium chloride (for fluoride ions) solutions. Table IV compares halide ion radii of sodium, potassium, and ammonium salts and shows that the diffusion of an anion is affected by the cationic species present.

When r is used for determining unknown concentrations of a cation or an anion in a compound, the anion must always be identical when the cation is being determined, and vice versa—e.g., consistent results will not be obtained if silver nitrate is used for standardization and the unknown is silver cyanide, silver sulfate, etc.

Diffusion radii of 0.1N solutions of individual cation nitrates were measured after 15 minutes in gelatin and found to follow the following sequence in order of greater diffusion distance:

$$\text{Zn(II)} > \text{Hg(I)} > \text{Co(II)} > \text{Cr(III)} > \text{Cd(II)} > \text{Au(III)} > \text{Ni(II)}$$

$$\text{Fe(II)} > \text{Hg(II)} > \text{Fe(III)} > \text{Mn(II)} > \text{Ag(I)} > \text{Ti(IV)} > \text{Cu(I)}$$

$$\text{Cu(II)} > \text{UO}_2\text{(II)} > \text{Pb(II)} > \text{Al(III)}$$

Interaction between ions and the diffusion media interferes with normal diffusion. This has been observed with mercury(I), bismuth(III), and iron(III) in gelatin. Bismuth(III) forms white bismuth hydroxide and iron(III) brown chelate complexes of amino acids. Nevertheless, iron(III) appears to follow normal diffusion according to Fick's law. Kelcosol arrests copper(II), copper(I), gold(III), nickel(II), aluminum(III), titanium(IV), cadmium(II), and zinc(II). Some ions diffuse preferentially at faster rates in some media than in others. A case in point is shown in Figure 5, which compares the slopes obtained for chlorine (as potassium chloride) after 15 minutes' diffusion in Kelcosol and in gelatin.

Single substances do not diffuse in gels as a compound but as anions and cations, as can be seen from Table V, which compares r (for 0.1N solutions after 15 minutes) of cations and anions in single compounds.

Table III. Separation of Cation Pairs by Selective Diffusion in Elvanol

Compounds Diffused	Developer	Colors, Ions, and Diameters of Zones: Inner	Outer
$HgCl_2$, $Cu(NO_3)_2$	Oxine	Cloudy lemon yellow, Cu^{++}, Hg^{++}	Transparent yellow, Hg^{++}
$HAuCl_4$, $HgCl_2$	Oxine	Purple, Au^{+++}, Hg^{++}	Transparent yellow, Hg^{++}
$Fe(NO_3)_3$, $HgCl_2$	Oxine	Gold-brown center surrounded by olive green ring, Fe^{+++}, Hg^{++}	Yellow, tinged with dull green from ring around iron zone, Hg^{++}
$Fe(NO_3)_3$, $Cu(NO_3)_2$	Oxine	Gold-brown center surrounded by olive green ring, Fe^{+++}, Cu^{++}	Dirty yellow-green, Cu^{++}

Table IV. Diameters of Diffusion Zones of Anions

(15-minute periods)

Compound	Kelcosol 0.1N	Kelcosol 0.05N	Kelcosol 0.01N	Kelcosol 0.005N	Gelatin 0.1N	Gelatin 0.05N	Gelatin 0.01N	Gelatin 0.005N
NaCl	12.0	10.9	8.4	...	7.0	5.5	4.9	4.9
NaBr	14.6	13.0	11.9	10.7	15.5	14.0	13.2	11.9
NaI	16.5	10.5	9.1	9.0	9.0	7.0		
NaF	6.9	6.2	5.9	...	7.8	7.4	7.1	6.9
NH_4Cl	9.7	8.0	7.7	...	7.2	6.3	5.2	5.0
NH_4Br	15.9	14.8	14.0	13.0	15.0	13.0	12.0	11.0
NH_4F	9.0	8.0	7.0	6.9	8.5	7.9	7.1	5.8
KCl	11.0	9.6	7.2	6.3	7.9	7.2	7.0	6.5
KBr	18.0	16.8	14.3	13.0	13.5	12.5	12.5	10.9
KI	17.5	16.5	15.2	13.0	7.5	5.3	4.5	...
KF	8.2	7.9	6.9	...	7.6	7.3	6.6	...

Table V. Diffusion of Compounds as Ions in Gelatin (G) and Kelcosol (K)

Compound	Cation Distance, Mm. K	Cation Distance, Mm. G	Cation Developer	Anion Distance, Mm. K	Anion Distance, Mm. G	Anion Developer
$BaCl_2$	10.4	10.8	SO_4^{-2}	8.5	9.2	Ag^+
$FeCl_3$	7.0	7.5	$Fe(CN)_6^{-4}$	9.2	7.0	Ag^+
$NiSO_4$	11.5	10.2	Rubeanic acid	9.8	6.2	Ba^{++}
$CoCl_2$	12.5	9.0	$Fe(CN)_6^{-4}$	..	7.2	Ag^+
$HgCl_2$	11.3	6.5	KI	12.4	7.0	Ag^+

DISCUSSION

Qualitative diffusion procedures resolve cations partially. When a cation mixture is placed centrally on a gelatin surface, each component migrates under the influence of the chemical concentrations or potential gradient. The

zone of each component extends from the starting position to the leading boundary. Each cation contaminates the zone of every more slowly diffusing cation; therefore, only a small fraction of the fastest migrating ion can be separated from others. Figure 6 illustrates this for cations A, B, and C.

A component may be retained by a zone in preference to another component if it is heavier, thus diffusing more slowly. It will be retained in preference if it also undergoes a secondary reaction with the diffusion media. This is strictly true only when the cations are of equal concentration in the mixture being resolved, and when they have a common anion.

Individual cations follow Fick's law of diffusion. When time is held constant and r and the concentration, c, of the ion in question are varied, solution of Fick's equation gives:

$$\int_{c_1}^{c_2} \frac{dc}{c} = \frac{K}{D} \int_{r_1}^{r_2} dr =$$

$$\log \frac{c_2}{c_1} = \frac{K}{D}(r_2 - r_1) \quad (1)$$

where r_1 is the diffusion radius traveled for an ion of concentration c_1, and r_2 is the radius for an ion of concentration c_2. K is a constant peculiar to the time interval of the diffusion process and varies inversely with t. D is the diffusion constant. Equation 1 when obeyed by an ion gives straight lines when log c is plotted against r at various time intervals, as is shown in Figures 2, 3, and 5. Equation 2 represents the solution of Fick's law when r and t are the variables and c is held constant.

$$DK \int_{t_1}^{t_2} dt = \int_{r_1}^{r_2} dr =$$

$$DK(t_2 - t_1) = r_2 - r_1 \quad (2)$$

K in this case varies directly with c.

Einstein has shown that the diffusion constant, D, may be expressed as

$$D = RT/6\pi\eta rN \quad (3)$$

where R is the gas constant, T is the absolute temperature, N is Avogadro's number, η is the viscosity of the diffusion media, and r is the mean radius of the ions.

A single ion will follow Fick's law, but not when mixed with other ions of the same charge. Thus studying Figure 2, one would expect when resolving a mixture of 0.1N solutions of mercury(I), lead(II), and silver(I) nitrates that the outer zone would contain mercury(I), the middle, mercury-(I) and silver(I), and the inner all three cations. Actually silver(I) is in the outer zone, lead(II) and silver(I) are in the middle zone, and all three cations are in the inner zone (*1*). It is obvious that mutual effects radically alter the diffusion of an individual ion.

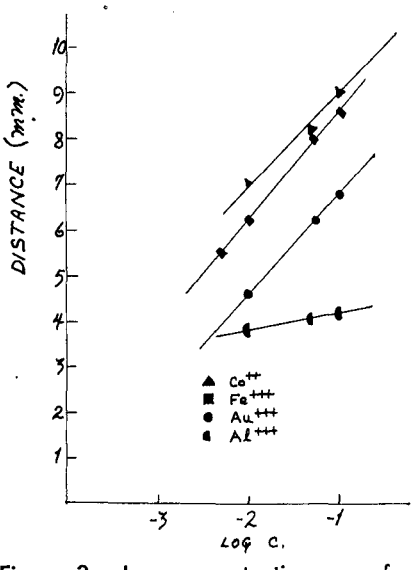


Figure 3. Log concentration *vs.* r for group III cations after 15 minutes' diffusion in gelatin

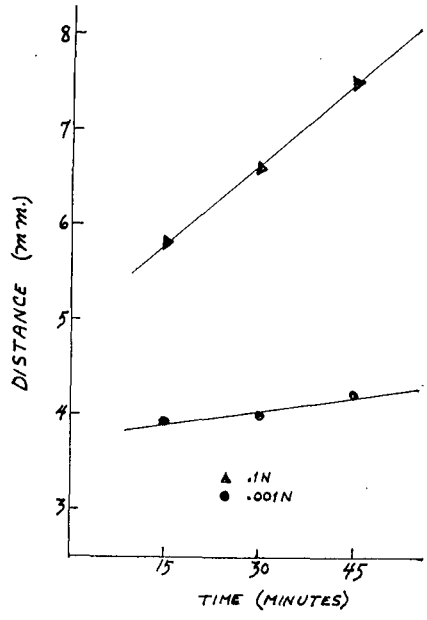


Figure 4. Plots of r *vs.* t for 0.1N and 0.001N solutions of silver nitrate diffusing in gelatin

CONCLUSION

Mixtures of cations may be resolved partially by differential diffusion in gels. The concentration of ions may be determined by measuring the distances traveled in a fixed length of time and comparing them with standards. To determine a cation's or an anion's concentration by this technique, the same compound must be used to obtain valid results. A single substances diffuses as separate entities of its respective cation and anion constituents.

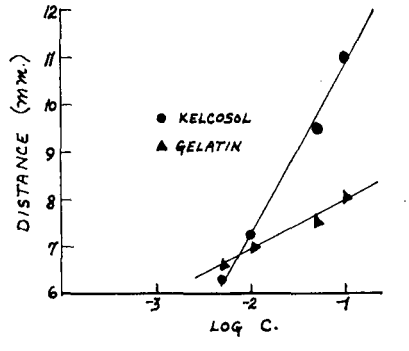


Figure 5. Selectivity of diffusion media

Comparison of chloride ion (from KCl) diffusing in Kelcosol and gelatin

ACKNOWLEDGMENT

The following students who worked

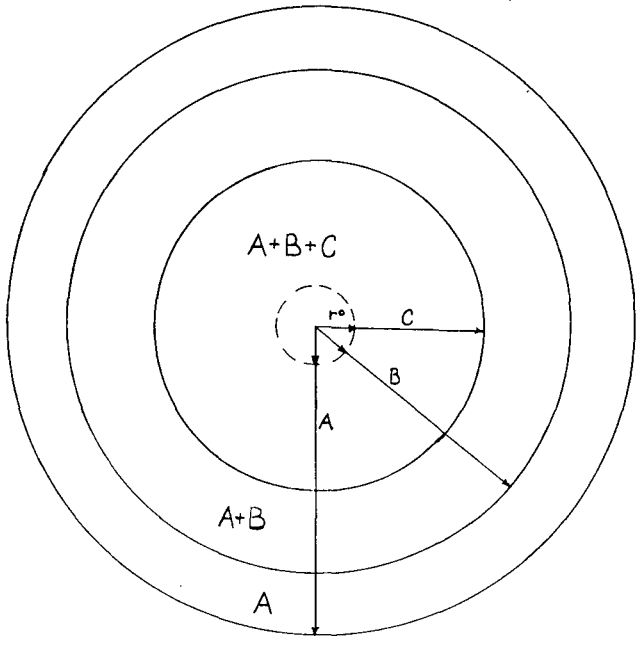


Figure 6. Schematic drawing of cross contamination of three cations, A, B, and C

on these diffusions received NaCURP certificates from Handbook Publishers, Inc., for their satisfactory solution of an undergraduate research program: Larry A. Teter, Fresno State College, Fresno, Calif.; Richard L. Hill, James W. Short, and William G. Wilkerson, University of Texas, Austin, Tex.; Gloria Defina, Paula Fetsko, and Constance Bourdeau, St. Joseph College, Emmitsburg, Md.; and Samuel Hirsch Jacobson, Boston University, Boston, Mass.

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RECEIVED for review March 7, 1958. Accepted December 31, 1958. Division of Analytical Chemistry, Symposium on Separation Processes through Differential Migration Analysis, 134th Meeting, ACS, Chicago, Ill., September 1958. Work done under the auspices of the National Cooperative Undergraduate Chemical Research Program (NaCURP).

Carrier Displacement Chromatography on Ion Exchange Resins

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▶ **Certain volatile bases assume intermediate positions in the order of displacement of amino acids on cation exchange resins and thereby convert partial into complete separations. Acids can do the same on anion exchange resins. A paramount criterion in the search for these selective agents or carriers is the dissociation constant, but aromaticity and size are also considered. Some of these carrier substances may be used in a second way. If a resin is first saturated with an appropriate acid or base, amino acids may separate by differential migration, if displaced into and through the zone of saturated resin. This method of column development has distinct advantages over those using buffers or strong acids. The need for clarification of the terminology and the mechanisms of ion exchange chromatography of ampholytes are discussed.**

WHEN solutes that bind firmly to an immobile phase are able to strip completely or displace more weakly bound substances, zones "push" one another down a column all at the same rate, the final displacing agent acting like a piston (*3*). This is diagrammed in Figure 1, where the curves represent concentrations of solute along a column. Even when individual affinities for the stationary phase differ greatly (*A* and *B*), pure zones are always separated by a mixed zone, the lower limiting size of which is governed by the mechanical imperfections of column operation. It is theoretically impossible to recover any substance completely in pure form.

Mixed zones tend to be larger when relative affinities differ to a lesser degree (*B* and *C*). Often solutes compete almost equally for the stationary phase (*D* and *E*) and nearly all of one or both substances is mixed with the other. Unlike most types of chromatography, the relative size of a mixed zone does not decrease as less material is chromatographed. Conversely, addition of more material does not increase the absolute sizes of mixed zones beyond certain maxima and the relative quantity of mixed material therefore becomes less. Because of these characteristics displacement chromatography of amino acids has been successful when very large mixtures—e.g., 280 grams of hydrolyzed egg albumin (*25*)—are separated.

Even when two solutes differ but slightly in their affinity for the solid phase (*D* and *E*, Figure 1) they may be separated by displacement chromatography if enough of a third substance with an intermediate affinity is interposed. Such interposed displacers, called "carriers," have been used in absorption chromatography (*9, 12, 15, 17, 18, 28, 34*).

Recently (*5*) carriers were used to expand the methodology of Partridge and coworkers (*20–27*) in the separation by displacement chromatography of mixtures of amino acids on ion exchange resins. The carriers were nonamphoteric bases and acids, all easily separated from the amino acids by evaporation or ion exchange. The observation of Davies (*7*) that with bases as well as acids the stronger displace the weaker on ion exchange columns made the search for carriers much easier. Although the displacement sequence of a group of substances cannot always be predicted from their dissociation constants, (*24, 25*), experience is a reliable guide in evaluating the magnitudes of the effects of molecular size and aromaticity in altering the expected displacement order.

Some groups of three or more amino acids have dissociation constants and other properties so nearly alike that when the group is chromatographed by displacement there is little tendency for one to act as a carrier to separate the others. Because of this the hope of finding other carriers to separate these groups of very similar amino acids is not great. However, the individual members of such groups are often separable by differential migration, if a resin column is first regenerated with the carrier initially used to displace the group and the amino acid mixture is then displaced through the column. In this case the carrier becomes a competitive developer.

Partridge and Brimley (*22, 24*) had demonstrated that some amino acids that fail to separate on a sulfonic acid resin may sometimes do so on an anion exchanger. These workers (*23*) and others (*10, 19*) had also improved some separations by raising temperatures. Cooling may also improve a separation (*5*). These factors were brought to bear in developing the empirically successful schemes (*5*) for separation of synthetic amino acid mixtures. The present paper illustrates the resolution of an acid hydrolyzate of protein.

EXPERIMENTAL

Hydrolysis. Rat muscle protein was precipitated, washed with 1% picric acid, and dried. After correction for picric acid content and ash, the sample weighed 2.34 grams. It was hydrolyzed by refluxing 24 hours in 25 ml. of 6*N* hydrochloric acid. After one treatment to near dryness in a rotary evaporator, the residue was redissolved in 50 ml. of water and filtered.

Chromatography. The mixture was then subjected to chromatography as described in Figure 2, which is a flow diagram of the separation plan with projections of the paper chromatograms of the fractions of effluent, all taken at 20-minute intervals. The type of resin of each of the six columns, the volumes and lengths of the resin beds, the displacing solutions, the temperatures of operations, the fraction numbers, and the volumes of the pooled effluent appear with the appropriate paper chromatograms. The latter were developed with 1-butanol–glacial acetic acid–water solvent (4:1:1). Chromatograms of the long ninhydrin-negative "breaks" between and preceding some amino acids were deleted from the figure, but the volume of discarded effluent is designated.

Many of the technical details have been described (*5*). If desired, very wide separations can be attained with carriers. This is emphasized in the primary fractionation, which was carried out on a column larger than previously used and twice the usual quantity of each carrier (30 mmoles). The first two carriers, 1-isopropylpyrazole and pyrazole (each 0.2*M*), were first added, followed by the amino acid mixture. When the third carrier, pyridine (0.1*M*), was added, a precipitate, presumably of tyrosine and cystine, appeared in the resin bed. No attempt had been made to precipitate these slightly soluble amino acids before the hydrolyzate was put on the resin. After about 300 ml. of water, added to the pyridine, had run through the column, the precipitate redissolved and the remaining carriers were added in 0.1*M* solution. The water accounts for the large volume of effluent between Groups I and II. Seemingly because this separation was carried out during very warm weather, cystine, which had always emerged at the end of Group II and had trailed across the pyrazole break, now appeared in Group III. The advantage of this is evident, and probably warrants the use of a heated column.

In three secondary separations columns of Dowex 50 were first saturated with one of the three weak bases, 1-isopropylpyrazole, pyrazole, or nicotinamide; each had been used as a carrier in the preliminary fractionation. For the two remaining separations Dowex 2 was first saturated with the weak acid, succinimide. The amino acid mixtures were displaced into the zone of carrier—resin from a short segment of hydrogen form resin in the case of the Dowex 50 columns or from hydroxide form resin in the case of the Dowex 2 columns. This sharpens and concentrates a mixture before it enters the zone where separation will occur.

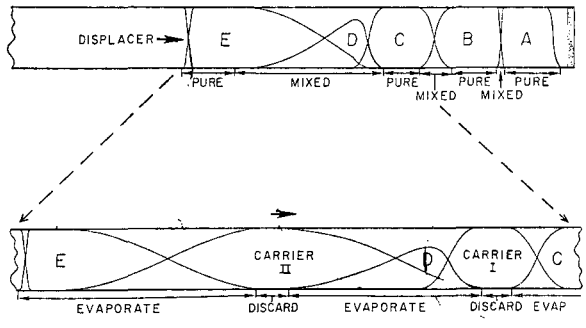


Figure 1. Diagram of concentrations in zones *A* to *E* in displacement chromatography and effect of carriers in promoting separation

The further separation of Group I was as given (*5*), except that a trace of hydroxyproline appeared well separated from proline. Threonine and serine separation was improved. The separation of Group II was unchanged, except that no attempt was made to separate the small final mixture of alanine and glycine by recycling. It is clear from the combined results of this separation and the previous one (*5*) that Group III, cystine, phenylalanine, tyrosine, and tryptophan all separate completely on Dowex 50 saturated with nicotinamide.

Recovery. The yields of twice recrystallized amino acids appear on Figure 2. Recovery from the crude protein was about 55% when corrected for the water of hydrolysis. This was lower than previously found for synthetic mixtures and lower than would be expected from a purer protein. Impurities such as carbohydrate, known to be present in the residue, not only detract from the true sample weight but also catalyze the decomposition of some amino acids during hydrolysis (*2*). Because this separation was merely to acquire enough of each amino acid for an isotope analysis, little effort was made to get high recoveries of the more abundant amino acids.

Tissue Extracts. This method has been applied with success to the isolation of free amino acids from picric acid extracts of several 1-kg. batches of rat muscle. With little extra labor several substances not present in protein may be obtained in pure form from these extracts. Taurine, creatine, creatinine, anserine, and carnosine are the more abundant of such compounds.

Taurine passes through the original desalting column (*4*) and can be recrystallized directly after the effluent is reduced in volume. Creatine follows pyrazole in the primary separation and is mixed with Group III. Much of it crystallized directly from the effluent and the rest may be crystallized by holding back the amino acids of Group III on a small column of Dowex 2–hydroxide, reducing this effluent to small volume, and then adding alcohol. Creatinine follows Group III and may be obtained in an analogous manner. Carnosine and anserine follow histidine and the three are mixed. They may all be separated by passing the mixture through a column of Dowex 50 saturated with picoline or with imidazole (details to be published).

DISCUSSION

Selection of Carriers. The practical aspects of carrier displacement chromatography on ion exchange resins are straightforward. Although one can distinguish at least three factors that determine the order of displacement of a group of substances from a cation resin exchange column, the most important is basic strength. Partridge and Brimley (*24*, *25*) found that if a margin of 0.2 pK unit is allowed, all but one of 24 amino acids emerge from a cation exchange resin in the same order as the relevant pK values (the dissociations that convert the positively charged ampholytes into neutral molecules). The exception was phenylalanine, which came off more than a full pK unit "late." Tyrosine and tryptophan are retarded even more (*5*), the latter (pK_1, 2.38) not being very cleanly displaced even by pyridine (pK, 5.2). Aromaticity is, therefore, associated with the most conspicuous "anomalies" (*25*) in the displacement order. Size is well correlated with smaller deviations—i.e., those that seem equivalent to 0.1 to 0.3 pK unit. Ammonia (pK, 9.27) cleanly separates lysine (pK_2, 8.95) and arginine (pK_2, 9.04), while valine (pK_1, 2.32), a larger molecule, follows alanine (pK_1, 2.34).

To select a carrier that may be useful in separating two compounds, one searches for a substance that has a dissociation constant between the two; the allowance given for size and aromatic character is dictated by preceden; and experience. The carrier compound

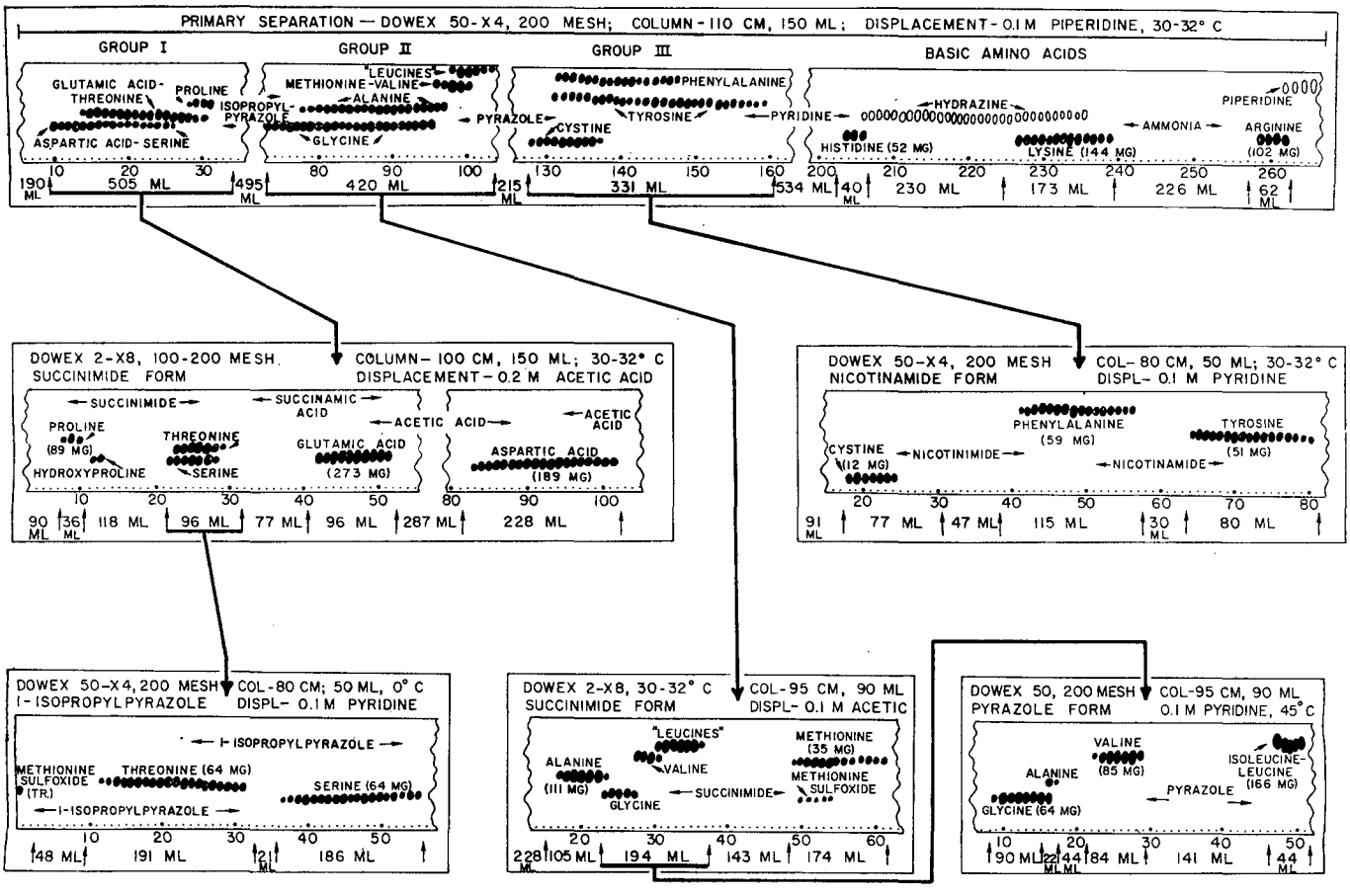


Figure 2. Scheme of separation of the amino acids from an acid hydrolyzate of muscle protein

Each block contains the operating data and results of a single column

must also be soluble, stable, and removable by simple methods. If these criteria are met, the separation has good chance of success.

Terminology. Some confusion currently exists over the classification and terminology of the different types of ion exchange chromatography. The terms "elution," "elution analysis," "elution development," "development," "development analysis," "displacement," "displacement analysis," and "displacement development" are all used (*1, 3, 6, 8, 14, 26, 29, 30, 32, 33*) and often seem to have overlapping meanings. Some authors (*6*, p. 14; *8, 29, 32*) regard elution or elution analysis as a process distinct from displacement; others (*1, 6*, p. 246; *16, 30*) look upon displacement as a type of elution or use the terms interchangeably. Part of the confusion arose when the terminology introduced by Tiselius (*33*) for nonionic chromatography was applied to ion exchange chromatography, the complications of which are even more exaggerated when acid-base complexing is involved (*13*) as is the case when amino acids are chromatographed.

By all criteria the primary separation here is one of displacement development, but the remaining separations involve differential migration in whole or in part and this gives them one important aspect of elution analysis. However, some authors (*8, 29*) restrict the terms "elution" and "elution analysis" to separations that employ agents with lesser affinities for the immobile phase than are possessed by the solutes being separated. These agents thereby function by washing through the moving zones of solute. In the majority of the separations described here, the opposite is true. The moving zones wash through the developing agent already on the column and the solutes in the zones then compete for both protons and the resin. Only water washes through the solutes. The latter are actually displaced from behind by more of the developing agent. This state of affairs also exists in separations where the developing solutions are buffers (*11, 19*). The foregoing merely points out the need for a precise and more comprehensive classification of ion exchange chromatography.

Types of Column Development. Three distinct types of developing solutions—viz., strong acids (*10, 31*), buffers (*11, 19*) and very weak bases (*5*)—may be used to separate neutral amino acids by differential migration on cation exchange resins. Some of the practical differences and similarities are noteworthy.

For reasons not as yet clear the capacity of a given column and the concentrations of amino acid in the effluent seem to be considerably greater when the developer is a weak base than when mineral acids or buffers are used. This allows one to isolate solid amino acids without evaporating so much water. A related advantage is that the progress of a column may be followed at least qualitatively by simple ninhydrin spot tests on filter paper, a procedure which is barely sensitive enough when concentrations are very low. Smaller volumes of effluent also mean that the amount of impurity from solubilized resin is lessened. The weak bases used are much easier to remove than buffer salts and are less apt to damage unstable compounds than mineral acids.

The striking similarity seen with the three types of column development is in the order of emergence of the protein amino acids. It is not surprising that this order, highly correlated with basic strength (*7*), is nearly identical with buffers (*11, 19*) and weak bases (*5, 24, 25*), because both types of agent can be primarily thought of as mobilizing the ampholytes by removing protons and thus rendering them neutral and free to move.

It is not so immediately obvious why

the order of emergence from a cation exchange column is nearly the same when the developing agent is a strong acid. Here virtually all of the amino acids in solution and on the resin possess a positive charge and therefore move down the column mainly in that form. However, in the case of two amino acids held to a sulfonic acid resin, all else equal, a competing hydrogen ion will have a better chance of dislodging the amino acid that has the greatest tendency to become neutral by losing a proton from its own carboxyl group. It is not clear why the effect of cyclic or aromatic structure is so much greater with this type of development than with the other two. On columns developed with hydrochloric acid (*10, 31*) phenylalanine follows not only the neutral amino acids but the basic ones as well.

ACKNOWLEDGMENT

The author is most grateful to R. T. Markiw for performing the chromatographic separations and to the U. S. Public Health Service for partial financial support.

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RECEIVED for review December 1, 1958. Accepted February 18, 1959.

Isolation of Cytoplasmic Particulates from Animal Tissues by Density-Gradient Centrifugation

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➤ Density-gradient centrifugation has been used to study the distribution of enzymes and other components of mammalian tissues. The technique permits the construction of curves relating intracellular distribution to particle size, and is particularly useful in comparing normal and abnormal tissues.

DIFFERENTIAL centrifugation (*6*) has been widely used during the past decade to obtain subcellular fractions of cells in sufficient quantity for chemical analysis and enzyme assay. A piece of tissue is homogenized in a suitable dispersing medium, and then centrifuged several times at progressively increasing speeds to yield fractions containing principally nuclei, mitochondria, and microsomes. However, it is virtually impossible to prepare these subcellular fractions in a sufficient homogeneous state, and many techniques of subfractionation have been devised.

Because the rate of sedimentation of the particles is a function of their size, a much better separation can be made by layering the homogenate over a column of liquid of slightly higher density than would be possible by conventional methods of differential centrifugation. The use of a density gradient to stabilize the boundaries of the sedimenting material is an essential part of this procedure. Anderson has discussed in considerable detail both the historical development and the principles of density-gradient centrifugation, or "gradient differential centrifugation," as he prefers to call it (*1*).

In the work described here, the density of the particles was greater than the maximum density of the gradient column. If the maximum density of the column is greater than that of any of the particles, after sufficiently long centrifugation at high speed the particles will reach an equilibrium position in the tube where their density equals that of the suspending medium. This principle has been used to separate epinephrine-containing particles of the adrenal medulla from mitochondria (*2*); the two types of particles are similar in volume but differ in density.

Most of this work has been carried out with gradients prepared by successively layering 9-ml. portions of 30, 25, 20, 15, and 10% sucrose solutions into a 50-ml. plastic centrifuge tube (inside diameter 2.8 cm.). Each solution contained 0.0003*M* disodium (ethylenedinitrilo)-tetraacetate, pH 7.4, to decrease the possibility of agglutination by calcium ions. The tube was stored at 1° C., usually for 48 hours; during this time

there was sufficient diffusion between layers to form a continuous gradient. Actually any time between 24 and 120 hours seemed to be satisfactory. The details of preparation and layering of the tissue homogenate, centrifugation, removal of layers, and assays for various cellular components have been described (*8, 11*).

In more recent work (*10*), solutions of sucrose plus polyvinylpyrrolidone (PVP) have been used. The sucrose concentration was kept constant at 0.25*M*, while the polyvinylpyrrolidone concentration was varied from 6 to 18%. Because of its high molecular weight, polyvinylpyrrolidone does not appreciably alter the osmotic pressure of the medium. It has been recommended for use in preparation of cellular particulates for electron microscopy. In addition, extraction of enzymes from particulates is minimized by the use of polyvinylpyrrolidone. It has some disadvantages, however, in that assays for total protein are complicated by its presence, and its high viscosity necessitates the use of much higher centrifugal forces (20,000 × *g* instead of 2000 × *g*) to achieve the same rate of sedimentation observed in the sucrose gradient.

Calculation of Particle Sizes. The rate at which a particle sediments in a centrifugal field is given by:

$$\frac{dL}{dt} = \frac{1}{18}\frac{d^2\omega^2 L(\rho_p - \rho_s)}{\eta} \qquad (1)$$

where d is the particle diameter, ω is the angular velocity, L is the distance from the axis of centrifugation to the particle, ρ_p is the density of the particle, and ρ_s and η are the density and viscosity of the suspending medium.

In the presence of a concentration gradient, however, empirical correction factors must be introduced to correct for the changes in density and viscosity of the medium, and for the effect of changing osmotic pressure on the particle density and diameter. Thus;

$$\rho_s = \rho_o + \alpha(L - L_o) \qquad (2)$$

$$\eta = \eta_o + \beta(L - L_o) + \gamma(L - L_o)^2 + \delta(L - L_o)^3 \qquad (3)$$

$$\rho_p = \rho_o' + \epsilon(L - L_o) \qquad (4)$$

$$d = d_o[1 + \zeta(L - L_o) + \theta(L - L_o)^2] \qquad (5)$$

where ρ_o, η_o, ρ_o', and d_o are the density of the medium, viscosity of the medium, density of the particle, and diameter of the particle at a distance L_o from the axis of centrifugation to the meniscus. The coefficients in Equations 2 and 3 were obtained from measurements of the viscosities and densities of sucrose solutions at 1° C.; those for Equations 4 and 5 were estimated from data presented by Tedeschi and Harris (*7*).

Substitution into Equation 1 gives:

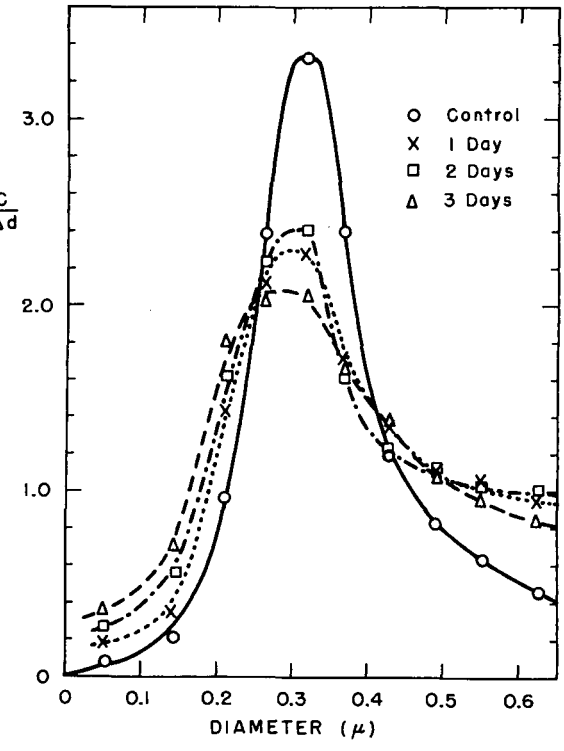


Figure 1. Effect of 600 r total-body x-radiation on intracellular distribution of cytochrome oxidase in rat thymus

Rats sacrificed 1, 2, and 3 days after irradiation. Fractionation carried out using sucrose-PVP gradients

$$\frac{dL}{dt} = \frac{\omega^2 L(d_o[1 + \zeta(L - L_o) + \theta(L - L_o)^2])^2}{18} \frac{([\rho_o' + \epsilon(L - L_o)] - [\rho_o + \alpha(L - L_o)])}{\eta_o + \beta(L - L_o) + \gamma(L - L_o)^2 + \delta(L - L_o)^3} \qquad (6)$$

This equation was integrated graphically, and for various combinations of ω and t the values of d_o were obtained for the 12 values of L chosen, the distances from the axis of centrifugation to the boundaries of the zones.

When polyvinylpyrrolidone was used to produce the gradient, the changes in osmotic pressure were negligible; further, the change in viscosity could be described adequately by a binomial rather than a trinomial. Thus Equation 6 simplifies to:

$$\frac{dL}{dt} = \frac{1}{18}\frac{d^2\omega^2 L(\rho_p - [\rho_o + \alpha(L - L_o)^2])}{\eta_o + \beta(L - L_o) + \gamma(L - L_o)^2} \qquad (7)$$

which is readily integrated to give:

$$d^2 = \frac{18}{\omega^2 t}\left[\frac{\eta_o - \beta L_o + \gamma L_o^2}{\rho_p - \rho_o + \alpha L_o} \ln \frac{L_n}{L_o} + \left(\frac{\eta_o - \beta L_o + \gamma L_o'}{\rho_p - \rho_o + \alpha L_o} + \frac{\beta - \gamma L_o}{\alpha} + \frac{\gamma(\rho_p - \rho_o)}{\alpha^2}\right) \ln \frac{\rho_p - \rho_o}{\rho_p - \rho_o - \alpha(L - L_o)} - \frac{\gamma}{\alpha}(L_n - L_o)\right] \qquad (8)$$

All the constants for evaluation of Equations 6 and 8 can be obtained with good accuracy except for the density of the particles. This problem is discussed elsewhere (*8, 10, 11*).

In this treatment it is assumed that the particles are spherical. This assumption is not completely warranted, as mitochondria are generally somewhat elongated. The departure from sphericity, however, is not so great that the error involved exceeds 5%. Particle sizes are presented as the equivalent spherical diameters—i.e., the diameter of a sphere which would have sedimentation characteristics identical with those of the particles actually studied.

On the basis of the sizes of particles predicted in each zone under the conditions employed, and of the concentration of a cellular component in each zone (after correction for side-wall sedimentation (*11*), one can construct curves relating the distribution of that component to the size of particles with which it is associated (*5*).

RESULTS

Observations on particulates of mammalian liver have been described in considerable detail (*4, 8–13*). The results may be summarized as follows. Distribution curves of various enzymes in rat liver are of at least four types. The first, typified by succinic dehydrogenase and cytochrome oxidase, shows maximum activity at an equivalent spherical diameter of 0.75 micron (mitochondria). The second, of which esterase is characteristic, is predominantly associated with particulates of about 0.12 micron diameter (microsomes). Bimodal distribution curves were obtained for diphosphopyridine nucleotide–cytochrome c reductase, arginase, and adenosine triphosphatase, the two maxima occurring at 0.12 and 0.75 micron. Finally, uricase and catalase are associated with particles of 0.44 micron diameter; this cyto-

plasmic component has not been adequately characterized cytologically. Whether it is identical with the "lysosome" fraction (*3*) is not certain; the distribution curve for acid phosphatase is similar to that of uricase (*12*) but not identical.

The principal usefulness of this method has been the comparison of tissue of normal animals with those that have been treated in various ways. Thus enzyme distributions in livers of rats have been studied after partial hepatectomy (*11*) or carbon tetrachloride treatment (*13*), livers of hypoxic guinea pigs (*4*), and livers of tumor-bearing mice (*9*). As an example of the applicability to other tissues, Figure 1 shows the distribution of cytochrome oxidase in the thymus of normal rats and rats sacrificed 1, 2, and 3 days after exposure to 600 r total-body x-radiation. The size associated with maximum activity was decreased slightly but consistently in the irradiated tissue, and the shape of the distribution curve was markedly altered. The mechanism by which x-radiation produces this change is not altogether clear.

DISCUSSION

Density-gradient centrifugation has provided a method for estimating the intracellular distribution of enzymes and other cellular components in terms of the size of the cytoplasmic particulates with which they are associated. Such a procedure obviates the necessity of assigning morphologic labels to subcellular fractions of known heterogeneity.

The method has certain drawbacks. Only a limited amount of material can be handled in one centrifuge tube; the author has used homogenate-gradient volume ratios of 1 to 10 or 1 to 12. It is obvious that the lower the ratio the better the resolution will be; and the smaller the amount of material available for analysis. The necessity for making certain assumptions about osmotic pressure gradients, sphericity of the particulates, particle density, and side-wall sedimentation has been mentioned. It is clear, also, that the homogenization process common to all differential centrifugation techniques, involving the disruption of cells by shearing and the liberation of cellular components into solutions of sucrose, is decidedly unphysiological.

The author has used the procedure to measure the sizes of polystyrene latex spheres of 0.5 to 3.0 micron diameter. These experiments, carried out in collaboration with H. E. Kubitschek of this laboratory, will be reported elsewhere; very close agreement has been obtained between sizes of particles estimated by this procedure with those measured optically.

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RECEIVED for review December 29, 1958. Accepted February 5, 1959. Based on material presented before the Division of Analytical Chemistry, Symposium on Separation Processes through Differential Migration Analysis, 134th Meeting, ACS, Chicago, Ill., September 1958. Work performed under the auspices of the U. S. Atomic Energy Commission.

Isolation of Carotenoids, Coumestrol, Chlorogenic Acid, and Antibiotics

Application of Countercurrent Distribution

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▶ **The application of countercurrent distribution technique to biologically active materials is reviewed briefly and examples showing its uses are given. These include carotenoids, isolated from oranges; a plant estrogen, coumestrol, isolated from Ladino clover; an isomer of chlorogenic acid from peaches; and the antibiotics, aterrimins, from *Bacillus subtilis* var. *aterrimus*.**

THE isolation and identification of small amounts of biologically active constituents from plant or animal materials by the classic methods of extraction, precipitation, and crystallization are often next to impossible. Chromatography, or selective adsorption in the wider sense—column, paper, ion exchange—has served admirably with some substances, but the separation of similar entities by these techniques is often unsuccessful.

Countercurrent distribution (CCD) may often accomplish the separation and, by prior knowledge of the different components' behavior, also serve to identify each. This operation has been practical only recently, since the development of integrated and automatic equipment for multiple transfers. Hundreds or even thousands of individual equilibrations are now possible within a few hours or days. This makes the high resolving power of the method usable for many separations and analyses. Craig and Craig (*4*) have been largely responsible for much of the development in this field.

Compounds that have been resolved by countercurrent distribution include various glycerides in natural fats, steroid hormones, amino acids, protein fragments, organic acids, dyes, and chemical intermediates (*10*).

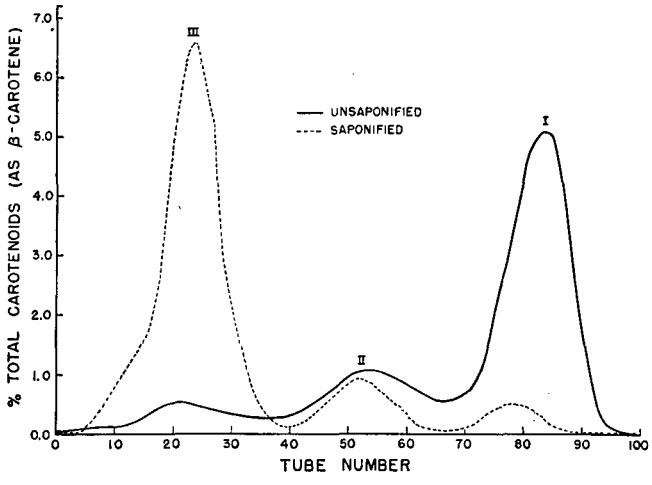


Figure 1. Countercurrent distribution of carotenoids from Valencia orange juice
System. Skellysolve B–absolute methanol

Color was measured at 440 mμ and results calculated from conversion table for β-carotene

ADVANTAGES AND LIMITATIONS OF METHOD

In both countercurrent distribution and partition chromatography, a moving phase containing the material to be fractionated is passed along a stationary phase at such a rate that equilibrium conditions are approached. In countercurrent distribution a stepwise transfer of the moving phase occurs; thus it can be thought of as "discontinuous partition chromatography." The lower phase is stationary, while the upper phase moves in discrete steps as rapidly as the two phases will separate after each contact. The equilibrium distribution of each solute between the two phases determines its retardation in passing through the train of tubes and consequently its final position at the end of a distribution run.

Countercurrent distribution is especially well adapted to the handling of unstable compounds. Changes in acidity or alkalinity are unnecessary. Any reagent added can be removed easily, usually under mild conditions. Practically everything can be recovered. If a particular solvent system fails to give the required separation, it can be evaporated and another tried. Although prior knowledge of the nature of the compound sought is unnecessary to effect the separation, knowledge of some property (chemical, physical, or biological) is required to locate the active fraction after separation.

Some obvious difficulties are encountered. Poorly soluble, highly polar, or extremely nonpolar compounds may cause trouble. Proteins having molecular weights in excess of 6000 have not been successfully distributed. Selection of the proper solvent system is often the most difficult problem. A solvent system that breaks into two phases quickly is very desirable. Emulsions can be very troublesome; they may slow the operation or render it impossible. Temperature changes may alter distribution coefficients markedly; for best results a constant temperature room is recommended.

Solubility data may be misleading for use in predicting distribution of a solute between two phases. Distribution in a partially saturated solution may be different from that in a nearly saturated solution. If a particular solvent system does not separate a pair of desired components completely, the initial distribution is carried out and all material is discarded except the two wanted fractions. The tubes are refilled and the last tube is connected to the zero tube. The system can now be recycled almost *ad infinitum* until the separation is achieved. This method is economical of solvents, requiring only the initial charging. Solvents must be free of residue or freshly redistilled. An excellent summary of mathematical models used in countercurrent distribution studies is presented by Weisiger (*10*).

Workers in this laboratory have made extensive use of countercurrent distribution in separating carotenoids, (*5–7*), a plant estrogen (*1, 2*), chlorogenic acid (*3*), and antibiotics (*8*).

CAROTENOIDS

Carotenoids have been separated and studied for years by column chromatography Discrete fractions have been recovered by selective elution or extrusion of the column and separate elution of carved-out portions. Much was accomplished by these techniques, but separation of carotenoids having two, three, and more hydroxyl or epoxide groups per molecule was less successful. The carotenes (hydrocarbons) are readily separated by chromatography after the xanthophylls are removed by a separation with petroleum ether and 90% methanol. The chromatographic separation of the xanthophylls is much less satisfactory. It was found that countercurrent distribution could be used to separate the xanthophylls into a number of fractions, which were then much more readily separable by chromatography. Therefore, countercurrent distribution has been exploited in studies of these pigments of citrus fruits and other plant products (*5*). The method has an additional advantage, in that the appearance of a particular pigment in a given fraction suggests the nature of the functional groups.

A simple system of methanol–Skellysolve B gave some fractionation of unsaponified carotenoids of orange juice (Figure 1); the saponified carotenoids were separated clearly into three portions. Before saponification, carotenes and fully esterified xanthophylls were found in fraction 1; after saponification, only carotenes remained. Fraction II, after saponification, contained monohydroxy compounds such as cryptoxanthin. Fraction III contained di- and polyhydroxy carotenoids and increased markedly as a result of saponification.

In these trials, samples containing up to 100 mg. of total carotenoids were used for distribution in the countercurrent distribution apparatus; 10 ml. of each solvent phase per tube was used. A larger amount of sample could have been used with the methanol–Skellysolve B solvent pair, because the phases separated easily, but other solvent systems emulsified badly when too much crude material was distributed.

With benzene–Skellysolve B–87% methanol a much more complete resolution was obtained (*5*) (Figure 2). The carotenes, cryptoxanthin, and a hydroxy-α-carotene-like substance were carried nearly to the last tube (fractions I and II). Fraction IIIA was shown by further chromatographic and spectrophotometric studies to be lutein and zeaxanthin, while IIIB was shown to contain five separate compounds—two isomers of antheraxanthin and three isomers of mutatoxanthin. Fraction IIIC contained two isomers of violaxanthin, and three of luteoxanthin, plus auroxanthin. The slowest-moving fraction, IIID, contained valenciaxanthin, sinesiaxanthin, two isomers of trollixanthin, valenciachrome, and trollichrome. Thus the primary separation of a very complex mixture was achieved by countercurrent distribution.

The technique was applied to elm leaves with similar results. With leaf extracts the unsaponified extract caused too much emulsion for countercurrent distribution, except with Skellysolve B–99% methanol. However, in this system chlorophylls obscured and contaminated the carotenoids. The presence of colorless hydrocarbons and alcohols also caused emulsions, but after saponification and partitioning between petroleum ether and 90% methanol the hypophasic fraction was distributed successfully (Figure 3). The diols were similar to those of orange juice, as was the small amount of monoether diols and the diether diols. The polyols were neoxanthin and isomers.

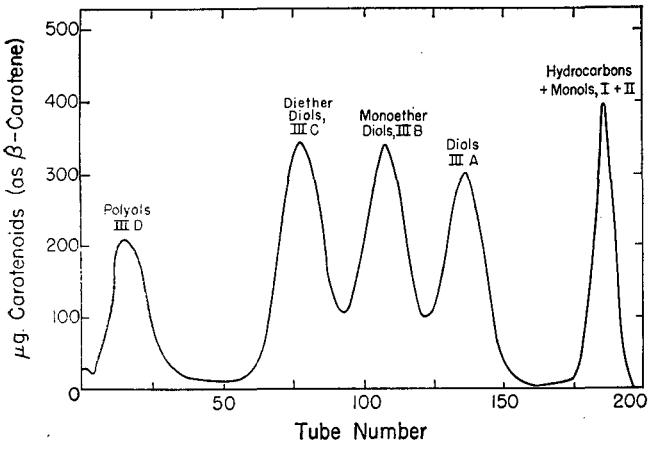


Figure 2. Countercurrent distribution of carotenoids from Valencia orange juice
System. Skellysolve B–benzene–87% methanol

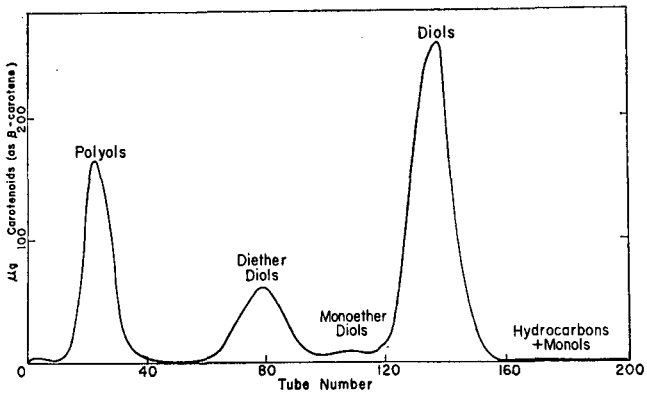


Figure 3. Countercurrent distribution of hypophasic carotenoids from elm leaves
System. Benzene–Skellysolve B–87% methanol

Table I. Solvent Systems Used in Isolating an Estrogen

Solvent System	Solvents and Proportions by Volume	No. of Transfers	Position of Estrogen in Tubes
A	Acetone–ether–water–Skellysolve B (10:5:5:2)	100	69–90
B	Chloroform–carbon tetrachloride–methanol–water (2:2:3:2)	100	56–80
C	Methanol–benzene–ether–water (4:4:1:1)	100	30–60
D	Skellysolve B–ethyl acetate–methanol–carbon tetrachloride–water (1:1:1:1:1)	100	25–58
E	Acetone–carbon tetrachloride–water (2:1:1)	100	40–76
F	Acetone–carbon tetrachloride–water–methanol (10:5:5:1)	280	30–60

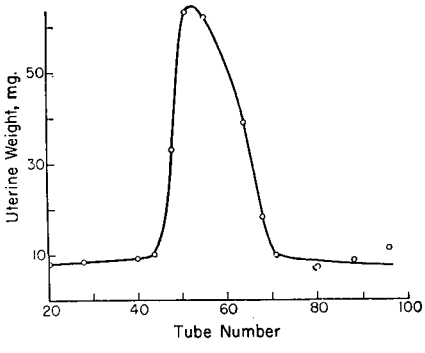


Figure 4. Countercurrent distribution of coumestrol from Ladino clover

COUMESTROL

Reports of abnormal response in animals pastured on Ladino clover have described nonnormal lambing in ewes, increased rate of gain in fattening lambs, and stimulated milk production in dairy cows. These symptoms suggested presence of an estrogen in the plants.

Assays employing immature female mice indicated high estrogenic activity in the clover. Fractionation of the plant material yielded a lipide extract which contained the estrogen, a very forbidding greenish black tarry mass.

A preliminary purification of this crude concentrate was accomplished by extraction with dilute aqueous alkali, acidification of the aqueous extract, and re-extraction of the acid solution with ether. This treatment removed the great bulk of fat-soluble impurities and resulted in about 100-fold concentration of the estrogenic activity. However, the estrogen still represented only a few per cent of the dried weight of this purified concentrate.

Application of countercurrent distribution was of utmost importance in finally obtaining a pure crystalline product. Successive application of five separate solvent systems was necessary before the pure material was obtained (Table I). As much as 100 grams of concentrate was distributed in the large 100-tube countercurrent distribution apparatus which holds 100 ml. of each solvent phase per tube.

To locate the position of the estrogen after a distribution, the contents of selected tubes taken at intervals were evaporated to dryness, and a weighed aliquot was fed to immature mice. The increase in uterine weight plotted against the corresponding tube number of the fraction tested located the estrogen. A typical assay curve with solvent B is shown in Figure 4.

Discovery that the estrogenic compound exhibited a strong bluish fluorescence under ultraviolet light aided the work immeasurably. Small samples from the countercurrent distribution apparatus were observed after migration on chromatostrips (*9*) or paper chromatograms.

Following isolation of the estrogen it was characterized and synthesized. The name coumestrol was assigned to the compound because of its coumarin-like structure, which differs from that of any of the previously isolated plant estrogens. It is about 30 times as estrogenic as the previously isolated plant estrogen, genistein.

CHLOROGENIC ACIDS

In studies of the enzymatic browning of fruits a new isomer of chlorogenic acid, "neochlorogenic acid," was isolated from peaches (*3*) by countercurrent distribution. A butanol extract of lyophilized peach puree was distributed between ethyl acetate and 2*M* phosphate buffer, pH 3.0. Four fractions were obtained. The first consisted of anthocyanins plus polymeric materials; the second, neochlorogenic acid; the third, chlorogenic acid; and the fourth, polymeric unknowns. Removal of sodium ions on an ion exchanger caused much loss of the neochlorogenic acid, but enough remained to obtain physical constants. This compound is thought to be coupled through the 1- or 4-position on the carboxylated ring.

ANTIBIOTICS

Countercurrent distribution has also been employed to isolate two antibiotics from bacterial cultures. A pure material, Subtilin A, has been obtained from the peptide complex recognized as Subtilin some years ago. Another antibiotic was obtained from *Bacillus subtilis* var. *aterrimus* and named "aterrimin" (*8*). Two compounds were recognized

in this complex as nonnitrogenous unsaturated lactones. Crude preparations of aterrimin are being sold for use in poultry feeds.

These examples illustrate the value of countercurrent distribution in separating widely different, biologically active materials from plant and animal sources. As information accumulates, its use will, no doubt, be expanded to serve many other purposes as well.

ACKNOWLEDGMENT

The authors are indebted to J. W. Corse and J. C. Lewis for aid in assembling data on chlorogenic acid and antibiotics.

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RECEIVED for review November 6, 1958. Accepted March 6, 1959. Mention of specific products does not imply endorsement by the Department of Agriculture.

Examination of Oxyacids of Phosphorus by Electrochromatography

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▶ **Differential electrical migration in moist paper is an effective method for resolution of mixtures of the oxyacids of phosphorus. Certain separations, such as the isolation of pyrophosphate, have been improved by addition of chemical precipitants, as zinc salts, to the background electrolytic solution. The addition of radioactive tracers before migration and the use of neutron activation after migration have facilitated the location and estimation of the various oxyacids of phosphorus separated in the moist paper. Migration sequences, mixed migrations, and comparative migrations in the same sheet of paper have provided a convenient basis for identification of the acids separated from mixtures. Some common oxyacids of phosphorus separated by a single migration in acetic acid are, in order of decreasing mobility, trimetaphosphate, tetrametaphosphate, tripolyphosphate, pyrophosphate, hypophosphorous, phosphorous, phosphoric, and condensed phosphoric acids. This sequence also varied with variation of the solvents. The technique has been applied in tests for the purity of radioactive phosphorus tracers, and for detection of the alterations of phosphorus compounds exposed to heat or neutrons.**

BECAUSE OF the importance of phosphorus compounds in nature (*3, 36, 60, 65*), analysis of mixtures of the oxyacids of phosphorus is essential to many nuclear (*32*), chemical (*19*), and biological investigations (*21, 26, 68*). Many chemical procedures that have been devised for the detection (*7, 11, 28, 43, 69*), estimation (*8, 9*), and separation (*27, 35, 38, 46*) of the phosphorus oxyacids, including colorimetry (*20, 34, 37*), titrimetry (*2, 22, 44, 63*), and precipitation (*35, 42–44, 66*), are not widely applicable to complex mixtures, particularly to those containing interfering substances. Differential migration methods such as paper chromatography have served for the examination of various oxyacids (*1, 13, 31*), some condensed acids (*10, 15–17, 29, 59, 67*), and certain phosphate esters (*23*), especially when supplemented by sensitive colorimetric methods for the detection of the separated substances (*24*). Many of these applications of the paper chromatographic method have been summarized recently by Hettler (*24*). Anion exchange chromatography has also been utilized for the separation of many phosphorus compounds in acid solutions (*6, 12, 25, 33*). The assay of phosphorus compounds from physical properties such as x-ray diffraction (*41, 45*), viscosity (*14, 58, 61*), optical properties (*4, 62*), melting point (*40, 70*), and ultracentrifugation (*30*) does not permit resolution of the mixtures. With the exception of chromatography (anion exchange or paper), these methods provide very little information about the unknown constituents of mixtures or about the minor constituents.

Promising separations of various oxyacids of phosphorus have been obtained by differential electrical migration in lactic acid solution stabilized with soft filter paper (*39*). This method has served for the examination of the neutron-activated acids (*53, 57*), for separation of phosphate, pyrophosphate, phosphite, and hypophosphite (*55*), condensed phosphates from radioactive (tracer) phosphate ($P^{32}O_4$) (*50*), and esters (*47, 64*), and for demonstrating that slowly migrating condensed phosphates are formed by the neutron irradiation of phosphate (*51, 52*), by γ-ray irradiation of phosphate, and by heating phosphate (*53*). The electrical mobility of the oxyacids varied with the hydrogen ion concentration (*18, 48*), buffer (*48*), and electro-osmotic transport (*18*).

As an extension of earlier studies (*49, 50, 55, 57*), electrochromatography has now been modified in several ways both for examination of the simple oxyacids of phosphorus and for examination of the complex condensed phosphoric acids. The distance of the electrical migration has been made to exceed that usually produced by flow of solvent in conventional paper chromatography. This more extensive migration has facilitated the separation of the slowly migrating components of the mixtures. The background electrolytic solution has been varied and reagents have been added to improve certain separations and to stabilize some of the condensed phosphoric acids. Some of the reagents have been added as zones in the migration system (*57*), so that they transmit certain acids of phosphorus but retain others. These modifications of the method have made possible the separation and detection of

various condensed phosphoric acids for which there are, as yet, no specific physical or chemical tests. These methods have facilitated the separation of the radioactive acids which were present in "carrier-free" condition as well as those present in larger quantities.

Because of the wide applicability and the great sensitivity of these electrochromatographic methods for examination of the oxyacids of phosphorus, the principal analytical techniques are described herein. Special applications of the techniques are being studied.

EXPERIMENTAL

In the author's electrochromatographic procedure, the mixtures of the phosphorus compounds were resolved by differential electrical migration from a narrow zone in moist filter paper. The resolved substances, separated as a series of zones, were located by spraying with molybdic acid followed by exposure to ultraviolet light (*5*) or by exposure of the paper to neutrons (*55, 69*) followed by scanning with a Geiger-Müller counter or by radioautography (*50*). The quantity of each component was estimated by counting techniques, and the individual constituents were recovered by elution of the active regions of the paper with water. The substances separated electrochromatographically were identified by comparison with authentic compounds submitted to electrical migration in the same sheet of paper.

For the determination of the migration behavior of the various phosphorus compounds, an apparatus was devised and the commercial filter paper purified by washing with acid (*54*). The filter paper sheets for the migrations were about 6 feet long (2 meters). For the migration studies, the paper was first moistened with 0.1*M* acetic acid. Aqueous solutions of the compounds (about 50 μl. of about 0.01*M* solutions) were placed in small zones on spots near the cathode end of the moist paper. The paper was then covered with the polyethylene sheets, and direct current potential, either 5 volts per cm. for 17 to 20 hours or 10 to 15 volts per cm. for 2 to 4 hours, was applied. The ends of the paper were then cut off, and the paper was uncovered and allowed to dry in air.

For the detection and location of the radioactive products, radioautographs were made with Kodak No Screen x-ray film. The separated substances were identified by reference to the migration or sequence of separate spots of authentic nonradioactive preparations, which were located by neutron activation, or alternatively, by the addition of radioactive tracers before the migration. For activation, the dried paper was placed on a sheet of polyethylene, and the two sheets were rolled together, providing a cylinder about 2 inches in diameter. This rolled paper was wrapped in another sheet of polyethylene and placed in the thermal column of the reactor for 2 days at a neutron flux of approximately 10^{12} neutrons per sq. cm. per second. The paper was then removed from the reactor and permitted to "cool" for some 7 to 14 days to reduce the background of the paper itself. It was then radioautographed, using Kodak No Screen x-ray film. With this activation technique, the lower limit of detectability of phosphorus in the paper was found to be 0.001 γ of phosphorus in 50 μl. of solution.

RESULTS AND DISCUSSION

Studies of the migration of phosphorus compounds were complicated by the complexity of the oxyacids of phosphorus. With respect to the state of oxidation, these acids fall into two principal groups, fully oxidized and partially reduced. Within each group the acids may be classified with respect to the degree and nature of condensation (Table I). Sequences and separability were established with authentic,

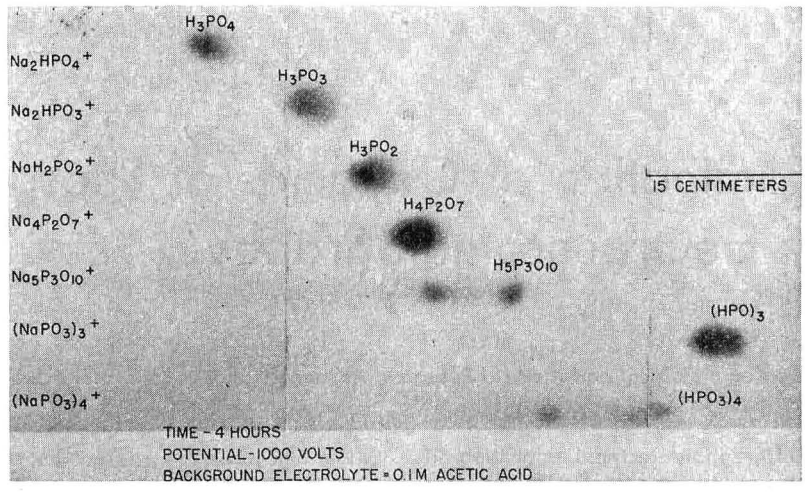


Figure 1. Radioautograph illustrating comparative electrochromatographic migration of oxyacids from preparations of inactive Na_2HPO_4, Na_2HPO_3, NaH_2PO_2, $Na_4P_2O_7$, $Na_5P_3O_{10}$, $(NaPO_3)_3$, and $(NaPO_3)_4$

The inactive substances in the electrochromatogram were activated by exposure of the paper to neutrons, and the radioautograph was made of this activated electrochromatogram. Background solution 0.1*M* acetic acid, time 20 hours, d.c. potential about 5 volts per cm.

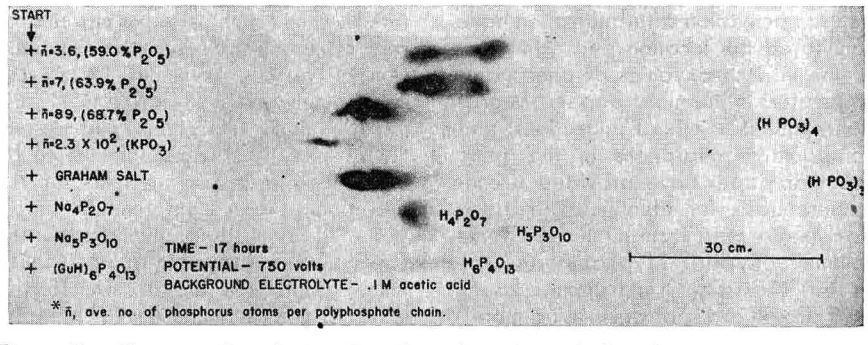


Figure 2. Comparative electromigration of condensed phosphates

All condensation products except that from KPO_3 and guanidine (Gu) tetrapolyphosphate were formed by heating sodium salts with varying ratios of Na_2O and P_2O_5

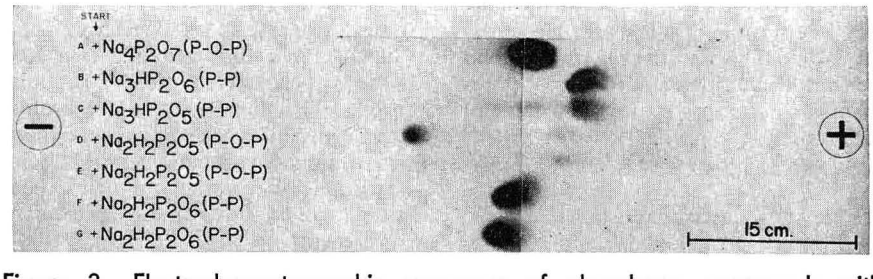


Figure 3. Electrochromatographic sequences of phosphorus compounds with P—O—P and P—P bonds

Time 17 hours. Potential 750 volts. Background electrolyte 0.1*M* acetic acid

nonradioactive phosphorus compounds which were submitted to electrical migration and located by autoradiography after neutron activation (Figure 1). Some of the very complex condensation products with P—O—P bonds (Figure 2) and some compounds with P—P bonds (Figure 3) also exhibited characteristic migration rates. These differential migration rates serve for the separation of mixtures of many of these compounds. There was no simple relationship between the molecular structure of the oxyacids and their separability by electrical migration.

Even though certain compounds migrate at nearly the same rate—e.g., pyrophosphate and tripolyphosphate—a mixture of the two may be resolved by addition of a suitable reagent, such as zinc ions, to the migration medium (*57*). For example, with adequate concentrations of zinc ions (0.05*M*), labeled pyrophosphate, prepared by neutron irradiation of the sodium salt (*49*), remained at the starting point, as shown in Figure 4. But at lower concentrations, the radioactive zone migrated slowly.

Various oxidation products of neutron-activated yellow phosphorus were separated by electrochromatography (Figure 5). They were identified by parallel migrations of authentic, nonradioactive preparations which were then activated and located by radioautography. As shown by the figure the principal oxidation products were H_3PO_4, H_3PO_3, H_3PO_2, and a nonmigrating substance with the properties of red phosphorus.

Through the use of labeled preparations, the concentration ranges at which the separations were effective were determined. The maximum concentrations were about 0.1*M*, or roughly equal to the ionic concentration of the background solution (*56*). With 50 μl. of 0.05*M* solution of phosphate, the zone contained 8×10^{-8} gram of phosphorus.

The minimum concentrations at which separations were effective lay below the limits of the detectability of the phosphorus compounds. With carrier-free tracers, about 3.5×10^{-14} gram of phosphorus-32 in 50 μl. could be detected by scanning after the migration. With nonradioactive phosphorus compounds, about 1×10^{-9} gram of phosphorus could be detected by radioautography after migration and neutron activation of the electrochromatogram.

For the separation of larger quantities of chemical substances, the apparatus was modified through the use of thick pads of paper. By using these, calcium and phosphate ions have been separated in some 4 or 5 ml. of 0.05*M* solution (*50*).

Electrochromatographic analysis revealed that carefully prepared preparations of phosphorus compounds were sometimes mixtures of several substances (*52*). As an example, a sample of highly radioactive, carrier-free orthophosphate obtained from Oak Ridge National Laboratory yielded pyrophosphate and a nonmigrating component in addition to phosphate. The separation of these components is illustrated in Figure 6. A zone trailing the phosphate zone has also been observed in other preparations (*50*).

Electrochromatographic analysis revealed that great care must be exercised

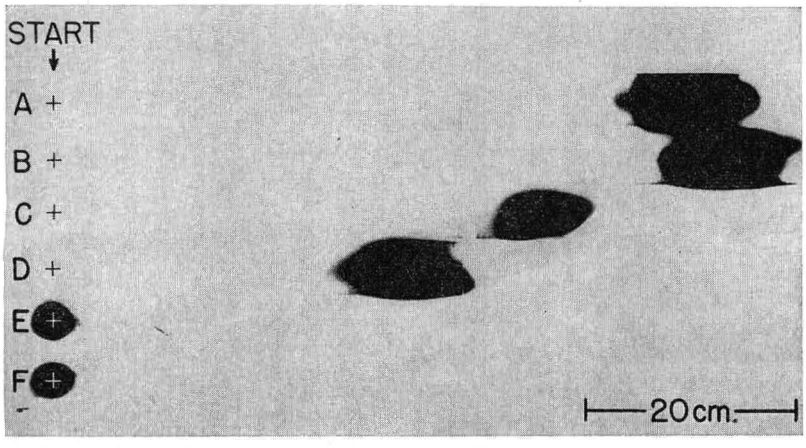


Figure 4. Radioautograph of electrochromatogram showing migration of pyrophosphate ions in the background electrolytic solution of lactic acid plus various concentrations of zinc ions

Each of the narrow paper strips contained 50 μl. of 0.1*M* radioactive phosphorus $Na_4P_2O_7$; the background solution consisted of 0.1*M* lactic acid plus zinc acetate: *A*, none; *B* 0.001*M*; *C*, 0.05*M*; D, 0.01*M*; E, 0.5*M*; F, 0.1*M*. Time 18 1/2 hours. Potential 500 volts per 100 cm.

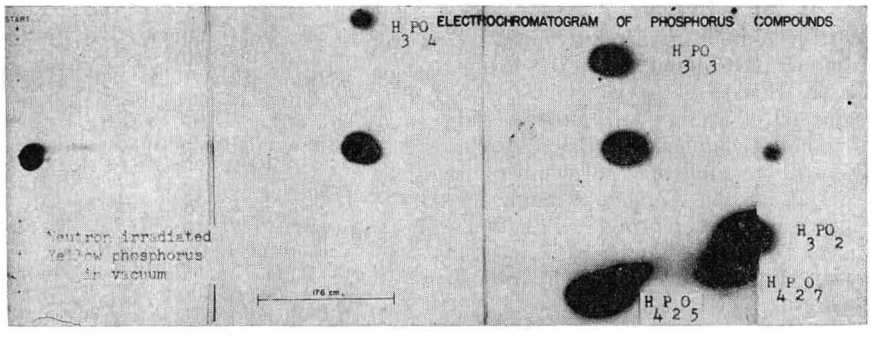


Figure 5. Radioautograph illustrating comparative electrochromatographic migration of acids from authentic preparations of H_3PO_4, Na_2HPO_3, NaH_2PO_2, $Na_4P_2O_7$, $Na_2H_2P_2O_5$, $Na_2H_2P_2O_6$, and of neutron-irradiated yellow phosphorus dissolved in 0.1*M* HNO_3

The zones of the authentic phosphorus compounds were located after activation of the electrochromatogram with neutrons. Background solution 0.1*M* acetic acid, time 20 hours, d.c. potential about 5 volts per cm.

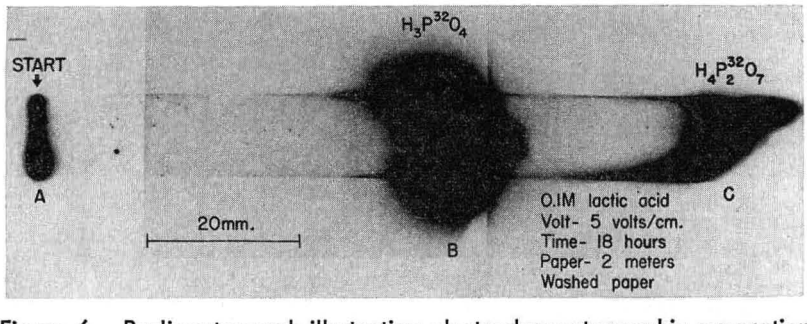


Figure 6. Radioautograph illustrating electrochromatographic separation of carrier-free radioactive phosphate from Oak Ridge National Laboratory

Unidentified product *A* remained at starting point. *B* was subsequently identified as $H_3P^{32}O_4$ and C as $H_4P_2{}^{32}O_7$

Table I. Oxyacids of Phosphorus Classified with Respect to Oxidation State and Degree of Polymerization

A. Completely oxidized forms
1. Monomolecular phosphates
   Orthophosphate, H_3PO_4
   Metaphosphate, HPO_3
2. Condensed linear phosphates (P-O-P bonds)
   Pyrophosphate, $H_4P_2O_7$
   Tripolyphosphate, $H_5P_3O_{10}$
   Tetrapolyphosphate, $H_6P_4O_{13}$
3. Condensed cyclic phosphates (P-O-P bonds)
   Trimetaphosphate, $(HPO_3)_3$
   Tetrametaphosphate, $(HPO_3)_4$

B. Reduced forms
1. Monomolecular oxyacids
   Hypophosphite, H_3PO_2
   Phosphite, H_3PO_3
2. Condensed oxyacids (P-O-P bonds)
   Pyrophosphite, $H_4P_2O_5$
   Isohypophosphate, $H_4P_2O_6$
3. Condensed oxyacids (P-P bonds)
   Hypophosphate, $H_4P_2O_6$
   Diphosphite, $H_4P_2O_5$

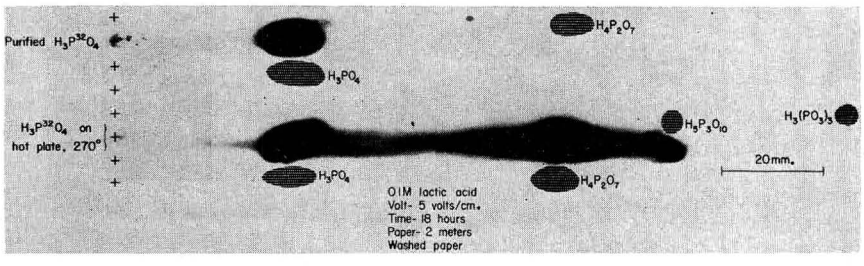


Figure 7. Radioautograph illustrating electrochromatographic examination of $H_3P^{32}O_4$ purified by ion exchange and heated in a beaker on a hot plate at 270° for 0.5 hour

Dark areas represent locations of radioactive products. Hatched circles represent positions of authentic acids of phosphorus added as 0.01 M solutions of their salts and located with molybdate reagent after the migration

in the preparation and handling of the oxyacids of phosphorus. When solutions of radioactive phosphate, purified by anion exchange chromatography, were evaporated, even a very short overheating of the residue produced a variety of condensation products. These products, separated and identified by differential electrical migration, proved to be pyrophosphate and other condensation polymers (Figure 7).

These investigations show that electrochromatography is a remarkably sensitive and effective technique for the examination of oxyacids of phosphorus. It serves for the resolution of mixtures, purification of tracers, identification of the various substances, and detection of the reactions produced by various conditions, such as heat, oxidation, and neutron irradiation.

ACKNOWLEDGMENT

The author expresses appreciation to H. H. Strain for advice and encouragement in the development of these methods. These studies were also aided in a material way by John R. Van Wazer, Peter G. Arvan, and Edward J. Griffith, Monsanto Chemical Co.; by Russell N. Bell and Lowell E. Netherton, Victor Chemical Works; by Oscar T. Quimby, Procter and Gamble Co.; Therald Moeller, University of Illinois; by Ernest Leininger, Michigan State University; by B. Raistrick, Scottish Agricultural Industries, Ltd., Edinburgh, Scotland; and by Bruno Blaser, Henkel Co., Düsseldorf, Germany, all of whom contributed special phosphorus compounds in this research. The author is indebted to members of the staff at the Argonne Reactor for supervising the neutron irradiations and to Thomas Doody for assistance in constructing a vacuum dehydration apparatus.

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RECEIVED for review January 12, 1959. Accepted February 26, 1959.

Separation of Antibodies by Starch Zone Electrophoresis

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▶ **Rabbit antisera against sheep red cells were fractionated by starch zone electrophoresis to ascertain the distribution of antibody in the serum proteins and ascertain if the number of antibody species was dependent on the immunization schedule. In antisera obtained early in immunization the antibody activity migrated as a single peak between β- and γ-globulins and sedimented with a globulin fraction of molecular weight about 1,000,000; late in immunization a second antibody migrated in the more slowly moving γ-globulins and sedimented with a fraction of molecular weight about 160,000. Physically distinct antibodies of the same serological specificity may be induced by a given antigen and the ratio in which they appear is a function of the immunization schedule.**

ZONE ELECTROPHORESIS in a starch-supporting medium is useful in resolving a complex mixture of proteins into its individual components. The chief advantages of the method over other preparative electrophoretic methods are: the relatively large amounts of material that can be separated in a single operation, and the comparative ease and simplicity of the experimental procedure involved. As with all electrophoretic methods, the degree of resolution attainable depends on the nature of the proteins involved and, particularly, the net electrical charge that these bear in a buffer of given pH and ionic strength. Since its introduction by Kunkel and Slater (*4*) to study the lipoproteins of blood serum, the method has been used in the analysis of virus inhibitors (*10*), bacterial enzymes (*6*), antibodies against diphtheria toxoid (*2*), serum proteins (*1*), and many others.

The work reported here involved the characterization of rabbit antibodies against sheep red blood cells formed after immunization with sheep red cells or other material such as guinea pig kidney. These antibodies, hemolysins, have the property of lysing sheep red cells when mixed with complement, a normal serum component. Hemolysins are particularly useful in immunological studies, because the amount of hemoglobin released by the lysed red cells can be measured accurately and conveniently by photometric methods. In general, hemolysins of two types occur; one has a molecular weight of

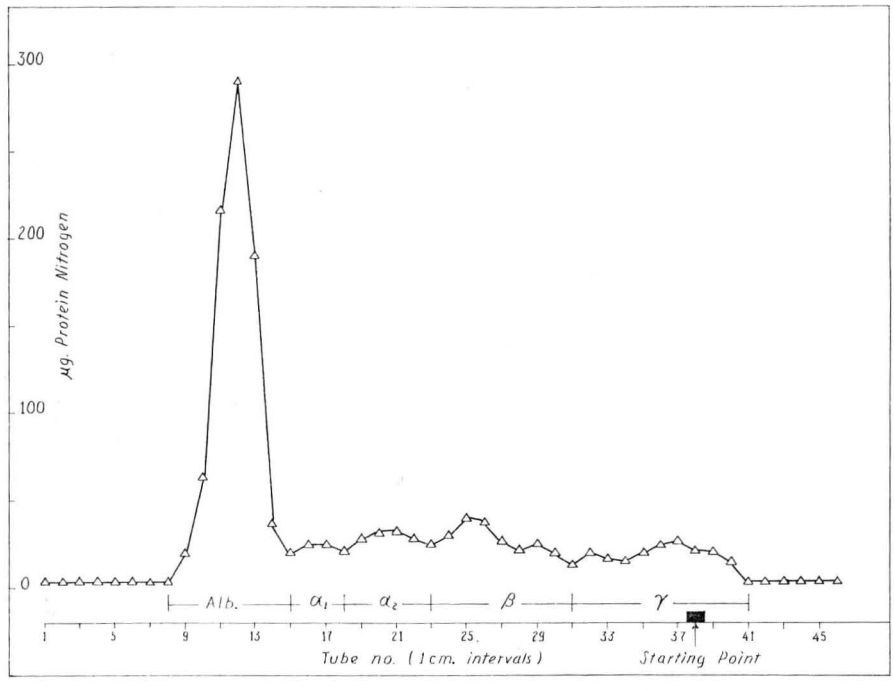


Figure 1. Starch zone electrophoresis of a normal rabbit serum

In this and all other figures electrophoresis was performed in pH 8.6 veronal buffer, 0.1 ionic strength (7)

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about 1,000,000 and is highly hemolytic, whereas the other is a smaller globulin of ordinary size (about 160,000) and is poorly hemolytic. The latter is often measured by its ability to combine with the red cell, as expressed in 50% combining units (see Figure 3). The purpose of this work was twofold: to ascertain the electrophoretic distribution of antibody activity in the serum proteins, and to ascertain whether antisera obtained during the early and late periods of immunization contained the same number of antibody species. The results illustrate the applicability of starch zone electrophoresis to a particular problem. It is hoped, however, that they also illustrate the generality of the method to problems involving the separation of biologically active substances in complex mixtures.

RESULTS AND DISCUSSION

The procedures used in preparing early and late rabbit antisera against various antigens and the measurement of antibody activity have been described (*7–9*). The lytic activity of antibody is defined in terms of 50% hemolysin units. One 50% hemolysin unit is that amount of serum or serum fraction which, in the presence of four 50% guinea pig complement units, will lyse 50% of a standardized number of red cells in 30 minutes at 37° C.

All starch zone electrophoresis experiments were performed in pH 8.6 veronal buffer, 0.1 ionic strength. The apparatus used differs only in minor respects from that described by Kunkel and Slater (*3, 4*). In a typical experiment, about 2 ml. of undiluted serum were mixed with dry potato starch to form a thin slurry and were carefully poured into a rectangular slit cut transversely 8 cm. from one end of a starch block 46 × 18 cm. in area and about 0.5 cm. thick. The starch block was equilibrated with buffer for about 2 hours at 5° C., after which an electromotive force of 300 volts was applied for 48 hours. At the end of electrophoresis the starch block was allowed to dry partially and was partitioned into 1-cm. segments. Each segment was eluted with veronal buffer and the eluates were tested for protein content by Lowry *et al.* (*5*) method and for antibody activity. Protein recovery ranged from 90 to 102% of the starting material, and antibody recovery from 70 to 95%.

Figure 1 shows the results with a normal rabbit serum. As compared to moving boundary electrophoresis of the same serum, the starch method yielded a greater number of subfractions and higher percentage values for γ-globulin and lower ones for albumin. The first difference is undoubtedly a result of the longer migration path of the starch block as compared to the path length of the Tiselius cell. The second difference

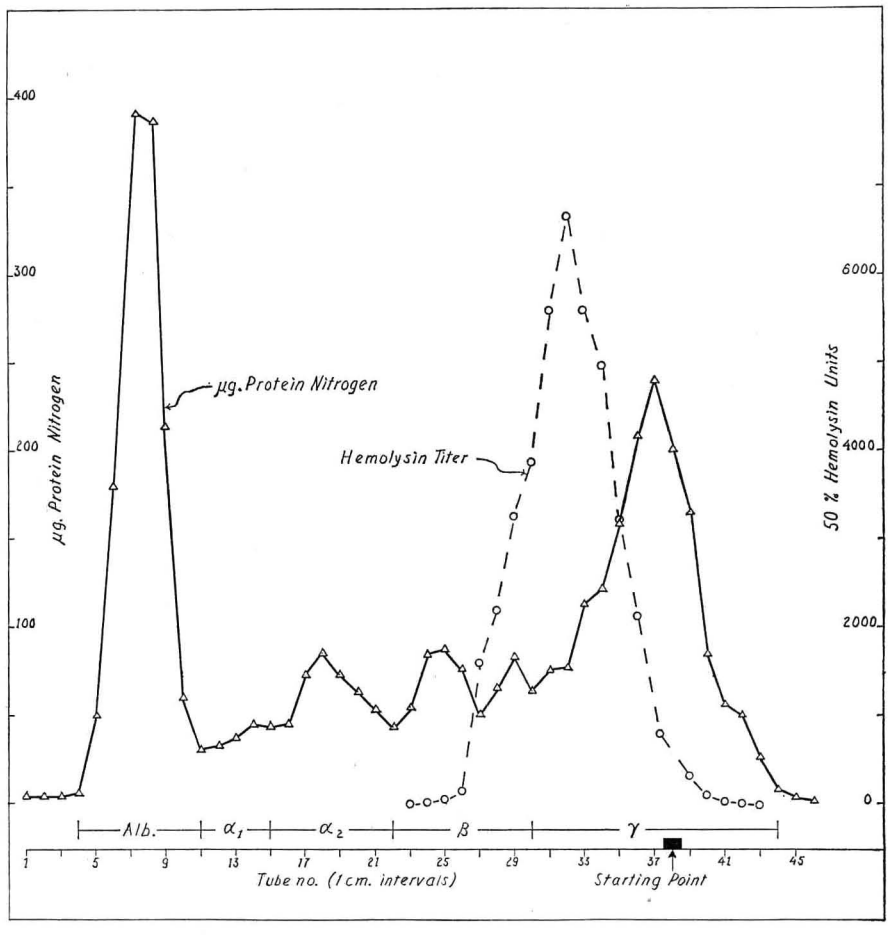


Figure 2. Starch zone electrophoresis of an antiserum against sheep red cells obtained during early stage of immunization

Antibody activity represented by a single peak centered between β- and γ-globulin fractions (7)

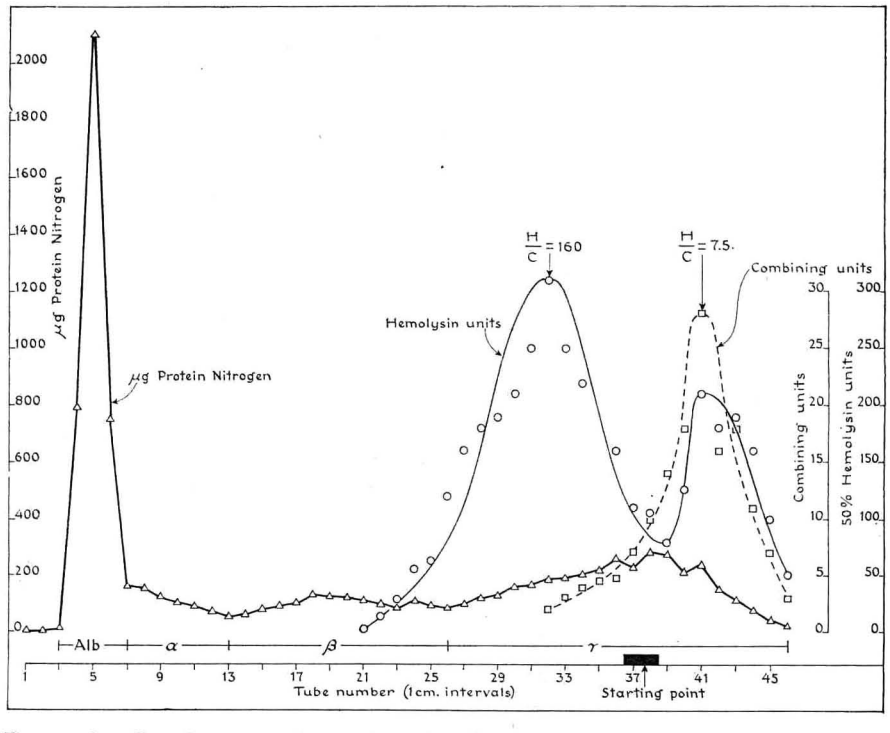


Figure 3. Starch zone electrophoresis of antiserum against sheep red cells obtained during late stage of immunization

Two peaks of antibody activity. Curve marked combining units parallels distribution of second antibody and indicates that two antibodies are serologically very closely related (9)

reflects the fact that all serum proteins do not contain the same number of tyrosine and tryptophan residues and hence would not be expected to give equal color reactions with the phenol reagent used in determining the amount of protein.

Figure 2 illustrates the distribution of antibody activity in a rabbit antiserum obtained during the early stage of immunization against sheep red cells. The salient features are: the single, symmetrical peak of antibody activity which was distributed between the β- and γ-globulins, and the peak of antibody activity which was not associated with a corresponding peak of protein concentration. The first point is significant because it demonstrates that an antibody may be distributed in more than one globulin fraction simply because its electrophoretic distribution overlaps neighboring fractions. The second point shows that the antibody activity was found in only a small fraction of the total protein in spite of the great increase of the γ-globulin fraction. This conclusion was further supported by the observation that absorption with specific antigen removed only about 0.2% of the total protein (*7*).

During the late stage of immunization against sheep red cells, the electrophoretic distribution of antibody activity differs markedly from that observed in the early stage (Figure 3). The most striking feature is the appearance of a second antibody in the more slowly migrating γ-globulins. The two antibodies are serologically very closely related, as evidenced by the ability of the second antibody to inhibit the reaction of antigen with the first antibody. This relationship is shown in Figure 3 by the curve of antibody activity measured in combining units. In Table I are summarized the properties of the two antibody fractions which were further characterized by moving boundary electrophoresis and ultracentrifugal analysis.

Table I. Some Properties of Rabbit Antibodies against Sheep Red Cells

Property	Antibody Components: Faster migrating	Antibody Components: Slower migrating
Appearance in immunization	Early	Late
Electrophoretic mobility[a]	-2.1μ	-1.2μ
Approximate sedimentation constant[b]	18 *S*	6 *S*

[a] In pH 8.6 veronal buffer, 0.1 ionic strength. Mobility, μ, expressed in units of $\mu \times 10^5$ sq. cm. volt^{-1} sec.$^{-1}$.
[b] In pH 8.6 veronal buffer, 0.1 ionic strength. Fractions adjusted to contain about 1% protein.

It is possible to increase the resolving power of the electrophoretic method by using it with another differential migration method. Thus electrophoretic separation of fractions obtained by preliminary high-speed centrifugation can furnish information about the relative size of two or more different proteins. Figure 4 presents some results obtained with late anti-sheep cell sera produced by immunizing with guinea pig kidney antigen and human Type A red blood cells (*8*).

Each antiserum was diluted with an equal volume of pH 8.6 veronal buffer and subjected to an average centrifugal force of 105,000 × g for 210 minutes. Each 11-ml. centrifuge tube was then partitioned into top (5-ml.) middle (5-ml.), and bottom (1-ml.) fractions. The three fractions were adjusted to the same protein concentration and were simultaneously subjected to electrophoresis in a starch block divided into three compartments.

To conserve space, only the globulin portions of interest are shown in Figure 4. In antiserum produced by immunizing with guinea pig kidney antigen, two antibody peaks may be seen. The faster moving antibody is clearly associated with a fraction of large molecular weight as evidenced by the progressively greater increase in antibody activity per microgram of protein nitrogen from top to bottom fractions (0.3 to 10.0). The more slowly moving antibody is similarly observed to be associated with a smaller protein, as the ratio of antibody activity per microgram of protein nitrogen was about the same in all three fractions. On the other hand, the antibody activity of human antiserum appears to be associated solely with the fraction of large molecular weight.

The immunological significance of these findings is not discussed here; they may be taken as illustrations of the

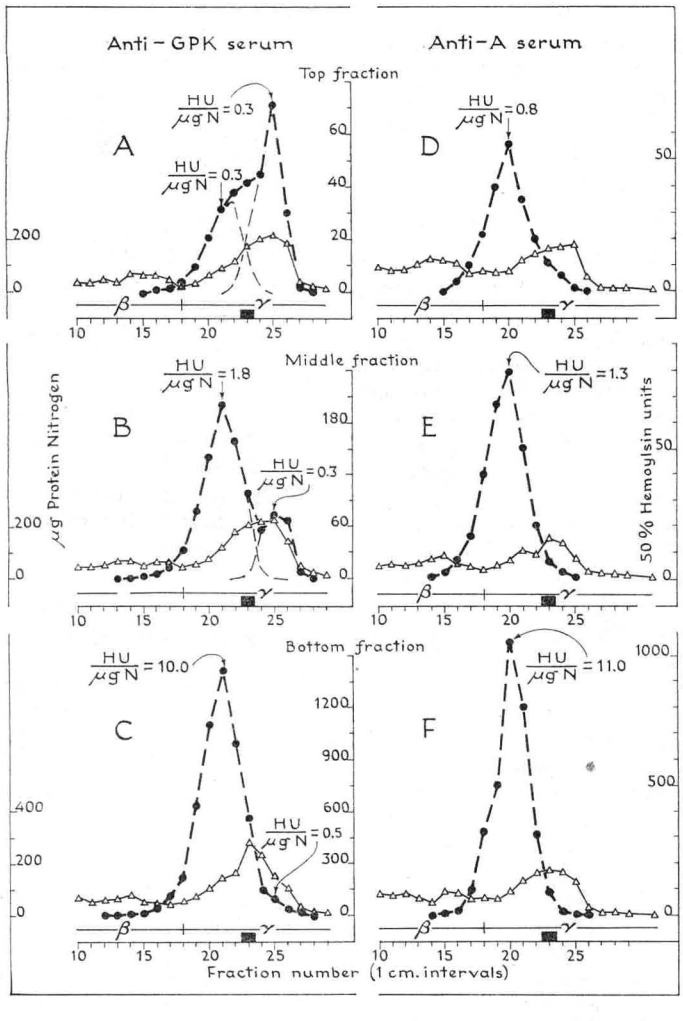


Figure 4. Starch zone electrophoresis of antisera formed after immunizing with guinea pig kidney (anti-GKP) and human Type A red blood cells (anti-A) obtained during late stage of immunization

Antisera first fractionated by high-speed centrifugation and three centrifugal fractions simultaneously separated in same starch block. Large and small antibody present in anti-GPK and only large antibody in anti-A (8)

type of information that can be obtained by a relatively simple and useful electrophoretic method.

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RECEIVED for review November 18, 1958. Accepted March 13, 1959. Contribution 1534, Sterling Chemistry Laboratory, Yale University. Work done under contract AT(11-1)-175 between the U. S. Atomic Energy Commission and the University of Chicago and aided by grants from the Dr. Wallace C. and Clara A. Abbot Memorial Fund of the University of Chicago.

Separations by Continuous Electrochromatography

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▶ Electrochromatography (continuous-flow curtain electrophoresis) is a comprehensive method in the analysis and preparative fractionation of crude labile biological materials and other complex systems. The operating principles, illustrations of typical experiments, and a discussion of some special problems emphasize its special properties. Particular emphasis is placed on the individuality of a given sample pattern and its value in correlation with special biological activities and other properties of the sample or feed stock; such a correlation ensures quality control in large scale industrial processing.

THE over-all operating principles and methodology of electrochromatography have been discussed by many investigators, including Grassmann and Hannig (*4*), Durrum (*3*), Strain (*12*), and Karler (*6*). Although "electrochromatography" is used throughout this paper, the longer expression "continuous-flow curtain electrophoresis" is more accurate; "electrochromatography" is shorter and emphasizes the fact that chromatographic or surface forces are always operative in the curtain or stabilized medium.

Electrochromatographic methods thus far developed are embodied in a variety of instruments consisting of a filter paper curtain or a packed cell, down which a fluid medium or background electrolyte is continuously fed and across which a continuous direct electrical current is passed; thus, we have an experimental situation in which the electrophoretic forces (electrical current flow or voltage gradient) act at right angles to the chromatographic or adsorptive forces (fluid flow). If a molecule or particle is now introduced in the upper part of this curtain or cell, it will move down the curtain with a definite reproducible angle under the influence of these two forces and will come off the drip points or take-offs provided at the bottom. Under proper conditions, different compounds will move through the curtain or cell at different angles, depending on their respective over-all physicochemical characteristics; thus, each substance will build up its own path and come off free of the other constituents in the original sample. The various constituents or fractions are collected, and the individual fractions can be put through this apparatus again (recycled) for further purification, or subjected to other physical, chemical, biological, or clinical methods of study.

In contrast to analytical electrochromatography, continuous electrochromatography involves the continuous addition of samples, the establishment of a stable unchanging pattern through the cell or curtain, and its maintenance throughout the course of fractionation. This stable pattern or "spectrum" presented by a given sample under a specific set of experimental conditions is distinctively individualistic for each sample preparation; Figure 1 illustrates such a pattern as obtained with the biological stain acid fuchsin. Any change in over-all physicochemical properties of the sample or in the experimental conditions will be ultimately reflected in changes in the specific fractionating pattern established in the curtain or cell. Shifts or changes in an established pattern will of course detract from the efficiency or resolution of the fractionation process. The individuality and stability of a specific fractionating pattern are the key to the full realization of the potential of continuous electrochromatography as a research and commercial processing operation.

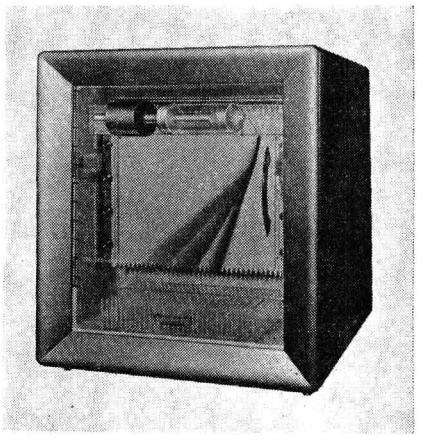


Figure 1. Stable fractionating pattern or "spectrum" of components produced by continuous electrochromatography of acid fuchsin stain using a modern preparative apparatus

Some of the preparative aspects of continuous electrochromatography may be illustrated by an early experiment involving the fractionation of a whole crude bacterial homogenate with the simultaneous recovery of no less than six biologically active components (enzymes). The over-all fractionating pattern in so far as the protein distribution on the curtain is concerned is illustrated in Figure 2. The complexity of the sample provides an overlapping, irregular continuum of the many protein as well as other constituents existing in this protoplasmic system.

A summary of the analytical and assay results (Figure 3), however, emphasizes the fractionating effectiveness of continuous electrochromatography. The simultaneous isolation with high yields

of at least six semipurified labile entities such as enzymes directly from a very crude preparation illustrates some of the preparative advantages of electrochromatography. In addition, most complex preparations such as this one present highly colored bands in the pattern which immediately characterize that preparation and facilitate checking the position and stability characteristics of the fractionating pattern during the experiment.

WORK WITH PLAGUE TOXIN

One of the biological preparations most thoroughly studied has been crude and purified plague toxin. Ajl and coworkers (*1*) first explored the possibility of preparative fractionation of plague toxin. Later studies by Ajl and coworkers (*2*) and by Spivack and Karler (*11*) represented an attempt to use continuous electrochromatography more comprehensively as a single method for larger scale preparations with higher recoveries. The work of Spivack and Karler is reviewed to illustrate several other features of electrochromatography.

As with the crude bacterial homogenate described above, the crude plague toxin preparations presented a wide spectrum of proteins and other constituents. Figure 4 illustrates this heterogeneity and localizes the position of all the protein which is plague toxin. In these crude preparations a daylight visible yellow component which gave a yellow fluorescence with an ultraviolet light was invariably found associated with the peak of the plague toxin. The routine fractionation procedure involved pooling of the three fractions containing the bulk of the plague toxin and recycling this pool under the same or different fractionating conditions until a toxin of required chemical, immunochemical, and biochemical purity was obtained. With each recycle of the toxin there was a corresponding diminution of the yellow pigment. As the work with various preparations progressed, this decrease in pigmentation was found to be an accurate measure of the degree of purification which had been effected with each passage of plague toxin through the curtain. It was thus possible to minimize the use of expensive, time-consuming biological assay techniques. It became possible by mere visual observation of the fractionating pattern to predict the purification factors and the biological activities (within accepted experimental limits) at every stage in the purification procedure.

The fractionating characteristics of highly purified plague toxin are illustrated in Figure 5. This phase in purification was achieved just at that point

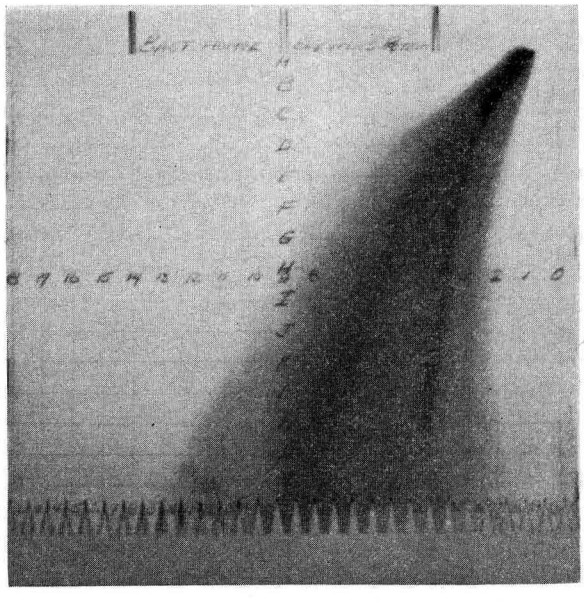


Figure 2. Fractionating pattern of the whole homogenate of *Pseudomonas fluorescens*

A representative sample was left on the curtain and stained for protein using bromophenol blue

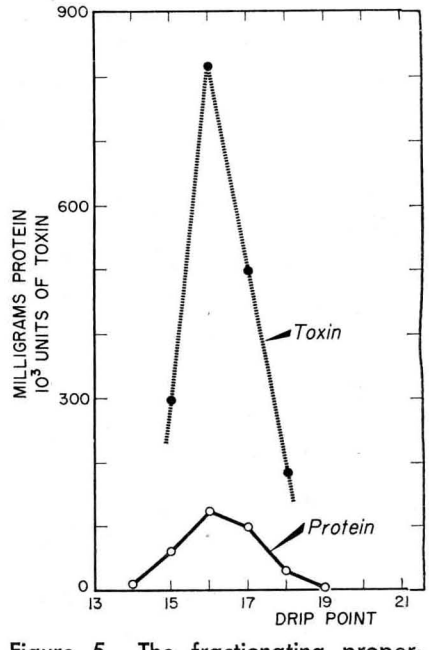


Figure 5. The fractionating properties of highly purified plague toxin (*P. pestis*) after four passes through a preparative electrochromatographic apparatus

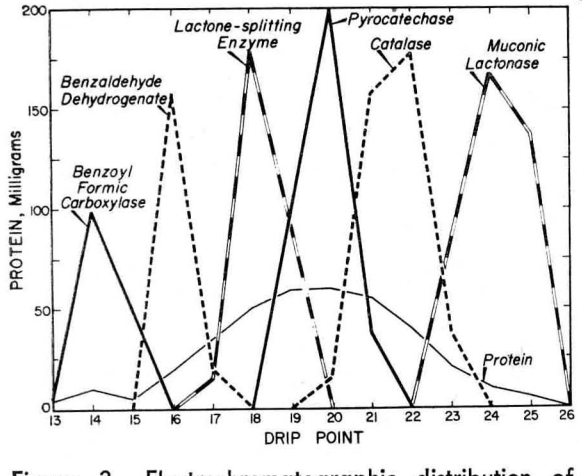


Figure 3. Electrochromatographic distribution of six enzymes from the whole homogenate of *Pseudomonas fluorescens*

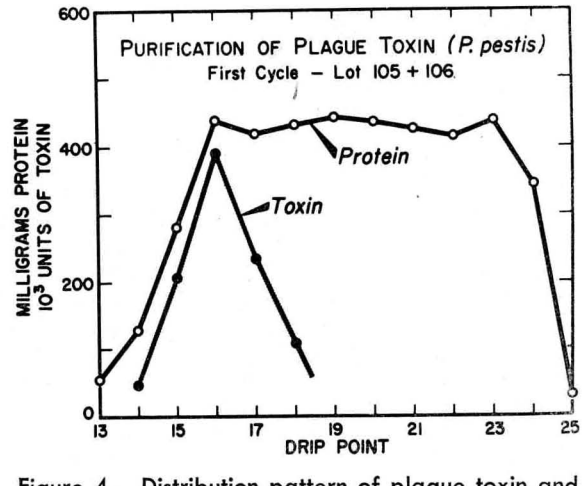


Figure 4. Distribution pattern of plague toxin and associated proteins obtained from the first pass (or cycle) of crude plague toxin (*P. pestis*) through a preparative electrochromatographic apparatus

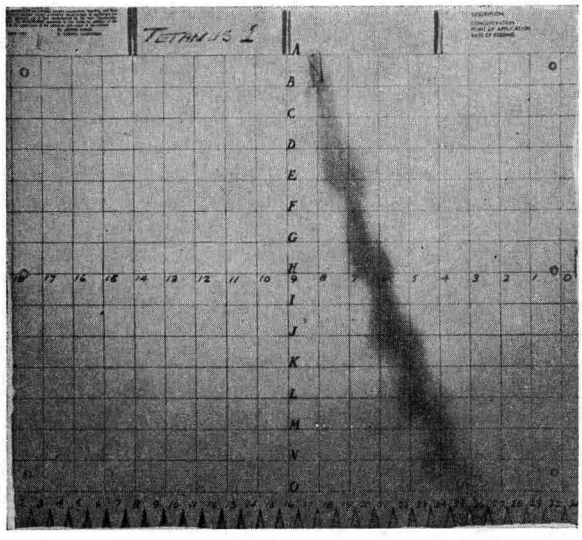


Figure 6. Untreated curtain obtained by the electrochromatography of partially purified tetanus toxoid

Irregularity of dark band is a result of intermittent interference by a trace gellike constituent

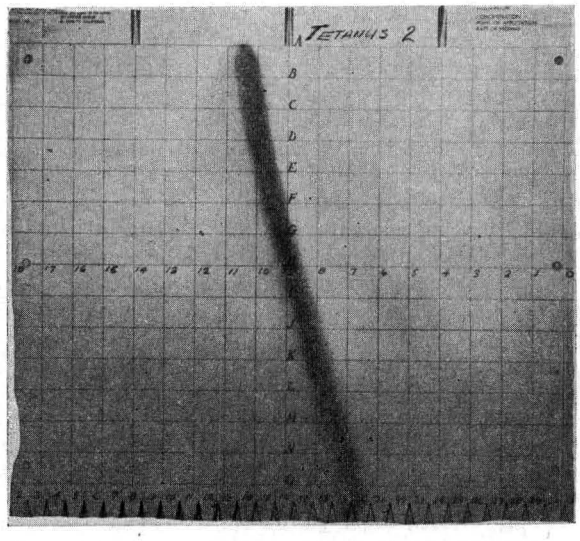


Figure 7. Untreated curtain obtained with tetanus toxoid after removal of interfering gel constituent (cf. Figure 6)

when the yellow contaminating pigment had been completely removed from the observable fractionating pattern of the curtain. This early correlation of a physical fractionating pattern with biological activity and chemical and physical properties has proved to be a common characteristic in the electrochromatographic analysis and processing of nearly all complex samples. In those few cases where natural pigments or other indicator compounds have been absent or nonspecific, some noninterfering harmless dye or other indicator may often be added to the system; it is then possible to establish an artificial but valid physical picture for evaluating the stability and general operating characteristics of the electrochromatographic pattern and for correlating that physical pattern with the purification process as described above for plague toxin.

TECHNIQUE OF OVERFEEDING

In addition to correlation of the fractionating patterns with biological activities, the work with plague toxin resulted in the development of the technique of overfeeding of a crude preparation in order to increase the throughput capacity of a given experimental system appreciably with nominal loss in over-all resolving power. With the whole crude plague toxin preparation it was possible to increase the apparent optimal rate of sample addition of 5 ml. per hour to 15 ml. per hour with approximately a 25% loss in resolving power. With this increase in the rate of sample addition the comparatively narrow area of application becomes three or four times as broad, thus forming a large elliptically shaped area or "blob" within which it is possible to observe the formation of the various components or bands and their crossing over to assume their expected, normal positions on the curtain.

This technique works for other complex, crude preparations (such as whole homogenized red blood cells) during their initial passage through a preparative electrochromatographic apparatus. Attempts to extend it to semipurified and purified fractions, as might be done in recycling, thus far have not proved successful. Although the exact mechanism involved in the successful overfeeding of crude preparations has not been ascertained, the technique has frequently proved a convenient way to increase the throughput capacity of an electrochromatographic instrument. This has been especially helpful where the nature of the starting samples has necessitated repeated recycling of a pertinent component.

WORK WITH TOXOIDS

Work with toxoids and toxins as a group has provided much information which has helped develop further the methodology of continuous electrochromatography. Work with tetanus toxoid preparations provides useful illustrations of the difficulties that may be encountered in this type of fractionation. Figure 6 is a photograph of a partially purified tetanus toxoid preparation having a gray color. The irregular pigmented band is illustrative of the interference by trace gel constituents frequently found in crude biological preparations. Their effect upon the fractionating pattern may be a simple physical one, in which the gel either increases, continuously carrying with it increasing amounts of the sample, or builds up intermittently with subsequent movement down the curtain in the direction of the general pattern. The latter case is illustrated by this tetanus toxoid preparation. Frequently these gel constituents can be removed by centrifugation or by more careful and complete dissolution of the crude preparation.

The result of such a successful removal from this tetanus toxoid preparation is illustrated in Figure 7. Subsequent analysis showed that the experiment was unsuccessful, in that there was no real fractionation of the protein, the toxoid, and the contaminating gray pigment. Examination of this starting sample showed it was composed of not less than 30% of a buffer salt. This order of concentration of a given ion or salt may sometimes interfere seriously with the fractionation mechanism of continuous electrochromatography.

The simplest way to handle such a possibility was to dialyze away the bulk of the buffer impurity and repeat the fractionation without further experimental changes. The result of this buffer removal is illustrated in Figure 8: The analytical data describing the fractions obtained from this curtain are summarized in Figure 9. The contaminating pigment has now been removed from the bulk of the protein and the toxoid protein has been coming off at the other side of the protein spectrum. This extreme type of interference by one or more associated small ions or salts has usually proved to be minimal, or at least less extensive than was the case with this partially purified toxoid preparation. Normally the electrochromatographic process automatically effects the necessary desalting of the sample shortly after application to

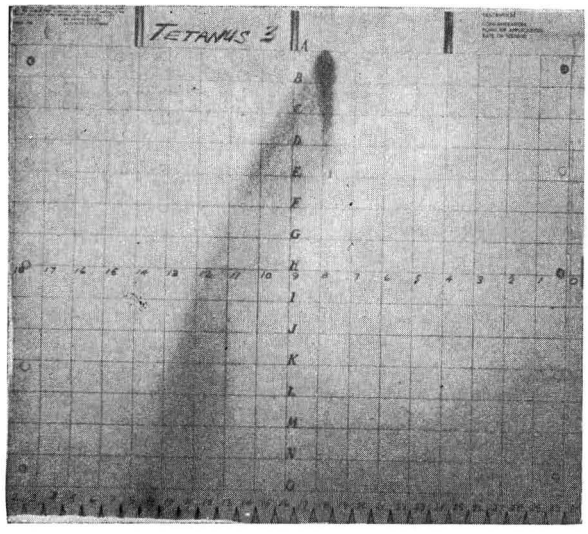


Figure 8. Untreated curtain obtained with tetanus toxoid after removal of both interfering gel constituent and contaminating buffer salt (cf. Figures 6 and 7)

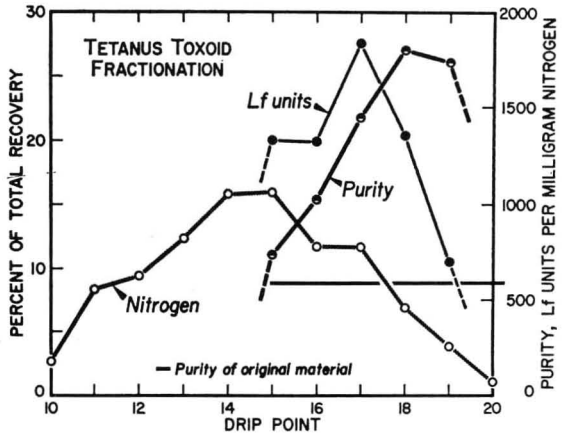


Figure 9. Distribution of protein (nitrogen), toxoid activity (*Lf* units), and purity (ratio of *Lf* to protein) obtained from curtain illustrated in Figure 8

the curtain. Where desalting is inadequate under a given set of experimental conditions, the sample may be dialyzed as described above—a procedure initially recommended by Durrum (*3*). Where dialysis is inconvenient or intolerable, the sample may still be processed, using more power, or the unresolved fractions may be recycled until true fractionation becomes operative. Presumably the explanation for such a breakdown in the fractionation process lies in the establishment of a limited region of high conductivity or very low voltage drop and hence little or no fractionation; perhaps it is best to visualize the movement of current across a region of minimal voltage drop —a region corresponding to the restricted position of the unfractionated sample band on the curtain.

The work with toxoids is also illustrative of the preparative potential of continuous electrochromatography. Extrapolation of the 24-hour preparative experiments upon which Figure 9 is based shows that with suitable modification of the laboratory apparatus illustrated in Figure 1 it should be possible to process a 500-gram batch of a crude toxoid or vaccine with unprecedented purification factors within 2 to 4 weeks. Although on a weight basis such an amount of sample may seem nominal, the toxoid or vaccine processed may be equivalent to one-half billion units of biological activity. After making allowances for discarding 10 or 20% of the biological activity, to secure unusually pure toxoid or vaccine, and keeping in mind that only four or five units of activity may be required per dose of vaccine, it can be seen that sufficient vaccine has been produced to vaccinate approximately 100,000,000 people. With this type of preparative experiment we are only one step removed from routine, automatic continuous processing using large scale industrial types of fractionating equipment.

The significance of the stable equilibrated pattern so necessary for successful continuous electrochromatography takes on added importance when the method is projected into full scale industrial production. Such stability provides inherent modern quality control of product, irrespective of the length of the processing period. This high degree of quality control should eliminate the variability found in customary batch processing and minimize expensive, time-consuming assays. Unlimited processing with continuous, automatic quality control will almost certainly lead to large scale industrial usage of continuous electrochromatography.

Most of the fractionations thus far effected by continuous electrochromatography have been restricted to comparatively highly charged or ionic sample systems processed through a curtain washed with an ionic medium such as buffer solutions. Recently, however, Kirk (*7*) fractionated successfully certain type-specific antigens using distilled or redistilled water as background medium on a thoroughly washed filter paper curtain. Although Paul and Durrum (*10*) and McDonald and Williamson (*9*) have established the feasibility of one-dimensional (paper strip) electrophoretic separations of highly charged particles in low ionic systems, this preliminary work represents an extension of continuous electrochromatography to the fractionation of noncharged molecules or particles in a comparatively nonionic medium. Current work in this laboratory involves the fractionation of nonpolar samples in nonpolar media such as the hydrocarbons or esters. On the basis of such work done by Lipsky and Landowne (*8*) in the chromatographic fractionation of fatty acids with ester solvents and in view of the pioneering work of Haugaard and Kroner (*5*), who used standard paper chromatographic solvent systems with their electrochromatographic system, there are no *a priori* or theoretical reasons why continuous electrochromatography cannot be extended to hydrocarbon, lipide, and other nonpolar substances within a reasonable time.

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RECEIVED for review December 17, 1958. Accepted March 17, 1959.

Separation of Lipides by Gas-Liquid Chromatography

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▶ **A gas-liquid chromatographic method which utilizes an ionization detection system and high efficiency capillary columns has been developed for the analysis of complex mixtures containing long-chain saturated and unsaturated acid esters. The theoretical plate efficiency of certain capillary columns approached 1000 plates per foot. The separation of certain cis-trans isomers—i.e., methyl elaidate from methyl oleate—was accomplished. The minimum quantity of organic vapor detectable was approximately 10^{-15} mole.**

THE technique of gas chromatography has made possible, for the first time, the convenient separation, isolation, and analysis of certain complex mixtures of fatty acids of biological origin (*3*). Conventional packed columns coated with relatively nonpolar heat-stable hydrocarbon or silicone compounds were employed in early studies concerned with the composition of substances containing long-chain saturated and unsaturated fatty acid methyl esters. Under these circumstances, the more polar components—i.e., fatty acids that contained the greatest degree of unsaturation—emerged from the column before the corresponding saturated acid ester of the same carbon chain length. Certain of the C-18 series of esters were satisfactorily separated only when many of the operational parameters involved in the technique of high temperature gas chromatography were stringently controlled. The recent introduction of a new class of stationary liquids has greatly simplified the resolution of closely related long-chain acid esters (*5, 6, 13*) and has extended the range of analysis to include compounds containing 26 or more carbons (*4, 7*).

Following the screening of a variety of polar polymers synthesized under varying laboratory conditions, it became possible to draw some degree of correlation between the nature of the chemical composition of the liquid phase and the facility and speed with which the individual components of higher molecular weight saturated and polyunsaturated acids could be resolved. When the sebacate and azelate polyesters of propylene, butylene, and diethylene glycol were used as stationary liquids, the long-chain acid esters rapidly emerged from the column. There was complete separation of linoleate and linolenate bands. Peculiarly, the resolution of stearate from oleate did not readily occur. When additional ester and ether linkages were introduced into the molecule by reaction of the shorter chain dicarboxylic acids—i.e., adipate, glutarate, and succinate—with diethylene glycol to form more polar polymers of molecular weights extending from 3200 to 30,000, unsaturated acid esters were easily separated from the saturated members of the same carbon length under a wide variety of operating conditions (*7*). Examples of the unique versatility of certain of these polymers to function as effective stationary liquids for the analysis of fatty acids can be noted in Figures 1 to 4.

In all instances, Celite 545, 60 to 80 mesh, was coated by mixing with a 10% chloroform solution of a succinate polyester of diethylene glycol (laboratory batch LAC-4-R777; molecular weight 4450; acid number, 0.21; and viscosities of 1292 and 61,500 centistokes at 100° and 38° C., respectively.) At the unusually low column temperature of 125° C. (Figure 1) the unsaturated components were retarded and emerged from the column after the corresponding saturated acid ester. Under these conditions, the time required for analysis was relatively long and stearate was not separated from oleate. When the operating temperatures and the column length were increased (Figures 2 to 4), the retention times of the various components were appreciably shortened and stearate was now readily separated from oleate.

Batches of the same polymer may vary greatly in their performance as stationary liquids for the analysis of fatty acid esters. The purity of starting materials, the mode of preparation, and experimental conditions during synthesis are factors which can lead to variations in the physical and chemical properties of these substances. Excellent results were obtained when columns containing this type of liquid phase were "preconditioned" for 48 to 72 hours prior to use and the operating temperatures of the column were maintained below 190° C. Under these conditions, the interaction of the sample vapor with the stationary phase (transesterification) was minimal and the columns could be used for 3 to 4 weeks before losses in resolving power were noted.

Despite advances in the analysis of fatty acids by gas chromatography, the resolution obtained by the use of the conventional packed column is still limited and the separation of certain interesting fatty acids—i.e., the positional and stereo isomers—is difficult or impossible. A new concept in gas chromatography was recently introduced by Golay (*2*), who suggested the use of columns made by coating the inner surface of long narrow-bore capillary tubing with a thin layer of stationary phase. Such columns are reported to have efficiencies far exceeding those of packed columns. Their use, however, requires a highly sensitive detector, because the maximum load of any single substance which can be applied without loss of resolving power is in the order of 0.1 γ. One of the few devices known at this time to possess sufficient sensitivity for use with these columns is that developed by Lovelock (*11*). The present study was conducted in an effort to obtain high efficiency capillary columns for the analysis of complex mixtures of fatty acids by using a modified form of this detector. The operational characteristics of this type of sensing system will be described elsewhere (*12*).

THE DETECTOR

The operation of the argon detector depends upon the ionization of organic molecules by collision with metastable argon atoms. The original device (*10*) as developed for use with packed columns consisted of a small metal ionization chamber to which a high potential was applied. It contained an α or β source of ionizing radiation and had a volume between 3 and 10 ml. When argon was used as the carrier gas and entered the chamber from the column, the primary electrons set free in the argon were accelerated by the applied potential to velocities sufficient to excite large numbers of the argon atoms to their metastable state.

$$\text{Argon}^{\circ} + \alpha\text{- or } \beta\text{-rays} \rightarrow \text{argon}^{+} + e^{-} \text{ (primary)}$$

$$\text{Argon}^{\circ} + e^{-} + \text{high voltage} \rightarrow \text{argon}^{*} \text{ (11.6 e.v.) (metastable state)}$$

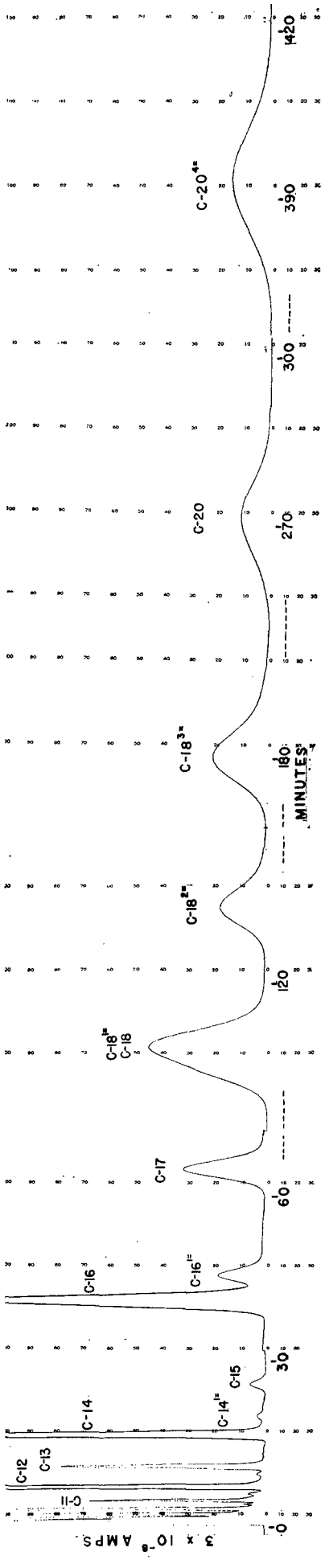


Figure 1. Separation of fatty acid esters on 6-foot column of 10% diethylene glycol succinate polymer on 60–80 mesh Celite 545

Temp. 125° C. Flow 50 ml./min. argon

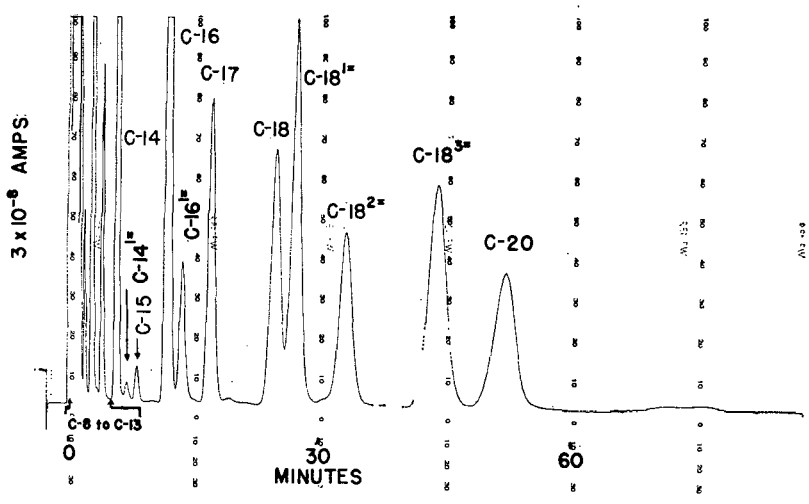


Figure 2. Separation of fatty acid esters on 9-foot column of 10% diethylene glycol succinate polymer on 60–80 mesh Celite 545

Temp 158° C. Flow 60 ml./min. argon

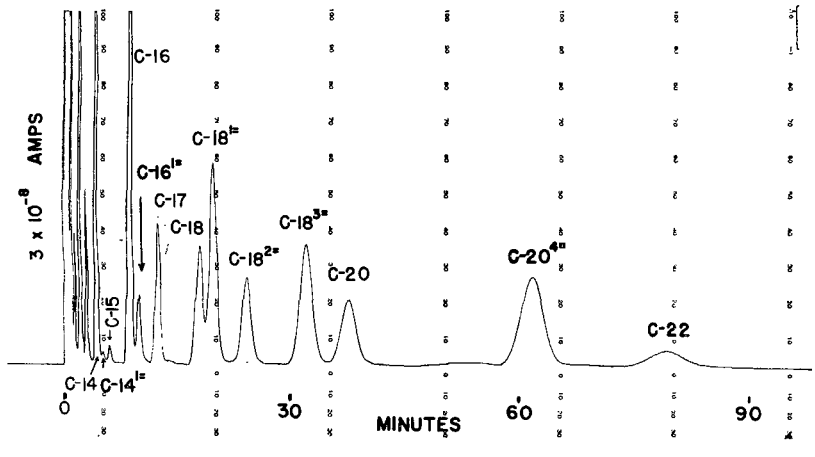


Figure 3. Separation of fatty acid esters on 9-foot column of 10% diethylene glycol succinate polymer on 60–80 mesh Celite 545

Temp. 168° C. Flow 70 ml./min. argon

Subsequently when an organic vapor emerging from the chromatographic column enters the argon-filled chamber, ionizing collisions take place which release secondary electrons. These electrons, when collected at the anode, give rise to an increase in ionization current which is related to the vapor concentration. They also generate more metastable atoms, replacing those lost on collision with the vapor molecules.

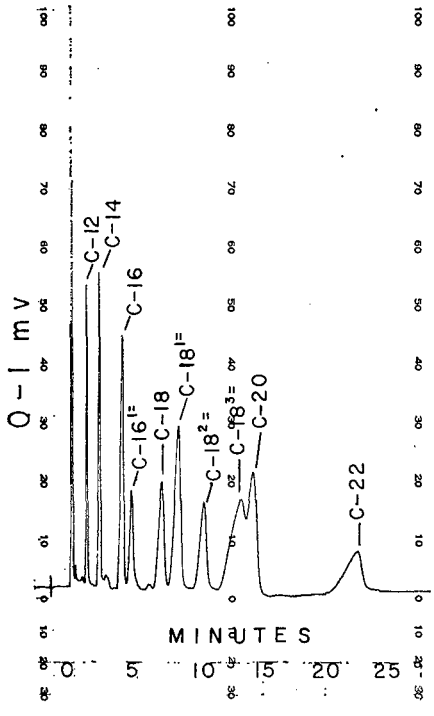
$$\text{Organic vapor} + \text{argon}^* \rightarrow \text{organic vapor}^+ + e^- (\text{secondary}) + \text{argon}^\circ$$

$$e^- + \text{argon}^\circ \rightarrow \text{argon}^*$$

$$e^- \xrightarrow{\text{to anode}} \text{current in amperes}$$

The original device was highly sensitive and would detect as little as 10^{-13} mole of most organic substances.

Because the rate of gas flow through capillary columns may be as low as 0.5 ml. per minute, fast-moving components may emerge from the column in a volume as small as a few tenths of 1 μl. Thus a detector for capillary columns must not only be highly sensitive but also possess a small effective volume, preferably in the range of 1 to 10 μl. The effective volume of the modified detector (*12*) employed in this investigation was reduced in two ways. With a chamber 1 ml. in volume a scavenging gas flow of 25 to 50 ml. per minute was



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Figure 4. Separation of fatty acid esters on 8-foot column containing 10% diethylene glycol succinate polymer on Celite 545, 60–80 mesh

Temp. 190° C. Flow 180 ml./min. helium. Detector, thermal conductivity unit

swept through the detector at a rate sufficient to reduce its time constant to about 1 second. This expedient, although effective in reducing the volume sufficiently for use with a capillary column, is disadvantageous in that the vapor entering the chamber is greatly diluted and sensitivity is lost in proportion to the total of the scavenging and capillary gas flow rates. This

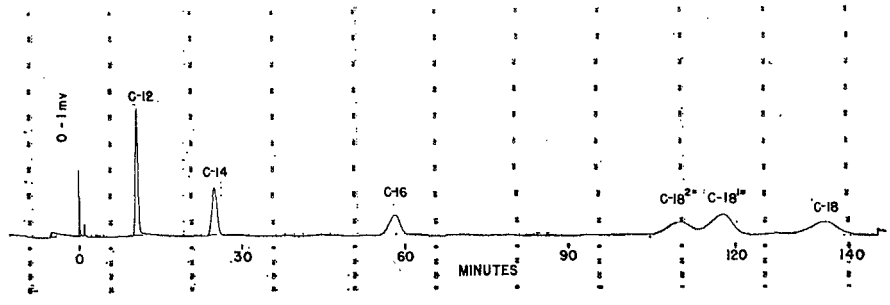


Figure 6. Separation on conventional powder-packed 8-foot column containing 20% Apiezon L on Celite 545, 140–170 mesh

Temp. 196° C. Flow 132 ml./min. helium. Detector, thermal conductivity unit

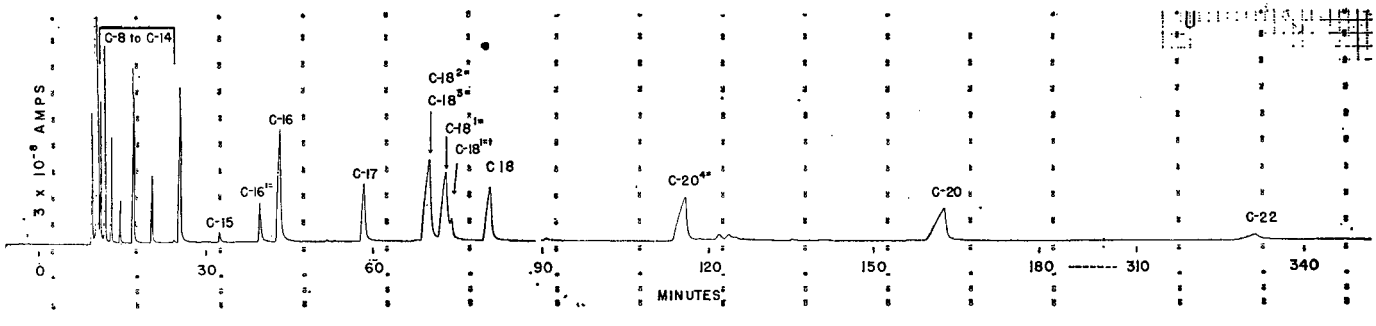


Figure 5. Separation of known mixture of fatty acids on 200-foot capillary column 0.010-inch i.d., coated with Apiezon L

Temp. 240° C. Pressure, 20 p.s.i. argon

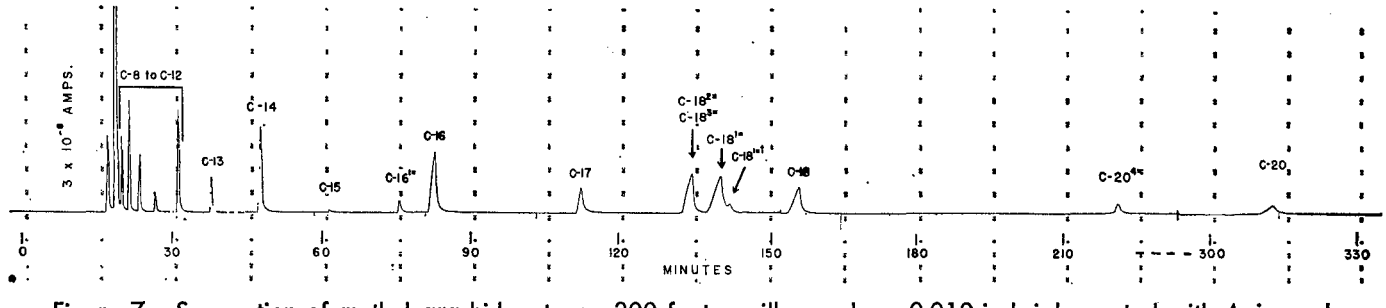


Figure 7. Separation of methyl arachidonate on 200-foot capillary column 0.010-inch i.d., coated with Apiezon L

Temp. 240° C. Pressure, 10 p.s.i. argon

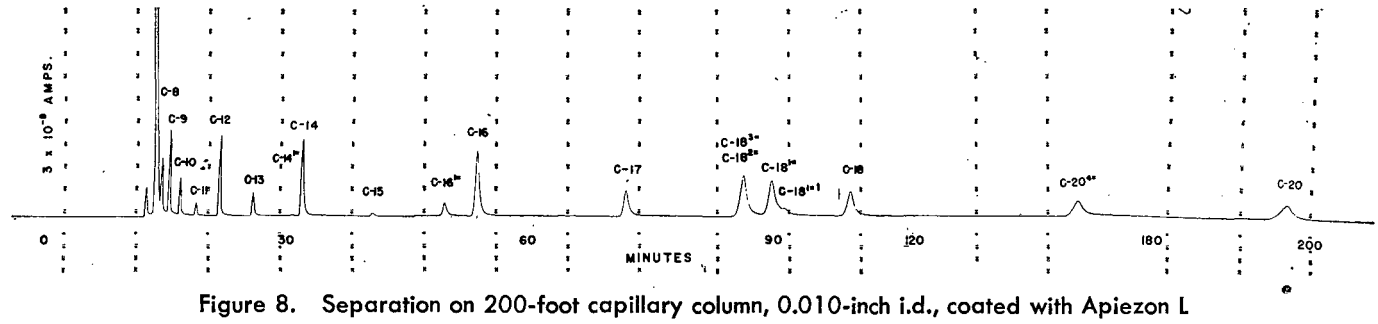


Figure 8. Separation on 200-foot capillary column, 0.010-inch i.d., coated with Apiezon L

Temp. 240° C. Pressure, 16 p.s.i. argon. Strontium-90 detector

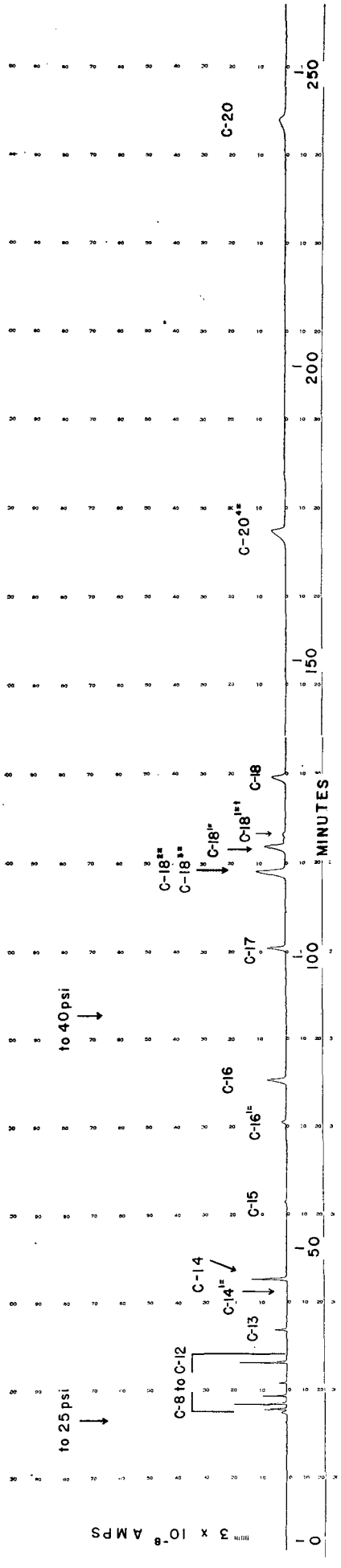


Figure 9. Pressure programming of a 200-foot column 0.010 inch in inside diameter coated with Apiezon L

Temp. 207° C. Starting pressure 10 p.s.i. argon

Table I.[a] Analyses with Detector of Early Design

Methyl Ester	Comp., %	Retention Time, Min.	S_f[b]	Calcd. Theor. Plates
Octanoate	4.1	2.8	0.04	21,400
Nonanoate	6.8	4.4	0.07	25,400
Decanoate	3.5	6.4	0.10	30,600
Undecanoate	1.2	9.6	0.15	40,000
Laurate	9.0	14.1	0.21	55,400
Tridecanoate	2.9	20.6	0.31	60,200
Myristate	10.7	30.6	0.47	64,100
Pentadecanoate	0.2	44.4	0.68	80,800
Palmitoleate	1.4	58.6	0.89	101,800
Palmitate	14.2	65.6	1.00	36,800
Margarate	4.4	95.1	1.45	94,500
Linolenate	7.5	118	1.80	...
Linoleate	3.7	...	...	...
Oleate	14.9	124	1.89	31,800
Elaidate	2.2	125	1.91	76,500
Stearate	8.4	139	2.12	59,200
Arachidonate	1.7	204	3.11	200,000
Arachidate	3.2	296	4.52	128,000

[a] Separation factor based on methyl palmitate equal to 1.00.
[b] 200-foot capillary column, 0.010-inch i.d., coated with Apiezon L. Temperature, 240° C. Pressure, 10 p.s.i. argon (Figure 7).

loss of sensitivity can be avoided, if the flow from the capillary column is led into the chamber by a metal tube of small diameter, which also serves as the anode. Under these conditions, the greater part of the electrical field resides within a few diameters of the surface of the tube inlet and this is the only part of the chamber where the primary electrons are sufficiently accelerated to generate metastable atoms. Thus, when the sample vapor from the column enters the region where there is a high density of metastable atoms, it is able to react and generate secondary electrons before it is appreciably diluted by the scavenging gas flow.

In practice, the primary electrons were generated in the chamber by including a sealed source containing approximately 50 μc. of radium embedded in a strip of silver foil. The current in the chamber with pure argon flowing was 2×10^{-8} ampere and the minimum detectable quantity of organic vapor was approximately 10^{-14} mole for a signal-noise ratio of 2 to 1. If a β-particle source is used—i.e., 10 mc. of strontium-90—the noise level is one tenth and the sensitivity is 10 times higher, extending the range to 10^{-15} mole.

A preliminary study (*9*) indicates the possibility of further increasing the over-all versatility of the system by employing ultrapure helium as a carrier gas. Under the circumstances, advantage is taken of the higher excitation potentials of the metastable states of helium (~19 and 21 e.v.) which would provide a constant response to all molecular species, including the permanent gases. Preliminary tests, using helium purified by diffusion through fine glass tubing, indicated that the use of pure helium as a carrier gas would extend the range of the detector in the manner expected.

CAPILLARY COLUMN

The columns used in these studies were 100- to 200-foot lengths of capillary tubing 0.010 inch in inside diameter. The outside diameter was selected at 0.062 inch to facilitate metal to metal connections. The material was Type 304 seamless annealed stainless steel. The columns were filled with the stationary liquid by forcing 10 to 20 ml. of a 2% chloroform solution of the liquid phase under 40 p.s.i. gas cylinder pressure into the capillary from a short thick glass tube connected to one end of the capillary according to the technique of Desty (*1*). The liquid may be moved more rapidly and conveniently into the capillary by means of a hydraulic pump which is capable of delivering fluid at 20,000 p.s.i. Once the capillary tube was filled, coating was effected by carefully blowing the solution out of the column at room temperature at 40 p.s.i. gas pressure. After the solution had emerged from the column, the gas flow was continued for several hours, to evaporate the last traces of solvent remaining within the capillary. The columns were then ready for use. Columns coated in this manner with thermostable liquid phases have been maintained at 240° C. for 2 to 3 months before losses in efficiency became evident.

SAMPLE INTRODUCTION

The maximum quantity of any single component which can be applied to a capillary column without reducing reso-

lution is on the order of 0.1 γ. Even with complex mixtures of biological origin, the optimum sample load is in the region of 1 γ or less. Application of such small loads by syringes or pipets of the conventional type used in gas chromatography is, at the present stage, difficult and inconvenient. Thus, in this investigation, the sample was introduced by means of a micrometer syringe through a silicone rubber seal into a simple T-shaped glass bypass device, which was isolated from the column compartment and maintained at 300° C. in order to provide instantaneous volatilization of the organic substances. In this manner, approximately 99.9% of the volatilized sample was vented to the atmosphere. The remainder, approximately 1 γ, entered the capillary column.

RESULTS AND DISCUSSION

A chromatogram illustrating the separation of a known mixture of fatty acids on a 200-foot capillary column coated with Apiezon L and operated at 240° C. with an inlet pressure of 20 p.s.i. is illustrated in Figure 5. The chromatograph depicted in Figure 6, which was obtained by using a conventional powder-packed column and Catharometer, is presented for comparison. Efficiencies extending up to 171,000 (Figure 5, methyl behenate) and 200,000 theoretical plates (Figure 7, methyl arachidonate) were obtained on the capillary columns. Under these circumstances, despite the higher operating temperatures and low flow rates, the cis-trans isomers, methyl elaidate and methyl oleate (*8*), were readily separated. Although stearate and oleate are sharply resolved, linoleate did not separate from linolenate.

The range of analysis can be extended over that obtained with conventional columns. In Figure 6, the stearate peak is greatly broadened and finally emerges from the column at 140 minutes. In contrast, the stearate band in Figure 5 is sharply defined and has a retention time of 77.5 minutes. Elevation of the temperature of the packed columns to 240° C. caused complete loss of resolution of the C-18 series of acids.

The analyses shown in Table I and Figures 5 and 7 were made using a detector of early design. It can be assumed that these columns have been overloaded. The number of theoretical plates increased in time, except where

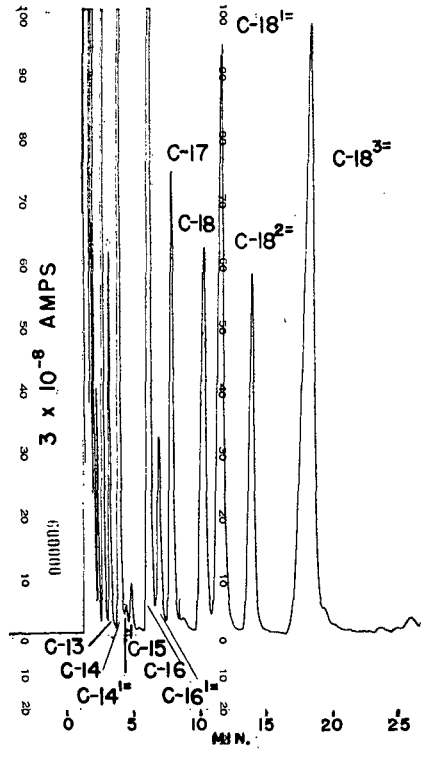


Figure 10. Analysis of known mixtures of C-8 to C-22 acid esters on 100-foot capillary column 0.010 inch in inside diameter, coated with diethylene glycol glutarate polyester

Temp. 193° C. Pressure 28 p.s.i. argon

an individual component of the mixture was present in a concentration of over approximately 8%. The overloaded bands typically have a leading front and a sharp back, when Apiezon L is used as a stationary phase. The overload phenomenon was particularly noticeable during the analysis of fatty acid esters.

The separation of hydrocarbons by capillary columns is a much easier problem. With hydrocarbons, loads nearly 100 times greater than that used with fatty acid esters did not overload the columns. By improvements in cell design, the sensitivity was increased approximately tenfold and it was possible to reduce the sample load in proportion. Under these conditions (Figure 8) there is little or no sign of overload and the final few bands emerging from the column are symmetrical. Unfortunately, when this particular analysis was performed the column had been in use for over 3 months and its efficiency had decreased significantly.

One of the unique features of the ionization detection system employed in this investigation is the relative insensitivity of the base line ("standing current") to changes in pressure and temperature. One example of this is illustrated in Figure 9, where the inlet pressure was increased twice during the analysis without appreciable effect on the base line. The same stability may be noted during "temperature programming" on a well conditioned column coated with a heat-stable liquid phase. Slight shifts in the base line, when they do occur, are almost invariably due to the "bleeding" of small quantities of stationary liquid from the column.

The analysis of a known mixture of acid esters extending from C-8 to C-22 on a 100-foot capillary column coated with a glutarate polyester of diethylene glycol is depicted in Figure 10. Separation of the added saturated and unsaturated components was rapid and complete. Despite the fact that the efficiency of the column was extremely poor (2800 theoretical plates for methyl stearate), the separation factor, α, for stearate and oleate was excellent at 1.13 (*7*). Unfortunately, it was not possible to resolve elaidate from oleate under these conditions.

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RECEIVED for review February 8, 1959. Accepted February 26, 1959. Work supported by grants from the National Heart Institute of the National Institutes of Health, National Dairy Council, and Nutrition Foundation.

END OF SYMPOSIUM

Variable Gradient Device for Chromatography

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▶ **The gradient devices described in the literature do not offer the flexibility in adjustment that is required in many chromatographic applications. A mixer that can produce nearly any gradient without change in the apparatus consists of a series of identical mixing chambers which contain eluent of varying composition in hydrostatic equilibrium. The initial concentration of each eluent and its position in the series are the controlling factors in the shape of the gradient. Quantitative relationships, as well as construction details, are presented.**

SINCE the introduction of gradient elution to chromatography several years ago, there have been many applications of the general technique in the fractionation of mixtures of substances having a wide range of elution requirements, and several methods for the production of gradients have appeared in the literature (*1–13*). With the exception of those which can utilize differential pumping rates (*9, 12*), all require a change in at least a part of the apparatus for each change in the shape of the gradient.

The need for a convenient means of making small changes in specific portions of the elution gradient in order to improve resolution in certain regions of the chromatogram, as well as for presenting the gradient data in a manner to permit ready duplication by others, led to the development of a variable gradient mixer (Varigrad) made up of a number of identical chambers connected in series and arranged in compact form.

Two arrangements employed in this laboratory are shown in Figure 1. In use, all the chambers are in hydrostatic equilibrium which is constantly being re-established in response to the removal of liquid from a chamber at one end of the series. A central motor drives a simple mechanism which stirs the liquid in each chamber. It is obvious that this system is part of a homologous series, of which Parr's two-chambered apparatus (*11*) is the first member. Because the authors' first model was of the square type shown in Figure 1, a nine-chambered system was a logical choice, and it has proved satisfactory in both mechanical performance and flexibility in the production of gradients.

SIMPLE GRADIENTS

Any number of the nine chambers can be used in combination by closing the appropriate connecting channel. If solution of concentration L (representing the limit concentration) is placed in the last chamber of a given combination, and the others are filled with water, liquid emerging from the first chamber will change in composition according to the curves shown in Figure 2. With L in chamber 2 of a two-chambered system, a linear gradient will be obtained. With L in chamber 3 of a three-chambered system, a concave gradient results, and the curvature increases progressively as the number of chambers is increased. The concentration, C, emerging from the mixer at any point is given by:

$$\frac{C}{L} = \left(\frac{v}{V}\right)^{N-1} \qquad (1)$$

where v is the volume of liquid which has emerged up to that point, V is the total volume of liquid originally in the mixer, and N is the number of chambers in the system.

COMPOUND GRADIENTS

If solution of concentration L is placed in a chamber which is not the first or the last of a system, curves of an entirely different sort are obtained. This is demonstrated in Figure 3, which presents all the curves resulting from single-chamber contributions in a nine-chambered series. Those produced by placing L in chamber 2 or in chamber 8 are mirror images of each other; the same is true for 3 and 7, and 4 and 6. A symmetrical curve is obtained with L in chamber 5.

These gradients can be regarded as the contributions of individual chambers to the compound gradients which the mixer is intended to produce. They can be determined empirically or calculated by the following equation:

$$\frac{C}{L} = \frac{(N-1)!}{(N-n)!(n-1)!}\left(1 - \frac{v}{V}\right)^{N-n}\left(\frac{v}{V}\right)^{n-1} \qquad (2)$$

in which N is the total number of chambers in the system, and n is the number of the chamber which contains

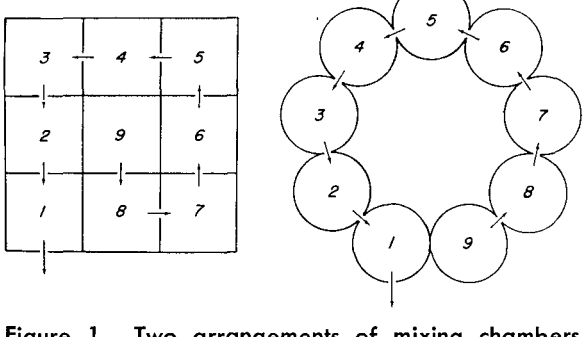


Figure 1. Two arrangements of mixing chambers, showing direction of flow

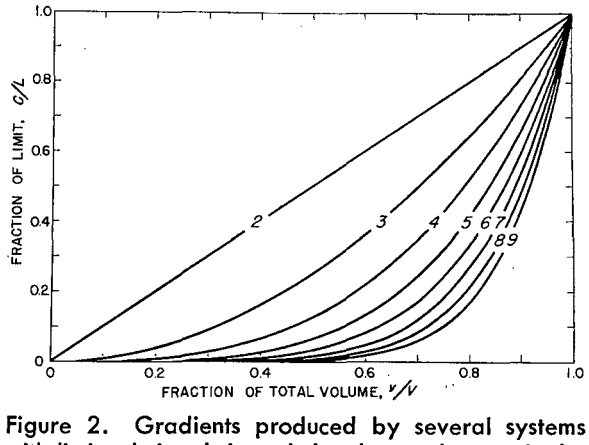


Figure 2. Gradients produced by several systems with limit solution, L, in end chamber and water in the rest

Number of chambers in each system is indicated

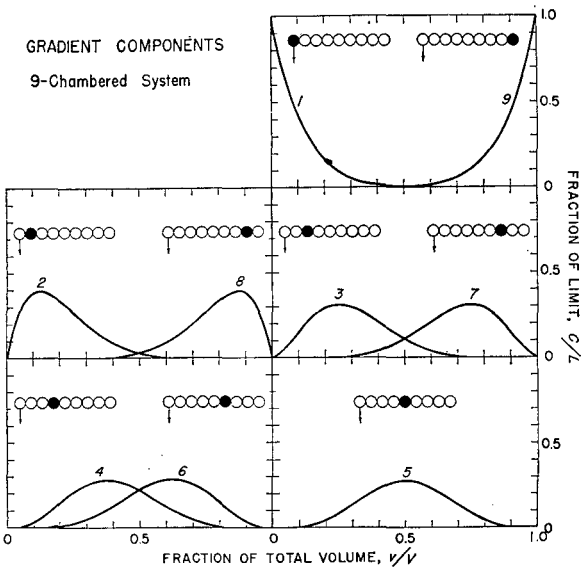


Figure 3. Single-chamber contributions in nine-chambered system

Blacked-in circles represent the position of solution *L*; other chambers are filled with water

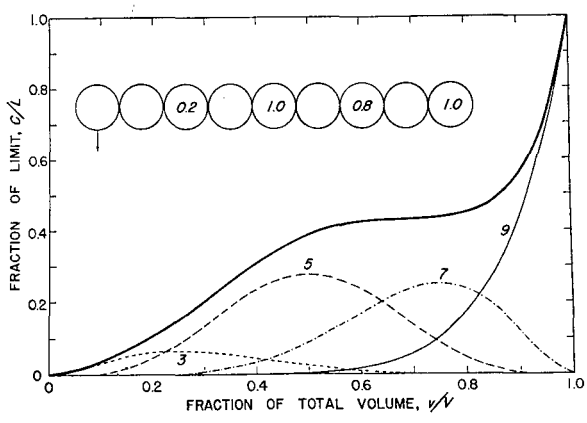


Figure 4. Formation of a compound gradient by the summation of the contributions of four chambers in a nine-chambered system

Numbers within circles at top represent concentrations, relative to *L*, charged to corresponding chambers. Other chambers contain water. Numbered curves represent resulting contributions of individual chambers indicated. Heavy curve is sum of curves below it.

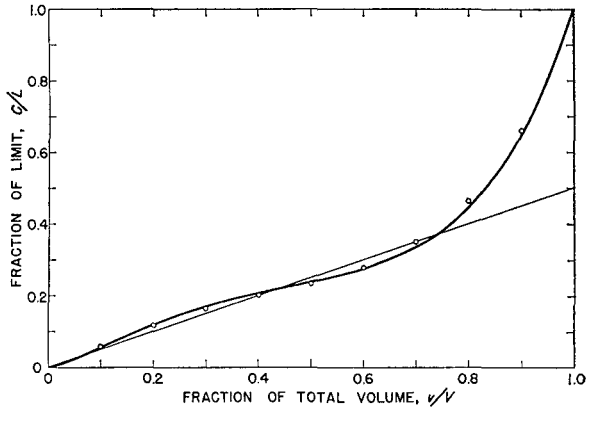


Figure 5. Illustration of a method for matching a preselected gradient, represented by the heavy line

L. The numbering begins at the chamber from which the liquid is withdrawn. Table I presents calculated values for the contributions of individual chambers in nine-, eight-, seven-, six-, five-, four-, and three-chambered systems.

Some idea of the effect of using larger numbers of chambers can be obtained very easily by sketching out the shape of the symmetrical curve which is always produced when *L* is in the middle chamber of an odd-numbered series. The sharpness of such a curve, as measured by the ratio of the concentration emerging at $v/V = 0.5$ to that emerging at $v/V = 0.25$, is given by:

$$\frac{C_{0.5}}{C_{0.25}} = (4/3)^{N-n} \qquad (3)$$

For a nine-chambered system ($N - n = 4$) this ratio is 3.16; for a 17-chambered system ($N - n = 8$) it would be 10. The actual concentration at the mid-point, relative to *L*, is the product of the following series:

$$\frac{C_{0.5}}{L} = \frac{1}{2} \cdot \frac{3}{4} \cdot \frac{5}{6} \cdots\cdots \frac{(N-2)}{(N-1)} \qquad (4)$$

Whereas the shape ratio increases exponentially with increasing number of chambers, the maximum height decreases by a diminishing factor.

If values are required for the maxima of the unsymmetrical curves, Equation 2 must be used, but the calculation is somewhat simplified by knowledge of the positions of the maxima:

$$\left(\frac{v}{V}\right)_{max} = \frac{n-1}{N-1} \qquad (5)$$

The summation of the contributions of four chambers in a nine-chambered system to form a compound gradient is illustrated in Figure 4. In this case, 0.2 of the limit concentration was placed in chamber 3, 0.8 in chamber 7, and the full limit concentration in 5 and 9, with water in all the rest.

In this summation it would not be necessary to regard a starting concentration above zero as a base line to be subtracted from the limit, as the chambers make their contributions independently. The starting concentration would then be the concentration placed in chamber 1, and its contribution would be calculated in the usual way.

In chromatographic applications the shape of the required gradient is usually determined by a succession of trials. Therefore, convenience in calculating the exact curve is less important than convenience in adjusting the shape to fit requirements indicated by previous chromatograms. Such adjustment can be accomplished by increasing or decreasing the concentrations in individual chambers, and when a suitable gradient has been achieved, it can be recorded by simply listing the concentrations used. These can be presented as molar values, but it is convenient to express them as fractions or percentages of the limit concentration. Thus, for example, 0, 2, 2, 8, 8, 8, 15, 30, and 100 define a particular shape in general terms, and a particular gradient is completely defined when the limit concentration is also specified.

For graphical presentation the gradient can be calculated from the concentration data, or determined empirically. Picric acid is a convenient solute for this purpose, because its absorbance at 355 mμ is a linear function of concentration in the range which can be read in a spectrophotometer.

When a mixture is being chromatographed for the first time, it is convenient to use a linear gradient with a limit concentration well beyond the anticipated maximum elution requirement. Such a gradient can, of course, be obtained by using only two of the chambers. For larger volumes a similar result can be achieved in a multichambered system by distributing the chamber concentrations in a linear manner, with the starting concentration in chamber 1.

If the chromatogram resulting from the use of a linear gradient thus obtained is crowded in some portions and spread out excessively in others, the concentrations in the individual chambers which

Table I. Calculated Contributions of Individual Chambers in Systems Comprising Several Chambers

	3 Chambers		4 Chambers		5 Chambers			6 Chambers			
v/V^a	3[b]	2	4	3	5	4	3	6	5	4	
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.00
0.05	0.002	0.095	0.000	0.007	0.000	0.000	0.014	0.000	0.000	0.001	0.95
0.10	0.010	0.180	0.001	0.027	0.000	0.004	0.049	0.000	0.000	0.008	0.90
0.15	0.022	0.255	0.003	0.057	0.000	0.012	0.098	0.000	0.002	0.024	0.85
0.20	0.040	0.320	0.008	0.096	0.002	0.026	0.154	0.000	0.006	0.051	0.80
0.25	0.062	0.375	0.016	0.141	0.004	0.047	0.211	0.001	0.015	0.088	0.75
0.30	0.090	0.420	0.027	0.189	0.008	0.076	0.265	0.002	0.028	0.132	0.70
0.35	0.122	0.455	0.043	0.239	0.015	0.112	0.310	0.005	0.049	0.181	0.65
0.40	0.160	0.480	0.064	0.288	0.026	0.154	0.346	0.010	0.077	0.230	0.60
0.45	0.202	0.495	0.091	0.334	0.041	0.200	0.368	0.018	0.113	0.276	0.55
0.50	0.250	0.500	0.125	0.375	0.062	0.250	0.375	0.031	0.156	0.312	0.50
0.55	0.302	0.495	0.166	0.408	0.092	0.300	0.368	0.050	0.206	0.337	0.45
0.60	0.360	0.480	0.216	0.432	0.130	0.346	0.346	0.078	0.259	0.346	0.40
0.65	0.422	0.455	0.275	0.444	0.178	0.384	0.310	0.116	0.312	0.336	0.35
0.70	0.490	0.420	0.343	0.441	0.240	0.412	0.265	0.168	0.360	0.309	0.30
0.75	0.562	0.375	0.422	0.422	0.316	0.422	0.211	0.237	0.396	0.264	0.25
0.80	0.640	0.320	0.512	0.384	0.410	0.410	0.154	0.328	0.410	0.205	0.20
0.85	0.722	0.255	0.614	0.325	0.522	0.368	0.098	0.444	0.392	0.138	0.15
0.90	0.810	0.180	0.729	0.243	0.656	0.292	0.049	0.590	0.328	0.073	0.10
0.95	0.902	0.095	0.857	0.135	0.814	0.172	0.014	0.774	0.204	0.021	0.05
1.00	1.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	0.00
	1[b]	2	1	2	1	2	3	1	2	3	v/V^a

	7 Chambers				8 Chambers				9 Chambers					
v/V^a	7[b]	6	5	4	8	7	6	5	9	8	7	6	5	
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.00
0.05	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.95
0.10	0.000	0.000	0.001	0.015	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.005	0.90
0.15	0.000	0.000	0.006	0.042	0.000	0.000	0.001	0.011	0.000	0.000	0.000	0.003	0.018	0.85
0.20	0.000	0.002	0.015	0.082	0.000	0.000	0.004	0.029	0.000	0.000	0.001	0.009	0.046	0.80
0.25	0.000	0.004	0.033	0.132	0.000	0.001	0.012	0.058	0.000	0.000	0.003	0.023	0.086	0.75
0.30	0.001	0.010	0.060	0.185	0.000	0.004	0.025	0.097	0.000	0.001	0.010	0.047	0.136	0.70
0.35	0.002	0.020	0.095	0.236	0.001	0.008	0.047	0.144	0.000	0.003	0.022	0.081	0.187	0.65
0.40	0.004	0.037	0.138	0.276	0.002	0.017	0.077	0.194	0.001	0.008	0.041	0.123	0.233	0.60
0.45	0.008	0.061	0.186	0.303	0.004	0.032	0.117	0.239	0.002	0.016	0.070	0.171	0.263	0.55
0.50	0.016	0.094	0.234	0.312	0.008	0.055	0.164	0.273	0.004	0.031	0.109	0.218	0.273	0.50
0.55	0.028	0.136	0.278	0.303	0.015	0.087	0.214	0.292	0.008	0.055	0.157	0.257	0.263	0.45
0.60	0.047	0.187	0.311	0.276	0.028	0.130	0.261	0.290	0.017	0.090	0.209	0.279	0.233	0.40
0.65	0.075	0.244	0.328	0.236	0.049	0.185	0.298	0.268	0.032	0.137	0.259	0.279	0.187	0.35
0.70	0.118	0.303	0.324	0.185	0.082	0.248	0.318	0.227	0.058	0.198	0.297	0.254	0.136	0.30
0.75	0.178	0.356	0.297	0.132	0.133	0.312	0.311	0.173	0.100	0.266	0.312	0.207	0.086	0.25
0.80	0.262	0.393	0.246	0.082	0.210	0.367	0.275	0.115	0.168	0.336	0.293	0.147	0.046	0.20
0.85	0.377	0.399	0.176	0.042	0.321	0.396	0.210	0.062	0.272	0.385	0.238	0.084	0.018	0.15
0.90	0.531	0.354	0.098	0.015	0.478	0.372	0.124	0.023	0.430	0.383	0.149	0.033	0.005	0.10
0.95	0.735	0.232	0.030	0.002	0.698	0.257	0.041	0.004	0.663	0.279	0.051	0.005	0.000	0.05
1.00	1.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.00
	1[b]	2	3	4	1	2	3	4	1	2	3	4	5	v/V^a

[a] Scale at left is to be used for chamber numbers appearing at top of column, that at right for chamber numbers appearing at bottom. Each column thus describes a pair of mirror images or, in some cases, a symmetrical curve.

[b] Numbers above or below columns refer to chamber initially containing solution having a concentration of unity. Initial concentration in other chambers of a given series is zero.

dominate those portions of the chromatogram can be decreased or increased to provide a more favorable distribution of eluting power in the next experiment. In cases where the early portions are crowded and require more or less uniform expansion, whereas the later peaks need sharpening, it is convenient to retain the linear form of the gradient in the first part, but with a lower slope, and swing up to the original limit in the later portions. This is readily achieved by reducing the concentrations of all the chambers except the first and last by some constant factor.

A method for matching the shape of a preselected gradient, using a nine-chambered mixer, is shown in Figure 5. A thin straight line has been drawn to approximate the major portion of the desired gradient. The intercept of this line on the ordinate at the right establishes a tentative limit from which chamber concentrations can be calculated for the production of the gradient represented by the thin straight line in the manner described above. By substituting the limit concentration of the desired curve in chamber 9 and adjusting the concentrations of chambers 3 and 6 to provide the required increment and decrement at single points on the curve dominated by those chambers, a set of chamber concentrations is obtained which will produce the gradient shown by the open circles.

In estimating the decrease in the concentration in chamber 6 that is required to lower the gradient to the heavy curve, one must also take into account the effect, at that point, of the large concentration increase in chamber 9. The point selected is that at which the chamber (in this case, 6) makes its maximum contribution, and the required change is calculated from

$$\Delta C = \frac{\Delta m}{m} C \qquad (6)$$

where m is the maximum contribution provided by concentration C, and Δm is the total change desired in this contribution.

GENERAL CHARACTERISTICS

The shape of the normalized gradient is independent of the total volume, permitting easy scaling up or down of chromatographic experiments, often with the same mixer. If desired, a single apparatus can be used to supply identical gradients simultaneously to several columns by pumping liquid from chamber 1 to all the columns at equal rates.

Any number of independent gradients of different molecular or ionic species can

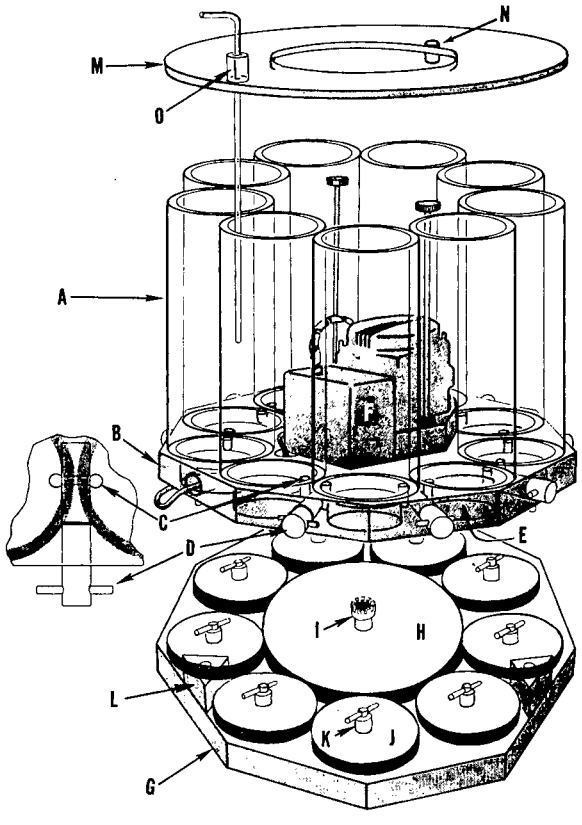


Figure 6. Nine-chambered Varigrad

A. Nine sections of Lucite tubing (6 × 2 1/4 I.D., 1/8 inch wall) cemented to top surface of plate B and to each other
B. Lucite plate 5/8 inch thick. Nine-sided polygon inscribed in 10-inch circle. Holes in central area for ventilation and for motor shaft not shown
C. Holes (3/16-inch diameter) drilled halfway into plate B to connect with sides of hole for stopcock D (see insert)
D. Eight Teflon stopcock plugs snugly fitting 3/8-inch reamed holes. Each plug fitted with neoprene O-ring and Lucite handle. Hole (1/8-inch diameter) through plug connects vertical holes C (see insert)
E. Recesses (1 1/2-inch diameter) extending 1/2 inch from lower surface of plate B. Magnet assemblies, K, turn in these spaces
F. Motor (KYC-23RB, 150 r.p.m., Bodine Electric Co., 2288 West Ohio St., Chicago 12, Ill.) and condenser mounted on Lucite block held to plate B by two long-shanked thumbscrews. Shaft fitted with coupling to engage I within 3/4-inch hole in center of plate B
G. Base of driving unit. Lucite (3/8 inch thick); same shape as plate B
H. Central driving disk, 5 1/4 inches, 1/4 inch thick
I. Multijaw coupling (FA5 Boston Gear Works, Quincy 71, Mass.) to engage similar component attached to shaft of motor (see F)
J. Disk, 2 1/4-inch diameter, attached to shaft. Brass washer between disk and bearing
K. Lucite shaft (1 3/16 × 1/4 inch diameter) turning in bearing (1/4-inch I.D., 5/8-inch O.D., Fafnir S1K7) mounted in base. Alnico V magnet (1 × 1/8 inch, Arnold Engineering Co., Marengo, Ill.) inserted through hole at top end of each shaft and held in place by section of soft plastic tubing compressed between disk and magnet
L. Three Lucite spacers, 1/2 inch high, cemented to G. Holes, 3/8-inch diameter, in top surfaces accommodate loosely three slightly tapered Lucite pegs (1/4 × 3/8 inch diameter) attached to bottom of plate B
M. Cover, 10 × 1/8 inch Lucite disk with 4 3/4-inch diameter hole in center for ventilation, to rest loosely on chambers, A
N. Lucite peg (1/4-inch diameter) attached to underside of cover, M, to fit into inside angle between two cylinders when cover is in place
O. Lucite rod (1 × 1/2 inch diameter) with central hole to position loosely fitting glass or plastic outlet tube at outermost point of inner wall of chamber

be produced simultaneously. For example, gradients of the acidic and basic components of buffers can be developed which satisfy independently selected specifications for pH and total molarity, and a gradient of a new buffer species can be used to compensate for the diminishing effectiveness of an initial species as the pH of the total gradient changes. Similarly, protective molecules can be caused to increase in concentration as some critical region (of pH, ionic strength, etc.) is approached.

Unless prolonged interruption of stirring occurs accidentally during an experiment, the gradients produced by this type of apparatus will be free of abrupt changes in composition, such as might occur in systems in which mixing of two or more streams of liquid is effected in a small volume. Moreover, because geometry is the determining factor, the gradients are highly reproducible, if stirring is adequate and hydrostatic equilibrium is maintained.

In any system operating by hydrostatic equilibration, allowance must be made for differences in density. This is most conveniently done by reducing the volume charged to each chamber in proportion to its density relative to the lowest density in the system. The error thus introduced is small for most cases involving aqueous buffers, but large deviations from the theoretical can be expected when liquids of widely different densities are employed. However, this does not preclude the use of hydrostatic equilibration in such cases, since the resulting gradients are adjustable and reproducible. If rigorous adherence to the theoretical curve is required, hydrostatic equilibration can be replaced with uniform piston displacement. This should be particularly advantageous for applications on a micro scale, where hydrostatic equilibration can be expected to offer difficulties.

With or without such modifications, devices based on the principle described may find application in other fields. Precisely controlled density gradients of many shapes should be useful in ultracentrifugation studies of rapidly sedimenting particles. Also, the ability to produce an unlimited number of independent gradients of different molecular species suggests applications in investigations concerned with the determination of optimal conditions in multicomponent systems.

DESIGN AND CONSTRUCTION

Devices based on the principle described can take many forms, but certain requirements must be met in the design and construction of each, if performance is to follow theory.

The chambers should be as nearly identical as possible with respect to cross-sectional area at any given level. If variation must be allowed, the chambers should be arranged in a sequence in which the diameters increase in the direction of increasing density of liquid charged, so that the errors introduced by volume variation will reduce those resulting from the initial hydrostatic adjustment.

The channels connecting the chambers must be large enough to permit rapid hydrostatic equilibration and to prevent blocking by very small air bubbles, yet their volume should be insignificant relative to the volume of the adjacent vessels. The channels must be lower than the floor of the chambers in order to prevent the laminar exchange of liquids of differing density which would otherwise occur even with the chamber contents in hydrostatic equilibrium.

Mixing at the bottom must be very efficient, and the height of the chambers should not be so great as to prevent adequate vertical transfer. Vortices should be kept as small as possible, consistent with adequate stirring, and all the vortices should be similar in size. Small stirrers made of soft iron wire—e.g., 1-inch sections of paper clip wire—covered with thin glass tubing from melting point tubes are recommended for use in the chambers of magnetically driven mixers. Polyethylene-covered stirrers have a tendency to develop a rough surface after long use, with consequent occasional sticking. The distance between the stirrers and the driving magnets

A. Nine sections of Lucite tubing ($3\frac{1}{4} \times 1\frac{1}{8}$ I.D., $\frac{3}{16}$ inch wall cemented to plate *B* and to each other. Outer walls milled flat at points of contact to reduce distance between inner walls of adjacent chambers to $\frac{1}{4}$ inch

B. Lucite plate, $\frac{3}{16}$ inch thick, $5\frac{1}{8}$-inch diameter

C. Hole ($\frac{3}{16}$-inch diameter) drilled through plate *B*. Channel, $\frac{3}{16}$ inch wide and $\frac{1}{16}$ inch deep, milled into bottom surface to connect holes in adjacent chambers

D. Lucite strips ($1\frac{1}{8} \times \frac{3}{8} \times \frac{1}{16}$ inch) cemented to underside of plate *B* to enclose channels connecting holes

E. Three $\frac{1}{4} \times \frac{1}{2}$ inch diameter Lucite pegs cemented to underside of plate *B*

F. Aluminum disk ($5\frac{1}{8} \times \frac{1}{8}$ inch) attached to $\frac{1}{2}$-inch diameter steel rod. Pegs *E* fit loosely into $\frac{17}{32}$-inch holes in disk

G. Thumbscrews, with locknuts, to level mixer

H. Haydon heavy duty inductor timing motor, 30 r.p.m. (Haydon Manufacturing Co., Torrington, Conn.)

I. Lucite disk ($5\frac{1}{8} \times \frac{1}{4}$ inch) fitted with bearings ($\frac{1}{4}$-inch I.D., $\frac{5}{8}$-inch O. D., Fafnir S1K7). Leveling bubble on upper surface and two $\frac{1}{4}$-inch pegs on lower surface (to position disk) are not shown

J. Lucite driving disk, about 3-inch diameter and $\frac{1}{8}$ inch thick, with straight knurl on edge. See text

K. Section of rubber tubing forced onto shaft of paddle to serve as friction disk (about $\frac{5}{8}$ inch diameter). Separated from bearing by brass washer

L. Lucite paddle ($3\frac{3}{16} \times \frac{5}{8} \times \frac{1}{16}$ inch, 45° twist) cemented to notched end of $1 \times \frac{1}{4}$ inch diameter Lucite shaft. Teflon washer between top of paddle and underside of disk *I*

M. Lucite rod (about $\frac{7}{32}$-inch diameter) to match, in the unstirred chamber, the displacement of liquid by paddles in the other chambers

N. Lucite rod ($\frac{3}{8} \times \frac{5}{16}$ inch diameter) with central hole to accommodate glass or plastic outlet tube. For clarity, this is shown in position above chamber 8; in normal use the tube would be inserted into chamber 1

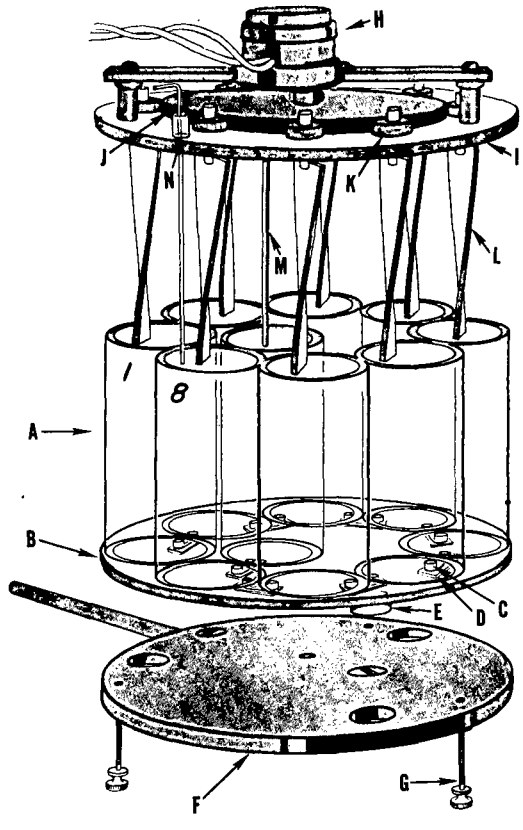


Figure 7. Paddle-stirred model

should be as small as possible to afford a strong, reliable coupling.

Although mixing is not required in the last chamber, the liquid in this chamber should be stirred in the same way as that in the others, in order to avoid, in magnetic mixers, inequalities in contribution as the vortices increase with decreasing depth of liquid. This is of little importance in paddle-stirred mixers, but these require provision for the displacement of liquid in the unstirred chamber to match the volume displaced by the paddles in each of the other chambers.

Figure 6 illustrates the construction details of a nine-chambered Varigrad having a capacity of 3400 ml. Lucite was used, because this mixer was intended for use with aqueous solutions; other materials should be employed when organic solvents are involved.

The central driving disk, *H*, is made of sponge rubber supported between two 5-inch Lucite disks, the lower $\frac{1}{8}$ inch thick and the upper $\frac{1}{16}$ inch. This sandwich rests on a brass disk ($2 \times \frac{1}{16}$ inch) soldered to a brass shaft having a $\frac{1}{2}$-inch diameter below the disk to fit a Fafnir S5K bearing $\frac{1}{2}$ inch in inside diameter, and $1\frac{1}{8}$ inches in outside diameter and a $\frac{1}{4}$-inch diameter above it to fit the coupling. Compression of the sponge rubber when the coupling is attached binds the disk assembly to the shaft.

Very serviceable outer disks, J, have been made by sandwiching three or four pieces of heavy canvas between two 2-inch disks of Lucite, the lower of which ($\frac{1}{8}$ inch thick) was cemented to the $\frac{1}{4}$-inch diameter Lucite shaft. Foam or sponge rubber can also be used. Polyurethane foam wore down too rapidly. Presumably, plastic or fiber gears can be used instead of friction disks, if suitable sizes are available.

In the construction of the driving mechanism care should be taken to provide adequate space between the rotating disks and the lower surface of the plate mounted above them, but the magnets should be as close as possible to the tops of the recesses in the plate. It is advisable to drill a few large holes through the central area of the plate to aid the circulation of air past the motor.

A paddle-stirred model with a capacity of 430 ml. is shown in Figure 7. Lacking stopcocks, it is simpler to construct but less convenient to use.

While the chambers are being filled, the connecting channels are stoppered with eight $4\frac{1}{2} \times \frac{3}{32}$ inch stainless steel rods fitted at the lower end with a short section of $\frac{3}{16}$-inch diameter vinyl tubing which projects about $\frac{1}{8}$ inch beyond the steel. Pressure on the rod after insertion of the tubing into one of the holes forces the slightly tapered end of the rod downward, compressing the tubing against the side of the hole.

As in the case of the magnetically stirred mixer, the details of construction of the driving mechanism can be varied. In existing models a Lucite disk $\frac{1}{8}$ inch thick has been used for *J*, supported on a 2-inch brass disk attached to a $\frac{1}{4}$-inch brass hub. The small outer disks were sections of heavy-walled rubber tubing of suitable diameter. The pressure between the large and small disks should be just sufficient to assure contact at all times. Excessive pressure puts an unnecessary load on the marginally powered motor.

The mixers shown in Figures 6 and 7 were designed for use at atmospheric pressure, with pumps feeding the emergent liquid to chromatographic columns. For this reason simple, loosely fitting covers were employed. If, however, flow is to be induced by air pressure or by gravity with a Mariotte-tube control, airtight covers fitted with connecting channels must be provided.

In preparation of the Varigrad for use, the channels should be flushed with water to displace air before the stopcocks (lightly greased with silicone lubricant) are closed. After the chambers have been charged with their respective solutions, the vertical connecting holes should be examined for air bubbles. It is advisable, moreover, to watch the liquid levels for a short period after the start of an experiment until it becomes evident that the meniscus is falling in every chamber.

When a stopcock is being greased, it is necessary to open a very small vent hole, extending from the innermost tip of the stopcock hole to the bottom of plate *B* (Figure 6), to permit the escape of occluded air and excess grease. This vent must then be resealed with a drop of

plastic cement. If silicone grease is employed, regreasing will be only infrequently required. Because the major function of the stopcocks is the isolation of the chambers during the short filling operation, slow leaks between the chambers are of no consequence. The O-rings effectively prevent leakage to the exterior. On the other hand, if one or more of the chambers is to remain empty during an experiment, the closed stopcock should be supplemented with a stopper of the type described above for use in the absence of stopcocks.

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RECEIVED for review November 19, 1958. Accepted February 9, 1959. Presented in part before the Federation of American Societies for Experimental Biology, Philadelphia, Pa., April 1958.

Automatic Derivative Potentiometric and Spectrophotometric Titrations of Organic Acids

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▶ **Nonaqueous titrations of carboxylic acids, sulfonamides, imides, mercaptans, phenols, and enols can be performed rapidly and accurately with a single titrant-solvent combination and automatic derivative potentiometric or spectrophotometric end point termination. The acids are titrated in acetone solvent with tri-*n*-butylmethylammonium hydroxide titrant. The applicability of many electrode pairs and indicators to potentiometric and spectrophotometric procedures, respectively, was investigated, and the most suitable ones applied to the automatic titration of a variety of acids. The end point reproducibilities are within 0.01 ml. of titrant, the buret reading error, and the results show that the automatic derivative procedure is an accurate method for determining percentage purity or neutralization equivalents of organic acids.**

RECENT literature has contained several reports on the titration of acids in relatively inert solvents (*1–4, 6, 7*). Most titrations of organic acidic materials are currently performed by recording the titration curves (*1*) or by a manual potentiometric procedure (*4*). Investigations in this laboratory of the automation of titrations by a derivative technique (*10–13*) demonstrate that a wide variety of acids can be titrated rapidly and accurately using acetone solvent, tri - *n* - butylmethylammonium hydroxide titrant, and either automatic derivative potentiometric (*10*) or automatic derivative spectrophotometric (*13*) end point termination.

Many electrode systems were studied to determine which was most suited for automatic derivative potentiometric end point detection in acetone solvent. A platinum (10% rhodium) indicator electrode *vs.* graphite reference electrode connected directly to the input of the commercially available Sargent-Malmstadt automatic titrator (*15*) was suitable in titrating automatically all except the very weakest acidic materials, such as phenol. Such materials can be titrated automatically using azo Violet indicator and the derivative spectrophotometric end point detection method. Other indicators were studied in acetone solvent and the most suitable were selected and tested using automatic derivative spectrophotometric end point termination. The same indicators are suitable for manual visual end point detection in acetone solvent.

The precision of the automatically obtained results was generally within 0.01 ml. of titrant, the reading error for the buret used. The automatic titration procedure is rapid and accurate for determining percentage purity or neutralization equivalents for organic acids.

Fritz and Yamamura (*4*) used acetone and Bruss and Wyld (*1*) used methyl isobutyl ketone (4-methyl-2-pentanone) as solvent for the titration of organic acidic materials using quaternary ammonium hydroxides as titrants. Malmstadt and Vassallo described the use of acetone-water solvent for the quantitative determination of perchloric-acetic acid mixtures (*13*) and sulfuric-hydrochloric and sulfuric-nitric acid mixtures (*12*). Acetone was selected for this study because of its availability, low cost, purity, and general solvent characteristics.

APPARATUS AND MATERIALS

TITRATOR. Automatic derivative potentiometric and spectrophotometric titrations can be performed using a modified titration stand (*13*) for the Sargent-Malmstadt titrator, but the new commercially available Spectro-Electro titration stand (E. H. Sargent & Co., Chicago, Ill.) is more convenient for switching to either potentiometric or spectrophotometric end point termination, with simple polarity, electrode and filter selection, and an easy method for the insertion and removal of the titration vessel. The photoconductive detector circuit was used for the spectrophotometric procedure and the platinum (10% rhodium) *vs.* graphite electrode pair for the potentiometric procedure.

RECORDER. Model G-10 Varian (Varian Associates), 100-mv. recording potentiometer.

BURET. A 10-ml., self-zeroing, Teflon stopcock buret (Fischer & Porter Co.) was filled with titrant from an elevated bottle through the side arm which was fitted with a Teflon stopcock, and all interconnections were made with either Teflon or polyethylene tubing.

BURET VALVE. Gum rubber tubing with a pinch-off solenoid is commonly used for starting and stopping the delivery of titrant, but the tubing is attacked by the nonaqueous titrant and requires frequent replacement.

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Table I. Indicator Colors[a] in Various Acetonic Buffers

Aqueous pH Interval	Indicator	0.01N Perchloric Acid	Perchloric Acid[b]	Nitric Acid[b]	Acetone	Benzoic Acid	Anthranilic Acid[b]	O-NO_2 Phenol[b]	Phenol[b]	Excess Base
		Glass *vs.* Calmel, Mv.								
		+685	+580	+200	−150	−250	−350	−450	−580	−750
...	Azo Violet	y	y	y	y	y	p	r	r	v
9.3–10.5	Thymolphthalein	c	c	c	c	c	c	c	b	b
8.3–10.0	Phenolphthalein	c	c	c	c	c	c	c	r	r
8.0–9.6	Thymol blue	r	o	y	y	y	y	y	b	b
12.0–14.0	Acid Fuchsin	r	r	r	r	r	r	r	y	y
...	Aceto red[c]	y	y	y	y	y	y	p	r	r
9.8–11.6	1-Naphtholbenzein	g	y	y	y	y	y	b	b	b
6.0–7.6	Bromothymol blue	y	y	y	y	y	g	g-b	b	b
...	Azo Violet I	y	y	y	y	y	p	r	r	v
4.8–6.4	Chlorophenol red	y	y	y	y	p	r	r	r	r
3.0–4.6	Bromophenol blue	y	y	y	y	b	b	b	b	b
3.8–5.4	Bromocresol green	y	y	y	g	b	b	b	b	b
3.7–5.2	Alizarin Red S	y	y	y	v	v	v	v	v	v
10.0–11.0	Aniline Blue	b	b	b	b	o	o	o	o	o
...	Methyl blue	b	b	b	b	r	r	r	r	r
6.8–8.0	Neutral red	r	r	r	y	y	y	y	y	y
4.4–6.2	Methyl red	r	o	o	y	y	y	y	y	y
3.1–4.4	Methyl orange	r	o	y	y	y	y	y	y	y
3.0–5.0	Congo red	b	v	r	r	r	r	r	r	r
1.2–2.3	Metanil yellow	r	y	y	y	y	y	y	y	y
0.0–2.0	Malachite green HCl	g	g	c	c	c	c	c	c	c
0.1–3.2	Methyl violet	b	v	v	v	v	v	v	v	v
0.8–2.6	Crystal violet	b	v	v	v	v	v	v	v	v

[a] Color code. b, blue; c, colorless; g, green; o, orange; p, pink; r, red; v, violet; y, yellow.
[b] Half neutralized.
[c] Diazotization product of *p*-acetoaniline and *o*-cresol.

However, neoprene tubing, now supplied with the titrator, has the necessary flexibility and chemical properties. The recently described automatic stopcock twister (*9*) replaced the tubing-solenoid system in this work.

ELECTRODES. Nickel, Chromel, tungsten, rhodium, copper, silver, palladium, platinum (10% rhodium), tin, iron, titanium, antimony, and platinum wires or rods sealed in glass tubes; a general-purpose Beckman glass electrode (Beckman Instruments, Inc.); a Sargent calomel electrode modified with saturated methanolic potassium chloride to replace the aqueous solution; and a graphite electrode made by stripping a few inches of wood from one end of a 9 H pencil. These electrodes were used in various combinations as described.

INDICATORS. Commercially available indicator materials were made up as 0.1% solutions in absolute methanol.

SOLVENT. Reagent grade acetone.

ORGANIC ACIDS. Various reagent grade and C.P. acids analyzed as received.

TITRANT. The titrant is tri-*n*-butylmethylammonium hydroxide in about 80% benzene–20% 2-propanol. Forty grams of silver nitrate were dissolved in 250 to 300 ml. of demineralized water and to this were added 100 ml. of 25% sodium hydroxide, which was prepared from a saturated aqueous solution of sodium hydroxide. The resulting silver oxide precipitate was washed by decantation with ten 100-ml. portions of water, collected on a filter, and washed twice with water, six times with absolute methanol, and finally with one 100-ml. portion of 2-propanol.

The moist silver oxide was added to a previously prepared solution of 32.8 grams of tri-*n*-butylmethylammonium iodide (*12*) in 90 ml. of 2-propanol contained in a glass-stoppered 300-ml. flask. After the flask had been shaken for 15 minutes, the mixture was poured through a sintered-glass filter funnel into a dark storage bottle. The flask was rinsed with three 25-ml. portions of 2-propanol, and the filtrate in the dark bottle was made up to 1 liter with dry benzene.

This titrant, protected from carbon dioxide and moisture by an Ascarite tube, was stable for more than 1 month.

Moist silver oxide was used because it reacts much faster than dry material. 2-Propanol, being less acidic, was used rather than methanol (*7*).

ELECTRODES FOR AUTOMATIC DERIVATIVE POTENTIOMETRIC TITRATION

Various types of reference and indicator electrodes can be used with the automatic derivative titrator, even though their absolute potentials shift from one titration to the next. The platinum (10% rhodium) *vs.* graphite and other electrode pairs were tested for applicability in the titration of organic acidic materials using acetone solvent and tri - *n* - butylmethylammonium hydroxide titrant dissolved in about 80% benzene–20% 2-propanol. No advantage was apparent for any electrode pair over the platinum (10% rhodium) *vs.* the pencil lead reference electrode which is available, simple, small, and reliable for the automatic titration of a variety of acids in acetone solvent and applicable for previously described methods (*10, 12, 15*). None of the electrode pairs was suitable for titrating phenol itself without amplifying the signal prior to feeding into the derivative titrator.

Recorded potentiometric titration curves using various electrode pairs are shown in Figures 1 to 4, for the titration of 5-ml. aliquots of 0.1*N* benzoic acid in 50 ml. of acetone. The titrant was added continuously at a rate of about 2 ml. per minute. For the automatic derivative titrations an electrode pair was connected directly to the input of the titrator, except for the glass *vs.* calomel pair, which was connected via a pH meter.

The electrode materials (Figure 1) respond rapidly and provide sharp changes of potential at the equivalence point. All of these materials can be used as indicator electrodes *vs.* the modified calomel electrode. The electrode materials (Figure 2) change potential rather slowly in the equivalence point region, and these electrodes have possibilities as reference electrodes to replace the calomel electrode and salt bridge. Figure 3 shows the potentiometric curves for platinum (10% rhodium), nickel, and Chromel *vs.* graphite, and it is apparent that the pencil lead acts as a good reference electrode for automatic derivative titrations, yielding titration curves similar to those with the calomel reference electrode.

Rather unusual titration curves are obtained using either platinum (10% rhodium), nickel, or Chromel *vs.* tungsten as the electrode system, as shown in Figure 4. The equivalence points on these curves are marked with an

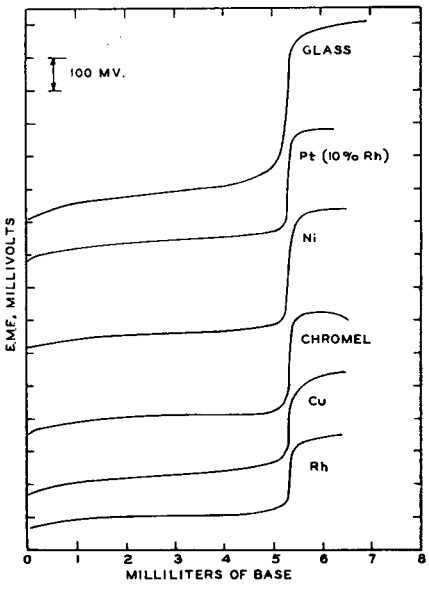


Figure 1. Recorded curves for titration of benzoic acid

Various indicator electrodes *vs.* calomel reference electrode

arrow. There is no attempt to explain the shapes of these titration curves, but they are suitable for automatic derivative end point termination The potential across the electrodes is fed directly into the derivative titrator and the titration is terminated automatically at the equivalence point (marked by the arrow).

INDICATORS FOR AUTOMATIC DERIVATIVE SPECTROPHOTOMETRIC TITRATION

Most of the present color indicators for nonaqueous titrimetry have been used on an empirical basis. Some answers as to choice of an indicator can be obtained from the literature, but most investigations were done to determine the relative strengths of acids in various solvents (*5*, *8*). Only phenolphthalein, thymolphthalein, and thymol blue have received much attention as indicators in inert solvents (*14*). A study was made as to which indicators function in acetone solvent and their applicability in titrating various acids.

Some pseudobuffer systems were prepared which covered a wide range of acidity; 0.1*N* solutions of different acids were half neutralized with 0.1*N* tri-*n*-butylmethylammonium hydroxide and diluted tenfold with acetone to form buffers which were 0.005*N* in both acid and salt. The acids used, in order of decreasing relative acidity, were perchloric, nitric, benzoic, anthranilic, *o*-nitrophenol, and phenol. Solutions of 0.01*N* perchloric acid, 0.01*N* tri-*n*-butylmethylammonium hydroxide, and acetone were also used to find the color of each indicator in these solutions.

To 10 ml. of each of the above solutions were added 2 drops of 0.1% indicator. The colors of the indicators in the various solutions are shown in Table I. Other indicators which function in water but did not have a pronounced color change in acetone were eosin (0.0 to 3.0), *m*-cresol purple (1.2 to 2.8), phenol red (6.8 to 8.4), and Tropeolin 0 (11.1 to 12.7).

The choice of indicator for a specific titration was made with the aid of Table I.

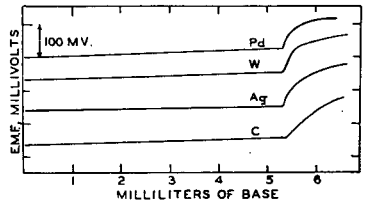


Figure 2. Recorded curves for titration of benzoic acid

Calomel reference electrode *vs.* various materials with characteristics suitable as automatic derivative reference electrodes

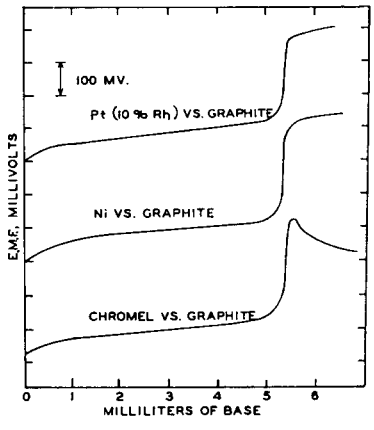


Figure 3. Recorded curves for titration of benzoic acid

Platinum (10% rhodium), nickel, and Chromel indicator electrodes *vs.* graphite reference electrode

PROCEDURE

A sample containing approximately 0.5 meq. of acid is dissolved in a small quantity of a suitable solvent, 40 ml. of acetone are added, and it is titrated with standard tri-*n*-butylmethylammonium hydroxide titrant. Automatic end point termination can be performed by either the derivative potentiometric or derivative spectrophotometric procedure. The samples may be dissolved in many solvents—alcohols, ketones, ethers, hydrocarbons, or chlorinated hydrocarbons, as long as the final titration medium contains at least 90% acetone. Water can be used, except for very weak acids such as enols and phenols.

Acetic acid in regular reagent grade acetone causes a small titration blank which is determined and subtracted from the total volume of titrant used for titration of the acid. The milliliter acetone blank is usually about 0.01 ml. of 0.1*N* titrant per 10 ml. of acetone used, and is most easily determined by the manual titration of a 50-ml. aliquot of acetone to the thymolphthalein end point.

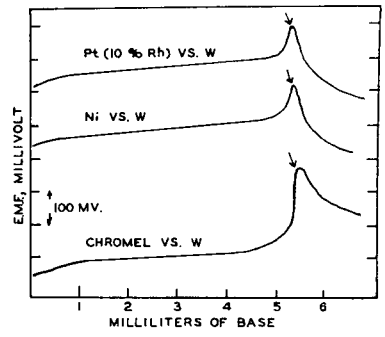


Figure 4. Recorded curves for titration of benzoic acid

Platinum (10% rhodium), nickel, and Chromel electrodes *vs.* tungsten electrode

Automatic Derivative Potentiometric Titration. A platinum (10% rhodium) indicator electrode is connected directly to the grounded cathode (black lead in cable) and the graphite reference electrode to the grid (red lead in cable) of the Sargent-Malmstadt automatic derivative titrator. A sample contained in a small beaker is inserted into the titrator; the delivery of titrant, previously adjusted to a rate of about 2 ml. per minute, and the stirring are started by pushing the automatic titration button; upon automatic termination at the end point, the volume is read and recorded. If a second end point is to be obtained for a mixture, the button is pushed again and the buret read after the automatic termination.

Automatic Derivative Spectrophotometric Titration. The correct filter and polarity positions are set on the Spectro-electrotitration unit as determined by the indicator to be used (Table II). Two drops of a suitable indicator are added to the sample contained in a small beaker, the beaker is inserted into the titrator, the start button is pushed, the titration automatically terminated at the end point, and the volume of titrant delivered is recorded. It is important that the correct stirrer be used in the titrator, so that the stirring is efficient but that no air whipped into the solution.

CALCULATIONS

The per cent purity of a known acid is calculated using Equation 1, and the neutralization equivalent of an unknown acid by Equation 2.

$$\% \text{ purity} = \frac{N_B (V_B - V_o) W_e}{10\ W} \qquad (1)$$

Neutralization equivalent =

$$\frac{1000\,W}{N_B\,(V_B - V_o)} \quad (2)$$

where N_B is the normality of base, V_B the milliliters of base from start of titration to the end point, V_o the milliliters of base to titrate impurity in the acetone, W_e the gram equivalent weight of acid, and W the grams of sample titrated.

RESULTS

The precision for both the automatic derivative potentiometric and spectrophotometric methods is illustrated (Table III) for a series of 10 titrations each for a relatively strong acid, benzoic, and a weak acid, succinimide. The standard deviation in all cases is about the reading error for the buret used.

The percentage purity for each of several organic acids, mostly reagent grade, was determined by automatic derivative end point termination (Table IV). The platinum (10% rhodium) *vs.* graphite electrode pair was used for the potentiometric procedure; for the spectrophotometric procedure azo Violet indicator was used for the acids succinimide through mercaptobenzothiazole, and thymolphthalein indicator used for benzoic acid and the last four acids in Table IV.

Determination of mixtures of mineral acids by automatic derivative potentiometric and spectrophotometric titration has recently been described (*12*). Mixtures of salicylic acid and methyl salicylate were titrated by the potentiometric procedure in this study (Table V). The accuracy of the results was limited because of the small volumes of titrant used. The reproducibility for each mixture was within the buret reading errors.

Table II. Selected Indicators for Various Acid Types

Acid Type[a]	Indicator	Color Change	Filter, Mμ	Polarity Position[b]
Very weak acids	Azo Violet	Red-blue	600	2
Substituted acetic acids	Thymolphthalein	Colorless-blue	575	2
Substituted benzoic acids	Thymol blue	Yellow-blue	575	2
Mineral acids	Neutral red	Red-yellow	550	1

[a] Nitrated phenols may be similar in strength to any of these acids. Acidic strength of phenols increases directly with number of nitro groups and depends somewhat upon their position in benzene ring.
[b] Polarity switch position 1 connects output from photoconductive detector circuit so that a decrease in absorbance at the end point provides a positive grid voltage swing at the input to the derivative control unit; position 2 gives correct connection for an increase of absorbance at the end point for the preselected wave length.

Table III. Reproducibility of Automatic Derivative Nonaqueous Titrations

Titrant for 5 Ml. 0.1*N* Benzoic Acid, Ml.		Titrant for 5 Ml. 0.1*N* Succinimide Acid, Ml.	
Potentiometric	Spectrophotometric[a]	Potentiometric	Spectrophotometric[b]
5.250	5.260	5.255	5.265
5.255	5.255	5.260	5.255
5.235	5.260	5.255	5.260
5.250	5.265	5.260	5.260
5.255	5.255	5.250	5.250
5.265	5.250	5.250	5.260
5.255	5.255	5.255	5.260
5.250	5.270	5.255	5.255
5.260	5.250	5.260	5.265
5.250	5.260	5.265	5.270
Av. 5.253	5.258	5.256	5.260
Std. dev. 0.008	0.006	0.005	0.006

[a] Thymolphthalein indicator.
[b] Azo Violet indicator.

Table IV. Percentage Purity of Various Organic Acids Determined by Automatic Derivative Titration in Acetone Solvent

Acid	Purity, % Potentiometric	Purity, % Spectrophotometric
Benzoic	Standard	
Succinimide	100.1	100.0
Phthalimide	100.3	100.3
Dibenzoylmethane	100.3	99.8
Phenol	...	99.6
Dodecylmercaptan	99.5	98.9
Mercaptobenzothiazole	97.3[a]	97.5[a]
Phenylacetic acid	100.1	100.0
p-NO_2(benzenesulfonamide)	100.0	100.0
m-NO_2 phenol	100.3	100.0
p-NO_2 benzoic acid	99.5	99.1

[a] Producer's specification, 97.5%.

Table V. Automatic Derivative Potentiometric Titration of Mixtures of Salicylic Acid and Methyl Salicylate

Salicylic Acid, Meq.		Methyl Salicylate, Meq.	
Taken	Found	Taken	Found
0.285	0.284	...	...
...	...	0.230	0.230
0.381	0.380	0.230	0.230
0.190	0.191	0.460	0.457
0.381	0.382	0.060	0.060
0.190	0.192	0.341	0.338
0.047	0.046	0.569	0.570
0.285	0.285	0.341	0.340

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RECEIVED for review April 11, 1958. Accepted January 30, 1959. Part of this material from a thesis presented by D. A. Vassallo in partial fulfillment of the requirements for the degree of doctor of philosophy, University of Illinois, Urbana, Ill., February 1958. Research supported in part by Standard Oil of Indiana Fellowship and in part by the U. S. Air Force under contract No. AF 18(603)-137, monitored by the Air Force Office of Scientific Research, Air Research and Development Command.

Gas Chromatographic Determination of Fuel Dilution in Lubricating Oils

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▶ A gas chromatographic method has been developed for measuring fuel dilution in crankcase oil. The apparatus consists of a preheater block which flashes the diluent from the oil into a helium stream, a precolumn which condenses oil entrained in the helium flow, and a gas chromatographic column which resolves and measures the amounts of diluent. Results are based on the use of an internal standard placed in known concentration in each determination. Extensive calibration tests indicate that the chromatographic method is the most sensitive and accurate analysis yet available for measuring fuel dilution. Supplementary advantages over distillation analyses are that small samples are used, the test is simple, the volatility range of the diluent is indicated, and the chromatographic chart provides a permanent record.

THE amount of gasoline found in crankcase oil may be used as a criterion of automobile engine performance. Dissolved gasoline lowers motor oil viscosity and causes an increase in oil consumption. Section Q-1, Technical Committee B, Committee D-2 of the American Society for Testing Materials has been studying determination of fuel dilution by distillation methods for many years. From extensive cooperative testing of distillation methods in several laboratories (*3*), it has been shown that: distillation methods give results lower than the true values, analysis is influenced by the nature and amounts of diluent and by the composition of the base oil, and reproducibility and repeatability of the distillation methods are poor.

With the aid of a simple attachment for a conventional gas chromatographic apparatus, a method for determining fuel dilution has been developed. The method has good precision, accuracy, and sensitivity. Results also give an indication of the nature of the diluent fraction. The test is simple and inexpensive. A single analysis requires 1 hour of instrument time and about 0.5 hour of operator time.

APPARATUS

The sample containing an internal standard is injected into a preheater block which flashes the diluent and standard into a stream of helium. The gas then goes into a precolumn which removes oil entrained in the helium. The helium plus the vaporized diluent and standard then enter a chromatographic unit where the diluent and standard are resolved and measured. A schematic drawing of the entire apparatus is shown in Figure 1.

Preheater Block. A high temperature method has been chosen for removing diluent fractions from oil. Helium is used to sweep volatilized fractions directly into a chromatographic column. A cutaway drawing of the heater block used to remove fuel fractions from oil is shown in Figure 2. The heater block has been designed with a high thermal capacity to ensure good heat transfer to the sample.

Four 125-watt strip heaters are bolted to the sides of the brass block. The heaters are connected in series and are controlled through a variable transformer. Fuel dilution determinations are made at block temperatures of 315° C. which correspond to a transformer setting of 65 volts. Settings of 110 volts may be used to bring the block quickly up to test temperature. The lowest temperature at which gasoline fractions are all immediately volatilized is 315° C. No peaks are observed for oil fractions or for products due to degradation of oil or polymer additives below this temperature.

The threaded plug at the base of the preheater block provides a method of removing solids from the system. In practice, it has been found that small amounts of solids do not affect the method or results. Moreover, solids accumulate slowly so that the block needs to be cleaned out no more than once every 50 runs. The threaded plug makes a tight seal with or without the use of a Teflon gasket.

The high operational temperatures for the preheater block require that it be extensively insulated. The block fits conveniently within a 2-liter borosilicate glass beaker. The sides and the base of the beaker are packed with asbestos and glass wool. The top of the beaker has been trimmed so that the valves on the preheater block may be easily operated.

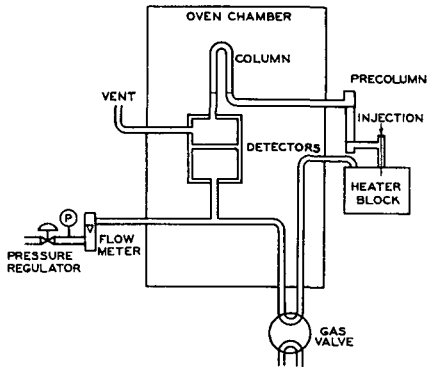


Figure 1. Apparatus for fuel dilution tests

Precolumn. Early research showed that oil samples are carried out of the preheater block by entrainment in the helium stream. The entrained oil is carried into the column where it condenses and dissolves in the packing, resulting in a marked change in the retention times and in the resolving power of the column. In cases of extreme contamination, column blockage occurs.

To protect the column packing, a vertical 4-inch precolumn has been installed between the preheater block and the Fractometer. The precolumn traps oil fractions which have been carried from the block. The position of the precolumn is shown in Figures 1 and 2. The column is constructed from conventional pipe, $^1/_2$ inch in outside diameter. The precolumn and adjacent lines are kept at a temperature well below that of the block and slightly higher than that of the analyzer column. Nine feet of Fiberglas-covered Nichrome wire (1.55 ohms per foot) is wrapped evenly about the helium line between the preheater block and the Fractometer inlet. A potential of 33 volts, established by a variable transformer, gives a satisfactory precolumn temperature of 175° C. Higher precolumn temperatures allow oil to be carried through into the unit; lower temperatures tend to retard unduly the diluent fractions.

To minimize vapor condensation and diffusion, short heated lines are used to connect the block, the precolumn, and the Fractometer. The precolumn packing is the same as that employed in the Fractometer and can hold up to 1.5 ml. of oil without disturbing experimental

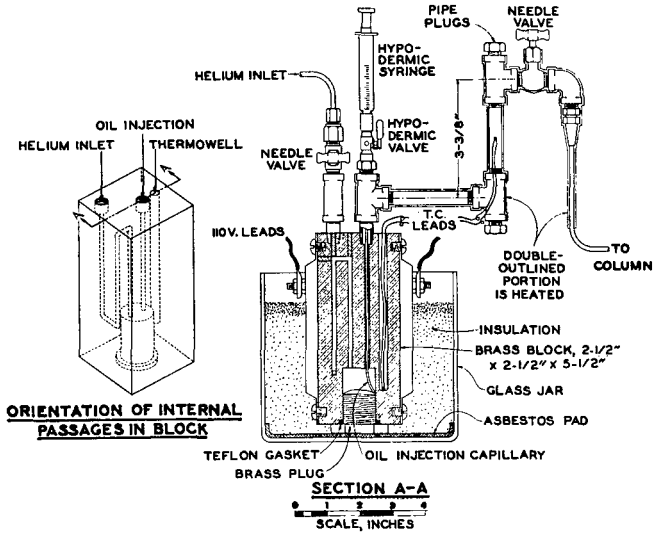


Figure 2. Preheater block for fuel dilution apparatus

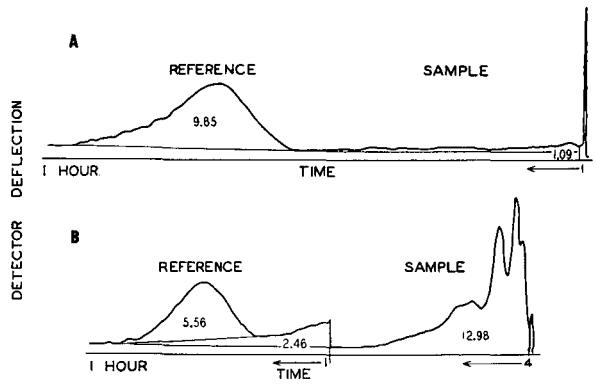


Figure 3. Chromatographic analyses in base oil

A. 0.10% premium gasoline, analysis 0.11%
B. 10.00% premium gasoline, analysis 9.78%

results. Beyond this amount, abnormal retention times and blockage of flow are observed. Therefore, the column packing is systematically replaced every three runs. To repack the precolumn, the helium flow is diverted by first turning the gas sample valve to vent. The Hoke valves adjacent to the precolumn are then turned off. Next, pipe plugs at either end of the column are unscrewed. The glass wool is then removed at each end of the precolumn, and the packing is rammed out by using a small diameter rod.

To replace the precolumn packing, glass wool is loosely fitted at the base of the precolumn. A small funnel is helpful in pouring the packing into the precolumn. A glass wool plug is placed at the top of the lightly packed column. The pipe plugs are then replaced, and the helium flow is again routed through the block and precolumn. Some care must be taken to prevent packing from entering and blocking the side arms. Packing replacement takes about 3 minutes. The heater on the precolumn need not be turned off for this operation.

To measure the temperature of the preheater block, a $^1/_8$-inch hole is drilled several inches into the top of the brass block. A Chromel-Alumel thermocouple is inserted in the hole to record block temperatures. Another thermocouple is placed against the barrel of the precolumn. Readings taken at this position have been shown to represent true precolumn temperatures. Temperatures of the block and precolumn are read from a single potentiometer box which is connected to the thermocouples through a heavy-gage, double-throw, knife switch.

Gas Chromatographic Apparatus. A Perkin-Elmer Vapor Fractometer Model 154B was used. Choice of the column packing, length, operating temperature, and other specifications is arbitrary; many variations are possible. The chief reasons for the particular conditions given here are that they give a reasonable elution time (about 1 hour) and some resolution for diluent components.

The column is 2 feet long and is made from copper tubing $^1/_4$ inch in outside diameter. The column temperature is 150° C. The column packing is 20 to 40 mesh size, Johns-Manville C-22 insulating brick, coated with 15 grams of 128° to 130° F. melting point paraffin wax per 100 grams of brick. The helium valve on the unit is adjusted at pressures between 10 and 20 p.s.i. to produce rotameter readings between 12 and 15. This corresponds to helium flow rates of 500 to 700 ml. per minute. The high flow rates keep run times below 1 hour. The potential on the detectors is set at 8 volts.

METHOD

An internal standard is used to determine the amount of diluent present. Two reference compounds have been used satisfactorily as standards. Each gives a useful retention time and is readily available in sufficient purity. *n*-Dodecane has a retention time of about 40 minutes and is used for many samples. When samples contain diluent less volatile than *n*-dodecane, 2-methylnaphthalene with a retention time of about 1 hour is used. The internal standards are made up in oil so that solid reference compounds may be used and small amounts of the reference may be accurately measured volumetrically. Reference solutions are made up with 10 grams of reference in 100 ml. of base oil plus reference. The base oil is conventional lubricating oil that has been heated under vacuum to remove any low boiling contaminants.

Unused samples of some compounded motor oils give a single peak on the recorder chart with a retention time of less than 30 seconds. Presumably this peak is associated with an oil additive. All diluent fractions tested have retention times greater than 30 seconds. Therefore, components which appear at less than 30 seconds are disregarded. Measurements on unused oils showed no detectable peaks with resolution times between 30 seconds and 2 hours. The base oil therefore does not interfere with the analysis. All species in the gasoline boiling range which are found in used oils are arbitrarily classified as fuel dilution.

A graduated hypodermic syringe is used to blend carefully a 10-ml. aliquot of test oil with 1 ml. of reference solution. The mixture is agitated in a closed bottle at room temperature for 30 seconds. By using a clean syringe with a long needle of large diameter, 0.25 to 0.5 ml. of sample is removed from the bottle. A 2-ml. B-D Yale syringe with a Luer-Lok fitting is satisfactory for sample handling. The needle on the syringe is detached, and the tip of the syringe is turned up. The plunger is then pushed in until essentially all air is removed and the proper amount of sample remains in the syringe. The syringe is next screwed onto the Luer-Lok fitting on the block. If the entire fuel dilution apparatus has come to thermal equilibrium, the sample may then be injected.

To inject a sample, the hypodermic syringe and plunger are held firmly, while the small valve leading to the block is opened by turning the stem 90°. When the valve is open, internal helium pressure tends to push out the hypodermic plunger. When this impulse is felt, the plunger is pushed down and 0.25 to 0.5 ml. of sample is injected. While the plunger is held down, the inlet valve is closed. The syringe may then be removed and cleaned.

The thermocouples in the block, precolumn, and Perkin-Elmer unit are used to judge when the dilution apparatus is in thermal equilibrium. In practice, the recorder is the most sensitive indicator for thermal equilibrium. When the pen record is smooth, and without drift with time at high sensitivity settings, a dilution sample may be injected.

The Perkin-Elmer unit is commonly run at an attenuation setting of one. Any reduction in sensitivity which may be necessary to keep the pen on

Table I. Calibration Runs on Dilution Samples of Known Concentration

Gasoline, 50% Bottoms	Made Up Concn., %[a]	Av. for 10 Runs, %[a]	Abs. Error	Std. Dev.
Regular	0.117	0.107	−0.010	0.034
Premium	0.110	0.103	−0.007	0.018
Regular	1.003	0.98	−0.023	0.081
Premium	0.992	0.94	−0.052	0.092
Regular	5.004	5.10	+0.096	0.34
Premium	5.006	5.02	+0.014	0.26
Regular	10.02	10.09	+0.07	0.26
Premium	10.07	10.23	+0.16	0.52

[a] 1% dilution is 1 gram of diluent in 100 ml. of oil plus diluent.

Table II. ASTM Cooperative Fuel Dilution Samples

ASTM Sample	Base Oil		Diluent		Fuel Dilution, Volume in Volume, %		
	Winter	Summer	20% bottoms	Whole gasoline	Method I, steam distn.	Method II, vacuum distn.	Method III, gas chromatography
Q-1-N		X	X		4.0	5.9	6.3
Q-1-O		X	X		13.5	16.5	16.6
Q-1-P		X		X	3.0	5.0	7.5
Q-1-Q		X		X	9.5	13.5	16.9
Q-1-R	X		X		2.7	5.2	5.1
Q-1-S	X		X		13.8	15.8	19.7
Q-1-T	X			X	2.7	4.7	5.7
Q-1-U	X			X	9.6	12.2	12.1

Table III. Analysis of Regular Fuel Dilution Samples

Gas Chromatography		Steam Distn., Ml./Ml., %	Difference, Ml./Ml., %
Gram/ml.	Ml./ml., %		
1.68×10^{-2}	2.1	1.2	−0.9
2.77	3.4	2.5	−0.9
10.17	12.4	10.2	−2.2
5.06	6.2	5.0	−1.2
0.90	1.1	0.4	−0.7
0.49	0.6	0.1	−0.5
0.66	0.8	Trace	−0.8
7.26	8.9	6.0	−2.9

chart occurs within the first 5 minutes of a run. The highest concentration of diluent fractions is commonly found in this region. From 5 minutes to the termination of the run, operator time may be spent in preparing samples and in integrating areas on the charts from previous determinations.

All diluent fractions are integrated as one area. For convenience, the dilution area may be arbitrarily divided up. Of course, where recorder sensitivity has been changed, areas must be integrated separately. Commonly, the base line for a dilution run is identical with the base line on the chart. In cases where the pen does not start or terminate at zero, careful judgment must be used in drawing the base line. There can be little uncertainty in the base line when the line is drawn through the initial and final pen positions. The pen often returns to the base line at midrun, which is after the gasoline fractions and prior to the reference. When this is true, the base line for the diluent and reference are the same. However, when diluent fractions dribble out into the dodecane region, the base line for the reference is drawn on a regular descending slope of the gasoline peak. No significant error is caused by this procedure as the distribution of compounds and isomers in the higher molecular weight range is such that there is no resolution in this region.

The concentration of diluent in oil is calculated by dividing the area for the diluent by the area of the reference peak. The quotient gives directly the concentration in grams of diluent per 100 ml. of oil; 1 gram in 100 ml. is called 1% dilution. No correction is entered for the differences in the thermal conductivities of the vapors involved. Thermal conductivities of all species in gasoline are known to be very similar. Moreover, calibration of the method with samples of known diluent concentration indicates that conductivities need not be considered as a source of error. The analyses may be easily adapted to other types of detection systems. For example, the carbon dioxide conversion technique would yield results which would be nearly independent of calibration errors.

After the final peak for a run has appeared on the recorder chart, the residual oil in the block is removed. This is accomplished by attaching tubing from a vacuum bottle to the Luer-Lok fitting. With vacuum on the sample inlet valve, the valve is opened for about 5 seconds. The oil is sucked up the tubing and is observed as a small amount of froth in the bottle. Only a small fraction of the total oil sample is recovered in this way as most of the oil is entrained in the helium stream. The entrained oil is condensed and recovered in the precolumn packing.

RESULTS

Three types of test samples were used in the development of the method: samples of known diluent concentration, ASTM cooperative samples, and randomly selected samples sent in for dilution analyses by distillation.

Samples of Known Concentration. Eight synthetic fuel dilution blends of known composition have been tested by the chromatographic method. The base oil for these blends was an unused 10–30 SAE multigrade oil. A standard and premium gasoline were distilled and the 50% bottoms used as diluent. The diluent samples which were made up at four concentration levels were analyzed in random fashion. Table I lists these results. In similar gas chromatographic analyses, peak areas have been found to agree well with known weight percentages. Therefore, the results are presented in units of weight of diluent per volume of oil. For each sample the average deviation of results is larger than the absolute error, which indicates that there are no consistent errors in the determination. Three per cent of all values were rejected on the basis of probability theory. The suspected values in each case differed from the mean of the rest of the array by four times the average deviation. As experience with the method was gained, the number of rejected results diminished rapidly.

Figure 3 shows typical results obtained on samples containing 0.1% and 10.0% dilution. The initial peak is caused by a pressure surge and/or air and is disregarded. The numbers under the curves are the planimetered areas for the diluent and reference in square inches. The numbers on the base line are sensitivity settings. The record illustrates the sensitivity and versatility of the method. The planimetered area representing 0.1% dilution is large enough so that it is safe to predict that dilutions can be detected to below 0.05%.

ASTM Samples. In 1957, eight laboratories participated in a cooperative program on eight fuel dilution samples. Two distillation analyses were employed: Method I was a conventional steam distillation (*1*); Method II was essentially a reduced

pressure distillation somewhat similar to ASTM D 1160 (*2*). Two base oils and two types of diluent were used. One diluent was a whole gasoline, the other a 20% bottoms fraction from the distillation of gasoline. These samples were run in quadruplicate by the gas chromatographic method. API gravities reported for the diluent were used to convert the gas chromatographic results to volume-in-volume concentrations to permit direct comparisons. Table II lists sample composition and results and shows that gas chromatographic values are generally higher than distillation results. Distillation procedures in no case give significantly higher diluent concentrations than chromatography. Results on sample Q-1-S seem abnormally high, and perhaps the sample tested in this work has become contaminated.

It is difficult to remove all diluent by distillation. Sample Q-1-R was heated to 300° F. for 4 hours and all vapors were removed which came off with the pressure reduced to 1 mm. of mercury. After this treatment, about 1% of diluent still was present in the oil.

Regular Dilution Samples. Eight randomly selected samples of used lubricating oil, submitted for dilution analysis by ASTM distillation D 322-55T (*1*), were determined by the gas chromatographic method. Using an assumed density for diluent of 0.82, gas chromatographic results were converted to volume-in-volume units. Results for these samples are tabulated in Table III. The chromatographic charts obtained were similar to those for the synthetic and ASTM samples. Again the chromatographic results were consistently higher than those obtained by steam distillation.

CONCLUSIONS

Results indicate that the chromatographic technique has improved the sensitivity, precision, and accuracy of fuel dilution analyses. Moreover, there are several additional advantages of this method over distillation procedures. Samples as small as 0.25 ml. may be run by weighing in the internal standard. Therefore, oil dilution samples may be periodically extracted during a continuous engine test without appreciably altering engine conditions.

If desired, resolution of the diluent can be increased by use of longer columns and lower temperatures than used in this work. This makes it possible to study individual fuel components that appear as diluent. The chromatographic chart also provides a permanent record of the nature and amounts of diluent.

This general technique has proved to be very useful for a variety of analytical problems.

LITERATURE CITED

(1) Am. Soc. Testing Materials, Philadelphia, Pa., "ASTM Standards," p. 169, Method D 322-55T, 1958.
(2) *Ibid.*, Appendix I, p. 1076.
(3) *ASTM Bull.*, to be published.

RECEIVED for review July 25, 1958. Accepted October 17, 1958. Division of Petroleum Chemistry, 134th Meeting, ACS, Chicago, Ill., September 1958.

Gas Chromatographic Determination of Dissolved Oxygen in Lubricating Oil

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▶ **A rapid quantitative method for the determination of oxygen in lubricating oil, utilizing gas chromatographic techniques, was developed in connection with a program relating engine wear to the amount of oxygen present in lubricating oil.**

In the recently developed chromatographic method for oxygen determination in lubricating oil, a special column effects a preliminary separation of the dissolved gases from the oil. Then the mixture of gases is passed through a Molecular Sieve analyzing column, which separates the individual components so that oxygen can be determined. The total analysis time is approximately 10 minutes. The lower limit of detection of oxygen is 0.01 volume %. The standard deviation of a single determination is 2.2% of the mean. The method can be extended to include the determination of other dissolved gases, such as nitrogen. The only known interference is argon, which has the same retention time as oxygen.

EXPERIMENTAL

Apparatus. The apparatus consists of a commercial gas chromatographic instrument modified with a bypass arrangement. The preliminary column, a glass U-tube, is attached to the bypass arrangement as shown in Figure 1. One leg of the glass U-tube is fitted with two coarse-porosity fritted disks 5 inches apart. A sampling port for injection of the oil sample is located midway between the disks. The diameter of the sample chamber is 0.5 inch. The analyzer column, a section of copper tubing 20 feet long and 0.25 inch in outside diameter, is packed with Linde 14 × 30 mesh Type 5A Molecular Sieve. The recorder is a 0 to 5 mv. strip chart which is operated at 60 inches per hour.

Procedure. The instrument is thermostatted to 75° C. and is operated with a helium carrier gas flow rate of 50 ml. per minute. The glass U-tube is wrapped with heating tape and also maintained at 75° C. It is attached to the bypass assembly, and the entire assembly is purged with helium. Then the stopcocks of the assembly are adjusted so that the glass U-tube is in the carrier gas stream of the instrument. When the instrument has reached equilibrium, as evidenced by a stable base line on the recorder, the oil sample is injected into the

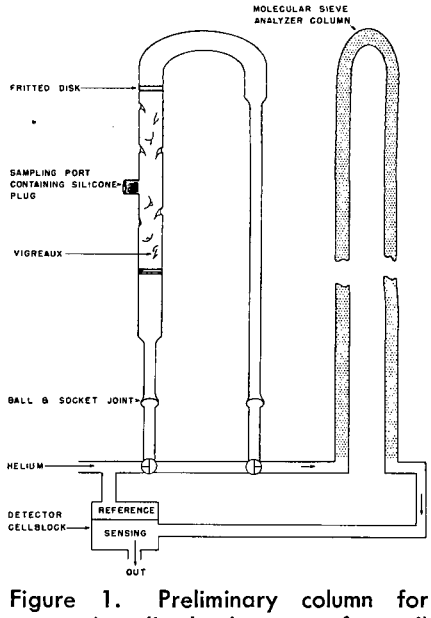


Figure 1. Preliminary column for separating dissolved oxygen from oil

Table I. Determination of Oxygen in Typical Lubricating Oil

Sample Size, Ml.	Temp. of U-Tube, ° C.	Volume % Oxygen Found
1.0	25	1.57
		1.60
		1.39[a]
0.25	75	1.70
		1.65
		1.80
1.0	75	1.72
		1.71
		1.73[a]
		1.70[b]
		1.73
		1.74
		1.77
2.0	75	1.82
		1.79

[a] Sample added to 1 ml. of oxygen-free oil in U-tube.
[b] Sample added to 2 ml. of oxygen-free oil in U-tube.

sampling port of the glass U-tube. The retention times of oxygen and nitrogen are approximately 6 and 9 minutes, respectively. Upon completion of the analysis, the glass U-tube is removed from the bypass arrangement and rinsed with hexanes to remove the oil sample. Calibration data are obtained by using pure oxygen as a standard. The sensitivity of the instrument is such that a peak area of about 16 square inches is obtained for 0.1 ml. of oxygen at standard temperature and pressure. The sensitivity is checked daily to minimize errors caused by any instrumental variations. Peak areas are measured with a planimeter.

RESULTS

Table I shows the values of oxygen obtained from an oil sample that was saturated with air. Preliminary studies showed that oxygen was not quantitatively removed from the oil when the glass U-tube was operated at room temperature and that the peaks on the chromatogram tended to tail slightly. With large oil samples, the tailing was more pronounced and the results were low. When the glass U-tube was heated to 75° C., no tailing was observed, and results were consistent over the range of sample sizes tested.

Preliminary columns of this type serve two purposes: The analysis can be performed without contaminating the analyzer column with high-boiling components, and speed of analysis is increased as only specific compounds reach the analyzer column.

RECEIVED for review November 10, 1958. Accepted January 8, 1959.

Paper Chromatography of 2,4-Dinitrophenylhydrazones

Resolution of 2-Alkanone, *n*-Alkanal, Alk-2-enal, and Alk-2,4-dienal Derivatives

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▶ A rapid paper chromatographic procedure for separating mixtures of 2,4-dinitrophenylhydrazones, 2-alkanones, *n*-alkanals, alk-2-enals, and alk-2,4-dienals of chain lengths up to 12 to 14 carbon atoms is described. Any member of the four homologous series studied, with the exception of acetone, ethanal, propanal, butanal, and propenal, can be identified. The procedure consisted of resolving into classes by ascending development with petroleum ether on untreated filter paper. Acetone separated with the *n*-alkanal class and methanal and ethanal with the alk-2-enal class. Earlier paper chromatography systems for the separation of *n*-alkanals were used to separate class groups into individual compounds. The inseparable mixtures were acetone-propanal, acetone-butanal, and ethanal-propenal.

A NUMBER of paper chromatographic systems are capable of separating a limited number of members of a homologous series of 2,4-dinitrophenylhydrazones. Ellis, Gaddis, and Currie (*3*) described a rapid ascending paper chromatographic procedure for separation of the C_1 to C_{14} *n*-aliphatic saturated aldehyde series, which was believed applicable to other homologous series of aliphatic monocarbonyls. However, it is extremely difficult to separate mixtures composed of different homologous series. Forss, Dunstone, and Stark (*5*) reported comparative R_f values of the four homologous series on the paper chromatographic system of Huelin (*9*) and Meigh (*15*); with the exception of acetone, values were similar for 2-alkanone C_n, alkanal C_{n+1}, alk-2-enal C_{n+2}, and alk-2,4-dienal C_{n+4}. Nonaka, Pippen, and Bailey (*17*) found a different R_f value alignment with the paper chromatographic system of Lynn, Steele, and Staple (*13*). Bassette, Day, and Keeney (*1*) separated homologous series easily on partition columns of nitromethane-hexane-Celite. However, mixtures of homologous series of 2-alkanones (except acetone), alkanals, and alk-2-enals were resolved into characteristic groups of C_n, C_{n+1}, and C_{n+2}, respectively. Monty (*16*) has reported a differential spectrophotometric determination of saturated aldehydes and ketones separated on a modified Kramer and van Duin (*12*) partition column. Pippen and coworkers (*18*, *19*) separated some of these inseparable groups with silicic acid–Celite adsorption columns, and by repeated chromatography of fractions isolated a number of aliphatic monocarbonyl derivatives from chicken tissue. Forss and Dunstone (*4*) have shown that adsorption columns are relatively destructive and may cause rearrangements.

This paper describes the extension of the paper chromatographic method of Ellis, Gaddis, and Currie (*3*) to other homologous series. A rapid new paper chromatographic method for the separation of mixtures of the four homologous series into classes is reported. The separation was referred to briefly by Gaddis and Ellis (*7*) and Ellis *et al.* (*3*) and this work was necessary to determine its effectiveness. The method (*8*) has since been used in a preliminary study of changes in the proportions of volatile monocarbonyl classes obtained from oxidizing fat.

SOLVENTS AND REAGENTS

ACS grades of carbon tetrachloride,

benzene, and methanol, and Skellysolve C (boiling point 91° to 95°) (Skelly Oil Co.) were purified as described (*3*). Technical grade petroleum ether was distilled, and the fraction boiling at 37° to 40° C. was used. Propylene glycol, U.S.P., and vaseline (white petroleum jelly, Blue seal, Chesebrough Manufacturing Co.) were also used.

MATERIALS AND EQUIPMENT

Materials and equipment have been described (*3*). Whatman No. 3 filter paper sheets were cut in the machine direction into tapering strips $10^1/_2 \times 1^3/_8 \times 1$ inches. Chromatographic chambers consisted of 300 × 38 mm. culture tubes. Mercury traps were used to regulate vapor pressure in the chromatographic chamber as described by Ellis, Gaddis, and Curry (*3*).

EXPERIMENTAL

Authentic C_1 to C_{14} alkanal 2,4-dinitrophenylhydrazones were prepared as described (*3*). Authentic C_5–C_{10} alk-2-enal 2,4-dinitrophenylhydrazones and C_5 to C_{12} alk-2,4-dienal 2,4-dinitrophenylhydrazones were used. C_3 and C_4 alk-2-enals were obtained from commercial sources. The C_{11} and C_{12} 2-enal homologs were prepared from C_9 and C_{10} saturated aldehydes by condensation with malonic acid (*6*), reduction with lithium aluminum hydride (*6*), and oxidation of the unsaturated alcohol by the method of Delaby and Guillot-Allègre (*2*). C_3 to C_9, C_{11} and C_{13} methyl ketone 2,4-dinitrophenylhydrazones were prepared from commercially obtained ketones. Hydrazone derivatives were prepared by the method of Iddles *et al.* (*10*), and purified by repeated recrystallization until a constant melting point was obtained.

Stock solutions in carbon tetrachloride of each hydrazone were prepared which contained the equivalent of approximately 25 mg. per liter (25 γ per ml.). Suitable volumes were taken from each stock solution to make up 100-ml. solutions of 30 μmoles per liter concentration. Aliquots of these solutions were used in the operations described below.

Paper chromatographic separations of homologous series were made as described (*3*). Various mixtures were prepared by mixing equal volumes of the proper solutions (30 μmole per liter concentration). The following formula was used to determine the volume of solution to spot a paper strip.

$$x \text{ ml.} = \frac{1.5\,n}{6\,ay}$$

where n = number of compounds in the mixture, a = absorbance at maximum of the solution, $y = 1$ for saturated aldehydes and methyl ketones, and $y = 2$ for alk-2-enals and alk-2,4-dienals. The capacity of the paper was much lower for 2-enals and 2,4-dienals, and a further reduction in the quantity applied was necessary, in addition to the decrease afforded by their higher extinction coefficients. Benzene was used in spotting the paper strips.

Resolution of mixtures of different homologous series was achieved by ascending solvent development on untreated Whatman No. 3 paper strips with 5.00 ml. of petroleum ether. The technique was otherwise the same, in every respect, as the method of separation of a homologous series (*3*). Because of the volatility of the solvent, chromatograms were usually run at 4° C. However, successful separations were obtained at temperatures as high as 20° C. Chromatograms were complete in about $1^1/_4$ hours. Amounts spotted on the paper were calculated by the simplified formula:

$$x \text{ ml.} = \frac{1.5}{a}$$

Separated spots were extracted for spectrophotometric measurements or application to other systems. The methods used and precautions necessary to remove impregnating agents have been described (*3*).

Table I. Paper Chromatography of 2-Alkanone, Alk-2-enal, and Alk-2,4-dienal Classes

2-Alkanone 2,4-dinitrophenylhydrazones	R_F[a] I Amount, γ	II Mixture	III Individual	IV Single extd. from I	V Mixture II extd. and authentic
Propylene glycol (20%) and Skellysolve-methanol (System 1)					
C_3	2.88	0.63	0.63	0.64	0.63
C_4	3.05	0.81	0.81	0.81	0.80
C_5	3.22	0.94	0.94	0.92	0.92
Alk-2-enal 2,4-dinitrophenylhydrazones					
C_3	0.99	0.38	0.38	0.39	0.39
C_4	1.05	0.49	0.50	0.48	0.50
C_5	1.11	0.67	0.67	0.67	0.68
C_6	1.17	0.80	0.80	0.82	0.83
C_7	1.23	0.92	0.92	0.92	0.93
Alk-2,4-dienal 2,4-dinitrophenylhydrazones					
C_5	0.73	0.45	0.46	0.45	0.46
C_6	0.77	0.56	0.55	0.56	0.56
C_7	0.81	0.73	0.73	0.74	0.74
C_8	0.85	0.85	0.85	0.85	0.85
C_9	0.89	0.94	0.95	0.93	0.95
Vaseline (7%) and aqueous methanol (System 2)					
2-Alkanone 2,4-dinitrophenylhydrazones					
C_6	3.39	0.72	0.72	0.73	0.72
C_7	3.56	0.65	0.65	0.65	0.65
C_8	3.72	0.59	0.60	0.60	0.58
C_9	3.89	0.51	0.51	0.52	0.51
C_{11}	4.23	0.36	0.38	0.37	0.36
C_{13}	4.60	0.24	0.24	0.24	0.24
Alk-2-enal 2,4-dinitrophenylhydrazones					
C_7	1.23	0.76	0.73	0.73	0.73
C_8	1.29	0.70	0.68	0.68	0.68
C_9	1.35	0.65	0.62	0.61	0.62
C_{10}	1.41	0.57	0.55	0.55	0.54
C_{11}	1.47	0.49	0.46	0.46	0.46
C_{12}	1.53	0.42	0.39	0.39	0.39
Alk-2,4-dienal 2,4-dinitrophenylhydrazones					
C_9	0.89	0.67	0.67	0.66	0.66
C_{10}	0.93	0.61	0.61	0.60	0.60
C_{11}	0.97	0.54	0.55	0.55	0.55
C_{12}	1.01	0.47	0.47	0.46	0.47

[a] Based on averages of 3 or more paper strips.

RESULTS AND DISCUSSION

Table I shows the R_F values obtained in the separation of homologous series of 2-alkanones, alk-2-enals, and alk-2 4-dienal 2,4-dinitrophenylhydrazones on propylene glycol–Skellysolve C–methanol and vaseline–aqueous methanol

Table II. Comparative R_F's of Four Homologous Series

	Paper Chromatography							
	Propylene Glycol				Vaseline			
R_F Values	2-Alkanone C_3–C_5	Alkanal C_1–C_6	Alk-2-enal C_3–C_7	Alk-2,4-dienal C_5–C_9	2-Alkanone C_6–C_9 C_{11}–C_{13}	Alkanal C_7–C_{14}	Alk-2-enal C_7–C_{12}	Alk-2,4-dienal C_9–C_{12}
1.00								
0.98								
0.96								
0.94				C_9				
0.92	C_5							
0.90		C_6	C_7					
0.88								
0.86								
0.84				C_8				
0.82								
0.80	C_4							
0.78		C_5	C_6					
0.76								
0.74				C_7		C_7	C_7	
0.72					C_6			
0.70							C_8	C_9
0.68			C_5			C_8		
0.66					C_7			
0.64		C_4					C_9	C_{10}
0.62	C_3					C_9		
0.60					C_8			
0.58								
0.56				C_6		C_{10}	C_{10}	C_{11}
0.54								
0.52			C_4		C_9			
0.50		C_3						
0.48							C_{11}	C_{12}
0.46						C_{11}		
0.44								
0.42				C_5			C_{12}	
0.40						C_{12}		
0.38					C_{11}			
0.36			C_3					
0.34		C_2						
0.32						C_{13}		
0.30								
0.28								
0.26						C_{14}		
0.24					C_{13}			
0.22		C_1						
0.20								
0.18								
0.16								
0.14								
0.12								
0.10								

systems. The separation was evaluated as described (*3*) for saturated aldehydes.

Table II compares the R_F values of individual compounds separated from homologous series of the four classes of monocarbonyl 2,4-dinitrophenylhydrazones. The closeness of R_F values of 2-alkanone C_n, alkanal C_{n+1}, alk-2-enal C_{n+2}, and alk-2,4-dienal C_{n+4} on the propylene glycol–Skellysolve C–methanol system agrees with the observations of Forss, Dunstone, and Stark (*5*) and follows the characteristic inseparable group pattern described by Bassette, Day, and Keeney (*1*). The R_F values indicated that mixtures should be separable into these characteristic groups. A mixture of 2-alkanones C_4 to C_5, alkanals C_2 to C_6, alk-2-enals C_3 to C_7, and alk-2,4-dienals C_5 to C_9, theoretically separable to five groups, separated into five spots on propylene glycol–impregnated paper. The spots were large and closely spaced and had R_F values of 0.93, 0.82, 0.68, 0.52, and 0.36. This separation was not further investigated.

The R_F alignment on the vaseline–aqueous methanol system was different. Those compounds with closely similar R_F values had the pattern of methyl ketone C_n, saturated aldehyde C_{n+2}, 2-enal C_{n+2}, and 2,4-dienal C_{n+3}. However, the spacing was so close on this system that a full mixture of all four classes would be very difficult to separate into characteristic groups.

The resolution of combinations of single compounds from each of the four classes is shown in Table III. Evaluation of the separation into classes was made by determination of the absorption maxima and rechromatography on propylene glycol or vaseline-impregnated paper with simultaneous determinations of the R_F values of individual authentic compounds. Results indicate the capabilities and limitations of the paper chromatographic method for separation into classes. The method did not separate saturated aldehydes C_1 or C_2 from 2-enal C_3, or any other 2-enal. Similarly, methyl ketone C_3 (acetone) was not separated from saturated aldehydes C_3 or C_4 or any higher molecular weight saturated aldehyde. Acetone, however, was readily separated from saturated aldehydes C_1 or C_2. In the absence of methyl ketone C_3 and saturated aldehydes C_1 and C_2, the method apparently will separate any combination of compounds of the four classes, including the characteristic inseparable groups (*1*).

Saturated aldehyde C_1 and 2-enals, inseparable by the class method, were readily separated on the propylene glycol–Skellysolve C–methanol paper chromatographic system. However, saturated aldehyde C_2 could not be separated from 2-enal C_3 on that system. Similarly, acetone was not separated from either propanal or butanal on the propylene glycol system.

Table IV shows the resolution of a mixture of four homologous series in the absence of acetone, methanal, and ethanal. Evaluation of the resolution into classes was made by separation of each class into individual compounds and identification with authentic mixtures. The value of the total procedure in the rapid identification of appropriate unknown mixtures is obvious.

Similar data are given in Table V for a complete mixture of four homologous series. The presence of acetone, methanal, and ethanal derivatives in a mixture of 2,4-dinitrophenylhydrazones can be detected by their tendency to partition between carbon tetrachloride and a 2*N* hydrochloric acid solution of 2,4 - dinitrophenylhydrazine (*8*). The behavior of acetone, methanal, and ethanal was as indicated in the preceding study of mixtures of single members of the homologous series (Table III). Acetone separates in the saturated aldehyde class, and methanal and ethanal in the 2-enal class. Complications from this resulted in the formation of an ethanal-propenal mixture and apparent acetone-propanal and acetone-butanal mixtures. The data in Tables III and V strongly suggest a distribution of acetone between propanal and butanal. Application of alcoholic alkali fading studies (*3, 11*) and the method of Monty (*16*) should be useful in the detection and determination of such mixtures. The binary mixtures are theoretically separable by repeated chromatography and, among the many paper chromatographic methods (*3, 14*) for separation of 2,4-dinitrophenylhydrazones, there are doubtless several capable of resolving these mixtures (*17*).

Table III. Resolution of Individual Members of Homologous Series

Mixture	Max., $M\mu$ (CCl_4) Authentic Compounds	Paper Chromatography Untreated Paper-Petm. Et_2O. Sepn. Mixture of Classes R_F Values	Max., $m\mu$ (CCl_4) Material Sepd. into Classes	Paper Chromatography Propylene Glycol System: Spots from class sepn. R_F values	Paper Chromatography Propylene Glycol System: Authentic individual compounds, R_F values	Paper Chromatography Vaseline System: Spots from class sepn. R_F values	Paper Chromatography Vaseline System: Authentic individual compounds, R_F values
Alkanal C_1	330	0.35	340–6	0.21	0.21		
Alk-2-enal C_3	354			0.44	0.44		
Alkanal C_2	343	0.35	346–9	0.42	0.38		
Alk-2-enal C_3	354				0.42		
Alk-2,4-dienal C_5	370–5	0.22	365–70	0.50	0.50		
2-Alkanone C_3	349	0.54	343–6	0.65	0.66		
Alkanal C_3	343				0.56		
Alk-2-enal C_4	358–61	0.35	358	0.59	0.56		
Alk-2,4-dienal C_6	380	0.21	370–5	0.60	0.61		
2-Alkanone C_3	349	0.48	346–9	0.73	0.66		
Alkanal C_4	343				0.73		
Alk-2-enal C_5	365	0.34	355–8	0.78	0.76		
Alk-2,4-dienal C_7	380	0.22	375–80	0.80	0.80		
Alkanal C_3	343	0.47	343	0.56	0.56		
Alk-2-enal C_4	358–61	0.34	358–65	0.56	0.56		
Alk-2,4-dienal C_6	380	0.22	375–80	0.61	0.61		
2-Alkanone C_4	349–52	0.57	349	0.83	0.85		
Alkanal C_4	343–6	0.47	343	0.73	0.72		
Alk-2-enal C_5	365	0.37	358–65	0.75	0.73		
Alk-2,4-dienal C_7	380	0.24	375–80	0.79	0.80		
2-Alkanone C_4	349–52	0.58	349	0.85	0.85		
Alkanal C_5	343–6	0.50	346–9	0.83	0.83		
Alk-2-enal C_6	365	0.38	361–5	0.86	0.86		
Alk-2,4,-dienal C_8	380	0.25	375–80	0.91	0.91		
2-Alkanone C_8	352	0.58	352			0.59	0.58
Alkanal C_9	343–6	0.48	346			0.58	0.58
Alk-2-enal C_{10}	365	0.36	361			0.56	0.56
Alk-2,4-dienal C_{12}	380	0.24	375–80			0.48	0.47
2-Alkanone C_9	352	0.55	349–52			0.48	0.48
Alkanal C_9	343–6	0.45	346			0.58	0.58
Alk-2-enal C_{10}	365	0.31	358–61			0.54	0.53
2-Alkanone C_9	352	0.55	349–52			0.49	0.48
Alkanal C_{10}	346	0.45	346			0.52	0.52
Alk-2-enal C_{11}	365	0.36	358–61			0.47	0.46

Table IV. Separation of Mixture of Four Homologous Series

Mixture of Members of 4 Homologous Series	Paper Chromatography: Untreated paper petm. Et_2O R_F values spots	Propylene Glycol–Skellysolve C–Methanol: R_F values unknown mixture	Propylene Glycol–Skellysolve C–Methanol: R_F values mixture authentic	Authentic Compounds Mixture	Unseparated Spots from Propylene Glycol System	Paper Chromatography, Vaseline–Aqueous Methanol: R_F values unknown mixture	Paper Chromatography, Vaseline–Aqueous Methanol: R_F values authentic mixture	Authentic Compounds Mixture
2-Alkanone C_4–C_9, C_{11}, C_{13} Alkanal C_3–C_{14} Alk-2-enal C_3–C_{12} Alk-2,4-dienal C_5–C_{12}	0.55	1.00				0.70	0.71	C_6-2-alkanone
		0.92	0.92	C_5 2-alkanone		0.63	0.64	C_7
		0.79	0.77	C_4		0.56	0.56	C_8
						0.49	0.50	C_9
						0.33	0.35	C_{11}
						0.21	0.23	C_{13}
	0.46	1.00				0.73	0.73	C_7-alkanal
		0.92	0.91	C_6-alkanal		0.65	0.66	C_8
		0.83	0.82	C_5		0.58	0.58	C_9
		0.73	0.71	C_4		0.51	0.51	C_{10}
		0.55	0.55	C_3		0.42	0.43	C_{11}
						0.36	0.36	C_{12}
						0.29	0.29	C_{13}
						0.22	0.23	C_{14}
	0.35	1.00					0.74	C_7-alk-2-enal
		0.91	0.91	C_7-alk-2-enal		0.67	0.68	C_8
		0.79	0.79	C_6		0.61	0.62	C_9
		0.67	0.65	C_5		0.55	0.55	C_{10}
		0.50	0.48	C_4		0.47	0.48	C_{11}
		0.39	0.38	C_3		0.39	0.41	C_{12}
	0.24	1.00					0.66	C_9-alk-2,4-dienal
		0.92	0.92	C_9-alk-2,4-dienal		0.59	0.60	C_{10}
		0.79	0.80	C_8	M	0.51	0.53	C_{11}
		0.67	0.68	C_7		0.44	0.45	C_{12}
		0.50	0.49	C_6				
		0.42	0.43	C_5				

Table V. Separation of Complete Mixture of Four Homologous Series

Mixture of Members of 4 Homologous Series	Paper Chromatography: Untreated paper petm. Et_2O R_F values of spots	Paper Chromatography: Vaseline–Aqueous Methanol R_F Values: Unknown mixture	Vaseline–Aqueous Methanol R_F Values: Authentic mixture	Authentic Compounds Mixture	Unseparated Spots from Vaseline System	Paper Chromatography, Propylene Glycol–Skelly C–Methanol R_F Values: Unknown mixture	Propylene Glycol–Skelly C–Methanol R_F Values: Authentic mixture	Authentic Compounds Mixture	Paper Chromatography, Propylene Glycol–Skelly C–Methanol R_F Values, Authentic Mixture	Authentic Compounds Mixture
		0.91				0.91	0.92	C_5-2-alkanone		
		0.79					0.77	C_4		
							0.61	C_3		
		0.71	0.71	C_6-2-alkanone						
		0.64	0.64	C_7						
	0.59	0.57	0.56	C_8						
		0.50	0.50	C_9						
		0.35	0.35	C_{11}						
		0.22	0.23	C_{13}						
						0.91	0.92	C_6-alkanal	0.91	C_6 alkanal
						0.83	0.81	C_5	0.82	C_5
		0.92				0.72		C_4	0.71	C_4
		0.73	0.73	C_7-alkanal		0.56	0.70	C_3-2 alkanone		
2-Alkanone		0.66	0.67	C_8			0.55	C_3-alkanal	0.55	C_3
C_3–C_9, C_{11}, C_{13}		0.59	0.58	C_9						
Alkanal		0.52	0.52	C_{10}			0.35	C_2	0.35	C_2
C_1–C_{14}	0.47	0.44	0.44	C_{11}			0.21	C_1	0.21	C_1
Alk-2-enal		0.36	0.36	C_{12}						
C_3–C_{12}		0.30	0.30	C_{13}						
Alk-2,4-dienal		0.23	0.23	C_{14}		0.81	0.80	C_6-alk-2-enal	0.80	C_6 alk-2-enal
C_5–C_{12}						0.69	0.67	C_5	0.67	C_5
		0.91				0.52	0.52	C_4	0.52	C_4
		0.74	0.74	C_7-alk-2-enal				C_3	0.35	C_3
		0.67	0.68	C_8				C_2-alkanal		
		0.61	0.62	C_9		0.39	0.39			
	0.35	0.54	0.55	C_{10}		0.21	0.21	C_1		
		0.48	0.48	C_{11}						
		0.41	0.41	C_{12}						
						0.80	0.80	C_8-alk-2,4-dienal		
		0.90				0.70	0.68	C_7		
		0.65	0.66	C_9-alk-2,4-dienal		0.52	0.49	C_6		
		0.60	0.60	C_{10}		0.44	0.44	C_5		
	0.23	0.53	0.53	C_{11}						
		0.45	0.45	C_{12}						

These methods appear adapted to quantitative application in spite of the small quantities involved, and the sensitivity of the compounds. 2,4-Dienals particularly appeared unstable, and fractions left exposed to light and air decreased in absorbance values and in absorption maxima (*17*). In paper chromatography there was always some material that remained in the origin, particularly on untreated paper. Nonmobile material was much smaller in amount on propylene glycol- and vaseline-impregnated paper. A mixture was made up of 2,4-dinitrophenylhydrazones of methyl ketones (C_4 to C_9, C_{11} and C_{13}), saturated aldehydes (C_3 to C_{14}), 2-enal (C_3 to C_{12}), and 2,4-dienal (C_5 to C_{12}) and separated into classes by chromatography on untreated paper. This mixture contained, on the basis of maximum absorbance, 24.6, 20.8, 24.1, and 30.5%, respectively. Proportions found were 25.3, 24.6, 24.0, and 26.1%, respectively. Total recovery of mobile material was 70.9%; this was equivalent to recoveries of 73.0, 81.0, 70.7, and 59.9%, respectively. Nonmobile material had a maximum at 346 to 52 mμ and amounted to 22.4% of the total amount of material recovered. In another experiment the four classes were each applied separately to untreated paper and chromatographed. Recoveries of mobile material were 86, 84, 66, and 74%, respectively. Material left at the origins made total recoveries 95, 93, 87, and 91%, respectively. The maxima of these nonmobile materials were 320 to 46, 320, 340 to 46, and 352 to 58 mμ. The quantitative application of these procedures requires further study.

ACKNOWLEDGMENT

The authors thank E. L. Pippen of the Western Utilization Research and Development Division for the samples of authentic C_5 to C_{10} alk-2-enal 2,4-dinitrophenylhydrazones and C_5 to C_{12} alk-2,4-dienal 2,4-dinitrophenylhydrazones, and George T. Currie of this laboratory for spectrophotometric measurements.

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RECEIVED for review June 11, 1958. Accepted December 3, 1958. Mention of specific commercial materials or equipment does not constitute recommendation for their use above similar materials and equipment of equal value.

Separation and Identification of *n*-Alkanals, 2-Alkanones, 2-Alkenals, and 2,4-Alkadienals by Paper Chromatography and Direct Spectrophotometric Examination

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▶ **A sensitive micromethod for identification of 2,4-dinitrophenylhydrazones of 2-alkanones, C_3–C_9; *n*-alkanals, C_1–C_{14}, *n*-alk-2-enals, C_3–C_{11}; and *n*-alka-2,4-dienals, C_5–C_{12} involves paper chromatography and spectrophotometric examination of the 2,4-dinitrophenylhydrazones directly on the chromatographed spot. Spectrum analysis of the chromatographed spots is carried out rapidly and easily on as little as 5 γ of material per spot and each class of 2,4-dinitrophenylhydrazone exhibits a characteristic maximum.**

THE paper chromatographic method of Lynn, Steele, and Staple (*9*) as adapted for micro separations was suitable for separation of the 2,4-dinitrophenylhydrazones of lower and higher homologs of not only the 2-alkanones and *n*-alkanals, but also the *n*-alk-2-enals and *n*-alka-2,4-dienals. However, there was a similarity in the migratory properties of certain members from each class. Therefore, during chromatography of mixtures of unknown composition, a criterion for identification in addition to migratory property was desired. The characteristic maximum that each class of 2,4-dinitrophenylhydrazone mentioned above exhibits in the ultraviolet absorption spectrum (*1, 7, 16, 18*) provides an additional criterion for identification. Such spectra are usually determined from a solution of the 2,4-dinitrophenylhydrazones in an appropriate solvent; hence use of this procedure for identifying a 2,4-dinitrophenylhydrazone entails extraction of the 2,4-dinitrophenylhydrazone from the paper. Previous workers (*2, 12, 17*) have obtained spectra of various substances directly from paper chromatograms. It was feasible to determine characteristic absorption spectra of 2,4 - dinitrophenylhydrazones directly from spots on the chromatogram and the data thus obtained for authentic 2,4-dinitrophenylhydrazones as well as their migratory properties are reported.

EXPERIMENTAL

Preparation of 2,4-Dinitrophenylhydrazones. The hydrazones of the *n*-alkanals were prepared from commercially available carbonyl compounds, as were some of the 2-alkanones. Other 2-alkanones were synthesized by the method of de Benniville (*3*). The *n*-alk-2-enols were synthesized by the method of Martin, Schepartz, and Daubert (*10*) or Kuhn

and Grundmann (*8*). The *n*-alka-2,4-dienals were prepared by the method of Pippen and Nonaka (*14*). The procedure by which the 2,4-dinitrophenylhydrazones were made has been reported (*13, 15*).

Chromatography of 2,4-Dinitrophenylhydrazones. The basic paper chromatographic method developed by Lynn, Steele, and Staple (*9*) was adapted as follows: A sheet of Whatman No. 1 filter paper $18^1/_4 \times 22^1/_2$ inches impregnated with a solution of 10% 2-phenoxyethanol in acetone (v./v.) is air-dried for 10 to 20 minutes before the 2,4-dinitrophenylhydrazones are spotted. Standard solutions of 2,4-dinitrophenylhydrazones, whose concentrations are about 5 mg. per ml. of chloroform, are applied to the paper using a 1-μl. self-filling micropipet. The paper is then placed in a Chromatocab Model A (Research Equipment Corp.) and developed $15^1/_2$ hours with *n*-heptane saturated with 2-phenoxyethanol. Descending chromatography is used throughout. Equilibration of the paper with the developing solvent prior to development is not necessary. In addition, the percentage of 2-phenoxyethanol in the impregnating solution can be increased or decreased to change the rate of movement of the 2,4-dinitrophenylhydrazones. Increasing the percentage of 2-phenoxyethanol tends to decrease the rate of movement and the opposite effect is observed by decreasing the percentage of impregnant.

On each chromatogram, *n*-heptanal-2,4-dinitrophenylhydrazone is applied to the paper as a tag compound so that a record of mobility, R_h, can be obtained. Mobility, R_h, is expressed as the ratio of the distance moved by a 2,4-dinitrophenylhydrazone to the distance traveled by *n*-heptanal-2,4-dinitrophenylhydrazone.

Spectrophotometric Examination. The 2,4-dinitrophenylhydrazone spot on the developed chromatogram was cut from the paper and placed over the sample aperture of the Cary Model 14 spectrophotometer. The absorption spectrum was determined in the wave length range between 300 and 450 mμ.

RESULTS AND DISCUSSION

Figure 1 is a plot of mobility values obtained from numerous chromatograms and illustrates that the members of the various series having the same carbon chain length separate readily from one another and that each homolog of a series lies on a near linear curve. The first member of each series except the 2-alkanones does not fall on the linear curve exhibited by the other homologs. The *n*-alkanals, in addition to the anomalous behavior of the first member, exhibit a slope change with the *n*-heptanal 2,4-dinitrophenylhydrazone being the pivot point. Because the solvent front runs off the paper, mobility was expressed as R_h, the ratio of the distance moved by a 2,4-dinitrophenylhydrazone to the distance moved by the 2,4-dinitrophenylhydrazone of *n*-heptanal.

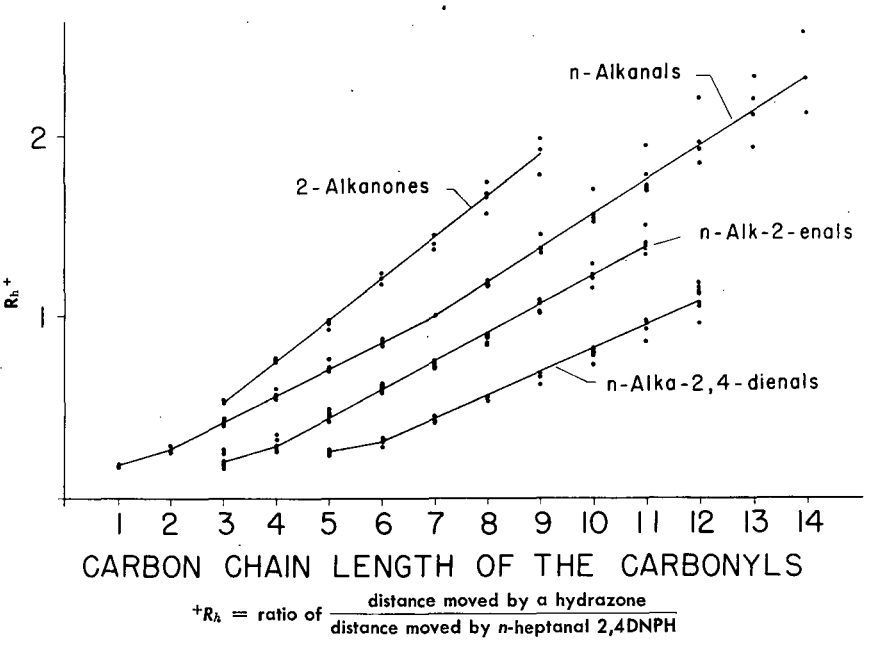


Figure 1. Mobility of 2,4-dinitrophenylhydrazones

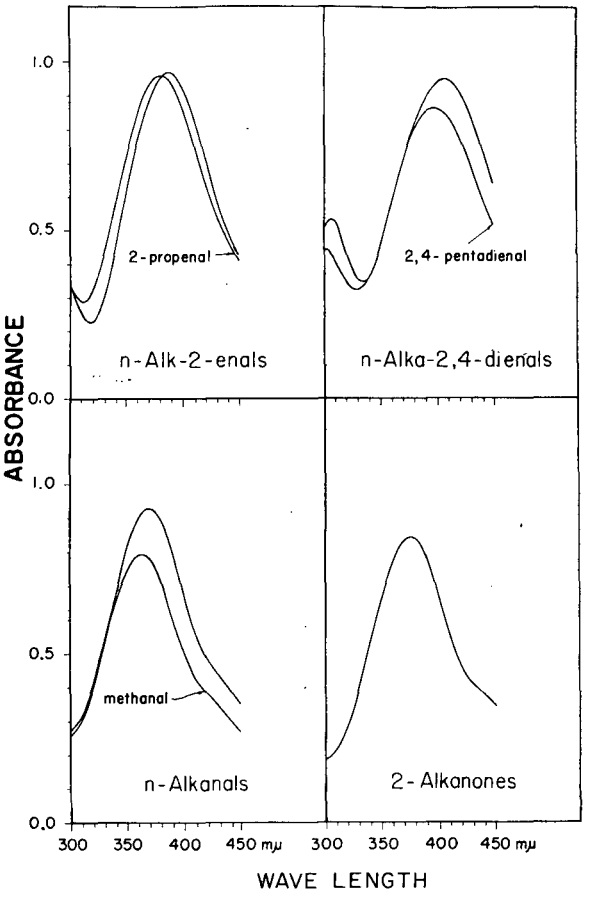


Figure 2. Typical spectra of 2,4-dinitrophenylhydrazones on paper chromatograms

Comparisons of the R_h values indicate that it is difficult to separate mixtures composed of members of the different homologous series. The hydrazones with similar R_h values are 2-alkanone C_n, alkanal C_{n+1}, alk-2-enal C_{n+3}, and alka-2, 4-dienal C_{n+5} for the lower homologs. While the relative alignment between alkanals, alk-2-enal, and alka-2, 4-dienal remain constant, the R_h overlap between the 2-alkanones and alkanals gradually shifts to $C_n : C_{n+3}$ for the higher homologs. Forss, Dunstone, and Stark (*4*) compared the migratory properties of the four homologous series using the paper chromatographic system of Huelin (*6*) and Meigh (*11*) and found the characteristic inseparable R_f groups to be 2-alkanone C_n, alkanal C_{n+1}, alk-2-enal C_{n+2}, and alka-2,4-dienal C_{n+4}. Likewise Gaddis and Ellis (*5*) using a propylene glycol–Skellysolve C–methanol paper (Skelly Oil Co.) chromatographic system obtained the same alignment as did Forss, Dunstone, and Stark (*4*) for the lower homologs and

2-alkanone C_n, alkanal C_{n+2}, alk-2-enal C_{n+2}, and alka-2,4-dienal C_{n+3} for the higher members using their vaseline–aqueous methanol system.

Figure 2 illustrates typical 2,4-dinitrophenylhydrazone spectra as determined by direct spectrophotometric examination of a chromatographed spot for the different classes of carbonyl compounds. Separate curves are included for the 2,4-dinitrophenylhydrazones of methanal (formaldehyde), 2-propenal (acrolein), and 2,4-pentadienal because these first members of their particular series differ somewhat from the general curve characteristic of the remainder of the series. Acetone 2,4-dinitrophenylhydrazone on the other hand exhibits a curve similar to the rest of its homologs; therefore a separate curve was not included. In addition to different maxima the typical spectrum of a *n*-alk-2-enal has both a maximum and a minimum above 300 mμ, which set it apart from the other classes, while the *n*-alka-2,4-dienals have two maxima and a minimum above 300 mμ. Both the *n*-alkanals and the 2-alkanones have nearly identical curves except where the maxima occur and where the shoulder occurs above 400 mμ. The maxima of the 2,4-dinitrophenylhydrazones shown in Figure 3 are somewhat higher than those reported in the literature (*1, 7, 16, 18*) for comparable types of 2,4-dinitrophenylhydrazones in chloroform or ethyl alcohol solvents. Figure 3 illustrates that 2,4-dodecadienal-2,4-dinitrophenylhydrazone when spotted on untreated paper has a λ_{max} at 392 mμ, which is the maximum commonly observed from this type of compound. However, when the same compound is examined in the presence of 2-phenoxyethanol either on paper or in solution, the spectral maximum shifts to 404 mμ and is due to a solvent effect exerted by 2-phenoxyethanol.

Table I lists the maxima and the minima as determined for authentic 2,4-dinitrophenylhydrazones on the developed chromatogram. The data show that there is no overlapping of the maxima between classes and that there can be no mistake in determining the class of these 2,4-dinitrophenylhydrazones.

There is, however, danger of deterioration of the *n*-alka-2,4-dienals if the chromatograms are exposed to air and light. Table I also lists the maxima and minima changes obtained after the spots were exposed to the laboratory atmosphere for 24 hours. Such changes can be controlled so that it is not necessary to determine the spectrum immediately after the chromatogram is developed. One way is to separate the chromatograms in a folder by some means such as a polyethylene sheet to prevent diffusion of the hydrazones from paper to paper.

In applying the method to unknown mixtures it is necessary, except for simple or fortuitous mixtures which are completely resolved by paper chromatography, to carry out preliminary fractionation by methods such as column chromatography. This yields simpler mixtures which are more likely to be amenable to resolution by paper chromatography. Care in interpreting results is necessary if derivatives, other than those described in this study, are present. The 2,4-dinitrophenylhydrazones of the branched-chain carbonyl compounds 2-methylpropanal, 3-methyl butanal, 3-methyl-2-butanone, and 4-methyl-2-pentanone gave spectral curves identical to those of the *n*-alkanal and 2-alkanone 2,4-dinitrophenylhydrazones, respectively. Fortunately, 2,4-dinitrophenylhydrazones of branched-chain compounds of the iso type which have been chromatographed migrate slightly faster than the corresponding straight-chain derivative having the same number of carbon atoms, and somewhat slower than the next higher straight-chain homolog.

In dealing with unknown mixtures the determination of migratory and spectral properties does not necessarily establish unequivocal identity. However, it is obvious from the above results that absorption spectra determined directly from paper chromatograms supply information which helps characterize the unknown.

Table I. Maxima and Minima of Homologous Series of 2,4-Dinitrophenylhydrazones as Determined Directly on Paper Chromatograms

Carbon Chain Length	*n*-Alkanals Max., mμ	*n*-Alk-2-enals Max., mμ	*n*-Alk-2-enals Min., mμ	2-Alkanones Max., mμ	*n*-Alka-2,4-dienals Max. 1, mμ	Max. 1,[a] mμ	Min.,[b] mμ	Max. 2,[b] mμ
C_1	362							
C_2	368							
C_3	370	381	312	373–374				
C_4	370	388	315–316	374–375				
C_5	370	388	316	375	397	391–392	327	303
C_6	370	388–389	316	375	402	388–389	331–332	307–308
C_7	370	388	316	375	403–404	390–391	333	307–308
C_8	370	389–390	316	375	403–404	391	333–334	306–307
C_9	370	389	316–317	375	403–404	390–391	334	307–308
C_{10}	370	389	316		404	391	333–334	308
C_{11}		389	316		403–404	390	333	308
C_{12}	370				403–404	390	333	308

[a] 2,4-Dinitrophenylhydrazone spots exposed to air 24 hours.
[b] These points either disappeared or were obscure after exposure to air 24 hours.

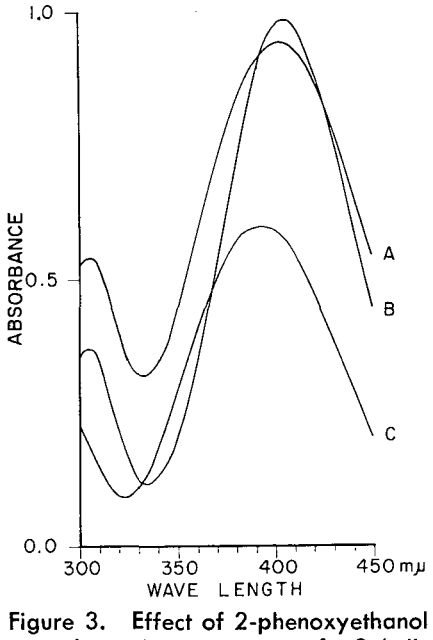


Figure 3. Effect of 2-phenoxyethanol on absorption spectra of 2,4-dinitrophenylhydrazones

A. 2,4-dodecadienal 2,4-dinitrophenylhydrazone on 2-phenoxyethanol–impregnated paper
B. 2,4-dodecadienal 2,4-dinitrophenylhydrazone in solution of 2-phenoxyethanol
C. 2,4-dodecadienal 2,4-dinitrophenylhydrazone on untreated filter paper

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RECEIVED for review July 15, 1958. Accepted December 2, 1958. Mention of specific products does not constitute endorsement by the Department of Agriculture over similar products not mentioned.

Photoelectric Technique for Spectrophotometry between 194 and 225 Millimicrons

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▶ To meet the requirements for accuracy in measuring absorption of light in the far-ultraviolet, a spectrophotometer has been developed for use between 194 and 225 mμ, with a constant half-intensity band width of 1.5 mμ. An RCA developmental photomultiplier Type C7180 was employed as the photoelectric detecting device. As the effective threshold of the photocathode was determined to be 240 mμ in this system, stray light of longer wave lengths could not be detected and stray light of shorter wave lengths was not found. The sensitivity of the instrument is sufficient to detect increments of 0.1% transmittance in solutions of absorbance approaching 2.0, when read with a water reference, and approaching 4.0, when read with a reference having an absorbance of about 2.0 with respect to water.

THE primary instrumental problem in spectrophotometry in the far-ultraviolet is concerned with the spectral purity of the incident light (*3*). The approach to the desired end—elimination of stray light—used in instruments designed to solve this problem has been the spectral purification of the incident light by double monochromation and detection of the transmitted light with a photodevice employing an S-5, S-13, or S-19 surface such as the 1P28 photomultiplier (*7*). The approach described is complete elimination from the measured light of stray light of wave lengths longer than the threshold value of the photocathode by the use of a solar-blind tube photomultiplier, with appropriate amplification of the photoelectric current. In this case double monochromation would only reduce the intensity of the monochromated light. For certain purposes monochromation, although expensive, may be adequate; for others it will not suffice.

In Figure 1 three independent curves have been plotted together for convenience in illustrating certain interrelationships which are basic to the thesis of this paper.

A gives the points which show the relative intensities of the xenon arc, 150 watts, as a function of wave length, the intensity at 700 mμ being plotted arbitrarily as 100% (*5*). It is apparent that the relative intensity is minimal in the far-ultraviolet; the ratio of visible to far-ultraviolet light is very large.

B shows the relative response of the 1P28 photomultiplier as a function of wave length observed when a xenon arc and a Leiss single monochromator were used; this tube is sensitive to a wide range of wave lengths and the response is much greater to visible than to ultraviolet light. In fact, the far-ultraviolet lies at the extreme limit of wave lengths detectable by the 1P28 photomultiplier.

C shows the analogous relative response of the C7180 photomultiplier as a function of wave length.

Two important considerations emerge: No light was detected having wave lengths longer than 240 mμ, and maximal response was found at 200 mμ, in the optimal region for work in the far-ultraviolet. It is apparent from *B* that the 1P28 photomultiplier is not suitable for use in the far-ultraviolet when any measurable amount of stray white light is present in the monochromated incident beam.

Stray visible light is not eliminated completely by double monochromation, although it is claimed that in certain instruments its intensity is cut by a factor of 10^3, with significant loss also in the intensity of the monochromated light. The incident light entering the cuvette is inevitably composed of two parts, monochromatic and stray light. In the far-ultraviolet the intensity of the monochromatic light decreases sharply with decreasing wave lengths (with instruments employing crystal quartz optics and double monochromation), but the intensity of the stray light remains essentially constant. As the incident light is passed through an ultraviolet-absorbing chromophore, part of the monochromatic light, in an absorption band, is absorbed, but none of the stray light. This means that the light emerging from the cuvette will necessarily contain a larger percentage of stray light than did the light entering the cuvette and the percentage of stray light in the measured light will vary with the concentration of the chromophore. Therefore errors in absorption data may become apparent at short wave lengths, especially in solutions of high absorbance, such as are encountered when enzymic activities are studied by means of difference spectra.

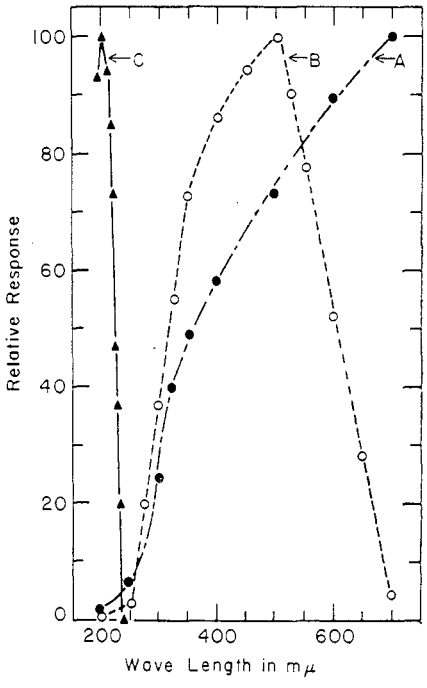


Figure 1. Basic interrelationships

A. Relative intensity of Xe arc as a function of wave length
B and C. Relative response of 1P28 and C7180 developmental photomultipliers used with a Leiss single monochromator and Xe arc as light source

PHOTOMULTIPLIER

The photoelectric equation indicates that the energy required to cause the emission of photoelectrons from the cathode of a photoelectric device will be equal to or greater than its threshold value. Several solar-blind ultraviolet multiplier phototubes have been described by Dunkelman (*4*) and solar-blind photocells by Zworykin and Ramberg (*7*). The apparent threshold value for the RCA developmental photomultiplier as used in this system was found to be 240 mμ (*C*, Figure 1); the ultimate value given by the manufacturer is about 280 mμ. A practical working limit on the long wave-length side of the spectral region under investigation is 225 to 230 mμ. On the short wave length end the practical limit is about 194 mμ, and is determined by such factors as the in-

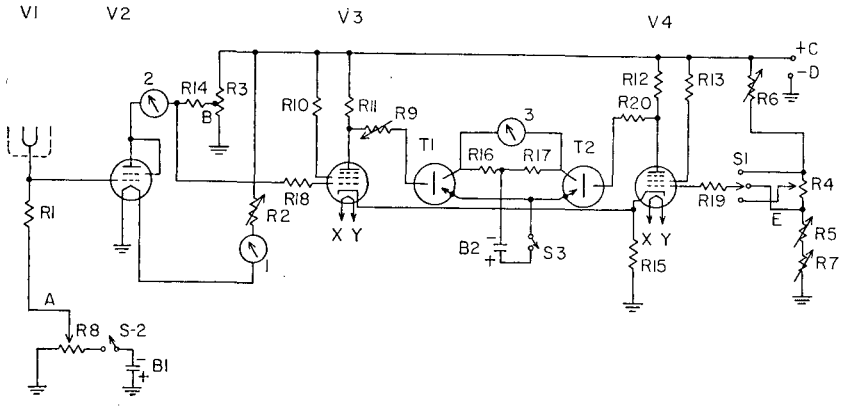


Figure 2. Schematic of direct current amplifier

B_1, B_2. 1.5-volt electrochem. cell
M_1. 10-ma. d.c. meter
M_2, M_3. 20-μa. d.c. meter
R_1. 10^9 — 10^{11} ohms
R_2, R_3. 50 kilo-ohms, 2-watt var.
R_4. 1-kilo-ohm Helipot
R_5. 1-kilo-ohm Helipot
R_6. 500-kilo-ohm 2-watt var.
R_7. 50-kilo-ohm, 2-watt var.
R_8. 20-kilo-ohm, 0.5-watt var.
R_9. 1–4-megaohm, 0.5-watt var.
R_{10}, R_{11}, R_{12}, R_{13}. 50-kilo-ohm, 2-watt
R_{14}. 1.0-megaohm, 0.5-watt
R_{15}. 1.1-kilo-ohm, 2-watt
R_{16}, R_{17}. 5-kilo-ohm, 0.5-watt
R_{18}, R_{19}. 1.5-megaohm, 0.5-watt
R_{20}. 500-kilo-ohm, 0.5-watt
S_1. Selector switch, 1 pole, 3 positions
S_2, S_3. SPST toggle switch
T_1, T_2. CK722
V_1, C7180 photomultiplier (only anode shown)
V_2. CK5889
V_3, V_4. 6AU6WA

creasing opacity of the prism, envelopes, and cuvettes, and the absorption of light by the atmosphere.

The C7180 developmental photomultiplier may be mounted in any position and does not require protection from ordinary room light. It cannot be installed without circuit changes in instruments using the 1P28 or 1P21 photomultiplier.

High Voltage Power Supply. The line-operated power supply to the dynodes of the photomultiplier supplied voltages ranging between 250 and 3500. Protection was provided for work with high voltages. Batteries lack sufficient versatility to be used in this system. Suitable commercial high voltage power supplies are available.

Dark Current. Dark current has been discussed by Zworykin and Ramberg (*7*). It is increased as the amplification of the photomultiplier is increased. In practice, the effect of dark current may be reduced by using a more intense light source, requiring less amplification, and eliminated by using alternating current amplification with a pulsed light source. The manufacturer indicates that the dark current of the C7180 developmental photomultiplier will not exceed 1×10^{-8} ampere at room temperature and 2500 volts. The dark current of the tube in present use was 1×10^{-11} ampere at room temperature and 1500 volts. It is not known what variations may be expected among different tubes.

CUVETTES

The data shown in Figure 3 were taken with crystal quartz cells having a 1-cm. light path. They were matched throughout the far-ultraviolet and shown to be free from borate, the impurity found in most fused silica cuvettes, which introduces errors into absorption curves due to its absorption and fluorescence (*3*, *6*).

A "special silica type C" (Corning Glass Works) was demonstrated to be free from fluorescent impurities and to have significantly higher transmittance in the far-ultraviolet than crystal quartz. An unselected sample 1 cm. thick transmitted 77% of the incident light at 200 mμ; four samples of commercial fused quartz of equal thickness transmitted 4, 7, 14, and 34%, respectively. Cells made of special silica type C should be ideal.

Care of Cuvettes. The matching of cells should be checked frequently, because scratches due to improper handling or cleaning will introduce error. The cells should not be allowed to stand when they contain any solution or solvent other than water. Alkalies and concentrated acids etch the cells sufficiently to reduce their transmittance in the far-ultraviolet and cause differences in matched sets. For cleaning, it is recommended that cuvettes be scrubbed gently in 2*N* hydrochloric acid with a tightly wound cotton swab (Q tip). After rinsing, the outer surfaces are dried with a bird's-eye cotton cloth which has been washed about nine times with a detergent.

MONOCHROMATOR

The Leiss single monochromator was used (Carl Leiss, Berlin, Germany, through the Photovolt Co.). It has a Littrow crystal quartz prism, two compensating aluminized parabolic mirrors for collimating the light and directing it into the exit slit, a rectangular slit (which may be opened to 3 mm., with a device for regulating the height of the image), an optical path 22.5 cm. from prism to slit, and no quartz windows. It was calibrated with the lines of mercury appearing in the region under investigation.

Slit Width. The smaller the half-intensity band width the greater will be the resolving power of the instrument; hence more fine structure may be observed with small slits. In taking absorption spectra the half-intensity band width was maintained nearly constant at 1.5 mμ by adjusting the slit width at intervals of 5 mμ. The theoretical slit width required was calculated from the known focal distance and data relating the rotation of the Littrow prism with wave length.

AMPLIFICATION

Light Source. The amplification required for the anode current of the C7180 photomultiplier depends upon the light source and the dynode supply voltage. Any suitable source of ultraviolet light may be used, such as the xenon or mercury-xenon arcs or the hydrogen discharge lamp. Sources of ultraviolet light have been compared by Baum and Dunkelman (*1*). Care in handling xenon lamps is indicated because they are said to develop a pressure of 20 atm. when in use. Absorption spectra taken with the light sources mentioned are similar.

The xenon arc was mounted in a housing having an internal liquid cooling system. Light and heat shields protect both instrument and operator.

Schematic of Amplifier. The schematic of the amplifier in present use is given in Figure 2. It is based in principle upon the Wheatstone bridge, in which R_{11} and R_{12} are arms with fixed resistance, V_3 is the unknown resistance, V_4 the calibrated resistance, and M_3 a null-indicating device.

Regardless of the light source, a large anode load resistor, R_1 (10^9 ohms or larger), is required. An electrometer tube, V_2, is used in the first stage of direct or alternating current amplification and provides an input with high impedance. The housing of the photomultiplier was designed so that the leads from both the controlling grid of V_2 and load resistor could be connected directly to the anode pin of the photomultiplier, to minimize stray capacitance. Additional amplification is necessary in the form of matched pairs of transistors, T_1 and T_2, and of tubes, V_3 and V_4.

Essential to the operation of the instrument are: a dark current control, R_5; a sensitivity control, R_9; a shutter to cut the incident light from the optical path (not shown); a variable power supply for adjusting the amplification of the photomultiplier (not shown); a potentiometer, R_4, for reading per cent transmittance; and a selector switch, S_1, which selects the connection of the

grid voltage of V_4 with the potentiometer.

Sensitivity Control. Sensitivity is controlled by the variable current-limiting resistor, R_9. R_{20} is used to limit the current flowing through the null indicator for its protection. This method of sensitivity control is independent of the incident radiation. The sensitivity used should be no greater than required for accuracy in the region in which readings are made. Between 100 and 25% transmittance minimal sensitivity may be used; below 10% maximal is required.

Measuring Device. Measurements as per cent transmittance are made on R_4 with a G. W. Borg micropotentiometer, Model 205, with a direct-reading microdial, Model 1307, calibrated in 1000 divisions. With this equipment (Allied Radio Corp., 100 North Western Ave., Chicago, Ill.) measurements may be made directly to the nearest 0.1% transmittance—i.e., they will not vary from the true value by more than ±0.05% transmittance. This degree of precision has no significance in solutions of low absorbance, but becomes a factor which limits accuracy as the absorbance approaches 2.0.

Readings on a linear scale as per cent transmittance were preferred to those on a logarithmic scale as absorbance because of the greater accuracy and availability of the linear scale and variable resistors.

Test of Amplifier. Before the amplifier is used initially certain adjustments, tests, and calibrations should be made.

The filaments of V_3 and V_4 should be aged. Before the amplifier's power supply is turned on, S_2 is closed, S_3 opened; R_3 is set at ground potential, R_8 at maximal resistance, and R_5 at half-maximal resistance. Tests of voltage are made with a VTVM at test points A to F. The negative test probe is connected to the grounded point, D, with the exception of point A, where the polarity is reversed. M_1, which measures the filament current of V_2, is regulated by R_2 (current 6 ma). M_2, which measures the plate current of V_2, is adjusted with R_3 (current 7 μa.). S_1 should be set at the less positive position. With S_3 closed and R_9 set at minimal resistance, R_6 should be adjusted so that M_3 reads zero. For calibration a potentiometer is required as input source. With the positive pole connected to test point D and the negative to the controlling grid of V_2, the reading on M_3 should be null when the potentiometer is set at −0.66 volt; if necessary, it is adjusted with R_5. The authors use a differential of 0.34 volt across R_1 as 100% transmittance because this value gives a satisfactory balance between light and amplifier. With S_1 at the position of more positive potential (100% transmittance) and the potentiometer set at −1.00 volt, the reading on M_3 should be null. If it is not, R_6 and R_7 should be adjusted, with test point E maintained at 5.7 volts. The amplifier should be calibrated by determining the per cent transmittance on the microdial as a function of the potential applied to the controlling grid of V_2. The authors found the curve linear, with standard deviation 0.006.

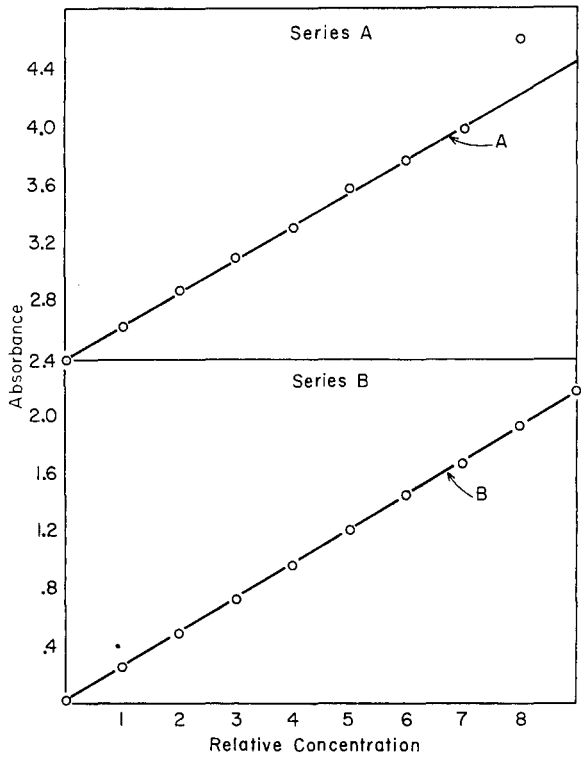


Figure 3. Absorbance, referred to water, of aqueous solutions of uridylic acid as a function of concentration, read at 194 mμ, with a xenon arc as light source

Points for curve B were read with water in reference cell; for curve A with a solution of absorbance 2.38 in reference cell

OPERATION

The amplifier and power supply reach stability in 6 hours, the xenon or mercury-xenon arc in 1 hour. For each wave length the slit is adjusted to give the desired half-intensity band width. With S_1 in the more positive position (dark current), M_3 is adjusted to null. With S_1 in the less positive position (100% transmittance), the reference cell is brought into position, the shutter opened, and M_3 adjusted to null by varying the gain of the photomultiplier. With S_1 connected to the arm of R_4 (% transmittance) the experimental cell is brought into position and R_4 is varied until M_3 reads null. The reading on the microdial is recorded as per cent transmittance.

PERFORMANCE

Figure 3 shows the results of an experiment designed to test the performance of the spectrophotometer and its potentiality for use in taking difference spectra in the far-ultraviolet.

A stock aqueous solution of uridylic acid was prepared with absorbance 4.76 at 194 mμ (solution A). A second stock solution was made by dilution of the first with an equal volume of water (solution B). A series of dilutions of each stock solution was made such that their theoretical relative concentrations were those indicated in Figure 3. The per cent transmittance of light with half-intensity band with 1.5 mμ, centered at 194 mμ, was determined with a xenon arc as source. In the B series the reference cell contained glass-distilled water; in A the reference was the dilute stock standard, absorbance 2.38.

Stray light of wave length longer than 240 mμ was not detectable by the photomultiplier (C, Figure 1). It remained to demonstrate whether stray light of wave length shorter than 240 mμ was present in the measured light. In the absence of all stray light the absorbances of the solutions illustrated in Figure 3 should be a linear function of their relative concentrations. The points indicated on A and B represent experimental values; the curves were drawn to conform with the least-square law for a straight line. The standard deviation for B was 0.01, for A 0.02. On B the observed conformity of the last point is somewhat fortuitous. Even without this point there is satisfactory correlation between absorbance and concentration (Beer's law) until the former approaches 2.0, with water in the reference cell.

In the reference cuvette of series A (absorbance 2.38) more than 99% of the incident selected monochromatic light was absorbed by the chromophore. Therefore in the experimental cuvette less than 1% could serve as incident light for measuring increase in absorption due to increase in concentration above that appearing in the reference cell. If there were present, in the measured light, any significant amount of stray light of wave lengths detectable by the photomulti-

plier, this fact should become apparent in these stringent circumstances and result in deviation from linearity. By this criterion no measurable amount of stray ultraviolet light was found in the system. The somewhat greater standard deviation found for A than for B is attributable largely to the fact that the extremely small photocurrents measured required maximal amplification, resulting in larger values for the dark current. Nevertheless, absorbances approaching 4.0 (referred back to water) have been determined with a fair degree of accuracy. The last point shown on A, with an expected absorbance of 4.2, is in serious error, and illustrates the limitation of the instrument, as used. It has not been included in the calculations. This limitation may be minimized by reduction of the dark current (*7*).

SUMMARY

The spectrophotometer described is capable of making accurate measurements of the absorption of ultraviolet light of wave length as short as 194 mμ. This technique may be used to characterize compounds by their absorption spectra in the far-ultraviolet, and follow the course of reactions in this region by difference spectra.

Absorption spectra in the far-ultraviolet of certain biologically important compounds are presented elsewhere (*2*).

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RECEIVED for review August 11, 1958. Accepted December 15, 1958. Investigation supported by a grant A-646(C) from the National Institutes of Health.

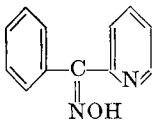
Spectrophotometric Determination of Palladium with Phenyl-alpha-pyridyl Ketoxime

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▶ **Phenyl-α-pyridyl ketoxime was found to be a highly selective and sensitive reagent for palladium. The palladium complex of the ketoxime has two characteristic absorption maxima, one at 410 mμ and the other at 340 mμ. At these wave lengths the photometric error is a minimum in the concentration ranges 2 to 10 and 1.5 to 8 p.p.m., respectively. The molar extinction coefficient of the palladium chelate is 3 × 10⁴ at 410 mμ and 5 × 10⁴ at 340 mμ. The reagent also forms complexes with iron, cobalt, nickel, and copper, which are all soluble in organic solvents. The interference due to these ions can be eliminated by using (ethylenedinitrilo)tetraacetic acid (EDTA). Gold and cyanide are the only interfering substances.**

PHENYL-α-PYRIDYL ketoxime(I) was reported (*5*) as a



I

new chelating agent for a number of cations. It was found to be highly selective for palladium(II), with which it reacted to form a yellow, water-insoluble chelate of definite composition. An analysis of the palladium complex showed that palladium and the reagent combine in the ratio of 1 mole to 2 moles.

The various reagents and procedures for the spectrophotometric determination of palladium were discussed by Beamish and McBryde (*1*, *2*) in two excellent review articles. West (*7*) and Mellon and Boltz (*4*) summarized the reagents used for the spectrophotometric determination of platinum metals during the last few years.

The principal difficulty encountered in many of the proposed methods for palladium is the interference of the bivalent ions of the transition metals, particularly the colored ones, and of other metals of the platinum group. Usually the interference of the metals other than the platinum metals can be eliminated by using EDTA.

Phenyl-α-pyridyl ketoxime formed stable colored complexes with nickel(II), cobalt(II), iron(II), copper(II), gold(III), and palladium(II). All were precipitated at a definite pH, from the aqueous phase and extracted with organic solvents such as chloroform and carbon tetrachloride. The efficiency of precipitation and extraction for most of the chelates from the aqueous phase was best between pH 6 and 11. However, the incipient precipitation started at a much lower pH. The palladium complex began to precipitate at pH 2. With the exception of copper(II), the spectral characteristics of the complexes were the same in both aqueous and nonaqueous phases. Figures 1 and 2 show the absorption spectra of a number of complexes in chloroform, and also that of copper in the aqueous phase. The present paper describes the procedure for the spectrophotometric determination of palladium, using phenyl-α-pyridyl ketoxime.

EXPERIMENTAL

Instruments. Spectral studies and light adsorbance measurements were made with a Beckman Model DK I recording spectrophotometer. Matched silica cells of 1-cm. light path were used. pH measurements were made with a Beckman Model G pH meter.

Preparation of Phenyl-α-pyridyl Ketoxime. Hydroxylamine hydrochloride (50 grams) dissolved in a minimum volume of water was added to 200 ml. of a 10% sodium hydroxide solution. To this were added 20 grams of 2-benzoylpyridine dissolved in 300 ml. of 95% ethyl alcohol. The mixture was refluxed for 8 hours and evaporated on a water bath until all the solid separated as a crust on the top and the liquid was brownish. Sometimes the oxime separated as an oil which solidified on cooling. The magenta solid was filtered and weighed (yield 30 grams). The crude product was crystallized several times from 95% ethyl alcohol until the oxime was obtained as a white crystalline substance melting at 161° C. Two or three more crops of crystals were obtained from the mother liquor. The combined yield was 70% of the theoretical. The

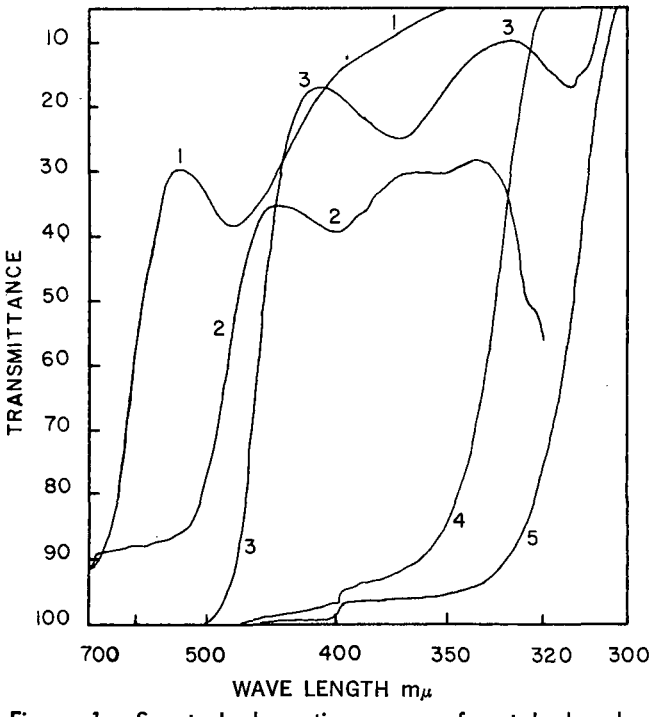


Figure 1. Spectral absorption curves of metal phenyl-α-pyridyl ketoximates in chloroform

1. Fe^{++} (12 p.p.m.)
2. Au^{++} (20 p.p.m.)
3. Pd^{+} (10 p.p.m.)
4. Reagent in $CHCl_3$
5. Reagent in ethyl alcohol

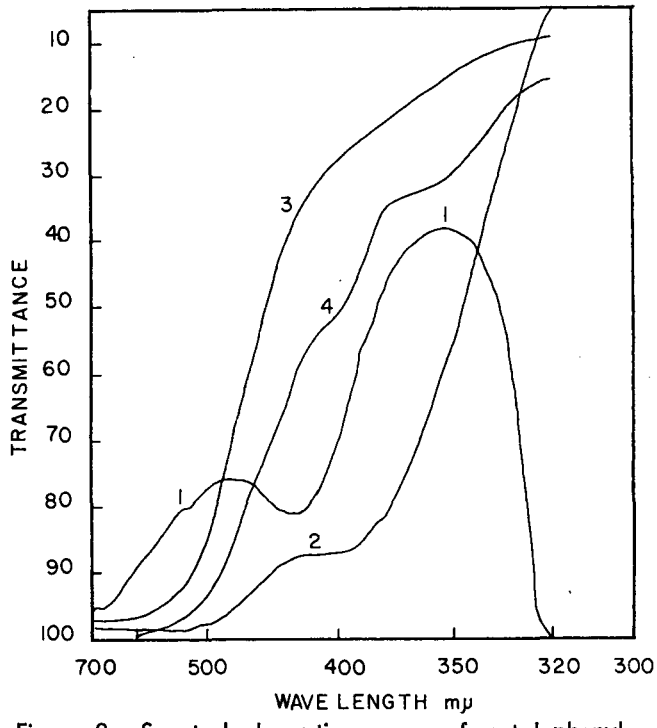


Figure 2. Spectral absorption curves of metal phenyl-α-pyridyl ketoximates in chloroform

1. Cu^{++} (24 p.p.m.)
2. Cu^{++} in H_2O (24 p.p.m.)
3. Co^{++} (4.8 p.p.m.)
4. Ni^{++} (4 p.p.m.)

analysis of phenyl-α-pyridyl ketoxime was as follows: Found: C, 72.95%; H, 5.10%; N, 14.20%. Calculated: C, 72.71%; H, 5.08%; N, 14.14%.

Standard Solutions. All chemicals were of analytical reagent grade.

The palladium solution was prepared from palladium(II) chloride and was 4*M* with respect to hydrochloric acid. It was standardized gravimetrically by precipitating palladium with dimethylglyoxime.

The solutions of other cations were prepared from their sulfates, nitrates, and chlorides and estimated by standard procedures. The buffers were prepared according to Vogel (*6*).

Reagent Solution. A 1% solution of phenyl-α-pyridyl ketoxime in 95% ethyl alcohol was used as the reagent.

Adsorption Spectra of Metal Phenyl-α-pyridyl Ketoximates. Preliminary studies indicated that for most of the cations, chelate formation and extraction of the chelates by organic solvents were most favorable between pH 6 and 11. Solutions for the study of the spectral adsorbance were prepared as follows. Depending upon the intensity of adsorption by the complex, 100 to 600 γ of the cation (4 to 24 p.p.m. in the final solution measured) were treated with 2 ml. of a 1% solution of the reagent in a 50-ml. beaker and diluted with water to about 15 ml. The pH of the solution was adjusted between 8.5 and 10 with sodium carbonate solution and recorded with a pH meter. The solution was then transferred to a 50-ml. separatory funnel using a minimum volume of water to wash the beaker. The volume of the aqueous phase was maintained at about 20 ml., and 10 ml. of chloroform was added to it. The two phases were mixed by vigorous shaking and allowed to separate. Two extractions were performed. The volume of the chloroform extract was made up to 25 ml. with chloroform. The aqueous phase was evaporated almost to dryness and tested for the particular cation by applying the appropriate spot test. The aqueous phase was free of the cation being extracted after the second extraction. The solution for the study of adsorption by copper(II) complex in water was made as above; the volume, however, was made to 25 ml. with water. Figures 1 and 2 show the spectral curves and Table I summarizes wave lengths at the peaks. A chloroform blank was prepared by diluting 2 ml. of the reagent solution to 20 ml. with water, adjusting the pH to 9, and extracting twice with chloroform. The final volume was 25 ml. with the chloroform. An aqueous blank was prepared by diluting 2 ml. of the reagent to 20 ml. with water, adjusting the pH to 9, and diluting to 25 ml. with water. Absorption studies were made against proper blank solutions.

Figure 1 shows the spectral characteristic of the reagent in chloroform and in ethyl alcohol *vs.* chloroform and ethyl alcohol, respectively.

Influence of pH on Chelate Formation and Extraction. The influence of pH on the formation and extraction of palladium phenyl-α-pyridyl ketoximate was studied. Concentration of palladium (10 p.p.m.) and the reagent and the procedure adopted for preparing solutions were the same as in the spectral absorption study. In one series of experiments, the pH was adjusted by the addition of different volumes of sodium carbonate solution (Figure 3). In another series, after pH had been adjusted to the desired value, it was stabilized by the addition of 2 ml. of a buffer in order to determine the most desirable buffer system. Acetate, phosphate, borate, ammonia–ammonium chloride, and alkali carbonate buffers were used. Only the phosphate interfered seriously with the absorption peak in the ultraviolet region; in the visible region it was unaffected (Figure 3).

Efficiency of Extraction. A number of solutions of each cation (500 γ) in 20 ml. were prepared and adjusted to a different pH between 2 and 11. Each solution was treated with 2 ml. of the reagent solution and extracted one to three times with chloroform. The aqueous phases were evaporated almost to dryness and tested for the cations. Aqueous phases were free of palladium(II), iron(II), cobalt(II), and nickel(II), after the first extraction. Gold(III) and copper(II) were completely removed by two extractions in the above pH range. This was further

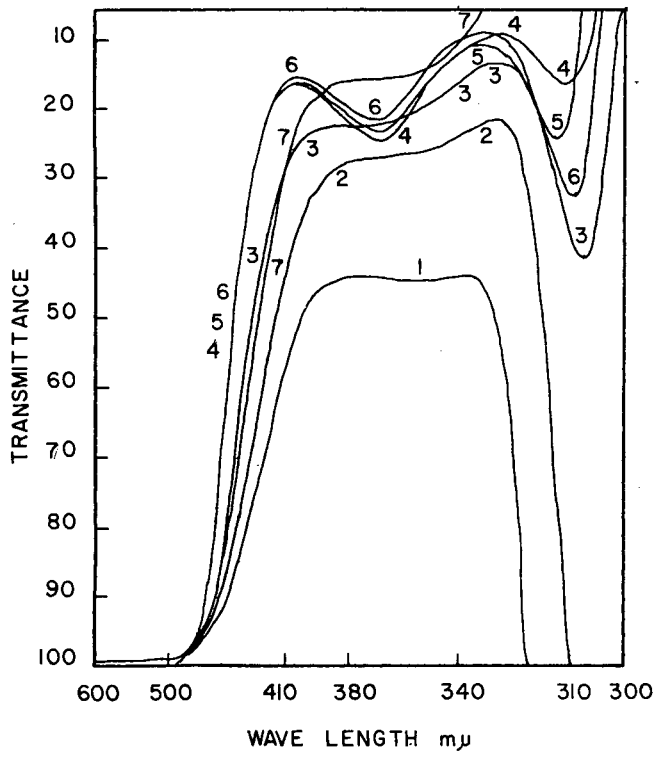


Figure 3. Spectral curves of palladium phenyl-α-pyridyl ketoximate in chloroform extracted from aqueous phase of different pH

1. pH 1.7
2. pH 5.0
3. pH 11.5
4. pH 6.4
5. pH 9.3
6. pH 10.8
7. pH 7.5

Phosphate buffer

Table I. Wave Lengths of Light Absorption Peaks of a Number of Metal Phenyl-α-pyridyl Ketoximates in Chloroform

Cation	Absorption Peaks, Mμ Wave Length	Rapid Increase in Absorption after	Figure and Curve No.
Fe^{++}	530	460 mμ	Fig. 1, curve 1
Au^{++}	450		2
Cu^{++}	475, 350		Fig. 2, curve 1
Cu (in H_2O)	415	390 mμ	2
Co^{++}		600 mμ	3
Ni^{++}		550 mμ	4
Pd^{++}		410, 317	Fig. 1, curve 3
Reagent (in $CHCl_3$)		360 mμ	4
Reagent (in C_2H_5OH)		340 mμ	5

verified by measuring the absorbance of the chloroform phase after each extraction.

Beer's Law. The spectral curves show that palladium phenyl-α-pyridyl ketoximate exhibits two distinct and characteristic absorption peaks at 410 and 327 mμ. Beer's law was studied at both these wave lengths in the concentration range of 0.5 to 14 p.p.m. using a cell of 1-cm. light path.

One milliliter of the reagent solution was added to the palladium solution contained in a 25-ml. beaker. The volume of the mixture was made up with water to nearly 10 ml. and the pH of the solution was adjusted between 8 and 9 with a sodium carbonate solution. The solution was then transferred to a 50-ml. separatory funnel using a minimum volume of water to wash the beaker. The solution was extracted three times, using 5 ml. of chloroform each time and the final volume of the extract was made up with chloroform, to 25 ml. Absorbance of the chloroform extract was measured at 410 and 340 mμ.

The concentration range for minimum photometric error was between 1.5 and 8 p.p.m. at 340 mμ and between 2 and 10 p.p.m. at 410 mμ. However, this range can be varied by changing the length of the light path through the solution. The average molar extinction coefficient of the chelate was found to be 3×10^4 at 410 mμ and 5×10^4 at 340 mμ.

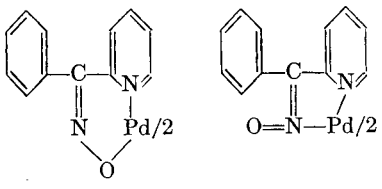
Effect of Other Ions. Binary mixtures were prepared containing 100 γ of palladium and 5 mg. of a second cation. The mixture was treated with 2 ml. of the reagent solution and was diluted with water to approximately 10 ml. The extraction was performed three times using 5 ml. of chloroform each time. The combined extracts were diluted to 25 ml. with chloroform. Absorbance was measured at both 410 and 340 mμ. Beryllium, magnesium, strontium, barium, zinc, cadmium, mercury(II), aluminum, lead, bismuth(III), vanadyl(II), manganese(II), iron(III), ruthenium(VI), rhodium(III), osmium(VI), iridium(N), platinum(V), and silver ions were not extracted and hence did not interfere. Interference due to copper(II), iron(II), cobalt(II), and nickel(II) was eliminated by using 2 ml. of 0.1*M* EDTA solution. EDTA did not interfere when absorbance was measured at 410 mμ. Interference by EDTA when absorbance was measured at 340 mμ was eliminated by treating the blank with an identical amount of EDTA. Bismuth(III) and iron(III) tended to hydrolyze and the hydroxides usually separated at the interface of the two phases. The suspensions were eliminated by filtration. Gold(III) was the only seriously interfering cation. Among the common complexing agents such as citrate, oxalate, tartrate, and cyanide, only cyanide interfered seriously.

Effect of Reagent, Temperature, and Time. At the optimum pH even a 1000 gold excess of reagent was without any adverse effect at 410 mμ. At 340 mμ, the absorbance was higher because of the absorption of the reagent. This was completely eliminated by treating the blank similarly. Variation in temperature of the chloroform extract between 0° and 45° C. did not affect the color intensity. Reaction and maximum color development were instantaneous. There was no fading of color in 2 weeks.

DISCUSSION

Phenyl-α-pyridyl ketoxime was found to be a sensitive and selective reagent for palladium. The procedure for the spectrophotometric determination of palladium is not rigorous. It is possible to separate palladium, using phenyl-α-pyridyl ketoxime, from a number of elements with which palladium is usually associated. The curves in Figure 3 indicate that between pH 6.4 and 10.8 the structure of the chelate formed is the same. Preliminary spectrophotometric studies (*3*) and analysis of the precipitate indicated that palladium and the reagent combined in the ratio of 1 to 2 moles. However, these studies did not reveal whether the palladium complex exists as structure II or III.

The results of the investigation on the likely structure of the palladium chelate and other analytical applications of phenyl-α-pyridyl ketoxime will be published later.



II III

ACKNOWLEDGMENT

Grateful thanks are expressed to Philip W. West for allowing the author to use the facilities of his laboratories. Partial financial support by the Division of Research Grants, Public Health Service, is thankfully acknowledged. Reilly Tar and Chemical Corp. made a gift of 1 pound of 2-benzoylpyridine.

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RECEIVED for review July 30, 1958. Accepted December 11, 1958.

Tracer Investigation of Iridium-Platinum Separation by Anion Exchange

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▶ **The separation of 10-mg. quantities of iridium and platinum by the use of Dowex A-1 anion resin has been investigated using radiotracer techniques. Elution curves are exhibited for iridium(III), platinum(II), and platinum(IV) recovery from resin columns, and a procedure is reported for the rapid separation of iridium and platinum.**

THE separation of platinum and iridium by ion exchange has been the subject of a number of investigations (*2–7*). Procedures involving anion exchange have been particularly appealing because of the extremely stable halide complexes formed by these elements. However, these methods were limited in that large volumes of eluent (and consequently long elution times) were required; in particular, anionic platinum complexes were bonded by the resin to such an extent that quantitative elutions were difficult to obtain. In a recent article, Berman and McBryde (*1*) have reported the successful recovery of fractional milligram quantities of platinum as chloroplatinate ion from Amberlite IRA-400 anion exchange resin, using perchloric acid as eluent. Independent work in this laboratory using radiotracer techniques to investigate the separation of 10-mg. quantities of iridium and platinum with Dowex A-1 anion exchange resin has yielded similar results. A procedure has been developed permitting the rapid separation of radiochemically pure platinum from iridium.

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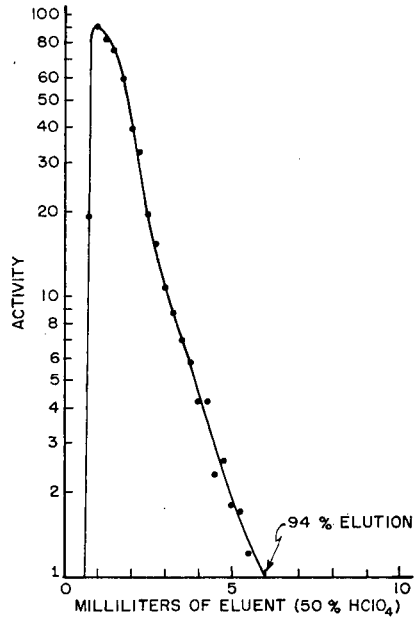


Figure 1. Elution curve of chloroplatinate with 50% perchloric acid

Platinum, 10 mg.

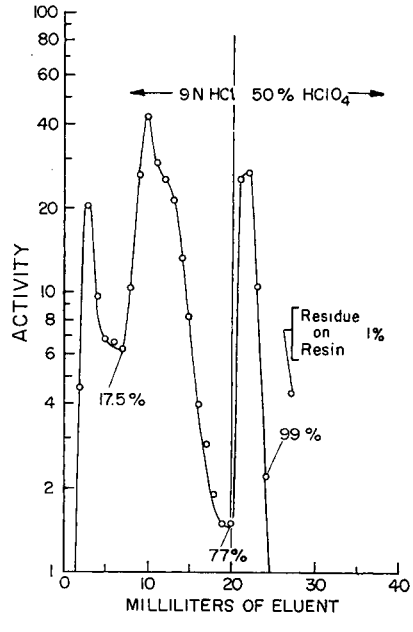


Figure 2. Elution curve of mixed chloroplatinite and chloroplatinate

Platinum, 10 mg.

APPARATUS AND REAGENTS

Dowex 1 resin, 200 to 400 mesh size, 10% cross-linked (Dow Chemical Co., Midland, Mich.), was employed in columns 5 mm. in diameter by 5 cm. in length, with column free volume of 0.75 ml. Before use, the columns were washed with 5 column volumes of 9*N* hydrochloric acid, followed by 5 column volumes of water. All elutions were carried out at 80° C., at a rate of 1 ml. per minute. Temperature was controlled by circulating water from a thermostatted reservoir through jacketed columns. The cyclotron-produced activities 2.7-day platinum-191 and 4.3-day platinum-193m served as the platinum tracer and pile-produced 75-day iridium-192 as the iridium tracer. As 63 to 66 k.e.v. x-ray and harder gamma radiations are associated with these tracers, the activities of all solutions and of the resin itself were directly counted with a sodium iodide scintillation spectrometer set to record quanta with an energy greater than 45 k.e.v. The fractions to be counted were of standard volume and were contained in standard size test tubes mounted in standard geometry with respect to the detector.

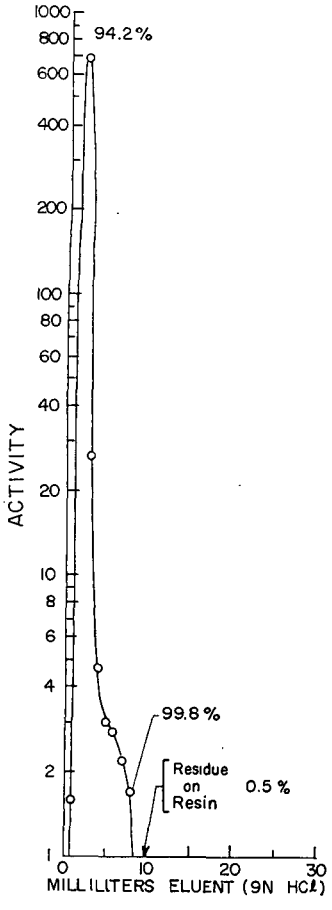


Figure 3. Elution curve of chloroiridate(III) with 9N hydrochloric acid

Iridium, 10 mg.

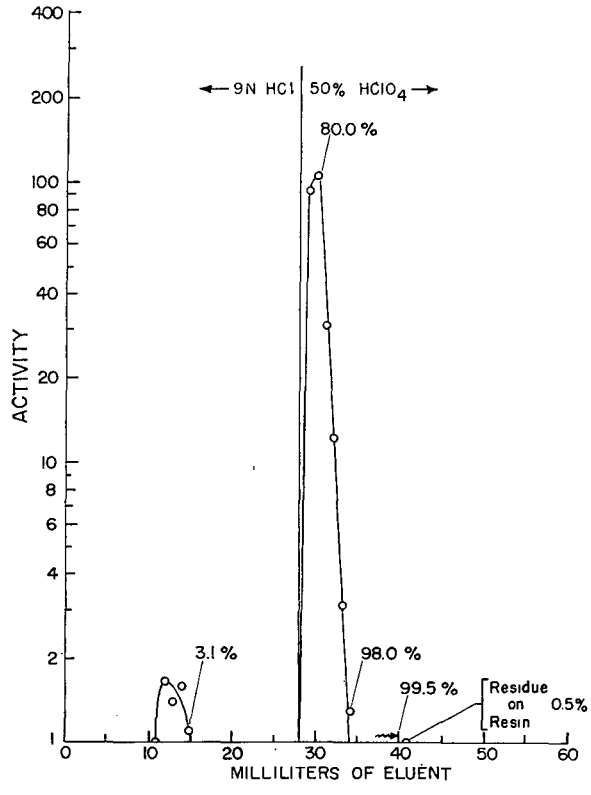


Figure 4. Elution curve of chloroplatinate after reduction with hydroxylamine hydrochloride

Platinum, 10 mg.

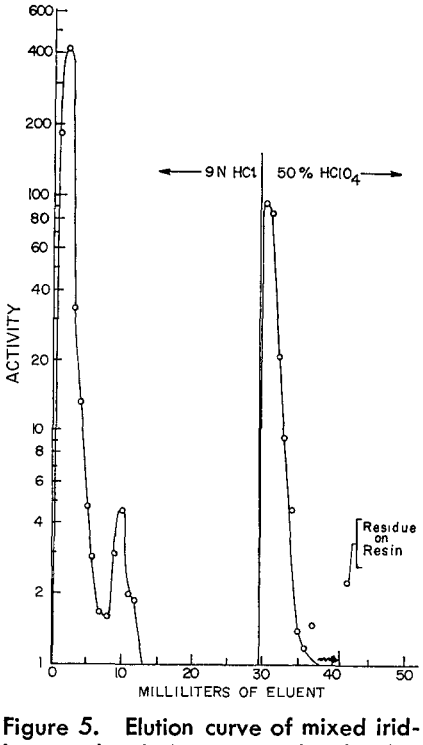


Figure 5. Elution curve of mixed iridium and platinum sample showing both tracers

Iridium and platinum, 10 mg. each
First count (1-17-58)

Carrier solutions, containing 5.0 mg. per ml. of quadrivalent iridium and platinum, were prepared from weighed amounts of potassium chloroiridate(IV) and platinum metal. Ten-milligram portions of metal from the carrier solutions were used, to which approximately 5×10^4 c.p.m. of the appropriate tracer were added. Before use, 2 ml. of 9*N* hydrochloric acid and 0.5 ml. of 30% hydrogen peroxide were added and the solutions were taken to dryness to ensure that both tracer and carrier were initially in the quadrivalent oxidation state. Samples were then redissolved in 1 ml. of hot hydrochloric acid or water. All solutions were counted prior to adsorption on the resin.

EXPERIMENTAL

Platinum(II) and (IV) Elution. Adsorption and desorption studies were first carried out with platinum as the chloroplatinate ion. Platinum in 10-mg. quantities was adsorbed onto the column quantitatively from 1 ml. of 3*N* hydrochloric acid. Ninety-four per cent of the adsorbed platinum was eluted from the column, using 4 ml. of 50% perchloric acid collected in 0.2-ml. fractions, which were diluted to 2 ml. for counting purposes. The elution curve shown in Figure 1 is consistent with elution limited by desorption kinetics.

The behavior of the chloroplatinite ion was also characterized. Ten milligrams of platinum, initially present as chloroplatinate in 1 ml. of water, were partially reduced by heating with 0.25 ml. of 10% hydroxylamine hydrochloride for 5 minutes in a boiling water bath. After adsorption onto the column from this solution, elution was carried out with 19 ml. of 9*N* hydrochloric acid which removed 77% of the platinum, indicating partial reduction of this platinum from chloroplatinate to chloroplatinite. The remaining chloroplatinate ion was eluted with 5 ml. of 50% perchloric acid and over-all platinum recovery was 99%. The two-peaked structure observed in the hydrochloric acid elution of chloroplatinite (Figure 2) is reproducible.

Iridium(III) and (IV) Elution. The adsorption and elution properties of iridium in the tri- and quadrivalent oxidation states were next determined at 80° C. The chloroiridate(IV) ion was very strongly adsorbed on the Dowex 1 column from 3*N* hydrochloric acid and could not be readily removed with hydrochloric or perchloric acids. Chloroiridate(III) ion, formed by prolonged reduction of chloroiridate(IV) with hydroxylamine hydrochloride, was more weakly adsorbed and could be easily eluted with hydrochloric acid. A series of elutions carried out with various concentrations of hydrochloric acid indicated that 9*N* hydrochloric acid eluted the chloroiridate(III) more readily than 3*N* or 6*N* acid. Figure 3 shows the elution curve obtained after adsorption of 10 mg. of trivalent iridium; 99.8% of the iridium was removed using 8 ml. of 9*N* hydrochloric acid. These results are similar to those reported by Kraus and coworkers (4) at room temperature.

Preferential Iridium(IV) Reduction. An investigation of the preferential reduction of quadrivalent iridium in the presence of quadrivalent platinum was next undertaken. The reduction of both chloro complex ions was found to be inhibited at higher hydrochloric acid concentrations. A series of experiments indicated that a hydrochloric acid concentration of 3*N* is optimal for preferential reduction of the iridium(IV) by hydroxylamine hydrochloride with minimum reduction of the platinum; at higher hydrochloric acid concentrations only partial reduction of the iridium(IV) was achieved.

Separation of Iridium-Platinum Mixtures. Ten milligrams of labeled platinum as chloroplatinate and 10 mg. of iridium as chloroiridate(IV) in 2 ml. of 3*N* hydrochloric acid were reduced with 0.25 ml. of 10% hydroxylamine hydrochloride for 2 minutes in a boiling water bath. At this point, the dark brown chloroiridate(IV) color was no longer present, showing that reduction to chloroiridate(III) was complete. After reduction and adsorption of the mixed iridium and platinum, 28 ml. of 9*N* hydrochloric acid were passed through the column, followed by 11 ml. of 50% perchloric acid. With this procedure, only 3% of the platinum was reduced and eluted with 9*N* hydrochloric acid, 96% was eluted with perchloric acid, and 0.5% of the platinum remained on the column (Figure 4).

To verify the purity of the platinum fraction obtained by the last procedure, a separation of 10-mg. quantities of labeled iridium and labeled platinum was attempted. Reduction was carried out in 2 ml. of 3*N* hydrochloric acid with 0.25 ml. of 10% hydroxylamine hydrochloride for 3 minutes in a boiling water bath. Elution was first made with 29 ml. of 9*N* hydrochloric acid, followed by 50% perchloric acid. Figure 5 shows the elution curve from activity determinations made immediately after separation; the second peak observed in the hydrochloric acid eluent is due to a small amount of chloroplatinite. Figure 6 was obtained from activity determinations made on the same eluent fractions one month (7 platinum tracer half lives) after separation. No long-lived iridium activity above 10% of counter background was observed in the perchloric acid eluent, indicating

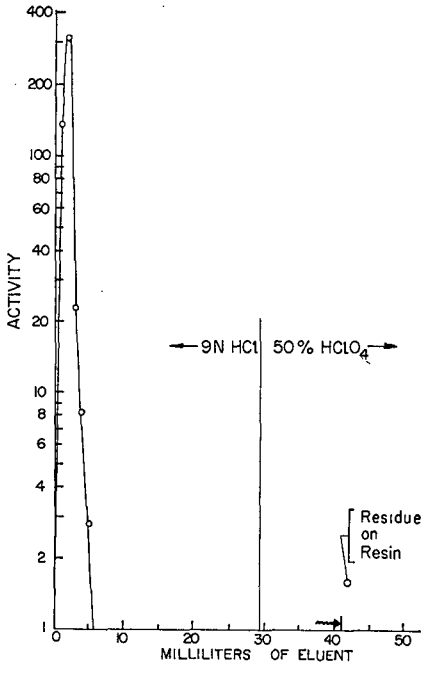


Figure 6. Elution curve of mixed iridium and platinum sample after decay of platinum tracer

Iridium and platinum, 10 mg. each
Second count (2-15-58)

that the purity of the platinum from this procedure is greater than 99%.

DISCUSSION

The method now being used in this laboratory for iridium and platinum separations is as follows:

Add 2 ml. of concentrated hydrochloric acid to 2 ml. of solution containing 10 mg. of platinum and 10 mg. of iridium; boil just to dryness. Add 2 ml. of 9*N* hydrochloric acid and 0.5 ml. of 30% hydrogen peroxide; again boil just to dryness.

Dissolve residue in 1 ml. of 3*N* hydrochloric acid and 5 drops of hydroxylamine hydrochloride (10%); heat for 3 minutes in a boiling water bath. Place on Dowex 1 column (temp. 80° C.) and elute iridium with 9*N* hydrochloric acid.

Elute platinum with 50% perchloric acid.

This procedure has afforded excellent fractionation of 41-hour iridium-188 and its long-lived parent, 10.3-day platinum-188.

Because degradation of the resin by hot perchloric acid could possibly account for the elution of the chloroplatinate ion, resin capacity measurements were undertaken. The capacity of resin samples which had and had not been exposed to 50% perchloric acid for 15 minutes at 80° C. was determined by converting the samples to the chloride form with an excess of hydrochloric acid, washing the resin free of excess hydrochloric acid, and determining the chloride on the resin using the Volhard method. No decrease in the capacity of the resin was observed, indicating stability of the resin under the elution conditions.

Preliminary experiments attempting elution of chloroplatinate ion with 3.5*M* lithium perchlorate have given elution curves comparable to those obtained with 3.5*M* (25%) perchloric acid. These results demonstrate the dependence of the elution upon displacement of the chloroplatinate ion by the perchlorate ion.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the cooperation of T. D. Thomas, Chemistry Division, University of California Radiation Laboratory, Berkeley.

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RECEIVED for review July 30, 1958. Accepted December 9, 1958. Taken in part from a thesis submitted by D. D. Busch in May 1957 to the Department of Chemistry, Princeton University, in partial fulfillment of the requirements for the bachelor of arts degree. Work supported in part by the U. S. Atomic Energy Commission and the Higgins Scientific Trust Fund.

Principles of End Point Detection in Chelometric Titrations Using Metallochromic Indicators

Characterization of End Point Sharpness

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▶ The optimal use of metallochromic indicators in chelometric titrations requires a thorough understanding of the factors involved. Unlike acid-base titrations, the interplay of a large number of equilibria must be considered. From pM-pH or pY-pH diagrams the sharpness of an end point may be quantitatively characterized by only two end point indices and the end point sharpness under different conditions may be readily compared. Such diagrams are useful in predicting the possibility of selective titrations and the feasibility of activating an indicator response for the titration of one metal ion by the addition of a second. The principles outlined are helpful in the search for new chelometric methods and indicators.

CHELOMETRIC titrations occupy an increasing role in the analysis of metal-ion mixtures. To widen the scope of these applications, new indicators and titrants are being studied and proposed. In the evaluation of metallochromic indicators, two important but different characteristics of the end point must be distinguished and considered. First, a large degree of contrast must exist between the colors of the indicator before and after the end point. Second, this indicator color change must be sharp—i.e., produced by a minimum increment of titrant. In the literature, these two characteristics have often been confused.

No quantitative scale has been set up for describing these two characteristics of end point quality. This article proposes a numerical characterization of the second characteristic, end point sharpness. The relationship between such sharpness and the significant parameters involved in a titration are derived to create a method for selecting titration conditions from theoretical considerations. A quantitative characterization of the color contrast of an end point and the theoretical aspects of screening dyes will be the subject of a separate communication

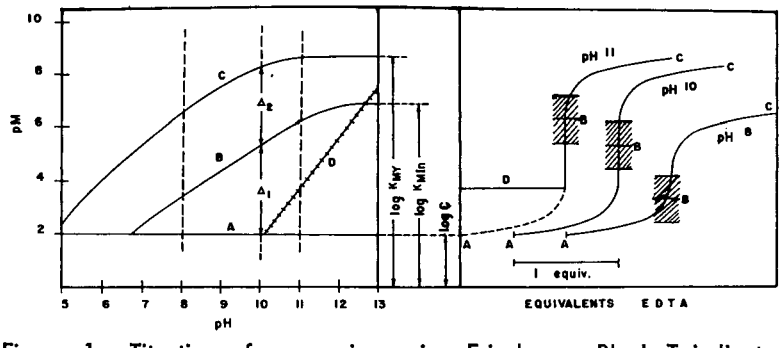


Figure 1. Titration of magnesium using Eriochrome Black T indicator

Left. pM-pH diagram
Right. Corresponding pM-titration curves

The sharpness of the end point in any titration with an indicator may be illustrated by a plot of the color change of the indicator *vs.* the equivalents of titrant. In acid-base titrations, the shape of such a curve depends on only a few equilibrium constants and characterization of the end point sharpness is relatively simple. In chelometric titrations, the number of pertinent equilibria is considerably larger. The end point sharpness depends on the stability constants of the metal chelonates and the metal-indicator complexes, on the concentrations of metal ion and indicator, and on pH, buffer, masking agents, and other cations present in solution. Color change curves have been calculated for a number of highly specified systems (*3, 4, 16*). Although the methods of calculation are of general applicability, separate calculations are required for each set of conditions. This procedure is tedious and the interplay of the various factors and equilibria is difficult to perceive.

In this paper, pM-pH and pY-pH diagrams present the quantitative significance of the factors involved. From such diagrams two end point indices can be readily obtained whose numerical values allow immediate quantitative characterization of the color change in the titration, independent of the system being considered. Because such diagrams are directly related to color change curves, they allow rapid prediction of optimum titration conditions. Other applications are in predicting the conditions necessary for selective titrations and in testing the feasibility of activating an indicator response for the titration of one metal by the addition of a second activating metal ion.

pM-TITRATION CURVES AND pM-pH DIAGRAMS

As in pH titration of acids and bases, metal ion titrations may be generally represented by pM titration curves. Examples of such curves are shown in Figure 1, right, for the (ethylenedinitrilo)tetraacetic acid (EDTA) titration of magnesium at various pH values. The initial pM is characterized by point *A* and the pM after the titration by point *C* where the solution is 100% overtitrated. The shaded areas indicate the region of the color change of Eriochrome Black T indicator, *B* corresponding to the point of 50% color change. Because the indicator forms a 1 to 1 complex with the metal ion, the fractional color change from 0.1 to 0.9 covers about 2 pM units. If the total pM break is large, the situation is ideal when the 50% color change point, *B*, coincides with the inflection point of the pM curve because, at this point, a maximum color change is obtained by a minimum increment of titrant. The titration curve at pH 10 is not too far from this ideal case.

The curves at pH 8 and 11 illustrate cases of dragging color changes; the end

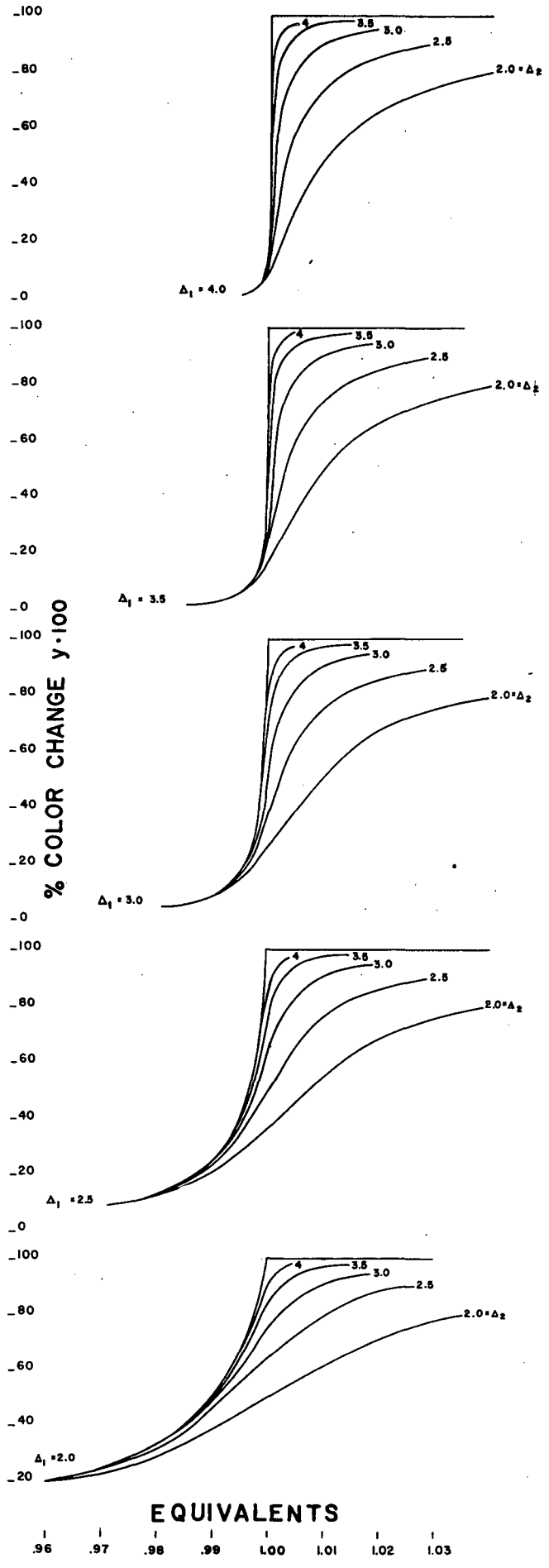


Figure 2. Color change curves for various end point indices

point in these cases tends to be early or late (pH 8 or 11). The pertinent points, *A*, *B*, and *C* are usually strongly pH-dependent under practical titration conditions. It is, therefore, advantageous to plot them as a function of pH in a pM-pH diagram (left side of Figure 1). This construction can be readily achieved with the help of the constants involved in the pertinent equilibria.

CONSTRUCTION OF pM-pH DIAGRAMS

Pertinent Equilibria. The construction of pM-pH diagrams requires the knowledge of the equilibrium constants for all the pertinent reactions. At this time these constants are known for only a few common metal-indicator reactions but are abundant for the various chelon-metal reactions (*2*). The determination of the metal-indicator stability constants offers a fruitful field for future investigations. The most basic equilibria are given by

$$M + Y \rightleftharpoons MY \qquad (1)$$

$$M + In \rightleftharpoons MIn \qquad (2)$$

For simplicity, the charges are omitted. The symbols are: M, metal ion; Y, chelon; In, indicator.

The corresponding equilibrium constants are:

$$K_{MY} = \frac{[MY]}{[M]\,[Y]} \qquad (3)$$

$$K_{MIn} = \frac{[MIn]}{[M]\,[In]} \qquad (4)$$

The concentrations, [Y] or [In], of the completely ionized forms are related to the stoichiometric concentrations [Y]′ or [In]′. These latter quantities correspond to the sum of the concentrations of the unmetallized species in all forms of ionization,

$$[Y]' = [Y] + [HY] + [H_2Y] + \ldots$$

$$[In]' = [In] + [HIn] + [H_2In] + \ldots$$

The concentration of the free form [Y] or [In] is related to the stoichiometric concentration of the unmetallated species by:

$$[Y] = \frac{[Y]'}{\alpha_Y} \qquad (5)$$

$$[In] = \frac{[In]'}{\alpha_{In}} \qquad (6)$$

where

$$\alpha = 1 + \frac{[H^+]}{k_n} + \frac{[H^+]^2}{k_n k_{n-1}} + \ldots + \frac{[H^+]^n}{\pi_n k_n} \qquad (7)$$

where k_n are the acidity constants of the chelating agent or indicator.

The effective equilibria reactions may be represented:

$$M + H_aY \rightleftharpoons MY + aH^+ \quad (8)$$

$$M + H_bIn \rightleftharpoons MIn + bH^+ \quad (9)$$

whose effective stability constants are then given by:

$$K'_Y = \frac{K_Y}{\alpha_Y} \text{ and } K'_{In} = \frac{K_{In}}{\alpha_{In}} \quad (10)$$

The other pertinent equilibria and their effect are described in separate sections.

Construction of Fundamental pM-pH Diagram. A simple pM-pH diagram such as given in Figure 1 for the titration of magnesium with EDTA using Eriochrome Black T (CI 203) as indicator, is readily constructed by the use of Equations 11, 12, and 13.

The initial pM, represented by line A is defined:

$$pM_A = -\log C_M \quad (11)$$

where C_M is the total concentration of metal ion. Equation 11 is valid only where no precipitating or complexing agents are present, or, if present, are not reactive with the metal ion being considered. Thus for the titration of 0.01M magnesium illustrated in Figure 1, pM_A has a constant value of 2 in the pH range up to pH 10.2, the pH at which formation of magnesium hydroxide commences in a 0.01M solution. At pH values greater than 10.2, the pMg increases with increasing pH with a slope, pM/pH, of 2, as illustrated by line D.

The hydrolysis constant, $\log K_{Mg(OH)_2} = 9.8$, as obtained from potential-pH measurements with the third class electrode system:

$$Hg|Hg\text{-}EGTA^{-2}, Mg\text{-}EGTA^{-2}, Mg^{+2}$$

[EGTA = ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid]. The electrode system works as pMg electrode within certain pH ranges (*15*).

Line B corresponds to the pM value at which the indicator is present 50% in free dye form and 50% in metal-indicator complex form. For a 1 to 1 metal-indicator complex, the pM at the 50% color change point, B, is equal to the logarithm of the effective stability constant of the metal-indicator complex:

$$pM_B = \log K_{MIn} - \log \alpha_{In} = \log K'_{MIn} \quad (12)$$

Thus at high pH values (>12)where the indicator exists in the free base form ([In] = [In]') and α_{In} equals 1, the pM_B equals the logarithm of the stability constant, $\log K_{MIn} = 7.0$ (magnesium–Eriochrome Black T complex), as illustrated in Figure 1. As the pH is decreased, a pH region occurs where the free indicator (In) converts into the monohydrogen form (HIn) and the pM_B value decreases with a slope, $\partial pM_B/\partial pH$, of 1. At an even lower pH region, where the monohydrogen form (HIn) of the indicator converts into the dihydrogen form (H_2In), a second break occurs in the pM-pH diagram, below which the slope, $\partial pM_B/\partial pH$, becomes 2. In Figure 1 this latter region is only partially visible. The breaks occur at pH values corresponding to the pk_n values of the indicator; for Eriochrome Black T, $pk_1 = 6.3$ and $pk_2 = 11.6$ and the equation for line B is

$$pM_B = 7 - \log (1 + [H^+] \times 10^{11.6} + [H^+] \times 10^{17.9})$$

At pH 6.8, lines B and A intersect; above this intersection the metal-indicator complex is stable, whereas below the hydrogen ions outcompete the metal for the indicator and the metal-indicator complex is unstable. The difference between lines B and A (or D) is actually a measure of the stability of the metal-indicator complex and Figure 1 shows that the stability of the magnesium–Eriochrome Black T complex increases with increasing pH but dissociates again above pH 12.

Line C represents the pM value at the point in the titration 100% past the end point (equimolar amounts of metal chelonate and chelon). The general equation for the pM of line C is

$$pM_C = \log K_{MY} - \log \alpha_Y = \log K'_{MY} \quad (13)$$

At high pH values (>10) where the chelon, EDTA, exists in the free base form ([Y] = [Y]') and α_Y equals 1, the pM_C equals $\log K_{MY}$ (= 8.8) as illustrated in Figure 1. As the pH is decreased, regions of pH occur where the various acid forms (HY, H_2Y) become predominant and the value of pM_C drops in a manner analogous to that of the pM_B curve, except that the breaks occur at different pH values because $pk_1 = 2.0$, $pk_2 = 2.76$, $pk_3 = 6.16$, $pk_4 = 10.26$. The equation for line C (for the magnesium-EDTA complex) is given by

$$pM_C = 8.8 - \log (1 + [H^+] \times 10^{10.26} + [H^+]^2 \times 10^{16.42} + \dots)$$

The terms omitted in the above equation are negligible in the pH region above 5. Line C intersects line A at a pH somewhat below 5; at pH values below this intersection the magnesium-EDTA complex is unstable, and at pH values above, the complex is stable. The distance between lines C and A (or D) is actually a measure of its stability. EDTA will dissolve magnesium hydroxide in the pH region from 10 to 13 and this is readily predicted from Figure 1 where line C is above D in this pH region.

pM-pH DIAGRAMS AND COLOR CHANGE CURVES

In a pM-pH diagram, the distance between lines A and C represents, at any pH, the extent of the total pM break. The occurrence of the color change—early, at the equivalence point, or late—is determined by the position of line B between A and C and may be characterized by the pM differences, Δ_1 and Δ_2:

$$\Delta_1 = pM_B - pM_A = \log K'_{MIn} + \log C_M = \log K_{MIn} - \alpha_{MIn} + \log C_M \quad (14)$$

$$\Delta_2 = \log K'_{MY} - \log K'_{MIn} = \log K_{MY} - \log K_{MIn} - \log \alpha_Y + \log \alpha_{In} \quad (15)$$

Δ_1 is a measure of the tightness of the metal-indicator complex as described in the previous section and Δ_2 expresses the extent to which the chelon displaces the indicator from the metal-indicator complex. Most important is the fact that Δ_1 and Δ_2, which henceforth are termed end point indices, define completely the sharpness of an indicator end point, if the indicator concentration is relatively small. The sharpness of an end point may, therefore, be characterized simply by two numbers—3.4 and 3.0, meaning that $\Delta_1 = 3.4$ and $\Delta_2 = 3.0$.

The example of Figure 1 shows that early end points are obtained if Δ_1 is small and Δ_2 is large (pH 8), but late end points are obtained if Δ_2 is small and Δ_1 is large (pH 11). The sharpest end points will be obtained with both Δ_1 and Δ_2 large. These end point indices, obtained from a pM-pH diagram, allow a simple but quantitative comparison of the end point sharpness under varying conditions of pH, type of indicator and chelon, buffer, concentration of metal ion, and related parameters.

This relationship is obtained by combining Equations 14 and 15 with 16. Equation 16, derived by Fortuin, Karsten, and Kies (*4*) and later by Flaschka and Khalafalla (*3*), represents the titration curve (color change *vs.* equivalents of chelon added) of a direct chelometric titration using a metallochromic indicator.

$$a = 1 - \left(\frac{1-y}{y}\right)\frac{1}{C_M K'_{MIn}} + \left(\frac{y}{1-y}\right)\frac{K'_{MIn}}{K'_{MY}} - \frac{[MIn]}{C_M} - \frac{K'_{MIn}(C_{In} - [MIn])}{K'_{MY} C_M} - \frac{1}{C_M K'_{MY}} \quad (16)$$

where a corresponds to the equivalents of chelon added; y, the fractional color change (fraction of free indicator); C_M, total metal ion concentration (titrated plus untitrated); C_{In}, total indicator concentration; [MIn], concentration of the metal indicator complex; K'_{MY}, effective stability constant of metal

chelonate, K'_{MIn}, effective stability constant of metal indicator complex.

Thus in terms of the end point indices, the equation is:

$$a = 1 - \left(\frac{1-y}{y}\right)\frac{1}{10^{\Delta_1}} + \left(\frac{y}{1-y}\right)\frac{1}{10^{\Delta_2}} - \frac{1}{C_M 10^{\Delta_2}}\left(10^{\Delta_2}[MIn] + C_{In} - [MIn]\right) - \frac{1}{10^{\Delta_1}+10^{\Delta_2}} \quad (17)$$

Because the product $C_M K'_{MY}$, which is equal to $10^{\Delta_1+\Delta_2}$, has to be at least 10^4 to 10^5 to obtain a usable end point break, the last term of Equation 17 may be neglected without introducing an error in a of more than 0.1%. Furthermore, in the fourth term, [MIn] may be neglected compared to 10^{Δ_2}[MIn]. Thus, after rearranging the term involving the indicator concentration, a becomes

$$a = 1 - \left(\frac{1-y}{y}\right)\frac{1}{10^{\Delta_1}} + \left(\frac{y}{1-y}\right)\frac{1}{10^{\Delta_2}} - \frac{C_{In}}{10^{\Delta_2}C_M}\left[1 + 10^{\Delta_2}(1-y)\right] \quad (18)$$

The color change during the course of the titration depends only on the parameters Δ_1, Δ_2 and the ratio C_{In}/C_M. Furthermore, if the ratio of the concentration of indicator to the ion being titrated is 10^{-4} or less, the last term of Equation 18 contributes less than 0.1% to a and may be neglected. Under this condition, the only parameters which govern the color change curve are Δ_1 and Δ_2.

$$a = 1 - \left(\frac{1-y}{y}\right)\frac{1}{10^{\Delta_1}} + \left(\frac{y}{1-y}\right)\frac{1}{10^{\Delta_2}} \quad (19)$$

Because Δ_1 and Δ_2 occur in Equation 19 in additive terms, their influence on the color change curve is easily separated and interpreted. The term containing Δ_1 occurs with a negative sign and thus determines the sharpness of the color change prior to the equivalence point. Δ_2 determines the sharpness of the color change after the equivalence point. Or, dragging before the equivalence point is caused by insufficient stability of the metal-indicator complex and dragging after this point by insufficient displacement power of the chelon towards the metal-indicator complex.

By using Equation 19, the color change curves were calculated for various combinations of Δ_1 and Δ_2 and the results are given in Figure 2. An important fact is that these color change curves are perfectly general and, therefore, completely independent of the particular system being studied. Accordingly, if the end point indices of any system are known—e.g., from a pM-pH diagram—the color change expected during the titration can be predicted from the corresponding curves in Figure 2.

SHARPNESS OF INDICATOR END POINT

The sharpness of the indicator end point is readily evaluated from Figure 2, where the color change curves for end point indices, Δ_1 and Δ_2, are plotted (assuming the indicator concentration negligible). The larger the Δ-values, the sharper the end point. With both end point indices equal to 4, the fractional color change from 0.2 to 0.8 occurs within less than 0.1% of added titrant, which is about the maximum precision expected from most volumetric procedures. Indices larger than 4 give no practical improvement. On the other hand, for values less than 2, the end point drags appreciably. However, an end point with a low index on one side may be improved with a high index on the other side. Because Δ_1 decreases with dilution, initially high Δ_1 indicates the possibility of titrations in very dilute solutions.

The color generally need not undergo a change as wide as from 0.2 to 0.8 to be easily detected visually. The exact range of color change which the eye detects best is a complex function of the tristimulus values of the two colors involved and varies according to the metal and the indicator. With most unscreened indicators it is convenient for the observer to follow the disappearance of the color of the metal-indicator complex. The disappearance of the last tinge of this color detectable visually occurs within a certain fixed range of the color change. In using Eriochrome Black T, for example, the color becomes (to the eye) pure blue at a color change of about 0.70 to 0.85. In these instances it is usually desirable to have an asymmetrical color change curve, especially if the total pM break is small ($\Delta_1 + \Delta_2 = 5$ to 6 pM units). With a very large total pM break, asymmetry causes little improvement because the end point is already sharp. The significance of skewed color change curves has been too frequently neglected.

The sensitivity or steepness of the color change curve, dy/da, as a function of the value y of the color change may be obtained by differentiation of Equation 19.

$$\frac{dy}{da} = \left[\frac{1}{10^{\Delta_1}y^2} + \frac{1}{10^{\Delta_2}(1-y)^2}\right]^{-1} \quad (20)$$

Figure 3 shows the sensitivity of the color change for various asymmetries of color change curve for which the sum of the indices is 6 and the color change value at the equivalence point is indicated by a short vertical line. Only with the symmetrical curve do the equivalence point and the point of maximum sensitivity coincide. For the asymmetrical curves, a small error occurs whose

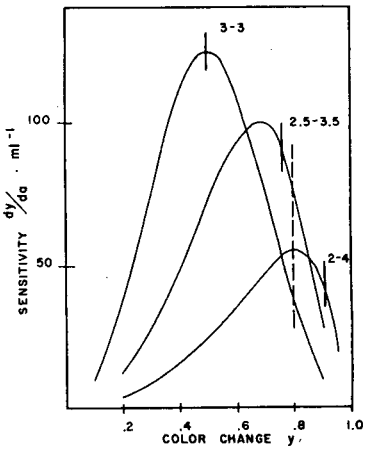


Figure 3. Sensitivity of color change

Negligible indicator concentration
Equivalence points (short vertical lines)

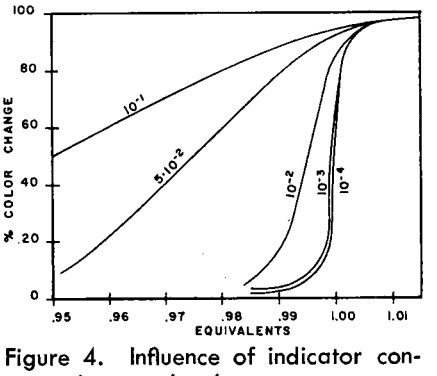


Figure 4. Influence of indicator concentration on color change curve

$\Delta_1 = 3.5$, $\Delta_2 = 3.5$

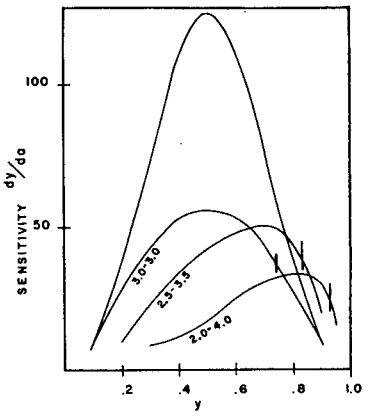


Figure 5. Sensitivity of color change with 1% relative indicator concentration

Equivalence point (short vertical lines)

value is 0.16 and 0.26% for indices of 2.5-3.5 and 2-4, respectively.

Consider an indicator where the value of 80% color change (dotted line in Figure 3) is best detected by the operator. In this case, maximum sensitivity occurs with the asymmetrical distribution 2.5 and 3.5 of the end point indices. For a symmetrical color change curve (3.0

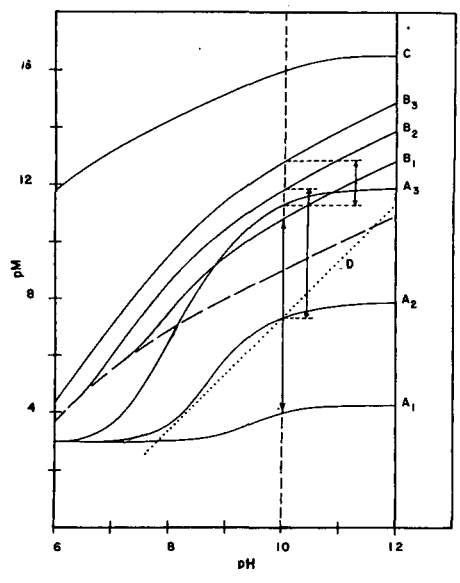


Figure 6. Effect of buffer on end point indices. Effect of ammonia on titration of $10^{-3}M$ zinc with EDTA, Eriochrome Blue Black R indicator

Subscript 1, $10^{-2}M$
2, $10^{-1}M$
3, 1 M ammonia
– – – Indicator color change in absence of ammonia
. . . Zinc hydroxide precipitation. Arrows indicate Δ_1 for three ammonia concentrations, pH 10

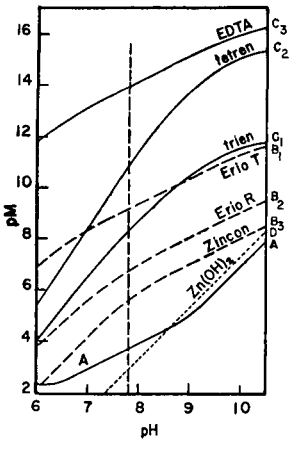


Figure 7. Matching indicator with titrant

Titration of 0.005M zinc in 0.1M triethanolamine buffer using various chelons and indicators

and 3.0), the sensitivity is much lower than when the indices are 2.0 and 4.0.

The maximum absolute—e.g., to a spectrophotometer—sensitivity is obtained with a symmetrical color change ($\Delta_1 = \Delta_2$) and at a color change of 0.5. With small end point indices (small total pM break) this point is often difficult to detect visually because of the gradual color change. However, when the indicator is perfectly screened, the color change occurs between complementary colors and because the color is gray at the 50% color change point, the equivalence point is accurately and sensitively observed.

Because the stability constants under varying titration and ionic strength conditions differ by several tenths of pK unit from the literature values, the color change curves derived from Δ-values are approximations for the color change curves encountered in an actual titration.

INFLUENCE OF INDICATOR CONCENTRATION

In very dilute metal ion solutions the concentration of the indicator may actually total as much as a few per cent of the metal ion concentration in order that a sufficient color intensity be obtained. In such cases, several drops of the titrant may actually be required to titrate the metal-indicator complex and the sharpness of the end point is appreciably decreased. For calculation of the color change curve in such cases, the last term of Equation 18 has to be considered. For practical conditions where $\Delta_2 \geq 2$, the correction term for the indicator concentration may be simplified to:

$$\gamma = (y - 1)\frac{C_{In}}{C_M}$$

The (negative!) correction term γ is added linearly to the right side of Equation 19 and is a simple function of the ratio of the indicator to metal ion concentration. The correction is illustrated in Figure 4 for the case of $\Delta_1 = \Delta_2 = 3.5$. The influence of an indicator concentration $C_{In} = 10^{-3}C_M$ is practically nondetectable in a volumetric procedure which has a precision of 0.1%.

The lowest concentration of indicator which may be used in a titration is limited by its visibility, generally about 0.5 to $2 \times 10^{-5}M$ in a 250-ml. Erlenmeyer flask. The exact minimum concentration depends on factors such as absorptivity, tristimulus value, eye perception of the operator, light source, length of light path through the titration vessel, and colored impurities. In a $10^{-2}M$ solution of sample the indicator correction may be neglected, but becomes increasingly important in more dilute solutions. In the titration of a $10^{-4}M$ solution of metal ion, an end point type which is very sharp in $10^{-2}M$ solution drags considerably because C_{In} is as large as 0.1 C_M. Under such circumstances the end point sharpness depends very little on the end point indices.

The indicator concentration is also a significant factor in the titration of small volumes because, to compensate for the small length of light path, unusually high concentrations of indicator may have been employed.

The influence of the indicator concentration on the sensitivity of the color change is illustrated in Figure 5. The top curve shows the sensitivity with negligible indicator concentration for the symmetrical case 3 and 3 (as Figure 3). The bottom family of curves represents the sensitivity for various end point indices in the presence of 1% of indicator ($C_{In} = 0.01C_M$) and illustrates the leveling of the sensitivity caused by the presence of high indicator concentration. Also the difference between the point of maximum color change and the equivalence point (vertical lines in the diagram) is larger when the amount of indicator is high. The differences are 0.50, 0.47, and 0.43 for the cases 3-3, 2.5-3.5, and 2-4,respectively.

TITRATION CURVES IN PRESENCE OF PRECIPITATE

From equilibrium considerations, the chelometric titration of a precipitated metal ion is possible in many cases. Although equilibrium is usually reached slowly in a titration which involves a precipitate, and such procedures are generally avoided, it is of interest to discuss briefly the color change curves in such titrations.

In alkaline solution, most metal ions precipitate as hydroxides,

$$M + xOH \rightleftharpoons \underline{M(OH)_x} \qquad (21)$$

$$K_{M(OH)x} = \frac{[H]^x}{[M]K_w^x} \qquad (22)$$

For a solution in equilibrium with a hydroxide precipitate a lower limit is imposed on the pM according to

$$pM_D = \log K_{M(OH)_x} - xpK_w + xpH \qquad (23)$$

In pM-pH diagrams this pM_D limit is represented by a line D (Figure 1). During the titration the pM value remains constant until all the hydroxide is dissolved, then the pM follows the titration curve which would have been obtained had no hydrolysis taken place. This is illustrated in Figure 1 for the titration of magnesium where line D is determined by the formation of magnesium hydroxide. At pH 11, the initial pM is equal to 3.9 and magnesium hydroxide is precipitated (provided the solution is more concentrated than $10^{-3.9}M$). It follows from Figure 1 (right), that a reasonable color change still may be obtained if the titration is carried out slowly enough to ensure equilibrium. At a pH of 12.5, however, the initial pM would be 7 and the magnesium is practically completely masked.

The color change of the indicator remains at a constant value during titration (until the precipitate disappears) as

may be calculated from Equation 4 after inserting the solubility product of the precipitate. However, in practice the dye frequently forms a lake with the precipitate and the total color will be different.

EFFECT OF BUFFER AND COMPLEXING AGENTS

Buffers are employed in chelometric titrations, not only to keep the pH constant, but also to prevent hydrolysis and subsequent precipitation of the metal ion. In cases where the buffer does not prevent precipitation, an additional auxiliary complexing agent is added to the solution (addition of tartrate for the titration of lead in ammonia buffer). An ideal buffer should have a complexing power just sufficient to keep the metal ion in solution, thereby necessitating only a minimum decrease in the initial metal ion activity.

The complexing properties of buffers may affect the pM values to a considerable extent, not only at the beginning of the titration, but also at the color change and past the end point.

For a theoretical estimation of the end point sharpness under these circumstances, the pM-pH plot must be constructed not from Equations 11, 12, and 13 but from Equations 27, 30, and 33 which take into account the various effects of the buffer.

Effect on pM_A. Metal ions in the presence of a complexing species, X, undergo the general reaction

$$M + mX \rightleftharpoons MX_m \qquad (24)$$

the corresponding equilibrium constant being

$$K_{MX_m} = \frac{[MX_m]}{[M]\,[X]^m} \qquad (25)$$

The initial concentration of the free (hydrated) metal ion in the presence of the complexing species, X, may be represented by

$$M = \frac{C_M}{\beta_{MX}} \qquad (26)$$

where β_{MX} is a factor depending on pH, acidity constants of species X, the complex constant K_{MX_m}, and concentration of the complexing species. In the absence of complexing agents, β_{MX} is equal to unity. In the presence of a buffer the initial pM is:

$$pM_A = -\log C_M + \log \beta_{MX} \qquad (27)$$

Effect of pM_B. In certain cases, a mixed complex between metal ion, indicator, and buffer may form

$$M + In + X \rightleftharpoons MInX \qquad (28)$$

and

$$K_{MInX} = \frac{[MInX]}{[M]\,[In]\,[X]} \qquad (29)$$

a compound of this type is $ZnInNH_3$, with In = Eriochrome Black T (CI 203) or Eriochrome Blue Black R (CI 202). In such a case the color change occurs at a higher pM value

$$pM_B = \log \frac{K_{MInX}}{\alpha_{In}\alpha_X} + \log [X] \qquad (30)$$

where α_X takes the acid-base properties of the buffer into account and is defined by Equation 7.

Effect of pM_C. Mixed complexes between metal ion, chelon, and another complexing ligand have been observed in several cases.

$$M + Y + X \rightleftharpoons MYX \qquad (31)$$

$$K_{MYX} = \frac{[MYX]}{[M]\,[Y]\,[X]} \qquad (32)$$

The stability of such mixed complexes is larger than the stability of the simple metal chelonate and as a result a larger pM break is obtained. The pM at 100% past the end point then becomes

$$pM_C = \log K_{MYX} + \log X + \log \alpha_Y \alpha_X \qquad (33)$$

Mixed complexes of this type are frequently formed by the EDTA complexes of mercury(II) and cobalt(III)—$HgYNH_3$, $HgYNH_2CH_2CH_2NH_2$, HgYPy (Py = pyridine), HgYCl, CoYCNS (*10, 15, 17, 18*).

Titration of Zinc in Ammonia Buffer. The influence of various concentrations of ammonia on the titration of zinc using Eriochrome Blue Black R (CI 202) is shown in Figure 6.

In the initial solution zinc is complexed by ammonia and a mixture of zinc ammine complexes $Zn(NH_3)^{++}$, $Zn(NH_3)_2^{++}$, $Zn(NH_3)_3^{++}$, and $Zn(NH_3)_4^{++}$ exists. The pM may be obtained from Equation 27 with

$$\beta_{MX} = 1 + K_1\left(\frac{C_X k_a}{k_a + [H^+]}\right) + K_1K_2\left(\frac{C_X k_a}{k_a + [H^+]}\right)^2 + K_1K_2K_3\left(\frac{C_X k_a}{k_a + [H^+]}\right)^3 + K_1K_2K_3K_4\left(\frac{C_X k_a}{k_a + [H^+]}\right)^4$$

where K_1 ($10^{2.2}$), K_2($10^{2.3}$), K_3 ($10^{2.3}$), and K_4 ($10^{2.0}$) are the stability constants of stepwise complex formation (*1*), k_a, is the acidity constant of ammonia, and C_X the total ammonia concentration. The graph shows that the initial pM_A increases greatly with increasing ammonia concentration (lines A_1, A_2, and A_3).

The pM_B for 50% color change of the indicator Eriochrome Blue Black R, $\log K_{ZnIn} = 12.5$ (*5*), in the absence of ammonia is shown as a dotted line in Figure 6. In the presence of ammonia the indicator forms the complex $ZnInNH_3$, $\log K_{ZnInNH_3} = 16.4$ (*6*), and the corresponding pM_B is given by Equation 30. The lines B_1, B_2, and B_3 represent the 50% color change at various ammonia concentrations. Because the zinc-EDTA complex does not react with ammonia, line *C* remains unaltered and Equation 13 applies.

The effect of the ammonia concentration on the end point sharpness of this titration may be evaluated by calculating the pM difference between the appropriate curves of Figure 6. At pH 10 the following values for the end point indices are obtained:

Ammonia Concentration (*M*)	$\Delta_1 - \Delta_2$
0	6.0–7.0
0.01	6.8–5.2
0.1	4.5–4.1
1.0	1.6–3.1

The data show that excessive concentrations of ammonia cause a strong decrease of the end point sharpness. On the other hand, line *D* indicates that at least about 0.1*M* ammonia should be present to prevent hydroxide precipitation. Although the titration with less ammonia buffer would give higher end point indices, this condition is not desirable because of the presence of precpitate.

If no mixed indicator complex would form, 0.1*M* ammonia would have given a poor end point ($\Delta_1 = 1.6$) and 1*M* ammonia would actually have displaced the zinc from the indicator.

COMPARISON OF END POINTS WITH VARIOUS CHELONS AND INDICATORS

The quality of an end point in the titration of a given metal ion depends both on the indicator and the chelon. A given indicator may yield excellent results with one titrant but poor results with another. Yet a second indicator may give good results with both titrants.

By a pM-pH diagram, the properties of an end point with different chelons and indicators may be readily compared *a priori*. In Figure 7 the characteristic lines *A*, *B*, *C*, and *D* are given for the titration of zinc in triethanolamine buffer with the chelons—EDTA, tetraethylenepentamine (tetren) [$\log K_{ZnY} = 15.4$ (*9*), $pk_3 = 7.9$, $pk_4 = 9.1$, $pk_5 = 9.9$ (*7*)] and triethylenetetramine (trien). The $\log K_{Zn(OH)_2} = 15.4$ for calculating line *D* was obtained from potential pH measurements with the third class electrode system: $Hg|HgEGTA^{-2}$, $ZnEGTA^{-2}$, Zn^{+2}.

The lines of 50% color change (*B*) apply to the indicators Eriochrome Black T ($\log K_{ZnIn} = 13.0$), Eriochrome Blue Black R, and zincon. For a pH of 7.8, the optimal buffer region of triethenolamine, the end point indices read from the diagram are as listed below. From a consideration of the end point indices, the behavior noted in Table I was expected. Experimental experience is in agreement with the end point properties predicted from the diagram.

TITRATION OF MIXTURE OF CHELONS

Curves for the titration of mixtures of chelons with a given metal ion titrant,

M, and the optimum conditions for selective titrations may be readily evaluated from pM-pH diagrams. Because the metal ion is used as a titrant, the pM value would be infinity at the beginning of the titration and would then drop as the titration proceeds.

Consider the titration of a mixture of EDTA and tetren with zinc ions, the course of the titration being followed by a pZn electrode. The principles involved are illustrated by Figure 7. At pH 10, for example, the pM starting at infinity would drop as the titration proceeds and a pM buffering region would occur at the pM values in the region of the lines C (pM 15 to 16) for EDTA and tetren with no clear pM break between them. Finally, after the titration of the sum of EDTA and tetren, a distinct pM break of about 7 units would occur.

At pH 6, however, EDTA could be titrated selectively in the presence of tetren with a pM break of about 6 units. This theoretical consideration shows that a selective visual titration of EDTA at pH 6 would be feasible if an indicator of suitable stability with zinc (color change at pM 9) could be found.

Table I. Prediction of End Point Sharpness for Chelometric Titration of Zinc (pH 7.8)

Indicator	Titrant	Δ_1	Δ_2	Comments and Expected Behavior
Erio T	EDTA	5.4	4.8	Very sharp
	tetren	5.4	1.7	Dragging after end point, titrate until first red tinge appears
	trien	5.4	−0.8	No end point
Erio R	EDTA	3.0	7.2	Sharp; titrate until last blue tinge disappears
	tetren	3.0	4.1	Sharp; titrate until last blue tinge disappears
	trien	3.0	1.5	Poor; dragging before and after end point
Zincon	EDTA	1.9	8.4	Dragging before end point; titrate until pure yellow
	tetren	1.9	5.3	Dragging before end point, titrate until pure yellow
	trien	1.9	2.7	Poor; dragging before and after end point

pY-pH DIAGRAMS

pM-pH diagrams are useful in evaluating the effect of factors such as pH, buffer, indicator, and chelon on the titration curve of a single metal ion. However, where more than one metal ion is present (such as titration of multicomponent mixtures, substitution titrations, sensitization of an indicator for the titration of one metal ion by addition of a second activating metal ion, and masking of metal ions) information concerning the relative influence of the competitive equilibria may be more clearly elucidated from a pY-pH plot.

The reason for this is general. The titration curve in any titration may be represented by a plot of p(titrate) or of p(titrant) *vs.* the titrant volume. For instance, the titration of acetate with acid may be plotted with p(acetate) or pH as the ordinate. Although there are no basic differences or theoretical advantages between the two presentations, a pH plot is commonly made because of its more general applicability and ease of measurement.

In the titration of mixtures, the situation is more complicated and the choice of presentation is important. In a plot of p(titrate), the number of breaks appearing on the graph depends on which presentation is employed and also, for the p(titrate) plot, on which titrated species is selected as the master variable. For example, in the titration of a mixture of acetate ion and ammonia with acid as titrant, one break will be obtained in a p(acetate) plot whereas two will be obtained in a p(ammonia) or pH plot. The full number of breaks is always obtained in a plot of the titrated species which reacts first with the titrant; this is actually the basis of the use of a mercury electrode in chelometric titrations (*12*).

However, a p(titrant) plot is much more general because this type function exhibits the corresponding breaks. This technique is generally adopted in acid and base titrations because, in the titration of mixtures of acids with alkali as titrant, a pH plot is equivalent to a p(titrant) plot because the p(titrant), pOH, is equal to $pK_w - pH$.

The titration curve of a single metal ion may be suitably represented in a pM plot; in contrast, for a titration involving more than one metal ion, the breaks and their correct sequence are best represented in a p(titrant)—pY— plot. The reverse case, a titration of several chelating agents with one metal ion, is best illustrated by a pM plot which corresponds in this case to a p(titrant) plot, an example of which has been described.

Because the titrant species may exist in several forms—i.e., complexed, hydrated—depending on the solution conditions, the use of the ultimate (or effective) titrant as the master variable is more general and offers the real basis for a unified treatment of the titration curve. By the ultimate titrant is meant that ingredient which actually limits the conversion of titrate species from the form present before to the form present after titration. Because the ultimate titrant species is usually present in a combined form, a measure of its true activity usually is not possible and consequently some practical parameter for its measurement is substituted.

CONSTRUCTION OF pY-pH DIAGRAMS

Line A cannot, as in the case of pM graphs, be represented by the initial condition of the sample because the pY here is infinity. The system prior to the end point is therefore characterized by the pY at half-titration:

$$pY_A = \log K_{MY} - \log \beta_{MX} \quad (34)$$

The pY_B at 50% color change point of the indicator is obtained by combination of Equations 3 and 12. The term [MY] is set equal to C_M because, under practical titration conditions, the color change should occur very near to this stoichiometric point.

$$pY_B = \log K_{MY} + \log \alpha_{In} - \log C_M - \log K_{MIn} \quad (35)$$

Line C represents the point in the titration 100% past the end point. Here [Y] is equal to C_M and

$$pY_C = \log \alpha_Y - \log C_M \quad (36)$$

The end point indices, Δ_1 and Δ_2, for characterization of the color change sharpness may be obtained in the same way from pY-pH diagrams as from pM-pH plots.

$$\Delta_1 = pM_B - pM_A = pY_A - pY_B \quad (37)$$

$$\Delta_2 = pM_C - pM_B = pY_B - pY_C \quad (38)$$

In a pY-pH diagram, only line A is independent of the metal ion concentration, lines B and C depending on the metal ion concentration C_M. This dependency is just opposite to that existing in pM-pH diagrams.

SELECTIVE TITRATIONS

Selectivity in the chelometric titration of a mixture of metal ions is of considerable importance and pY-pH diagrams offer an excellent unified basis for judging the degree of selectivity and for elucidating the influence of the various factors which govern it. One usual method of selective titrations employs the inherent differences between the stability constants of the various metal chelonates. When the difference is sufficient, the metal ion which forms the more stable chelonate will be titrated first, and a pY and pM break occur before the second metal ion reacts to any practical extent with the titrant. Such end points often are easily detected with physical methods such as potentiometry, amperometry, and spectrophotometry.

For visual end point indication using metallochromic indicators, certain additional requirements must be fulfilled. First, the stability of the indicator complex of the metal ion to be titrated must be of a rather specified nature. Second, the indicator should not form, under the titration conditions employed, a complex with the other metal ions.

These conditions are outlined on the pY diagram, Figure 8, for the titration of zinc in the presence of calcium or magnesium with EDTA using pyridine buffer pH 6. Figure 8, left, shows the characteristic lines A, B, and C as defined in the previous section. The pyridine zinc complex is so weak that line A remains practically unaltered; the curvature of line A near pH 8 is caused by the hydrolysis of the zinc ion. The indicator, 7-(4-sulfo-1-naphthylazo)-8-quinolinol-5-sulfonic acid [log K_{ZnIn} = 6.9, pk_1 = 3.0, pk_2 = 7.0 (*13*)], forms a complex with zinc above pH 3.5 (the intersection of lines A and B) but no appreciable complex formation occurs with the alkaline earth ions at pH 6 (*13*). The metal-EDTA complexes are stable at pH values above the intersection points of the lines A and C. The pY titration curve for the titration of a zinc solution (pH 6) in the absence of alkaline earth ions is shown as curve I on the right side of Figure 8 with the color change indicated by the shaded area. The end point indices obtained from the pY-pH diagram are

$$\Delta_1 = pY_{A,Zn} - pY_B = 2.9$$

and

$$\Delta_2 = pY_B - pY_C = 5.9$$

indicating a satisfactory titration condition according to Figure 2.

The end point indices at other pH values can be ascertained from Figure 8 and the expected color change curves obtained from Figure 2. For the titration of zinc alone, the sharpest color change curve should be obtained near pH 7. In the presence of calcium, pH 6 gives better results.

The pY titration curve in the presence of an equal amount of calcium (pH 6) is illustrated as line II on the right side of Figure 8. The end point break for the titration of the zinc occurs between the lines A for zinc and A for calcium. After the titration of calcium only a very small pY break occurs, between line A for calcium and line C. The end point index Δ_1, in the zinc titration, does not change by the addition of calcium.

The second end point index Δ_2 is now obtained as the difference between pY_B and $pY_{A,Ca}$ and is equal to 2.9, considerably lower than the Δ_2 obtained in the absence of calcium ion. The titration of zinc in the presence of calcium ion is nevertheless possible although the end point will not be so sharp (see Figure 2).

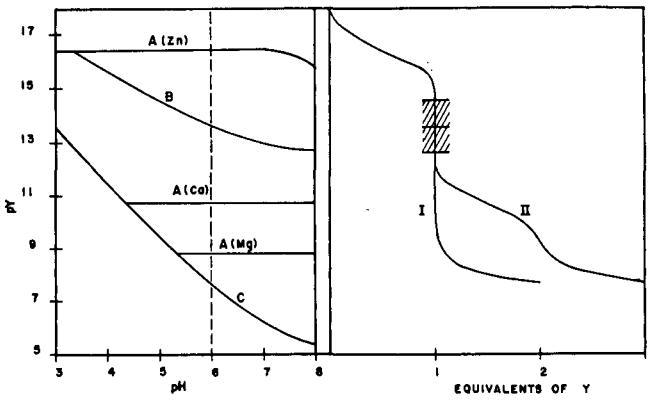


Figure 8. Selective titration of zinc ($10^{-3}M$) in presence of equal amount of calcium

Pyridine buffer, EDTA titrant, indicator: 7-(4-sulfo-1-naphthylazo)-8-quinolinol-5-sulfonic acid

From the pY-pH diagram the choice of pH 6 was made to yield a symmetrical color change curve ($\Delta_1 = \Delta_2$) because the maximum over-all break ($\Delta_1 + \Delta_2$) is constant over the pH range from 4.5 to 7. Line C indicates pY at 100% past the end point in a titration of zinc alone while the lines Ca and Mg indicate the pY at 50% past the end point in a titration in the presence of an equimolar amount of one of these alkaline earth ions.

The sharpness of the end point depends on the concentration of the alkaline earth ions present in solution. This effect also may be evaluated from a pY-pH diagram. In the presence of a molar concentration of magnesium ion, tenfold greater than that of zinc, line Mg in Figure 8 is shifted upwards by one pY unit. A hundredfold excess would shift the Mg line up two units and the sharpness of the zinc titration in this case ($\Delta_1 = 2.9$, $\Delta_2 = 2.8$) would be comparable to that for zinc in the presence of an equimolar quantity of calcium ($\Delta_1 = 2.9$, $\Delta_2 = 2.9$). Concentrations of calcium much greater than that of zinc produce drawn-out color changes beyond the end point—e.g., Δ_2 is small.

MASKING

The selective titration of a metal ion, M, in a mixture is possible (disregarding rate effects) only when the effective stabilities of the chelonates of the other metal ions are sufficiently lower than that of M. By adding suitable quantities of appropriate masking agents, the required difference in the effective stability constants, and thus selectivity, can often be achieved.

Generally, a given masking agent is effective for the desired purpose only under specified conditions of pH and then only with a given metal indicator and a given titrant. The best choice of these variables has usually been evaluated from many trial-and-error experiments. However, the behavior of a masking agent under various conditions may be predicted from a theoretical consideration of the equilibria involved and other applications become apparent. This theoretical approach is greatly simplified and the results more easily visualized when the various effective equilibria are plotted in a pY-pH diagram. The optimal set of conditions (such as choice of indicator, titrant, pH, buffer) may then be read from the diagram as well as the advantages and limitations of a masking agent.

As an example, consider the application of tetraethylenepentamine (tetren) as a masking agent. Figure 9 shows the pY-pH diagram for titrations with EDTA in the presence of $0.1M$ tetren. The effect of the masking agent is perfectly analogous to the complex effect and is treated identically. pY_A, the half-titration point, is expressed by Equation 34, the factor β_{MX} taking into account the complexing effect of the masking agent X. Tetren forms 1 to 1 complexes with metal ions (*9*) and the term log β_{MX}, which must be subtracted from the horizontal lines representing the stability (log K_{MX}) of EDTA complexes of the metal, is equal to log K_{MX} − log α_X + log C_X. The diagram indicates that mercury(II), copper, nickel, and zinc are masked in the alkaline region (pH 10), because their curves (dashed lines) drop below pY_C, the pY value beyond the end point. Cadmium also is masked, but less effectively. At this pH, lead, calcium, and magnesium may be titrated in the presence of those other metal ions.

The diagram also reveals the masking properties of tetren at other pH values. In acid solution (pH 5) only copper and mercury are masked, and a titration of lead is possible in the presence of copper, mercury, magnesium, and small quantities of calcium. The titration of zinc, nickel, or cadmium in the presence of copper and mercury at pH 5 is less favorable. Triethylenetetramine (trien) is superior to tetraethylenepentamine as a

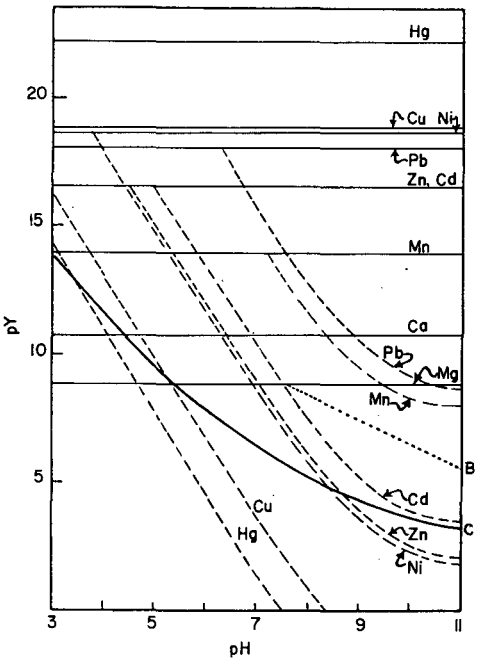


Figure 9. Masking with tetraethylenepentamine

pY-pH diagram for EDTA titration of $10^{-3}M$ metal ions in presence of 0.1 M tetraethylenepentamine
– – – Effect of masking agent (formation of hydrogen complexes neglected)
. . . Color change of magnesium–Eriochrome T complex
— (Horizontal) values of log K_{M-EDTA}

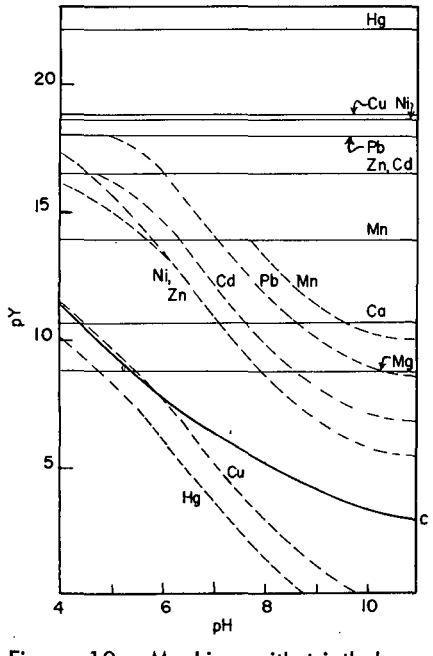


Figure 10. Masking with triethylenetetramine

pY-pH diagram for titration of $10^{-3}M$ metal ions in presence of 0.1 M triethylenetetramine
– – – Effect of masking agent (formation of hydrogen complexes taken into account)

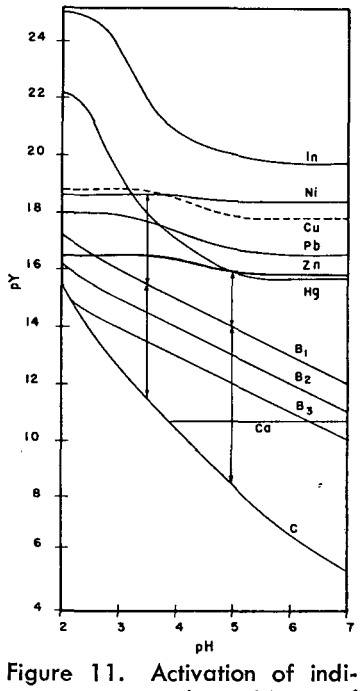


Figure 11. Activation of indicator response by addition of second metal ion

EDTA titrations of $10^{-2}M$ metal ion solutions in 0.1 M acetate buffer using PAN indicator
B_1 $10^{-4}M$ copper present
B_2 $10^{-3}M$
B_3 $10^{-2}M$

masking agent for this purpose (Figure 10).

Although a metal ion is masked in respect to the titrant, it is not necessarily masked in respect to the indicator. In selective titrations where metallochromic indicators are employed, the indicator must combine only with the metal ion to be titrated. This point may be checked by comparing the effective stability constants of the metal-indicator complexes in the presence of the masking agent or, as is often better, by a quick experiment.

The existence of mixed complexes MInX (where X is the masking agent) complicates the situation. For example, the red nickel–Eriochrome Black T complex (which does not react with EDTA) turns more violet upon the addition of tetren. This mixed complex also does not react with EDTA and the indicator is blocked by nickel even in the presence of tetren. Thus the titration of magnesium in the presence of nickel, where the latter is masked with tetren, is not possible using Eriochrome Black T. With metalphthalein, however, the titration is possible.

The sharpness of an indicator end point in the presence of a masking agent may be evaluated from the pY-pH diagram in the usual way. The curve, for example, corresponding to the color change of magnesium–Eriochrome Black T is shown as a dotted line in Figure 9.

Some experimental results using polyamines as masking agents are shown in Table II. About 1 ml. of the commercial polyamine (Union Carbide Chemicals Co.) was added to the sample (20 ml.) for titrations in alkaline medium. For titrations in acid solution, the pH of the polyamine was adjusted to about 5 prior to the addition of the sample. The polyamine serves also as a buffer in these cases. The titrations were carried out with 0.01*M* EDTA. The amount of nickel, copper, and cobalt masked by polyamines is limited in a practical sense because the intense color of the corresponding polyamine complexes obscures the color change of the indicator.

The horizontal solid lines in Figures 9 and 10 represent the log K_{MY} values of the metal-EDTA chelonates. The actual pY_A values are equal to these log K_{MY} values only when the effects of dissociation of MY by acid, formation of metal-chelonate derivatives, hydrolysis of metal ion, and complexation of metal ion by buffer or masking agents are negligible. For example, the pH effect causes the pY_A to increase and blend into line *C* at lower pH values, but has no effect on pY_A at pH values above those where pY_A and *C* intersect.

Because pY-pH diagrams predict the reaction sequence of metal ions with titrant, they can be utilized for establishing conditions for selective photometric titrations. At pH 10, calcium (also manganese, lead, and magnesium) can be selectively titrated with EDTA in the presence of nickel, mercury, and copper, provided tetren is added as a partial masking agent. After the calcium reaction, nickel (but not copper or mercury) will react. At pH 7, lead (or manganese) may be selectively titrated in the presence of copper, calcium, mercury, and nickel. The calcium and nickel will cotitrate in the next phase of the titration without interference from copper or mercury. A number of other permutations using different partial masking agents, pH values, and chelon titrants extend the scope of this approach.

SENSITIZATION OF METALLOCHROMIC INDICATOR WITH ANOTHER METAL ION

The titration of a metal ion, M_1, which forms too weak a complex with an indicator may often be achieved, with a

Table II. Masking with Polyamines

Ion Titrated	Indicator	Condition, pH	Ion Masked, Mmole	Taken, Mmole	Found, Mmole
		Tetraethylenepentamine, approx. 0.1*M*			
Mg(II)	Erio T	NH_3, 10	0.1 Hg(II)	0.1005	0.1008
			1.0		0.1003
			0.1 Zn(II)		0.1004
			1.0		0.1001
			0.1 Cu(II)		0.1008
			0.1 Cd(II)		0.1001
			0.1 Cd(II)		0.1007
			0.1 Ni(II)		[a]
			0.1 Co(II)		[a]
Mg(II)	Metalphthalein	NH_3, 10	0.1 Ni(II)	0.1005	0.102[b]
			0.1 Co(II)		0.102[b]
					0.103[b]
Ba(II)	Methyl thymol blue	NaOH, 12	0.1 Cd(II)	0.0985	0.0983
			0.1 Zn(II)		0.0983
			1.0		0.98[c]
			0.1 Co(II)		0.098[c]
Pb(II)	Methyl thymol blue	NH_3, 12 + tartrate	0.1 Ni(II)	0.0540	0.0545
			0.1 Hg(II)	0.0952	0.0954
			0.1 Zn(II)		0.0953
		Triethylenetetramine, approx. 0.1*M*			
Pb(II)	Xylenol orange	Hexamethylenetetramine, 5	0.1 Hg(II)	0.0952	0.0950
			0.05 Cu(II)	0.0540	0.0545
Zn(II)	Xylenol orange	Hexamethylenetetramine, 5	0.1 Hg(II)	0.1004	0.1008

[a] Indicator is blocked.
[b] Poor color change.
[c] Indistinct end point.

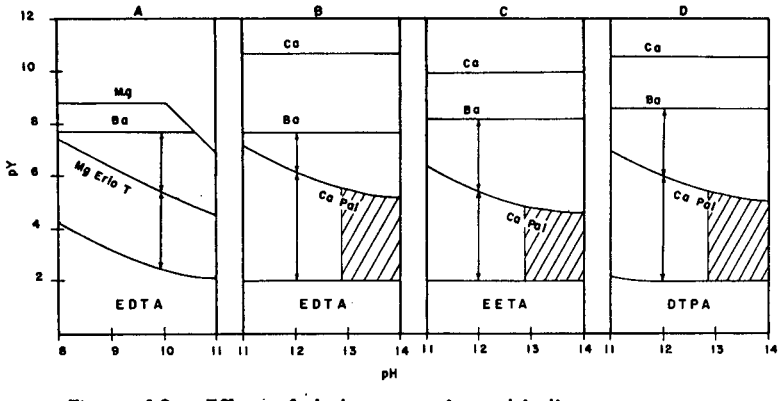


Figure 12. Effect of chelon on activated indicator response

A. $\Delta_1 = 2.4, \Delta_2 = 3.0$
B. $\Delta_1 = 1.6, \Delta_2 = 4.1$
C. $\Delta_1 = 2.8, \Delta_2 = 3.4$
D. $\Delta_1 = 2.6, \Delta_2 = 4.0$

very good end point, by adding a small amount of a second metal ion M_2 (or its chelonate). The metal ion M_2 is one which by itself gives a good color change in a titration with this indicator. Examples are the titration of heavy metal ions with PAN [1-(2-pyridylazo)-2-naphthol] or 1-naphthyl azoxine in the presence of a few drops of copper-EDTA solution and the titration of calcium with Eriochrome Black T after addition of a few per cent of magnesium-EDTA.

A basic requirement is that the reaction

$$M_1 + In + M_2Y \rightarrow M_1Y + M_2In \quad (39)$$

takes place extensively in the direction as written. This reaction may occur in the desired direction even if the chelonate M_2Y is more stable than M_1Y because the free energy change of the over-all Reaction 39 involves also the formation of the stable indicator complex, M_2In. At the end point the indicator is liberated from M_2In as Reaction 39 is reversed.

The quality of the end point depends strongly on the exact ease with which the reaction is reversed. This in turn depends on the concentration of the activating metal ion M_2 (or its chelonate), on the stability constants involved, and the pH. These factors are discussed using the copper-PAN and and calcium–Palatine Fast Blue GGNA (Pr 144) systems as examples. The same principles may be applied for the selection and development of new indicator systems of this type (*11*).

Figure 11 shows the pY_A curves corresponding to the half-titration point for a number of metal ions in acetate buffer [log $K_{Hg(OAc)_2}$ = 8.5]. The dotted line represents copper. The lines B_1, B_2, and B_3 designate pY_B for 50% color change of the copper-PAN indicator system for three concentrations of copper (10^{-4}, 10^{-3}, and 10^{-2}, respectively). For PAN, log K_{CuPAN} = 16.0 (20% dioxane), pk_1 = 1.9 and pk_2 = 12.2 according to Pease and Williams (*8*). The end point sharpness in the titration of a $10^{-2}M$ solution of copper in 0.1*M* acetate at different pH values may be evaluated from the curves, Cu, B_3, and *C* exactly as explained in the previous section. At pH 5, the end point indices 5.9 and 3.5 indicate a very sharp color change which occurs in practice.

The other metal ions listed in Figure 11 give with PAN no or such weak complexes that the copper-PAN complex is formed preferentially (Reaction 39).

The characterization of the end point sharpness for these metal ions using copper-PAN is more complicated than for the titration of copper alone and the determination and use of the end point index Δ_1 involve certain reservations. As a first approximation, Δ_1 may be chosen as the difference between the lower pY_A line of the metal ions involved and the indicator line, pY_B, corresponding to the particular concentration of the activating metal ion (copper). For titration of 0.01*M* lead in the presence of $10^{-4}M$ copper, Δ_1 is taken as the pY difference between the lines Pb and B_1. For an analogous titration of indium, Δ_1 is taken as the pY difference between the lines Cu and B_1.

The error introduced by this choice depends on the relative concentrations and relative effective chelonate stability constants of the titrated and the activating metal ion. A rigorous treatment would be more complicated than desirable for the scope of this publication. However, an end point index Δ_1 so determined represents the least sharpness to be expected and the actual end point sharpness may be slightly better.

Effect of pH. Figure 11 shows that for the titration of nickel in the presence of $10^{-4}M$ copper, sharp end points will be obtained at pH values of 3.5 (end point indices 3.1 and 4.0) and above. At lower pH values the end point will become sluggish. For zinc, the minimum pH under the same conditions is about 5 (end point indices 1.9 and 5.5).

Effect of Concentration of Activating Metal Ion. The concentration of the activating metal ion—with constant

concentration of the indicator—may have a pronounced effect on the end point. When the copper concentration of $10^{-3}M$ (instead of 10^{-4}) is employed (line B_2) for the titration of $10^{-2}M$ zinc, the end point is considerably improved. At pH 5 this corresponds to a change in the end point indices from 1.9 and 5.5 to 2.9 and 4.5. At pH 4.5, the end point would be sluggish with $10^{-4}M$ copper present (1.6 and 5.0); with $10^{-3}M$ copper, the end point is improved (2.6 and 4.0). A further tenfold increase in the copper concentration ($10^{-2}M$) would not yield any worthwhile improvement (3.6 and 3.0). In the titration of nickel, an increase of the copper concentration from 10^{-4} to $10^{-3}M$, does not improve the end point. These considerations show that the optimum amount of copper-EDTA to be added to the sample depends upon several factors and that the use of a prepared copper-PAN complex where the amount of copper is fixed is not recommended.

Effect of Chelon. At pH 12 the indicator Palatine Fast Blue GGNA (Prototype No. 144) gives an exceptionally sharp color change in the titration of calcium with EDTA (sharpness 4.6 and 4.1 in $0.01M$ solution). For this indicator, log $K_{CaIn} = 7.5$, $pk_1 = 8.9$, $pk_2 = 12.9$ (*6*). A pY-pH diagram for this system is given in Figure 12*B*. This end point is sharper than that of the magnesium–Eriochrome Black T system (Figure 12, *A*) (3.4 and 3.0 in $0.01M$ in solution). It might appear that the calcium–Palatine Fast Blue system offers advantages for end point indication in the titration of barium over the customary titration of barium employing an equimolar amount of magnesium-EDTA and Eriochrome Black T (at pH 10). However, very poor end points are obtained.

This paradoxical behavior is readily interpreted using Figure 12, *A* and *B*. The sharpness of the barium titration with the magnesium–Eriochrome Black T system is 2.4 and 3.0, and with the calcium–Palatine Fast Blue system 1.6 and 4.1. In the second case the color change drags before the end point although the metal indicator complex employed is stronger.

Application of a different chelon represents a frequently overlooked but important parameter which will often greatly improve the end point. Either or both of two conditions must exist. In the barium titration, the barium complex of the selected chelon should be more stable than that of EDTA (line Ba rises) or the calcium complex of the selected chelon should be weaker (lines Ca and Ca-Pal drop). With both effects present, the end point will improve even further.

Figure 12, *C* and *D*, show the diagrams for the chelons β,β-diamino diethyl ether-*N*,*N*′-tetraacetic acid (EETA) and diethylenetriaminepentaacetic acid (DTPA) where the end point sharpness is 2.8 and 3.4 and 2.6 and 4.0, respectively. A successful titration method for barium (and for sulfate indirectly) was developed from these considerations (*6*). For DPTA, log $K_{CaY} = 10.6$, log $K_{BaY} = 8.6$, $pk_5 = 10.5$ (*14*, *19*).

The shaded areas on Figure 12 indicate the region where Palatine Fast Blue turns pink. Because the calcium complex is also pink, a useful visual end point cannot be observed at high pH although the values for the end point index are good.

EXTENSIONS

Overlooked Equilibria. Although metal-indicator and metal-dye complexes of the simple type MIn and MY, are the ones most frequently encountered in chelometry, other complexes exist. With chelons, complexes such as MHY and MH_2Y form in acid solution, MYOH and $MY(OH)_2$ in alkaline solution. In addition many currently employed metallochromic indicators form complexes with metal ions in a ratio other than 1 to 1. For example, pyrocatechol violet and the phthalein and sulfophthalein derivatives (metalphthalein, methyl thymol blue, and xylenol orange) form the complexes M_2In, MHIn, and MIn; some dyes may form complexes of the type MIn_2. For such instances, the equations given here for the construction of the diagrams have to be correspondingly altered and extended.

Acid-Base Titrations. The considerations concerning the sharpness of an indicator color change may also be applied for color changes in pH titrations. The color change curves may be obtained from Figure 2 with the help of the end point indices, Δ_1 and Δ_2 evaluated as the differences between characteristic points in the pH titration. The situation is much simpler than in the case of metal ion titrations.

At half-titration, the pH_A is equal to the pK_a of the titrated base

$$pH_A = pK_a \quad (40)$$

The indicator color change occurs at

$$pH_B = pK_{In} \quad (41)$$

at 100% past the end point, the characteristic point is

$$pH_C = -\log C \quad (42)$$

where C is the total concentration of the titrated species. Thus:

$$\Delta_1 = pH_A - pH_B = pK_a - pK_{In} \quad (43)$$

$$\Delta_2 = pH_B - pH_C = pK_{In} + \log C \quad (44)$$

Similar relationships can be obtained for multicomponent and successive titrations.

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RECEIVED for review July 28, 1958. Accepted December 23, 1958. Work supported by The Research Corp.

Trace Determination of Copper and Cobalt

By Chelate Chromatography and Dithizone Photometry

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▶ **The work reported is an outgrowth of a study in extending the applicability of the chromatographic adsorption analysis of metal chelates to trace analysis. Several metal diethyldithiocarbamates have been separated on an activated silica adsorbent. After chromatographic separation the dithiocarbamates are converted to the corresponding diphenylthiocarbazone (dithizone) complexes and determined photometrically. A photometric dithizone method for cobalt has been developed to be used in conjunction with appropriate separatory techniques. Practical separations of microgram quantities of copper and cobalt from several important interferences have been effected for both synthetic and natural samples. Apparatus requirements are easily met and the procedure is not unduly complex.**

In recent years a number of applications of chelating agents in chromatographic separations have been reported. In general, however, these applications have been of a qualitative nature, and difficulties of spot evaluation (in paper chromatography) and band overlap (in column separations) have minimized the quantitative applications.

In 1943, Erämetsä (*7*) reported the use of an organic reagent in the chromatographic adsorption analysis of metallic cations. Several diphenylthiocarbazone compounds were prepared and their behavior on activated alumina was observed. While this study was again essentially qualitative in nature, it pointed the way to further investigation. Later studies (*1, 4, 6, 12*) have indicated that the chromatographic separation of metallic chelates may have quantitative potentialities. The work of Hilliard and Freiser (*9*) is especially significant from this standpoint. Applications to trace analysis have been limited, and the present study was undertaken to investigate the use of the chromatographic adsorption technique as a separatory method and as a means of removing interferences in the microgram region.

CHROMATOGRAPHY

Preliminary investigation of several chelating agents and a variety of adsorbents led to several generalizations. Activated silicic acid seems to be the adsorbent most adaptable to this type of trace analysis, especially from the standpoint of zone detection. Extractability, stability, and high color sensitivity of the chelates formed, as well as nonspecificity of the reagent, were observed to be important properties of a chelating agent applicable to this type of separation.

The work of Al-Mahdi and Wilson (*1*) indicated that the chromatographic adsorption analysis of certain diethyldithiocarbamates might be applied to a trace method of determination of these metals. While some metals were quantitatively recoverable, the separation of ternary mixtures of copper, cobalt, and nickel chelates was not satisfactory.

Bode (*3*) has studied the extractability of diethyldithiocarbamates as a function of acidity. Metal ions quantitatively extracted from aqueous solution by sodium diethyldithiocarbamate into carbon tetrachloride over pH range of 5 to 11 are: silver(I), bismuth(III), cadmium(II), cobalt(III), copper(II), iron(III) (incomplete above pH 10), mercury(II), nickel(II), lead(II), palladium(II), tellurium(I), thallium(III), and zinc(II).

Preliminary work showed that while all chelates of diethyldithiocarbamate studied (Table I) were strongly adsorbed from carbon tetrachloride solution by activated silica, the copper and cobalt compounds could be separated by migration with chloroform–carbon tetrachloride developers. Of the metals which commonly interfere in the trace determination of copper and cobalt, only the nickel and bismuth chelates migrated appreciably upon passage of the developer. Separations of copper and cobalt diethyldithiocarbamates on activated alumina were found to be poorer generally than those on activated silicic acid. The order of elution for the copper and cobalt compounds, using the same solvent and developer, was reversed when activated alumina was the adsorbent employed.

The nickel diethyldithiocarbamate, a source of considerable difficulty when separations on alumina are attempted, undergoes rather extensive decomposition at the top of the silica adsorbent upon passage of the developing solvent. During the passage of the developer, a small quantity of either a decomposition product or undecomposed nickel chelate migrates between the copper and cobalt zones in a diffuse band shown to contain nickel. This behavior is most apparent when large quantities of the nickel chelate are adsorbed. The decomposition was shown not to be a result of oxygen adsorbed on the column or of impurities in the solvents, but was noted to be more extensive in chloroform than in carbon tetrachloride and to be a function of band "concentration."

The iron(III) chelate, adsorbed initially in a narrow black band, separated into two zones upon passage of the developer. The rates of migration of both were extremely slow. The colors of the adsorbed cadmium and zinc complexes were observed to fade slowly, possibly also a result of decomposition. The mechanisms of these apparent decompositions were not investigated.

The concentration of chloroform in carbon tetrachloride giving the most effective separation of copper and cobalt diethyldithiocarbamates on activated silicic acid was determined by measurement of the R-values at varying concentrations, using a graduated column.

$$R = \frac{\text{movement (in milliliters) of zone}}{\text{volume (in milliliters) of solvent}}$$

when R_f refers to the leading edge of the zone and R_t refers to the trailing edge of the zone.

As shown in Table II, the most effective separation was accomplished by using a 50% (by volume) chloroform in carbon tetrachloride solvent. The difference between the zone front migration rates becomes greater as the percentage of chloroform increases. Above 50%, however, the copper chelate zone becomes so diffuse (reflected by R_f —

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R_t) that any advantage in increased difference in zone front migration rates is destroyed by zone widening. Using a 50% (by volume) chloroform–carbon tetrachloride developer, the initially adsorbed copper and cobalt diethyldithiocarbamates could be resolved into well-defined zones on the silica adsorbent and could be eluted in small volumes of solution. Table I summarizes the behavior of some diethyldithiocarbamates under these conditions.

Table I. Column Behavior (Activated SiO_2) of Diethyldithiocarbamates

Diethyldithiocarbamate	Zone Color	Development with 50% $CHCl_3$ in CCl_4
Cu(II)	Brown	R_f 0.16
Co(III)	Green	R_f 0.057
Ni(II)	Light brown	Decomposition, migration between Cu and Co bands
Fe(III)	Green-black	No migration. Possible decomposition
Zn(II)	Blue-green	No migration
Cd(II)	Blue	No migration
Bi(III)	Yellow	R_f 0.06

PHOTOMETRY

In this work the most straightforward procedure would have been the photometric evaluation of the chelates directly upon elution. While such methods are available (*2*, *3*), they are somewhat lacking in sensitivity. Hence the procedure adopted was to destroy the eluted diethyldithiocarbamates, convert to the corresponding diphenylthiocarbazone compounds, and measure the latter by photometric means. Diphenylthiocarbazone (dithizone) was selected because of the high photometric sensitivity of its chelates, and because copper may be extracted into organic solvents by dithizone from dilute acid solutions, thus minimizing interferences from most other metals. Elimination of any possible nickel interferences is especially advantageous. Further, but a single reagent solution is required for both the copper and cobalt determinations. For the copper analysis, a calibration curve (Beer's law plot) for the copper dithizonate-dithizone system was developed, using the method of Liebhafsky and Winslow (*10*) with minor modifications. Once the original curve had been established, a standard and the unknown solution were carried through the analysis together, by the procedure given below. The average deviation was found to be ±0.004 absorbance unit, corresponding to a concentration uncertainty of ±0.013 γ of copper per milliliter.

Several reasons may be cited for the lack of a photometric dithizone method for the determination of cobalt. First, dithizone is a relatively nonspecific reagent, especially in basic solution. However, in the present work, the lack of specificity would not constitute a drawback because of the prior chromatographic separation. Also, the cobalt dithizonate must be extracted from a basic solution, and under strongly basic conditions the color decays, possibly as a result of the formation of the feebly colored enol tautomer (*8*). When the extraction is carried out from alkaline solution the removal of excess dithizone from the organic phase is a function of pH. Thus varying amounts of dithizone would be present along with the chelate in the organic phase, and a mixed color method would not appear feasible without very careful pH control.

Figure 1 shows the absorbance versus pH of extraction curve for the extraction of cobalt by dithizone into carbon tetrachloride. The region of low slope in the pH range 9.4 to 10.5 permitted the development of a photometric method for cobalt. The curve shape was found not to change with variations in the cobalt concentration, being simply displaced to lower absorbance values with decrease in the cobalt concentration. At pH 7.5 there is a considerable amount of dithizone reagent, in addition to the extracted cobalt complex, in the carbon tetrachloride phase which absorbs significantly at 545 mμ. As the pH of the aqueous phase increases, the amount of dithizone transferred to the aqueous phase also increases, so that the organic phase exhibits decreasing absorbancies. As the pH reaches a value of about 9.5,

Table II. Elution of Diethyldithiocarbamates with Chloroform in Carbon Tetrachloride

Volume % $CHCl_3$ in CCl_4	Copper Chelate		Cobalt Chelate	
	R_f	R_t	R_f	R_t
0	0.004	0.004	0.002	0.000
10	0.015	0.011	0.015	0.008
20	0.029	0.020	0.026	0.020
30	0.042	0.032	0.034	0.034
40	0.12	0.055	0.045	0.037
50	0.16	0.10	0.057	0.047
60	[a]	[a]	0.084	0.072

[a] Band becomes increasingly diffuse above 60% $CHCl_3$ in CCl_4.

the major portion of the dithizone has been removed and the absorbance values remain relatively constant, as most of the absorption is due to the cobalt complex. At pH 10.5 the curve begins to decay, probably as a result of the formation of an enol tautomer. The curve for the reagent itself shows a similar decay with pH, showing increased removal of the dithizone from the carbon tetrachloride phase. The change of slope in the reagent curve at pH 9.7 may be due to air oxidation of the reagent, which becomes more serious as the basicity of the aqueous phase increases. The effect of change in the pH of the buffer on the absorption spectrum and the absorption maximum of the pure cobalt dithizonate in carbon tetrachloride was found to be negligible. The cobalt complex forms immediately upon shaking in the carbon tetrachloride phase and no special precautions concerning stability toward light are necessary.

The degree to which pH and reagent concentration had to be controlled in order to validate relocation of an original calibration curve by simultaneous extraction of a standard was investigated. The effect of variation in pH is shown in Figure 2. The data indicate that only approximate reproduction of the buffer pH should be necessary between pH 9.5 and 10.4. Although, ideally, variations in reagent concentration at constant pH should not affect the slope of the calibration curve, Beer's law deviations and variations of the pH and volume of the aqueous phase could make such changes significant. Figure 3 shows two calibration curves determined with dif-

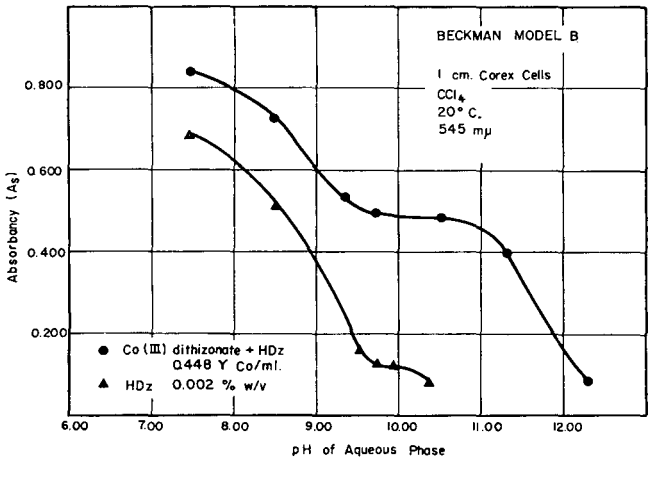


Figure 1. Absorbance vs. pH of extraction curve for extraction of cobalt by dithizone into carbon tetrachloride

ferent initial reagent concentrations. This variance is readily controlled within the necessary limits by weighing the solid reagent, and by appropriate volumetric dilution. The reagent concentration selected is believed to be an optimum, since it provides a stoichiometric excess yet minimizes difficulties caused by a large surplus. The final working curve shows a linear relationship between absorbancy (at 545 mμ) and cobalt concentration from 0.2 to 0.8 γ per ml.

The accuracy and reproducibility of this method are dependent upon essentially the same factors as govern the copper procedure, in addition to the pH and reagent concentration variables. An added source of error arises from the increased tendency for reagent oxidation in basic solution. This difficulty is minimized by shaking for the shortest time necessary for the attainment of equilibrium. The average deviation was found to be ±0.004 absorbance unit corresponding to a concentration uncertainty of ±0.005 γ of copper per ml. This method, while highly sensitive, is applicable only in conjunction with adequate separatory methods.

EXPERIMENTAL PROCEDURE

Apparatus. Column. The chromatographic columns were constructed of borosilicate glass, 10 mm. in diameter, and about 30 cm. in length, with a three-way stopcock at the bottom. One outlet was used to collect the effluent and the other was connected by polyethylene tubing to a 125-ml. separatory funnel. A 2.5-cm.-thick glass-wool plug was used to support the adsorbent. One hundred-milliliter dropping funnels were connected to the column top by means of ground-glass joints. A stem protruded 2.5 cm. below the joint and created an air lock at the column top, thus allowing the liquid level to remain constant.

pH Meter. A Beckman Model H-2 pH meter was used in conjunction with glass and saturated calomel electrodes for all pH measurements. The meter was standardized with a buffer solution of pH 10.00.

Spectrophotometer. A Beckman Model B spectrophotometer with matched 1-cm. Corex cells was employed in the photometric measurements.

Drying Tube. An 8-cm. long, 10-mm. glass tube was prepared with a sintered-glass disk at one end. This tube was partially filled with 1 to 2 grams of reagent grade anhydrous sodium sulfate for drying carbon tetrachloride extracts.

Glassware. All volumetric glassware was calibrated by appropriate means. Short-stemmed 125-ml. separatory funnels were used for all extractions. These were rinsed with dithizone reagent and triply distilled water before use.

Reagents. For the quantitative studies, Mallinckrodt silicic acid (Analytical Reagent D-616) was employed. The material used had the following sieve analysis: 100 mesh, 1% retained; 150 mesh, 74% through; 200 mesh, 30% through. This product allowed a reasonable flow rate and is highly adsorptive. The R values for the diethyldithiocarbamates were obtained with this reagent. It was activated at 500° C. for 24 hours when utilized for the separation of copper and cobalt diethyldithiocarbamates.

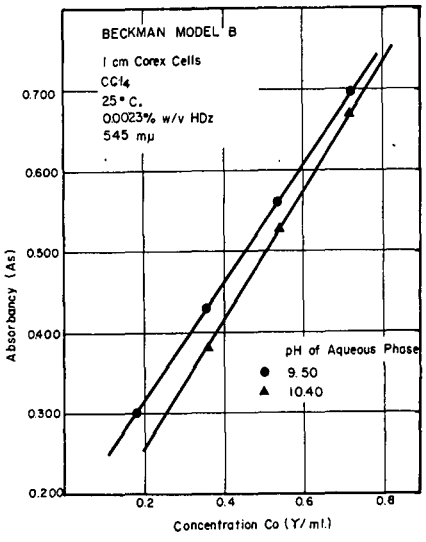


Figure 2. Effect of variation in pH in validating relocation of an original calibration curve by simultaneous extraction of a standard

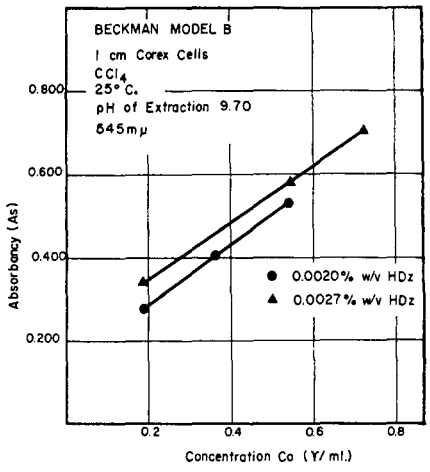


Figure 3. Calibration curves determined with different initial reagent concentrations

Solvents. The U.S.P. carbon tetrachloride used in chromatographic work and related extractions was redistilled in an all-glass system and dried with anhydrous sodium sulfate before use. Merck reagent grade chloroform was shaken with anhydrous calcium chloride, and the supernatant liquid was filtered. This chloroform was passed through a column filled with 20 grams of activated silicic acid and again filtered before use as a developing solvent. Merck reagent carbon tetrachloride was used in extractions for photometric measurement.

Distilled Water. Triply distilled water from a Precision Scientific three-stage still was used in preparing all aqueous solutions and in rinsing cleaned glassware.

Buffer Solutions. All buffer solutions were prepared in triply distilled water from reagent quality ammonium chloride and ammonium hydroxide. They were stored in polyethylene containers and checked with the pH meter at regular intervals.

Hydroxylamine Hydrochloride, 10 grams in 100 ml. of triply distilled water.

Metal Salt Solutions. Standard solutions of copper(II), nickel(II), and cobalt(II) were prepared from the reagent grade nitrates in triply distilled water. The solutions were standardized by appropriate means and diluted volumetrically to yield concentrations of about 10 γ of metal ion per milliliter.

Dithizone Solutions. The C.P. diphenylthiocarbazone was dissolved in reagent carbon tetrachloride to prepare a 0.01% w./v. stock solution. After filtration, the stock reagent was stored at 0° C. This was found to stabilize the reagent solution very well. All reagent solutions were prepared by dilution of the stock with reagent carbon tetrachloride.

Sodium Pyrophosphate Solution. Ten grams of sodium pyrophosphate in 100 ml. of water provided a nearly saturated solution. The solution was purified by adding 1.0 ml. of 0.1% sodium diethyldithiocarbamate solution and extracting any heavy metal chelates with two or three small portions of carbon tetrachloride. Filtration through a previously moistened filter paper removed most of the dispersed droplets of carbon tetrachloride, and the solution was stored in a polyethylene bottle. Fresh solutions were prepared periodically because of slow hydrolysis of the pyrophosphate.

Sodium Diethyldithiocarbamate Solution. A 0.1% solution was prepared by dissolving 0.1 gram of the reagent (Eastman) in triply distilled water and diluting to 100 ml. The reagent was stored in the dark and was newly prepared each week.

Sodium Hydroxide Solutions. Fifty per cent by weight and 1.0F sodium hydroxide solutions were prepared by dissolving the requisite quantity of reagent grade material in triply distilled water. The solutions were stored in tightly capped polyethylene bottles.

General Procedure. Twenty-five milliliters of the ammonium hydroxide–ammonium chloride buffer (pH 9.6 to 9.8) and 15 ml. of 10% sodium pyrophosphate solution were added to 125-ml. separatory funnels. Known amounts of the standard cobalt and copper solutions were added, together with the interfering elements in solution form. After addition of 2.0 ml. of the 0.1% sodium diethyldithiocarbamate solution, the mixture was allowed to stand for a minute. Next, a 2- to 3-ml. portion of carbon tetrachloride was added and the funnels were shaken for 3 minutes. After separation of the layers, the extract was passed through the drying tube. The shaking was twice repeated with carbon tetrachloride

and the sodium sulfate in the drying tube was finally rinsed with 1 ml. of carbon tetrachloride. The total volume of extract was kept to about 10 ml.

Three grams of activated silicic acid, slurried with carbon tetrachloride, produced an adsorbent bed about 7.5 cm. deep. The minimum depth allowed was 6.5 cm., as a shorter depth did not permit satisfactory separations. The maximum depth used was 10 cm. Deeper beds required more time for elution and tended to give more diffuse bands. The carbon tetrachloride extract of the diethyldithiocarbamate was transferred to the column. A constant flow rate of about 0.5 ml. per minute was used in adsorbing the chelate mixture on the column. Adsorption was completed by two or three rinsings with carbon tetrachloride.

The 50% chloroform–carbon tetrachloride developer was then passed through the column at a flow rate held between 0.3 to 0.4 ml. per minute. The total elution time for both zones varied between 1 and 2 hours, depending upon the column length and the flow rate of the developer.

The two chelate zones, upon elution, were collected in 25-ml. Erlenmeyer flasks. The organic solvents were evaporated and to the residues were added 0.75 ml. of concentrated sulfuric acid and 0.3 ml. of 60% perchloric acid. These solutions were heated at 200° to 250° C. until the solution became colorless. After cooling, the solutions were diluted with an equal volume of triply distilled water, and the acid was neutralized by the dropwise addition of 50% sodium hydroxide solution, using methyl red indicator. The solution was brought back to the acid side by the addition of one drop of concentrated sulfuric acid. A 1*F* solution of sodium hydroxide was added until the indicator again turned yellow. The cobalt sample was then diluted with triply distilled water for subsequent colorimetric analysis. To the copper sample was added one drop of concentrated hydrochloric acid, and the sample was diluted. If more than about 7 γ of cobalt or of copper were estimated to be present, the solutions were transferred to 25-ml. volumetric flasks and diluted to the mark. The photometric analysis of each sample was accomplished according to the following procedures.

Copper. Twenty milliliters of 0.03*F* hydrochloric acid were added to each of two 125-ml. separatory funnels. Two drops of 10% hydroxylamine hydrochloride solution were added to each funnel, in order to minimize reagent oxidation. One milliliter of a standard copper solution (10.3 γ of copper per ml.) was added to one funnel and an appropriate aliquot of the sample solution to the other. Exactly 10 ml. of the dithizone reagent were added to each funnel and both were shaken for 10 minutes. (The shaking time necessary for attainment of equilibrium may vary with the purity of the carbon tetrachloride.) After the layers had separated, a small volume of the organic phase in each funnel was withdrawn, and its absorbance was measured at 510 mμ, using carbon tetrachloride as the reference material. The original calibration curve was readjusted by using the absorbancy of the standard, and the concentration of the unknown was obtained from this curve. This technique eliminates errors arising from variation in reagent concentration which is otherwise difficult to control.

Cobalt. Exactly 25 ml. of an ammonium hydroxide–ammonium chloride buffer (pH 9.60 to 9.80) were added to each of two 125-ml. separatory funnels. A calibrated pipet was used to deliver an accurate volume of standard cobalt solution (8.95 γ of cobalt per ml.) to one of the funnels, and an aliquot of the sample was added to the other. An equivalent volume of triply distilled water was added to the standard to maintain near equality of the aqueous volumes. Precisely 10.00 ml. of the dithizone reagent (0.0021 to 0.0027% w./v.) were added to each funnel. After shaking for a maximum of 5 minutes, the layers were allowed to separate, and a portion of the organic phase was withdrawn. Absorbancy values at 545 mμ were determined using carbon tetrachloride as the reference. The original calibration curve was readjusted with the absorbance of the standard and the concentration of the unknown was then calculated.

INTERFERENCES

To study the nature and extent of the various metal interferences, synthetic samples containing standard amounts of copper and cobalt together with varying amounts of interfering metals were prepared. The results of the analysis of such samples may be summarized as follows.

Nickel. Amounts of nickel up to about 50 γ caused no difficulty. The elution of the cobalt diethyldithiocarbamate band is very sharp and only very small amounts of nickel will accompany it. In the pH range of 9.6 to 9.8, the range selected for the extraction of the cobalt dithizone complex, the photometric interference of any traces of nickel is very small. At these pH values it was found from the pH *vs.* absorbance curve of the nickel chelate that the absorbance of the nickel compound is almost negligible. A higher pH of extraction may also be used, thus further decreasing any possible interference from nickel. It is probable that considerably more than 50 γ of nickel can be tolerated but excessive amounts would be likely to give somewhat high recoveries for cobalt.

Iron and Manganese. The addition of pyrophosphate to the ammonium hydroxide—ammonium chloride buffer prior to extraction of the diethyldithiocarbamates was effective in preventing the interference of 10-mg. quantities of iron and 5-mg. quantities of manganese. It is certain that much larger quantities of these metals can be present without causing significant error. No manganese chelate was extracted and only a very small amount of the iron(III) diethyldithiocarbamate was visible at the top of the column after extraction of copper and cobalt under these conditions.

Table III. Analysis of Synthetic Samples Containing Various Interferences

Interference	Copper, γ Added	Copper, γ Recovered	Cobalt, γ Added	Cobalt, γ Recovered
None	10	10	9	7
1.5 g. $Na_4P_2O_7$ 2.0 g. Na_2SO_4	10	10	18	17
1.5 g. $Na_4P_2O_7$ 2.0 g. Na_2SO_4	10	11	5	4
10 γ Ni	10	12	9	10
10 γ Ni	11	11	10	11
20 γ Ni	5	6	18	18
20 γ Ni	21	22	5	7
20 γ Ni	10	12	4	4
51 γ Ni	10	11	9	7[a]
5 mg. Mn	21	20	9	9
10 mg. Fe	10	11	9	9
10 mg. Zn	5	7	9	9
0.1 mg. Cd	10	9	9	10
1.0 mg. Bi	10	14[b]	18	36[c]
1.0 mg. Bi	10	11	18	36[c]

[a] Typical result of insufficient oxidation before photometric determination.
[b] Typical result of excessive amount of NaOH in neutralization.
[c] Bismuth interference.

Zinc and Cadmium. Both zinc and cadmium form white insoluble compounds with sodium diethyldithiocarbamate in basic solution. These compounds are but slightly extracted by carbon tetrachloride. Moderate quantities of zinc and small amounts of cadmium could be present in an ammoniacal buffer (pH 9.68) containing sodium pyrophosphate. No interference was noted from 10 mg. of zinc and 0.1 mg. of cadmium in this procedure. Any of the chelates extracted remained at the column top.

Bismuth. Bismuth forms a yellow diethyldithiocarbamate which is readily soluble in carbon tetrachloride, and, when present in more than microgram quantities, seriously interferes in the photometric determination of copper with diethyldithiocarbamate (*5*). As may be seen in Table III, in this procedure 1 mg. of bismuth contributed to a high recovery of cobalt only. The high copper recovery was the result of a deliberate excess of sodium hydroxide in the neutralization step (trace metal contamination) rather than an actual bismuth interference. The high values for cobalt result from a small quantity of bismuth extracted from the sample, forming a yellow-green zone which migrated with the cobalt zone. These two bands could not be resolved with

Table IV. Red Clover Analysis

Sample No.	Sample Weight, Grams	Copper, P.P.M. Present	Copper, P.P.M. Found	Cobalt, P.P.M. Present	Cobalt, P.P.M. Found
971	1.04	17.0	15.7	8.8	7.5
	1.13	17.0	15.2	8.2	6.8
	1.34	17.0	16.6	..	..
982	1.55	11.5	11.6	5.9	5.9
	1.64	11.5	11.5	5.9	6.2
	1.52	11.5	12.4	..	..
	1.05	11.5	11.5	..	..
Average error.	±0.3 γ copper, ±0.3 γ cobalt.				

the developer used. The bismuth is also extracted by dithizone in ammoniacal solution, thus interfering in the colorimetric determination of cobalt. It should be possible to overcome the interference of bismuth with cobalt in the final step, because cobalt is not extracted by dithizone from a solution containing a reducing agent. (In the presence of bismuth it is necessary to add sufficient diethyldithiocarbamate reagent to complex the bismuth as well as the copper and cobalt.) Two aliquots of the oxidized cobalt-bismuth fraction, one containing hydroxylamine, could be taken for photometric analysis. The absorbancy readings (545 mμ) of the extract from the aliquot containing the reducing agent subtracted from the absorbance value of the other extract should give a correct value for cobalt.

Table III summarizes some representative analyses for copper and cobalt in the presence of various common interferences.

APPLICATION

To test the practical applicability of the method, samples of plant material were analyzed. Several red clover samples which had been analyzed spectrographically were obtained. In some cases the cobalt content was too low to permit chemical analysis, and standard amounts of cobalt were added to the samples after digestion. A simple digestion procedure which should be applicable to biological and plant materials not containing excessive amounts of silica was adopted, in part, from a procedure suggested by McNaught (*11*).

To 1 gram of the dry clover in a 100-ml. Kjeldahl flask were added 2 ml. of distilled water and 1 ml. of concentrated nitric acid. The mixture was boiled gently until the volume was about 1.5 ml., and then cooled. A 2-ml. volume of concentrated sulfuric acid was added, the solution was cooled, and 1 ml. of concentrated nitric acid was added. The treatment was repeated until the liquid remained colorless after evaporation to sulfur trioxide fumes. All but about 0.5 ml. of the sulfuric acid was fumed off and the solution was diluted 2 to 1 with triply distilled water.

The neutralization was carried out directly in the Kjeldahl flask. A few drops of 50% sodium hydroxide and three drops of 0.02% methyl red indicator were added. The 50% sodium hydroxide solution was added until the indicator turned yellow and the walls of the flask were washed down with small portions of triply distilled water. A drop of concentrated sulfuric acid was added, and the solution was rendered slightly basic again by dropwise addition of 1*F* sodium hydroxide.

The resulting solutions were added to 125-ml. separatory funnels for extraction. The copper and cobalt were then extracted, chromatographically separated, oxidized, and determined colorimetrically as previously described.

Table IV tabulates the results obtained.

ACKNOWLEDGMENT

The authors are indebted to C. B. Smith, Plant Nutrition Laboratory, for the analyzed clover samples.

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RECEIVED for review April 11, 1958. Accepted December 10, 1958.

Rapid Method for Determination of Small Amounts of Carbon Monoxide in Gas Mixtures

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▶ **A rapid and simple method for the determination of carbon monoxide in gas mixtures converts carbon monoxide to carbon dioxide by using decomposed silver permanganate as a catalyst. The carbon dioxide formed is absorbed in an Ascarite tube and determined gravimetrically. With 500-ml. gas samples, concentrations of carbon monoxide in the order of 0.1 to 1.5% can be determined. By increasing the sample size the lower limits of this method can be extended. The method is well suited to working conditions encountered in a plant control laboratory.**

THE determination of small amounts of carbon monoxide in gas mixtures has great industrial importance. Because of its high toxicity, traces must be determined in many industrial processes, mine atmospheres, exhaust gases, etc. In a number of petrochemical processes the amounts of carbon monoxide in reactor tail gases indicate the efficiency of the chemical process and the degree of catalyst poisoning. Exact values of carbon monoxide, usually below a 1% level, are required for process evaluation and control in such cases.

The classical Orsat method (*7*) for gas analysis is not sufficiently accurate and precise in this concentration range; therefore, various iodine pentoxide

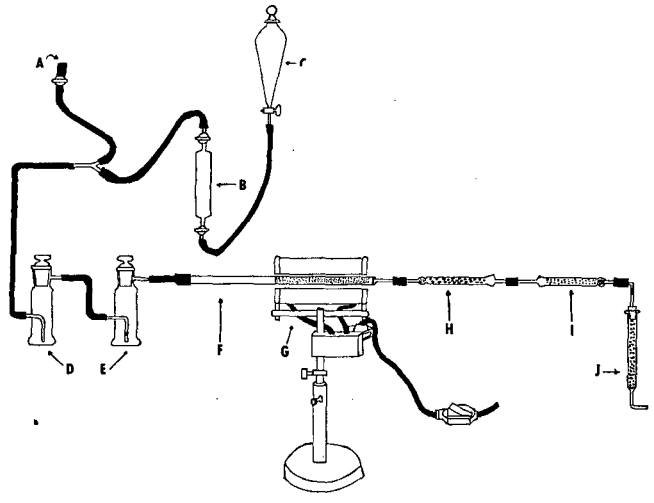


Figure 1. Apparatus for determination of carbon monoxide

A. Connection to oxygen tank
B. Gas sample bottle
C. Separatory funnel
D. Mersil absorption bulb
E. Anhydrone-Ascarite absorption bulb
F. Combustion tube
G. Microheater
H. Anhydrone absorption tube
I. Ascarite absorption tube
J. Guard tube

methods (*1*, *2*) are generally used for an accurate determination of very small amounts of carbon monoxide. However, these methods can hardly be used in plant control operations because of their complexity.

To eliminate these difficulties and in view of the increasing volume of work in an industrial laboratory, a simple and economical method for carbon monoxide determinations in gas mixtures adaptable to plant control operations was sought.

Körbl (*4*, *5*) in his fundamental study of analytical applications of thermally decomposed silver permanganate, indicated that it was a good catalyst for the conversion of carbon monoxide to carbon dioxide. This suggested utilizing decomposed silver permanganate for the quantitative determination of carbon monoxide. Katz and Katzman (*3*) used granular silver permanganate as a catalyst for carbon monoxide. They determined amounts of carbon monoxide by measuring the heat generated during the oxidation reaction.

In the proposed method, decomposed silver permanganate is used to oxidize carbon monoxide to carbon dioxide in a short combustion tube heated at 450° to 500° C. The carbon dioxide is later absorbed in an Ascarite absorption tube and determined gravimetrically by classical microanalytical techniques. The gas mixtures analyzed contained oxygen, nitrogen, carbon dioxide, carbon monoxide, and unsaturated hydrocarbons. Determination of carbon monoxide is usually required in such industrial gas mixtures. The drying and purifying train eliminates interfering substances such as carbon dioxide, unsaturated hydrocarbons, and moisture. To decrease the back pressure in the system, only solid absorbants were used.

REAGENTS

Thermally Decomposed Silver Permanganate (*6*). Silver permanganate is prepared by dissolving 92.0 grams of potassium permanganate in 2 liters of distilled water (90° to 100° C.) and adding 102.0 grams of silver nitrate. The solution is left overnight, and the precipitate of silver permanganate is filtered on a medium porosity fritted-glass funnel using suction, washed with 1 liter of distilled water, and then dried in a vacuum oven at 60° to 70° C. Crystalline silver permanganate should be handled with extreme care because of its explosive nature when in contact with organic material. It is then thermally decomposed in two stages.

The first stage of decomposition is conducted in a test tube containing 1 to 2 grams of silver permanganate. The tube is heated with a microburner in one spot, and when the temperature reaches 120° C., the material decomposes spontaneously with a hissing noise. The decomposed product is an amorphous mass with a volume three times greater than that of the original crystalline silver permanganate.

The second stage of decomposition is rather slow and can be accomplished in an oven heated at 500° C. After being heated for 1 to 2 hours, thermally decomposed silver permanganate is ready for use in the combustion tube. The composition of the thermally decomposed silver permanganate corresponds to the formula, $AgMnO_2$. This material is safe to handle.

Mersil. This reagent (*1*) is prepared by dissolving 164 grams of red mercuric oxide in 715 ml. of cold 29% sulfuric acid. To this solution 480 grams of magnesium sulfate heptahydrate are added and then dissolved. The solution is filtered and mixed with sufficient dried, purified silica gel to give a uniformly moist mass. The slight excess of liquid is drained by combined pressure and suction, and the mass is dried at 100° C. until the particles are just free-flowing.

Oxygen is supplied from a cylinder of prepurified oxygen with a reducing valve.

APPARATUS

Separatory Funnel. A 1-liter separatory funnel filled with saturated sodium chloride solution is used to displace the gas in the sample bottle.

Absorption Unit for Unsaturated Hydrocarbons. A Stetson-Norton gas absorption bulb is filled with Mersil. A 5-mm. plug of borosilicate glass wool is placed on the bottom and on the top of the Mersil filling. The ground joint of the bulb is sealed with a film of Kröenig cement.

Absorption Unit for Carbon Dioxide and Water. A Stetson-Norton gas absorption bulb is filled with equal volumes of Ascarite and Anhydrone, which are separated by a 5-mm. borosilicate glass wool divider.

Combustion Tube (Vycor). A combustion tube 200 mm. long and 9 mm. in outside diameter is used. One end of the tube is drawn out to form a tip 3 cm. long, 3 mm. in outside diameter. A section of asbestos fiber 5 mm. long is inserted into the tip, followed by a 10-cm. layer of decomposed silver permanganate. A 10-mm. plug of asbestos on this retains the packing.

Combustion Furnace. This is a standard electrical microheater, required for a conventional carbon and hydrogen combustion train (ACS microspecification) used for the oxidation of impurities in commercial oxygen. It maintains a temperature of approximately 500° C. without a rheostat. A regular Bunsen burner with a wing tip may be used as a substitute heating unit.

An Anhydrone tube is used to remove traces of water. A semimicro absorption tube (Fieser, Arthur H. Thomas Co.'s specification) with a ground-in hollow-glass stopper, over-all length 140 mm., is filled with Anhydrone. Small plugs of cotton are placed in the tips of the absorption tube. The ground-glass joint is sealed with Kröenig cement.

Ascarite Tube. A semimicro absorption tube is filled with Ascarite in the same manner as above.

Guard Tube. Any of several varieties of absorption tubes filled with Anhydrone and Ascarite may be used to protect the Ascarite tube from the atmosphere.

Assembling the Apparatus. A diagrammatic sketch of the apparatus is shown in Figure 1. Scimatco (Fisher Scientific Co.) rubber tubing (1 cm. in outside diameter) is used to make all the glass connections, except to the Ascarite, Anhydrone, and guard tubes, where heavy-walled rubber (micro) tubing is used.

Table I. Determination of Carbon Monoxide in Synthetic Gas Mixtures

Carbon Monoxide, %			Dev. from Av.
Present	Found	Recovery	Recovery
1.512	1.508	99.74	0.16
1.531	1.540	100.73	0.83
1.210	1.208	99.83	0.07
1.221	1.230	100.73	0.83
1.045	1.042	99.71	0.19
1.051	1.045	99.43	0.47
0.742	0.742	100 00	0.10
0.754	0.772	102.40	2.50
0.540	0.536	99.25	0.65
0.554	0.563	100.73	0.83
0.330	0.324	98.18	1.72
0.320	0.314	98.12	1.78

Av. and std. dev. 99.90 ± 0.85%.
Sample size used, 500 ml.

Table II. Determination of Carbon Monoxide in Synthetic Gas Mixtures

(Higher and lower concentration ranges)

Sample Size, Ml.	Carbon Monoxide, % Present	Found	Recovery
100	5.000	5.026[a]	100.52
2000	0.050	0.058[a]	116.00

[a] Each figure is average of three determinations.

PROCEDURE

The microheater is turned on and allowed to reach operating temperature, 450° to 500° C. The apparatus is purged with oxygen (20 to 40 ml. per minute) for 10 to 15 minutes. Meanwhile the Ascarite absorption tube is wiped with a chamois cloth and weighed on a semimicro or microanalytical balance. A trace of glycerol is added to the tips of the Ascarite absorption tube, and it is connected to the Anhydrone and guard tube. The flow of oxygen is stopped and the stopcock of the sample bottle adjoining the combustion train opened. The stopcock which connected the sample bottle to the separatory funnel is then opened and adjusted to permit liquid displacement of the gas at a rate of 20 to 30 ml. per minute. The solution of sodium chloride displaces the gas in the sample bottle by gravity, forcing the gas mixture through the combustion train. When the sample has been completely displaced, the stopcocks are closed. The system is then purged with oxygen for 15 minutes. After this time the flow of oxygen is stopped and the Ascarite tube disconnected from the assembly, wiped with the chamois, and weighed. The increase in weight of the Ascarite tube is proportional to the carbon monoxide in the sample. An increase in weight of 1 mg. is equal to 0.501 ml. of carbon monoxide.

Calculation.

$$\% \text{ CO} = \frac{A \times 0.501 \times 100}{V \text{ (ml.)}}$$

where

A = increase in weight of Ascarite tube, milligrams
V = volume of gas sample, milliliters, at standard pressure and temperature

RESULTS AND DISCUSSION

A series of synthetic gas mixtures containing nitrogen (75 to 78%), oxygen (18 to 21%), carbon dioxide (0.03 to 1.0%), unsaturated hydrocarbons (1.0 to 5.0%), and carbon monoxide (0.3 to 1.5%) was prepared and analyzed for carbon monoxide content. The accuracy of the analysis in the indicated range is to ±0.85%. The results are presented in Table I. Periodical analyses of the same gas mixtures except for carbon monoxide indicated no blank value.

Because this method is based on a gravimetric principle, the analysis of gas mixtures containing higher than 1.5% concentrations of carbon monoxide would give better accuracy and precision than the analysis of mixtures with lower concentrations. Smaller sample sizes are indicated in such cases. When carbon monoxide is present in less than 0.1% concentrations, proportionally larger samples should be used to obtain satisfactory accuracy and precision.

The results of triplicate analyses of gas mixtures are presented in Table II.

A liquid nitrogen trap can be incorporated in the assembly. This will freeze out saturated hydrocarbon varieties. A number of purifying and drying trains for specific gas mixtures as described by Adams and Simmons (*1*) may be used with this method.

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RECEIVED for review February 14, 1958. Accepted December 16, 1958.

Photometric Determination of Tantalum with Phenylfluorone

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▶A photometric phenylfluorone method for the determination of traces of tantalum has been developed. The interference of other metals is eliminated by virtually isolating the tantalum by solvent extraction from hydrofluoric-hydrochloric acid solution with methyl isobutyl ketone and by masking with (ethylenedinitrilo)tetraacetic acid during the subsequent color development.

DURING development of a photometric method for germanium it was found that tantalum and several other metals form red compounds of various intensities and hues with phenylfluorone (*1*). It seemed worthwhile to determine whether the color reaction could be made the basis of a practical method for the photometric determination of tantalum. It appeared probable that, by virtually isolating the tantalum by extraction with methyl isobutyl ketone (*2*) and by masking any remaining interference with (ethylenedinitrilo)tetraacetic acid (EDTA), a satisfactory determination of tantalum could be made. This has proved to be true.

REAGENTS

Standard Tantalum Solution (100 γ of tantalum per ml.). Transfer

0.0500 gram of pure tantalum metal plus 2 ml. of hydrofluoric acid to a covered platinum crucible. Add nitric acid dropwise with gentle heating until dissolution of the metal is complete and brown fumes have been expelled. Cool, dilute to 500.0 ml., and store in a polyethylene bottle.

Buffer Solution (pH 4.5). Dissolve 500 grams of ammonium acetate in 500 ml. of water. Add 700 ml. of acetic acid followed by 2 grams of benzoic acid (as preservative) dissolved in 20 ml. of methanol. Dilute to 2 liters. Transfer 10 grams of Knox powdered gelatin to 1 liter of water in a beaker and heat in an 80° to 90° C. hot water bath, with occasional stirring, until the gelatin has dissolved. Cool. Mix the buffer and gelatin solutions and adjust the pH of the mixed solution with the aid of a pH meter to 4.5 by the addition of acetic acid or ammonium hydroxide.

Phenylfluorone Solution. Transfer 0.0500 gram of phenylfluorone (2,3,7-trihydroxy-9-phenyl-6-fluorone) to a 100-ml. beaker. Add 50 ml. of methanol followed by 1 ml. of hydrochloric acid and then stir until dissolved. Transfer to a 500-ml. volumetric flask and dilute to the mark with methanol.

PREPARATION OF CALIBRATION CURVE

Working individually, transfer 0, 0.40, 0.80, and 1.20 ml. of standard tantalum solution (100 γ of tantalum per ml.) to a 100-ml. platinum dish. Float the dish in an 80° to 90° C. water bath and allow the tantalum solution to evaporate just to dryness with the aid of a gentle jet of air. Pipet 1 ml. of hydrofluoric acid (1 + 99) from a polyethylene bottle to the dish and heat and swirl on a low temperature hot plate until the tantalum compound dissolves. Cool, add 5 ml. of 10% w./v. aqueous solution of the disodium salt of EDTA, swirl, and pour into a 50-ml. volumetric flask. Add 10 ml. of pH 4.5 buffer solution to the dish, swirl, and pour to the flask. Pipet 10.0 ml. of phenylfluorone solution to the flask, swirl to mix, and then allow to stand 30 minutes. Add 5 ml. of a saturated aqueous solution of boric acid to minimize the corrosive action of the hydrofluoric acid on the absorption cell, dilute to the mark with acetic acid (1 + 9), and mix. Immediately measure photometrically at 530 mμ in a 1-cm. absorption cell using water as the reference solution. Without delay rinse out the absorption cell containing the sample.

ANALYSIS OF SAMPLE

If the sample solution to be analyzed is acid, evaporate to dryness in a 100-ml. platinum dish. If sulfuric or perchloric acids are present, continue the evaporation on a low flame until evolution of acid fumes just ceases. If the sample solution to be analyzed is alkaline, acidify it with hydrofluoric acid in a platinum dish and then evaporate to dryness. If sexivalent chromium is present in either instance add 30% hydrogen peroxide dropwise to the acid solution until the yellow color of the chromate disappears, before evaporating the solution to dryness.

Cool, add 5 ml. of hydrofluoric-hydrochloric acid solution (20 ml. of hydrofluoric acid plus 20 ml. of hydrochloric acid plus 360 ml. of water in a polyethylene bottle). Heat while swirling on a low temperature hot plate until the residue has dissolved. Cool, pour into a 60-ml. separatory funnel, and wash-transfer with an additional 5-ml. portion of the mixed acids.

Add 5 ml. of methyl isobutyl ketone, stopper, shake vigorously for 30 seconds, and allow the two layers to separate. Drain the lower layer to a 50-ml. conical flask until the ketone layer just reaches the top of the bore of the stopcock. Pour the ketone into a clean 100-ml. platinum dish. Repeat the extraction and add the second 5-ml. ketone extract to the same dish. Evaporate the combined ketone extracts to dryness on a hot water bath under an air jet.

Add 0.25 ml. of perchloric acid plus 0.25 ml. of nitric acid and heat on a low temperature hot plate until copious fumes of perchloric acid are being evolved. Finally add 0.25 ml. of nitric acid and evaporate to complete dryness. Cool, add 1 ml. of hydrofluoric acid (1 + 99), stir with a platinum rod, and warm to dissolve the residue. [If removal of chromium has not been complete, add a drop of 30% hydrogen peroxide, evaporate to dryness under an air jet, and repeat the solution in 1 ml. of hydrofluoric acid (1 + 99).] Continue as directed in Preparation of Calibration Curve.

DISCUSSION

Masking agents such as thioglycolic acid, tiron, and hydrogen peroxide cannot be used in the above method because they complex tantalum too strongly. In contrast, the complexing action of EDTA on tantalum is much less than it is on most other metals. By arranging to make the color development at a low pH and in the presence of an excess of EDTA the interference in the photometric phenylfluorone determination of tantalum can be markedly restricted. In fact tests have shown that, of the metals listed in Table I, only germanium, tellurium, titanium, sexivalent chromium, trivalent antimony, and niobium interfere. The extent of this interference can be seen in Table II. To obtain these data an aliquot of standard tantalum solution plus an aliquot of a standard solution of the metal being tested was evaporated to dryness in a platinum dish and then treated as directed in Preparation of Calibration Curve. Strangely, the interference of tellurium, titanium, and niobium appears to be greater in the presence of tantalum than in its absence.

The interference shown in Table II can be eliminated by isolating the tantalum by the methyl isobutyl ketone solvent extraction method before attempting its determination. About 92% of the tantalum can be recovered with a single extraction or about 98% with a double extraction. Small amounts of certain metals accompany the tantalum but there appears to be very little extraction of the six interfering metals with the exception of sexivalent chromium. Extraction of the latter can be virtually eliminated by previous reduction to the trivalent state. By using the ketone extraction, the tantalum determination can be made completely specific as shown in Table I. To obtain these data, an aliquot of standard tantalum solution plus aliquots of standard solutions of several other metals were evaporated to dryness in a platinum dish and then analyzed for tantalum as directed in Analysis of Sample.

If the sample to be analyzed for tantalum contains more than about 0.5 mg. of such severely interfering metals as germanium, tellurium, or titanium it may be advisable to make an acid wash of the combined ketone extracts by shaking for 30 seconds with 5 ml. of the hydrofluoric-hydrochloric acid solution, allowing to settle, and then discarding the lower layer. An alternative procedure, in the analysis of samples containing appreciable amounts of germanium, would be to expel traces of this metal, that may have accompanied the tantalum in the extraction,

Table I. Specificity of the Proposed Method for Tantalum

Metals Added, 100 γ	Tantalum Found, γ
Ta, Na, K, Cs, Rb, Li, La	98
Ta, Ga, Hg, Au, Bi, Pt, Rh	98
Ta, P, W, Re, Mo, Si, Se	100
Ta, Fe, Mn, Pr, Hf, Th, Ru	99
Ta, Al, V, Co, Ir, Zr, Cu	100
Ta, Zn, Pb, Tl, U, Os, In	99
Ta, Sn, Be, Os, Cd, Ni, Cr(III)	100
Ta, Ce, Nb, Ba, Ca, Mg, B, Y	98
Ta, Ag, Pd, Sc, Sr, Sm	99
Ta, Ti, Te, Nb, Ge, Sb(III)	100
Ta, Nb (500 γ)	101
Ta, Ti (500 γ)	98
Ta, Te (500 γ)	98
Ta, Ge (500 γ)	100
Ta, Sb(III) (500 γ)	98
Ta, Sb(V) (500 γ)	99
Ta, Cr(VI) (500 γ)	100

Table II. Interference in Photometric Determination of Tantalum

Metals Added, 100 γ	Tantalum Found, γ
Ge + Ta	350
Te + Ta	168
Ti + Ta	120
Cr(VI) + Ta	123
Sb(III) + Ta	120
Nb + Ta	105
Nb + Ta	137[a]

[a] 1000 γ of Nb + 100 γ of Ta used.

by adding 1 ml. of hydrochloric acid to the dry residue and evaporating to dryness just before the color development.

The optimum wave length for the photometric determination of tantalum is 530 mμ, since the relative absorption of the colored tantalum compound versus the reagent background is maximum at this wave length. The calibration curve obtained at 530 mμ is linear and transmittances ranging from 85% for the reagent blank to 15% for 120 micrograms of tantalum are obtained when a 1-cm. absorption cell is used.

The intensity of the color of the tantalum-phenylfluorone solutions increases slowly with time even after a 30-minute color development. The rate of color development is influenced by such factors as the pH and the concentration of fluoride, EDTA, buffer, and phenylfluorone. All these variables must be closely controlled if reproducible results are to be obtained. When such control is maintained, satisfactory quantitative determination of tantalum is possible.

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RECEIVED for review August 25, 1958. Accepted December 9, 1958.

Hydrocarbon Compound—Type Analysis of Automobile Exhaust Gases by Mass Spectrometry

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▶ **A method of hydrocarbon-type analysis of gaseous mixtures such as automobile exhaust gases is based on mass spectrometry. Mercuric perchlorate and perchloric acid on granular firebrick were used to remove olefins and acetylenes in the gas phase. The olefinic components were removed by taking an aliquot of the gaseous sample into the mass spectrometer through an absorption column packed with mercuric perchlorate–treated firebrick. By comparing this spectrum with that of an untreated sample, a clean separation of the spectra of paraffinic and olefinic compounds could be made. The results are interpreted in terms of the hydrocarbon types—paraffins, cycloparaffins, mono-olefins, acetylenes, and cyclo-olefins, and aromatics.**

MASS SPECTROMETRY has been successfully applied to the analysis of complex mixtures of hydrocarbons, including gasoline. Brown's (*1*) comprehensive treatment of this subject, included a rather complete bibliography. Walker and O'Hara applied mass spectrometry to the analysis of automobile exhaust gases (*4*). Success depends on whether each component or type of compound yields a significantly different pattern from the others present in the mixture. In Brown's treatment, compounds of general classes were grouped together, giving four major compound types which were determined as groups of compounds. These are aliphatic paraffins, mono-olefins and cycloparaffins, aromatics, and "coda" (consisting of cyclomono-olefins, diolefins, and acetylenes).

The purpose of this work was to develop a method for distinguishing between cycloparaffins and mono-olefins in gaseous mixtures by mass spectrometry. The mass spectral patterns are too similar to allow interpretation of the spectra in terms of these two types of compounds. Brown (*1*) suggested bromination or nitrosation for differentiating between mono-olefins and cycloparaffins in the case of condensed samples. An attempt to adapt a bromination procedure to the analysis of automobile exhaust gases failed because part of the paraffins, and all of the olefins, were lost in contacting the gases with an aqueous bromine solution.

The work of Young, Pratt, and Biale (*5*), in which aqueous mercuric perchlorate solution was used to trap ethylene produced by ripening fruit, suggested a solution. Mercuric perchlorate is capable of holding ethylene and other olefins through π-bonding of the mercury with the unsaturated bond of the olefin. It was correctly assumed that solid mercuric perchlorate might serve equally well. Mercuric perchlorate and perchloric acid on the surface of 40- to 60-mesh Johns-Manville firebrick effectively remove olefins from a gaseous hydrocarbon mixture. This method, combined with mass spectroscopy, makes it possible to differentiate between olefins and cycloparaffins.

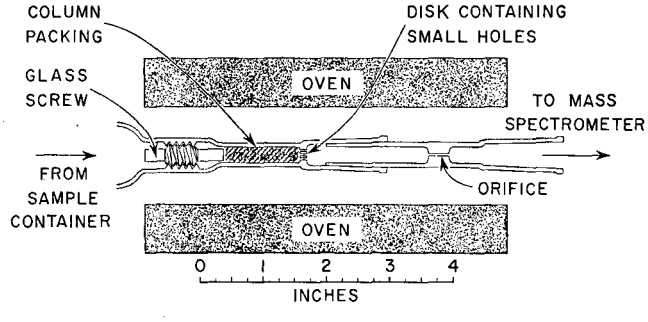


Figure 1. Olefin absorption tube

EXPERIMENTAL

Several synthetic mixtures containing a limited number of saturated and unsaturated aliphatic hydrocarbons and sufficient air to give hydrocarbon concentrations similar to the total hydrocarbon concentration present in automobile exhaust gases were prepared in 1-liter stainless steel cylinders. These cylinders were maintained at 100° C. throughout the experiments. The mass spectral pattern for an aliquot of each mixture was quantitatively in agreement with that expected on the basis of the individual patterns for these components. A Consolidated Electrodynamics Corp. Model 21-401 mass spectrometer, which measures m/e^+ values up to 115, was used.

A second aliquot of the synthetic mixture was introduced into the mass spectrometer gas handling system through a 40- to 60-mesh mercuric perchlorate–treated Johns-Manville firebrick column at various temperatures at the rate of 110 cc. per minute. This olefin absorption column is shown in Figure 1. The column packing was 15 mm. in length and 7 mm. in diameter, weighed approximately 0.2 gram

and was held in place by a loose glass screw. The mercuric perchlorate–treated brick was prepared by mixing one part by weight of 40- to 60-mesh firebrick with one part by weight of a solution that was 1*M* in mercuric perchlorate and 2*M* in perchloric acid. The moist brick was spread thinly and dried for 1 hour at 110° C. This mercuric perchlorate–treated brick was stored in a stoppered glass bottle until used. There was no evidence of loss of activity due to storage. The flow rate through the adsorption column was controlled by a critical orifice placed between the column and the mass spectrometer inlet. Both the column and the orifice were well inside the furnace and were maintained at the furnace temperature.

To minimize adsorption effects and to study the capacity of the column packing, the system was flushed for a predetermined time with the Hoke valve (Hoke, Inc.) of the cylinder open and the stopcock to the mass spectrometer vacuum source open. The flow through the system was controlled by the orifice during this flushing operation and also during the actual introduction of the sample. The adsorption efficiency of the column for olefins in a synthetic mixture was studied at several temperatures with the flow rate and flushing time held constant to determine the effect of temperature. Results of this study are summarized in Table I.

The capacity of the column for adsorption of olefins was studied at 100° C., with a flow rate of 110 cc. per minute and with various flushing times. These results, based on data in Table III, are given in Table II. In a similar study with a second synthetic mixture containing 0.0609, 0.0834, 0.0496, 0.0503, 0.0766, and 0.0758 mole % of methylcyclohexane, methylcyclopentane, 2,4,4-trimethyl-2-pentene, 2,4,4-trimethyl-1-pentene, cyclohexene, and 2-methyl-1,3-butadiene, respectively, for flushing times of 0.2, 1, 2, and 3 minutes, the olefin and coda compounds were quantitatively removed. However, for flushing times appreciably longer than 3 minutes, the column became depleted and olefin and coda compounds passed through the column. A freshly packed column must be used for each experiment.

A number of compounds were tested individually to determine whether they passed through the mercuric perchlorate–brick column at 100° C. into the mass spectrometer. The results of these tests, with the results for mixtures, are summarized in Table IV. Including the compounds from Tables I, II, and IV, there were six paraffins, three cycloparaffins, seven mono-olefins, and three coda compounds.

The olefinic-type compounds were quantitatively removed from the gas stream and the paraffinic types were quantitatively passed through the column. It was concluded that the method was sufficiently specific to make this type of split of the components of automobile exhaust gases. Aromatic compounds are not quantitatively removed from the gas stream by the mercuric perchlorate column. Because of this it was most convenient to use characteristic peaks of aromatics from the pattern of the untreated aliquot. The conditions chosen for the studies of automobile exhaust gases and gasoline-air mixtures were those found most successful for synthetic mixtures. The mercuric perchlorate–brick column was maintained at 100° C., the flow rate during the flushing and sampling operations was 110 cc. per min., controlled by a critical orifice, and a flushing time of 1 minute was used.

Experiments with a column packed with untreated 40- to 60-mesh firebrick gave results that were quantitatively identical with those for aliquots introduced directly into the mass spectrometer. The automobile exhaust gas samples were collected and stored in previously evacuated 1-liter, stainless steel cylinders. The cylinders were maintained at 100° C. during collection, storage, and analysis to prevent loss of some of the less volatile components through condensation. Similar precautions were taken with synthetic samples.

Before the spectral patterns were interpreted, they were all corrected for the mass spectrometer background and normalized to comparable sensitivities. In the case of the automobile exhaust gas samples, normalization was based on the argon peak at an m/e^+ ratio of 40. This introduced a slight error due to the small contribution of certain hydrocarbons, but was more satisfactory than normalization based on the nitrogen peak at an m/e^+ ratio of 28. The argon sensitivity at m/e^+ of 40 was 540 divisions per micron.

INTERPRETATION OF MASS SPECTRAL DATA

For complex hydrocarbon mixtures it is generally impossible to set up and solve the necessary set of simultaneous equations that would give a unique solution. However, as Brown (*1*) has demonstrated, it is possible to analyze the patterns according to the compound types, such as paraffins, cycloparaffins, mono-olefins, coda, and aromatics. In this case, a somewhat simplified treatment of the data was made possible by experimentally splitting the patterns according to groups of compound types. These groups are paraffins and cycloparaffins, olefins and coda, and aromatics. This was accomplished by determining mass spectral patterns for two aliquots of the sample. One aliquot was introduced directly into the mass spectrometer and a second aliquot was introduced through a mercuric perchlorate column which selectively removed the olefins and coda compounds and part of the aromatics.

Fortunately, there is very little contribution by the simpler aromatic compounds to the mass spectral peaks that are characteristic of paraffins, cycloparaffins, olefins, and coda compounds, and very little contribution to the aromatic compound patterns by compounds of the other groups. It is true that coda compounds may have small contributions at the aromatic peaks. In the case of automobile exhaust gases these contributions may be ignored because of the relatively small total concentrations of coda compounds compared with the concentrations of aromatic compounds. This makes it possible to use certain peaks characteristic of aromatics from the pattern of the whole sample for the deter-

Table I. Effect of Temperature on Removal of Olefins by Mercuric Perchlorate–Brick Column

(Flow rate, 110 cc./min.; flushing time, 1 min.; sample, synthetic hydrocarbon–air mixture[a])

Column Temp., ° C.	Result
25	Apparent increase of 50% in paraffins over reference sample concentrations. Probably due to adsorption during flushing period and desorption during sampling
60	Adsorption and release of paraffins similar to 25° C. experiment. Smaller increase in paraffins
100	Mass spectral pattern identical with that for paraffinic components of the mixture

[a]

Component	Mole %
n-Pentane	0.0990
2-Methylpentane	0.0929
3-Methylpentane	0.0925
n-1-Octene	0.0784
n-2-Hexene	0.0980
2-Methyl-2-butene	0.1120

Table II. Effect of Flushing Time through Mercuric Perchlorate–Brick Column on Removal of Olefinic Compound Types

(Temp., 100° C.; flow rate, 110 cc./min.; sample, synthetic hydrocarbon–air mixture[a])

Flushing Time, Min.	Results (Patterns Given in Table III)
1	Mass spectral pattern for paraffinic materials only
2, 4, 8,	Patterns became complicated by compounds not originally present and by preferential elution of certain olefins. This effect increased with increasing flushing time

[a]

Component	Mole %
n-Pentane	0.193
2-Methylpentane	0.179
3-Methylpentane	0.178
n-1-Octene	0.152
n-2-Hexene	0.189
2-Methyl-2-butene	0.216

Table III. Mass Spectral Patterns for Synthetic Hydrocarbon–Air Mixture in Table II

	Peak Heights				
m/e^+	Untreated aliquot	$Hg(ClO_4)_2$ 1 min.	$Hg(ClO_4)_2$ 2 min.	$Hg(ClO_4)_2$ 4 min.	Air sample (background)
2	8.0	6.7	7.6	6.4	7.8
15	4.9	5.0	7.5	10.0	1.4
18	208	1550	1257	1188	189
25	1.3	1.1	1.2	1.3	1.0
26	11.5	8.6	10.3	10.9	6.0
27	75.6	48.9	58.2	65.1	10.1
28	26310	24960	24390	24570	27480
32	5830	5500	5390	5440	6040
39	54.0	20.6	34.0	44.4	1.2
40	449	420	412	419	458
41	154.8	85.8	118.2	145.5	2.3
42	173.7	98.7	121.2	139.5	1.5
43	230	216	245	290	3.3
44	49.4	53.7	53.9	56.5	40.5
53	14.6	3.1	6.1	8.7	0.4
54	8.8	1.0	3.7	5.6	0.4
55	170.1	12.9	59.3	89.4	1.5
56	102.9	60.9	73.5	83.4	1.0
57	101.4	99.9	100.8	101.4	1.5
58	4.5	15.3	19.5	22.1	0.2
67	6.2	0.8	2.5	3.6	0.4
68	2.7	0.5	1.2	2.0	0.3
69	31.6	1.7	18.7	33.1	0.8
70	62.7	9.7	24.3	31.7	1.4
71	40.6	39.4	40.0	40.5	0.3
72	11.0	11.6	11.6	11.5	0.5
81	0.8	0.8	1.2	1.9	0.4
82	1.3	0.5	0.7	1.0	0.3
83	8.0	0.8	3.6	8.5	0.8
84	29.4	0.7	18.7	34.6	0.3
97	1.1	0.4	1.2	1.3	0.3

Table V. Mass Spectral Sensitivities for C_3^+ Hydrocarbons[a]

Sensitivities (fractional parts of *n*-butane m/e^+ 43 peak height per micron of sample pressure)

Hydrocarbon Type	Σ43	Σ_c41	Σ_o41	Σ53	Σ77
C_3^+ paraffins	1.70	0.55	..	..	..
Cycloparaffins	0.18	1.60	..	..	..
C_3^+ mono-olefins	..	..	1.05	0.08	..
C_3^+ coda	..	..	0.37	0.84	..
Aromatics	..	..	..	..	2.00

Notation	Peak Heights Summed, m/e^+ Ratios	Pattern[b] to Which Summation Applies
Σ43	43, 57	Paraffins and cycloparaffins
Σ_c41	41, 55, 69	Paraffins and cycloparaffins
Σ_o41	41, 55	Olefins and coda
Σ53	53, 67	Olefins and coda
Σ77	77, 78, 91, 92, 105, 106	Aromatics

[a] Subscript $_3^+$ refers to number of carbon atoms in hydrocarbon molecules.
[b] Paraffin and cycloparaffin pattern was obtained for an aliquot from which olefins were removed. Olefin and coda pattern was obtained by difference between total pattern for an aliquot and a second aliquot from which olefin and coda compounds were removed. Aromatics pattern was taken from untreated aliquot.

mination of the concentration of aromatics. The pattern resulting from the aliquot from which the olefin and coda compounds were removed represents the paraffins and cycloparaffins, with little interference from aromatics at the peaks characteristic of paraffinic type compounds.

The pattern representing the olefins and coda compounds is equal to the difference between the pattern for the untreated aliquot and for the aliquot from which the olefin and coda compounds were removed.

The method of analysis of mass spectral data was similar to that used by Brown (*1*). He pointed out that spectra of paraffins show strong peaks at m/e^+ ratios of 43, 57, 71, 85, and 99; spectra of mono-olefins and cycloparaffins show strong peaks at m/e^+ ratios of 41, 55, 69, 83, and 97; spectra of cycloolefins, diolefins, and acetylenes at m/e^+ ratios of 53, 67, 68, 81, 82, 95, and 96; and spectra of aromatics at m/e^+ ratios of 77, 78, 79, 91, 92, 105, 106, 119, 120, 133, and 134.

Table V lists the mass spectral peaks used in this study to represent various classes of compounds and the relative sensitivites for the summations of peak heights necessary to resolve patterns according to compound types. These sensitivities were based on calibrations for a

Table IV. Compounds Studied for Mercuric Perchlorate–Brick Column Adsorption

Not Adsorbed	Partially Adsorbed	Adsorbed
Methane	Benzene	Ethylene
Ethane	Toluene	Acetylene
Methyl-cyclo-pentane	Xylenes	2-Methyl-2-butene
		n-1-Hexene
Methyl-cyclo-hexane		*n*-2-Hexene
		Cyclohexene
Cyclohexane		2,4,4-Trimethyl-2-pentene
n-Pentane		2,4,4-Trimethyl-1-pentene
n-Hexane		2-Methyl-1,3-butadiene
2-Methyl-pentane		*n*-1-Octene
3-Methyl-pentane		

representative group of hydrocarbons of each type, using a Consolidated Electrodynamics Corp. Model 21-401 mass spectrometer. They were consistent with the more expensive data given in the tabulations of the American Petroleum Institute Research Project 44. They were also consistent with the sensitivities used by Brown (*1*) for the analysis of liquid hydrocarbon mixtures.

The following equations were used for a quantitative estimation of the various hydrocarbon types based on the mass spectral patterns, using the mercuric perchlorate method of splitting the sample. Equations 1, 2, 3, and 4 were used to calculate pressures of saturates and apply to paraffin and cycloparaffin patterns.

$$C_3^+ \text{ paraffins, microns} = (0.61\ \Sigma 43 - 0.069\ \Sigma_c 41)A \quad (1)$$

$$\text{Cycloparaffins, microns} = (0.65\ \Sigma_c 41 - 0.20\ \Sigma 43)A \quad (2)$$

$$\text{Methane, microns} = 2.30\ [(15 \text{ peak height}) - 0.007\ (\Sigma_c 41 + \Sigma_{43}) - 0.50\ (26 \text{ peak height})]A \quad (3)$$

$$\text{Ethane, microns} = 6.33\ [(26 \text{ peak height}) - 0.01\ (\Sigma_c 41 + \Sigma 43)]A \quad (4)$$

Equations 5, 6, 7, and 8 were used to calculate pressures of unsaturates and apply to olefin and coda patterns.

$$C_3^+ \text{ olefins, microns} = (0.99\ \Sigma_o 41 - 0.44\ \Sigma 53)A \quad (5)$$

$$C_3^+ \text{ coda, microns} = (1.24\ \Sigma 53 - 0.094\ \Sigma_o 41)A \quad (6)$$

$$\text{Ethylene, microns} = [(27 \text{ peak height} - 0.18\ \Sigma_o 41)2.48 - (26 \text{ peak height} - 0.036\ \Sigma_o 41)0.077]A \quad (7)$$

$$\text{Acetylene, microns} = [(26 \text{ peak height} - 0.036\ \Sigma_o 41)1.17 - (27 \text{ peak height} - 0.18\ \Sigma_o 41)1.05]A \quad (8)$$

Equation 9 was used to calculate aromatics and applies to aromatic patterns.

$$\text{Aromatics, microns} = (0.50\ \Sigma 77)A \quad (9)$$

A in Equations 1 through 9 is the reciprocal of the n-butane sensitivity at an m/e^+ ratio of 43. Propyne is not included in the C_3^+ coda equation. Its determination on the basis of the peak at an m/e^+ ratio of 39 is complicated by contributions from most of the other classes of compounds. In addition to C_3^+ hydrocarbons, methane, ethane, ethylene, and acetylene can be determined by applying appropriate corrections for the C_3^+ hydrocarbon contributions to the peaks with m/e^+ ratios of 15, 26, and 27. The presence of nitrogen in automobile exhaust gases makes the m/e^+ 28 peak unusable. The inverse solutions for the simultaneous equations are all given in terms of the mass spectral sensitivity for n-butane at an m/e^+ ratio of 43, making possible the direct application of these equations to data from mass spectrometers that give similar cracking patterns.

ANALYSIS OF AUTOMOBILE EXHAUST GASES

This method of analysis of automobile exhaust gases has been used extensively in this laboratory. The experimental part is simple to perform and the calculations can be performed manually. An IBM computer (International Business Machines Corp.) has been used for comprehensive studies. The time from the beginning of sample collection to the completion of the calculation need not be greater than 2 to 3 hours.

Mass spectral data, obtained by the mercuric perchlorate adsorption method, for a typical modern gasoline and a 1955 model automobile operated under conditions of idling, acceleration, deceleration, and crusing are given in Table VI. The total patterns for the untreated samples are the sums of the split patterns given in Table VI for each sample. Table VII interprets the data in Table VI in terms of individual components and compound types. A pressure of 90 microns in the mass spectrometer was used in each case and the mole per cent for each component is given by

$$\text{Mole per cent} = \frac{(\text{pressure in microns from Equations 1–9}) \times 100}{90 \text{ microns}} \qquad (10)$$

ANALYSIS OF RAW GASOLINES

Five gasolines were subjected to hydrocarbon-type analysis based on the mercuric perchlorate–mass spectral method and the fluorescence indicator adsorption chromatographic method (FIA) developed by Criddle and LeTourneau (*2*). The mass spectral patterns for the whole gasolines were determined on samples introduced into the mass spectrometer by the microburet technique of Taylor and Young (*3*). These patterns were used as reference patterns for dilute gasoline-air mixtures prepared in 1-liter stainless steel cylinders. The similarity of these reference patterns and those based on the gasoline-

Table VI. Mass Spectral Data for Automobile Exhaust Gases

	Peak Heights (Divisions)[a]							
	Idle		Acceleration		Deceleration		Cruising	
m/e^+	A[b]	B[c]	A[b]	B[c]	A[b]	B[c]	A[b]	B[c]
2	279.7	−13.0	465.8	−11.3	871.2	−79.2	383.4	−8.6
15	2.4	2.4	8.9	−0.1	27.8	−1.2	7.1	−0.1
18	2274	−406	2363	−616	2430	−957	1475	−588
25	0.1	1.4	0.5	2.0	0.4	11.4	0.7	1.7
26	3.1	10.5	4.8	10.5	5.2	69.1	4.0	10.6
27	13.5	5.4	12.9	6.4	16.6	22.3	10.9	4.7
28	32980	−327	31440	−315	31850	465	31210	97.4
32	678.3	−21.2	103.6	42.5	528.0	0.1	90.5	3.3
39	1.8	3.5	0.8	3.0	4.2	11.2	0.9	3.2
40	479.6	0.0	479.6	0.0	479.6	0.0	479.6	0.0
41	4.2	4.4	2.5	3.6	11.4	12.6	2.2	4.1
42	4.7	0.4	2.7	1.6	10.9	5.5	2.1	2.0
43	8.9	−0.5	5.8	−0.2	23.2	−1.6	5.1	−0.1
44	5968	−61	5428	−280	4731	−205	5529	−318
53	0.2	0.7	0.2	0.9	0.9	2.6	0.2	0.7
55	2.4	2.3	1.5	1.2	6.9	5.6	0.9	1.5
56	3.0	1.6	2.0	0.7	9.6	2.9	1.3	1.1
57	3.8	0.1	2.0	0.3	10.4	−0.1	2.0	−0.1
67	0.7	0.7	0.1	0.8	0.7	3.3	0.4	0.3
69	1.4	1.3	0.8	0.5	3.8	2.3	0.7	0.4
70	1.6	1.3	1.1	0.4	5.6	1.5	0.5	0.4
71	1.7	−0.9	0.4	0.1	4.2	−0.4	0.6	−0.1
	Aromatics							
77	0.1		0.7		3.1		0.7	
78	1.6		2.0		9.5		2.2	
91	4.6		3.4		14.2		3.5	
92	2.0		1.8		6.1		1.3	
105	1.5		0.5		5.0		0.8	
106	1.7		0.9		3.9		1.2	

[a] Mass spectrometer operated with ion current of 90 μa. and sample pressure of 90 microns. Butane 43 peak sensitivity was 533 divisions per micron.
[b] Paraffin and cycloparaffin pattern.
[c] Olefin and coda pattern.

Table VII. Composition of Automobile Exhaust Gases

(Mole %)

Hydrocarbon Type	Operating Conditions			
	Idle	Acceleration	Deceleration	Cruising
Methane	0.003	0.030	0.116	0.023
Ethane	Present[a]	Present[a]	Present[a]	Present[a]
Ethylene	0.020	0.027	0.087	0.017
Acetylene	0.016	0.013	0.125	0.017
C_3^+paraffins	0.015	0.009	0.040	0.008
Cycloparaffins	0.005	0.003	0.016	0.002
C_3^+ mono-olefins	0.012	0.008	0.032	0.010
C_3^+ coda	0.002	0.003	0.012	0.001
Aromatics	0.012	0.010	0.044	0.010

[a] Quantitative estimation of ethane was made impossible by variations in mass spectrometer background at m/e^+ ratio of 26. Maximum value could be determined on basis of data in Table VI and Equation 9.

Table VIII. Gasoline Analysis

	Components					
	Saturates		Unsaturates		Aromatics	
Gasoline Sample	Mass spec., mole %	FIA, vol. %	Mass spec., mole %	FIA, vol. %	Mass spec., mole %	FIA, vol. %
1	87.3	93.8	0.6	0.3	12.1	5.9
2	73.6	76.4	16.4	14.0	10.0	9.6
3	53.3	60.4	26.7	17.5	20.0	22.1
4	57.2	51.7	30.7	32.5	12.1	15.8
5	29.4	34.0	49.4	38.1	21.2	27.9

air mixtures is evidence that there were no serious losses in the latter case. The complete analyses were based on the gasoline-air mixtures. Gasoline concentrations of 20 to 25 mg. per liter of air at 100° C. at atmospheric pressure were used, giving total hydrocarbon concentrations of approximately 0.5 mole %. These concentrations were purposely made somewhat higher than those found in actual automobile exhaust gas samples to reduce the uncertainty in experimental measurements.

The hydrocarbon types in these gasoline-air mixtures were determined on the basis of Equations 1 through 9. For comparison with the results on the liquid gasoline by the FIA method, the paraffins and cycloparaffins were assumed to represent saturates; the olefins and coda compounds were assumed to represent unsaturates, and the aromatics were compared directly. The agreement in Table VIII between the mass spectral results and FIA results is satisfactory considering the approximations involved. No attempt was made to introduce a mole to volume factor, nor were the mass spectral coefficients for the various compound types adjusted to fit gasoline type compounds rather than those predominant in automobile exhaust gases.

ACKNOWLEDGMENT

The mass spectral determinations were performed by Lydia Peters and W. C. Crawford. The exhaust gas samples were collected by R. W. Gates and I. S. Yaffe under the direction of W. C. Thuman. The author is indebted to Harold Eding for assistance and advice in interpretation of the mass spectra.

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RECEIVED for review February 15, 1958. Accepted December 11, 1958. Work supported by The Western Oil and Gas Association.

Identification of Position Isomers of Some Stearic Acid Derivatives by Infrared Spectroscopy

Study of *trans* 6- through 11-Octadecenoic Acids and Corresponding Contiguously Substituted Dihydroxystearic Acids in the Crystalline State

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▶**The position of the double bond in *trans* 6- through 11-octadecenoic acids can be determined by studying the equally spaced absorption bands in the 1180- to 1350-cm.$^{-1}$ region. This band progression, which in the case of *n*-saturated fatty acids depends on the total chain length, is related to the chain segment next to the carboxyl group. The corresponding dihydroxy acids can be distinguished by studying the 800- to 1200-cm.$^{-1}$ region. The high and low- melting series show distinct differences. Spectral variations within the low- or high-melting series are small, but in many cases sufficient to permit positive identification.**

JONES, McKay, and Sinclair have pointed out that in the infrared absorption spectra of crystalline fatty acids a progression of uniformly spaced bands is observed in the 1180- to 1350-cm.$^{-1}$ region and that the number of bands in the progression is related to chain length (*4*). For *cis*-unsaturated acids the progression becomes irregular, but for *trans*-9,10-octadecenoic acid, the region is similar to one of the short chain saturated acids—e.g., lauric—suggesting independent behavior of the two chain sections (*4*). Corish and Chapman (*2*) found similar bands in the spectra of lower homologs (C_2 to C_{12}), although the regularity seems to decrease with decreasing chain length. Meiklejohn *et al.* (*6*) have found that in the higher members the number of bands in the progression is approximately equal to half the number of carbons in the chain. Jones, McKay and Sinclair (*4*) suggested that the bands arise from the rocking and/or twisting vibrations of the CH_2 groups. Ferguson (*3*) noticed that the single crystal spectra of eicosanoic acid obtained with polarized radiation by Cole and Jones (*1*) do not show parallel polarization of the progression bands, as would be expected for wagging or twisting modes. Meyer and Schuette (*7*), who studied over 300 long-chain compounds, observed that all isomeric cis and trans forms of the octadecenoic acids gave fewer bands in the 1180- to 1850-cm.$^{-1}$ region than was observed for stearic acid. As the double bond approached the terminal methyl group, these bands increased in number.

Five position isomers of *trans*-octadecenoic acid have been examined for correlations between the band progression and the position of the double bond. The results indicate that the number of bands in the progression series is related to the (total) length of the chain segment next to the carboxyl group. This offers a way to determine the location of the double bond in *trans*-monounsaturated fatty acids. In the particular series studied, the results are unambiguous. One sample (elaidic acid) was studied with polarized radiation to pinpoint the progression bands and locate the 1300-cm.$^{-1}$ region carboxyl band. The results indicate that the weak bands on the high frequency side of the progression region are of a different nature than the four main bands and that—contrary to saturated acids and particularly, eicosanoic acid (*1*)—no distinct carboxyl band is found in this region.

Experimental. The fatty acids have been described (*9*). The infra-

red spectra were obtained using a Beckman Model IR-3 instrument equipped with sodium chloride optics and a Perkin-Elmer Model 21 instrument equipped with sodium chloride optics and a silver chloride polarizer. The latter was used to study the sample of *trans*-9,10-octadecenoic acid with polarized radiation. *trans*-6,7-; *trans*-7,8-; *trans*-8,9-; *trans*-9,10-, and *trans*-11,12-octadecenoic acids were studied as potassium bromide pellets. These were prepared by grinding about 1.5 mg. of the dry solid sample together with about 300 mg. of dry potassium bromide for 2 minutes in a vibrator-grinder and pressing the resulting mixture in a $^1/_2$-inch diameter die by applying a total force of 20,000 pounds for 5 minutes.

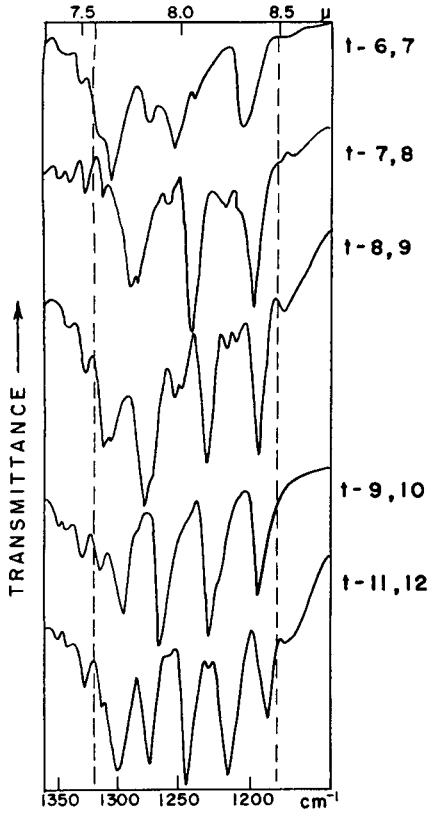


Figure 1. 1175- to 1350-cm.$^{-1}$ region of *trans*-octadecenoic acids

An oriented sample of the *trans*-9,10-acid was prepared by melting the substance between two rock salt plates and subsequent cooling, using a temperature gradient. The resulting oriented film was studied with the electric vector first parallel and then perpendicular to the direction of crystal growth.

Results and Discussion. Figures 1 and 2 illustrate that in the region roughly between 1180 and 1320 cm.$^{-1}$ there exists a series of relatively strong, equally spaced bands just as in the case of saturated acids. The "rule of two" relationship observed by Meiklejohn and coworkers (*6*) apparently applies to the first segment of the hydrocarbon chains in the *trans*-unsaturated acids studied. The number of these bands is equal to $n/2$ for the even members and to $(n - 1)/2$ for the odd members, if n is the number of carbon atoms in the chain segment between the carboxyl group and the double bond, the end atoms of the segment included. Some weaker, irregular bands are also observed, but the regular spacing and higher relative intensity of the progression bands makes their recognition relatively easy. Although in the saturated fatty acids the number of bands is indicative of the whole chain length, in unsaturated acids, the bands indicate the length of the chain segment next to the carboxyl group. This is illustrated by a comparison of Figures 2 and 3. Figure 3 shows schematically the approximate positions and relative intensities of the progression bands of the C_6 to C_{12} saturated acids. The data are computed from absorption curves published recently by Corish and Chapman (*2*) and obtained by them on solid samples using a low temperature cell. Data for the C_{11} saturated acid and *trans*-10,11-octadecenoic acid were not available. The estimated band positions for these compounds are indicated by broken lines in Figures 2 and 3.

On the high frequency side of the band progression of the unsaturated acids there exists another progression of much weaker intensity (Figures 1 and 2). These bands are not influenced by the position of the double bond and appear at about the same positions for all odd and even acids studied. They should not be included in the progression which is related to the length of the chain segments.

The terminal chain segments (from the double bond to the methyl group) do not seem to give rise to regularly spaced medium intensity bands. In this aspect they behave much like unsubstituted *n*-paraffins which show progression bands of considerably lower intensity and less regular spacings than the corresponding fatty acids. Some weak bands occurring between the main progression bands, and partially overlapping with them, might be caused by the terminal segments, but no conclusions seem possible at present, except that the double bond effectively interrupts the chains without having a noticeable activating effect.

Complications may be encountered in determinations of the lengths of chain segments by the band progression method of which the first is the problem of polymorphism. Jones, McKay, and Sinclair (*4*) have shown that in the case of stearic acid, various polymorphic forms absorb differently in the infrared, including the progression region. In the case of *trans*-octadecenoic acids, no polymorphism has been reported (*5*). No irregularities which might be caused by different crystal configurations have been found in this laboratory. Nevertheless, polymorphism is a potential source of trouble and should

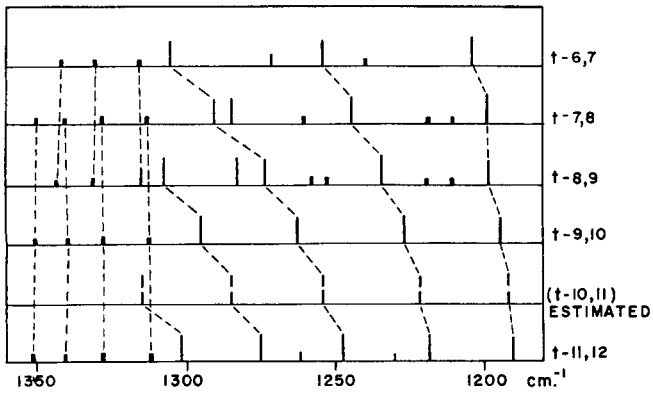


Figure 2. Schematic presentation of absorption bands of *trans*-octadecenoic acids in 1175- to 1350-cm.$^{-1}$ region

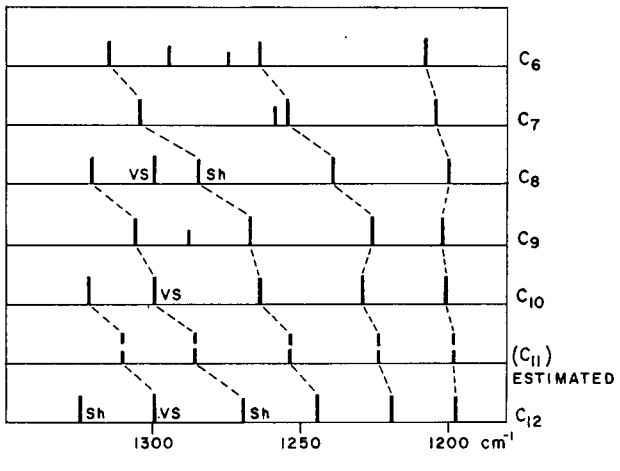


Figure 3. Schematic presentation of absorption bands of C_6 to C_{12} saturated fatty acids in 1175- to 1350-cm.$^{-1}$ region

VS. Very strong SH. Shoulder

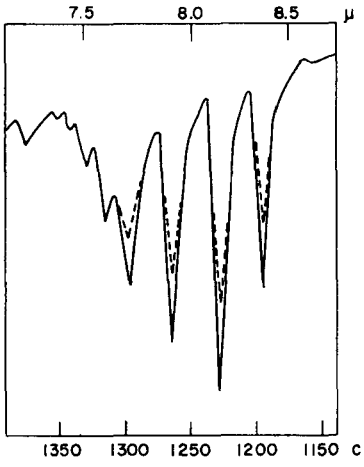


Figure 4. 1150- to 1350-cm.$^{-1}$ region of *trans*-9,10-octadecenoic acid observed with polarized radiation

— Electric vector parallel to direction of crystal growth
--- Electric vector perpendicular to direction of crystal growth

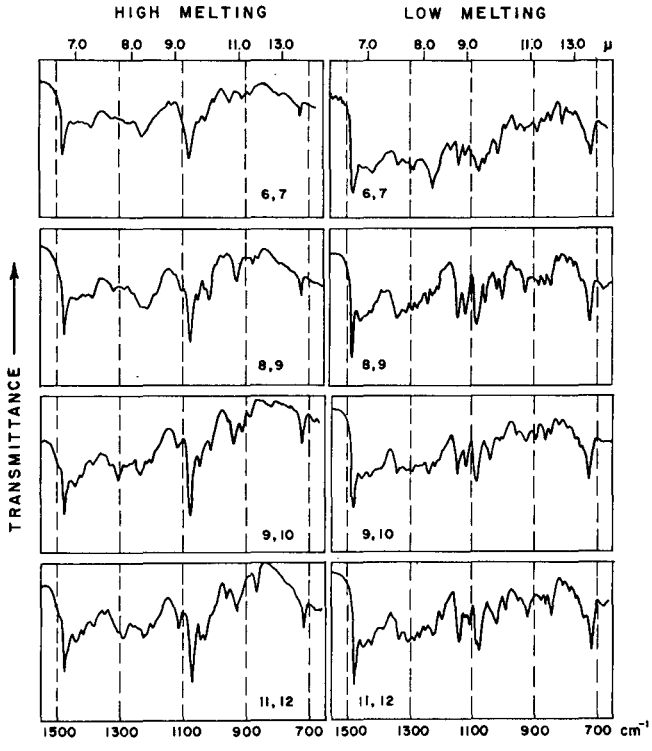


Figure 5. 1550- to 700-cm.$^{-1}$ region of high and low melting 6,7-, 8,9-, 9,10-, 11,12-dihydroxystearic acids

be considered in attacking a new group of compounds.

Also, in most fatty acid spectra there is a strong carboxyl band around 1300 cm.$^{-1}$ which overlaps with the progression and sometimes renders the counting of the significant bands difficult. Figure 3 shows the presence of such a band in several of the saturated acids. In the series of octadecenoic acids no band which could obviously be assigned to the carboxyl group was found. Cole and Jones (*1*) have shown that in eicosanoic acid, the 1300-cm.$^{-1}$ carboxyl band is polarized differently from the progression bands. In *trans*-9,10-octadecenoic acid, on the other hand, all four major bands in the progression region show the same polarization, whereas the weak bands on the high frequency side of the region show no polarization under the same conditions (Figure 4). This seems to indicate that the carboxyl band is relatively weak in this series and does not overlap with the bands of the progression.

A further problem is the setting of the frequency limits of the progression region. For the saturated acids, Meiklejohn *et al.* (*6*) have somewhat arbitrarily chosen the region between 7.43 and 8.47 microns (about 1346 to 1180 cm.$^{-1}$). This is not appropriate in this series of unsaturated compounds. The weak bands in the 1300- to 1350-cm.$^{-1}$ region should be excluded because the spacings are irregular, the positions do not change with chain length, the intensity is considerably lower, and the polarization characteristics are different. These observations indicate that no universally valid progression region can be defined. For each group of compounds the bands belonging to the progression should be identified separately by applying the above criteria.

Progression bands are usually assigned to coupled —CH_2— wagging and/or twisting vibrations (*4*, *6*). Ferguson (*3*) has recently pointed out that for eicosanoic acid the polarization data (*1*) are not in agreement with such an assignment. The results obtained by Meiklejohn *et al.* (*6*) on saturated acids and obtained in this laboratory on the octadecenoic acids indicate that the number of bands is closely related to the total length of the chain (or chain segment) rather than the number of CH_2 units. The same conclusion is suggested by the data of Corish and Chapman (*2*).

DISTINGUISHING BETWEEN POSITION ISOMERS OF CONTIGUOUSLY SUBSTITUTED DIHYDROXYSTEARIC ACID

Hydroxylation results in a different kind of modification of the basic structure than unsaturation. The chain of single-bonded carbon atoms is preserved, but the regular packing of the individual molecules is altered by the voluminous hydroxyl groups as well as by the new hydrogen bonds introduced. A different type of change in the band progression, which depends on the packing of the molecules as well as on the length of chain segments (*8*) would be expected. Specific COH absorption bands should occur around 3400 and 1000 cm.$^{-1}$ The exact positions of these bands would be expected to depend on the position of the hydroxyl groups and on the hydrogen bonds formed.

Experimental. The preparation of the high and low melting dihydroxystearic acids has been described (*9*). Infrared spectra of the 6,7-, 8,9-, 9,10-, and 11,12-members of the high and low melting series were obtained on a Perkin-Elmer Model 21 instrument by using the scanning and sampling techniques described. Some of the potassium bromide pellets were foggy and caused excessive scattering. Increasing the pressing time from 5 to 10 minutes eliminated this difficulty. Prolonged grinding (up to 10 minutes) was also tried, but resulted in loss of detail in the resulting spectra, probably because of a breakdown of crystallinity. Polymorphism is not ruled out in the dihydroxy acids, although care was taken to prepare all the samples by identical procedures (*9*).

Results and Discussion. All hydroxylated acids show strong absorption around 3400 cm.$^{-1}$ and a series of medium strength bands between 1000 and 1200 cm.$^{-1}$, corresponding to the O—H stretching vibrations and the C—O— stretching and/or deformation vibrations, respectively. The 1180- to 1350-cm.$^{-1}$ region shows only weak bands with no apparent regularity.

The infrared spectra are extremely similar above 1550 cm.$^{-1}$, showing a broad, unresolved O—H band around 3400 cm.$^{-1}$, C—H stretching bands around 2900 cm.$^{-1}$ and the carboxyl band at 1710 cm.$^{-1}$ Figure 5 gives the observed spectra in the 700- to 1550-cm.$^{-1}$ region. The most obvious differences between the various isomers occur between 1000 and 1150 cm.$^{-1}$,

in a region commonly associated with COH vibrations. Members of the high melting series are clearly distinguishable from the corresponding low melting substances by a comparison of the absorption patterns in this region. The low melting members have a more complex structure, as might be expected from the complicated interactions and hydrogen bonds, which are possible if both groups are on the same side of the chain. (Similar complexities might be expected in the OH stretching region if investigated under higher resolution. Current studies with sodium chloride optics showed merely the presence of a broad unresolved band which was similar for all compounds.) The differences between the individual members within one series are small, but sufficient to allow identification and differentiation in a number of instances. No regularities comparable with the band progression of unsaturated acids could be detected.

Aside from the bands which could be associated with the hydroxyl groups, puzzling phenomena appear on bands commonly associated with the carboxyl group. Thus, the strong band usually appearing around 1300 cm.$^{-1}$ is occasionally shifted to 1225 cm.$^{-1}$ and the band around 925 cm.$^{-1}$ is split into a number of weaker bands, giving rise to a pattern which appears to be different for each compound. Together with the 1000- to 1200-cm.$^{-1}$ region this furnishes sufficiently distinctive fingerprint patterns to allow the identification of each individual isomer by infrared spectra.

The position isomers of contiguously substituted dihydroxystearic acid could not be identified by clear-cut regularities like the *trans*-monounsaturated acids, but in the region roughly between 800 and 1200 cm.$^{-1}$, there is enough spectral detail to allow identification on a fingerprint basis.

ACKNOWLEDGMENT

The author is indebted to H. B. Knight and Daniel Swern of this laboratory, and to J. B. Brown, Ohio State University, for the samples of hydroxylated and unsaturated acids. Thanks are also due to G. C. Nutting for useful suggestions and to Anne Smith and Carl T. Leander, Jr., for help in the experimental work.

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RECEIVED for review February 28, 1958. Accepted December 18, 1958. Presented in part at the Ninth Annual Conference of Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., March 1958. Mention of specific products does not imply endorsement by the department to the possible detriment of others not mentioned.

Spectrophotometric Determination of Residual Δ^4-3-Ketosteroids in Bulk $\Delta^{1,4}$-3-Ketosteroids

Differential Rates of Thiosemicarbazone Formation

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▶ **Chemical or microbiological production of $\Delta^{1,4}$-3-ketosteroids from Δ^4-3-ketosteroids requires a method for measuring small amounts of residual Δ^4-3-ketosteroids in $\Delta^{1,4}$-3-ketosteroids. A spectrophotometric procedure is based on the difference in rates of thiosemicarbazone formation. Analysis of standard mixtures of representative steroid pairs indicates a mean deviation for per cent Δ^4-3-ketosteroid of $\pm$0.04 over the range 0 to 6%.**

RECENTLY, $\Delta^{1,4}$-3-ketosteroids, such as prednisolone and 6α-methylprednisolone, have assumed commercial importance because of their high glucocorticoid and anti-inflammatory activities (*1, 6, 8, 11*). Their production involves chemical (*5*) or microbiological (*7*) dehydrogenation of appropriate Δ^4-3-ketosteroids and a method for the determination of residual Δ^4-3-ketosteroids in $\Delta^{1,4}$-3-ketosteroids is needed.

The reaction of thiosemicarbazide with $\Delta^{1,4}$-3-ketosteroids proceeds very slowly compared to Δ^4-3-ketosteroids. The determination of small amounts of Δ^4-3-ketosteroids in $\Delta^{1,4}$-3-ketosteroids is based on these differences in rates of thiosemicarbazone formation. The procedure utilizes a modification of the spectrophotometric technique of Talbot, Ulick, Koupreianow, and Zygmuntowicz (*10*) based on the observation of Evans and Gillam (*4*) that excess thiosemicarbazide does not interfere in the determination of thiosemicarbazones of α-β unsaturated ketones.

REAGENTS

Thiosemicarbazide Hydrochloride, 0.1*M*. A sample of 0.9114 gram of thiosemicarbazide is dissolved in 50 ml. of 0.2*N* hydrochloric acid and the resulting solution is diluted to 100 ml. with water. This reagent is stable at room temperature for several months.

Standard Steroid Mixtures. A standard solution of the appropriate $\Delta^{1,4}$-3-ketosteroid in 95% ethyl alcohol is prepared to contain about 0.10 mg. per ml. Standard mixtures are also prepared which contain, in addition to the $\Delta^{1,4}$-3-ketosteroid at the above concentration, approximately 1, 2, 3, 4, and 5% of the corresponding Δ^4-3-ketosteroid.

APPARATUS

Glass-Stoppered Test Tubes. Prepared by sealing the ends of ₹ 19/38 outside ground joints.

Constant Temperature Water Bath, 25° C.

Spectrophotometer. A Beckman Model DU spectrophotometer equipped with a jacketed cell compartment maintained at 25° C. was used. Such thermostatic control should not be necessary in an air-conditioned laboratory. Spectra

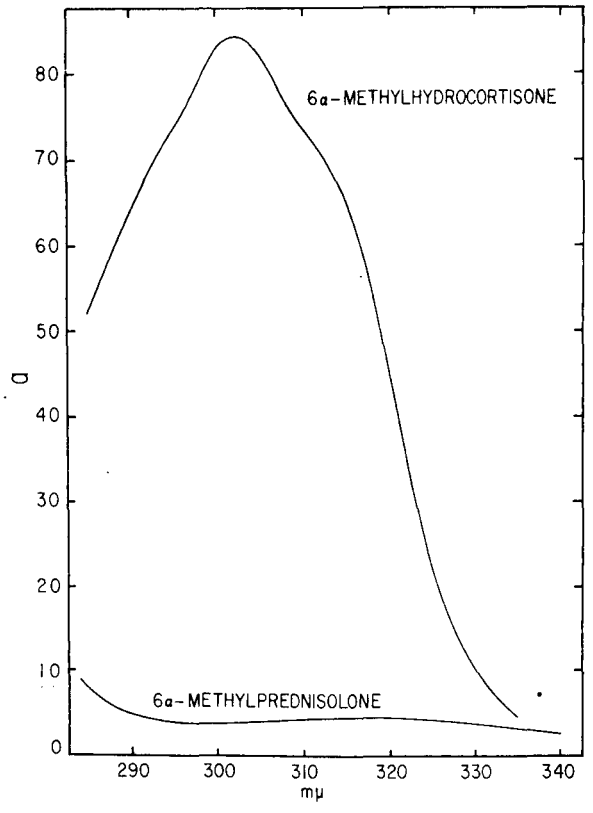


Figure 1. Absorption spectra of thiosemicarbazones from 6α-methylhydrocortisone and 6α-methylprednisolone (2-hour reaction)

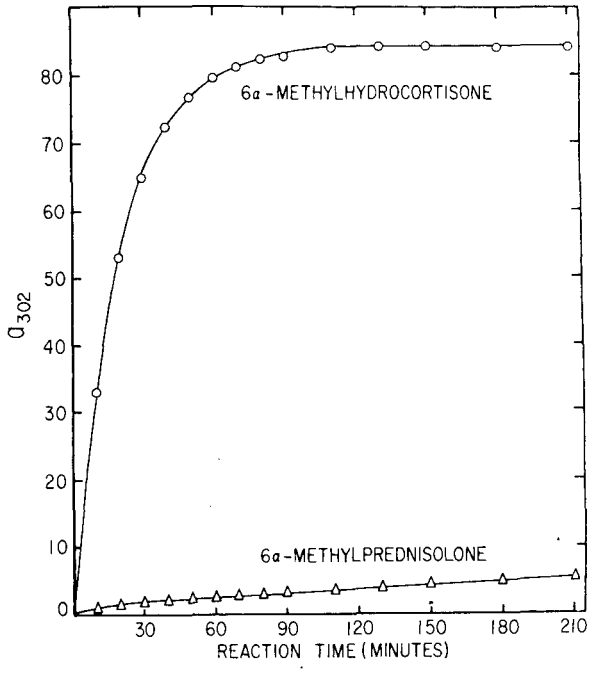


Figure 2. Production of thiosemicarbazones from 6α-methylhydrocortisone and 6α-methylprednisolone at 25° C.

Table I. Determination of Δ^4-3-Ketosteroids in Mixtures with $\Delta^{1,4}$-3-Ketosteroids

Δ^4-3-Ketosteroid, %					
Hydrocortisone in Prednisolone			6α-Methylhydrocortisone in 6α-Methylprednisolone		
Taken	Found	Dev.	Taken	Found	Dev.
0	−0.03	−0.03	0	−0.01	−0.01
	−0.01	−0.01		0.00	0.00
1.04	1.17	+0.13	1.28	1.27	−0.01
	1.07	+0.03		1.34	+0.06
2.04	2.05	+0.01	2.54	2.53	−0.01
	2.01	−0.03		2.49	−0.05
3.04	3.17	+0.13	3.76	3.74	−0.02
	3.03	−0.01		3.74	−0.02
4.01	4.02	+0.01	4.96	4.96	0.00
	4.03	+0.02		4.90	−0.06
4.97	4.95	−0.02	6.12	6.10	−0.02
	4.96	−0.01		6.31	+0.19
		Mean dev. ±0.04			

Table II. Determination of 6α-Methylhydrocortisone in Bulk 6α-Methylprednisolone

Sample	6α-Methylhydrocortisone, %
1	2.82, 2.97
2	0.20, 0.10
3	0.14, 0.06
4	0.22, 0.30
5	1.54, 1.61
6	0.41, 0.32
7	0.77, 0.71

were obtained with a Cary Model 11 recording spectrophotometer.

PROCEDURE

Approximately 25 mg. of the $\Delta^{1,4}$-3-ketosteroid are accurately weighed into a 50-ml. volumetric flask and dissolved and diluted to volume with 95% ethyl alcohol. A 5-ml. aliquot is diluted to 25 ml. with 95% ethyl alcohol.

A 5-ml. aliquot of the diluted sample solution is transferred to a glass-stoppered test tube and the tube immersed in the water bath at 25° C. for 15 to 20 minutes. One milliliter of the thiosemicarbazide hydrochloride reagent, also at the bath temperature, is added, the solution is thoroughly mixed, and the stoppered tube is again immersed in the bath. After 120 ± 2 minutes, the absorbance of the solution is determined at 302 mμ *vs.* a reagent blank.

At the same time, 5-ml. aliquots of the standard mixtures are transferred to separate glass-stoppered test tubes and carried through the procedure above.

Absorbance at 302 mμ per mg. of steroid per ml. of the diluted sample solution, a, is calculated for the unknown and for the standard mixtures. A plot of a *vs.* per cent Δ^4-3-ketosteroid in the standard mixtures is made. A straight line is fitted to these points and the slope, m, and the intercept, b are determined. The per cent Δ^4-3-ketosteroid in the unknown is calculated from the equation:

$$\%\Delta^4 = \frac{a - b}{m} \quad (1)$$

RESULTS AND DISCUSSION

This procedure is based on the marked difference in rates of reaction of Δ^4-3-ketosteroids and $\Delta^{1,4}$-3-ketosteroids with thiosemicarbazide hydrochloride. Δ^4-3-Ketosteroids react completely within 2 hours at 25° C. giving thiosemicarbazones with absorption maxima at 302 mμ. $\Delta^{1,4}$-3-Ketosteroids react only slowly. After 2 hours the resulting thiosemicarbazones have absorptivities at 302 mμ which are 4 to 5% of those from the corresponding Δ^4-3-ketosteroids and which are increasing at the rate of about 0.5% per minute (Figures 1 and 2). The use of buffers to take advantage of the general acid catalysis of thiosemicarbazone formation (*3*, *9*) greatly increases the reaction rates (*2*).

However, the conditions as described provide the most practical differentiation of the two classes of ketosteroids involved.

Calibration curves prepared as described above are strictly linear. Day-to-day variations in the slope and intercept of such curves necessitate the inclusion of standard mixtures in parallel with unknowns for maximum accuracy. These variations appear to reflect the effect of minor temperature differences on the reaction rate of the $\Delta^{1,4}$-3-ketosteroid involved.

Results from the application of the method to known mixtures of typical steroid pairs are given in Table I. A mean deviation for per cent Δ^4-3-ketosteroid of $\pm$ 0.04 is obtained over the range 0 to 6%. Similar results have been obtained with steroid intermediates. Analysis of a group of typical 6α-methylprednisolone samples for per cent 6α-methylhydrocortisone has given a mean deviation from the average of duplicates of $\pm$ 0.05 (Table II).

The method is based on the assumption of a two-component system. Non-steroidal material will therefore act as a negative interference and give a low value for Δ^4-3-ketosteroid. A determination of total steroid by ultraviolet spectroscopy or other applicable methods corrects for such interference. The procedure appears to be general for Δ^4-3-ketosteroids (*2, 10*) and consequently will not differentiate mixtures of such steroids. The Δ^4-3-ketosteroid range of 0 to 6% has been useful for the analysis of bulk preparations. Other ranges may by accommodated by suitable adjustment of sample sizes and appropriate calibration.

If reaction mixtures are prepared in groups of three at approximately 10-minute intervals, 15 samples may be run by a single operator in about 3 hours, exclusive of the time required for sample preparation. This procedure, therefore, provides a rapid, sensitive, and precise measure of small amounts of Δ^4-3-ketosteroids in bulk $\Delta^{1,4}$-3-ketosteroids.

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RECEIVED for review July 23, 1958. Accepted December 11, 1958.

Determination of Bound Styrene in Raw and Cured Polymers by Nitration

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▶ **Although existing methods for the determination of styrene content of polymers yielded excellent results with pure raw polymers, no satisfactory method was available for cured stocks or carbon black masterbatches. A spectrophotometric method has been developed which is applicable to raw and cured stocks without modification, is comparatively simple, requires about 3 man-hours per determination, and yields excellent results in the hands of a laboratory technician. The polymer is nitrated with concentrated nitric acid to yield *p*-nitrobenzoic acid as the main product. Results with more than 2000 samples indicate accuracy and precision to about ±1% relative.**

THE three most widely used methods for the determination of bound styrene are index of refraction (*2, 4, 10*), infrared absorption (*3, 7, 9*), and ultraviolet absorption (*6, 12, 13*). For accurate results raw polymers undergoing analysis must be free from carbon black, fillers, antioxidants, soap, fatty acids, and gel. When cured stocks are being analyzed, the cured polymer must be separated in a soluble form from all other ingredients in the stock. Losses of polymer may result from degradation of the polymer by the solvent or from adsorption of the polymer on the filter aids used to remove carbon blacks and fillers. Among the other methods reported in the literature are x-ray diffraction (*8*), mass spectrometry (*11*), reaction rates with nitric-sulfuric acid mixture (*14*), and density measurements (*15*).

The nitration procedure described was suggested by the qualitative method in the British User's Memorandum (*5*) and by the ASTM method (*1*). Materials most likely to cause erroneous results may be removed by extraction, and those that remain unextracted (carbon black, fillers, and other polymers) interfere slightly, if at all.

When polymers containing bound styrene react with an excess of nitric acid, the polymer is first nitrated and then oxidized. The main product is *p*-nitrobenzoic acid as shown by the ultraviolet absorption spectrum, the melting point, and the neutral equivalent.

The accuracy and precision are very satisfactory, especially for raw polymers. Calibration by a series of standard polymers, provided by the National Bureau of Standards, shows average agreement for styrene content of 0.2%. The precision is of the same order of magnitude.

APPARATUS AND MATERIALS

The instrument was a Beckman Model DU spectrophotometer, with silica cells having an optical path of 1.000 cm. The slit widths for the wave lengths concerned were 285 mμ, 0.720 mm.; 273.75 mμ, 0.813 mm.; and 265 mμ, 0.878 mm.

The method was calibrated using samples of styrene-butadiene copolymers accurately analyzed by the National Bureau of Standards for carbon and hydrogen. The styrene contents as derived from these values ranged from 0 to 53%. Later work with mixtures of

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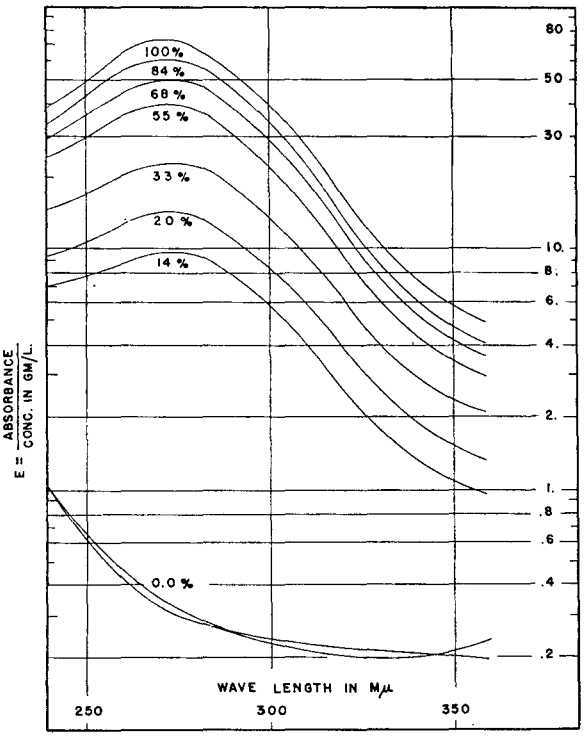


Figure 1. Spectra of material extractable from reaction of styrene-butadiene copolymers and nitric acid

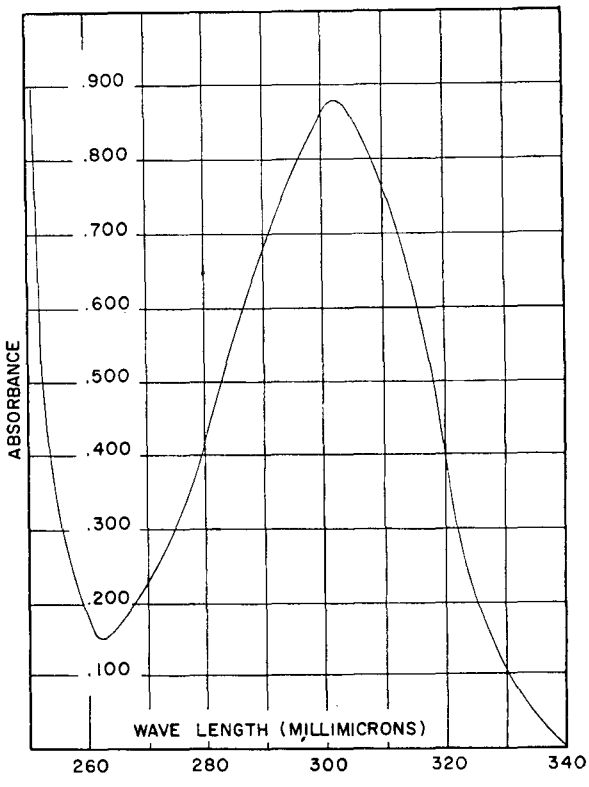


Figure 3. Ultraviolet absorption spectrum of sodium nitrate from nitric acid and sodium hydroxide

polystyrene and polybutadiene show that calibration using such mixtures would be just as satisfactory. The ultraviolet absorption spectra of material extracted from the reaction of styrene-butadiene copolymers and nitric acid are given in Figure 1.

Reagents. Concentrated nitric acid, Baker's analyzed reagent grade, 69.9%, specific gravity 1.420. Sodium hydroxide pellets, reagent grade, 5*N*, 200 grams of sodium hydroxide per liter (distilled water); 0.1*N*, 4 grams of sodium hydroxide per liter (distilled water).

Anhydrous diethyl ether, analytical reagent grade.

PROCEDURE

Weigh a sample of chloroform-extracted cured stock, or alcohol-extracted raw stock, not exceeding 0.25 gram. Put the weighed sample in a 125-ml. standard-taper flask and add 20 ml. of concentrated (69 to 71%) nitric acid. Add a few boiling chips, and insert a water-cooled Graham condenser.

Graham Ful-Jak condensers (Scientific Glass Apparatus Co., Catalog No. J932-4) are better than Allihn condensers in the digestion apparatus. They reduce the loss of nitric acid and the air oxidation of *p*-nitrobenzoic acid during digestion.

Place on a cold electric hot plate. Turn on the heat and allow the sample to reflux fairly vigorously for at least 16 hours (see Figure 2). After the first hour the space in the flask above the boiling acid should contain only slight brown fumes, most of the brown fumes being well up in the condenser.

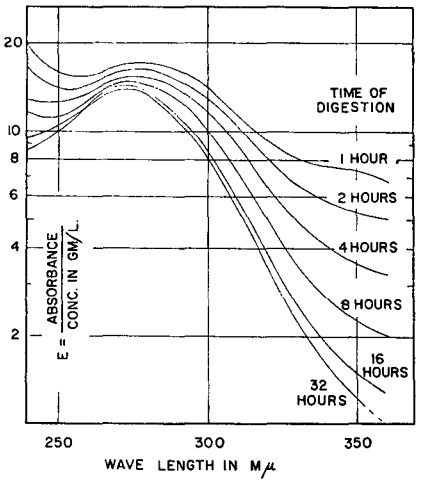


Figure 2. Ultraviolet absorption spectra of material extractable from reaction of SBR rubber and nitric acid

Pour 10 to 20 ml. of distilled water into the top of the condenser and turn off the heat. The water will be slowly drawn down into the flask, washing the condenser. Allow the reaction mixture to cool enough to permit handling the flask. Disconnect the condenser and rinse the standard-taper joint with distilled water. Transfer the contents into a 400-ml. beaker and chill to below room temperature.

With stirring, add 50 ml. of 5*N* sodium hydroxide solution (20 grams of sodium hydroxide pellets in 100 ml. of water). Because this amount is insufficient for neutralization, the solution should be still "very strongly acid" to Eimer & Amend's Alkacid test paper or similar indicator paper. Chill the beaker and contents to below room temperature and transfer the contents, with appropriate washing, to a 500-ml. separatory funnel. Extract the solution three times with separate 50-ml. portions of ether. Combine the ether extracts in a 500-ml. separatory funnel and wash with a 50-ml. portion of distilled water to remove any interference from nitric acid (see Figure 3).

Transfer the combined ether extracts to a 400-ml. beaker containing a few grams of anhydrous sodium sulfate. Transfer the ether extract to a second separatory funnel. Extract the accumulated ether extracts with three or more portions of approximately 0.1*N* sodium hydroxide solution, collecting the aqueous extracts in a 250-ml. volumetric flask. Fill the volumetric flask to the line, and mix well. With a 25-ml. pipet and a 250-ml. volumetric flask, dilute the alkali extract 1 to 10, using 0.1*N* sodium hydroxide as diluent. With 0.1*N* alkali in the blank cell, and the diluted alkali extract in the sample cell, measure the absorbances at 265, 273.75, and 285 mμ, with a Beckman Model DU spectrophotometer. From the absorbance values and sample weight calculate the styrene content.

THEORY

It is assumed that when a butadiene-styrene polymer is nitrated, and the nitration products are examined for ultraviolet absorption, the absorption due to the styrene nitration products is proportional to the styrene content of the polymer; the absorption due to the butadiene nitration products is proportional to the butadiene content.

For a pure raw polymer:

$$a_s \text{ (polymer)} = Xa_s\text{(styrene)} + (1 - X)a_s\text{ (butadiene)} \quad (1)$$

where X and $(1 - X)$ are the fractions of styrene and butadiene, respectively, and a_s is the absorptivity for the material concerned. Absorptivity equals A_s/bc, where A_s is the absorbance of the solution, b is the optical path in centimeters, and c is the concentration in grams per liter.

RESULTS

a_s (butadiene) was determined by the nitration and extraction of polybutadiene. The values at 265, 273.75, and 285 mμ were 0.373, 0.310, and 0.265, respectively.

a_s (styrene) was determined by nitration and extraction of a series of samples accurately analyzed by the National Bureau of Standards for carbon and hydrogen content. The styrene contents derived from these values ranged from 0 to 53%. Although these samples were prepared by a commerical company, they were available to participating members in the government synthetic rubber program carried on during the World War II.

These values should be determined for each instrument used because of differences in slit widths.

Three wave lengths were used in developing the present method because interfering materials might be present. Lack of agreement at the three wave lengths indicates the presence of interferences. If interferences are present, the method cannot be considered to be accurate for that particular sample.

Although *p*-nitrobenzoic acid is the major nitration product, it is not the only material present. Consequently, the absorptivity for *p*-nitrobenzoic acid could not be utilized.

When the absorptivities are inserted in Equation 1, Equations 2, 3, and 4 result.

$$\% \text{ styrene} = 1.52\, a_{s265} - 0.56 \quad (2)$$

$$\% \text{ styrene} = 1.44\, a_{s253.75} - 0.45 \quad (3)$$

$$\% \text{ styrene} = 1.60\, a_{s285} - 0.42 \quad (4)$$

Table II shows the results when Equations 2, 3, and 4 were applied.

INTERFERENCES

The following materials interfere slightly or not at all: smoked sheet, natural rubber reclaim, Paracril B, poly(methyl methacrylate), polyisoprene, polyethylene, polypropylene, neoprene, and carbon black.

Interferences include accelerators, antioxidants, aromatic compounds, and certain heterocyclics. The compounding ingredients including oils would be readily removed by extraction. Such materials as α-methylstyrene, vinyl toluenes, ethylstyrenes, and divinylbenzenes will yield similar nitration products and cannot be distinguished from styrene by this method.

APPLICABILITY

Although the method was developed specifically for styrene-butadiene copolymers, it is applicable to styrene-acrylonitrile resins, terpolymers, graft polymers, and blends.

Table I. Absorptivity Values for Styrene

Sample	Styrene Content, %	a_s 265 mμ	a_s 273.75 mμ	a_s 285 mμ
X-454	8.58	5.85	6.14	5.67
		5.88	6.23	5.76
X-478	22.61	15.14	16.11	14.62
		15.14	16.11	14.68
MS660	36.26	23.87	25.20	22.61
		23.87	25.46	23.01
MS662	42.98	28.60	30.16	26.85
		28.63	30.32	27.29
MS661	53.09	35.00	36.78	33.40
		35.38	37.04	33.62
Polystyrene	100.00	67.00	70.84	62.10
		67.43	71.12	62.16
Weighted av.		66.27	69.71	62.63

Table II. Apparent Styrene Content of Raw Polymers

Sample	Styrene, % Present	Styrene, % Found	Error, % Absolute	Error, % Relative
NE-1	0.00	0.01	0.01	..
		0.01	0.01	..
X-454	8.58	8.46	0.12	1.40
		8.57	0.01	0.12
X-478	22.61	22.72	0.11	0.49
		22.76	0.15	0.66
MS-660	36.26	35.78	0.48	1.32
		36.11	0.15	0.41
MS-662	42.98	42.81	0.17	0.40
		43.14	0.16	0.37
MS-661	53.09	52.72	0.37	0.70
		53.16	0.07	0.13
Polystyrene	100.0	100.59	0.59	0.59
		101.01	1.01	1.01
		Av.	0.24	0.63

Table III. Apparent Styrene Content of Cured Stocks

Sample	Styrene, % Present	Styrene, % Found	Error, % Absolute	Error, % Relative
JEN-1	0.00	0.65	0.65	..
		0.55	0.55	..
JEN-2	3.34	3.15	0.19	5.68
		3.61	0.27	8.08
JEN-3	6.68	6.81	0.13	1.95
		6.61	0.07	1.05
JEN-4	10.01	10.40	0.39	3.90
		10.20	0.19	1.90
JEN-5	13.35	13.65	0.30	2.55
		12.95	0.40	3.00
			0.31	3.48

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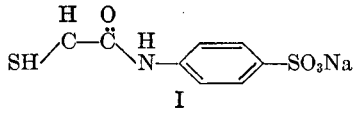
RECEIVED for review November 26, 1957. Accepted January 23, 1959. Division of Rubber Chemistry, Symposium on Physical Testing and Analysis, 132nd Meeting, ACS, New York, N. Y., September 1957.

Sodium-p-(mercaptoacetamido)benzene Sulfonate as Colorimetric Reagent for Cobalt

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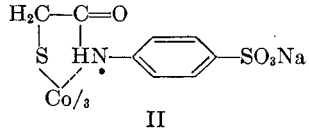
▶Sodium - *p* - (mercaptoacetamido)-benzene sulfonate is a highly selective and sensitive reagent for the direct determination of cobalt(II). With cobalt(II) it gives a brown-red water-soluble complex in the pH range of 6.5 to 7.5 with an absorbance peak at 475 mμ and a brown complex in the pH range 8.5 to 11.0 with an absorbance peak at 390 mμ. The system obeys Beer's law in the concentration range of 0.3 to 2.0 p.p.m. of cobalt and is stable toward time and temperature. The commonly associated elements do not interfere. Nickel gives a light brown unstable complex with the reagent and interferes. The reagent compares favorably with the others used for this purpose.

THE use of thioglycollanilides as gravimetric reagents for cobalt(II) has been described (*3, 4, 12, 17*). Their high selectivity toward cobalt and fairly high sensitivity suggested that the sulfonic acid derivative of such a compound, either as such or in the form of the sodium salt, should prove a useful colorimetric reagent for cobalt. Sodium - *p* - (mercaptoacetamido)benzene sulfonate(I)



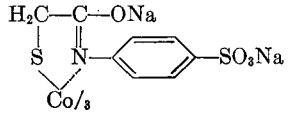
I

is a very good colorimetric reagent for cobalt. It gives a water-soluble brown-red complex in the pH range of 6.5 to 7.5 with an absorbance peak at 475 mμ. The empirical formula of the complex, determined by applying Job's continuous variation method (*9*), is CoR_3, where *R* stands for the reagent. It can be best represented by the chelate structure (II)



II

In the pH range of 8.5 to 11.0, a brown complex is formed with an absorbance peak at 390 mμ. The empirical formula of this complex is also CoR_3. At higher pH, enolization of the keto group appears to take place (*8*) and the complex may be represented as:



In the pH range of 7.5 to 8.5, both forms of the cobalt complex seem to exist.

The presence of diverse ions does not interfere in the direct determination of cobalt. However, some of the reagent is consumed by diverse cations and gives colorless complexes and additional reagent should be used. Under similar conditions, nickel(II) also forms a light brown complex. Although it is unstable toward time, heat, and acid, complete removal of the interference is not possible. However, both cobalt and nickel can be determined simultaneously. Iron(II) and (III) also form a light brown complex. A modified procedure is given to remove this interference.

The reagent in solid state is very stable. In solution it is oxidized by atmospheric oxygen to disulfide (*8*), and should be properly stored or, preferably, freshly prepared before use.

EXPERIMENTAL

PREPARATION OF REAGENT. Several methods (*1, 2, 12, 18, 19*) for preparing thioglycollanilides have been described. In this investigation, a modification of the method of Misra and Guha Sircar (*12*) was used. It consists essentially in refluxing an aromatic amine with mercaptoacetic acid between 110° and 120° C. in an atmosphere of carbon dioxide. Sulfanilic acid is not acylated with mercaptoacetic acid under these conditions. However, its sodium salt can be easily acylated, because the basic characteristics of the amino group are completely masked by the presence of an acidic sulfonic group in the case of sulfanilic acid.

To 9.7 grams of twice crystallized and dried sodium sulfanilate in a hard glass boiling tube were added 6 ml. of 80% mercaptoacetic acid. The reaction is exothermic. It was heated in an oil bath kept between 120° and 130° C. An apparatus to give a continuous flow of carbon dioxide over the mass was made and fitted with a short air condenser. The mass started frothing, and the water vapor formed during the reaction was swept along with the carbon dioxide. In about 3 hours the mass solidified. The air condenser was removed and the tube was further heated for about half an hour to expel the unreacted excess of mercaptoacetic acid. The solid mass was removed, rubbed to a fine powder in a pestle mortar with 99% ethyl alcohol, and filtered under suction. It was boiled with a large excess of 99% ethyl alcohol and filtered hot under suction. This operation was repeated to remove any adhering mercaptoacetic acid. Sodium-*p*-(mercaptoacetamido)benzene sulfonate was dried at 120° C. and the yield was about 8 grams.

Analysis found, N, 5.12; S, 22.40%. $C_8H_8O_4S_2NNa.H_2O$ requires N, 4.88; S, 22.29%.

REAGENT SOLUTION. A 1.0% w./v. solution of the reagent in distilled water was prepared fresh each time.

STANDARD COBALT SOLUTION. Approximately 4.6 grams of reagent grade cobalt nitrate hexahydrate were dissolved in 250 ml. of water. Cobalt content was determined gravimetrically using 1-nitroso-2-naphthol. It was diluted to give 16 γ of cobalt per ml.

BUFFER SOLUTIONS. The first solution of pH 8.7 to 8.8, was prepared by dissolving 30.92 grams of boric acid, 37.27 grams of potassium chloride, and 6.56 grams of sodium hydroxide in 1 liter of water. The second, of pH 7.0 to 7.2, was prepared by dissolving 68 grams of potassium dihydrogen phosphate and 15.73 grams of sodium hydroxide in 1 liter of water.

SOLUTIONS OF DIVERSE IONS. Reagent grade soluble salts were dissolved in water to give 0.1% w./v. solution of the respective ion.

INSTRUMENTS. Absorbance measurements were made with a Spectronic-20 colorimeter using 1-cm. cuvettes (Bausch & Lomb Optical Co.). pH measurements were made with a Beckman pH meter Model H-2. Visual color comparisons were made in 50-ml. Nessler tubes of standard type.

Sodium-*p*-(mercaptoacetamido) benzene sulfonate is a white crystalline compound, highly soluble in water and only slightly soluble in alcohol, acetone, and other organic solvents. It is

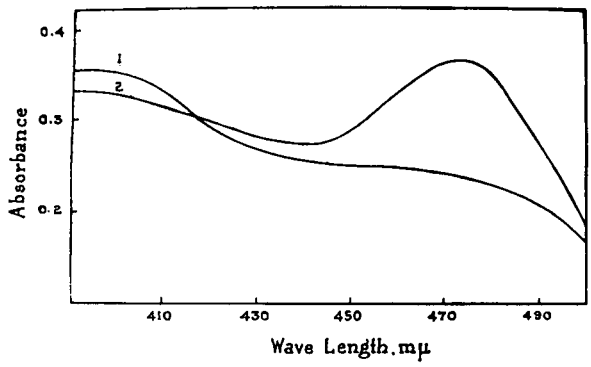


Figure 1. Spectral characteristics of cobalt complex

1.6 p.p.m. cobalt

1. pH 8.8
2. pH 7.0

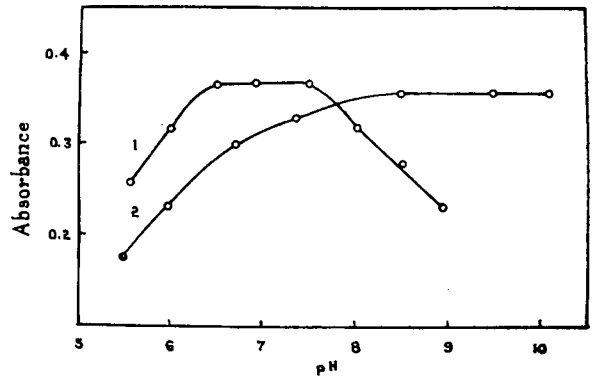


Figure 2. Effect of pH

1. At 475 mμ
2. At 390 mμ

quantitatively oxidized by iodine and this reaction has been used for its direct volumetric estimation. The purity of the compound and its stability in solution have been determined by titrating it against standard iodine solution. The compound in solution is stable for 6 hours and is oxidized to the extent of 7% in 30 hours, 13% in 53 hours, and 18% in 80 hours.

Spectral Characteristics of Cobalt Complex. Five milliliters each of standard cobalt solution, containing 16 p.p.m. of cobalt, were taken in two 50-ml. flasks. To both were added 2 ml. of 10% sodium potassium tartrate and 5 ml. of 1% reagent solution. Varying amounts of 0.1% sodium hydroxide solution were added so that pH in one case was about 7.0 and in the other, about 8.8. The pH can also be adjusted by adding 5 ml. of the buffer solutions. The solutions were made up to volume and the absorbance, at different wave lengths, was measured after about 1 hour. Because the reagent solution has no absorbance in the visible range, water was used as the blank.

Figure 1 gives the absorbance curves of cobalt complex at pH 7.0 and 8.8. At pH 7.0 the color of the complex is brown-red and gives an absorbance peak at 475 mμ. At pH 8.8 the color is brown and there is a gradual fall in the absorbance from 390 mμ toward the higher wave lengths.

Effect of pH. A series of solutions was prepared as described and varying amounts of 1% sodium hydroxide solution were added so that the final pH of the solutions ranged between 5.0 and 11.0. Absorbance was measured after 1 hour at both 475 and 390 mμ.

Figure 2 gives the effect of change of pH on absorbance. At 475 mμ the constant maximum absorbance is between 6.5 and 7.5, and at 390 between 8.5 and 11.0. The absorbance at 475 mμ in the pH range of 6.5 to 7.5 is slightly more than at 390 in the pH range of 8.5 to 11.0.

The stability of the complex at a lower pH after its formation at a higher pH was studied. After all the solutions had been added as described above, it is diluted to about 40 ml. and 10% v./v. hydrochloric acid was added so that the final pH after making up the volume was about 2.0. The color intensity remained unaltered for 15 minutes.

Reagent Concentration and Mole Ratio. A series of solutions was prepared in which the mole ratio of cobalt to reagent was from 1 to 2 to 1 to 80. In one set of experiments, the pH was kept at about 7.0 and in another, at about 8.8. Absorbance was measured after 1 hour at 475 mμ in the former and at 390 mμ in the latter. There was a very gradual rise in absorbance indicating that the complex is in a highly dissociated form in solution. At pH 7.0 full development of color is ensured at a 1 to 60 ratio of cobalt to reagent and at pH 8.8, at a 1 to 45 ratio.

Rate of Reaction and Stability of Complex. Experiments were conducted both at pH 7.0 and 8.8 and, at intervals, the absorbance was measured at 475 and 390 mμ, respectively. The complete development of color at pH 7.0 took about 30 minutes and at pH 8.8, 50 minutes. The time for complete development of color also depends upon the excess of reagent added. Thus at pH 7.0, by keeping the cobalt to reagent ratio at 1 to 300, the time was reduced to 15 minutes. The colors at both pH values were stable for 10 hours and showed an increase in the intensity by 5% after 24 hours.

Effect of Heating. The solutions were heated on a boiling water bath for times varying from 1 to 15 minutes before making up to volume. They were cooled immediately to room temperature. At higher temperatures the colors faded out but reappeared on cooling. After making up the volume, the absorbance was measured without further waiting. The complete development of color took about 5 minutes of heating. Heating for more than 12 minutes gave lower results. The colors obtained by this procedure are slightly more intense than those obtained by keeping the solutions for definite intervals at room temperature.

Beer's Law. The color development obeys Beer's law in both the pH ranges of 6.5 to 7.5 and 8.5 to 11.0. The concentration range in the former case at 475 mμ is 0.29 to 2.0 and in the latter case at 390 mμ is 0.3 to 2.0 p.p.m. of cobalt.

Sensitivity of Reaction. Fifty-milliliter solutions containing 2 ml. of reagent solution, 1 ml. of potassium dihydrogen phosphate buffer (pH 7.0), and 0.1 to 1.0 p.p.m. of cobalt were prepared in Nessler tubes and kept for 30 minutes for full development of color. Cobalt (0.11 p.p.m.) was easily distinguishable from a blank. Thus the sensitivity of the color reaction could be taken to be 1 part of cobalt in 9,000,000 parts of solution. Spot plate sensitivity was determined to be 0.1 γ of cobalt in 0.15 ml. of solution.

Recommended Procedure. Cobalt can be determined colorimetrically in the pH range of 6.5 to 7.5 at 475 mμ and pH 8.5 to 11.0 at 390 mμ. In the lower range, the specificity and sensitivity of the reagent are greater and the possibility of precipitation of the hydroxides of heavy metals is less than in the higher. Hence, the lower pH range was preferred. Here also two slightly different procedures were adopted.

Procedure I. To a cobalt solution containing 15 to 100 γ of the metal in a 50-ml. flask are added 2 ml. of 10% sodium potassium tartrate solution, 5 ml. of buffer solution (pH 7.0), and 5 ml. of 1% reagent solution. The pH can also be adjusted by adding 0.1% sodium hydroxide solution. The solution is made up to volume and absorbance is measured, after 30 minutes, at 475 mμ, using water as the blank.

Procedure II: The sample is prepared as described above except that before making up to volume, it is kept in a boiling water bath for 5 to 7 minutes, cooled to room temperature in a water trough, and then made up to vol-

Table I. Tolerance of Diverse Ions

Ion	Added as	Concn., P.P.M.
Zn(II)	Acetate	200
Mn(II)	Chloride	200
Cu(II)	Sulfate	150
Fe(II)	Sulfate	150
Fe(III)	Sulfate	150
Al(III)	Sulfate	300
Cr(III)	Sulfate	150
Ag(I)	Nitrate	150
Pd(II)	Chloride	5
Tungstate	Sodium	150
Molybdate	Sodium	150

ume. Absorbance is measured, without further waiting, at 475 mμ. This procedure gives slightly better results and takes less time.

Tolerance of Diverse Ions. In slightly acidic or alkaline medium zinc(II), manganese(II), aluminum(III), chromium(III), bismuth(III), mercury(II), lead(II), antimony(III), tin(II), titanium(IV), cerium(IV), zirconium oxide(II), uranium(IV), uranyl(II), thorium(IV), vanadium(III), platinum(IV), gold(III), and tungstate do not give any color reaction with the reagent and are not likely to interfere in the colorimetric determination of cobalt. Silver(I) gives a white precipitate with the reagent which is soluble in an excess of the latter. Molybdate in acid medium gives a yellow color, which changes to green, and finally to blue, on standing. The reagent appears to reduce molybdate to molybdenum blue. Palladium(II) in acidic medium gives a fairly intense and stable yellow. Copper(II) gives a bluish black color which disappears on adding an excess of the reagent. Nickel(II), iron(II), and (III) give light brown unstable complexes.

For studying the interference of diverse ions in the determination of cobalt. Procedure II was followed. Table I summarizes the tolerance of diverse ions as parts per million of ions. Except for palladium(II), where the limiting concentration has been found out, the tolerance beyond the limit mentioned in Table I has not been tested.

Some reagent is consumed by the diverse ions and an extra amount should be used. For every milliliter of 0.1% solution of diverse ion, 2 ml. of 1% reagent solution should be added, except in the case of chromium(III) where 4 ml. of the reagent should be used.

Direct determination of cobalt in the presence of nickel is not possible. The former is separated from the latter by precipitating it as potassium cobaltinitrite (*10*). It is then dissolved in 2*N* sulfuric acid and is estimated colorimetrically by the procedure given above.

Cobalt in Presence of Iron. Iron(II) and (III) interfere in the usual procedure. This interference is eliminated by bringing the pH of the solution to between 2 and 4 by adding dilute hydrochloric acid.

The sample is prepared as described. After it has been cooled to room temperature, it is diluted to about 40 ml., and 1 to 1.5 ml. of 10% v./v. hydrochloric acid are added, so that the pH after making up the volume is between 2 and 4. Absorbance should be measured within 15 minutes because the color is not stable at a low pH for a longer period.

DISCUSSION

Several colorimetric methods have been described for the determination of cobalt. 1-Nitroso-2-naphthol (*16*), 2-nitroso-1-naphthol (*21*), nitroso-R salt (*11*), 2-nitroso-1-naphthol-4-sulfonic acid (*19*), ammonium thiocyanate (*16*), terpyridyl (*13*), *o*-nitrosophenol (*7*), *o*-nitrosoresorcinol (*14*), 3-nitrososalicylic acid (*15*), and diethylthiocarbamate (*6*) are the reagents commonly used. Only a few are briefly discussed.

The most widely used reagent is nitroso-R salt. However, the time of heating after the reagent and the nitric acid have been added and the final concentration of the acid are important factors which affect the intensity of the color. Recently, the use of 2-nitroso-1-naphthol-4-sulfonic acid, which has an identical chelating system has been reported. However, most of the common cations produce precipitates during the process, which must be removed by repeated centrifuging and washing. 3-Nitrososalicylic acid has been reported to be more specific than *o*-nitrosophenol and more convenient than *o*-nitrosoresorcinol and has been used for the separation and codetermination of cobalt and nickel. However, the cobalt complex must be extracted with petroleum ether before measuring the absorbance. Because of its similarity to the authors' reagent, reference is made to *o*-(mercaptoacetamido)-*p*-nitrophenol (*5*) which has been reported as a colorimetric reagent for cobalt. The low solubility of the reagent in water and that of the complex even in alcohol are serious drawbacks with this compound.

In many respects sodium-*p*-(mercaptoacetamido)benzene sulfonate is superior to the reagents listed and can be used for the direct determination of cobalt in presence of almost all the commonly associated diverse ions. Its high sensitivity and selectivity may class it among the better reagents for cobalt.

ACKNOWLEDGMENT

The authors express their gratitude to Shri Bhim Sen, Principal, Government College, Ajmer, for providing facilities in the college.

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RECEIVED for review May 28, 1958. Accepted December 8, 1958.

Ion Exchange Separations

Chromatographic Separations Based on Ion Charge

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▶ **A rapid chromatographic separation is based on the greater affinity of a cation exchange resin for a trivalent than for a bivalent metal ion. A solution of ethylenediammonium chloride serves as the eluting agent. Conditions are worked out for separation of zinc and lanthanum, and the effect of variables is studied. This type of separation should be applicable to a wide variety of analytical problems.**

THE affinity of cation exchange resins for metal ions increases with the magnitude of the charge on the ion. This effect is appreciable and is more pronounced in dilute solutions. However, only a few workers have utilized this phenomenon to effect chromatographic separations of cations of different charge. Examples reported include separation of traces of lithium from larger amounts of calcium (*4*), separation of magnesium and calcium from alkali metals (*8*), calcium from yttrium (*7*), rare earths from thorium (*5*), and thorium from uranium (*1*). All of these methods involve differential elution of the metal ions with hydrochloric acid. Kakihana (*6*) separated beryllium and aluminum using a cation exchange column in the calcium form. The aluminum is retained by the column but most of the beryllium passes through. The remainder of the beryllium is eluted from the column with dilute calcium chloride.

The current authors found that cations of different charge can be conveniently separated on a cation exchange column using a solution of an ethylenediammonium salt as the eluting agent. The ethylenediammonium ion, being bivalent, permits separations to be accomplished with a significantly lower concentration of eluent than is necessary when an acid is used as the eluent. For example, a separation of zinc and lanthanum that requires elution with 1*M* hydrochloric acid can be carried out successfully with a 0.05 to 0.1*M* ethylenediammonium solution. Using the latter eluent, pH adjustment and titration of the metal ion in the effluent is facilitated.

Separations based on differences in ion charge are worthy of more attention than they have received. Some advantages are:

The technique is simple and requires a minimum of apparatus and chemicals.

Separations are fairly rapid and require little manipulation.

The eluting reagent does not usually interfere with subsequent analytical operations.

Separations are quantitative.

The method should be capable of broad application.

In the present work, conditions for the ion exchange separation of zinc and lanthanum are established, and the effect of some important variables are studied. This information should lay the ground work for other separations of this type. It is expected that a bivalent metal ion can be separated from almost any tri- or quadrivalent metal ion.

EXPERIMENTAL

Ion Exchange Resin. Use Dowex 50 × 8 resin. Remove the fine particles by backwashing with water in a large column. Before using commercial resin, purify by placing in a large column and washing successively with 10% ammonium citrate (pH 3 to 3.5), 3*M* hydrochloric acid, and water. Convert the resin to the ethylenediammonium form by treatment with an excess of ethylenediammonium chloride, pH 3. Wash with successive portions of water, ethyl alcohol, and acetone, then remove from the column and air dry.

Ion Exchange Column. Use conventional ion exchange columns of 12- or 21-mm. inside diameter. To avoid disturbing the top portion of the resin during elution, add eluent dropwise from a 125-ml. cylindrical separatory funnel inserted in the top of the ion exchange column through a 1-hole rubber stopper. In some cases it is necessary to elevate the eluent reservoir above the column in order to increase the pressure head enough to obtain the desired flow rate.

General Procedure. Partly fill the column with water, and slowly add dry ion exchange resin in the ethylenediammonium form until the resin bed reaches the desired height. Run a little 0.1*M* ethylenediammonium chloride solution through the column to avoid resin volume changes during the elution, then add the sample of zinc or lanthanum (usually 25 ml. of a 0.01*M* solution, pH 3 or below). Allow this solution to flow at a rate not exceeding 5 ml. per minute until the liquid level is about 2 cm. from the top of the resin bed. At this point, elute the column with ethylenediammonium chloride (usually 0.1*M*, pH 3) and collect 10-ml. fractions of the effluent. Determine the metal ion content of each fraction by titration with (ethylenedinitrilo)tetraacetate (EDTA). Titrate zinc with 0.05*M* EDTA using naphthyl azoxine indicator (*2*); titrate lanthanum using arsenazo indicator (*3*).

After each ion exchange separation, transfer the resin to a used resin container. When sufficient used resin has accumulated, regenerate in a large column using ammonium citrate and hydrochloric acid as before. Convert to the ethylenediammonium form, wash, air dry, and store for future use.

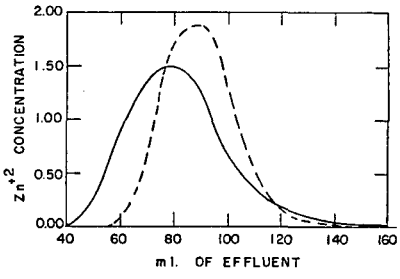


Figure 1. Effect of resin particle size on elution of zinc

Column, 12 mm. i.d. × 16 cm.
Zinc, 0.25 mmole
Flow rate, 5 ml. per minute
Eluent, 0.1*M* ethylenediammonium chloride (pH 3)
——— 50 to 100 mesh resin
– – – 100 to 200 mesh resin

EFFECT OF VARYING CONDITIONS

Resin Size. As shown in Figure 1, the elution curves for zinc are more symmetrical and show less tailing of the bands with 100 to 200 mesh resin than with 50 to 100 mesh. Also, the lanthanum breaks through on an 8-cm. column at 210 ml. with 50 to 100 mesh resin compared to 270 ml. with 100 to 200 mesh. A satisfactory flow rate can be achieved with 100 to 200 mesh resin, but a finer resin would seriously impair the flow rate.

Table I. Movement of Bands under Various Conditions

Standard conditions: 12-mm. i.d. column, 0.25 mmole of metal ion, 100 to 200 mesh resin, flow rate 5 ml./minute, 0.1*M* ethylenediammonium chloride (pH 3) eluent

Resin Height, Cm.	Condition Varied	U[a] for Zn, Ml.	Rate of Zn Peak Travel, Cm./Ml.	U[b] for La, Ml.	Rate of La Band Edge Travel, Cm./Ml.
8	Standard	50	0.20	270	0.019
16	conditions	90		700	
8	50 to 100	40	0.20	210	0.019
16	mesh resin	80		630	
8	Flow rate	50	0.20	280	0.017
16	2.5 ml./min.	90		750	
8	0.15*M*	40	0.32	140	0.032
16	EnH_2^{++}	65		390	
8	0.20*M*	35	0.32	90	0.160
16	EnH_2^{++}	60		140	
8	pH 2.0	50	0.27	270	0.017
16	eluent	80		750	
8	pH 1.5	50	0.27	290	0.018
16	eluent	80		730	
8	21-mm. i.d.	150	0.08	1200	
16	column	250			

[a] Volume of effluent when zinc concentration is maximum.
[b] Volume of effluent at leading edge of lanthanum band.

Table II. Effect of Metal Ion Concentration

Conditions: 12-mm. i.d. column, 100 to 200 mesh resin, flow rate 5 ml./minute, 0.1*M* ethylenediammonium chloride (pH 3) eluent

Metal Ion Added, *M* (25 Ml.)	Column Length, Cm.	Ml. of Eluent To Elute Completely Zn^{++}	Ml. of Eluent When First La^{+++} Appears
0.01	8	90	270
0.05	8	100	180
0.10	8	110	80
0.01	16	140	700
0.05	16	140	540
0.10	16	170	280

Table III. Quantitative Separation of Mixtures

Conditions: 12-mm. i.d. column, 100 to 200 mesh resin, flow rate 5 ml./minute, 0.1*M* ethylenediammonium chloride (pH 3) eluent

Sample Compn., Mmoles	Column Length, Cm.	Effluent Collected, Ml.	Recovery of M^{++}, %
0.25 Zn, 0.05 La	8	120	99.4
0.25 Zn, 0.25 La	8	120	100.0[a]
0.25 Zn, 1.25 La	8	130	100.1
0.25 Zn, 2.50 La	16	180	100.0
			101.0
		200	99.8
			100.1
0.0025 UO_2, 0.25 Th	4	150	98.4

[a] La recovery 100.4% after elution from column with 1*M* ethylenediammonium chloride.

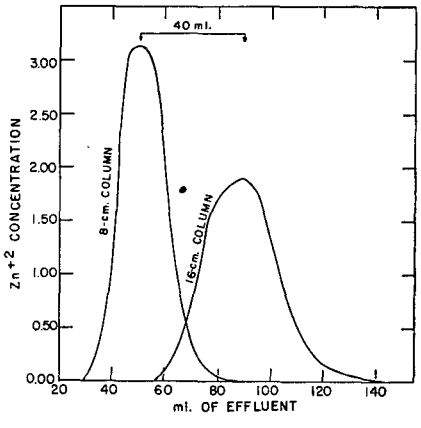


Figure 2. Effect of column length on elution of zinc

Column, 12 mm. i.d.
Zinc, 0.25 mmole
Resin, 100 to 200 mesh
Flow rate, 5 ml. per minute
Eluent, 0.1M ethylenediammonium chloride (pH 3)

Ethylenediammonium Concentration. Using 100 to 200 mesh resin and a flow rate of 5 ml. per minute, 90 ml. of 0.1*M* ethylenediammonium chloride is required to completely elute 0.25 mmole of zinc from an 8-cm. column. Under the same conditions, 270 ml. of effluent is collected before the first lanthanum is eluted from the column. As a result of these experiments, 0.1*M* ethylenediammonium chloride was chosen as the best concentration for the separation of zinc and lanthanum on a rather short column.

Flow Rate. A flow rate of about 5 ml. per minute on the 12-mm. inside diameter column seems to be the optimum. A flow rate of 8 ml. per minute causes some tailing of the elution curves. A 2.5 ml. per minute rate gives elution curves for zinc and breakthrough volumes for lanthanum that are almost identical to those obtained at the 5 ml. per minute flow rate.

Rate of Band Movement. The rate at which the zinc band travels down the column was calculated from the elution curves of zinc from 8- and 16-cm. columns (Figure 2). The difference between volumes of eluent at the peak of the two curves represents the volume of eluent required to move the zinc band a distance of 8 cm. Likewise, the rate of travel of the front edge of the lanthanum band was calculated from the difference in breakthrough volumes on the 8- and 16-cm. column. The rate of band travel under various conditions is tabulated in Table I.

Column Length. As shown in Figure 2, the zinc band flattens out somewhat and becomes slightly less concentrated as it travels a greater column length. However, owing to the difference in rate of travel of the zinc and lanthanum, the bands become more widely separated on a longer column. Speed and convenience dictate that the column should only be as long as required to permit complete elution of zinc and allow a suitable margin before lanthanum appears in the effluent collected.

Column Diameter. A column of greater diameter permits the separation of larger quantities of di- and trivalent ions. The elution curves for zinc on a 21-mm. inside diameter column have been determined. This column has a cross-sectional area approximately three times that of the smaller column used (12-mm. inside diameter). The volume of eluent required for the larger column is greater, but the time required for separation is no longer because of the faster flow rate (15 ml. per minute).

pH. Lowering the pH of the eluent from 3.0 to 2.0 or 1.5 affects the elution of zinc and lanthanum only slightly. The rate of movement of zinc as a function of pH is reported in Table I.

Concentration of Metal Ion. Most of the experiments were carried out with 0.25 mmole of zinc or lanthanum added to the column as a 0.01*M* solution. The effect of a twofold or even a fivefold increase in zinc concentration is small. Increasing the lanthanum concentration, however, causes the lanthanum to break through sooner. Data are given in Table II.

QUANTITATIVE RESULTS AND SCOPE

Quantitative results are given in Table III for the separation and deter-

mination of zinc in mixtures containing varying amounts of lanthanum. Separation of 0.25 mmole of zinc from an equal or fivefold lanthanum concentration is satisfactory on an 8-cm. column, but a 10 to 1 lanthanum to zinc ratio requires a 16-cm. column.

Many other bivalent metal ions behave in a manner similar to zinc on elution with an ethylenediammonium solution. Results indicate that 125 ml. or less of 0.1*M* ethylenediammonium chloride will completely elute any of the following divalent ions from an 8-cm. column: calcium, cupric, ferrous, mercuric, magnesium, manganous, nickelous, plumbous, or uranyl. A slight modification of conditions may be required for quantitative separation of some of these ions from trivalent metals. For example, copper(II) elutes like zinc using pH 2 eluent, but gives a badly tailed curve when eluted with pH 3 ethylenediammonium chloride solution.

Other rare earth ions are eluted more rapidly than lanthanum, but a separation from bivalent ions should still be feasible. Thorium elutes at a slower rate than lanthanum; therefore, separation of a bivalent metal from thorium is quite easy. With proper choice of conditions, it should be possible to separate lanthanum and other trivalent metals from thorium.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Gerald R. Umbreit who originally suggested the use of the ethylenediammonium ion as an eluting agent.

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RECEIVED for review September 29, 1958. Accepted December 22, 1958. Contribution No. 667 of the Ames Laboratory of the U. S. Atomic Energy Commission.

Acetylacetone as Analytical Extraction Reagent

Calculation of Successive Formation Constants from Extraction Data

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▶ **Acetylacetone has been employed as both a chelating agent and solvent in solvent extraction studies. To determine the formation constants, the extraction behavior of seven acetylacetonates was studied. Allowing for the presence of charged intermediates, the successive formation constants for nine acetylacetonates were calculated. These show close agreement with values obtained potentiometrically. In the case of copper(II) acetylacetonate, theoretical calculation of the extraction curve over the pH range 0 to 5 has produced a curve that is duplicated in an experimentally obtained curve.**

SOLVENT extraction as a tool of analytical chemistry enjoys a favored position because of its ease, simplicity, speed, and wide scope (*14*). Acetylacetone (2,4-pentanedione) has been the subject of a number of systematic studies (*12*, *13*, *17*, *18*, *21*, *22*). In most cases attempts have been made to understand the system by applying theoretical principles of extraction and chelate formation.

In acetylacetone-water system, the formation constants obtained from the extraction data when the existence of charged intermediates in the aqueous phase has been neglected have shown only an approximate correlation with potentiometric measurements. With a better understanding of the nature of species present in the aqueous phase, it has been possible to calculate the successive formation constants for the acetylacetonates.

REAGENTS

Commercial acetylacetone was purified as described by Steinbach and Freiser (*22*).

Standard Metal Ion Solutions. The stock solution of copper sulfate was standardized by electrodeposition and diluted for use.

Pure hafnium metal (loaned by the Atomic Energy Commission) was dissolved in aqua regia and the weight of the metal in solution was found by difference. The solution was evaporated to dryness and the residue taken up in concentrated sulfuric acid. The solution was made up to a suitable volume to give a solution containing 7.00 mg. of hafnium per ml.

A weighed quantity of uranium oxide, U_3O_8, was dissolved in nitric acid, fumed with sulfuric acid, and made up to volume to give a stock solution containing 5.00 mg. of uranium per ml.

A weighed quantity of reagent grade zinc oxide was carefully dissolved in dilute sulfuric acid and made up to volume to give a solution containing 10.00 mg. of zinc per ml.

A weighed quantity of zirconium oxychloride tetrahydrate was dissolved in water to give a solution containing 2.50 mg. of zirconium per ml.

Preparation of Acetylacetonates. Copper, lead, and uranyl acetylacetonates were prepared as described by Krishen and Freiser (*12*).

A solution of hafnium, prepared by dissolving hafnium metal in aqua regia, was mixed with 10% sodium carbonate solution and acetylacetone at 15° C. The precipitate that formed on standing was filtered and recrystallized from hot benzene. Melting point, reported value, 194–6° C. (*7*); found, 194–6° C.

Technical iron(III) acetylacetonate as obtained from the Union Carbide and Carbon Corp. was recrystallized from 95% alcohol and dried for 1 hour at 120° C. Melting point, reported value, 179° C. (*6*); found, 178–9° C.

Technical zinc acetylacetonate as obtained from the Union Carbide Corp. was recrystallized from hot 95% alcohol. A sample was dissolved in nitric acid and zinc determined polarographically. Per cent zinc, theoretical, 24.80; found, 24.8.

An aqueous solution of zirconium

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oxychloride tetrahydrate was mixed with a mixture of 10% sodium carbonate solution and acetylacetone at 15° C. The resulting precipitate was recrystallized from absolute alcohol. Melting point, reported value, 194.5–6° C. (*2*); found, 194–6° C.

EXTRACTION PROCEDURE

For the equilibrium studies, the organic phase was prepared by dissolving weighed amounts of recrystallized copper(II), iron(III), lead(II), and zinc(II) acetylacetonates in water-saturated acetylacetone to give about $10^{-3}M$ solutions in each case. The aqueous phase was prepared by adjusting the pH with sulfuric acid and sodium hydroxide and saturating with acetylacetone. In the case of hafnium, uranium, and zirconium, the metal ion was originally in the aqueous phase and the volume was made up to 25 ml. finally. Ten milliliters of each of the two phases was pipetted into a dry 50-ml. volumetric flask and shaken for 2 hours on a mechanical shaker and a water bath thermostatted at 25° C. When the extraction was complete, the pH of the aqueous phase was measured with a Beckman Model G pH meter. A suitable aliquot of either of the phases was pipetted out for determination of metal concentration.

ANALYTICAL METHODS

The methods for determination of copper, lead, and uranium have been described (*12*).

HAFNIUM AND ZIRCONIUM. A suitable aliquot of the acetylacetone layer was kept over a few milligrams of sodium carbonate to remove traces of acid, then 2 ml. of 1% solution of 8-quinolinol in 95% alcohol was added and the absorbance read at 395 mμ against a blank of acetylacetone treated similarly. The amount of the metal was read from standard curves prepared by taking various amounts of the standard solutions of the acetylacetonates in water-saturated acetylacetone.

IRON. The organic phase was filtered through a filter paper and made up to a suitable volume and the absorbance read against water-saturated acetylacetone at 440 mμ. The concentration was obtained from the standard curve prepared by reading the absorbance of similar solutions containing weighed amounts of iron(III) acetylacetonate.

ZINC. One milliliter of the acetylacetone layer, after extraction, was dissolved in water, its pH adjusted to between 5 and 9, and the volume made up to 100 ml. The solution was shaken with three successive 10-ml. portions of carbon tetrachloride to remove acetylacetone. Ten milliliters of the solution was shaken with 10 ml. of 0.001% diphenylthiocarbazone in carbon tetrachloride and the absorbance of the carbon tetrachloride layer read at 520 mμ using the dithizone solution as the blank. The amount of zinc was determined using the standard curve prepared by taking 1 ml. of various concentrations of zinc acetylacetonate in acetylacetone.

In the aqueous phase, zinc was determined polarographically using 1*M* ammonium hydroxide and 1*M* ammonium chloride as the supporting electrolyte (*1*). A 0.002% solution of Triton X-100 (Rohm & Haas) was used as the maximum suppressor. The half-wave potential was −1.33 volts *vs.* S.C.E. The standard curve was prepared by using standard zinc sulfate solution.

Table I.

[$K_1 = 10^{8.31}$ (*8*), $K_i = 10^{-8.95}$, and $(HR)_w = 1.7\ M$]

pH	1st Term	2nd Term
0	1	$10^{-0.5}$
1	10^{-2}	$10^{-1.5}$
2	10^{-4}	$10^{-2.5}$
3	10^{-6}	$10^{-3.5}$

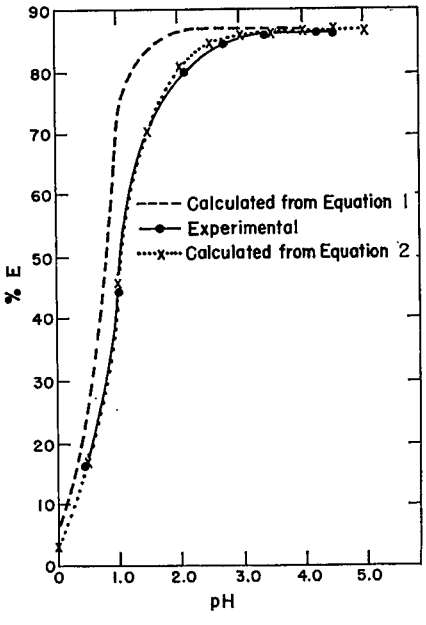


Figure 1. Comparison of calculated and expermental extraction of copper acetylacetonate

DISCUSSION OF RESULTS

Under the experimental conditions employed, similar equilibrium status was attained irrespective of whether the metal species were originally in the organic or the aqueous phase (*11, 12*). In some solvent extraction systems, the course of the chelate extractions may be described by the following equation:

$$\frac{E}{100 - E} = D = \frac{K_{D_e} K_i^n K_f (HR)_w^n}{(H^+)^n} = \frac{K''}{(H^+)^n} \qquad (1)$$

where

E = per cent of metal extracted into the organic phase

D = distribution ratio of the metal

K_{D_e} = distribution coefficient

K_i = ionization constant for the reagent

K_f = formation constant of the chelate

$(HR)_w$ = concentration of the reagent in aqueous phase

n = charge on the metal ion

This simplified equation is a very reasonable approximation and gives correct slopes for the extracton curves in dithizone or 8-quinolinol (oxine) extraction systems. In those cases the value of $(HR)_w$, the concentration of the reagent in the aqueous phase, is rather low (about $10^{-4}M$) and the ionization constant is also low for oxine $K_{a_1} K_{a_2} = 10^{-14.83}$ (*15*). The effect of the presence of charged chelates of type MR^+ can be neglected. In the acetylacetone-water system, the conditions are not comparable. Thus, the concentration of the reagent in water is very much higher, 1.7*M*, the ionization constant is high, $10^{-8.95}$, and the distribution coefficients, K_{D_e}, are lower. Under these conditions the effect of the presence of charged intermediates cannot be neglected. The extraction equation must be modified to give:

$$D = \frac{(K_{D_e} - D)K_1 \ldots_n (HR)_w^n K_i^n}{(H^+)^n + K_i (HR)_w K_i (H^+)^{n-1} + \ldots\ldots + K_1 \ldots_{(n-1)} (HR)_w^{n-1} K_i^{n-1} (H^+)} \qquad (2)$$

To get a curve which conforms to Equation 1, the second and the subsequent terms in the denominator of Equation 2 have to be neglected. Considering the case of a simple divalent ion like copper(II), where the denominator has only two terms, the deviation from Equation 1 becomes evident (Table I).

The second term becomes progressively more important as pH increases, so that beyond a pH value of 1, it is the first term that may be neglected.

In the case of copper acetylacetonate, the log of the over-all formation constant has a value of 15.16 (*8*), the K_{D_e} has been found to be 6.5, so that it is possible to calculate the theoretical value for extraction at various pH values. These values in the pH range 0 to 5.0 were calculated on the basis of both Equations 1 and 2. A comparison of these two theoretical curves with the experimentally obtained curve (Figure 1) makes clear the need for considering the complete equation. Using Equation 2 this not only gives the correct slope throughout the pH range, but the theoretical and the experimental curves are found to be identical within the experimental errors.

When other metal ions are considered, where more than one intermediate charged chelate can exist, the effect of each succeeding term in the denominator becomes increasingly more important even at lower pH values. Thus, deviations from the slope calculated from

Equation 1 were found in each case, the limiting value of the slope being attained in only a few cases. The slope was found to be nearer that calculated for a monovalent metal in most cases. This observation is supported by results reported by Steinbach (*20–22*). In the cases he considered, even where the slope was close to the calculated value, the agreement was reasonable in only a limited range of pH values.

Rydberg (*16*) studied the extraction of thorium(IV) acetylacetonate with benzene using significantly lower concentrations of acetylacetone in water. The decreased reagent concentration should decrease the value of the second and the succeeding terms in the denominator of Equation 2. However, close agreement between the calculated and experimental values of extraction was possible only when the presence of the charged chelates was taken into account.

Distribution Coefficients and Stability Constants. To calculate the formation constants for the acetylacetonates, the distribution equation may be rearranged to give:

$$K_{1\ldots n} = \frac{D(H^+)^n + K_1(HR)_w K_i(H^+)^{n-1} + \ldots + K_{1\ldots(n-1)}(HR)_w{}^{n-1}K_i{}^{n-1}(H^+)}{(K_{D_e} - D)(HR)_w{}^n K_i{}^n} \quad (3)$$

At 50% extraction, the distribution ratio, D, equals unity. If as an approximation, all terms except $(H^+)^n$ are neglected in the numerator, an approximate value for the over-all formation constant for the acetylacetonates may be calculated from the experimental value of $pH_{1/2}$ (the pH for 50% extraction) and the maximum extraction reached, K_{D_e}. The values for the formation constants calculated in this manner are shown in Table II.

Although the conditions of temperature, metal ion concentration, and ionic strength are not identical, some agreement is apparent between the values of the formation constants obtained from the extraction and potentiometric (*8–10*) methods.

In the case of iron(III) acetylacetonate, the exact value of K_{D_e} cannot be determined as the extraction approximates 100%, and some very sensitive technique of analysis, like that of radiometric estimation, would have to be utilized for determining the ratio of the metal in the organic and the aqueous phases. It was through the use of this technique only that Rydberg (*16*) was able to calculate the K_{D_e} for thorium acetylacetonate in a water-benzene system. In water-acetylacetone system, similar limitations affect the determination of K_{D_e} for cerium(III), indium(III), and thorium(IV) acetylacetonates (*11, 20*).

Table II. Approximate Formation Constants for Acetylacetonates

Ion	$pH_{1/2}$	K_{D_e}	log K_f	Comparison
Aluminum(III)[a]	1.75	9.5	19.9	22.3 (*9*)
Beryllium(II)[a]	0.67	41	14.5	14.63 (*8*)
Copper(II)	1.10	6.5	14.5	15.16 (*8*)
Gallium(III)[a]	1.20	28.6	21.0	23.6 (*9*)
Hafnium(IV)	1.75	6.6	27.0	
Iron(III)	0.07	1000[b]	21.0	26.2 (*9*)
Lead(II)	5.65	4.9	6	
Uranyl	1.66	65.5	13.9	14.15 (*8*)
Zinc(II)	5.30	1.8	5	8.81 (*10*)
Zirconium(IV)	1.50	2.5	28	

[a] From Steinbach (*20*).
[b] Assumed value.

Table III. Formation Constants for Acetylacetonates (25° C.)

Ion	Log K_1	Log K_2	Log K_3	Log K_4	Log K_f
Aluminum(III)[a]	8.25	7.35	5.91		21.51
Comparison (30° C.)	8.6	7.9	5.8		22.3 (*9*)
Beryllium(II)[a]	8.17	6.37			14.54
Comparison (20° C.)	7.88	6.75			14.63 (*8*)
Copper(II)	8.96	6.84			15.80
Comparison (20° C.)	8.31	6.85			15.16 (*8*)
Gallium(III)[a]	7.79	7.48	6.29		21.56
Comparison (30° C.)	9.5	8.4	5.7		23.6 (*9*)
Hafnium(IV)	8.73	6.64	6.38	6.35	28.10
Iron(III)[b]	9.27	8.71	5.89		23.87
Comparison (30° C.)	9.8	9.0	7.4		26.2 (*9*)
Lead(I)	4.51	2.49			7.00
Uranyl	8.87	5.26			14.13
Comparison (30° C.)	7.66	6.49			14.15 (*8*)
Zinc(II)	5.06	3.66			8.72
Comparison (30° C.)	4.98	3.83			8.81 (*10*)
Zirconium(IV)	8.38	7.58	7.26	6.86	30.08

[a] Calculated from experimental data from Steinbach (*20*).
[b] Based on assumed value of $K_{D_e} = 1000$.

The value of K_{D_e}, in cases where it is low, would be determined with sufficient precision by the extraction technique to permit calculation of the formation constants.

The effect of the presence of the charged chelates would reasonably be more important where K_{D_e} is low. However, even in the case of thorium acetylacetonate, where the K_{D_e} is high [315 + 30 in water-benzene system (*16*)], the presence of the intermediate charged chelates cannot be neglected (*19*) and the approximate equation does not give correct values for the formation constants. Similar observations have been made in other systems (*3–5*).

Where the value of K_{D_e} has been determined, the stepwise formation constants can be calculated if the complete equation is applied. There would be n number of unknowns for a metal ion with a charge n, due to the successive formation constants, K_1, K_2, ... K_n. To get those values, n equations would be required. These can be obtained from the extraction curve by finding the distribution ratio, D, at different pH values.

Mathematically, it is possible to calculate even K_{D_e} by utilizing $(n + 1)$ equations. However, the precision of the experimental data does not justify the calculation of this quantity. Thus in the case of iron(III) acetylacetonates, varying values of K_{D_e} were obtained when data from different experimental points were used. Slight variation in the extraction value from say 99.6 to 99.9% alters the K_{D_e} value from 249 to 999.

In the case of zinc acetylacetonate, the K_{D_e} is only 1.8, $pH_{1/2}$ is 5.3, and the value obtained from the extraction data is considerably lower than that obtained from the potentiometric measurements (Table II). Steinbach (*20*) had considered this K_{D_e} to be erroneous because of the possibility of hydrolysis of zinc.

However, the acetylacetonate can be expected to be more stable than the hydroxide and even at pH 7, the relative concentrations of the hydroxyl ions ($10^{-7}M$) and acetylacetonate ($10^{-1.7}M$) would favor the formation of the acetylacetonate. As such the value of 1.8 for K_{D_e} for zinc acetylacetonate cannot be considered erroneous and the major factor to be considered is the presence of the intermediate chelates. Taking two values of extraction at different pH values (D = 1.00, pH = 5.30; D = 0.25, pH = 4.35) from the curve obtained experimentally, the formation constants for the chelate and the intermediate have been calculated. These calculations give a value of 8.7 for the logarithm of the over-all formation constant, K_f, while log K_1 and log K_2 are 5.1 and 3.7, respectively. These values may be compared with the values obtained potentiometrically—8.81, 4.98, and 3.83 (*10*).

When metal ions with an oxidation number of 3 or 4 are considered, the solution of the various equations requires the application of determinants. These calculations have been made for a number of acetylacetonates (Table III).

On allowing for the intermediates, the maximum change in log K_f (+3.78) took place in the case of zinc where the K_{D_e} is the lowest (1.8) and $pH_{1/2}$ is the second highest (5.30) and the least changes, +0.04 and +0.23, took place in the case of beryllium and uranyl where the K_{D_e} values are the highest (41 and 65.7) and the $pH_{1/2}$ among the lowest (0.67 and 1.66).

Thus the effect of the presence of charged intermediates is enhanced when K_{D_e} is low or the extractions take place at higher pH values.

In addition to the presence of charged chelates, the theoretical calculations will be affected by hydrolysis and changes in the complexity of the molecular species. However in the system studied, the close agreement between the formation constants obtained from extraction and potentiometric studies (Table III), suggests that interference from such causes is negligible. In the case of zinc(II) acetylacetonate, hydrolysis can be ruled out as a factor of any consequence. The low concentrations of metal ions ($10^{-3}M$) used in this study, along with the fact that the extraction behavior of copper(II) acetylacetonate has been correctly predicted (Figure 1) from the value of the formation constants obtained independently by potentiometric methods, indicate that increases in molecular complexity, if any, are not significant.

ACKNOWLEDGMENT

The authors gratefully acknowledge the financial assistance of the U. S. Atomic Energy Commission.

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RECEIVED for review August 7, 1958. Accepted December 8, 1958.

Polychromatic Technique for the Identification of Amino Acids on Paper Chromatograms

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▶ A simplified polychromatic technique was developed to study the amino acid composition of protein hydrolyzates; the previous two-dimensional chromatographic systems were not entirely satisfactory, because of the time consumed and the inherent variables encountered in determining the reproducibility of R_f values. Twenty common, naturally occurring amino acids in amounts as low as 1.2 γ were completely differentiated after 6 hours of resolution when sprayed with ninhydrin–cupric nitrate (N-CN) and were readily identified by their characteristic color complexes. This method was equally satisfactory in the detection of single amino acids or mixtures on a unidimensional chromatogram in n-butyl alcohol–acetic acid–water (4:1:5 ratio) solvent system, although some possessed only slightly different R_f's—i.e., leucine-isoleucine.

PAPER chromatographic techniques have contributed much toward simplifying the analysis of numerous multicomponent systems, such as mixtures of sugars, amino acids, and inorganic ions (*4*). Many methods for the identification of amino acids on a paper strip chromatogram are satisfactory. However, this report presents a simplified procedure that was developed in these laboratories.

Investigations have recently been conducted to find a selective indicator to identify amino acids. Many indicators containing ninhydrin have been used, but with the exception of the yellow color of proline, they do not differentiate shades of red, violet, and purple. The excellent procedure of Hardy *et al.* was not applicable to the present problem because of its limited application (identification of 11 commonly occurring amino acids), such as the differentiation of leucine from isoleucine (*6*). In the quest for a polychromatic method for the identification of amino acids, released from a protein moiety, the following indicators were employed: 0.25% w./v. ninhydrin in acetone (*17*), 0.3% ninhydrin in 95% ethyl alcohol (*12*), 0.2% ninhydrin in water-saturated *n*-butyl alcohol (*5*), 4% ninhydrin in pyridine (*15*), and the method reported by Levy in 1953 (*8*). None of these reagents proved to be as selective to color formation as the reagent described, which is a modification of the ninhydrin-copper complex method employed by Levy and Chung (*8*).

EXPERIMENTAL

Two standard amino acid mixtures containing 10 amino acids in each were

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ห้องสมุด กรมวิทยาศาสตร์

prepared. Group I contained 50 mg. of the amino acid in 5 ml. of 0.28*N* hydrochloric acid. Group II contained 50 mg. of the amino acid in 5 ml. of 0.32*N* hydrochloric acid.

Group I	
1. Lysine	6. Valine
2. Aspartic acid	7. Trytophan
3. Glycine	8. Phenylalanine
4. Threonine	9. Leucine
5. Proline	10. Isoleucine

Group II	
1. Glutamic acid	6. Arginine
2. Alanine	7. Cystine
3. Tyrosine	8. Cysteine
4. Histidine	9. Asparagine
5. Methionine	10. Serine

The ninhydrin–cupric nitrate indicator (N-CN) consisted of two solutions, I and II, combined in a ratio of 25 to 1.5 just before using. Solution I contained 0.2% ninhydrin (anhydrous 1,2,-3-triketohydrindene) in 50 ml. of absolute ethyl alcohol, 10 ml. of glacial acetic acid, and 2 ml. of 2,4,6-collidine. Solution II was a 1% solution of cupric nitrate trihydrate (analytical reagent Mallinckrodt Chemical Works) in absolute alcohol.

Ten microliter aliquots of the individual amino acids as the hydrochlorides in Groups 1 and 2 and an equal mixture of the two groups were placed on Whatman No. 1 filter paper. The strips were placed in a glass chromatographic chamber containing *n*-butyl alcohol–acetic acid water (4:1:5) as the solvent (*11*). Using descending chromatography the strips were developed for 6 to 7 hours, the time needed for the solvent front to migrate 32 to 35 cm. during each run. The strips were then removed, dried in an oven for 5 minutes at 104° to 110° C., sprayed with the N-CN indicator, and placed in an oven for 1.5 to 2 minutes at 105° C.

RESULTS

The resulting color formations on a white background are unique for each amino acid studied, with the exception of glutamic acid and alanine, which both yielded purple spots, but of different intensity—i.e., alanine is much darker if equal concentrations are present. Arginine and valine also form a purple spot, but their rates of flow are so dissimilar that identification is very readily achieved.

Table I describes the color of the amino acid spots formed after using this indicator, immediately upon removal from the drying oven. Within 10 to 15 minutes the bright blue ring of tryptophan disappears. With few other exceptions, the remaining amino acids are color stable for about 30 minutes; then slight changes occur, such as a pink ring forming around lysine and serine. After these changes in color occur and if the strips are kept in the dark, the color remains stable for 1 to 2 months, other than slight fading. The background becomes a yellowish green after 5 or 6 days and the intensity of this color increases with time.

Table I. Colors of Amino Acids after Use of N-CN Indicator[a]

Compound	Color
Cystine	Gray
Lysine	Reddish brown, pink ring forms upon standing
Histidine	Light brown with dark brown ring, inside a yellow ring
Asparagine	Golden
Arginine	Dark purple
Serine	Greenish brown, red ring forms upon standing
Aspartic acid	Light blue (if removed from the oven too soon, aspartic acid will be bright green)
Glycine	Orange brown with bright orange ring
Threonine	Greenish brown, changes to purplish brown upon standing
Glutamic acid	Purple, fades slightly upon standing
Alanine	Dark purple
Proline	Light green with yellow ring
Cysteine	Gray
Tyrosine	Light brown
Valine	Purple
Methionine	Grayish purple with yellow ring
Tryptophan	Brown with bright blue ring (ring fades rapidly)
Isoleucine	Light blue
Phenylalanine	Greenish yellow
Leucine	Light purple with yellow ring

[a] Amino acids listed in order of separation after resolution in *n*-butyl alcohol–acetic acid–water (4:1:5) ratio on Whatman No. 1 filter paper.

Table II. Evaluation of Chromatograms Developed with Different Solvents and Sprayed with the N-CN Color Reagent

Solvent	Grading[a]
1. *n*-Butyl alcohol–pyridine-water, 3:2:1.5 (*16*)	3_+
2. *n*-Butyl alcohol–pyridine-water, 4:5:1 (*9*)	2_+
3. *n*-Butyl alcohol–water, 85:15 (*14*)	4_+
4. *n*-Butyl alcohol–saturated with 2N NH_4OH (*7*)	2_+
5. *n*-Butyl alcohol–dioxane, 4:1, saturated with water (*3*)	3_+
6. 70% ethyl alcohol (*10*)	3_+
7. Pyridine–water, 65:35 (*2*).	2_+
8. *m*–Cresol saturated with water (*5*)	1_+
9. 2,4-Lutidine–collidine–water 1:1:2 (*1*)	3_+
10. *tert*-Butyl alcohol–ethyl methyl ketone–diethylamine–water, 40:40:20:4 (*13*)	2_+[b]

[a] Method of grading ranges from 1 to 4_+ and is derived by comparison with butyl alcohol–acetic acid–water (4:1:5) which is arbitrarily assigned a 4_+ value.
[b] Leaves dark background.

The sensitivity of this indicator was determined by diluting the 1% standard amino acid solution to 0.50, 0.25, 0.13, 0.06, and 0.03%, placing from 2- to 10-μl. aliquots of the various concentrations on a strip, and chromatographing them. Asparagine, proline, and phenylalanine could be detected by position alone with 2 μl. of a 0.06% concentration. However, as a very faint yellow color was produced which was not typical of the original 1% solutions, these amino acids could be identified by a characteristic color at this level only with an 8 μl. application of 0.06% concentration. The remaining amino acids could be identified by color alone at the 2 μl. to 0.06% level, but below this concentration, the colors were atypical of the 1% solutions, fading to yellow as the concentration was decreased.

The effect of developing solvents other than the *n*-butyl alcohol–acetic acid–water system on the selective color formation of the N-CN indicator was studied in the same manner. The results using various developing solvents were graded by comparing them with the *n*-butyl alcohol–acetic acid–water system (Table II).

DISCUSSION

The great advantage of paper chromatography lies in its simplicity and the relatively small expense of the equipment. Paper chromatography can be further simplified for the detection of amino acids by the polychromatic method. This technique virtually eliminates the time-consuming two-dimensional chromatography for the study of complex mixtures and provides a sensitive indicator for the identification of amino acids in concentrations as low as 1.2 γ. Although the quantities of amino acids required to give a visible spot on the chromatogram are dependent on a number of factors, the N-CN color reagent is equally satisfactory for the differentiation of 20 different amino acids either individually or in a mixture at levels of 0.06%. Although as noted in Table I both cystine and cysteine appear as gray spots when sprayed with ninhydrin–cupric nitrate (N-CN), there is no difficulty in identifying them, because of the marked differences in rate of flow. Lysine, histidine, and asparagine

(and the other combinations with the one exception of cystine-cysteine) in a similar mixture of 20 amino acids appear frequently as homogeneous spots, when chromatographed by conventional techniques. However, this method indicates that there are sufficient differences in their R_f values within a given spot to warrant the selective identification of each of the amino acids by their color complexes.

This technique has been employed routinely in these laboratories for the identification of amino acids in hydrolyzates of the beta hemolytic streptococci fraction. It was of help in distinguishing between the troublesome leucine and isoleucine in amino acid mixtures. Maximum resolution is the most outstanding feature of two-dimensional chromatography. However, this degree of resolution is not required when the polychromatic procedure is employed, even though some of the amino acids possess relatively similar R_f values.

In unidimensional chromatography lysine, histidine, and asparagine butyl alcohol–acetic acid–water, 4:1:5 ratio), when sprayed with ninhydrin, appear as a homogeneous substance. Each amino acid, however, can be readily distinguished after a minimum of 6 hours of resolution when sprayed with N-CN. Other combinations of amino acids selected on the basis of the similarity of R_f values, such as cystine-cysteine, glycine-aspartic acid, arginine-serine, threonine-glutamic acid-alanine, tyrosine-valine-methionine, tryptophan-phenylalanine, and isoleucine-leucine also were studied to determine if they could likewise be detected after a minimum of 6 hours of resolution. These mixtures of amino acids would also appear as homogeneous mixtures and give singular spots when treated with the ninhydrin reagents. However, when treated as outlined, each amino acid could be characterized by its color complex.

A single application provides a far superior chromatogram than using two sprays to effect only semipolychromatic results.

Perhaps the most outstanding advantage of this method is that the interfering factors such as abnormalities occurring as a result of ionizable substances, cation concentration, the rate of movement of solvent, and temperature, are of no major significance; in two-dimensional chromatography they would be of considerable importance in the reproducibility of R_f values for the identification of amino acids.

ACKNOWLEDGMENT

The authors express their appreciation to Jack H. Davis and Irwin Schultz, MC USNR, for helpful suggestions in the preparation of this manuscript.

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RECEIVED for review April 9, 1958. Accepted November 14, 1958. From Research Project NM 52 06 04.4, Bureau of Medicine and Surgery, Navy Department, Washington 25, D. C. The views and opinions expressed herein are those of the authors and do not necessarily reflect those of the Navy Department or the Naval Service at large.

Titrimetric Determination of Alkyl Mercaptan–Dialkyl Sulfide and Alkyl Mercaptan–Alkyl Disulfide Mixtures

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▶ **Mixtures of *n*-alkyl mercaptans and dialkyl sulfides can be determined titrimetrically using two aliquots. The first aliquot is titrated for mercaptan with iodine, and the second, after treatment with basic acrylonitrile, is acidified and titrated with bromate-bromide solution. The amount of dialkyl sulfide is found by the difference. The results agree to better than 1%. Mixtures of *n*-alkyl mercaptans and alkyl disulfides are determined iodimetrically. Mercaptan alone is titrated first, and then the total mercaptan is titrated after the reduction of alkyl disulfide with zinc in an acetic-hydrochloric acid–alcohol mixture. Alkyl disulfides with higher molecular weights than propyl disulfide are recovered quantitatively.**

ACCURATE TITRIMETRIC METHODS for determination of mercaptans (thiols) have been developed using iodine (*7*) or silver nitrate (*3, 8, 11*). The determination of alkyl sulfides by bromate-bromide standard solutions has been described by Siggia and Edsberg (*10*). However, samples containing a mixture of alkyl sulfide and alkyl mercaptan in excess of 10% cannot be analyzed satisfactorily, because of the slow and incomplete oxidation of mercaptan and alkyl sulfide with bromine and the interference of alcohol in exhaustive oxidation. This difficulty can be overcome by using the addition reaction of acrylonitrile to alkyl mercaptan to yield sulfide, which is readily oxidized to sulfoxide. The reactions of acrylonitrile and alkyl mercaptans have been studied by Earle (*2*) and Hurd and Gershbein (*4*), and the determination of acrylonitrile and alpha-beta-unsaturated compounds has been described by Beesing *et al.* (*1*).

Mixtures of alkyl mercaptans and alkyl disulfides in the absence of alcohol have been determined by bromine oxidation as described by Siggia and Eds-

berg (*10*), and in the presence of alcohol using a Jones reductor as described by Kolthoff *et al.* (*9*). The exhaustive oxidation method is not applicable in presence of alcohol. The results for disulfide obtained with a Jones reductor are about 4% low. Better results are obtained by reducing alkyl disulfides under refluxing conditions with amalgamated zinc and acetic-hydrochloric acid–ethyl alcohol mixtures. The reduction of alkyl disulfides by zinc in acetic acid has been described recently by Karchmer and Walker (*6*).

This paper describes conditions under which mixtures of alkyl mercaptan–dialkyl sulfide and alkyl mercaptan–alkyl disulfide can be determined accurately using standard iodine and bromate solutions. The results obtained by these methods agree to better than 1%.

REAGENTS AND APPARATUS

Master solutions were prepared by weighing out approximately 0.025 mole of Eastman White Label alkyl mercaptan, dialkyl sulfide, or alkyl disulfide and diluting to 250 ml. with 95% alcohol or glacial acetic acid. The glacial acetic acid solutions were used for exhaustive oxidation titrations with bromate. Mixtures were prepared by pipetting out the desired amounts of the master solutions.

The concentration of mercaptan in the master solution was determined iodometrically. The concentration of alkyl sulfide in the master solution was obtained using standard bromate-bromide titrant. The alkyl disulfide concentration was derived primarily from the weighed amount. However, the results were checked against the titration of the disulfide after reduction to mercaptan or by exhaustive oxidation in the absence of alcohol.

Approximately 0.1*N* potassium triiodide solution was prepared by dissolving iodine in potassium iodide solution and standardizing against arsenious oxide.

The standard 0.1*N* bromate-bromide solution was prepared by weighing analytical grade potassium bromate and adding at least a sevenfold excess of potassium bromide. The normality was checked against arsenious oxide.

Acrylonitrile used for the condensation reaction with alkyl mercaptans was obtained from the Matheson Co. It was used without further dilution.

The alkyl disulfide solutions were reduced in a refluxing flask containing a water-cooled condenser and a cold finger cooled by an ice-salt mixture. The latter attachment is necessary when using ethyl and propyl disulfides.

PROCEDURE

Alkyl Mercaptan–Dialkyl Sulfide Mixtures. Two equal aliquots of the alkyl mercaptan–dialkyl sulfide mixture were placed in separate flasks. The contents were diluted with a sufficient amount (30 to 50 ml.) of alcohol to keep the alkyl mercaptan and dialkyl sulfide in solution throughout the titration. One of the aliquots was acidified with approximately 2.0 ml. of acetic acid; mercaptan was then titrated with the standard iodine solution until the color of free iodine appeared. For small amounts of mercaptan a 10-ml. buret was used to enable the titrant to be measured within ±0.01 ml.

Alkyl mercaptans with higher molecular weights than propyl mercaptan could be titrated in the absence of oxygen in acidic potassium iodide solution with the standard bromate-bromide. This mercaptan solution was deaerated using pieces of dry ice. The results were somewhat lower than in the direct iodometric determination. When this method is used, only one standard solution is necessary for determination of mercaptan and sulfide.

The contents of the second flask were made alkaline with approximately 3 drops of 10% potassium hydroxide, then acrylonitrile was added from a 1-ml. graduated pipet. The volume of acrylonitrile to be added was determined by the amount of mercaptan present. Approximately a twofold excess as compared to mercaptan was usually sufficient. However, the volume of acrylonitrile is not critical, as long as there is an excess and provided blank determinations are run using the same amount of acrylonitrile.

After the addition of acrylonitrile the solution was stirred for about 2 minutes to ensure completion of the reaction. It was then acidified with approximately 15 ml. of glacial acetic acid containing about 3 ml. of concentrated hydrochloric acid and titrated with the standard bromate-bromide solution to the first appearance of free bromine throughout the solution for at least 30 seconds. The end point could be observed best in a white light against a white background. Under these conditions the blank volume of 0.1*N* bromate-bromide necessary to impart a yellow coloration to the solution did not exceed 0.1 ml. In the presence of approximately 0.2 ml. of acrylonitrile the blank required about 0.15 ml.

The amount of alkyl sulfide present in the mixture was calculated from the two titrations as follows:

$$\text{Alkyl sulfide, grams} = \text{meq.}_{BrO_3^- - Br^-} - 2(\text{meq.}_{I_2^-}) \times E$$

where milliequivalents of bromine is for the second titration corrected for the blank, milliequivalents of iodine is for the first titration corrected for the blank, and E is the equivalent weight of sulfide.

Alkyl Mercaptan–Alkyl Disulfide Mixtures. Two equal aliquots of the alkyl mercaptan–alkyl disulfide mixture were used; one was placed in a volumetric flask and the other in a refluxing flask. The first aliquot was titrated with standard iodine solution until the appearance of free iodine color. Alkyl disulfide in the second aliquot was reduced with 20-mesh granulated zinc under refluxing conditions in a mixture of alcohol and acetic and hydrochloric acids. Satisfactory results were obtained using a mixture containing approximately 25 ml. of alcohol, 5 ml. of acetic acid, and 0.5 ml. of hydrochloric acid. The reduction of *n*-alkyl disulfides was complete in about 30 minutes.

The refluxing flask was then chilled in an ice bath, after which the condenser and cold finger were rinsed and the contents transferred to the titration flask. Total mercaptan was then titrated with iodine.

For the reduction of ethyl disulfide special precautions were taken to prevent the loss of ethyl mercaptan and ethyl disulfide by evaporation. The gases escaping from the cold finger were allowed to bubble through the iodine solution. However, the losses of ethyl mercaptan were considerable. Tertiary alkyl disulfides could not be reduced and determined by this method. Mercaptans boiling higher than ethyl mercaptan were recovered quantitatively. The amount of disulfide in the mixtures was determined as follows:

$$N_{I_2} - (\text{ml.}_2 - \text{ml.}_1) \times \text{eq. wt. of disulfide} = \text{grams of disulfide}$$

Results obtained by this method were low by approximately 1%.

RESULTS AND DISCUSSION

The determination of alkyl mercaptans and dialkyl sulfides in the presence of alcohol cannot be performed using exhaustive oxidation with bromate-bromide in strongly acid solution because of the oxidation of alcohol. However, in the absence of alcohol the oxidation proceeds slowly to completion in the following manner:

$$RSH + 3Br_2 + 2H_2O = RSO_2^+ + 6Br^- + 5H^+ \quad (1)$$

$$RSR + Br_2 + H_2O = RSOR + 2Br^- + 2H^+ \quad (2)$$

$$RSOR + Br_2 + H_2O = RSO_2R + 2Br^- + 2H^+ \quad (3)$$

The first and third reactions are slow and incomplete at room temperature, but can be speeded by heating. In the presence of alcohol the determination is based on the condensation reaction of alkyl mercaptan with acrylonitrile

$$RSH + CH_2 = CHCN \xrightarrow{(OH)^-} RS{-}CH_2{-}CH_2{-}CN \quad (4)$$

The resulting sulfide is readily oxidized in dilute acid solution by bromine according to Equation 2. It is titrated to the first appearance of free bromine throughout the solution. The double bond in acrylonitrile is not brominated readily. The end point can be determined to within ±0.03 ml.

The average values of five determinations for mixtures of alkyl mercaptans and dialkyl sulfides are summarized in Table I. The results for alkyl mercaptan in the absence of dialkyl sulfide determined by iodine or by bromate-

Table I. Titration of Alkyl Mercaptan–Dialkyl Sulfide Mixtures

(Results are average of five determinations)

Sample	Millimoles of Alkyl Mercaptan Added	Millimoles of Alkyl Mercaptan Found by Iodometric method	Millimoles of Alkyl Mercaptan Found by Acrylonitrile condensation, and bromate titration	Millimoles of Dialkyl Sulfide Added	Millimoles of Dialkyl Sulfide Found by bromate titration[a]
Ethyl mercaptan	0.9574	0.956	0.957	..	..
Mixture IA[b]	0.9574	0.956	..	0.2470	0.244
Mixture IB[b]	0.4787	0.477	..	0.6175	0.615
Mixture IC[b]	0.1915	0.190	..	1.2350	1.227
Diethyl sulfide	..	..	..	1.2350	1.236
n-Propyl mercaptan	1.1130	1.109	1.115	..	..
Mixture IIA[c]	1.1130	1.107	..	0.2469	0.245
Mixture IIB[c]	0.5665	0.556	..	0.6172	0.616
Mixture IIC[c]	0.2226	0.221	..	1.2340	1.232
Dipropyl sulfide	..	..	..	1.2340	1.233
n-Butyl mercaptan	1.0640	1.061	1.065	..	..
Mixture IIIA[d]	1.0640	1.059	..	0.1844	0.183
Mixture IIIB[d]	0.5320	0.531	..	0.4611	0.460
Mixture IIIC[d]	0.2128	0.211	..	0.9222	0.921
Dibutyl sulfide	..	..	..	0.9222	0.921
n-Octyl mercaptan	0.9015	0.901	0.901	..	..
Mixture IVA[e]	0.9015	0.901	..	0.1844	0.183
Mixture IVB[e]	0.4507	0.449	..	0.4611	0.460
Mixture IVC[e]	0.1803	0.179	..	0.9222	0.921

[a] Millimoles of dialkyl sulfide obtained from $[(meq.)_{BrO_3^-} - 2(meq.)_{I_3^-}]/2$.
[b] Mixture I, ethyl mercaptan and diethyl sulfide.
[c] Mixture II, *n*-propyl mercaptan and dipropyl sulfide.
[d] Mixture III, *n*-butyl mercaptan and dibutyl sulfide.
[e] Mixture IV, *n*-octyl mercaptan and dibutyl sulfide.

bromide titrations, after the condensation of mercaptan with acrylonitrile, are in good agreement. The end point can be reproduced readily to ±0.03 ml., yielding reproducibility better than 1% for the 10-ml. volume of titrant used.

The results for dialkyl sulfide in the presence of mercaptan can be reproduced to approximately 1% or better if small amounts are present. The results are in better agreement with higher molecular weight dialkyl sulfides than with diethyl sulfide.

The presence of acrylonitrile affects the blank correction somewhat. The change of volume of acrylonitrile from 0.2 to 0.5 ml. increases the blank correction by approximately 0.1 ml. of 0.1*N* bromate. The condensation of acrylonitrile with mercaptan is rapid and complete in alkaline solution at pH 12 or above.

Normal and secondary mercaptans can be titrated with the standard iodine solution. Tertiary mercaptans should be titrated argentometrically. Hydrogen sulfide and all the substances which can be oxidized by iodine will interfere. Bromate-bromide titrations can be used in the absence of all the substances which can be oxidized by bromine and all unsaturated compounds prone to bromination. Dialkyl sulfides can be oxidized to sulfoxides without difficulty, and the end points are rather sharp and reproducible.

Mixtures of hydrogen sulfide, mercaptan, and dialkyl sulfide can be titrated potentiometrically with alcoholic silver nitrate to yield the results for mercaptan and hydrogen sulfide simultaneously (*5*). Dialkyl sulfide then can be determined by bromate-bromide titration after the separation of hydrogen sulfide and mercaptan as cadmium sulfide and cadmium mercaptide, respectively. This separation can be avoided by condensing hydrogen sulfide and mercaptan with acrylonitrile and titrating all the dialkyl sulfides with the standard bromate-bromide solution. However, the results for the dialkyl sulfide are continuously low and reproducibility is rather poor. The difficulty arises in condensation of hydrogen sulfide with acrylonitrile. Ordinarily it is necessary to have about a fivefold excess of acrylonitrile and a condensation time of about 30 minutes instead of 2 minutes as is used for mercaptans.

Alkyl disulfide in mixtures of alkyl mercaptan and alcohol cannot be determined by exhaustive oxidation with bromine. However, the resulting mercaptan can be determined after the reduction of the disulfide with zinc in an acetic-hydrochloric acid–alcohol mixture with an accuracy as good as for mercaptans alone. Determinations of mixtures of alkyl disulfide and alkyl mercaptan mixtures are summarized in Table II.

Table II. Titration of Alkyl Mercaptan–Alkyl Disulfide Mixtures

(Results are average of five determinations)

	Millimoles of Mercaptan Added	Millimoles of Mercaptan Found by iodometric method	Millimoles of Disulfide Added	Millimoles of Disulfide Found by Iodometric method	Millimoles of Disulfide Found by Exhaustive oxidation with bromate[a]
Ethyl disulfide	..	..	0.713	0.645	0.680
n-Propyl disulfide	..	..	1.244	1.235	1.207
Mixture IA[b]	0.2226	0.221	1.244	1.231	..
Mixture IB[b]	1.1130	1.107	0.248	0.245	..
n-Butyl disulfide	..	..	1.105	1.098	1.079
Mixture IIA[c]	0.2128	0.212	1.105	1.096	..
Mixture IIB[c]	1.0640	1.059	0.221	0.219	..
n-Octyl disulfide	..	..	1.175	1.170	1.145
Mixture IIIA[d]	0.1803	0.179	1.175	1.169	..
Mixture IIIC[d]	0.9015	0.901	0.235	0.232	..
Isopropyl disulfide[e]	..	..	1.251	1.238	1.215
tert-Butyl disulfide[e]	..	..	0.978	0.592[e]	0.959

[a] Solutions have no alcohol.
[b] Mixture I, *n*-propyl disulfide and *n*-propyl mercaptan.
[c] Mixture II, *n*-butyl disulfide and *n*-butyl mercaptan.
[d] Mixture III, *n*-octyl disulfide and *n*-octyl mercaptan.
[e] Resulting *tert*-butyl mercaptan is titrated with silver nitrate.

The results for butyl and octyl disulfides determined (after reduction with zinc) with the standard iodine solution can be readily reproduced to within ±0.1 ml. with a recovery better than 99%. The results for ethyl and propyl disulfides are low. The recovery for ethyl disulfide is about 94% and that for propyl disulfide about 98.5%. This is probably caused by the volatilization of mercaptans or disulfides and incomplete reduction of the corresponding disulfides. However, with proper care propyl disulfide can be determined iodometrically with better accuracy than by using exhaustive oxidation with bromine. Tertiary alkyl disulfides cannot be reduced satisfactorily and, therefore, cannot be determined by this method.

The determination of mixtures of alkyl mercaptans, dialkyl sulfides, and

alkyl disulfides in the presence of alcohol cannot be achieved by direct titrimetric methods without the removal of mercaptan and the reduced disulfide as silver mercaptides. In these mixtures the condensation of mercaptan with acrylonitrile after the reduction of disulfide with zinc does not proceed even in presence of ethylenediaminetetraacetic acid, and thus dialkyl sulfide cannot be determined satisfactorily by this method.

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RECEIVED for review June 25, 1958. Accepted December 3, 1958.

Spectrophotometric Determination of Traces of Nickel with 4-Isopropyl-1,2-cyclohexanedionedioxime

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▶ The use of xylene rather than chloroform to extract the nickel(II)–4-isopropyl-1,2-cyclohexanedionedioxime chelate results in increased sensitivity in the spectrophotometric determination of nickel. The extraction coefficient in xylene is more than five times as great as that in chloroform, and the low solubility of xylene in aqueous solution permits the use of much larger phase ratios (aqueous to organic). The system conforms to Beer's law for concentrations of nickel from 1 to 12 γ of nickel per ml. of xylene. The precision of the method is 2%. Iron, cobalt, and copper interfere seriously; however, their interference can be minimized so that weight ratios of metal to nickel of 20, 2, and 8, respectively, can be tolerated. Concentrations of nickel from 0.005 to 100 p.p.m. were determined in water, alkali metals, and several analytical reagents. Recoveries in excess of 95% were obtained when known amounts of nickel were extracted from aqueous solutions of relatively high ionic strength at aqueous-to-organic volume ratios as high as 300 to 1.

REAGENTS for the determination of nickel have been adequately reviewed by Hooker and Banks (*6*), who noted that the vicinal dioximes are examples of a nearly ideal reagent for the determination of nickel. Of the several substituted cyclohexanedionedioximes recommended as reagents for the determination of nickel by gravimetric, spectrophotometric, and titrimetric procedures, these authors suggested 4-isopropyl-1,2-cyclohexanedionedioxime as the best reagent for the spectrophotometric determination of nickel. They suggested that extraction of the nickel chelate from large volumes of aqueous solution into chloroform offers the most sensitive method yet reported for the determination of nickel.

This report is concerned with further studies of 4-isopropyl-1,2-cyclohexanedionedioxime as a reagent for nickel. With xylene as an extractant for the nickel chelate, the reagent has been applied to the determination of 10 γ of nickel in as much as 500 ml. of aqueous solution with a coefficient of variation of 2%. This order of sensitivity makes the method ideally suited for application to such samples as liquid metals, purified water, and reagent chemicals.

Following the completion of this investigation, Blundy and Simpson (*1*) published a method applying 4-methyl-1,2-cyclohexanedionedioxime to the determination of nickel with toluene as the solvent for the nickel chelate.

APPARATUS

All absorbance measurements were made in 1.00-cm. Corex cells. The absorption spectra were recorded with a Cary automatic recording spectrophotometer, Model 14-M, and absorbance measurements were made with a Beckman Model DU spectrophotometer.

REAGENTS

Prepare a standard nickel(II) solution by dissolving 5 grams of nickel shot in hydrochloric acid and diluting the resulting solution to 500 ml. Determine the concentration of nickel gravimetrically (*3*). The solution used for this study contained 9.97 mg. per ml. Make appropriate dilutions of this stock solution to yield standard solutions of lower nickel concentration.

Prepare a 0.004*M* solution of the chromogenic reagent, 4-isopropyl-1,2-cyclohexanedionedioxime [synthesized according to the procedure of Hooker and Banks (*6*)], by stirring 80 mg. of the reagent with 100 ml. of water for 24 hours and then filtering off the excess reagent. The 0.004*M* solution is a saturated aqueous solution of the dioxime (*6*).

Xylene, hexane, chloroform, benzene, and toluene, all reagent grade.

Ammonium acetate solution, approximately 10*M*.

Sodium fluoride solution, 10 mg. of fluoride per ml.

Sodium thiocyanate solution, 10% (w./v.).

Potassium cyanide solution, 10% (w./v.).

Hydroxylamine hydrochloride solution, 10% (w./v.), prepared fresh daily.

Prepare a solution of Sulfi-Down (stabilized thioacetamide, distributed by A. Daigger and Co., Chicago, Ill.) by dissolving 2.5 grams of the solid reagent in 50 ml. of water and heating to 80° ± 5° C. Cool immediately to prevent evolution of hydrogen sulfide. Store in a tightly stoppered flask and prepare fresh daily.

PROCEDURE

Transfer a volume of solution which contains 10 to 150 γ of nickel to a separatory funnel of appropriate size. Adjust to approximately pH 7 and add sufficient 10*M* ammonium acetate to make the final solution 1*M* with respect to acetate. For a final aqueous volume of 100 ml. or less, add 2 ml. of the saturated dioxime solution. For each additional 100 ml. of aqueous solution add another 2 ml. of dioxime solution. After 30 minutes, add 10 ml. of xylene; a smaller volume may be used if the amount of nickel is very low. Shake the contents of the funnel

vigorously for 2 minutes; longer equilibration periods may be necessary for aqueous volumes in excess of 500 ml.

After the phases have separated, discard the aqueous phase. Measure the absorbance of the xylene solution of the chelate at 383 mμ versus the xylene extract of a blank solution prepared in the same manner as the sample. On the basis of standards which contain 1 to 12 γ per ml. of nickel in the organic phase, the coefficient of variation of this method is less than 2%.

EXPERIMENTAL

Extractants for Nickel Chelate. Hooker and Banks (*6*) extracted nickel after precipitation as the 4-isopropyl-1,2-cyclohexanedionedioxime chelate from a neutral solution buffered with ammonium acetate. To effect quantitative extraction from large volumes of solution, they carried out repeated extractions with small volumes of chloroform, which were then combined and diluted to an appropriate volume.

Other solvents were investigated in an attempt to accomplish quantitative extraction of the chelate into the organic phase with a single equilibration. The criteria on which the selection of the solvent was based were a large extraction coefficient of the chelate and a negligible mutual solubility with the aqueous phase. Preliminary screening tests of various solvents were carried out by extracting, with a single 10-ml. portion of the solvent, solutions which contained 50 γ of nickel that had been precipitated with a solution of 4-isopropyl-1,2-cyclohexanedionedioxime (*6*). The extent of extraction was estimated either on the color of the chelate remaining in the aqueous phase or on the color of a second extract. Of the solvents tested, chloroform, benzene, and xylene appeared to be satisfactory (Table I).

Tests were carried out to determine the distribution of nickel after extraction of the chelate into each of these solvents by precipitating 8.5 μmoles of nickel with 21 μmoles of the reagent [a 25% excess of that required to form the $Ni(C_9H_{14}N_2O_2)_2$ complex] (*2*). The nickel chelate was then extracted into 10 ml. of the solvent from 100 ml. of aqueous solution buffered at pH 7 by the addition of 0.1 mole of ammonium acetate. The aqueous solution also contained 100 mg. of fluoride to complex any iron that might be present. After the phases were separated, the organic phase was discarded and the aqueous phase was centrifuged to remove the last traces of the organic phase.

The concentration of nickel in the aqueous phase was determined by adding 2 ml. of a saturated solution of the dioxime in water, extracting the remaining chelate into 5 ml. of the appropriate solvent, and measuring the absorbance of these extracts at 383 mμ. The concentration of nickel was obtained from a standard curve prepared by extracting known quantities of the nickel chelate into each of these solvents. The chelate conformed to Beer's law for concentrations of nickel of 0.5 to 10 γ per ml. for all three solvents. The extraction coefficients of nickel—i.e., the ratio of the concentration of nickel in the organic phase to the concentration of nickel in the aqueous phase—for the three solvents were 210 for chloroform, 320 for benzene, and >1000 for xylene.

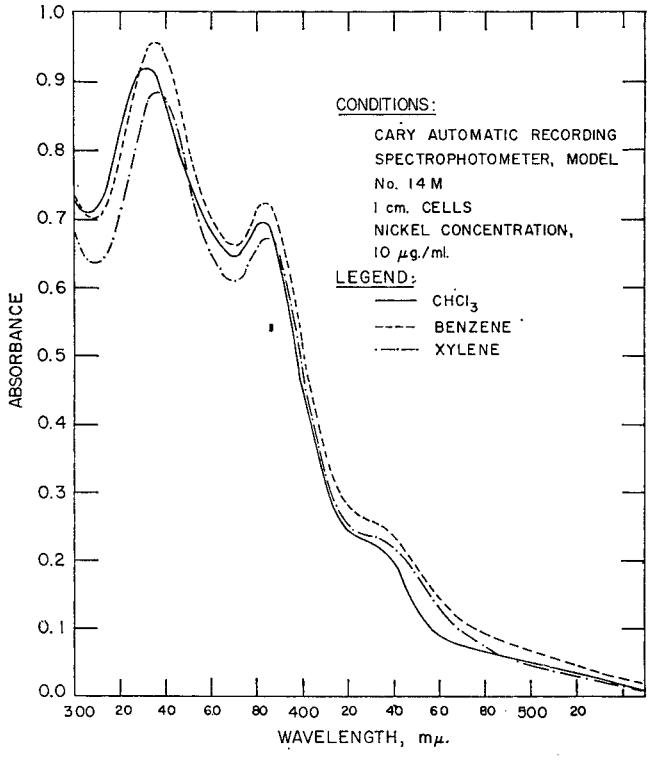


Figure 1. Absorption spectra of nickel-(II)-4-isopropyl-1,2-cyclohexanedionedioxime chelate in chloroform, benzene, and xylene

Xylene was selected as the most suitable solvent because it had the largest extraction coefficient. For extraction under conditions of high aqueous-to-organic volume ratios, xylene offers three additional advantages: low solubility in water (*7*), rapid rate of extraction of the chelate, and sharp phase separation.

The absorption spectrum of the chelate in each of the three solvents is shown in Figure 1. For all three solvents the spectra are of the same general shape and exhibit absorption maxima at 385 and at approximately 335 mμ. The position of the maximum at the shorter wave length shifts somewhat with different solvents.

Effect of pH of Aqueous Phase on Extraction. In the presence of a small excess of reagent, the extraction coefficient reached a maximum value (approximately 1000) when the pH of the solution was 7 or 8. The value of the coefficient decreased rapidly at pH values lower than 6 and higher than 9, to about 100 at pH 5 and pH 10. The decrease in the coefficient at high pH values may have been caused in part by the large amount of ammonium hydroxide required to adjust the pH. For the sake of convenience, pH 7 with 1*M* ammonium acetate as the buffer solution was chosen as the optimum acidity. Hooker and Banks recommend extraction from an acetate-buffered solution.

Table I. Comparison of Solvents as Extractants for Nickel Chelate

Solvent	Comments
Chloroform	Approximately 95% extracted rapidly to yield yellow solution
Carbon tetrachloride	Approximately 50% extracted slowly to yield yellow solution
Perchloroethylene	No apparent extraction; some chelate transferred as red precipitate
Butyl acetate	No apparent extraction; some chelate transferred as red precipitate
n-Amyl alcohol	
Paraffinic hydrocarbons	
Aromatic hydrocarbons	
Benzene	Rapid, quantitative extraction of chelate to yield yellow solution
Toluene	Extraction similar to benzene, but less rapid
Xylene	Very rapid, quantitative extraction of chelate to yield yellow solution

Solubility of Reagent in Xylene. The extraction coefficient of the chelating agent was determined at pH 7 and 5. The amount of reagent remaining in the aqueous phase was determined by measuring the absorbance of xylene extracts obtained after the addition of excess nickel. The absorption spectra of these extracts coincided with those of extracts from solutions that contained excess reagent. When 15 mg. of reagent was initially present in the aqueous phase, the coefficient was 0.46 at pH 7 as compared to 8.3 at pH 5. Therefore, at pH 7 and at aqueous-to-organic phase ratios of 10 to 1, practically all the chelating agent remains in the aqueous phase.

Extraction Efficiency at Extreme Phase Ratios. In order to obtain a more precise estimation of the extraction coefficient so that a limiting phase ratio for quantitative extraction could be estimated, equilibration measurements were carried out with increased quantities of nickel and reduced concentrations of excess reagent. Solutions which contained 17 μmoles of nickel as the chelate in 10 ml. of xylene (prepared by direct dissolution of the dried chelate) were equilibrated with 100 ml. of aqueous solutions to which 0 to 6.9 μmoles of reagent had been added. Following equilibration, the concentration of nickel in the aqueous phase was determined as previously described.

The formation and extraction of the chelate can be represented by the equation

$$Ni^{++}{}_a + 2RH_{2_a} \rightleftharpoons Ni(RH)_{2_o} + 2H^+{}_a \quad (1)$$

where RH_2 = 4-isopropyl-1,2-cyclohexanedionedioxime.

Under the conditions of these experiments the reactants are present in relatively low concentrations. Therefore, all activity coefficients should remain constant, and the concentration equilibrium constant may be expressed as:

$$K = \frac{[Ni(RH)_2]_o[H^+]_a^2}{[Ni^{++}]_a[RH_2]_a^2} = \frac{[Ni(RH)_2]_o[H^+]_a^2}{[Ni^{++}]_a\left\{\frac{R_a + 2[Ni^{++}]_a}{1 + \frac{k_1}{[H^+]_a} + \frac{k_1k_2}{[H^+]_a^2} + \frac{D}{P}}\right\}^2} \quad (2)$$

in which the concentration of the undissociated reagent, RH_{2a}, has been expressed in terms of the excess reagent before extraction, R_a, by algebraic solution of conventional equilibrium expressions in which k_1 and k_2 are the first and second ionization constants of the reagent, D is the distribution ratio of the reagent between the organic and aqueous phases, and P is the phase ratio. Since these equilibrations were carried out under conditions of constant phase ratio and acidity, the value of the terms, $\frac{k_1}{[H^+]_a}$, $\frac{k_1k_2}{[H^+]_a^2}$, and $\frac{D}{P}$, in Equation 2 are constant; therefore, a practical concentration equilibrium constant, k', which is a function of the hydrogen ion concentration and the phase ratio, may be defined as follows:

$$K'(P, [H^+]) = \frac{[Ni(RH)_2]_o}{[Ni^{++}]_a\{R_a + 2[Ni^{++}]_a\}^2} \quad (3)$$

Values of K', calculated on the assumption that no undissociated chelate remains in the aqueous phase after extraction, are given in Table II. In view of the low concentrations of nickel remaining in the aqueous phase and the inherent limitations of phase separation, the agreement between the values of the equilibrium constant is satisfactory. The value of the equilibrium constant, approximately 3×10^{13}, should not be materially different at the concentration of reagent specified in the analytical procedure.

From Equation 3 it is obvious that the extraction coefficient, E_a°, is given by the expression

$$E_a^\circ(R_a, [H^+], P) = K'\{R_a + 2[Ni^{++}]_a\}^2 \quad (4)$$

Under the usual conditions of analytical measurements, the concentration of uncomplexed nickel is negligible and the value of the extraction coefficient is equal approximately to $K'(R_a)^2$. As it has been determined that the reagent remains substantially in the aqueous phase when extractions are carried out at pH 7 and at a phase ratio of 10, the value of $\frac{D}{P}$ is negligible and K' and E_a° are therefore independent of the phase ratio at ratios of 10 or greater. However, it is necessary to increase the amount of reagent when larger aqueous volumes are used in order to maintain the same concentration of reagent in the aqueous phase.

In the recommended procedure, at least 2 ml. of the saturated solution of the reagent is specified. If the quantity of nickel corresponds to the upper limit of the concentration range for precise photometric measurements (150 γ), the concentration of excess reagent, R_a, is about $3 \times 10^{-6}M$. From Equation 4, the extraction coefficient, E_a°, is calculated to be about 25,000. If 50 γ or less of nickel is present, a larger excess of reagent is present, and the extraction coefficient, E_a°, exceeds 100,000.

Table II. Effect of Excess Reagent on Equilibrium Constant of Nickel(II)–4-Isopropyl-1,2-Cyclohexanedionedioxime Chelate in Xylene

(Nickel added, 17.1 μmoles; aqueous-to-organic phase ratio, 10; pH of aqueous phase, 7; 1M ammonium acetate)

Excess Reagent Added, μmoles per Liter	Ni Concn. in Aq. Phase after Equilibration, μmoles per Liter	E_a°	Equilibrium Constant, K', (Liter per Mole)^2 $\times 10^{-13}$
0	3.21	530	1.3
3.0	1.71	1000	2.4
3.0	2.08	820	1.6
11.8	0.22	~8000	5.2[a]
59	0.14	>10000	0.35[a]

[a] Concentration of nickel in aqueous phase based on absorbance measurements less than 0.010.

INTERFERENCES

The effect of 100-mg. quantities of several cations and anions on the determination of 15 γ of nickel by the extraction of the nickel chelate into chloroform was investigated by Hooker and Banks. They reported serious interference only from cobalt(II), copper(II), iron(II), iron(III), and ruthenium(III). Except for ruthenium, which is easily removed by volatilization (*5*), suitable masking agents or extraction procedures were proposed for the elimination of these interferences. They recommended extracting the iron(III) as the cupferrate, complexing the cobalt as the hexacyanocobaltate(III) ion, and complexing the copper as the dithiocyanocuprate(I) ion.

Of the interferences reported by Hooker and Banks, iron, cobalt, and copper were of primary importance in this investigation. When the chelate is extracted into xylene, the interference of these substances is more serious than that reported by Hooker and Banks, probably due to increased effectiveness of the solvent. Cobalt and copper yield xylene extracts with absorbances proportional to the concentration of the interfering substances. The interference of iron is not proportional to its concentration because it is partially hydrolyzed in the aqueous phase.

Because separations were considered to be impractical for large volumes of sample solution, the use of masking agents to eliminate the interference of iron was investigated. Of the complexing agents investigated—fluoride, citrate, tartrate, and *o*-phenanthroline—fluoride was the only reagent which, when added to the aqueous solution, would reduce the interference of iron without reducing the effectiveness of the

Table III. Effect of Interfering Ions

(Nickel, 50 γ; aqueous-to-organic phase ratio, 10; reagent concentration, 0.00008*M*)

Interfering Ion	Tolerance Limit without Masking Agent, γ		Masking Agent	Tolerance Limit with Masking Agent, γ	
	2% error	5% error		2% error	5% error
Co(III)	4	10	H_2O_2, KCN	100	
Cu(II)	2	4	Sulfide	400	800
Fe(II)	2	5	...		
Fe(III)	2	5	Fluoride		
			0.1 gram	200	
			0.2 gram	400	
			0.6 gram	1000	2000

Table IV. Determination of Nickel in Diverse Samples

(All samples extracted with 10 ml. of xylene)

Material	Sample Weight, Grams	Vol. of Aqueous Phase, Ml.	Nickel Found		Recovery of Added Nickel	
			γ	P.p.m.	γ	%
Tap water	500	600	4.1	0.008		
Demineralized water + 100 γ Ni	1000	1200			99	99
Reagent grade HCl						
A	300	100[a]	6.0	0.020		
B	900	100[a]	5.2	0.005		
Sodium-potassium alloy						
A	3.1	200	8.3	2.7		
B	2.4	200	9.7	4.0		
Lithium metal						
A	11.2	600	1.6	0.14		
B	61.0	2800	6.2	0.098		
Sodium chloride	100	350	6.5	0.065		
Potassium chloride + 50 γ Ni	100	500	2.1	0.021	49	98
Lithium chloride[b] + 50 γ Ni	375	3000			48	96

[a] Evaporated to very small volumes to remove free acid.
[b] Lithium chloride solution extracted to remove any nickel prior to nickel addition.

nickel extraction. The tolerance limits for iron in the presence of various concentrations of fluoride are reported in Table III.

As much as 100 γ of cobalt produced no measurable interference when converted to the hexacyanocobaltate(III) ion according to the procedure of Feigl and Kapulitzas (*4*), in which the cobalt is oxidized with peroxide in the presence of cyanide, and the excess cyanide is destroyed with formaldehyde to decompose the nickel-cyanide complex.

While copper can be eliminated as the dithiocyanocuprate(I) ion, it was impossible to form this complex and the hexafluoroferrate(III) complex in the same solution. As no satisfactory reagent was found for masking copper(II) in the aqueous phase, the copper and nickel chelates were extracted into the xylene, and the interference of copper was eliminated by washing the xylene extract with 5 ml. of a slightly acidic solution of thioacetamide. The copper, along with some of the nickel, was extracted into the aqueous thioacetamide solution. The nickel was returned to the xylene phase by adding 1 ml. of reagent solution and 5 ml. of 10*M* ammonium acetate and re-equilibrating the solutions. The copper remained in the aqueous phase as suspended copper sulfide.

Because this procedure introduces a significant increase in the absorbance of the extracts, samples must be compared to reference extracts that have been prepared in exactly the same manner. As much as 800 γ of copper can be removed from the organic phase by a single wash with a solution of thioacetamide. However, an amount of chelating agent sufficient to complex the copper and nickel completely and to provide excess reagent must be added to the solution, because the copper competes with the nickel for the chelating agent.

Similarly, iron, which cannot be removed from the extracts by washing with a neutral fluoride solution, can be complexed with fluoride after removal from the xylene by extraction with an acidic aqueous solution. This technique must be used to remove the interference of iron from extracts of sample solutions that contain constituents which are not compatible with large amounts of fluoride ions—e.g., lithium or calcium. In general, since iron is a common contaminant and may be present in concentrations sufficient to compete with nickel for the dioxime, it is usually desirable to complex the iron(III) by direct addition of fluoride to the solution of the sample prior to the extraction of the copper and nickel chelates.

The precision of the method is not altered by addition of fluoride; however, since procedures for the elimination of the interference of cobalt and copper reduce the precision of the method from 2% to 5%, the use of these procedures should be restricted to samples containing these contaminants. Because of the large volumes of solution which may be analyzed by this procedure, extreme care must be exercised to prevent contamination by copper. Maximum tolerance limits of the interferences are reported in Table III.

APPLICATIONS

The results of analyses of diverse samples, as well as recoveries of added nickel, are shown in Table IV. For all samples except those which contain lithium, fluoride was added to the aqueous phase to complex any iron present, following which the organic extract was washed with an acidified solution of hydrogen sulfide to remove any copper. The organic extracts from the lithium samples were washed with an acidified fluoride solution after separation of the phases, because addition of fluoride ion to the aqueous phase would precipitate lithium fluoride.

The recovery of known amounts of nickel is satisfactory, particularly in the analysis of lithium chloride. The success of this extraction at the unfavorable phase ratio of 300 to 1 lends considerable credence to the validity of the results shown, despite the fact that the amount of nickel available in some of the samples was less than the recommended minimum. The nickel concentrations in the samples shown in Table IV are reasonable values for materials of these types.

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RECEIVED for review June 19, 1958. Accepted December 1, 1958. Division of Analytical Chemistry, 134th Meeting, ACS, Chicago, Ill., September 1958. Work carried out under contract No. W-7405-eng-26 at Oak Ridge National Laboratory, operated by Union Carbide Nuclear Co., a division of Union Carbide Corp., for the Atomic Energy Commission.

Mass Spectrometric Analysis of Aliphatic Amides

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▶ **The mass spectra of 35 aliphatic amides have been tabulated and correlated. The amides are divided into three classes: primary, secondary, and tertiary. Fragmentation and rearrangement ions typifying each of the classes are noted, in addition to spectral characteristics common to all three classes of the aliphatic amides. These observations are utilized for prediction and characterization of molecular structure.**

CORRELATION of the mass spectra of the aliphatic amides permits an additional type of compound to be more rapidly and reliably characterized in regard to its molecular structure. Previous correlations of this type for the aldehydes (*4*), esters (*5*), alcohols (*3*), ketones (*11*), halogens (*10*), acids (*7*), and sulfides and mercaptans (*8*) have provided valuable information which facilitates the elucidation of molecular structure using mass spectral data.

The mass spectra of 35 aliphatic amides are tabulated and correlated. Fragmentation and rearrangement ions which are useful in structural identification are discussed.

EXPERIMENTAL

The spectra were obtained on a 90° sector mass spectrometer (*1*), which had a 200° C. heated inlet system (*2*).

The amides were the purest available either commercially or by synthesis. No attempts toward further purification were made.

RESULTS

The amides are classified as

primary $\left(R-\overset{O}{\overset{\|}{C}}-NH_2\right)$,

secondary $\left(R-\overset{O}{\overset{\|}{C}}-NH-R'\right)$,

and tertiary $\left(R-\overset{O}{\overset{\|}{C}}-N\begin{matrix}R''\\R''\end{matrix}\right)$.

Molecular ions are detected in all of the amide spectra examined. Molecular ion contributions vary from the highest peak in the spectra of several of the lower molecular weight amides to a minimum of 0.5% of the most intense ion current for several cases. The higher molecular weight secondary and tertiary amides give a molecular ion contribution, which is 1 to 5% of the most intense ion current in the spectrum, making possible rapid determination of molecular weight.

Several cleavages, which are of secondary importance for the amides as a whole, are especially significant for the lower molecular weight amides. The molecular ion or a fragment which can be correlated with cleavage of one of the bonds alpha to the carbonyl group is often the most intense ion in the spectrum when the alkyl chain substituted on the carbonyl and the alkyl chain(s) substituted on the nitrogen atom have no gamma carbon atom. Exceptions are observed for *N*-ethyl acetamide and *N*,*N*-diethyl acetamide.

Primary Amides. The spectra of the primary amides $\left(R-\overset{O}{\overset{\|}{C}}-NH_2\right)$ are given in Table I.

The mass spectra of the primary amides are characterized by an ion of *m/e* 59 ($C_2H_5NO^+$), which is the most intense ion in the spectrum. This rearrangement ion can be correlated with cleavage of the carbon-carbon bond beta to the carbonyl accompanied by the rearrangement of a hydrogen atom to the fragment containing the nitrogen atom.

$$R-\overset{\oplus}{C}H-CH_2-\!\!\!\sim\!\!\!-CH_2-\overset{O}{\overset{\|}{C}}-NH_2 \rightarrow C_2H_5NO^+ (m/e\ 59)$$

This beta cleavage accompanied by hydrogen rearrangement has been previously observed to be a prominent spectral characteristic of the aliphatic aldehydes [*m/e* 44 ($C_2H_4O^+$)] (*4*), and the rearrangement ion of the amides, like the aldehydes, affords resultant stable structures (*9*). Intense rearrangement ions corresponding to similar cleavage are frequently observed for the aliphatic acids (*7*) [*m/e* 60 ($C_2H_4O_2^+$)], the aliphatic cyanides [*m/e* 41 ($C_2H_3N^+$)], and the ketones (*11*).

Branching of the alkyl portion of the molecule is more difficult to specify; however, in general, cleavage occurs more readily at the bonds adjacent to branching than at carbon-carbon bonds along an unbranched chain.

Several amides of higher molecular weight give *m/e* 72 peaks of secondary importance, corresponding to cleavage of the carbon-carbon bond gamma to the carbonyl.

Secondary Amides. The spectra which were obtained of the six secondary amides $\left(R-\overset{O}{\overset{\|}{C}}-NH-R'\right)$ are given in Table II.

Only one of the secondary amides—*N*-butyl octadecanamide—has an alkyl chain, which contains a gamma carbon atom, attached to the carbonyl. The most intense peak in the *N*-butyl octadecanamide spectrum is a rearrangement ion of *m/e* 115 ($C_6H_{13}NO^+$), which can be correlated with cleavage similar to that of the primary amides.

$$C_{14}H_{29}-\overset{\oplus}{C}H-CH_2-\!\!\!\sim\!\!\!-CH_2-\overset{O}{\overset{\|}{C}}-NH- \rightarrow C_6H_{13}NO^+ (m/e\ 115)$$

(C_4H_9 →)

This cleavage and rearrangement are expected to be important for other secondary amides having a carbon atom gamma to the carbonyl.

Another mass spectral feature characterizing the secondary amides is an intense *m/e* 30 (CH_4N^+) peak, which is in many cases the most intense peak in the spectrum. The *m/e* 30 peak can be correlated with a rearrangement ion resulting from cleavage of the carbonyl carbon–nitrogen bond and cleavage of the carbon-carbon bond beta to the nitrogen atom, accompanied by the rearrangement of a hydrogen atom to the nitrogen-containing fragment. For example, *N*-ethyl acetamide (*a*), *N*-butyl acetamide (*b*), and *N*-isobutyl acetamide (*c*), give *m/e* 30 as the most intense peak in their spectra.

(*a*) $CH_3-\overset{O}{\overset{\|}{C}}-\!\!\!\sim\!\!\!-\overset{\oplus}{N}HCH_2-\!\!\!\sim\!\!\!-CH_3 \rightarrow CH_4N^+ (m/e\ 30)$

(*b*) $CH_3-\overset{O}{\overset{\|}{C}}-\!\!\!\sim\!\!\!-\overset{\oplus}{N}HCH_2-\!\!\!\sim\!\!\!-$

$CH_2CH_2CH_3 \rightarrow CH_4N^+ (m/e\ 30)$

(*c*) $CH_3-\overset{O}{\overset{\|}{C}}-\!\!\!\sim\!\!\!-\overset{\oplus}{N}HCH_2-\!\!\!\sim\!\!\!-$

$CH(CH_3)_2 \rightarrow CH_4N^+ (m/e\ 30)$

This *m/e* 30 rearrangement ion is comparable to rearrangement ions observed in aliphatic amine spectra (*6*).

Tertiary Amides. The spectra of the tertiary amides $\left(R-\overset{O}{\overset{\|}{C}}-N\begin{matrix}R''\\R''\end{matrix}\right)$ are given in Table III.

The major contributions in the spectra of the tertiary amides are correlated with the rearrangement ions

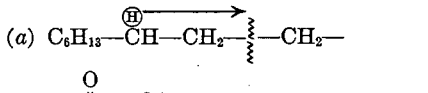
Table I. Mass Spectra of Primary Amides

Molecular	Form	Acet	Propion	*n*-Butyr	Isobutyr	*n*-Valer	Isovaler	Trimethyl acet	Capro	4-Methylvaler	Non	γ-*tert*-butyl capro	γ-*sec*-butyl capro	Dodecan	Hexadecan	Stear[b]
weight	45	59	73	87	87	101	101	101	115	115	157	171	171	199	255	283
Source[a]	EK	EK	D	EK	EK	EK	D	A	EK	EK	EK	D	D	A	A	EK
m/e	Relative Intensity															
15	1	23	1	1	2	0.7	1	1	0.4	0.3	0.2	0.2	0.2	0.4	0.1	2
27	12	2	14	14	20	8	6	6	7	8	4	3	4	5	3	5
28	10	7	12	5	9	5	2	~4	2	4	3	1	2	25[c]	40[c]	~34
29	28	2	26	4	4	14	10	32	6	9	5	6	9	10	7	7
30	0.9	2	2	1	0.9	1	0.8	1	0.6	0.8	0.4	0.4	0.8	1	0.7	1
31	1	3	0.1	0.5	0.1	0.6	0.7	0.1	0.4	0.5	0.2	0.1	0.2	0.3	0.2	0.5
41	0.3	16	1	17	32	13	17	47	9	15	9	11	16	18	14	16
42	2	28	2	9	10	5	6	4	5	5	3	2	3	4	3	4
43	12	57	3	30	60	11	20	2	24	22	13	11	18	22	21	27
44	27	76	100	64	100	38	31	16	27	33	13	8	15	12	7	15
45	100	2	5	3	6	2	1	1	1	2	0.6	0.3	0.7	1	1	2
55		...	7	7	8	6	1	3	6	8	6	9	17	11	13	16
56		0.1	3	0.4	0.9	2	2	6	1	3	1	2	2	3	6	5
57		0.1	18	0.2	0.2	11	12	100	2	16	5	16	15	13	16	18
58		0.2	1	0.2	0.6	0.7	1	8	0.2	2	0.3	1	1	2	1	2
59		100	3	100	26	100	100	3	100	100	100	100	100	100	100	100
60		4	0.1	3	0.7	3	3	0.2	3	3	5	4	8	9	12	13
71			0.2	8	6	...	...	0.1	2	2	2	4	9	4	6	8
72			11	18	40	19	0.5	...	18	33	27	7	46	34	44	44
73			56	1	2	3	3	...	4	7	5	2	10	8	10	9
74			3	...	0.1	0.1	0.2	...	0.2	0.5	0.3	0.2	0.6	0.8	1	3
85				...	...	3	2	0.3	...	0.1	0.3	0.1	0.2	2	4	5
86				3	4	3	11	9	9	7	6	0.8	2	7	7	7
87				3	23	0.2	0.6	0.5	0.5	0.7	0.5	0.1	0.2	1	2	2
88				1	2	...	...	...	...	...	...	0.2	0.1	0.1	0.6	0.5
99						...	...	...	1	0.9	0.1	0.2	0.2	0.6	1	2
100						0.8	1	0.5	0.3	8	1	2	4	2	2	2
101						0.6	2	18	...	0.5	0.4	0.3	0.3	2	2	3
114									0.4	0.6	4	21	27	5	7	6
115									0.5	0.6	0.5	11	3	2	3	6
116									0.7	1	...	4	1	0.2	0.8	2
127											...	...	0.1	0.2	0.5	1
128											2	0.1	0.2	5	7	9
129											0.3	...	...	2	3	3
141											0.2	...	...	0.2	0.4	1
142											0.1	0.6	2	1	1	2
143											...	0.1	0.2	0.5	0.6	0.9
156											0.1	2	0.4	3	1	1
157											0.9	0.3	...	1	0.7	0.5
169												...	0.1	...	0.2	1
170												...	...	2	2	3
171												0.5	0.7	0.7	0.8	0.8
184														...	1	2
185														...	0.8	1
197														0.1	1	3
198														0.3	2	4
199														1	0.6	0.9
212															4	1
213															1	0.4
226															2	1
227															0.6	0.3
240															0.4	5
241															0.2	1
255															3	0.6
283																4
P[d]	100	100	56	3	23	0.6	2	18	0.5	0.6	0.9	0.5	0.7	1	3	4
S/S_{tol}[e]	0.65	0.57	0.85	0.59	0.69	0.99	0.98	1.0	~0.88	0.86	1.2	0.96	0.69	0.33	0.22	0.12

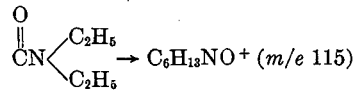
[a] EK = Eastman Kodak; D = Dow Chemical Co. (compounds listed are not necessarily commercially available); A = Aldrich Chemical Co.
[b] Some background contributing.
[c] Air background present.
[d] *P* = molecular ion (parent peak).
[e] $S/S_{tol} = \dfrac{\text{scale divisions } A}{\text{mg. of } A} \Big/ \dfrac{\text{scale divisions toluene}}{\text{mg. of toluene}}$.

previously noted for the primary and secondary amides.

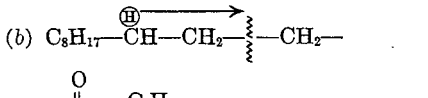
Two of the tertiary amides—*N,N*-diethyl decanamide (*a*) and *N,N*-diethyl dodecanamide (*b*)—give the most intense peak in their spectra at *m/e* 115. The formation of this rearrangement ion corresponds to cleavage of the carbon-carbon bond beta to the carbonyl, accompanied by the rearrangement of a hydrogen atom to the amide portion of the molecule.



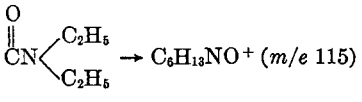
(*a*) $C_6H_{13}-\overset{\textcircled{H}}{\overset{\frown}{CH}}-CH_2-\cdots-CH_2-$



$\rightarrow C_6H_{13}NO^+$ (*m/e* 115)



(*b*) $C_8H_{17}-\overset{\textcircled{H}}{\overset{\frown}{CH}}-CH_2-\cdots-CH_2-$

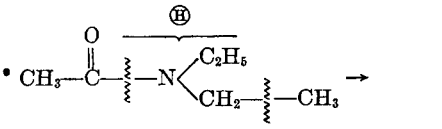


$\rightarrow C_6H_{13}NO^+$ (*m/e* 115)

The majority of the remainder of the tertiary amides examined give the most intense peak in their spectra at masses corresponding to cleavage similar to that proposed in the case of the *m/e* 30 rearrangement ion of the secondary amides. In other words, cleavage of the nitrogen–carbonyl carbon bond and one of the carbon-carbon bonds beta to the nitrogen atom, accompanied by the rearrangement of a hydrogen atom to the nitrogen-containing portion of the molecule, is consistent with the rearrangement ions observed.

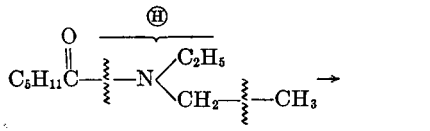
Several tertiary amides having different alkyl substitutions illustrate the formation of this rearrangement ion.

(*a*) *N,N*-diethyl acetamide



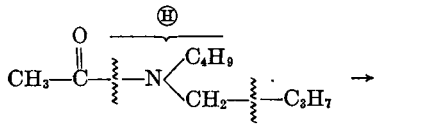
$\rightarrow C_3H_8N^+$ (*m/e* 58)

N,N-diethyl hexanamide



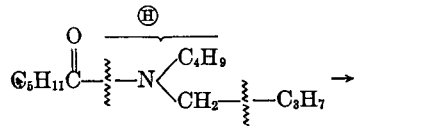
$\rightarrow C_3H_8N^+$ (*m/e* 58)

(*b*) *N,N*-dibutyl acetamide



$\rightarrow C_5H_{12}N^+$ (*m/e* 86)

N,N-dibutyl hexanamide



$\rightarrow C_5H_{12}N^+$ (*m/e* 86)

Table II. Mass Spectra of Secondary Amides

	N-Methyl Form	*N*-Methyl Acet	*N*-Ethyl Acet	*N*-Butyl Acet	*N*-Isobutyl Acet	*N*-Butyl Octadecan
Molecular weight	59	73	87	115	115	339
Source[a]	EK	D	D	EK	D	D
m/e	Relative Intensity					
15	7	23	10	4	4	0.3
27	3	2	6	4	3	3
28	34	17	8	5	4	7
29	13	3	13	5	4	8
30	54	67	100	100	100	13
31	2	17	2	3	4	0.4
41	3	3	3	7	7	14
42	1	10	11	4	3	3
43	1	83	59	34	31	18
44	1	4	48	19	3	12
45	0.1	9	2	2	1	0.7
55	0.3	0.7	0.1	2	2	13
56	0.4	0.9	0.3	3	4	4
57	0.4	0.4	0.1	2	2	23
58	8	40	0.1	4	13	3
59	100	1	4	0.6	0.6	2
60	3	1	1	10	18	4
71		0.3	0.1	0.3	0.3	6
72		0.4	14	29	31	6
73		100	0.6	21	6	20
74		4	...	1	1	9
85			...	...	...	3
86			0.5	9	0.6	7
87			72	1	0.1	3
88			5	...	...	0.3
99				0.4	...	1
100				6	12	11
101				0.4	0.8	1
114				0.2	0.1	0.7
115				11	11	100
116				2	2	8
128						23
129						4
142						4
143						0.9
156						2
157						1
170						4
171						1
184						4
185						0.8
197						1
198						3
199						0.6
212						2
213						3
226						2
227						0.4
239						2
240						2
241						0.4
268						2
282						1
~295						2
~311						2
~339						2
P[b]	100	100	72	11	11	2
S/S_{tol}[c]	0.98	0.49	0.70	0.68	0.98	0.45

[a] EK = Eastman Kodak; D = Dow Chemical Co. (compounds listed are not necessarily commercially available).

[b] *P* = molecular ion (parent peak).

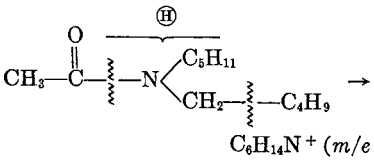
[c] $S/S_{tol} = \dfrac{\text{scale divisions } A}{\text{mg. of } A} \Big/ \dfrac{\text{scale divisions toluene}}{\text{mg. of toluene}}$.

Table III. Mass Spectra of Tertiary Amides

m/e	N,N-Dimethyl Form	N,N-Dimethyl Acet	N,N-Diethyl Form	N,N-Diethyl Acet	N,N-Dibutyl Acet	N,N-Diethyl Hexan	N,N-Dipentyl Acet	N,N-Diethyl Decan	N,N-Dibutyl Hexan	N,N-Dipentyl Hexan	N,N-Diethyl Dodecan	N,N-Dioctyl Acet	N,N-Dioctyl Hexan
Molecular weight	73	87	101	115	171	171	199	227	227	255	255	283	339
Source[a]	D	D	D	D	D	D	D	EK	D	D	D	D	D
	Relative Intensity												
15	11	11	5	5	2	2	2	0.3	0.4	0.3	0.4	0.5	0.5
27	2	1	15	5	3	16	5	4	4	3	3	2	3
28	12	5	25	6	2	8	3	~4	3	2	4	4	8
29	6	1	28	7	7	28	~8	13	7	7	10	5	8
30	14	8	77	14	12	6	25	3	5	8	3	11	10
31	0.5	0.5	1	0.5	0.4	1	0.6	0.1	0.3	0.2	0.1	0.3	0.4
41	3	2	4	2	5	22	15	11	11	11	9	10	14
42	24	19	10	7	3	15	6	5	5	4	4	4	5
43	6	46	3	31	19	41	47	13	18	35	12	29	37
44	55	100	47	31	15	29	32	16	8	15	11	26	22
45	2	23	11	6	2	7	2	0.5	1	0.7	0.7	2	2
55	0.1	2	0.7	0.4	2	13	5	9	5	6	9	6	10
56	1	2	5	3	2	9	5	4	3	3	3	4	4
57	0.1	0.2	1	0.8	5	7	3	8	11	2	8	8	12
58	5	1	84	100	0.8	100	11	54	1	4	37	2	3
59	0.2	0.1	3	4	0.1	5	0.6	4	0.3	0.3	2	0.3	0.6
60	...	2	0.2	4	1	49	4	0.3	8	1	1	3	7
71	0.1	...	0.5	0.5	0.3	13	~5	5	8	13	3	3	12
72	7	15	17	21	6	35	~5	24	3	3	17	5	4
73	100	0.9	7	1	5	30	5	8	7	2	8	6	10
74	5	0.1	0.8	0.3	0.3	8	1	5	0.8	0.3	4	0.7	1
85		...	3	0.8	0.3	0.4	1	2	0.4	0.7	2	0.7	2
86		0.2	36	3	100	13	25	10	100	7	8	10	6
87		69	2	~1	10	17	7	8	9	4	6	9	7
88		4	0.2	0.1	0.4	3	0.5	0.5	0.4	0.3	0.5	0.6	0.6
99			0.2	0.2	0.8	8	0.6	0.1	7	8	0.4	0.5	8
100			2	6	7	47	100	40	9	100	33	3	4
101			100	0.4	0.6	4	~7	3	0.9	7	3	2	3
114				0.1	4	5	65	2	5	50	2	8	13
115				41	0.3	71	5	100	7	4	100	1	2
116				4	0.7	5	0.3	7	0.8	1	7	0.1	1
127					...	0.1	0.3	0.2	0.2	0.7	0.1	0.5	0.7
128					23	30	35	33	21	24	29	6	6
129					3	4	3	5	4	8	5	0.9	3
141					...	...	0.4	...	0.1	0.5	0.1	0.2	0.5
142					2	9	36	7	4	16	6	100	100
143					0.2	1	4	1	0.9	3	1	11	12
155					...	...	0.3	0.7	0.2	0.4	0.1	1	1
156					6	1	27	2	14	7	2	8	11
157					0.6	0.1	3	0.6	2	1	0.9	1	2
170					0.1	0.9	12	4	0.7	3	3	12	13
171					5	8	1	0.6	5	0.4	0.7	2	5
184							4	2	9	11	2	52	39
185							0.7	0.4	1	2	0.4	7	6
198							0.4	2	3	4	2	5	7
199							6	0.8	0.4	3	0.4	0.7	1
212								0.7	0.7	7	1	5	5
213								0.1	0.1	1	0.3	0.8	1
226								1	0.5	4	2	2	3
227								6	4	0.7	0.6	0.5	1
240										0.9	0.6	5	12
241										0.2	0.1	0.8	2
255										4	4	0.7	0.9
268												17	38
282												0.8	2
283												3	3
~296													8
~310													3
~339													2
P[b]	100	69	100	41	5	8	6	6	4	4	4	3	2
S/S_{tol}[c]	0.84	0.62	0.29	0.71	0.95	0.38	0.45	0.66	0.79	0.57	0.38	0.53	0.29

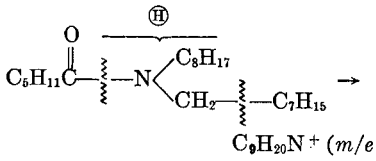
[a] D = Dow Chemical Co. (compounds listed are not necessarily commercially available); EK = Eastman Kodak.
[b] P = molecular ion (parent peak).
[c] $S/S_{tol} = \frac{\text{scale divisions } A}{\text{mg. of } A} \Big/ \frac{\text{scale divisions toluene}}{\text{mg. of toluene}}$.

(c) *N,N*-dipentyl acetamide



$C_6H_{14}N^+$ (*m/e* 100)

(d) *N,N*-dioctyl hexanamide



$C_9H_{20}N^+$ (*m/e* 142)

AMIDE IDENTIFICATION

An unknown aliphatic amide may be characterized by its mass spectrum as follows:

1. The peak at highest mass in the spectrum designates the molecular weight of the amide.

2. If the most intense ion current in the spectrum corresponds to the $C_nH_{2n+1}NO$ series (*m/e* 59, 115, etc.), this is a molecular ion or a rearrangement ion corresponding to cleavage of the carbon-carbon bond beta to the carbonyl group, accompanied by the rearrangement of a hydrogen atom.

When $n = 2$ and *m/e* 59 (not a molecular ion) is the most intense peak in the spectrum, a primary amide is necessitated. When n is greater than 2, either a secondary or tertiary amide is indicated, and additional information may be obtained from the $C_nH_{2n+2}N$ series (see 3).

3. If the most intense ion current in the spectrum falls in the $C_nH_{2n+2}N$ series (*m/e* 30, 58, 86, 100, 142, etc.), the rearrangement ion can be correlated with cleavage of the nitrogen–carbonyl carbon bond and the carbon-carbon bond beta to the nitrogen atom, accompanied by hydrogen rearrangement to the nitrogen-containing fragment. A maximum *m/e* 30 peak ($n = 1$) designates a secondary amide. A maximum contribution in the $C_nH_{2n+2}N$ series, when n is greater than 1, indicates, in general, a tertiary amide.

The study of tertiary amides was limited by the availability of compounds to cases where the alkyl substitutions on the nitrogen atom were identical.

In the cases examined, a maximum spectral contribution in the $C_nH_{2n+2}N$ series when $n = 3$ designates an *N,N*-diethyl amide; $n = 5$, an *N,N*-dibutyl amide; $n = 6$, an *N,N*-dipentyl amide; and $n = 9$, an *N,N*-dioctyl amide.

The mass-to-charge ratios used for amide identification, particularly in the $C_nH_{2n+2}N$ series, are also characteristic of other types of structures; therefore, relatively pure compounds should be used when the above rules are applied for characterization of the amides.

ACKNOWLEDGMENT

The author extends sincere thanks to R. S. Gohlke, V. J. Caldecourt, and F. W. McLafferty for aid and advice in the preparation of this manuscript.

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RECEIVED for review January 8, 1958. Accepted January 15, 1959.

Determination of Traces of Mercury in Biological Material by Catalytic Action of Mercuric Ions

Microelectrolysis of Mercury

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▶ **Determination of traces of mercury in biological material was based on the catalytic action of mercuric ions on the reaction of potassium ferrocyanide with nitrosobenzene. The absorbance of the violet product, $[Fe(CN)_5(C_6H_5NO)]^{---}$, at a fixed reaction time, depends on mercuric ion concentration. The organic substance was ashed with nitric acid, and the mercury was oxidized with bromine vapor and extracted from the solution by electrolysis. The deposit on the electrode was dissolved and mercury determined by its catalytic action. In the concentration interval 2.7×10^{-7} to 10^{-5} mole per liter of mercury, in urine and blood, respectively, the relative standard error was fairly constant and amounted to about 20%.**

THE catalytic action of mercuric ions on the reaction of potassium ferrocyanide with nitrosobenzene in aqueous solution and the mechanism of the reaction have been described (*2*, *3*). The absorbance of the violet product, $[Fe(CN)_5(C_6H_5NO)]^{---}$, at a fixed reaction time depends on the concentration of mercuric ions. Thus mercuric ion can be determined by its catalytic action. Application of this method to the determination of traces of mercury in the atmosphere and in mercury ore ash has been reported (*1*, *4*). Besides mercuric and mercurous ions, the ions of gold, and to some extent of platinum and silver, show catalytic action with decreased intensity, but these metals are seldom present in biological material. [In earlier work (*4*) it was reported that there is no catalytic action of silver ions, but small catalytic action was subsequently observed.]

The analysis of biological material includes ashing of the organic matter,

the extraction of traces of mercury, and its determination. The ashing is still a problem. A survey on destruction of organic matter was recently published (*6*); Middleton and Stuckey (7) used nitric acid in addition to small quantities of other reagents. In this work, a complete ashing of the organic matter was obtained using nitric acid only. After ashing, mercury was expelled by heating, mixed with bromine vapor, absorbed in bromine water, and deposited from the solution rich in electrolytes by electrolysis, and the deposit dissolved in bromine water. After evaporation of the excess bromine, a solution containing traces of mercuric ions free from foreign electrolytes was obtained. The catalytic action of the mercuric ions in such a solution can be measured without the difficulties which at a higher ionic strength might arise from a negative salt effect, because the charges of ferrocyanide and mercuric ions have opposite signs.

EXPERIMENTAL

Materials. The details of the preparation of aqueous solutions of nitrosobenzene have been described (*1*). The potassium ferrocyanide trihydrate used was Merck & Co., Inc., analytical grade. Aqueous solutions were freshly prepared by diluting a 0.2*M* solution which was used as a stock solution for a few days only and kept in darkness. The water used before the electrolysis, as well as the nitric acid, was redistilled because traces of other metals will coprecipitate with mercury on the cathode, and later react with potassium ferrocyanide, giving insoluble ferrocyanides. It was noticed, in accordance with earlier observations (*10*), that analytical grade nitric acid, as well as other analytical reagents, contains traces of mercury.

All glassware used was borosilicate glass.

Apparatus. A Unicam S.P. 500 and a Hilger H700 quartz spectrophotometers were used for the spectrophotometric measurements (glass cells 10-mm.). The absorbance was measured at 528 mμ—at the maximum absorbance of $[Fe(CN)_5(C_6H_5NO)]^{---}$ (*2*).

Ashing of Biological Material. The apparatus is shown in Figure 1. Biological material (5 ml. of blood or 25 ml. of urine) was added to flask *A* of about 200-ml. capacity and made of glass resistant to higher temperatures (about 600° C.), or of quartz. *B* is a water-cooled absorption cell of about 120-ml. capacity. The inlet glass tube has a small sintered G_1 glass filter of about 0.5-sq. cm. area at the end. *C* is a reservoir for bromine and *D* is an absorption cell containing sodium hydroxide solution for the absorption of excess bromine. It is a modification of the original Moldawskij apparatus for oxidation and absorption of atmospheric mercury (*9*).

To 25 ml. of urine in flask *A* were added 4 ml. of concentrated redistilled nitric acid. About 4 ml. of redistilled water were added to absorption cell *B*. A stream of fresh air (about 0.4 liter per minute), free of mercury, purified by passing through acid potassium permanganate solution, was led through the apparatus by an aspirator attached at *E*. A small stream of air (one to two bubbles per second) was led into the apparatus at *C*, pulling bromine vapors from the reservoir with liquid bromine, which also enabled counting of the bubbles. The flask with the sample was heated carefully on an open flame. In about 45 minutes the solution had evaporated almost to dryness. The heating was carefully continued because carbonization soon took place and was followed by evolution of gases. The ashing was achieved in about 10 minutes. The white residue was then heated for 1.5 hours at about 600° C. to force mercury salts to sublimate into absorption cell *B*.

To a 5-ml. sample of blood was added concentrated redistilled nitric acid in portions of 5, 1, 1, 0.5, and 0.5 ml. over about a 3-hour period. After the addition of nitric acid was completed, the solution was heated carefully almost to dryness. A small quantity of pyrolysis products will pass into absorption chamber *B* and coagulate, forming a gummy ball about 3 mm. in diameter. (The gummy pyrolysis products are smaller in the case of urine but the passing of gases was not disturbed when G_1 glass filter was used.) When the blood was completely ashed, a residue brown from iron remained. The temperature was then increased to about 600° C. and the heating continued for 1.5 hours. The absorption solution was transferred to a beaker and the absorption cell and the glass tubes were rinsed three times with 5 ml. of redistilled water. The solution was evaporated to about 4 ml. There is almost no danger of losing mercury by this evaporation because of the high ionic strength of the solution.

Microelectrolysis with Platinum Electrodes. The apparatus is shown in Figure 2. The cathode and anode were platinum wires with a diameter of 0.6 and 0.1 mm., respectively. Before use the electrodes should be heated for a few minutes in a flame. The cathode reached almost to the bottom of the cell and the length of the anode was adjusted to produce a current of 40 to 80 ma. at an e.m.f. of 3.5 to 4.0 volts. If the current is stronger, the rapid evolution of hydrogen could remove the deposit from the cathode. About 8 ml. of the solution was usually electrolyzed for 24 to 30 hours. The evolution of gases resulted in adequate stirring. The mushroom-type condenser reduces the loss of liquid. The conditions of the electrolysis become steady in a short time, so that the electrolysis can be left going overnight. When electrolysis is completed, the cathode should be removed from the solution without interrupting the applied e.m.f. to prevent the nitric acid from dissolving the mercury. Mercury deposited on the electrode was readily

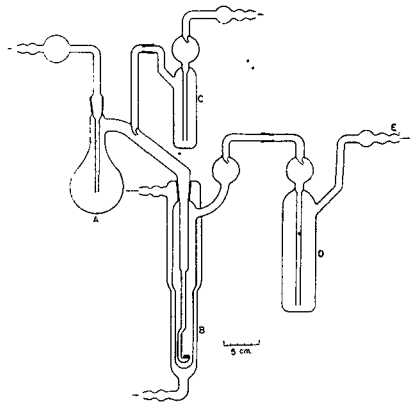


Figure 1. Apparatus for ashing biological material containing traces of mercury and for absorption of mercury vapors

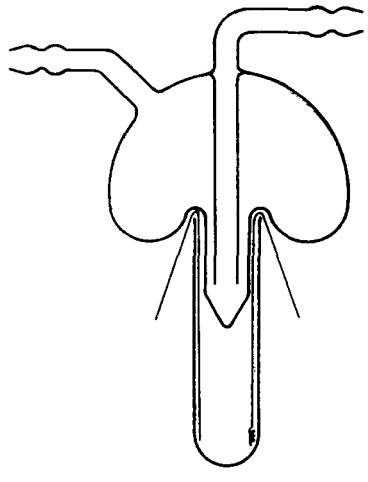


Figure 2. Apparatus for microelectrolysis of mercury

dissolved in a few minutes in about 2 ml. of lukewarm saturated bromine water. Excess bromine was removed by careful evaporation on a water bath or by using an infrared lamp until no odor of bromine could be detected. Because the solution was not free of electrolytes, the possibility of mercury loss was increased.

Under these conditions, a platinum cathode was adequate despite the fact that the tendency of mercury to alloy with platinum is weak, so that the deposit is not very adherent. This is a disadvantage when the cathode must be dried before weighing, as in the gravimetric analysis, for which a gold cathode is recommended (*8*).

Catalytic Determination of Mercuric Ions. Potassium ferrocyanide was $5 \times 10^{-4}M$ and nitrosobenzene $4.2 \times 10^{-4}M$. At these concentrations the uncatalyzed reaction is negligible (*2*, *4*). About 2 ml. of the solution left after the electrolysis were quantitatively transferred to a graduated 5-ml. cylinder, using about 1.5 ml. of distilled water. Five drops of acetate buffer solution of pH 4.1, 3 drops of 0.1*N*

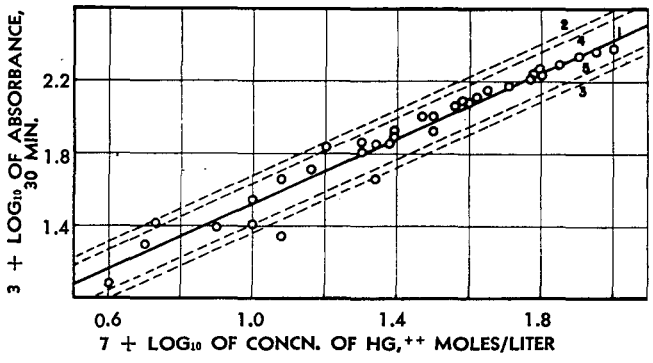


Figure 3. Linear dependence of $\log_{10}E_{30}$ on $\log_{10}C_{Hg}{}^{++}$. E_{30} is absorbance (at 528 mμ) of $[Fe(CN)_5(C_6C_5NO)]^{-3}$ after 30 minutes

2 and 3, and 3 and 4. 95% confidence limits for $m = 1$ and $m = 2$, respectively
m. Number of parallel measurements giving absorbance

sodium hydroxide, and 0.50 ml. of 0.0042M (0.4445 gram per liter) nitrosobenzene solution were added and the volume was made up with distilled water to 4.5 ml. Under such conditions, acetate buffer solution could ensure the initial pH of 4.1 (*1–4*). The graduated cylinder was immersed in a water thermostat at 20° ± 0.05° C. and after thermal equilibrium was reached, 0.50 ml. of 0.005M potassium ferrocyanide solution, thermally equilibrated, was added. The reaction was carried out in faint diffuse light. The absorbance was determined after 30 minutes at 528 mμ, using distilled water as a reference solution.

STATISTICAL TREATMENT OF RESULTS

Calibration Curve (34 analyses). The absorbances were plotted as a function of known concentrations of mercuric ions, added to urine or blood, respectively. The final concentration of mercuric ions ranged from 4×10^{-7} to 10^{-5} mole per liter. A series of 34 measurements was treated statistically in the same way as the determination of mercury in the atmosphere (*1*). Equation 1

$$3 + \log_{10} E_{30} = 0.906(\log_{10} C_{Hg}{}^{++} + 7) + 0.617 \quad (1)$$

is the equation of the straight line obtained by plotting $\log_{10}E_{30}$ against $\log_{10}C_{Hg}{}^{++}$ as shown in Figure 3. The equation was calculated using the method of least squares. The relative standard errors of the concentration of mercuric ions, calculated according to Davies (*5*) and Youden (*11*) as described (*1*), is a fairly constant value and amounted to about +21 and −19% for $m = 1$, and +15 and −13% for $m = 2$ in the concentration interval 2.7×10^{-7} to 10^{-5} mole per liter of mercuric ion. m is the number of independent parallel measurements on the basis of which the absorbance E_{30} was determined.

From Equation 1 it follows that the unknown concentration of mercuric ions should be calculated according to the equation

$$\log_{10} C_{Hg}{}^{++} = 1.104 \log_{10} E_{30} - 4.37 \quad (2)$$

This equation has all parameters very similar to the equation previously calculated for the determination of mercury in the atmosphere ($\log_{10}C_{Hg}{}^{++} = 1.112 \log_{10}E_{30} - 4.427$) (*1*). The differences are caused mainly by small systematic losses of mercury during the ashing of biological material.

Although the accuracy of the measurements is not very high, it is adequate for practical purposes. The main source of error lies in the procedure for the destruction of the large amount of organic matter which contains only minute quantities of mercury. To show that this ashing is the main source of error, known quantities of mercuric chloride were added to urine and blood and a calibration curve was made which omitted the process of ashing. This was replaced by the addition of 2 ml. of concentrated redistilled nitric acid to the urine and 5 ml. to the blood, and the solutions were heated for a few minutes at about 90° C., using a small condenser, to achieve a partial destruction of the proteins. A series of 10 such determinations was treated statistically. In the concentration interval 10^{-6} to 10^{-5} mole per liter of mercuric ions, the relative standard error amounted to about +11 and −10% for $m = 1$, and +9 and −8% for $m = 2$.

The determination of mercury in biological material would be much simpler if ashing of the organic matter could be replaced by partial destruction of the proteins, as described. Stock pointed out (*10*) that mercury covalently bonded to the organic compounds in urine could not be precipitated by hydrogen sulfide. He could precipitate only one quarter of the mercury present in urine if he did not previously destroy the organic matter. It was found in this investigation that after a partial hydrolysis of the proteins in urine and blood with nitric acid, the subsequent electrolysis will extract most of the mercury present in urine and blood, as shown in Table I. In these experiments the material contaminated with mercury was blood and urine from workers in the Yugoslav mercury mine in Idria. In mercury poisoning there is an increased possibility that the mercury will form complexes with organic substances. Analyses were also performed for comparison after a complete destruction of the organic matter. Although the determinations of mercury were always lower than after complete ashing of the organic matter, the differences were not great and an estimate of mercury in blood and urine can be obtained without the tedious process of ashing.

Microelectrolysis with Gold Cathode. Determinations were also made using gold wire for the cathode and platinum wire for the anode. The deposit of mercury on a gold electrode is more adherent than on a platinum electrode, so that an electric current up to 200 ma. could be used, which enabled completion of electrolysis in less than 24 hours. Bromine water could not be used for dissolving the mercury deposited on the gold wire

Table I. Determination of Mercury in Blood and Urine of Workers in Mercury Mine

Person	Specimen	Mercury Found, Moles per Liter × 10^6: After complete ashing	Upon addition of HNO_3	No treatment prior to electrolysis
1	Urine	2.50	2.10	
2		0.47	0.40	0.41
3	Blood	1.90	1.73	
4		0.98	0.92	
5	Urine	3.95		1.50
		3.75		
5	Blood	1.40	1.35	

1, 3, 4, 5. Exposure to mercury vapors.
2. Exposure to HgO.

because gold also dissolved to some extent and having itself a small catalytic action, interferred with that of mercury.

Mercury was dissolved in about 10 minutes in concentrated nitric acid (3 drops). The solution was transferred to a graduated 25-ml. cylinder, using about 19 ml. of distilled water. Concentrated sodium hydroxide solution was added to obtain a weakly acid solution (pH about 5). Nitrosobenzene solution was added (2.5 ml. of 0.0042*M* solution) and 5 drops of an acetate buffer solution of pH 4.1. (Only a small quantity of the buffer solution was used to keep the negative salt effect as low as possible.) The volume of the solution was made up to 22.5 ml. and the cylinder was immersed in a water thermostat at 20° ± 0.05° C., then 2.5 ml. of thermally equilibrated potassium ferrocyanide solution (0.005 *M*) were added, and the determination was carried out as described.

Six determinations were made after a complete destruction of the organic matter in urine. The accuracy of the procedure seems to be similar to the one obtained with platinum electrodes. A statistical treatment on a larger number of determinations will be made in due course.

ACKNOWLEDGMENT

The authors thank H. B. Iveković for helpful discussions.

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RECEIVED for review August 14, 1958. Accepted December 10, 1958.

Accurate Determination of Uranium in the Presence of Small Amounts of Molybdenum

Lead Reductor and Stannous Chloride Methods

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▶ **The conditions have been determined under which the lead reductor may be used for the accurate determination of uranium containing more than 50 p.p.m. of molybdenum. Results are low and erratic when the widely accepted method of preparing samples in perchloric acid is used. Accurate and precise results are obtained only in the absence of perchloric acid and, therefore, preparation of samples by dissolving in nitric acid and fuming with sulfuric acid is recommended. In addition, the application of the stannous chloride method to the determination of uranium in the presence of up to 6.6% molybdenum has been studied. Accurate results are obtained if the molybdenum content is separately determined and a correction is applied to the basis of a three-electron change. Previous work had suggested a two-electron change for the molybdenum. The effects of other common interferences are evaluated.**

MOLYBDENUM, and other elements that are reduced to air unstable states in the Jones reductor, interfere in the determination of uranium by the usual Jones reductor method (*6*, *8*) either by consumption of reagent or by inducing oxidation of uranium(IV) to uranium(VI) during aeration. Sill and Peterson (*8*) have shown that interference of this latter type can be reduced or eliminated by using a modification of the lead reductor method (*1*). The modified method consists of fuming the sample to dryness with either sulfuric or perchloric acid, taking up the residue in 30% hydrochloric acid, and passing this solution through a properly prepared lead column. The effluent is either collected in ferric chloride and titrated with dichromate to a diphenylamine sulfonate end point, or collected in phosphoric acid and titrated with ceric sulfate to a ferroin end point. Fuming with perchloric acid is recommended over fuming with sulfuric acid to eliminate serious bumping caused by the separation of salts.

In determining uranium in the pure oxide (U_3O_8) in this laboratory the method suggested by Sill and Peterson gave reproducible results which checked well with other data on the same oxide. However, when the method was tried on some metal samples, the results were low and erratic. Further investigation showed that reproducible and accurate results could be obtained on pure oxide fumed with either perchloric or sulfuric acid and on metal fumed with sulfuric acid, but not on the same metal if it was fumed with perchloric acid.

Iron, molybdenum, and silicon were the impurities which were significantly higher in the metal than in the oxide. To determine whether any one, or a combination of these, affected the uranium determination, a series of experiments was run to study the interactions among these three impurity elements and the fuming agent (perchloric or sulfuric acid).

In a limited survey of the effects of interferences on the stannous chloride method, Main (*4*) found that the titration of 100 mg. of uranium oxide, (U_3O_8) containing 7 mg. of molybdenum consumed 21% more titrant than the uranium itself. This corresponds to a two-electron change for the molybdenum.

In Main's (*4*) procedure the uranyl ion is reduced by stannous chloride in a hot, concentrated hydrochloric acid solution containing iron catalyst and phosphoric acid. After cooling, the excess stannous chloride is oxidized with excess mercuric chloride. Ferric chloride, sulfuric acid, phosphoric acid, and diphenylaminesulfonic acid indicator are added and the solution is

titrated with 0.02*N* potassium dichromate. This procedure is much faster than the solid reductor method, but in practice is applicable only to metals and other samples in which the uranium content is approximately known so that the proper volume of stannous chloride can be calculated.

Because the stannous chloride method offers advantages in speed and does not present the problems encountered with the contamination of solid reductors, a study was made of the molybdenum interference to determine if it were sufficiently reproducible to permit a correction to be made, without sacrificing accuracy in the uranium analysis. During this study some unanticipated results were found.

EXPERIMENTAL

Reagents. All uranium samples were prepared from MS-ST U_3O_8 (99.93% pure). Distilled water and reagent grade chemicals were used. Solutions of potassium dichromate were prepared from reagent grade potassium dichromate and standardized against MS-ST U_3O_8.

Apparatus. Potentiometric titrations were performed with a Beckman Model G pH meter and a platinum-saturated calomel electrode pair. The titrant was stored in a constant temperature bath (30° ± 0.5° C.) and pumped to a water-jacketed 100-ml. buret for titrations. Thus, errors due to density changes were virtually eliminated.

The lead reductor was prepared by placing a glass wool plug at the bottom of a 45 × 2 cm. glass column and filling the column about two thirds full with reagent grade, granular lead. Before use the lead was washed until bright with 3*N* hydrochloric acid. When not in use, the reductor was filled with 3*N* hydrochloric acid containing 0.1% ferric iron. It has been reported (*5*) that unless this iron is present, the first determination each day is several tenths of 1% low. Before use each day the reductor was washed with 0.8*N* hydrochloric acid, and between samples it was rinsed with distilled water to remove lead sulfate.

Lead Reductor Method. Some of the oxide (U_3O_8) samples were dissolved in concentrated nitric acid and fumed to dryness with sulfuric acid, while the rest were dissolved and fumed to dryness with perchloric acid. The residues were taken up in 25 ml. of water, 10 ml. of concentrated sulfuric acid, and 15 ml. of concentrated hydrochloric acid. The cooled uranium solutions were then washed through the lead reductor at a rate of 15 to 20 ml. per minute with 0.8*N* hydrochloric acid. The reduced solutions were caught under excess ferric sulfate.

The ferrous iron formed by reaction of the ferric sulfate with uranium plus impurities was then titrated with 0.05*N* potassium dichromate. The end points for the samples fumed with sulfuric acid were detected potentiometrically. However, the end-point potentials were not well defined for the samples fumed with perchloric acid and they were titrated to a visual diphenylamine sulfonate end point. For these titrations, 10 ml. of phosphoric acid were added to improve the end point.

Results. The main purpose of this investigation was to determine why low and erratic results were obtained when uranium metal samples were analyzed by the lead reductor method after fuming with perchloric acid. Previous work here and at other laboratories (*5*, *8*) had not indicated that any difficulty of this type should be encountered. To interpret the results in terms of accuracy as well as of precision, the work described here was done with uranium oxide samples of known impurity content. The only known differences between the metals that gave low and erratic results and those that gave apparently satisfactory results were the higher iron, molybdenum, and silicon contents of the former. To determine whether any one, or a combination, of these impurities interfered, various amounts and combinations of these impurities were added to the pure uranium oxide and the uranium was determined by the lead reductor method. The average recovery for 29 samples that were fumed with sulfuric acid, and that contained up to 2000 p.p.m. of molybdenum, 1000 p.p.m. of iron, and 200 p.p.m. of silicon was 100.00% with a standard deviation of ±0.03%.

From these data it is apparent immediately, that up to 2000 p.p.m. of molybdenum, 1000 p.p.m. of iron, and 200 p.p.m. of silicon do not interfere, if the samples are fumed in sulfuric acid. The titrations, of course, must be corrected for iron and molybdenum on the basis of one- and three-electron changes, respectively. However, low and erratic results are obtained when samples fumed in perchloric acid contain as much as 100 p.p.m. of molybdenum; Table I.

It was of interest to determine next, the minimum concentration of molybdenum that causes low and erratic results. Table II shows the results obtained on samples fumed with perchloric acid, when the molybdenum content was varied from 20 to 500 p.p.m. The first indication of erratic results was at the 100 p.p.m. level so that the tolerance level appears to be at 50 p.p.m. No studies have been made to determine whether the sample size affects the tolerance level of molybdenum. Finally, to gain some knowledge of the mechanism of this interference, an experiment was designed which would indicate the step in the procedure at which the interference occurred. To do this, pure uranium oxide, samples (0.6 gram) were combined with 100 γ of molybdenum at various stages of the procedure. The results are tabulated in Table III. It is clear from these data that low and erratic results are obtained when the uranium and molybdenum are passed through the reductor simultaneously in perchloric acid solution.

This behavior could be explained on the basis that the molybdenum-catalyzed reduction of perchlorate by lead forms a product that oxidizes uranium(IV), thereby preventing the

Table I. Effect of Impurities on Lead Reductor Method with Perchloric Acid Fuming

Impurity Added, P.P.M.	U_3O_8, Gram		U_3O_8 Recovered, %
	Taken	Found	
200 Fe	0.6004	0.6002	99.97
	0.6000	0.6002	100.03
200 Si	0.5995	0.5997	100.03
	0.6008	0.6005	99.95
200 Fe, 200 Si	0.5990	0.5992	100.03
	0.5995	0.5994	99.98
100 Mo	0.6001	0.5975	99.57
	0.6007	0.5984	99.62
100 Mo, 100 Fe	0.6000	0.5973	99.55
	0.6016	0.5994	99.63
100 Mo, 200 Fe	0.5993	0.5965	99.53
	0.6003	0.5984	99.68
200 Mo	0.6016	0.6002	99.77
	0.6011	0.5951	99.00
	0.5993	0.5910	98.62
	0.6007	0.5850	97.39
200 Mo, 100 Fe	0.5997	0.5963	99.43
	0.6010	0.5959	99.15
200 Mo, 200 Fe	0.6001	0.5981	99.67
	0.6006	0.5959	99.22
	0.5988	0.5920	98.86
	0.6013	0.5974	99.35
200 Mo, 200 Si	0.6004	0.5996	99.87
	0.6010	0.5954	99.07
200 Mo, 200 Fe, 200 Si	0.5993	0.5966	99.55
	0.6009	0.5977	99.47
	0.6008	0.5990	99.70
	0.6006	0.5976	99.50

Table II. Interference of Molybdenum in Uranium Determination by Lead Reductor Method[a]

Mo Added, P.P.M.	U_3O_8 Taken, Gram	U_3O_8 Found, Gram	U_3O_8 Recovered, %
20	0.6010	0.6011	100.02
	0.6005	0.6006	100.02
50	0.6009	0.6011	100.03
	0.6007	0.6006	99.98
100	0.6001	0.5975	99.57
	0.6007	0.5984	99.62
150	0.5997	0.5989	99.87
	0.5993	0.5980	99.78
500	0.6001	0.5943	99.03
	0.6021	0.5972	99.18

[a] The uranium-molybdenum samples were dissolved and fumed with $HClO_4$. Results corrected for molybdenum added.

Table III. Effect of Adding Molybdenum to Uranium at Various Steps in Lead Reductor Procedure

Step Prior to Which 0.5 Gram of U and 100 γ of Mo Are Combined	Uranium Recovered, %
Fuming with $HClO_4$	99.60
	99.67
	99.27
	99.10
Reduction	99.38
	99.39
	99.42
	99.38
Reaction with ferric sulfate	100.00
	99.93
	99.80
	100.00
Titration	100.02
	99.98
	99.98
	100.02

complete reduction of uranium(VI). This type of molybdenum-catalyzed reduction has been shown by Haight to take place in the reduction of perchlorate by tin(II) (*3*) and at the dropping mercury electrode (*2*).

Stannous Chloride Method. Using the procedure of Main (*4*) for samples containing from 0.09 to 0.6 gram of uranium oxide, a heavy gel formed immediately after the reduction and attempts to titrate these samples gave fading end points and erratic results. The addition of enough sulfuric acid to dissolve the gel obscured the end point. Although the gel appeared to be uranium(IV) phosphate, attempts to prevent its formation by dilution of the phosphoric acid were unsuccessful. Schreyer and Baes had reported (*7*) that precipitation of uranium(IV) phosphate could be prevented by adjusting the acidity of the solution to 4.5*M* in sulfuric acid. The procedure was therefore modified to include the addition of 5 ml. of concentrated sulfuric acid prior to the phosphoric acid. No further gel formation was encountered. With this modification, the sulfuric acid was omitted from the acid mixture that is added prior to the titration. A standard solution of 0.5*N* potassium dichromate was used for all titrations and a sample size of 0.6 gram of uranium oxide was found most convenient to achieve the desired accuracy.

Table IV. Effect of Molybdenum on Determination of Uranium by Stannous Chloride Method

U Present, Gram	Mo Added, γ	0.05031 *N* Titrant, Ml.[a]	Mo, Corr., Ml.	Theoretical Titer, %
0.5084	200	85.01	0.12	100.00
0.5085	400	85.17	0.25	100.02
0.5083	600	85.25	0.37	100.01
0.5085	1000	85.54	0.62	100.02
0.5082	2000	86.09	1.24	99.99

[a] After subtraction of 1.01 ml. blank.

Table V. Comparison of Uranium Analyses in the Presence of Molybdenum by Jones Reductor and Stannous Chloride Methods

No. of Samples	Molybdenum Concn., P.P.M.[a]	Uranium Av. % Normalized		Coefficient of Variation,[b] %	
		$SnCl_2$	J. R.	$SnCl_2$	J. R.
8	<50	100.00	99.97	0.05	0.06
6	50–99	99.99	99.67	0.02	0.17
13	100–300	100.00	99.52	0.03	0.36

[a] Determined by colorimetric (thiocyanate) method.
[b] Calculated by combining results on aliquots from each sample.

Table VI. Effects of Impurities on Stannous Chloride Method

Impurity Element	Impurity, Grams per 100 Grams U	No. of Titrations	Av. Error, %	Av. Electron Change	Standard Deviation of Error, %
Al	2.0	1	−0.01	...	...
Ca	2.0	1	0.00	...	...
Cr	0.05	1	−0.03	...	...
	0.20	2	−0.09	...	±0.13
	2.0	3	−0.19	...	±0.07
Cu	0.05	1	−0.04	...	...
	0.20	2	−0.05	...	±0.03
	2.0	3	+1.73	0.4	±0.03
Mo	0.04	2	+0.16	3.3	±0.03
	0.08	1	+0.31	3.1	...
	0.12	1	+0.44	3.0	...
	0.20	6	+0.74	3.0	±0.02
	0.40	3	+1.45	3.0	0.00
	6.6	3	+24.49	3.0	±0.05
Ni	2.0	1	−0.01	...	...
Ti	0.05	1	+0.04	0.3	...
	0.20	1	+0.24	0.5	...
	2.0	2	+2.48	0.5	±0.25
V	0.02	2	+0.04	0.8	±0.01
	0.05	5	+0.06	0.5	±0.06
	0.10	5	+0.07	0.3	±0.10
	0.20	2	+0.40	0.8	±0.03
	2.0	2	+4.50	1.0	±0.01
W	0.05	1	+0.04	1.2	...
	0.20	1	+0.07	0.5	...
	2.0	4	+0.52	0.4	±0.12
Zn	2.0	1	0.00	...	...
Zr	0.05	1	0.00	...	...
	0.20	1	−0.04	...	...
	2.0	1	−0.15	...	...

Results. Effect of Molybdenum. The effect of varying the amount of molybdenum upon the determination of uranium is shown in Table IV. It is clear from these data that a stoichiometric correction for molybdenum based upon a three-electron change can be made over the range 0.04 to 0.4%. Table VI indicates that the correction is applicable also to 6.6% molybdenum.

A thorough comparison of this method with the Jones reductor method was then made on routine metal samples run in a control laboratory. All results obtained by the stannous chloride method were corrected for molybdenum on the basis of a three-electron change. The Jones reductor results could not be corrected, because results were usually low due to induced oxidation (*8*). The comparison is shown in Table V. While results by the Jones reductor method become erratic when the molybdenum concentration increases above 50 p.p.m., the stannous chloride method remains unaffected. Likewise, the average uranium analysis by the Jones reductor method decreases as the molybdenum increases above 50 p.p.m. The analyses by the stannous chloride method gave results that were consistent with results obtained before and after molybdenum was present as an impurity. They were also consistent with the results on the other analyzed impurities. The "normal" uranium concentration is taken as 100.00%.

Effect of Other Impurities. In Main's (*4*) study of interferences in the stannous chloride method, bismuth, cerium, cobalt, manganese, and tungsten did not appear to interfere, while

copper, molybdenum, titanium, and vanadium gave high results. Some of this work was repeated, to determine if the interferences were stoichiometric, and some additional elements were studied with the results shown in Table VI.

Aluminum, calcium, nickel, and zinc in amounts up to 2% cause no interference. Chromium causes slightly low results. Titrating in an inert atmosphere and varying the time of standing prior to titration did not eliminate the error. Copper, when present in 2% concentration, causes a reproducible, but unexplained positive error. Titanium and tungsten are reduced to indefinite valence states and are subsequently titrated giving high results. At the suggestion of H. H. Willard, fluoride was added to complex the titanium(IV). The addition of 10 ml. of saturated sodium fluoride solution to samples containing 0.2 and 2.0% titanium reduced the errors by about half, but did not eliminate them. Vanadium gives high results corresponding to approximately a one-electron reduction. However, the end point is poor in the presence of vanadium, causing erratic results. Zirconium phosphate precipitates at a 2% zirconium concentration and apparently some uranium is coprecipitated to give low results.

VARIATIONS IN METHOD. Main's (*4*) procedure was modified by the addition of sulfuric acid to prevent formation of a uranous phosphate gel. Another variable studied in some detail was the time of standing between reduction and titration. Samples containing excess stannous chloride for as long as 3 hours gave accurate titrations. Results on samples that had stood for up to 7 hours tended to be low, while samples standing overnight had no excess stannous chloride left. Erratic results were obtained when perchloric acid was present during the reduction. This was expected in view of the known tendency for stannous chloride to reduce the perchlorate ion in the presence of molybdenum (*3*) and other catalysts.

Main attributes the low results which were frequently obtained on samples requiring more than 30 ml. of titrant to the air oxidation of the ferrous iron during titration. He suggests adding dry ice to the titration flask to provide an oxygen-free atmosphere during the titration. Early in this study, a comparison of titrations run with and without the dry ice indicated no difference although the titrations required over 80 ml. of 0.05*N* dichromate. The titrant was added rapidly up to the end point.

ACKNOWLEDGMENT

The use of the stannous chloride method was suggested by H. C. Anderson following a visit to the University of California Radiation Laboratory, Livermore Research Laboratory, where Main's method was discussed.

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RECEIVED for review April 24, 1958. Accepted December 8, 1958. Work done under AEC Contract No. AT(29-1)-1106.

Resolution of Isomeric Xylenes by Gas-Liquid Chromatography

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▶ Several new polar stationary liquid substrates are suitable for the gas chromatographic resolution of *m*- and *p*-xylenes in aromatic mixtures. By using these liquids on aqua regia–treated C-22 firebrick or Chromosorb, separations can be effected on short columns in reasonable emergence times. The aqua regia treatment allows the use of finer particle sizes, longer columns, and higher flow rates because of the lower pressure drop across the column.

AROMATIC mixtures containing benzene, toluene, ethylbenzene, and xylenes are currently analyzed by infrared (*7*) and ultraviolet (*1*) spectroscopy. Since 1952 (*4*), gas-liquid chromatography has also been used to analyze aromatics. Although most C_6–C_8 aromatics can be resolved by gas chromatography, *m*- and *p*-xylene have resisted practically all attempts at separation.

The current approach involved a study of liquid substrates and the inert solid support. Suitable stationary liquid phases were selected to give at least a noticeable resolution of *m*- and *p*-xylene; efficient columns using these liquids were then prepared by studying such factors as inert supports, particle size, flow rates, and percentage of stationary phase on the support.

MATERIALS AND REAGENTS

Aqua Regia–Treated Firebrick. One hundred grams of C-22 firebrick (Johns-Manville) of 40 to 60 mesh were added to 300 ml. of aqua regia. The mixture was heated so that a mild reaction continued for about 1 hour. After cooling, the acid was removed by adding water and decanting several times. Then 300 ml. of dilute sodium hydroxide (5%) were added and decanted to neutralize any remaining acid, and finally the firebrick was thoroughly water-washed on a 60-mesh screen to remove remaining fines. The support was dried in an oven at 110° C. overnight and rescreened to the desired mesh size. A 20% loss in weight occurred during the over-all process.

Stationary Liquid Substrates. Ethylene carbonate–1-nitronaphthalene mixture. This substrate is prepared by mixing equal quantities of ethylene carbonate and 1-nitronaphthalene and heating to 60° C.

Dimethylsulfolane.

1-Chloronaphthalene. All stationary liquids were put on the support in a 15 to 100 ratio. Methylene chloride was the solvent used to apply the substrate to the solid support.

EXPERIMENTAL

The first stationary liquid phase to produce a separation of the xylene isomers was recently reported by Zlatkis, O'Brien, and Scholly (*9*): benzyl-

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Table I. Relative Retention Volume Ratios and Separation Factors for Various Liquid Substrates

(15% w./w.)

	B.P., ° C.	[a]	[b]	[c]	[d]
Benzene	80.1	1.00	1.00	1.00	1.00
Toluene	110.6	2.05	2.28	2.35	3.30
Ethylbenzene	136.2	3.93	4.65	5.00	8.96
p-Xylene	138.4	4.14	5.37	5.45	10.30
m-Xylene	139.1	4.27	5.65	5.75	10.92
o-Xylene	144.4	5.25	7.30	7.73	14.39
S_{12} (*p*- and *m*-xylene)		0.031	0.052	0.055	0.060
Q (*p*- and *m*-xylene)		24.2	9.80	9.35	10.93
R (*p*- and *m*-xylene)		0.76	0.51	0.52	0.66

[a] 16-ft. benzyldiphenyl on 60 to 80 mesh untreated Chromosorb, 100° C., 100 ml./min. He.

[b] 8-ft. ethylene carbonate–1-nitronaphthalene on 40 to 60 mesh aqua regia–treated C-22 firebrick, 50° C., 200 ml./min. H_2.

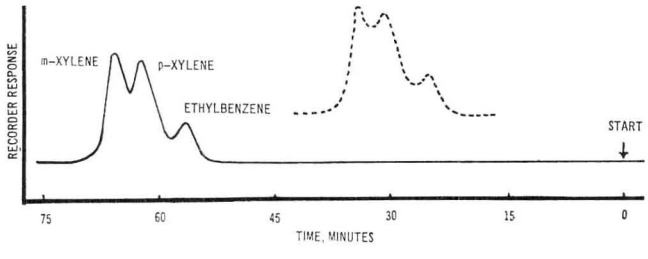
[c] 8-ft. dimethylsulfolane on 40 to 60 mesh aqua regia-treated C-22 firebrick, 50° C., 150 ml./min. H_2.

[d] 8-ft. 1-chloronaphthalene on 40 to 60 mesh aqua regia–treated Chromosorb, 50° C., 220 ml./min. H_2.

Figure 1. Chromatogram of aromatics using dimethylsulfolane as stationary liquid

—Aqua regia–treated C-22 firebrick

---Untreated C-22 firebrick



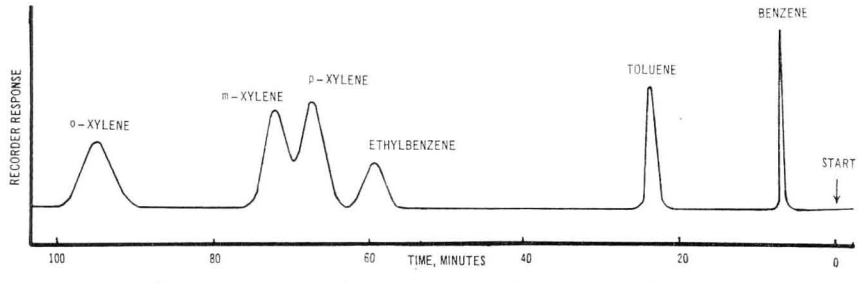


Figure 2. Gas chromatogram of aromatic mixture

8-foot column of 15% 1-chloronaphthalene on 40 to 60 mesh aqua regia–treated chromosorb, temp. 40° C., 220 ml./min. hydrogen

diphenyl, an aromatic hydrocarbon. Column efficiencies of 9500 theoretical plates were necessary to produce a resolution of 0.76. Values for the relative peak separation, S_{12}, relative peak sharpness, Q, and resolution, R, shown in Table I are based on an equation developed by Jones and Kieselbach (*5*):

$$S_{12} = \frac{t_2 - t_1}{t_1}$$

where

$Q = t_x/w$
$R = QS_{12}$
t_2 = elution time of second peak
t_1 = elution time of first peak
t_x = elution time of a given peak
w = width of peak as measured at the base-line intercepts of the tangents to the peak

New suitable liquid stationary phases have been found for the separation of isomeric xylenes in aromatic mixtures. One of these polar compounds, ethylene carbonate, showed a small resolution of *m*- and *p*-xylene using columns with theoretical plate efficiencies of 1500. By using an aqua regia–treated firebrick, the peaks were sharpened and the resolution was increased. This resolution can also be enhanced by using a finer particle size of the support. The acid-treated firebrick allows longer columns, because there is considerably less pressure drop compared with untreated water- or acid-washed firebrick. Flow rates of 300 ml. per minute can be effected on coiled columns 25 feet × 1/4 inch containing 40- to 60-mesh acid-treated support by using inlet pressures of 30 p.s.i. This compares with 200 ml. per minute for similar columns containing untreated supports. When ethylenebenzene was present in the aromatic mixture, a mixed liquid phase was necessary to effect a resolution because ethylene carbonate will not separate *p*-xylene from ethylbenzene. 1-Nitronaphthalene was a good stationary phase for aromatics, and in particular, for *p*-xylene and ethylbenzene. This naphthalene derivative was mixed with ethylene carbonate in equal parts and the resultant mixture used as the liquid substrate. Both compounds are solid at ambient temperatures; however, the mixture melts at about 35° C. and is suitable for use in the temperature range 25° to 60°.

Another polar compound with characteristics similar to ethylene carbonate has been useful in resolving aromatic mixtures. This liquid substrate, dimethylsulfolane (Matheson, Coleman & Bell), has been used for the analysis of olefins (*3*, *6*) and can now be extended to C_6–C_8 aromatics. Retention volume ratios and operating conditions are shown in Table I. A chromatogram using this liquid is illustrated in Figure 1 and compares the difference in resolution due to the inert support. Dimethylsulfolane can resolve ethylbenzene from the other aromatics and shows an R value of 0.52 for *m*- and *p*-xylene using an 8-foot column.

The most efficient stationary liquid substrates studied for the resolution of xylenes have been the 1-halonaphthalenes (Distillation Products Industries). In particular, 1-chloronaphthalene will give an R of 0.66 for *m*- and *p*-xylene on an 8-foot column of 2500 theoretical plates in about 1 hour (Figure 2). The 1-bromo compound gives a resolution of 0.55, while 1-iodonaphthalene

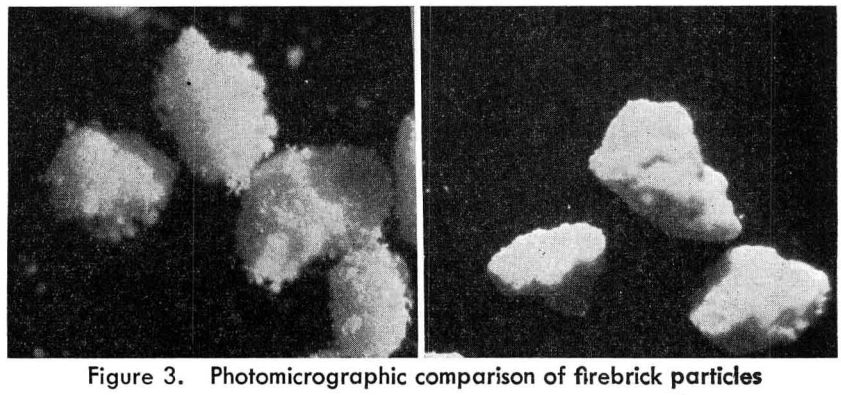


Figure 3. Photomicrographic comparison of firebrick particles

Left. Untreated C-22 firebrick
Right. Aqua regia–treated C-22 firebrick

yields a 0.33 resolution of the isomers under comparable conditions. The 1-iodo compound elutes the aromatics much faster than 1-chloronaphthalene. 1-Bromonaphthalene is intermediate in this effect. The ratio of retention volumes (separation factor) of *m*- and *p*-xylenes on the 1-chloronaphthalene column is 1.06 as indicated in Table I.

Other reagents have been useful in resolving the xylene isomers, although not to the same degree as 1-chloronaphthalene. These include propylene, trithioethylene and monothioethylene carbonates, 4 *p*-chloroacetophenone, and phenothioxin.

RESULTS AND DISCUSSION

An aromatic mixture containing benzene, toluene, ethylbenzene, *p*-, *m*-, and *o*-xylene was resolved on several substrates using the aqua regia–treated firebrick. Retention volume ratios, separation factors, and operating conditions are given in Table I for benzyldiphenyl, ethylene carbonate–1-nitronaphthalene, dimethylsulfolane, and 1-chloronaphthalene.

Ethylene carbonate, dimethylsulfolane, and 1-chloronaphthalene are polar compounds capable of separating *m*- and *p*-xylenes by gas-liquid chromatography. 1-Chloronaphthalene, which appears to be the best is readily available and can be used at temperatures up to 50° C. The percentage of liquid phase in the procedure used may vary from 5 to 15%. With long columns it would be advisable to use 5% to reduce elution times. A 15-foot column containing 5% dimethylsulfolane on 60 to 80 mesh treated firebrick produces an *R* value of 0.78 for these isomers.

The specially prepared aqua regia–treated firebrick offers several advantages in gas chromatography: It enhances resolution because all the fines are removed, it permits the use of finer mesh sizes and longer columns, thereby increasing resolving efficiency, and it considerably reduces pressure drop across the column.

The same effects are noted when Chromosorb is treated with aqua regia. The small fines adhering to the firebrick particles are removed by the acid treatment and the resulting product appears as tiny smooth boulders. The photomicrographs in Figure 3 compare the treated and untreated firebrick.

When long columns (20 feet) are used, it may be necessary to increase the flow rate of carrier gas to shorten retention times. Flow rates up to 300 ml. of hydrogen per minute have been satisfactory with no appreciable loss in resolution.

Columns of relatively low theoretical plate efficiencies can effect difficult separations in gas chromatography by the choice of a suitable stationary liquid (*8*). The use of an aqua regia–treated support is also preferable. The highly efficient columns described by Cheshire and Scott (*2*) might be further improved by using aqua regia–treated supports as well as the stationary phases described above.

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RECEIVED for review August 18, 1958. Accepted February 6, 1959. Division of Analytical Chemistry, 134th Meeting, ACS, Chicago, Ill., September 1958.

Separation and Microidentification of Metallic Ions by Solvent Extraction and Ring Oven Techniques

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▶ A scheme has been devised for the separation and identification of 35 metallic ions in a single drop of unknown, based on a combination of solvent extraction and ring oven techniques. The unknown is separated into four groups by placing the nonaqueous extracts of metal chlorides, thiocyanates, acetylacetonates, and diethyldithiocarbamates on filter paper and depositing them as rings by means of a ring oven. A fifth group is obtained by similar treatment of the aqueous solution of residual ions. The rings of deposited salts are cut into sectors and the individual ions are identified by means of spot tests. Complete separations and identification can be made in an hour.

In the usual methods of semimicro and micro qualitative analysis it is seldom possible to separate and identify several ions when present in a single drop of a solution. The precipitation, filtration, and washing of the precipitate dilute the test solution to such an extent that the ions are brought below their respective identification limits. In 1954, Weisz devised the ring oven technique (*3–5*) as a means of schematically analyzing a single drop of a solution containing more than one metallic ion. In this method, a filter paper is placed on an electrically heated ring and a drop of sample is added to the center of the paper. The addition of a reagent fixes some of the ions locally while others move to the periphery of the ring on account of the capillary action of the filter paper. Because of the rapid evaporation of the wash liquid as it approaches the heated surface, the ions washed out are collected in the form of a ring and are subsequently detected by spot tests. The separations themselves can be remarkably efficient. However, the success of the ring oven technique depends almost entirely on the specificity and sensitivity of the reagents unless very elaborate and comprehensive separation schemes are employed.

The purpose of the present work was to develop a simple microanalytical procedure using the ring oven method, employing a prior separation of metal ions into more or less definite groups through liquid-liquid extraction. Solvent extraction was chosen because of its greater efficiency, simplicity, and speed. In general, it is superior to the precipitation methods usually employed as it is free from coextraction, the analog of coprecipitation. It is also more rapid and may be simpler to perform in many cases.

Because samples in aqueous solution are the prime concern in the analysis of metal ions the solvent extraction in the present work implies the use of an aqueous-organic solvent pair. Morrison and Freiser have broadly classified (*2*)

the extraction systems on the basis of the nature of the extractable species—i.e., chelate extraction and ion association extraction. Of the various extraction systems known in the two catagories, chloride, thiocyanate, bromide, iodide, acetylacetone (2,4-pentanedione), diethyldithiocarbamate, diphenylthiocarbazone (dithizone), and 8-quinolinol systems were investigated. Organic solvents were so chosen as to ensure the most favorable distribution of the extractable species. Sometimes a mixture of two organic solvents was prepared after considering not only the distribution of the extractable species, but also their boiling points, degrees of miscibility, relative specific gravities, and tendencies to form emulsions. Efficiency of separation was determined only on a qualitative basis.

Among the ion association extraction systems, chlorides and thiocyanates were most useful in the present work, as the rings produced were colorless and the excess chloride and thiocyanate ions did not interfere in the subsequent stages of analysis. The fluoride system was not sufficiently versatile, while in the bromide and iodide systems, the rings produced by the extracted ions were too highly colored, presumably owing to the decomposition of the extracted metal complexes.

In the chelate systems, acetylacetone and diethyldithiocarbamate were preferred on account of their versatility and the colorless rings they produced. Acetylacetone forms well defined chelates with a large number of metals and the solubility of acetylacetonates in organic solvents is much higher than that of most chelates used analytically. Extractions have been carried out with the acetylacetone system using this compound itself as solvent or with other organic liquids such as carbon tetrachloride, chloroform, and benzene as solvents. The chief advantages of using acetylacetone itself as solvent are that extractions can be made at a low pH and many acetylacetonates are more soluble in acetylacetone than in other organic solvents. Extractions with the diethyldithiocarbamate system are also done at a low pH. While diphenylthiocarbazone and 8-quinolinol are excellent chelating agents, they have the disadvantage of forming colored rings which interfere with subsequent spot testing.

PROCEDURE

The general technique adopted was extraction of a few drops of the aqueous test solution with an organic extractant under appropriate conditions of pH in the presence of suitable ion association or chelating agents. The organic and aqueous layers were kept in contact for sufficient time in a test tube or microbeaker to attain equilibrium; at least three extractions were made with each system to attain maximum efficiency.

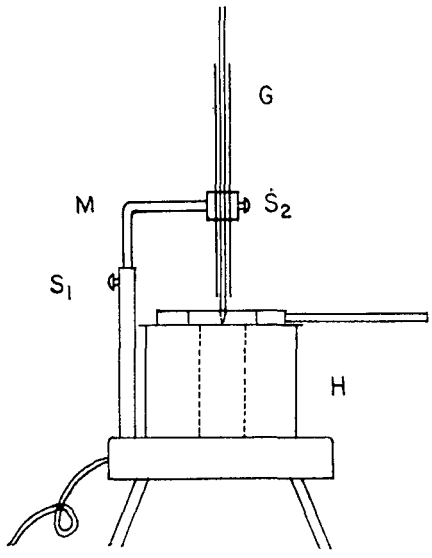


Figure 1. Ring oven

The separation of the two phases was carried out with an extraction pipet (*1*).

Only the first extract (organic phase) was subjected to analysis, which was done by transferring the extract of the test solution to the center of the filter paper with a capillary pipet inserted through the guide tube of the ring oven. The aluminum ring was placed on the paper to keep it in position. The spot was then treated with 0.1*N* hydrochloric acid which was absorbed in the filter paper, thus washing the extract concentrically to the hot surface of the oven where it was vaporized, leaving a deposit of transported salts. Five to ten washings served to concentrate the ions quantitatively and sharply in the ring zone.

The filter paper was then oven dried and cut into several sectors. Each single sector of filter paper, after having been developed by suitable reagents, showed a circular arc. Care was taken that the deposits gathered in the sharp ring zone were not redissolved during the course of identification with resultant loss in test sensitivity. The aqueous layer left after each group extraction was subjected to further extractions under different conditions, repeating the above procedure.

The metallic ions under investigation have been classified into four broad groups according to the systems of extraction employed (Table I).

EXPERIMENTAL

Apparatus and Reagents. The ring oven (Figure 1) consists of a cylindrical block of aluminum, *H*, 40 mm. high and 70 mm. in diameter with a bore hole of 22-mm. diameter. A heating element is installed inside the block and temperature is controlled by a suitable powerstat. Outside the cylinder is fixed an aluminum rod, *M*, adjustable by a screw, S_1. The rectangular aluminum rod, *M*, bears on its end a guide tube, *G*, about 50 mm. long, which is adjustable in height by means of a screw, S_2. The glass tube, *G*, serves as a guide for a capillary pipet. The guide tube must stand vertically and point exactly to the center of the bore hole. It ends about 2 to 3 mm. over the surface of the heating block, *H*. An aluminum ring of 30-mm. inner diameter is placed over the filter paper to keep it in place. The temperature at the surface of the heating block should be about 80° to 115° C.

Reagents for the detection of individual metal ions have been listed under the respective procedures.

Chloride Group. A few drops of the aqueous (1%) test solution are treated with two drops of concentrated hydrochloric acid. The final strength of the acid should be approximately 7*N*. Extraction is performed with 2 ml. of a 2 to 1 mixture of methyl isobutyl ketone and *n*-amyl acetate, and the extract is washed 4 to 5 times into the ring with 0.1*N* hydrochloric acid. The ring is then cut into eight segments. Although a single extraction suffices for identification purposes, one or two additional extractions may be advisable in each case to ensure complete removal of group members. For example, if iron is present in large amounts, enough of it will remain after a single extraction of the chlorides to appear in and interfere with subsequent separations and tests. The tests applied for the different ions are as follows:

Gold(III). A portion of the ring is treated with a freshly prepared 1% solution of stannous chloride. Blackening of the ring indicates the presence of gold.

Molybdenum(VI). A portion of the ring is acidified with hydrochloric acid and treated with a 0.1% solution of diphenylcarbazide. A violet stain indicates that molybdenum is present.

Iron(II). If gold is present, the ring is treated with a 1% solution of ammonium thiocyanate. A red coloration appears if iron is present. If gold is absent, a 1% solution of potassium ferrocyanide may be used as the reagent. A blue ring is formed if iron is present. Because an insoluble precipitate is produced, the stain is more sharply defined and the test is more sensitive.

Antimony(V) and Arsenic(III). A portion of the ring is made into pulp with dilute sulfuric acid in a test tube, and a few grains of metallic zinc are added. A piece of paper impregnated with silver nitrate is placed over the top of the test tube. In the presence of antimony or arsenic, a black stain is formed on the filter paper.

A part of the circular arc, as close as possible to the deposit, is cut out and placed on a spot plate. A few drops of hydrochloric acid (1 to 1) and one drop of 5% potassium iodide are added, then mixed by blowing with a pipet. A drop of a 0.5% Rhodamine B solution is added and the solution is mixed again. A blue-violet precipitate indicates antimony. A blank test is carried out for comparison.

Gallium(III). A sector of the filter paper is treated with a 0.02% solution of morin in alcohol. A yellowish green

fluorescence under ultraviolet radiation is indicative of the presence of gallium.

VANADIUM(V). A portion of the ring is treated with a 20% solution of sodium fluoride. When dry, it is treated with 1% solution of 8-quinolinol in acetic acid. A dark brown ring indicates the presence of vanadium.

Thiocyanate Group. The aqueous extract from the previous operation is treated with 3 drops of 7*M* ammonium thiocyanate. This is now extracted with 2 to 3 ml. of diethyl ether and washed into the ring with 4 to 5 portions of 0.1*N* hydrochloric acid. The metallic ions of this group are tested as follows:

COBALT(II). During the process of extraction a blue coloration of the organic phase is indicative of the presence of cobalt. This may be further confirmed by treating a portion of the ring with a 1% alcoholic solution of 1-nitroso-2-naphthol in an ammonical medium. A brown stain forms if cobalt is present.

ZINC(II). A portion of the ring is exposed for a minute to fumes of ammonia. It is then treated with a 0.01% diphenylthiocarbazone solution in carbon tetrachloride. A red ring indicates zinc is present.

BERYLLIUM(II) AND TIN(IV). The presence of either or both of these ions can be detected by the fluorescence test with 0.02% morin solution in ethyl alcohol. Tin can also be detected by treating the ring with a sodium sulfide solution. A yellow ring is obtained in the presence of tin.

Acetylacetone Group. The aqueous extract from the previous group is treated with 1 or 2 drops of dilute hydrochloric acid to provide a pH of approximately 2. This is then subjected to extraction by 2 ml. of acetylacetone. The aqueous and the organic layers should be kept in contact for 2 to 3 minutes, because some of the acetylacetonates are extracted slowly. Aluminum does not extract completely in acidic medium; therefore, after three extractions in acidic medium, a portion of the aqueous extract is treated with a few drops of 4*M* sodium hydroxide to adjust the pH to approximately 7. This is now extracted and washed into the ring as usual with dilute hydrochloric acid. Titanium, zirconium, and chromium are only 75% extracted in this group and so a part of these ions will appear in the next group also. Thorium is partially extracted. The ring made of the acidic extract is tested for copper(II), uranium(VI), zirconium(IV), and titanium(IV), while the ring made out of the second extract is tested for aluminum(III) only. The metallic ions of this group are detected as follows:

ALUMINUM(III). A portion of the ring is treated with a drop of dilute acetic acid followed by a drop of 0.02% morin solution in alcohol. A greenish fluorescence under ultraviolet radiation indicates the presence of aluminum. Zirconium interferes with the above test.

COPPER(II). A portion of the ring is exposed to ammonia fumes and treated with a 0.1% solution of dithio-oxamide in alcohol. A black ring appears when copper is present.

URANIUM(VI). A sector of the filter paper is treated with a 1% solution of potassium ferrocyanide. A brown ring indicates the presence of uranium. When another sector of the filter paper is treated with a drop of 5*N* sodium phosphate solution, a strong yellow fluorescence under the mercury lamp confirms the presence of uranium.

ZIRCONIUM(IV). Zirconium gives a fluorescent test with 0.02% morin solution even in acidic solutions. This test is not satisfactory if aluminum is present.

TITANIUM(IV). A sector of the filter paper is treated with a 1% aqueous solution of chromotropic salt solution. A deep reddish ring is obtained when titanium is present.

Diethyldithiocarbamate Group. The aqueous extract left from the previous operations is treated with a 2% solution of sodium diethyldithiocarbamate. The pH should be approximately 3. In most cases, a precipitate appears when the reagent is added, then dissolves when extracted with ether. As the reagent decomposes rapidly in an acidic medium, the extractions should be carried out without delay with an excess of the reagent. The ring formed may be of brownish color due to the decomposition of the reagent. The color can be destroyed on exposure to bromine vapors. Except for indium, the presence of members of this group can be detected by treatment with sulfide. Individual tests for the ions are as follows:

CADMIUM(II). A few drops of the organic extract are placed in the center of a filter paper, which is then exposed in hydrogen sulfide for about 2 minutes. The metal sulfides belonging to this group are precipitated and fixed in the center of the filter paper. After the paper is washed with dilute hydrochloric acid, the spot is exposed to bromine vapors and finally ammonia fumes. The filter paper is placed on the ring oven and the central spot washed with 0.1*N* ammonium hy-

Table I. Scheme of Separations

Chloride Group Separation

Test solution + 2 drops concd. HCl + 2 ml. methyl isobutyl ketone and *n*-amyl acetate (2:1 mixture)
Extract with an extraction pipet

Organic Phase (1)	Aqueous Phase (1)
Sb^{+5}, Au^{+3}, Fe^{+3}, V^{+5}, Mo^{+6}, Ga^{+3}, As^{+3}, Ge^{+4}, and Te^{+4} (Individual ions tested as described in Chloride Group)	Co^{+2}, Zn^{+2}, Be^{+2}, Sn^{+4}, Al^{+3}, Cr^{+3}, Cu^{+2}, U^{+6}, Zr^{+4}, Ti^{+4}, Ni^{+2}, Cd^{+2}, Bi^{+3}, Pb^{+2}, Hg^{+2}, Tl^{+1}, W^{+6}, Mn^{+2}, Se^{+4}, In^{+3}, Ba^{+2}, Sr^{+2}, Ca^{+2}, Mg^{+2}, Ce^{+3}, and Th^{+4}

Thiocyanate Group Separation

Add 3 drops of 7*M* NH_4CNS. Extract with 2 ml. of diethyl ether

Organic Phase (2)	Aqueous Phase (2)
Co^{+2}, Zn^{+2}, Be^{+2}, and Sn^{+4} (Individual ions tested as described in Thiocyanate Group)	Al^{+3}, Cr^{+3}, Cu^{+2}, U^{+6}, Zr^{+4}, Ti^{+4}, Ni^{+2} Cd^{+2}, Bi^{+3}, Pb^{+2}, Hg^{+2}, Tl^{+1}, W^{+6}, Mn^{+2}, Se^{+4}, In^{+3}, Ba^{+2}, Sr^{+2}, Ca^{+2}, Mg^{+2}, Ce^{+3}, and Th^{+4}

Acetylacetonate Group Separation

Add 2 ml. acetylacetone, wait for a few minutes, and extract.

Organic Phase (3)	Aqueous Phase (3)
Al^{+3}, Cr^{+3}, Cu^{+2}, U^{+6}, Zr^{+4}, and Ti^{+4} (Individual ions tested as described in Acetylacetone Group)	Ni^{+2}, Cd^{+2}, Bi^{+3}, Pb^{+2}, Hg^{+2}, Tl^{+1}, W^{+6}, Mn^{+2}, Se^{+4}, In^{+3}, Ba^{+2}, Sr^{+2}, Ca^{+2}, Mg^{+2}, Ce^{+3}, and Th^{+4}

Diethyldithiocarbamate Group Separation

Add 2 ml. of diethyldithiocarbamate solution. Extract immediately with diethyl ether.

Organic Phase (4)	Aqueous Phase (4)
Ni^{+2}, Cd^{+2}, Bi^{+3}, Pb^{+2}, Hg^{+2}, Tl^{+1}, W^{+6}, Mn^{+2}, Se^{+4}, and In^{+3} (Individual ions tested as described in Diethyldithiocarbamate Group)	Ba^{+2}, Sr^{+2}, Ca^{+2}, Mg^{+2}, Ce^{+3}, and Th^{+4} (Individual ions tested as described under Aqueous Extract)

droxide. Only cadmium ions are washed out to the ring. A portion of the ring is treated with sodium sulfide, and if a yellow coloration results, cadmium is present.

BISMUTH(III). A second portion of the extract is washed out to form a ring, a sector of which is then treated with a 1% solution of ammonium thiocyanate. A yellow stain indicates the presence of bismuth.

MERCURY(II). A second sector of the ring is treated with 4*N* hydrochloric acid. After the paper is dry, a drop of 0.01% of diphenylthiocarbazone in carbon tetrachloride is added. An orange stain indicates the presence of mercury. Another portion of the ring is treated with a 10% solution of malonic acid. It is dried and treated with a 0.1% solution of diphenylcarbazide. A blue stain confirms the presence of mercury.

SELENIUM(IV). A portion of the ring is treated with an acidic solution of hydrazine. A red ring forms within 20 minutes if selenic ion is present. Another portion of the ring is treated with a 1% solution of 3,3′-diaminobenzidine hydrochloride. The presence of selenium is confirmed by a red stain.

THALLIUM(I). A portion of the ring is treated with a concentrated solution of ammonium phosphate, then dried and treated with a 2*N* solution of potassium iodide. A yellow ring indicates the presence of thallium.

MANGANESE(II). A sector of the ring is treated with 20% solution of sodium fluoride, then dried. The ring is treated with 4*N* sodium hydroxide solution. A black coloration appears when manganese is present.

TUNGSTEN(VI). A part of the ring is treated with 6*N* hydrochloric acid, then dried and treated with a 10% solution of tannic acid. A brown stain is obtained when tungsten is present.

MAGNESIUM(II). A portion of the ring is treated with a 1% alkaline solution of Titan Yellow. A pink color appears in the presence of magnesium.

NICKEL(II). A sector of the filter paper is exposed to ammonia fumes for 30 seconds and then treated with a 1% solution of dimethylglyoxime. A red ring shows the presence of nickel.

LEAD(II). A portion of the ring is fumed over ammonia and sprayed with a freshly prepared dilute solution of rhodizonic acid. It is then fumed slowly over hydrochloric acid. If lead is present, the ring undergoes the following color changes: yellow, red, blue, and violet.

Aqueous Extract. The aqueous extract left after the four previous operations is used directly for analysis on the ring oven.

BARIUM(II), STRONTIUM(II), AND CALCIUM(II). A freshly prepared concentrated solution of rhodizonic acid gives colored rings with the three ions. This is a general test and the individual ions cannot be differentiated conveniently.

CERIUM(III). A sector of the filter paper is treated with a 1 to 1 solution of ammonia and hydrogen peroxide. A yellow ring indicates the presence of this ion.

ACKNOWLEDGMENT

The authors acknowledge the aid of the U. S. Army Office of Ordnance Research under whose program this research was conducted. Thanks are due to Herbert Weisz and Francois Tron for helpful discussions.

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RECEIVED for review June 19, 1958. Accepted December 3, 1958.

Rapid Paper Chromatographic Microassay of Free and Ester Cholesterol of Blood

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▶A convenient, rapid (2-hour) procedure, well suited for multiple assay, is described for the quantitative paper chromatographic separation and assay of micro amounts of free and ester cholesterol of blood serum. Quantitative recoveries of free, ester, and total cholesterol were obtained. Coefficients of variation of a single assay for these cholesterol fractions, duplicated on different days, averaged 3.5%. Results for total cholesterol agreed with those of each of two other methods within 2%.

A SIMPLE, rapid, quantitative paper chromatographic separation and assay of micro amounts of free and ester cholesterol of blood serum would be useful in studies of cholesterol metabolism.

The procedure described uses zinc carbonate–coated Whatman No. 1 filter paper with 1.5% ethyl ether in cyclohexane for the quantitative cholesterol assay of human blood sera. Amounts of cholesterol compounds chromatographed (15 to 75 γ each) were chosen to permit maximum sensitivity, while using ordinary spectrophotometric equipment.

MATERIALS AND EQUIPMENT

Analytical grade chemicals: ammonium carbonate, zinc carbonate, 25% ammonia, phosphomolybdic acid, ferric chloride hexahydrate, concentrated sulfuric acid, 95 to 97%, acetone, absolute ethyl alcohol, and ether, peroxide-free.

Chloroform, purified by shaking twice with 1*N* hydrochloric acid, washing with distilled water until the last wash is neutral, drying 24 hours over phosphorus pentoxide, redistilling, and taking the middle fraction. It is preserved in the dark over decolorizing charcoal (25 grams per liter of Norit XXX), which was previously dried in a vacuum oven at 120° for 48 hours. It is filtered and redistilled, preferably just before use, or else kept a maximum of 14 days in a dark bottle at room temperature.

Cyclohexane, spectral grade purity.

Glacial acetic acid, 99 to 100%, analytical grade (E. Merck A. G., Darmstadt). It must be free of glyoxylic acid, by test according to Langan (*7*).

Cholesterol, U. S. P., purified through the dibromide (*2*), recrystallized once from 1 to 1 methanol–ethyl ether, and dried under vacuum. Melting point (corr.) = 148.6–9.1°.

Cholesterol laurate, synthesized by the method of Swell and Treadwell (*12*) from cholesterol, purified as described, and lauroyl chloride (Eastman Kodak Co.), recrystallized three times from acetone, and dried under vacuum. Melting point (corr.) = 91.0–1.5°.

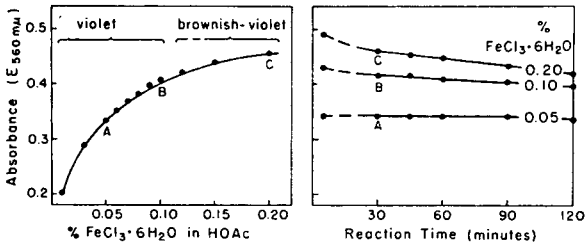


Figure 1. Effect of ferric chloride concentration on absorbance (left) and color stability (right)

Absorbance at 560 mμ of 60 γ of cholesterol in 3 ml. of $FeCl_3$–HOAc plus 2 ml. of concentrated H_2SO_4, read in Beckman DU. Left, E_{560} at 30 minutes

Cholesterol oleate (Armour & Co.), purified by chromatography on silicic acid, as described by Wycoff and Parsons (*13*), to remove the slight impurity which migrated on the paper chromatogram as free cholesterol, and dried under vacuum. Melting point (corr.) = 48.8–9.4°.

All cholesterol compounds were anhydrous. Each was found homogeneous by paper chromatographic characterization. Compounds were preserved in a vacuum desiccator—e.g., over phosphorous pentoxide, in a cold room at 0°.

Battery jar, 19 cm. high and 15 cm. in diameter, with top edge ground plane, for chromatography chamber. A heavy glass plate cover sealed without lubricant which might interfere in the assay.

Water bath at 70° and oxygen-free nitrogen for all solvent evaporations.

PROCEDURES

Extraction of Blood. Blood serum was extracted with 1 to 1 acetone–ethyl alcohol, in 1 to 25 dilution, as described by Sperry and Webb (*11*). An aliquot of the filtrate was concentrated by evaporation and kept at 0° until used. The last portion of the solvent was evaporated and the lipide-soluble portion of the residue dissolved in exactly 0.5 or 1 ml. of chloroform just prior to spotting the chromatogram.

Paper Chromatography. Ascending paper chromatography was carried out at 23° ± 2°. Whatman No. 1 filter paper was impregnated with zinc carbonate, according to Green, Marcinkiewicz, and Watt (*3*). The mobile phase was freshly prepared 1.5% (v./v.) ethyl ether in cyclohexane.

In preparation, the chamber was lined with filter paper and its floor covered to a suitable depth with the mobile phase, which was allowed to ascend the paper and saturate the atmosphere. At the same time, a piece of zinc carbonate paper was prewashed with purified chloroform by the ascending technique and allowed to air-dry for 1 minute. The region of the solvent front, containing numerous impurities, was discarded and the paper cut to 36 × 18 cm. The origin line was drawn 2 cm. from the long edge.

Solutions of blood extracts or of pure cholesterol compounds in chloroform were spotted with a 5-μl. pipet at approximately 3-cm. intervals, using 10 to 20 μl. of solution, containing 15 to 75 γ each of free and ester cholesterol, per spot. Appropriate blanks and standards were included, as described below.

The paper was placed on the floor of the chamber and the mobile phase allowed to ascend to a height of about 12 cm., which required 20 to 30 minutes. The paper was then dried in air for 1 minute.

Guide strips, including one with standards and one or more with blood extracts, were cut out of the paper and sprayed with a solution of 10% (w./v.) phosphomolybdic acid in absolute ethyl alcohol. (The spray reagent was freshly made or else protected from light.) Strips were placed in an oven at 130° for 20 to 30 seconds until blue-green spots of cholesterol compounds appeared.

As little as 0.1 γ of cholesterol (free or ester)—i. e., approximately 0.5 γ per sq. cm. of paper—is detectable.

Paper segments were cut from the blood lipide chromatograms, corresponding to guide strips which contained blood extracts. Areas taken for all ester segments were equal, as were those of all free cholesterol segments. Portions of unspotted sections of the chromatogram, which were identical in position and size to free and ester cholesterol segments, were taken for blanks.

Each paper segment was folded, placed in a test tube, and covered with chloroform, and the tube was shaken intermittently for 5 to 10 minutes to elute cholesterol compounds. The segments were then washed with additional chloroform and discarded. Eluates, including washings, were made up to approximately equal volume, usually 15 ml. (because of the possible effect of solvent on the subsequent cholesterol assay value).

Solvent was evaporated from the eluates, and exactly 3 ml. of 0.05% (w./v.) solution of ferric chloride hexahydrate in glacial acetic acid were added to each tube so as to wash down the inside surface. Tubes were stoppered (aluminum foil–covered corks), heated with gentle shaking for 15 seconds at 70° to ensure dissolving any residue, and cooled to room temperature.

Spectrophotometric Cholesterol Assay. Cholesterol was determined by the following modification of the Zlatkis method (*16*), chosen because of its simplicity and sensitivity.

The tube, containing the test sample dissolved in 3 ml. of 0.05% (w./v.) ferric chloride hexahydrate in glacial acetic acid, is tipped at a 45° angle. Exactly 2 ml. of concentrated sulfuric acid are added from a microburet so as to form a bottom layer. The solution is well mixed, by shaking or with a footed glass rod, and let stand at room temperature. After exactly 30 minutes, absorbance at 560 mμ is read with the Beckman spectrophotometer. Because bubbles form eventually, solutions are not transferred to cuvettes until immediately before reading. The cuvettes are thoroughly rinsed with distilled water and acetone and dried with nitrogen between readings.

Reagent blanks and standards were included with all analyses. For direct assay, standard solutions were prepared with glacial acetic acid (1 mg. of cholesterol per ml.) and freshly diluted before use with 0.05% ferric chloride hexahydrate in glacial acetic acid. For assay of chromatographed blood lipides, cholesterol standards were chromatographed exactly like the blood samples.

SPECTROPHOTOMETRIC CHOLESTEROL ASSAY

Zlatkis' reagent volumes of 3 and 2 ml. were kept, but the method was modified by dissolving the ferric chloride in glacial acetic rather than sulfuric acid.

Table I. Typical Data of Standard Curves for Pure Free and Ester Cholesterol

Quantity (as Cholesterol) per 5 Ml. of Color Solution, γ	Cholesterol			Cholesterol Laurate		
	No. of assays	Mean absorbance, 560 mμ[a]	Coeff. of variation, %	No. of assays	Mean absorbance, 560 mμ[a]	Coeff. of variation, %
15	3	0.083	1.9	4	0.081	1.8
30	8	0.164	1.6	8	0.160	2.2
60	12	0.326	1.4	11	0.317	1.1
120	3	0.646	0.8	4	0.630	1.0

[a] In assays 2 ml. of concd. H_2SO_4 were added to 3 ml. of 0.05% $FeCl_3.6H_2O$–glacial HOAc solution of cholesterol compound and mixed. Absorbance was determined in Beckman, Model DU with photomultiplier attachment, 1-cm. light path, at exactly 30 minutes.

This eliminates preparation of color reagent for each assay and gives a stable reagent (*6*, *15*).

Effect of Ferric Chloride Concentration. The optimum concentration of ferric chloride for these particular circumstances had to be determined, because it affects intensity and stability of color (*10*, *15*). The concentration of ferric chloride hexahydrate in glacial acetic acid was therefore varied over the range of 0.01 to 0.2% (w./v.) with the assay technique, otherwise, as described under Procedures, and the effect of this variation on both of these factors was noted. Results are shown in Figure 1. The absorbance increases over the whole range tested and does not plateau. Color stability falls off markedly.

Because the decrease in absorbance for the period, 30 to 120 minutes, is only 2% for the 0.05% solution, this concentration of ferric chloride was adopted, and absorbance was determined at exactly 30 minutes in all assays.

Readings were made at 560 mμ, as done by Zlatkis (*16*), because λ_{max} was nearly identical for various solutions in this series—562 to 565 mμ.

Standard Curves. Some calibration data for pure cholesterol and cholesterol laurate in this spectrophotometric method are summarized in Table I. In this series Beer's law was obeyed by both cholesterol and cholesterol laurate, and good precision is shown for both compounds over the range tested, coefficients of variation averaging 1.5%. Absorbance of cholesterol laurate averaged 98% that of free cholesterol.

Depth of Color. The following factors did not affect absorbance in cholesterol assay: a normal (±0.5%) or maximal (±2%) variation in quantity of sulfuric acid added by microburet; the presence of zinc(II) (as zinc chloride) in amounts of 100 γ per tube; and heating solutions of cholesterol compounds in 3 ml. of ferric chloride–acetic acid reagent for 15 seconds at 70°.

The presence of water or physiological saline, however, depresses color values significantly. Although not tested for the cholesterol assay procedure of this study, effects of their presence were determined for the similar assay technique of Henley (*6*). For example, the decrease of absorbance on adding one part of physiological saline to 100 parts of the color reaction-mixture averaged 3.4% for cholesterol, cholesterol laurate, and cholesterol oleate.

PAPER CHROMATOGRAPHY OF PURE CHOLESTEROL COMPOUNDS

Results with Chromatographed Standards. Some calibration data for pure cholesterol and cholesterol laurate, which were chromatographed both separately and in mixtures for use as standards during assay of blood sera, are given in Table II. Also given are control values for the same compounds spotted and directly eluted.

The values for control and chromatographed compounds are nearly identical. This shows that both cholesterol and cholesterol laurate are well separated and completely recovered following chromatography.

Comparison with Unchromatographed Standards. The data of Table II were collected at the same time as the data for standards, given in Table I, and results can be directly compared.

Absorbances (for 60 γ) of chromatographed cholesterol and cholesterol laurate (Table II) were, respectively, 6 and 2% higher than those of their opposite numbers in the standard curve (Table I), and experiments were made to determine the causes of these differences.

Comparison of standard curves was made for pure cholesterol, cholesterol laurate, and cholesterol oleate, when subjected to (*a*) chromatography and elution; (*b*) direct solution in chloroform (same quantity as used for elution) and transfer to ferric chloride–acetic acid solution; and (*c*) direct solution in

Table II. Calibration Data of Chromatographed Pure Free and Ester Cholesterol

Treatment	Cholesterol			Cholesterol Laurate		
	No. of trials	Mean absorbance, 560 mμ[a]	Coeff. of variation, %	No. of trials	Mean absorbance, 560 mμ[a]	Coeff. of variation, %
Chromatographed on $ZnCO_3$ paper and eluted	6	0.346	1.8	7	0.323	1.5
Spotted on $ZnCO_3$ paper and directly eluted (controls)	3	0.342	2.0	4	0.325	3.7

[a] Values for 60 γ of compound (as cholesterol) in 5 ml. of color solution with spectrophotometric assay as in Table I.

Table III. Results of Paper Chromatographic Cholesterol Assay of Blood Sera and Precision of Duplicates, Run on Separate Days

Sample No.	Subject: Sex	Subject: Age, years	Dates of Assay	Serum Cholesterol Values[a]: Total, mg. %	Mean, mg. %	Free, mg. %	Mean, mg. %	Ester, mg. %	Mean, mg. %	Free/total, %	Mean, %
1	F	26	10/28/57	144.7	146.4	40.5	40.9	104.2	105.5	28.0	27.9
			11/ 5/57	148.0		41.2		106.8		27.8	
2	F	23	10/25/57	176.3	181.4	51.8	50.6	124.5	130.8	29.4	27.9
			11/ 5/57	186.4		49.3		137.1		26.4	
3	M	16	10/25/57	134.1	135.8	35.2	35.4	98.9	100.4	26.2	26.1
			10/28/57	137.4		35.6		101.8		25.9	
4	M	19	10/31/57	169.5	164.5	43.4	42.8	126.1	121.8	25.6	26.0
			11/ 5/57	159.5		42.1		117.4		26.4	
5	M	17	10/31/57	126.2	126.8	36.9	36.4	89.3	90.4	29.2	28.7
			11/ 5/57	127.4		35.9		91.5		28.2	
6	M	23	10/31/57	150.2	145.9	42.7	42.7	107.5	103.2	28.2	29.2
			11/ 5/57	141.6		42.7		98.9		30.2	
Mean, mg. %				150.11		41.44		108.67		27.63	
Std. dev.,[b] mg. %				±5.00		±0.894		±5.23		±1.11	
Coef. of variation, %				3.3		2.2		4.8		4.0	

[a] Each value given represents mean for duplicate spots on a chromatogram.

[b] Std. dev. = $\sqrt{\frac{1}{2n}\Sigma(\text{difference between duplicates})^2}$

acetic acid and dilution with ferric chloride–acetic acid.

Results of this comparison seemed to establish that:

1. The differences between chromatographed and unchromatographed cholesterol and cholesterol laurate (6 and 2%, respectively) were demonstrable on repeated trials over a range of quantities—15 to 90 γ of cholesterol compounds.
2. A 2 to 4% positive error in absorbance, which was due to something in the spotting and eluting portions of the chromatographic process, occurred for all three cholesterol compounds. (This was shown by the 2 to 4% difference between results in (*a*) and (*b*) above, and by the lack of difference between chromatographed and directly eluted standards—Table II.)
3. Variable differences in absorbance, due to the use of chloroform as a transfer solvent, were found. For free cholesterol, cholesterol laurate, and cholesterol oleate, they were approximately +1, −2.5, and +1%, respectively [differences between results in (*b*) and (*c*) above].
4. Sums of effects for 2 and 3 could explain values for total effects, listed under 1.

Effect of Prewashing Zinc Carbonate Paper. In further experiments, prewashing of the zinc carbonate paper caused no increase in the absorbance of chromatographed cholesterol but did remove chromogenic impurities from the paper.

Absorbance at 560 mμ of a typical blank from paper, prewashed with purified chloroform, was 0.006 *vs.* 0.000 for a reagent blank.

Accuracy of Micropipet. The micropipet was checked and shown to deliver the correct volume of chloroform solution. Five-microliter portions of a fresh solution of crystalline β-carotene (Hoffmann-La Roche, Inc.) in chloroform were spotted and immediately eluted with petroleum ether. Absorbance at 453 mμ was determined. Values agreed with results of direct spectral assay, as well as known purity, of the β-carotene within 1%.

Possible Spotting Error. Further, spotting β-carotene from a small volume (0.5 ml.) of chloroform solution gave an apparent positive error of 1.5%, which could be ascribed to evaporation. Spotting two to four times, without cleaning the micropipet between, also gave an apparent positive error of less than 2%.

These effects may also have occurred in cholesterol chromatography and might account, in part, for the difference in absorbance, noted under 2 above.

The magnitude of and explanation for the differences in absorbance values for chromatographed cholesterol compounds (Table II) and unchromatographed ones (Table I) are not established with certainty. The differences are undoubtedly somewhat variable. (For example, use of chloroform, shown to be pure by gas chromatography, appeared to nullify the solvent transfer effect upon cholesterol compounds, noted under 3 above.)

Need for Chromatographed Cholesterol Standards. The cholesterol method requires inclusion of chromatographed standards to obtain best results.

Standards should consist of pure cholesterol, freshly dissolved in the same solvent as the unknowns and at similar concentrations. They should be spotted on the same paper, with the same pipet, and using the same volume of solution per spot as used for the unknowns.

A mean calibration factor, derived from standards of several consecutive days' runs, is preferred to one from a single chromatogram.

ASSAY OF HUMAN BLOOD SERA

Recovery of Added Cholesterol. Pure cholesterol or cholesterol oleate were added in amounts of 30 or 60 γ (as cholesterol) to blood extracts while spotting the chromatograms. Recoveries, based on simultaneously chromatographed pure cholesterol compounds, were quantitative (97.8, 100.0, and 101.2%).

Thus, admixture with blood lipide constituents during chromatography apparently does not affect chromogenic properties of chromatographed pure free or ester cholesterol, which supports their use as standards in blood assay.

Standard for Blood Ester Cholesterol. Because of the 6% difference between absorbances of the chromatographed compounds, pure cholesterol laurate and pure free cholesterol, (Table II), the choice of a standard for blood ester cholesterol rested on several factors. Cholesterol laurate, used for recovery tests because of its demonstrated purity, is not known to occur to any appreciable extent in human blood serum. Various cholesterol esters of blood (chiefly, cholesterol linoleate) were not available in pure form. Pure cholesterol oleate, an ester which does occur in blood, gave values (96 to 99 %) nearly equivalent to those of free cholesterol in various trials.

Therefore, free cholesterol was used as the standard in determining both free and ester cholesterol content in blood, and the calibration factor used was that for chromatographed free cholesterol (Table II).

Trial of Blood Assay. The method was tested on sera from six subjects (Swiss laboratory personnel), and each serum was extracted, chromatographed, and assayed for cholesterol on two separate days.

In each chromatogram, portions of serum lipide extracts, representing 32 μl. of serum per spot, were spotted in duplicate, with duplicate portions of pure cholesterol and cholesterol laurate—60 γ as cholesterol per spot. At least two unspotted sections for blanks and appropriate guide strips were included.

Direct elution controls for total cholesterol in blood serum were run concurrently with portions of the blood lipide extracts.

Results. The mean difference and standard deviation between direct elution and paper chromatographic values for total cholesterol for the six sera were 3.2 ± 4.97 mg. per 100 ml. of serum. Comparison of the calculated t (*14*) = 1.58 with the value of t for probability of 0.01, which is 4.032, shows that the values agree within experimental error, and no loss or gain of cholesterol compounds occurred during chromatography.

Other results are detailed in Table III.

Precision of Assay. The standard deviation of a single paper chromatographic assay was calculated by the formula

$$\text{Std. dev.} = \sqrt{\frac{1}{2n}\ (\text{sum of } d^2)}$$

where d is the difference between duplicate assay values and n is the number of pairs of duplicates.

The values of the coefficients of variation for a single assay (Table III) show very good precision, because they measure variance of two complete chromatographic assays, each carried out at different times. When six replicates of one sample were run on one chromatogram, the coefficients of variation of a single assay for free and for ester cholesterol were only 1.9 and 2.2%, respectively.

Comparative Assay by Other Methods. The six blood sera were analyzed for total cholesterol by two other methods:

For assays by the method of Abell *et al.* (*1*) absorbance was determined with the Beckman spectrophotometer at 625 mμ; the observed $E^{1\%}_{1\text{ cm.}}$ (625 mμ) for pure free cholesterol was 45.0.

In analyses by the procedure of Henley (*6*) absorbance was read in the spectrophotometer at 560 mμ at exactly 30 minutes. Values were corrected for the effect of saline on absorbance of cholesterol compounds, as noted above.

Results (Table IV) show that for total cholesterol, average differences between paper chromatographic results and those by each of two other methods are less than 2%. Application of the t test (*14*), as detailed in Table IV, indicates that results agree within experimental error.

Appearance of Blood Lipide Chromatogram. Figure 2 shows a chromatogram of the six blood lipide extracts. At the extreme left are spots, containing pure cholesterol (below) and pure

Table IV. Comparative Assays for Total Cholesterol by Paper Chromatography and Other Methods

Blood Serum No.	Serum Total Cholesterol, Mg./100 Ml.[a] Paper chromatography	Method of Abell (1)	Method of Henley (6)
1	146.4	149.1	143.3
2	181.4	179.4	171.0
3	135.8	134.4	133.4
4	164.5	162.4	161.5
5	126.8	132.8	129.7
6	145.9	144.2	144.8
Mean of differences from paper chromatography results, mg./100 ml.	...	−0.25	+2.78
Std. dev. of difference, mg./100 ml.	...	±3.35	±4.19
t[b]	...	0.18	1.6

[a] All values means of duplicate assays.
[b] t at D.F. = 5 and p = 0.01 is 4.032.

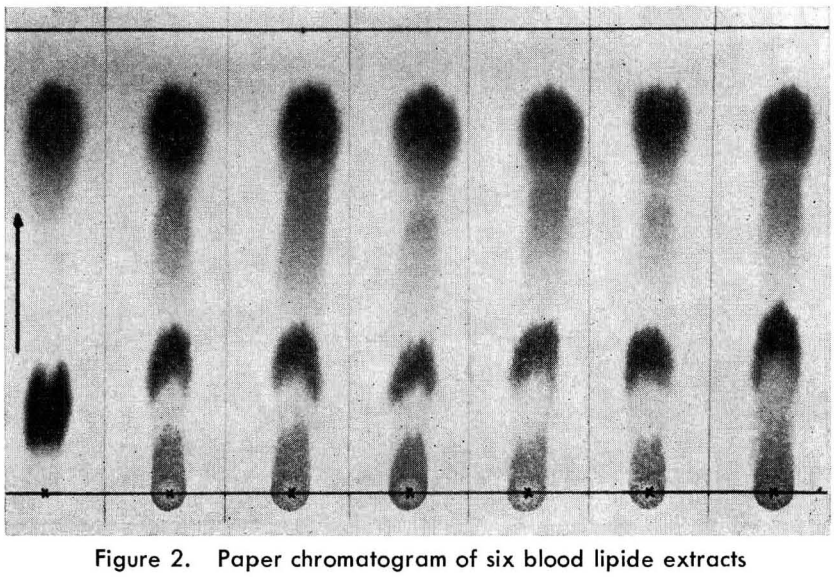


Figure 2. Paper chromatogram of six blood lipide extracts

Extreme left bottom, pure free cholesterol
Extreme left top, cholesterol laurate
System and staining as in text

cholesterol laurate (above). Good separation of free and ester cholesterol, with respective R_f's of approximately 0.2 and 0.8 for pure compounds, is shown. The free cholesterol of blood extracts has a slightly higher R_f due, probably, to accompanying blood lipides; therefore it is important to use replicate blood lipide extracts on the guide strips used for cutting paper chromatogram segments.

Possible Interference of Other Blood Constituents. The identity of the material in blood lipide extracts which, in addition to free and ester cholesterol, is stained by phosphomolybdic acid reagent (Figure 2) is not known. By elution and assay of chromatographic segments below and above free cholesterol, it was shown not to interfere in the spectrophotometric method for cholesterol used here.

α-Tocopherol and β-carotene, two blood lipide constituents which might conceivably interfere in the spectrophotometric cholesterol assay method, were tested. Although both react positively, their relatively insignificant amounts in blood preclude their interfering.

Bilirubin and bromide ion, which may occur in blood serum and interfere with the cholesterol determination with iron–acetic and sulfuric acid reagents (8, 9), should be separable by paper chromatography on zinc carbonate paper. When tested, they stayed at the origin, while free cholesterol (the cholesterol compound with the lowest R_f in this chromatographic system) usually migrates a significant distance.

Direct Chromatography of Serum. Attempts were made, with partial success, to chromatograph serum applied directly to zinc carbonate paper to eliminate prior extraction of the blood lipides.

Drying a serum spot (32 μl. of serum) on the paper allows about 10% each of free and ester cholesterol present in serum to migrate and separate well during subsequent chromatography. (This may be cholesterol which is not tightly bound in a lipo-protein complex.) The yield is increased to about two thirds of the total cholesterol in serum by brief prior treatment of the serum spot with such reagents as 1 to 1 acetone–ethyl alcohol or 1M sodium sulfate. Experiments are being continued, and it is hoped to achieve complete release and separation of free and ester cholesterol from a serum spot, possibly with use of a detergent.

In related trials, total cholesterol compounds in a spot of blood serum (32 μl.) applied to paper were completely eluted by immersing the paper in 7 ml. of absolute ethyl alcohol at 80° for 2 hours, followed by washing the paper with ethyl alcohol (8 ml.); this technique might serve for a simple assay of total cholesterol in blood serum.

After this paper was written, the report by Hansen and Dam (5) of a similar method came to the authors' attention. They extract 25 γ or less of cholesterol and its esters and chromatograph them on filter paper with iso-octane as the mobile phase. Their modified Zlatkis cholesterol method uses fresh color reagent for each assay and special microcuvettes.

In comparative trials, in which quantities (60 γ) of free and ester cholesterol such as used in the present work were spotted and chromatographed, separation was much clearer with the chromatographic system described here than with that of Hansen and Dam. Hansen has found (4) good separation of 60-γ amounts of cholesterol compounds with their system if applied, as described (5), as a 1.5-cm. streak. It appears that zinc carbonate–impregnated paper is advantageous in spotting and separating the larger amounts of cholestrol compounds.

The time of chromatography is the same for both systems, and neither introduces material into eluates, which interferes in subsequent spectrophotometric assay, as happens in other paper chromatographic procedures (5).

ACKNOWLEDGMENT

Apppreciation is expressed to E. Brauchli of F. Hoffmann-La Roche, Basel, for this helpful interest in the work, to M. Nancy Green for preparation of purified cholesterol and cholesterol laurate, and to A. Koenig for competent technical assistance.

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RECEIVED for review March 24, 1958. Accepted November 3, 1958. Presented in part before the Division of Biological Chemistry, Methods and Procedures, 133rd Meeting, ACS, San Francisco, Calif., April 1958. Work supported in part by grants from the John A. Hartford Memorial Fund, the Albert and Mary Lasker Foundation, New York, the Nutrition Foundation, Inc., New York, and the Fund for Research and Teaching, Department of Nutrition.

SCIENTIFIC COMMUNICATIONS

Solvent Effects on the Electro-oxidation of Iodide Ion

SIR: Recent voltammetric studies of the oxidation of iodide ion in acetonitrile at a rotating platinum electrode, by Kolthoff and Coetzee (*4*) and Popov and Geske (*6*), have shown that electro-oxidation to iodine proceeds in two steps. The first involves oxidation of iodide ion to triiodide ion and is a two-electron step; the second involves oxidation of triiodide ion to iodine and is a one-electron step:

$$6I^- = 2I_3^- + 4e$$
$$2I_3^- = 3I_2 + 2e$$

Because solvent effects on the oxidation-reduction potential of inorganic redox couples are, at present, receiving a great deal of attention, we wish to report additional data of interest on this oxidation, to call attention to solvent effects on the mechanism of electro-oxidation and electroreduction of inorganic substances.

Chronopotentiograms for the electro-oxidation of iodide ion at a platinum foil electrode in aqueous 0.1*M* sodium perchlorate indicated a single one-electron step for the formation of iodine, as expected (*1*, *5*). The $E_{1/4}$ and $0.059/n$ values were 0.51 volt. *vs.* S.C.E. and 0.057, respectively. One-step oxidation of iodide ion to iodine was also observed in: methanol, 1-butanol, 2-propanol, and acetic acid. Although ethylene glycol favored the two-step oxidation of iodide ion to iodine, the $E_{1/4}$ values for the two steps differed by only 0.1 volt. The following solvents along with acetonitrile were found to favor two-step oxidation of iodide ion to iodine: acetone, 1,2-dimethoxyethane, dimethyl sulfoxide, and acetic anhydride.

Electro-oxidation of iodide ion in pyridine was also found to proceed via a two-step mechanism but of a different type. The $E_{1/4}$ values are 0.37 and 0.89 volt *vs.* S.C.E., chronopotentiometric transition times indicate that the electrode reactions are

$$2I^- = I_2 + 2e$$
$$I_2 = 2I^+ + 2e$$

It is somewhat surprising that the oxidation should proceed by this mechanism, in view of the fact that the triiodide ion is known to be more stable than PyI_2 (*3*). Interestingly, the electro-oxidation of iodine in pyridine also proceeds via a two-step mechanism; the $E_{1/4}$ values are 0.5 and 0.9 volt *vs.* S.C.E. When a small amount of sodium iodide was added to the solution of iodine in pyridine, a three-step chronopotentiogram with $E_{1/4}$ values of about 0.35, 0.5, and 0.9 volt *vs.* S.C.E. was obtained. It appears that the first step in the electro-oxidation of iodine in pyridine involves oxidation of triiodide ion to iodine, followed by further oxidation of iodine to iodine(I) in the second step. This supports evidence previously presented for the following dissociation of iodine in pyridine (*2*, *7*).

$$2I_2 + Py = PyI^+ + I_3^-$$

The electro-oxidation of iodide ion in mixed solvent systems of acetone-water and 1 to 1 ethylene glycol–1,2-dimethoxyethane was found to proceed in two steps. In the case of acetone-water system, two steps were evident up to 60 to 70% water.

The mechanism favored by a particular solvent is evidently more subtle than could be explained on the basis of an aqueous *vs.* nonaqueous effect, as shown by the fact that the alcohols, acetic acid, and pyridine favor the same process as water; that pyridine, a base, and acetic acid, an acid, favor the same mechanism; that acetic acid and acetic anhydride favor different processes; and that, in general, solvents containing the OH group favor one-step electro-oxidation of iodide ion to iodine.

Table I lists the $E_{1/4}$ *vs.* S.C.E. values for the oxidation of iodide ion in the solvents studied.

Table I. $E_{1/4}$ *vs.* S.C.E. Values for Oxidation of Iodide Ion

Solvent (0.1*M* $NaClO_4$)	Step 1	Step 2
Water	0.51	
Methanol	0.40	
1-Butanol[a]	0.42	
2-Propanol[a]	0.42	
Acetic acid	0.44	
Ethylene glycol	0.42	0.52
Pyridine	0.37	0.89[b]
Acetonitrile	0.25	0.55
Acetone	0.22	0.62
Acetic anhydride	0.20	0.60
1,2-Dimethoxyethane	0.39	0.65
Dimethyl sulfoxide	0.48	0.70
1 to 1 acetone/water mixture	0.42	0.54
1 to 1 ethylene glycol–1,2-dimethoxyethane mixture	0.37	0.56

[a] 0.2*M* $LiClO_4$.
[b] $I_2 \rightarrow I^+$ step.

A more detailed report on this and related studies will be presented in the near future.

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RECEIVED for review January 27, 1959. Accepted March 2, 1959.

Electronic Controlled-Potential Coulometric Titrator

SIR: Circuit changes have been made to improve the performance of the electronic controlled-potential coulometric titrator [ANAL. CHEM. **31**, 488 (1959)].

CIRCUIT

1. Replace the 1000-ohm 0.5-watt resistor connected to the base of the transistor by a 4700-ohm 2-watt resistor.
2. Insert an M-500 (1N 1084) rectifier at the reduce-oxidize switch in the lead from the reduce-oxidize switch that goes to the 2.5-, 25-, and 250-ohm resistors. Connect the anode (arrow) terminal of the rectifier to the reduce-oxidize switch. The rectifier is thus added in series with the current metering circuit.
3. Return the ground connection from the common connection of the two 10,000-ohm resistors in the integrator bias network by a separate wire directly soldered to pin *E* of the power supply connector.
4. Return the ground for the integrating amplifier (pin 4 of connector 26-4200-16S B) to only the common connection of the two 10,000-ohm resistors in the integrator bias network.
5. Replace the 470,000-ohm resistor that is connected to the limit light by a 560,000-ohm resistor.
6. Connect a 0.1-μfd. Vit. Q 400-volt capacitor across the two terminals of the "titrate-off-reset" switch that are wired to the relay coils.
7. There should be a total of four wires attached directly to pin *E* of the power supply connector.
8. The resistor connected to pin *B* of the cell connector should be a 400,000-ohm 0.5% wire-wound resistor.

CONSTRUCTION

Note 9 (Table I) should read: "The integrator bias should be adjusted for essentially zero drift of the integral readout voltage at zero volt output of the integrator." The integrator bias may be adjusted and the titrator checked for drift by the following procedure:

1. Turn on the a.c. power switch on the GAP/R R-100A power supply.
2. After a delay of 1 minute, turn on the d.c. power switch on the GAP/R R-100A power supply.
3. Warm the instrument up for 15 minutes or longer.
4. Connect a readout device to the instrument.
5. Disconnect the cell leads.
6. Throw the titrate switch to the reset position.
7. Turn the reduce-oxidize switch to reduce.
8. Turn the readout switch to X10 position.
9. The readout device should indicate less than 0.0001 volt.
10. Throw the titrate switch to the titrate position.
11. After 5 minutes, measure the readout voltage. If it has changed more than 0.0001 volt, readjust the integrator bias potentiometer and repeat steps 6 through 11.
12. Repeat the drift measurement and integrator bias adjustment until the readout changes less than 0.0001 volt after 15 minutes in the titrate position.
13. Turn the reduce-oxidize switch to the oxidize position.
14. Throw the titrate switch to the reset position.
15. The readout device should indicate less than 0.0001 volt.
16. Throw the titrate switch to the titrate position.
17. After 15 minutes in the titrate position, the readout voltage should change less than 0.0001 volt.
18. If it is not possible to adjust the integrator bias potentiometer to meet these drift specifications, a leakage current exists at the input of the integrator. The leakage current must be eliminated. The most common sources of leakage currents are ground loops.

ABSOLUTE CALIBRATION PROCEDURE

Connect a 4-μfd. capacitor of good quality across the calibrated resistor.

IN-SERVICE OSCILLATION CHECK

Under some titration conditions the output of the control amplifier includes oscillations at about 60/kc. Oscillation of the control amplifier adversely affects the titration results. This can be eliminated by reducing the gain of the control amplifier loop. A simple and effective method is to increase the value of the resistor connected to the transistor base from 1000 to 4700 ohms. The titrator should be checked for the absence of oscillations when it is put into service. Connect a cell containing suitable electrolyte and a reversible redox sample to the titrator. Attach an oscilloscope between chassis ground and the working (isolated) electrode. Perform an oxidation and a reduction titration. Ascertain that the control amplifier does not oscillate during these titrations. A small amount of 60 c/s ripple is normal during thgh-current periods.

INITIAL-CURRENT LIMITING

The maximum initial current that may be allowed to pass through a specific titration cell without adverse effect on the analytical results seems to depend on cell and electrode geometry, stirring efficiency, and current density and varies with the ion being titrated. As a result, the 160-ohm current limiting resistor is included in the original circuit. If the amount of material titrated causes the initial current to be higher than the current that can be handled by the cell, the size of the current limiting resistor must be increased, the cell design changed, or the control potential initially set at a lower value until some of the sample has reacted and the titration completed at the full value of the control potential.

FRACTION OF CELL CURRENT INTEGRATED

In the original circuit 1/40,000 of the cell current is integrated. This is suitable for most coulometric titrations. It would be advantageous to increase this fraction to titrate high equivalent weight substances such as plutonium. This may be done by substituting a wire-wound resistor of lower value for the 400,000-ohm resistor connected to pin 1 of the integrating amplifier. As the 10-ohm resistor must handle high current, it is a wire-wound power resistor, not a precision resistor. However, the value of these resistors is stable over long periods.

TRANSISTOR

The transistor type used in the seven instruments constructed to date is a Motorola 2N375. If similar transistors having higher frequency response become available, it would be worthwhile to test their performance in this circuit. It would also be possible and desirable to simplify the circuit, if an NPN transistor could be switched in for reduction titrations in a circuit similar to that used for oxidation with the PNP transistor. At this time no NPN commercially available transistor has sufficient gain, current rating, and voltage ratings.

CHECKOUT PROCEDURE

A checkout and test procedure has been written for the electronic controlled-potential coulometric titrator (available from the authors upon request).

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Application of Ion Exchange to the Colorimetric Determination of Trace Amounts of Uranium Using Dibenzoylmethane

SIR: A method for ion exchange separation and colorimetric determination of uranium is reported, an extension of previous work (*3–5*). The uranium, complexed with dibenzoylmethane in 80% alcohol, is determined colorimetrically at 400 mμ. This method has been routinely applied to a large number of low-grade uranium samples.

EXPERIMENTAL

Reagents. Resins included Dowex 1, Dowex 2, Duolite A-101, and Amberlite IRA-400. Amberlite IRA-400, consisting of 40- to 60-mesh beads and purchased as Amberlite XE-117, Type 1 or 2, is recommended. A standard uranium solution containing 50 mg. of uranium oxide per liter was prepared from uranyl sulfate. All other chemicals were of reagent grade.

Apparatus. Absorption measurements were made with a Bausch & Lomb Spectronic 20 colorimeter with 0.5- and 1-inch cells. A Beckman Model G pH meter was used for pH control. A column similar to the one described (*4, 5*) was used, except that a 14/20 standard-taper ground-glass joint connected the dropping funnel and the column.

Procedure. The procedure for dissolution of the ores and separation of uranium by ion exchange is essentially as reported (*3–5*). The perchloric acid eluate containing the uranium is collected in 400-ml. beakers, which are placed on an asbestos-covered hot plate regulated at 250° to 300° and evaporated almost to dryness. The last traces of perchloric acid are removed by placing the beakers in a furnace regulated between 250° and 300° C. for 10 to 20 minutes. After cooling, the sides of the beakers are washed down with 10 ml. of distilled water and thoroughly policed, whereupon 25 ml. of 95% ethyl alcohol is added. One milliliter of a 1% solution of dibenzoylmethane in 95% alcohol is added and the solution brought to an apparent pH of 7.0 to 7.5 by regulation with 0.005*M* sodium hydroxide or sulfuric acid. This solution is transferred to a 50-ml. volumetric flask with 95% alcohol. The solutions are filtered through retentive filter paper such as Whatman No. 5 to remove traces of insoluble residue and absorbances are determined at 400 mμ. The blank is prepared from 1 ml. of 1% dibenzoylmethane in 50 ml. of 80% ethyl alcohol solution.

This procedure is satisfactory for nearly all types of uranium samples, including those containing thorium up to a thorium-uranium ratio of 5 to 1. If the thorium-uranium ratio is higher, it would be readily detected by abnormally high radiometric measurements. The procedure can be modified to handle samples containing up to 100 mg. of thorium by including a 100-ml. wash with 10*N* hydrochloric acid after the final wash with hot water and before elution with 1*M* perchloric acid. Excess water should be removed from the column before the hydrochloric acid wash.

For samples containing more than 100 mg. of thorium or calcium phosphate the procedure was altered to include a double column separation.

After solution of the ore, the sample is evaporated to dryness with hydrochloric acid, taken up in 50 ml. of 10*M* hydrochloric acid, and adsorbed on the chloride form of the resin. After the resin is washed with approximately 50 ml. of 10*M* hydrochloric acid, the sample is eluted with 100 ml. of hot 10% hydrochloric acid. Upon standing 10 minutes, the last of the eluate is blown out with air. Five milliliters of 9*M* sulfuric acid is added to the eluate, and it is evaporated to strong fumes of sulfur trioxide. After cooling, 100 ml. of distilled water is added and the solution boiled until the residue dissolves. After the pH is adjusted to about 1.2, the sample is treated with 10 to 30 ml. of 6% sulfurous acid and readsorbed on the sulfate resin.

Development. An investigation of anion exchange resins revealed that Amberlite IRA-400 or Duolite A-101 was the most satisfactory for both the standard procedure and the double column separation. The Dowex 1 and Dowex 2 resins could be eluted satisfactorily with 10% hydrochloric acid, but only about half of the uranium was recovered with 1*M* perchloric acid.

A number of other solvents can be substituted for ethyl alcohol. The most practical system investigated was isopropyl alcohol–water solutions limited between 50 and 80% alcohol.

Because the colored complex had to be developed in a neutral solution, all the acid-eluting agent had to be eliminated before color development. Relatively small amounts of acid caused excessive salt formation in the alcohol system and gave low results. The bulk of the perchloric acid can be easily removed by evaporation on a hot plate in a perchloric acid hood. Early attempts at removing the last traces of acid with a Bunsen flame gave erratic results. To explain this phenomenon, a series of experiments was performed, eliminating the last traces of perchloric acid in a controlled muffle furnace at various temperatures (Table I). The temperature is critical, as elevated temperatures convert some of the uranium into an insoluble form.

Table I. Removal of Last Traces of Perchloric Acid before Color Development

[0.20 mg. of uranium (U_3O_8) present]

Furnace Temp., °C.	Treatment, Min.	Uranium Found, Mg.	Av. Dev., Mg.
250	30	0.20	0.000
		0.20	0.000
300	30	0.20	0.000
		0.20	0.000
350	30	0.175	−0.025
		0.180	−0.015
400	30	0.173	−0.027
		0.185	−0.020
500	15	0.148	−0.052
		0.105	−0.095
500, 300[a]	15, 30	0.20	0.000
		0.20	0.000

[a] Sample 6 was heated for 15 minutes at 500° C., then redissolved, retreated with perchloric acid, and reheated for 30 minutes at 300° C.

DISCUSSION AND RESULTS

Interferences studied in addition to those previously reported (*1, 4*) were the effects of excessive amounts of thorium and calcium phosphate. In sulfate solutions thorium formed an anionic complex that was adsorbed by the resin. Samples containing more than 2 mg. of thorium showed a considerable residue of thorium salts after evaporation of the eluate, the amount increasing with thorium concentration. Concentrations in excess of 1 mg. interfered in the absorbance readings. For concentrations between 1 and 100

Table II. Standard Samples Run by Routine Procedure

Sample	Actual % (U_3O_8) Reported for Standard	Weight of Sample, G.	% U_3O_8 Found: Duplicate analyses	% U_3O_8 Found: Av.	Dev. of Av. from Reported Value
New Brunswick No. 1 (phosphate rock)	0.029	1.00	0.024 0.025	0.024	−0.005
		1.00	0.027[a] 0.027[a]	0.027	−0.002
AEC					
4	0.180	0.20[b]	0.180 0.180	0.180	0.000
5	0.114	0.25[b]	0.110 0.109	0.110	−0.004
8	0.064	1.00	0.063[c] 0.065[c]	0.064	0.000
9	0.109	0.25[b]	0.104 0.104	0.104	−0.005
Rubidoux Mtn. leucogranite	0.00071 (7.1 p.p.m.)	5.00	0.0006 0.0007	0.00065	−0.00006
		10.00	0.0007 0.0008	0.00075	0.00004
Natl. Lead					
1	0.015	1.00	0.0148 0.0152	0.015	0.000
2	0.019	1.00	0.0178 0.0182	0.018	−0.001
3	0.024	1.00	0.0225 0.0230	0.023	−0.001
4	0.012	1.00	0.0120 0.0116	0.012	0.000
5	0.012	1.00	0.0113 0.0110	0.011	−0.001

[a] Values obtained by double column technique.
[b] Aliquots of 1.00- or 1.25-gram samples.
[c] Sample dissolved using HF and HNO_3.

mg. per sample, a 100-ml. wash of 10*N* hydrochloric acid after adsorption usually eliminates the interference. For concentrations above 100 mg., the double column separation is recommended; thorium would not be a problem even in excessive amounts, as it is not adsorbed on the resin in 10*N* hydrochloric acid (*2*).

Phosphate rock containing in excess of 100 mg. of calcium phosphate per sample caused losses due to coprecipitation of uranium with calcium sulfate in the solution step. These losses were minimized by using the double column separation. In 10*N* hydrochloric acid, neither calcium nor phosphate was adsorbed on the resin. As uranium and iron were adsorbed, the effect of high concentrations of iron was studied. Iron concentrations in excess of 200 mg. per sample caused uranium leakage in a resin volume of 5 ml. In such cases the resin volume should be increased.

To determine the accuracy of the procedure, a series of uranium standards was analyzed, a majority obtained from the Colorado Plateau (Table II). The reported uranium content of the New Brunswick and AEC standards is a statistical average from the results of several independent laboratories. The leucogranite was supplied by the California Institute of Technology and analyzed in replicate by independent analysts using chemical extractions and ultraviolet fluorescence. The National Lead samples are a series of fluorometric standards from Grand Junction, Colo.

CONCLUSIONS

The procedure is adaptable to routine analyses and can be done with a minimum of expense and equipment, especially in laboratories where ion exchange is in use or a fluorometer is not available. Interferences from high thorium or calcium phosphate are minimized by double adsorption. Analytical detection may be expected for uranium concentrations as low as 0.0001%. The average deviation is 0.002% or better for uranium values between 0.1 and 0.01%. For uranium values between 0.01 and 0.001% the accuracy of the determination is to approximately ±10%.

ACKNOWLEDGMENT

The authors thank John N. Butler and his staff, Mackay School of Mines, for suggestions and encouragement.

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CONDUCTED under Contract No. AT(49-1)-624 between the U. S. Atomic Energy Commission and the University of Nevada, Mackay School of Mines.

Rapid Spectrophotometric Determination of Manganese-Triethanolamine and Peroxide Complexes of Manganese(III)—Correction

A PRIOR procedure by Bruno [*Accad. pugliese sci., Atti e relaz.* **11**, Pt. 2, 409 (1953)] for the spectrophotometric determination of manganese(II) (*sic*) in alkaline triethanolamine solution is acknowledged. This procedure recommended the determination at 625 mμ for solutions containing 5 to 30 γ of manganese per ml., but the nature of the reduction and the identity of the colored species were not elucidated.

The upper limit of 30 γ per ml. for the Bruno method corresponds to our concentration limit of 0.5m*M* [ANAL. CHEM. **31**, 146 (1959)], above which we could not effect the rapid quantitative air oxidation of the manganese(II). For more concentrated solutions, the manganese(III)-peroxide complex interferes and must be decomposed as described previously. The molar absorbance index of the manganese(III)-triethanolamine complex at 625 mμ is slightly greater than that at 438 mμ, but the latter wave length provides a more specific method because copper(II) and iron(II) do not interfere. In spite of their simplicity, neither of these procedures can be recommended for concentrations less than about 1m*M* because the molar absorbance of the manganese(III)-triethanolamine complex is not sufficiently large to give accurate results; more sensitive procedures are available for dilute solutions.

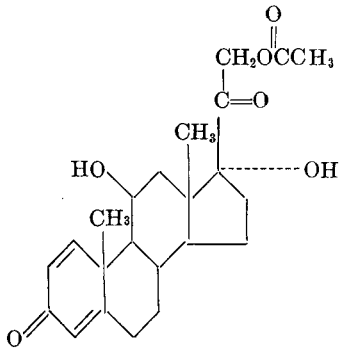
E. R. NIGHTINGALE, JR.

CRYSTALLOGRAPHIC DATA

181. Unit Cell Dimensions and X-Ray Powder Data of Prednisolone Acetate

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THIS note reports the results of an x-ray crystallographic study, both powder and single crystal, of prednisolone acetate,



a steroid of potential importance in the treatment of arthritis. The steroid (melting point 229° to 234° C.) was furnished by the Schering Corp.

Single crystals of prednisolone acetate were obtained by slow evaporation of its chloroform solution. They showed monoclinic prismatic habit. Rotation, $0kl$ and $h0l$ Weisenberg photographs were taken for two crystal specimens. The only systematic absences observed—$(0k0)$ absent for k odd—are indicative for the space group $P2_1$. The unit cell dimensions are: a = 9.07, b = 13.94, c = 8.58 A. ± 0.3%, β = 103.4°.

The experimental density determined by flotation is 1.27 grams per cc. The molecular weight calculated from these data and assuming two molecules per unit cell is 404. It compares well with the theoretical value of 402.5.

For identification purposes powder photographs of prednisolone acetate were also taken. In Table I the relative intensities and spacings of 21 lines are reported. The first 10 entries include all observable lines in this range of d values, the other entries only the stronger reflections.

Table I. Powder Data for Prednisolone Acetate, Co $K\alpha$ Radiation

Line	Intensity[a]	d, A.
1	m	8.81
2	vvw	8.32
3	vvw	7.46
4	s	7.11
5	m	6.92
6	m	6.18
7	vs	5.45
8	m	5.34
9	m	5.10
10	mw	4.89
11	m	4.29
12	mw	4.13
13	ms	3.98
14	vw	3.85
15	m	3.56
16	w	3.37
17	m	3.21
18	mw	3.11
19	w	2.93
20	m	2.77
21	m	2.66

[a] w = weak, m = medium, s = strong, v = very.

ACKNOWLEDGMENT

The author is grateful to the Schering Corp., Bloomfield, N. J., under whose support this study was carried out, for permission to publish the data, and to Dean Graham for growing the single crystals.

CRYSTALLOGRAPHIC data for publication in this section should be sent to W. C. McCrone, 501 East 32nd St., Chicago 16, Ill.

INFRARED QUANTITATIVE ANALYSIS DATA

Analysis of Detergent-Slurry Components

V. A. CIRILLO, The Atlantic Refining Co., Philadelphia, Pa. **CS-76**

Component No.	Component Name	Component Formula	Range %	Accuracy %	λ or ν B.L. Points	Slit (mm) Δλ or Δν	Concn. g/m Length mm
1	Benzene	C_6H_6	12–20	±0.3	14.57	1.10 0.109μ	0.04 0.100
2	Water	H_2O	30–60	±1.0	6.07	0.12 0.028μ	0.04 0.100
3	Methanol	CH_3OH	0–25	±0.3	9.63	0.56 0.066μ	0.04 0.100
4	Detergent sodium dodecyl benzene sulfonate	$C_{18}H_{29}SO_3Na$	15–25		9.88	0.56 0.063μ	0.04 0.100

Instrument: Beckman IR-4, NaCl prism
Sample Phase: Slurry or emulsion

Cell Windows: AgCl
Absorbance Measurement: Base line: Benzene Point: All others

Calculation: Inverse matrix

Solvents: Acetone and dioxane

Relative Absorbances[a]–Analytical Matrix:

Component/λ	14.57	6.07	9.63	9.88
1	26.8	0	1.07	0.03
2	0	13.6	1.82	1.90
33	0	0.12	33.0	3.60
4	0	1.80	5.11	4.59

Material Purity: Reference compounds 99+% pure
Comments:
Slurry is heated slightly until a solution results.
Two 1-ml. aliquots are weighed in 25-ml. volumetric flasks containing dioxane and acetone, respectively.
The benzene band at 14.57 microns differs from its usual position 14.86 microns in less polar solvents.
It is necessary to scan only the following regions:
Benzene. 15.0 to 14.0 microns, acetone solvent
Methanol detergent. 10.4 to 9.0 microns, acetone solvent
Water. 7.1 to 5.8 microns, acetone solvent

[a] Relative absorbances are given as the slope of the Beer's law concentration curves used expressed in terms of absorbance per 100% of constituent.

Determination of Polyacrylonitrile in Coatings

M. L. ADAMS and M. H. SWANN, Coating and Chemical Laboratory, Aberdeen Proving Ground, Md. **CS-77**

Component No.	Component Name	Component Formula	Range %	Accuracy %	λ or ν B. L. Pts.	Slit (mm) Δλ or Δν	Concn. mg/ml Length mm
1	Acrylonitrile	C_3H_3N	0–6	±0.3	4.48 4.90 (3) (comment 3)	0.060 0.0093μ	comment 2.02

Instrument: Perkin-Elmer Model 21, NaCl prism
Sample Phase: Dilution of resin solution with toluene

Cell Windows: NaCl
Absorbance Measurement: Base line__X__ Point_____

Calculation: Inverse matrix_____ Successive approx._____
Graphical__X__

Relative Absorbances[a]–Analytical Matrix:

Component/λ	4.48μ
1	5.99

Material Purity: Reference compound was an acrylonitrile-butadiene copolymer which was assayed by a standard Kjeldahl procedure.
Comments:
1 Quantitative toluene dilutions (by wt.) of the assayed acrylonitrile-butadiene copolymer were used to prepare standards.
2 Resin solutions that are too viscous are quantitatively diluted (by wt.) with toluene in such a way that the experimental absorbance is at 4.48 μ.
3 A line drawn parallel to the zero line and tangent to the absorption minimum at 4.90μ was used as a base line.
4 A rock salt plate was placed in the reference beam.

[a] Relative absorbances are given as the slope of the Beer's law concentration curves used expressed in terms of absorbance per 100% of constituent.

Determination of Naphthalene and Ethyl Chloride in Tetraethyllead

R. P. CURRY, Ethyl Corp., Baton Rouge, La. **CS-78**

Component No.	Component Name	Component Formula	Range %	Accuracy %	λ B.L. Pts.	Slit (mm) Δλ	Concn. % Length mm
1	Naphthalene	$C_{10}H_8$	0–1	0.03	12.80μ	0.381 0.069μ	100 0.103
2	Ethyl chloride	C_2H_5Cl	0–1	0.05	7.82μ	0.143 0.042μ	100 0.103

Instrument: Perkin-Elmer 21, NaCl prism
Sample Phase: Liquid

Cell Windows: NaCl
Absorbance Measurement: Base line_____ Point__X__

Calculation: Inverse matrix_____ Successive approx._____
Graphical __X__

Relative Absorbances[a]–Analytical Matrix

Component/λ	12.80μ	7.82μ
1	1.12	0.000
2	0.000	0.143

Material Purity: Reference compounds 99+% pure
Comments: A highly refined TEL sample was run for obtaining reference points; however, the following equations for base line absorbance were found to be satisfactory: A (naphthalene) = $A_{4.50}$ + 0.064; A (ethyl chloride) = $A_{4.50\mu}$ + 0.133. $A_{4.50\mu}$ is absorbance of sample compared to air in reference beam.

[a] Relative absorbances are given as the slope of the Beer's law concentration curves used expressed in terms of absorbance per 1% of constituent.

Determination of Acetate and Formate in Geranium Algerian and Geranium Bourbon

A. J. FENTON, The Procter and Gamble Co., Cincinnati, Ohio **CS-79**

Component No.	Component Name	Component Formula	Range %	Accuracy %	λ B.L. Points	Slit (mm) Δλ	Concn. g/l Length mm
1	Acetate (as geranyl acetate)	$C_{12}H_{20}O_2$	0–30	±0.3	8.6 7.57–8.92	0.319 0.038μ	200 0.05
2	Formate (as geranyl formate)	$C_{11}H_{18}O_2$	0–30	±0.3	8.1 7.57–8.92	0.300 0.040μ	200 0.05

Instrument: Perkin-Elmer Model 321, NaCl prism.
Sample Phase: Solution in toluene

Cell Windows: NaCl
Absorbance Measurement: Base line

Calculation: Graphical

Relative Absorbances–Analytical Matrix:

Component/λ	8.1μ	8.6μ
1	2.14	0
2	0	1.46

Material Purity: Reference compounds 99+% pure
Comments: Calibration curves made up using geranyl acetate and formate in toluene.

Determination of 1-Hexyne and 1-Hexene in Iso-octane Solution

R. P. CURRY, Ethyl Corp., Baton Rouge, La. **CS-80**

Component No.	Component Name	Component Formula	Range %	Accuracy %	λ or ν B.L. Pts.	(mm) Δλ or Δν	g/ml Length mm
1	1-Hexyne	C_6H_{10}	0–15	±0.3	3.05μ	0.046 0.028μ	2/10 0.114
2	1-Hexene	C_6H_{12}	0–20	±0.2 ±0.5	6.08μ 10.08μ	0.102 0.036μ 0.224 0.049μ	2/10 0.114

Instrument: Perkin-Elmer 21, NaCl prism
Sample Phase: Solution in iso-octane.

Cell Windows: NaCl
Absorbance Measurement: Base line_____ Point__X__

Calculation: Inverse matrix_____ Successive approx._____
Graphical__X__

Relative Absorbances[a]–Analytical Matrix:

Component/λ	3.05μ	6.08μ	10.08μ
1	3.04	0.000	0.000
2	0.000	2.69	3.82

Material Purity: Reference compounds 98+% pure
Comments: Other 1-alkynes and 1-alkenes will interfere. In the particular application involved, 1-hexyne was the only alkyne possible from the reaction. Two wave lengths are used for 1-hexene to check against other hexenes.

[a] Relative absorbances are given as the slope of the Beer's law concentration curves used expressed in terms of absorbance per 100% of constituent.

These data represent standard publication and submission is open to anyone in accordance with regulations of ANALYTICAL CHEMISTRY. The Coblentz Society is acting only as an aid to the journal.

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Device for Reading Double-Alignment Nomographs

J. G. Roof, Shell Development Co., Houston, Tex.

When a nomograph relates only three variables, a single setting of an index line will generally obtain a reading of the value sought. An opaque straightedge for alignment is unsatisfactory, as parallax is difficult to avoid, and extrapolation from the last plotted value to the edge of the rule is difficult, especially on nonlinear scales. A narrow, well defined line on the lower surface of a transparent rule or parallel to the long edge of a transparent drafting triangle facilitates reading of a nomograph (*1*).

An equation relating four variables is often presented as a double-alignment nomograph with a pivot axis. The advantages of a transparent rule can be incorporated into a simple device for reading such nomographs.

The guide (Figure 1) is essentially a transparent plastic rule having a line scribed longitudinally on the lower side, with a cross-hair pivot arrangement that permits shifting the pivot point within a range of about 2 inches along an extension of the index line, and rotating the rule about the pivot point. The device is assembled from four parts: body, spool core with lower flange, upper flange, and rubber O-ring. The spool fits within a longitudinal slot near one end of the body. The body is recessed on the lower side around the slot to sufficient depth to accept the lower flange of the spool and allow it to slide along the slot without objectionable drag by the O-ring. This O-ring is recessed in a circular groove in the lower flange of the spool, protruding only far enough to furnish a firm fix when the spool is pressed down against the nomograph. On its lower face the spool is scribed with two lines along diameters at right angles.

The top flange of the spool is a separate disk of plastic, attached to the core by pressing the two pieces together after solvent has been added to soften the two pieces. If care is taken, no air bubbles are formed at the interface. The core of the spool should be made slightly longer than might seem necessary, to extend above the body of the guide when the solvent is being added; otherwise solvent may touch the body and roughen the wall of the slot. The slot may be enlarged at one end, keyhole fashion, to permit placing the previously assembled spool in the slot; a retaining plug can then be placed in the enlarged end.

APPLICATION

In connection with one set of nomographs used in the determination of cyclic carbon in petroleum distillates (*3*) the guide is first placed on the nomograph so the index line passes through the determined values of the refractive index, n_D, and the density, d, of the test material, and the pivot axis, 100 y, crosses the slot of the guide (Figure 2). Then the body of the guide is held firmly against the nomograph while the intersection of the spool cross hairs is brought to the pivot axis by sliding the spool along its slot. The spool is pressed down firmly to fix its position on the pivot axis, and the body is rotated until the index line passes through the determined value of the molecular weight, M, of the test material. The cyclic carbon content is estimated directly from the intersection of the index line with the per cent C_R scale corresponding to positive or negative values of y.

In this application it may be necessary to rotate the body of the guide as much as 180° between the two settings. For maximum convenience, double-alignment nomographs can be arranged to minimize rotation, by "folding" so the pivot axis lies to one side of all other scales on the chart. Such rearrangement of Figure 2 leads to that shown in Figure 3. The nomograph is more compact, the necessity of swinging the body of the guide through the vertical is eliminated, and rotation through more than 90° is rare. When a chart is folded, it is desirable to indicate the sets of axes to be used simultaneously. Even when a two-color press is available, all axes and graduations should be printed in one color, to avoid errors in color register, but one pair of axes can bear titles and numbers in a second color. When printing in two colors is impractical, a broad line may be drawn parallel to each of the second pair of axes to set them off prominently (Figure 3). Chance of error may be serious when the numerical values on the chart are more nearly the same on two or more axes.

If it is inexpedient to fold a nomograph to give an external pivot axis, with proportional charts of the single-N or double-N type (*2*), a reading guide of adequate length with a pivot slot near the center of the body should be considered.

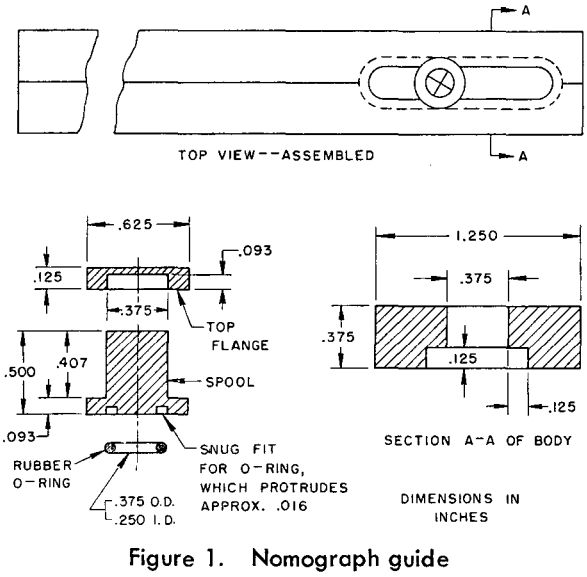


Figure 1. Nomograph guide

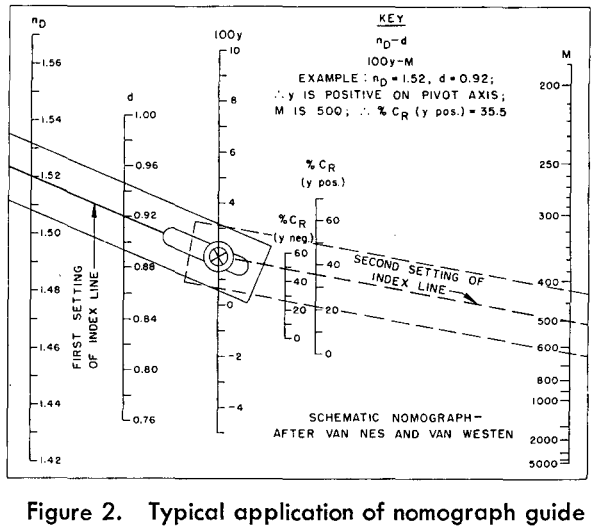


Figure 2. Typical application of nomograph guide

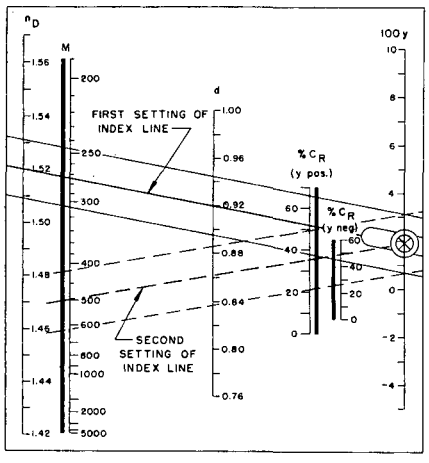


Figure 3. Example of folded nomograph

The device appears to have certain operating advantages over the nomograph compasses described by Van Nes and Van Westen (*3*). Essentially, their guide consists of two opaque straightedges connected by an open-center hinge fitted with a transparent window, which has cross hairs that mark the intersection of extensions of the two index lines, regardless of the angle subtended. Simultaneous setting of the first arm upon three values is required—the straightedge passes through the proper values, in the present example, of n_D and d, while the intersection of the cross hairs must fall upon the y axis. Once this first arm is properly placed, it is held in position while the second arm is rotated until its edge crosses the proper value of M; the corresponding value of per cent C_R is then read.

After the nomograph compass has been placed in its final position, a check reading on all five axes is possible. The present device is transparent, and the initial setting is made on only two points rather than three simultaneously. If one is willing to forego the latter convenience, the spool can be fitted into a closely fitting circular hole rather than the slot, simplifying the machine work required for construction.

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PUBLICATION 159, Exploration and Production Research Division, Shell Development Co., Houston, Tex.

Machine for Preparing Phosphors for the Fluorometric Determination of Uranium

R. E. Stevens, W. H. Wood, K. G. Goetz, and C. A. Horr, U. S. Geological Survey, Denver, Colo.

SMALL QUANTITIES of uranium are usually determined by measuring the fluorescence of a sodium or lithium fluoride–uranium phosphor. A serious handicap has been the difficulty of preparing the phosphors. When the flux is melted by manually holding a container of platinum or gold over an open flame, conditions of heating and cooling cannot be readily duplicated and fluorescence may vary widely.

Sadowski and Gentry (*5*) describe a fusion rack which prepares one phosphor at a time over a flame. Racks for preparing a large number of phosphors at one time have been used (*1*, *4*, *7*, *8*). The equipment consists of a single large burner over which 20 small dishes ($^7/_{16}$-inch internal diameter) containing uranium and flux can be heated at once. There is no adequate provision for stirring the melt. Variations in heat over different parts of the burner and in cooling rate from outside to inside positions are compensated by random arrangement of the unknowns, standards, and blanks. Although these racks are suitable for use with small dishes, they are not practical for supporting a large number of the larger dishes used by many laboratories.

Thatcher has designed a support for preparing three phosphors at a time in a muffle furnace (*6*). The melts are swirled by an externally mounted motor, which swings the support for the dishes in the furnace. Preparation of the phosphors in a muffle furnace is not completely satisfactory, however, because of the greater solubility of the platinum in the flux in the oxidizing atmosphere of the furnace, with consequent variability of results, as recognized by most users of the fluorometric method for uranium. Recently Michelson (*3*) described a device for drawing the dish through an electric tube furnace or burner at a repeatable rate.

The machine described here was designed for use in the procedures described by Grimaldi, May, and Fletcher (*2*), in which 2 grams of flux (45.5 parts by weight of sodium carbonate, 45.5 parts by weight of potassium carbonate, and 9 parts by weight of sodium fluoride) are used for making the phosphor, with platinum dishes of 38-mm. inside diameter. The 4-mm. flat lip around each dish holds the dish in a horizontal position in the mountings provided in the machine.

Phosphors prepared at one time in the machine show good reproducibility and separate runs show good agreement. Where precise values are wanted, each run can be standardized by including blanks and phosphors of known uranium content. The machine eliminates the tedious hand preparation of phosphors

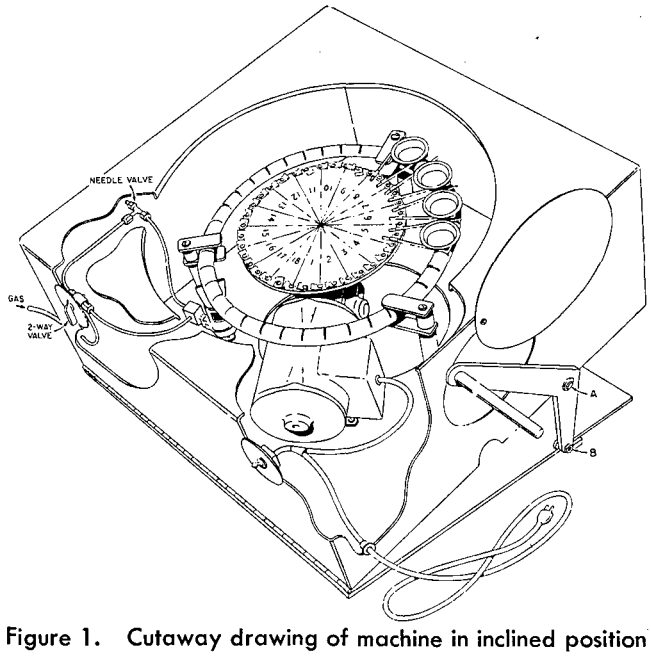


Figure 1. Cutaway drawing of machine in inclined position

Showing motor, mounting of dishes, inclining lever, etc., but only parts of dishes and mounting rods

and makes possible a significant increase in the number of samples run per unit of time by the method. Operation requires little skill. Preparation of phosphors with the machine is more than three times as rapid as preparing them one at a time by hand. Results are generally free from gross errors arising in the fusion process.

DESCRIPTION OF MACHINE

The essential feature of the machine is a metal plate which revolves above the ring burner at a fixed height and speed, with fused-silica rods mounted at the edge of the plate to support the platinum dishes.

The upper and lower base plates are $20^1/_2 \times 20^1/_2 \times {}^1/_4$ inch aluminum, hinged together as shown in Figure 1. Ten-inch ventilating holes are cut in the upper and lower base plates, with an uncut segment in the upper base plate for mounting the motor. A lever, pivoted at *A*, running to a stop, *B*, inclines the machine during the fusion period.

The dish-mounting plate is machined from an aluminum casting to $8^3/_4$-inch diameter, with a $1^1/_4 \times 2^3/_4$ inch hub for attachment to an electric motor. A $^1/_{20}$-hp., 110-volt alternating current motor rotates the dish-mounting plate, by means of a gear box, at 10.8 r.p.m. Cable clamps, $^1/_8 \times {}^3/_{16}$ inch, are attached to the upper surface of the plate for clamping the fused-silica mounting rods for the dishes.

The ring burner is formed from standard $^1/_2$-inch water pipe in a circle 11 inches in diameter, and the ends are welded. Slots, $^1/_{25}$ inch wide, $^1/_4$ inch deep, and spaced 1 inch apart, are cut on the upper surface of the ring. The valve assembly from a Fisher burner is welded to an opening on the bottom of the ring burner. The burner is held $^7/_8$ inch below the dish-mounting plate and centered by adjustable amounts. Two gas inlets are provided (Figure 1), one with unrestricted flow for initial melting of the flux and the other with an adjustable needle valve for heating the flux at or near its melting point. The gas is diverted to the latter inlet by means of the valve handle at the front of the machine.

The machine is encased in a metal box extending 2 inches above the ring burner, with a $16^1/_2$-inch diameter opening in the top. A cover prevents contamination when not in use.

If the gas pressure is too low, an extra gas inlet is added.

The manner of fastening the 3-mm. fused-silica mounting rods to the dish-mounting plate is shown in Figure 1.

The long arm of one L-shaped rod (3 inches long with a right-angle 1-inch arm) is placed under a cable clamp, the rod parallel with the radius of the dish-mounting plate and extending out so that the center of a dish, when mounted, is centered over the ring burner. It is securely fastened with the short arm extending to the right in the same plane as the surface of the plate. A straight 3-inch rod is then clamped to the right of the L-shaped rod, so that the bowl of the platinum dish fits the opening between the rods with a slight amount of play. The lips of the dish rest on the fused-silica rods. The remaining pairs of rods are similarly clamped in position, with equal spacing around the circumference of the dish-mounting plate, and all dishes equidistant from it. (For each dish to fit all positions precisely, the dishes should be shaped frequently to exact size with a wooden shaping mold.)

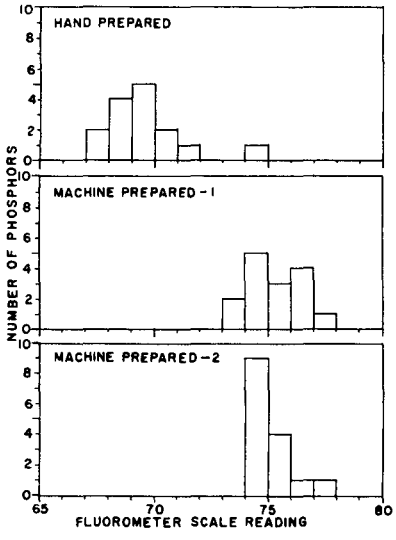


Figure 2. Reproducibility with hand- and machine-prepared phosphors

ADJUSTMENT OF FLAME

The high and low flames of the burner are first adjusted to the proper height; no further adjustment is normally needed.

With the machine in the horizontal position, 18 dishes, each containing 2 grams of flux (discarded phosphors can be used) are mounted in the machine. The motor is turned on. The flame indicator at the front of the box is turned to the high position, the aperture on the right of the box opened, the ring burner lighted, and the aperture closed. After the flux has sintered, the machine is put in the inclined position. The Fisher burner valve under the ring burner is adjusted so that the flux in the dishes melts completely in 1 to 2 minutes. The flame indicator is then turned to the low position, and the low adjustment screw, at the left of the box, is adjusted until there is a continuous small cloud of crystals in the molten flux.

OPERATING PROCEDURE

Place the 18 platinum dishes, each containing 2 grams of flux and uranium to be measured, over the ring burner with the machine in the horizontal position. Turn the motor on to rotate the dishes around the ring burner. Open the door at the right side of the box, turn the flame indicator to the high position, light the burner, and close the side door. Note the time. Continue heating in the horizontal position until the flux is well sintered, then place the machine in the inclined position by pulling the positioning lever at the right of the box until the stop is reached. Continue heating at full heat until most of the flux in the dishes has melted. Turn the flame indicator to the low position and continue heating until 5 minutes have elapsed from the time the flame was lighted. (Operating at the full heat of the ring burner during the entire heating period gave somewhat more erratic results.) Turn the flame adjustment to high. Immediately return the machine to the horizontal position with the positioning lever, and turn off the flame. Continue rotation of the dishes over the ring burner during a 10-minute cooling period. Remove the dishes and read the fluorescence of the phosphors with a fluorometer.

ADAPTATION TO DISHES OF DIFFERENT DEPTH

With the dish described by Grimaldi, May, and Fletcher (*2*) and with 2 grams of flux, the molten flux reaches nearly to the edge of the dish and covers the center when the machine is in the inclined position. This allows the molten flux to reach all surfaces in the dish, as the dishes revolve with the machine inclined. This is not so with deeper dishes, and design of the machine has to be changed for complete solution of the uranium in the dish into the molten flux. A second lever gives a steeper angle of inclination to the machine, so that the molten flux can reach nearly to the edge of the deeper dishes. A suggested routine with a machine modified for deeper dishes, following preliminary melting of the flux, would be to heat for 1 minute in the lower inclined position with the molten flux reaching the center of the dish, heat for 1 minute in the higher inclined position with the flux reaching nearly to the edge of the dish, heat another minute in the lower inclined position, raise the flame to full heat, put the machine in the horizontal position, turn off the gas, and cool.

COMPARISON OF PRECISION OF HAND-FUSED AND MACHINE-FUSED PHOSPHORS

Comparisons of hand fusion and machine fusion, made by a number of laboratory workers, showed a definite improvement in repeatability when a machine was used.

Figure 2 compares results after hand preparation and machine preparation of phosphors. These represent runs made in sequence, each of 18 phosphors (three blanks in each run are not shown), to illustrate repeatability of each method of preparation of phosphors containing 0.05 γ of uranium. The

machine-prepared phosphors showed better precision than those prepared by hand, and the two runs with the machine are essentially in agreement.

ACKNOWLEDGMENT

The authors thank Charles G. Bay and Leonard B. Riley for suggestions in the building of the phosphor-making machine, and A. G. King, Wayne Mountjoy, Irving Frost, and John C. Antweiler for confirmatory experiments establishing its usefulness.

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Protein Determination for Large Numbers of Samples

Gail Lorenz Miller, The Pioneering Research Division, Quartermaster Research and Engineering Center, Natick, Mass.

LOWRY, Rosebrough, Farr, and Randall's procedure for colorimetric determination of protein [*J. Biol. Chem.* **193**, 265 (1951)] is particularly convenient when only a few samples are determined at one time.

Two modifications in procedure make it possible to determine large numbers of samples. Use of a comparatively smaller volume of more concentrated alkaline copper reagent and a larger volume of more dilute Folin phenol reagent permits introduction of a proportionately larger volume of phenol reagent with sufficient force to ensure adequate preliminary mixing, and final mixing can be postponed until after the reagent has been added to all the samples. Heating the final mixtures of samples and reagents at 50° C. accelerates development, reducing the time to minutes.

One-milliliter aliquots of alkaline copper reagent composed of 10 parts of 10% sodium carbonate in 0.5*N* sodium hydroxide and 1 part of 0.5% copper sulfate in 1% potassium tartrate are added to 1-ml. aliquots of protein solution in colorimeter tubes 14 mm. in outside diameter. After the mixtures have stood for 10 minutes, 3-ml. aliquots of a 1 to 11 dilution of Folin phenol reagent are added to the samples as forcibly as practicable. The mixtures of samples and reagents are heated for 10 minutes at 50° C. in a constant temperature water bath. After the mixtures are cooled to room temperature, absorbance is read at wave lengths of 540 to 750 mμ, depending on the sensitivity required.

For addition of the alkaline copper reagent and the Folin phenol reagent, hand-operated plunger-type pipets or motor-driven automatic pipets are very useful. With automatic pipets, glass instead of stainless steel valves are required when the phenol reagent is used, because of the problem of corrosion.

Entirely analogous results were obtained with bovine serum albumin and with gelatin, although, as expected, the color with gelatin was about half that with bovine serum albumin. The reproducibility of analyses over the range of 0.04 to 0.20 mg. of protein in the modified test is, on the average, to 2%, essentially the same as in the original test.

Automatic Multistage Semimicro Zone Melting Apparatus

A. P. Ronald, Chemistry Section, Fisheries Research Board of Canada, Technological Station, Vancouver, B. C.

THE technique of zone melting, the mathematics (*1*, *9*) and principles (*2*) of which have been adequately described, is not entirely new in metallurgical purification (*8*, *9*, *13*), but is relatively new in organic chemistry. Röck (*11*) has purified benzene; Hesse and Schildknecht (*6*), fatty alcohols; and Handley and Herington (*3*), benzoic acid, pyrene, anthracene, and crysene. The limited success of chromatographic techniques in the sterol field suggested that a suitable apparatus might make zone melting applicable to problems in this laboratory.

This method for purifying organic compounds requires that a narrow molten band travel slowly down a vertical column of the solid substance in a glass tube; impurities are separated and normally concentrated at the bottom of the column. A horizontal tube may be used in metallurgy (*5*), but in organic separations when the molten zone passes and the material crystallizes the solid drains away from the side of the tube and seeps back.

No available apparatus (*3–7*, *11*, *12*) was entirely suitable for the purpose. An automatic macromodel (*5*) was investigated, but the disengagement of the drive motor by the system of pawl and ratchet was not clearly understood, and it did not appear suitable for adaptation to a micro scale. A zone melting apparatus could have been modified by changing the heater carriage to a sample carriage and building a bank of heaters through which the sample tubes could pass, but the apparatus described here was completed before the advertisement appeared (*10*). An apparatus (*7*) apparently similar to the commercial one has been described, but the mechanical operations are different. The apparatus described here is cheaper, smaller, and self-contained.

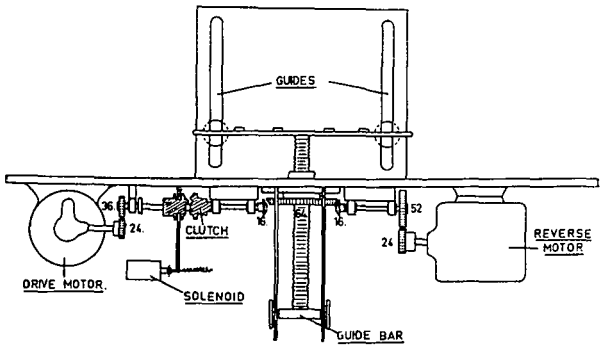
PRINCIPLE OF OPERATION

The apparatus (Figure 1) consists of a drive motor which raises a platform that supports four tubes containing the material to be purified. As the platform rises, the tubes pass through a heater which creates a well defined molten zone of material. The molten zone passes slowly down the column to the bottom. The platform reverses, travels quickly to its starting position, and again commences its slow ascent.

The slow drive speed for raising the platform is attained by a synchronous motor with a drive shaft speed of 120 revolutions per hour. This is reduced by external gears to 20 revolutions per hour. The last reduction is by a bevel gear with an internally threaded hub

Figure 1. General layout of mechanical components

Drive motor, Bodine Type NSY-12R
Reverse motor, Bodine Type CL-2
Clutch solenoid, GE magnetic switch solenoid 16252
Bevel gears, two-16-tooth, one-64-tooth
Spur gears, two-24-tooth, one-36-tooth, one-52-tooth



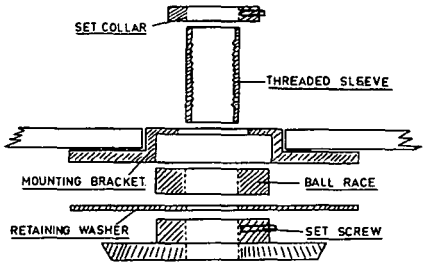


Figure 2. Exploded view of bevel gear assembly

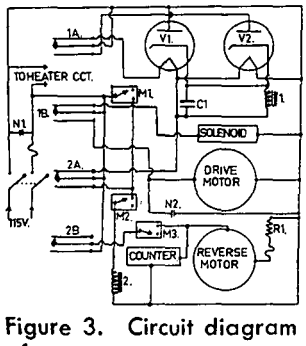


Figure 3. Circuit diagram of apparatus

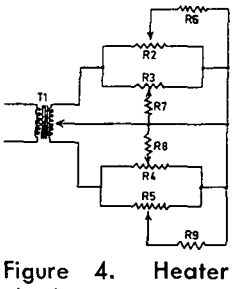


Figure 4. Heater circuit

(Figure 2), through which passes a $^3/_8$-inch-diameter rod with 16 threads to the inch, fixed by a guide bar attached at right angles to the rod and sliding in a slot so that it cannot rotate. The bevel gear on completing 16 revolutions raises the platform 1 inch. The gear turns 20 times in 1 hour; thus the platform travels 1.25 inches per hour.

AUTOMATIC RECYCLE. On reaching a predetermined height the guide bar attached to the bottom of the threaded rod lifts a lever which operates a microswitch. The power is cut off from the drive motor, which is then disengaged from the system through the action of a solenoid-operated dog clutch. The reverse motor cuts in, returning the platform, in 6 seconds, to its original position, where on striking another microswitch it is stopped. After about 15 seconds the clutch engages and the drive motor starts. The delay before commencement of the recycle is effected by incorporation of a delay mechanism in the circuit, installed to avoid damage to the gear system.

OPERATION OF APPARATUS

When the electronic delay circuit (Figure 3) is switched on, 15 seconds elapse before the relay coil, 1, is energized, cutting off, through the action of relay contact 1*A*, the filament voltage to tube *V*1 and making the plate voltage to tube *V*2. The relay is held on until the circuit to the coil is broken, at which point even immediate application of current to the circuit would not energize the coil until 15 seconds had elapsed —the time required for the filament of tube *V*1 to heat up and allow sufficient current to flow to energize the coil.

Relay contact 1*B* controls the action of the clutch solenoid and the drive motor. When coil 1 is not energized, the clutch solenoid is in operation. This disengages the drive motor from the system and closes microswitch *M*3, which is so positioned that the circuit to the reverse motor is not complete unless the clutch is fully disengaged. Even with the clutch disengaged the reverse motor will not act until its circuit has been completed. This can occur only in the following manner. Microswitch *M*1 is closed either by action of the bar attached to the threaded rod or by manual manipulation. Coil 2 is then energized and relay contacts 2*A* and 2*B* close and break the circuit to the delay unit through relay contact 2*A*. When the circuit to the delay unit is broken, 2*A* makes a circuit to its own coil; this is necessary, as microswitch *M*1 is opened as soon as the reverse motor comes into operation. Relay contact 2*B* makes a circuit to microswitch *M*3. When the filament of tube *V*2 has cooled sufficiently to cut off the power supply to coil 1, relay contact 1 opens, the drive motor stops, and the clutch disengages and completes the circuit through *M*3 to the reverse motor. The rod then travels down until the bar strikes microswitch *M*2, opening the self-holding circuit to coil 2. 2*A* and 2*B* open, the reverse motor stops, the circuit to the delay unit is made, and after 15 seconds coil 1 is again energized. The clutch engages, the drive motor starts, and a new cycle commences.

Table I. Values of Component Parts of Electrical Circuit

	Resistors
*R*1	100-ohm 50-watt
*R*2, *R*3, *R*4, *R*5	2-ohm 50-watt rheostats
*R*6, *R*7, *R*8, *R*9	Loop of No. 26 bare nickel-chromium wire
	Condensers
*C*1	50-mfd. electrolytic
*V*1, *V*2	117Z6GT, tubes converted to ordinary triode, doubling current-carrying capacity
	Relays
1	P & B Relay, KA 11D, 110-volt, d.c., D.P.D.T.
2	Guardian Relay, Series 200, 115-volt, a.c., D.P.D.T.
	Switches
*M*1, *M*2, *M*3	Unimax. Type 2 HBW-1, microswitch
On-off	10-ampere D.P.S.T. toggle switch
	Transformers
*T*1	Hammond, Type 1129R-60CY, 6-volt, 10-ampere center tapped
	Fuses
	Two 2-ampere, Type 3-AG
	Indicator Lights
*N*1, *N*2	Neon bulb, Type GE-NE 51, resistor-type mountings

CONSTRUCTION OF APPARATUS

The mechanical components were assembled on an aluminum plate 10 × 24 × $^3/_8$ inches. The bevel gear assembly was first manufactured and assembled and then mounted in the center of the plate. The reverse motor gear shaft was assembled, and the 16-tooth bevel gear was fastened to one end of a 2-inch length of $^1/_4$-inch stainless steel shaft, by means of a pin driven through the hub of the gear and through the shaft. The 52-tooth spur gear was fastened to the other end by drilling a $^1/_4$-inch hole in the gear and fitting it to the shaft, and a small hole was drilled parallel to the shaft so that it cut a keyway from the shaft and the gear. The hole was then tapped and a screw inserted which held the shaft and the gear firmly together. The gear assembly was mounted on $^1/_4$-inch brass pillow blocks, fastened to an aluminum mounting block of such thickness that the bevel gears meshed correctly. The slow-speed drive motor shaft was mounted by a similar procedure. A hub was brazed to each spur gear which was to be mounted on a motor shaft, a hole drilled and tapped on each hub, and a setscrew inserted. The gear was held in position on the drive shaft of the motor by the setscrew.

This allows easy mounting, simple adjustment, quick removal, and disengagement of any motor while tests are being carried out on the correct positioning of the electrical components of the system. If the gear system is not mounted properly and any tightness occurs, difficulty will be experienced in disengaging the clutch; careful alignment is necessary and the clutch teeth should be angled as much as possible. Microswitches *M*1 and *M*2 can be mounted in many ways. One convenient mount is on the side of a right-angled bracket, mounted on the outside of the guide bar support. A slot is cut in the side of the bracket to allow easy positioning of the microswitches. The mechanism of the microswitch is actuated through coming in contact with that part of the guide bar which protrudes through the guide bar support.

Each heater unit is constructed from a single loop of No. 26 bare nickel-chromium wire attached to copper rods approximately 5 inches long and 1/8 inch in diameter, and held in place by a Bakelite strip which provides both insulation and a means of adjusting the height of the heater. Each heater is controlled by a rheostat, wired in the manner of a voltage divider (Figure 4). The type of circuit used gave the best individual control for each heater. With all the heaters working at maximum temperature the load is about 13 amperes. However, the 6-volt center-tapped 10-ampere Hammond transformer worked satisfactorily and there was no indication of heating due to overloading.

To determine the effectiveness of the apparatus a trial run was carried out with mixtures of 1 and 0.1% 2,4-dinitrophenylhydrazine in naphthalene. Approximately 500 mg. of the mixture was heated and drawn into a preheated tube 25 cm. long (outside diameter 4 mm., inside diameter 2 mm.), to a height of about 15 cm. Five passes completely removed the 2,4-dinitrophenylhydrazine from approximately 90% of the 0.1% mixture and concentrated it in a 1-cm. zone above the point which the heater reached at the end of each run. Fifteen passes were required to remove the impurity completely from approximately 80% of the 1% mixture, concentration taking place in the bottom 3 cm. of the tube.

ACKNOWLEDGMENT

The author thanks D. R. Idler for suggesting this project, for advice during development of the equipment, and for assistance in preparing the manuscript, and F. C. Freeman for assistance in solving technical problems and helping to construct the prototype.

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A Compact Unit for Concentration of Multiple Solutions at Reduced Temperature

A. S. Meyer, J. C. Matthews, and P. Samarco, Schering Corp., Bloomfield, N. J.

THE unit described (Figure 1), permitting concentration of multiple solutions at reduced temperature, has been used in this laboratory for several years.

The tubes containing the solutes are attached to a holder, *K*, by means of a stainless steel spring which snugly surrounds the slot of the holder (Figure 2). This holder was designed for accommodating 12 tubes with a maximal diameter of 1 inch. (Smaller tubes are retained equally well by the spring, even if they are located adjacent to tubes of larger diameter.) If Erlenmeyer flasks are used frequently, it is better to space the openings farther apart; in the construction described, only alternate openings could be utilized with these flasks.

Air or nitrogen is introduced from the source, *G*, through a distributing manifold (*D*, Figure 3), 16 inches of lightweight rubber tubing 1/8 inch in internal diameter, *B*, and an 8-inch long rigid extension tube 7/32 inch in outside diameter, *I*, joined to a 3 1/2-inch long 18 gage stainless steel needle with a Yale adapter and square-cut needle end, *J*. The gas flow is directed to the individual needle by a stainless steel stopcock No. MSO 1, *C* (items *I*, *J*, and *C* are from Becton, Dickinson and Co.). The end of the needle is placed approximately 1/2 inch above the surface of the liquid to be evaporated. The extension, *I*, held in

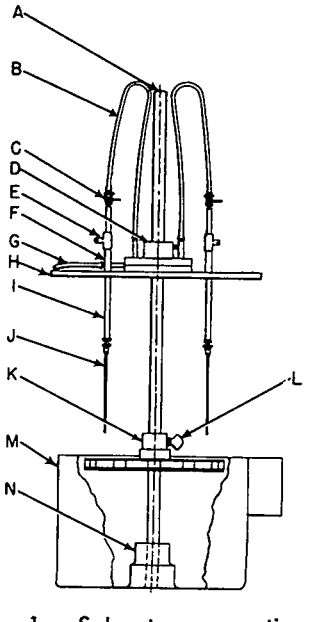


Figure 1. Solvent evaporation unit
Side view drawn to scale

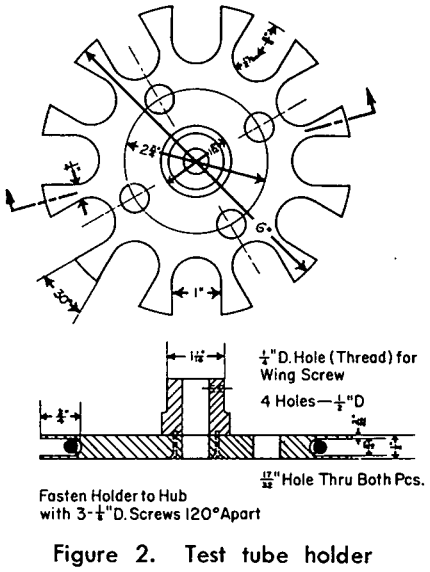


Figure 2. Test tube holder
Cross sections top and side, drawn to scale

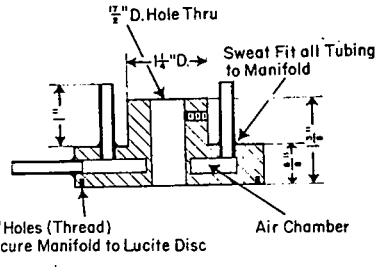


Figure 3. Manifold
Cross section, drawn to scale

place by a clip, E, passes through a perpendicularly adjusted stainless steel sleeve $1/4$ inch in internal diameter, F, which is push fitted into a Lucite disk of $1/4$ inch thickness, H. The latter serves at the same time as a cover. Lucite cover (fastened to the manifold, D) and tube holder K are, at a working distance of approximately $5\,1/2$ inches, both attached by a wing screw, L, to a $1/2 \times 22$ inch rod, A. This shaft rests in a 2-inch high copper block, N, with a cylindrical hole $1/32$ inch larger than the rod diameter and freely rotating. The shaft seat, N, is silver soldered onto the pan, M, in which the water is heated by a circular immersion heater and thermostatted at the desired temperature. If Erlenmeyer flasks are used, a wider pan is required or alternatively a bulge has to be welded to the front of the pan.

The advantages of this unit are obvious. For speedy operation a simple attachment of the tubes is required, here brought about by the use of a spring. A facile way of adjusting the needle end above the solvent surface has been achieved. The rotary arrangement is convenient and space saving. The general principles of this construction can easily be adapted for similar purposes. (A similar unit is now available from Organomation Associates, Turnpike Sta., Shrewsbury, Mass.).

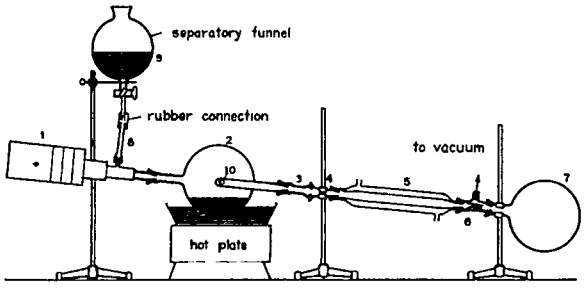
Continuous-Feed Rotating Evaporator

Gunter Zweig, Pesticide Residue Research Laboratory, University of California, Davis, Calif.

IN AN all-glass, continuous-feed rotating evaporator described by Kohn [ANAL. CHEM. **28**, 1061 (1956)] the evaporating flask was driven by a belt, made from a rubber band, connected to a pulley on a laboratory stirring motor. Because of difficulty due to slippage of the belt, another evaporator was assembled, which utilizes commonly available equipment. This evaporator has been in use in this laboratory for several months, concentrating large amounts of acetone and alcohol solutions of plant extracts.

The continuous-feed, rotating evaporator consists of a Rinco evaporator, 1; Rinco special condenser flask with ₹24/40 neck, 2; adapter with ₹24/40 inner joint and SB28/15 outer joint, 3; adapter with SB28/15 inner joint and ₹24/40 outer joint, 4; condenser with ₹24/40 joints, 5; vacuum adapter with ₹24/40 joints, 6; round-bottomed flask with 24/40 joint (500-ml. size), 7; ₹12/30 inner joints, 8; and 3-liter separatory funnel, 9.

Adapter 4 is clamped tightly to a ring stand, so that condenser flask 2 and adapter 3 are turning while the evaporator is in operation. The solution is fed from the separatory funnel at a constant rate, which is regulated so that the level of the liquid is constant in the condenser flask, which is actually serving as the evaporating flask. The level of liquid is kept 2 inches below the opening, 10, to prevent splashing. The receiving flask, 7, is kept at room temperature, so that vapors do not collect and operation may be continuous. All glass connections are well greased with silicone stopcock grease. By using a water aspirator at about 25 to 30 mm. of mercury pressure, 2 liters of an acetone extract from potatoes or green leaves may be concentrated to dryness in less than 1 hour. The concentrate is then transferred from the condenser flask with several washings of acetone.



Solutions containing mineral acids should not be concentrated, because the metal shaft of the Rinco evaporator will corrode slightly. Substitution of a stainless steel shaft overcomes this disadvantage, as the solution that passes through the metal shaft is at ambient temperature. The relatively high cost (about $100) may discourage routine use of this evaporator in some laboratories.

Base-Line and Maximum Value Methods in Infrared Quantitative Analysis

H. L. Cupples, Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md.

IN A STUDY of the susceptibility of infrared base-line methods to errors caused by overlapping bands of unknown constituents not present in the calibrating solutions, base-line determinations were made of γ-benzene hexachloride (BHC) in admixture with its *isomers*, which were considered for this purpose to be unknown impurities.

Spectra were taken in carbon disulfide solution on a single-beam recording spectrophotometer, and the relevant points converted to per cent transmittance. All base lines were drawn from the curve intersection with the ordinate at the selected wave length, parallel to the zero energy line. At 15 selected wave lengths in the 7.5- to 15.0-micron range, 27 base lines were established (two each at 12 of the wave lengths), and, using purified γ-BHC, graphical calibrations were determined for each base line. All these calibrations, therefore, were valid for a γ-BHC–carbon disulfide system; the base lines were drawn in a uniform manner, none being specially chosen for use in the presence of the isomers or any other specific impurities. Then γ-BHC was determined in a carbon disulfide solution containing 15.0 grams per liter of γ-BHC in admixture with 15.0 grams per liter of its isomers, the isomers being present in proportions approximately that of technical BHC.

Figure 1 shows determined amounts as percentages of the true amount. Of the 27 determinations only nine are within $\pm 10\%$ of the true amount, and many are seriously in error. It is evident that an indiscriminate application of base-line methods to mixtures of unknown qualitative composition may result in large errors. If, however, the spectra of the sample and known components are compared, and the analytical wave lengths and base lines chosen with reference to them, better results will be ob-

tained. The arrows in Figure 1 indicate wave lengths which might be so selected.

Maximum Value. At the same wave lengths, determinations were made of the absorbance of the solute, log I_0/I, in which I_0 is the intensity of the beam when the cell is filled with solvent and I is the corresponding intensity with the cell filled with sample or calibrating solution. At each wave length, γ-BHC was estimated by referring the absorbance of the sample to a corresponding γ-BHC calibration curve, with no correction for overlapping bands. The lowest of these estimations is the "maximum value," in this instance 101.2% of actual. This maximum value is not merely an uncorrected value, or approximation. It is also a value which, within experimental error of the absorbances, will equal or exceed the actual value, except for the very unusual situation (in the 7.5- to 15.0-micron range) where the analytical mode is weakened by molecular complexing of substances not present in the calibrating solutions.

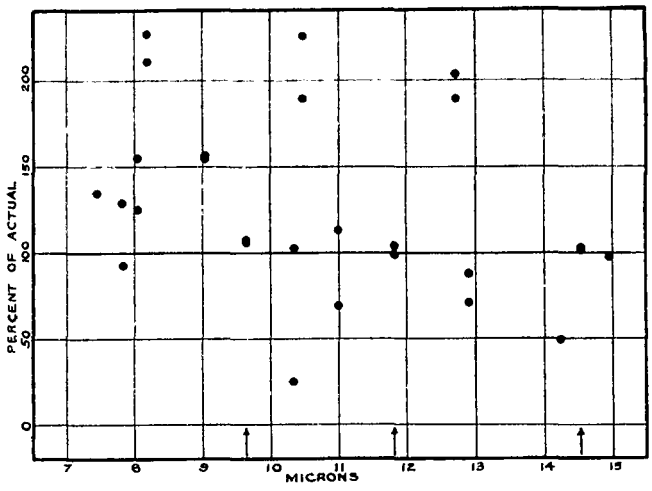


Figure 1. Determination of γ-BHC in BHC isomers by baseline–density methods

The maximum value technique has been applied with good results to the determination of dieldrin and aldrin (insecticides) in emulsifiable concentrates. It may be of substantial value in regulatory and control work which requires only evidence that the amount present is below a stated value.

Improved Method of Heating Chromatographic Columns

L. R. Bunney, Randall Phillips, and E. C. Freiling, U. S. Naval Radiological Defense Laboratory, San Francisco 24, Calif.

In investigations involving ion exchange column chromatography it was desired to operate columns above room temperature to increase the rate at which ion exchange equilibrium is approached. The range of 80° to 90° C. includes most usual operating temperatures, but some experience has been obtained at higher temperatures.

Three methods of heating have been used by numerous investigators: refluxing vapors of a suitable liquid, such as trichloroethylene (boiling point 87° C.), circulating water from a constant temperature bath, and flexible heating tapes wrapped around a liquid-filled jacket. These methods all have drawbacks.

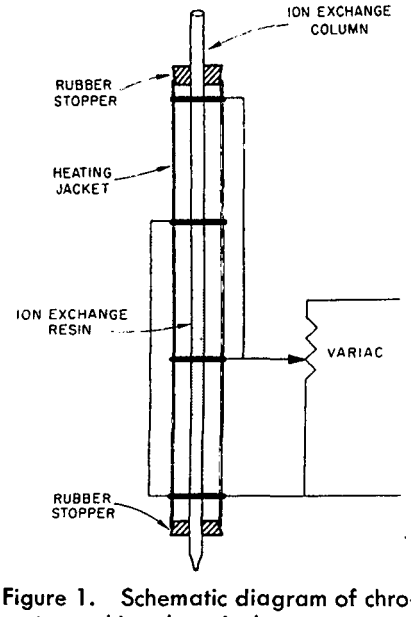


Figure 1. Schematic diagram of chromatographic column jacket

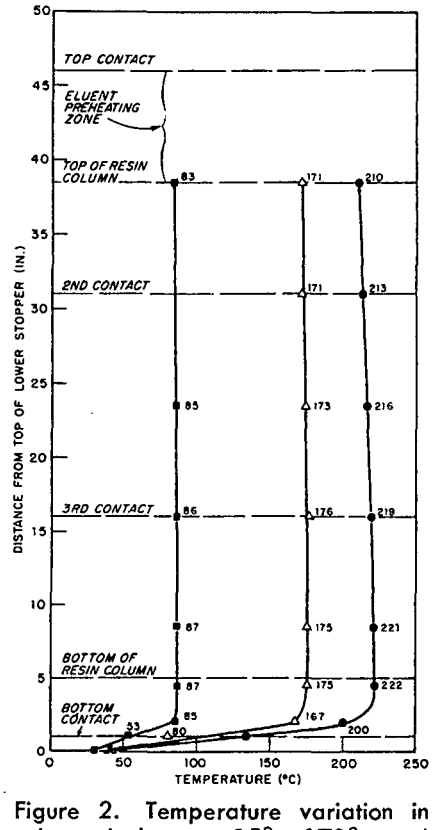
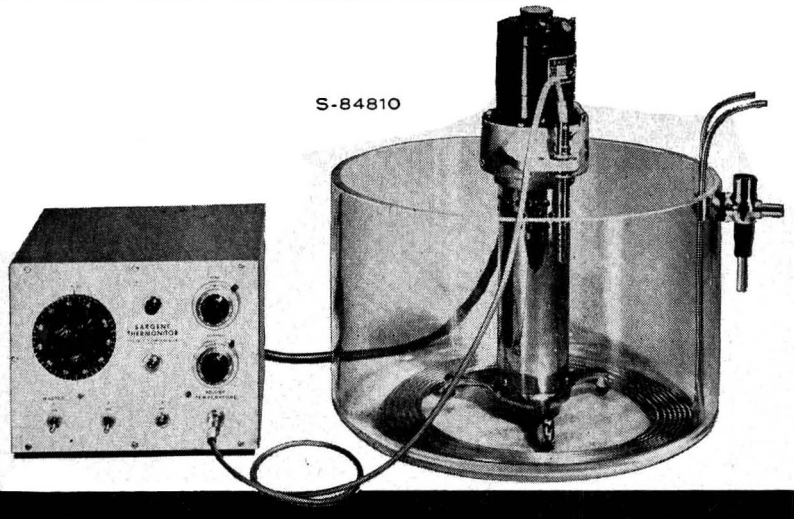


Figure 2. Temperature variation in column jacket at 85°, 173°, and 216° C.

A method that has been in use in this laboratory for over a year utilizes a column heating jacket of electrically conducting Pyrex tubing (Corning Glass Co., Corning, N. Y., Catalog No. 9340). The electrically conductive film, which acts as a resistance element, is on the outside surface of the tubing. This film is chemically inert, very hard, and transparent. Using this technique reduces the complexity of the equipment necessary for heated column chromatography and increases the reliability of the experimental setup. The shock hazard from the equipment is small, if care is taken with the polarity of the Variacs.

A 48-inch length of tubing 30 mm. in outside diameter, divided into three sections wired in parallel, is used as a heating jacket for an ion exchange column, 13 mm. in outside diameter, as shown in Figure 1. Water is used as a heat transfer agent between the two columns for temperatures below 90° C. For operating temperatures in the range of 90° to 220° C., Dow Corning 550 fluid has given the most satisfactory performance. The temperature is controlled by a Variac. The maximum service temperature of the tubing is 350° C.

The temperature variation along the length of the column under steady-state conditions is shown in Figure 2 for operating temperatures of 85° ± 2°, 173° ± 3°, and 216° ± 6° C.



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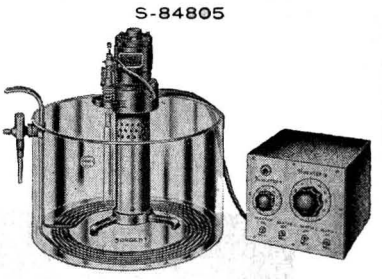
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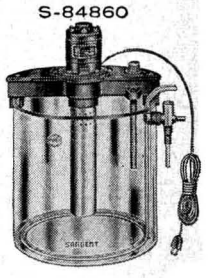
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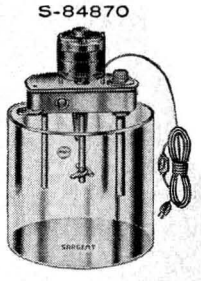
S-67424 VISCOSIMETER BATH—Kinematic, Double Wall, High Precision, 0.02°F, Sargent.

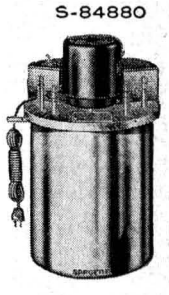
S-67428 VISCOSIMETER BATH—Thermonitor Controlled, Kinematic, Double Wall, High Precision, 0.02°F, Sargent.

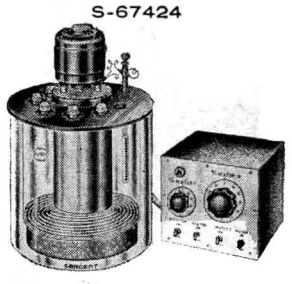
Write for Sargent Bulletin T on Temperature Controlling, Measuring and Recording Instruments

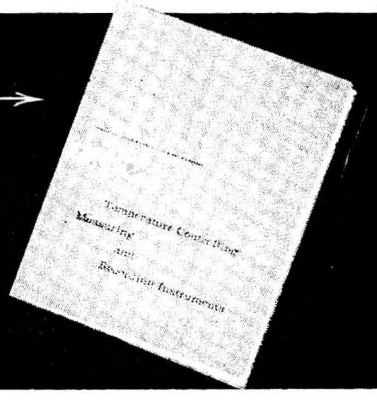












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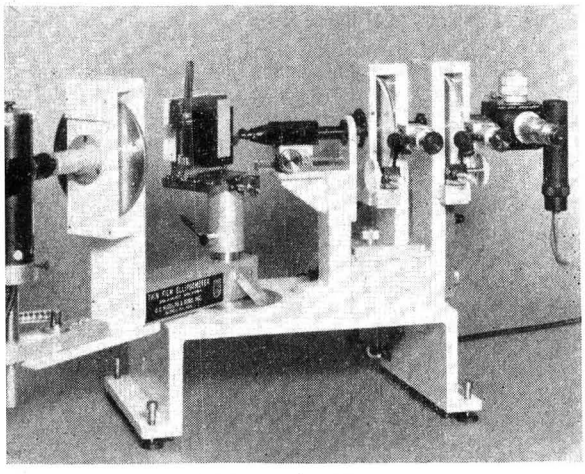
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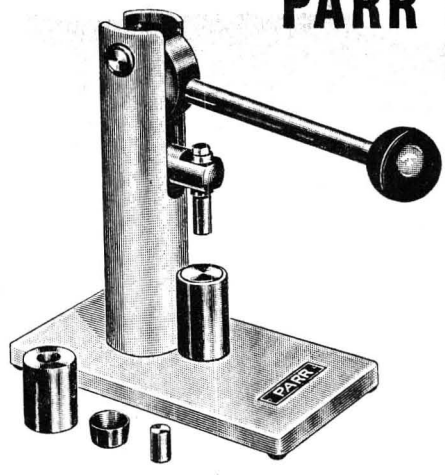
For applications, working characteristics and list prices, write for Bulletin 43-AC



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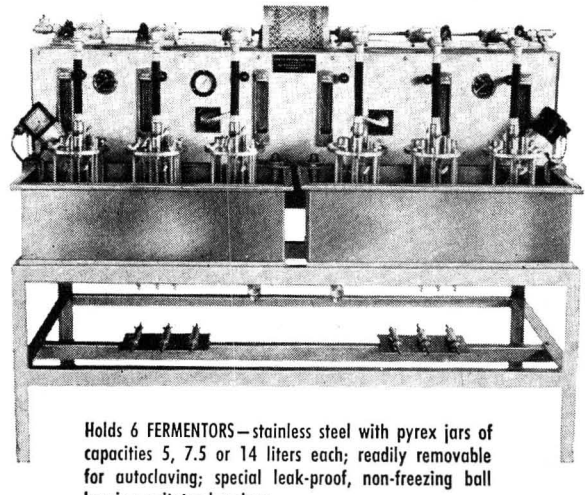
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NEW PRODUCTS

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Host of New Instruments Unveiled at Pittsburgh

PITTSBURGH—A large majority of the 75 exhibitors at the Exposition of Modern Laboratory Equipment brought forth new instruments, apparatus, and other items of interest to analytical chemists. The exposition was held in conjunction with the Annual Pittsburgh Conference on Analytical Chemistry & Applied Spectroscopy. It was visited by well over 3000 registrants during the 5 days of the meeting, March 2 to 6.

The editors, with the cooperation of the exhibitors, have attempted to present highlights of the items shown. Some items, it should be noted, were so new that neither photographs nor literature were available. These will be described in future issues.

The new items are described below:

Recording Analytical Balance

Wm. Ainsworth & Sons, Inc., is now making a new recording analytical balance with two differently colored pens that record both weight and temperature against time on the same chart. This balance follows by a year Ainsworth's first automatic recording balance—a one-pen model that records weight against time only.

The sample can be suspended from the balance and weighed in a furnace for thermogravimetric analysis. The balance, with a capacity of 200 grams, and temperature ranges of −25° C. to 525° C. or −50° C. to 1050° C., is accurate to plus or minus 1/10 mg. Weight and temperature readings are linear. **P-1**

Vacuum Balance

A vacuum, semimicro, automatic recording balance by Wm. Ainsworth & Sons, Inc., makes it possible to weigh in a vacuum or inert atmosphere. Also designed for thermogravimetry work, its balance beam, pans, and automatically controlled weights are in a small vacuum chamber.

Capacity is 100 grams on each pan; sensitivity is $^1/_{100}$ mg. The model has a two-pen recorder that records both temperature and weight on the same strip chart. **P-2**

UV Cuvettes

Aloe Scientific offers for first time round, matched, ultraviolet-transmitting cuvettes for use with Beckman DU spectrophotometers. Transmitting ultraviolet light down to 190 mμ, they compare favorably, Aloe says, with square "quartz" cuvettes in the 235 to 240 mμ range.

Measuring 50 mm. long and having an inside diameter of 10 mm., the cuvettes come packed in matched sets of four with a tolerance of $^1/_2$% T or by the dozen matched to a tolerance of 1% T. **P-3**

> The Advertisers' Product Guide (April II) incorrectly listed the heading Polarographs and Accessories. It should have read: Polarographic Equipment. Polarograph is a registered trade mark of E. H. Sargent & Co.

Spectrophosphorimeter

American Instrument has a new instrument that excites, resolves, measures, then records spectra and decay curves of phosphorescence. The Aminco-Keirs spectrophosphorimeter is designed mainly for study and concentration evaluation of solutions of unsaturated organic molecules.

Essential parts of the equipment are: a type A-46 high pressure water-cooled mercury arc with grating monochromator to excite samples, variable speed cylindrical phosphoroscope to isolate phosphorescent emission from any fluorescent or excitation emission, another grating monochromator to resolve the phosphorescent emission, and a photomultiplier to measure the phosphorescent intensity. **P-4**

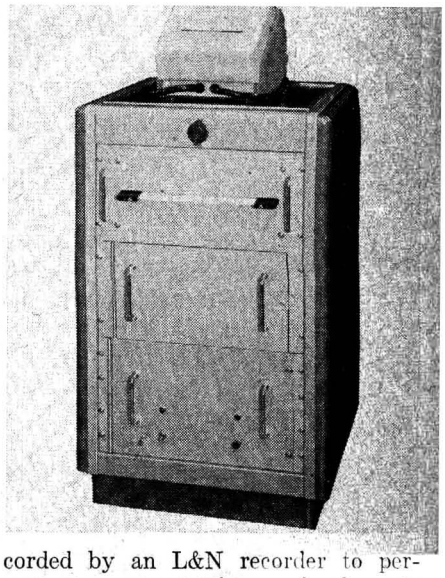
Digital Readout

Applied Physics Corp.'s new Cary digital readout, an accessory that may be connected to any Cary recording spectrophotometer, presents spectral data from the ultraviolet, visible, and near infrared in digital form.

Absorbance or transmittance data are automatically read-out at regular wave length intervals or at specific desired wave lengths. Accuracy, APC says, is as good as that of the basic spectrophotometer data. The system comprises an encoder, a power supply, junction box, parameter box, and switches, all housed in a single cabinet connected to the spectrophotometer by a multiconductor cable. **P-5**

ARL Computer

"Radically new" is the way Applied Research Laboratories describes its read-out computer, an instrument that prints analytical results simultaneously with Leeds & Northrup chart traces on standard ARL recording consoles. It provides for translation of voltages recorded by an L&N recorder to percentage concentrations of elements being determined. The proper decimal place for each element's concentration is encoded simultaneously.



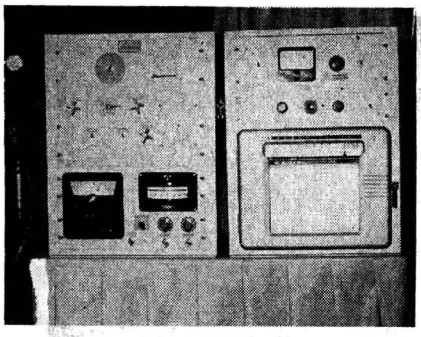
The instrument allows easy conversion of new and existing quantometers to computational and print-out instruments, says ARL. **P-6**

Infrared Spectrophotometer

The Model 2834 infrared spectrophotometer for oil and phenol analysis is available from Axler Associates. The firm, which stresses the instrument's low cost, says sensitivity compares to that in a prism-type research instrument. A portable unit, the 2834, permits analysis with cells having path lengths from 0.01 to 5 cm.—or any desired cell length made on special order. **P-7**

Portable Chromatograph

The new Model 20 gas-liquid chromatograph, put out by Wheelco Instruments Division of Barber-Colman, is a

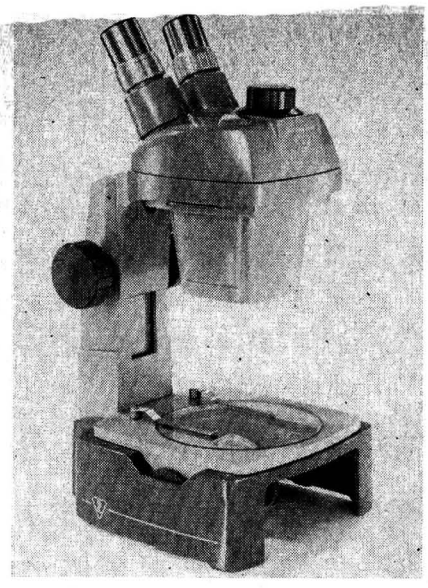


portable model suitable for detecting very small amounts of ionizable compounds. Detector time constant, depending on the relative flow rate of the carrier gas, may be as low as $^1/_4$ second when using a capillary column. **P-8**

Stereomicroscope

An entirely new line of stereomicroscopes being introduced by Bausch & Lomb Optical Co. features a completely dust-sealed optical system and "Stereo-Zoom" magnification—a new concept that makes possible a continuous flow of magnification, infinite within the range of the instrument.

The new line eliminates need for separate lenses and nosepieces, prevents blackout when changing power. A



power pod encloses the entire "eye" of the instrument—lenses, eyepieces, and mirrors—in a single unit, protecting it from dust and other foreign particles.

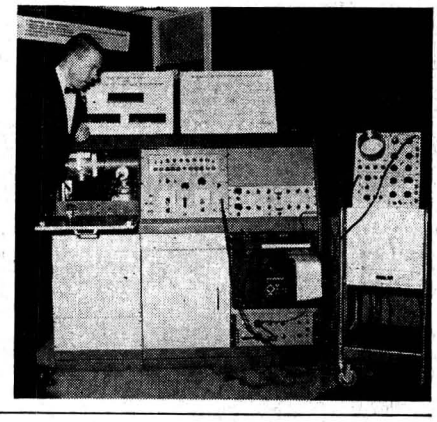
Working distance is constant at 4 inches without using lens attachments. A wide field of view lets the viewer see large parts of the specimen, permits him to work on the specimen quickly and precisely. **P-9**

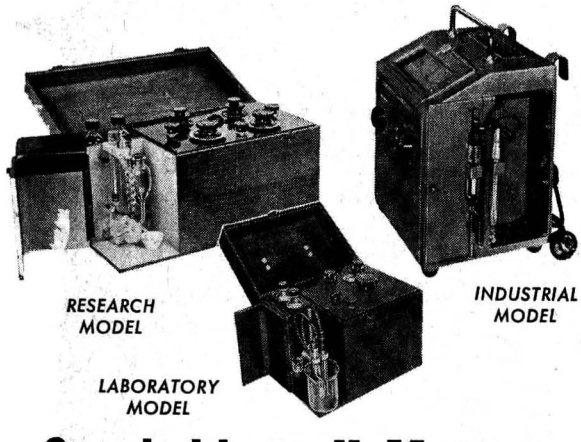
Prism-Grating Spectrophotometer

Beckman's Instruments Division is now making the IR-7 prism-grating infrared spectrophotometer with grating operation throughout the 2.5 to 16.6μ. Introduced a little over a year ago as a custom-made unit, the double-monochromator IR-7 is now going into regular commercial production (ANAL. CHEM., October 1958, page 91A). **P-10**

Analog Output System

The new analog output system, announced by Bendix Aviation Corp.,





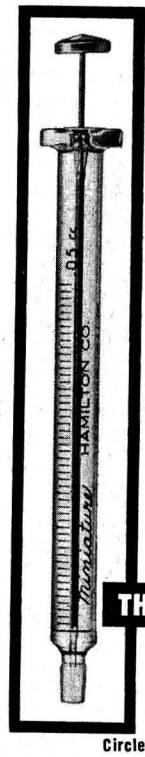
Cambridge pH Meters

The three portable models illustrated above incorporate new and important advantages. A.C. line operation eliminates battery nuisance. Laboratory and Research models use electron-ray tube for precise null-point indication. Industrial model is direct-reading, continuous-indicating; ruggedly built for plant use.

Accuracy: Research .02 pH, Laboratory .05 pH, Industrial .10 pH Cambridge pH equipment also includes single- and multi-point indicators and recorders. Send for Bulletin 910-MR.

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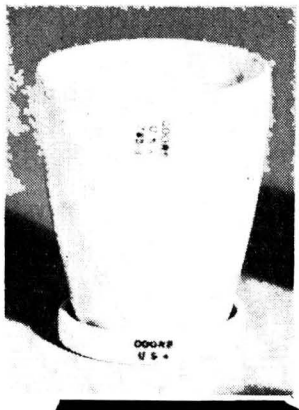
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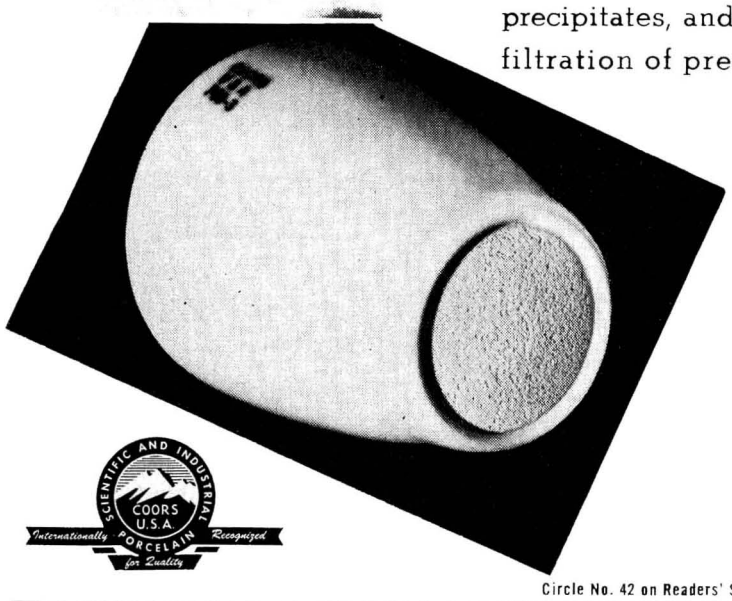
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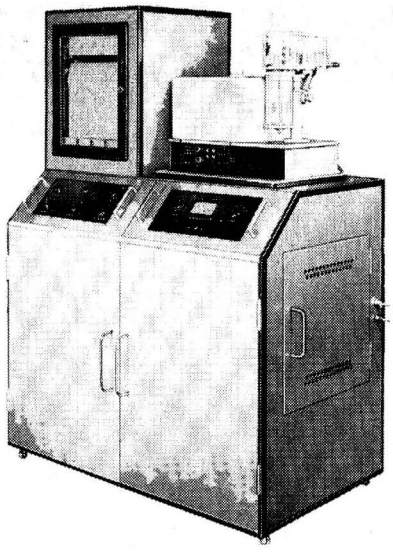
permits strip chart recording of either mass ratios or mass spectra when used with the firm's time-of-flight mass spectrometer. Sensitive enough to easily detect single ion impulses, it has a dynamic range of 1 million. From one to six channels can be incorporated into this output system. Ratio output has the advantage of being insensitive to all spectrometer variables that affect all peaks proportionately. **P-11**

Metallographic Equipment

Boder Scientific has taken on a new line of metallographic equipment produced in Denmark by Struers Scientific Instruments. Line includes three pregrinders, three electropolishers, four diamond-abrasion polishers, and polishing disks. It also handles the Struers hydraulic mounting press for embedding specimens in conductive or ordinary resins, as well as the resins. **P-12**

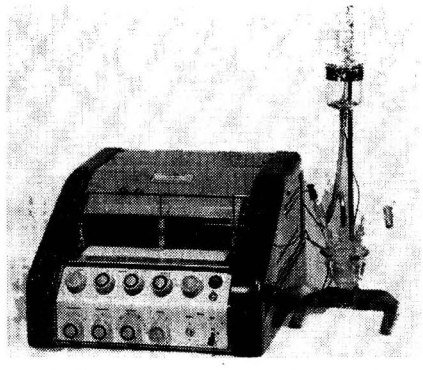
Turbidity Titrator

C. A. Brinkmann & Co. announces the AGFA turbidity titration photometer, a German instrument, used to determine molecular weight distribution in polymers. The photometer, which can be used in full daylight, operates on the single beam principle. Operations at temperatures up to 100° C. is possible since the chamber for the cuvette is well insulated, can be tempered accurately, usually by circulating water. **P-13**



Recording Polarimeter

Brinkmann is distributor of the Metrohm Polarecord E261, a Swiss direct-reading instrument for polarography, amperometric titration, and

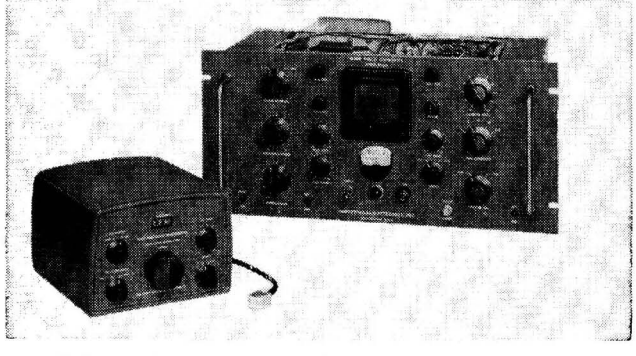


millivolt measurement. All controls are grouped on the front of the instrument, and the scales for current and voltage are in the same field of view. The graph is in horizontal position. Said to have high sensitivity, it is possible under favorable conditions to determine, for instance, metal ion concentrations to 10^{-7} molar. **P-14**

Rotating Viscometer

The electric rotational viscometer, distributed by Brinkmann, has a wide range of use, including advanced rheological experiments as well as short and accurate viscotests. It can be used as an indicating, recording, and controlling viscometer. Called the Rotovisco, the instrument is designed so that viscosity

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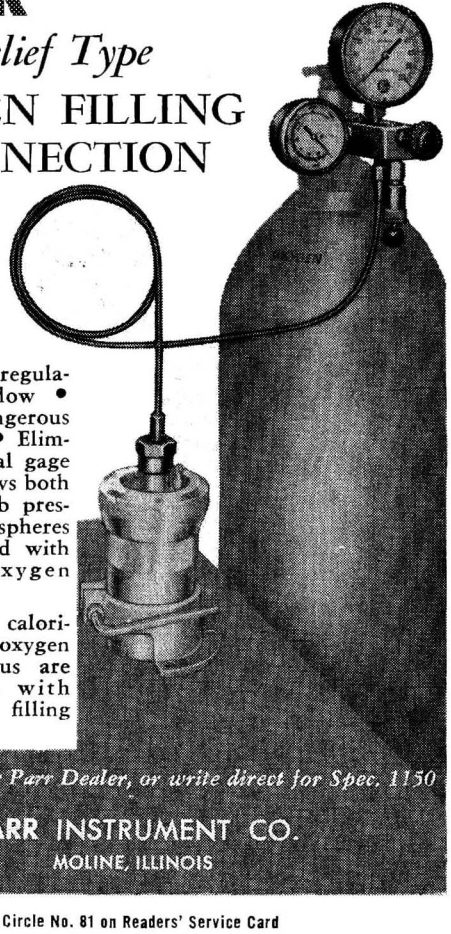
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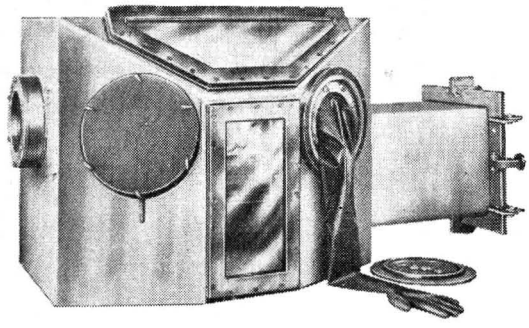
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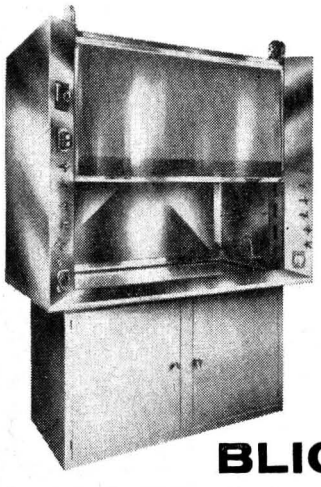
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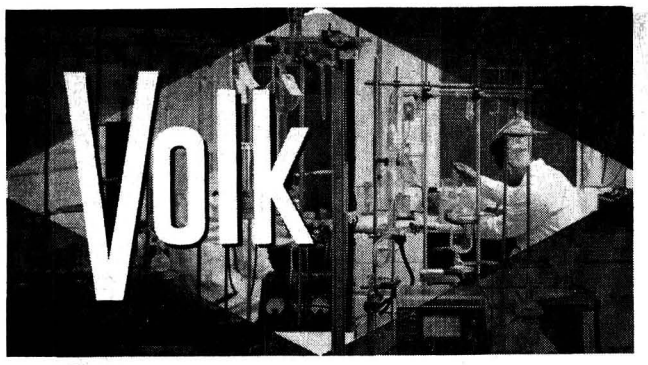
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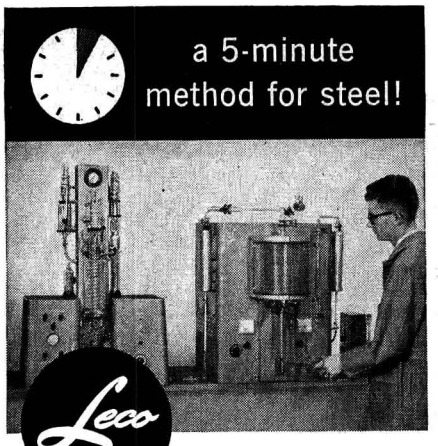
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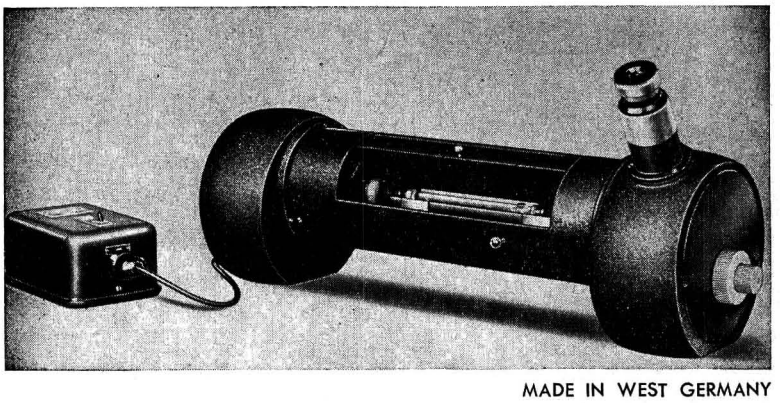
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Through observation telescope, the scales as well as the half-shade field of view can be observed.

Direct vernier readings can be made to 0.01° and estimated to 0.005°.

Moveable carrier, holding two polarimeter tubes, facilitates procedure of examinations.

Coaxially-mounted coarse and fine adjustment knobs and switch controlled by right hand resting on table-top.

Interchangeable built-in sodium or mercury spectral bulbs furnish strictly monochromatic light controlled by precision interference filters.

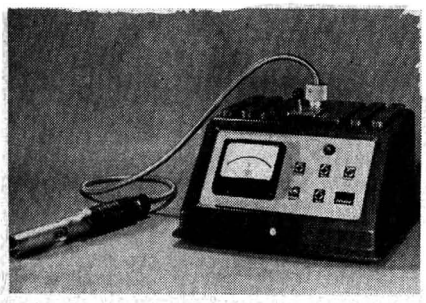
Newly designed precision polarimeter tubes ensure the absence of strain, furthering assurance of true measuring results.

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values measured are converted into electric values. Viscosity values are expressed in centipoises. **P-15**

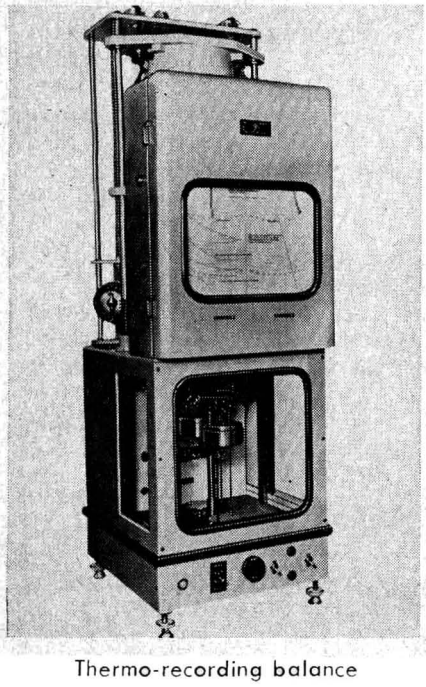
Precision Balance

The new Sartorious precision balance, called the Kilomat and handled by Brinkmann, lets the operator read the weight within seconds after releasing the beam. With a load range of 3000 grams, the entire mechanism is enclosed in metal housing. Controls are close together, and no manipulation of weights is needed for samples below 1000 grams. **P-16**

Induction Furnace

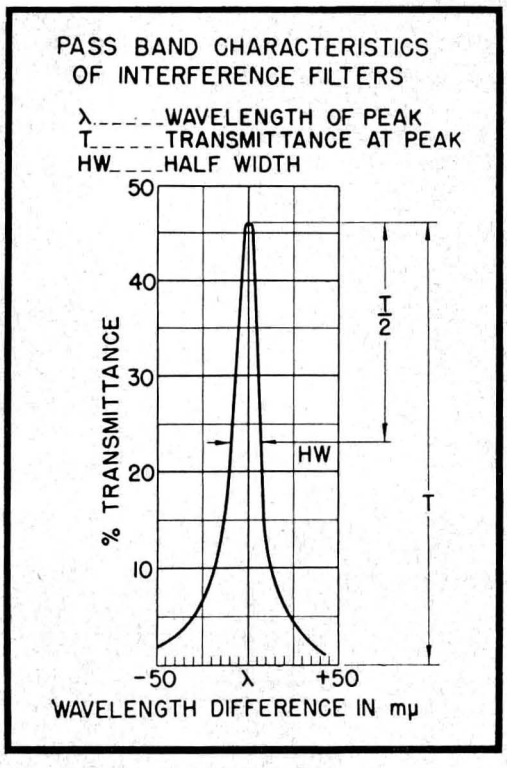
Burrell Corp. will market a new Combustron, a high frequency furnace for carbon and sulfur. It handles full factor weights of granular or pin-type samples, yielding hot, fluid fusions that push out carbon or sulfur with speed and accuracy.

The Combustron can be used in a horizontal or vertical position, depending on whether the analyst prefers boats or crucibles. For carbon work, a built-in catalyst tube serves for converting from the monoxide to the dioxide. **P-17**



Thermo-recording balance

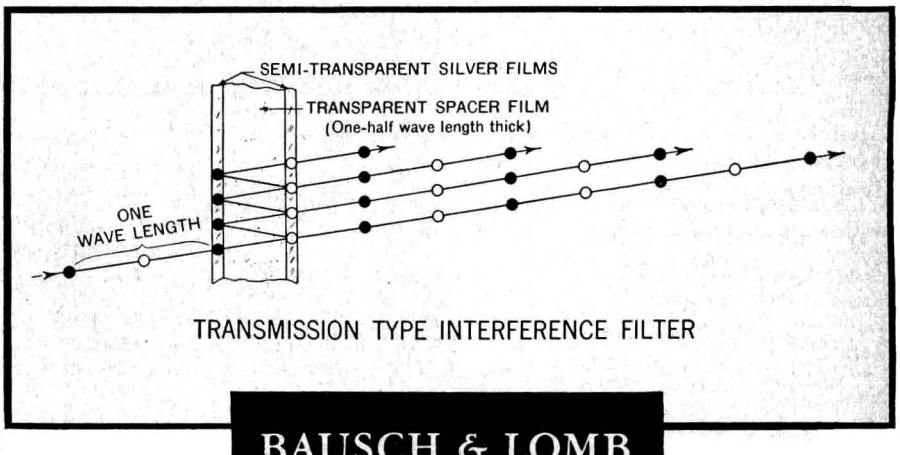
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WRITE for detailed Data Brochure D-248. Bausch & Lomb Optical Co., 60941 St. Paul Street, Rochester 2, New York.



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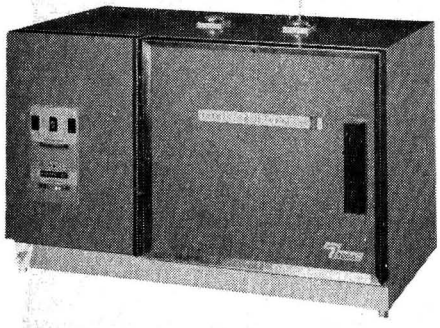
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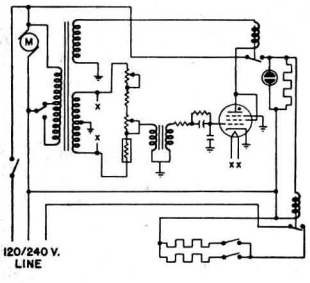
Total Re-Design for Freas Laboratory Ovens

Freas laboratory ovens have recently undergone a complete design change and now offer such innovations as an electronic control system, higher operating temperatures and advanced styling features which combine to provide greater operating dependability and convenience.



New Control Dependability

The most interesting feature of the new oven line is the electronic control system which utilizes a resistance thermostat acting through an electronic tube and sealed mercury relays. Moving parts—usually the cause of oven control failures in hydraulic thermostat systems—are completely eliminated. The simple circuitry of the new system is shown below.



Higher Operating Temperatures

Increased insulation, internal reinforcing and generally improved construction coupled with the electronic control now allow operating temperatures up to 325°C setting a new high in the laboratory oven field.

New Operating Convenience

The functionally grouped controls offer new ease of operation. The control dial is calibrated for direct temperature selection. The modern exteriors are easy to clean and maintain. Work load capacity and temperature uniformity in gravity convection models has been greatly increased by a unique airflow system.

Whether your needs are for standard ovens, vacuum, incubators, sterilizers, low temperatures, high temperatures (1000°F.) or special purpose models, you will find them all fully described in Bulletin #302—contact your Distributor or PRECISION.

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NEW PRODUCTS

Thermo-Recording Balance

The Stanton thermo-recording balance (Model TR 1) is announced by Burrell Corp. The instrument provides combined heating and weighing of materials under test and gives a continuous, permanent visual pen record of all changes. A cam, which can be modified, makes for a uniform heating rate. Sensitivity is 1 mg. Other models are available for thermo-recording or recording only. These have sensitivities of 1 mg. or 0.1 mg. **P-18**

Lab Balances

Burrell Corp. introduces three models of the Stanton Ultramatic single pan balance—the UM3, UM4, and UM5—which vary according to sensitivity. Dual release frees both hands for faster operation. Weight determination can be made in less than 30 seconds. Other advantages: air damping, direct projection reading, and full external weight loading up to 200 grams without use of loose weights. **P-19**

Beta Ray H/C Meter

Central Scientific has redesigned its beta ray H/C meter from a table model to a shock-mounted floor model. For determining the hydrogen or hydrogen-carbon ratios in hydrocarbons,



the new model is easier to read, weighs more accurately on a new built-in analytical balance, and gives a larger range of density measurements. The operating mechanism of the new model is mounted on sliding tracks for easy access. **P-20**

High-Temp. Chromatograph

The high-temperature chromatograph (Type 26-203), a modular instrument used for quantitative analyses of gases, liquids, and solids, can resolve and analyze materials with boiling points as high as 600° C. Temperature regulation can be held to ±0.5° C. of setting by a heavy duty, mercury thermostat.

Made by Consolidated Electrodynamics Corp., the 26-203 can be used in research and product analyses with many of the complex mixtures found in the petroleum, petrochemical, and chemical industries, according to the firm. **P-21**

Liquid Moisture Monitor

A new liquid moisture monitor, developed by Consolidated Electrodynamics Corp., measures the amount of moisture in liquids continuously, quickly, and automatically. Designated the CEC 26-312, the monitor can detect amounts as small as 1 p.p.m. Important application: monitoring trace moisture in jet fuel. To adapt to the electrolytic principle used, Consolidated introduced a stripping column ahead of the electrolytic cell to remove all moisture from the liquid sample. Monitor cannot be used with C_1 to C_5 alcohols, chemicals that react with water, or highly viscous liquids. **P-22**

Giant Flasks

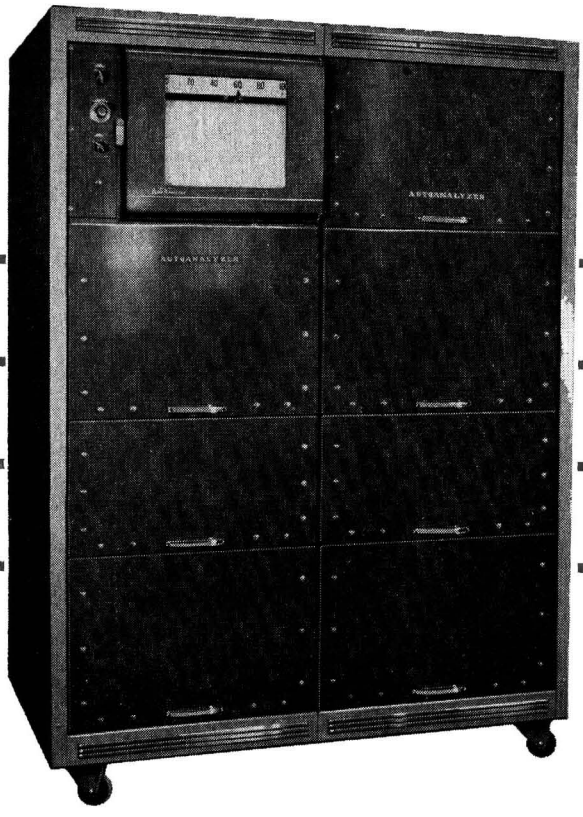
Corning Glass Works' large Pyrex reaction flasks come with capacities of 5, 10, and 20 gallons. Developed to meet the need in research labs for large capacity, these all-glass tank reactors have heavy walls, but are not recommended for vacuum or pressure use. Covers and other accessories also available. **P-23**

Hotpack Furnace

A new furnace using fibrous potassium titanate as insulation is offered by the Electric Hotpack Co. Under tests up to 2200° F., insulation did not shrink. Having a hot chamber volume of 230 cubic inches, furnace's over-all size is 1160 cubic inches. Heat-up time: 25 minutes. **P-24**

Gas Chromatograph

F&M Scientific Corp.'s new Model 202 linear programmed temperature gas chromatograph provides controlled heat on the detector to 300° C. and controlled temperature of column to



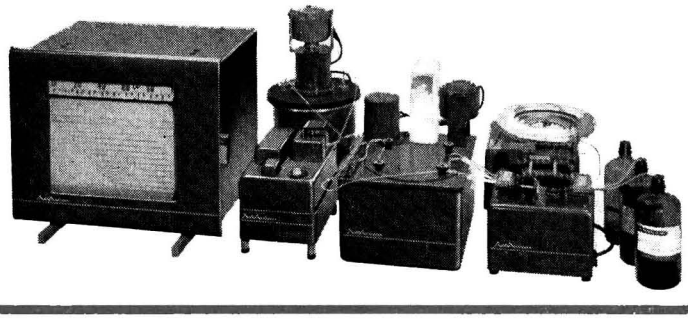
continuous automatic chemical analyses

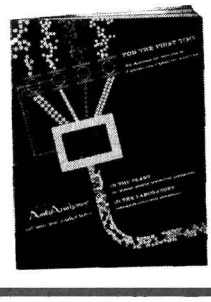
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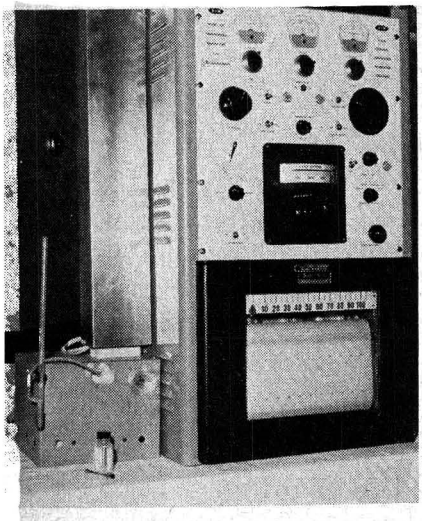




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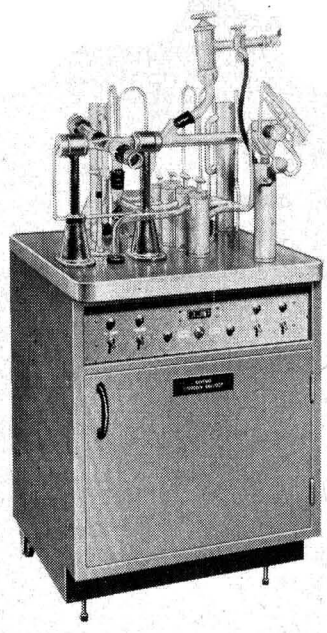
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400° C. It has separate heat for injection port up to 500° C.

The column temperature can be programmed linearly at nine heating rates from 3° C. minimum to 48° C. maximum. Said to be ideal for analyzing mixtures with wide-range boiling points, it also has the advantages of speed and versatility in that one can change column temperature and the column itself in minutes without changing base-line stability. It can be used on isothermal basis. **P-25**



Hydrogen Analyzer

The new Serfass hydrogen analyzer, handled by Fisher Scientific, uses the hot extraction method to detect and measure the amount of hydrogen in metals or other solids. Total gases can also be measured.

The machine needs no high voltage or water connections, is fast and accurate. A laboratory can run four to five samples an hour. Precision is ±0.2 p.p.m. in the 3 to 10 p.p.m. range found in vacuum-poured steels. Gas weight appears on a calibration chart. **P-26**

Peak Reader, Memory Device

Greenbrier Instruments' new peak reader and memory device acts as a data converter for use with chromatographic process analyzers to produce a continuous electrical signal proportional to the peak chromatographic signal for preselected components.

Developed by Phillips Petroleum, it produces a direct current e.m.f. output signal that can be used in data logging systems, computer, and control systems. Any number of memory units may be used with the basic unit by adding additional grating relays to the basic unit. **P-27**

"Speedi-Cell"

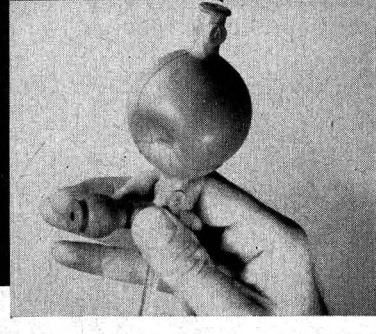
The chromatographic "Speedi-Cell," made by Labline Inc., is said to cut the quantitative analysis time of amino acids to two to three hours, as against the 48 to 96 hours required by standard ion exchange and paper chromatography methods.

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Safety Pipette Filler

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The new PROPIPETTE eliminates the dangerous practice of using the mouth to draw liquids into pipettes. It is simple to use and the operator soon becomes proficient so that liquids can be delivered quickly, precisely and safely. Measurement precision is extremely high (0.01cc). The instrument has three agate-ball valves which operate independently and the entire procedure can be done with only one hand.

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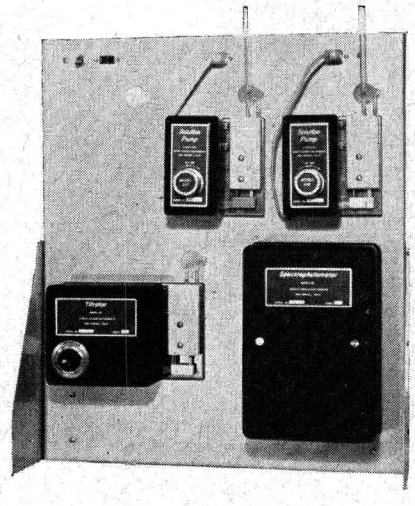
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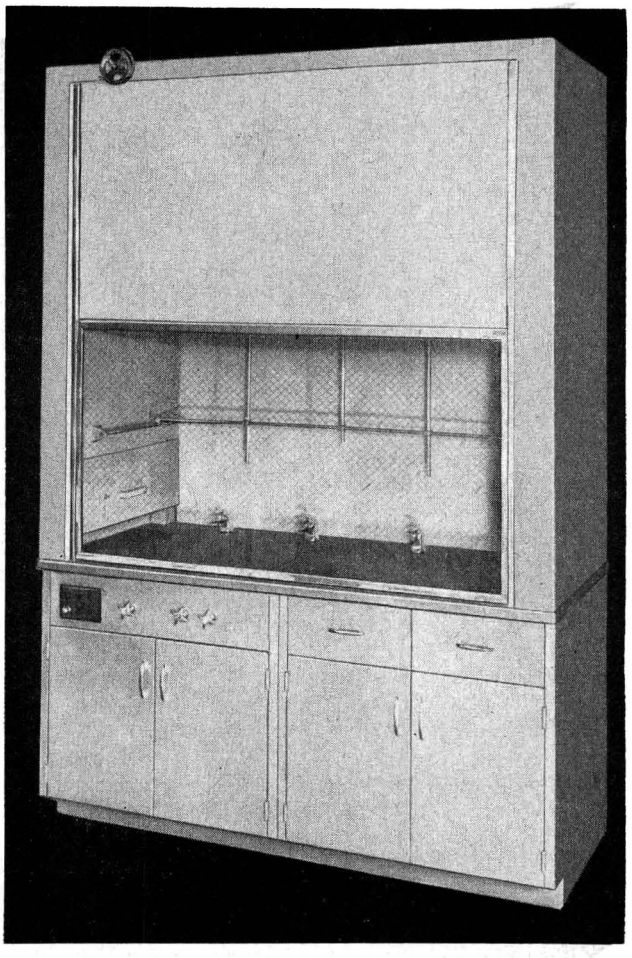
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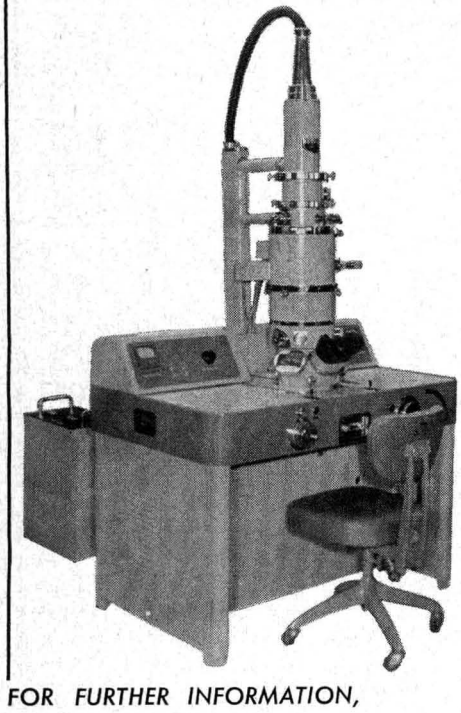
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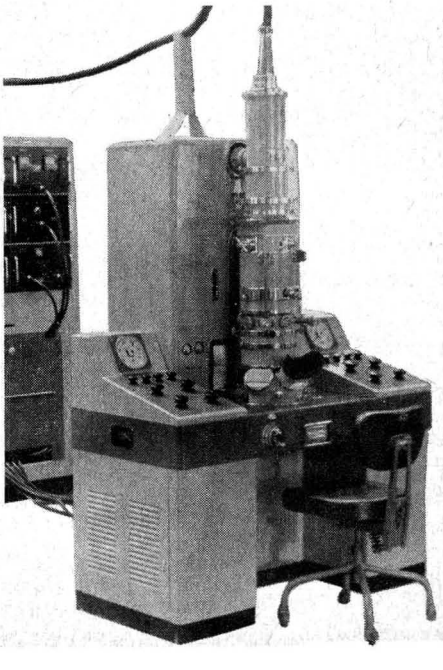
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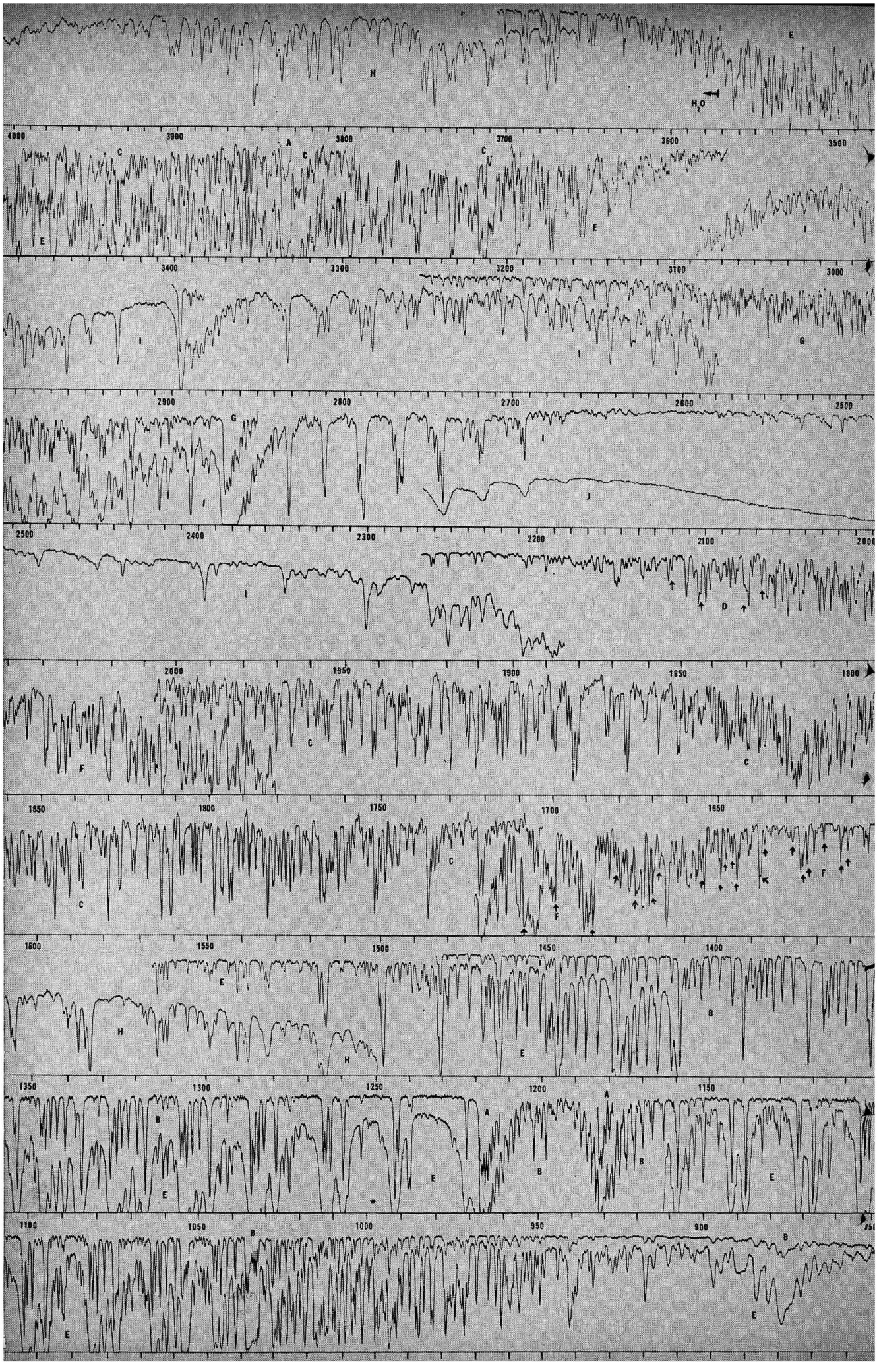
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SPEED: 0.2 cm^{-1}/min...600-2000 cm^{-1}
0.4 cm^{-1}/min...2000-4000 cm^{-1}
GAIN: 30-40%
PERIOD: 32 seconds

Spectrum Designation	*Cell Pressure*	*Cell Path Length*
A	7.5 mm	0.10 M
B	7.5 mm	1.00 M
C	7.5 mm	2.8 M
D	7.5 mm	8.2 M
E	75.0 mm	2.8 M
F	75.0 mm	4.6 M
G	75.0 mm	8.2 M
H	750.0 mm	2.8 M
I	750.0 mm	8.2 M
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Using a sealed stainless steel container and horizontal filter paper, papergrams are developed in shorter time because of more rapid solvent movement at 60° C. As many as 20 to 40 samples of amino acids can be analyzed in a day, using four cells. All parts of the cell can be removed for cleaning. **P-28**

Thermocups

Labline Inc.'s thermocups, with inner and outer containers made of stainless steel, can be used for low or high temperature baths, cold junction thermocouple jars, brittleness tests, and several other uses. Insulation is rigid foam polymer-type resin with 95% closed cell formation. Cups will resist most acids, alkalies, and common organic solvents. Five sizes available, with capacity ranging from 1 to 8 quarts. **P-29**

Oxygen Analyzer

A new vacuum fusion instrument determines oxygen in titanium in five minutes. Developed by Titanium Metals Corp. of America but made by Laboratory Equipment Corp., the apparatus consists of a furnace section, power supply, analytical system, and evacuation system. Sample goes into furnace where temperature is 2100° C. Gases are collected for two minutes. **P-30**

Pipet Control

A new pipetting device, the Pumpett automatic pipet control, is offered by Arthur S. LaPine & Co. The device makes it unnecessary to fill a lab pipet by mouth suction; a rubber bulb does the job.

For micro amounts, a small internal rubber bulb, depressed by a control knob screw, can be operated independently. The operator keeps liquid under constant observation at eye level. **P-31**

Graphite Tube Furnace

The TR25-212C18 graphite tube furnace is designed for rapid heating and maintaining uniform temperatures up to 5000° C. The unit, made by Lindberg Engineering Co., comes in a completely packaged unit of several components.

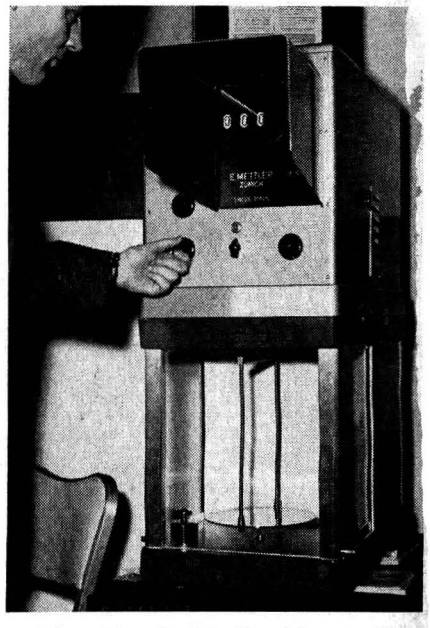
The element and terminal connections are housed in a gastight chamber, since graphite oxidizes quickly at high temperature. Saturable core reactor controls in conjunction with radiation pyrometer controls temperature accurately. **P-32**

Box Furnace

Lindberg Engineering Co.'s high temperature box furnace incorporates a one-piece No. 1 gage heating element made from high temperature alloy wire. A hinged, side mounted door pivots to the left; hence keeps hot side away from operator. Internal wiring, transformer, and power line terminal boards are concealed in the lower section of the furnace, but can be reached through a removable rear guard screen. **P-33**

Heavy-Load Balance

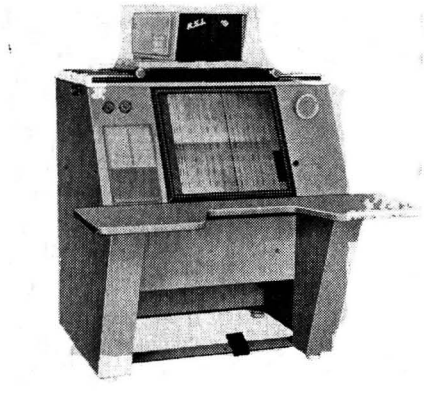
Two heavy-load balances—Models W5/11 and W8/11—are offered by Mettler Instrument Corp. Each has been developed for remote control and reading of substances dangerous to



health. The electrically driven weight mechanism can be operated from a control desk. The analyst can read the large optical scale from a 14-foot distance. **P-34**

Spec Reader

National Spectrographic Laboratories, Inc., announces a new spec reader



that features a "line of light" (dual collimating) system. Its reproducible flexibility enables stepwise settings for positive resetting of profile slit width, height, and rotation. It readily adapts to recording. Among other features: large screen with flat field, over 5 square feet of writing area, and plenty of knee and foot room. **P-35**

Vapor Fractometer

A new high resolution, high sensitivity instrument, said to represent a major advance in gas chromatography instrumentation, has been developed by Perkin-Elmer's Instrument Division. Called the Model 208 vapor fractometer, the company says it is the first commercial instrument that offers a flame ionization detector as a standard detector. It also incorporates the new high resolution Golay, or capillary columns, invented by P-E.



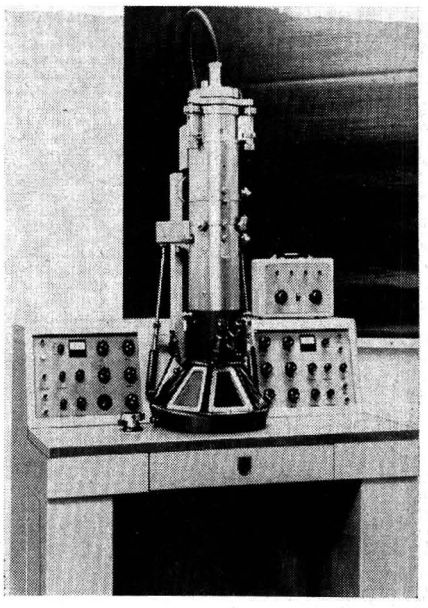
Design features let the fractometer pick up components at a level of about 10^{-9} gram. The 208 eliminates need for helium or the necessity of a balanced reference. Since flow, pressure, and reasonable temperature change do not effect it, it is possible to program these parameters in a run without influencing the base line. **P-36**

X-Ray Spectrograph

Philips Electronics, Inc.'s new Norelco portable x-ray spectrograph is designed for mobile applications in the field and around plants. Recommended uses: in-process control of metals, alloys, and chemicals; field and lab assaying; field checking of leaded fuels; identification and sorting of metals such as plate and bar stock; and gaging film and plating thickness. **P-37**

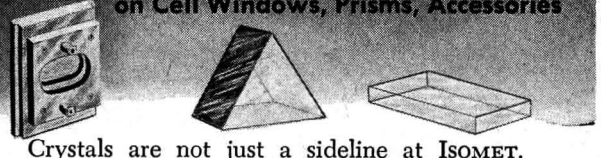
Electron Microscope

The Norelco EM 200 electron microscope provides 200,000x direct magnification and up to 2 million x by photographic methods. Announced by the Instruments Division, Philips Electronics, Inc., which guarantees resolving power of 10 A. or better, the EM 200 consists of a desk-type console and auxiliary cabinet. It uses accelerating voltages of 40, 60, 80, and 10 kv. which may be charged during operation.



The electron optical system includes a vertical column having six electro-

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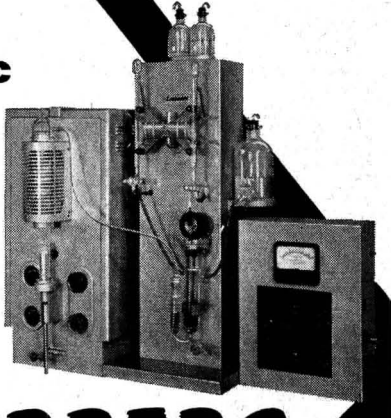


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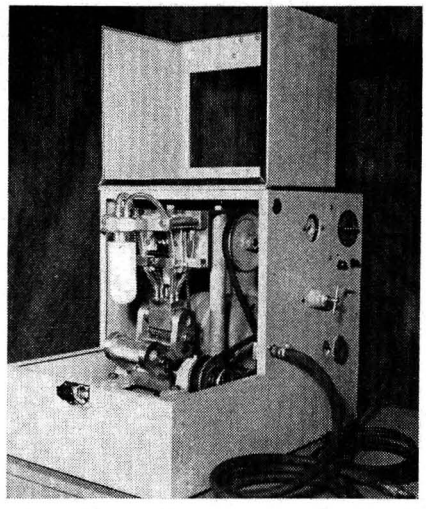
magnetic lenses. One, the double condenser lens, has an independent compensator and adjustable electromagnetic centering device. Wide field scanning permits quick specimen examination at 300x magnification.

The objective lens has adjustable, detachable pole pieces that allow the specimen to be inserted from the side. Both the objective and the condenser lenses have externally adjustable compensating mechanisms providing directional and amplitude correction for axial astigmatism. **P-38**

Particle Size Grinder

A new selective particle size grinder in sizes to accommodate both lab and production work is available from Pitchford Scientific Instruments Corp. Its significance lies in its ability to grind to a more uniform particle size. It does this by removing particles from the grinding means—rod-shaped pestles or balls of the same material as that to be ground—as soon as they reach the right size.

The cylindrical chamber in which grinding occurs can be equipped with particular screens of the right size. Located near both ends of the cylinder, the screens have a fitting with two hose



connections. One hose goes to a collector, the other to compressed air. A cam arrangement shuts off the proper hose in a sequence that causes air to be blown through the chamber to remove particles when they are small enough to pass through the screen.

The chamber, mounted on eccentric shaft driven by an electric motor, is propelled end ways with a conical-shaped motion at each end. This causes impact as well as mulling action like that done by mortar and pestle.

Production models can produce more than large ball mills of hundreds of gallons capacity, says Pitchford. In the paint industry, grinding of pigments and blending of pigment with vehicles can be done on the same unit, giving a finished product. **P-39**

pH Meter

The new Vibron pH meter (Model C33B), with a full scale deflection for 0.1 pH change, reads directly to 0.002 pH unit. This degree of discrimination, says the Milton Roy Co., is due to a Vibron electrometer (Model 33B) which maintains stability at ± 0.002 pH over a 12-hour period. Line operated, the pH meter has manual temperature compensation from 15° to 42° C. A wide range of electrodes is available. **P-40**

Recording Polarograph

A new recording polarograph for laboratory use has been developed by E. H. Sargent & Co. The Model XV is designed for the routine analyst, according to the firm, and includes facilities for application to all conventional polarography with full size recording and analytical accuracy. It permits direct alternate use as a recording potentiometer of the 2.5-mv. range. **P-41**

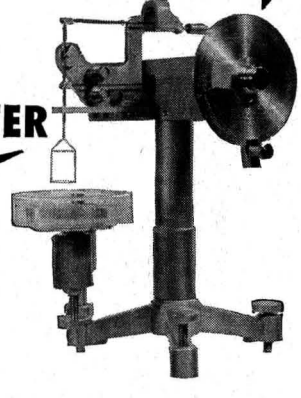
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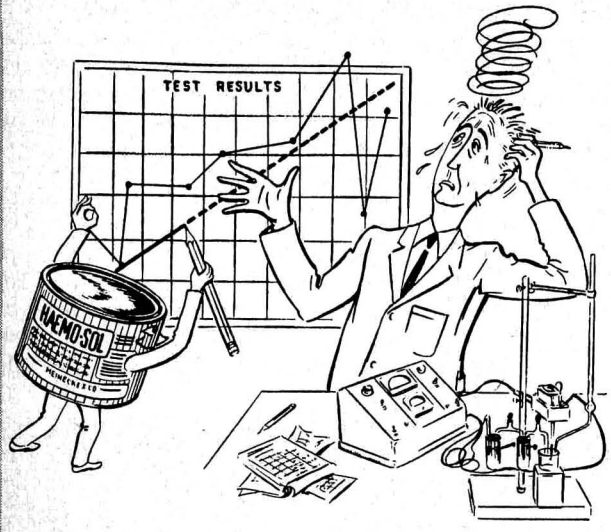
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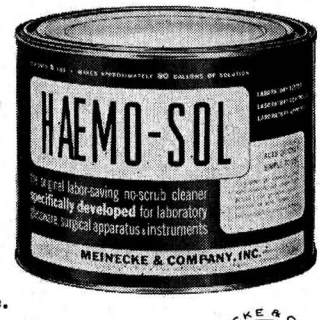
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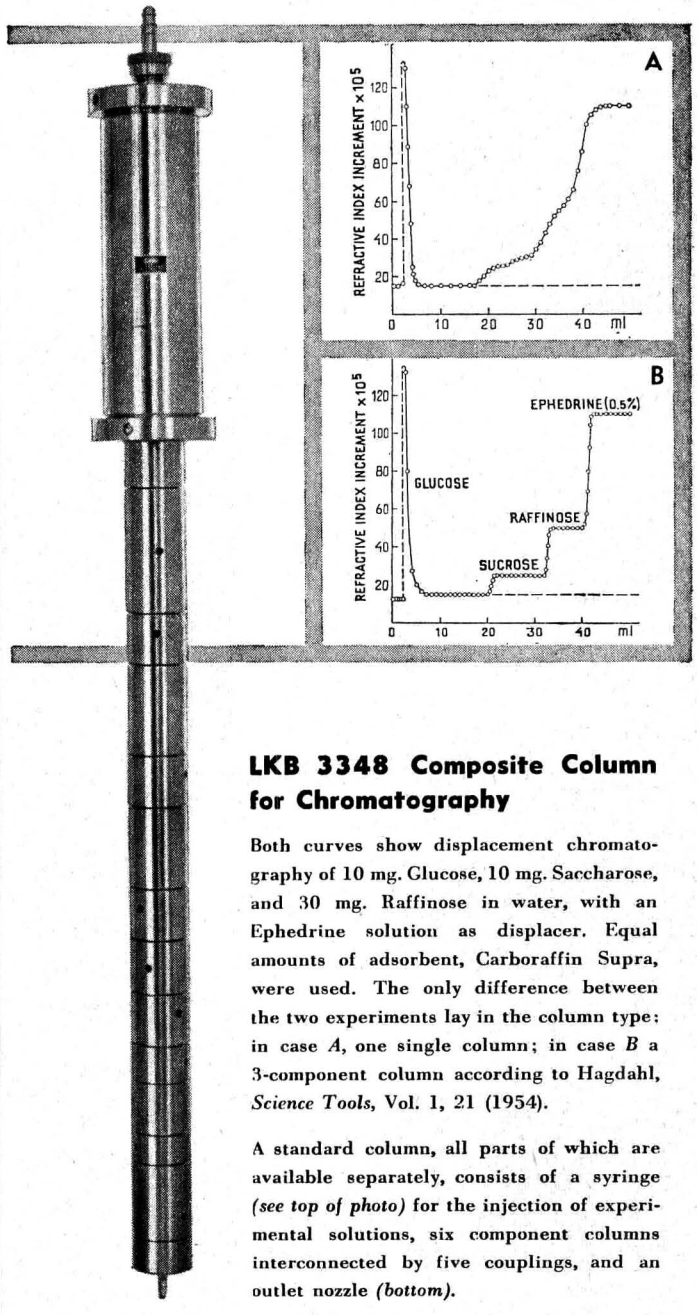
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Column Chromatography



LKB 3348 Composite Column for Chromatography

Both curves show displacement chromatography of 10 mg. Glucose, 10 mg. Saccharose, and 30 mg. Raffinose in water, with an Ephedrine solution as displacer. Equal amounts of adsorbent, Carboraffin Supra, were used. The only difference between the two experiments lay in the column type: in case *A*, one single column; in case *B* a 3-component column according to Hagdahl, *Science Tools*, Vol. 1, 21 (1954).

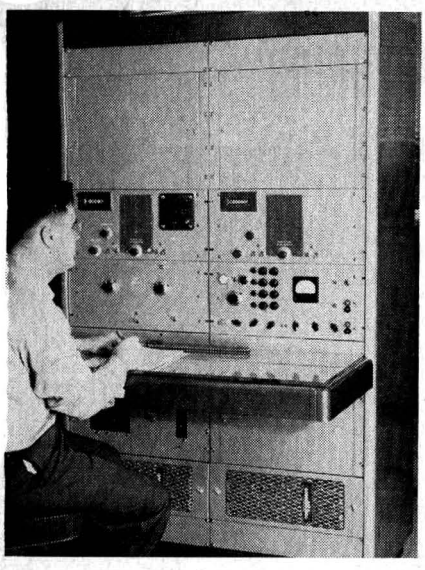
A standard column, all parts of which are available separately, consists of a syringe *(see top of photo)* for the injection of experimental solutions, six component columns interconnected by five couplings, and an outlet nozzle *(bottom)*.

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Nuclear nitrometer

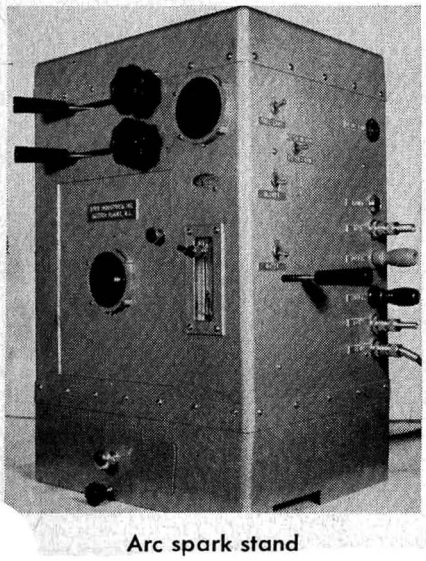
Lab Clamps

Scharr & Co. is now supplying a new line of Labasco laboratory clamps and Structo frames. Labasco says it surveyed a broad field to determine advances desired in clamp design. Improved features are: more strength resulting from a "C" clamp frame cross section resembling a structural beam, better clamping action, expanded utility, longer life, greater rigidity. **P-42**

Nuclear Nitrometer

A nuclear nitrometer that cuts time necessary to make nitrogen determinations from four hours to less than 15 minutes is announced by Schlumberger Well Surveying Corp.

The new instrument measures nitrogen in solids with an accuracy of ±2%. Measurement is made from gamma-ray spectra caused by bombarding nitrogen nuclei by fast neutron. No residual radioactivity results. **P-43**



Arc spark stand

Stopcock

Scientific Glass Apparatus Co. offers the new improved stopcock which now uses a Teflon plug with an exaggerated taper that completely eliminates binding, according to the company. And the borosilicate glass barrel is processed to make lubrication unnecessary. An adjustable nut, acting on a stainless steel spring, permits adjusting to the right tension for either pressure or vacuum use. **P-44**

Arc Spark Stand

Spex Industries, Inc., announces the new arc spark stand, of which the interior is almost free of components, except for the electrode jaws themselves. The main jaws open bilaterally.

An erect image of the electrodes is magnified four times in a variable density polaroid window. A 115-volt outlet is built into the unit, and all switches and outlets are on the side panel. The stand can fit almost any optical bench and spectrograph. **P-45**

CHROMATOGRAPHY OVEN



HAZARD-SAFE EFFICIENT

Here is the new forced-air chromatography oven developed by National Appliance for more efficient, safer drying of chromatograms. Includes all modern features:

Roll-out paper rack with removable stainless steel rods, capacity 10 sheets 18¼ " x 22½ ".

Interior completely stainless steel.

Blower operates independently of heater for room temperature drying, adjustable fresh air intake.

Dimensions, Exterior: 27 " wide x 23 " deep x 39 " high Interior: 21 " wide x 18½ " deep x 24" high.

Temperature range room to 120° C., accurate hydraulic thermostat.

Safety features: main switch prevents operation of elements without blower. Shaded-pole, non-sparking motor, switch, thermostat and pilot light in vapor-tight instrument panel. Element limited to 230°C. by safety thermostat.

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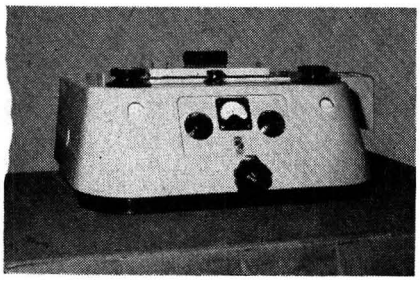
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Electrode Packer

Elpac, developed in South Africa and distributed here by Spex Industries, Inc., automatically loads graphite electrodes. The material can be packed into electrodes (three can be packed at one time) in seconds. Steel plungers are custom designed for the type of electrode used. This device is the first South African instrument to be distributed in the U. S. **P-46**

All-Purpose Spectrophotometer

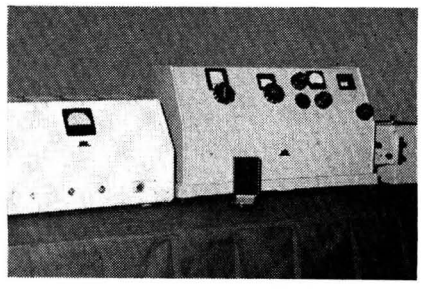
Unicam, an English company, offers a new all-purpose spectrophotometer



called the SP. 600. It has a Littrow monochromator with glass prism. Measurements are made by either absorption or transmission. Analysis of an unknown solution or transparent solid by transmission measurement in the visible and near ultraviolet regions is readily carried out, and intercomparisons of up to four samples is easily made. Complete absorption spectra may be plotted or readings made at one selected wave length. **P-47**

Quartz Spectrophotometer

The photoelectric quartz spectrophotometer—SP. 500—by Unicam of England is designed for qualitative and quantitative determinations by percentage absorption or transmission of light at wave lengths in the range 2000 to 10,000 A.



The basic instrument comprises a monochromator formed of rigid meehanite box casting which contains the optical components. The box is sealed to keep out dust and moisture. With a new prism, the wave length range of the SP. 500 is extended from 200 to 186 mμ. **P-48**

Modulation System

Varian Associates' V-4560 100-kc. field modulation and control unit has the purpose of raising the EPR spectrometer modulation to as high a value as practicable. The system provides the means for generation of the 100-kc. sweep field, and for amplification and phase detection of the EPR signal.

The multipurpose EPR cavity and connecting waveguide bend combine with the unit to form the 100-kc. modulating system, completely compatible with the present EPR spectrometer. The resulting increase in sensitivity over the present V-4500 EPR spectrometer, operating at modulation frequency of 400 cycles, is about 16. **P-49**

Variable Temperature System

The variable new temperature system, by Varian Associates, permits EPR samples to be observed over a wide range—from −185° C. (liquid N_2) to 300° C. A copper heat exchanger cools dry nitrogen gas to a temperature depending on the rate of flow.

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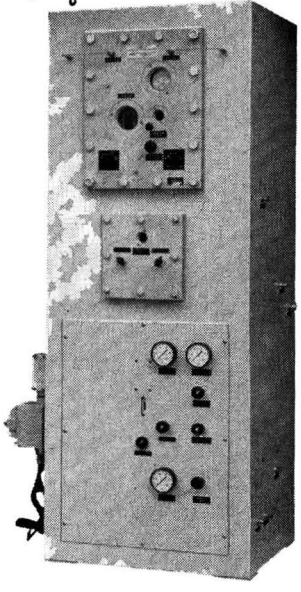
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A Vycor heater and transfer tube heats the cooled gas which then passes out the tube. It then makes contact within a ground ball and socket joint with a variable temperature quartz Dewar insert extending vertically through the cavity. A copper-constantan thermocouple joint mounted in the insert, measures the temperature of the gas entering the cavity. **P-50**

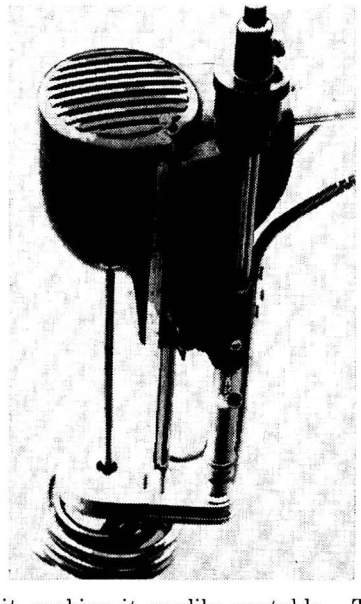
G-11A Recorder

The G-11A strip-chart recorder with thermocouple input chassis gives the user the capability of direct measurement and recording of temperature information from thermocouples. The T2 chassis can handle all common thermocouples, says Varian Associates.

Seven standard range elements are now available and each range element provides two calibrations—the 0° referenced Fahrenheit calibration and the corresponding centigrade equivalent. **P-51**

Constant Temperature Circulator

A redesigned Bronwill constant temperature circulator is announced by Will Corp. It consolidates all elements of the former model into one compact



unit, making it readily portable. The Bronwill converts any suitable container into a bath closely controlled to ±0.01° C., as well as an external circulation system driven by a centrifugal pump on the motor shaft. **P-52**

Safety Pipet

Will Corp. announces six models of the Kern-Exelo safety pipet, with automatic pump. Designed to stop accidents from old fashioned mouth-suction pipets, the Kern-Exelo is easy to operate in five steps: place finger over aperture in cap; raise barrel, drawing liquid past graduations; remove finger, pushing barrel back to original position; replace finger, stopping flow at graduation mark; remove finger, allowing liquid to flow out. **P-53**

Flat Stopcocks

Will Corp. is distributing Kern-Exelo borosilicate glass stopcocks. Tested in England, the flat stopcocks consist of a body and key, mounted together with a spindle and retaining nut. Said to need no lubrication, they will not leak even under high vacuum. Available with bore diameters of 2, 3, and 6 mm. **P-54**

Hydrogen Analyzer

A new apparatus for measuring the amount of dissolved hydrogen in metals and other solid materials is announced by Fisher Scientific Co. The company says it is capable of determining hydrogen in a typical 1-gram metal sample over a range of 0.2 to 1000 p.p.m., with a precision of ±0.2 p.p.m. Four or five



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steel samples an hour is a normal schedule, it adds, and other metals can be run at a faster rate. The water-cooled unit takes 15 amperes at 115 volts, 60 cycles. It is mounted on a steel cabinet 26 × 24 × 33 inches high. **P-55**

OH-37 Duplex Recorder

This recorder, announced by General Electric, is a compact, general-purpose recorder for plotting two related electrical quantities side by side on a single chart. It is housed in a 15-inch high cast aluminum case, is designed for semi-flush panel mounting. Recorder is available with a complete set of rate gears. **P-56**

Automatic Voltage Calibrator

The Model 55 automatic calibration unit, made by Beckman, is designed to fill requirements for highly accurate signal source for checking the accuracy of voltage signals. For manual use, as well as automatic, the 55 has application in the calibration setup and check-out of oscillographs, recording potentiometers, telemetry systems, and data handling systems. **P-57**

Panel Meters

A new line of 6-inch panel meters (a.c. and d.c. models), with sensitivities beginning with 0 to 5 ma. or 0 to 5 mv., is available from Assembly Products, Inc. The meters, called Model 661, may be mounted in any kind of panel, since their steel backs shield them from magnetic fields. **P-58**

Briefly Noted

Laboratory Homogenizer. Model C-8 processes samples from 30 cc. to 8 gallons per hour, operating at pressure range from 100 to 3000 p.s.i. C. W. Logeman Co. **P-59**

Bottle Holder-Carrier. Protects glass bottles containing acids and other hazardous liquids. Terne plate body lined with polyester urethane padding absorbs shocks. Protectoseal Co. **P-60**

Neutron Detectors. Boron trifluoride detectors range in size from 42 inch long small diameter probes to commonly-joined multiple units. Nuclear-Chicago Corp. **P-61**

Linear Amplifier. Model 358-I is said to recover from 4000 overload in 8 microseconds. Over-all gain is 70,000 with stability of 0.9% for line voltage 105 to 130 volts. Maximum count rate is 250,000 per second random. A previous item on the instrument erroneously stated overload recovery to be 8 seconds. Franklin Electronics, Inc. **P-62**

NEW CHEMICALS

CHEMICALS
REAGENTS
MATERIALS

Ion Exchange Resin

Samples of a chelating resin, said to be the first commercially available, can be obtained from The Dow Chemical Co. According to the company, Dowex chelating resin A-1 will remove traces of heavy metals such as iron, copper, or zinc from concentrated solutions of alkali and alkaline earth metal salts. Also, alkaline earth cations may be removed from concentrated solutions of alkali metal cations. Exceptionally *high selectivity* makes possible the separation of many closely related ionic species, such as nickel from cobalt and copper from nickel. The resin is said to be easily regenerated by mineral acids, and to have the same stability and sphericity characteristics as Dow's other polystyrene-base ion exchange resins. **C-1**

Spectroscopic Rod

National Carbon Co. announces new graphite spectroscopic rod designed for greater reproducibility. Test show, says National, these advantages: less deviation (improved precision of determinations); greater uniformity from end to end, from rod to rod; greater density and homogeneity; and better machinability. **C-2**

Polyamino Acids

Polyamino acids, a recently synthesized series of biochemicals, are now available commercially from Mann Research Laboratories, Inc. Those available include poly-L-aspartic acid, poly-L-glutamic acid, poly-L-hydroxyproline, poly-L-lysine hydrochloride, poly-L-lysine hydrobromide, poly-D-lysine hydrochloride, poly-L-proline, and polysarcosine. In addition to separate purchase, Mann's polyamino acid kit No. 405, which includes five of the above materials, is also available. The company says the compounds are of interest especially in protein studies, and enzyme and blood chemistry. **C-3**

Carbon Electrodes

Carbon electrodes, made by United Carbon Products Co., are said to be expertly machined and purified to provide increased sensitivity of d.c. arch techniques with excellent reproducibility. Nine shapes are available. **C-4**

Magnesium Oxide

A special grade of magnesium oxide powder offered by Fisher Scientific Co. is said to have exceptionally high purity, small particle size, and low reactivity. Electron photomicrographs of the M-300 electronic-grade oxide show that individual particles are under 0.1 micron in diameter. Sedimentation studies on a typical lot showed that 55% of the oxide agglomerates are less than 1.2 microns in diameter, and at least 99.5% are under 20 microns. **C-5**

New Available Diamines

A variety of new diamines has been added to those available from The Ames Laboratories, Inc. Among these are *N*-methylethylenediamine, *N*-benzyl-*N*,*N'*-dimethylethylenediamine, and tetramethyl-1,6-hexanediamine. Also included are several chelating agents: disalicylidene ethylenediamine, disalicyclidene propylenediamine, and disalicylidene-1,3-propanediamine. The chemicals, of high purity, are suggested by the company as intermediates in preparation of chelating agents, pharmaceuticals, insecticides, metal deactivators, plastic foaming and curing agents, and for other uses. In all, 12 new materials are listed. **C-6**

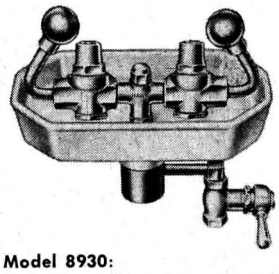
Rare Earth Oxides

Two catalog sheets issued by Research Chemicals, Inc., provide a description of the rare earth oxides and ytterbium metal now available from the company. In the case of the rare earth oxides, a physical description is given which includes color, crystal structure, lattice constant, rare earth–oxide interatomic distance, melting point, and density. The sheet concerning ytterbium metal lists about a dozen physical properties and gives a short discussion of corrosion resistance. **C-7**

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Model 8930:
Basic eye-wash model; enameled iron bowl, chrome plated brass heads. HAWS also offers eye and face wash fountains and drench showers.

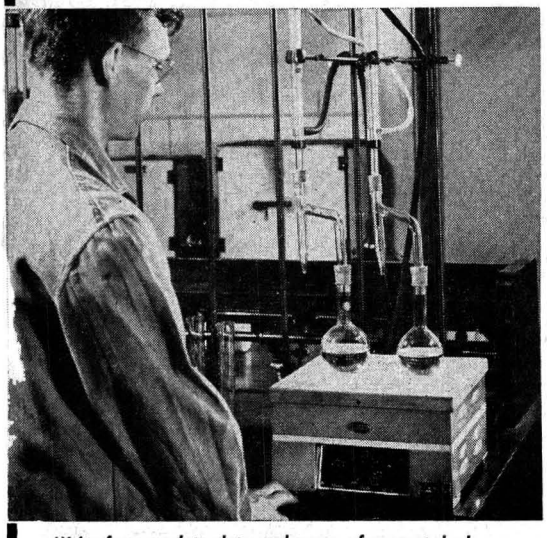
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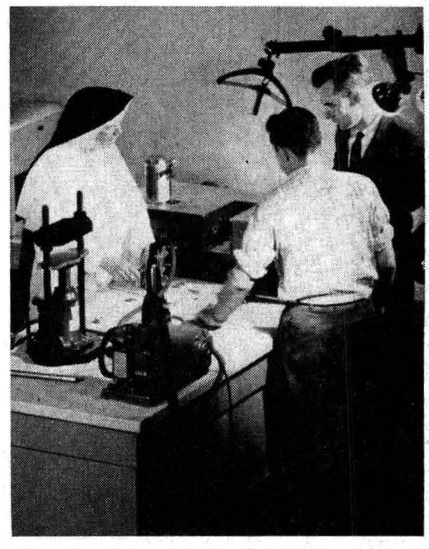
Modern design and precision workmanship set the Adams Analytical Centrifuge apart from other economy machines. Compact (10″ diameter) and light (11¾ lbs.), the unit is strongly built and has a chemical-resistant finish. Head accommodates six 5 ml. tubes. Motor operates on 110-115 volts, 60 cycles, AC, and oilite bearings require no lubrication for two years.

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MANUFACTURERS' LITERATURE



Scene from new film "Infrared Spectroscopy"

Infrared Spectroscopy Film. A 36-minute, 16 mm. color and sound film on the theory and practice of infrared instrumental analysis was produced by Rensselaer Polytechnic Institute for the Perkin-Elmer Corp., Norwalk, Conn. The film is available for purchase or rental. **L-1**

Diamine Chemicals. Catalog lists new fine chemicals suggested as intermediates, prices included. The Ames Laboratories, Inc. **L-2**

Organic Bromo Compounds. Four-page price list includes 28 organic bromo compounds and 12 oximes. Beacon Chemical Industries, Inc. (*Price list 50B*). **L-3**

Sonic Energy Cleaning Appraisal. Report on sonic energy tells what it is, how it operates, how it can be used, and how to evaluate it. Bendix Aviation Corp. (*How to Appraise Sonic Energy Cleaning*). **L-4**

Chemical Data. Three new bulletins describe properties, preparation, and applications of molybdenum hexacarbonyl, tungsten hexacarbonyl, and tungsten hexachloride. Bibliography included. Climax Molybdenum Co. **L-5**

Organic Chemicals. New catalog lists characteristics and specifications of organic chemicals. Four pages. Also lists other company literature. Emery Industries, Inc. (*Sept. 1958 catalog*). **L-6**

MANUFACTURERS' LITERATURE

Corn Syrups and Sugars. Booklet of 45 pages describes manufacture, properties, equipment, and handling of corn syrups, corn syrup solids, crude corn sugar, and dextrose. Has 12 tables in analytical section. Illustrated. Corn Industries Research Foundation, Inc. (*Corn Syrups and Sugars*). **L-7**

Spectrophotometer. Four-page leaflet describes Model 62 spectrophotometer for measuring color density or light transmission between 410 and 690 $m\mu$ in a flow stream. Harold Kruger Instruments. **L-8**

Guarded Wheatstone Bridge. Four-page data sheet gives specifications and a table of maximum applied voltages for the 4735 Guarded Wheatstone Bridge. Leeds & Northrup Co. (*Data sheet E-53 [1A]*). **L-9**

Superspeed Centrifuges. New 16-page catalog describes superspeed automatic and refrigerated centrifuges and accessories. Lourdes Instrument Corp. (*Cat. 159*). **L-10**

Pushbutton Actuators. Data sheet shows the new 12MA series for basic switches. Chart gives typical requirements and applications. Micro Switch. (*Data sheet 155*). **L-11**

Manual Sample Changer. Literature describes Model 3056 manual sample changer, for use when measuring radioactive samples. Nuclear-Chicago Corp. **L-12**

Radioisotopes for Mixing Efficiency. Four-page bulletin describes evaluations, advantages, and practical applications in use of radioactive materials for mixing. Nuclear-Chicago Corp. (*Tech. Bull. 2*). **L-13**

Spectrophotometer. Sixteen-page catalog describes Model 4000-A spectrophotometer for ultraviolet to near infrared, and operating in three wave length ranges from 200 to 2800 $m\mu$. Illustrated. Perkin-Elmer Corp. **L-14**

Electron Microscope Booklet. New 12-page booklet discusses magnification, resolution, specimen preparation, and fields of application of electron microscopes. Philips Electronics, Inc. (*Questions and Answers on Electron Microscopes*). **L-15**

New Chromatography Instrument. Four-page bulletin describes Hycalog Chronofrac, a gas chromatography unit for stream or separate sample analysis which detects by catalytic combustion and uses air as carrier. Precision Scientific Development Co. (*Bull. 605*). **L-16**



One of many assembly lines of skilled operators employing AO CYCLOPTIC Stereoscopic Microscopes to assemble advanced germanium computer transistors at Texas Instruments vast Semiconductor —Components division in Dallas, Texas.

Here's how Texas Instruments mass produces precision transistors

with AO's CYCLOPTIC Microscope

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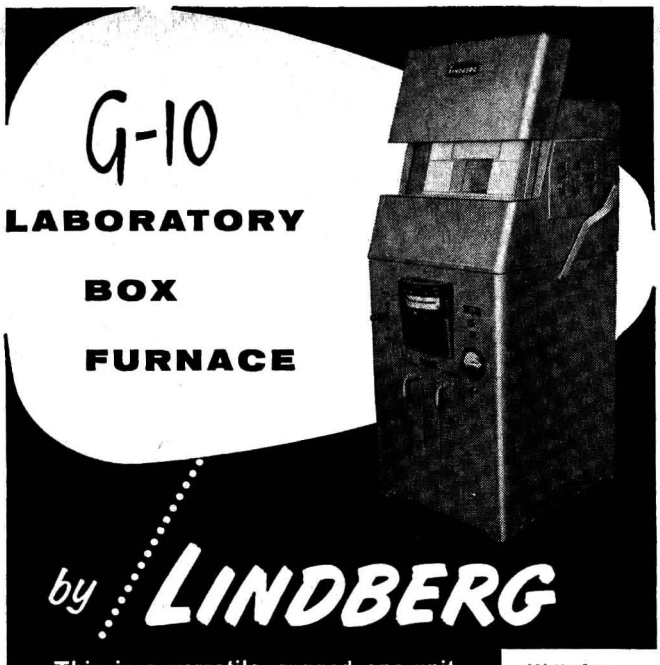
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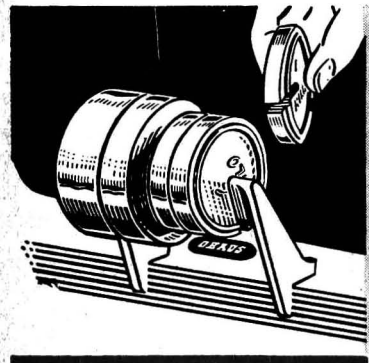
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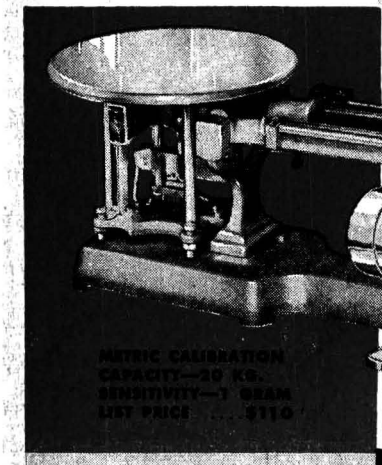
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MANUFACTURERS' LITERATURE

Crystal Counter Chart. New chart gives working range for crystals and counters in x-ray spectrograph applications. Philips Electronics, Inc. **L-17**

Flash Point Recorder. Four-page bulletin explains recorder which draws sample from stream, operates continuously to record results, and makes flash point tests at intervals averaging three minutes. Precision Scientific Development Co. (*Bull. 702*). **L-18**

MiniPumps. Four-page bulletin describes line of controlled volume MiniPumps for metering chemicals in minute volumes against pressures. Milton Roy Co. (*Bull. 1257-1*). **L-19**

Wheat Moisture Determinations. Material study report describes moisture determinations in wheat grain, wheat flour, and tempered wheat by Model 104 NMR Analyzer. Calibration curves included. Schlumberger Well Surveying Corp. (*Material Study Report 10.58*). **L-20**

Moisture Content in Urea. Material study report describes and gives calibration curves for measurement of moisture content in urea by Model 104 NMR Analyzer. Schlumberger Well Surveying Corp. (*Material Study Report 25.58*). **L-21**

Temperature-Millivolt Tables. New chart converts temperature to millivolt values for eight thermocouple calibrations. Thermo Electric Co., Inc. **L-22**

Laboratory Equipment. Four-page bulletin describes stirrers, heating tapes and mantles, steel beakers, and similar items. University Apparatus Co. **L-23**

Instruments for Testing. Four-page bulletin tells about selected testing instruments and applications for them. Thwing-Albert Instrument Co. (*Facts on Testing*). **L-24**

Semiconductor Preforms. Four-page bulletin describes semiconductor preforms for alloy junctions in germanium and silicon devices, and includes analyses of alloys and importance of minimal impurities. Accurate Specialties, Inc. (*Tech. Bull. A-68*). **L-25**

Laboratory Glassware. Forty-page catalog supplement lists new Pyrex and Vycor brand laboratory ware and includes about 700 custom made items. Corning Glass Works. (*Supplement 3*). **L-26**

Twin Film Service. Twelve-page pamphlet outlines Twin Film service, for providing regular reports on radiation dosimetry for personnel protection. Tracerlab, Inc. **L-27**

ACETATES

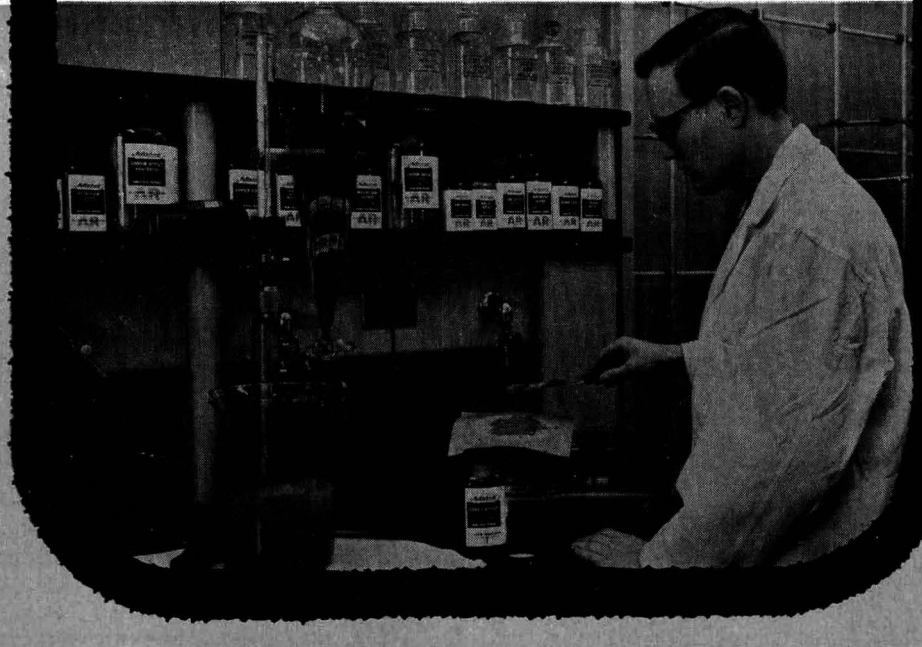
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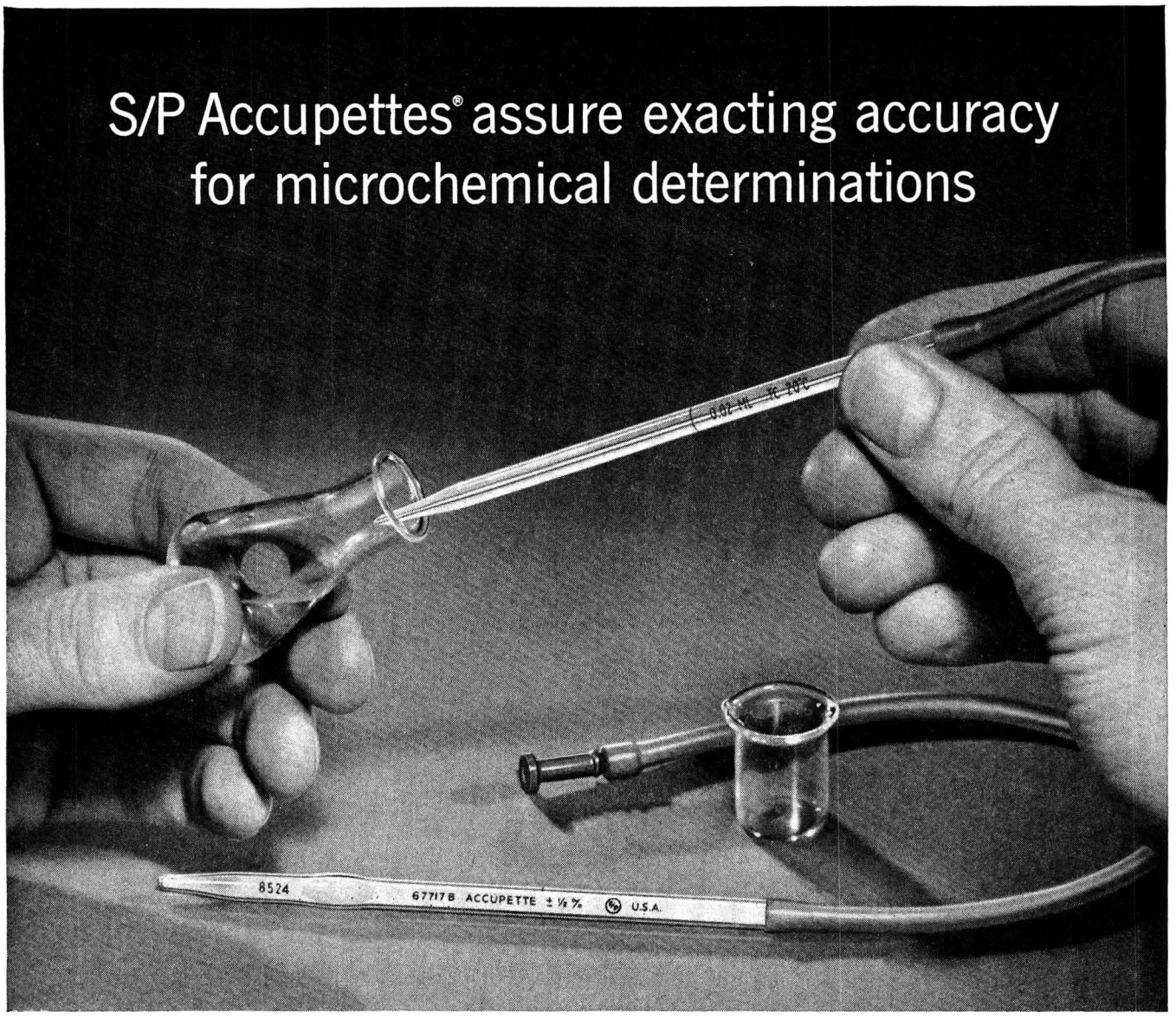
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For your convenience—short "refreshers" on last month's ads. Obtain free information on any of the products or services described by circling the corresponding key numbers in the "Product Capsules" portion of the reply card immediately preceding this section.

Accelerators. Bulletin on 400,000-volt "Van de Graaff" positive-ion accelerator that yields, 10^{10} neutrons per second. High Voltage Engineering Corp. **64A**

Analyzers. Bulletin on low cost gas chromatography vapor phase analyzer from Central Scientific Co. **10A**

Analyzers. Davis Instruments offers literature on continuous and portable gas analysis instruments. **18A**

Analyzers. 3 models from NRC Equipment Corp. analyze: oxygen, hydrogen, and nitrogen of most metals; hydrogen in titanium and zirconium; and hydrogen in steel. **25A**

Analyzers. Bulletin from Phoenix Precision Instrument Co. on amino acid analyzer for separation, identification and quantitative analysis. **76A**

Analyzers. 8-page brochure from Technicon Controls, Inc. on the "Auto-Analyzer" that provides continuous automatic chemical analyses. **84A**

Apparatus. Circle indicated numbers for bulletins on following Laboratory Glass & Instruments Corp. apparatus: universal model evaporator, 20A-1; evapo-mix, 20A-2; variable speed shaker, 20A-3; multi-dialyzer, 20A-4; and zone electrophoresis apparatus, 20A-5. **20A-1 to 5**

Apparatus. Circle indicated numbers for info on following Thermo Electric Mfg. Co. apparatus: furnace, 32A-1; temperature controller, 32A-2; hot plate, 32A-3; stir-plate, 32A-4; flask heater, 32A-5; and portable pyrometer, 32A-6. **32A-1 to 6**

Apparatus. Circle indicated numbers for bulletins on following Wm. Boekel & Co., Inc. apparatus: Pensky-Martens closed tester, 90A-1; water bath, 90A-2; pipet washer with rack, 90A-3; and pipet dryer, 90A-4. **90A-1 to 4**

Atomic Energy Books. Brochure describes volumes on the proceedings of the second international conference on the peaceful uses of atomic energy. United Nations. **69A**

Balances. Circle 6A for info on Wm. Ainsworth & Sons, Inc. automatic recording, analytical, semi-micro, and micro balances and weights. **6A**

Balances. Cahn Instrument Co. offers complete information on new electrobalance. **9A**

Balances. Bulletin on complete line of balances and weights offered by Ohaus Scale Corp. **46A**

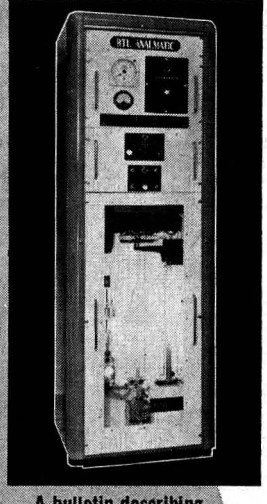
Balances. Circle 50A for complete file on Mettler Instrument Corp. products and services. Balances are based on the principle of weighing by substitution. **50A**

Balances. Circle 70A for complete catalog on Voland and Sons, Inc. line of balances. **70A**

(*Continued on page 112 A*)

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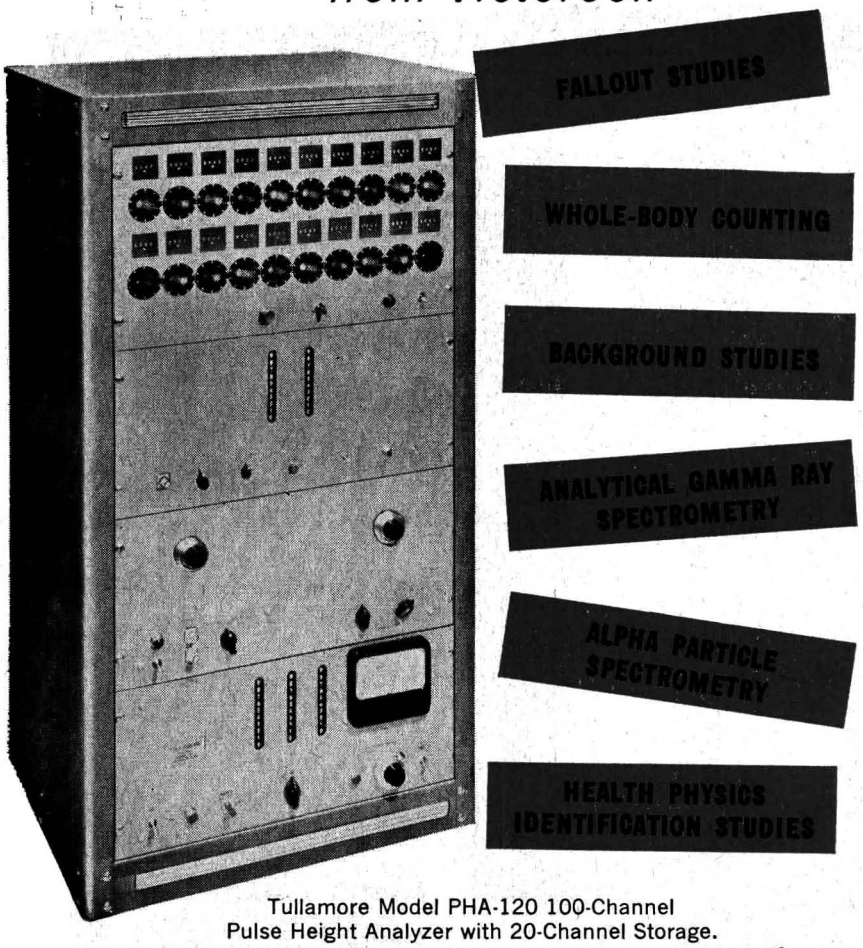
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Product Capsules

Blenders. New Patterson-Kelley Co., Inc. bulletin describes 4 "Twin-Shell" blenders: standard, yoke, intensifier, and liquid-solids. **67A**

Burets. Scientific Industries, Inc. offers bulletin on new ultra-buret. **30A**

Cells. Circle 35A for info on line of glass absorption cells made by Klett Mfg. Co. **35A**

Cells. Pyrocell Mfg. Co. offers info on fused optical quartz absorption cells for spectrophotometers. **66A**

Cells. Absorption cells available in pyrex, quartz, corex, and optical glass from Phoenix Precision Instrument Co. **77A**

Centrifuges. Clay-Adams offers info on an efficient, high-speed centrifuge for micro-analysis. **12A**

Centrifuges. Circle 27A for data from International Equipment Co. on new high-speed refrigerated centrifuge with automatic acceleration and added accessories. **27A**

Centrifuges. Circle 81A for catalog from Lourdes Instrument Corp., on automatic, super-speed refrigerated centrifuge. **81A**

Centrifuges. Ivan Sorvall, Inc. offers catalog on complete line of centrifuges and laboratory instruments. **51A**

Chromatography Apparatus. New F & M Scientific Corp. programmed temperature apparatus analyzes low, medium, and high boiling compounds from a single run. **15A**

Chromatography Papers. Circle 8A for samples of Whatman special papers for chromatography. H. Reeve Angel & Co. Inc. **8A**

Cleaners, Glassware. "Haemo-Sol" cleans lab glassware and apparatus. Circle 21A for sample and literature from Meinecke & Co., Inc. **21A**

Counters. Info from Coulter Industrial Sales Co. on counter that electronically amplifies, scales, and counts "particle-pulses." **53A**

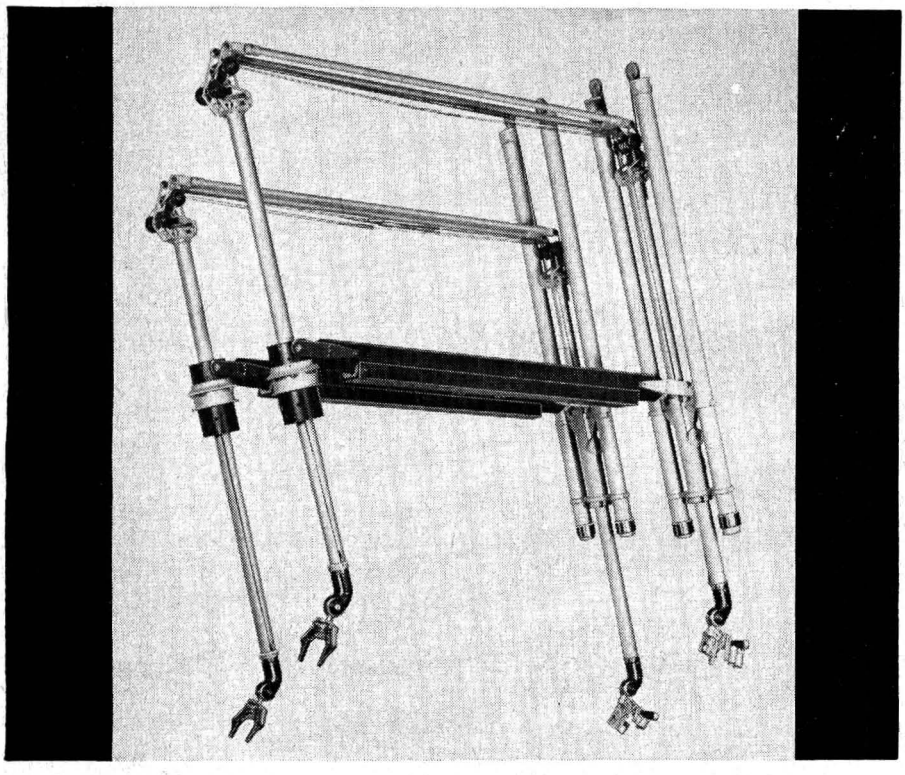
Crystals. Info from Crystals, Inc. on sodium iodide, sodium chloride, potassium bromide, anthracene, and stilbene crystals. **85A**

De-Ionizers. Illinois Water Treatment Co. offers info on clear plastic cartridge de-ionizer. **89A**

Detectors. Info from Gow-Mac Instrument Co. on detectors for vapor phase chromatography. **16A**

(*Continued on page 114 A*)

Handle Radioisotopes With Fingertip Precision



CRL's model 7 Master-Slave Manipulator

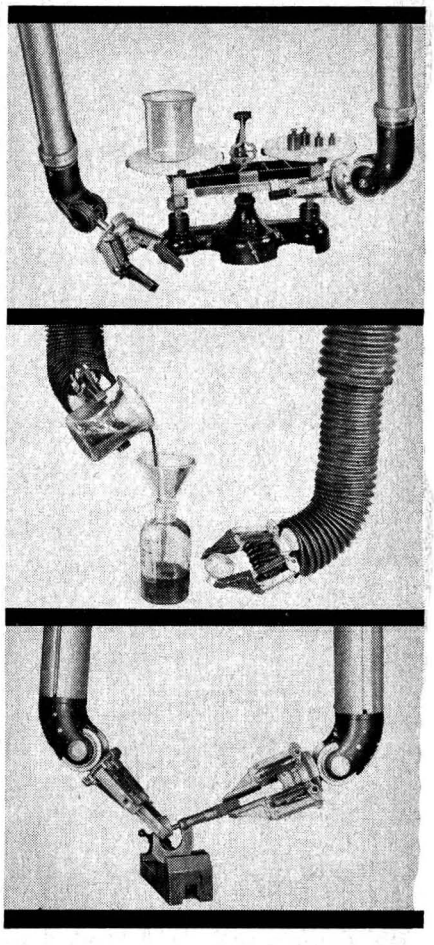
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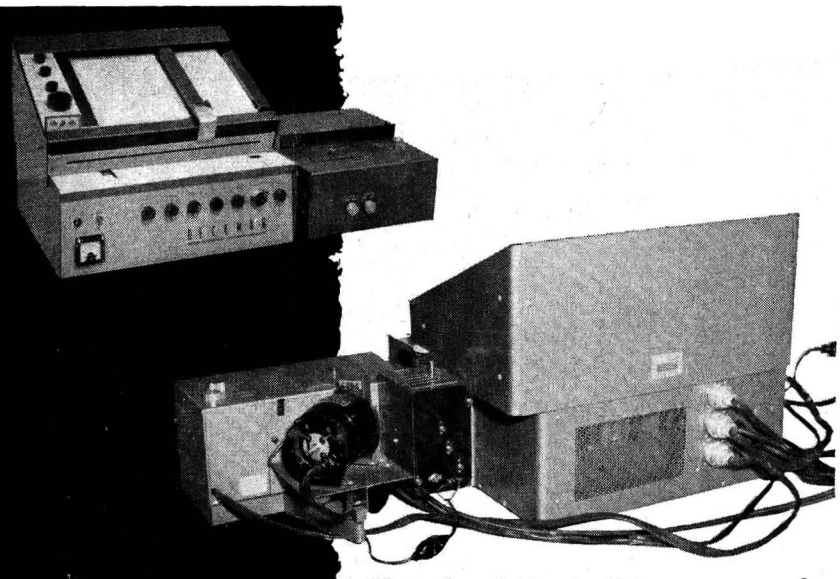
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Circle No. 102 on Readers' Service Card

Product Capsules

Electrodes. United Carbon Products Co. offers data on new preformed carbon electrodes. **34A**

Electrophoresis Apparatus. Bulletin on Kern Co. portable, speedy micro-electrophoresis apparatus. **73A**

Evaporators. Circle 19A-1 for bulletin on new portable flash evaporator. Circle 19A-2 for colorimeter bulletin. Laboratory Glass & Instruments Corp. **19A-1, 2**

Fluorimeters. Klett fluorimeters determine thiamin, riboflavin, and other substances which fluoresce in solution. Klett Mfg. Co. **60A-2**

Fluorine Gas. Circle 43A for Baker & Adamson product data sheet, "Handling Elemental Fluorine Gas in the Laboratory." General Chemical Div., Allied Chemical Corp. **43A**

Fluorometers. Circle 61A-1 for bulletin on photoelectric fluorometer. Circle 61A-2 for bulletin on spectrofluorometer. Farrand Optical Co., Inc. **61A-1, 2**

Freeze-Drying Unit. Scientific Products, Div. of American Hospital Supply Corp. offers catalog featuring a compact, portable, mechanically refrigerated freeze-drying unit. **7A**

Furniture. Circle 57A-1 for catalog on complete line of laboratory furniture and equipment. Circle 57A-2 for fume hood catalog. Duralab Equipment Corp. **57A-1, 2**

Furniture. Circle 83A for new catalog on "Steelab" laboratory furniture. Laboratory Furniture Co., Inc. **83A**

Generators. Texas Nuclear Corp. offers info on new low cost neutron generator. **4A**

Heating Equipment. Circle 37A for catalog on "Briskeat" flexible electric tapes, jackets and blankets. Briscoe Mfg. Co. **37A**

Instruments. Circle 1A for detailed specs on complete line of Carl Zeiss, Inc. scientific instruments. **1A**

Instruments. Circle indicated numbers for bulletins on following: line-operated pH meter model 110, 38A-1; line-operated pH meter model 115, 38A-2; battery-operated pH meter, 38A-3; reflection meter, 38A-4; electronic densitometer, 38A-5; multiplier fluorescence meter, 38A-6; colorimeter model 402-E, 38A-7; colorimeter model 450, 38A-8; and colorimeter model 401, 38A-9. Photovolt Corp. **38A-1 to 9**

(Continued on page 116 A)

Klett . . .

Photometers

Klett-Summerson

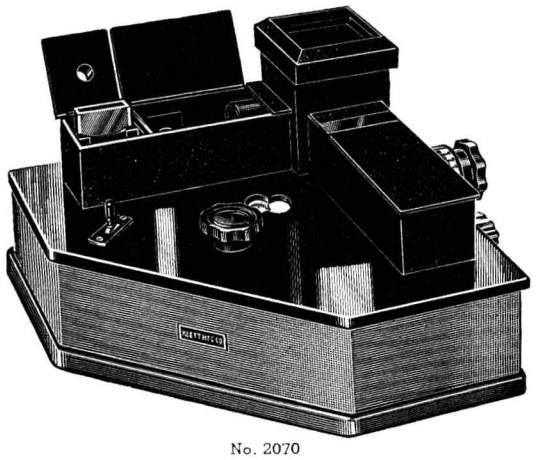


Photoelectric Glass Cell Colorimeter

No. 900-3

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The Klett Fluorimeter



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Designed for the rapid and accurate determination of thiamin, riboflavin, and other substances which fluoresce in solution. The sensitivity and stability are such that it has been found particularly useful in determining very small amounts of these substances.

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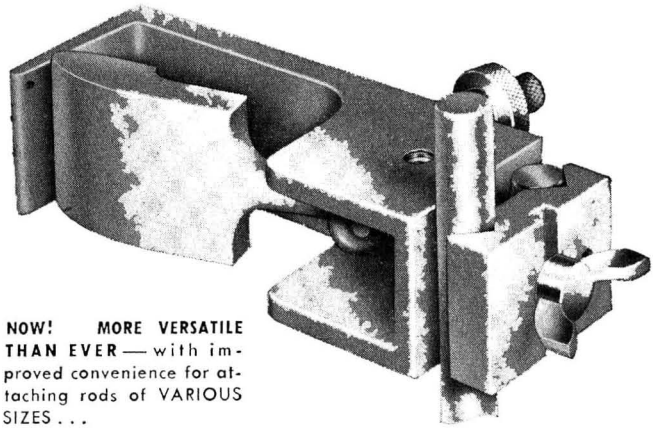
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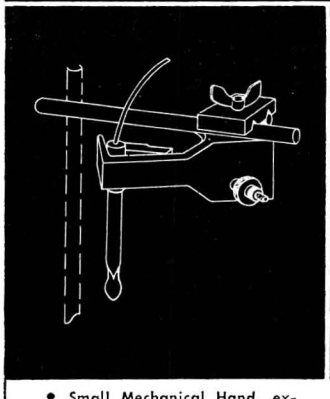
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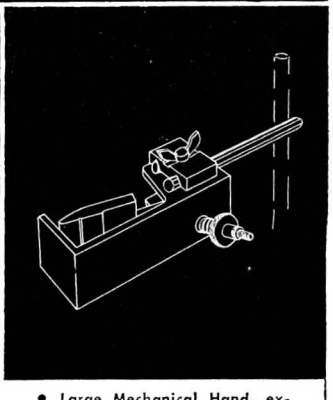
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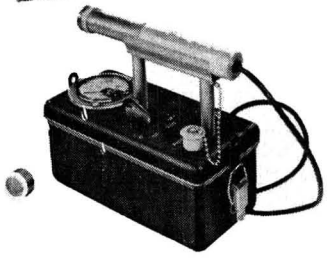
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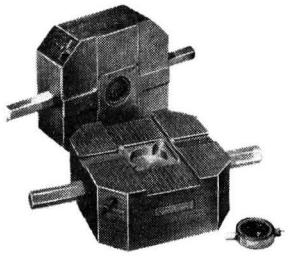
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Product Capsules

Instruments. Circle indicated numbers for info on following: model 221 double-beam spectrophotometer, 71A-1; vapor fractometer, 71A-2; infracord spectrophotometer, 71A-3; model 21 double-beam infrared spectrophotometer, 71A-4; universal spectrometer, 71A-5; model 4000-A spectrophotometer, 71A-6; flame photometer, 71A-7; and Tiselius electrophoresis, 71A-8. Instrument Div., Perkin-Elmer Corp. **71A-1 to 8**

Melting Point Apparatus. Info from Arthur H. Thomas Co. on capillary melting point apparatus with periscopic thermometer reader. **33A**

Microscopes. Chicago Apparatus Co. offers data sheet on its x-ray microscope. Magnifications to 1500X. **45A**

Moisture Testers. Circle 40A for bulletins and reprints of published applications on the C. W. Brabender volatiles moisture tester. **40A**

Mortars and Pestles. Coors Porcelain Co. mortars and pestles are available in 6 styles and 49 sizes. **13A**

Oxygen Bombs. Circle 39A for info on Parr Instrument Co. self-sealing oxygen bombs in 6 styles. **39A**

Photochemical Equipment. Engelhard Industries, Inc. offers 16-page brochure, "Photosensitization," that details facts on Hanovia Lamp Div. photochemical equipment. **68A**

Photometers. New Phoenix Precision Instrument Co. universal light scattering photometer measures absolute turbidity, dissymmetry, depolarization. **79A**

Photometers. Info on new accessories for Phoenix Precision Instrument Co. light scattering photometer that permits measurements of materials solid at room temperature. **80A**

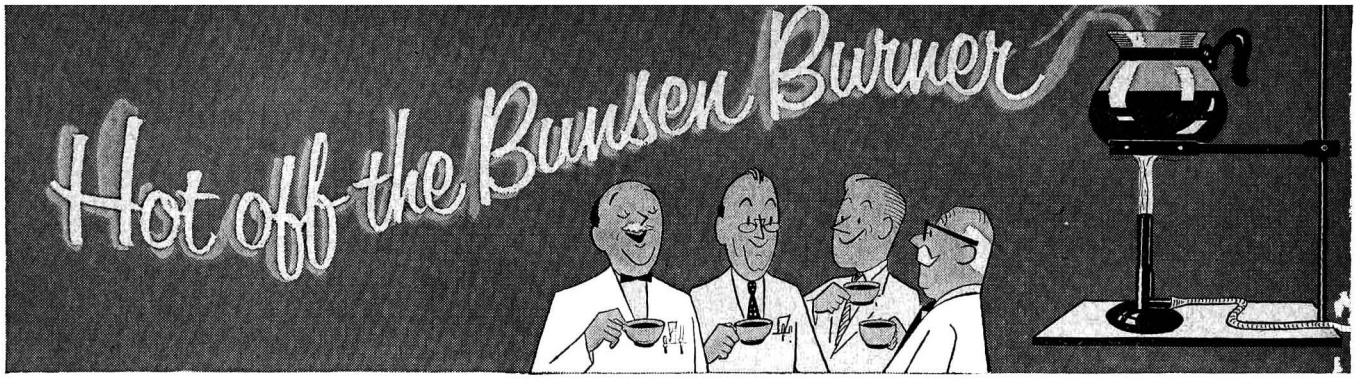
Pipet Fillers. Flexible bulb "Propipette" holds set level, fits any pipet, operates with one hand. Instrumentation Associates. **86A**

Polarographs. Standard Scientific Supply Corp. offers info on the cathode ray "Polarotrace" with electrode stand. **31A**

Presses. Literature from Fred S. Carver Inc. on standard 10 ton press and 20 ton models. **47A**

Reagents. Circle indicated numbers for following: 16-page booklet, "Column Partition Chromatography," 22A-1; reagents for washing semiconductors, 22A-2; ethylenediaminotetraacetic acid, 22A-3; and Schain test reagent, 22A-4. Merck & Co., Inc. **22A-1 to 4**

(Continued on page 118 A)



Among chemists, the morning coffee-break is a well-established custom. During these informal symposia worthwhile data often are exchanged. We should like to sit in and tell you about some of our many hundreds of MERCK LABORATORY CHEMICALS.

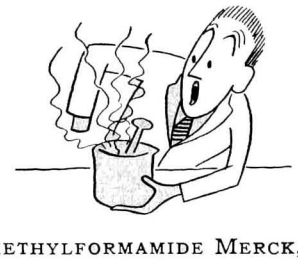
COLLECTOR'S ITEM



Human foibles know no bounds. Some people like to read dictionaries; others collect catalogs. For the latter we'd like to call attention to the latest edition of the MERCK LABORATORY CHEMICALS CATALOG. This opus, our twentieth in fifty years, is physically bigger (6" x 9" pages), easier to read, and arranged according to A.C.S. nomenclature.

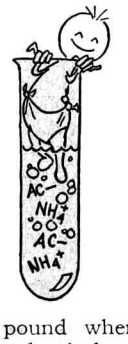
For the edification of our friends, the catalog gives specs on all our reagents, including many organic solvents. Ranges of useful transmittance are listed for the spectro solvents. This year's catalog is an extra-galactic effort. Let us know how many copies you need and they will be sent to you—gratis, of course.

UNIVERSAL ORGANIC SOLVENT



DIMETHYLFORMAMIDE MERCK, like money, has unusually wide appeal. Its low evaporation rate and ability to lure complex organics into solution have made it popular with all kinds of chromatographers. Feed chemists on the other hand report Dimethylformamide is frankly indispensable in the determination of NiCarbazin, a new type of drug used to prevent coccidiosis in poultry. Also, protein chemists are intrigued—finding Dimethylformamide with its high dielectric properties dandy as a reaction solvent for amino acids. In fact, if it's organic and tough to dissolve, reach for the Dimethylformamide and watch your fingers, Doctor! Skeptics should send for our technical data sheet.

AMMONIUM ACETATE—PRECURSOR OF LIFE?

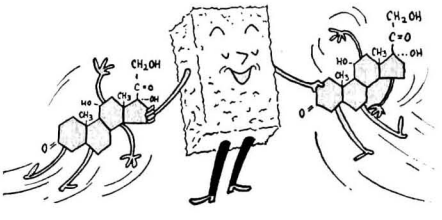


It's been postulated that inanimate matter made the jump to life via the effects of ionizing radiation on solutions of simple chemical compounds. An interesting bit of evidence to support this concept involves the use of Merck Ammonium Acetate. Aqueous dilute solutions of this relatively simple compound when exposed in ubiquitous polyethylene bags to beta-radiation of 2, 10, 20, 30 and 50 Mrep in a van de Graff electron accelerator resulted in the synthesis of glycine, aspartic acid and an unknown amino acid believed to be diaminosuccinic acid.

Though yields of these AA-inspired amino acids could hardly be described as heavy, they were anything but intangible. This is not to imply that under certain circumstances Ammonium Acetate does not have an ephemeral side. Used as an eluting agent for ion-exchange resins, AA leaves no residue on evaporation—a happy, practical thought for chromatographers.

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Product Capsules

Reagents. G. Frederick Smith Chemical Co., Inc. offers info on analytical reagents, specialty chemicals, and primary standards. **56A**

Reagents. Eastern Chemical Corp. offers info on complete line of organic reagents. **88A**

Reagents. Circle 62A-1 for info on polarographic reagents, 62A-2 for info on tetra-*n*-butylammonium hydroxide, and 62A-3 for solution of sodium biphenyl. Southwestern Analytical Chemicals. **62A-1, 2, 3**

Recorders. E. H. Sargent & Co. offers bulletin on multi-purpose recorder that measures current and voltage and all other quantities which can be transposed into potential or current signals. **28A**

Refractometers. Bulletin on new Phoenix Precision Instrument Co. automatic process refractometer that provides end-point control through continuous stream analysis. **74A**

Refractometers. Bulletin on Phoenix Precision Instrument Co. automatic recording chromatographic refractometer. **75A**

Refractometers. Circle 78A for info on new Phoenix Precision Instrument Co. differential refractometer. **78A**

Refrigerated Bath. Bulletin from Wilkens-Anderson Co. on refrigerated bath for controlled cooling and heating. —10° C. to +65° C. **54A**

Sample Changers. Packard Instrument Co., Inc. offers bulletin on "Auto-Gamma" sample changer that provides completely automatic scintillation counting. **26A**

Scintillation Phosphors. Circle 49A-1 for 40-page book on scintillation phosphors. Circle 49A-2 for 36-page book on optical crystals. The Harshaw Chemical Co. **49A-1, 2**

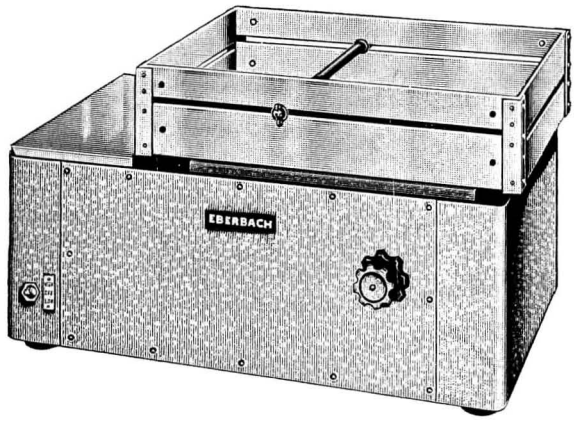
Spectrographs. Philips Electronics, Inc. offers information on portable x-ray spectrograph that provides element identity, alloy sorting, and plating thickness. **87A**

Spectrography Equipment. Bausch & Lomb Optical Co. offers info on its complete line of spectrography equipment and service. **63A**

Spectrometers. Circle 3A for catalog on single channel, automatic, and multichannel scintillation spectrometers from Baird-Atomic, Inc. **3A**

(Continued on page 120 A)

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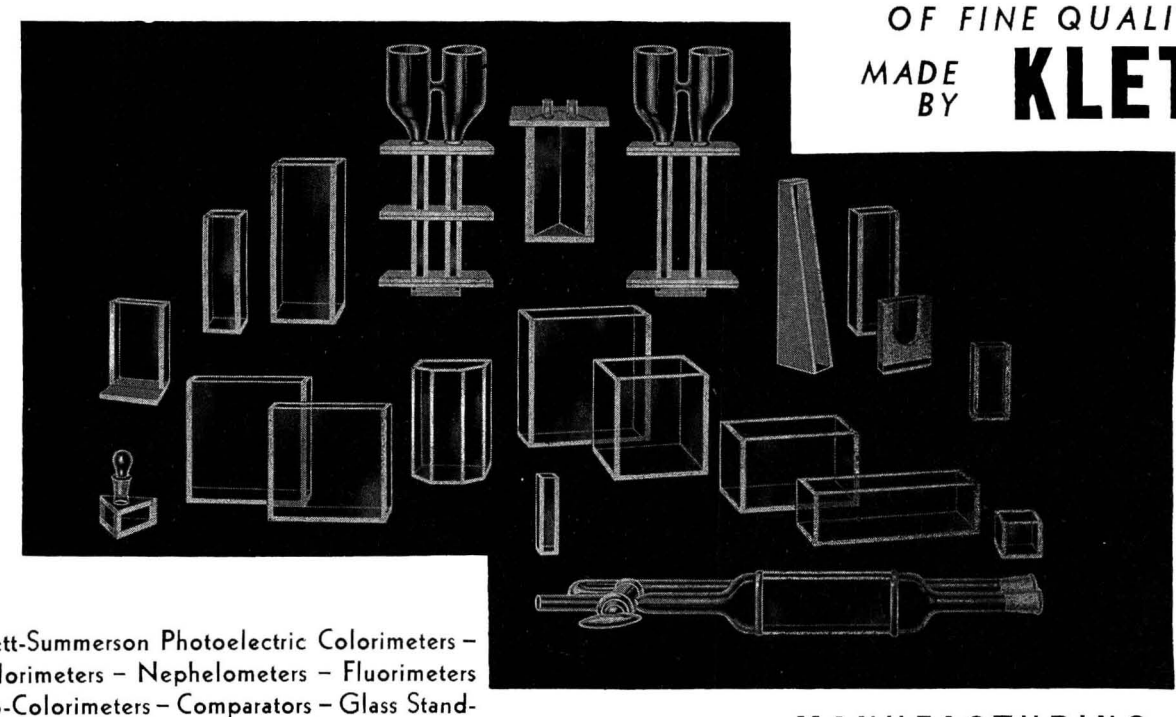
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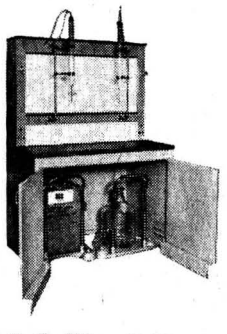


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Product Capsules

Spectrophotometers. New Perkin-Elmer Corp. model 4000-A spectrophotometer features double monochromator, and micro-potentiometer 100% line compensation system. Circle 72A for complete data. **72A**

Stopcocks. General Laboratory Supply Co. offers info on the "Tapnik" diaphragm plastic buret stopcock. **44A-1**

Supplies. Harshaw Scientific offers a one source market for furniture, glassware, apparatus, chemicals or equipment. **2A**

Supplies. Scientific Glass Apparatus Co. Inc. offers info on its apparatus, instruments, glassware, chemicals, supplies, and repairs. **29A**

Supplies. Will Corp. offers complete planning service in selecting laboratory apparatus. **82A**

Supplies. Circle 58A for Macalaster Bicknell house organ, "MBCO News." **58A**

Supplies. New York Laboratory Supply Co., Inc. serves industry with all laboratory requirements. Circle 52A for info on instruments and apparatus. **52A**

Thermometers. Atkins Technical, Inc. offers info on the "Thermophil," a thermistor thermometer. **55A-1**

Thermometers. Tri-R Instruments offers catalog covering electronic thermometers and compact magnetic stirrers. **42A-1**

Titrators. The Emil Greiner Co. offers info on Manostat microtitrator that detects end point electrometrically. **17A**

Titrators. Bulletin on the Central Scientific Company's new "Color Matic" end point detecter and volumatic syringe. **11A**

Ware, Glass. Circle 5A for literature on the complete line of Ace Glass Inc. laboratory glassware. **5A**

Ware, Glass. Corning Glass Works offers supplement No. 3 to its full line catalog. Lists 912 new Pyrex brand glassware products. **14A**

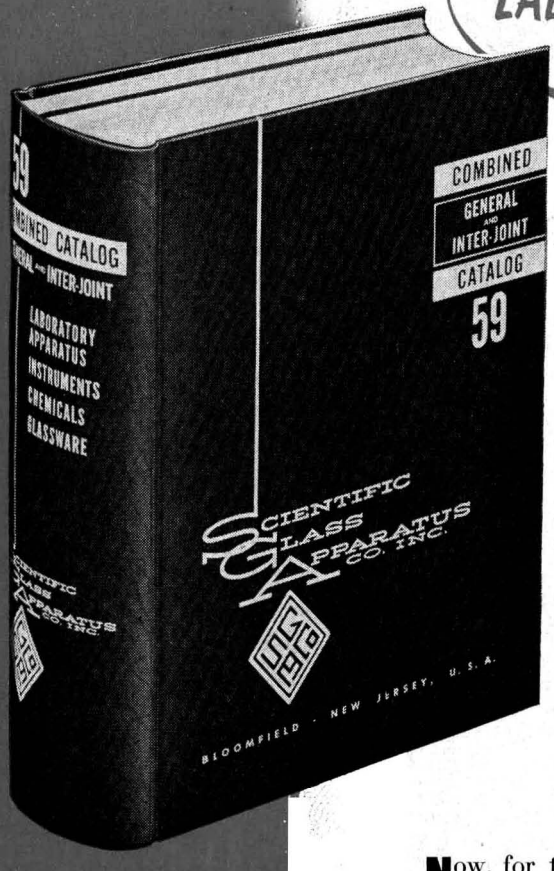
Ware, Plastic. Circle 23A for the Nalge Co., Inc. catalog on unbreakable, chemically resistant "Nalgene" labware. **23A**

Ware, Plastic. Bel-Art Products offers catalog on its complete line of plastic laboratory ware. **36A**

Ware, Fused Quartz. Technical data on "Vitreosil" fused quartz ware from Thermal American Fused Quartz Co., Inc. **24A**

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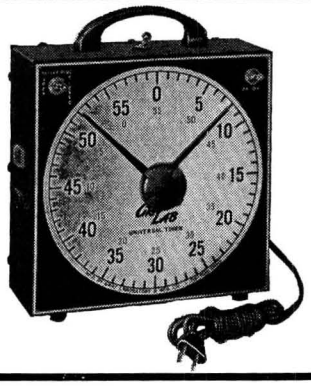
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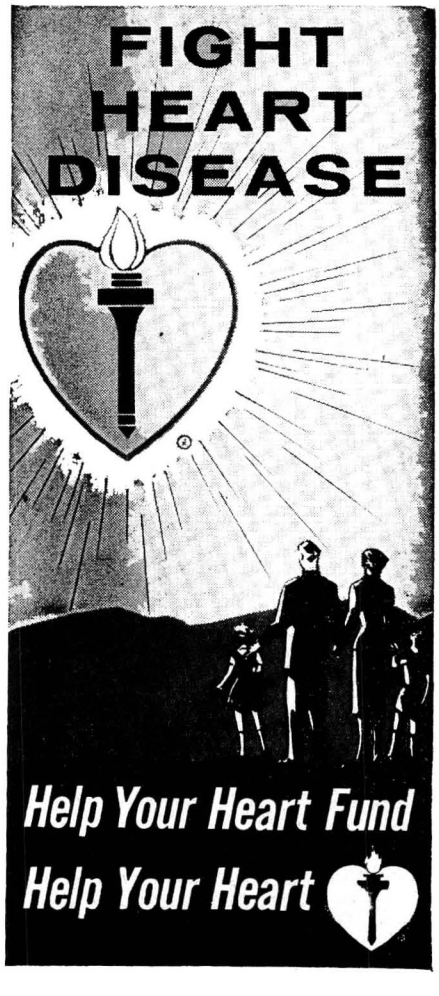
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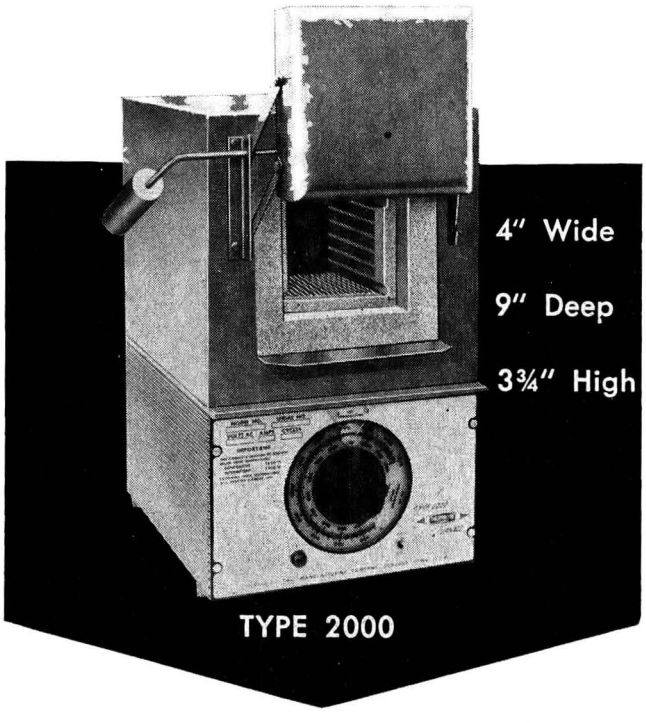
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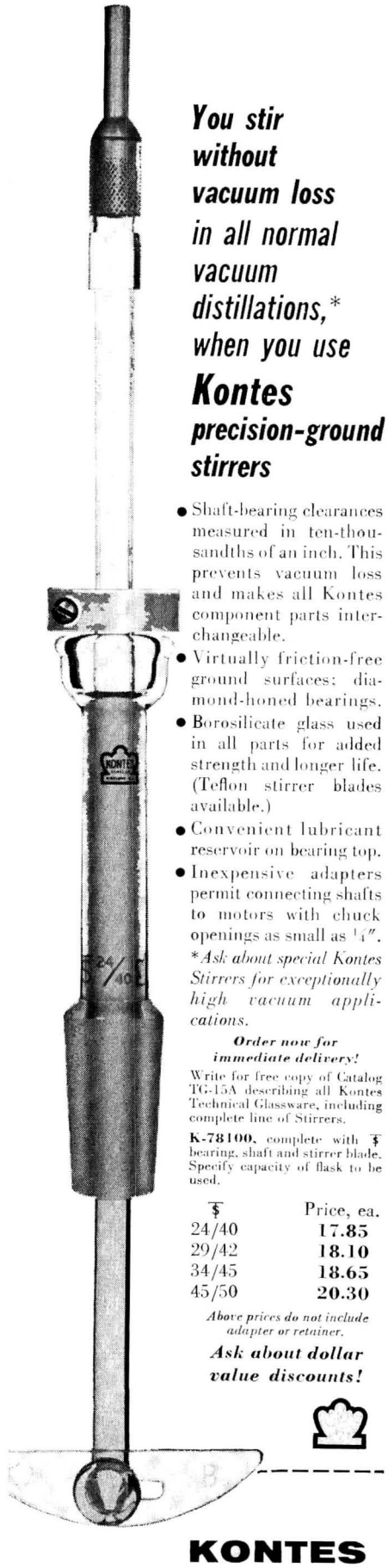
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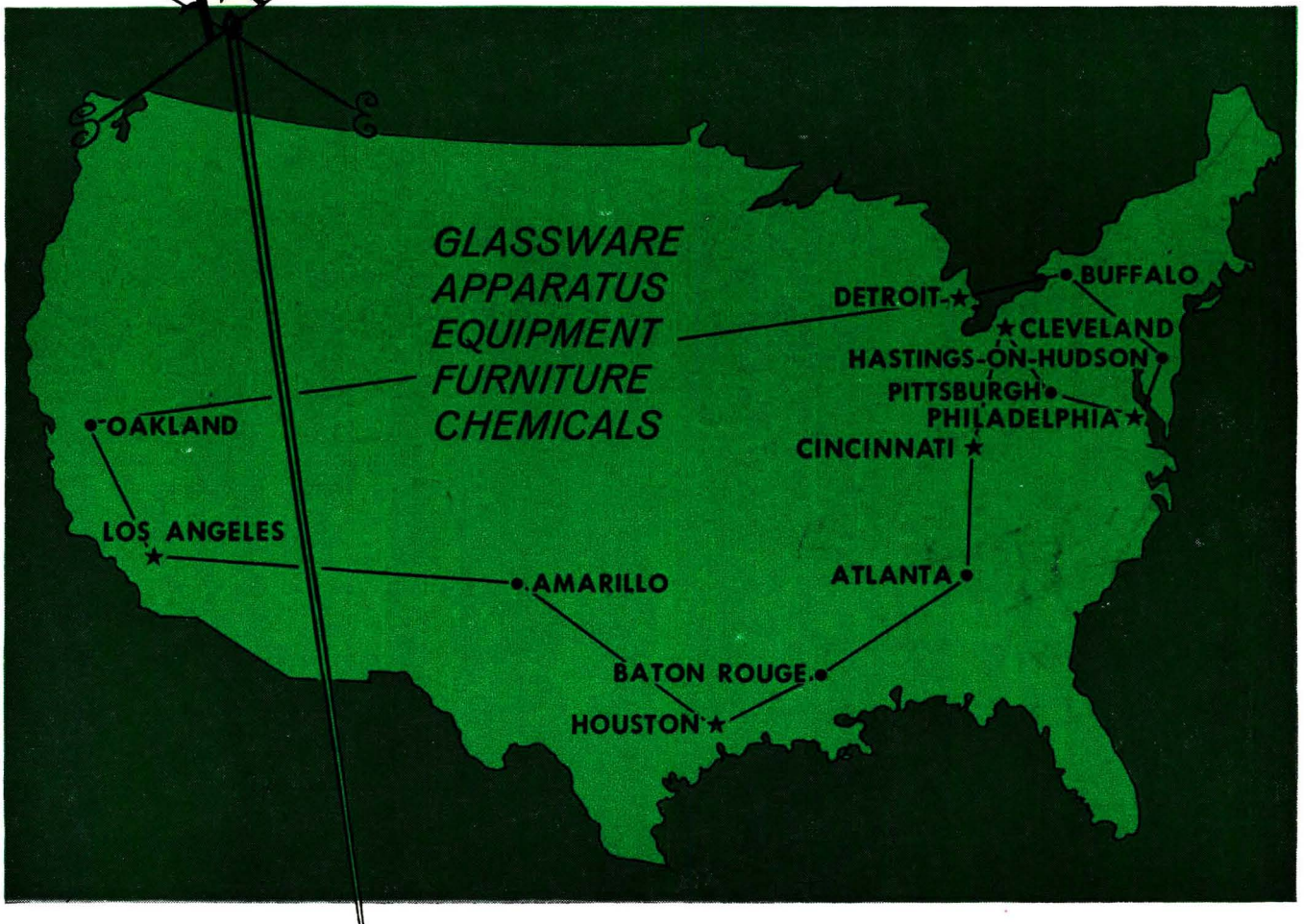
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