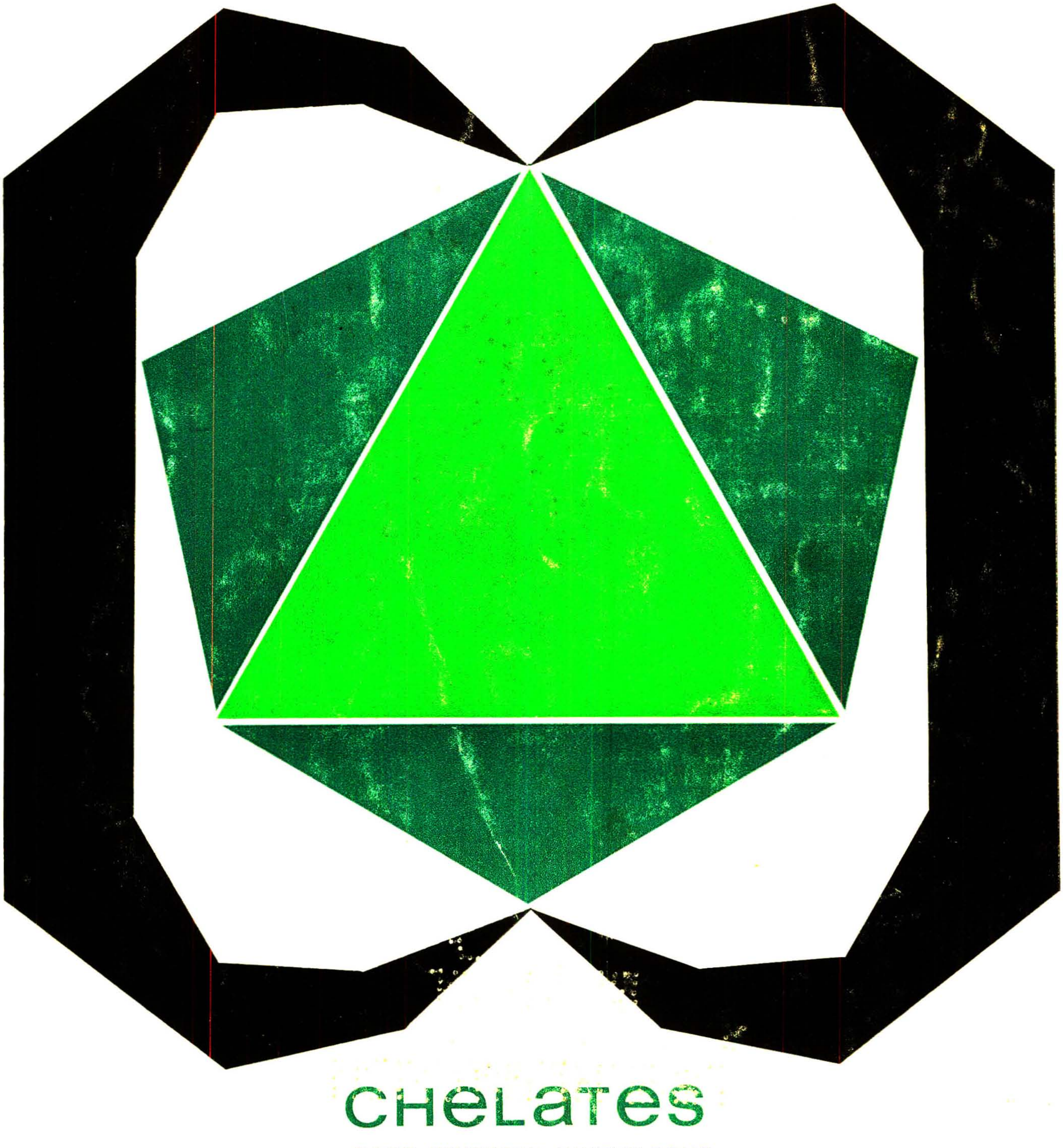
DECEMBER 1963

ANALYTICAL CHEMISTRY



CHELATES

PAGE NINETEEN NINETY-FOUR

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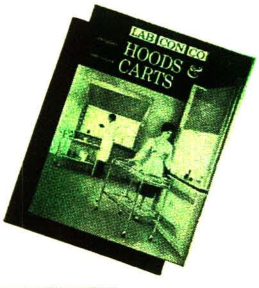

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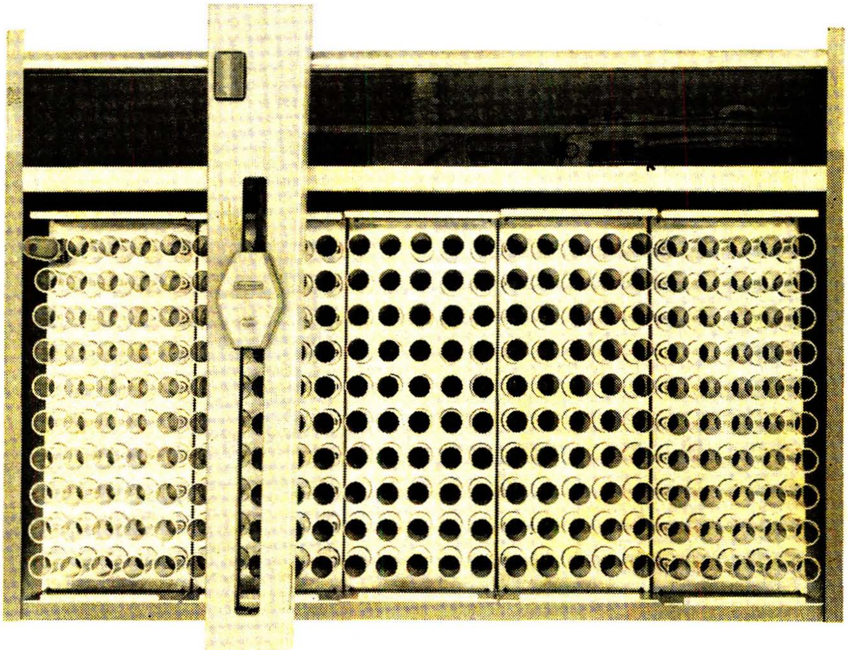

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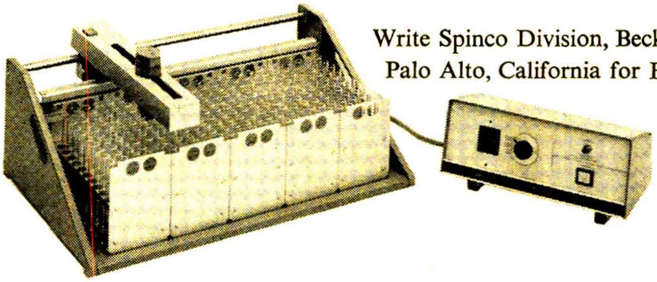

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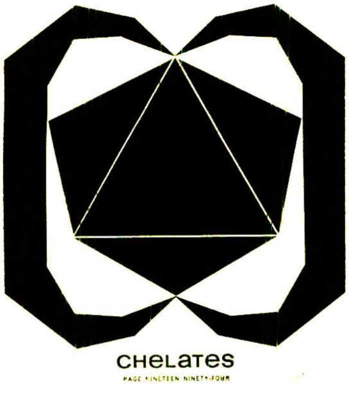
VOLUME 35, NO. 13 DECEMBER 1963

ANALYTICAL CHEMISTRY



This Month's Cover is based on the theme of the 16th Annual Analytical Summer Symposium: metal chelates. A summary of this meeting, held in June at The University of Arizona, begins on page 1994.

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Issued November 22, 1963

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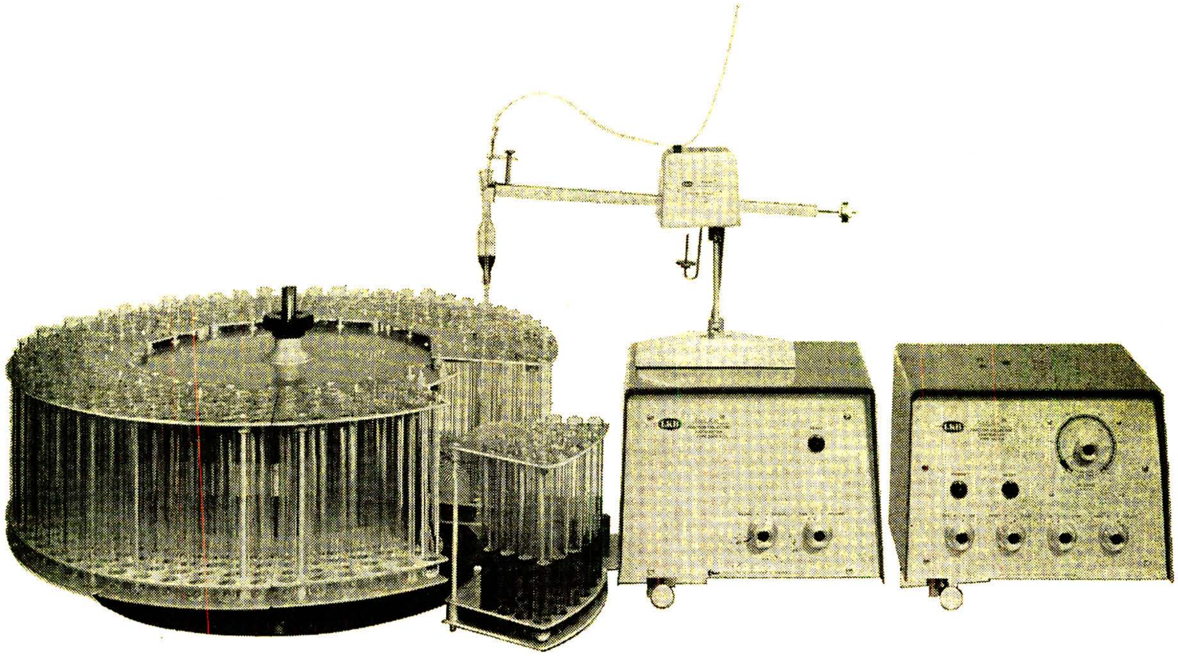

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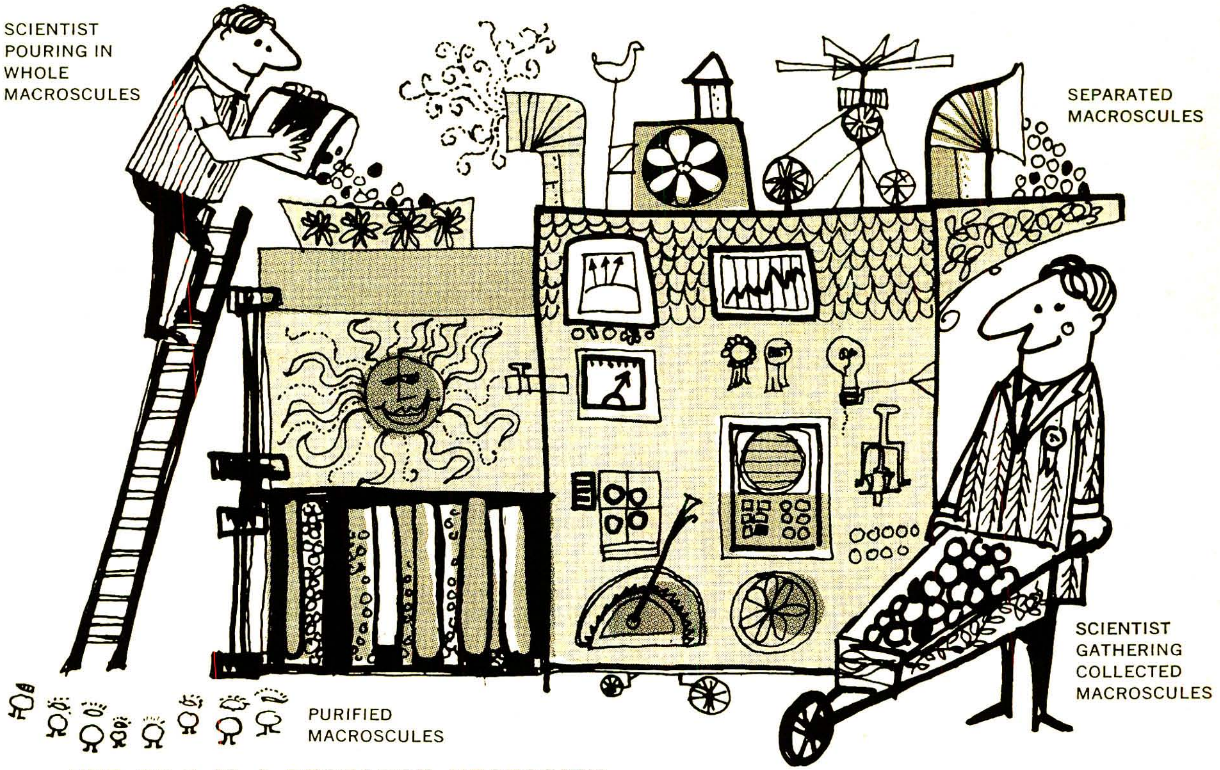

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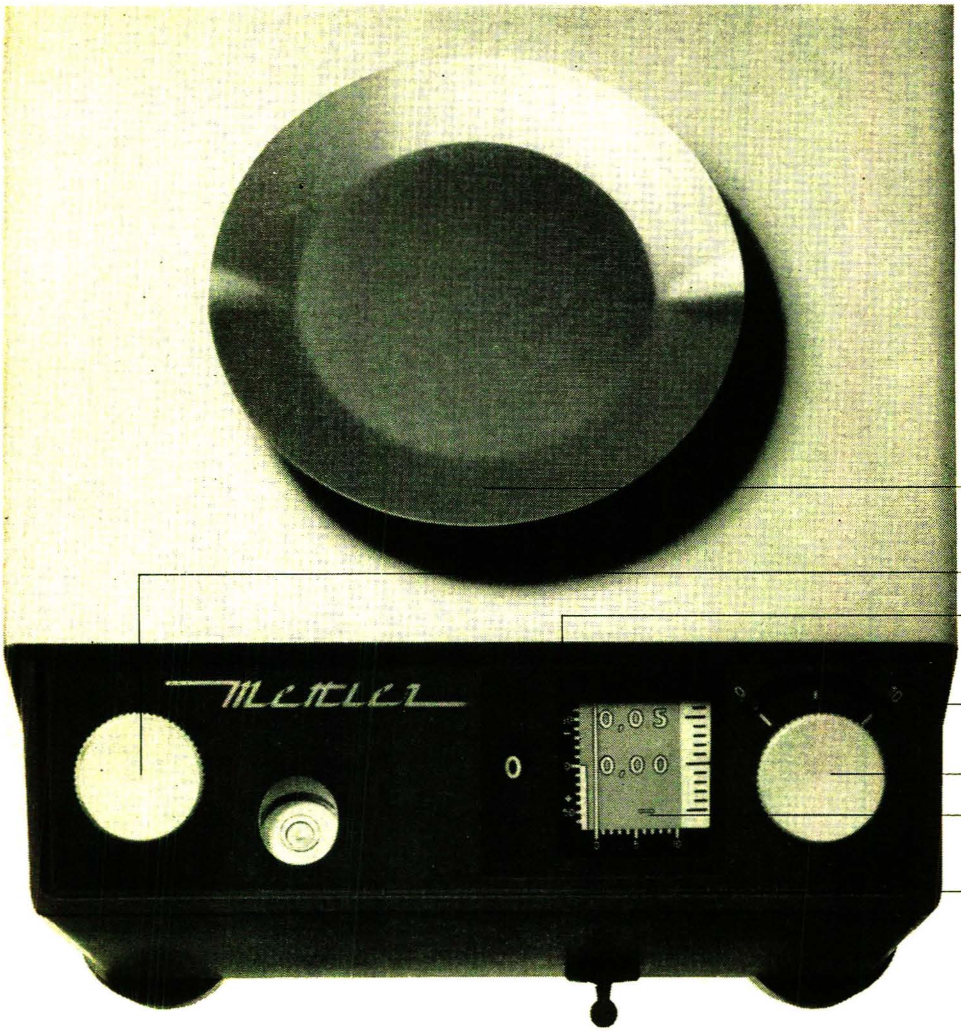
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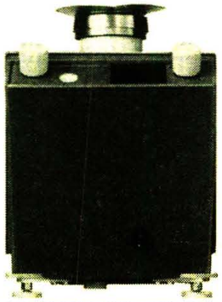

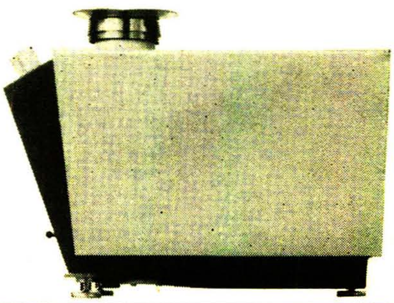

AC BRIEFS

Highlights of the scientific and technical articles in this issue

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Metal Chelates in Analytical Chemistry. A Report of the 16th Annual Analytical Symposium

Various aspects of the role of metal chelates in analytical chemistry are discussed. Charged metal chelates are used in titrimetry, as masking agents, and in ion exchange separations. Uncharged metal chelates are the basis of separations by precipitation, solvent extraction, and chromatography. Colorimetric, spectrophotometric, and radiochemical procedures for metal ions are outlined.

QUINTUS FERNANDO, HENRY FREISER, and EDWARD N. WISE, Department of Chemistry, University of Arizona, Tucson, Ariz.

Anal. Chem. 35, 1994 (1963)

Kinetic Origin of Tailing in Chromatography

Equations are given for an idealized model for tailing. Concentration profiles are obtained and plotted for both elution and nonelution chromatography. Tailing may originate when a sorption site exists which holds molecules for a time equal to that necessary for one quarter of the zone to pass by.

J. CALVIN GIDDINGS, Department of Chemistry, University of Utah, Salt Lake City 12, Utah

Anal. Chem. 35, 1999 (1963)

Fluorine-Containing Polymers as Solid Supports in Gas Chromatography

To establish conditions for optimum separation, the effects of flow rate, temperature, sample size, type of polymer, and both kind and concentration of liquid phase were studied. Superior peak symmetry can often be obtained for highly polar compounds using fluorocarbon and chlorofluorocarbon supports.

J. J. KIRKLAND, Industrial and Biochemicals Department, E. I. du Pont de Nemours & Co., Wilmington, Del.

Anal. Chem. 35, 2003 (1963)

Contribution of Interfacial Resistance to Band Broadening in Chromatography

The contribution of the resistance to mass transfer at the interface to the plate height has been evaluated for the basic forms of chromatography. It is shown that with the exception of liquid-liquid chromatography, this effect is negligible in comparison with other band-broadening mechanisms normally encountered.

G. J. KRIGE and VICTOR PRETORIUS, Department of Physical and Theoretical Chemistry, University of Pretoria, Pretoria, South Africa

Anal. Chem. 35, 2009 (1963)

A Combustion–Gas Chromatographic Method for the Simultaneous Determination of Carbon and Sulfur in Ferrous Metals

Carbon and sulfur are determined on a silica gel column after combustion. Combustion products are eluted by temperature programming. A thermal conductivity detector and an integrator are used for detection and recording. Complete analysis time is 17 minutes. Standard deviations ranged from 0.02 to 0.001 for carbon in the 0.011 to 3.28% range. Sulfur compositions from 0.011 to 0.329% gave standard deviations of 0.001 to 0.012.

W. K. STUCKEY and J. M. WALKER, Department of Chemistry, Kansas State College of Pittsburg, Pittsburg, Kan.

Anal. Chem. 35, 2015 (1963)

Carbon Determination in Ferrous Metals by Gas Chromatography

The method permits detection of 0.0005% carbon. Eight different NBS steel and iron samples with carbon contents between 0.011 to 3.28% were run. The time required is 20 minutes per sample.

J. M. WALKER, and C. W. KUO, Department of Chemistry, Kansas State College of Pittsburg, Pittsburg, Kan.

Anal. Chem. 35, 2017 (1963)

Programmed Gradient Elution Chromatography with the Steroid Analyzer

A gradient pumping system which permits the polarity of the eluting solvent to be varied at will is used for the controlled separation of adrenocortical hormones. Examples are given of the use of selected programmed gradients with seven adrenocortical hormones and beef adrenal extract.

DANIEL FRANCOIS, DAVID F. JOHNSON, and ERICH HEFTMANN, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Md.

Anal. Chem. 35, 2019 (1963)

Quantitative Analysis of Aromatic Hydrocarbons by Capillary Gas Chromatography

Quantitative analysis of C_6 through C_{11} aromatics is accomplished using polar substrates in capillary columns. Comparative elution data and spectra of several polar substrates are given. The study includes over 30 available compounds in the previously mentioned range.

JOHN Q. WALKER, Barber-Colman Co., Pasadena, Tex., DAN L. AHLBERG, Research Division, Signal Oil and Gas Co., Houston 12, Tex.

Anal. Chem. 35, 2022 (1963)

Qualitative Analyses of Naphthalenes by Capillary Gas Chromatography

The analyses of naphthalenes, monosubstituted naphthalenes, and disubstituted naphthalenes through C_{12} were investigated using combination analytical techniques. A gas chromatograph with capillary columns, a high temperature flame ionization detector, and a special heated inlet system resolved the 15 naphthalene compounds investigated.

JOHN Q. WALKER, Barber-Colman Co., Pasadena, Tex., DAN L. AHLBERG, Research Division, Signal Oil and Gas Co., Houston, Tex.

Anal. Chem. 35, 2028 (1963)

Column Partition Chromatography of Vitamins A and D on Fluoropak 80

The stationary phase is Fluoropak 80 impregnated with iso-octane and the mobile phase is aqueous methanol. The system is reversed-phase column chromatography. Examples of the use of this column for the separation of vitamins A from D are given.

PHILIP S. CHEN, Jr., A. RAYMOND TEREPKA, and NANCY REMSEN, Department of Radiation Biology, University of Rochester, N. Y.

Anal. Chem. 35, 2030 (1963)

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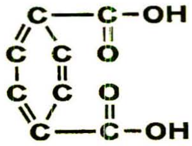

Terephthalic acid

Form ....*white powder*
Melting point ....*300°C. (sublimes)*
Acid number *672-676*

Surely the sun hasn't shone on the last thing in terephthalic acid polymers. For those still confident and searching, we use this space to let you know that we have it available commercially.

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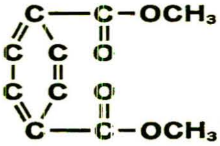

Dimethyl terephthalate

Form ....*white solid*
Melting point ....*140.6°C.*
Free acidity (as TPA) *0.05%*

As companion to B47 we offer its daughter for those who do not wish to bother preparing their own dimethyl ester. The small, neat, white briquettes make it handy in reactions such as transesterifications with polyhydric alcohols to produce thermoplastic polyesters.

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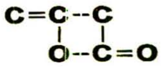
Kingsport, Tennessee **B48**



Diketene

Form ....*colorless liquid*
Boiling point, 760 mm. ....*127°C.*
Solubility ....*organic solvents*

When talk gets around to reactive chemicals, someone always mentions diketene. With good reason—there isn't much it won't react with. Promising for synthesis of dyes, drugs, pesticides.

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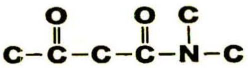

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N,N-Dimethyl acetoacetamide

Form ....*liquid*
Boiling point, 760 mm. ....*220°C.*
Assay ....*98.0% min.*

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C–C=C–C–OH

Crotyl alcohol

Form ....*colorless liquid*
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Boiling point, 760 mm. ....*121-122°C.*

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Kingsport, Tennessee **B55**

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Selective Separation of Polycyclic Aromatic Compounds by Countercurrent Distribution with a Solvent System Containing Tetramethyluric Acid

Distribution coefficients measured for other representative polycyclic aromatic hydrocarbons and heterocyclic compounds indicate the general utility of the separation method.

JAMES D. MOLD, THOMAS B. WALKER, and LEE G. VEASEY, Research Department, Liggett and Myers Tobacco Co., Durham, N. C.

Anal. Chem. 35, 2071 (1963)

A General Method for the Chromatographic Separation of Nonionic Surface-Active Agents and Related Materials

Separation is achieved using silica gel as adsorbent. Elution is accomplished successively with chloroform and mixtures of chloroform and ethyl ether, acetone, or methanol. Results obtained with a number of three- and four-component mixtures are described. General rule that more hydrophilic material requires more polar solvent to elute is followed.

MILTON J. ROSEN, Department of Chemistry, Brooklyn College of the City University of New York, Brooklyn, N. Y.

Anal. Chem. 35, 2074 (1963)

Separation and Determination of Iron(II) and Iron(III) with Anthranilic Acid Using Solvent Extraction and Spectrophotometry

Iron(III) forms a precipitate with anthranilic acid which can be extracted into organic liquids, leaving iron(II) behind. Absorbance of the iron(III) anthranilate is proportional to the amount of iron(III) present. Relative error for the method is 2%.

DONALD L. DINSEL and THOMAS R. SWEET, Department of Chemistry, McPherson Chemical Laboratory, The Ohio State University, Columbus 10, Ohio

Anal. Chem. 35, 2077 (1963)

Liquid-Liquid Extraction of Cesium with 2-Thenoyltrifluoroacetone

Recent developments for the inhibition of hydrolysis of 2-thenoyltrifluoroacetone provide the basis for quantitative extraction of cesium under a variety of conditions. Method is applicable to either trace or macro quantities of cesium. Applications for both analytical chemist and separations technologist are proposed.

PETER CROWTHER and FLETCHER L. MOORE, Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Anal. Chem. 35, 2081 (1963)

Adsorption of the Elements on Inorganic Ion Exchangers from Nitrate Media

The distribution coefficients of 60 metal ions were measured for hydrous zirconium oxide, zirconium phosphate, zirconium tungstate, and zirconium molybdate ion exchangers from nitrate media over the pH range of 1 to 5. Separations of analytical interest are given.

WILLIAM J. MAECK, MAXINE E. KUSSY, and JAMES E. REIN, Atomic Energy Division, Phillips Petroleum Co., Idaho Falls, Idaho

Anal. Chem. 35, 2086 (1963)

The Application of Stripping Analysis to the Determination of Silver(I) Using Graphite Electrodes

The wax-impregnated graphite electrode was used in these studies. The method was applied to solutions of silver ion as dilute as $4.0 \times 10^{-9}M$. The general characteristics of graphite electrodes were evaluated.

S. P. PERONE, Department of Chemistry, Purdue University, Lafayette, Ind.

Anal. Chem. 35, 2091 (1963)

Polarographic Determination of Nitrite as 4-Nitroso-2,6-xylenol

The described method permits the simultaneous determination of nitrite and nitrate in samples containing a large excess of the latter. The overall reproducibility of the method is less than 1% (expressed as relative standard deviation).

A. M. HARTLEY and R. M. BLY, Department of Chemistry and Chemical Engineering, University of Illinois, Urbana, Ill.

Anal. Chem. 35, 2094 (1963)

The Tubular Platinum Electrode

The construction and operation of a tubular platinum electrode are described. Electrochemical measurements can be made on a solution flowing through it. Sensitivity is high, electroactive substances being detectable at concentrations below $10^{-8}M$.

W. J. BLAEDEL, C. L. OLSON, and L. R. SHARMA, Chemistry Department, University of Wisconsin, Madison, Wis.

Anal. Chem. 35, 2100 (1963)

Polarography in Fused Alkali Metaphosphates

Polarograms were obtained with a cell consisting of a platinum microelectrode inserted in a melt contained in a platinum crucible. The platinum crucible served as a massive and nonpolarizable anode. Twenty oxides and compounds were studied.

ROY D. CATON and HARRY FREUND, Department of Chemistry, Oregon State University, Corvallis, Ore.

Anal. Chem. 35, 2103 (1963)

Polarography of Lanthanum(III), Praseodymium(III), and Ytterbium(III) in Anhydrous Ethylenediamine

Anhydrous Yb(III), La(III), and Pr(III) acetates in anhydrous ethylenediamine gave a double irreversible wave for Yb (-0.411, -0.747 volt) and single irreversible waves for La and Pr (-0.565, 0.571 volt).

LARRY C. HALL and DAVID A. FLANIGAN, Department of Chemistry, Vanderbilt, Nashville, Tenn.

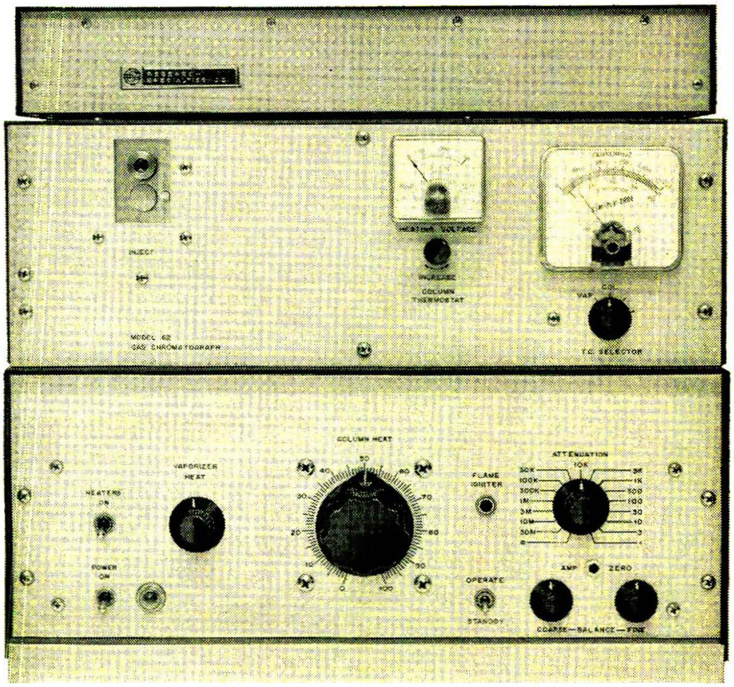
Anal. Chem. 35, 2108 (1963)

Anodic Stripping Voltammetry of Gold and Silver with Carbon Paste Electrodes

In the described procedure, the effects of electrode material, electrode area, supporting electrolyte, deposition potential, deposition time, deposition solution volume, stirring rate, and anodic voltage scan rate are discussed. Determinations of as little as 1.0 p.p.b. of gold and 0.25 p.p.b. of silver were made with about 10% relative error.

EMMETT S. JACOBS, Jackson Laboratory, E. I. du Pont de Nemours & Co., Inc., Wilmington 99, Del.

Anal. Chem. 25, 2112 (1963)



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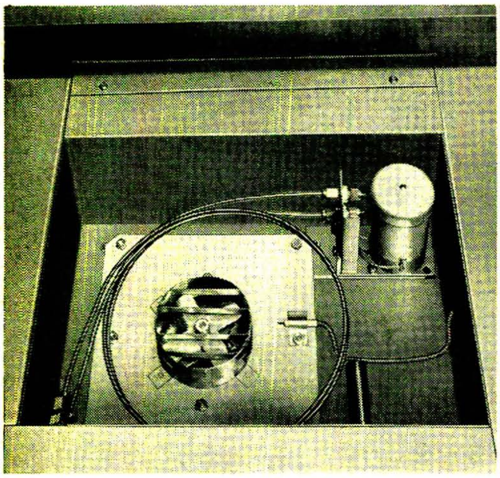

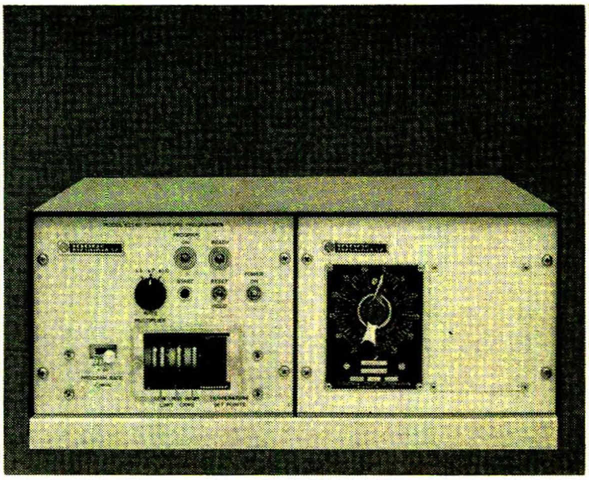

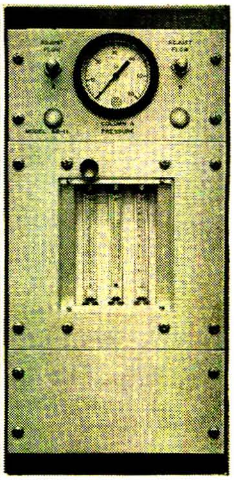

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Determination of Some Heavy Metal Ions by Complexometric Titration with Sodium Azide Indicator

Copper and iron are determined in acid solution by titration against (ethylenedinitrilo)tetraacetic acid (EDTA) using sodium azide as an indicator. Zn, Al, and Ni can also be determined by back-titrating the excess EDTA added against a standard copper solution, using the azide as an indicator.

F. G. SHERIF and B. I. RAAFAT, Chemistry Department, Faculty of Science, University of Alexandria, Alexandria, Egypt, U.A.R.

Anal. Chem. 35, 2116 (1963)

Extraction of Submicrogram Amounts of Molybdenum with Cupferron-Chloroform Using Molybdenum-99

The extraction gives a distribution ratio of over 200 between chloroform and aqueous phases. Over 90% of Mo can be separated in one extraction when only 0.1 μg. is present in 1 liter of aqueous phase. Bone can be ashed at temperatures up to 850° C. without loss of Mo.

W. B. HEALY and W. J. McCABE, Soil Bureau, and Institute of Nuclear Sciences, Department of Scientific and Industrial Research, Wellington, New Zealand

Anal. Chem. 35, 2117 (1963)

Spectrophotmetric Estimation of Copper (I) Using Rubeanic Acid

The molecular formula of the complex is determined by the method of continuous variation and the probable structure of the complex is indicated. The method is applicable in a concentration range of 2 to 12 p.p.m. of Cu(I).

AGNES PAUL, Alagappa Chettiar College of Technology, University of Madras, Madras, India

Anal. Chem. 35, 2119 (1963)

Indirect Ultraviolet Spectrophotometric Determination of Silicon

After extraction of the heteropoly acid with pentanol–diethyl ether, the molybdosilicic acid is stripped with a basic buffer solution. The absorbance of the aqueous molybdate solution is measured at 230 or 210 mμ.

LOUIS TRUDELL and D. F. BOLTZ, Department of Chemistry, Wayne State University, Detroit 2, Mich.

Anal. Chem. 35, 2122 (1963)

Visible Absorption Characteristics of the Bis-(2,9-dimethyl-1,10-phenanthroline)–and Bis-(4,4′,6,6′-tetramethyl-2,2′-bipyridine)–Copper(I) Ions

The absorbing species in solution for the determination of copper using 2,9-dimethyl-1,10-phenanthroline (dmp) and 4,4′,6,6′-tetramethyl-2,2′-bipyridine (tmb) are the bis complex ions, $[Cu(dmp)_2]^+$ and $[Cu(tmb)_2]^+$, respectively. Interferences are discussed.

J. R. HALL, M. R. LITZOW, and R. A. PLOWMAN, Chemistry Department, University of Queensland, Queensland, Australia

Anal. Chem. 35, 2124 (1963)

Purity Examination of Silicon and Germanium Halides by Long-Path Infrared Spectrophotometry

Dissolved gases and volatile compounds, and especially organic contaminants, can be detected by the described method. Impurities in the range 1 to 200 p.p.m. can be detected by examination of the infrared absorption spectrum of 10-cm. liquid layers of silicon and germanium tetrahalides.

MYRON J. RAND, Bell Telephone Laboratories, Inc., Allentown, Pa.

Anal. Chem. 35, 2126 (1963)

Sensitive and Selective Spectrophotometric Reaction for Determination of Trace Amounts of Calcium

Calcium down to the 0.1-p.p.m. level is determined using Calcichrome, cyclo-tris-7-(1-azo-8-hydroxynaphthalene-3, 6-disulfonic acid. At pH 12 and 615 mμ, the procedure has a molar absorptivity of 7600.

MANOLITA HERRERO-LANCINA and T. S. WEST, Chemistry Department, The University of Birmingham, Birmingham 15, England

Anal. Chem. 35, 2131 (1963)

Simultaneous Determination of Niobium and Tantalum by Neutron Activation Using Niobium-94*m* and Tantalum-182*m* and Rapid Radiochemical Separations

Samples are irradiated for 10 minutes in a neutron flux of 10^{12}*n*. cm.$^{-2}$ second^{-1}. After simultaneous separation of niobium and tantalum, niobium-free tantalum is extracted with 1-pentanol(*n*-amyl alcohol). Niobium and tantalum are determined in rock, stainless steel, and graphite samples.

CHONG K. KIM and W. WAYNE MEINKE, Department of Chemistry, University of Michigan, Ann Arbor, Mich.

Anal. Chem. 35, 2135 (1963)

Evaluation of Some Analytical Procedures for Niobium-Base Alloys

Conventional chemical and vacuum fusion procedures for carbon, hydrogen, and oxygen are evaluated. Chemical and optical spectrographic solution procedures for determination of alloying amounts of Mo, W, Ti, and Zr are discussed. Data from round-robin analyses of samples from the three niobium alloys are examined.

J. P. McKAVENEY, Central Research Laboratory, Crucible Steel Co. of America, Pittsburgh 13, Pa.

Anal. Chem. 35, 2139 (1963)

Method and Apparatus for Determination of Small Isotopic Oxygen Variations in Beryllium Oxide

The described method was tested for 1- to 5-mg. samples of finely ground BeO containing 0.2 to 2.0 atom per cent of the oxygen as O^{18}. The standard deviation among 19 successive samples was ±0.002 when the O^{18} abundance was about 0.2%.

R. A. MEYER, S. B. AUSTERMAN, and D. G. SWARTHOUT, North American Aviation Science Center, North American Aviation, Inc., Canoga Park, Calif.

Anal. Chem. 35, 2144 (1963)


7
who says Stoppers can't be improved?

A Mass Scale Based on CH$_2$ = 14.0000 for High Resolution Mass Spectrometry of Organic Compounds

The advantage of this scale is that ions differing by one or more CH$_2$ groups have the same mass defect. Tables of mass defects for combinations of H, C^{12}, C^{13}, N, O, S^{32}, and S^{34} are given.

EDWARD KENDRICK, Analytical Research Division, Esso Research and Engineering Co., Linden, N. J.

Anal. Chem. 35, 2146 (1963)

A Solid State Digitizer for Mass Spectrometers

Digitizer accepts ion peaks and acceleration voltage signals, converts the analog signals to digital, and presents the data on punched paper tape and printed output. Designed primarily for use in round-the-clock operations, the digitizer results in savings in labor and cost, and increases the number of samples handled by 66%. Standard deviations are ±0.1 mass number up to mass 125 and ±0.25 mass number up to mass 250.

E. M. THOMASON, Instrument Department, Hydrocarbons Division, Monsanto Chemical Co., Texas City, Texas

Anal. Chem. 35, 2155 (1963)

Ultramicrodetermination of Iodine by a Rapid Automatic Reaction-Rate Method

The method is based on the Sandell-Kolthoff reaction, in which a trace of iodine or iodide acts as catalyst for the reduction of Ce(IV) in the presence of As(III). Iodine in the range of 0.015 to 0.45 μg. was determined with a relative error of about 1 to 2%.

H. V. MALMSTADT and T. P. HADJIIOANNOU, Department of Chemistry and Chemical Engineering, University of Illinois, Urbana, Ill.

Anal. Chem. 35, 2157 (1963)

A Simple Instrument for the Direct Determination of Dielectric Constants of Liquids over a Wide Range of Values

A solid state instrument which uses the capacity-substitution method is described. Dielectric constants of materials with ϵ values of up to 35 can be determined with a relative error of less than 1%. Typical results with 12 liquids are presented. Drift and reproducibility are discussd, as well as sensitivity.

P. P. MALONEY and R. NELSON SMITH, Department of Chemistry, Pomona College, Claremont, Calif.

Anal. Chem. 35, 2161 (1963)

Determination of Moisture in Ion Exchange Resins by Karl Fischer Reagent

Analysis is carried out by direct titration in methanol or pyridine solvent. Data are given comparing the Karl Fischer titration results with zylol distillation. The data obtained by adding known amounts of H$_2$O and titrating are given.

FRANK X. POLLIO, Rohm & Haas Co., Philadelphia, Pa.

Anal. Chem. 35, 2164 (1963)

Correction of Luminescence Spectra and Calculation of Quantum Efficiencies Using Computer Techniques

A method for the calibration of an Aminco-Kiers Spectrophosphorimeter with fluorescence attachment is described. A computer program was written to correct recorded spectra and provide a printout of the corrected spectrum in units of quanta per unit frequency interval *vs.* frequency.

HARRY V. DRUSHEL, A. L. SOMMERS, and ROBERT C. COX, Esso Research Laboratories, Humble Oil and Refining Co., Baton Rouge, La.

Anal. Chem. 35, 2166 (1963)

Determination of Propylene in Ethylene-Propylene Copolymers by Infrared Spectrometry

Copolymers of ethylene and propylene yield vinyl and vinylidene unsaturation on pyrolysis. The ratio of the infrared absorption of the vinyl groups to that of the vinylidene groups varies with the mole fraction of propylene in ethylene-propylene copolymers.

JAMES E. BROWN, MAX TRYON, and JOHN MANDEL, National Bureau of Standards, Washington 25, D. C.

Anal. Chem. 35, 2172 (1963)

Flame Photometric Microdetermination of Boron in Organoboron Compounds

The method requires decomposition of weighed samples, preparation of standard-added solution and dilute sample solution, and measurement with a flame spectrophotometer. The relative error is within 1% of the theory.

TAMOTSU YOSHIZAKI, Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka, Japan

Anal. Chem. 35, 2177 (1963)

Spectrophotometric Determination of Nitrogen in Total Micro-Kjeldahl Digests. Application of Phenol-Hypochlorite Reaction to Microgram Amounts of Ammonia in Total Digest of Biological Material

One to 15 μg. of nitrogen (as ammonia) can be determined by the described method. Absorptivity of colored solutions is constant with NH$_3$ concentration from 0.1 to 1.5 μg. per ml. Those solutions too dark for direct spectrophotometric determination may be diluted without loss of linearity.

LEWIS T. MANN, Jr., Laboratory of Chemical Pathology, Department of Pathology, Harvard Medical School, Boston 15, Mass.

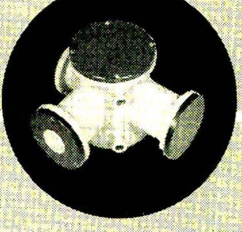
Anal. Chem. 35, 2179 (1963)

Sensitive New Methods for Autocatalytic Spectrophotometric Determination of Nitrite through Free-Radical Chromogens

Some new methods more sensitive than others reported in the literature are presented. Evidence shows that free radicals are obtained in all the procedures. Advantages and disadvantages are discussed and suggestions are given for the application of the procedures to solutions containing dyes, large amounts of sulfite, or minute amounts of nitrite.

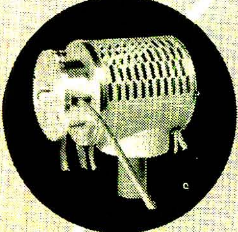
EUGENE SAWICKI, T. W. STANLEY, JOHN PFAFF, and HENRY JOHNSON, Division of Air Pollution, Robert A. Taft Sanitary Engineering Center, Public Health Service, Cincinnati 26, Ohio.

Anal. Chem. 35, 2183 (1963)

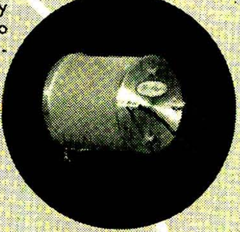


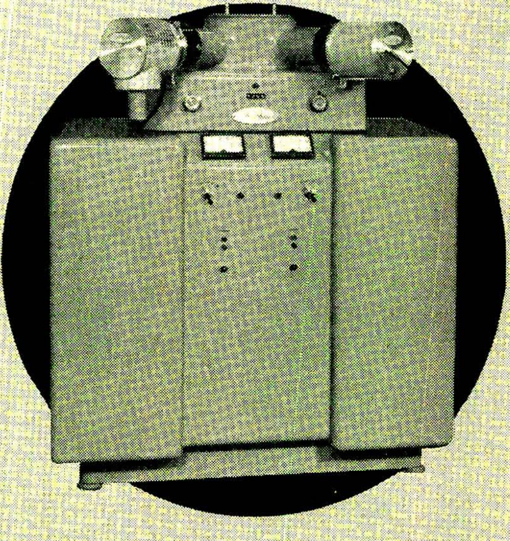
DUAL POSITION DETECTOR. Two position mount simplifies reflectance, fluorescence or absorption studies ● includes high sensitivity photomultiplier tube.

LIGHT SOURCE. High energy discharge lamp ● Hinteregger type ● flow-through gas system ● handles 1,000 watts of energy continuously, up to 8,000 watts intermittently ● water and forced air cooled.

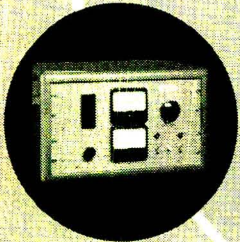


DETECTOR. Vacuum mounted ● high sensitivity photomultiplier tube mounted external to vacuum ● detects radiation through a wavelength range from 500A to 6,000A.

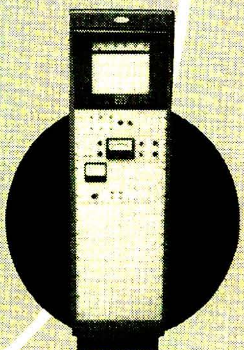




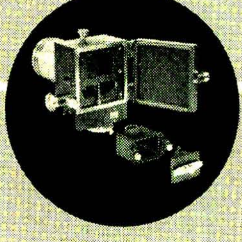
HALF METER OR ONE METER VACUUM U. V. SCANNING MONOCHROMATOR, Seya-Namioka type. This table or console model, with standard equipment including grating, slit assemblies, scanning drive, pumps and gauges, is designed and constructed to work the wavelength range from 500A to 6,000A.



POWER SUPPLY. High voltage dual mode AC power supply ● for use with light source ● operated as Michigan type, air gap controlled spark or as an AC arc.



DETECTOR ELECTRONIC SYSTEM. One package contains regulated variable high voltage DC power supply ● linear electrometer with bucking current source ● strip chart recorder (0.4) ● remote control panel.



FILM HOLDER. Daylight loaded into spectrometer ● interchangeable with standard slit assembly ● uses 35mm film ● covers approximately 1,700A in one exposure.

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BRIEFS

Direct Quantitative Isolation of Monocarbonyl Compounds from Fats and Oils

The procedures were used in determining the carbonyl constituents of fats, oils, extracts of cheese, and whole milk powders. Theoretically all carbonyl compounds capable of forming a 2,4-dinitrophenylhydrazone under the given conditions are isolated lipide-free.

D. P. SCHWARTZ, H. S. HALLER, and MARK KEENEY, Dairy Products Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Washington 25, D. C.

Anal. Chem. 35, 2191 (1963)

Critical Study of Different Methods for Determination of Chlorite

Methods for the determination of hypochlorite and chlorate with special reference to their behavior for chlorite determinations were studied. A procedure using ammonium sulfate for chlorite determination was developed.

M. H. HASHMI and A. A. AYAZ, West Regional Laboratories, Pakistan Council of Scientific & Industrial Research, Lahore, Pakistan

Anal. Chem. 35, 2194 (1963)

Determination of Normal Alpha-Olefins by Hydrobromination

Normal alpha-olefins in olefinic mixtures within the C_{12}–C_{18} range may be determined with a relative error of 1.3%. Normal alpha-olefins are converted by hydrobromination in the presence of lauroyl peroxide to normal primary alkyl bromides, which can be separated on Molecular Sieves.

JOSEPH C. SUATONI, Gulf Research and Development Co., Pittsburgh, Pa.

Anal. Chem. 35, 2196 (1963)

Reflectance Fluorescence Spectra of Aromatic Compounds in Potassium Bromide Pellets

The measurements were made in the concentration range of 0.00002 to 0.2 mole of solid aromatic compound per gram of KBr. For some hydrocarbons the appearance of the spectra were concentration-dependent.

B. L. VAN DUUREN and C. E. BARDI, Institute of Industrial Medicine, New York University Medical Center, New York, N. Y.

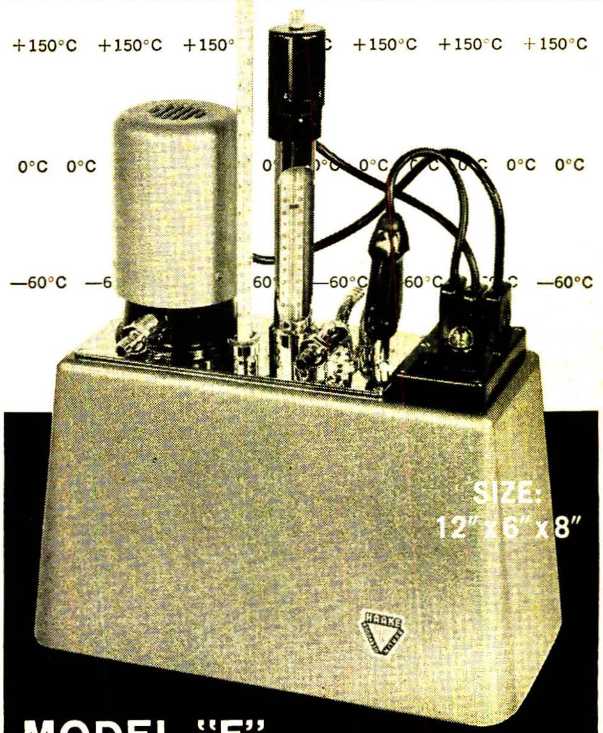
Anal. Chem. 35, 2198 (1963)

Oxygen Determination in Rocks, Minerals, and Water by Neutron Activation

Two-gram samples of rocks and minerals are used to determine oxygen from the reaction $O^{16}(n,p)N^{16}$. The method permits about 80 analyses per day and is nondestructive. Relative standard deviation is 0.39%. Interferences from F and B in rocks are taken into account.

ALEXIS VOLBORTH and H. E. BANTA, Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tenn.

Anal. Chem. 35, 2203 (1963)



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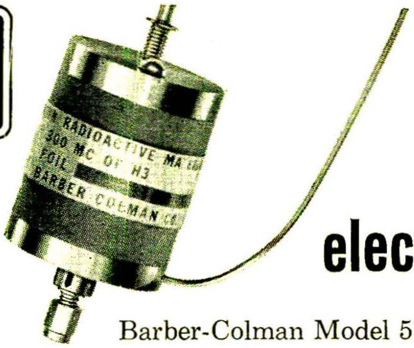


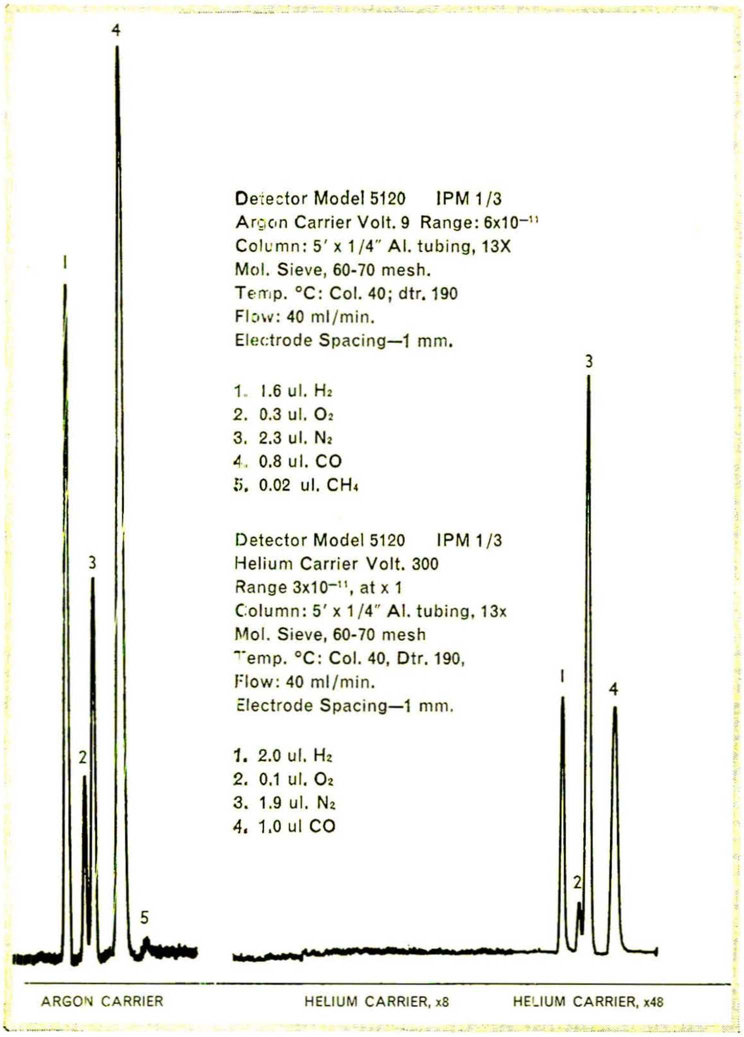
New detector adjusts for cross section or electron attachment response

Barber-Colman Model 5120 Gas Chromatography Detector achieves optimum response to electro-negative compounds and permanent gases by adjustment of the 1 cm diameter electrodes over an inter-electrode spacing range of 1 to 25 mm.

This outstanding feature provides several distinct modes of operation, giving the user the capabilities of many detectors for the price of one.

Excellent sensitivity and linearity are obtained with H_2 carrier gas in the cross section mode. Still higher sensitivities to permanent gases with slight non-linearity is obtained with Helium or Argon carriers. The chromatograms below demonstrate typical response to permanent gases and electro-negative compounds.

Model 5120 Detector is usable with all Barber-Colman instruments, including the new Series 5000 Selecta-System. Write to Barber-Colman for further data.

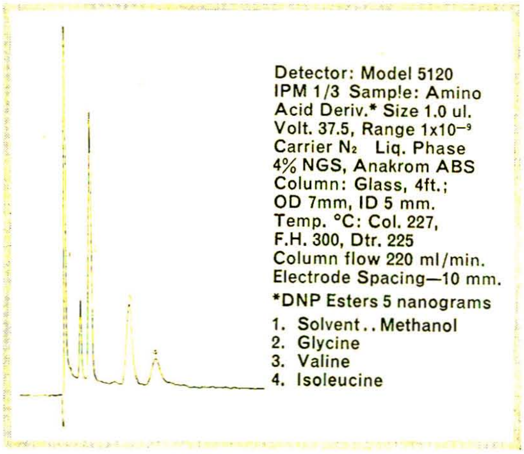


Detector Model 5120 IPM 1/3
Argon Carrier Volt. 9 Range: 6x10^{-11}
Column: 5′ x 1/4″ Al. tubing, 13X
Mol. Sieve, 60-70 mesh.
Temp. °C: Col. 40; dtr. 190
Flow: 40 ml/min.
Electrode Spacing—1 mm.

1. 1.6 ul. H_2
2. 0.3 ul. O_2
3. 2.3 ul. N_2
4. 0.8 ul. CO
5. 0.02 ul. CH_4

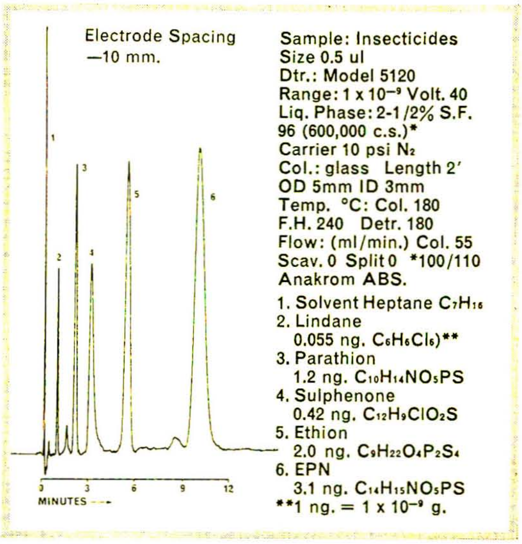
Detector Model 5120 IPM 1/3
Helium Carrier Volt. 300
Range 3x10^{-11}, at x 1
Column: 5′ x 1/4″ Al. tubing, 13x
Mol. Sieve, 60-70 mesh
Temp. °C: Col. 40, Dtr. 190,
Flow: 40 ml/min.
Electrode Spacing—1 mm.

1. 2.0 ul. H_2
2. 0.1 ul. O_2
3. 1.9 ul. N_2
4. 1.0 ul CO



Detector: Model 5120
IPM 1/3 Sample: Amino Acid Deriv.* Size 1.0 ul.
Volt. 37.5, Range 1x10^{-9}
Carrier N_2 Liq. Phase 4% NGS, Anakrom ABS
Column: Glass, 4ft.;
OD 7mm, ID 5 mm.
Temp. °C: Col. 227,
F.H. 300, Dtr. 225
Column flow 220 ml/min.
Electrode Spacing—10 mm.
*DNP Esters 5 nanograms

1. Solvent.. Methanol
2. Glycine
3. Valine
4. Isoleucine



Sample: Insecticides
Size 0.5 ul
Dtr.: Model 5120
Range: 1 x 10^{-9} Volt. 40
Liq. Phase: 2-1/2% S.F. 96 (600,000 c.s.)*
Carrier 10 psi N_2
Col.: glass Length 2′
OD 5mm ID 3mm
Temp. °C: Col. 180
F.H. 240 Detr. 180
Flow: (ml/min.) Col. 55
Scav. 0 Split 0 *100/110
Anakrom ABS.

1. Solvent Heptane C_7H_{16}
2. Lindane
   0.055 ng. $C_6H_6Cl_6$)**
3. Parathion
   1.2 ng. $C_{10}H_{14}NO_5PS$
4. Sulphenone
   0.42 ng. $C_{12}H_9ClO_2S$
5. Ethion
   2.0 ng. $C_9H_{22}O_4P_2S_4$
6. EPN
   3.1 ng. $C_{14}H_{15}NO_5PS$

**1 ng. = 1 x 10^{-9} g.

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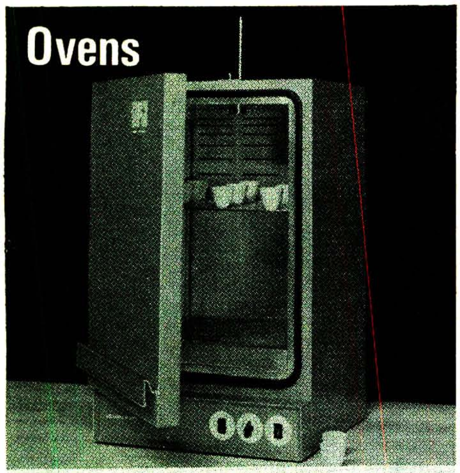
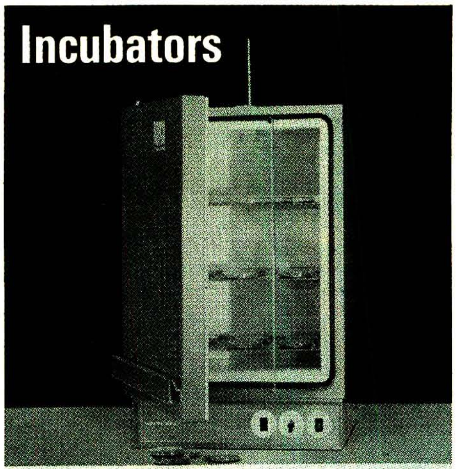
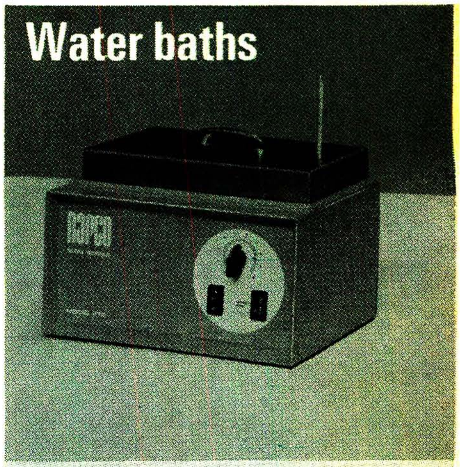
Ovens



Incubators



Water baths



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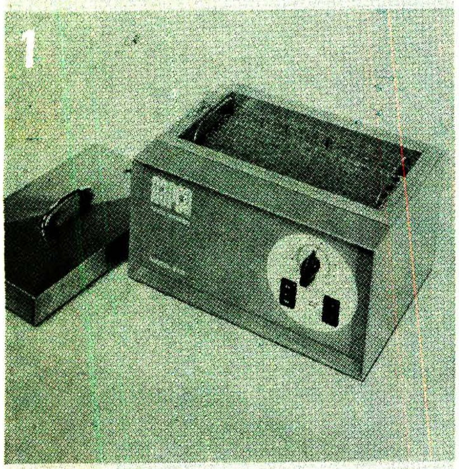

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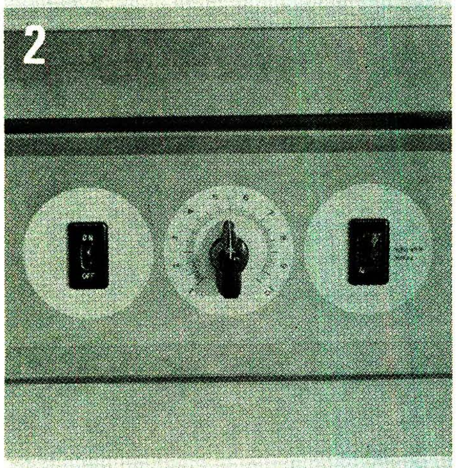

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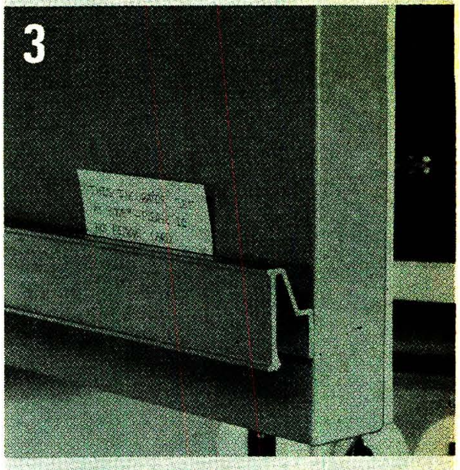

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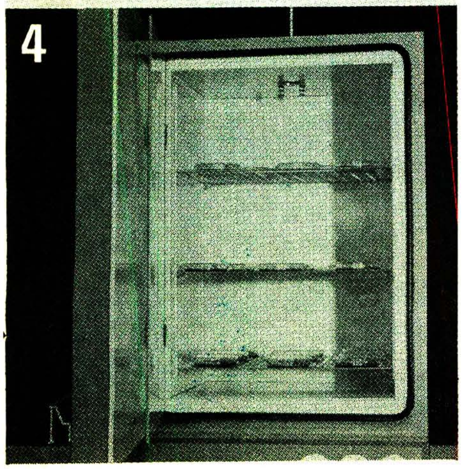

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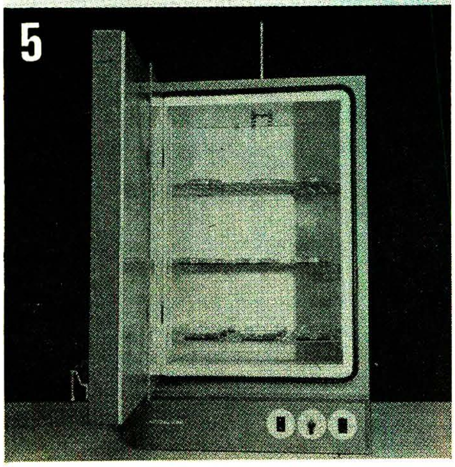

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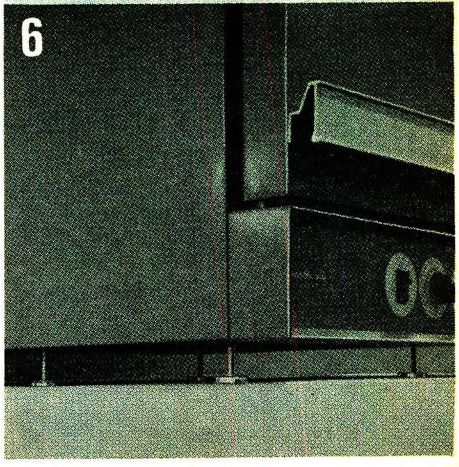

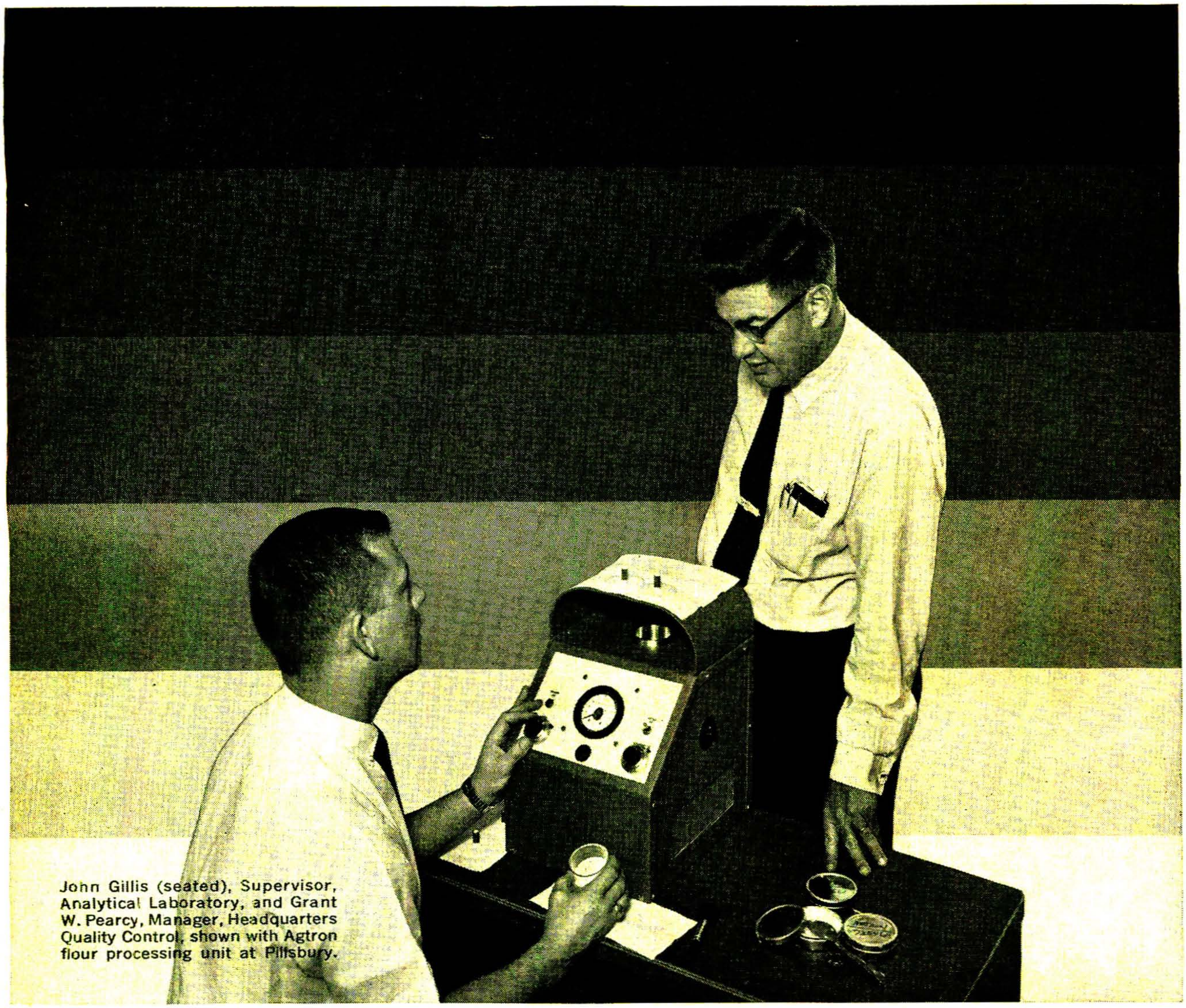

John Gillis (seated), Supervisor, Analytical Laboratory, and Grant W. Pearcy, Manager, Headquarters Quality Control, shown with Agtron flour processing unit at Pillsbury.

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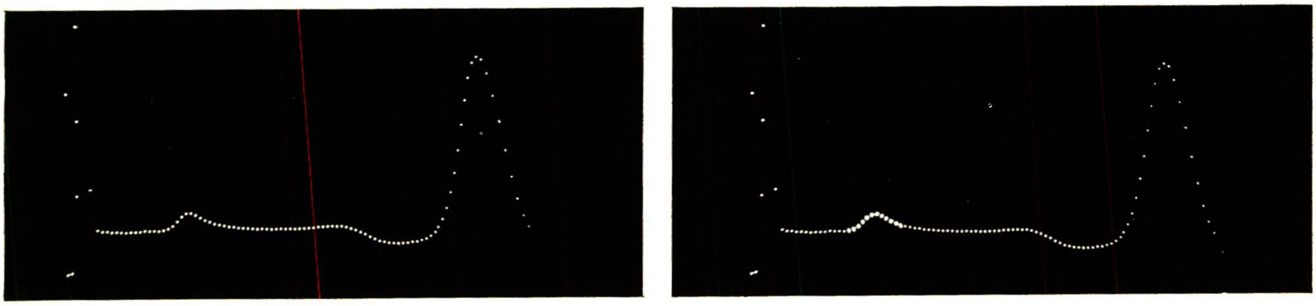

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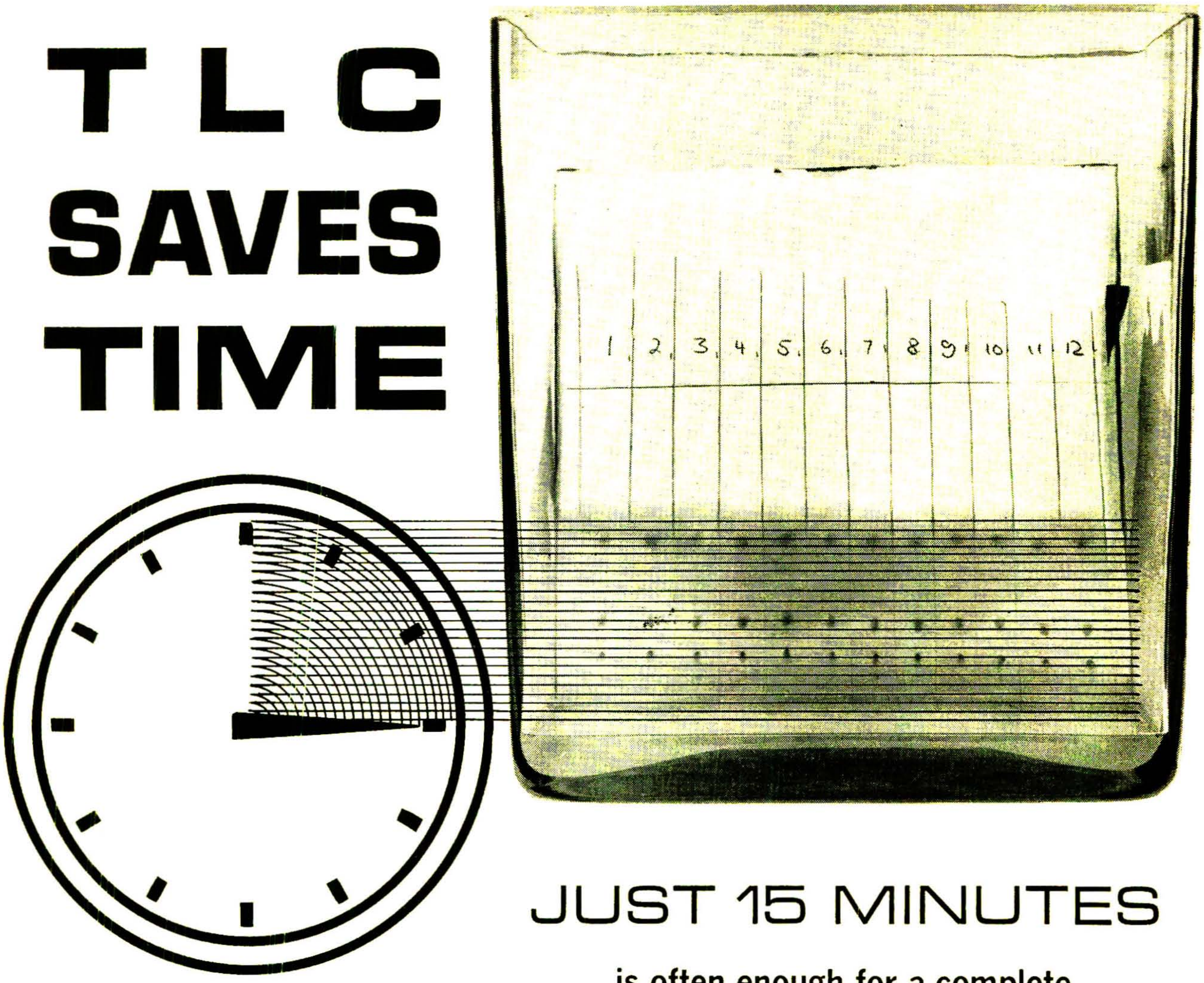

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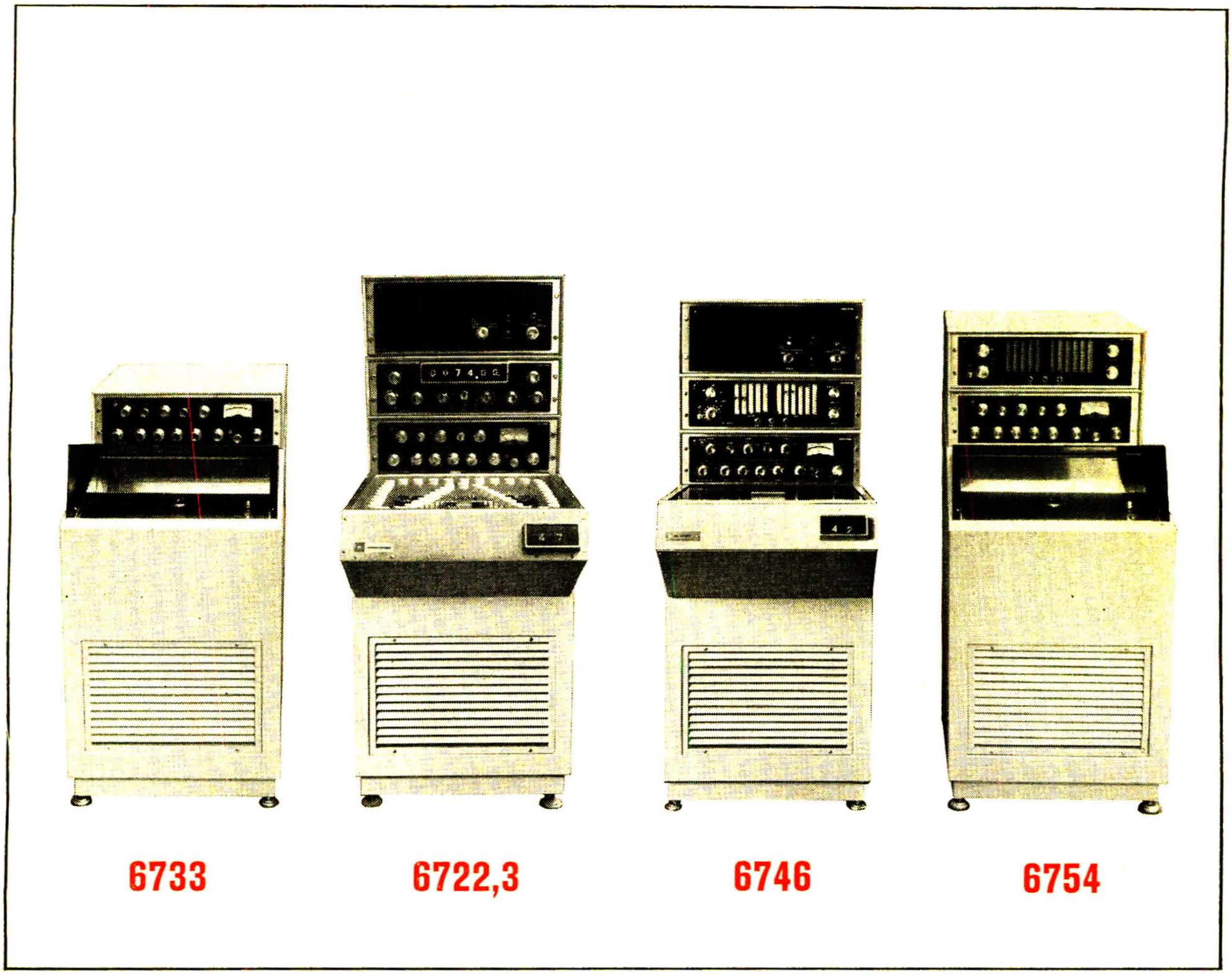
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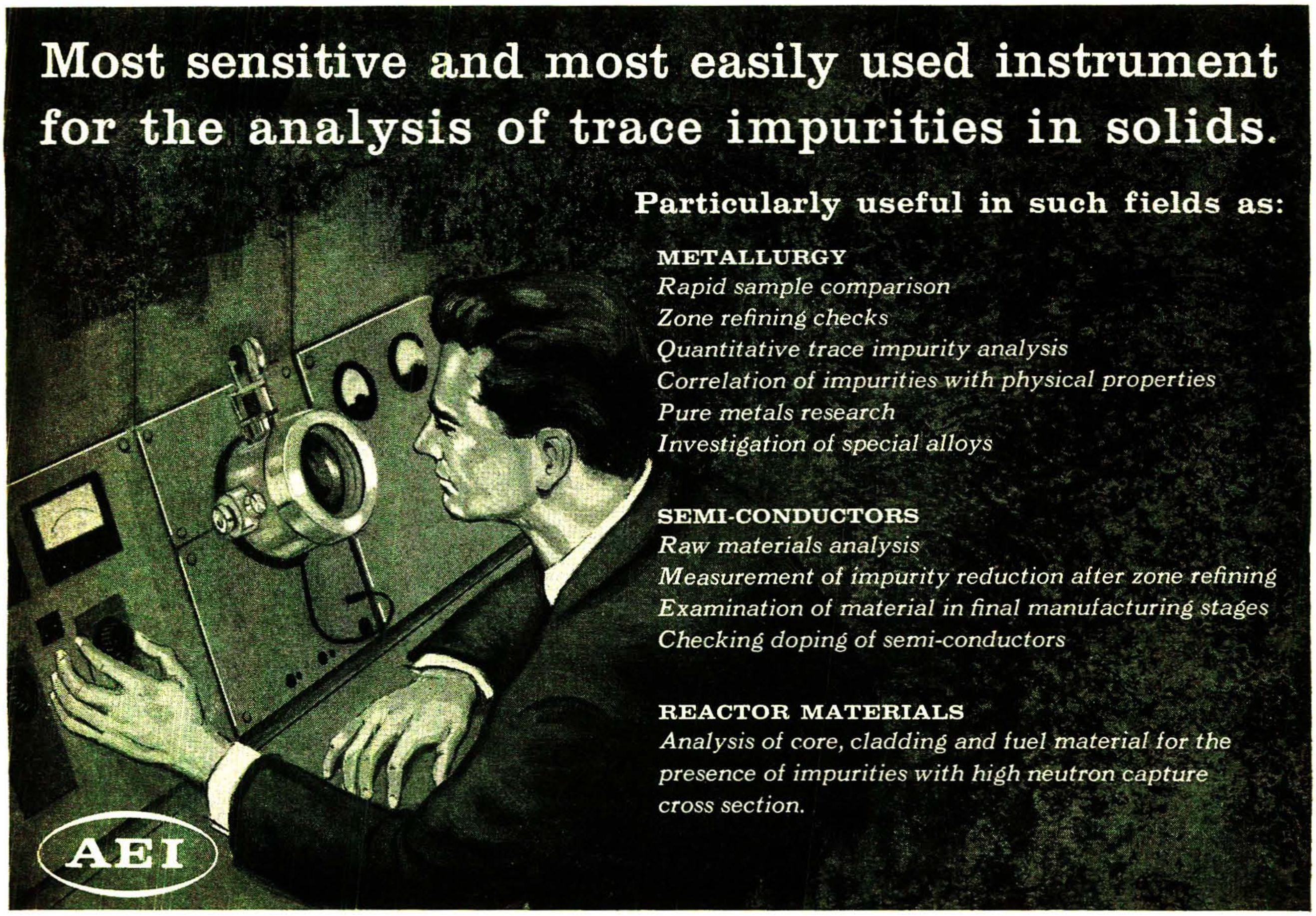

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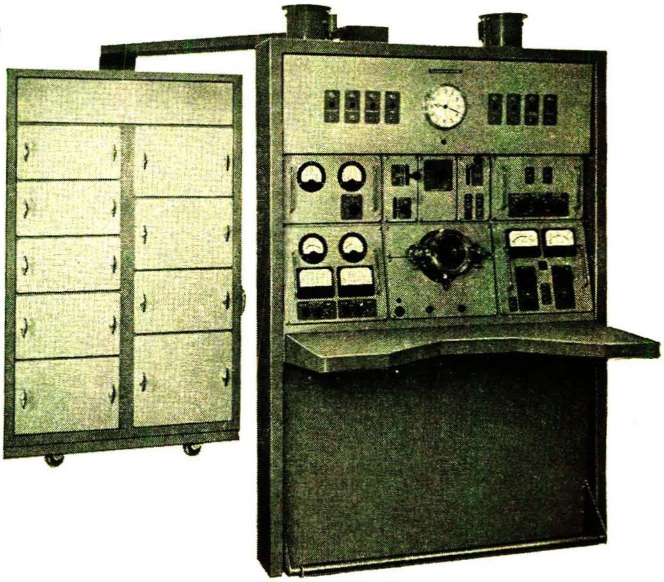

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The Analysis of a Profession

by **David N. Hume,** *Professor of Chemistry, Massachusetts Institute of Technology, Cambridge 39, Mass.*

This Report for Analytical Chemists is the text of the Fisher Award Address given on April 2, 1963 at the Spring National ACS Meeting in Los Angeles by the recipient, Dr. David N. Hume. In the address, Dr. Hume discusses the nature of analytical chemistry as a separate discipline and the responsibilities of the teacher of analytical chemistry

ONE OF THE MOST difficult problems facing the analytical chemist today is that of explaining to others just what analytical chemistry is. Much of the difficulty derives from changes in the nature of the profession and the fact that a given word may have a whole spectrum of meanings. Even the very general definition of an analytical chemist as one who determines the composition of matter (in contrast to the synthetic chemist who prepares, purifies, and isolates chemical compounds) leads to a paradox if we compare the synthetic organic chemist determining the structure of his product by infrared, NMR, and mass spectrometry with the analyst preparing, purifying, and isolating silver chloride preparatory to weighing it. The increasing complexity of modern chemistry is to some extent the cause of this confusion, as is the fact that a chemist seldom works on only *one* branch of the subject, more often combining the techniques and approaches of several. The subdivision of chemistry into several fields—analytical, inorganic, organic, and physical, for example—has, nonetheless, real utility, even though precise definitions of these fields are difficult to formulate. An unfortunate consequence is, however, that without a clear definition the individual substitutes an image, and this image may be distorted or even damaging.

We shall perhaps make faster progress in our thinking if we avoid the problem of trying to define chemistry as a whole, and instead merely note its fluidity and, in particular, the haziness of the borderline between it and physics. Let us view the practice of chemistry as the attacking of chemical problems, and divide these problems into those of a preparative nature and those involving the properties of chemical systems. There is an ever-growing array of methods applicable to chemical problems, and I would like to suggest that a good practical description of the modern professional analytical chemist is one who is a specialist in the methodology of solving problems having to do with the properties of chemical systems. I specify the professional analytical chemist because it makes a very great difference whether one is a highly educated specialist or a trained technician. The matter of images enters here. If the image of the technician is confused with that of the professional, there can be trouble.

We all know that most chemical analyses are done not by analytical chemists but by technicians, just as most organic syntheses are done not by the organic chemists but by chemical operators. In the latter instance, there is no problem of confused images. The chemical operator wears coveralls and a hard hat, adds reagents with a long-handled shovel, and attends to his duties in an easily recognizable production area. There is no difficulty in distinguishing him from the research synthetic chemist in the laboratory. The analytical technician, on the other hand, may wear the same white coat and use the same instruments in the very same laboratory as the research analytical chemist. With outward appearances so much alike, it is very easy for observers to get an entirely false image of the analytical chemist, particularly if their own experience with analytical chemistry was limited to an exposure to traditional qualitative and quantitative analysis taught in the sophomore year.

If one attempts to clarify the nature of modern analytical chemistry by describing some of the research currently under way in this field, he may run into another type of conceptual difficulty. The listener, after hearing about kinetics of electrode reactions, equilibrium constants in low dielectric constant solvents, and the mechanism of excitation in flame spectroscopy, is likely to say, "But what you are doing is really physical chemistry. Why don't you call yourself a physical chemist?" But we are not physical chemists because, although the phenomena may often be the same, the outlook is quite different. This is well illustrated by a specific example.

One of my colleagues, a physical chemist, enjoys studying the vapor-liquid composition of three-component systems as a function of temperature. He views such a problem as having two parts. The first is the annoying, but necessary, matter of having to find—or worse yet—develop methods suitable for the determination of his components in each phase with a precision and accuracy sufficient for his purpose. Once this unpleasant (to him) preliminary is out of the way, he can proceed to gather very precise data with loving care and then fit equations to it—an activity which gives him great personal satisfaction. I also see the problems as having two parts of dissimilar attractiveness. For me the interesting part, the aspect of the problem that represents a real challenge and calls for imagination, orginality, and creativity, is the development of the method which makes a solution to the problem possible. Once this is done, the "pure" research is over and there remains only the "applied" gathering of data and calculation of results, an activity which I find quite unexciting. Here is a real difference in outlook, and it is one thing which characterizes the analytical chemist and makes it necessary to consider analytical chemistry as a discipline separate from the other branches of chemistry.

The analytical chemist must be a realist. He does not have his quarters in an ivory tower. He is concerned with "the analysis of things as they are," as one of my predecessors so aptly put it. This concern with real systems implies no slight upon theory. The analytical chemist needs and applies all the theory he can get, but he is forced by the nature of his activities to recognize that much of the theory available today is of limited applicability in real situations.

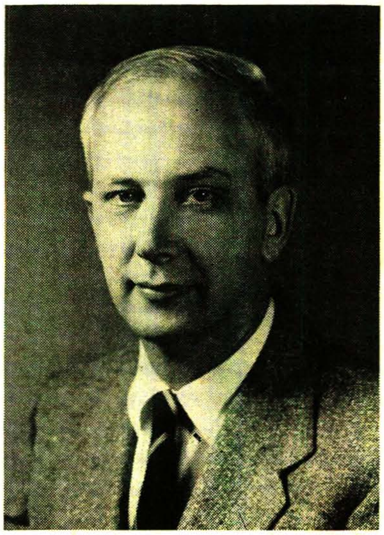
Analytical Chemistry as a Separate Discipline

It is important that analytical chemists themselves realize that analytical chemistry is a separate discipline and why it is different. It is of concern to us that other chemists realize the distinction, because out of an understanding of the nature of modern analytical chemistry will come an increasing respect for it. It is therefore essential that students of chemistry become aware that analytical chemistry is a separate discipline and that it is an important, an interesting, and a rewarding field of professional specialization.

This thought focuses our attention on some of the responsibilities of the teacher of analytical chemistry. It is, first of all, his responsibility to have a clear conception of his profession in order that he may impart it to his students. As an analytical chemist, he should have a feeling of identification with the profession, and he should give his students a feeling for its distinctiveness and its importance. He also needs to make his students aware that there are worthwhile scientific activities outside of teaching and academic research. The three branches of our profession—academic, industrial, and governmental—are equally honorable, and educators need to recognize this. The teacher of chemistry needs contact with industry to give him a balanced outlook on chemistry as a whole. Without such an outlook, he is likely to be capable in only part of his subject.

Failure to give adequate recognition to the chemist in industry is, alas, a not uncommon sin among teachers. There is at least one well-known and otherwise reputable institution in which the graduate students are all but told outright that to go into the chemical industry would make them second-class citizens! I think this is a crime. Some of the best minds in chemistry are in the chemical industry. This is not just because of money. Industry is the source of extremely interesting and stimulating problems. Some of the best work that is done comes from non-academic laboratories, and very real opportunities exist there. For many individuals, too, there is far greater personal satisfaction possible from being creative in a dynamic, modern research, development, and production group than in "pure" research where a problem and its outcome may not really matter to anyone else. We should not forget that were it not for the practical side of chemistry—that is, the chemical and pharmaceutical industry—our Society would not be enjoying a membership approaching 100,000. Chemists would stand in science, both in numbers and in status, down with the ichthyologists and herpetolo-

Dr. David N. Hume, Fisher Award winner for 1963, is professor of chemistry at Massachusetts Institute of Technology. Active in a number of research areas, Dr. Hume has published over 80 papers in such fields as polarography, complex ion equilibria, thermometric and photometric titrations, radiochemical analysis, and statistical methods. Dr. Hume has also been extremely active in modernizing the teaching of analytical chemistry and has been influential in revising both the undergraduate and graduate curricula in analytical chemistry at MIT. Dr. Hume was born in 1917 in Vancouver, B. C. He received his B.A. in 1939 and his M.A. in 1940 from the University of California, Los Angeles, and his Ph.D. in 1943 from the University of Minnesota. During the war, Dr. Hume worked with the Manhattan Project at the University of Chicago, and at the Clinton Laboratories, Oak Ridge, Tenn. After the war, Dr. Hume taught at the University of Kansas until 1947 when he joined MIT. Dr. Hume is author of the review "Polarographic Theory, Instrumentation, and Methodology" for ANALYTICAL CHEMISTRY. He has served as a member of the advisory board of ANALYTICAL CHEMISTRY and is an editor for the McGraw-Hill Advanced Chemistry Series. He is a member of the ACS, Sigma Xi, Phi Lambda Upsilon, and a Fellow of the American Academy of Arts and Sciences.



gists. If you do not know who the ichthyologists and herpetologists are, I have made my point.

To return to the responsibilities of the teacher of analytical chemistry, it is clear that a prime responsibility is to teach analytical chemistry as it is actually practiced today. It is a modern subject and a subject requiring more background and sophistication than has been attained by the average sophomore. The traditional approach which devotes over half the time spent on analytical chemistry to classical quantitative inorganic analysis in the sophomore year is clearly not adequate for present day needs. If analytical chemistry is to be taught as methods for attacking chemical problems, the student must be far enough along to know what chemical problems are, and he must have the background for understanding methods based on physical and physico-chemical, as well as chemical principles. This suggests that beginning analytical chemistry should be taught at a level more advanced than is possible in the traditional spot in the sophomore year. The addition of a semester of instrumental methods in the senior year is not a sufficient solution. The undergraduate's time is too short and too valuable to permit an entire year or even a semester to be devoted to the traditional techniques alone.

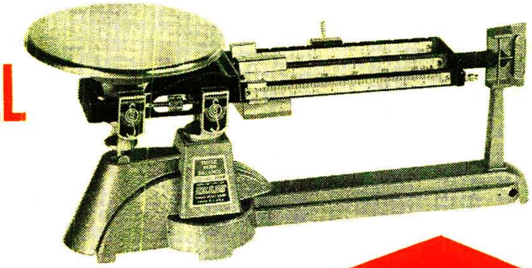
Approaches in Teaching Analytical Chemistry

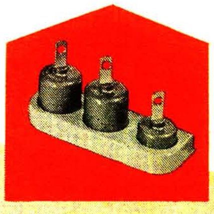
There are various approaches to the teaching of chemistry which make it possible to do something about this problem. The great diversity of sizes and objectives in our educational institutions makes it unlikely that there is any one plan which is suitable for all. I am firmly convinced that there are many paths to excellence, as well as to mediocrity, and it is not appropriate that I go into an analysis of all the various educational experiments now in operation. I should, however, like to mention what are perhaps the two extremes. One of these is the technique of "integrating" analytical chemistry into the entire curriculum, with the idea that analytical principles be learned in context rather than separated from the rest of chemistry. Unfortunately, the integration may take the form of obliteration. A department that is unsuccessful in its efforts to hire a capable analytical chemist may divide up the responsibility for analytical instruction among the remaining members of the staff and then, to make a virtue out of necessity, cite theoretical educational advantages in defense of its expediency. Whatever the particular circumstances may be, the result is almost inevitably the same: analytical instruction is neglected and the student leaves with a serious deficiency in his chemical education.

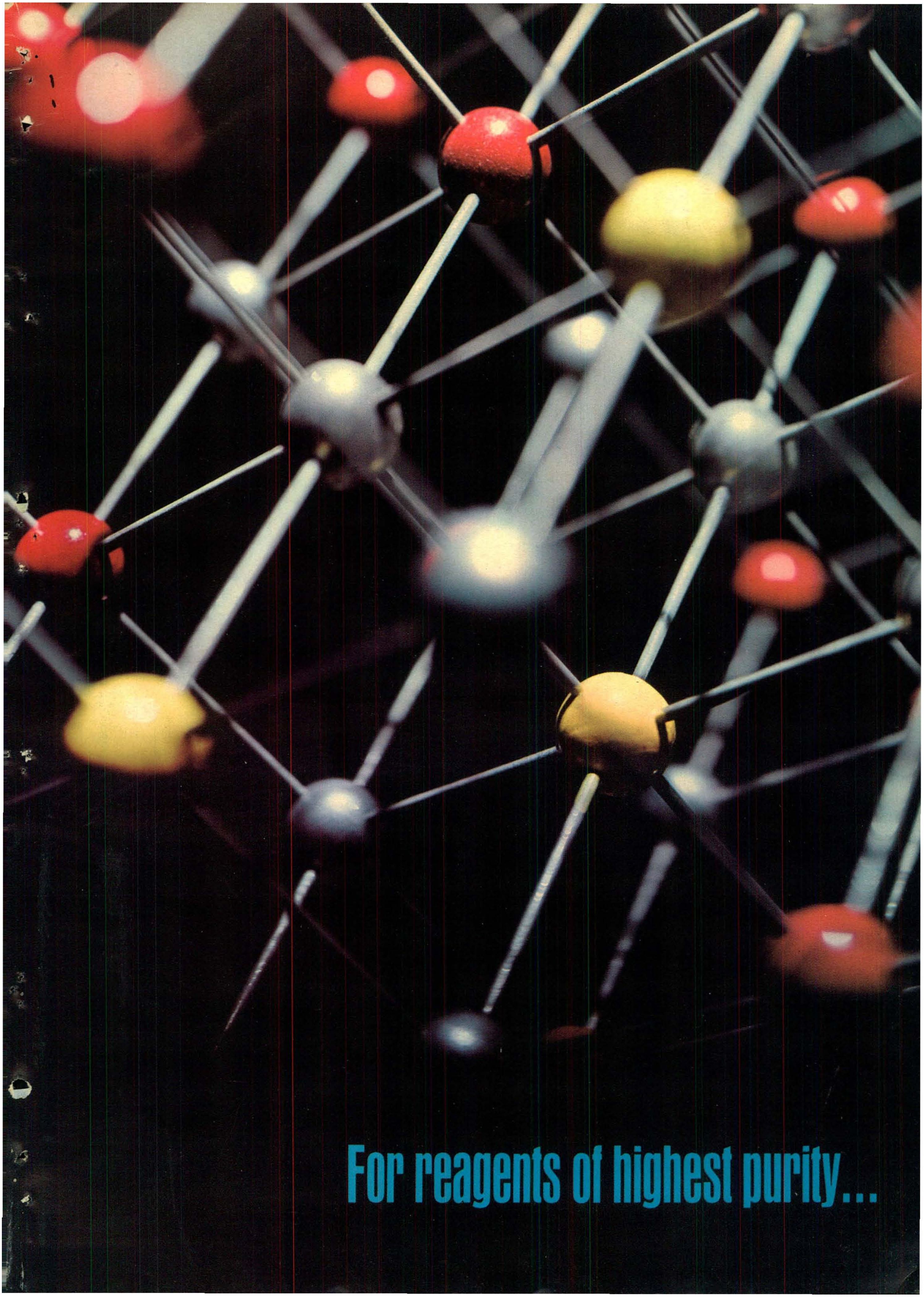
The other extreme is the approach taken by MIT, the University of Illinois, and a number of other institutions, most of which are large enough to have several professional analytical chemists on staff. Analytical chemistry is not introduced until the student has had a good grounding in the other branches of chemistry, and in mathematics and physics as well. Analytical chemistry is then taught as an advanced subject, making full use of all that has come before. The advantages of this are numerous and obvious. Analytical chemistry can be taught as it is actually practiced. Organic and inorganic analysis can be given equal status. The theoretical basis of analytical methods need not be slighted. The application to all branches of chemistry is immediately evident to the student, and he finds it a stimulating synthesis of the theoretical and practical. The rapid spread of this approach to the teaching of analytical chemistry is a most encouraging development, and it promises to do a lot toward alleviating the most crucial problem which faces the profession at the moment, that of interesting undergraduates in going on to graduate work in the field. The demand for Ph.D. analytical chemists is intense and continuous. The problem has been to transmit this information to the undergraduate and make him aware of the opportunity which it affords.

Placement of analytical chemistry at an advanced level in the curriculum gives the teacher an opportunity to present a truly interesting and worthwhile course. It is a course which, quite naturally, draws from the stream of current analytical research, and in this connection the importance of the teacher being himself involved in analytical research must be stressed. The contribution which a balanced graduate research program can make to the improvement of undergraduate teaching needs to be recognized and utilized. The enthusiastic teacher, who knows the significance of his subject and who maintains its freshness by drawing on today's research discoveries today, is not likely to find lack of student interest a problem.

Chemistry as a whole is undergoing rapid change and, in some respects, the field of analytical chemistry more than the others. Such change does not come without dislocations and uncertainties, inconveniences and upsets. Some observers, focusing on particular aspects of the present state of transition, have expressed concern for the future of our profession and there has been talk of a crisis in analytical chemistry. I do not subscribe to any such pessimistic view. If one looks at the present status of analytical chemistry in the light of any sort of historical perspective, if he compares the number, importance, opportunities, recognition, and relative pecuniary reward of analytical chemists today with those of twenty years ago, he sees that as a whole we are prospering. There are problems—of course! Progress never comes without its difficulties. But many of these problems were always with us, and among the new ones it is encouraging to note that a number of them are actually problems of growth. There is much to be done and much that can be done to improve the state and the status of analytical chemistry. A great deal of it involves taking a positive approach toward our subject and informing people what it is and why it is significant. If we fail to do this and our status declines, we have only ourselves to blame. If, on the other hand, we put aside our pessimism and emphasize the positive, we actively contribute to the improvement of our professional standing. END ■



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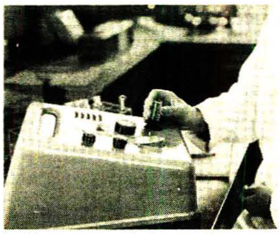
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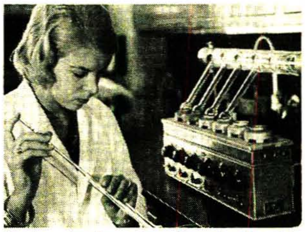
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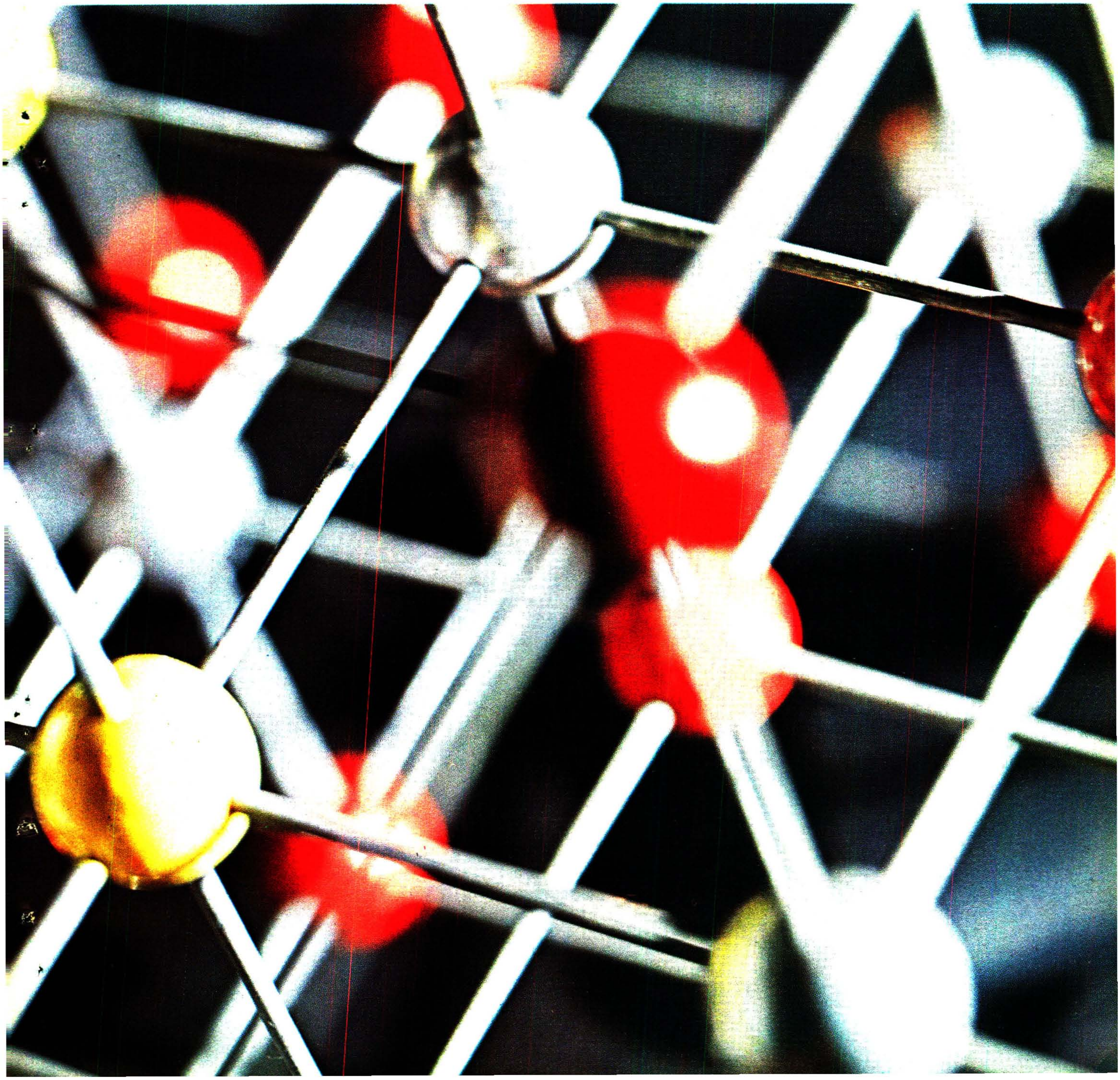

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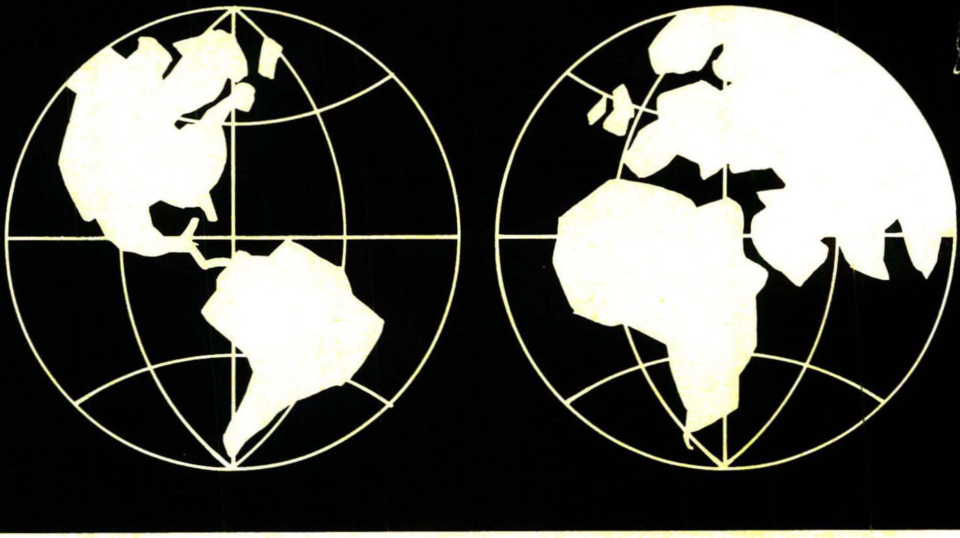

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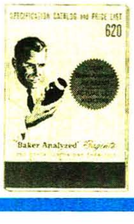

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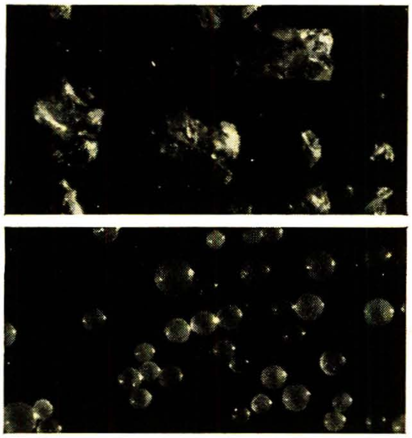

Figure 1. SEPHADEX was formerly supplied in the form of irregularly shaped particles as shown in top photograph. Now available in spherical beads as shown below, SEPHADEX facilitates packing of columns and greatly increases speed of operations.

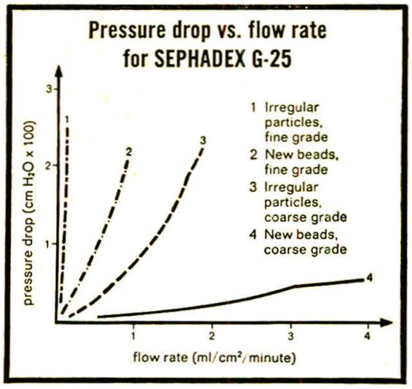

Figure 2. The curves above depict pressure-drop/flow-rate functions for columns using a 50 cm bed of SEPHADEX G-25. For given hydrostatic heads, note that the new spherical beads give up to tenfold greater effluent deliveries. Analytical procedures are hastened and preparative operations are put within economically practical engineering ranges.

Available forms of new SEPHADEX beads

SEPHADEX Type	Exclusion Limit (MW)	Grade	Size (microns)	Bed Volume ml/g
*G-25	5,000	coarse fine	100-300 20-80	5
G-50	10,000	coarse fine	100-300 20-80	10
*G-75	50,000	one grade	40-120	12-15
G-100	100,000	one grade	40-120	15-20
G-200	200,000	one grade	40-120	30-40

*SEPHADEX G-25 (fine) and G-75 will not be available before October, 1963.

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CHEMISTRY

For full information on the use of SEPHADEX in gel filtration, send the coupon with your letterhead.

(Inquiries outside the Western Hemisphere should be directed to PHARMACIA, Uppsala, Sweden.)

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Department BD, 501 Fifth Avenue, New York 17, New York

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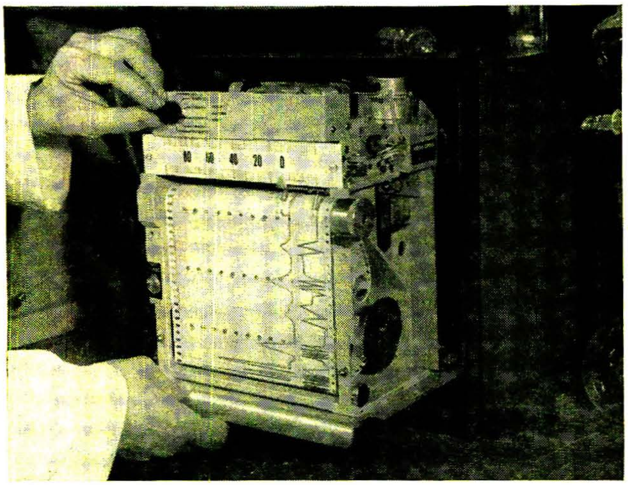
THE *ELECTRONIK 18* RECORDER



The *ElectroniK 18* Recorder is a reliable, economical instrument with all the features you need for laboratory work. Now in use on such applications as chromatography, spectrometry and precision cryosurgery—to name but a few—it is suitable for all but the most demanding high-precision analytical assignments.

A *new* option, the manual quick chart speed changer, is particularly valuable for analytical work: it permits the technician to speed up the strip chart movement when the record needs to be "spread out" to show additional detail during an analysis. This is done with the flick of a knob; with no interruption of the record as chart speed is changed.

Check the specifications and options listed below to convince yourself that the *ElectroniK 18* is the instrument to meet your analytical needs.

STANDARD SPECIFICATIONS

Calibrated Accuracy: ± 0.3% of span
Dead Band: ± 0.2%
Impedance: Up to 25,000 ohms source
Dynamic Response: Flat to 4 cps at 10% p-p
Pen Speed: 1-second full scale
Rebalancing Element: Honeywell standard slidewire
Balancing Motor: Honeywell standard sectionalized motor
Amplifier: Fully transistorized
Constant Voltage Supply: Zener diode
Adjustments: Variable gain and damping controls
Chart: Calibrated width 6 inches, 126 feet long
Chart Drive: Single base speeds from 1" to 120" per hour with ½ and 2 times base speed change gears provided.

OPTIONS

New, Improved Integrator: Two options (1) Model 215-1 Disc Integrator, for pen readout on right side of chart, or (2) Model 315 Disc Integrator, providing pulse signal output for actuation of electrical or mechanical counters, printers, and stepping motors, and to feed electronic data systems. **Price (1) $585.00; (2) $515.00.**

New Chart Speed Changer: Quick manual chart speed selector, providing the following speeds:

0.5 min./inch	5 min./inch
1.0 min./inch	10 min./inch
2.5 min./inch	

Price, $100.00.

Adjustable Range Unit: Recorder attachment for use where instrument must measure a wide range of emf's from a variety of signal sources. Has adjustable span, 5 mv to 12.5 volts, plus suppression adjustment. **Price, $450.00.**

Event Marker (Operation) Pen: One or two operation pens available for time correlation or event noting. **Price, $50.00 per pen.**

Transmitting Slidewire: Useful for remote transmission, integration, control, etc. **Price, $35.00.**

Auxiliary Contacts: For alarm signalling or sequencing analytical operations. **Price, $35.00 for first switch, $15 for each additional switch.**

Chart Tear-Off Bar: Chart can be removed in convenient lengths by tearing off against bar. **Price, $5.00.**

PREAMPLIFIER UNIT
(Deviation Amplifier)
Addition of this unit permits the **ElectroniK 18** to record microvolt ranges, as required for differential thermal analysis. **Price, $550.00**

3 BASIC TYPES COVER MOST ANALYTICAL USES

Type I—Model #18301856-01-37-0-000-060-07-053
Range: —.05 to 1 mv
Chart: 100-252-N, calibrated —.05 to 0 to 10, 10 time lines/inch, green grid lines.
Chart Speed: Manual change gears for: 30, 60, 120"/hr.
Price: $600.00
If tear-off bar is not needed, so specify and deduct $5.00.

Type II—Model #18301856-01-37-0-000-(560)-07-053
Range and Chart: Same as Type I above
Chart Speed: 5 speed shift giving: 10, 5, 2.5, 1.0 and 0.5 min. per inch
Price: $700.00
If tear-off bar is not needed, so specify and deduct $5.00.

Type III—Model #18301856-01-37-0-000-(560)-07-053
Range: —0.263 to 1 mv reversed
Chart: 100-253-NR, calibrated 10 to 0 over lower 4.75", 10 time lines/inch, green grid lines
Chart Speed: 5 speed shift giving: 10, 5, 2.5, 1.0 and 0.5 min. per inch.
Integrator: Designed for mounting Disc Instrument model 215-1
Prices: Instrument, $700.00
Integrator, $585.00.

If tear-off bar is not needed, so specify and deduct $5.00. Contact your local Honeywell sales office for other details or write Honeywell, Wayne & Windrim Avenues, Philadelphia 44, Pa.

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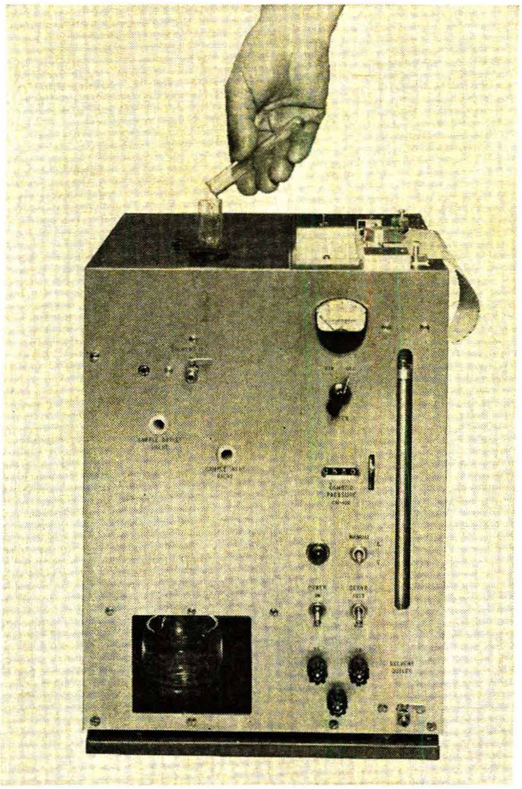
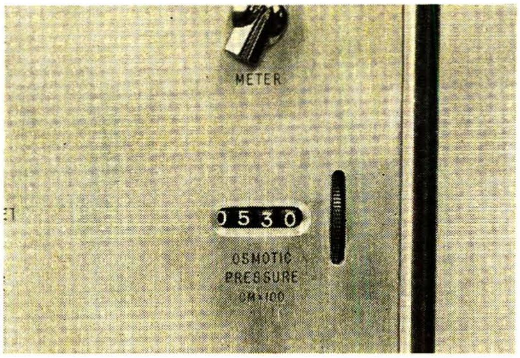
Sample in at 10:05



Osmotic Pressure at 10:15



Number-Average Molecular Weight Minutes Later

It's that fast in routine work with Hallikainen's new Model 1361 Automatic Osmometer.* And as easy as it looks. You pour sample into a convenient external receptacle. 5 to 10 minutes later, you read osmotic pressure on a direct-reading digital dial. Simple inlet and outlet valves let you change samples quickly for serial analysis. You can read four concentrations and calculate the number-average molecular weight of a polymer sample in less than an hour.

Model 1361's null-seeking servo-mechanism rapidly equilibrates pressure between sample and solvent in the osmometer cell, sparing you the hours-long wait of conventional membrane osmometry. With speed comes the accuracy of digital read-out that clicks off changes down to 0.01 cm of solvent over a 10 cm range.

Sensitivity of the instrument and its speed of measurement let you observe membrane permeation by low molecular weight compounds as it happens. A syringe-full of fresh solvent quickly flushes out the cell and restores initial membrane condition. It takes 20 minutes, at the most, to install a new membrane.

The complete Automatic Osmometer, with built-in chart recorder, is packaged in a trim 12 by 15 by 13-inch cabinet. The standard model operates at 35° C. An accessory kit converts it to 135° C operation. The product bulletin is contained entirely within four highly informative pages. Write for your copy today from:

**Shell Development Company design*

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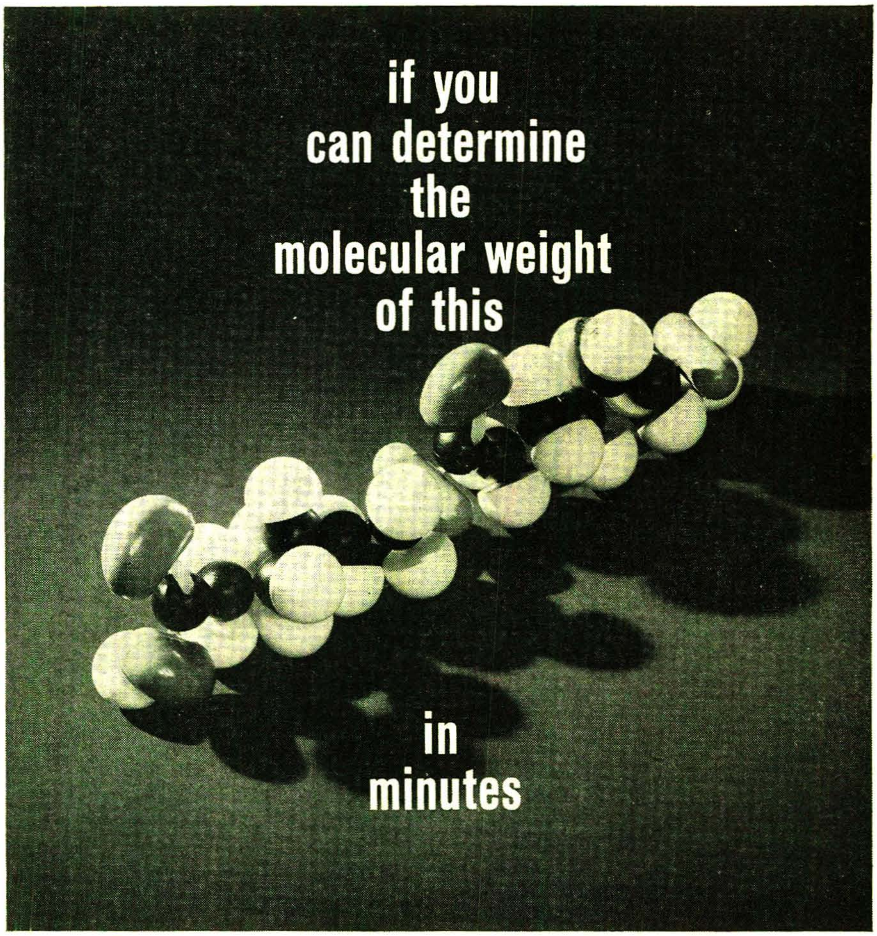

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15 Industrial Polymer Labs
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Polyvinylidene Chlorides
Polymethyl Methacrylates

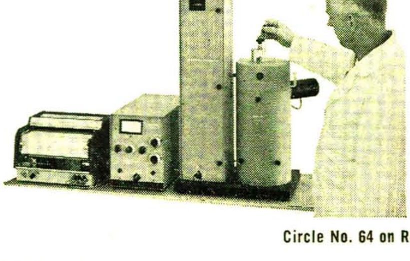
* Names on request

Mechrolab High-Speed Membrane Osmometers have been in daily service for many months by users such as those at left, to determine molecular weights of natural and synthetic polymers. Complete and accurate determinations based on four concentrations are made in **minutes**, not in hours or days.

Series 500 High-Speed Servo-Osmometers are designed for the number-average molecular weight range of 20,000 to 1,000,000, using organic or aqueous solutions.

Three models are available, covering a wide range of temperatures; Model 501, for general polymer work, from 25°-65°C; Model 502, for solutes which dissolve only at elevated temperatures, from 25°-130°C; and Model 503, 5°-65°C, for natural polymers which denature at ambient temperatures.

Interested? Would you like to join the others on our list? Start by writing now for literature. If you wish, a demonstration will be arranged in your area. Write to 1062 Linda Vista Avenue, Mountain View 4, Calif.



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Winter Gordon Research Conference

THE WINTER GORDON RESEARCH CONFERENCES will be held from January 27 to February 7, 1964, at The Miramar Hotel, Santa Barbara, Calif. The conferences, designed to stimulate research, hold informal type meetings composed of scheduled lectures and discussion groups.

The Winter Conferences consist of two programs—one on polymers, and one on electrochemistry: electrode processes.

Attendance at the Conferences is limited to approximately 100. Individuals interested in attending should write the office of the director, Dr. W. George Parks, Director, Gordon Research Conferences, University of Rhode Island, Kingston, R. I., and request application forms.

PROGRAM

POLYMERS

Maurice L. Huggins, Chairman

Bruno H. Zimm, Vice Chairman

January 27

Recent Progress in Polymer Research, H. Mark.
Organochemical and Analytical Studies of Polymers, W. Kern.

January 28

The Structure of Crystalline Polymers, I. Nitta.
Association Complexes of Polymers, F. E. Bailey.

January 29

High Temperature Polymers, T. W. Campbell.
Polyaromatics, C. S. Marvel.
The Role of Oxygen in the Formation and Degradation of Polymers, F. R. Mayo.

January 30

Equation of State of Polymer Liquids; Glass Transitions, A. J. Navlik and R. Simha.
Subject to Be Announced, A. A. Berlin.
Polymerization of Cyclic Olefins, G. Natta, G. Mazzanti, and G. Dall'Asta.

January 31

Toward a Theoretical Strategy for the Chemistry of Polymerization, K. Fukui.
Subject to Be Announced, S. E. Bresler.

ELECTROCHEMISTRY: ELECTRODE REACTIONS

Ralph N. Adams, Chairman

Richard Buck, Vice Chairman

February 3

Double Layer and Electrode Kinetics, Paul Delahay.
Cation Effects on Electrode Kinetics, Lucien Gierst.
Rotated Disk Electrodes, A. C. Riddiford.

February 4

Anion Effects on Potential Distribution at a Germanium Electrode, P. J. Boddy.
Electrochemical Studies of Rapid Homogeneous Chemical Reactions Employing AC Techniques, Donald Smith.

February 5

Coulometry Applied to Electrode Mechanism Studies, Allen J. Bard.
Electron Exchange Reactions, Henry Taube.
Photo Currents at Mercury Electrodes, G. C. Barker.

February 6

Kinetics of Hydrogen and Deuterium Discharge on Platinum, Manfred Breiter.
Chemical Reactions of Solvated Electrons, Leon Dorfman.

February 7

Electron Exchange Reactions, N. Sutin.

AAAS Annual Meeting

The annual meeting of the American Association for the Advancement of Science will be held this year in Cleveland from December 26 to 30. Of particular interest to chemists is the program of Section C—Chemistry. Two symposia are planned; chemistry of radical ions will be held December 27. A symposium on the chemistry of the excited state will be held on December 30. Both of these symposia will be co-sponsored by the Cleveland Section of the American Chemical Society. More information is available from Dr. S. L. Meisel, Socony Mobil Oil Co., Paulsboro, N. J.

LSU Symposium

The 17th Annual International Symposium on Modern Methods of Analytical Chemistry will be held at Louisiana State University, Baton Rouge, January 27 to 30, 1964. The program will consist of invited speakers only. Each of the speakers will have two hours to discuss topics of current interest. Time will be provided for questions and informal discussion.

The LSU Symposia are provided as a means for giving exhaustive surveys of current topics. Outstanding authorities in the various fields concerned are brought in to discuss the history, theoretical aspects, instrumentation, techniques, applications, and future developments of their specialties. The programs are conducted on an informal basis so that a free exchange of ideas between delegates and speakers is obtained. The LSU Symposia are unique in providing contact between distinguished foreign and American chemists.

In addition to the technical program, a display of modern laboratory equipment will be provided by leading apparatus manufacturing and supply houses.

Registration fee, not including tickets for the mixer and banquet, is $15.00. Student registration is $2.00, but does not include banquet tickets.

Housing is available on the campus in the newly renovated Adult Education Center, Pleasant Hall. Persons desiring to stay in downtown hotels or motels should make their own reservations.

All correspondence concerning registration and housing should be addressed to Short Courses and Conferences, General Extension Division, LSU. Questions concerning the program should be addressed to Dr. Philip W. West, Chemistry Department, LSU, Baton Rouge, Louisiana.

PROGRAM

Onium Ions as Analytical Reagents; Some New Analytical Reagents and Techniques. Harold Affsprung, University of Oklahoma.
Activation Analysis; Ultramicro Methods of Analysis. I. P. Alimarin, Lomonosov Moscow University.
Gas Liquid Chromatography. Stephen Dal Nogare, Du Pont, Wilmington, Del.
Recent Developments in Chelometry. Hermann Flaschka, Georgia Institute of Technology.
Internal Reflection Spectroscopy. N. J. Harrick, Philips Laboratory, Irvington-on-Hudson, N. Y.
Electroanalytical Studies in Molten Salts. Gleb Mamantov, University of Tennessee.
Compleximetric Methods of Analysis. Rudolf Pribil, Czechoslovak Academy of Sciences, Prague.
Electron Diffraction Applied to Polymers; Interference Microscopy. Robert G. Scott, Du Pont, Wilmington, Del.
Nuclear Magnetic Resonance. James N. Shoolery, Varian Associates, Palo Alto, Calif.
Reagents for the Precipitation of the Nitrate Ion; Some Recent Developments in Analytical Chemistry. William I. Stephen, The University, Edgbaston, Birmingham, England.

Electronics Course

A two-week course on the principles of electronics and the use of electronic instrumentation systems in research will be presented in San Francisco, January 27 to February 7, 1964, by the University of California's Engineering and Sciences Extension. The course is designed to provide a working knowledge of fundamental theory and analytical formulations, as well as a background for assessing the capabilities of instrumentation systems and adapting them to research requirements.

Among the topics to be covered are electrical and nonelectrical signals; complex signal wave forms; analysis of linear and nonlinear devices; amplitude modulation and demodulation; electron devices and electric circuits; signal transfer properties; and examples of a variety of instrumentation systems, including those used in nuclear magnetic resonance spectroscopy and infrared spectrophotometry.

Further information and registration forms may be obtained from Engineering and Sciences Extension, University of California, Berkeley, Calif. 94720.

Industry Items

Cenco Instruments Corp., 6450 W. Cortland St., Chicago 35, Ill., has acquired the **Phoenix Precision Instrument Co.** of Philadelphia. Phoenix is a developer and manufacturer of specialized instruments for laboratory research and on-stream control in production of pharmaceuticals, serums, etc.

Houston Instrument Corp., 4950 Terminal Ave., Bellaire 101, Texas, and **Auto Data, Inc.,** San Diego, Calif., a subsidiary, have merged. Auto Data will become a division of Houston Instrument Corp.

Lab-Line Instruments, Inc., has moved from Chicago to 15th and Bloomingdale Aves., Melrose Park, Ill. 60160.

Technical Measurement Corp., makers of nuclear and biomedical research instrumentation, has opened a new office to serve the Midwest. The facility will contain a complete spare parts inventory and laboratory and test personnel for instrument repairs and modification. The address is 5508 S. Brainard Ave., LaGrange, Ill.

Volk Radiochemical Co., Skokie, Ill., and **Isotopes Specialties Co.,** Burbank, Calif., have merged. Marketing responsibility for both organizations has been assumed by Volk.

CALENDAR OF EVENTS

Dec. 5 to 7	**19th Southwest Regional Meeting.** Shamrock Hilton Hotel, Houston, Texas. *Contact:* M. A. Mosesman, Research and Development, Humble Oil and Refining Co., P. O. Box 4255, Baytown, Texas.
Dec. 12	**American Microchemical Society.** Chemists Club, New York City. Subject: F. D. A. Concepts of Acceptable Analytical Procedures for Standards, Frank H. Wiley. *Contact:* R. A. Hofstader, Esso Research and Engineering Co., Linden, N. J.
Dec. 26 to 30	**American Association for the Advancement of Science Annual Meeting.** Cleveland, Ohio. *Contact:* Dr. S. L. Meisel, Socony Mobil Oil Co., Paulsboro, N. J. Page 43 A, Dec.

Coming Events

Jan. 7 to 9—Tenth National Symposium on Reliability and Control. Statler Hilton Hotel, Washington, D. C. *Contact:* L. S. Gephart, Lockheed Missiles and Space Co., Dept. 64-01, Bldg. 104, Sunnyvale, Calif.

Jan. 24 to 25—First Annual Microscopy Symposium. Fontainebleau Motor Hotel, New Orleans, La., Sponsor: Louisiana Society for Electron Microscopy. *Contact:* C. J. Arceneaux, Ethyl Corp., P. O. Box 341, Baton Rouge, La. 70821.

Jan. 27 to Feb. 7—Winter Gordon Research Conferences. The Miramar Hotel, Santa Barbara, Calif. *Contact:* Dr. W. George Parks, Director, Gordon Research Conferences, University of Rhode Island, Kingston, R. I. Page 43 A, Dec.

Feb. 3 to 4—Research Conference on Gas Chromatography. University of California, Los Angeles, Calif. *Contact:* H. L. Tallman, Physical Sciences Extension, Room 6532, Engineering Bldg., University of California, Los Angeles 24, Calif. Page 68 A, Oct.

Feb. 3 to 7—ASTM International Conference on Materials and ASTM Committee Week. Sheraton Hotel, Philadelphia, Pa., *Contact:* American Society for Testing and Materials, 1916 Race St., Philadelphia 3, Pa.

Feb. 27 to 29—Fifth Omnibus Conference on Experimental Aspects of NMR Spectroscopy. Mellon Institute, Pittsburgh, Pa. *Contact:* Mellon Institute, 4400 Fifth Ave., Pittsburgh, Pa.

Mar. 2 to 6—Fifteenth Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. Penn-Sheraton Hotel, Pittsburgh, Pa. *Contact:* Rudolph B. Fricioni, Allegheny Ludlum Steel Corp., Corporate Quality Control Laboratory, Research Center, Brackenridge, Pa. Page 81 A, Sept.

Mar. 23 to 26—Second International Symposium on Advances in Gas Chromatography. Sheraton Lincoln Hotel, Houston, Texas. *Contact:* Prof. A. Zlatkis, Dept. of Chemistry, University of Houston, Texas. Page 81 A, Sept.

Apr. 19 to 21—Second Annual Oak Ridge Radioisotope Conference. Gatlinburg Civic Auditorium and Riverside Motor Lodge, Gatlinburg, Tenn. *Contact:* Overman, Special Training Division, Oak Ridge Institute of Nuclear Studies, P. O. Box 117, Oak Ridge, Tenn. Page 49 A, Nov.

Apr. 22 to 25—Fourth Rare Earth Research Conference. Camelback Inn, Phoenix, Ariz. *Contact:* Dr. LeRoy Eyring, Dept. of Chemistry, Arizona State University, Tempe, Ariz. Page 67 A, Oct.

May 7 to 8—Fourth Conference on Vacuum Microbalance Techniques. Mellon Institute, 4400 Fifth Ave., Pittsburgh, Pa. *Contact:* Mr. F. A. Brassart, Westinghouse Research and Development Center, Beulah Rd., Pittsburgh 35, Pa. Page 67 A, Oct.

May 20 to 23—Symposium on Modern Methods for the Analysis of Organic Compounds. Eindhoven, Holland. Sponsors: German Chemical Society and Royal Dutch Chemical Society. *Contact:* Gesellschaft Deutscher Chemiker, Geschäftsstelle, 6000 Frankfurt (Main), Postfach 9075, Germany. Page 47 A, Nov.

May 25 to 29—Society of Physical Chemists of France, 40th annual meeting. Bordeaux, France. Subject: Structure of Polyatomic Radicals. *Contact:* Prof. Guy Emschwiller, Société de Chemie Physique, 10 rue Vauquelin, Paris 5°, France, Page 75 A, Sept.

June 2 to 5—15th Annual Mid-America Symposium on Spectroscopy. Sheraton-Chicago Hotel, Chicago, Ill. *Contact:* Elwin N. Davis, Sinclair Research, Inc., 400 E. Sibley Blvd., Harvey, Ill.

June 22 to 26—Third International Congress of the International Federation of Societies of Cosmetic Chemists. New York City. *Contact:* Walter Wynne, 321 West 44th St., Room 700, New York 36, N. Y. Page 62 A, August.

July 19 to 25—Third International Congress of Polarography. Southampton University, England. *Contact:* Dr. D. A. Pantony, Dept. of Metallurgy, Royal School of Mines, Prince Consort Rd., London S. W. 7, England. Page 74 A, Sept.

Aug. 26 to Sept. 3—Third European Regional Conference on Electron Microscopy. Prague, Czechoslovakia. *Contact:* 3rd European Regional Conference on Electron Microscopy, Prague 1964, Organizing Committee, Albertov 4, Prague 2, Czechoslovakia. Page 75 A, Sept.

Sept. 14 to 18—International Conference on Mass Spectrometry. Paris, France. Sponsors: ASTM Committee E-14, G. A. M. S. and the Hydrocarbon Research Group. *Contact:* Secretariat du G. A. M. S., 1, rue Gaston Boissier, Paris XV, France. Page 67 A, Oct.

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PRODUCT	SIZE	MERCK	BRAND B	BRAND M
Chloroform Reagent (A.C.S.)	5 pt.	$1.19	$1.24	$1.24
Acetone Reagent (A.C.S.)	8 pt.	$ .48	$ .54	$ .52
Methyl Alcohol Anhydrous Reagent (A.C.S.)	8 pt.	$ .44	$ .48	$ .48
Isopropyl Alcohol Reagent	8 pt.	$ .51	$ .56	$ .53
Carbon Tetrachloride Reagent (A.C.S.)	5 pt.	$ .97	$1.05	$1.00
Toluene Reagent (A.C.S.)	8 pt.	$ .45	$ .49	$ .48

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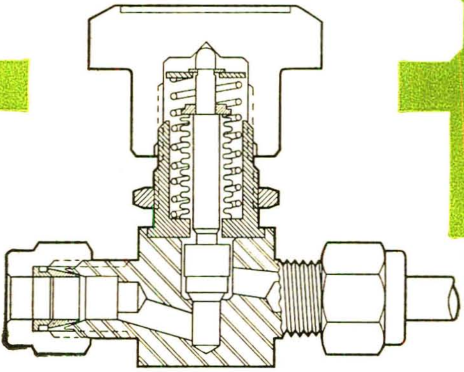
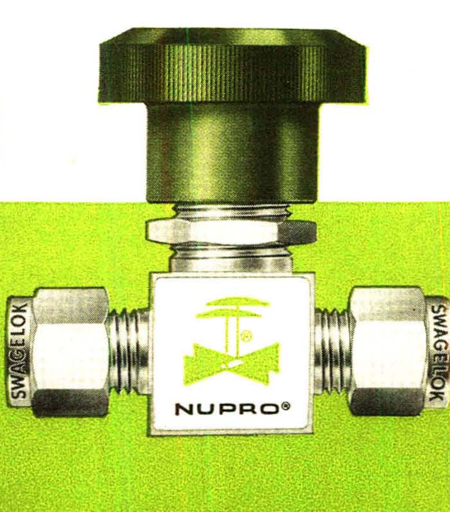
BRASS BELLOWS VALVE





The NUPRO Brass Bellows Valve is a new, compact, hermetically sealed valve for use in all applications where absolute leak tightness is necessary.

Helium leak-tight shutoff is obtained with a Teflon coated stem tip which insures the advantages of a soft sealing valve without incurring the usual disadvantages.

This NUPRO Valve can be used at pressures to 600 p.s.i. and temperatures to 300° F. Panel mounting and back mounting provide outstanding installation flexibility unusual in such a compact valve.

SPECIAL FEATURES

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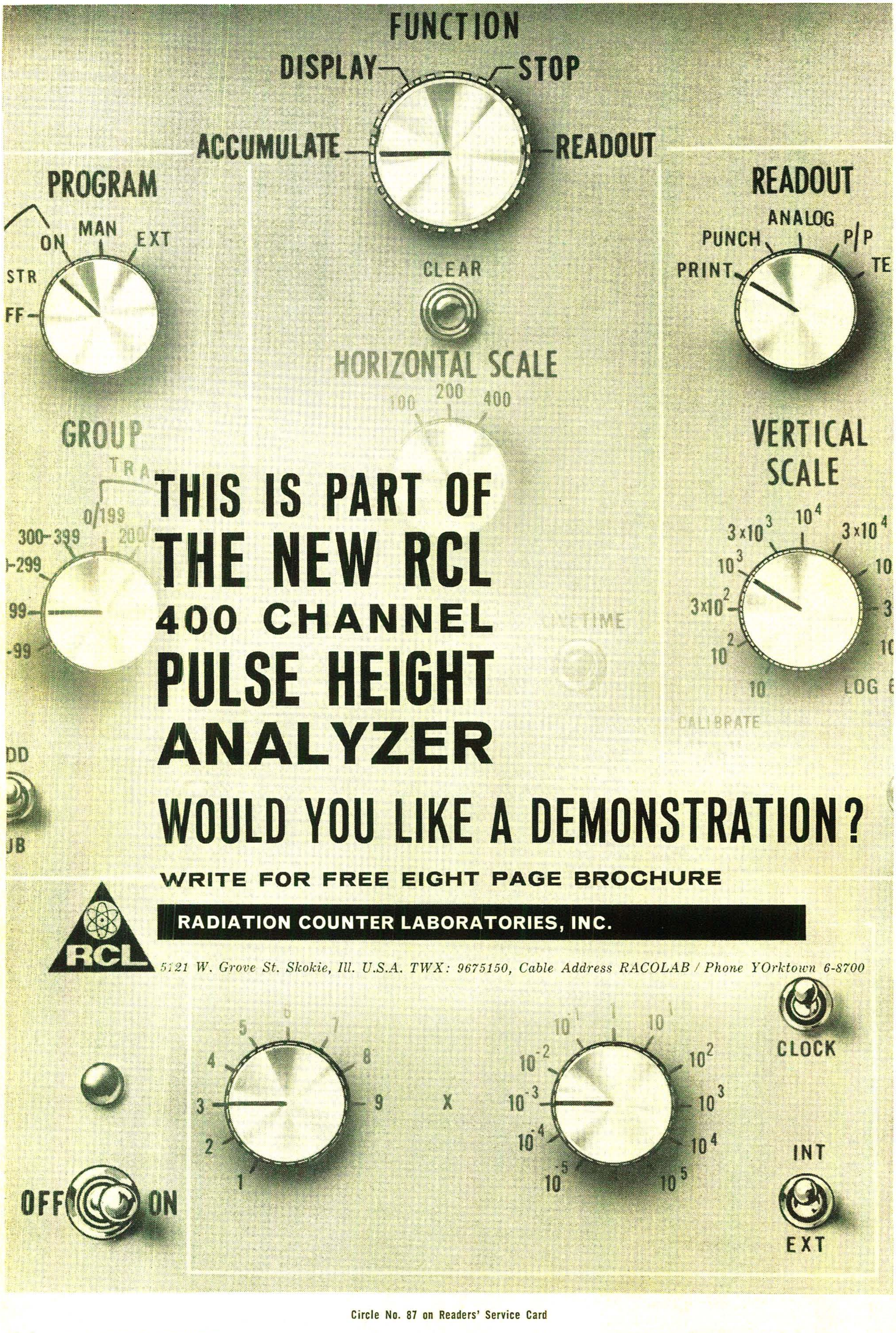
*DuPont **Crawford Fitting Company trademark



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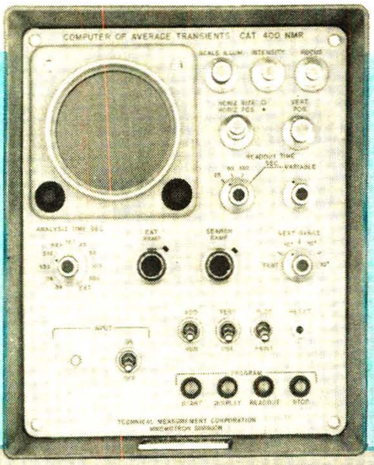
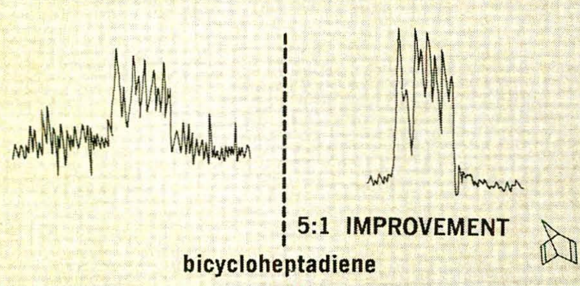
Important News for NMR Spectroscopists

NEW LOW-COST MNEMOTRON COMPUTER USES AVERAGING TECHNIQUE TO GREATLY INCREASE SIGNAL-TO-NOISE RATIO



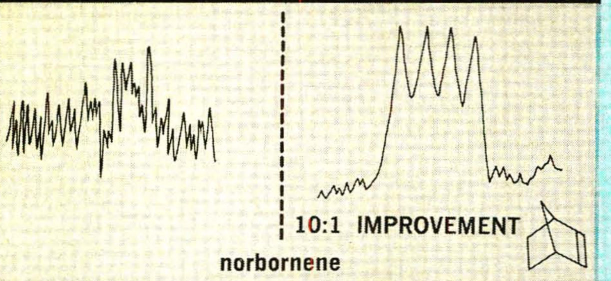
TYPICAL RESULTS ACHIEVED THROUGH USE OF CAT 400 NMR



5:1 IMPROVEMENT

bicycloheptadiene

WITHOUT CAT 400 NMR **WITH CAT 400 NMR**



10:1 IMPROVEMENT

norbornene

WITHOUT CAT 400 NMR **WITH CAT 400 NMR**

- Shown above are ^{13}C patterns of the olefinic hydrogens from ^{13}C in natural abundance. [P. Laszlo and P. von R. Schleyer, J. Am. Chem. Soc. 85, 2017 (1963)].
- Development of the technique for using the CAT in chemical applications of NMR and ESR is described by L. C. Allen and L. F. Johnson, J. Am. Chem. Soc. 85, 2668 (1963). These authors summed 2500 spectral traces in CAT 400 and obtained a resultant spectrum for 0.004% ethyl benzene in CCl_4 having the same appearance found for a 0.2% solution with a single trace.
- O. Jardetzky, N. G. Wade and J. J. Fischer, Nature, 197, 183 (1963) report detection of 0.005 M DPN (diphosphopyridine nucleotide) in D_2O (alcohol dehydrogenase) — an experiment that illustrates the importance of the averaging technique in allowing the investigator to work with concentrations usually found in biological systems.

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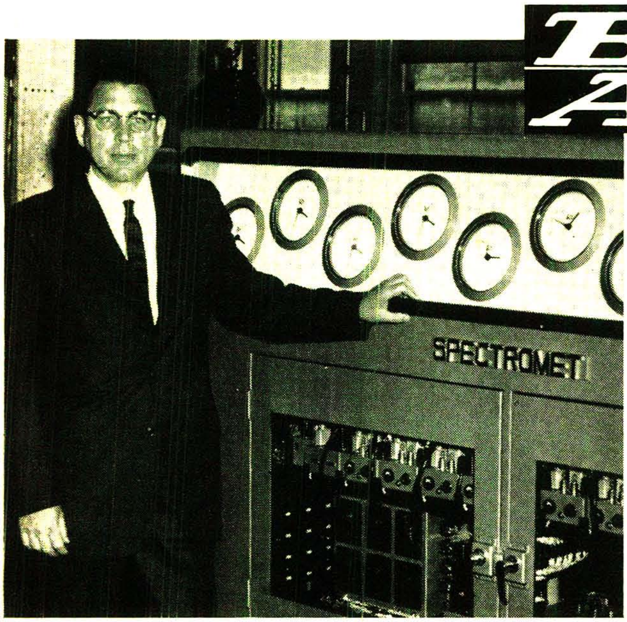

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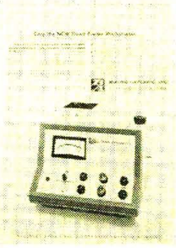

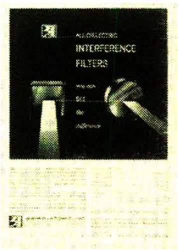
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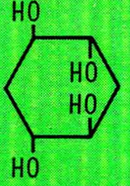
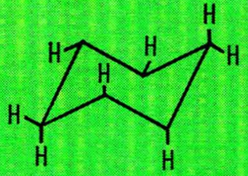
NO. 8 A-60 AT WORK SERIES

The assignment of the correct configurations to the various stereoisomers which correspond to a particular structure is one of the most difficult problems of organic chemistry. In many cases, NMR provides an elegant and convenient method for making such assignments.

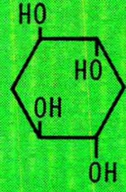
For example, the structure, 1, 2, 4, 5-cyclohexanetetrol, corresponds to five different configurations, differing in the arrangement (cis or trans) of the four OH groups. All five of these stereoisomers have been isolated and their configurations established by NMR.[1]



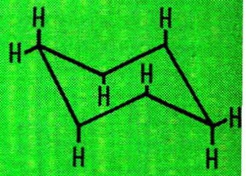
(A)



(A′)

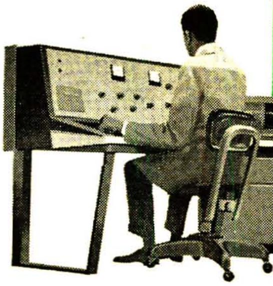


(B)



(B′)

Shown above are the configurations of the two possible trans/trans 1, 2, 4, 5-cyclohexanetetrols (A and B). Also shown (A′ and B′) are the chair conformations corresponding to A and B. The A-60 NMR spectra shown were obtained from the two isomers resulting from a reaction expected to give only the trans/trans arrangement. The problem, then, was to assign each isomer the proper configuration, that is, either (A) or (B).



NMR

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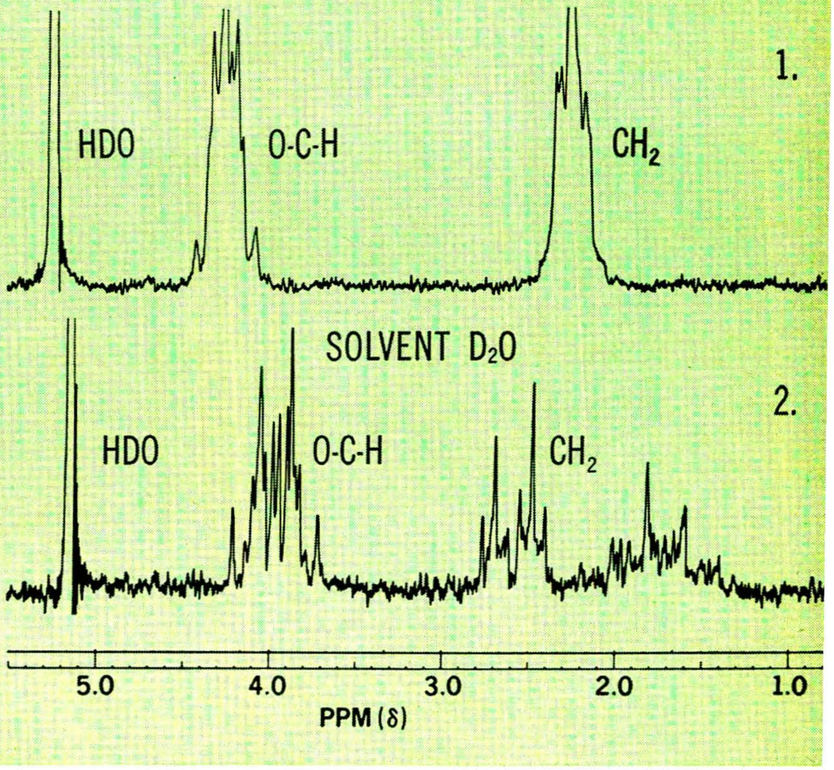
In spectrum 1 the signals from the four methylene protons appear in a narrow (width less than 22 cps) pattern at 2.33 ppm downfield from external tetramethylsilane. At 4.26 ppm is a similar pattern due to the four O-C-H ring protons. The narrowness of these two patterns reveals that little if any chemical shift exists among the four methylene protons or among the four O-C-H ring protons. This lack of chemical shift suggests that rapid chair-chair interconversion is taking place resulting in equivalence of the four protons in each group on a time-average basis. Only the diaxialdiequatorial chair conformation A′ would present such a situation since ring inversion converts it to a conformation identical with the original.

Spectrum 2 can be interpreted in terms of conformation B′ which has four equatorial OH and on inversion gives a highly unfavored conformer. The patterns at 1.85 and 2.67 are assigned to the axial and equatorial protons respectively of the methylene groups, which is verified by the appearance of these patterns. The mere demonstration that the methylene protons are non-equivalent suffices to allow the isomer giving this spectrum to be assigned configuration B.



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[1] For details see McCasland, Furuta, Johnson and Shoolery, J. Org. Chem. **28**, 894, (1963) and references therein.

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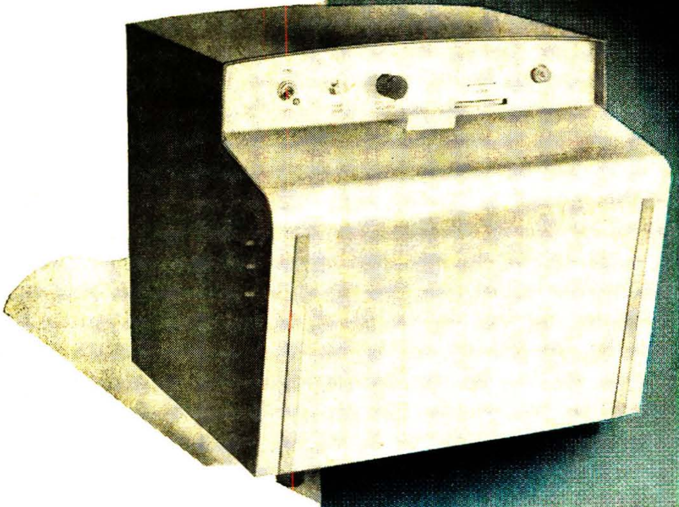

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*"*An Automated Fluorometric Method for the Determination of Serum Magnesium*" John B. Hill, Department of Pharmacology, University of North Carolina Medical School, Chapel Hill, N. C. Annals of New York Academy of Sciences, Vol. 102, Oct. 1962
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NEW BOOKS

Particle Size: Measurement, Interpretation, and Application. *R. R. Irani and C. F. Callis. viii + 165 pages. John Wiley and Sons, Inc., 605 Third Ave., New York 16, N. Y. 1963. $7.50.*

Reviewed by Emil S. Palik, General Electric Company, Lamp Metals and Components Department, Cleveland, Ohio

The objective of this latest book on particle size measurements is to fulfill the need for a single reference work "which would give an analyst a simple choice of the preferred technique for particle size measurement of a specific material" and, in addition, to aid in the interpretation of the results. In 10 chapters the authors attain this objective with a clearly-written style, well-documented material, and ample graphs and tables. The chapter headings are: Application of Particle Size Distribution Measurements, Definitions, Methods of Data Presentation, Distribution Functions Applicable to Particle Size Distributions, Sedimentation Techniques, Microscopy, Sieving, Miscellaneous Techniques, Comparison of Particle Size Distribution Data from Various Methods, and Procedure for Choosing the Appropriate Method of Particle Size Measurement.

The book is up-to-date with literature references and methods and contains numerous references to commercially available instruments. The book appears to be a compromise between the detailed comprehensive volumes and the abbreviated texts that have appeared during the past decade. The authors have placed some emphasis on work which is described in detail in the literature but have not made extensive critical evaluations in all cases. This limitation is undoubtedly necessary in a book which covers a wide rapidly growing field and should be borne in mind by readers. This book differs from its predecessors by having an entire chapter devoted to a procedure for selecting the appropriate method of particle size measurement. Although the scheme presented is fairly comprehensive, it can be only a guide. The reader will of necessity need to treat each sample on an individual basis, and most certainly the choice of method will be largely dictated by considerations unique to the reader.

The book has been written to appeal to both the novice and the more experienced worker, although in the reviewer's opinion its primary value will be for newcomers in the field. The authors have not devoted any sections to the measurement of pore volume and pore volume distributions which, like surface area and particle size, are properties that can have a significant effect on the behavior of particles. The authors do not mention or describe all of the sedimentation devices available, particularly other forms of the pipet, turbidimeter, and sedimentation balance; however, their coverage is more thorough in current methods than any other publication known to the reviewer.

A large number of examples are from the authors' own research. However, first hand experience with some of the instruments is lacking, and this probably accounts for the lack of critical analysis for these instruments. A large section is devoted to sedimentation techniques, and here the authors have gone into considerable detail on methods and calculations. The table of dispersants for selected materials is brief, but their tests for proper dispersion are elucidated in some detail.

The type is clear and easy to read. More sketches or, preferably, photographs of the instruments described would have made the book more useful. The binding and illustrations are good and the charts and graphs have been well chosen and clearly presented. Standard conventions have been employed in the graphical data presentations. A few mistakes and typographical errors were observed, but these do not detract significantly from the reading. In the mathematical derivations, the authors have chosen symbols which are unlike those employed in some other texts, but this should not cause the reader any difficulty.

This book reflects the wide experience and high level of competence of the authors, and the range and balance of their presentation is commendable. In view of this, the reviewer recommends this newest book to the particle size community as a worthwhile addition to the growing number of books on this subject.

Inorganic Thermogravimetric Analysis, 2nd and Revised Ed. *Clement Duval. Translated by Ralph E. Oesper. xv + 722 pages. American Elsevier Publishing Co., Inc., 52 Vanderbilt Ave., New York 17, N. Y. 1963. $22.*

Reviewed by C. B. Murphy, Advanced Technology Laboratories, General Electric Co., Schenectady 5, N. Y.

In contrast to the original edition (1953), which was a compilation of the work of Professor Duval and his students, this edition is completely revised and represents a fairly comprehensive treatment of the literature up to, and including, 1960. Some 2200 references are cited.

The book is divided into two parts. Part One consists of chapters devoted to a historical review of thermogravimetry, deflection type thermobalances, null type thermobalances, and precautions to be observed in application of the technique. Seventy-nine chapters, each devoted to an element, from lithium to americium, together with a brief section, organic chemistry, constitute Part Two. References are cited at the end of each chapter. The material covered has been cross-indexed—i.e., barium sulfate is covered in the chapter on barium, with a notation to this effect in the chapter on sulfur.

Twenty-one applications of thermogravimetry are cited. However, some of these are very unique—i.e., the determination of the atomic weight of carbon through the thermolysis of calcium oxalate. Isothermal experimentation is briefly mentioned, and, then, not in connection with high temperature oxidation of metals, where significant work has been performed. The application of the thermobalance to the study of reaction kinetics disappointingly was given half a page. The chapter on organic materials in no way reflects the amount of work that has been accomplished, particularly with polymeric materials. It would appear that this last chapter could have been omitted and, more appropriately been substituted by chapters devoted to clays and minerals.

The book reflects Professor Duval's interests in the thermal properties of precipitates. It is from this point of view that most of the materials are considered. The analytical chemist concerned with gravimetric procedures will find the temperatures for treatment of his precipitates well established.

In spite of some shortcomings, the compilation of data on the inorganic compounds will make this book a valuable reference for those interested in the thermal properties of materials.

Thin Film Chromatography. *E. Vernon Truter. xi + 205 pages. Interscience Publishers, Inc., 605 Third Ave., New York 16, N. Y. 1963. $7.00.*

Reviewed by ***B. Z. Senkowski, Assistant to the Director of Products Control, Hoffmann-La Roche, Inc., Nutley 10, N. J.***

The general acceptance and widespread use of "Thin Film Chromatography" is evidenced by the increasing number of publications utilizing this method. The more universally accepted term for this procedure appears to be thin layer chromatography. For the sake of uniform nomenclature, it would appear advantageous to standardize in favor of the term thin layer chromatography.

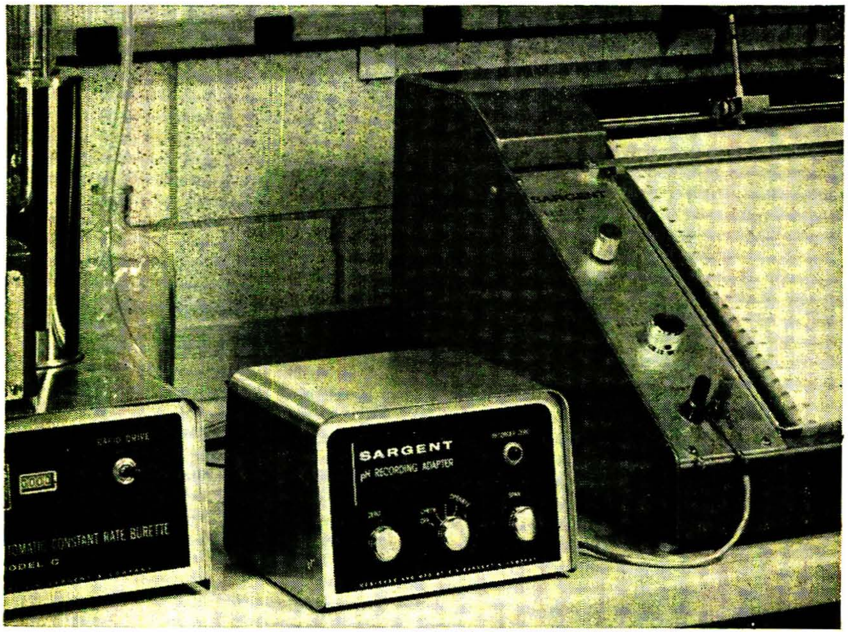
Truter's book, in English, contains 205 pages and serves as a general survey of the equipment and operational techniques on this timely subject. Part I (57 pages) deals with every phase of thin layer chromatography from the clean glass plate through the recording of the chromatogram. The factors which influence the mobility of the sample on the plate are lightly discussed, such as adsorbents, application, development sample size, detection, and reproducibility of the R_f values.

The author states that the optimum sample load to be applied to a plate should be about 10 μg. or less. This is in many cases an impractical limitation when studies concerning purity are carried out. Part II (64 pages) deals with Special Techniques and describes the different procedures used in developing a chromatogram, pH effects, and normal and reversed phase partitioning in thin layer chromatography. Brief mention is made of ionophoresis as applied to thin layer chromatography. Under Identification, the author discusses the use of functional group reactions on the chromatoplate and chromatography of derivatives to help characterize an unknown sample. This section (10 pages) is very well written and would be helpful to anyone working in this area. An adequate treatment of the quantitative evaluation of chromatographed samples is presented.

In the reviewer's estimation, some of the repetition that exists in Part I and Part II regarding development and detection could have been eliminated through consolidation of the related information. Part III (81 pages) describes application of thin layer chromatography and contains a section on the Resolution of Groups of Related Compounds (51 pages). This latter topic is particularly well organized and contains a number of the important contributions that have appeared in the literature such as the work on medicinal compounds, alkaloids, and amino acids.

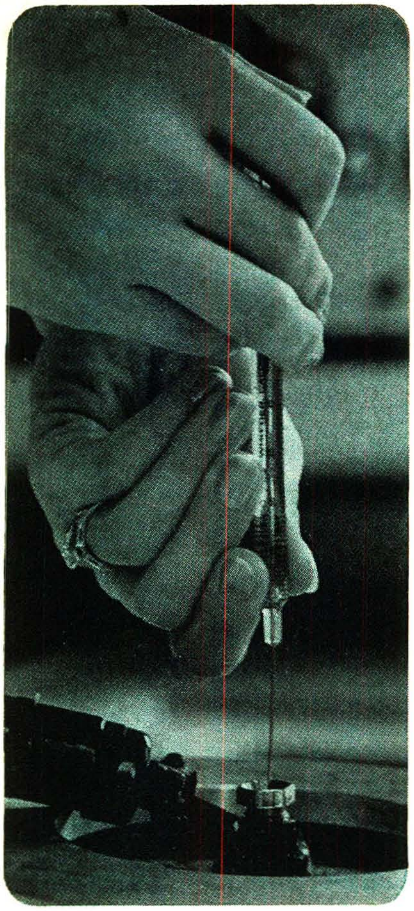
The book lists 282 references including a number that appeared in 1962. Many of the journals are not readily accessible to the average laboratory.

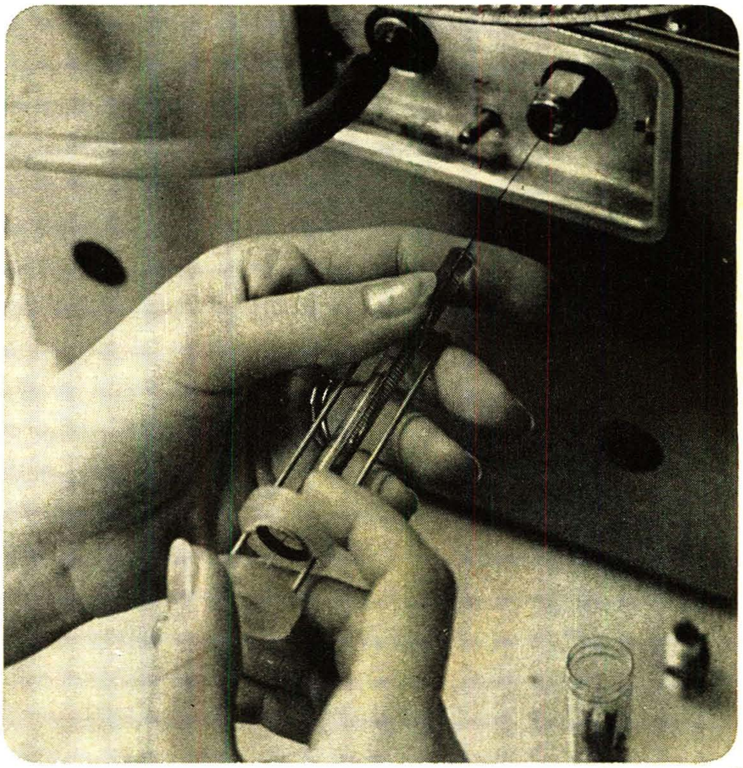
This concise volume is recommended to those who wish to introduce thin layer chromatography to their work.

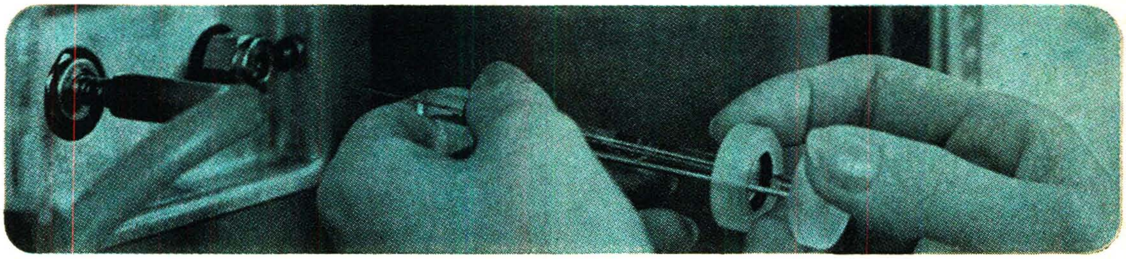


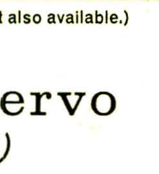
Infrared Spectra of Inorganic and Coordination Compounds. *Kazuo Nakamoto. xii + 328 pages. John Wiley & Sons, Inc., 440 Park Ave., South, New York 16, N. Y. 1963. $9.50.*

Reviewed by ***Stephen E. Wiberley, Department of Chemistry, Rensselaer Polytechnic Institute, Troy, N. Y.***









NEW BOOKS

Many texts dealing with infrared spectrometry have appeared in the past few years, but only one has dealt exclusively with inorganic compounds, and that book was bibliographic in nature. Hence, this book which critically reviews infrared spectra of various inorganic compounds fills a definite void.

The first section of the book deals with the theory of normal vibrations in a definitive fashion. Purposely and wisely omitted are subjects (such as rotational spectra and experimental techniques) already well covered by other authors.

The discussion of inorganic compounds is developed in a logical fashion starting with diatomic and triatomic molecules and then leading into the point groups of more complex symmetry.

The final section of the book treats coordination compounds.

The author does not attempt to cover all the references to the infrared spectra of inorganic compounds but has chosen examples to illustrate particular concepts. His interpretation of the various spectra are not always as detailed as Herzberg's classical text, which treats both inorganic and organic molecules, but the main spectral features are emphasized in a concise and informative manner. There are several useful group frequency charts and, of course, the omnipresent group character tables.

The author's writing is direct, clear, and to the point. This reviewer recommends this book as a valuable addition to the library of chemists interested in the spectra and structure of inorganic and coordination compounds.

Composition Tables. Data for Compounds Containing C, H, N, O, S. *George H. Stout. xi + 391 pages. W. A. Benjamin, Inc., 2465 Broadway, New York 25, N. Y. 1963. $6.*

These tables were planned to eliminate repetitive calculation of theoretical values of organic compounds. The ranges of the elements included are: C, 1 to 40; H, about C/2 to 2C + 2 + N; O, 0 to 10; N, 0 to 5.

All the molecular and atomic weights given as well as the per cent compositions, were calculated using the 1961 atomic weights of the IUPAC and are based on carbon-12 as a standard. They were calculated on an IBM 709 computer.

A set of preliminary tables provides multiples of atomic weights for the calculation of analyses of compounds

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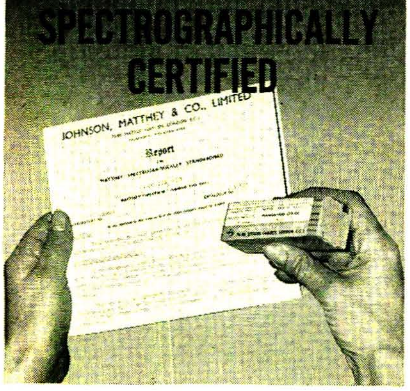

not included in the main tables, as well as multiples of functional group weights for the calculation of theoretical results for group analyses.

Electrochemistry. Theoretical Principles and Practical Applications. *Giulio Milazzo. Translated by P. J. Mill. xv + 708 pages. American Elsevier Publishing Co., Inc., 52 Vanderbilt Ave., New York 17, N. Y. $20.*

This is the first English edition translated from the Italian manuscript and brought up to date. The aim of the book is to set out the fundamental concepts of electrochemistry in the simplest and most readily accessible form. It is suitable for non-specialists but presupposes a certain minimum familiarity with chemical and physico-chemical concepts. Included are chapters on electrolytes and electrolytic conduction, Galvanic cells, electrolysis and electrochemical kinetics in aqueous solutions, analytical applications, the electrochemistry of colloids and electrokinetic phenomena, general considerations about electrochemical plants, electrometallurgy in aqueous solutions, nonmetallurgical electrolytic processes, electrolysis in molten electrolytes, practical primary cells and storage batteries, and the electrochemistry of gases.

Organic Electronic Spectral Data. *Vol. IV. 1958–1959. J. P. Phillips, F. C. Nachod, editors. 1179 pages. Interscience Publishers, Inc., 605 Third Ave., New York 16, N. Y. 1963. $20.*

Volumes I and II covering the periods, 1946–52 and 1953–55, appeared in 1961. Volume III covering 1960–61 is now being prepared.

The spectral data in this volume were abstracted from journals listed in the reference section. All entries are organized according to the molecular formula index used by Chemical Abstracts and Beilstein. For the compounds listed, the following information is given: name and formula; solvent used; wavelength values in millimicrons for all maxima, shoulders, and inflections, and the logarithms of the corresponding molar absorptivities in parentheses; and code numbers referring to the journal, page number, and year.

Spectrophotometric Analysis of Drugs Including Atlas of Spectra. *Irving Sunshine and S. R. Gerber. xvii + 235 pages. Charles C Thomas, Bannerstone House, 301–327 East Lawrence Ave., Springfield, Ill. 1963. $10.50.*

The ultraviolet and infrared absorption data of 150 drugs are presented in this volume. Narcotics are not included since these are well documented in the literature.

A screening procedure is presented for isolating the drugs from blood for further analysis. It is based on solvent extractions of blood whose proteins are precipitated by zinc salts in alkaline medium. The ultraviolet absorption data can be obtained using this extract. This material is reextracted and used for KBr pellet infrared analysis. The infrared absorption spectra are given as chloroform solutions and as KBr pellets.

The ultraviolet absorption spectra index lists the drugs and indicates the wavelength of their absorption maximums in mμ, other absorption peaks of the same drug, absorption minimums, and page number for the spectra. The UV spectra show two curves; one for the acid form of the drug and one for the basic form.

The infrared absorption data were obtained using a double beam recording Perkin-Elmer Model 21 spectrophotometer.

Gas Chromatography 1962. *M. van Swaay, editor. lii + 411 pages. Butterworth, Inc., 7235 Wisconsin Ave., Washington 14, D. C. 1963. $19.50.*

This book contains the proceedings of the fourth symposium organized by the Fachgruppe Analytische Chemie of the Gesellschaft Deutscher Chemiker and the Gas Chromatography Discussion Group of the Hydrocarbon Research Group of the Institute of Petroleum, held at the Auditorium Maximum, Hamburg, Germany, from June 13 to 16, 1962.

Opening addresses and lectures given by H. Kienitz, W. Drexelius, R. Sieverts, R. Kuhn, A. J. P. Martin, and A. V. Kiselev are included. The first section contains eight papers on theoretical considerations in gas liquid and gas solid chromatography. Section II, 10 papers, is concerned with apparatus and techniques including programmed chromatography, capillary columns, mass spectrometric identification, and process control using gas chromatography. The last section contains nine papers on applications.

The volume contains the panel discussions and includes the discussions that ensued after presentation of the individual papers.

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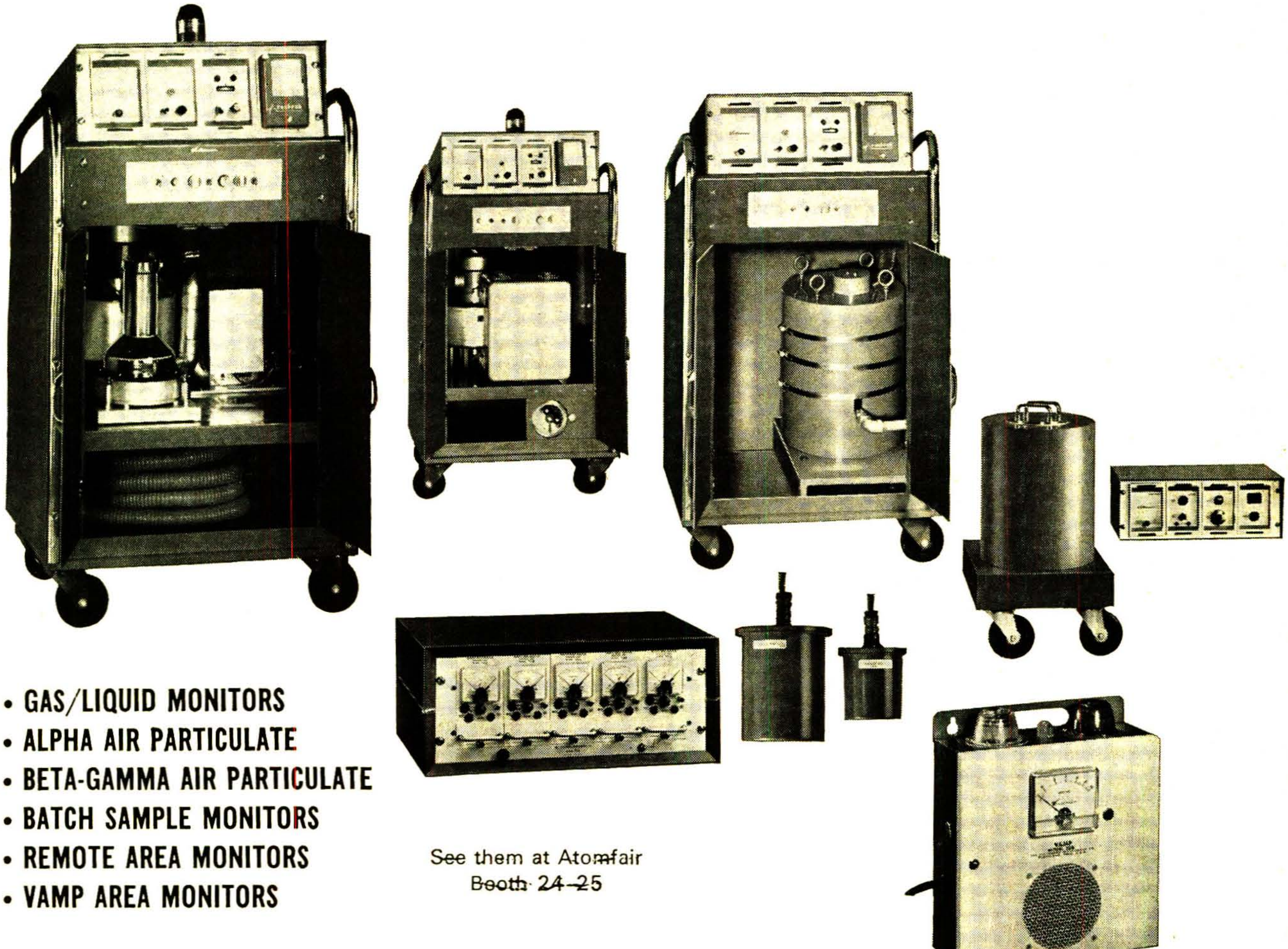

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NEW BOOKS

Analytical Chemistry published a symposium summary written by R. S. Juvet, Jr., page 77 A, September 1962.

The Identification of Molecular Spectra. *3rd edition. R. W. B. Pearse and A. G. Gaydon. xi + 347 pages. John Wiley & Sons, Inc., 605 Third Ave., New York 16, N. Y. 1963. $18.50.*

New data, recorded since the second edition was published in 1950, through the end of 1961 have been included in this volume. There are 84 molecules which appear for the first time, observations and information have been extended for 135 molecules, and tables of persistent heads have been revised and increased by the addition of about 350 new entries.

The wavelength region considered is from 10,000 to 2000 A. Recorded data from all diatomic molecules, and the triatomic and more complex molecules with well-defined banded structures are included. The first section lists the strongest heads of the more persistent and better known band systems of each molecule in order of wavelength, together with information as to origin, intensity in various sources, and appearance. The second section consists of individual lists of band heads for each system of each molecule with notes related to the data.

In addition, ten pages are devoted to procedures and precautions to be used in identifying molecular spectra. A number of photographs show the more frequently encountered band systems. Finally, the book contains a table of persistent lines of the elements, and author, and subject indexes.

Advances in X-Ray Analysis. *Vol. 6. William M. Mueller, Marie Fay, editors. xi + 480 pages. Plenum Press, Inc., 227 West 17th St., New York 11, N. Y. 1963. $17.50.*

This is the newest volume in a continuing series reporting the proceedings of the annual conferences on applications of x-ray analysis held at the Denver Research Institute. This book contains the papers presented at the conference held August 8 to 10, 1962. Topics covered include x-ray diffraction and fluorescence; analysis of radioactive samples; analysis at cryogenic and high temperatures, and in vacuum; instrumentation and accessories; x-ray diffraction methods for studying aerosols; on-stream analysis; nondestructive testing; and other aspects of x-ray analysis.

U. S. Government Publications

The following can be obtained from the Office of Technical Services, U. S. Dept. of Commerce, Washington, D. C. 20230

Infrared Technology and Nondestructive Testing. *B. G. Martin, of Army Redstone Arsenal for the U. S. Army. February 1963. 30 pages. Order AD 402 888. $1.00.*

This report discusses a promising nondestructive testing device for detection of flaws in materials, based on measurement of infrared radiation. General feasibility has been proved in the laboratory, and the device is now being developed for the nondestructive testing of solid fuel rocket motors, primarily for unbonded flaws.

Effect of Pressure on the Thermal Conductivity of a Gas. *C. C. Mintner of U. S. Naval Research Laboratory. February 1963. 24 pages. Order 298 997. 75 cents.*

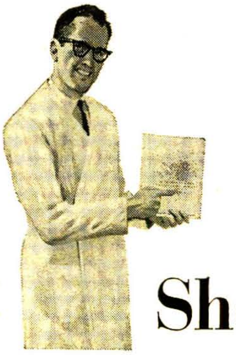
This report describes research that indicates that mean temperature increases as the pressure of a gas is decreased and therefore at a given temperature the thermal conductivity of a gas decreases as the pressure is reduced.

Application of the Internal Standardization Method for the Gas Chromatographic Determination of Drying Oil Fatty Acids. *G. G. Esposito, U. S. Army Aberdeen Proving Ground. March 1963. 11 pages. Order AD 402 178. 50 cents.*

Army research aimed at determining a suitable method for obtaining quantitative data from the chromatographic separation of methyl esters prepared from oils used in organic coating indicates that gas chromatography using an internal standard is adequate.

Precision Temperature Control of Air and Oil Baths. *N. L. Hensinger, of Sandia Corp., for the U. S. Atomic Energy Commission. March 1963. 21 pages. Order SCTM 71-63. 50 cents.*

Development of a new precision temperature control device for air and oil baths is disclosed. The controller in combination with a carefully designed air bath, has achieved temperature control to ±0.003° C. In com-



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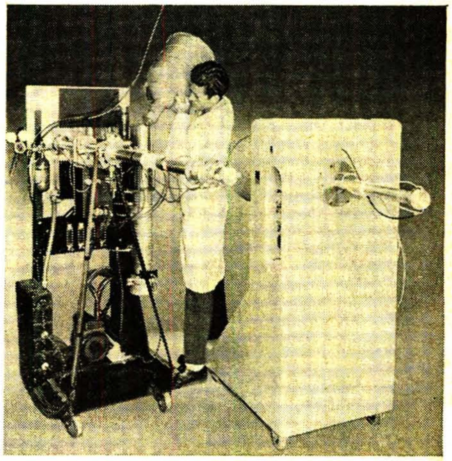

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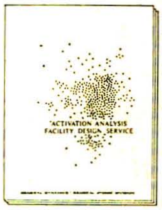

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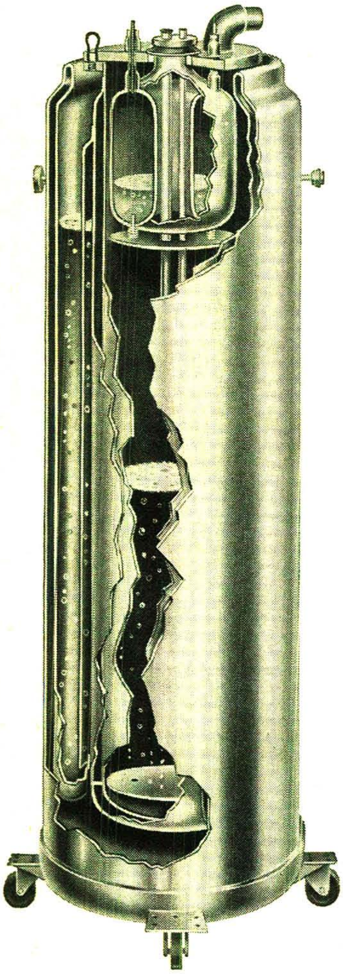


bination with a carefully designed oil circulation system, temperature control to ±0.02° C. has been achieved in large oil baths.

A Versatile Six-Inch Radius Mass Spectrometer for Isotopic Analysis of Solids, Liquids, or Gases. *H. O. Finley et al. New Brunswick Laboratory, for the U. S. Atomic Energy Commission. April 1963. 37 pages. Order NBL 191. 75 cents.*

A six-inch radius mass spectrometer has been designed for use in low-mass work such as for boron and lithium analysis. The design of the analyzer tube ends permits rapid exchange of ion sources and collectors with a minimum of physical change or loss of instrument time. The ion source and vacuum system valve arrangement permit fast interchange of samples.

Ultracentrifuge Photoelectric Scanner, Split-Beam. *K. W. Lamers, Lawrence Radiation Laboratory for the U. S. Atomic Energy Commission. October 1962. 63 pages. Order UCRL 10499. $1.75.*

An electromechanical scanner is described which can be used for displaying absorbance changes when a light-absorbing material such as a virus, protein, or nucleic acid is sedimenting in the centrifugal field of an ultracentrifuge.

A Versatile Spectrometer Magnet Power Supply. *W. A. Groppe, Union Carbide Nuclear Co., for the U. S. Atomic Energy Commission. April 1963. 25 pages. Order Y 1414. 50 cents.*

This newly designed mass spectrometer magnet power supply offers good regulation, low drift, and a sufficient range of current. It can be used with both 6-inch and 12-inch spectrometers.

A Plug-In Type Mass Spectrometer Surface Ionization Source for Use with a Vacuum Lock. *W. W. Rice, Union Carbide Nuclear Co., for the U. S. Atomic Energy Commission. January 1963. 15 pages. Order Y 1415. 50 cents.*

A plug-in type ionization source has been designed and built to be used with existing vacuum locks and Nier-type sources of the 6-inch and 12-inch mass spectrometers.

Analytical Chemistry Division, Annual Progress Report. *Period ending December 31, 1962. ORNL for the U. S. Atomic Energy Commission. 153 pages. Order ORNL-3397. $2.75.*

Developments in three major areas of research are presented in this report: analytical research, analytical development, and service analyses. Subjects in the report include analytical instrumentation, chemical analysis of advanced reactor fuels, special research problems, the gas cooled reactor project, radiation effects, nuclear and radiochemical analyses, organic and inorganic preparations, ionic analyses, infrared studies, and process analyses.

A Contribution to the Determination of Beta and Gamma Cellulose in Cellulose Rayon Pulps. *W. Piela et al. Paper Review, Communique No. 100 of the Pulp and Paper Research Institute, No. 1, 1958. Translated from Polish. 7 pages. Order 61-31266. 50 cents.*

Four chemical methods for finding the content of beta cellulose and gamma cellulose rayon pulps are reviewed in this Polish translation. The four are: Cross and Bevan; Czech; Swedish; and a combined method.

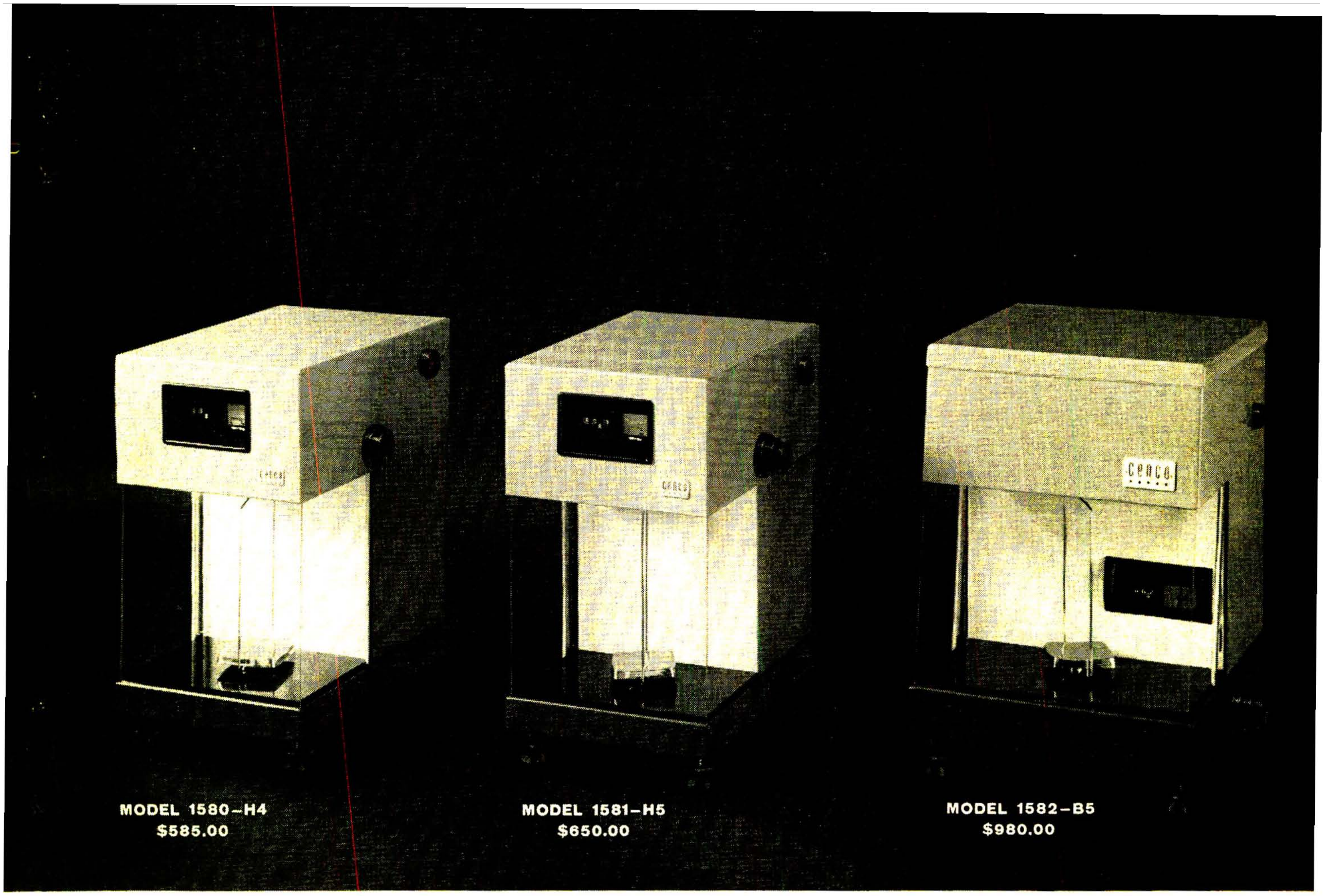
The Analysis of Polyphenyl Radiolysis Products by Gas Chromatograph and Other Methods. *W. W. West, California Research Corp., for the U. S. Atomic Energy Commission. October 1962. 35 pages. Order TID-17508. $1.*

Polyphenyls that have decomposed under the heat and radiation of a nuclear reactor were analyzed by gas chromatography. Gases, liquids, and solids (up through the hexaphenyls) were successfully analyzed. Two other methods, using the spectrometer as a gas chromatographic detector, and adsorption chromatography, were also considered.

Application of Distillation Techniques to Radiochemical Separations. *J. R. DeVoe. National Academy of Sciences–National Research Council. August 1962. 29 pages. Order NAS-NS 3108. 50 cents.*

This is another publication in the Nuclear Science Series. After discus-

(*Continued on page 68 A*)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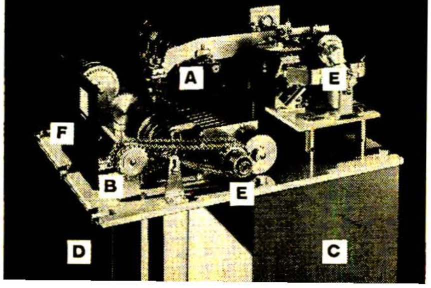

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There are two different versions of this low cost laboratory workhorse.

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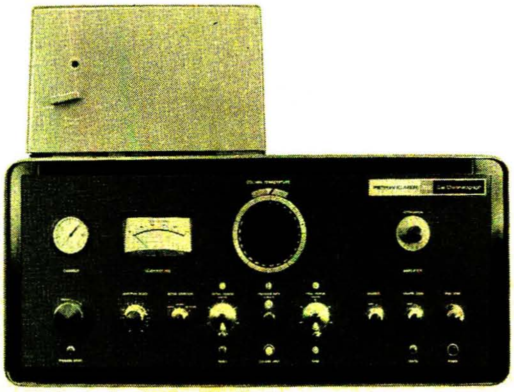
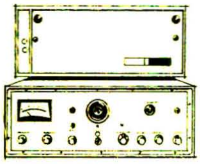
This sophisticated gas chromatograph combines the most modern advances in instrument design and performance with utmost simplicity of operation. Whether you use it in the research laboratory for precise elution time measurements, or in the routine control laboratory for dozens of daily automatic analyses, its unmatched speed, resolution, reliability and precision open new areas of information on virtually any organic mixture amenable to gas chromatographic separation. It adds automatic reset and programming features to an unparalleled accuracy of temperature control, to provide—for the first time—dependable unattended operation.



MODEL 800 SERIES

BASIC UNIT $3195



800 DIFFERENTIAL FLAME. The basic instrument in this series features the Dynathermal oven design with fully proportional temperature control and Perkin-Elmer's exclusive Differential Flame Ionization Detector. For dual or single column, packed or capillary operation, precise linear temperature programming, sample collection—*the ultimate in versatility.*

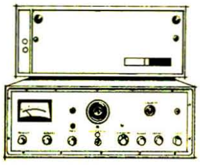
800 DIFFERENTIAL FLAME/HOT WIRE. The Hot Wire Detector in this combination is installed in a separate temperature-controlled oven, and may be used independently of, or in parallel with, the basic Differential Flame Ionization Detector.

800 DIFFERENTIAL FLAME/ELECTRON CAPTURE. In this version, both the basic Differential Flame Ionization Detector and the Electron Capture Detector—in its separate thermostatted oven—are mounted at the same time. Pulser power supply is optional.

800 DIFFERENTIAL FLAME/MICRO CROSS-SECTION. The newest Perkin-Elmer detector system, the Micro Cross-Section Detector, is mounted in a separate thermostatted oven, in addition to the Differential Flame Detector. It may be interchanged with either the Hot Wire or the Electron Capture Detector described above.

MODEL 801 SERIES

BASIC UNIT $3195



801 DIFFERENTIAL FLAME. Designed to meet the needs of *medical, biomedical and pesticide residue applications,* this instrument retains all the control advantages of the Model 800, but features an *all-glass system,* including "on column" injection in either of the dual columns. A major breakthrough is the new all-glass removable injection block, which permits the analysis of "dirty" samples: i.e., anaesthesia gases in blood, pesticide residue extracts.

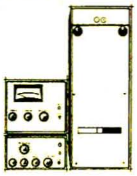
801 DIFFERENTIAL FLAME/HOT WIRE. As with the standard Model 800, the Model 801 can be equipped with an accessory Hot Wire Detector installed in a separate temperature-controlled oven, to permit dual-column thermal conductivity operation.

801 DIFFERENTIAL FLAME/ELECTRON CAPTURE. Particularly useful for pesticide residue analysis and silyl ether derivatives of steroids. The Electron Capture Detector can be installed at the same time as the dual Flame Ionization Detector. Again, pulser power supply is optional.

801 DIFFERENTIAL FLAME/MICRO CROSS-SECTION. Also available with this version is our newest detector system, the Micro Cross-Section Detector, mounted in a separate thermostatted oven.

MODEL 810 SERIES

BASIC UNIT $1695



810 DIFFERENTIAL FLAME. This new series, of *modular design,* offers—for the first time—dual column operation, with the high-sensitivity Differential Flame Ionization Detector, at an *extremely low price.* Included in the basic instrument, in addition to the Flame Detector, are the "ballistic" temperature programmer, and separate temperature control for the dual injection blocks, with the proven Dynathermal oven concept.

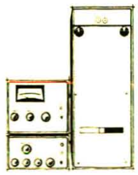
810 DIFFERENTIAL FLAME/HOT WIRE. The Hot Wire Detector in this combination is installed in a separate temperature-controlled oven, and may be used independently of, or in parallel with, the Differential Flame Ionization Detector.

810 DIFFERENTIAL FLAME/ELECTRON CAPTURE. In this version, both the additional Electron Capture Detector—in its separate thermostatted oven—and the Differential Flame Detector of the basic instrument are mounted at the same time. A pulser power supply is optional.

810 DIFFERENTIAL FLAME/MICRO CROSS-SECTION. The Micro Cross-Section Detector is mounted in a separate modular thermostatted oven, and is installed at the same time as the Differential Flame Ionization Detector which is basic to the instrument. It may be readily interchanged with the Electron Capture and Hot Wire Detectors described above.

MODEL
811
SERIES

BASIC UNIT $1695



811 DIFFERENTIAL FLAME. The *low-cost* way to achieve outstanding performance in *medical, biomedical and pesticide residue studies* where glass columns are specified. As in the Model 801, this unit features an all-glass injection and column system, coupled with modular design and Differential Flame Ionization Detector in the basic instrument.

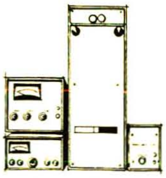
811 DIFFERENTIAL FLAME/HOT WIRE. Like the Model 810, the Model 811 permits the attachment of a modular, accessory Hot Wire Detector, complete in its own separate temperature-controlled oven, permitting dual-column thermal conductivity operation.

811 DIFFERENTIAL FLAME/ELECTRON CAPTURE. In critical pesticide residue analyses, as well as bio-medical determinations of the silyl ether derivatives of steroids, the Model 811 provides low-cost proficiency. The Electron Capture Detector can be installed at the same time as the basic Differential Flame Ionization Detector.

811 DIFFERENTIAL FLAME/MICRO CROSS-SECTION. Also available with this version is our newest detector system, the Micro Cross-Section Detector, mounted in a separate thermostatted oven. It is interchangeable with the Hot Wire and Electron Capture Detectors described in the two paragraphs above.

MODEL
820
SERIES

BASIC UNIT $1495



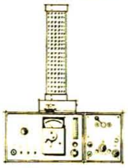
820 HOT WIRE. This instrument has the same features as the Model 810. The principal difference is that the Model 820 is equipped with the Dual Hot Wire Detector and control unit. Separate temperature control for dual injection block, columns and detector.

820 ELECTRON CAPTURE. The Electron Capture Detector module is interchanged with the basic Hot Wire Detector module. The addition of the Ionization Detector Electrometer Amplifier module completes this unit.

820 MICRO CROSS-SECTION. The Micro Cross-Section Detector is interchanged with the Hot Wire Detector module.

MODEL
222P

BASIC UNIT $2800



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MODEL
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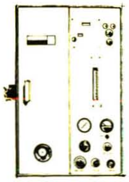

154D THERMISTOR. Here is the *world standard* for gas chromatography. The reliability of this model has been proved by the thousands of instruments installed and operating in the field today. The Model 154D features a precisely-thermostatted, circulating air bath oven, and thermistor thermal conductivity detector.

154D THERMISTOR/FLAME. With the thermistor detector and the flame ionization detector usable independently, in series, or in parallel.

154D THERMISTOR/COLUMN SWITCHING. Three columns may be installed in the instrument and used independently or in series. Columns may be switched in and out while operating, and all columns may be backflushed.

MODEL
154L
SERIES

BASIC UNIT $950

154L THERMISTOR. The *lowest-cost* precision-engineered gas chromatograph on the market today. Of the same oven design as the Model 154D and with the same thermistor detector, the Model 154L is ideal for routine analysis.

154L THERMISTOR/COLUMN SWITCHING. Ideal for Natural Gas Analysis where columns must be switched during analysis and heavy ends backflushed.

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4 **Micro-Reactor Accessory.** This is essentially a micro-pilot plant with heated reaction chamber and direct introduction into the gas chromatograph. Particularly useful for surveying activities of catalysts as reflected in the composition of reaction products.

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6 **Recorders.** Perkin-Elmer's selection includes ten models, all leading makes; also, the correct chart paper for each chromatogram.

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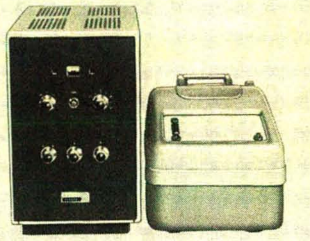

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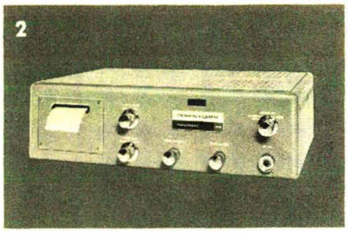

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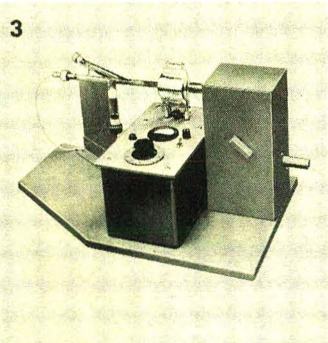

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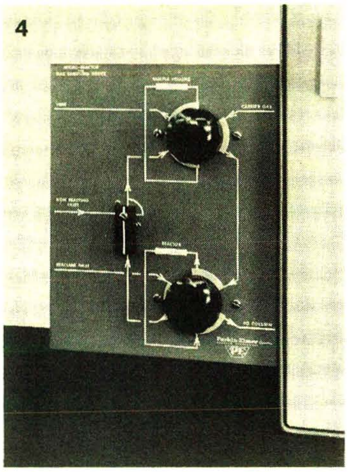

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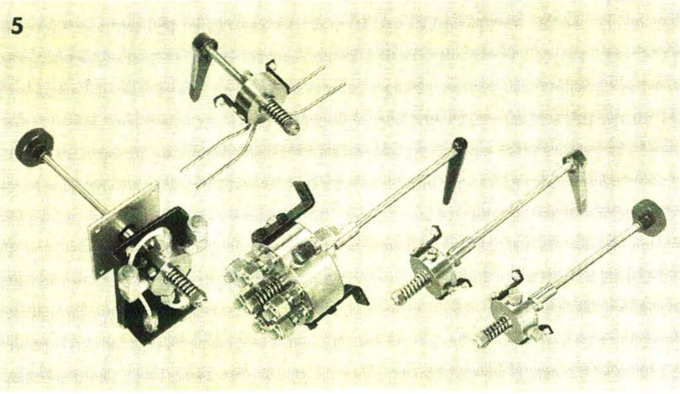

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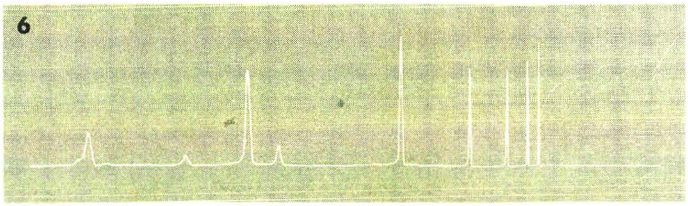

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❻ Same pH and millivolt ranges as Model 110. Readable to 0.03 pH unit. Temperature control adjustable from 20° to 100°C.

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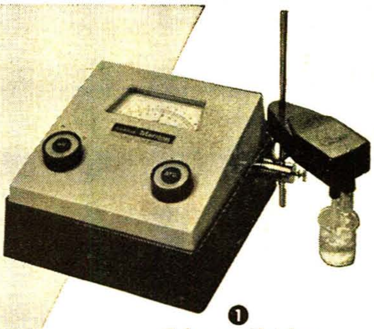

❶ Coleman Metrion H-5305X—$139.00

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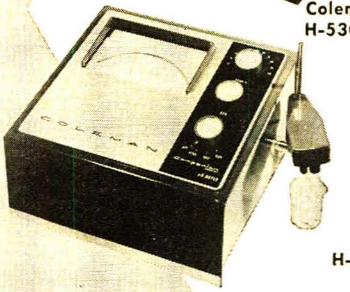

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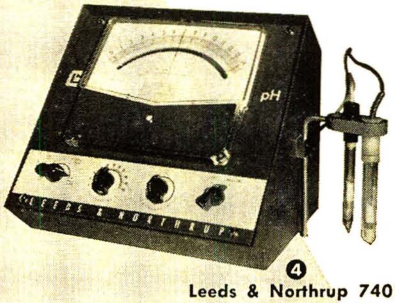

❹ Leeds & Northrup 7401 H-4901X—$330.00

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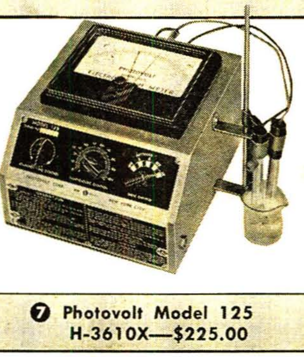

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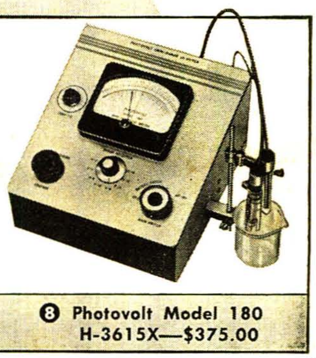

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The following can be obtained from the Superintendent of Documents, U. S. Government Printing Office, Washington, D. C. 20402.

Experimental Statistics. *Mary Gibbons Natrella. National Bureau of Standards Handbook 91. August 1, 1963. 504 pages. $4.25.*

This handbook contains in a single volume a collection of statistical procedures that are useful in the design, development, and testing of materials; the evaluation of equipment performance; and the conduct and interpretation of scientific experiments.

Proceedings of the 1962 Standards Laboratory Conference. *National Bureau of Standards Miscellaneous Publication 248. August 16, 1963. 254 pages. $1.75.*

This publication consists of the proceedings of the first national meeting of the National Conference of Standards Laboratories, which convened in Boulder, Colo., August 8–10, 1962. The publication carries the text of the more than 30 papers presented, plus transcriptions of discussion panels. Also included is a report of the business and information session, with its panel on standards laboratory information dissemination.

Research on Crystal Growth and Characterization at the National Bureau of Standards During the Period January to June 1963. *H. Steffen Peiser, editor. National Bureau of Standards Technical Note 197. September 23, 1963. 43 pages. 30 cents.*

This technical note summarizes individual and collaborative National Bureau of Standards research activities in the second six months period of a special research program on the growth and characterization of crystals.

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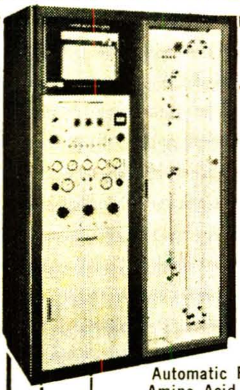
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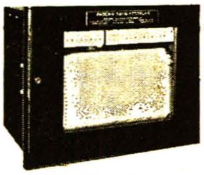
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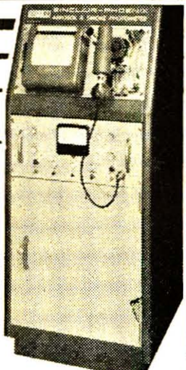
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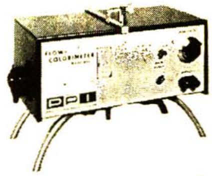
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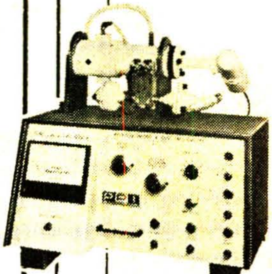
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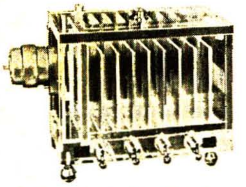
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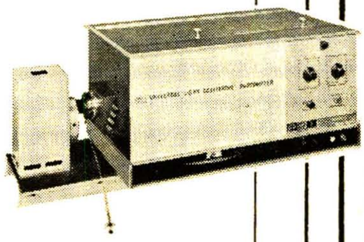
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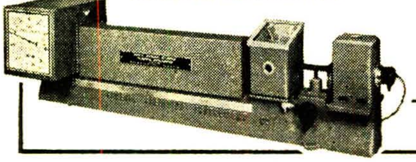
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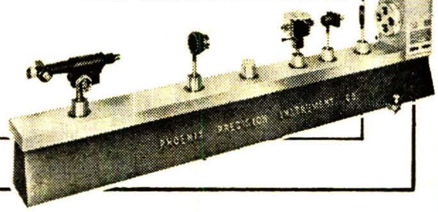
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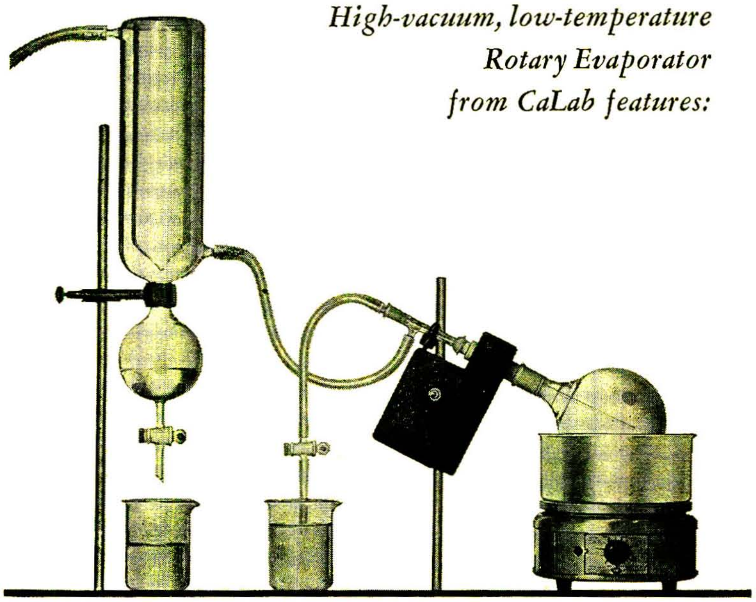

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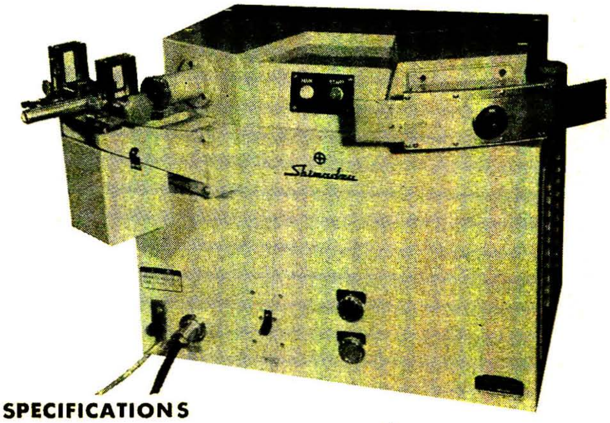

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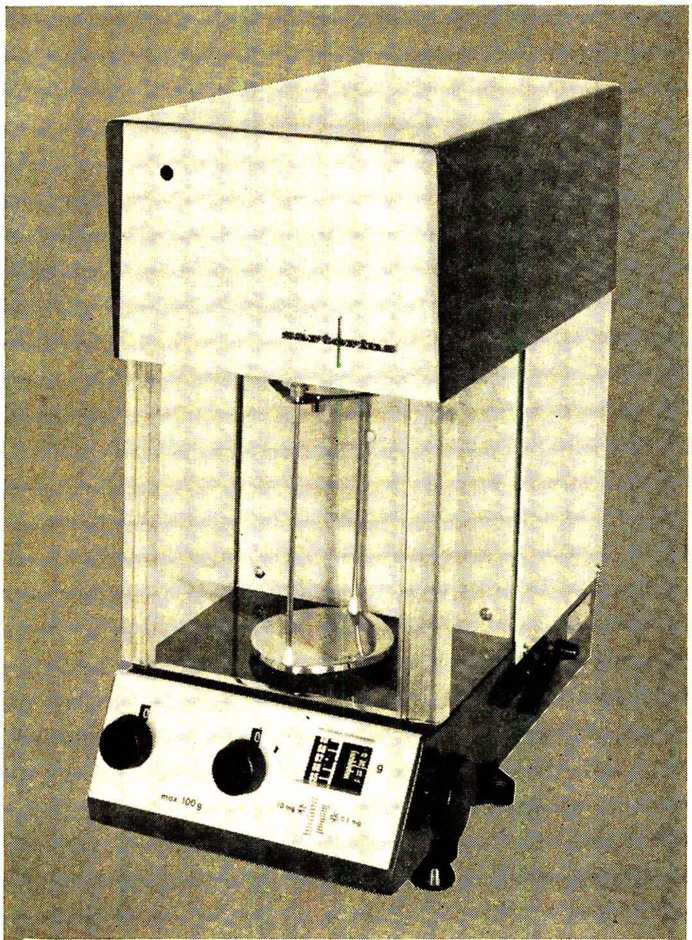

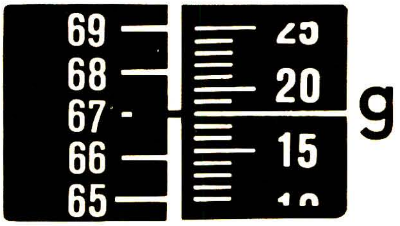

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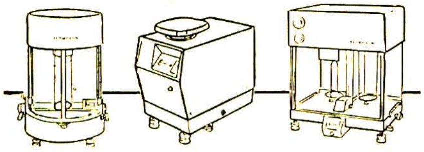

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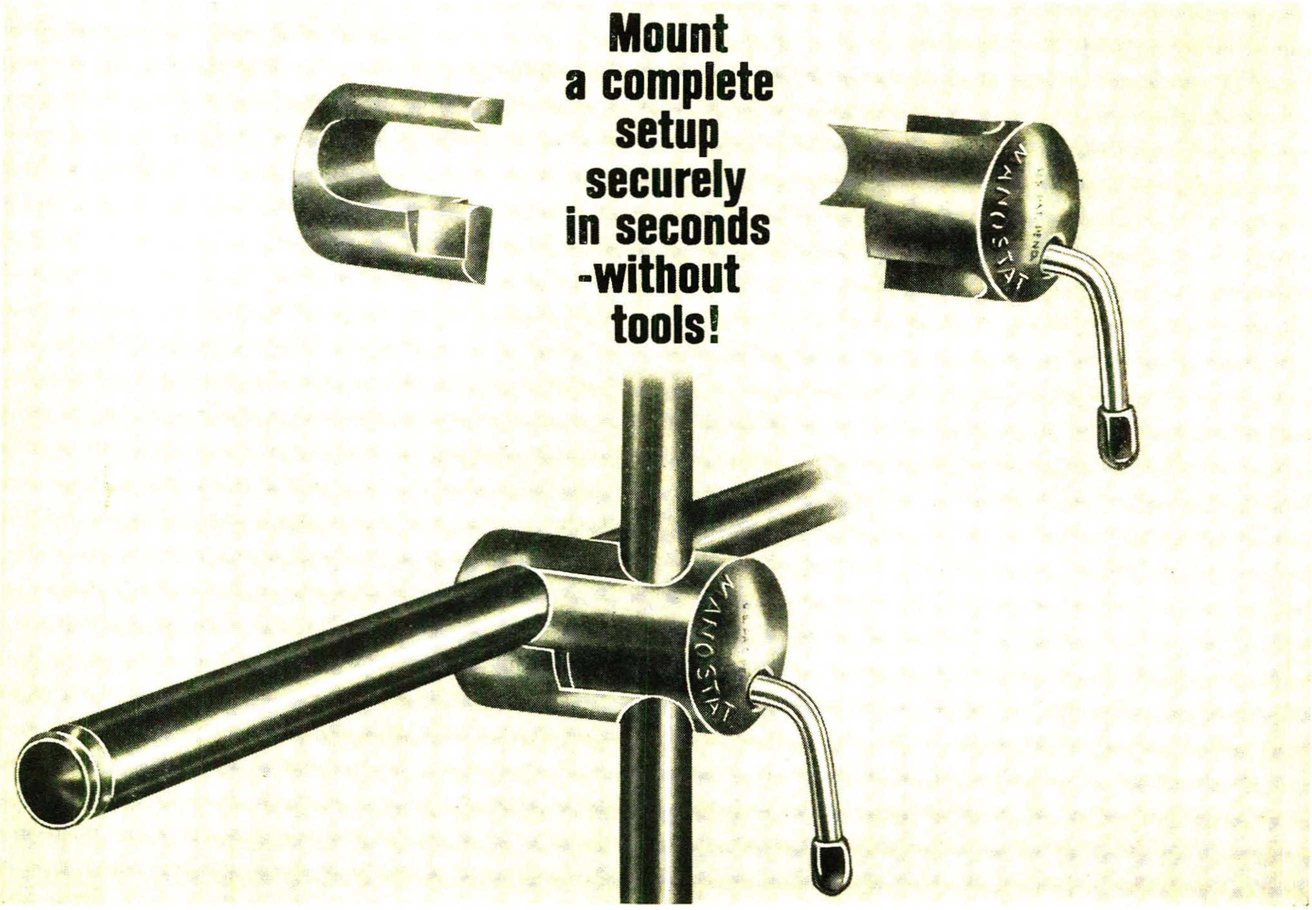
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the EDITOR'S column

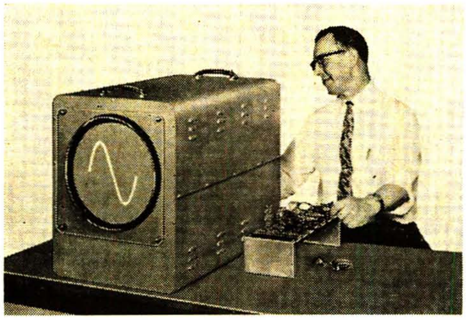
L. T. Hallett, Editor

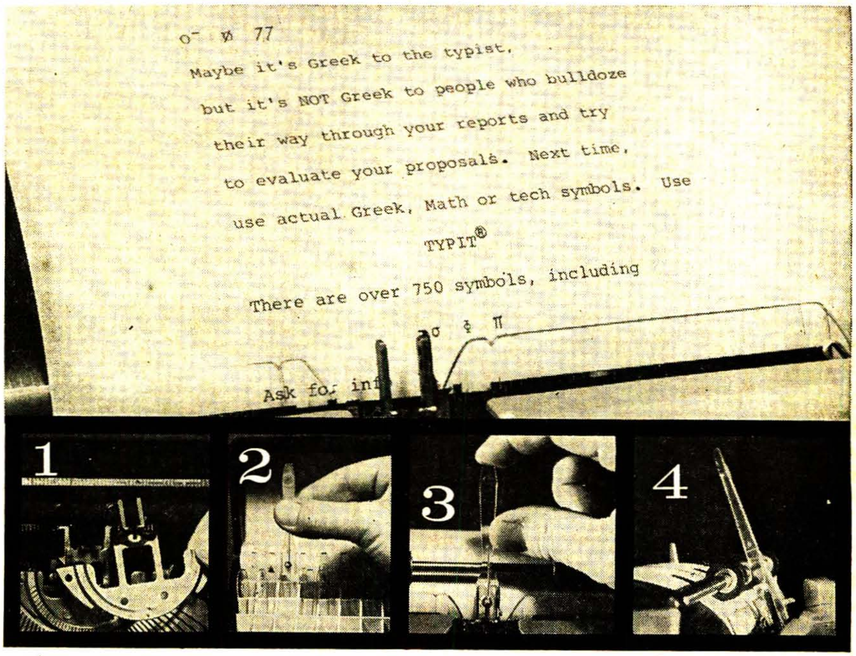
SOME INTERESTING APPLICATIONS of instrumental analysis to legal problems were described at the Symposium on Instrumental Methods of Analysis at the 77th Annual Meeting of the Association of Official Agricultural Chemists held in Washington, D. C., in October.

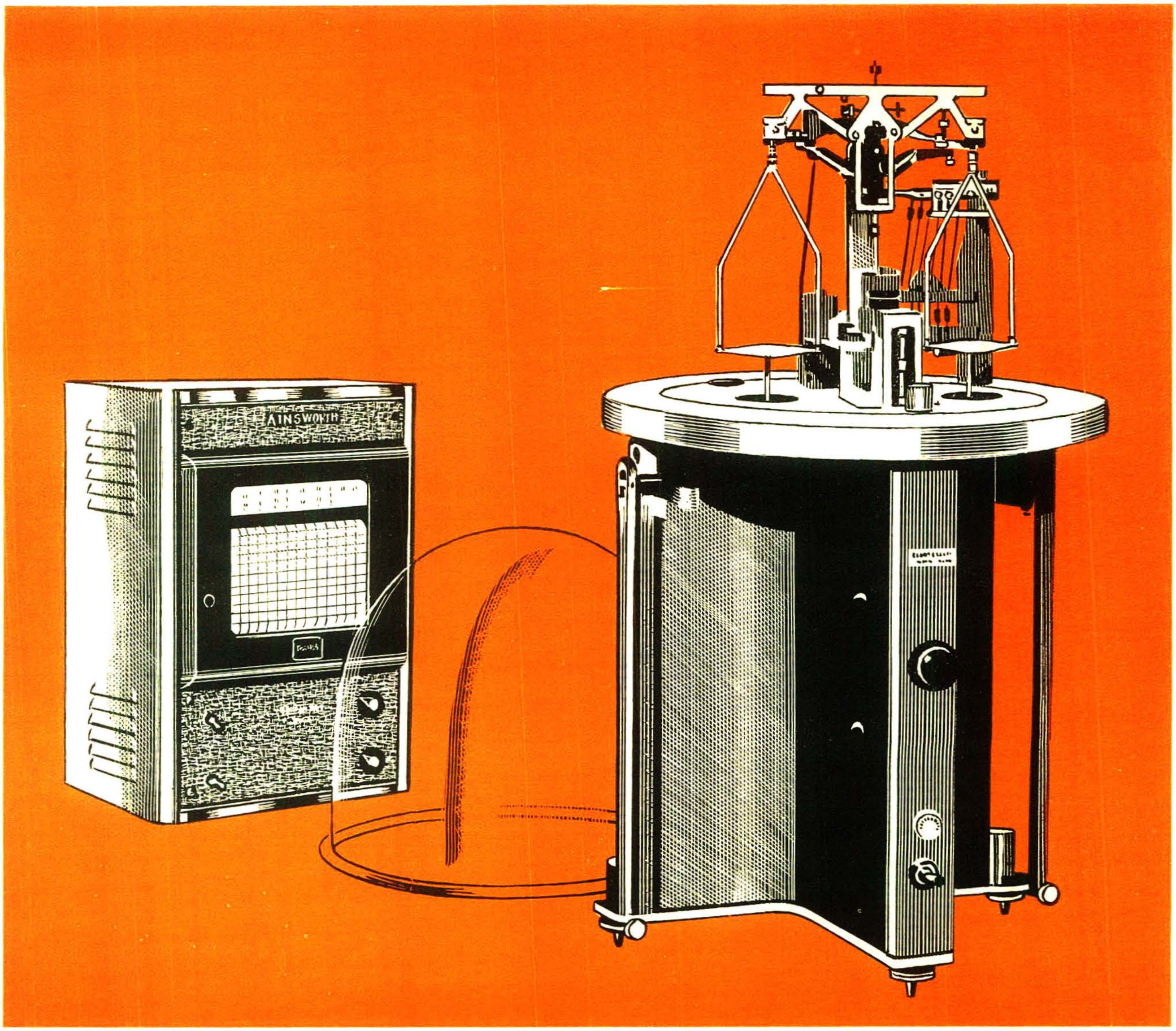
Melvin Lerner, Chief Chemist of the U. S. Customs Laboratory in Baltimore, Md., described three types of products for which gas chromatographic analysis has been especially valuable.

Gas chromatography has been extremely helpful in the identification of alcoholic beverages which have been shipped to the U. S. Chemists at the U. S. Customs Laboratory have examined a wide range of liquors with gas chromatography, and in many cases, types, brands, and even the country where the liquor sample was produced can be distinguished. In the case of a certain French brandy, for example, 23 identifiable gas chromatographic peaks have been found.

The problem of distinguishing Cuban tobacco from the tobacco of other countries, has, in a large measure, been solved by gas chromatography. In the gas chromatographic analysis of tobacco, the U. S. Customs Laboratory chemists extract the air dried tobacco leaf with water and then petroleum ether. Several hundred samples of tobacco from many parts of the world have been analyzed. By using an argon ionization detector and SE-30 or General Electric XE-61 as the column material, it is possible to distinguish Cuban tobacco from tobacco grown in other countries, as Cuban tobacco contains mostly low boiling constituents, while tobaccos from other countries have more flavor constituents and more high boiling constituents. It



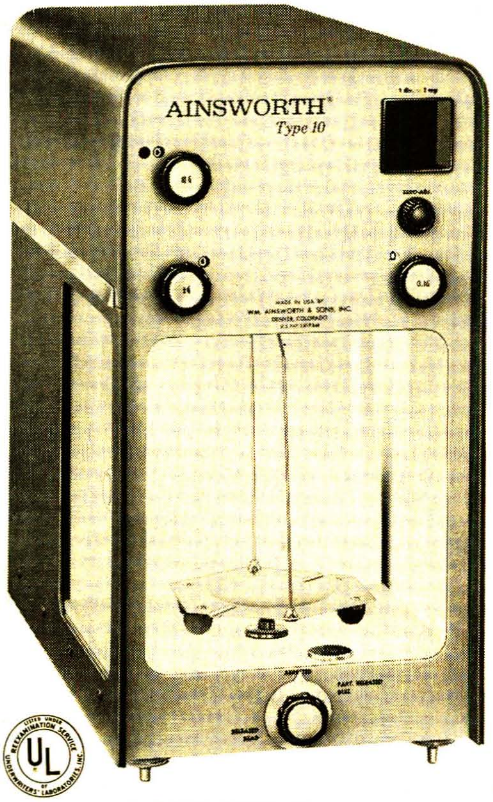


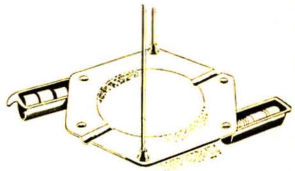

AINSWORTH

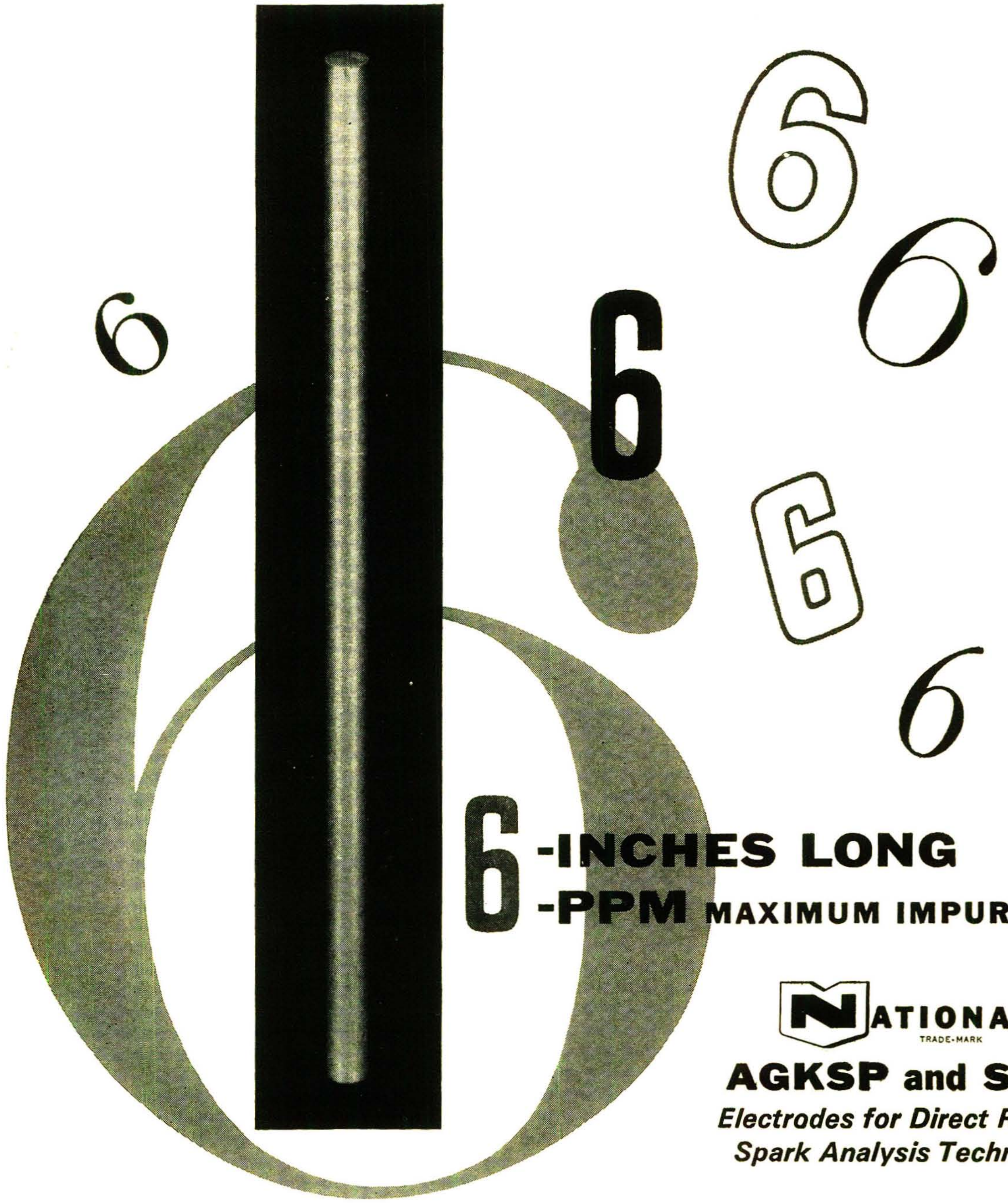
is even possible to distinguish Cuban grown tobacco from Cuban seed, American-grown tobacco from American seed, American-grown tobacco from Cuban seed, and tobacco grown in Honduras (a country with a climate greatly similar to Cuba) from Cuban seed with gas chromatography.

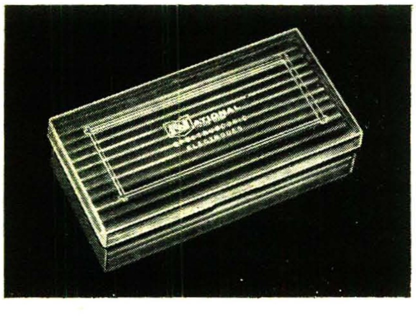
In narcotics analysis, gas chromatography is used primarily as a separation tool. After a preliminary separation on the argon ionization chromatograph, the eluant is collected in potassium bromide powder. Then the components are identified with the infrared spectrophotometer. In the analysis of marihuana, a combination of gas chromatography and infrared analysis has enabled the chemist to identify and determine the concentration of tetrahydrocannabinol, the important ingredient in the narcotic. According to Mr. Lerner, recent studies in Germany using thin layer chromatography and infrared spectroscopy have led to the discovery of three different tetrahydrocannabinols present in marihuana.

Ultraviolet spectrophotometry is one instrumental technique that has been extremely valuable in determining whether a race horse or dog has been drugged, according to Dr. John A. Herculson, Chief Chemist of the Maryland Racing Commission, Baltimore, Md. Over 150 drugs can be determined by UV spectrophotometry. In cases where mixtures contain compounds with similar UV peaks, UV spectrophotometry has been supplemented with column chromatography, gas chromatography, thin layer chromatography, and crystal identification to determine the constituents. Samples are customarily taken from the winning animal's saliva and urine. Over 70 different drugs have been detected in the urine of winning race horses over the years. Quantities of drugs found in race horse urine are small varying from 3 to 100 micrograms, which stresses the need for careful analytical work by the racing commission chemist.









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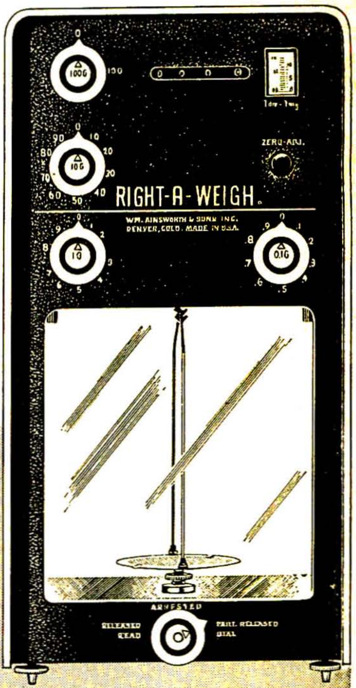

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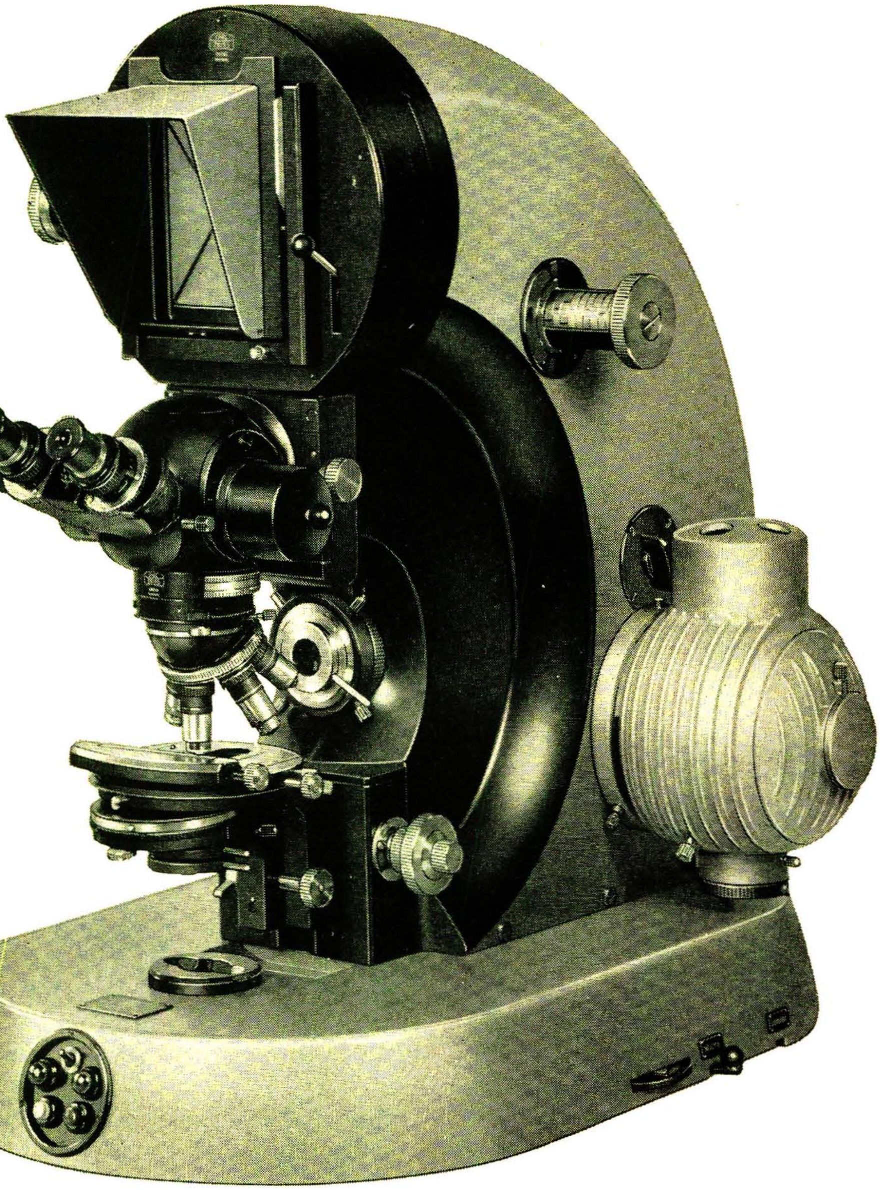

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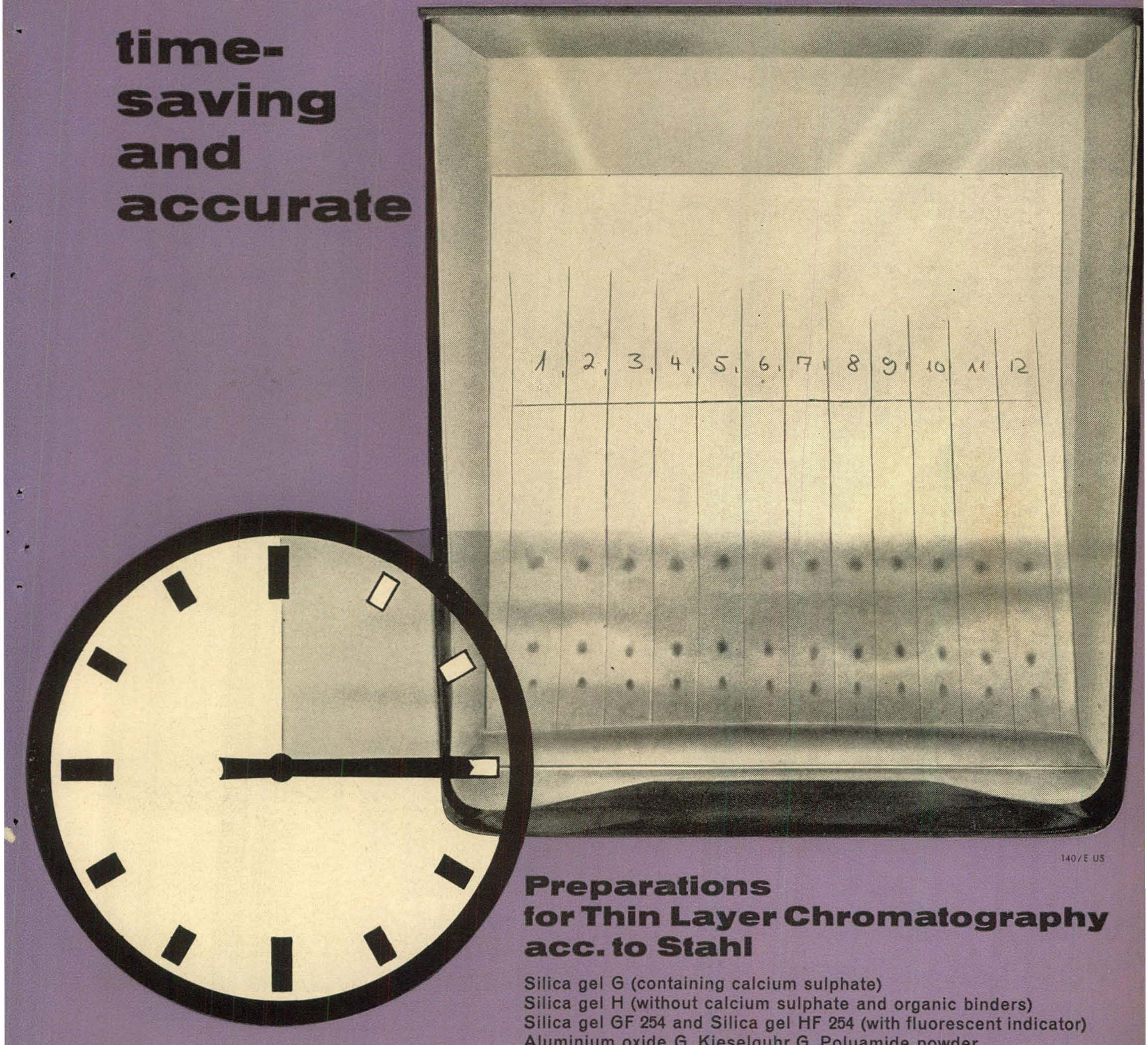
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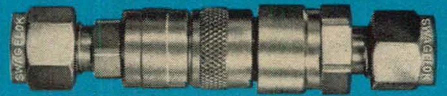

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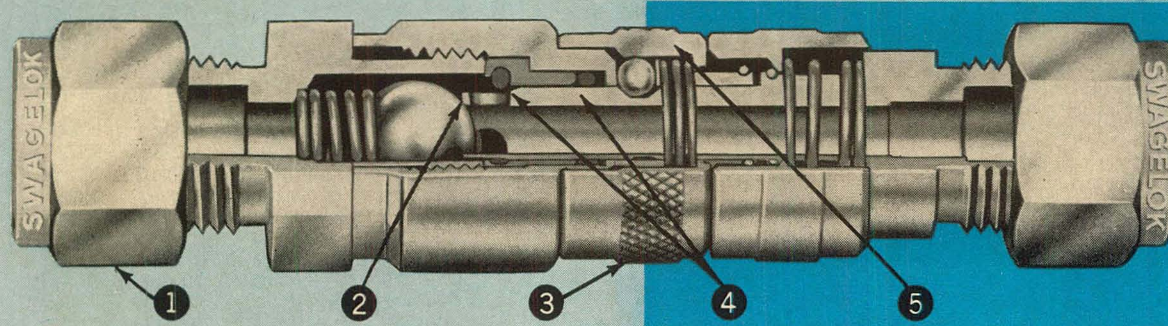

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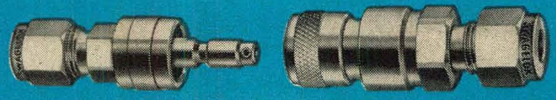
FOR A LEAK-PROOF VACUUM-TIGHT SEAL

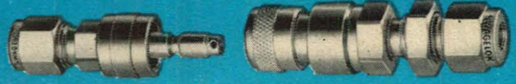
TUBE TO PIPE WITH SINGLE END SHUT-OFF

TUBE TO PIPE WITH DOUBLE END SHUT-OFF



TUBE TO TUBE WITH SINGLE END SHUT-OFF

TUBE TO TUBE WITH DOUBLE END SHUT-OFF



TUBE TO BULKHEAD TUBE WITH SINGLE END SHUT-OFF

TUBE TO BULKHEAD TUBE WITH DOUBLE END SHUT-OFF

SWAGELOK Quick-Connects can be used with metal as well as plastic tubing.

SWAGELOK
QUICK-CONNECT WITH SINGLE END SHUT-OFF

Swagelok Quick-Connects are suggested for use with portable equipment and bulkhead panel applications. They are furnished through local distributors in sizes for ¼" through ⅜" O.D. tube, available in stainless steel and brass. The Swagelok Quick-Connect Fitting is normally furnished with Buna "N" O Rings. Other O Ring materials are available for special applications.

SWAGELOK
QUICK-CONNECT WITH DOUBLE END SHUT-OFF

Swagelok Quick-Connects with unique double end shut-off, for use on hydraulic or pneumatic lines, contain instant acting seals in both parts of the unit which completely prevent loss of any pressure from either end of the line when the fitting is disconnected. Flow is resumed instantly when the male and female units (stem assembly and body assembly) are connected.

The SWAGELOK end of the Quick-Connect is assembled on the tube the same as any standard SWAGELOK Fitting. No special preparation of the tubing is necessary.

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884 East 140th Street—Cleveland 10, Ohio
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Your local Swagelok sales and service representative can provide immediate delivery of quick-connects.

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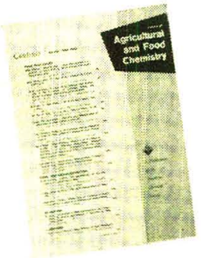

This scientific journal presents basic and applied research contributions and occasional review papers in the fast-moving fields of agricultural and food processing chemistry. Each bimonthly issue contains 25 to 30 authoritative papers in the inter-related areas of plant nutrients (fertilizers) and growth regulators, pesticides, food additives, food processing, and the biochemistry of nutrition.

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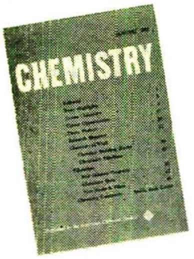

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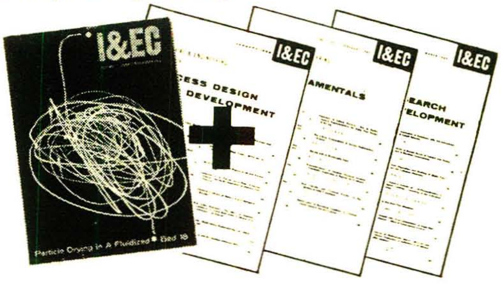

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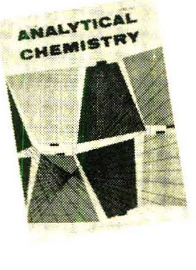

covers all phases of quantitative and qualitative analysis, both traditional "wet" and instrumental, with staff-written or originated articles and original research contributions; describes new apparatus, chemicals, equipment, instruments, products, reagents; shows new laboratory designs; contains a wealth of professional information for analytical chemists in all fields. Publishes Analytical Reviews and Buyers' Guide issue each April.

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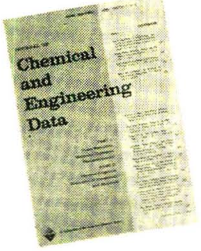

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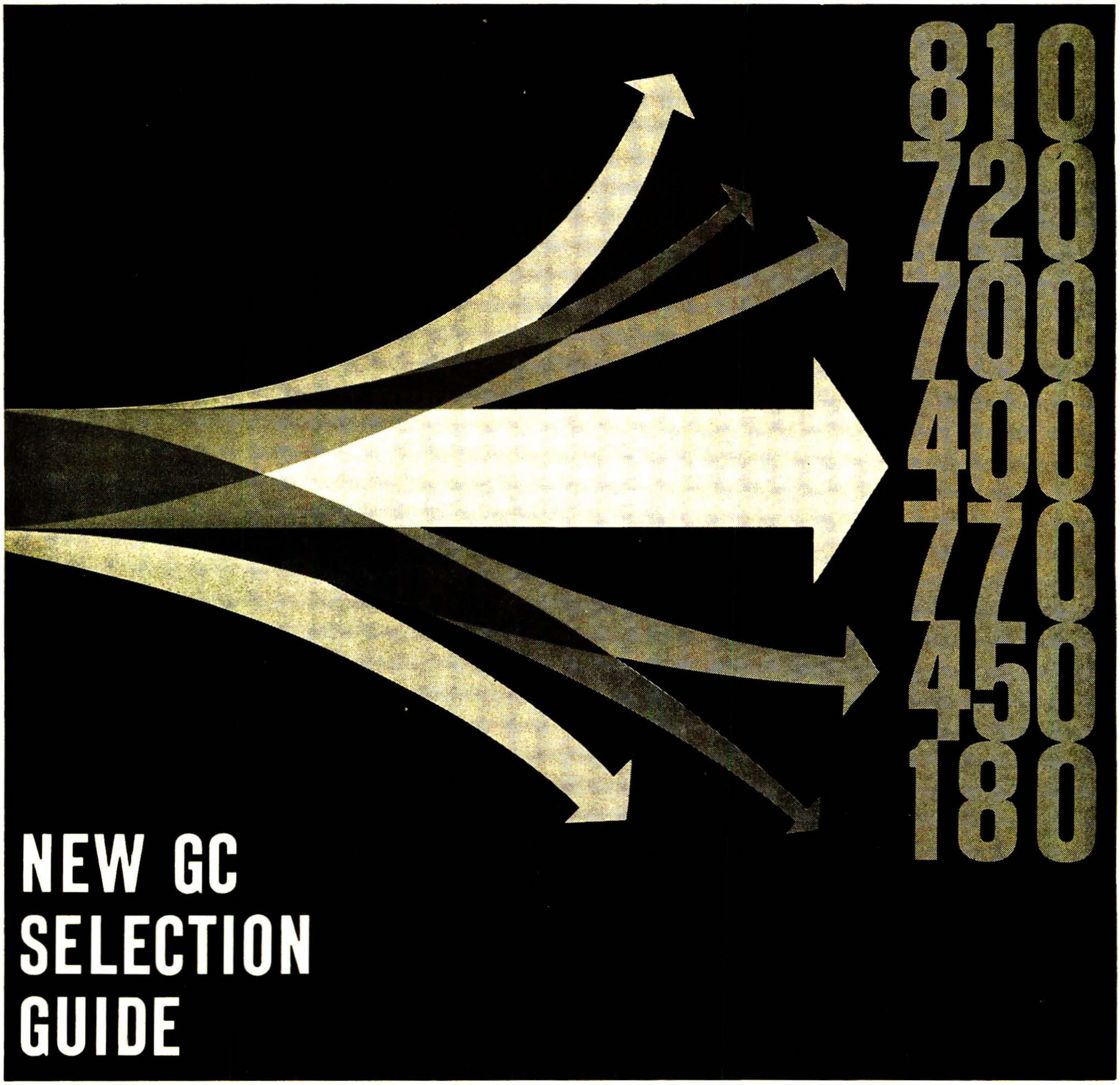
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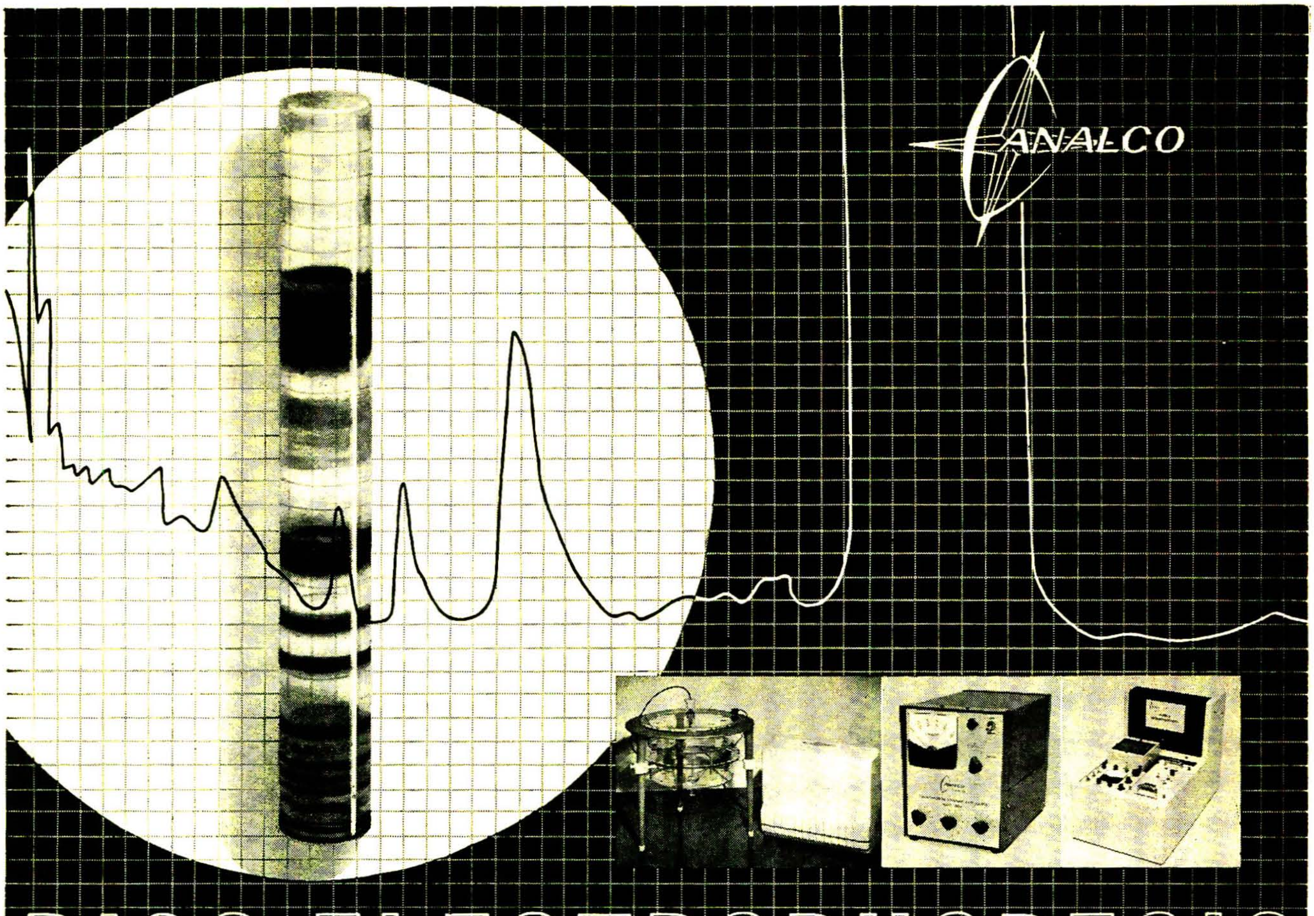

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Eight-Story Building Houses Abbott's Drug and Chemical Research Activities

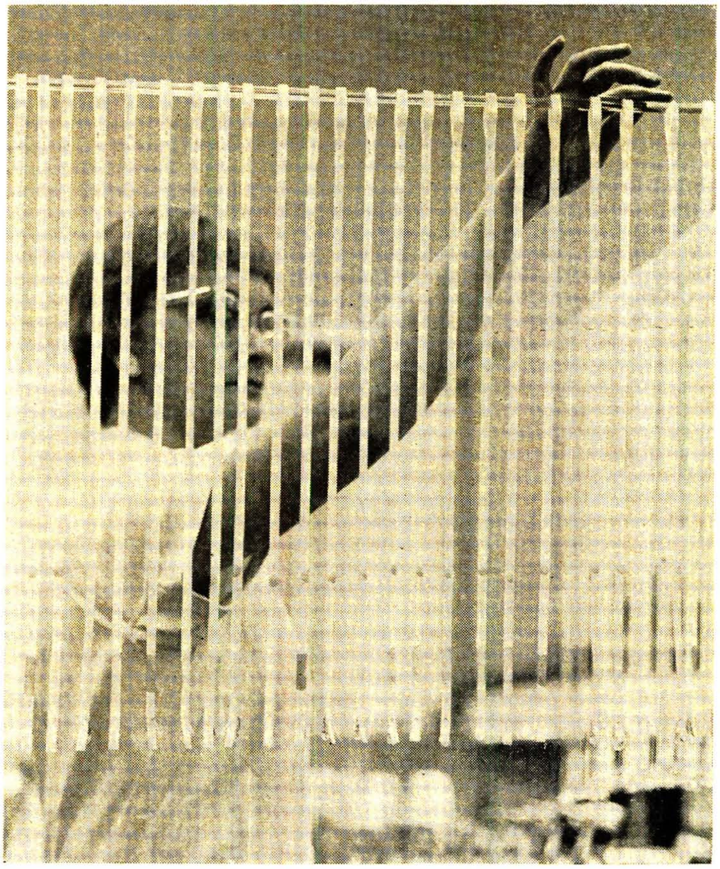
AN EIGHT-STORY GLASS AND ALUMINUM STRUCTURE at Abbott Laboratories' North Chicago, Ill., headquarters provides the center for the company's drug and chemical research activities as well as a large portion of the control and development functions.

When dedicated two years ago, the new structure containing 225,000 square feet, doubled the space available for the company's scientific facilities. About 650 persons, more than half of them professional scientists, are active in the Abbott research and development efforts. During the last five years there has been a doubling of the research and development budget and personnel. The company's R and D costs were $10.4 million in 1962 and are expected to rise to $11.6 million in 1963.

In the new building, laboratories generally are arranged along each side of a central service section containing pipes and conduits. This allows great flexibility in arranging or moving laboratories as need arises. Interior walls of laboratories and offices are constructed of metal framework and panels that can be moved easily and inexpensively if changes should become necessary.

A four-story wing to the building is constructed so that it can be expanded upward in the future. Eight-story additions also can be erected that would result in a building three times as large as the present eight-story wing.

Current projects under way in the Abbott research center include diseases of the heart and circulatory systems, mental illnesses, infectious diseases, metabolic studies, allergies, endocrinology, animal health products and industrial chemicals. Receiving more emphasis than ever before are basic investigations of nucleic acids, the chemical nature of cancer, biochemistry of antibiotics, enzymes, brain metabolism, nerve regeneration, and body defense mechanisms.

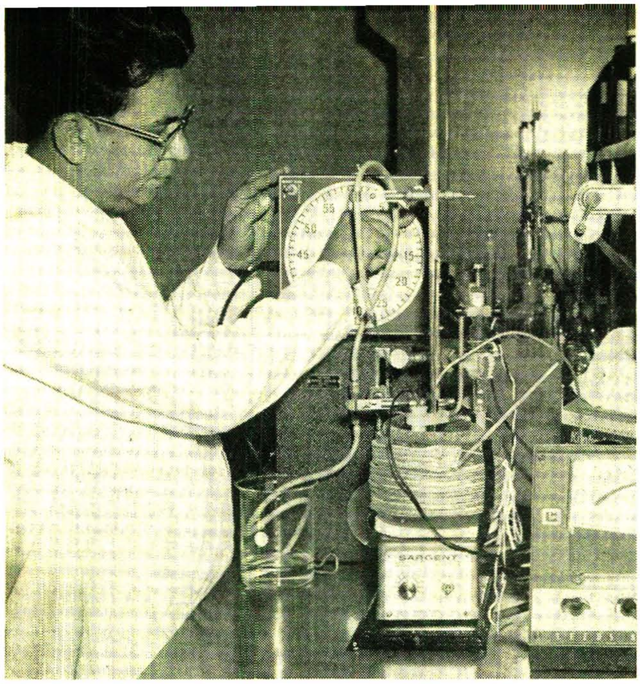


Continuous screening for new antibiotic-producing micro-organisms from soil samples collected throughout the world is speeded by quick identification of families of micro-organisms that have merely been "rediscovered." Paper chromatography, using several solvents, gives routine identification of antibiotics and sorts out promising new ones for testing

Analytical services are diversified throughout various departments at Abbott Laboratories. As a consequence, much of the analytical work in the early research stages of the development of a potential new product is conducted in the research department concerned. This not only provides faster analysis but is partly based on the philosophy that process knowledge is essential for proper interpretation of analytical results. However, instruments and services requiring specialized skills such as x-ray fluorescence and diffraction, emission spectrography, infrared, nuclear magnetic resonance, and microchemistry are centralized.

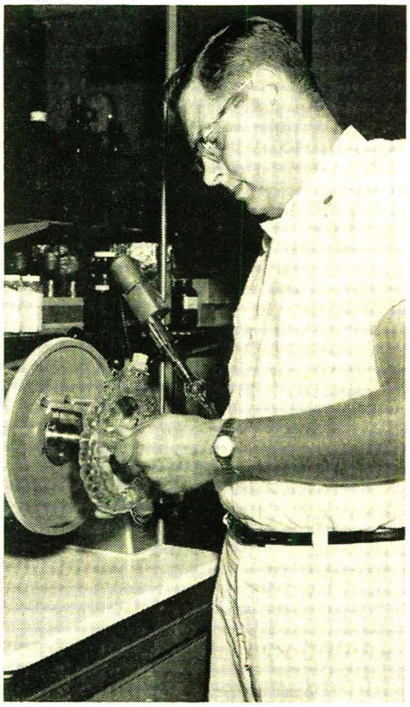
As the product proceeds to the marketing stage, more of the analytical research and requests for service become centralized in the Analytical Research Department which is part of the Quality Control Division. The Analytical Research Department has three major functions: first to develop suitable analytical methods for the Chemical Control Department, second to provide analytical control for compounds under clinical investigation, and third to provide analytical services to other areas within the company.

Because analytical problems vary depending on the type of sample, the department is organized into groups to handle the analysis of drugs and chemicals, formulations, trace analysis, and development samples. Primary emphasis is placed on using the best technique to solve the problem rather than fitting the problem to a specific technique. Although instruments are used extensively as a means to an end, much use is made of wet chemistry.



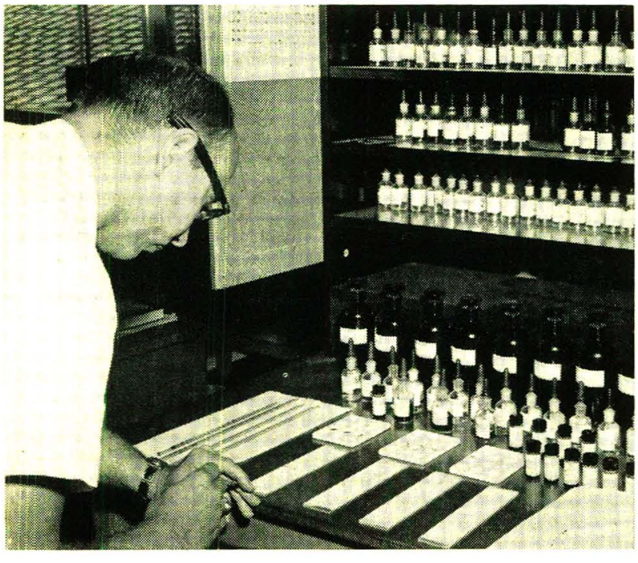
◄ **The operation of a buffer evaluation apparatus** is observed by an analytical chemist. The apparatus tests the effectiveness of buffer compounds in maintaining the pH in body fluids

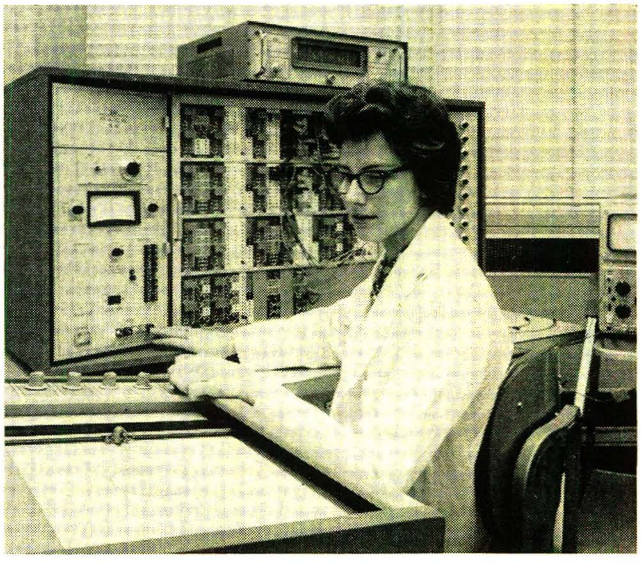
An Erweka tester is being evaluated for its control and research applications to the determination of release time of sustained release preparations. The preparation is treated with a solution that simulates gastrointestinal tract fluid in the body. With the instrument, samples of the solution are withdrawn at timed intervals to determine concentration of active ingredients released ►



Thin layer chromatographic techniques and qualitative tests for various functional groups are used to characterize and identify drugs and their degradation products ▼

Analog computer simulates biological systems for research purposes. On the computer is an equation representing a system that returns to normal (for example a heart beat that slows down to its usual pace after having speeded up because of exercise or excitement). The pattern is traced at left on paper and at far right on the oscilloscope ▼

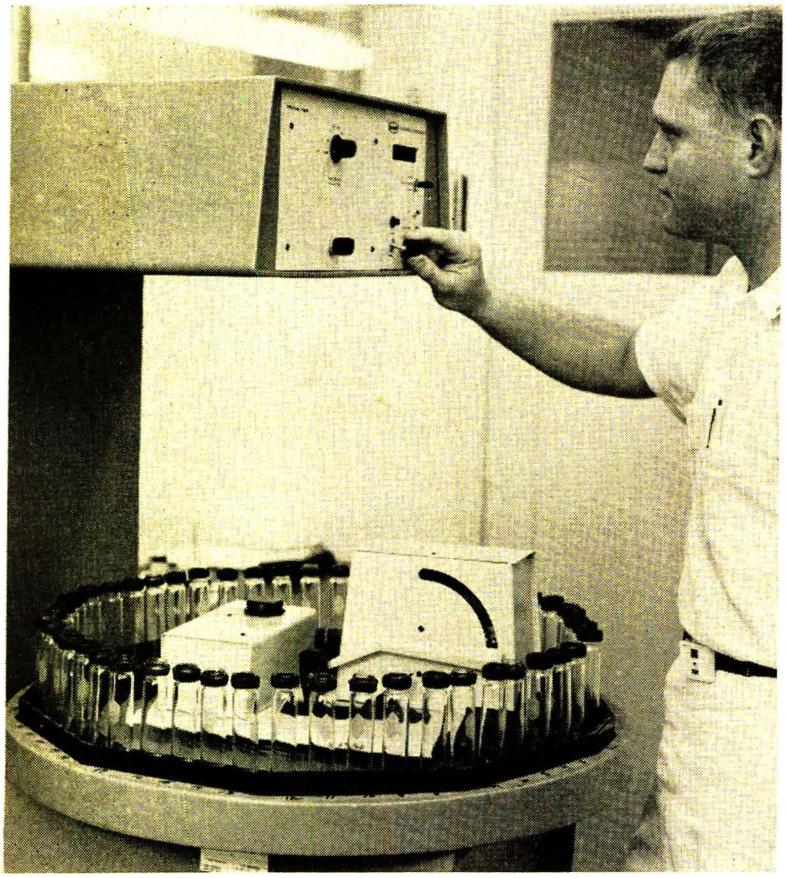


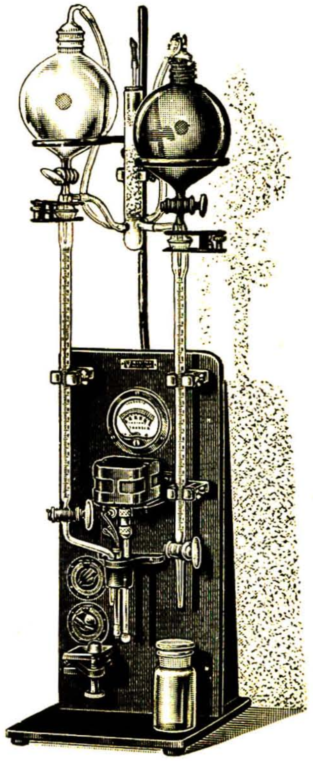


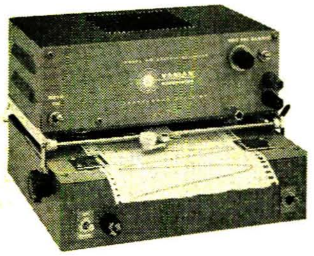
Newest addition to the Abbott Research Center is this eight-story building. A full penthouse accommodates ventilating and air conditioning equipment for the entire building. Basement contains lecture hall, centralized storerooms and laboratory services, and glass blowing shop in addition to other laboratories. Not shown is four-story wing

LABORATORY OF THE MONTH

Radio-iodine-131 samples, from diagnostic studies, are automatically counted by a gamma programmer, and results are printed on tape. Efficiency and versatility of this type of instrumentation in radiochemical analysis release the analytical chemist for nonroutine work







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Varian was the first to bring you such a sensitive, low-cost potentiometer recorder. Thousands in use constantly prove the G-10's accuracy and reliability for every recording chore within its capability. This sensitive, rugged instrument features: 10 to 100 mv d.c. full scale; select one or two chart speeds from 1/2"/hr. to 16"/min.; 1% accuracy; pen speed 1 or 2½ seconds full scale. For full specifications and prices, write to RECORDER DIVISION.

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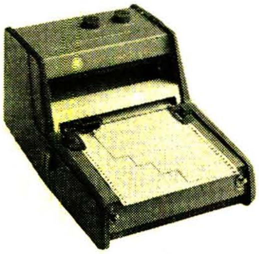

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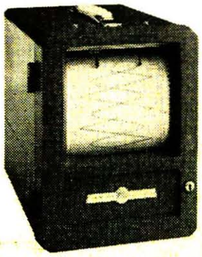

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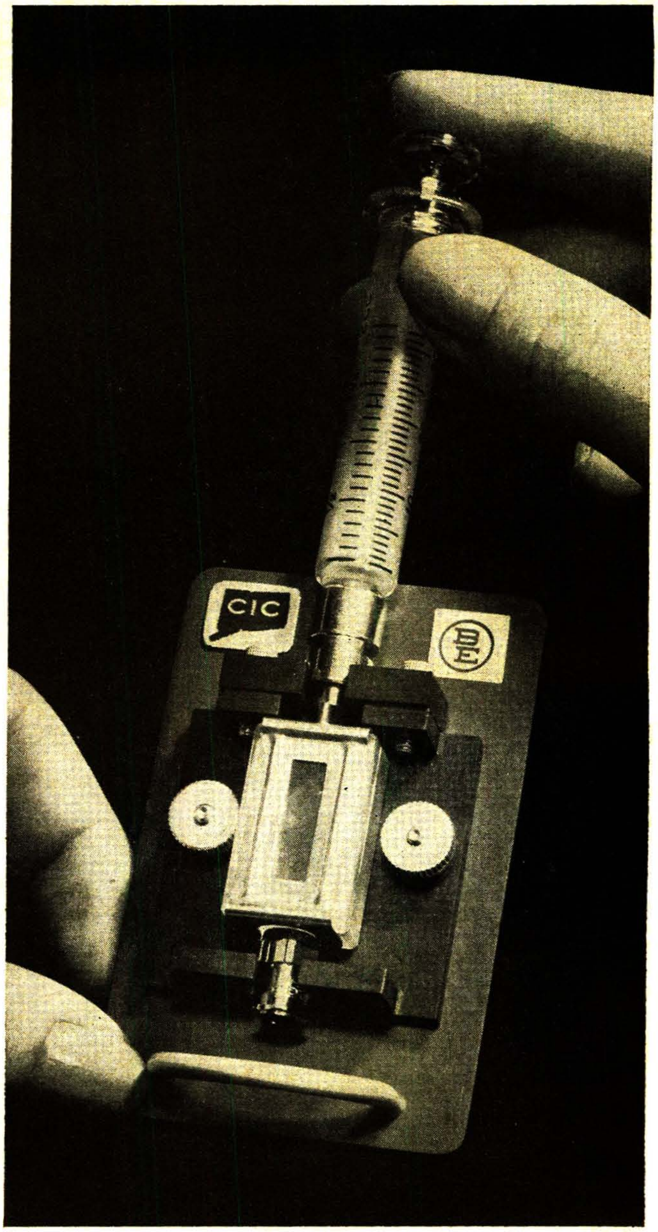

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Here is a general purpose, permanently-sealed, liquid absorption cell designed for handling small volume samples in all pathlengths and window materials. Amalgamated seal of either silver-mercury or lead-mercury provides insurance against leakage. New Luer-Lok* fittings take ordinary vaccine or Luer syringe and eliminate spilling, smearing, make cleaning easier. This cell utilizes the minimum amount of crystal and reduces cost of rebuilding by ready replacement of complete inserts which are available. Inserts, as designed, need no additional spacers, gaskets or bolts.

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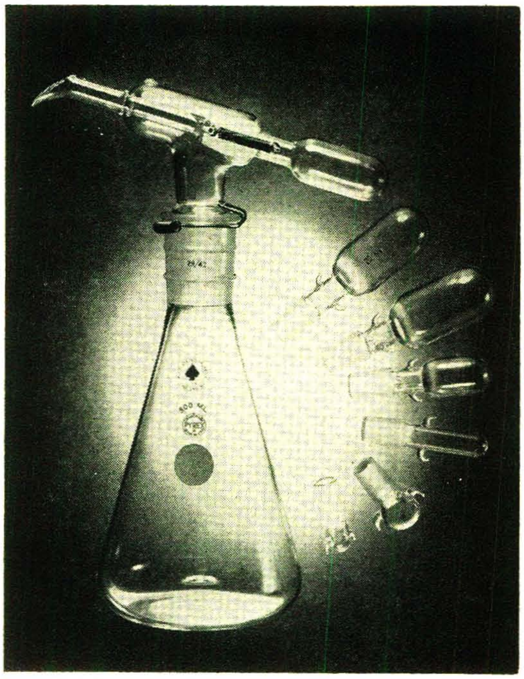
- **Short Tipping Angle.**



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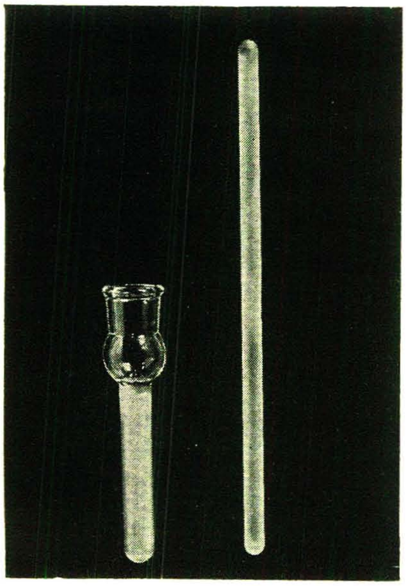

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SPECIFICATIONS: Ground section of barrel 82 mm. Overall length of barrel 125 mm., length of ground rod 240 mm. Barrel clearance of .005 to .008" meets G.S.A. specs.

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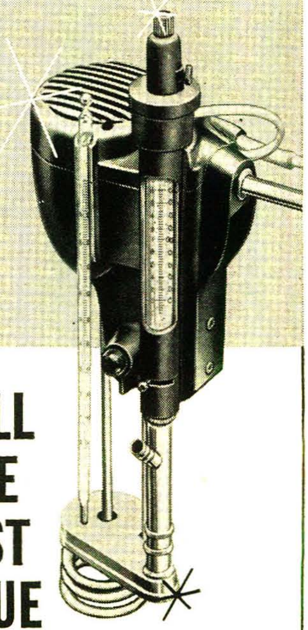

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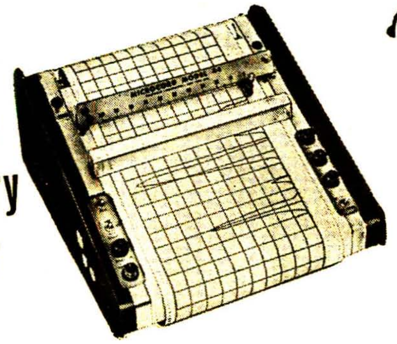
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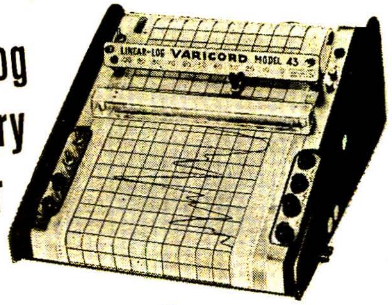
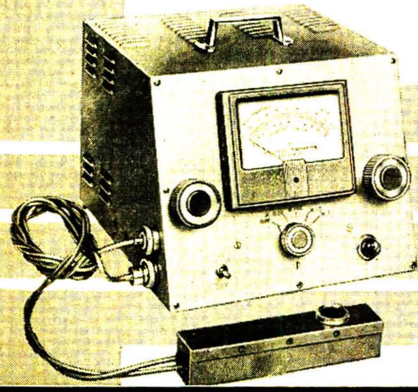
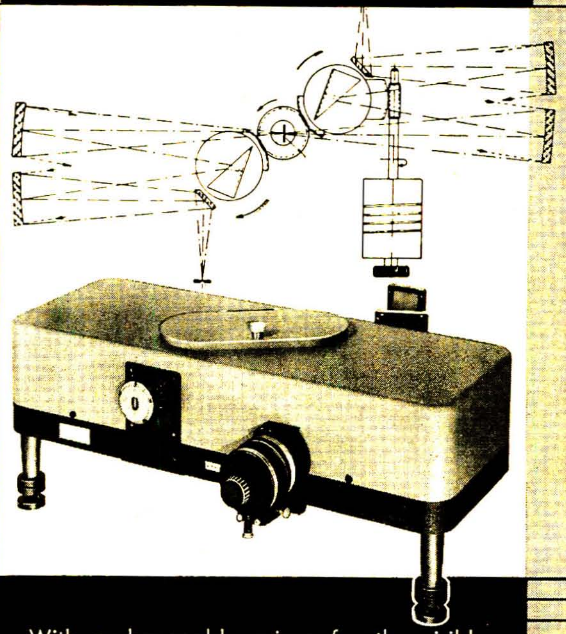

LINEAR-LOG VARICORD MODEL 43





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>
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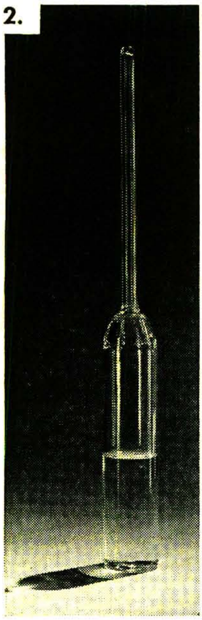
Upon receipt of the gentleman's barb we switched over to the method we use for making *Fluorescein Dibutyrate* (EASTMAN 8965, $6.75 for 25 g.). This other dibutyryl ester of a dye is employed in a similar scheme for determining lipase fluorometrically in the presence of other esterases (*Anal. Chem. 35*, 588). Now they're both clean of free dye. Only honest men can afford to advertise like this. Honest and successful men. And above all, modest.

2.



◀ which might be 5 ml under N_2 of

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EASTMAN G5082 Hexyl Acetate
EASTMAN G467 Methanol
EASTMAN G2381 2-Methoxyethanol
EASTMAN G946 Methylcyclohexane
EASTMAN G1263 4-Methylcyclohexene
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The "G" stands for gas chromatography, the means of final purification that gives us strong confidence that these are the purest of all liquid EASTMAN Organic Chemicals and a strong suspicion that they are the purest liquids available from a commercial house. Sure, with nothing better to do for a few months and nobody asking questions about outlays for equipment, a good physical chemist could work up a sneer at that word "commercial." He could possibly also work up 5 ml of any of these compounds constituted of molecules more homogeneous in some respect or other than ours.

Ours carry a green label. They are intended as standards. You may conceive of less static uses. We expect that each transaction will involve individual correspondence and explanation of the g. c. curve for the particular batch, a document we shall furnish with the vial. It's knowing precisely what you are getting in the vial that has to be worth the price. Neither the liquid nor the information is worth much without the other. The cost to us of any discussion that does not result in an order is on the house.

The list of "G" liquids may well have lengthened by the time you read this.

3. *Rhodamine B* (EASTMAN 4453, $3.30 for 10 g.), which has at least the following 30 uses, as described in the indicated *Chemical Abstracts* references:

1. Determination of Sb (*41*, 1947c; *50*, 1516a; *54*, 10657c; *54*, 9606b; *21*, 1779^{2})
2. Determination of Au (*44*, 4820b; *50*, 2357g; *54*, 22160i)
3. Determination of Tl (*44*, 4820b; *48*, 2514g; *52*, 6063b; *56*, 4097a)
4. Determination of Ga (*49*, 10118g; *50*, 2357e)
5. Determination of Fe (*45*, 8936b)
6. Determination of W (*52*, 971b)
7. Determination of Cd (*53*, 18743a)
8. Determination of Ta (*53*, 7857i)
9. Determination of U (*54*, 1177d)
10. Determination of Pu (*55*, 5212d)
11. Determination of Re (*56*, 9296)
12. Determination of Zn (*56*, 7976g)
13. Fluorescent indicator in fatty acid determination (*46*, 7793e)
14. Determination of molecular weights of polymer chains by inhibition of fluorescence (*48*, 7986h)
15. Fluorescent indicator in determination of $S^{=}$, $S_2O_3^{=}$, and SCN^{-} (*41*, 3392d)
16. Indicator for critical micelle concentration of soaps (*49*, 694f)
17. Fluorescence indicator in iodometry, bromometry, and titration by $KMnO_4$ (*45*, 8936b; *32*, 7367^{3}; *33*, 2062^{7}; *35*, 1722^{6})
18. Fluorescent spot test for enolizable nitro compounds (*49*, 7451d)
19. Selective stain for plasma in living plant cells (*45*, 5770g)
20. Vital stain with transient specificity for mitochondria (*51*, 5199f)
21. Determination of dead yeast cells (*52*, 17611c)
22. Paper pulp differentiation (*52*, 9597d)
23. Fluorescent protein tracer (*53*, 10493b)
24. Hypochlorite determination in presence of chlorates (*53*, 8941c)
25. Acid-base indicator in glacial acetic acid (*54*, 4243a)
26. Determination of cracks and splits in objects by u-v fluorescence (*55*, 6056h)
27. Vital stain for tannin in *Spirogyra* cell and for vacuoles in higher plants (*55*, 2815c)
28. Indicator of water translocation through cortex of plants (*55*, 23696c)
29. Lipid determination in nerves (*56*, 662h)
30. Vital stain strongly stored in nucleus membrane of some plant cells but not in nucleus interior (*33*, 1356^{9})

4. In this day and age of pH meters and other modern devices, when one would expect a slack demand for that old familiar wall chart showing pH ranges and color changes of 57 EASTMAN pH Indicators and would be quite wrong, an updated version thereof with 61.

5. Some 4100 other compounds detailed in "EASTMAN Organic Chemicals List No. 43."

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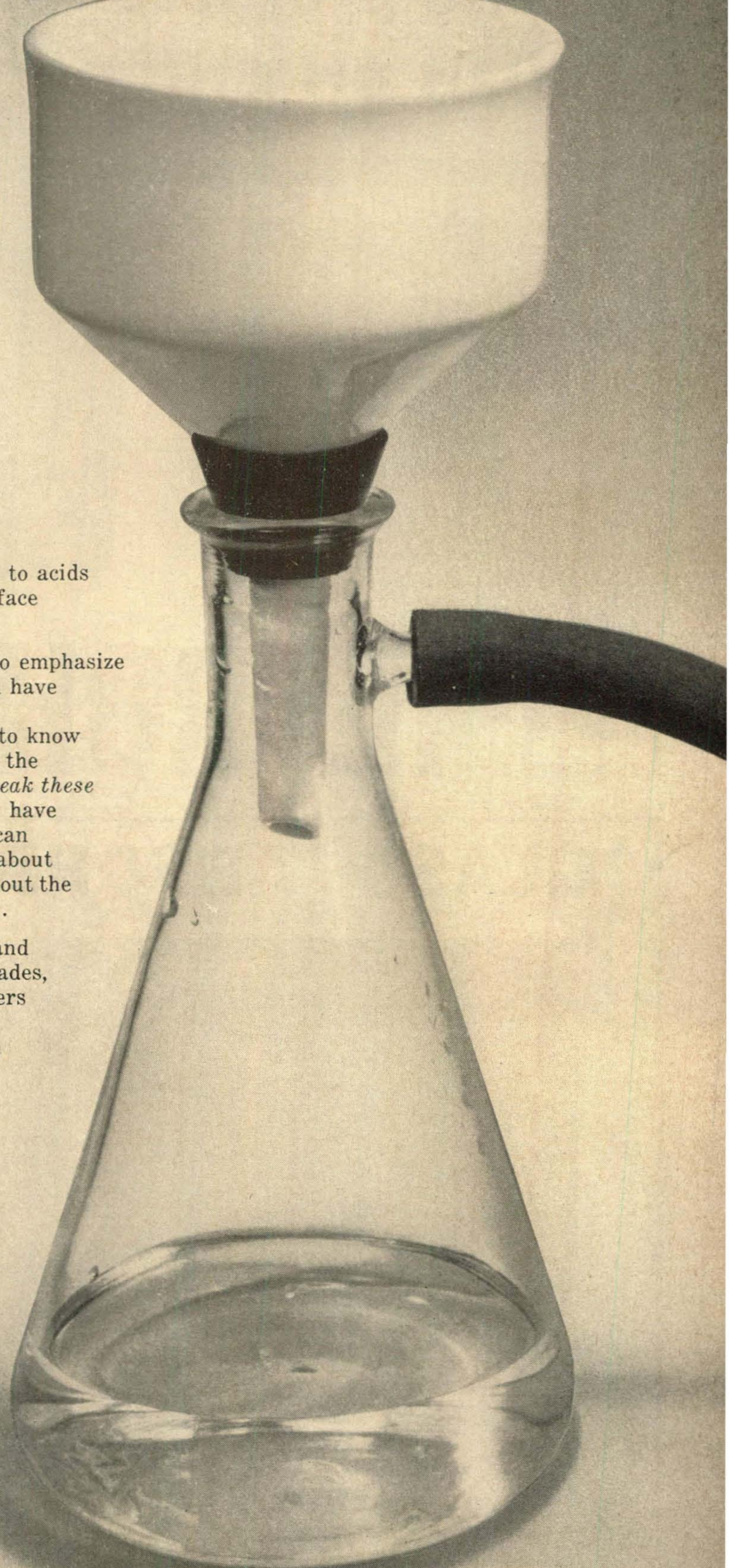

When you need high wet strength, resistance to acids and alkalis, freedom from lint and a hard surface for scraping precipitates.

The illustration of the Buchner is supposed to emphasize the problem of filtration under suction. If you have ever had a filter paper break in the middle of one of these operations you will be happy to know you could have prevented this by using one of the Whatman hardened grades. *We defy you to break these under normal laboratory suction.* Also, if you have to remove the precipitate from the paper you can scrape away with a spatula without worrying about including fibers. (If you are more concerned about the filtrate, *relax*; it won't have any fibers either).

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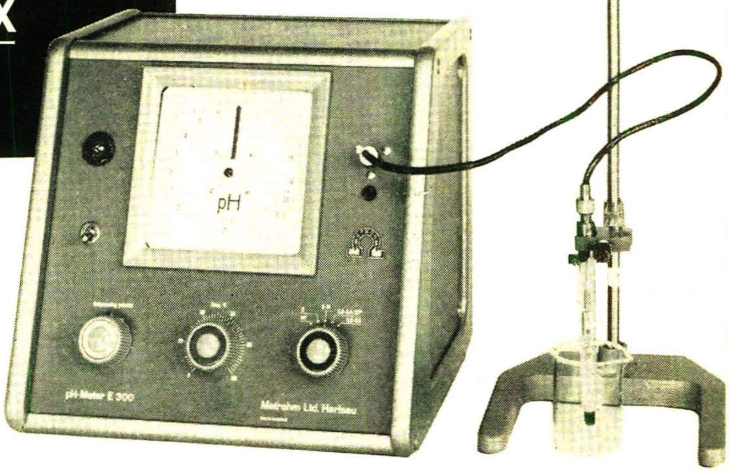
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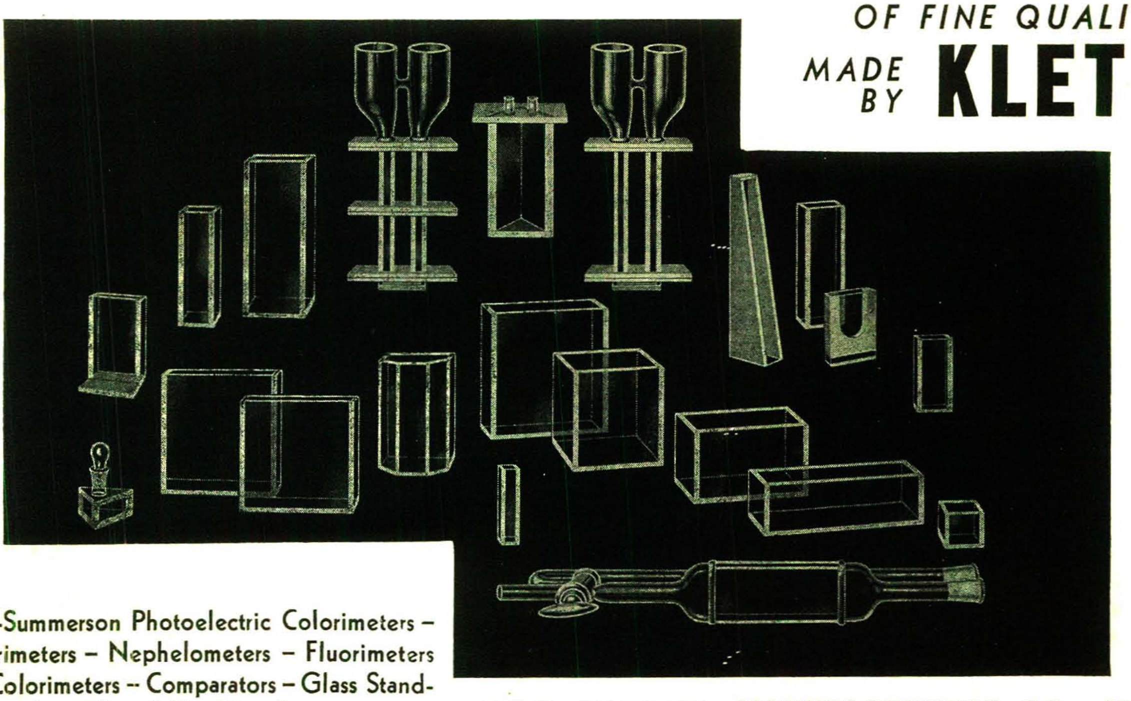

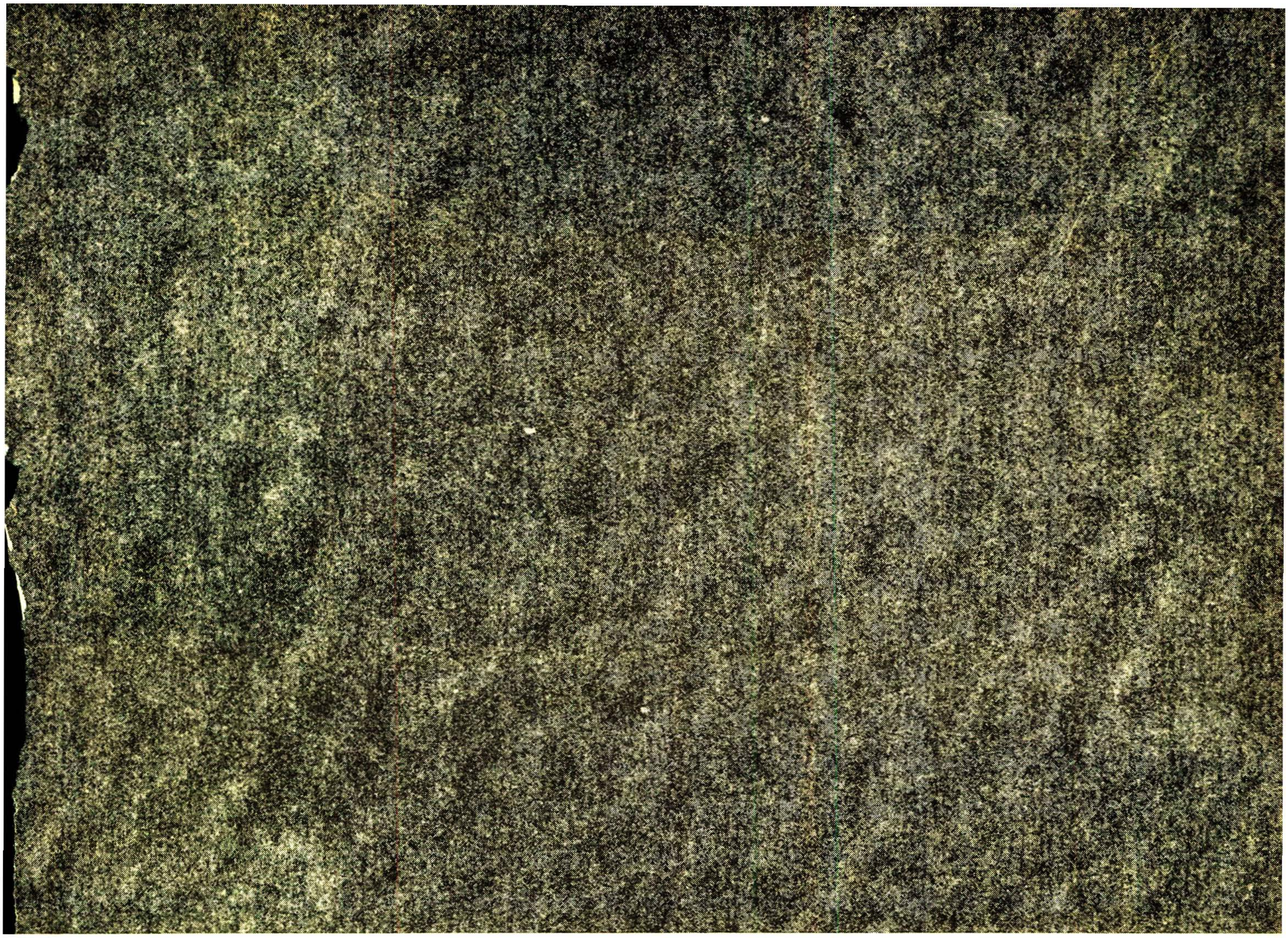

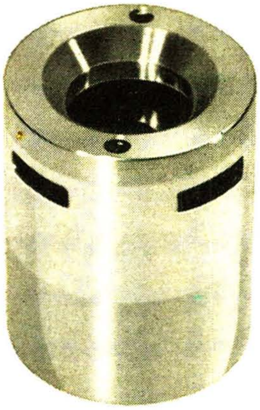

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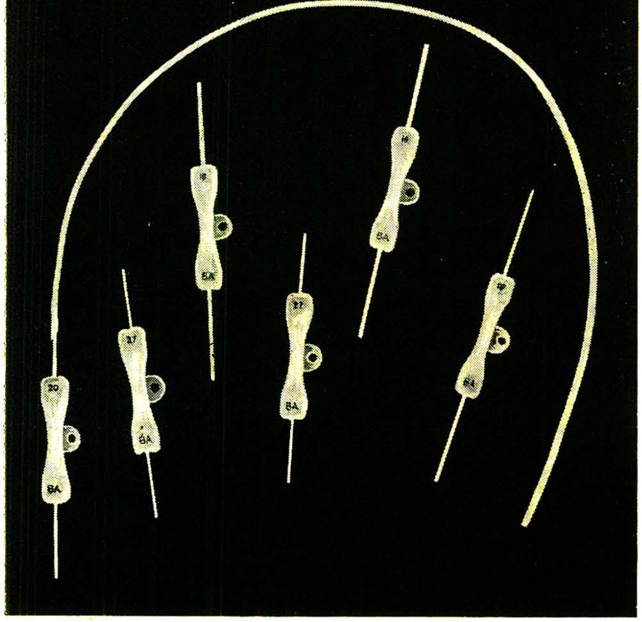

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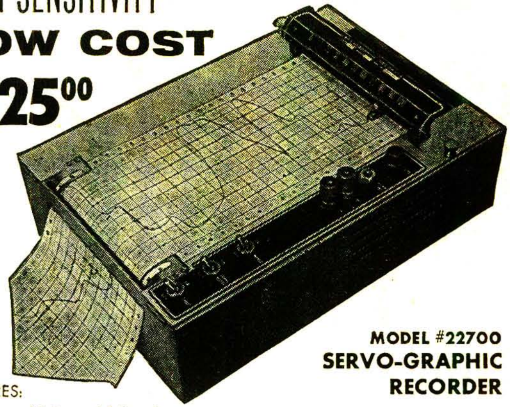

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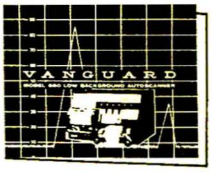
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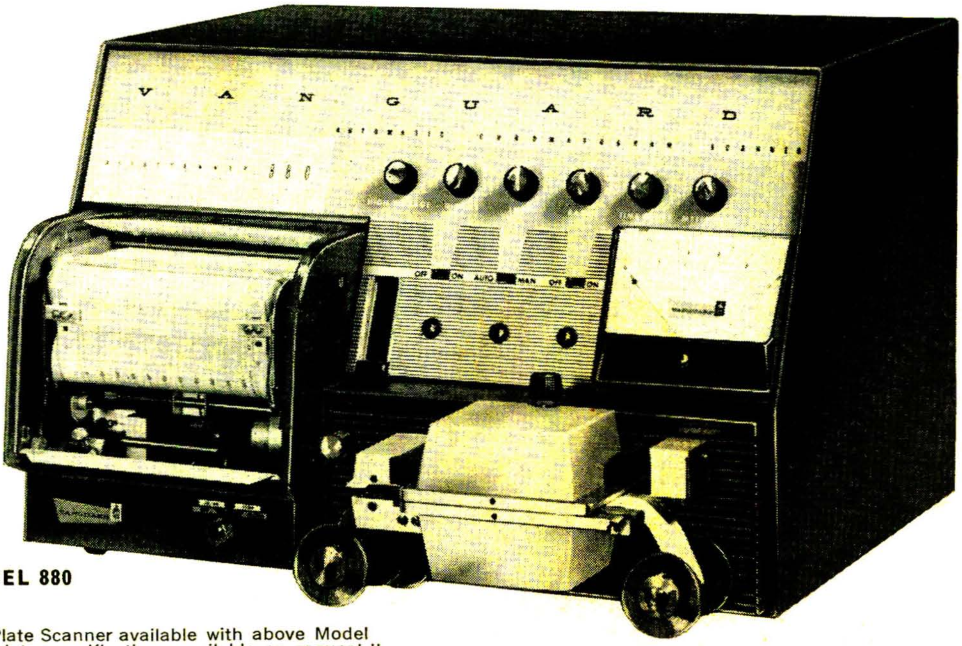
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INSTRUMENTATION

by Ralph H. Muller

Developments in Electronics

At the close of this year, we wish to discuss some revolutionary developments in electronics which are already beginning to influence instrument design. For more than a decade, electronic design has been increasingly threatened with what the experts call the "tyranny of numbers." In the early 1950's, an advanced computer contained somewhat over 1000 active devices. By the end of the decade this number had risen to the order of 100,000. Equipments are now in development with a device count in the many millions. For every active element (tube or transistor) there are passive devices, inductors, capacitors, resistors, wires, etc. For a long time, we have had microminiaturization, in which drastic reductions in size and weight have effected many economies including cost. However, these things cannot be continued without limit and do not evade the essential "tyranny of numbers." The inevitable job of assembling, maintaining, and interconnecting these tiny elements presents formidable problems, not the least of which is the matter of reliability.

MICROELECTRONICS

As J. A. Morton, Vice President, Bell Telephone Laboratories, Inc., remarked a few years ago: "The aim of electronics is not simply to reproduce physically the elegance of classical circuit mathematics—rather, it is to perform desired electronic system functions as directly and as simply as possible from the basic structure of matter."

In a recent monograph, "Microelectronics, Theory, Design and Fabrication," (383 + xix pages, McGraw-Hill Book Co., Inc., New York, N. Y., publishers, edited by Edward Keonjian), these topics are discussed by fourteen authorities. This book is a remarkable example of a publication devoted to a field, progress in which is so rapid that much of what is described will be ancient history a year from now.

Quoting again from the foreword to this book, Morton says: "The functional device approach exploits our potential ability to perform electronic circuit functions by going directly to the physics of solids without being impeded by classical concepts of circuit elements. Increasingly, we can expect the invention and development of physically simple single devices which will replace circuits having large numbers of classical elements.

"A few functional devices have been in use for many years, though not dignified by that name. The piezoelectric crystal, as a resonator, is equivalent to an assembly of coils, capacitors, resistors and connections, but nowhere within the crystal can one identify this part as a coil or that part as a capacitor. In the semiconductor area, newer examples of functional devices are the *pnpn* and Esaki diodes and the *pnpn* shift register and counter. With other materials, too, we have demonstrated the feasibility of performing complex logic and memory functions directly in a monolithic wafer of ferrite, cryogenic or ferroelectric material, with corresponding reductions in individual component–connection count per function."

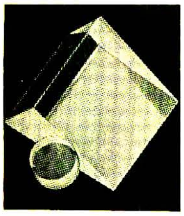
MOLECULAR ENGINEERING

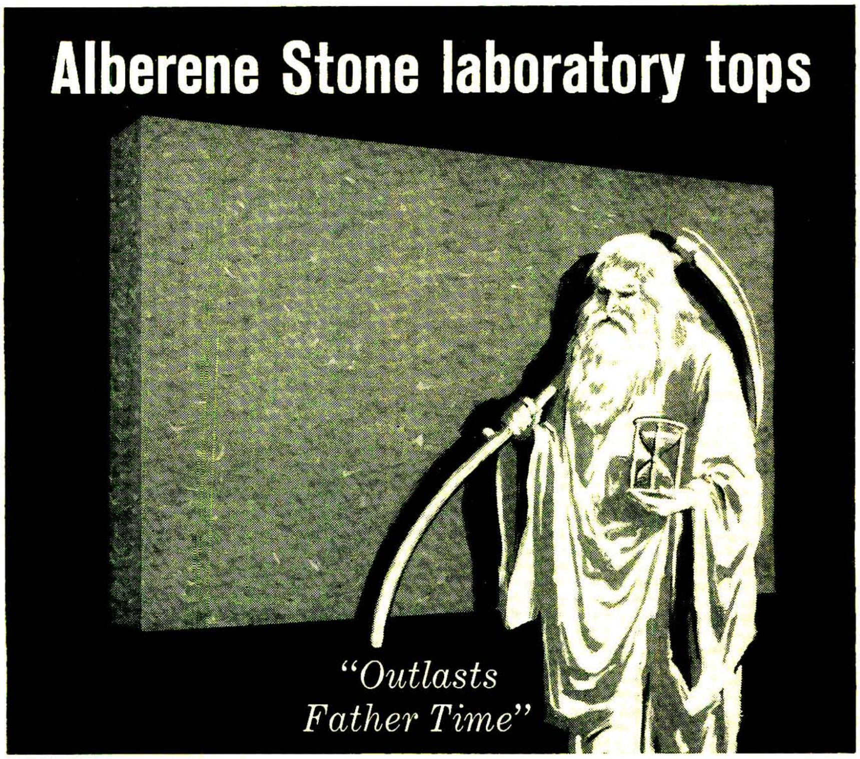
A closely related discussion, which deals primarily with materials, is to be found in a special report entitled, "Materials for Space-Age Electronics" [*Electronics* **36**, No. 43, 37 (1963)]. Current developments and applications for such materials as gallium arsenide, gallium phosphide, gallium and indium antimonide, indium arsenide, boron nitride and phosphide, the titanates, and a host of other compounds. These substances are not strangers to the chemist but the electronics people have bold plans for their more widespread use in what they call "molecular engineering." The organic chemist is by no means out of this picture. Rare earth chelates such as europium trifluoro thenoyl acetonate have been used as laser materials and made it possible to produce stimulated emission in liquids and plastics. Pumping energy is absorbed in the organic portion of the molecule and transferred into the central europium atom to produce the characteristic emission.

The current attitude seems to be that one should not look for semiconductive properties per se in organic compounds, but rather to see how they can be used to perform electronic functions. Some of the properties that may be useful are piezoelectric, piezoresistive, photochromatic, electrochromatic, dielectric properties, acoustic interactions, and infrared transitions.

SEMICONDUCTORS

New tricks are not lacking in the application of semiconductor devices to





passive elements. One of the neatest of these is the raising of Q values in a tank circuit (LC) by neutralizing the equivalent resistance of the tank circuit with a semiconductor negative resistance element. As described by Carl D. Todd [*Electronics* 36, No. 40, 30 (1963)] such negative resistance elements (NRE) can be paralleled with a tank circuit to neutralize its equivalent resistance. When the NRE is adjusted for infinite Q, it leads to sinusoidal oscillations in the circuit. Editorial comment on this paper suggests that "getting rid of resistance merely by introducing negative resistance to cancel it seems so obvious as to be unreal. It's like a science fiction plot to use negative gravity to hold the space ship aloft, yet for tank circuits it works." As author Todd explains, the utility of the scheme resides in the fact that "many resonant circuits require a higher Q than practical LC components can give. This is especially true for the lower frequencies where relatively large inductances are necessary and where a coil having sufficiently high Q is often too heavy." Many refinements of the general principle are described among which is the matter of temperature compensation. For one NRE it was possible to obtain stability of the order of 5 parts per million per degree Centigrade over a temperature range of −20° to +100° C.

ELECTROMECHANICAL DEVICES

There are constant improvements in precision and size-reduction in electromechanical devices. A good example is the stepping motor. The device is like a synchronous motor in principle, except that its rotor does not revolve smoothly and continuously when the motor is energized. Instead, on command from the input, the rotor travels an incremental step, stops instantly and locks magnetically in position. When a signal of opposite polarity is applied, the rotor advances another precise step, delivering torque in exact proportion to and at the same rate as the input.

Sigma Instruments of Braintree 85, Mass., announces an electromagnetic drive of this sort, the Cyclonome, which delivers torque in precise 18° steps at rates up to 1000 steps per second with p to 5-inch-ounces of torque with no standby power to maintain high holding torque. It has only one moving part with no catches, ratchets, or escapements. Sizes are as small as 1 cubic inch and require only the simplest input circuitry. The device is suitable for chart and tape drives analog-digital converting, impulse counting and, for step servos, in remote positioning and in timing.

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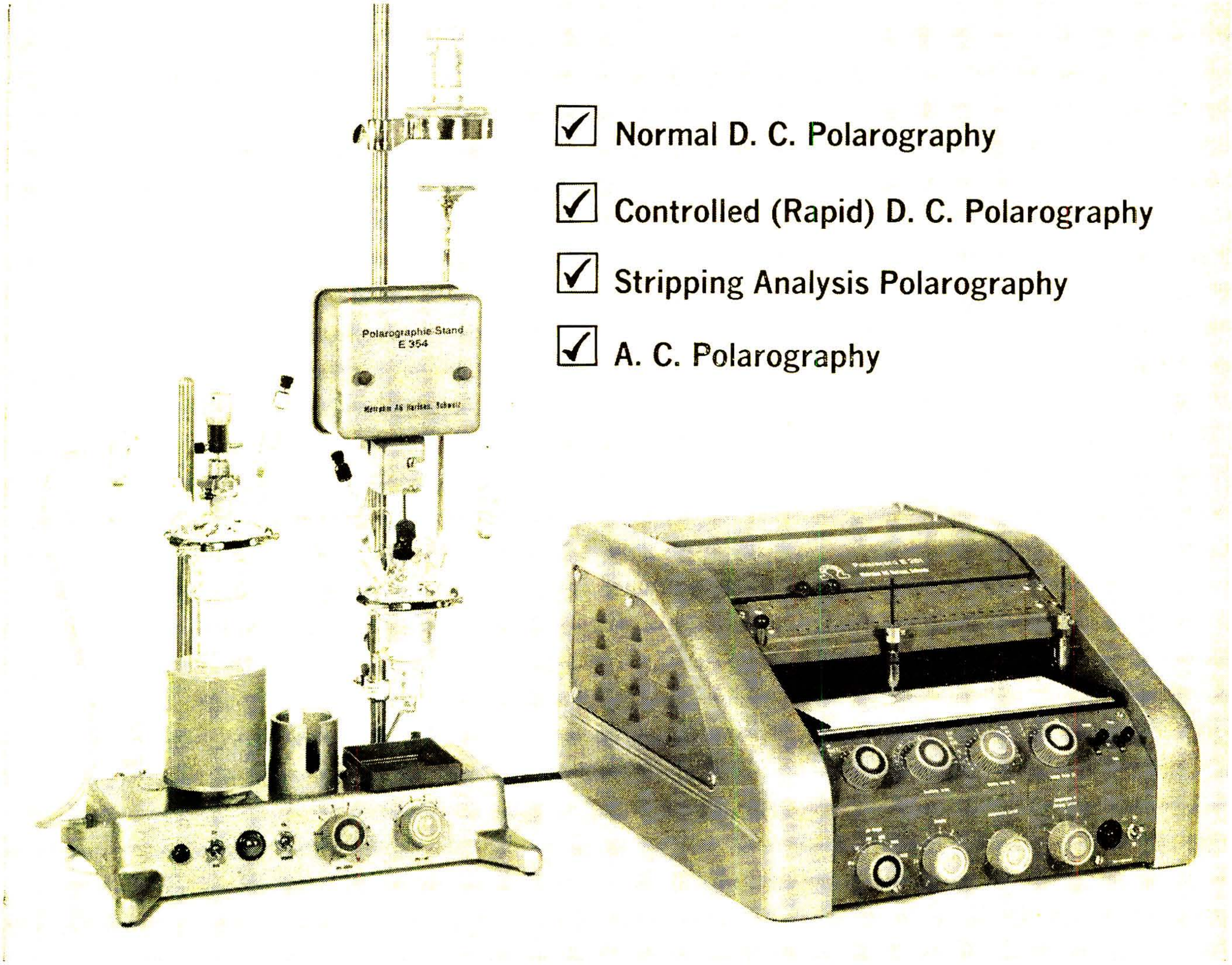

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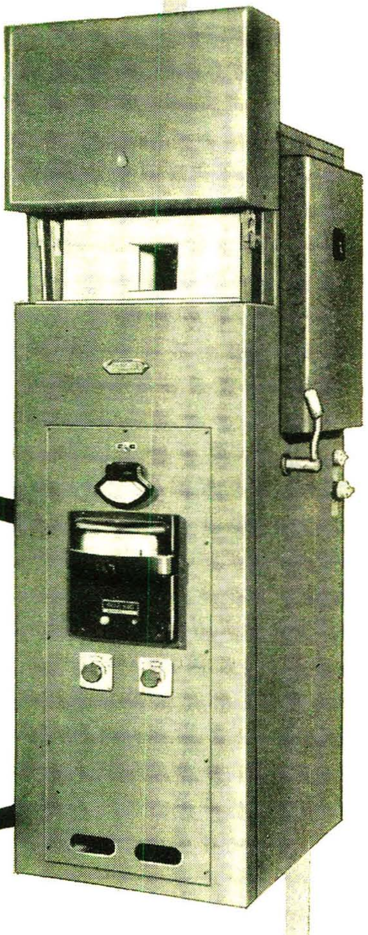

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Another convenient method, when using pipettes of 20 lambdas capacity or larger, is to discard the bulb assembly and, holding the pipette with the fingers (or a pair of tweezers), fill it by capillary attraction. Drop the pipette into a test tube containing the diluent and stopper. A short vigorous shaking will wash the contents of the pipette into the diluent.

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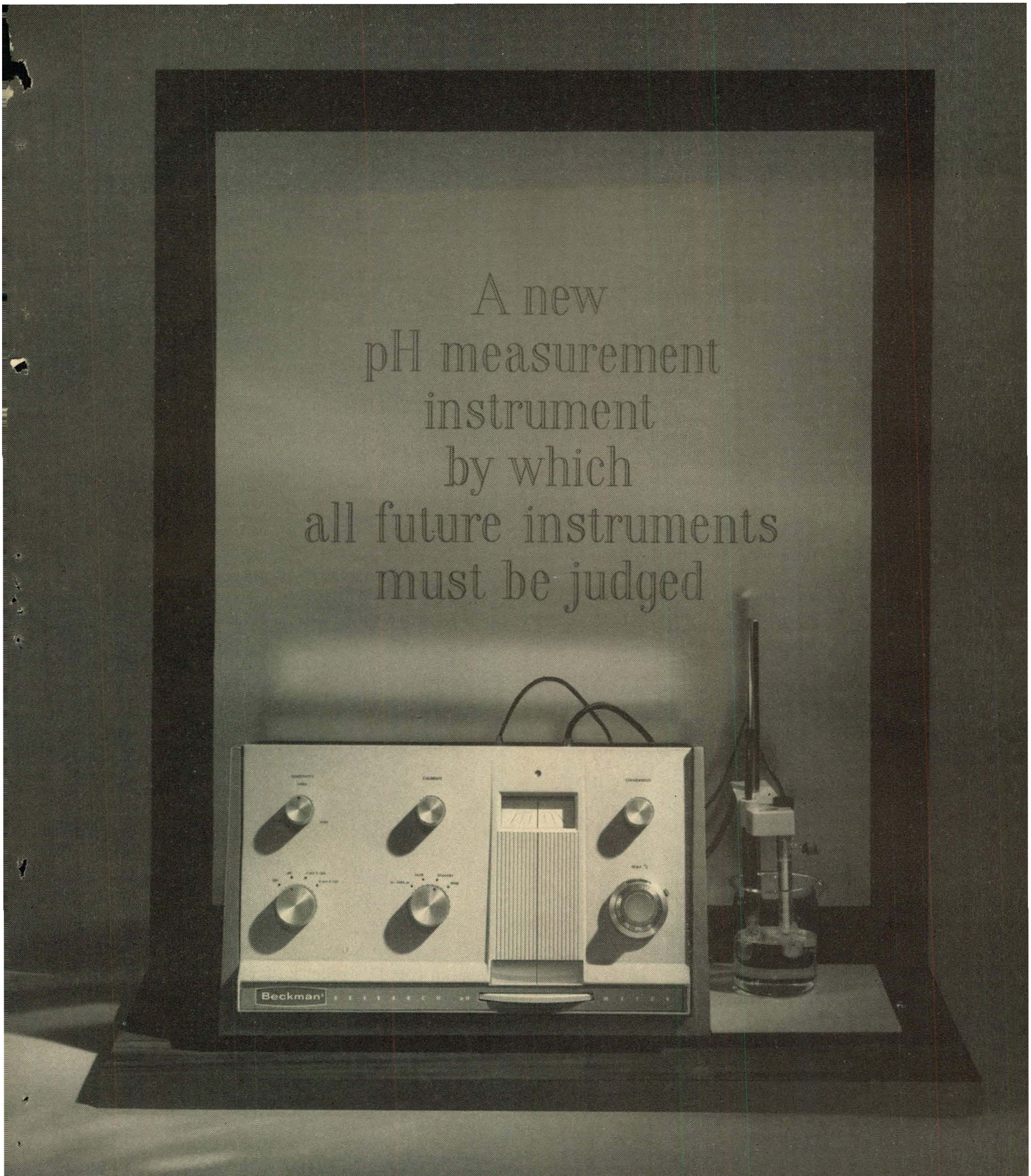

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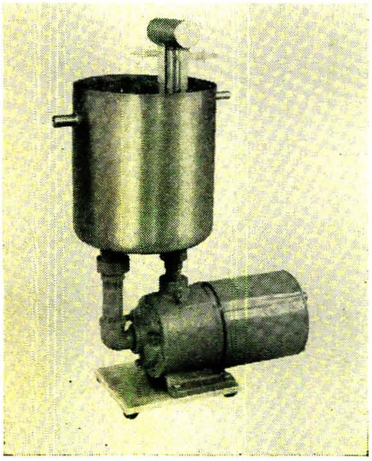

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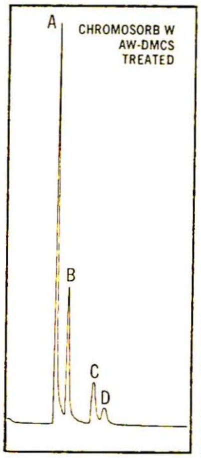

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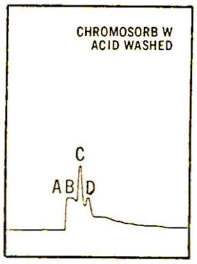

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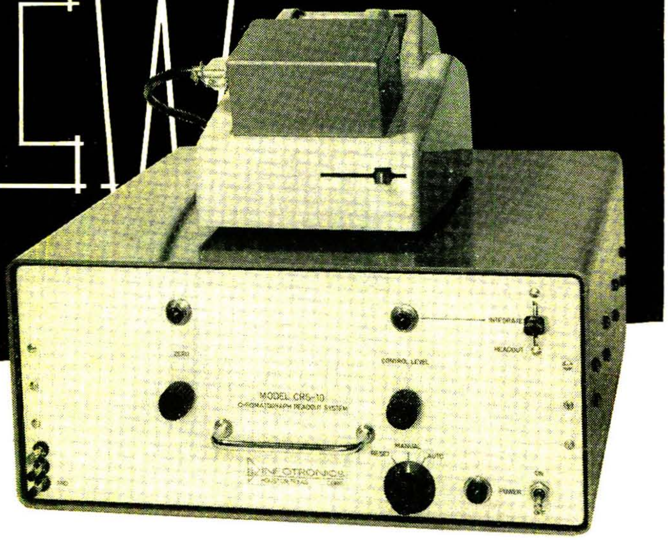

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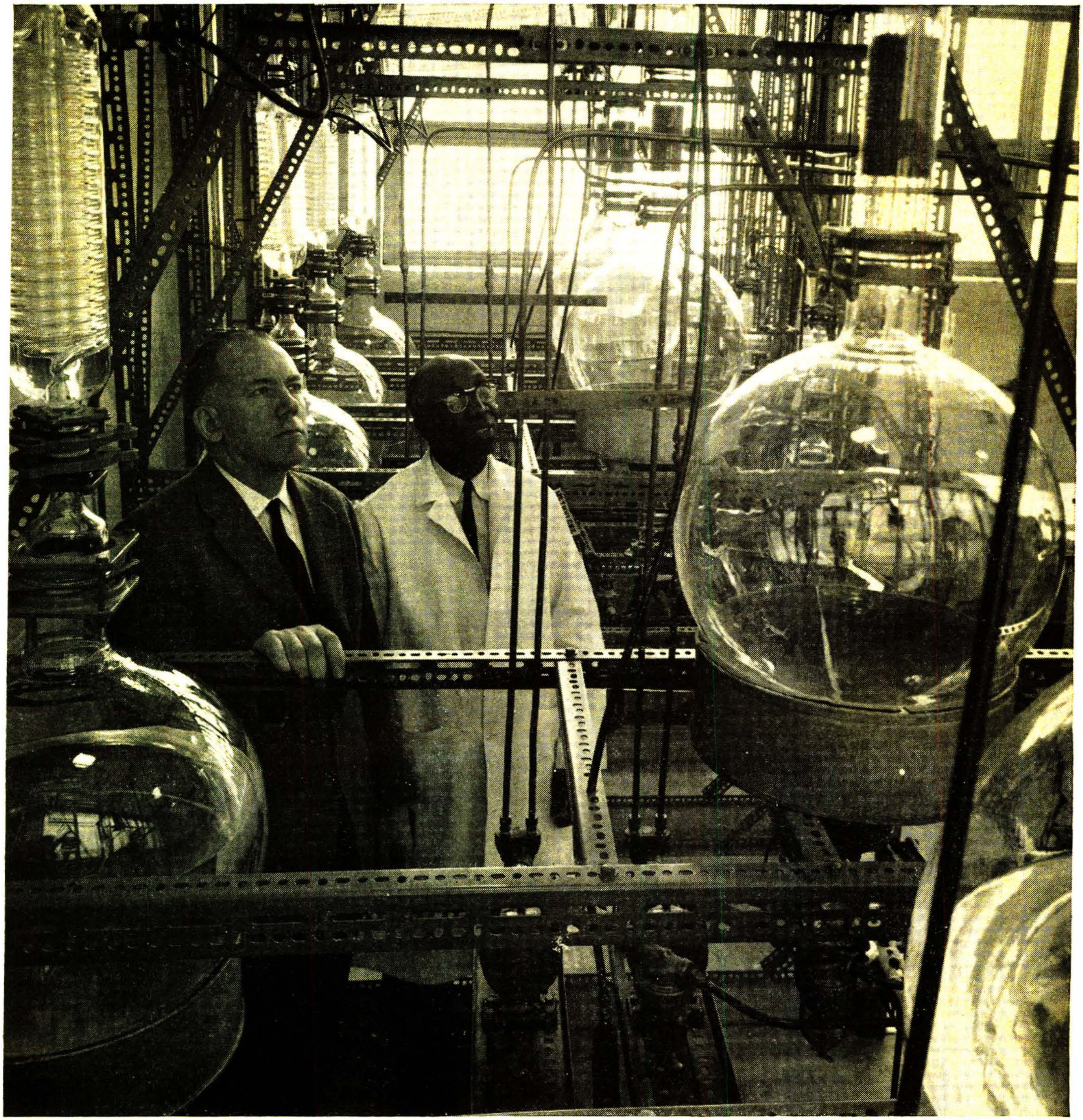

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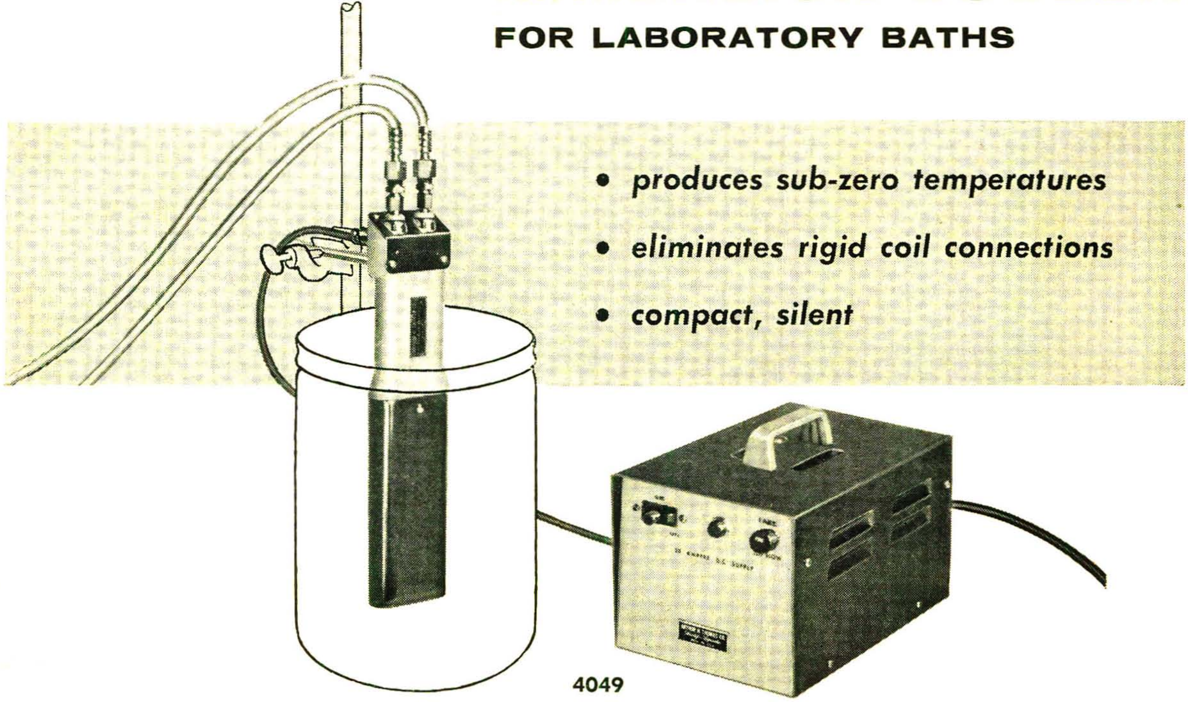

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December 1963, Vol. 35, No. 13
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Anonymous Reviewers

A QUESTION SOMETIMES asked by authors is, "Why do some reviewers prefer to remain anonymous?" One of our readers has again called our attention to this problem by quoting from Eyring's Presidential Address made at the 145th ACS National Meeting in New York to the effect that anonymous reviewers can choose this means to be needlessly critical and unfair to an author's research paper.

In the case of this journal, 30% of the reviewers prefer to remain anonymous. We believe that many authors have the misconception that the reviewer makes the final decision as to whether his paper should be published. This, of course, is not the case. The reviewers help the editor and his staff to arrive at a fair appraisal of the work. It often happens that of the two reviewers chosen, one is overly critical and another says only that the paper should be published without change. The editor here has a choice of consulting a third reviewer, but he more likely will decide to send the critical and anonymous comments to the author and ask him to defend his research. If, in the editor's opinion, the author's rebuttal is satisfactory, the paper is accepted and the reviewer overruled. The reviewer is usually made aware of the decision and sent a copy of the author's rebuttal. In this give and take, the author is not at a disadvantage, even though the reviewer prefers to remain anonymous. In most cases we feel that there are good reasons for the anonymity.

Our reader was concerned that perhaps we had not given sufficient thought to this problem. We assure him and our authors that we are constantly concerned with arriving at a fair decision. We believe our review system is a sound one if the editor is alert to his responsibilities. His is the final decision.

L. T. Hallett

Metal Chelates in Analytical Chemistry

A Report of the 16th Annual Analytical Symposium

QUINTUS FERNANDO, HENRY FREISER, and EDWARD N. WISE

Department of Chemistry, University of Arizona, Tucson, Ariz.

▶ Chelating agents are of great utility in almost every aspect of the analysis of metals. Ligands which form charged metal chelates are used in titrimetry, as masking agents, or in separations based on the ion exchange principle. Ligands which form uncharged metal chelates are used as the basis of analytical separations such as precipitation, solvent extraction, and chromatography which includes gas phase chromatography for volatile chelates. Since many chelates are characteristically colored, chelating agents are sensitive and selective reagents in colorimetric and spectrophotometric procedures for metal ions. An understanding of the factors relating structure to analytical behavior makes it possible to design new chelating agents having more useful characteristics for particular applications. At the Sixteenth Summer Symposium held at the University of Arizona, June 19–21, 1963, various aspects of the role of metal chelates in analytical chemistry were discussed.

EDTA AND SIMILAR REAGENTS

A NUMBER OF SPEAKERS stressed the importance of factors underlying the design and proper use of chelating agents. Charles N. Reilley, of the University of North Carolina, in his discussion of the theory of metal titrations with EDTA and similar titrants pointed out the need to consider the role of kinetics of chelate formation and dissociation as well as the equilibrium aspects of such reactions. Careful distinction between end point reactions blocked by unfavorable equilibria and those blocked by slow kinetics is important since each responds to different types of treatment. A problem arising from kinetically blocked end points can sometimes be solved by working at increased temperatures, whereas a thermodynamically unfavorable system will not be improved significantly by this means.

Reilley pointed out that despite the paucity of quantitative kinetic data, a great deal of insight can be gained from a consideration of the vast number of qualitative observations on the rates of end point reactions in the literature. A reasonable prediction of the feasibility of many end point reactions can be made by considering the nature of the molecular structure of both the chelating titrant and the metallochromic indicator. In direct titrations, where the end point is reached by the replacement of the metallochromic indicator coordinated to the metal ion by the titrant molecule, the structure of the metallochromic indicator is of prime importance. The rate of reaction of the metal-indicator complex with the titrant becomes slower as the number of metal coordination sites in the indicator molecule decreases. Since reduction in the number of coordination sites results in lower stabilities of the metal complexes, a balance must be struck in the design of the indicator between these opposing tendencies. For example, the *o,o*-dihydroxyazonaphthalene indicators are likely to give sluggish end points when used with transition metals and small highly charged cations and are useful primarily with divalent nontransition metal ions. Faster end point reactions are observed when one of the coordinating hydroxy groups is replaced by a heterocyclic nitrogen atom as in 4-(2-pyridylazo)resorcinol (PAR). Still faster than PAR are the azo-substituted 8-Quinolinol indicators—e.g., 8-hydroxy-7-(4 sulfo-1-naphthylazo)-5-quinoline sulfonic acid(SNAZOXS). Here, the lower stabilities of metal-SNAZOXS complexes resulting from the bidentate nature of the indicator are sufficiently offset by the formation of 2:1 indicator-metal complexes of higher stability. The bidentate nature of the chelation sites of Pyrocatechol Violet certainly contributes to the remarkable lability of the metal-indicator complexes and hence to the rapid end points observed with this useful indicator.

Variation in the structure of the indicator not involving the chelation sites can also significantly influence the rate of end point reactions. Replacement of a naphthol nucleus in the Erio R structure which is blocked by nickel even at elevated temperatures, by a substituted pyrazolone nucleus forms Erio Red B which gives excellent end points with Ni-EDTA titrations at 70° C. Substituents that are electron-withdrawing or that offer steric hindrance to chelation also increase lability as well as reduce stability. Indicator impurities such as oxidized or reduced forms of the dye or metal contaminants are frequently responsible for sluggish end points.

In discussing the role of the structure of the titrant on end point reaction rates, Reilley emphasized the importance of steric effects. Although 1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid (DCyTA) reacts more rapidly with $Al(H_2O)_6^{+3}$ than EDTA, it gives sluggish end points with nickel and copper indicator complexes. This he attributed to the hindrance of the rigid cyclic backbone structure of DCyTA to the favorable orientation for attack of the chelated metal indicator complex, but this is not so critical with the aquated metal ion where orientation effects are less important. The pH can change the titrant structure, and for this reason largely has a direct bearing on the rate of the end point reaction.

The nature of the metal ion being titrated also plays a part in the rate of the end point reaction. In general, the smaller the ionic radius and the larger the charge on the metal ion, the more likely is its end point reaction to be slow. With transition metals, the situation becomes more complex; chromium (III) is notorious for its slow substitution reactions and nickel is generally slower than copper. With such ions use of auxiliary complexing agents can sometimes remedy sluggish end point reactions. The addition of *o*-phenanthroline to the normally blocked Cu-Xylenol Orange–EDTA end point results in a rapid reversible end point. This may arise from the formation of either an intermediate Cu–phenanthroline or a mixed complex. The deliberate addition of 1 to 5% of a foreign metal ion to a metal ion before chelometric titration often serves to eliminate a sluggish end point since in effect the end point reaction of the sensitizing metal is substituted for that of the metal being titrated. Thus with appropriate choice of sensitizing metal ion, an indicator can be used for a whole series of metals, which either give insufficient color contrast or blocked end points with the free indicator. Thus nearly every metal that can be determined by EDTA can be determined by using Cu-sensitized 1-(2-pyridylazo)-2-naphthol (PAN) as indicator.

Marked acceleration of end point reactions of EDTA is often found when a mixed solvent is used in place of water. This effect correlates better with a decrease in EDTA solubility than with dielectric constant.

In contrast to direct titrations, the success of many back-titrations depends on nonattainment of one or more equilibria; here the basic problem is the reduction of the rate at which these undesirable equilibria are attained. The back-titration of Al–EDTA with Cu and SNAZOXS indicator would fail if Cu, Al, EDTA, and SNAZOXS were equilibrated since the aluminum would be largely displaced from the complex by the more strongly chelated copper, destroying the stoichiometry of the titration and blocking the indicator. A result of this inverse relationship between direct and back-titrations is that the opposite of what was said about direct titrations can often be applied to back-titrations. DCyTA, for example, is rather poor as a direct titrant because of the slowness with which it undergoes exchange with metal-indicator complexes, yet is excellent in back-titrations because of the sluggishness with which DCyTA chelates exchange with indicators.

Finally, Reilley mentioned the usefulness of NMR techniques in the study of the structure, solution equilibria, and even reaction kinetics of metal chelates. From such measurements, the acid dissociation constants and protonation sites of chelating titrants can often be determined. Further, by observing the pH dependency of certain proton chemical shifts in solutions containing the ligand and the nonparamagnetic metal ion, metal chelate formation constants may be determined. Qualitative information about the kinetics of chelate reactions can sometimes be obtained by an analysis of the splitting patterns or band width changes in NMR spectra of chelates containing metals having an appreciable quantity of a suitable magnetic isotope.

H. Flaschka of the Georgia Institute of Technology discussed the application of photometric titration as a means of increasing the sensitivity of chelometric titrations. Photometric titrations, in contrast to pH or other potentiometric titrations, are linear in that the quantity measured as the titration proceeds is directly proportional to the concentration rather than being logarithmically related. The shapes of such titration curves consist of two line segments easily extrapolated to meet at the end point instead of the sigmoid shape associated with logarithmic titration curves. The sharpness of the end point in a logarithmic titration is much more dependent on the magnitude of the equilibrium constant of the titration reaction than is that of the linear titration since in the latter the straight line segments are obtained in the presence of a large excess of one of the reactants.

Two general types of photometric indicators may be distinguished by the general shape obtained in a photometric titration. First, the step indicator is one which is added in a very small amount and changes color at the end point resulting in a step-shaped titration curve. Second, the term "slope" of "self" indicator is applied when any of the species involved in the titration absorbs. Titration curves of self-indicating systems show only one change of slope which is less abrupt than the slope changes seen for step indicators. Self-indicating systems can be used in place of metallochromic indicators for nonindicating systems, thereby eliminating some of the problems mentioned above associated with these dyes. Furthermore, greater selectivity can be achieved with "slope" than with "step" indicators. For example, when Ca and Mg are titrated with EDTA using Erio T in the usual fashion as a step indicator only the sum of Ca and Mg is obtained. If, however, the Erio T is added in excess of the amount of Mg present, then a slope titration results in which the magnesium indicator complex acts as a self-indicator. The titration curve will be nearly horizontal until all of the Ca reacts with EDTA. Further addition of titrant will change the absorbance of the solution as the Mg-indicator complex reacts with EDTA. When all the Mg has reacted, no further color change occurs and the titration curve again becomes horizontal. Thus, the two slope changes in the titration curve permit the analysis of Ca and Mg in each other's presence. Using this approach, Flaschka was able to develop a method for the determination of both Ca and Mg in a single drop of blood serum. A method for the determination of Cd and Zn in the presence of one another in an ammoniacal solution using Cu as a slope indicator and ethyleneglycol - bis - (B - aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA) as titrant was also described. In ammoniacal medium the order of the conditional formation constants of EGTA complexes is Cd $>$ Cu $\geq$ Zn. Thus, Cd is titrated first and the slope changes when either Cu alone or Cu together with Zn starts to complex. By this means Cd can be determined in the presence of several hundredfold excess of Zn. Using slope titrations, as many as four metal ions have been determined in a single solution.

Robert L. Pecsok of the University of California at Los Angeles discussed the effects of chelation on electrochemical behavior and, conversely, the interpretation of such behavior to elucidate the nature and properties of metal chelates. The formation constant of the Cr(II)–EDTA complex was evaluated for the first time using the potentiometric technique modified to obviate the difficulty imposed by the rapid oxidation of Cr(II). Simulated titration curves were obtained by preparing mixtures containing the proper amounts of EDTA, acid, and/or base, and enough KCl to bring the ionic strength to $0.1M$. After thorough deaeration of each of these mixtures, the appropriate amount of Cr(II) was added. Equilibrium was reached within 30 seconds and the solutions were stable for at least 30 minutes. The formation constant for CrY^{-2} as calculated from the simulated titration data ($\log K_f = 13.61$) showed a tendency to increase with decreasing pH. This trend necessitated the assumption of the formation of a protonated species $CrHY^-$ having a pK_a of 3.00 similar to that found for the rest of the divalent first row transition metal ions. The constancy of the pK_a values could be explained only by the assumption of a quinquedentate structure of CrY^{-2}. The CrY^{-2} was shown to have a d^4 configuration with four unpaired electrons from magnetic susceptibility measurements.

The polarography of CrY^{-2} was studied and a one-electron reversible anodic wave observed in the pH range 4 to 10. The half-wave potential was independent of both total Cr and EDTA concentrations which proved the absence of polymeric species and the presence of one EDTA per Cr in both the oxidized and reduced complexes. The half-wave potential of the anodic wave was independent of pH in the range 4 to 6 ($E_{1/2} = -1.227$ volts *vs.* SCE) but became more negative with pH values below 4. This indicates that the neutral CrY^{-2} is being oxidized in the pH range 4 to 6; below pH 4 the protonated $CrHY^-$ is the species oxidized. The polarographic behavior in the alkaline region indicates the presence and participation of hydroxy complexes. From the value of $E_{1/2}$ in the pH range 4 to 6, and the K_f of CrY^{-2}, the value of the formation constant of CrY^- was calculated as $\log K_f = 23.40$.

G. J. Abel, Jr., of the American Smelting and Refinery Co.'s Central Research Laboratories, in his discussion of the application of EDTA to the analysis of nonferrous materials, indicated that EDTA procedures were used for the analysis of one or more elements present in virtually every alloy produced as well as in the many raw materials and by-products associated with the nonferrous industry. He then outlined a number of procedures which were adapted to suit these needs.

Lead is one of the basic elements which is determined in brass, bronze, solder, white metals, drosses, and ores after separation as lead sulfate. Tin, antimony, and arsenic are removed by hydrobromic acid–bromine volatilization and the precipitated lead sulfate is separated by filtration. The precipitate is dissolved in ammonium acetate and titrated with EDTA at pH 10 after the addition of tartrate to prevent hydrolysis of the lead, and cyanide to mask any occluded divalent metal ions. Eriochrome Black T is used as the indicator with the addition of magnesium as indicator ion.

Zinc is determined in brass, bronze, diecast, slush metal, silver solders, and by-products by EDTA titration; in fact, every zinc determination, excepting low or trace amounts, is performed titrimetrically with EDTA. The titration is generally performed at pH 10 after masking divalent metal ions with cyanide and demasking zinc with formaldehyde. Eriochrome Black T is used as the metal-indicator. Preliminary separation will depend upon the matrix. A general method of separation is based on a thiocyanate extraction.

Zinc in aluminum-base alloys may be separated by passing a solution 0.75*M* in hydrochloric acid through a column of Dowex 1. The adsorbed zinc is eluted with water and titrated.

Zinc oxide is determined in zinc dust by masking the metallic zinc by amalgamation with mercury. The zinc oxide is dissolved simultaneously in ammonium hydroxide–ammonium chloride solution and titrated as before.

Nickel is determined in its alloys by EDTA titration after preliminary separation of the nickel with dimethylglyoxime. The precipitate is separated by filtration and dissolved in hydrochloric acid and hydrogen peroxide to destroy the dimethylglyoxime. The solution containing the nickel is titrated with EDTA at pH 11 using murexide indicator.

A general procedure for determining aluminum in all alloys is based on the removal of interfering elements by extraction with sodium diethyldithiocarbamate. The sample solution, buffered to pH 5.5 is treated with sodium diethyldithiocarbamate and the carbamates are extracted with chloroform and discarded. The aqueous layer is treated with excess EDTA and boiled to destroy the excess carbamate as well as to complete the EDTA reaction. The cooled solution is back-titrated with standard zinc solution at pH 5.5 using xylenol orange indicator. Chromium is the only interference encountered, but its presence is obvious from the lavender color of the Cr-EDTA complex. A rapid method for the determination of aluminum in zinc-base alloys depends upon the displacement of EDTA from an aluminum–EDTA complex by fluoride.

Magnesium, one of the earliest elements determined by EDTA titration, is determined in aluminum and zinc-base alloys by titration at pH 10 with Eriochrome Black T indicator. The type of separation and masking agents used depends on the alloy. Zinc-base alloys are analyzed directly for magnesium by masking zinc, copper, nickel, and ferrous iron with cyanide, and aluminum with triethanolamine. Titrations of magnesium when aluminum is present are best conducted rapidly in cold solutions to prevent blocking of the indicator by aluminum.

Details of the analysis of fusible alloys containing bismuth, lead, cadmium, indium, and tin which were an analyst's nightmare before the advent of EDTA were also described by Abel.

The determination of indium with EDTA can be performed at pH 3 to 5 or pH 10 depending on the interfering elements. Xylenol orange is used as indicator at pH 3 to 5 and Eriochrome Black T with magnesium indicator ion at pH 10. The reaction of indium is slow near the end point unless the solution is hot. Indium can be separated from interferences by extraction of its bromide complex or its acetylacetonate.

Molybdenum in ores, concentrates, and leach solutions can be determined by an indirect EDTA method. The molybdenum is precipitated as lead molybdate at pH 4 to 5 and filtered. The lead molybdate precipitate is dissolved in sodium hydroxide and sodium tartrate, neutralized, and buffered to pH 10. The lead is now titrated with EDTA using Eriochrome Black T and magnesium ion as indicator.

NEW CHELATING AGENTS

R. Belcher of the University of Birmingham, England described a study of the reactions of *p*-dimethylaminobenzalrhodanine with silver in which over 30 similar compounds have been synthesized. From the work done so far, it would appear that the complexing process is by no means as simple as was first supposed; moreover, the silver reaction can take place even though the imino hydrogen is absent.

The discovery of EDTA as a titrant led to extensive investigations to find new indicators. This in turn led to the further examination of these indicators as spectrophotometric reagents. For example, alizarin complexon was found by Belcher and his coworkers to give a strong chelate with cerium(III) ions, which further formed a 1:1:1 complex with fluoride. This complex is blue whereas the Ce(III) complex is red. This provided the first direct color reaction for the fluoride ion, and further interesting studies of this reagent have been made.

The dyestuff Fast Sulfon Black F was first studied as an EDTA indicator for copper. Its highly selective reaction suggested that it might have useful properties as a spectrophotometric reagent for the determination of copper. Although there are already a host of valuable spectrophotometric copper reagents, this reagent has some advantages. In these studies the only metal whose interference could not be masked was beryllium. The beryllium color still persists in the presence of cyanide which masks copper satisfactorily. Accordingly, this provides a specific colorimetric reaction for beryllium.

Another reagent which was first synthesized as an indicator for the titration of calcium with EDTA was obtained by diazotizing H-acid. This compound is very suitable as a spectrophotometric reagent for the determination of calcium, and from the tests carried out so far appears to have advantages over previously recommended reagents.

The compound 4(2-pyridylazo) resorcinol has been studied by various investigators as a spectrophotometric reagent. Conditions have now been established in which only niobium reacts specifically in the presence of most other metals including tantalum.

The compound bromopyrogallol red is a valuable spectrophotometric reagent for the determination of niobium and also silver. It is possible to enhance the sensitivity of the silver reaction by the formation of a ternary complex.

In the course of earlier investigations in the field of *o,o*-dihydroxyazo compounds by Harvey Diehl and coworkers at Iowa State University there was uncovered a compound which gives a color with magnesium but not with calcium. The magnesium compound proved fluorescent and provided a basis for both a fluorometric and a colorimetric method for magnesium. The tolerance for calcium in both is exceptionally large. The various aspects of the system such as absorption, excitation and fluorescence spectra, effect of pH, effect of excess reagent, effect of alcohol concentration in the solvent, have been investigated and the methods have been applied successfully to the determination of magnesium in water, serum, limestone, iron ore, and cement. The reaction of various hydroxyazo compounds, prepared during the work, toward beryllium has been investigated also. The structure of *p*-nitrophenylazoorcinol, rather widely used for the spectrophotometric determination by beryllium, has been established and the nature of the reaction with beryllium worked out. The unsatisfactory character of this reagent suggests several possibilities for synthesizing an improved reagent.

The usefulness of the *vic*-dioximes as selective analytical reagents has been steadily increasing since the time they were first proposed for this purpose in 1905 by Tschugaeff. The *vic*-dioximes available today are widely used for separations involving precipitations and extractions. These reagents are also extensively used for the gravimetric, titrimetric, and spectrophotometric determination of various metals. These reagents are commonly used for the gravimetric determination of nickel and palladium. The *vic*-dioximes have also been used as the basis for alkalimetric, oxidimetric, complexometric, amperometric, nonaqueous, and heterometric methods of analysis. Rhenium, iron, nickel, cobalt, and copper are among the metals which can be determined spectrophotometrically with the *vic*-dioximes. The isomerization, crystal structure, nomenclature, and the analytical applications of the *vic*-dioximes were discussed by Charles V. Banks of Iowa State University.

The chemical, magnetic susceptibility, infrared, x-ray diffraction, and thermodynamic evidence concerning the structure of the insoluble metal(II)-*vic*-dioxime coordination compounds were reviewed. The crystal structures of these compounds were correlated with such properties as solubility product constant, intrinsic solubility, overall stability constant, and absorption spectra. The nature and stability of the soluble coordination complexes formed between nickel ions and *vic*-dioximes in alkaline media were discussed. These fundamental studies have been very beneficial in helping one to decide how to "tailor-make" new and improved *vic*-dioximes for specific analytical applications.

8-Mercaptoquinoline, a hygroscopic blue liquid which forms a crystalline red dihydrate, was introduced first in 1944 by Taylor who stated that it could not be a useful analytical reagent because of ready oxidation to the bis-8-quinolyldisulfide. However, it has been found by Freiser and coworkers that with reasonable precautions, the oxidation can be largely eliminated. Not until recently has 8-mercaptoquinoline received much attention in analytical applications.

Since 8-mercaptoquinoline would appear to have potentialities as useful and as versatile as 8-quinolinol, a study of the formation constants of metal chelates of 8-mercaptoquinoline was undertaken by the University of Arizona group. Although 8-mercaptoquinoline is significantly more acidic than 8-quinolinol, its chelates are as stable (similar K_f values) and form at lower pH values (higher proton exchange constants) than do the 8-quinolinol chelates. The analytical significance of these results was discussed.

An equilibrium study of another interesting system was presented by H. P. Gregor of the Polytechnic Institute of Brooklyn, who described a number of the properties of a typical metal–polyelectrolyte system and their utility in analytical chemistry. The polymer employed was 100,000 mol. wt. poly-*N*-vinylimidazole (PVI), a commercially available material having certain properties analogous to those of many bio-polymers.

The binding of a metallic cation such as zinc to this polymer can be measured most readily by pH titrations, and thermodynamic binding constants or displacement constants may be evaluated by the general procedure of Bjerrum as adapted to polymeric systems by Gregor and coworkers. On the assumption that the activity coefficient of counter ions is determined principally by the mean activity coefficient of the supporting electrolyte, it was shown that thermodynamic formation constants determined by potentiometric titrations and by equilibrium dialysis experiments agreed within experimental error.

This polymer shows a maximum coordination number of four with Zn(II) and Cu(II). Formation constants were calculated from data obtained in the presence of a high concentration of neutral salt and at a high equivalent ratio of polymer to metal ion. The successive binding constants for the zinc–polyvinylimidazole system increased as the number of ligands attached to the metal ions increased. The stoichiometry of PVI–Zn complexes was studied by precipitating the polymer in the presence of different ratios of polymer to zinc.

The turbidity of Zn–PVI complexes was studied by light-scattering, and the turbidity increased as polymer was added at a constant metal ion concentration; at higher polymer concentrations the turbidity decreased. When an excess of metal ion was present, it acted as a cross-linking agent and high molecular weights were obtained, ones which decreased as more polymer was added to the system with a resultant shift to intramolecular as opposed to intermolecular cross-linking.

The mixed complexes of Cu(II), PVI, and nitrilotriacetic acid (NTA) were studied by the technique of equilibrium dialysis. For this system the overall formation constant was the same as that predicted from the individual constants, within experimental error. The mixed complex contained equimolar amounts of PVI, Cu(II), and NTA.

Several analytical applications of metal-polyelectrolyte complexes were discussed. The equilibrium dialysis procedure is simple and should allow one to compare the relative binding power of ligands directly. Since a low concentration of polymer ($10^{-5}N$) forms quite turbid solutions with many metals, a simple nephelometric procedure for their estimation might be devised. The high specific turbidity of polyelectrolytes should make these procedures quite sensitive. The use of metal polyelectrolytes in solvent extraction techniques is also an interesting possibility because by the addition of hydrophobic side chains one can reduce the solubility of the polymer in water to insignificant levels.

CHELATES IN SOLVENT EXTRACTION

The theory of the solvent extraction of metal chelates was developed in considerable detail by both David Dryssen of the Royal Institute of Technology, at Stockholm, and George Schweitzer of the University of Tennessee. Equations were developed for the various equilibria involved, and methods for identifying the dominant organic and aqueous species were described. Using these relationships, various means of controlling extractions were discussed. Included were rate relations, temperature effects, effects of the presence of aqueous complexes, and effects of alterations in the organic solvent.

A comparison of some extraction constants or pH_{50} values with the constants for the first steps in the mononuclear hydrolysis of the metal ion was made. Dryssen noted that as the extraction constant of a reagent decreased, the spread of pH_{50} values of the metals increased. Effects of the metal ion concentration such as polynuclear hydrolysis, precipitation of the metal chelate, and radiocolloid formation were discussed.

The formation of adducts in the organic phase with the uncharged extractable metal chelate, MA_N, and of some mixed solvent effects were treated. Finally, Dryssen discussed the extraction of mixed chelates and some practical applications of mixed chelate extraction.

Chelate extractions with TTA were reviewed by Oscar Menis of Nuclear Materials and Equipment Corp. Parameters, including hydrogen ion concentration, solvent, and aqueous environment were evaluated in terms of their enhancing, complexing, and/or kinetic effects. The information was classified according to these variables to provide guides in the selection of specific methods.

Enhancement effects in several systems were described. A general term, synergistic effect, has recently gained wide usage to describe the influence of two or more factors on the magnitude of the distribution ratio of a metal complex. A comparison was given of the various mechanisms for agents which produce a greater combined result than when acting individually such as adduct formation, solvent coordination, and mixed chelate formation. In addition, the utilization of the competitive action of complexing ligands in the aqueous media for separating groups of metal chelates was discussed.

Separations of the actinide and rare earth elements, as well as iron, aluminum, and copper were presented under the various aspects mentioned previously. Thus in the case of thorium, the utilization of acetate aids in the extraction of thorium by a mechanism of adduct formation. It was postulated that the thorium–TTA–acetate molecule is the extracted species and served to enhance the extraction. On the other hand, in the case of rare earths, lanthanum and others, the pH of extraction is a function of the acetate concentration, shifting the extraction of these elements to higher pH values in high acetate solutions. In this manner a sharp separation of thorium and elements of the rare earth group can be achieved. The solvent type, whether polar or nonpolar, plays a role in separations. This is especially significant when a polar solvent such as hexone is used. For example, hexone in the absence of TTA does not extract thorium while aluminum is extracted. Furthermore, the combined effect of hexone and TTA is synergistic in that aluminum can be extracted at pH 0. The extraction of iron and copper–TTA from an acetate medium is very rapid. Similarly, the rate of extraction of aluminum is much greater from an acetate medium than it is from a strong acid medium.

Finally, an example was given of the simultaneous determination of several rare earths based on the careful control of solution parameters.

CHELATES IN OTHER SEPARATION METHODS

Philip W. West of Louisiana State University discussed the application of complexing agents in masking interfering metal ions for the development of greater selectivity in metal separations and determinations.

The usefulness of metal chelates in radiochemical separations was amply demonstrated by H. L. Finston of Brookhaven National Laboratory who discussed in detail procedures in which chelating agents were applied to the separations of radioisotopes. Rapid solvent extraction procedures necessary for the isolation and study of short-lived radionuclides were pointed out. Decontamination factors of greater than 10^{12} have been obtained in the separation of uranium from associated fission products after neutron irradiation, by extraction with dibenzoylmethane in the presence of DCyTA.

Since the various fractions for radiochemical studies are very pure it is possible to apply nonspecific methods of chemical yield determination of the initially added carrier. Furthermore, the widespread use of well-type scintillation counters for liquid samples obviates gravimetric yield determinations. EDTA reacts with so many cations that it is possible to use it as a single reagent for convenient and extremely rapid chemical yield determinations in many different procedures.

Application of a semimicro volumetric technique has enabled the determination of Ba in 30 seconds, the titration being carried out directly in the counting tube. R. L. Sievers of Wright-Patterson Air Force Base, Ohio, discussed the use of volatile metal chelates in the separation and analysis of metals by gas phase chromatography. Metal chelates of acetylacetone, trifluoroacetylacetone, and hexafluoroacetylacetone were studied. A number of chelates can be eluted at column temperatures far below their boiling points and in most cases below or near their melting points. A definite trend was observed in the relative ease of elution. Chelates of the fluorine-containing β-diketones are considerably more volatile and can be eluted at much lower column temperatures than corresponding complexes of acetylacetone. Column temperatures as low as 30° C. have been used in separations of hexafluoroacetylacetonato complexes.

Trifluoroacetylacetonato chelates of beryllium(II), aluminum(III), gallium (III), indium(III), chromium(III), iron (III), copper(II), rhodium(III), zirconium(IV), and hafnium(IV) have been eluted successfully. Separations of several mixtures of these complexes have been achieved. Examples are the separations of the aluminum(III), gallium(III), and indium(III), and the aluminum(III), chromium(III), rhodium(III), and zirconium(IV) complexes.

The gas chromatographic approach is well suited for trace metal analysis in addition to conventional analysis. The fluorine-containing chelates are susceptible to detection with highly sensitive electron capture detectors. Recent studies have shown that quantities of halogen-containing organic compounds on the order of 10^{-12} gram can be detected by electron capture. This emphasized the extraordinary potential of the technique in trace analysis. Gas chromatography was used to separate *cis* and *trans* isomers and optical isomers of metal chelates. Optical isomers were separated on columns which contained optically active stationary phases.

J. Coleman of Harvard Medical School discussed his work with B. Vallee on the inhibition of metalloenzymes by chelating agents. Metalloenzymes are proteins which contain metal atoms firmly bound to the protein. When isolated in completely purified form, the ratios of metal to protein and activity to protein become constant and the ratio of moles of metal per mole of enzyme becomes an integral number. The role of the metal was described as that of a reactive group of the enzyme molecule involved in activity which is lost when the metal atom is either removed or interacts with chelating agents to form a mixed complex.

Kinetic Origin of Tailing in Chromatography

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► Tailing may be assumed to originate as a kinetic phenomenon when the sorption isotherm is linear. The various mechanisms for this are discussed. Equations are then presented for an idealized model for tailing, first proposed by Giddings and Eyring in 1955. Concentration profiles are obtained and plotted for both elution and nonelution chromatography. Tailing may originate when a sorption site exists which holds molecules for a time equal to that necessary for one quarter of the zone to pass by. In gas liquid chromatography this is equivalent to having a small percentage of the stationary liquid in pores with seven to eight times the normal diffusion distance.

TAILING in chromatography may appear as anything from a slight zone asymmetry up to a severe zone distortion with a considerable mass of material stringing out behind the bulk of the zone. Most zones are at least slightly affected by this phenomenon. The harmful effects of tailing are in rough proportion to the excess spreading of the zone rearward, a spreading which can overlap or mask other zones.

Tailing is usually blamed on a nonlinear adsorption isotherm. This is rather natural, because the earliest theories of chromatography (*3*, *13*), showed that nonlinear adsorption isotherms were sources of very high asymmetry. Recent work with ultrasensitive detectors in gas chromatography has shown, however, that tailing often exists when solute concentrations are well below the point where a noticeable departure from linearity occurs. It is thus necessary to explain certain occurrences of tailing in terms of a linear picture of chromatography.

The first explanation for tailing in the presence of linear sorption was apparently given by Giddings and Eyring in 1955 (*7*). A kinetic mechanism, in which adsorption occurs on two types of sites, was postulated. It was assumed that the second type of site, with a high adsorption energy, was relatively scarce. Molecules would not often be "captured" by such a site, but when adsorption did occur the molecules would be held for a considerable time. When desorption finally occurred, the bulk of the zone would already have passed over. Desorption would thus occur into the trailing part of the zone and the resulting buildup of concentration would appear as a tailing phenomenon. We may call this kind of site a "tail-producing site."

Keller and Giddings (*10*) have calculated concentration profiles for zones which are influenced by a slow, reversible chemical reaction between chromatographic migrants. Such a reaction may occur between isomers, etc., as migration proceeds. The resulting profiles frequently show tailing (as well as "fronting"). It was thus demonstrated that tailing may have a kinetic origin even in the presence of a linear isotherm.

A qualitative discussion of tailing in capillary gas liquid chromatography has been given by Golay (*8*). The assumed mechanism is the absorption and holdup of solute molecules in excess droplets of liquid. This concept is apparently the same as that proposed by Giddings and Eyring, except that the slowness of desorption is caused by an excessive diffusion distance rather than by a high-energy adsorption site.

Another source of tailing in the linear range is the presence of excess dead volume in the system, usually in the injection or detection units. The explanation is much like the others given above: A quantity of solute can be shunted into dead pockets, where a sufficient time elapses before it escapes to cause tailing. Johnson and Stross (*9*) have shown tailing profiles which originate with a large detector volume within which perfect mixing is assumed.

No other mechanism in the linear range has been postulated which would give a significant degree of tailing [a normal elution profile will always show a slight positive skew (*7*), but for narrow zones this is not significant]. In the nonlinear range, of no direct interest here, a number of mechanisms for tailing have been proposed. These include the effect of the adsorption isotherm, volume changes caused by sorption (*1*), and temperature changes caused by sorption (*6*, *12*).

THEORY

The theory given here will implement the original suggestion by Giddings and Eyring. Although a two-site theory has been extensively developed (*5*, *7*, *11*), it is too difficult to apply in rigorous form, particularly when a more attractive alternative exists. As shown by Keller and Giddings (*10*), the fast exchange reaction (as on the first or "normal" type of site) leads to an effective diffusion process whose spreading influence can be readily calculated. This is superimposed on the effect of the slow exchange process on the tail-producing site. The latter must be calculated by rigorous theory.

It will be assumed that the first-order adsorption and desorption rate constants on the tail-producing site are k_a and k_d, respectively. Thus $1/k_a$ will be the mean time required for a molecule to adsorb on this type of site and $1/k_d$ will be the mean time required for a molecule, once adsorbed, to detach itself. It will further be assumed that all of the solute is in the mobile phase as migration begins.

Elution Development. It has been shown (*7*) that the elution profile corresponding to the above assumptions is

$$P(t_s) = \left(\frac{k_a k_d t_m}{t_s}\right)^{1/2} e^{-k_a t_m - k_d t_s} I_1\left(\sqrt{4 k_a k_d t_m t_s}\right) \quad (1)$$

where t_s is the (variable) time spent adsorbed on the tail-producing site, and is thus the time measured from the appearance of a normal peak—i.e., a peak undisturbed by slow adsorption. An inert peak (the "air" peak in gas chromatography) requires a fixed time, t_m, to migrate through the column. The quantity $I_1(X)$ is a Bessel function of imaginary argument.

Equation 1 is more conveniently written in terms of the reduced time variable, y, where $y = t_s/t_m$. The dimensionless parameters, a_1 and a_2, are equal to $k_a t_m$ and $k_d t_m$, respectively. With this change of terms and variables Equation 1 becomes

$$P(y) = \left(\frac{a_1 a_2}{y}\right)^{1/2} e^{-a_1 - a_2 y} I_1\left(\sqrt{4 a_1 a_2 y}\right) \quad (2)$$

The molecules which escape through the column without ever being adsorbed on those sites are not allowed for in Equation 2. These molecules appear as a narrow pulse at time $t_s = 0$ and the ideal profile contributed by them, analogous to Equation 2, is

$$P'(y) = e^{-a_1} \delta(y) \quad (3)$$

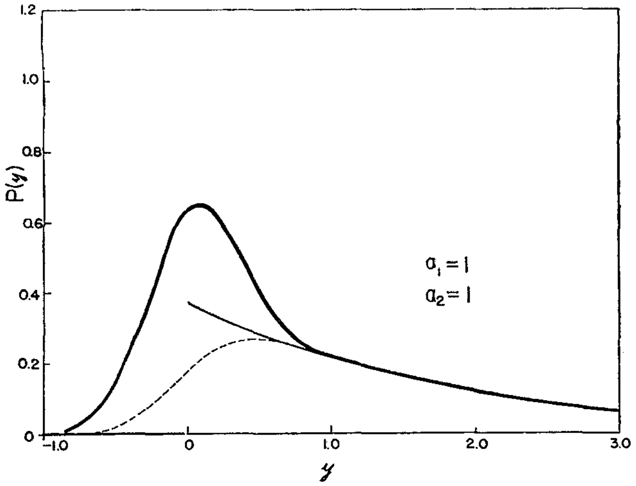

Figure 1. Elution profile with $a_1 = 1$ and $a_2 = 1$

In this and subsequent profiles the light solid line is a direct consequence of the tail-producing sites, the dashed line is the same except as it is smeared by column processes, and the heavy solid line is the final concentration profile

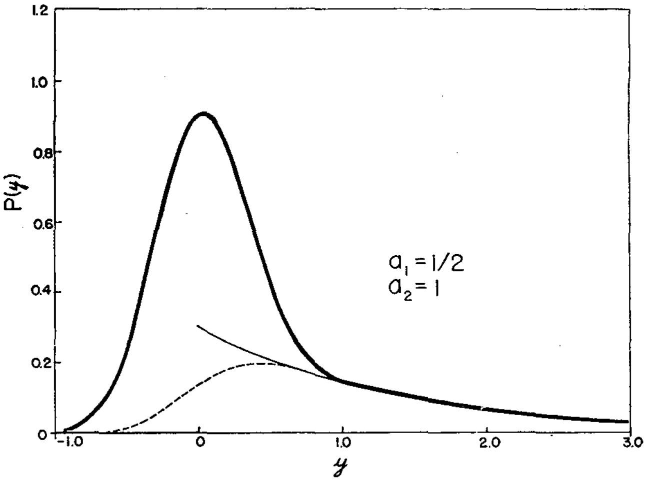

Figure 2. Elution profile with $a_2 = {}^1/_2$ and $a_1 = 1$

where the delta function, $\delta(y)$, signifies an infinitely thin pulse at $y = 0$. The addition of Equations 2 and 3 yields a concentration profile normalized to unit area. This undisturbed profile must now be modified to allow for the effective diffusion processes of the column. This is a simple matter for that part of the concentration profile found in Equation 3, since a Gaussian profile is found immediately. A numerical procedure must be used for $P(y)$. The Schmidt method (*2*) has been used for this purpose here. The degree of effective diffusion which will occur will be related to the number, N, of theoretical plates in the column (excluding the effects of second-site adsorption). This is discussed below.

t_s is the time measured from the appearance of the center of the undisturbed chromatographic peak. This peak will, however, require a time t (the elution time) after injection to make its appearance. Thus our coordinate systems using t_s or y have their origins a time t after injection.

The effective diffusion of solute is related to both t and the number, N, of plates in the column. Thus the standard deviation, τ, on the time scale (which may be used as the parameter-characterizing diffusion) is given by $\tau = t/\sqrt{N} = (t_m/\sqrt{N})(t/t_m)$. The ratio t_m/t is simply the R value (zone velocity over carrier velocity) for the undisturbed component peak. Thus $\tau = t_m/R\sqrt{N}$. The standard deviation, σ_y, along the y axis is given by $\sigma_y = \tau/t_m$. Thus

$$\sigma_y = 1/R\sqrt{N} \qquad (4)$$

Thus if one chooses reasonable values for R and N, the diffusion parameter, σ_y, is determined and the calculations can be made accordingly.

Nonelution Chromatography. The nonelution or on-column zone profile resulting from slow second-site kinetics is given by the sum of the following equations (*10*).

$$P_3(x) = b \exp[-a(1 - x) - bx] \; I_0\sqrt{4abx(1 - x)} \qquad (5)$$

$$P_4(x) = [abx/(1 - x)]^{1/2} \exp[-a(1 - x) - bx] \, I_1\sqrt{4abx(1 - x)} \qquad (6)$$

$$P_5(x) = e^{-b}\,\delta(1) \qquad (7)$$

where x is the fraction of the total time, t, spent unattached to the second site and thus $1 - x$ is the fraction of time spent adsorbed on this type of site. Since the molecule is migrating normally when unattached, x may be thought of as a reduced distance coordinate representing the distance traveled relative to that of the undisturbed zone. The zone profile calculated from these equations will, then, simply represent the distribution of component along the column length, $x = 0$ and 1 being the initial and final positions of the undisturbed zone, respectively.

The kinetic parameters of Equations 5, 6, and 7 are $a = k_d t$ and $b = k_a R t$ (the R appears in the last expression because only the fraction R of unattached molecules is free to react, the rest being adsorbed on the normal sites). Both I_0 and I_1 are Bessel functions.

The effective diffusion processes of the column, to be superimposed on the profile discussed above, is once again related to the number of plates generated in migrating to the final position a distance L along the column. The standard deviation, σ, developed by an undisturbed zone, is given by $L/\sqrt{N}$ in this case. The standard deviation in x, σ_x, is modified to σ/L because of the change in scale factor. Thus

$$\sigma_x = 1/\sqrt{N} \qquad (8)$$

Once N is fixed at a reasonable value, the extent of diffusion can be calculated in accordance with the σ_x obtained.

RESULTS

Calculations have been made which indicate the degree of tailing caused by slow sorption-desorption processes. The computed profiles are shown in Figures 1 to 6.

Elution Chromatography. Figures 1 to 4 pertain to elution chromatography. Different kinetic parameters, a_1 and a_2, are used in the calculations, but the columns from which these profiles might be expected are otherwise assumed to be identical. Except for the disturbance which leads to tailing, each column is assumed to have a thousand plates, $N = 1000$, and the R value is assumed to be 0.1. A change in these values would alter the details of the profiles but the main features would be unchanged.

Each figure consists of three curves. The curve with the sharp discontinuity at $y = 0$ is that calculated from Equation 2 for those molecules which are captured at least once by the second kind of site. The dotted line is the profile obtained from this through a diffusion process corresponding to 1000 plates (Equation 4). The heavy line is the final profile, a sum of the dotted line and the Gaussian resulting from the diffusion of the spike in Equation 3.

The zero on the y scale corresponds to the emergence of the undisturbed peak. Since y is a measure of time in units of the dead time, t_m, and since $R = 0.1$, the initial injection occurs at $y = -10$

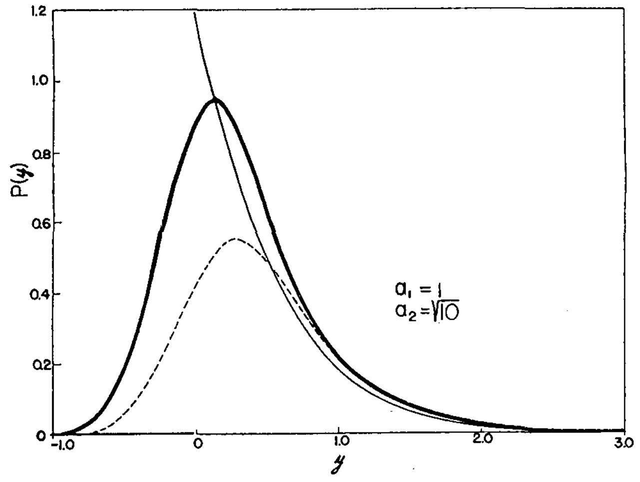

Figure 3. Elution profile with $a_1 = 1$ and $a_2 = \sqrt{10}$

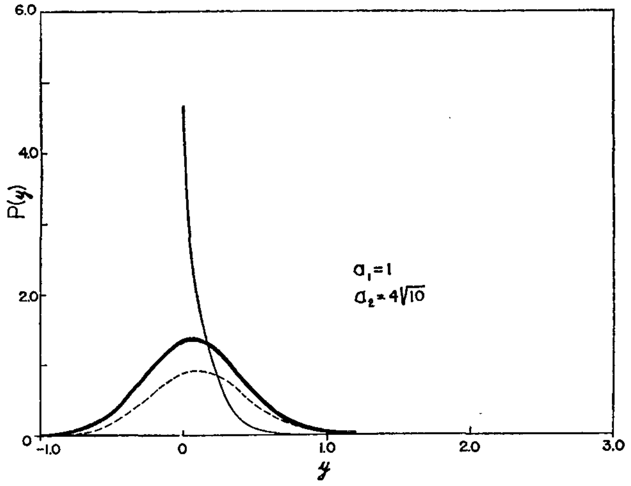

Figure 4. Elution profile with $a_1 = 1$ and $a_2 = 4\sqrt{10}$

for all these cases. This fact gives some perspective to the type of chromatograph this column would yield.

Figure 1 shows a very significant degree of tailing. This profile is calculated with $a_1 = a_2 = 1$. These parameters, interpreted simply, mean that 63% of the molecules become attached at least once to the tail-producing site and that 37% of the molecules pass through the column without attachment. A molecule, once attached, requires a mean time t_m for desorption. It is this slowness of desorption which produces tailing, for in the time a molecule remains attached to this site an inert peak passes through the entire column length. This explains why the tail in Figure 1 is spread out over several y units, each y unit being the passage time of the inert peak. In this same time of attachment the bulk of the component zone migrates through $^1/_{10}$ (since $R = 0.1$) of the column length. Since at its widest point (just before elution) the zone width, 4σ, is only $^1/_8$ of the column length, a molecule lost to a tail-producing site from the center of the zone will not ordinarily emerge again until the zone is well past. It is molecules of this sort which form a tail by desorbing to the rear of the zone.

Figure 2 is similar to Figure 1. The only difference is that a_1 has been changed from 1 to $^1/_2$. This means that more molecules (61% instead of 37%) migrate through the column without being disturbed by the tail-producing sites. The concentration in the tail (controlled by a_1) is thus less, but its length (controlled by a_2) is just as great.

In Figure 3 a_2 has been increased from 1 to $\sqrt{10}$. This increase in desorption rate reduces the length of the tail. The tailing is still noticeable, however. The value $\sqrt{10}$ was chosen for a_2 in order to make k_d equal $1/\tau$, where τ is the standard deviation of the undisturbed peak in time units just before its elution. The fact that k_d equals $1/\tau$ can be shown by writing $k_d = a_2/t_m = \sqrt{10}/t_m$, and $1/\tau = R\sqrt{N}/t_m = \sqrt{10}/t_m$. If a different column with some other N is chosen, we would expect a similar profile as long as $k_d = 1/\tau$, an equality that can be arranged by letting $a_2 = R\sqrt{N}$.

Figure 4 shows a profile in which a_2 has been increased to $4\sqrt{10}$—i.e., $k_d = 4/\tau$. Very little tailing is evident. This was anticipated, since a molecule held up by a tenacious site for the time $1/k_d$, or $\tau/4$, desorbs again before a very large fraction of the zone has gone by. Figures 3 and 4 taken together show that as a rough rule tailing is not a major concern unless $k_d \leq 1/\tau$ or $a_2 \leq \sqrt{N}/R$.

Magnitude of Rate Constant for Tail-Producing Sites. Throughout this paper we have emphasized the wide difference existing between tail-producing sites and normal sites. It is worth calculating the extent of that difference in at least one limiting case.

If zone spreading in the undisturbed zone is controlled by adsorption and desorption on the normal sites (rather than by longitudinal diffusion, etc.), the column plate height is (*4*)

$$H = 2R(1 - R)v/k_d' \qquad (9)$$

where v is the mean velocity of the mobile phase and k_d' is the rate constant for desorption from the normal site. Since $a_2 = k_d t_m$, the ratio of k_d' to k_d is

$$k_d'/k_d = 2R(1 - R)N/a_2 \qquad (10)$$

where N has been used in place of L/H. The critical value of a_2, from above, is $R\sqrt{N}$, which combined with Equation 10 gives

$$k_d'/k_d = 2(1 - R)\sqrt{N} \qquad (11)$$

Thus for the parameters used in this paper, $N = 1000$ and $R = 0.1$, the tail-producing site must have a desorption rate some 58 times slower than normal in order to be significant. If factors other than adsorption-desorption rates control the plate height, the discrepancy would need to be even greater.

Application to Gas Liquid Chromatography. Some degree of tailing is encountered in most applications of gas liquid chromatography in spite of the fact that ultrasensitive ionization detectors have led to an ever-decreasing sample size. Assuming that this tailing may often have a kinetic origin, we can postulate two mechanisms which may be responsible. First a few active sites on the solid support may lead to tailing. All active adsorption sites, it should be emphasized, will not lead to tailing under linear conditions, but only those which succeed in retarding solute for a time comparable to τ, as discussed above.

The second mechanism for tailing may originate in liquid diffusion. The units of liquid which cause retention are distributed in a complex geometrical way, presumably with a wide variation in physical dimensions. Any unit of liquid held in, say, a long narrow pore will equilibrate slowly with external solute because of the large effective diffusion distance, d. The equilibration time increases with d^2 and thus the effective desorption constant, k_d, is proportional to $1/d^2$. (Even though desorption is no longer a single-step process, the essential nature of desorption is little changed.) Thus if one is looking for a pool of liquid which will produce tailing, and which must therefore have a k_d value 58 or so times smaller than average, one finds that the effective diffusion distance must be seven to eight times the normal distance. The relative amount of stationary phase which must be tied up in these abnor-

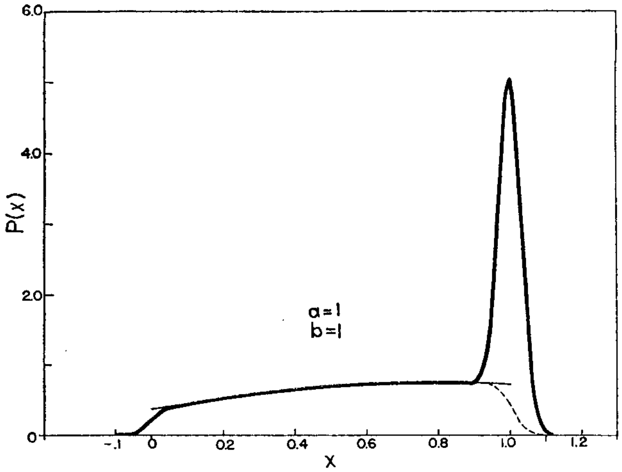

Figure 5. Nonelution profile with $a = 1$ and $b = 1$

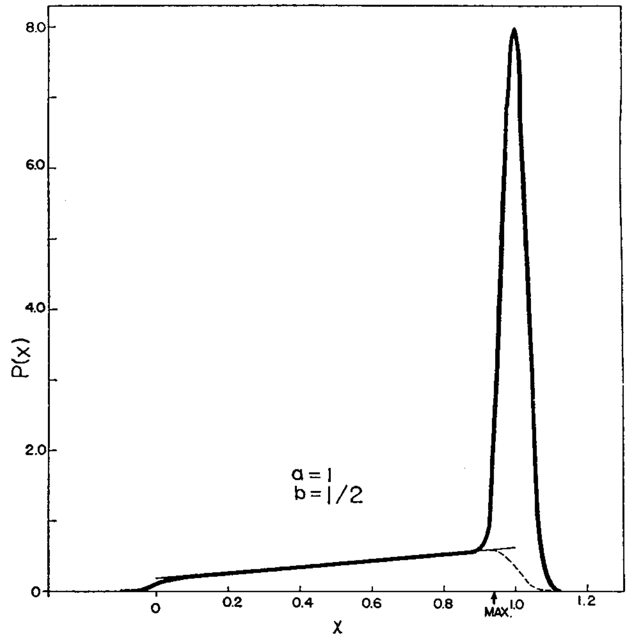

Figure 6. Nonelution profile with $a = 1$ and $b = {}^1/_2$

mally large units is the additional retention time, τ, caused by these units divided by the normal retention time, t, minus t_m. This fraction is $1/(1 - R)\sqrt{N}$. For the parameters used above this is 0.035—i.e., 3.5% of the liquid should be found in large units. This requirement could probably be relaxed somewhat, since tailing would undoubtedly be discernable if a_l were several times less than the value used in Figure 3. Probably 1 to 2% liquid would suffice. The nature of such liquid—whether it occurs in elongated pores, caverns with restricted entry, or large units formed from small ones running together—is highly uncertain at this point.

Nonelution Chromatography. Figures 5 and 6 show concentration profiles for nonelution chromatography. Once again a column is assumed which generates 1000 plates and the R value is 0.1. Figure 5 is plotted with the parameters $a = 1$ and $b = 1$. The tailing is more prominent than in Figure 5 where the sorption rate is cut in half, $b = {}^1/_2$, and a remains at unity. The gross features of these profiles are very similar to those for elution. Also in common with the elution case, the tail will hold up longer when the desorption rate parameter, a, is small, and contain more material when the sorption rate parameter, b, is large. The only significant difference between elution and nonelution cases lies in the termination of the tail; the elution tail diminishes gradually to zero, while the nonelution tail (for the parameters used here) is cut off rather sharply at the origin of migration. In paper chromatography a very weak spot may be left at the origin. This is presumably due to adsorption taking place before development is started, a case not covered in the equations given here.

CONCLUSIONS

Tailing can originate as a kinetic effect even with a linear isotherm. It is not clear how often this occurs. One of the objects of the present work is to define the nature of the kinetic effect so that it can be more readily identified in experimental work. Two main points distinguish the kinetic effect from other effects, particularly nonlinear ones:

Nonlinear tailing will decrease as the sample size is lowered, whereas kinetic tailing will be little affected by such changes.

Kinetic tailing will be especially prominent at high velocities. If one takes two zones of equal width at half height, one at a selected high velocity where rate effects dominate and another at a low velocity where longitudinal diffusion effects are of equal magnitude, the high-velocity zone will show more tailing. Nonlinear tailing will show little difference.

ACKNOWLEDGMENT

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Fluorine-Containing Polymers as Solid Supports in Gas Chromatography

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▶ Certain fluorine-containing polymers have been critically evaluated as solid supports for gas chromatography. The effects of flow rate, temperature, sample size, type of polymer, and both kind and concentration of liquid phase were studied to establish conditions for optimum separations. Columns with fluorine-containing polymer supports can be made almost as efficient as typical diatomaceous earth columns. In addition, superior peak symmetry can often be obtained for highly polar compounds by using fluorocarbon and chlorofluorocarbon supports. Columns made with fluorine-containing polymer supports often show nonlinear specific retention volume *vs.* per cent liquid substrate plots. This phenomenon can be explained by the spreading behavior of substrate liquid on the support. Differences in spreading are only encountered when the surface energy of the liquid is higher than that of the surface.

WHEN ANALYZING AQUEOUS SAMPLES or other highly polar materials by gas chromatography, it is often advantageous to use fluorine-containing polymers for the column support (*1–4, 6, 17*). Columns made with these materials generally produce symmetrical chromatographic peaks for highly polar compounds, whereas tailing peaks often occur if diatomaceous earth supports are used. While various applications of fluorocarbon and chlorofluorocarbon supports have been mentioned in the literature, no critical study of these materials as gas chromatographic supports has been reported. There has also been considerable discrepancy in the literature regarding the general utility of various types of fluorocarbons (*4, 17*), and often essential technique details have not been included.

After this investigation was concluded, evaluation of a porous Teflon was reported by Staszewski and Janak (*16*). Some of the physical characteristics ascribed to this support are strikingly different from that of Teflon-6 (E. I. du Pont de Nemours & Co., registered trademark for TFE fluorocarbon resin), said to be the source material; however, some of the conclusions regarding the use of this support agree with certain results obtained during the study reported herein. No details of the preparation of porous Teflon columns were given. More recently, Landault and Guiochon (*12*) have also described some of the gas chromatographic characteristics of Teflon-6; details of column preparation were included.

In this paper, an attempt is made to compare critically the gas chromatographic characteristics of the major types of fluorocarbon and chlorofluorocarbon supports that have been previously mentioned in the literature. Particular emphasis is placed on the experimental techniques necessary to obtain optimum performance from each of these materials. Factors investigated during this work include types of support, effect of amount of liquid phase on column efficiency and specific retention volumes, effects of flow rate and temperature, type of liquid phase, and type and amount of sample. An attempt is made to explain some of the unusual nonlinear specific retention volume phenomena often associated with the use of fluorine-containing polymer supports.

EXPERIMENTAL

Apparatus. An F & M Scientific Corp. Model 300 gas chromatograph was employed for all measurements. Helium carrier gas was purified by passage through a type 5A Molecular Sieve trap.

Liquid Phases. Squalane, Carbowax 400 and diglycerol were selected as liquid phases to represent a range of types. Squalane is a nonpolar liquid, Carbowax is of intermediate polarity, and diglycerol is a highly polar material.

Test Sample. The test mixture used throughout this study was composed of acetone (13.6% by weight), ethyl acetate (30.9%), water (22.9%) and *n*-butanol (32.6%). These solutes illustrate a variety of polar types. Unless otherwise indicated, 1 μl. of the test mixture was injected into the chromatograph with a 10-μl. Hamilton microsyringe.

Supports. The supports studied during this work are listed in Table I.

Fluoropak-80 was obtained from The Fluorocarbon Co., Fullerton, Calif. This granular type fluorocarbon resin has a sponge-like structure of relatively low surface area. Particles are somewhat rod-like, irregular, do not easily aggregate, and have only a slight tendency to develop troublesome static charges during handling.

Full-range Teflon-6 powder, obtained from the Plastics Department, E. I. du Pont de Nemours & Co., Wilmington, Del., was used as received. Sized Teflon-6 (40 to 60 mesh) was obtained by carefully sieving full-range material, as described below. Teflon-6 is composed of fragile aggregates that tend to develop static charges easily. This polymer contains a significant number of minute pores which contribute to its relatively high surface area.

The chlorofluorocarbon used in this study was a 50- to 80-mesh fraction sized from type 3081 Kel-F granular molding powder, obtained from Minnesota Mining & Manufacturing Co., Jersey City, N. J. The fairly uniform Kel-F particles appear to be aggregates of smaller units. The granules are physically rather hard, and do not tend to aggregate easily. This material can be handled similarly to diatomaceous earth supports during sizing and column preparation.

Columns. The columns used were U-shaped, $^1/_4$-inch o.d., $^3/_{16}$-inch i.d. stainless steel tubing, 1 meter long. Kel-F and Fluoropak-80 were coated using standard solvent evaporation procedures. Dichloromethane was used as a solvent for squalane and Carbowax 400, and hot methanol for diglycerol. The solvents were removed by careful heating on a steam bath, with gentle mixing, until the mixtures were dry. Columns of these supports were packed with vibration. The amount of liquid

Table I. Fluorine-Containing Polymer Supports

Type	Designation	Surface area, m²/g.[a]
Kel-F	3081 Powder, full-range	2.2
Fluoropak-80	Full-range	1.3
Teflon-6	Lot #80185, full-range	10.9
Sized Teflon-6	40–60 mesh	10.5

[a] Nitrogen flow technique (*14*); a sample of Teflon-6 carefully characterized by conventional B.E.T. measurements was used as a standard. The surface area of another sample of Teflon-6 has been reported as 11.7 m²/g. (*8*).

substrates on the support was within 1%, relative to the indicated values.

The fragile nature of Teflon-6 type supports requires that special techniques be used to prepare satisfactory column packing. Teflon-6 powder, as received, is sometimes quite lumpy and unsuited for column preparation. This difficulty is overcome by placing the material in a round ice cream-type cardboard container, chilling the contents to about 0° C., and vigorously shaking. This treatment generally puts the powder in a free flowing, non-aggregated state. Teflon-6 can be screened satisfactorily by first chilling to 0° C.; temperatures lower than this should usually be avoided, since pickup of water is sometimes excessive.

The recommended procedure for packing columns with Teflon-6 type support is as follows. Dissolve the liquid phase in a highly volatile solvent such as acetone, ether, or dichloromethane, and add the solution to the support contained in a shallow evaporating dish. Remove the solvent carefully without the use of heat. A satisfactory technique is to continuously turn over portions of the mixture gently with a spatula while a stream of dry nitrogen is rapidly flowing across the dish. Evaporation of the solvent must be carried out slowly. The evaporative cooling of the volatile solvent should maintain the desired temperature. If this coating operation is not properly carried out, rod-like aggregate particles sometimes are formed. If the coated packing appears lumpy, it should be carefully screened after chilling.

To prepare the chromatographic column, cool the packing to about 0° C. Close the lower end of the column tubing with a small plug of glass wool. Slowly add the chilled packing material through a glass funnel, while vibrating the column by holding it on the half-flattened shaft of a stirring motor. The vibration action is sufficient to ensure a homogeneous packing if the packing material is kept cool during the filling process. Additional tapping of the column is usually undesirable, since the packing may tend to aggregate into "plugs" which may channel the flow of gas. After completely filling the column in this manner, close the open end with a small glass wool plug. A 1-meter column of 20% liquid on Teflon-6 should contain approximately 11 to 12 grams of packing. Such a column requires an input pressure of 4 p.s.i. helium to produce a flow rate of 50 to 60 cc. per minute at column outlet. All columns were conditioned at the operating temperature, with carrier gas flowing, for several hours before use.

The improved handling characteristics of cooled Teflon-6 packing should be emphasized. Presumably, the reason for this is that Teflon has a transition point at about 19° C. The lower temperature state is significantly harder and has much less tendency to aggregate.

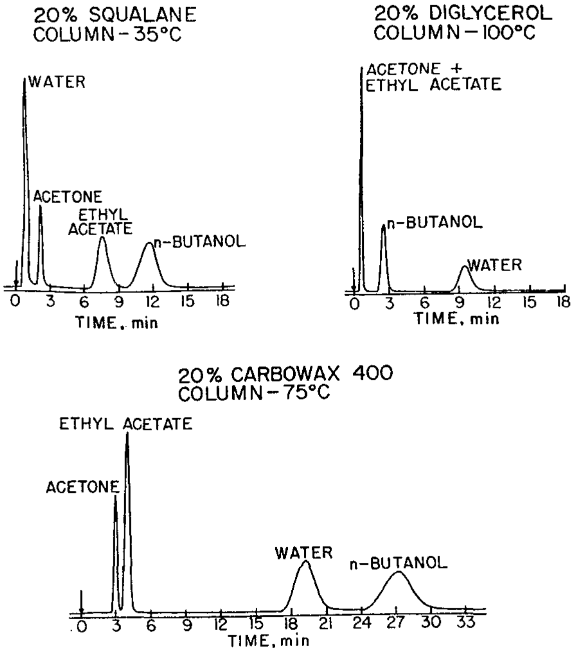


Figure 1. Separation of test mixture with various liquid phases

Support: full-range Teflon-6
Carrier flow: 50 cc./min.

Because Teflon-6 is a spongy, fragile material, it was initially feared that columns made with this support might have serious channeling problems, which would result in very poor column performance. However, Kieselbach of Du Pont has recently prepared a column with Teflon-6 support according to the techniques described in this paper, and has obtained a symmetrical peak for air using high speed equipment and specialized techniques which he has developed for this purpose (*11*). This, together with the data presented herein, indicates that properly prepared columns with Teflon-6 supports are not subject to serious channeling.

Technique. All data were obtained with the mixture of the solutes, unless otherwise noted. Retention times reported were reproducible to about 2% or better in most cases. Specific retention volumes were corrected for column "dead" space, temperature, and column pressure drop, and are reported as corrected retention volume cubic centimeters per gram of coated support.

RESULTS AND DISCUSSION

Effect of Amount and Type of Liquid Phase on Retention Volume. To study the effect of fluorine-containing column supports on specific retention volumes, a series of columns was prepared with varying amounts of each of the three liquid substrates. Figure 1 shows chromatograms of the test sample mixtures separated on columns made with full-range Teflon-6 support. As illustrated, the elution order of the test solutes is drastically dependent on the partitioning liquid. Water is eluted first when squalane is used as a substrate, and last with diglycerol. Carbowax 400 separates the various components in the order of boiling points.

Data obtained on gas chromatographic columns with diatomaceous earth supports show a linear relationship between specific retention volume and the amount of liquid substrate, excepting certain adsorption effects at very low loadings (*7*, *15*). This implies that the substrate in a heavily loaded column is just as effective, per unit weight of liquid, as is that on a lightly loaded column. This also suggests that the physical distribution of the liquid substrate is the same on a heavily loaded support as on a lightly loaded one. The situation is somewhat analogous to putting a few drops of water on a clean glass plate *vs.* putting a considerable number of drops on the glass. As long as there is room enough for each drop to spread to its maximum extent, the amount of liquid surface presented by each unit volume of water is the same, and does not depend upon how much of the glass was covered with water droplets.

Now consider what happens if most of the glass plate is covered with a wax film, but a few clean glass spots are left uncovered. If a few drops of water are put on the clean glass areas, they will still spread out and behave as they did on the completely clean glass plate. If more water drops are put on the glass than can be held on the few clean spots, however, the remainder must sit on the wax-covered areas. These drops will not spread out to give a high ratio of liquid surface per volume as before, but will sit as little balls on the waxed surface.

If, instead of water, drops of hexane are used, spreading on either the cleaned or waxed portions of the glass plate would be good in all areas.

This hypothetical experiment demonstrates an important difference in the spreading behavior of liquids on high energy *vs.* low energy surfaces. The clean glass represents a surface of

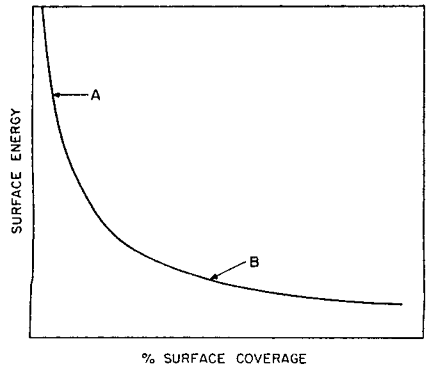


Figure 2. Surface energy plot for solids

relatively high energy, while the waxed glass represents a low energy surface. The experiment illustrates that spreading differences are only encountered when the surface energy of the liquid is higher than that of the solid.

The heterogeneous nature of the surface of solids is well known. A typical plot relating the surface energy of a solid as a function of the percentage of the surface covered, either by gas or liquid, is shown in Figure 2. As indicated, there is a significant portion of the solid surface which has much higher energy than that which is characteristic of the major portion—i.e., the homogeneous surface—of the solid, which has the surface energy value approached asymptotically at high coverage in Figure 2. This figure diagrammatically illustrates the heterogeneous character of the surface of solids, and is analogous to the wax coated glass plate just described. The heterogeneous energy profile of the solid surface can be equated to the glass plate which has been virtually covered with low surface energy, but still contains patches of clean glass surface of high surface energy.

With conventional diatomaceous earth column supports, this liquid spreading problem is seldom encountered, since siliceous surfaces usually have a much higher energy than the liquid substrates which are placed upon them (*9*). With fluorine-containing polymer supports, however, a situation analogous to the water on the glass plate can arise. The surface energy of these polymers is generally lower than most of the liquids commonly used as gas chromatographic liquids.

The low surface energy of fluorine-containing polymer supports produces some unique gas chromatographic effects. While diatomaceous earth supports demonstrate a linear relationship between specific retention volume and the amount of liquid phase, excepting certain adsorption effects at very low loadings (*15*), fluorine-containing polymer supports often show a disproportionate change in specific retention volume with increase in the amount of liquid phase. The spreading behavior of high surface energy liquids on low energy surfaces can be used to explain these phenomena.

Diglycerol, which has a very high surface energy, characterized by point *A* on the curve in Figure 2, shows a specific retention volume pattern on fluorine-containing polymer supports which is considerably different from that found for liquid substrates of lower surface energy. As can be seen by the diglycerol plot in Figure 3, the relative specific retention volume curve shows a rapidly decreasing slope up to about 2% loading. Above this point, the specific retention volume plot is linear. The diglycerol spreading phenomenon in this instance is analogous to the case where water droplets are placed on a glass plate which has been mostly covered with a wax film. Below about 2% loading, a significant fraction of the diglycerol apparently has been adsorbed on the support surface in the form of a film of relatively high partitioning coefficient, resulting from good wetting of the higher energy patches of the support surface. As illustrated by point *A* in the plot in Figure 2, there are apparently only a few sites on Kel-F with surface energy higher than that of diglycerol. Therefore, these sites are quickly covered with only 2% diglycerol. The spreading of diglycerol at these high energy sites gives a high ratio of liquid surface per unit of volume (as in the case of water dropped on a clean glass plate); hence, the liquid at these sites has a relatively high partitioning coefficient.

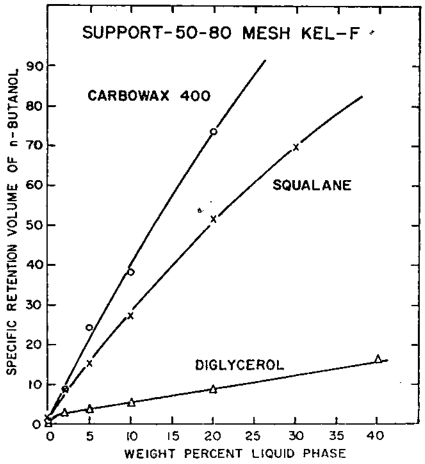


Figure 3. Effect of type of liquid phase on specific retention volumes

When more than about 2% diglycerol is placed on Kel-F, the liquid does not spread on the remaining support surface in the same manner, because of the large difference in the surface energies of the liquid and the support. (This is analogous to the waxed glass plate experiment with the Kel-F representing the waxed glass surface and diglycerol representing the water droplets.) The liquid deposited on this surface apparently has a lower partitioning coefficient than the liquid which was initially deposited at the sites of higher energy. Thus, the nonlinear portion of the specific retention volume curve shown in Figure 3 can probably be ascribed to the selective adsorption of most of the high surface energy diglycerol on the high energy sites of the support, so that at about 2% loading, these sites have been essentially covered. Since the remainder of the surface has an energy lower than that of diglycerol, additional liquid deposits homogeneously as nonspreading droplets, resulting in a surface which produces a linearly increasing specific retention volume as the amount of liquid is increased.

Differences in spreading due to surface energy effects are further magnified when Teflon is used as a support. Since the surface energy of this material is about 19 dynes per sq. cm., compared with about 31 dynes per sq. cm. for that of Kel-F (*18*), very few commonly used chromatographic substrates have a surface energy which is low enough to spread on Teflon. Materials such as squalane and Carbowax 400 might have a surface energy which would correspond to point *B* on the curve in Figure 2. As indicated on the plot, however, there are a considerable number of sites of high surface energy which are easily wetted by these substrates of lower energy. The films that have spread on these higher energy sites have a relatively high partitioning coefficient because of the high surface-to-volume ratio of the liquid. Since the

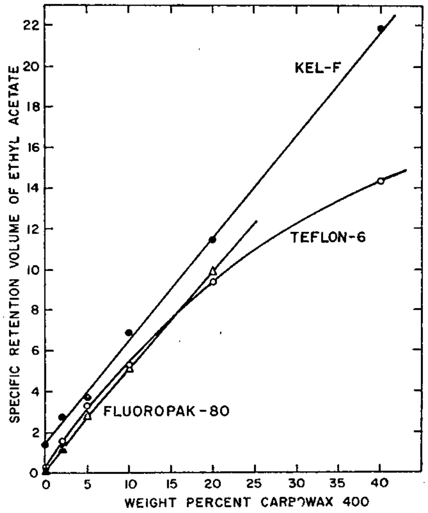


Figure 4. Effect of amount of liquid phase on specific retention volumes

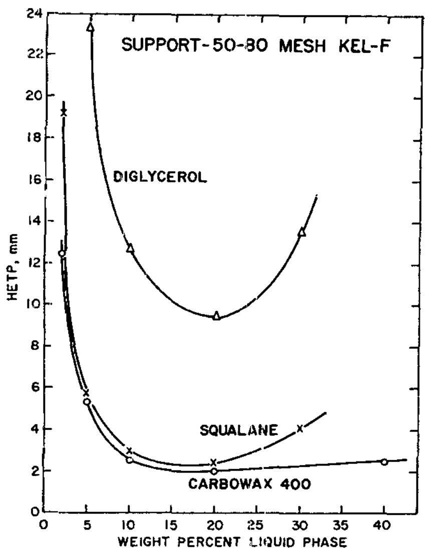


Figure 5. Effect of amount and type of liquid phase on column efficiency

Solute: *n*-butanol
Carrier flow: 50 cc./min.
Column temperature: 75° C.

surface energy of liquids represented by point *B* in Figure 2 is not much different from the surface energy of the homogeneous support surface, some liquid may also be simultaneously deposited on the homogeneous support surface. However, at these sites where the surface energy of the liquid is higher than that of the solid, the liquid does not spread efficiently, and the resulting liquid has a lower partitioning coefficient than at high surface energy sites of better spreading. A continuation of the coating process would, therefore, result in a decreasingly efficient partitioning surface as the weight ratio of substrate at sites of superior spreading decreases. Such a process results in a specific retention volume–weight per cent liquid curve of continuously decreasing slope, such as that of the Teflon-6 plot shown in Figure 4. Plots similar to the ethyl acetate curve in Figure 4 were also obtained for the other test solutes used in this study. Experimental points were determined with zero liquid phase for all solutes.

The ethyl acetate specific retention volume plot of Kel-F in Figure 4 is linear, indicating basic differences between fluorocarbon and chlorofluorocarbon in regard to the distribution of the Carbowax 400 liquid phase. The situation is analogous to the hypothetical experiment involving the wetting of both clean and wax covered areas of a glass plate with hexane. Carbowax 400 apparently spreads effectively on Kel-F, because the surface energy of the solid is higher than the surface energy of Carbowax 400. This is in contrast to the Teflon-6, the surface of which is mostly of a lower energy than Carbowax 400. It would seem that Kel-F has been wetted with a liquid of "constant partition coefficient" when ethyl acetate is used as the solute.

Kel-F with zero liquid phase demonstrates a small but significant retention of ethyl acetate. While Teflon shows negligible retention of the solutes in Figure 4, data presented in a later section indicate that some adsorption is apparent if the sample size is decreased sufficiently.

While a linear specific retention volume–weight per cent liquid phase relationship is found for ethyl acetate (Figure 4), data for *n*-butanol obtained simultaneously on the same column show a nonlinear relationship. It would, therefore, appear that the "effective" liquid film is different for the two solutes as they pass through the column. This could occur as the result of local changes in the spreading of the partitioning liquid as the solute bands pass through the column (*5*). It is likely that the passage of *n*-butanol, a compound of high surface energy, down a column might cause significant changes in the spreading of the low surface energy substrates, such as squalane.

Since the surface area of Fluoropak 80 is only about one eighth that of Teflon-6, it might be expected that the effects of spreading of liquids on these supports might be different. Fluoropak 80 undoubtedly has a significantly smaller number of surface sites of energy higher than that of a liquid such as Carbowax 400. It appears reasonable, therefore, that the change in the partitioning coefficient of any liquid film placed on Fluoropak 80 might be less in magnitude than that found for Teflon-6. This is supported by the Fluoropak 80 data shown in Figure 4, which show only a very slight nonlinearity.

Effect of Type and Amount of Liquid Phase on Column Efficiency. The efficiency of columns prepared with fluorine-containing polymer supports can also be significantly affected by the type of liquid used as substrate. The type of liquid phase is a much more critical parameter with these supports than it is with diatomaceous earths, since fluorine-containing polymers are not as readily wetted by most organic liquids. As can be seen in Figure 5, columns made with Carbowax 400 or squalane show much smaller optimum *HETP* values than those obtained from a diglycerol column. The steepness of the diglycerol curve beyond the minimum *HETP* suggests that as more liquid is added, increasingly thick pools are formed, making the column progressively more liquid diffusion–limiting. The higher viscosity of diglycerol could also partly account for the higher *HETP* obtained on columns with this liquid.

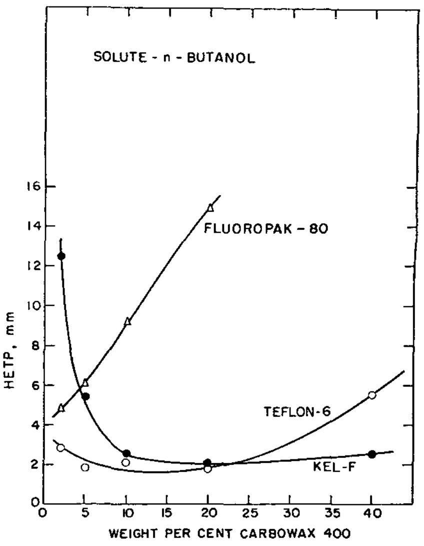


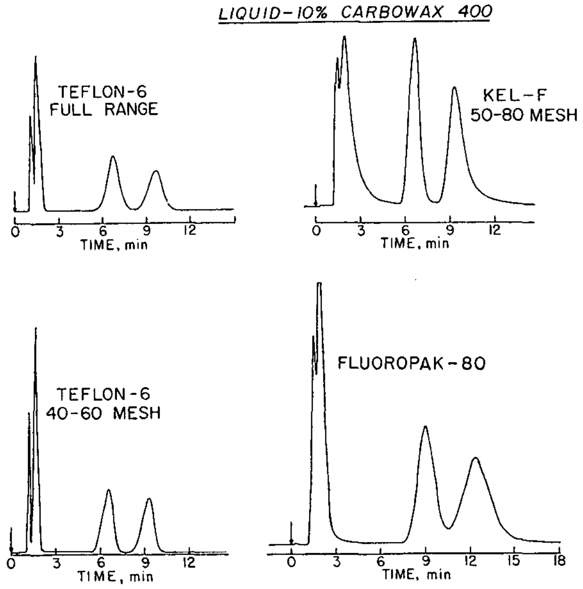
Figure 6. Effect of weight per cent liquid phase on column efficiency

Column temperature: 75° C.
Carrier flow rate: 50 cc./min.

The *HETP* curves for squalane and Carbowax 400 in Figure 5 are similar in shape to those which can be obtained with diatomaceous earth supports. The higher efficiency of the squalane and Carbowax 400 columns indicates better wetting of the support by these liquids than with diglycerol. Squalane and Carbowax 400 also show less increase in *HETP* at high loading than does diglycerol. This suggests that liquid mass transfer limitations of these two liquids are not greatly affecting column efficiency at the carrier gas flow rate used.

As shown by the data in Figures 5 and 6, optimum *HETP* values for Kel-F columns are produced when 15 to 20% by weight of liquid phase is used. Teflon-6 columns give optimum *HETP* when loaded with Carbowax 400 at the 20% level, as shown in Figure 6. Other liquids with similar surface energies probably will show a corresponding *HETP* pattern. However, use of liquid substrates with surface energies grossly different from Carbowax 400 may require a different loading to produce optimum *HETP*. The data in Figure 6 also indicate that optimum *HETP* values for Fluoropak 80 are obtained with very low loadings, perhaps 2% or less, presumably because of the relatively low surface area of this material.

Columns with Kel-F supports show a smaller rise in *HETP* when the amount of liquid loading is increased beyond the optimum than do columns prepared with Teflon-6. This effect, shown in Figure 6, cannot be explained by amount of available surface, since the surface area of Kel-F is only about $^1/_5$ that of Teflon-6. A reasonable



◀ **Figure 7. Effect of type of support on separation of test mixture**

Column temperature: 75° C.
Carrier flow: 50 cc./min.

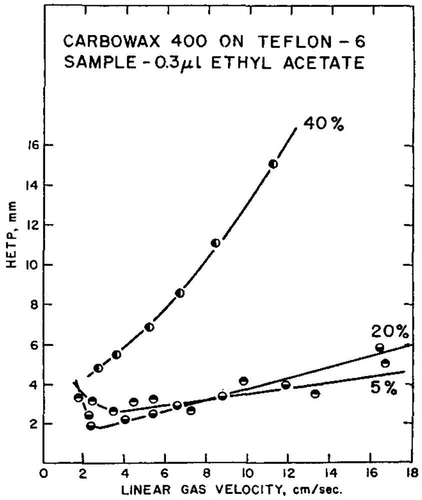


Figure 8. Effect of linear gas velocity on column efficiency at various liquid loadings

Column temperature: 75° C.
Full-range Teflon-6

explanation for the effect involves the superior spreading of the liquid on Kel-F.

A comparison of the practical effectiveness of the various supports studied is shown in Figure 7. Several useful conclusions can be drawn from these chromatograms. For instance, the sized Teflon-6 column is clearly superior to the column prepared from full-range Teflon-6, effectively demonstrating the desirability of using sized support. While the Kel-F separation in Figure 7 shows a poorer separation of acetone and ethyl acetate than that obtained with Teflon-6, the column efficiency for water and *n*-butanol is approximately the same. Tailing of some of the peaks in the Kel-F curve is also evident, suggesting small but significant adsorption of certain solutes by this support. Fluoropak-80 affords a much poorer separation of the test mixture than the other fluorocarbons. This may be due, in part, to the fact that the 10% loading used was probably not optimum for this support. The lower column efficiency could also be an effect of the poor packing homogeneity. Columns made with both fluorocarbon supports show minimal peak tailing effects in Figure 7, indicating insignificant adsorption of the test compounds.

Effect of Carrier Gas Velocity on Column Efficiency. The optimum carrier gas velocity for columns made with both full-range and sized Teflon-6 support is somewhat lower than is usually the case for comparable diatomaceous earth columns. The data in Figure 8 show that the smallest *HETP* for 5 and 20% Carbowax 400 columns is obtained with a helium carrier gas velocity of about 3 to 5 cm. per second. With 40% liquid loading, the column is strongly liquid diffusion–limiting. As indicated by the steep rise in the *HETP* curve with increasing carrier gas velocity, optimum performance is obtained at a very low flow rate.

The optimum efficiency of columns properly prepared from sized Teflon-6 compares favorably with the efficiency of columns made from conventional diatomaceous earth supports, as illustrated in Figure 9. The efficiency of the full-range Teflon-6 column is significantly poorer than that of the column prepared from sized material, emphasizing the importance of packing homogeneity. The *HETP* for *n*-butanol at the optimum carrier gas velocity for each of the support materials was found to be as follows:

Support	*HETP*, mm.
Fluoropak-80, full-range	3.6
Kel-F, 50–80-mesh	2.6
Teflon-6, full-range	2.3
Teflon-6, 40–60-mesh	1.7
Chromosorb W, 80–100-mesh	1.1

The data in Figure 9 show that Kel-F might be the preferred fluorocarbon support, should very high flow rates need to be used. Similar data were obtained using water as the solute.

Effect of Sample Size. The effect that sample size has on the efficiency of fluorine-containing polymer columns can be significantly influenced by the type of liquid employed. As shown in Figure 10, a sharp increase in *HETP* occurs as polar solutes of progressively less than about 100 µg. are chromatographed on a nonpolar column of 20% squalane on Teflon-6. This is undoubtedly due to adsorption by the support, which produces a nonlinear isotherm and peak tailing, the greatest relative effect being apparent with the smallest sample. The increasing *HETP* values for samples larger than about 100 µg. probably can be attributed to the progressively more diffuse sample plug placed on the inlet of the column, and/or the increasing tendency to overload the packing.

The absence of adsorption effects for the Carbowax 400 data in Figure 10 indicates that active adsorption sites have been effectively eliminated by the addition of this substrate. The significantly larger retention volume (higher partition coefficient) of *n*-butanol probably accounts for the very small increase in *HETP* as larger amounts of this solute are chromatographed. Adverse equipment geometry effects, such as unwanted diffusion effects in the vaporizer and detector and

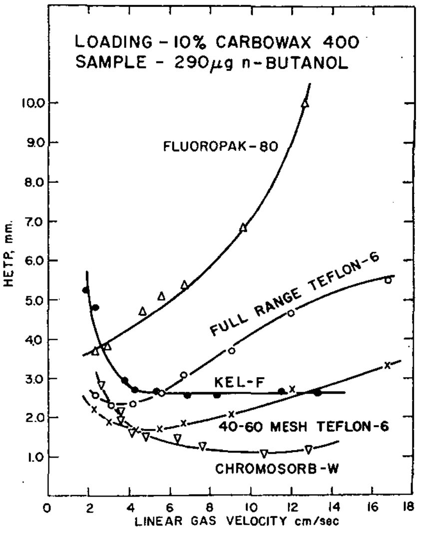


Figure 9. Effect of linear gas velocity on column efficiency

Column temperature: 75° C.

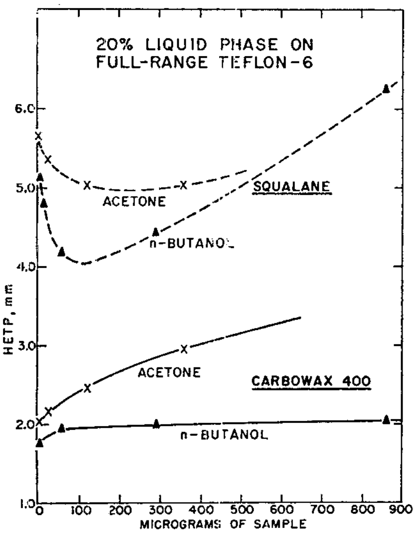


Figure 10. Effect of sample size on column efficiency

Squalane column temperature: 35° C.
Carbowax 400 column temperature: 75° C.
Carrier flow: 50 cc./min.

the tendency to overload the column, are of less significance for *n*-butanol than for a compound of low partition coefficient, such as acetone. However, these effects probably account for the steeper slope of the acetone curve.

The effect of sample size on specific retention volumes also correlates with the effects on column efficiency just discussed. When 1 to 2 μg. of acetone are chromatographed in a squalane–Kel-F column, a 10% increase in the specific retention volumes is noted over that found for sample sizes larger than about 100 μg. With the Carbowax 400–Kel-F column, however, less than 5% increase in specific retention volume is exhibited when very small samples are chromatographed. This again demonstrates the reduced adsorption effects which take place when polar partitioning liquids are used.

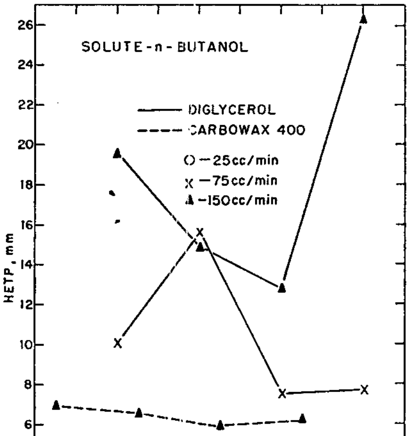


Figure 11. Effect of temperature on column efficiency

Support: full-range Teflon-6

While Teflon-6 is not a completely inert support, it is one of the least adsorptive materials now available. The somewhat larger adsorptive effects demonstrated by Kel-F powder are believed to be due, at least in part, to surface carboxylic acid groups. This is supported by the observation that methylene blue indicator is readily adsorbed to Kel-F powder from a dilute *n*-propanol solution. Preliminary tests have indicated that the adsorption effects of Kel-F can sometimes be reduced by treatment with alcoholic potassium hydroxide.

Effect of Temperature on Column Efficiency. Some interesting effects on column efficiency occur as the temperature is varied on Teflon-6 columns prepared with 20% by weight of the different test liquid phases. As seen in Figure 11, *HETP* data obtained on the Carbowax 400 column at a fixed carrier gas flow rate show very little variation throughout the temperature range studied. Very similar results were also obtained with a 20% squalane column throughout the temperature range of 35° to 90° C. However, the diglycerol column presents a different picture. At very low flow rates, temperature change has a minor effect; the efficiency of a 20% diglycerol column approaches that shown by the other phases tested. When the flow rate is increased, however, the *HETP* of the column drastically increases, and large changes in column efficiency take place as the column temperature is varied.

Table II. Optimum Operation of Supports

Type	Optimum liquid phase, Wt. %	Optimum carrier linear gas velocity, cm./sec.
Teflon-6	15–20	3–5
Fluoropak-80	2–5	ca. 2
Kel-F	15–20	7–15

The data in Figure 11 again indicate that Carbowax 400 and squalane are dispersed on Teflon-6 in thinner, more uniform partitioning films than is diglycerol. This liquid probably is dispersed on the polymer in relatively large droplets or deep pools, because of poor wetting of the support. As a result of this, the efficiency of the diglycerol column at the higher flow rates is drastically limited by mass transfer in the liquid. The decrease in *HETP* at intermediate temperatures is probably due to the improved wetting of the Teflon surface by diglycerol as the viscosity is decreased. The rapid rise in *HETP* of the upper curve may be explained by a combination of the low partition coefficient of the liquid as a result of the higher temperature, together with increasingly poor liquid mass transfer characteristics as the flow rate is increased.

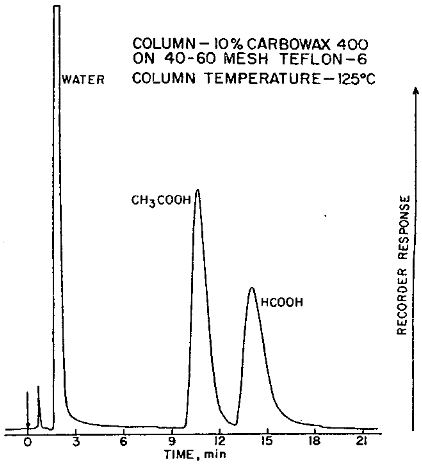


Figure 12. Separation of organic acids in aqueous solution

Carrier flow: 50 cc./min.

Conditions for Optimum Column Operation. Conditions for optimum column operation of the various fluorine-containing polymer supports have been concluded from the data obtained during this study, and are summarized in Table II. These conditions have been generalized somewhat; however, they have proved to be a useful guide. Different optimum conditions may be found for particular separations.

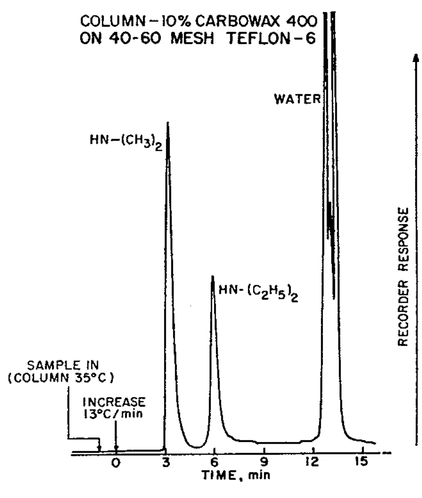


Figure 13. Separation of alkylamines in water

Carrier flow: 50 cc./min.

APPLICATIONS

To test the applicability of the techniques developed during this study, model separations of highly polar compounds of different chemical types were devised. Figure 12 shows the separation of an equal mixture of water, acetic acid, and formic acid obtained on a column of 10% Carbowax 400 on 40- to 60-mesh Teflon-6. Good peak symmetry was obtained without the use of acidic agents in the substrate to overcome dimerization of solute acids (*10, 13*). Approximately 500 theoretical plates are shown for both the acetic and formic acid peaks. Almost 1000 theoretical plates per meter have been obtained with Teflon-6 columns in more favorable separations.

The versatility of the Carbowax-400–Teflon-6 column is shown in the separation of a dilute aqueous mixture of highly basic compounds, dimethylamine and diethylamine, as illustrated in Figure 13. This is a programmed temperature separation with the column held at 35° C. for sample injection, followed by a temperature increase of 13° C. per minute.

In addition to the obvious advantages of using fluorine-containing polymer supports for the analysis of highly polar compounds, it is sometimes desirable to use these materials for the analysis of certain compounds which are catalytically decomposed by diatomaceous earth supports.

Fluorine-containing polymer supports can also be effectively used with high boiling samples. Temperature limitations are approximately 160° C. for Kel-F and about 275° C. for Teflon, although some minor changes in the characteristics of Teflon-6 columns have been said to take place at high temperatures (*12*).

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Contribution of Interfacial Resistance to Band Broadening in Chromatography

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▼ The contribution of the resistance to mass transfer at the interface to the plate height has been evaluated for the four basic forms of chromatography. It has been shown that, with the exception of liquid-liquid chromatography, this effect would be negligible in comparison with the other band-broadening mechanisms in most of the situations normally encountered in practice.

The proposal originally put forward by Khan (*18, 19*) of a significant contribution to band broadening in gas chromatography arising from a resistance to mass transfer across the phase interface has led to further theoretical and experimental studies of the mechanism of this process. Experimental investigations of the phenomena taking place at the gas liquid interface have been contradictory. Hazeldean and Scott (*14*) have, for instance, concluded that the contribution to the plate height is negligible in gas liquid chromatography in comparison with other band-broadening mechanisms, whereas Martin (*21*) has contended that adsorption of the solute on the surface of the stationary liquid phase can be significant. This would suggest that under certain circumstances interfacial resistance is an important cause of peak spreading. In view of the difficulty of clearly distinguishing by experimental means alone, a possible interfacial mechanism from other band-broadening processes, this lack of agreement is not surprising and one must conclude that—at least until the generally accepted band-broadening mechanisms are more perfectly understood—the contribution of an interfacial band-broadening mechanism can best be assessed on theoretical grounds.

Three theoretical expressions have been proposed. According to Khan (*18*) the contribution to the plate height of a resistance to mass transfer at the gas liquid interface is

$$H_{ir}^{gl} = \frac{2k'd_f u}{(1 + k')^2 k_d} \qquad (1)$$

A more general expression has been derived by Giddings (*10*)

$$H_{ir}^{gl+lp} = 2X_g u \left[\frac{(1 - X_s)^2}{X_s k_{sg}} + \frac{(X_b + X_p)^2}{X_s k_{sb}} + \frac{X_p}{k_{pb}}\right] \qquad (2)$$

in which both gas liquid and liquid solid interfaces are taken into account. It is shown below that these two equations are equivalent under similar circumstances. Finally, for a gas solid interface (*10*)

$$H_{ir}^{gp} = \frac{2k'u}{(1 + k')^2 k_{pg}} \qquad (3)$$

The main drawback of these expressions is that actual values of the parameters involved are not easily obtainable for practical chromatographic systems, so that H_{ir} cannot be estimated in such cases. In the present paper an attempt has been made to remove this difficulty and hence to estimate H_{ir} for each of the four basic types of chromatography.

GAS SOLID CHROMATOGRAPHY

Since (*2, 11*)

$$(k_{pg})^{-1} = \tau = \tau_o e^{Q_a/RT} \tag{4}$$

and, according to Cremer (*6*),

$$k' = \frac{\beta S_c \tau}{\epsilon'} \sqrt{\frac{RT}{2\pi M}} \tag{5}$$

Equation 3 may be written as

$$H_{ir}{}^{gp} = \frac{2(k')^2\epsilon'}{(1 + k')^2\beta S_c} \sqrt{\frac{2\pi M}{RT}}\, u \tag{6}$$

From Equation 6 it is clear that $H_{ir}{}^{gp}$ increases as the surface area of the adsorbent decreases. S_c may be written as $S_o\ (1-\epsilon')$, where, for spherical particles with a smooth surface, $S_o = 6/d_p$ (*4*). The maximum value of $H_{ir}{}^{gp}$ is therefore given by

$$H_{ir}{}^{gp} = \frac{(k')^2 d_p\, \epsilon'}{\beta(1 + k')^2\, 3(1 - \epsilon')} \sqrt{\frac{2\pi M}{RT}}\, u \tag{7}$$

The relative importance of $H_{ir}{}^{gp}$ may be gauged by comparing it with other terms in the total plate height expression for gas solid chromatography, the most suitable being one which also includes the particle diameter. There are a number of such expressions; of these, that for the interparticle resistance to mass transfer would provide the best basis for comparison with $H_{ir}{}^{gp}$ since this mechanism is always present, and it is one of the smallest causes of band broadening. Although several expressions have been proposed to describe this mechanism, they all predict approximately the same contribution to the plate height (*1*). Consequently the simplest expression

$$H_{rm} = \frac{0.01\ (k')^2\, d_p{}^2\, u}{(1 + k')^2 D_g} \tag{8}$$

proposed by van Deemter (*7*) would serve the present purpose.

From Equations 7 and 8

$$\frac{H_{ir}{}^{gp}}{H_{rm}} = \frac{33.3\epsilon' D_g}{\beta(1 - \epsilon')d_p} \sqrt{\frac{2\pi M}{RT}} \tag{9}$$

Representative values of the quantities appearing in Equation 9 are: $D_g = 10^{-1}$ sq. cm. per second, $d_p = 2 \times 10^{-2}$ cm., $T = 273°$ A., $M = 50$, $\epsilon' = 0.4$. Ascribing the maximum value of unity to β, it is found that the ratio in Equation 9 is $\simeq 0.01$. Although this parameter may vary over a wide range, under normal circumstances the contribution of $H_{ir}{}^{gp}$ to the total plate height would be expected to be negligible in comparison with the other band-broadening processes.

LIQUID SOLID CHROMATOGRAPHY

It follows from Equation 2 and an expression similar to Equation 4 for k_{pb} that the contribution of interfacial resistance to band broadening at the liquid solid interface may be written as (*10*)

$$H_{ir}{}^{lp} = \frac{2k'}{(1 + k')^2}\, u\tau_o e^{Q_a/RT} \tag{10}$$

where X_g has been interpreted as the mole fraction of the solute in the mobile (liquid) phase. Unfortunately, a simple expression which is similar to Equation 5 relating k' to τ is extremely difficult to derive because of the complexity of the liquid structure. However, the relative importance of $H_{ir}{}^{lp}$ may be assessed by comparing it with the expression for H_{rm} proposed by van Deemter. From Equations 8 and 10 it is seen that

$$\frac{H_{ir}{}^{lp}}{H_{rm}} = \frac{200\ D_l\tau_o e^{Q_a/RT}}{k' d_p{}^2} \tag{11}$$

Representative values of the quantities in Equation 11 are: $D_l = 3 \times 10^{-5}$ sq. cm. per second, $d_p = 2 \times 10^{-2}$ cm., $\tau_o = 10^{-13}$ second, and $T = 300°$ A. Q_a rarely exceeds 13 kcal. per mole (*12*), and even if k' is taken as unity (which would probably be an underestimation for $Q_a = 13$ kcal. per mole) the ratio in Equation 11 is $\cong 0.004$. One may therefore conclude that $H_{ir}{}^{lp}$ would be important only under exceptional circumstances.

GAS LIQUID CHROMATOGRAPHY

There is experimental evidence (*9*) to show that if the supporting medium in gas liquid chromatography is properly prepared, and sufficient liquid loadings are employed, the amount of solute which is adsorbed on the surface of the support material is negligible. Consequently it may be reasonably assumed that in such cases $X_p \cong 0$, and hence Equation 2 reduces to

$$H_{ir}{}^{gl} = 2X_g u \left[\frac{(1 - X_g)^2}{X_s k_{sg}} + \frac{X_b{}^2}{X_s k_{sb}}\right] \tag{12}$$

where k_{sg} is the rate constant for desorption from the liquid interfacial region into the gas phase, and is given by an expression similar to Equation 4 which may be written as

$$(k_{sg})^{-1} = \tau_o e^{(E_e + P\Delta V/RT)} \tag{13}$$

To calculate E_e it is necessary to know the nature of the molecular interactions between the solute and its surroundings in the adsorbed and gas states. It is assumed that in the adsorbed state the only interactions which need be considered are those between neighboring molecules, and that the gas is ideal. The number of nearest neighbors of an adsorbed molecule will depend on whether it is adsorbed on top of the liquid surface or in the topmost liquid layer; both regions are considered as contributing to the interfacial region. It will be assumed that solute gas molecules either have sufficient energy to penetrate the topmost layer of the liquid phase or they are reflected back into the gas phase. The validity of this assumption, as well as the various expressions employed below to calculate the value of k_{sg} will be tested later by applying the model to the theoretical estimation of Ostwald coefficients. Now the number of nearest neighbors of a solute molecule on the surface, z', is usually $^3/_4$ of its neighbors in the bulk of the solution, z (*28*). One may calculate z if it is assumed that all the molecules are spherical, and that the solution is sufficiently dilute for each solute molecule to be entirely surrounded by solvent molecules. Under these conditions, if a molecule of radius r_i is completely surrounded by molecules of radius r_j,

$$z = \frac{4}{3} z' = \frac{4(r_i{}^2 + 2r_i r_j)}{r_j{}^2} \tag{14}$$

The intermolecular attraction energy of a pair of unlike molecules may be calculated by summing the van der Waals expressions (*17*)

$$E_K = \frac{2\mu_i{}^2\mu_j{}^2}{3(r_i + r_j)^6 kT} \tag{15}$$

$$E_D = \frac{2\mu_i{}^2\alpha_j}{(r_i + r_j)^6} \tag{16}$$

$$E_L = \frac{3\alpha_i\alpha_j\, I_i I_j}{2(r_i + r_j)^6(I_i + I_j)} = \frac{3\ \alpha_i\alpha_j\nu_i{}^0\nu_j{}^0 h}{2(r_i + r_j)^6(\nu_i{}^0 + \nu_j{}^0)} \tag{17}$$

for the Keesom, Debye, and London energies, respectively. These expressions yield an estimate of the total intermolecular energy—i.e., including both attractive and repulsive energies—only if an "effective" value of r is used. For this purpose a slightly modified form of the expression given by Clusius and Weigand is employed (*26*)

$$r = \left(\frac{M}{6.93\rho N_o}\right)^{1/3} \tag{18}$$

An expression due to Onsager (*31*),

$$\mu^2 = 4.96\ kTr^3 \times \left[\frac{(2\epsilon + n_D{}^2)(\epsilon + 2)}{3\epsilon(n_D{}^2 + 2)}\right]\left(\frac{\epsilon - 1}{\epsilon + 2} - \frac{n_D{}^2 - 1}{n_D{}^2 + 2}\right) = 6.846 \times 10^{-16} Tr^3 A \tag{19}$$

has been used to estimate the dipole moments in Equations 15 and 16. The radius, r, in Equation 19 is that defined by Equation 18. A is a function of ϵ and n_D only.

The polarizability is calculated from the Lorentz-Lorenz expression (31), using Equation 18, as

$$\alpha = 1.654\left(\frac{n_D^2 - 1}{n_D^2 + 2}\right)r^3 = 1.654\,Br^3 \quad (20)$$

where parameter B is a function of n_D only.

Values of I or $\nu°$ are not easily obtained. However, the value of I for most organic molecules lies between 200 and 250 kcal. per mole (17) and consequently an average value of I of 225 kcal. per mole ($\nu° = 2.41 \times 10^{15}$ second^{-1}) may be used without introducing undue error. From Equations 14 to 20, using this value of I, it is found that

$$\frac{E_e}{RT} = 49.23\left(A_1A_2 + A_2B_1 + A_1B_2 + \frac{1.45 \times 10^4 B_1B_2}{T}\right)\left[\frac{y^4(2 + y)}{(1 + y)^6}\right] \quad (21)$$

where y is given by r_1/r_2.

The parameter τ_o in Equation 13 is the reciprocal of the vibration frequency of the surface molecules (2) of the liquid. An expression has been derived (23) for this frequency in a condensed system of molecules obeying Mie's potential as

$$\frac{1}{\tau_o} = \frac{1}{2\,\pi a_e}\left(\frac{48E_o}{m_r}\right)^{1/2} \quad (22)$$

where the exponents of the intermolecular distance in the attractive and repulsive terms of Mie's potential have been taken as 6 and 12, respectively (23). The reduced mass of the vibrating system is given by

$$m_r = \frac{m_i m_j z'}{m_i + m_j z'} \quad (23)$$

when a molecule of mass m_i is vibrating with respect to z' molecules of mass m_j. For the present purpose E_o is the energy of interaction of a solvent molecule with nine nearest neighbors, and a_e may be taken as $2r_2$. From Equations 22 and 23, with an expression similar to Equation 21 for E_o, it follows that τ_o may be written as

$$\frac{1}{\tau_o} = \frac{1.61 \times 10^4}{(2r_2)M_2^{1/2}}(A_2^2T + 2A_2B_2T + 1.45 \times 10^4B_2^2)^{1/2} \quad (24)$$

The rate constant, k_{sg}, as given by Equation 13 can now be expressed in terms of easily determined parameters. E_e is calculated from Equation 21; $P\Delta V = RT$ per mole; τ_o is calculated from Equation 24. Making the relevant substitutions,

$$k_{sg} = \frac{5.92 \times 10^3}{(2r_2)M_2^{1/2}}(A_2^2T + 2A_2B_2T + 1.45 \times 10^4B_2^2)^{1/2}$$
$$\exp\left\{-49.23\left(A_1A_2 + A_1B_2 + A_2B_1 + \frac{1.45 \times 10^4B_1B_2}{T}\right)\left[\frac{y^4(2 + y)}{(1 + y)^6}\right]\right\} \quad (25)$$

The factor k_{sb} may be considered as the rate constant for diffusion away from the interfacial region into the bulk liquid phase. Diffusion in solutions may be treated by the theory of absolute reaction rates (13), which postulates an activated complex for diffusion situated between the initial and final equilibrium positions of the diffusing solute molecule. A solute molecule which diffuses from the bulk of a solution onto the surface loses the stabilization resulting from the greater number of neighbors which it had in the bulk, but the entropy of the system increases because of the vibration of a molecule perpendicular to the surface. The former effect is denoted by $\Delta E'$, the latter by $T\Delta S'$. If ΔF_b is the free energy for diffusion in the bulk liquid phase, it follows from the theory of absolute reaction rates that

$$k_{sb} = \frac{kT}{h}\,e^{-(\Delta F_b - \Delta E' + T\Delta S')/RT} \quad (26)$$

For diffusion in the bulk liquid phase the volume and entropy changes are negligible and, hence, from the well-known definition of ΔF_b, $\Delta F_b \cong \Delta E_b$, the activation energy for diffusion in the bulk liquid phase. Since a molecule has $^3/_4$ as many neighbors on the surface as in the bulk of the solution, $\Delta E' \cong {}^1/_4\Delta E_b$. Furthermore, it has been suggested that ΔE_b is a constant fraction, usually $^1/_3$, of the energy of vaporization per mole, ΔE_v. Equation 26 may then be written as

$$k_{sb} = \frac{kT}{h}\,e^{-(1/4\,\Delta E_v + T\Delta S')/RT} \quad (27)$$

ΔE_v can be calculated from Equation 21 by setting $y = 1$, $A_1 = A_2$, and $B_1 = B_2$. Thus,

$$\Delta E_v = 2.31RT \times \left(A_2^2 + 2A_2B_2 + \frac{1.45 \times 10^4B_2^2}{T}\right) \quad (28)$$

The entropy due to vibration perpendicular to the surface with a frequency ν_s can be calculated from statistical thermodynamics as (3)

$$\Delta S' = R\left[\frac{h\nu_s}{kT}\left(e^{h\nu_s/kT} - 1\right)^{-1} - \ln(1 - e^{-h\nu_s/kT})\right] \quad (29)$$

It is a matter of some conjecture whether the activated complex possesses this additional vibrational entropy or not. The barrier for diffusion into the bulk liquid will be higher if it does not, and since the minimum value of the rate constant is more important, it will be assumed that it does not. One may then calculate k_{sb} from Equations 27 to 29, ν_s, which is equal to $1/\tau_o$, being obtained from Equation 22. In this case, E_o will be given by the interaction between a solute molecule and its z' surrounding solvent molecules, and is described by an expression similar to Equation 21.

Table I. Physical Properties of PEG 400 and Silicone Oil at 25° C.

	PEG 400	Silicone oil
M	400	550
ρ, g./cc.	1.0	0.97
ϵ	11.40	4.00
n_D	1.465	1.405
$r \times 10^8$ cm.	4.58	5.14
A_2	1.179	0.320
B_2	0.276	0.245

In order to estimate the value of H_{ir}^{gl} given by Equation 12, it only remains to calculate the mole fractions of the solute in the different regions. To calculate X_s, it is assumed that a completely homogeneous solution of solute in solvent is formed. If the liquid forms a uniform film of thickness d_f on the supporting medium, it follows that

$$\frac{X_s}{X_b} = \frac{2r_2}{d_f} \quad (30)$$

Usually $2r_2 \ll d_f$, and consequently $X_s \ll X_b$. Under these conditions, $X_g \cong 1/(1 + k')$ and $X_b \cong k'/(1 + k')$. Substitution of Equation 30 into Equation 12 then yields

$$H_{ir}^{gl} = \frac{2k'}{(1 + k')^2}\left(\frac{1}{k_{sg}} + \frac{1}{k_{sb}}\right)\frac{d_f}{2r_2}\,u \quad (31)$$

where k_{sg} and k_{sb} are functions of parameters A and B which, for the more common molecules, may be calculated from tabulated values of ϵ and n_D. These data are, however, not generally available for the stationary phases commonly employed in chromatography. Consequently, approximate values of ϵ were determined for a representative polar and nonpolar stationary phase–viz., polyethylene glycol 400 (PEG 400) and silicone oil, respectively—by measuring the capacitance of a small cell filled with the liquid on an L-C bridge, and then obtaining the value of the dielectric constant from a calibration curve. Approximate values of n_D were determined with an Abbe refractometer using a white light source. The results, at 25° C., are given in Table I.

Values of the parameter $(2r_2)k_{sg}$, calculated from Equation 25 at 25° C., have been plotted as a function of y in Figures 1 and 2, using the data in Table I, for various values of A_1. B_1 has been chosen as 0.25 in both cases, this being a representative value for the solutes generally encountered in practice.

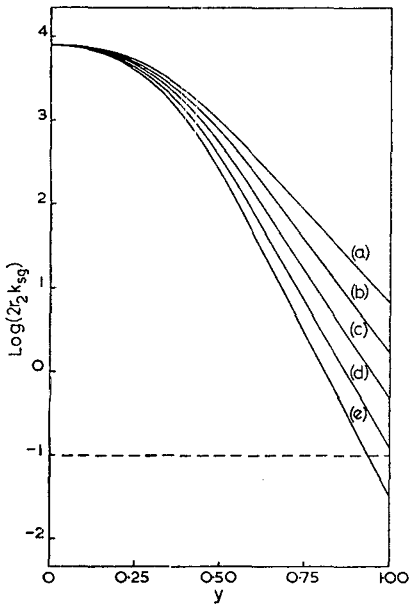

Figure 1. Variation of $(2r_2)k_{sg}$ with y at 25° C. for silicone oil

$B_1 = 0.25$
a. $A_1 = 0$
b. $A_1 = 1$
c. $A_1 = 2$
d. $A_1 = 3$
e. $A_1 = 4$

One may calculate k_{sb} from Equations 27, 28, and 29. From Equation 29, $\Delta S'$ increases with decreasing frequency of vibration, and hence, from Equation 22, with decreasing intermolecular interaction energy. An approximate lower limit of $(2r_2)k_{sb}$ was calculated by taking $A_1 = 0$, $B_1 = 0.25$, and $y = 0.25$, the value of v_s being determined by taking an average value of 100 for the mole weight of the sample. For PEG 400 and silicone oil, these values yield $(2r_2)k_{sb} = 500$ and 1000 cm. per second, respectively.

The relative importance of interfacial resistance as a mechanism giving rise to band spreading in gas liquid chromatographic columns can now be assessed by comparing the expression for H_{ir}^{gl} given by Equation 31 with that describing the resistance to mass transfer in the stationary liquid film, H_{rl}, which is also a function of d_f (*10*)

$$H_{rl} = \frac{2k'd_f^2u}{3(1 + k')^2D_l} \qquad (32)$$

From Equations 31 and 32,

$$\frac{H_{ir}^{gl}}{H_{rl}} = \frac{3D_l}{d_f}\left[\frac{1}{(2r_2)k_{sg}} + \frac{1}{(2r_2)k_{sb}}\right] \qquad (33)$$

Taking D_l as 3×10^{-5} sq. cm. per second and d_f as 10^{-3} cm., it follows from Equation 33 that $H_{ir}^{gl} \gtrsim H_{rl}$ when the factor in the brackets is $\gtrsim 10$ cm.$^{-1}$ second. From what has been said above it is clear that the contribution of $(2r_2)k_{sb}$ to H_{ir}^{gl} is negligible, and hence that desorption from the interfacial region into the gas phase is the rate-controlling mechanism in interfacial resistance for both polar and nonpolar stationary phases.

Figure 1 shows that for silicone oil $2r_2k_{sg} > 0.1$ cm. per second over almost the entire range of A_1 and y considered here, and it is to be expected that the effect of interfacial resistance is negligible in this case and, in general, whenever a nonpolar stationary phase is employed. Figure 2 shows that this conclusion does not hold for PEG 400—fairly large sections of the curves for $A_1 = 2$, 3, and 4 lie below the dashed line representing the limiting value of $(2r_2)k_{sg}$ for the values of the parameters chosen. Taking $B_1 = 0.25$ ($n_D = 1.41$), approximately the following combinations of ϵ and M/ρ yield $(2r_2)k_{sg} = 0.1$ cm. per second for PEG 400 at 25° C.: $\epsilon = 33$, $M/\rho = 120$; $\epsilon = 25$, $M/\rho = 170$; $\epsilon = 17$, $M/\rho = 230$; and $\epsilon = 9$, $M/\rho = 400$.

An investigation of tables of ϵ (*15*) reveals that none of the molecules with a dielectric constant less than 35 at 25° C. has these combinations of ϵ and M/ρ, although there are several borderline cases. It follows that $2r_2k_{sg}$ would always be greater than 0.1 cm. per second and consequently it is to be expected that $H_{ir}^{gl} \lesssim H_{rs}$ for the values of the parameters chosen. However, there are possibly solutes—e.g., water and glycerol—which have values of $\epsilon > 35$ at 25° C., for which the abovementioned conclusion might not hold. Such situations have not been included in Figures 1 and 2, since the theory given here would not be valid because the Onsager expression for the dipole moment, which determines A, no longer holds for associated liquids like these (*31*).

It is interesting to compare the present theory with that developed by Khan (*18*). From the above discussion it follows that for all practical purposes Equation 31 reduces to

$$H_{ir}^{gl} = \frac{2k' \quad d_fu}{(1 + k')^2(2r_2)k_{sg}} \qquad (34)$$

If Equation 34 is compared with Equation 1 it is seen that $k_d = (2r_2)k_{sg}$. The expression derived by Khan for k_d cannot be evaluated for the systems generally encountered in gas chromatography, whereas $(2r_2)k_{sg}$ can. However, Khan has calculated k_d for a hypothetical system consisting of chloroform as a stationary phase and acetone as a sample at 25° C. He found that $k_d = 0.002$ cm. per second. If $(2r_2)k_{sg}$ is calculated for the same system from Equation 25, its value at 25° C. is 0.49 cm. per second, which is considerably larger than Khan's value.

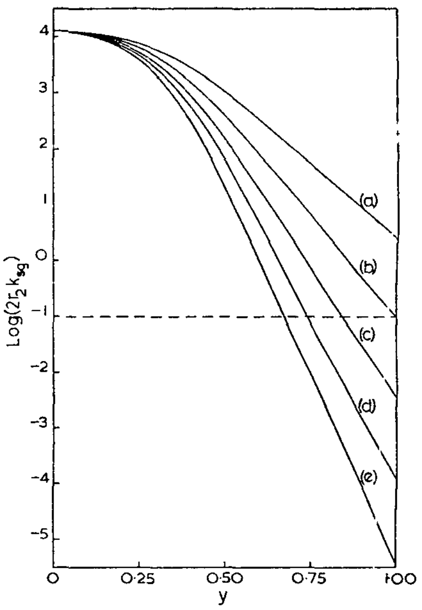

Figure 2. Variation of $(2r_2)k_{sg}$ with y at 25° C. for PEG 400

$B_1 = 0.25$
a. $A_1 = 0$
b. $A_1 = 1$
c. $A_1 = 2$
d. $A_1 = 3$
e. $A_1 = 4$

An indication of the reliability of the expressions derived in this paper can be obtained by using these expressions to estimate the Ostwald coefficients which have been accurately determined by experimental methods. The treatment is similar to that proposed by Moelwyn-Hughes (*27*).

Consider the solution of a gas in a liquid. The partial pressure of the gas, which is assumed to be ideal, is P_i. The gas molecules collide with the surface, and the number of collisions (cm.$^{-2}$ second^{-1}) is $c_{i_g}\bar{u}_i$, where, from the kinetic theory of gases,

$$\bar{u}_i = \sqrt{\frac{kT}{2\pi m_i}} \qquad (35)$$

To pass into the topmost liquid layer, a gas molecule must possess sufficient energy, say E_h, to force the liquid molecules apart, making a hole large enough in the surface to accommodate it. Molecules having energies less than E_h are assumed to be elastically reflected back into the gas phase. If $f(E_h/kT)$ is the probability that molecules possess an energy greater than or equal to E_h, the rate of condensation of gas molecules on the surface is, from the ideal gas law,

$$P_i(2\pi m_ikT)^{-1/2}f(E_h/kT) \qquad (36)$$

To evaporate into the gas phase, the condensed molecules must have sufficient energy to overcome the attractive forces of z' neighboring molecules; if this energy is denoted by E_e, the rate of evaporation is

$$c_{i_s}\, v_s\, f(E_e/kT) \qquad (37)$$

These two rates are equal at equilibrium. From Equation 30 it is clear that

$$c_{is} = 2r_j c_{ib} \qquad (38)$$

and from Equations 36, 37, and 38, at equilibrium,

$$\frac{P_i}{c_{ib}} = 2r_j\nu_e\, f(E_e/kT)\sqrt{2\pi m_i kT}/f(E_h/kT) \qquad (39)$$

Experimentally it is found that

$$P_i = K_H c_{ib} \qquad (40)$$

where K_H, the Henry's law constant, is a function of the temperature and the nature of the solute and solvent, but is usually independent of the composition of the solution and the pressure. Since the vapor is assumed to be ideal, $P_i = c_{ig}kT$ and Equation 40 may be written as

$$S = \frac{c_{ib}}{c_{ig}} = \frac{kT}{h} \qquad (41)$$

From Equations 39, 40, and 41,

$$S = f(E_h/kT)\sqrt{\frac{kT}{2\pi m_i}}\Big/ 2r_j\nu_e f(E_e/kT) \qquad (42)$$

S can be theoretically estimated if the probability factors can be evaluated. An approximate expression can be obtained by considering the system as analogous to one consisting of q feebly coupled oscillators. The total energy can then be expressed as the sum of $2q$ squared terms, and the probability that a molecule will have an energy of at least E_i is (*22*)

$$f(E_i/kT) = e^{-E_i/kT}(E_i/kT)^{q-1}/(q-1)! \qquad (43)$$

when $E_i \gg (q-1)kT$.

To evaluate Equation 43 for evaporation and condensation, E_e and E_h may be calculated from the model developed above. The value of q for evaporation can be obtained by assuming that the solute must possess the energy E_e in a direction perpendicular to the surface, and in a vibrational degree of freedom, which will be characterized by two squared terms for the energy. Consequently $q = 1$, and from Equation 43

$$f(E_e/kT) = e^{-E_e/kT} \qquad (44)$$

The corresponding expression can be obtained for condensation by assuming that the internal molecular motions of a molecule in the liquid and gas phase are the same. Consequently, to penetrate the topmost liquid layer, a gas molecule must obtain the energy from its three translational degrees of freedom—i.e., $q = {}^3/_2$ and $(q-1)! = {}^1/_2\sqrt{\pi}$. From Equation 43

$$f(E_h/kT) = 2e^{-E_h/kT}\sqrt{E_h/\pi kT} \qquad (45)$$

Table II. Comparison of Theoretical and Experimental Values of the Ostwald Coefficient

Liquid	Gas	$r_i \times 10^8$, cm.	$r_j \times 10^8$, cm.	$\alpha_j \times 10^{24}$, cc./mole	μ_j, Debye	$S_{\text{theor.}}$	$S_{\text{exptl.}}$	Ref.
Benzene	He	1.00	2.77	10.37	0	0.032	0.018	(*5*)
	Ne	1.17	(2.62)	(9.89)	...	0.025	0.026	(*5*)
	Ar	1.43	...	...	...	0.097	0.243	(*5*)
	N_2	1.50	...	...	...	0.079	0.108	(*5*)
	O_2	1.40	...	...	...	0.081	0.195	(*5*)
n-Hexane	He	1.00	3.15	11.84	0	0.071	0.045	(*5*)
	Ne	1.17	(2.78)	(11.48)	...	0.037	0.063	(*5*)
	Ar	1.43	...	...	...	0.051	0.494	(*5*)
Methanol	He	1.00	2.13	3.27	2.80	0.008	0.036	(*5*)
	N_2	1.50	(2.56)	(3.28)	...	0.034	0.165	(*20*)
	O_2	1.40	...	...	...	0.044	0.248	(*20*)
Acetone	N_2	1.50	2.60	6.38	3.50	0.114	0.182	(*20*)
	O_2	1.40	(3.12)	(6.13)	...	0.120	0.279	(*20*)

One can calculate ν_e from Equation 22, setting $E_o = E_e$ and $a_e = r_i + r_j$. From Equations 22, 42, 44, and 45

$$S = \sqrt{\frac{m_r E_h}{24 m_i E_e}}\,\frac{(r_i + r_j)}{r_j}\, e^{E_e - E_h/kT} \qquad (46)$$

This coefficient has been calculated for a series of simple polar and nonpolar substances at 25° C., and the values are given in Table II. The values of the various parameters were obtained as follows. E_h is calculated by multiplying the intermolecular energy of like molecules by z' obtained from Equation 14. The intermolecular energy may be taken to be ${}^1/_9$ of the latent heat of vaporization of the pure substance if this information is available. E_e was obtained from the van der Waals expressions using Equations 18 to 20 for the liquid molecule. The radii used for the inert gases are taken from the work of Uhlig (*32*), who also considered the energy required to form a hole in the surface of a liquid; van der Waals radii (*16*) were employed for oxygen and nitrogen. Tabulated values (*24*) of the polarizability and vibrational frequency, $\nu°$, of the gases have been used.

Table II shows that, in general, for polar, nonpolar, or even long-chain molecules, the model proposed predicts Ostwald coefficients to within a factor 5 of the accepted value. Of the systems studied only argon in *n*-hexane showed a greater divergence. The values of α included in parentheses were obtained by summing individual tabulated bond contributions to the total polarizability (*24*), and are almost identical to those calculated from Equation 20. The values of r_j in the parentheses were calculated by setting the latent heat of vaporization (*25*) equal to $9(E_K + E_D + E_L)$ and solving for r_j; the agreement with the predictions of Equation 18 is good, and if the value of 2.62×10^{-8} cm. is used for the radius of benzene, the values of S for He, Ne, Ar, N_2, and O_2 are 0.031, 0.027, 0.245, 0.204, and 0.193, respectively. Three of these are virtually identical to the experimentally determined value.

From these results it may be concluded that the estimated values of the contribution of interfacial resistance to band broadening in gas liquid chromatographic systems which have been given here are approximately correct. This would suggest that the theory developed by Khan overestimates the relative importance of this effect.

LIQUID-LIQUID CHROMATOGRAPHY

Theoretical and experimental work by Drickamer and his coworkers (*29, 30*) has shown that the rate of diffusion of a solute through a liquid-liquid interface can be as little as 10^{-6} of that in the bulk phases themselves. This would suggest that if a solute is injected into the mobile phase of liquid-liquid chromatographic system, an equilibrium distribution of the solute between the two phases is unlikely to be established. In fact, van Duin (*8*) has gone so far as to suggest that there is no effective penetration of the stationary phase, and that the process taking place is merely adsorption-desorption from the surface of the stationary liquid phase.

The high resistance to mass transfer across the interface in a liquid-liquid system may be theoretically explained in terms of the model presented in the previous section. Consider a solute molecule in the mobile phase of a liquid system. This molecule diffuses toward the liquid-liquid interface, and it may be shown (*13, 30*) that the ratio of the interfacial to bulk diffusion coefficients is given by

$$\frac{D_{if}}{D_{bs}} = e^{(\Delta E_{bs} - \Delta E_{if})/RT} \qquad (47)$$

if it is assumed that, to a first approximation, the molar volumes of the stationary and mobile phases are the same, and that the entropy and volume changes occurring on diffusion are negligible (*13*).

The ratio in Equation 47 may be calculated from a knowledge of the activation energies. ΔE_{b3} is approximately equal to $^1/_3$ of the latent heat of vaporization (*13*) and from Equation 28

$$\frac{\Delta E_{b3}}{RT} = 0.77\left(A_3^2 + 2A_3B_3 + \frac{1.45 \times 10^4 B_3^2}{T}\right) \quad (48)$$

The energy, ΔE_{if}, consists of three separate contributions. To penetrate the topmost layer of the stationary phase the solute molecule must not only have sufficient energy to free itself from the attractive potential field of $(^3/_4)z_3$ of the mobile phase molecules but must also be able to make a hole in the stationary liquid surface which is sufficiently large to accommodate it. Since in passing from one phase to the other a solute molecule is also under the attractive influence of approximately $(^1/_4)z_2$ molecules of the stationary liquid phase,

$$\Delta E_{if} = z_3'E_{13} + z_2'E_{22} - \frac{1}{3}z_2'E_{12} \quad (49)$$

The individual contributions to ΔE_{if} in Equation 49 follow directly from expressions similar to Equations 21 and 28 as

$$\frac{z_3'E_{13}}{RT} = 49.23\left(A_1A_3 + A_1B_3 + A_3B_1 + \frac{1.45 \times 10^4 B_1B_3}{T}\right)\left[\frac{(y')^4(2 + y')}{(1 + y')^6}\right] \quad (50)$$

$$\frac{z_2'E_{22}}{RT} = 0.77\left(A_2^2 + 2A_2B_2 + \frac{1.45 \times 10^4 B_2^2}{T}\right)y(y + 2) \quad (51)$$

and

$$\frac{z_2'E_{12}}{3RT} = 16.41\left(A_1A_2 + A_1B_2 + A_2B_1 + \frac{1.45 \times 10^4 B_1B_2}{T}\right)\left[\frac{y^4(2 + y)}{(1 + y)^6}\right] \quad (52)$$

where y' is given by r_1/r_3.

The large number of parameters appearing in Equations 49 to 52 make an estimation of the relative magnitude of the interfacial and bulk diffusion coefficients for a variety of substances rather tedious. For the present purpose we consider the simple situation where $y = y'$, $A_1 = A_2 = A_3$, and $B_1 = B_2 = B_3$. Representative values of y and B would be 0.5 and 0.25, respectively. Using these values, $T = 300°$ A., and A = 0, 1, 2, 3, and 4; the calculated values of D_{if}/D_{b3} are $\cong 0.14$, 0.06, 6×10^{-3}, 1.7×10^{-4}, and 1.4×10^{-6}, respectively. This is essentially in agreement with the results obtained by Sinfelt and Drickamer (*30*).

It may thus be concluded that in most liquid-liquid systems, particularly if the mobile phase is less polar than the stationary phase or the solute molecule is polar, $D_{if} \ll D_{b3}$, and hence that diffusion across the interface would be the rate-controlling mechanism. This would imply that under such circumstances an adsorption-desorption equilibrium is probably established at the surface, as has been suggested by van Duin (*8*).

To estimate the contribution of a surface adsorption-desorption process to the plate height one may use an expression similar to Equation 10. The relative importance of interfacial resistance may then be assessed by comparing it with the term describing the resistance to mass transfer in the mobile phase. The ratio of H_{ir}^{ll} to H_{rm} is clearly given by Equation 11 with H_{ir}^{lp} replaced by H_{ir}^{ll}.

The factor τ_o in this expression is given by Equation 24. The energy required by a solute molecule to desorb from the surface of the stationary phase is approximately equal to the energy needed to free itself from the attractive field of $(^1/_4)z_2$ molecules of the stationary phase plus that required to force $(^1/_4)z_3$ molecules of the mobile phase apart to make a hole large enough for the solute molecule to fit into. Since there is no volume change on desorption, it follows that

$$Q_a = E_e \cong \frac{1}{3}z_2'E_{12} + \frac{1}{3}z_3'E_{33} \quad (53)$$

In terms of the theory developed above one may calculate the first term in Equation 53 from Equation 52, whereas the second term is given by Equation 51 with subscript 2 replaced by 3 and y' substituted for y. Taking $D_l = 3 \times 10^{-5}$ sq. cm. per second, $d_p = 2 \times 10^{-2}$ cm., $k' = 1$, and $T = 300°$ A., and calculating τ_o and Q_a by assuming that $M_2 = 500$, $r_2 = 5 \times 10^{-8}$ cm., and $A_1 = A_2 = A_3 = 3$, $B_1 = B_2 = B_3 = 0.25$, and $y = y' = 1$—which can be regarded as extreme values—it is found that the ratio of H_{ir}^{ll} to H_{rm} in Equation 11 is $= 0.04$. It follows that when little effective penetration of the stationary phase takes place, the contribution of H_{ir}^{ll} to the total plate height in liquid-liquid chromatography is negligible in comparison with other band-broadening processes.

NOMENCLATURE

A = convenient parameter
a_e = equilibrium separation distance of vibrating system
B = convenient parameter
c_{ij} = concentration of solute i in region j
D = diffusion coefficient of solute
D_{if} = diffusion coefficient of solute through interface
d_f = thickness of stationary liquid film
d_p = particle diameter
E_D, E_K, E_L = Debye, Keesom, and London attraction energies
E_e = energy required by solute molecule to evaporate from liquid surface into mobile phase
E_{ij} = interaction energy between molecules i and j
E_h = energy required by solute gas molecule to penetrate surface of liquid
E_o = minimum potential energy of vibrating system
$\Delta E'$ = loss in energy of solute molecule on diffusing from bulk to surface of liquid
ΔE_b = energy for diffusion of solute in bulk liquid phase
ΔE_v = energy of vaporization of pure liquid
ΔF_b = free energy for diffusion of solute in bulk liquid phase
$f(E_i/kT)$ = probability that molecule possesses an energy $\geqslant E_i$
H_{ir} = contribution of interfacial resistance to plate height
H_{rl} = contribution of resistance to mass transfer in stationary liquid film to plate height
H_{rm} = contribution of interparticle resistance to mass transfer in mobile phase to plate height
h = Planck's constant
I = ionization potential
K_H = Henry's law constant
k = Boltzmann's constant
k' = ratio of mass of solute in stationary phase to mass in mobile phase at equilibrium
k_d = rate constant for desorption defined by Khan
k_{ij} = rate constant for desorption from ith into jth region or phase
M = molar weight
m = molecular weight
m_r = reduced mass of vibrating system
N_o = Avogadro's number
n_D = index of refraction for sodium D line
P = pressure
P_i = partial pressure of substance i
Q_a = amount of heat which must be added to system to desorb molecule
q = convenient parameter
R = gas constant
r = molecular radius
S = Ostwald coefficient
S_c = surface area of particles in column per unit column volume
S_o = surface area of particles in column per unit bed volume
$\Delta S'$ = gain in entropy of solute molecule on diffusing from bulk to surface of liquid
T = absolute temperature
u = carrier flow velocity

u_i = average value of component of velocity in given direction of ideal gas molecule of substance i
ΔV = volume change
X_i = mole fraction of solute in region i
y = ratio of radius of solute molecule to that of stationary phase molecule
y' = ratio of radius of solute molecule to that of mobile phase molecule
z = number of nearest neighbors of molecule in bulk liquid phase
z' = number of nearest neighbors of molecule in topmost layer of liquid
α = polarizability
β = fraction of molecules reflected from surface
ϵ = dielectric constant
ϵ' = interparticle porosity of column
μ = dipole moment
ν^0 = vibration frequency of zero-point motion
ν_s = vibration frequency of molecule perpendicular to surface
ρ = density
τ = time of adsorption
τ_o = convenient parameter
1, 2, 3 = subscripts denoting solute, stationary, and mobile phases, respectively
b, g, l, p, s = super- or subscripts denoting following regions or phases: bulk liquid, gas, liquid, packing material, liquid surface, respectively
i, j = convenient subscripts

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A Combustion—Gas Chromatographic Method for the Simultaneous Determination of Carbon and Sulfur in Ferrous Metals

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▶ A method is described in which the sample is combusted in a pressurized stream of oxygen with a high frequency induction furnace. The combustion products are swept onto a 4-foot silica gel column, which is then purged oxygen-free with helium, the combustion products eluted by temperature programming, detected by a thermal conductivity detector, and recorded on a strip chart recorder equipped with a Disc Chart Integrator. The analysis time, from sample firing through complete elution of the combustion products is 17 minutes. Carbon compositions analyzed were from 0.011 to 3.28%. Sulfur compositions ranged from 0.011 to 0.329%. The method could easily be adapted to a routine analysis with no change in accuracy or precision.

THE COMBUSTION of ferrous metals in an oxygen atmosphere and the subsequent analysis of the combustion products by various techniques has been reported for both carbon and sulfur (*1*, *4*, *8*, *10*). Recently, infrared spectrometry has been used for carbon determinations with results reproducible to 0.001% (*5*). In addition, the sulfur content of steels has been detected after combustion by neutron activation analysis (*2*) and by spectrophotometric means (*3*).

However, a simultaneous method for carbon and sulfur is the most desirable. Several methods have been reported for simultaneous carbon–sulfur analysis in steels involving titrations (*6*), but the instrumental approach appears to be preferred. The far ultraviolet region of the spectrum has been used to determine sulfur, phosphorus, carbon, and silicon. The mass spectrometer has also been used as a detector (*7*) and there is now an instrument commercially available using infrared means for detection.

Gas chromatography is an excellent means of detection and is ideal for this type of analysis. However, only recently have the techniques involved in elemental analysis of organic compounds been extended to the determination of carbon in ferrous metals (*11*). The proposed simultaneous method utilizes gas chromatographic separation of the combustion products and detection by a thermal conductivity detector.

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Table I. Results of Carbon–Sulfur Determinations

N.B.S. steel sample	C				S			
	N.B.S., %	Found, %	Std. dev.	Mean error, %	N.B.S., %	Found, %	Std. dev.	Mean error, %
82a	2.24	2.18	±0.02	−0.06	0.102	0.106	±0.005	+0.004
122d	3.28	3.17	±0.01	−0.11	0.092	0.084	±0.003	−0.008
16d	1.01	1.01	±0.01	0.00	0.033	0.039	±0.002	+0.006
129b	0.094	0.095	±0.002	+0.001	0.224	0.211	±0.012	−0.013
8i	0.077	0.074	±0.001	−0.003	0.064	0.060	±0.003	−0.004
10e	0.406	0.412	±0.005	+0.006	0.047	0.049	±0.001	+0.002
10g	0.240	0.245	±0.002	+0.005	0.109	0.014	±0.003	−0.005
55e	0.011	0.012	±0.001	+0.001	0.011	0.011	±0.001	0.000
170a	0.052	0.049	±0.002	−0.003	0.021	0.022	±0.002	+0.001
133a	0.120	0.127	±0.002	+0.007	0.329	0.346	±0.009	+0.017

EXPERIMENTAL

Apparatus and Materials. The system of Walker and Kuo (*11*) was employed with certain modifications (Figure 1). The quartz combustion tube and double O-ring seal described by Kuo, Bender, and Walker (*9*) allowed the samples to be combusted in a pressurized stream of oxygen. For this study, the brass components were rhodium plated. A stainless steel system was used from the quartz combustion tube through the silica gel column using $^1/_4$-inch tubing and Aminco stainless steel valves, Models 44-1585 and 45-4003. A few grams of W. A. Hammond Drierite Co. calcium sulfate was placed in the tubing immediately following the combustion chamber to prevent the possible adsorption of sulfur dioxide by moisture.

A 4-foot column was prepared using Matheson, Coleman and Bell chromatographic grade silica gel (60- to 80-mesh) and stainless steel tubing. A column oven was constructed of transcite with glass wool insulation, so that one side of the oven was mounted on hinges to allow easy access to the oven and to increase the cooling rate. The connections, including the blower and thermistor, were retained from an original F & M Scientific oven.

Procedure. A carefully timed procedure was followed for each determination. The helium two-stage regulator was first set for 16 p.s.i. and the flow rates of 60 ml. per minute on the sample side and 15 ml. per minute on the reference side checked. The recorder and integrator were turned on, the detector current set at 130 ma., and the system allowed 24 hours to equilibrize. Block temperature was 200° C. The oxygen regulator was then set for 16 p.s.i. This resulted in an oxygen flow rate of 67 ml. per minute. All crucibles were prefired for 15 minutes immediately before firing to ensure a carbon, sulfur-free blank. The prefiring was accomplished using 0.4 gram of 170a steel and 1.0 gram of tin accelerator. The prefired crucible was then charged with 0.500 ± 0.0005 gram of the steel sample and 1.0 gram of tin accelerator. After the charged crucible was reloaded into the furnace, V_1 was opened completely for 1 minute and the combustion tube purged. With the attenuator on the × 512 position, V_3 was closed simultaneously while V_2 was being opened. This allowed oxygen to pass through the column. The combustion was then initiated. After 7 minutes, the furnace was turned off and V_2 closed and V_3 opened simultaneously. After an additional 1 minute, 45 seconds, the oven door was closed and recorder chart drive started at 1 inch per minute.

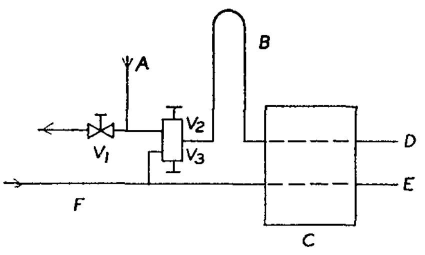

Figure 1. Schematic diagram of system

A. Oxygen plus oxidation products
B. Silica gel column
C. Detector block
D. Sample side
E. Reference side
F. Helium supply
V_1, V_2, V_3. Valves

For the most rapid and accurate determination, a special temperature program technique was employed which resulted in a nonlinear program. The oven temperature was initially 30° C. The indicator of the temperature programmer was set at 100° C. with a temperature setting of 42° per minute. The oven heater and the temperature programmer were activated simultaneously. The programming indicator begins increasing at 42° per minute. The oven temperature then increases at the maximum rate until the oven temperature and the programmer temperature coincide. From this temperature upward, the programming rate is 42° per minute. This technique gave a maximum increase in temperature up to 150° C. and resulted in the best carbon dioxide peak.

After both carbon dioxide and sulfur dioxide had been eluted from the column, the chart drive on the recorder and the temperature programmer were turned off. The indicator on the temperature controller was then set at 30° C. Approximately 15 minutes were allowed for the oven to cool from the maximum temperature of 350° C. and stabilize at 30° C. The periods during prefiring, column oven cooling, and sample combustion are more than adequate to carefully weigh all materials and record the results for each determination.

RESULTS

The results are shown in Table I. To correlate between samples of varying composition, a series of attenuation factors were evaluated, using the ×1 position as reference. The attenuator was first set on the ×1 position and the bridge set out of balance. An average value of counts per inch was found over a 5- to 10-minute period. The attenuator was then changed to the ×2 position and the average counts per inch again evaluated. The ratio of the counts per inch values on the two positions was the attenuation factor.

A program was written for a Royal Precision Electronic Computer LGP-30 to calculate results. A mean sensitivity of all samples was taken as a standard to calibrate the instrument and to calculate the carbon–sulfur percentage for each run. An average of five runs was taken as the per cent found. Standard deviations were computed for the five runs on each sample. The program allowed all results to be calculated in 15 minutes.

A combustion time of 7 minutes was found adequate for all samples except 122d, which contained the highest percentage of carbon. This sample was combusted 8 minutes. More erratic results were obtained with samples 122d and 82a compared to the other samples. Less uniform combustions are felt to be responsible.

CONCLUSION

The overall sensitivity showed 46,550 counts per mg. of carbon. One microgram of carbon would accordingly give a maximum deflection approximately 2% of full scale with the attenuator on the ×1 position. The apparatus showed a sulfur sensitivity of 20,710 counts per mg.

Since various compositions can be compensated by simply changing attenuations, the method is applicable to a wide range of concentrations. Also, no reagents or special techniques are necessary. The apparatus could easily be adapted, by means of solenoid valves and timers, so that only one master switch would be needed for the entire determination. The procedure would then consist only of loading the sample, activating the master switch, and reading the result. Use of calibration curves would result in an analysis requiring only about 5 minutes of a technician's time per run.

The time per analysis could be reduced by shortening the time required to elute the sulfur dioxide from the column. An oven capable of being programmed at a faster rate could conceivably reduce the total analysis time to 15 minutes or less.

ACKNOWLEDGMENT

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Carbon Determination in Ferrous Metals by Gas Chromatography

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▶ An extremely sensitive and highly precise method for the determination of carbon content in ferrous metals by gas chromatography was developed. Samples were combusted in a high frequency induction furnace, their gaseous products passed through a 4-foot 5-A. molecular sieve column, and detected by a thermoconductivity detector. The carbon dioxide was trapped in the column at 100° C. while oxygen was swept out by the helium carrier gas after complete combustion. The carbon dioxide peak came off the column at about 275° C. by means of temperature programming while its area was measured by a disk chart integrator. Eight different NBS steel and iron samples with carbon contents varying from 0.011 to 3.28% were run. This method permits detection of 0.0005% carbon at the maximum detector sensitivity. The time required for a single run is approximately 20 minutes. Some advantages of this technique are its simplicity of operation, broad detection range (0.0005 to 20% carbon), and high sensitivity.

THE PROCEDURE of carbon determination in ferrous metals has been well established. Many methods and their modifications have appeared in the literature (*1–3, 5, 11, 13*). They can be classified mainly as the following categories: the wet chemical method, the direct combustion method, the vacuum fusion (low-pressure combustion) method, the electroconductometric method, and the spectrographic and mass spectroscopic methods. The trend of development in this field is to seek a rapid and highly sensitive method which would enable one to detect not only very low carbon content, but also very high carbon content as well.

The gas chromatographic technique appears to be a step in the right direction. Although there has not been any published paper concerning the carbon determination in ferrous metals by gas chromatographic technique, papers were given at the 1962 and 1963 meetings of the Pittsburgh Conference of Analytical Chemistry and Applied Spectroscopy (*6, 8*). In addition, several workers did utilize this technique in the determination of carbon and hydrogen in organic compounds. Duswalt and Brandt (*4*) combusted their sample in an oxygen stream. Sundberg and Maresh (*12*) using copper oxide as internal oxidizing agent, burned their sample in a helium atmosphere. In both cases, the combusted gases were led through a liquid nitrogen trap and the carbon dioxide was trapped. Since silica gel columns were used in both cases, the liquid nitrogen trap is a necessity so as to get rid of the excess oxygen and to concentrate the gases for quick injection into the sample column. Recently, Nightingale and Walker (*9*) developed a simultaneous C–H–N determination using a gas chromatographic technique. They introduced the coupling of the high frequency induction furnace and the gas chromatograph. These workers made use of a 5 A. molecular sieve column and eliminated the troublesome liquid nitrogen trap. Still, an internal oxidizing agent, silver permanganate, was used instead of burning the sample in an oxygen atmosphere. Parsons, Pennington, and Walker (*10*) also utilized the high-frequency furnace–gas chromatograph combination in the determination of nitrogen.

The work described here is based on the retention of carbon dioxide by the 5 A. molecular sieve. The carbon dioxide in the combusted mixture was trapped and "stored" in the molecular sieve column under isothermal conditions while the excess oxygen passed on through. Helium, the carrier gas, was used to purge the oxygen from the column hence the need for an internal oxidizing agent and the liquid nitrogen trap was eliminated. The carbon dioxide peak was obtained through temperature programming.

EXPERIMENTAL

Apparatus and Materials. A schematic diagram of the entire system is shown in Figure 1. The apparatus consisted of a Leco high frequency induction furnace Model 523, a F & M Scientific Model 500 linear-programmed temperature gas chromatograph, with a 4-foot 5-A. molecular sieve column, and two Hevy Duty

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Electric Co. Multiple Unit tube furnaces.

Two $^3/_4$-inch copper tubes, filled with Mallinckrodt wire-form analytical reagent grade copper(ic) oxide, were heated by the tube furnaces which were set at the 3-3 position giving a temperature of 500° to 600° C. Linde hospital-grade oxygen and helium were run through the preheaters which oxidized and carbonaceous matter present in the gases. But the oxygen and helium were passed through two copper tubes, 24 inches long and $^3/_4$ inch in diameter, which were packed with G. F. Smith Chemical Co. reagent-grade magnesium perchlorate, Arthur W. Thomas Co. Ascarite (8–20 mesh) and Leco 501-60 specially prepared manganese dioxide. Any possible moisture, carbon dioxide, and sulfur dioxide in the flow lines was thereby removed.

The purified helium was then passed into the chromatographic unit as the carrier gas. The purified oxygen was introduced into the induction furnace. To maintain proper flow rate of oxygen through the molecular sieve column, a pressurized system was desired. Since the original Leco designed silicone rubber connection could hold only moderate pressures, a self-designed brass double "O" ring seal was incorporated into the oxidation chamber. This double "O" ring arrangement was first reported in a paper by Kuo, Bender, and Walker (*7*). Instead of the conventional Leco quartz combustion tube, a quartz tube with a length of $7^1/_2$ inches, and an outside diameter of $1^3/_8$ inches was used. All of the flow lines and connections were made of $^1/_4$-inch copper tubing and fittings. Needle valves were purchased from the Matheson Co. (No. 107). Leco 501-76 carbon-free tin accelerator (20–40 mesh), 528–35 crucibles, and 528–40 covers were used in this study.

Procedure. The following conditions were maintained during each analysis:

Helium flow rate at sample side: 80 ml. per minute at 100° C. column temperature. Helium flow rate at reference side: 50 ml. per minute at room temperature. Oxygen flow rate at sample side: 80 ml. per minute at 100° C. column temperature. Program rate: 42° C. per minute setting. Block temperature: 200° C. Injection port temperature: off, room temperature. Bridge setting: 130 ma. Temperature limit setting: 400° C. Oxygen pressure: 11 p.s.i. Helium pressure: 11 p.s.i. Attenuator setting: 2–128, depending on carbon content of samples.

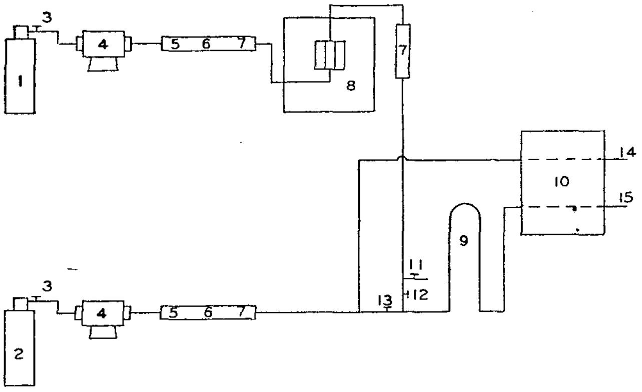

Figure 1. Schematic diagram of apparatus

1. Oxygen cylinder
2. Helium cylinder
3. Pressure regulators
4. Copper oxide tubes and preheaters
5. Magnesium perchlorate scrubber
6. Ascarite scrubber
7. Manganese dioxide scrubber
8. High frequency induction furnace
9. Molecular sieve column, 4 feet 5 A.
10. Block

11, 12, 13. Valves

14. Reference detector side
15. Sample detector side

With valves 11 and 13 opened and valve 12 closed, oxygen was released to the atmosphere and helium passed through the column, the sample detector, and into the atmosphere. The helium flow of the reference side was maintained constant at all times. The pen on the recorder was then set at its electrical zero by adjusting the control node. At this point, the disk chart integrator would show no area. The sample, 0.5 ± 0.001 gram, was weighed on a Mettler Gram-Atic Balance and mixed with 1.0 gram of Leco tin accelerator in the prefired crucible. The sample crucible was now loaded into the induction furnace with valve 11 open so the carbon dioxide from the air would have no chance of getting into the column. After making sure that all the carbon dioxide in the combustion chamber was purged out, valve 11 was then closed. At the same time, valve 13 was closed and valve 12 opened. Oxygen from the induction furnace was now passed through the column. The pen of the recorder would go out of scale due to the passage of oxygen through the sample detector. The induction furnace was now turned on and sample combusted. The combusted gases were passed through a manganese dioxide tube which retained all the sulfur dioxide in the gas mixture, and then into the 4-foot 5-A. molecular sieve column where carbon dioxide was trapped. The furnace was turned off after the oxidation was complete. The combustion time for each sample was found depending on the amount and the kind of samples in the crucible and varied from 10 to 12 minutes. Valve 12 was closed 2 minutes after the furnace was turned off so there would not be any carbon dioxide left behind in the oxidation chamber or in the copper connection. At the same time, valve 13 was opened and the helium carrier gas was sweeping the excess oxygen out of the column. In doing this, the pen of the recorder would slowly be drifting to lower scale and finally back to the original position—the electrical zero. This indicated that all of the oxygen was expelled. The temperature limit of the oven was now released and column temperature programming initiated. (To save time, once the time required for the pen to reach the equilibrium is observed, one can program it before the pen is completely back to zero, so long as the equilibrium will be reached just before the carbon dioxide peak shows up.) The area of the peak was read from the disk chart integrator. The over-all time required for each single run is approximately 20 minutes.

Table I. Results of Carbon Determination in Ferrous Metals by Gas Chromatography

NBS sample	Carbon, %		Mean error	Standard deviation
	NBS	Found		
55e	0.011	0.0102	−0.0008	±0.000104
170a	0.052	0.0524	+0.0004	±0.00015
133a	0.120	0.1178	−0.0022	±0.00056
10g	0.240	0.2345	−0.0055	±0.00110
10e	0.406	0.3968	−0.0092	±0.00309
10d	1.01	1.0269	+0.0169	±0.00200
82a	2.24	2.3239	+0.0839	±0.00750
122d	3.28	3.5666	+0.2866	±0.00877

RESULTS AND DISCUSSION

Eight kinds of NBS standard ferrous metals, with carbon contents ranging from 0.011 to 3.28%, were run, with results tabulated in Table I. For the lowest carbon sample, NBS 55e, the mean error is a −0.0008. For the highest carbon sample, NBS 122d, the mean error is a +0.2866. In actual unknown determination, a series of correction factors of counts × attenuation per p.p.m. carbon, must be found for individual gas chromatographic units by firing fractional weights of a standard sample at different attenuation settings. The latter was done for the work reported in this paper.

At its maximum sensitivity, attenuation of 1, 0.003% of carbon would give a full scale deflection. With this setting, one can easily detect 0.0005% of carbon. In fact, 1 gram of Leco tin in the prefired crucible has been fired in this system, an area with a peak height of about 0.5 inch was seen which indicates the presence of trace carbon in the Leco tin accelerator.

A major advantage of this technique is its broad detection range. Its lower limit is about 0.0005% while its upper limit (extrapolated) is of the order of 20% absolute carbon. Any sample with carbon content within this range can be determined simply by shifting the setting of the attenuator to the proper sensitivity. This method also eliminates the necessity of absorption cells, reagents, solutions, etc. Its simplicity and neatness in operation should be noted, including its suitability for routine analytical work. The materials necessary for connecting the induction furnace to the gas chromatographic unit would cost no more than a few dollars.

By using the quartz enclosed, carbon crucible instead of the porcelain crucible, this method can be applied to the microcarbon determinationf or organic compounds.

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Programmed Gradient Elution Chromatography with the Steroid Analyzer

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▶ Programmed separation of adrenocortical hormones by gradient elution chromatography with the steroid analyzer is described. Controlled separations are accomplished by means of a gradient pumping system, which permits the polarity of the eluting solvent mixture to be increased or decreased at will. The effect of selected programmed gradients on the separation of seven adrenocortical hormones and beef adrenal extract is demonstrated.

A COMPLETELY automatic device for analyzing adrenocortical hormones by gradient elution chromatography on columns has recently been developed in this laboratory (*1*). The steroid analyzer assays aliquots of eluate fractions and is capable of producing any desired elution gradient. A number of devices for programmed gradient elution have previously been described (*3*).

PRINCIPLE

The steroid analyzer, described in detail earlier (*1*), consists of two integral units. The gradient elution system operates independently of the cyclic operation of the remainder of the apparatus. A gradient cam, a metal replica of a plot of solvent ratio as ordinate *vs.* time as abscissa, is followed by the arm of a linear potentiometer. The position of this arm, as it traces the cam, governs the amounts of the two solvents, light petroleum ether (PE) and dichloromethane (DCM), that are individually pumped into a small mixing vessel. The mixture then flows through the column by gravity. A desirable feature of this differential pumping method of producing the elution gradient is that the concentration of one solvent in the other may be increased or decreased at will.

The remainder of the apparatus performs the automated procedures for dividing, collecting, drying, and analyzing the fractions by both ultraviolet spectrophotometry (UV) and colorimetry after reaction with Blue Tetrazolium (BT).

EXPERIMENTAL

The steroid analyzer was used as previously described, with the following exceptions. To determine the height of the potentiometer arm required for the elution of individual hormones, it was positioned manually for the preliminary experiments involving reversal of solvent ratios, as described below.

The column used differed slightly from that described in our original method for the quantitative determination of individual corticosteroids (*4*). The stationary phase was water, supported by a silicic acid column (Merck, dried at 100° for 3 hours). The ratio of support to stationary phase was 2.5 to 1 by weight. Identity and purity of fractions were monitored by thin-layer chromatography (*2*).

Stock solutions of 100 μg. per ml. of absolute ethanol were prepared from the following steroids: Δ^4-pregnen-21-ol-3,20-dione (Q), Δ^4-pregnen-21-ol-3,11,-20-trione (A), Δ^4-pregnene-11β,21-diol-3,20-dione (B), Δ^4-pregnene-17α,21-diol-3,20-dione (S), Δ^4-pregnene-17α,21-diol-3,-11,20-trione (E), Δ^4-pregnen-18-ol-11β,-21-diol-3,20-dione (Aldo.), and Δ^4-pregnene-11β,17α-21-triol-3,20-dione (F).

Adrenal cortex extract (Upjohn) with a biological activity equivalent to 100 μg. of F per ml. was dried under nitrogen and applied to the column without purification.

Samples of 5 μg. of each reference compound were automatically determined with an accuracy of 98 ± 2%.

Preliminary experiments with the seven adrenocortical hormones revealed that the solvent ratios required for the elution of each are critical. If the concentration of DCM in PE is decreased by as little as 1%, the elution pattern is altered, and a 5 to 10% decrease will retard the succeeding zones.

Table I lists the concentrations of DCM in PE which will elute or hold each steroid. Any given steroid is eluted by delivering to the column 30

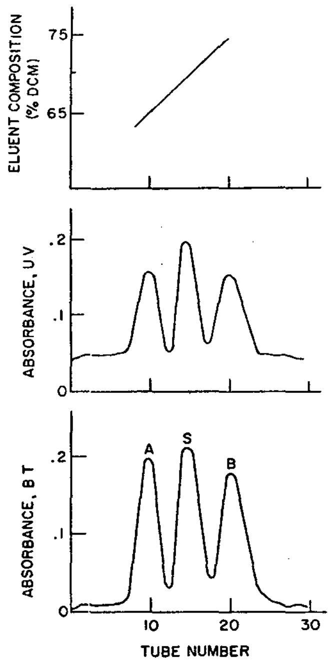

Figure 1. Separation of compounds A, S, and B using a linear gradient

Gradient change expressed as % DCM in PE per tube

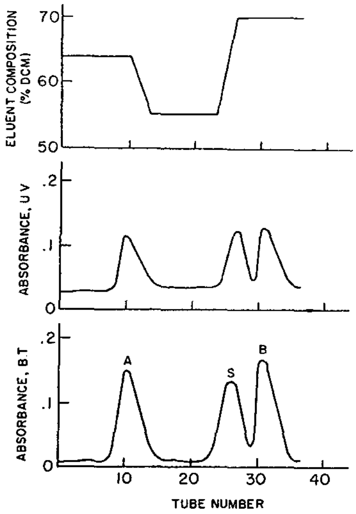

Figure 3. Programmed separation of compounds A, S, and B

Different gradient from Figure 2

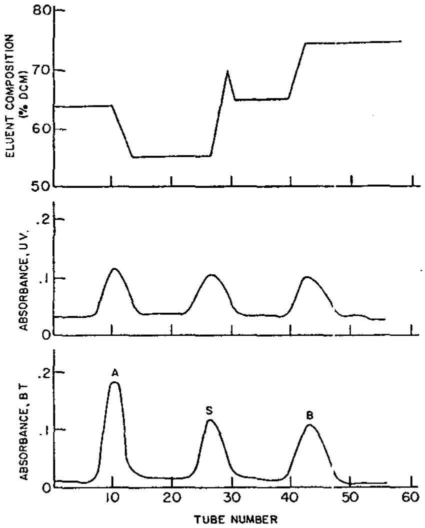

Figure 4. Programmed separation of compounds A, S, and B

Different gradient from Figure 2

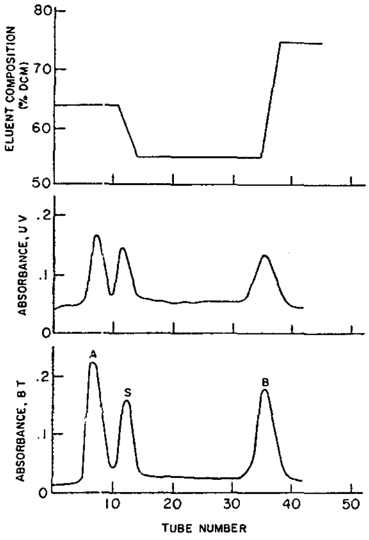

Figure 2. Programmed separation of compounds A, S, and B

Upper. Elution gradient, % DCM per tube
Lower. Absorbance per tube

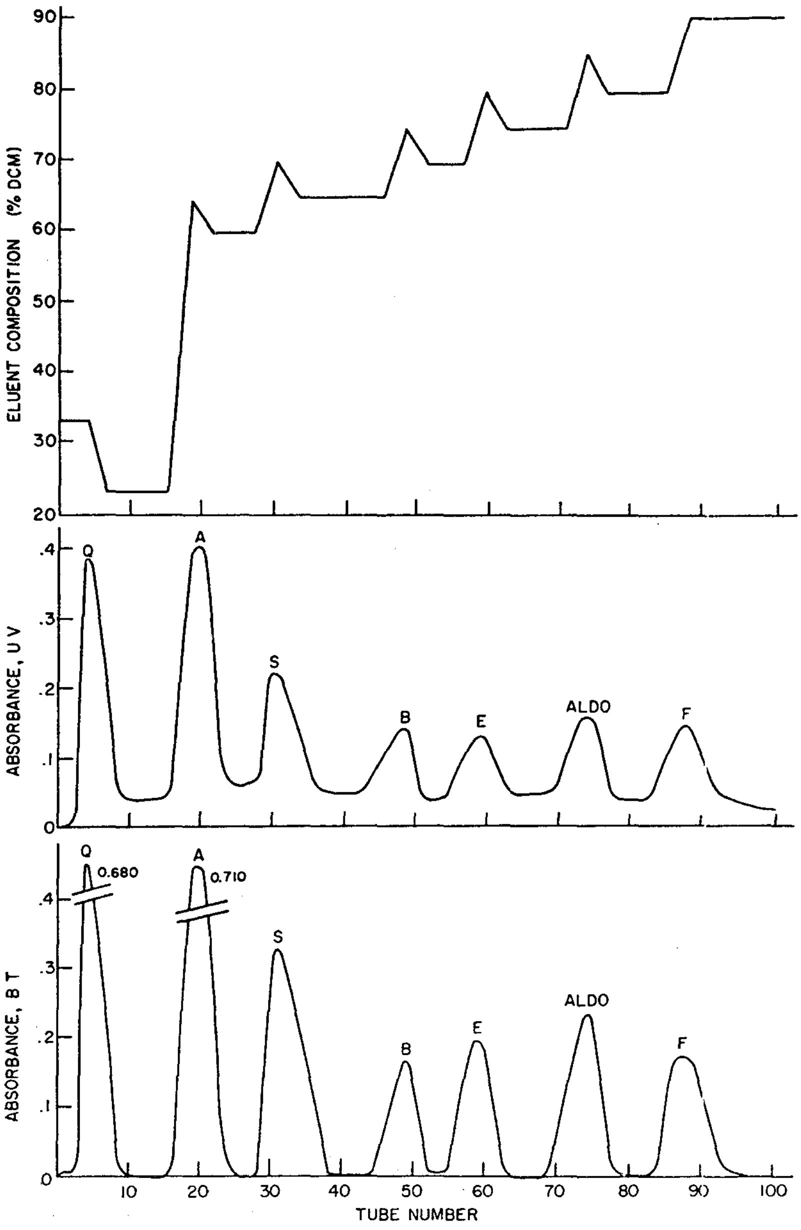

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Figure 5. Programmed separation of seven physiologically active adrenocortical hormones

Upper. Gradient change, % DCM in PE per tube
Lower. Absorbance per tube

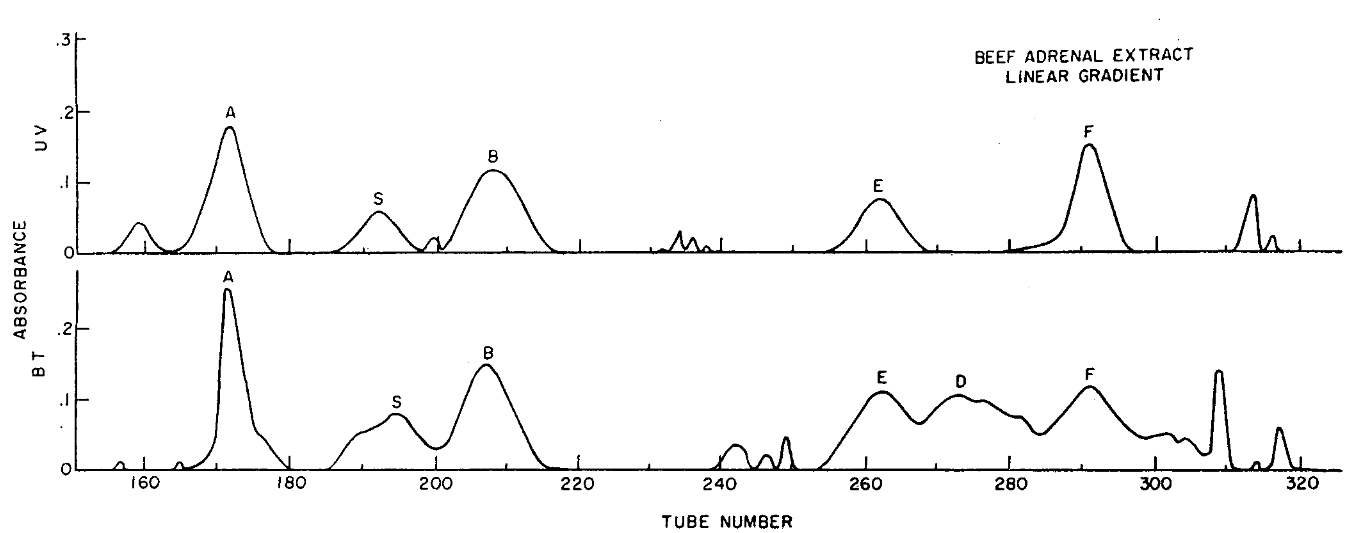

Figure 6. Separation of adrenal steroids in 6 ml. of beef adrenal extract

Linear gradient, started at 100% PE

ml. of solvent, having the composition shown under "Elute." This is equal to the holdup volume of the column. Thereafter, the polarity of the eluent is decreased below the composition shown under "Hold." The interval between the peaks is governed by the length of time that the solvent composition is kept below the "Hold" concentration.

Table I. Per Cent DCM in PE Required to Elute or Hold Individual Hormones

Compound	Elute	Hold
Q	33	20
A	64	55
S	70	60
B	75	65
E	80	70
Aldosterone	86	75
F	91	80

Figure 1 illustrates the separation achieved for a mixture of A, S, and B, using a linear gradient, concentration change of 0.66% per tube. Under these conditions, the three compounds are eluted in succession without intervals. The distance between peaks is readily controlled by selecting an appropriate elution program. In Figure 2, the interval between S and B has been increased by a drop in polarity of the eluent. In Figure 3, this is shown for the interval between A and S, and Figure 4 illustrates an extremely wide spacing between the three peaks, produced by a special elution program.

The programmed separation of all seven reference compounds is presented in Figure 5. The distance between peaks and the sharpness of each peak can be controlled by selecting an appropriate gradient curve. This analysis was accomplished in 17 hours, using a 10-minute collection cycle on the analyzer. Subsequently, the time was reduced to 8.5 hours by adjusting the collection time to 5-minute cycles.

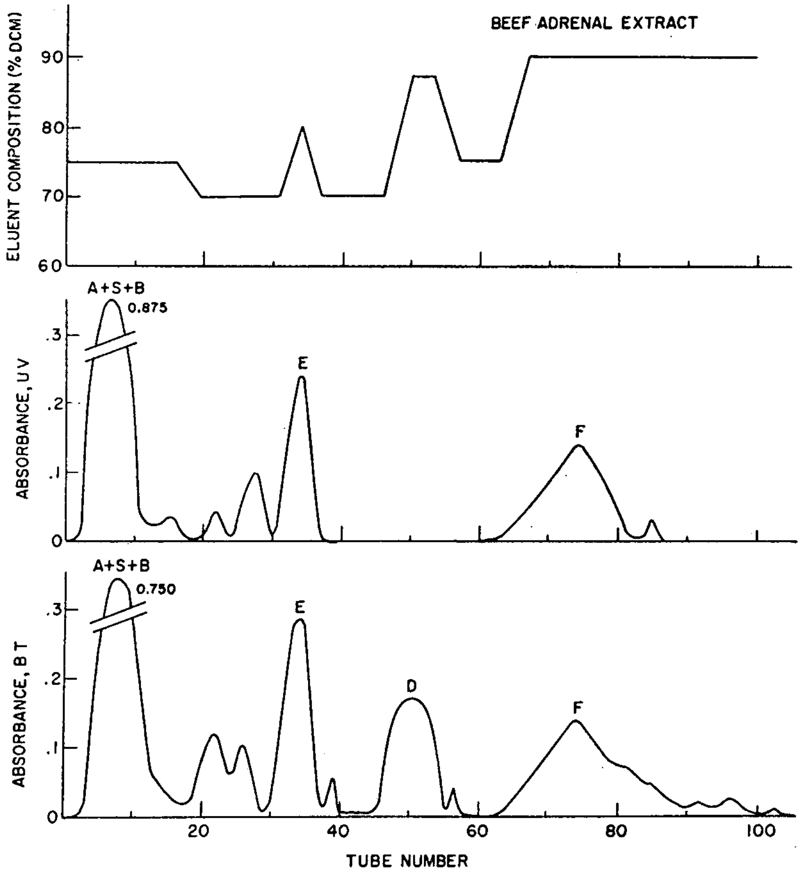

Figure 7. Programmed separation of adrenal steroids in 6 ml. of beef adrenal extract

Upper. Elution gradient, % DCM in PE per tube
Lower. Absorbance per tube

One of the prime advantages of column chromatograhy over other types of chromatography is its greater load capacity. This permits the use of samples large enough for the determination of minor components in the presence of excessive amounts of other components or impurities. Even so, analysis is often hampered by overlapping of closely related compounds, especially if such an overlap involves a minor component. Figure 6, representing the analysis of 6 ml. of adrenal extract, shows that the column is overloaded under the conditions used (linear gradient, concentration change of 0.333% per tube). Yet, this amount of sample is required for the determination of small peaks, such as those occurring between B and E and between E and F.

The problem was solved by programming the gradient elution as shown in Figure 7 (top). To save time, compounds A, S, and B were deliberately eluted in a single peak, while increasing the resolution of subsequent compounds, particularly the BT-reducing compound between E and F. This compound was shown by thin-layer chromatography to be Reichstein's Compound D (allopregnane - 3β,17α,21 - triol - 11,20 - dione). Thus, any portion of the elution pattern can be selectively compressed or expanded by programmed gradient elution.

The steroid analyzer (*1*) is a prototype instrument, representing a considerable investment of time and cost. The degree of automation achieved probably exceeds the requirements of a routine laboratory. However, the principle of gradient elution and particularly polarity reversal will undoubtedly be found generally useful for difficult resolution problems and may be applied either manually or by a simple combination of tape programming and dual pumping. Details of this simplified system will be described elsewhere.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical advice and assistance of Frank O. Anderson, Grant C. Riggle and John K. Cullen, Jr., Instrument Engineering and Development Branch, Division of Research Services. They thank William J. Haines, Upjohn Co., for the generous gift of beef adrenal extract.

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Quantitative Analysis of Aromatic Hydrocarbons by Capillary Gas Chromatography

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▶ The use of polar substrates with capillary columns has made the separation of *m*- and *p*-xylene less difficult. *m* - Bis(*m* - phenoxyphenoxy)benzene (b.p. 273° C. at 1-mm. pressure) can be utilized as a capillary column substrate for quantitative analysis of C_6 through C_{11} aromatics. If base line separation in the C_6 through C_9 region is desired, modification of the liquid phase with squalane is required. Comparative elution data and spectra of several polar substrates are given. Reliable quantitative analytical data in this aromatic range can be obtained using these columns in a gas chromatograph employing a linear sample splitter and high temperature flame ionization detector. This investigation includes over 30 available compounds in the C_6 through C_{11} range. Twenty-five of the first 28 aromatics were resolved in 17 minutes using this method.

THE analysis of aromatic hydrocarbons has been studied by a number of workers (*1-6*), and in the last three years the use of capillary columns has made the separation of *m*- and *p*-xylene less difficult. The results of the authors' investigation of several liquid phases are given in Table I.

The present paper deals with the utilization of *m*-bis(*m*-phenoxyphenoxy)-benzene (BPB) (b.p. 273° C. at 1-mm. pressure) as a capillary substrate. This material is easy to coat on capillary columns, is very stable, and can be utilized for the analysis of C_6 through C_{11} aromatics. If better than 90% separation in the C_6 through C_9 region is desired, modification with squalane (a relatively nonpolar substrate) is required. This, however, limits the column to this particular range because of excessive elution time for higher boiling materials. A discussion of column preparation, operating parameters, etc., illustrates that reliable quantitative analytical data in the C_6 through C_{11} aromatics range can be obtained using this column system on a gas chromatograph employing a linear sample splitter and ionization detector.

EXPERIMENTAL

Apparatus. Barber-Colman Model 20 and Model 61-C instruments equipped with linear stream splitters

Table I. Liquid Stationary Phases Investigated for Aromatic Separations

	Aromatic-paraffin resolution	Xylene resolution	Thermal stability
β,β'-Oxydipropionitrile	4	1	1
Carbowax 1500	3	2	3
Oxybis-2-ethyl benzoate with GE96 (100)	2	2	2
m-Bis(*m*-phenoxyphenoxy)benzene with squalane	2	4	3
m-Bis(*m*-phenoxyphenoxy)benzene	4	3	4

Rating system.

1. Poor
2. Fair
3. Satisfactory
4. Excellent

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[1] Present address, Wilkens Instrument and Research, Inc., Houston, Texas.

▶

Figure 1. Chromatogram of typical refinery 5° F. xylene fraction on modified BPB

70° C., 2.9 ml./min. helium flow

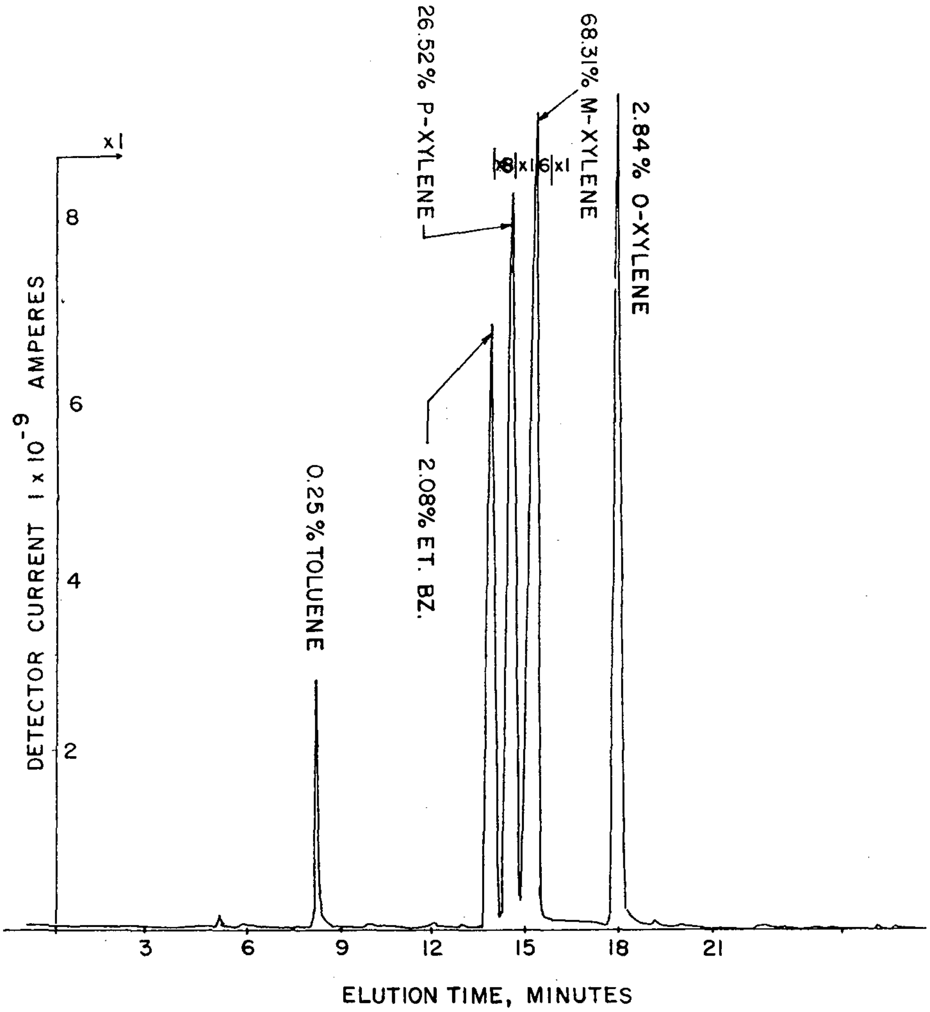
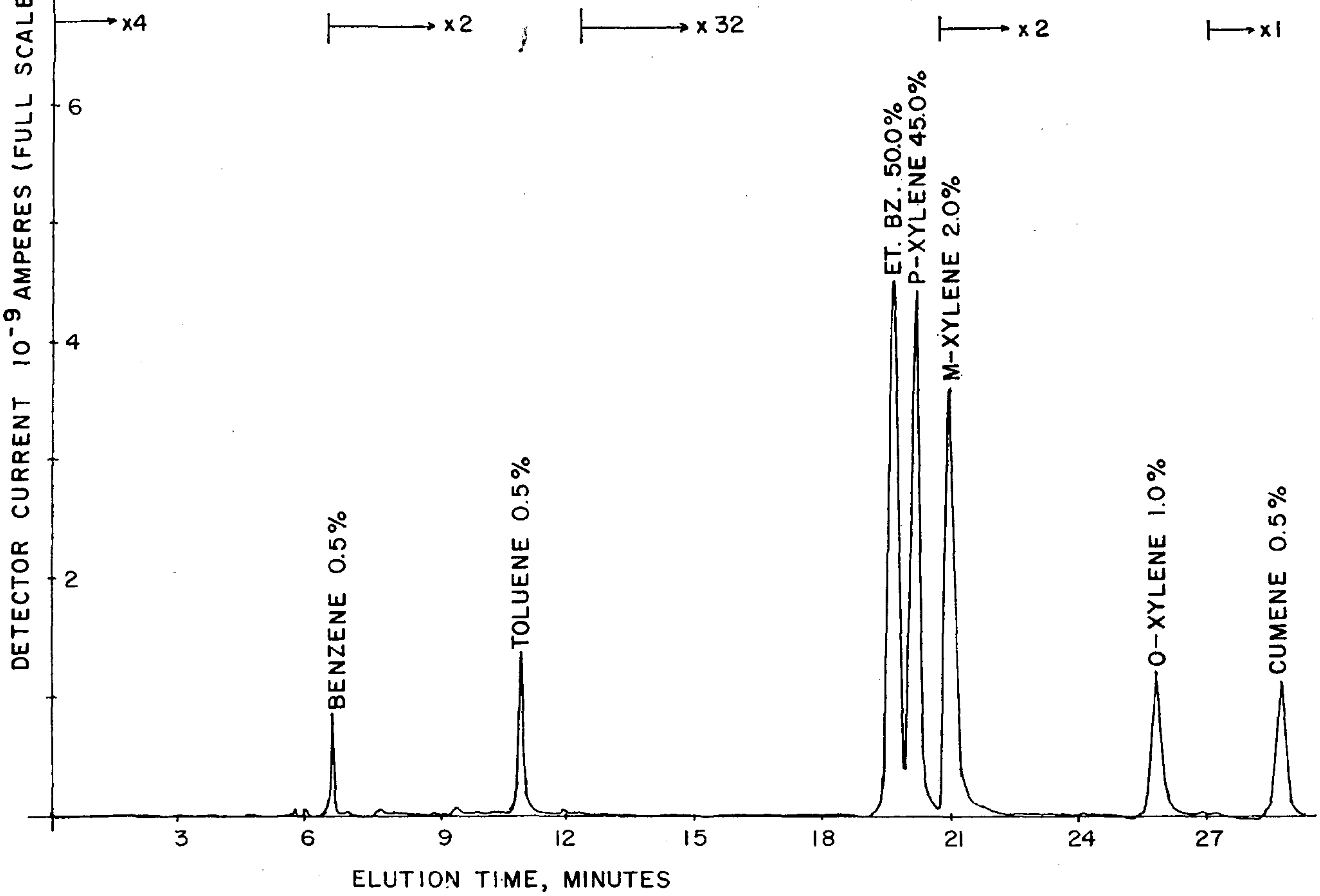


Figure 2. Chromatogram of high concentration of ethylbenzene and *p*-xylene on BPB

85° C., 2.0 ml./min. helium flow

▼



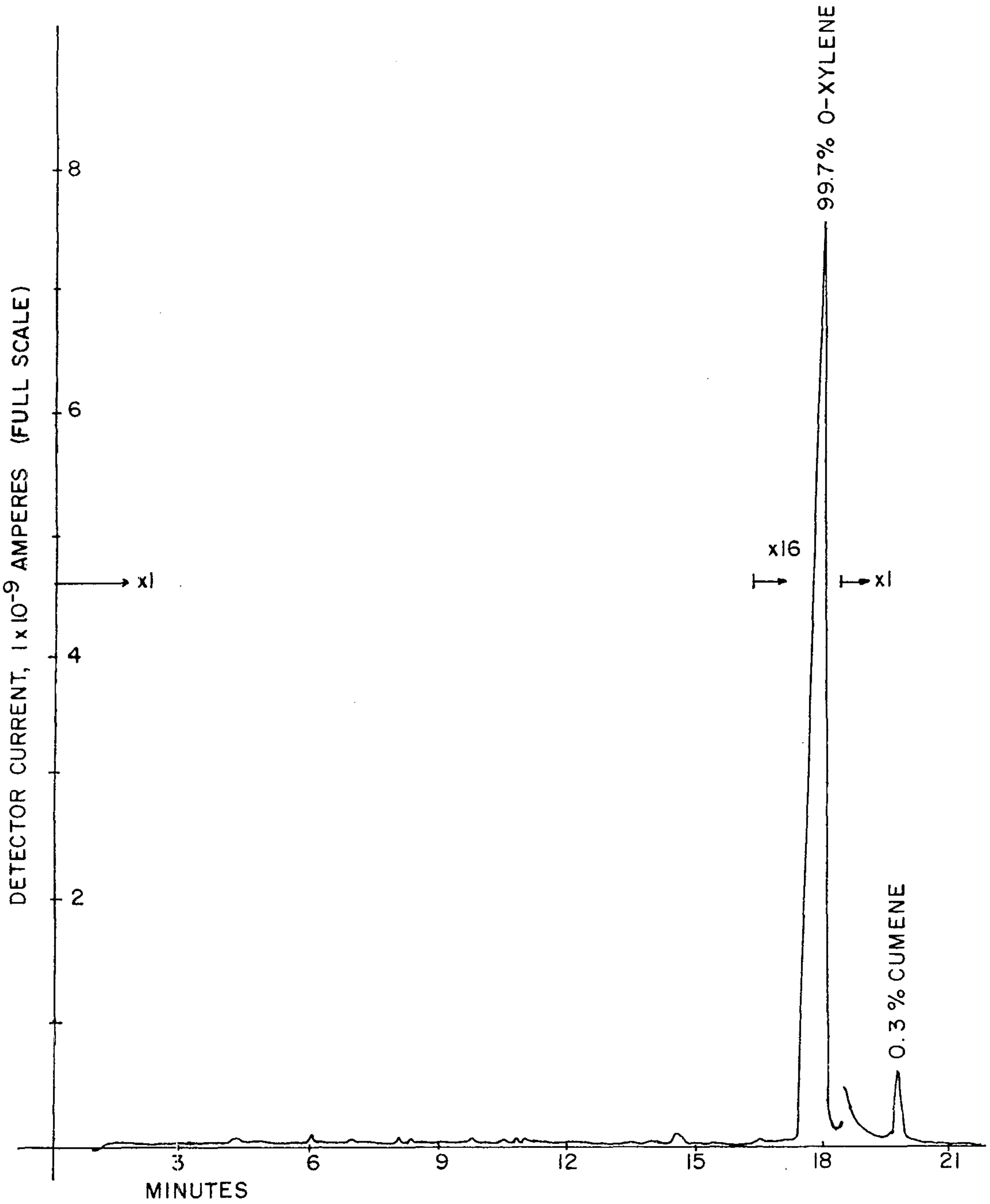


Figure 3. Chromatogram of high purity o-xylene on modified BPB

71° C., 2.9 ml./min. helium flow rate

and low temperature (room) and high temperature (over 200° C.) flame ionization detectors and −0.25- to +5.0-mv. recorders (2-second response time) were used in the development of this method. Disc Integrators capable of measuring at least 6000 c.p.m. were required for peak area measurements. Sample injection was with 1- and 10-µl. Hamilton syringes.

Preparation of Column. LIQUID SUBSTRATES. Modified *m*-bis(*m*-phenoxyphenoxy)benzene (BPB) (C_6 through C_9) aromatics (available from Eastman Organic Chemicals, Rochester, N. Y.). Dissolve 0.4 ml. of BPB and 0.1 ml. of squalane in 9.5 ml. of methylene chloride.

BPB (C_6 through C_{11}) aromatics. Dissolve 0.7 ml. of BPB in 9.3 ml. of methylene chloride.

COLUMN CLEANING AND COATING. The 200-foot × 0.01-inch i.d. stainless steel column was washed with at least three separate 5-ml. charges of pentane, one 5-ml. charge of methanol, and one 5-ml. charge of pure methylene chloride. The appropriate coating solution was then applied, using standard coating techniques.

Operating Procedure. The operating conditions were adjusted to data on each chromatogram. The helium flow rate was measured with a conventional soap film meter. An inlet pressure about 20 to 40 p.s.i.g. produced the flow rate. A clean, dry microsyringe was flushed several times with the sample to be analyzed. A 0.0005- to 0.002-ml. sample was injected into the chromatograph through a silicone septum. The exact size utilized depends upon the split ratio and relative electrometer gain. For linear quantitative results, maximum peak height should not exceed 7×10^{-9} and 7×10^{-8} ampere for the low and high temperature detectors, respectively. The time required for the analysis will vary, depending upon the boiling range of the sample and the operating conditions.

Operating Conditions. Electrometer sensitivity of 1×10^{-10} to 7×10^{-8} ampere was used for all experiments.

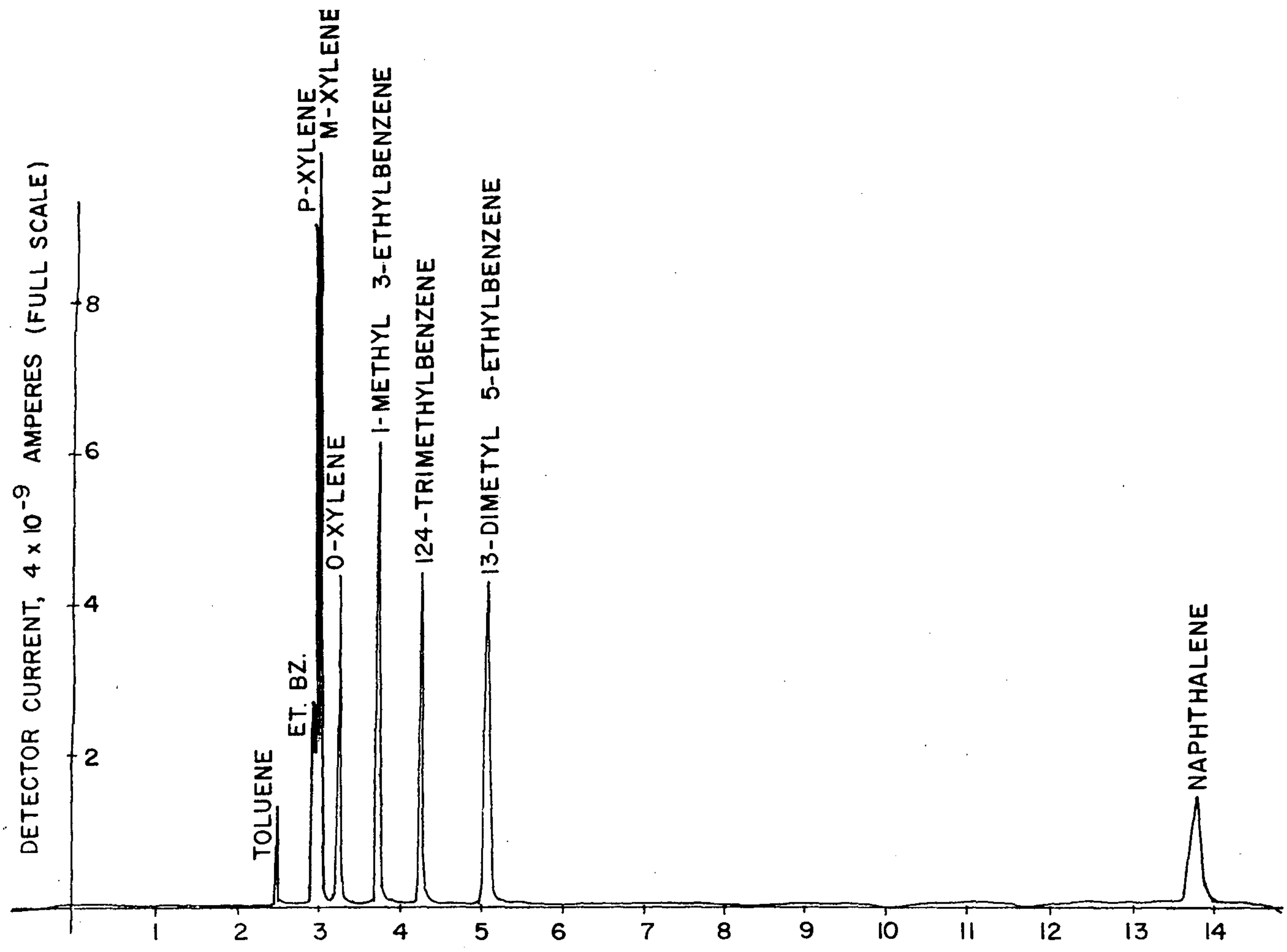


Figure 4. Chromatogram of wide boiling range aromatic blend on BPB

145° C., 6.0 ml./min. helium flow rate

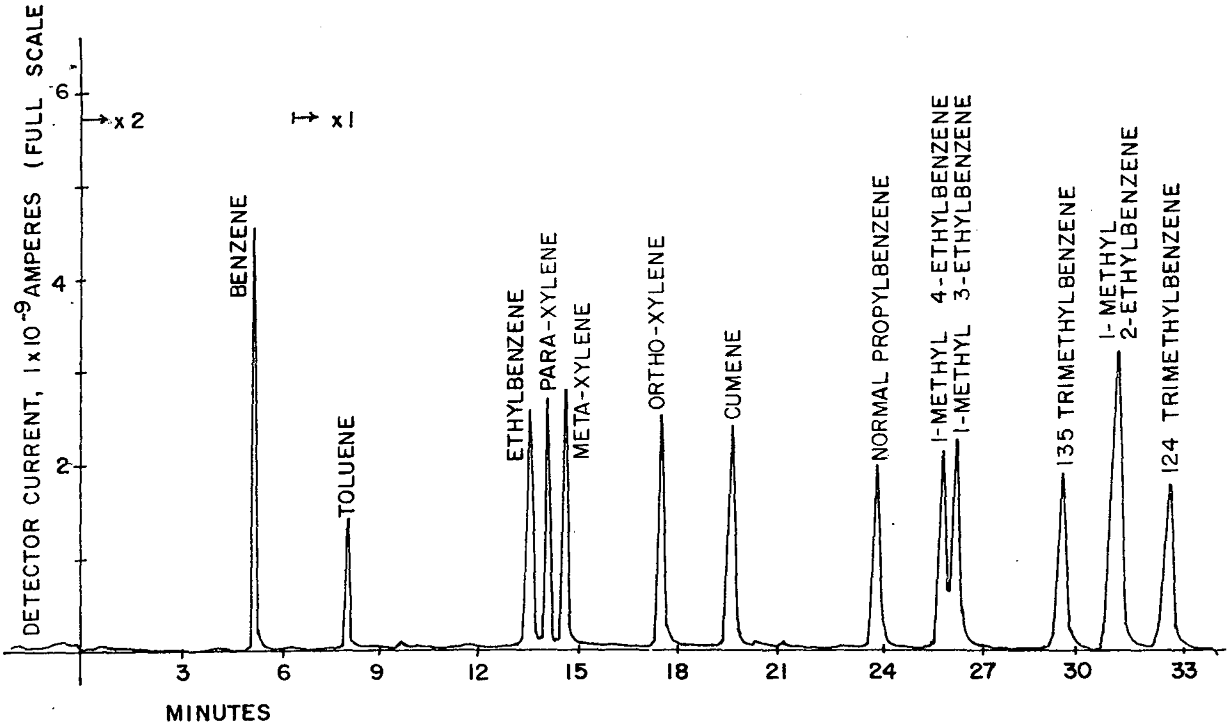


Figure 5. Chromatogram of C_6 to C_9 aromatics on modified BPB

70° C., 2.9 ml./min. helium flow rate

The flash heater and splitter heat temperatures were maintained at 300° ± 5° and 200° ± 2° C., respectively. The low temperature flame was at 40° C., while the high temperature flame was at 200° to 225° C. Split ratios were varied from 100:1 to 200:1. The column temperature and column flow rate are given on each chromatogram.

RESULTS

Various synthetic blends of the C_6 through C_{11} aromatics were run on BPB columns, utilizing both the Model 20 and the Model 61-C instruments, interchangeably.

Table II. Relative Retention Data

Peak No.	Component	Boiling point, ° C.	Relative time (benzene = 1.00) BPB/ squalane at 70° C.	BPB 105° C.	BPB 130° C.
1	Benzene	80.10	1.00	1.00	1.00
2	Toluene	110.62	1.55	1.37	1.14
3	Ethylbenzene	136.19	2.61	1.93	1.36
4	*p*-Xylene	138.35	2.71	1.96	1.36
5	*m*-Xylene	139.10	2.83	2.06	1.40
6	*o*-Xylene	144.41	3.40	2.31	1.51
7	Styrene	145.14	...	...	1.59
8	Isopropylbenzene	152.39	3.80	2.46	1.61
9	*n*-Propylbenzene	159.22	4.66	2.86	1.68
10	1-Methyl-3-ethylbenzene	161.30	5.13	3.00	1.75
11	1-Methyl-4-ethylbenzene	161.99	5.06	3.04	1.75
12	1,3,5-Trimethylbenzene	164.72	5.75	3.29	1.83
13	1-Methyl-2-ethylbenzene	165.15	6.08	3.52	1.92
14	*tert*-Butylbenzene	169.12	...	3.43	1.87
15	1,2,4-Trimethylbenzene	169.35	6.47	3.87	2.01
16	Isobutylbenzene	172.76	...	3.52	1.92
17	*sec*-Butylbenzene	173.30	...	3.68	1.96
18	Indane	177.82	...	...	1.59
19	1,2,3-Trimethylbenzene	176.08	...	4.75	2.34
20	*m*-Diethylbenzene	181.10	...	4.75	2.28
21	Indene	182.44	...	5.60	2.61
22	*n*-Butylbenzene	183.27	...	...	2.58
23	*o*-Diethylbenzene	183.42	...	5.35	2.53
24	*p*-Diethylbenzene	183.75	...	4.96	2.38
25	1,3-Diethyl-5-methylbenzene	183.75	...	...	2.50
26	1-Methyl-4-*tert*-butylbenzene	192.76	...	6.74	2.91
27	1,2,4,5-Tetramethylbenzene	196.80	...	7.81	3.72
28	Isopentylbenzene	198.90	...	5.77	2.69
29	Tetralin	207.57	...	...	5.01
30	Naphthalene	217.55	...	...	6.39
31	Biphenyl	255.00	...	...	16.61

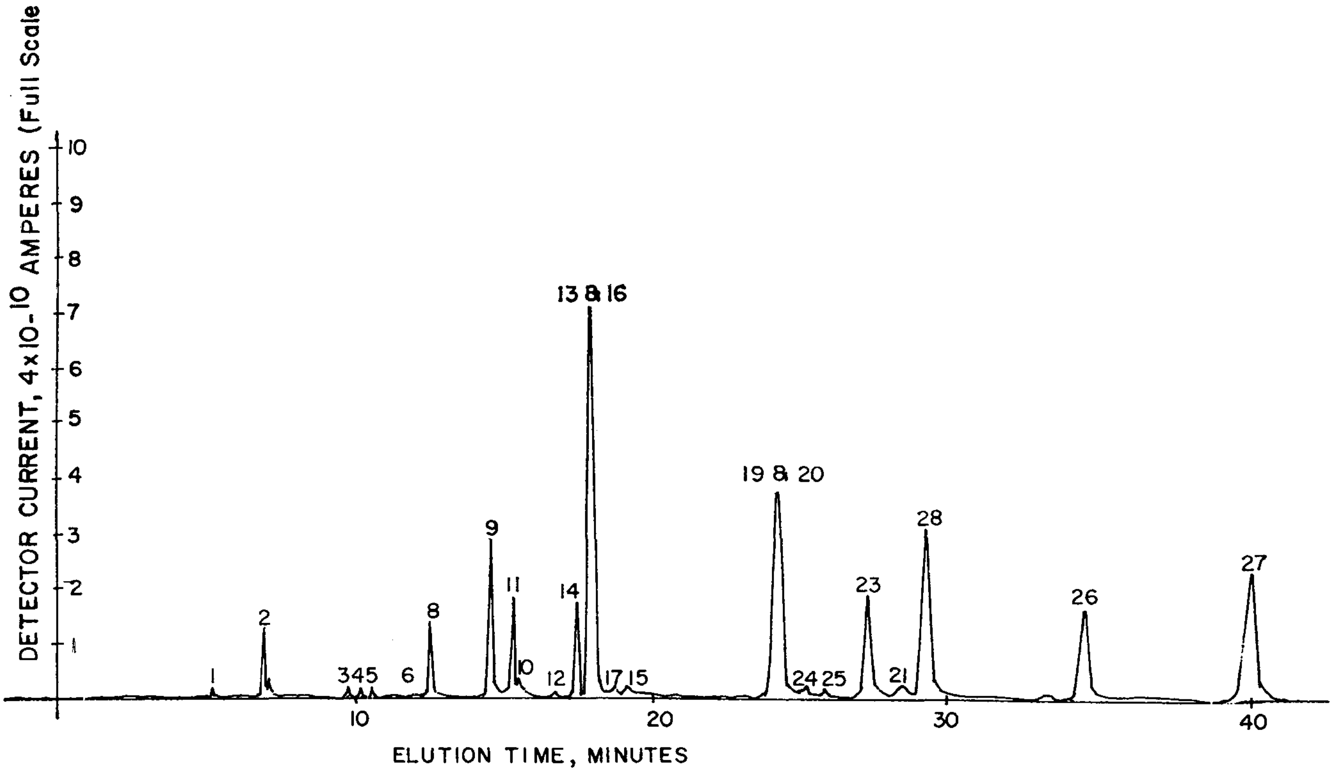


Figure 6. Chromatogram of C_6 to C_{10} aromatics on BPB

105° C., 2.0 ml./min. helium flow rate
Peak numbers refer to Table II

The data from these runs indicated that optimum (90% or better) separation of the ethylbenzene, *p*-xylene, and *m*-xylene could not be obtained on the pure BPB columns; therefore modification with squalane (a boiling point substrate) was necessary. The addition of the squalane caused some interference of trace C_{10} paraffins with the C_8 aromatics, as would be expected, but by running the same sample on modified and pure BPB columns, optimum information was obtained for the common 5° mixed-xylene cut of industry (Figures 1 and 2), and excellent data for high purity (Figure 3) isomers in this range.

The data also indicated that the upper operating temperature of the modified BPB was too low for materials above the C_9 range. Elution time was excessive and considerable tailing ensued. The single-phase BPB column temperature was elevated to reduce elution time, but some tailing was encountered when the low temperature flame detectors were used in the C_{10} to C_{11} range. By switching to the high temperature flame units it was possible to elute these materials in a shorter time with excellent peak shapes, reasonable precision of analytical data, and wider sample size range (Figure 4).

In following this particular system of evaluation of these columns, it was possible as well to determine the relative detector responses or sen-

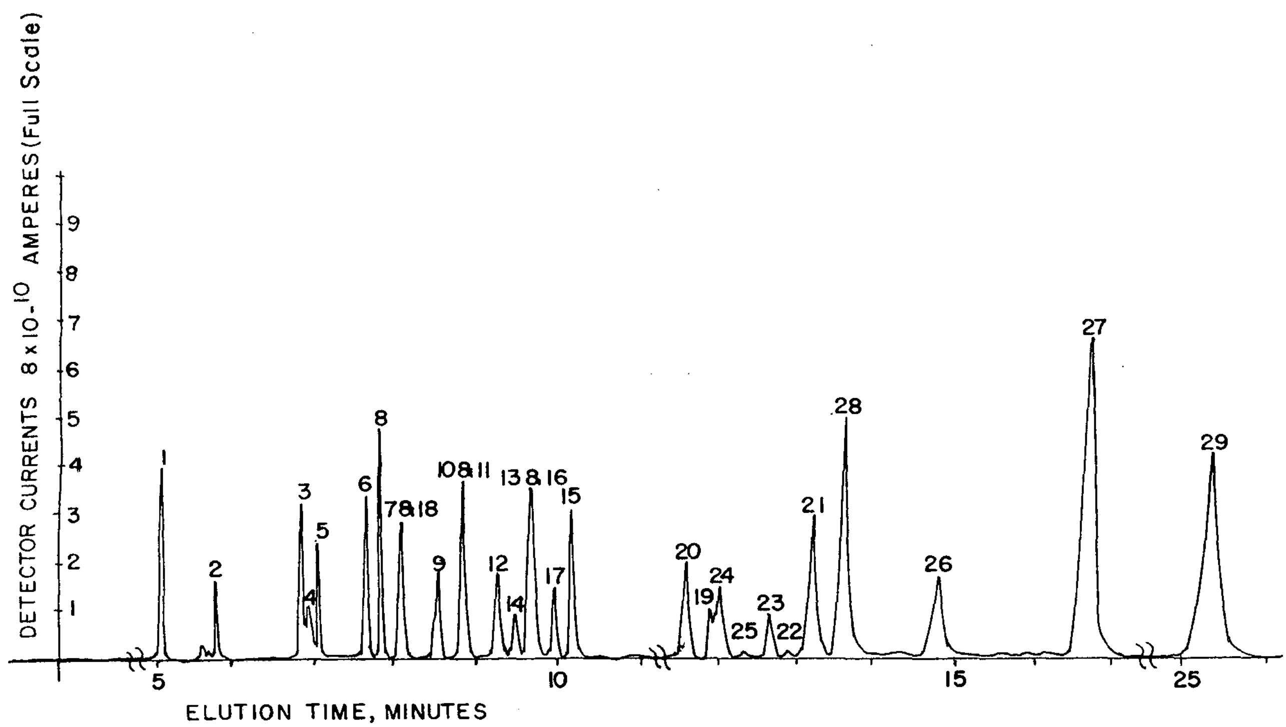


Figure 7. Chromatogram of C_6 to C_{11} aromatics on BPB

130° C., 2.0 ml./min. helium flow rate
Peak numbers refer to Table II

sitivity of four different hydrogen flame detectors on two separate instruments. These data indicated that the relative responses of the four were essentially the same within experimental error, but further studies are in progress. Since the high temperature unit works equally well with both the low temperature and high temperature columns, and is not subject to condensation of eluted high boilers, it is preferred for general use. The boiling point, elution time, and typical chromatograms of 31 aromatic compounds are listed in Table II and Figures 5, 6, and 7. The problem of repeatability was also investigated. Tables III and IV illustrate typical repeatability data obtained with the low temperature flame units on a mixture of relatively narrow boiling range and high temperature flame units on a mixture of wide boiling range. In obtaining these data, the sample size injected was varied within the range of 0.0005 to 0.002 ml. to ensure that no significant deviation occurred. The authors believe the repeatability of the wide boiling range material is limited by the count rate of the integrators used in this study.

DISCUSSION

By utilizing a combination of squalane, modified BPB, and a pure BPB substrate coated on a 200-foot × 0.01-inch i.d. stainless steel capillary column, it is possible to achieve excellent analytical results for the C_6 through C_{11} aromatics, if all instrument and operator techniques are critically examined. We have shown the method to be repeatable; therefore accuracy can be obtained by peak normalization. While flow rate and temperature are not critical functions of column life (pure BPB), this system contains so many close boiling components of the same general hydrocarbon type (aromatics and substituted aromatics) that optimum separations are a direct function oi operating conditions. Therefore, the particular boiling range of the samples encountered should be known, so that the proper column and operating conditions can be selected. The necessity for the combination of a linear sample splitter and high temperature ionization detector cannot be overemphasized.

ACKNOWLEDGMENT

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Table III. Repeatability Data[a] for Low Temperature Flame Ionization Detector

	Run 1	Run 2	Run 3
Benzene	0.5	0.5	0.5
Toluene	0.6	0.6	0.6
Ethylbenzene	45.4	45.4	45.4
p-Xylene	48.5	48.6	48.7
m-Xylene	3.0	3.0	3.0
o-Xylene	0.9	0.9	0.8
Cumene	0.6	0.6	0.5
n-Propylbenzene	0.5	0.4	0.5

[a] Based on peak area normalization.

Table IV. Repeatability Data[a] for High Temperature Flame Ionization Detector

Toluene	Durene	Naphthalene
63.0	9.3	27.7
63.0	9.5	27.5
63.5	9.4	27.1
63.1	9.4	27.5
63.3	9.1	27.6
63.1	9.3	27.6
63.5	9.0	27.5

[a] Based on peak area normalization.

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Qualitative Analysis of Naphthalenes by Capillary Gas Chromatography

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▶ The analysis of naphthalenes, monosubstituted naphthalenes, and disubstituted naphthalenes through C_{12} has been investigated using combination analytical techniques. A systematic survey was made to determine the conditions required to achieve rapid, accurate, analytical results on this mixed naphthalene cut by gas liquid chromatography alone. A gas chromatograph equipped with capillary columns, a high temperature flame ionization detector, and a special heated inlet system would resolve the 15 naphthalene compounds investigated. It was necessary to run the sample on two columns to achieve resolution of all components, but using only one column with *m*-bis(*m*-phenoxyphenoxy)benzene (BPB) gave more information than previously reported in the literature.

THE analysis of naphthalenes has been studied by a number of workers (*2*, *3*) and in the last few years the separation of the methylnaphthalenes has been commonplace. This paper deals with the utilization of polyphenyl ether. Apiezon L and Ucon 2000 were used as capillary substrates for the separation of the first 15 naturally occurring naphthalenes through 1,8-dimethylnaphthalene. Twelve of these components boil in a 12° range and their separation using capillary columns has not previously been reported.

Although we could not completely resolve all 15 components on one column, these separations are superior to those reported in the literature.

EXPERIMENTAL

Apparatus. A standard Barber-Colman Model 61-C instrument equipped with separately controllable flash heaters and stream splitters and high temperature flame-ionization detectors and −0.25- to 5.0-mv. recorders (2-second response time) were used in the development of this method. Sample injection was with 1- and 10-µl. Hamilton syringes.

Table I. Operating Conditions

Conditions	BPB	Apiezon L	Ucon 2000
Sensitivity	1×10^{-10} amp./5 mv.	1×10^{-10} amp./5 mv.	1×10^{-10} amp./mv.
Carrier gas	Helium	Helium	Helium
Column dimensions	225 feet × 0.01 inch	200 feet × 0.01 inch	200 feet × 0.01 inch
Column temperature, ° C.	175	156	165
Flash heater temperature, ° C.	325	310	350
Split temperature, ° C.	290	305	300
Detector temperature, ° C.	255	225	215
Sample size, µl.	0.6	0.8	0.8
Split ratio	125:1	200:1	170:1
Flow rate, ml./minute	6.0	9.0	5.0

Preparation of Column. LIQUID PHASES. *m*-Bis(*m*-phenoxyphenoxy)-benzene (BPB) is available from Eastman Organic Chemicals, Rochester, N. Y. Dissolve 0.9 ml. of *m*-bis-(*m*-phenoxyphenoxy)benzene in 9.1 ml. of methylene chloride.

Dissolve 1.0 ml. of Apiezon L (James G. Biddle Co., Philadelphia 7, Pa.) in 9.0 ml. of petroleum ether.

Dissolve 1.0 ml. of Ucon 2000 (Union Carbide Co., New York 17, N. Y.) in 9.0 ml. of petroleum ether.

COLUMN CLEANING AND COATING. The capillary column was attached to a source of high pressure (0 to 1000 p.s.i.g.) effluent gas, preferably argon. A 5- to 10-ml. reservoir was placed between the column and the regulator. The new steel was washed with at least three separate 5-ml. charges of pentane, one 5-ml. charge of methanol, and one 5-ml. charge of pure methylene chloride. Column was coated in the same way.

Procedure. The optimum operating conditions used in obtaining the illustrated chromatograms are listed in Table I. The helium flow rates were measured with a conventional soap film meter. An inlet pressure of about 20 to 40 p.s.i.g. produced the flow rate. A clean, dry microsyringe was flushed several times with the sample to be analyzed. The sample was injected into the chromatograph through a silicone septum. The exact size utilized depends upon the split ratio and relative electrometer gain. For linear quantitative results, maximum peak height should not exceed 7×10^{-8} ampere with this high temperature flame detector.

Table II. Relative Retention Data

Component	Boiling point, ° C.	BPB at 175° C.	Ucon 2000 at 165° C.	Apiezon L at 156° C.
Naphthalene	217.9	1.00	1.00	1.00
2-Methylnaphthalene	241.1	1.51	1.47	1.44
1-Methylnaphthalene	244.6	1.71	1.62	1.55
1-Ethylnaphthalene	258.7	2.17	2.08	2.05
2-Ethylnaphthalene	257.9	2.17	2.13	2.01
2,6-Dimethylnaphthalene	262.3	2.28	2.23	2.22
2,7-Dimethylnaphthalene	262.8	2.32	2.23	2.22
1,7-Dimethylnaphthalene	262.9	2.45	2.41	2.31
1,6-Dimethylnaphthalene	265.6	2.58	2.50	2.40
1,3-Dimethylnaphthalene	263	2.61	2.50	2.40
2,3-Dimethylnaphthalene	268	2.81	2.72	2.59
1,4-Dimethylnaphthalene	268	2.84	2.77	2.59
1,5-Dimethylnaphthalene	265	2.91	2.72	2.65
1,2-Dimethylnaphthalene	266	3.04	3.00	2.77
1,8-Dimethylnaphthalene	270	3.50	3.42	3.12

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[1] Present address, Wilkens Instrument and Research, Inc., Houston, Texas.

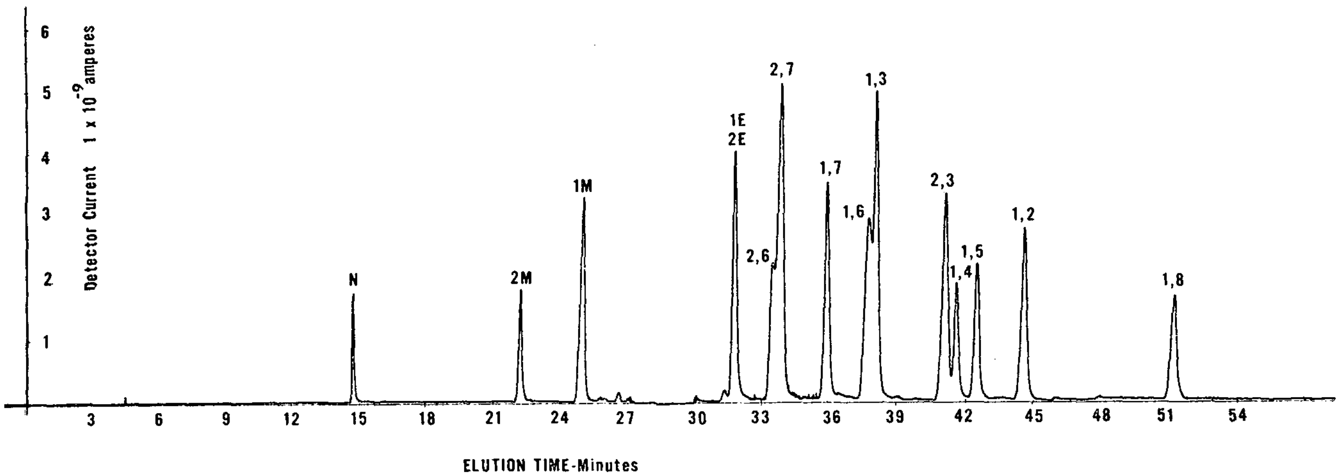

Figure 1. Separation of naphthalenes with *m*-bis(*m*-phenoxyphenoxy)benzene

RESULTS

The naphthalenes investigated included the first 15 naturally occurring compounds from naphthalene through 1,8-dimethylnaphthalene. King, Fabrizio, and Donnell (*3*) by using gas chromatography obtained 10 peaks from a similar mixture while investigating the composition of catalytic gas oils and tar distillates. Their apparatus consisted of packed columns and thermal conductivity detectors. This was a significant contribution in the analysis of high boiling components present in petroleum and tar oils; however, to complete the analysis, it was necessary to use other instrumental methods.

Since we had already investigated the use of the *m*-bis(*m*-phenoxyphenoxy)-benzene for the analysis of complex mixtures of aromatic hydrocarbons using high efficiency capillary column and high temperature flame ionization detectors (*1*), it seemed likely that separations could be obtained on naphthalene mixtures by this technique.

This liquid phase, coated on a 225-foot × 0.01-inch column, separated 13 of the 15 compounds (Figure 1). The 1-ethyl- and 2-ethylnaphthalenes were not separated under operating conditions which gave a reasonable analysis time. Although this was better than previously reported, it was readily apparent that a substrate of different characteristics—i.e., polarity—was necessary to effect the separation of the ethylnaphthalenes.

Apiezon L (a nonpolar substrate) was next investigated. This capillary column, 200-foot × 0.01-inch, resolved the 1-ethyl- and 2-ethylnaphthalenes, but

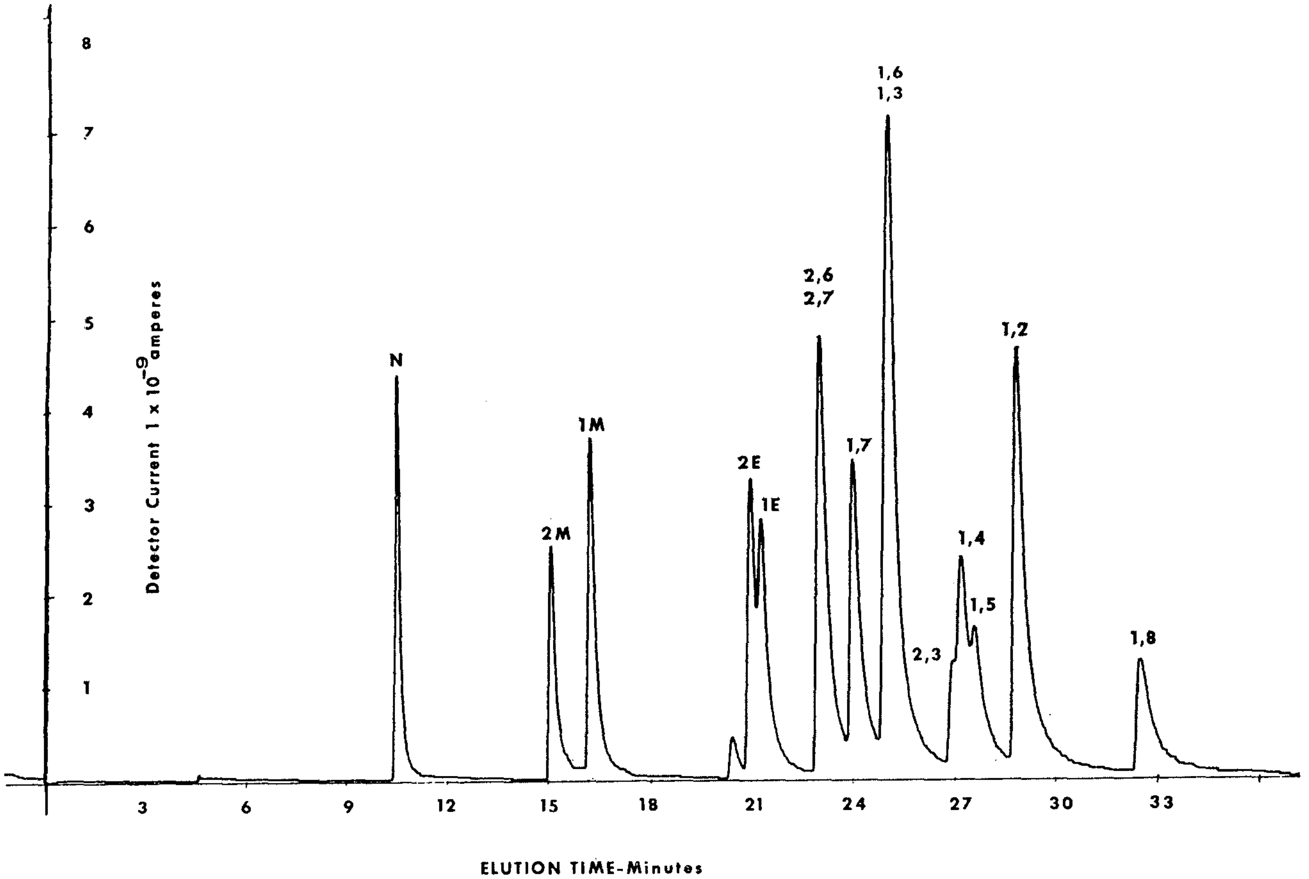

Figure 2. Separation of naphthalenes with Apiezon L

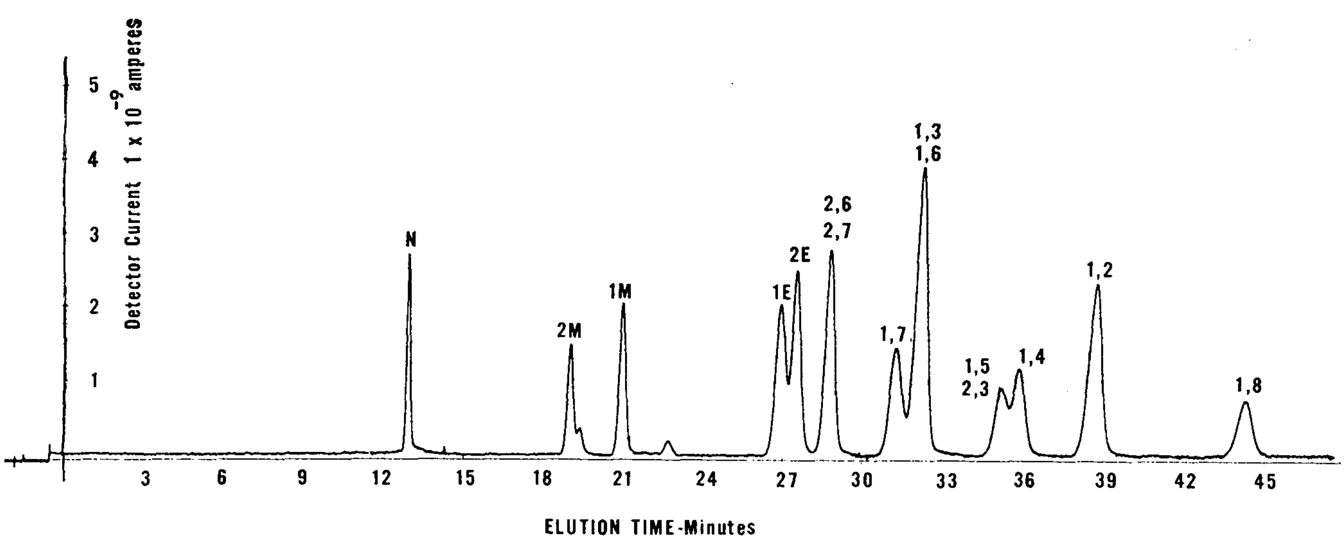

Figure 3. Separation of naphthalenes with Ucon 2000

tailed badly on all components (Figure 2). This seems characteristic of this column with this class of compounds, since it operates satisfactorily with a paraffin mixture in the same boiling range.

To achieve a separation without peak tailing, a number of substrates operable in this range (150° to 200° F.) were investigated. The Ucon 2000 200-foot × 0.01-inch column separated the 1-ethyl- and 2-ethylnaphthalenes without tailing and in a reasonable elution time (Figure 3). Retention data for these 15 components are tabulated in Table II.

In investigating the quantitative aspects of this system, it was found that these high boiling components could not be quantitatively split unless the temperature of the splitter tee was kept above the normal boiling point of the components being analyzed.

DISCUSSION

It was possible to achieve qualitative analytical results for the naphthalenes boiling through 270° C. by correlating the spectra obtained from the *m*-bis(*m*-phenoxyphenoxy)benzene column and the Ucon 2000 column, since neither column would separate all the points of interest. This method (with appropriate liquid phases) may be applied to other complex compound mixtures, which are similar in boiling point and structure.

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Column Partition Chromatography of Vitamins A and D on Fluoropak 80

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▶ A reversed-phase column chromatographic system allowing the separation of vitamin D from vitamin A is described. The stationary phase consisted of Fluoropak 80 impregnated with iso-octane and the mobile phase was aqueous methanol. Advantages include the use of easily volatile non-u.v.-absorbing solvents which facilitate spectrophotometry and liquid scintillation counting of eluted fractions. Examples illustrating the use of this column are presented.

Although a large variety of techniques are available for partition chromatography of polar compounds, relatively few systems have been described for such separation of nonpolar materials—e.g., vitamins A and D (*4*). Most methods for the latter substances rely on the theoretically less desirable adsorption systems (*1*).

This report introduces the use of Fluoropak 80 impregnated with iso-octane as a stationary phase for the column partition chromatography of vitamin D. Use of this support eliminates a serious drawback of available partition methods, namely the difficulty of obtaining separated material free of the solvents used in chromatography. The mobile phase employed in most of these studies has been 90% methanol.

The column has been used mostly for evaluating the purity of and for purifying radioactivity-labeled vitamin D_3 which may then be used for physiological or biochemical studies. In the latter studies, the Fluoropak column has been used to separate radioactive vitamin D_3 from vitamin A in tissue extracts.

EXPERIMENTAL

Apparatus. A 3-foot glass column (12 mm. i.d.) with fritted glass disk and Teflon stopcock was used with the GME Model V 15^2 fraction collector. Ultraviolet absorption was measured either on the collected fractions with the Beckman DK-2 recording spectrophotometer or between the column and *fraction collector* with the GME Model U.V. 265 I.F. recording attachment coupled to a Texas Instruments recorder. Radioactivity was counted in the TMC Model LP-1 liquid scintillation counter.

Reagents. Flouropak 80 (full range particle size) was purchased from the Fluorocarbon Co., 1206 East Ash Ave., Fullerton, Calif. Sieving revealed that practically all the particles were between 20 to 40 mesh, and material between these sizes was used. Iso-octane (2,2,4-trimethylpentane) was obtained from Phillips Petroleum Co., Bartlesville, Okla. The scintillator solution added to each sample for counting was 10 ml. of toluene containing 0.4% diphenyloxazole (DPO) and 0.01% 1,4-di[2-(5-phenyloxazolyl)]-benzene (POPOP).

Procedure. Equal volumes of iso-octane and 90% methanol were equilibrated. Some of the stationary liquid phase (iso-octane) was mixed with dry Fluoropak 80 to impregnate the solid support. The column was filled with iso-octane and the impregnated Fluoropak 80 added to a height of 80 cm. The solid support was then gently tamped with a glass rod until the column was 75 to 77 cm. The iso-octane level was lowered to the top of the Fluoropak and at least 100 ml. of mobile phase (90% methanol) was slowly run through and the sample applied in about 1 ml. of mobile phase. Several hundred μg. of unlabeled vitamin D_3 marker may be added to facilitate location to the vitamin D_3 zone when separating radioactive vitamin D_3 from tissue extracts. Elution was then begun and fractions of 5 or 10 ml. were collected. For radioactivity measurement, the test tube contents were transferred to a 10-dram glass vial, dried with air, dissolved in scintillator solution, and counted.

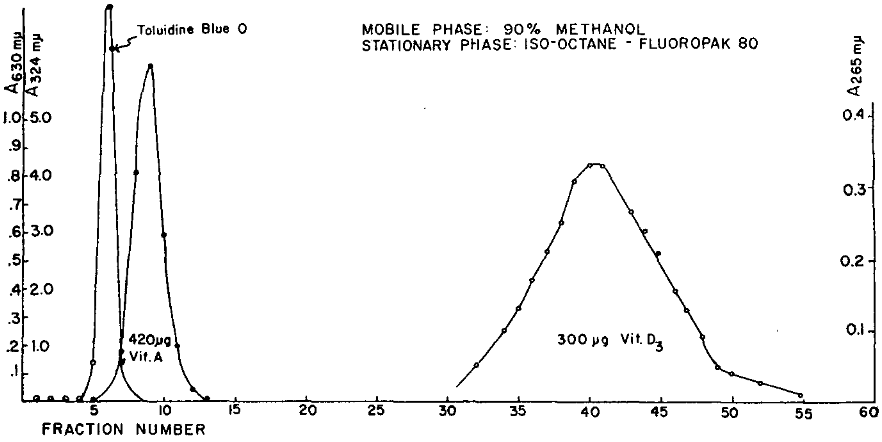

Figure 1. Separation of vitamins A and D_3 on Fluoropak 80

Table I. Recoveries of Vitamins A and D from Fluoropak 80 Column

Temperature	Sample	Added, μg.	Recovered, μg.	Recovered, %
4° C.	Vitamin D_3	300	295	98.3
	Vitamin D_3	3000	2985	99.6
	Vitamin A_1	420	412	98.2
25–30° C.	Vitamin D_3	1077	1007	93.5
	Vitamin D_3	1255	1155	92.0
	Vitamin D_3	940	896	95.4
	Vitamin A_1	2950	2150	73.0
	Vitamin D_2	400	388	97.2

RESULTS AND DISCUSSION

Figure 1 shows a typical separation of vitamin A_1 and D_3 standards. Toluidine Blue 0, which partitions exclusively into the methanol phase, was added simply to illustrate the solvent front, peak of which was at 30 ml. With 90% methanol, vitamin A emerged very soon after the solvent front, while the peak of vitamin D_3 occurred at 220 ml. As evident from the figure, separation of these two vitamins was excellent.

Stability of vitamin D on the Fluoropak 80 column was checked by running paper chromatograms of pooled column eluates in the systems described by Kodicek (*2*). The paper chromatograms were heated to 80° C. in an oven after dipping through 25% antimony trichloride/$CHCl_3$ (*3*). No colored or fluorescent breakdown products were observed.

Recoveries of vitamin D_3 (as well as vitamin A) were essentially quantitative at 4° C. (column set up in walk-in refrigerator), as shown in Table I. At summer room temperatures, recoveries of the less stable vitamin A were not so good as at the lower temperature, but vitamin D_3 recoveries always exceeded 92%.

A primary application of the Fluoropak column has been to purify radioactively labeled vitamin D_3 for physiological or biochemical studies. An example is illustrated in Figure 2 where 1 mg. of vitamin D_3-7-H^3 was run. The radiochemical purity of the vitamin D_3 exceeded 97% and the non-vitamin D_3 radioactivity was present in u.v.-absorbing material more polar than the vitamin and eluted earlier from the column.

The radioactive contaminant present with the original labeled vitamin D_3-7-H^3 could have arisen from breakdown of the vitamin D_3 itself. A variety of experimental conditions can cause destruction of vitamin D. While it is not the purpose of the present paper to present extensive data on this point, it is necessary to indicate that the Fluoropak column effectively separates vitamin D from its more polar breakdown products produced by ultraviolet irradiation, exposure to oxygen, and dispersion into water. An illustration of this type of separation is shown in Figure 3, where the last mentioned

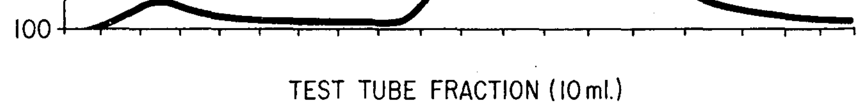

Figure 2. Vitamin D_3-7-H^3

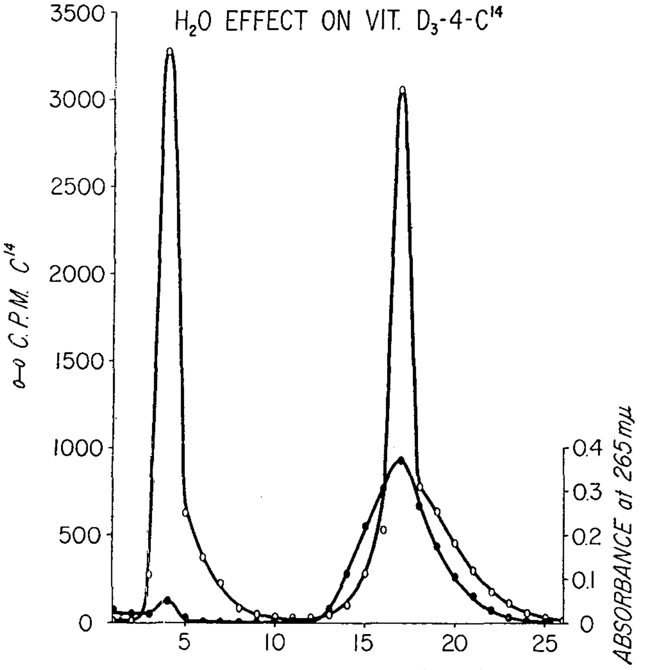

Figure 3. Effect of water on vitamin D_3-4-C^{14}

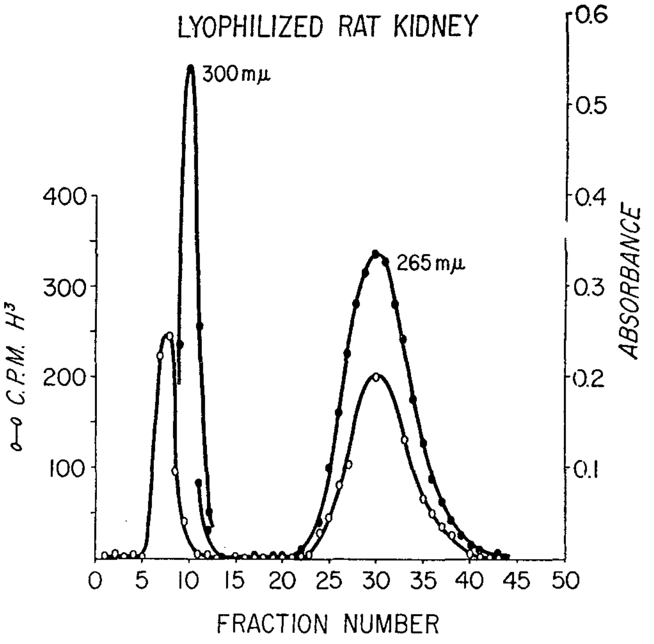

Figure 4. Lyophilized rat kidney with 400 μg. added vitamin D_3 marker

procedure was employed. Five μg. of vitamin D_3-4-C^{14} in 10 μl. of methanol was rapidly squirted into 25 ml. of water, the dispersion allowed to stand for 24 hours, dried under vacuum, and applied to the Fluoropak column. With concentrations of vitamin D large enough to be followed by u.v. absorption—e.g., 10 to 20 μg. per ml.—this breakdown could be observed as a loss in absorbance and shift in peak to longer wavelengths. Further details on studies of vitamin D stability will be reported elsewhere.

The Fluoropak column may also find application to physiological studies in the chromatography of tissue extracts. In preliminary experiments lyophilized or saponified rat tissues, extracted with chloroform (or dichloromethane) and ethyl acetate were used. Chromatographic eluates from liver extract prepared in the above manner exhibited an early peak of relatively polar u.v.-absorbing material running with and near vitamin A, as in Figure 4. Most of the absorbance was due to this vitamin or its breakdown products. In the region where vitamin D was eluted (130 to 250 ml.), practically no ultraviolet absorbance was evident with extracts of normal liver or other soft tissues. This indicated the futility of demonstrating normal tissue vitamin D levels by ultraviolet absorption alone but gave hope that more sensitive quantitation methods might be developed in the future for chemically estimating tissue vitamin D levels following a separation of this type.

Exhaustive surveys of either the stationary liquid phase or the mobile phase were not made, and perhaps other combinations of solvents can be developed for other purposes. The distribution ratio of vitamin D between 90% MeOH and iso-octane was about 0.22, rising to 0.5 at a concentration of 95% MeOH. Thus elution characteristics may be easily varied by altering the alcohol concentration of the mobile phase.

The liquid stationary phase was less amenable to changes. Without iso-octane, there must be some adsorption effects, for if methanol in concentrations of 70% or above was run over Fluoropak 80 powder, the sterols all remained in the alcohol. However, at 65% methanol separations between vitamins A and D were possible, although there was tailing and the solubilities of sterols in this concentration of methanol were poor.

The major advantages of the reversed-phase system described above are: the inert stationary support which does not decompose or otherwise contribute impurities to the eluates; use of solvents which are available in pure form at low cost and which are transparent in the visible and u.v. regions allowing direct absorbance measurements without evaporation or further purification; and use of solvents which are completely and readily volatile, facilitating the application of liquid scintillation counting or other analytical techniques to column eluates.

A disadvantage which may arise in attempting to chromatograph large amounts of crude extract is the necessity of dissolving the material in 90% methanol. Some extracts are rather poorly soluble in this solvent and may require preliminary purifications before running on Fluoropak 80.

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A High-Temperature Inlet Manifold for Coupling a Gas Chromatograph to the Time-of-Flight Mass Spectrometer

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▶ The usefulness of the gas chromatograph time-of-flight mass spectrometer is to a very great extent a function of the inlet system used to couple the two units. This paper describes an inlet system which was designed for applications to higher boiling materials, capable of sustained operation at 200° C. and intermittent operation up to 250° C. Internal volume of the manifold has been reduced to a minimum to provide resolution in the time-of-flight (TOF) manifold equivalent to the gas chromatograph (GC) resolution. Construction materials are of stainless steel and quick-acting toggle valves are employed. The valves are of bolted bonnet design and any part may be repaired or replaced without removing the valve body. The entire manifold is enclosed in an oven with an adjustable temperature control. The system has been used successfully for materials boiling from −128° to 314° C. and with concentrations in the p.p.m. range.

THE COMBINATION of the Bendix time-of-flight mass spectrometer with the gas chromatograph has proved to be an extremely useful and versatile qualitative tool. Two general schemes have been used to couple the two together—that is to take the GC effluent at or slightly above atmospheric pressure and introduce it into the TOF mass spectrometer which is under a pressure of 10^{-6} to 10^{-8} mm. of Hg. The first method proposed by Gohlke (*2*) in 1959 made use of a single leak which passed a fraction of the GC effluent continuously into the mass spectrometer and photographic recording of the spectra as they appeared on the oscilloscope screen. Ebert (*1*) used the more sophisticated analog output system for presentation of spectra and trapped sections of the GC effluent and then allowed the material to leak into the mass spectrometer.

We have used the second approach since the trapping technique allows one to obtain a higher quality spectrum and for very small components in a mixture, enhances the sensitivity. However, work with the Ebert manifold (*1*) demonstrated several design deficiencies. The large internal volume seriously degraded the resolution of the GC and lowered the sensitivity and resolution of the unit for the detection and identification of trace materials. The solenoid valves could not be maintained in a leak-free condition. The use of heating tape for temperature control resulted in local overheating and generally poor temperature regulation. The use of copper and brass as materials of construction resulted in corrosion. Accessibility of the constituent parts of the manifold for repair or replacement was poor. The maximum permissible temperature was approximately 150° C.

Because of the limited usefulness of the manifold described by Ebert (*1*), an inlet system for the GC-TOF unit which avoids the objections raised above was designed.

Design Considerations. Components constituting 0.1% or more of the sample can be passed directly into the mass spectrometer through a leak valve, but for components in smaller concentrations, the sample is so diluted with the helium carrier gas that a usable spectrum can be obtained only at the expense of the vacuum system. To circumvent this problem, the sample can be condensed with liquid nitrogen and the helium removed with an auxiliary vacuum system. On the basis of the above considerations, a trapping system must contain a minimum of two traps to be efficient, a direct-run trap and a cold trap.

Because of the desirability of obtaining a spectrum of every component as it emerges from the GC and thus avoiding time-consuming repeat runs, the addition of more traps seems advisable; however, there are several factors which completely negate any advantage of additional traps. Spectra can be obtained with the direct-run trap in less than one minute; consequently, more than one direct-run trap is of very limited usefulness and introduces larger maintenance problems. Furthermore, as a manifold increases in size and complexity, diffusion of the GC effluent markedly decreases the resolution available at the TOF mass spectrometer. The cold trap which is used for minor constituents must be located near the GC outlet as any diffusion of the small peaks results in loss of resolution. If a second cold trap is added, the sample must pass through two three-way valves before entering the trap, and as a result this trap is useful only for clearly resolved components. In work with higher boiling materials, the limiting factor with respect to the speed with which spectra can be obtained is the pumping speed of the mass spectrometer vacuum system and the over-all speed is not materially increased with more than two traps.

Maximum retention of the GC resolution is of paramount importance in the construction of an inlet manifold system; this requires connections of minimum internal volume. A second advantage of minimizing the internal volume of the manifold is the reduction of clean-up time of a trap after a sample has been trapped and its spectrum obtained.

The speed with which components emerge from the GC dictates the use of quick-acting valves which, in turn, must be capable of operation in an environment of at least 200° C. The mass spectrometer leak valves must also be of quick-acting design, and those described by Ebert (*1*) are satisfactory.

The applicability of mass spectroscopy is most severely limited by compounds having low vapor pressure as it is necessary to introduce the sample into the mass spectrometer as a vapor. Heating the inlet manifold will extend the range of the system; consequently, an oven was designed to maintain the inlet manifold at the desired temperature. The valves do not touch the oven, but are heated by radiation and convection.

The materials of construction were principally stainless steel and Teflon (E. I. du Pont de Nemours & Co.) TFE-fluorocarbon resin. The Teflon was confined to valve seats and gaskets. The area of Teflon fluorocarbon exposed directly to the mass spectrometer vacuum was extremely small (in the

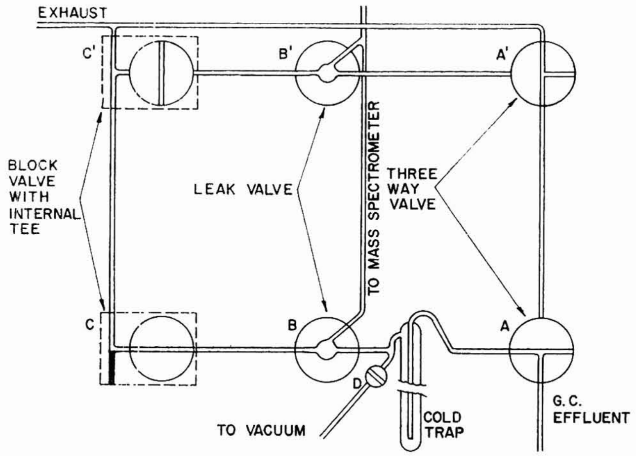

Figure 1. Schematic showing layout of inlet manifold

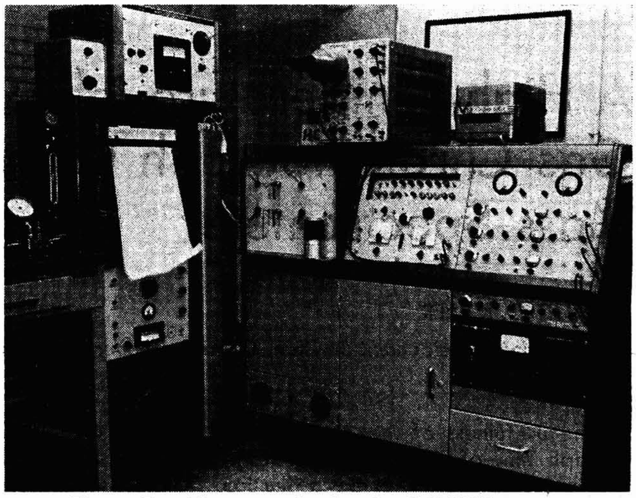

Figure 2. Photograph of entire GC-TOF system

order of 2 to 3 square mm.), and no decomposition products were noted in the mass spectrometer background. Copper and brass were rigorously excluded except for use in the exhaust line.

The cold trap must be heated after the helium is removed to increase the vapor pressure of the trapped constituent. Direct resistive heating was found to be rapid and convenient.

Sample Input System. The sample input system is designed so that a portion of the GC effluent can be isolated and then passed through a leak valve into the TOF mass spectrometer where its spectrum can be obtained. The leak valves are identical to those described by Ebert (*1*). The three-way valves are George W. Dahl Demi valves, code number SBT, constructed of stainless steel and with Teflon fluorocarbon seats for vacuum service. The valve ports were modified by the supplier so that the outlets were 180° and 90° from the inlet. The two-way valves were also supplied by the Dahl Company and are code number H1B. These are block values with an internal "tee." All of these valves are equipped with toggle actuation and are fabricated for panel mounting. The valves contained $^{1}/_{8}$-inch female NPT connections, and plugs were machined to completely fill the connections. The plugs were drilled with a 0.065-inch hole and connections were made between valves with $^{1}/_{8}$-inch stainless steel tubing of the same i.d. The joints were silver soldered.

The block valve, which controls the vacuum to the cold trap, is a modification of a standard Hoke high-temperature solenoid valve (series 90, normally closed). The valve body was drilled up through the seat so that a $^{1}/_{8}$-inch o.d. stainless steel tube could be inserted and silver soldered in place. A new valve seat was formed from the projecting tip of the $^{1}/_{8}$-inch tubing. What had been the original outlet side of the valve body was milled off and plugged. This modification resulted in a significant reduction of the cold trap volume. The pressure differential across this valve is small enough so that leakage presents no problem. The actuating coil was placed outside the oven to protect it from overheating. The entire manifold was mounted on a Transite board with the valve handles protruding through the front.

An aluminum box was fabricated to enclose the valve bodies and two 250-watt strip heaters were attached to the box. The output of these heaters is controlled by a Fenwal thermoregulator, Number 17002. The leak valve adjustments protrude through the back of the oven for convenience of adjustment, and access to the Fenwal temperature adjustment is provided. At 200° C., the temperature of the manifold was monitored at several points and a maximum temperature variation of 10° C. was observed.

To reduce the time required for cold trapping, the trap was constructed of thin-wall stainless steel tubing, the inner tube was 15 gauge with an i.d. of 0.054-inch and the outer tube was $^{3}/_{16}$-inch o.d. Means were devised to heat the trap by direct resistive heating. The output of a low-voltage, high-current transformer was attached to the ends of the trap and a toggle switch (spring-loaded in the off position) was installed in the 115-volt line to the transformer primary. The secondary leads are 12-gauge copper wire; however, these were attached to the trap with short lengths of 20-gauge copper wire. The 20-gauge connections prevent the electrical leads from acting as heat sinks after the trap has been heated.

This heating system allows a complete cold trapping cycle (trap evacuated, condense sample, pump off helium, warm trap, obtain spectrum, and pump out trap) to be completed in approximately 2 minutes for volatile material. The trap can be heated from −190° to 50° C. in 10 seconds with a 60-ampere current at 2.5 volts.

Figure 1 is a schematic of the manifold viewed from the back. The GC effluent enters the manifold at the three-way valve *A* by means of a 15-gauge 0.054-inch i.d. stainless steel tube. From *A*, the flow can be directed through the cold trap and out through *C* to exhaust. Alternately, the flow can be direct to *A'*, where it can be routed through the direct run trap or directly to exhaust. The leak valves (*B* and *B'*) are modified so that they are open to flow-through regardless of the position of the valve stem.

In operation, the cold trap is normally kept closed and evacuated; when it is desirable to trap a small fraction, the trap is cooled with liquid nitrogen, valve A is positioned so that flow is directed into the trap just as the peak emerges from the GC detector, as indicated by the GC recorder. After a slight delay, valve *C* is opened and the effluent is allowed to flow through the cold trap to exhaust. When sufficient material has been collected, the trap is closed by means of valves *A* and *C*, the helium is pumped off through *D*, *D* is closed, the trap is heated, and the sample is passed into the mass spectrometer through the leak valve *B*. The delay in opening *C* allows a small positive pressure to build up in the trap and thus prevents material from being pulled from the exhaust line back into the trap. Cleanup is accomplished by heat and vacuum.

For large fractions, the effluent is routed from *A* to *A'* and thence to *C'* and then to exhaust. As the peak reaches a maximum, *A'* and *C'* are closed and the sample admitted directly through *B'* to the mass spectrometer. After a satisfactory spectrum has been obtained, the trap is cleaned by purging with the GC effluent.

Figure 2 shows the entire GC-TOF unit with accessory equipment and the inlet manifold in place. The connection from the GC detector is visible at the lower left of the inlet manifold.

CONCLUSION

A high-temperature inlet manifold has been described for coupling a gas chromatograph to the time-of-flight mass spectrometer. The system has been in daily use for over a year and has been used for the identification of unknown materials on a routine basis during this time. Applications have covered materials boiling from −128°

to over 300° C. With high-temperature operation, thermal decomposition is a problem; however, compounds which have sufficient thermal stability to permit separation by GC do not generally decompose in the inlet system. Spectra of volatile material at the 5 to 10 p.p.m. concentration can readily be obtained using the conventional 20- to 50-µl. GC sample. The minimum volume design of the manifold gives resolution in the manifold comparable to that indicated by the GC detector.

Constant usage has demonstrated that the inlet system is free of maintenance problems and is applicable to a large number of organic materials.

ACKNOWLEDGMENT

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Gas Chromatography of Aqueous Solutions. Determination of Hydrocarbons and Halocarbons

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▶ The interference from water in the gas chromatographic analysis of aqueous solutions of hydrocarbons or halocarbons can be avoided by use of a precolumn which completely removes the water without absorbing these organic compounds. A column containing a mixture of nine parts of phosphorus pentoxide and one part of Desicote- and Siliclad-treated firebrick can be used to analyze over 50 successive 10-µl. samples.

Gas chromatographic analyses of aqueous organic solutions are usually hampered seriously by the large tailing of the water peak. This situation can be overcome by using a detector such as the hydrogen flame which is relatively insensitive to the presence of water vapor (*3*). However, there are times when this type of equipment is not available or cannot be used, either because of the potential hazard or because it is necessary to determine other components such as CO, CO_2, N_2, O_2, and N_2O which are not detected by the hydrogen flame detector.

Aqueous organic solutions can best be analyzed by gas chromatography when the water is removed by absorption or by reaction with a material to produce a more volatile and easily eluted gas. Kung, Whitney, and Cavagnol (*2*) have used a precolumn of calcium carbide to convert the water vapor to acetylene. The disadvantage of this technique lies in the possibility that the acetylene will mask other components in the solution.

Drying agents normally used in analytical chemistry have not been employed for the absorption of water injected into a gas chromatograph, probably because most of them are not efficient enough to remove all the water. Phosphorus pentoxide, however, is an excellent drying agent and experiments in this laboratory have shown that a precolumn of this material is capable of absorbing all the water contained in microliter samples injected into a gas chromatograph.

In the preparation of the absorption column described, the essential feature is the use of a special silicone-coated firebrick as an inert granular support for the powdered phosphorus pentoxide. Using a precolumn of this mixture to absorb water before it enters the partition column extends the life of the partition column, reveals components masked by the water peak, shortens the analysis time, and allows use of more specific partition columns for resolving the dissolved components.

EXPERIMENTAL

Apparatus. An F&M Scientific Corp., Model 119C, gas chromatograph, equipped with a 100,000-ohm thermistor detector and a stainless steel preheater was used in all analyses. The detector signal was supplied to a 1-mv. Brown recorder. All columns were prepared from 0.25-inch o.d. copper tubing and the connections were made with Swagelok fittings (Crawford Fitting Co., 8811 East 140th St., Cleveland 10, Ohio).

Gas Chromatograph Conditions. The gas chromatograph columns were composed of two parts: *A*, water absorption column; and *B*, partition column. The two columns were connected in series in the order *A-B* with the preheater attached to the water absorption column and the partition column attached to the detector. The preheater was maintained at 200° C. while the columns and detector cell were operated at 40° C. The flow of carrier gas, helium, was maintained at a rate of 40 ml. per minute.

Preparation of Phosphorus Pentoxide Absorption Column. The phosphorus pentoxide absorption column was prepared by packing a 16-inch length of copper tubing with a mixture of nine parts of phosphorus pentoxide powder and one part of 60- to 80-mesh firebrick which had been coated with Desicote (Beckman Instruments, Inc., Catalog No. 18772) and Siliclad (Clay-Adams Inc., Catalog No. J-600).

The firebrick used in the water absorption column was prepared by treating 200 grams of 60- to 80-mesh firebrick (F&M Scientific Corp.) with 50 ml. of Desicote. After this mixture was rolled for 2 hours, it was transferred to a Buchner funnel and washed with 200-ml. portions of acetone until the acetone filtrate was colorless. This treated firebrick was air-dried by suction on the Buchner funnel for one-half hour and then it was dried overnight in an oven at 100° C.

The Desicote-treated firebrick was mixed with 600 ml. of water containing 40 ml. of Siliclad solution in a similar manner with the exception that the treated firebrick was washed twice with water in place of acetone.

One gram of the Desicote- and Siliclad-treated firebrick was mixed with 9 grams of phosphorus pentoxide powder in a tightly capped 20-ml. capacity vial. A polyethylene funnel was attached by Tygon tubing to one end of a 16-inch length of copper tubing; the other end of the copper tubing was plugged with borosilicate glass wool and capped with a rubber septum. The vial was shaken for about 3 minutes, or until the firebrick and phosphorus pentoxide powder were thoroughly mixed. It was then uncapped and quickly inverted in the funnel. While the vial was held tightly in the funnel, the column was vibrated with a small vibrating tool. The flow of material from the funnel into the column was observed through the clear Tygon tubing.

The packing of a 16-inch length of

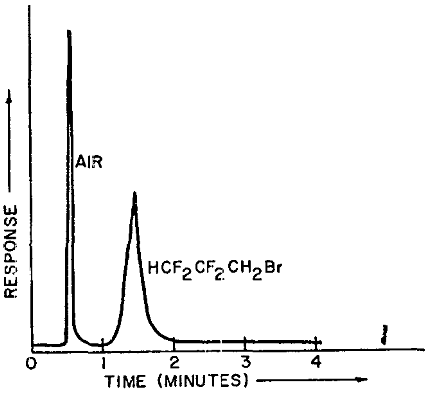

Figure 1. Chromatogram of aqueous organic solution with fresh absorption column

Column: A. P_2O_5 absorption precolumn
B. DC-710 oil partition column
Col. temp.: 40° C.
Detector: 100,000-ohm thermistor
He flow rate: 40 ml. per minute
Sample: 10-µl. aqueous solution

copper tubing was accomplished in about 3 minutes by means of this technique. When the packing was complete (approximately half of the material in the vial was needed to pack a 16-inch length of copper tubing), the open end of the tubing was plugged with borosilicate glass wool and capped with a rubber septum. Several such columns were prepared at one time and stored until needed.

Partition Column. The partition column used in this investigation was a 12-inch length of copper tubing packed with a mixture of 20% (w./w.) Dow Corning 710 Silicone oil and 80% (w./w.) 60- to 80-mesh firebrick which had been coated with Desicote.

Comparison of Desiccants. Silica gel, Drierite, Molecular Sieve 5A, alumina, diglycerol, and phosphorus pentoxide powder were each packed in gas chromatograph columns and were tested for their ability to absorb water from microliter samples of an aqueous solution injected into the gas chromatograph. Phosphorus pentoxide powder and diglycerol were the only desiccants which completely absorbed the water under these conditions. The diglycerol column had a very poor water absorbent capacity and it caused the retention times of organic compounds to change as water was absorbed.

Comparison of Inert Supports. Various inert support materials were mixed with phosphorus pentoxide powder in an effort to find a mixture which would not clog the column. The various materials which were tested are listed in Table I. Each of the absorbent mixtures was packed in a 16-inch length of copper tubing in the same manner as that described for the Siliclad- and Desicote-treated firebrick. The absorption columns were tested under identical gas chromatographic conditions by serially injecting 10-µl. samples of an aqueous solution containing 0.4% of halopropane (3-bromo-1,1,2,2-tetrafluoropropane; b.p. 74° C.).

RESULTS AND DISCUSSION

While there are a number of materials classified as drying agents, only those which could be packed in a gas chromatograph column and remain stable at elevated temperatures were considered. Phosphorus pentoxide was the only desiccant tested which completely absorbed the water injected into a glass chromatograph without absorbing halopropane.

However, phosphorus pentoxide is such a good desiccant that it was very difficult to pack the powder in a 0.25-inch o.d. gas chromatograph column. An even more serious problem occurred after the injection of approximately 0.05 ml. of water. The absorption of water by the phosphorus pentoxide turned the powder into a viscous liquid (phosphoric acid) which clogged the column and rendered the column useless for further analyses.

An inert material was mixed with the phosphorus pentoxide to prevent the absorption column from clogging. However, even with the use of an inert material as a carrier and diluent the absorption of water by the phosphorus pentoxide eventually produced a wet column which caused gas-flow disturbances. The gas-flow disturbances in the absorption column caused erratic noise signals. The injection of more water onto the absorption column caused an increase in the noise signal until it completely obscured the signal due to the halopropane. The breakthrough volume of an absorption column was arbitrarily set at the amount of water absorbed before the noise fluctuations became equal to the signal from 10^{-6} gram of halopropane (100 p.p.m. in a 10-µl. sample).

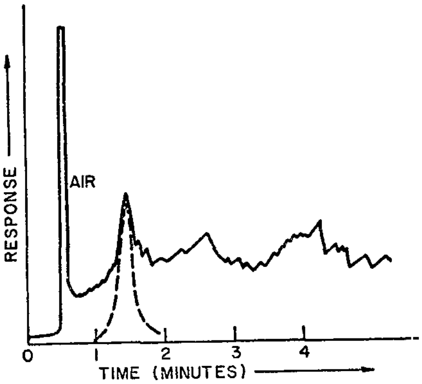

Figure 2. Chromatogram of aqueous organic solution after injection of 0.53 ml. of water

Column: A. P_2O_5 absorption precolumn
B. DC-710 oil partition column
Col. temp.: 40° C.
Detector: 100,000-ohm thermistor
He flow rate: 40 ml. per minute
Sample: 10-µl. aqueous solution

Table I. Performance of Phosphorus Pentoxide Absorption Columns with Various Supports

Support material	Wt. % support[a]	Breakthrough volume, ml. H_2O	Comment
Untreated firebrick, 60- to 80-mesh	10	0.30	Absorbed halopropane
Desicote-treated firebrick, 60- to 80-mesh	10	0.40	Easy to pack
Siliclad-treated firebrick, 60- to 80-mesh	10	0.40	Easy to pack
Siliclad- and Desicote-treated firebrick, 60- to 80-mesh	10	0.55	Easy to pack
Asbestos fibers	5	0.60	Very difficult to pack
Asbestos powder, 100 mesh	10	0.40	Absorbed halopropane
Glass beads, 60- to 80-mesh	25	0.50	Difficult to pack

[a] Phosphorus pentoxide powder comprised the remainder of the column packing.

The concentrations of absorbent, phosphorus pentoxide, and support shown in Table I represent the optimum combination for each support material to give the maximum breakthrough volume. Higher temperatures and greater carrier gas flow rates did not appreciably affect this volume. Greater column lengths also failed to increase the absorption capacity. While columns with diameters greater than 0.25 inch were not tested, it is very probable that they would give an increased breakthrough volume.

The Siliclad- and Desicote-treated firebrick was chosen as the inert support since the results, as given in Table I, clearly indicated that it has the best combination of absorber capacity and ease of packing. An absorption column ($^1/_4$ inch × 16 inches) containing 3.7 grams of phosphorus pentoxide had a breakthrough volume of 0.53 gram of water. This is equivalent to a 37.5% conversion of P_2O_5 to H_3PO_4. Approximately a dozen such columns have been used with an average breakthrough volume of 0.53 ± 0.03 ml. of water.

Figure 1 shows a typical chromatogram of an aqueous solution containing 100 p.p.m. of halopropane after injecting 52 successive 10-µl. samples. The standard deviation for this analysis is 1 p.p.m. Figure 2 shows a chromatogram obtained from the same column after the 53rd sample was injected. The noise level from the gas flow disturbance has completely obscured the halopropane peak which is represented by the dotted line.

The aqueous organic solutions can be easily and quickly analyzed by gas chromatography when the water is completely removed by absorption with a good desiccant. Furthermore, the desiccant can be used in a gas chromatographic column when it is mixed with an inert support such as silicone-coated firebrick.

An absorption column prepared from phosphorus pentoxide will interfere with the determination of ethyl alcohol, ether, and acetone since these components are partially absorbed by the phosphorus pentoxide power. However, materials such as saturated hydrocarbons and halogenated hydrocarbons are not absorbed by the phosphorus pentoxide.

An absorption column as described above has been used to allow the gas chromatographic determination of the solubility of halopropane in water. Also, a gas chromatographic procedure using this absorption column has been developed and is being used for the direct gas chromatographic determination of volatile anesthetics in whole blood (*1*).

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Analysis of Volatile Inorganic Fluorides by Gas Liquid Chromatography

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▶ **Specialized equipment and techniques were needed to apply gas liquid chromatography to the analysis of highly reactive, volatile inorganic fluorides. Poly(trifluoromonochloroethylene) oils (Kel-F) were suitable for use as stationary phases after chemical stabilization. Using the equipment developed, in-line analyses of plant streams containing uranium hexafluoride, chlorine trifluoride, chlorine, hydrogen fluoride, and other reactive gas have been made. To speed analysis, a split column chromatograph has been developed and automated.**

The atomic energy industry, by its demand for new and unusual materials, has been responsible for the development of a number of industrial processes which involve the handling of volatile inorganic halides, particularly fluorides. Probably the most impressive example is in the production and isotopic enrichment of uranium hexafluoride.

The analysis of process streams of these halides for the purposes of development or production presents a problem which is practically insoluble by analytical methods that depend upon the chemical reactivity of the gases, since hydrolysis, which is virtually the only method of attack, yields the same products from widely differing molecular species. In some cases it has proved possible to devise methods for the determination of a specific constituent of a mixture, but for general analysis, attempts have been made to employ physical rather than chemical methods. Vapor pressure measurements, selective adsorption, fractional distillation, sonic measurements (*6*), nuclear magnetic resonance (*12*, *23*), mass spectrometry (*1*, *2*), and infrared spectrometry (*16*, *26*) have all been tried with little or limited success because of the chemical reactivity of the compounds involved.

The advent of gas chromatography, however, offered the possibility of a general method of analysis with fewer restrictions than any other method, and development of the technique for the analysis of halide mixtures was commenced in 1954. This has been successful to the point where reliable, automatic, in-line analyzers have been produced to analyze almost any halide mixture, and a number of different aspects of the work haue been reported (*13–15*, *17*, *18*, *24*, *25*). This report presents a general account of the work and describes the techniques evolved to meet the difficulties of this application of gas chromatography.

EXPERIMENTAL

Apparatus. General Problems. Because of the reactivity of halides toward structural materials, moisture, and organic vapors, in all analytical work with these gases there is continual uncertainty as to the stability of the sample and, consequently, in the interpretation of results.

These factors had considerable influence on the design and operation of a chromatograph. A high standard of cleanliness was necessary in its construction, and every effort was made to keep extraneous materials out of the apparatus during its subsequent operation.

No material was found that was completely inert to all possible combinations of inorganic fluorides, although nickel, Monel, and aluminum, if suitably treated, and polymers such as Kel-F (Minnesota Mining & Mfg. Co.) and Teflon (E. I. du Pont de Nemours & Co.) were reasonably resistant.

To have fabricated all the components from nickel or Monel would have been extremely tedious and expensive. Fortunately, corrosion tests carried out on commercially available components of brass or copper protected by Electroless (*4*, *11*) nickel plate showed that the corrosion resistance imparted by this plate was at least as good as that of pure nickel.

For experimental purposes, plating was carried out at 98° ± 1° C. in a bath of the following composition (grams per liter): nickel chloride, 21.25; sodium hypophosphite, 25.85; succinic acid, 8.00; and sodium hydroxide, 3.75; adjusted to pH 5.5 with sodium hydroxide. Deposition was allowed to continue until the object appeared to be uniformly plated but was not otherwise controlled.

Nickel was used for all tubing carrying sample gases, joints being made in one of three ways. Permanent joints were made with commercial brass capillary fittings which were nickel-plated and hard-soldered with a silver-zinc solder; joints intended to be broken occasionally were made with commercial compression couplings, nickel-plated except for the soft copper ferrules; and joints intended to be broken frequently were made with nickel-plated, screwed vacuum unions (Edwards High Vacuum Co., Ltd.) incorporating fully trapped, neoprene O-

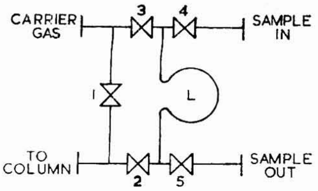

Figure 1. Arrangement of valves in sample introduction system

rings. All these joints gave satisfactory corrosion resistance and vacuum tightness even after heat cycling between room temperature and 100° C. Occasional replacement of the neoprene rings in the screwed unions was necessary. They hardened slowly by reaction with the gases diffusing into them but rarely failed as long as the joint was undisturbed. Teflon O-rings were not available during the course of this work.

Reasonable freedom from the decomposition of samples could be assured only if the internal surfaces of the apparatus were fully fluorinated before use and carefully preserved from contact with anything, including atmospheric moisture, that might destroy the fluoride film. All assemblies were, therefore, tested by conventional vacuum techniques. A limiting inleakage rate of 1 centiliter of gas at 1-micron pressure per second (clusec) was permitted for all components and was found to provide adequate protection from inleakage of moisture.

Since no known surface was completely inert, it was desirable to keep the elapsed time between withdrawal of a sample from a system and its analysis as short as possible and to keep the surface-to-volume ratio of any enclosure small. While it was possible to observe these precautions to some extent when samples were taken in prefluorinated nickel bottles for transport from process stream to chromatograph, a considerable time was expended in checking the vacuum standard of connections and fluorinating interconnecting pipework before a sample could be drawn from the plant or transferred to the chromatograph. The whole process might take four or five times as long as the eventual gas chromatographic analysis and, despite all precautions, the first aliquots drawn from the sample vessel into the chromatograph invariably showed evidence of reaction with traces of water. The only satisfactory method of sampling was to connect the chromatograph directly into the reaction vessel or process stream and to use some form of flow-through system for drawing samples into the instrument.

Carrier Gas. Both nitrogen and helium have been used with success. Because of the sensitivity of the samples to water vapor, it is necessary for the carrier gas to be kept extremely dry. It was found satisfactory to pass the gas as delivered from a cylinder, through a tube 4 feet 6 inches in length and $^5/_8$ inch in diameter containing Linde Molecular Sieve 5A ($^1/_{16}$-inch pellets). This drier, when activated by heating at 325° C. under vacuum for 3 hours before use, delivered gas with a moisture content of less than 5 volume parts per minute at a rate of 50 ml. per minute for several months before requiring regeneration. The column packing of Kel-F oil on a Kel-F support abstracts water cumulatively even from gas as dry as this, and when an appreciable time elapses between analyses this water must be removed before the next analysis is attempted.

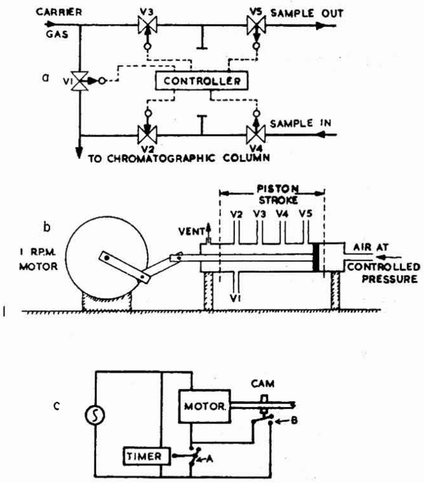

Figure 2. Automatic sample introduction system

a. Schematic diagram
b. Pneumatic operator
c. Electrical control

Sample Introduction. The only form of sample introduction system that has been found to work effectively is illustrated diagrammatically in Figure 1.

By closing valves 2 and 3 and opening 4 and 5, sample flows through the sample loop, *L*. By reversing the procedure, the sample in *L* is isolated and carried on to the column by the carrier gas. Valve 1 allows carrier gas to be bypassed to the column while valves 2 and 3 are closed. The dead spaces between valves 3 and 4, and 2 and 5, were made very small by the use of short lengths of capillary tubing for those connections. The volume of the sample loop was usually 15 ml.

The choice of valves was restricted, since they had to be vacuum-tight and to contain no lubricant or packing. Bellows-sealed refrigeration valves (Danfoss Mfg. Co.) have been used effectively, after nickel plating, for manual systems but are now unobtainable. The more expensive, specialized valves manufactured by Hoke, Inc., Englewood, N. J. (Types M482 and M484), were very satisfactory for this application. Figure 2, *a* and *b*, and Figure 3 show a system constructed for automatic operation by pneumatically driven Hoke valves. On test, this system showed a life in excess of *10,000 operations (18)*, and has given satisfactory service installed on in-line instruments. The system was actuated by the electrical circuit shown in Figure 2, *c*. A preset timing unit operates switch *A*, which starts the motor (Drayton Regulator and Instrument Co., Type R.Q.R., 1 r.p.m.). A cam within the motor then causes *B* to close and when *A* is subsequently opened by the timing unit, the motor continues to run, driving the piston shown in Figure 2, *b*, until it returns to its starting position, when *B* opens and the motor stops.

A considerable effort has been made to simplify the system by the use of multiway valves. Many types have been described, but for use with volatile halides only those that can operate unlubricated are suitable. In practice, the choice has been limited to valves of the type in which a flat plate of Teflon slides on a flat plate of nickel or Monel containing the valve ports. No commercial valves of this type have been found to be vacuum-tight, and specially manufactured valves based on a commercial design, though initially satisfactory, failed after a relatively few operations through galling of the Teflon.

Columns. Initially, no column packing materials were known that were resistant to the action of volatile fluorides, and both a support material and a stationary phase had to be found.

For the majority of analyses the lowest operating temperature for the column was fixed at about 50° C. to avoid condensation of uranium hexafluoride. Possible packings were examined at temperatures between 50° and 100° C. in contact with chlorine trifluoride diluted with an equal volume

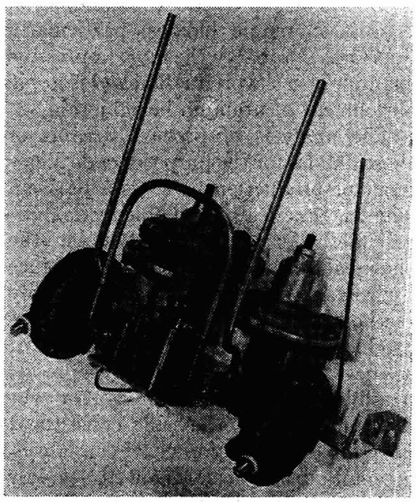

Figure 3. Automatic sample introduction system

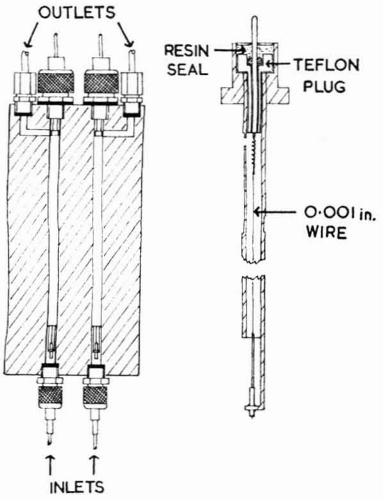

Figure 4. Katharometer and sensing element holder

of nitrogen at atmospheric pressure, and in contact with pure hydrogen fluoride at atmospheric pressure. These were the most reactive mixtures likely to be encountered. Only Kel-F or Teflon supports, coated with Kel-F oils, were sufficiently inert and reversible in their behavior to be used as packing materials (*17*). Of these, Kel-F was chosen as the support material, since the grade then available (Kel-F grade 300 low density molding powder) had a greater capacity for the liquid phase, absorbing 120% of its own weight before becoming tacky, compared with Teflon molding powder which absorbed only 80%. In some circumstances the large capacity was advantageous, but Teflon powder has been used successfully by Ellis (*7–9*).

The two Kel-F oils which were most suitable were Kel-F 10/200 and Kel-F 40. Though some of the less viscous oils gave an apparently increased separating power, their volatility was correspondingly higher, and pronounced bleeding of the columns limited their effective life and caused instability in the detector.

The column packing material was prepared by reducing the Kel-F powder to any required range of particle size by the use of a micro hammer mill (Glen Greston) followed by sieving. The powder was freed from metallic particles and grease by successive extractions, first with hot 5*N* hydrochloric acid, and then, after washing and drying, with Arcton 113 (Imperial Chemical Industries, Ltd.). The purified powder was slurried with a solution of the liquid phase in Arcton 113 and the solvent removed by gentle heating with continuous stirring. The prepared material was packed into the column in the normal manner.

A suggestion has recently been made (*21*) that column materials of this type can be classed as monophase gels and cannot be regarded as completely analogous to the normal concept of a coated inert particle. During our tests of different column materials some support for this concept was obtained, as it was discovered that the uncoated support showed some separative ability for halogens, but attempts to enhance this without the addition of liquid polymer were unsuccessful. However, there is also evidence that the Halocarbon–Kel-F powder used as a separating medium by Lysyj and Newton (*21*) is not the same as that used by us, because the order of elution of the gases HF, F_2, Cl_2, and ClF_3 is very different.

For general work the column and operating conditions defined in Table I have been selected as optimal, though small variations may be needed for particular analyses.

With the aim of reducing contact between the sample and the nickel tube to the minimum, the columns were initially packed in fairly wide tubes ($^1/_4$-inch i.d.). These have subsequently been reduced to $^3/_{16}$-inch i.d. and it appears that the effect, if any, of the nickel wall is not serious.

The use of capillary columns of Teflon or nickel for the analysis of volatile halides has been tried but the results were less satisfactory than when using packed columns (*25*). Moreover, the very small samples that are required for capillary columns increase the difficulties experienced with the sample introduction system.

Detectors. The gas density balance has the great advantage, in working with reactive materials, that the sensing elements do not come into contact with the constituents of the sample, and a Monel balance produced by A. J. P. Martin has been successfully used by Ellis (*7–9*) for analyses of this type. The katharometer detector, however, was considered to have the advantages of robustness and simplicity for plant applications, and has been developed to give a very satisfactory detector for use with reactive halides.

The present form of the katharometer is shown in Figure 4.

The sensing element itself consists of nickel wire 0.001 inch in diameter and $4^1/_2$ inches in length, supported between stouter pieces of nickel wire and tensioned to 5-gram weight by a small spiral spring of nickel wire 0.003 inch in diameter. All joints between wires are silver-soldered, and for ease of assembly and replacement the wires are supported in holders of $^1/_8$-inch-bore tubing which are a push-fit in the katharometer block. The life of the wires in normal use appears to be indefinite, provided the wire temperatures are not excessive. In practice, the current through the bridge has been limited to 200 ma. when nitrogen carrier gas was used or 300 ma. when helium was used. The sensitivity of the detector is compared with that of Gow-Mac katharometers in Table II.

A flame ionization detector has also been developed for the detection of volatile halides (*25*). Though generally it was more sensitive than the katharometer, it proved to have widely differing sensitivities for different compounds, and was particularly insensitive to hydrogen fluoride. It was also rendered unserviceable by the passage of uranium hexafluoride, and was not regarded as offering any general advantage over the katharometer, with which it is compared in Table II.

Table I. Optimum Column and Operating Conditions

Column support	Kel-F 300 low density molding powder
Oil	Kel-F 40
Loading, oil: support	1:5
Mesh size of support	178–211 microns
Column length	14 feet
Operating conditions	
Carrier gas	Nitrogen
Flow rate of gas	10 cc./minute
Temperature	90° C.

Table II. Comparison of Detector Sensitivities

Detector	Carrier gas	Sample gas	Bridge current, ma.	Sensitivity, mv. cc./μg. (*5*)
Katharometer				
Capenhurst	Argon	Oxygen	200	0.164
Gow-Mac tungsten wire, type 9285	Argon	Oxygen	150	0.435
Gow-Mac thermistor, type 9677	Argon	Oxygen	4.5	0.290
Capenhurst	Nitrogen	Chlorine	200	0.340
Capenhurst	Nitrogen	Chlorine trifluoride	200	0.135
Flame ionization				
Capenhurst	Argon	Chlorine	...	77.2
		Chlorine trifluoride	...	40.8
		Hydrogen fluoride	...	4.21

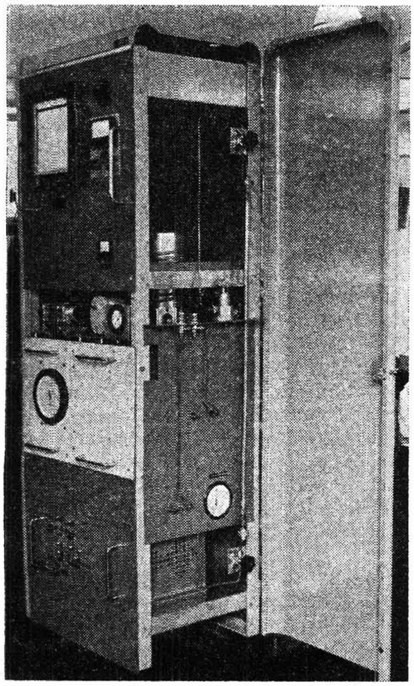

Figure 5. Complete chromatograph

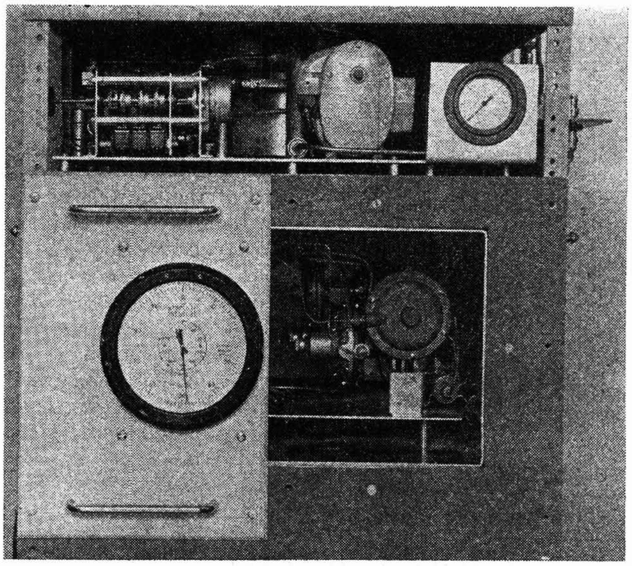

Figure 6. Close-up of timing system and hot box containing sample introduction system

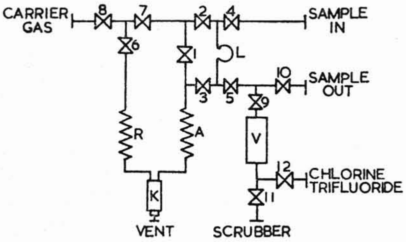

Figure 7. Schematic diagram of complete chromatograph and ancillary valves

By careful design of the heating and insulation of the katharometer it has been possible to reduce the spurious signals due to thermal changes to less than 2 μv. at the output of the bridge and to apply a moderate degree of amplification to increase the sensitivity. A Leeds and Northrup amplifier (Type 9835A) matched to a L. and N. Model H recorder has proved very satisfactory for this purpose.

Assembly of Chromatograph. The engineering of a robust, transportable in-line analyzer incorporating the special components detailed above has been fully described (*13*). Views of the complete instrument are shown in Figures 5 and 6, that in Figure 6 showing the automatic sampling system in its hotbox with the timing unit above. The katharometer is contained within the cylindrical heater that supports the columns, and this has been made visible in Figure 5 by removal of the cover which normally encloses it.

The flow system used is shown in Figure 7.

The carrier gas, dried by the Molecular Sieve column previously described, enters the apparatus through the pressure-reducing valve, 8. The flow is then divided equally by control valves 6 and 7, half passing through a reference column, *R*, and the other half passing via the sample introduction system (valves 1 to 5) to the analysis column, *A*. Both gas streams then enter the katharometer and are united before discharge to the vent. The balancing of the gas flows eliminates base line drift and reduces spurious peaks caused by fluctuations in the ambient atmospheric pressure or the carrier gas supply. Disturbances caused by bleeding of the column material were also reduced in this way.

The volume, *V*, was used when it was impossible to obtain flow-through samples. By evacuating this volume it was possible to sweep a relatively large volume of gas through the sample line and to remove products resulting from reactions with the walls before isolating the actual sample.

Valves 9 to 12 enabled fluorination of the lines to the sample connections to be made after the instrument had been connected up and enabled chlorine trifluoride samples to be introduced into the column for conditioning purposes as described later.

Experimental Procedure. The only differences required from normal technique when reactive fluorides are analyzed are related to the removal of all reactive material from apparatus and to problems associated with absorption.

When the chromatograph is connected into the reaction or process system, a vacuum test must be applied to all the connecting pipework, particularly on the upstream side of the sample introduction system. The interior surfaces of all sample lines and of the sample introduction system are then passivated by evacuating the system and introducing chlorine trifluoride to a pressure of 300 Torr. This is allowed to stand at the operating temperature of the system for at least one hour, or longer if possible, and eventually pumped away through a suitable scrubbing system. A rotary vacuum pump protected by a scrubber 30 inches in length and 2 inches in diameter containing granulated calcium chloride and sodium fluoride, followed by a glass trap cooled in liquid nitrogen, was adequate. Traces of explosive oxyhalogen compounds accumulate in the cold trap, which must be so arranged that it can be purged of its contents by a flow of nitrogen before it is allowed to return to room temperature.

After this treatment, no gas contaminated by water or organic vapor may be allowed to enter the lines unless the passivation treatment is repeated.

The column must next be freed from reactive material by using the sample-introduction system to inject successive samples of pure chlorine trifluoride at a pressure of 300 Torr at intervals of a few minutes. About six samples are injected and after they have been cleared from the column, two successive samples are injected at a suitable interval of time and the chromatograms examined. These should consist of a single large peak and should be identical. If these conditions are not satisfied, the above sequence of treatments is repeated as often as necessary until this result is achieved.

Chromatograms illust;ating typical progress in conditioning a column are illustrated in Figure 8. Chromatogram *a* was obtained when the column was partially conditioned and *b* when it was fully conditioned. Experience has shown that, if the sample gas is allowed to flow continuously through the sample introduction system, this rarely needs further conditioning. The column usually needs to be reconditioned daily

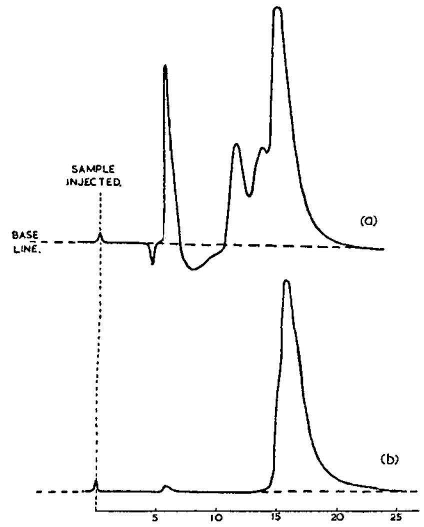

Figure 8. Chromatograms taken during stabilization

a. Unstabilized
b. Stabilized

by a few samples of chlorine trifluoride, the process being repeated until identical traces are obtained. The carrier gas is always kept running.

Volatile inorganic halides almost all exhibit strong physical or chemical sorption on surfaces. In gas chromatographic analysis this has the effect of falsifying chromatograms until all surfaces have been brought into sorption equilibrium with the sample. The net effect is that the apparent analysis of the sample is intermediate between the true composition and the composition of the previous sample. For small changes in sample composition the error is usually unimportant, and changes in the composition of process streams can be followed without difficulty. For large changes, however, such as are encountered when changing from conditioning the column to analysis, it is necessary to pass replicate samples through the column until consistent results are obtained.

In calibrating the chromatograph with pure substances, it is necessary also to guard against errors arising from side reactions and from adsorption effects in the dispensing system. The dispensing system should be conditioned with chlorine trifluoride at 300 Torr, and then equilibrated with a calibration mixture of similar concentration to that desired before the final mixture is introduced. Known pressures of individual gases are introduced into the sample system and the peak heights observed are plotted as a function of pressure. When it is required to calibrate to pressures of less than 10 Torr, it is convenient to use a known mixture of the sample gas with carrier gas.

RESULTS AND DISCUSSION

Calibration. Calibration graphs obtained as described above are shown in Figure 9. With the exception of hydrogen fluoride, which shows a pronounced curve, the graphs are linear. Experiments showed that in most mixtures there was no interaction between the gases which would result in a change of detector sensitivity, and that it was valid to equate partial pressures with the pressures indicated by the calibration graphs. However, in some cases, where samples of high pressure had to be taken if the partial pressure of a minor component was to be sufficient for its response to be measurable, anomalies were observed. It was then necessary to make a special calibration using mixtures of approximately the composition of the gas to be analyzed. These effects again were attributed mainly to adsorption and were particularly noticeable when small quantities of hydrogen fluoride were being analyzed.

Hydrogen fluoride was also anomalous, in that it was the only gas of the group which gave negative peaks when nitrogen was used as carrier gas.

Preparation of Gases for Calibration Purposes. The gases of interest do not form any regular homologous series, and it is therefore necessary to obtain a specimen of each gas in order to calibrate the chromatograph.

In general, bottled gases were used for calibration, where they were available. The purity was normally high, and small corrections for impurity content could be made by inspection of the chromatogram. Where the required gases were not available, recourse was had to the preparation of specimens. Mellor (*22*) provides much information on the preparation and properties of these compounds.

Many of the methods of preparation quoted in the literature were of considerable antiquity and the products, when examined by means of gas chromatography, were found to be grossly impure. However, the use of all-metal apparatus and purification by low temperature fractional distillation using vacuum techniques often enabled samples of sufficient purity to be obtained. Satisfactory results have been obtained with the methods described briefly below.

CHLORINE MONOFLUORIDE (*19; 22*, p. 149) (b.p. −100.1° C.). A buffer vessel of 5-liter capacity was connected through a reaction tube (2 feet × 1 inch diameter) packed with nickel wire to three tubular metal traps in series, each fitted with a Hoke valve. All the vessels were made from nickel or Monel and were connected by nickel pipes. The apparatus was first evacuated, and then conditioned by introducing chlorine trifluoride to a pressure of 100 to 200 Torr. The gaseous products of fluorination were pumped away and the buffer vessel was charged with a mixture of chlorine (100 Torr) and chlorine trifluoride (300 Torr). The gases were allowed to mix for 1 hour and then expanded at a rate of 50 to 80 ml. per minute (at 400 Torr) through the reaction tube, which was heated to 500° ± 20° C. in an electric tubular furnace. The product condensed in the first of the traps, which was cooled in liquid nitrogen. This trap was warmed in a mixture of solid carbon dioxide and trichloroethylene and the chlorine monofluoride distilled into the adjacent trap which was cooled in liquid nitrogen. The gas was stable and could be kept in a well fluorinated vessel for several weeks.

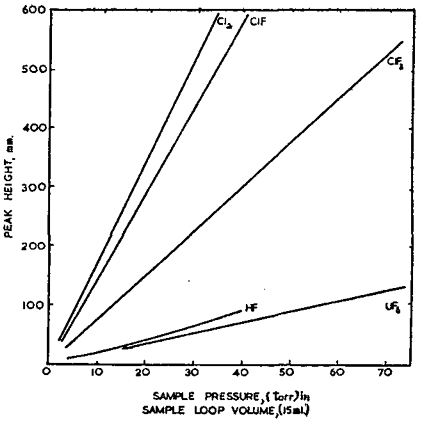

Figure 9. Calibration graphs

CHLORIDE DIOXIDE (*22*, p. 522) (b.p. +11° C.). Chlorine dioxide was made by passing a mixture of chlorine (10%) and nitrogen (90%) through two nickel towers (8 inches long and ½-inch diameter) packed with sodium chlorite. The reaction was carried out at room temperature and the product was collected in a metal sample bottle, which was initially evacuated. Decomposition within the bottle was rapid, and consecutive chromatograph traces showed increasing amounts of chlorine and oxygen.

To prevent breakdown on the chromatograph column the carrier gas flow was increased to 120 cc. per minute to reduce the resistance time within the column. The best results were obtained by trapping the sample directly into the sample loop of the chromatograph. It was not possible or desirable to store the samples for any length of time.

CHLORINE MONOXIDE (*22*, p. 514) (b.p. +2° C.). A mixture of nitrogen and chlorine in equal proportions was dried by passing it through a bubbler containing concentrated sulfuric acid and then passed through a reaction tower containing dry mercuric oxide at room temperature. The gas was subsequently dried again by passing through a tube of Anhydrone and the product collected in a glass trap at −78° C. The product was partially separated from chlorine by allowing the trap to warm slowly and trans-

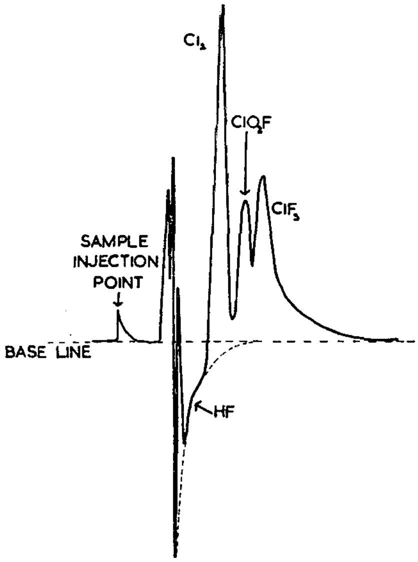

Figure 10. Chromatograms of mixture containing hydrogen fluoride

ferring the bulk of the chlorine to a colder trap. The product decomposed rapidly unless precautions were taken to prevent its exposure to light.

FLUORINE MONOXIDE (*22*, p. 189) (b.p. −144° C.). Fluorine from a cylinder was bubbled at a rate of 5 cc. per minute through a continuously replenished solution of 2% sodium hydroxide in water. The gas was then passed through two traps, the first chilled to −78° C. to remove water and the second to −190° C. The second trap was isolated and fluorine and oxygen were removed by evacuation, leaving a pale yellow liquid.

PERCHLORYL FLUORIDE (*10*; *22*, p. 183) (b.p. −47.5° C.). Fluorine was passed through two nickel towers containing potassium chlorate at a flow rate of 15 to 20 cc. per minute. The first tower (1 foot × 1 inch diameter) contained pellets of nickel gauze mixed with the salt, so that heat generated by reaction could be dissipated readily. Thermo-couples were inserted in the bed and the gas flow was regulated so that the temperature was maintained below 50° C. The second bed contained potassium chlorate only and was warmed to 50° C. to scavenge remaining traces of fluorine.

The product, a white solid, was condensed in a glass trap at liquid nitrogen temperature. It was then allowed to evaporate through two scrubbers, the first containing an aqueous solution of 10% sodium hydroxide and 5% sodium thiosulfate and the second sodium hydroxide pellets. The purified product was condensed in a chilled trap.

A similar method (*3*) wherein the potassium chlorate was maintained at −40° C. did not yield any product.

CHLORYL FLUORIDE (*22*, p. 183) (b.p. −6° C.). The apparatus was similar to that used for the preparation of perchloryl fluoride. Chlorine trifluoride was passed through the towers containing potassium chlorate and the product was condensed without purification in a nickel sample bottle cooled in liquid nitrogen. The flow rate was controlled at 15 to 20 cc. per minute and the temperature of the potassium chlorate not allowed to exceed 30° C. Chlorine was the main impurity and could be vaporized at low temperature to yield chloryl fluoride of 98% purity.

Precision. The precision of replicate measurements depends largely upon the precision of measurement of peak height, since integration of peak area has been little used in this work. In general, the over-all precision is better than ±2% expressed as a relative standard deviation and in favorable circumstances can reach ±1%.

The chromatographs were extremely stable, calibration figures obtained over intervals of 6 months or more lying within a few per cent of each other. This stability makes the technique particularly suitable for process monitoring or control.

Separations. The retention times of a number of halogen compounds relative to chlorine have been measured under normal operating conditions and are given in Table III.

The table shows that in general the retention time is related to the volatility of the substance concerned, a notable exception being hydrogen fluoride. With the column materials available there was considerable congestion among the species with short retention times. Moreover the columns were not as efficient in terms of HETP as those used for conventional organic separations (HETP for chlorine = 1 to 1.5 cm.) and overlapping of peaks was therefore observed. Attempts to improve column performance led to the statistical investigation of the column (*24*).

In general, the chromatographic behavior of gases on these columns was similar to that of organic gases on conventional columns. However, interactions between some parameters led to some anomalous behavior. For example, the usual resort of increasing column length to increase separation was shown to be only slightly beneficial.

Fortunately, the most difficult mixtures to separate, chlorine oxides and oxyfluorides in the presence of large amounts of chlorine and chlorine trifluoride, were rarely encountered. Qualitative indication of their presence was possible, but when quantitative analyses were required under laboratory conditions, infrared methods were used (*16*). The mixtures usually analyzed consisted of uranium hexafluoride, chlorine, chlorine trifluoride, hydrogen fluoride, chlorine monofluoride, and permanent gases. Of these, only hydrogen fluoride and chlorine monofluoride were difficult to resolve and if one or the other was present as a trace, quantitative estimation of this was difficult.

Hydrogen fluoride was anomalous in many respects. In addition to a thermal conductivity greater than that of nitrogen, it did not conform with the usual elution order, its calibration curve was nonlinear, and considerable tailing of the peak was always experienced. An example of a trace obtained from a sample containing hydrogen fluoride is shown in Figure 10. Where the main interest lay in the determination of gases other than hydrogen fluoride, it was sometimes possible to remove this gas, by passing the sample through a tube packed with sodium fluoride before admission into the sample loop.

Two explanations for the tailing phenomenon have been considered—either that hydrogen bond formation results in abnormal sorption where the sorbed species have a wider range of interaction energies than normally occur, or that partial separation of an equilibrium mixture of hydrogen fluoride polymers is taking place (*20*). It was not possible to differentiate between these postulates. On raising the temperature the tailing of the peak was reduced but both theories would indicate that this was to be expected. The retention time of the peak maximum

Table III. Retention Times of Halide Gases Relative to Chlorine

Operating conditions. Column temperature, 60° C.
Carrier gas, nitrogen at 48 cc./minute
Column length, 14 feet

Substance	Relative retention time	Boiling point/sublimation temperature, °C.
Uranium hexafluoride	6.0	+56.5
Bromine	2.6	+63
Chlorine monoxide	1.76	+2
Chlorine trifluoride	1.45	+11
Chlorine dioxide	1.45	+11
Chloryl fluoride	1.28	−6
Chlorine	1.0	−33.7
Perchloryl fluoride	0.85	−46
Hydrogen fluoride	0.60	+19
Chlorine monofluoride	0.48	−100
Fluorine monoxide	0.40	−144
Fluorine	Not retained	−188

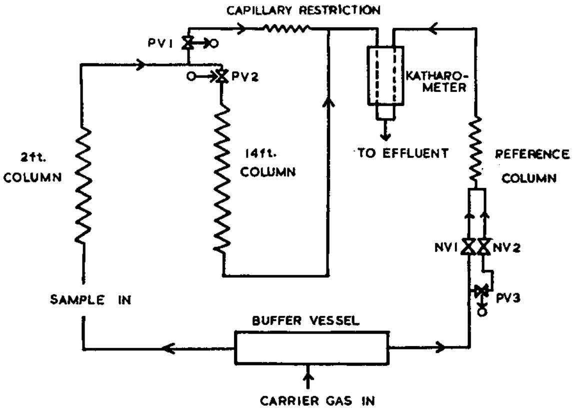

Figure 11. Schematic diagram of split-column chromatograph

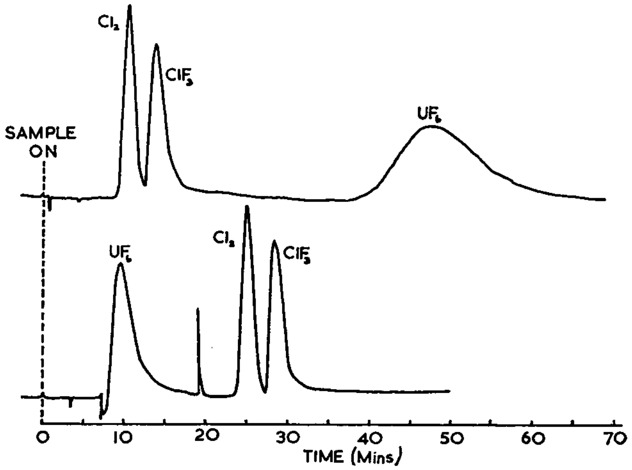

Figure 12. Chromatograms of samples containing uranium hexafluoride

a. Obtained with ordinary chromatograph
b. Obtained with split column chromatograph

varied slightly with the pressure. This would tend to support the polymer theory, as the composition of the equilibrium mixture of polymers would be expected to change with pressure.

Hypochlorous acid, which could be shown by infrared methods to be present always in trace amounts in the samples of chlorine monoxide used, never appeared as a separate peak on the chromatogram. On the introduction of hypochlorous acid solution into the chromatograph, the only peak observed corresponded to that of chlorine monoxide. It was thus concluded that the sorptive affinity for water of the column made it as effective a dehydrating agent as the chemical reagents used in some of the methods of manufacture of chlorine monoxide from hypochlorites.

Rapid Analysis of Mixtures Containing Uranium Hexafluoride. The comparatively long retention time of uranium hexafluoride made rapid analysis of mixtures containing this gas impossible, particularly if the separation of the lighter components had to be maintained. While temperature programming would have enabled the analyses to be completed more quickly, the design of the chromatograph did not readily lend itself to modification in this way and rapid cooling of the columns prior to the next sample injection would have been particularly difficult.

The problem was solved by the division of the column into two sections of different lengths. The system is schematically shown in Figure 11.

When the sample was introduced, the pneumatically operated valve, *PV*1, was closed and *PV*2 opened. Sufficient time was allowed for all the light components to be carried on to the longer column, while the hexafluoride remained on the short column. The pneumatic valves were then simultaneously operated, *PV*1 opening and *PV*2 closing, and the uranium hexafluoride was eluted directly to the katharometer with no flow through the longer column. After elution of the peak, the valves were restored to their original states and the light gases eluted. During this process the inlet pressure of the carrier gas remained constant and changes in the flow of gas through the detector resulted from the different impedances to gas flow offered by the two columns. This caused steps to appear in the base line signal from the katharometer. These were partially removed by inserting the capillary restriction shown in the bypass line, and were finally eliminated by changing the flow through the reference side by means of valve *PV*3, which was operated simultaneously with the other valves. Thus the flow through the needle valve, *NV*1, balanced that through the combined columns, and the flow through *NV*1 and *NV*2 balanced that through the short column only.

An example of the trace obtained with and without split column operation is shown in Figure 12. The holdup in the flow of the light components has no noticeable effect on their separation, and the sharpness of the response of the detector to the uranium hexafluoride is improved. After completion of the exploratory experiments, the system was automated so that the timer unit which caused the sample to be introduced also actuated the compressed air supply to the additional pneumatic valves at predetermined time intervals after sample introduction.

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Separation of Stereoisomers by Column Fractional Precipitation

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▶ Column fractional precipitation was successfully employed in separating 1,3,5-tri-(4-bromophenyl)benzene from 1,3,5-tri-(3-bromophenyl)benzene, o,p-quaterphenyl from *m,p*-quaterphenyl, and in determining the purity of a tribromodecaphenyl, 1,3-di-(2-chlorophenyl)-1,3-cyclohexadiene, and 1,3-di-(3-bromophenyl)benzene. Isomeric steroid mixtures of 5β-androstan-3α-ol-17-one and 5α-androstan-3α-ol-17-one and of 3α-hydroxycholestane and 3β-hydroxycholestane were separated, but a mixture of 5α-androstan-3,17-dione and 5β-androstan-3,17-dione could not be resolved.

Baker and Williams (*1*) described a column fractional precipitation technique which has been successfully applied to the fractionation of industrial types of polystyrene of large molecular-weight distribution and of polymers of narrow distribution. Separation is achieved by subjecting the polymer, supported on an inert packing of micro glass beads, to a simultaneous temperature and solvent gradient. A linear temperature gradient is maintained along the length of the column and the percentage of "good" solvent in the initial nonsolvent increases exponentially. At a particular temperature and solvent composition, the most soluble molecular-weight species will dissolve and move down the column to a lower temperature region where it will again precipitate. When the solvent composition has been sufficiently enriched with "good" solvent, the precipitate will redissolve and proceed down the column in a series of precipitation and dissolution steps. The material will leave the column as a saturated solution.

In a previous paper, Schulz and Purdy (*14*) derived equations for the average solvent composition and the interstitial column volume for a fractional precipitation column of the Baker and Williams type. This work shows that column fractional precipitation is not limited to the separation of polymers, but can be extended to the separation of other high molecular-weight organic substances.

EXPERIMENTAL

Reagents and Apparatus. Reagent grade chemicals were used without further purification. All polyphenyl samples were kindly made available by G. F. Woods of this laboratory. This steroid samples were provided by the Division of Biochemistry, Walter Reed Army Institute of Research. Commercial *p*-dioxane was purified by distillation from all-glass apparatus and was stored in the presence of sodium (*3*).

The fractional precipitation apparatus was built in this laboratory and has been described elsewhere (*14*). The temperature gradient of the apparatus was determined to be linear from 0° to 65° C. The measurement of the solvent gradient has been described in a previous paper (*14*).

All absorbance measurements were made either with a Beckman DK-1 or Model B spectrophotometer.

Procedure. Selection of Solvent Systems. The choice of the solvent system depended on preliminary solubility studies and turbidimetric titrations. Approximately 0.1 gram of the substance under investigation was dissolved in 5 ml. of good solvent. Nonsolvent was added with a pipet until turbidity was observed. The appearance of the precipitate and the reversibility of the precipitation process were noted. Promising solvent systems were further investigated by turbidimetric titrations.

Approximately 100 mg. of a single substance was weighed into a 100-ml. volumetric flask and diluted with good solvent. A 10-ml. aliquot of the solution was transferred to a 250-ml. Erlenmeyer flask and then equilibrated in a constant-temperature bath. The solution was titrated with nonsolvent from a 50-ml. buret to a visual turbidimetric end point. In many cases, the precipitate was redissolved by an additional 20 ml. of good solvent and titrated again. The titrations were run at temperatures varying by 10-degree intervals from 10° to 60° C. Plots of the solvent composition at the turbidimetric end point *vs.* temperature were constructed. In Figure 1 are shown the solubility curves of 3α-hydroxycholestane and 3β-hydroxycholestane as obtained from these turbidimetric titrations.

Fractionation Procedures. After preliminary solubility studies in various solvents and solvent pairs, the *p*-dioxane–water system was chosen to separate 1,3,5-tri-(3-bromophenyl)benzene from 1,3,5-tri-(4-bromophenyl)benzene, water being the nonsolvent. A 1:1 mixture of the two isomers was finely ground and samples ranging from 22 to 57 mg. were mixed with glass beads and transferred to the column. The 250-ml. mixing vessel was initially filled with either 33 or 45% *p*-dioxane in water and was surrounded by a heating mantle. Solvent, which was preheated to 50° C., was delivered to the column at a rate ranging from 6 to 10 ml./hour. The top of the column was heated to 63° C. Thirty to 39 fractions were automatically collected. The fractions were analyzed spectrophotometrically.

The purity of a tribromodecaphenyl was investigated in two stages. A 45.7-mg. sample was placed on the column and treated initially with a solvent mixture of 45% *p*-dioxane in water. Forty fractions were collected. A second sample of equal size was fractionated with solvent of a starting composition of 80% *p*-dioxane in water. Thirty-six fractions were collected. Ultraviolet spectra were obtained on 1-ml. aliquots of each of the fractions. Similar purity analyses were made for 1,3 - di - (2 - chlorophenyl) - 1,3 - cyclohexadiene and 1,3-di-(3-bromophenyl)-

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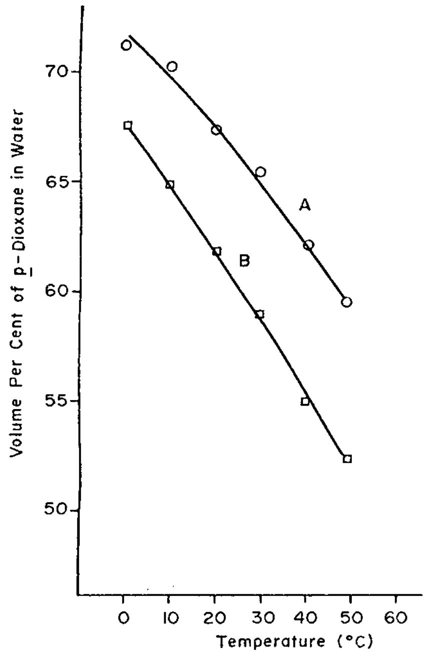


Figure 1. Solubility curves from turbidimetric titrations

A. 3α-Hydroxycholestane
B. 3β-Hydroxycholestane

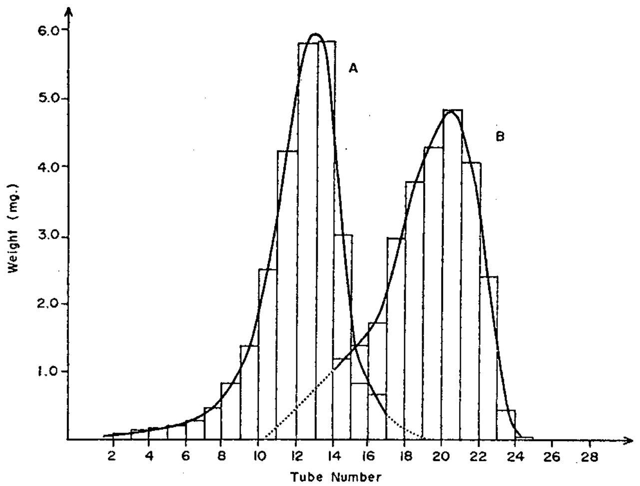
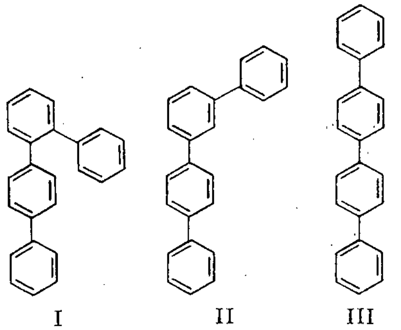


Figure 2. Separation of two isomeric polyphenyls

A. 1,3,5-Tri-(3-bromophenyl)benzene
B. 1,3,5-Tri-(4-bromophenyl)benzene

benzene. Since these latter two substances are highly viscous liquids, they could be mixed directly with the glass beads. A water-*n*-propanol solvent system was used in the latter two cases, with pure water initially in the 250-ml. mixing vessel. Fractions were analyzed spectrophotometrically.

Fractionation of a mixture of three quaterphenyls, *o,p* (I), *m,p* (II), and *p,p* (III) was investigated with an initial 10% *n*-propanol in water in the 250-ml. mixing vessel and a sample size of 26.4 mg. The three isomers were present in a weight ratio of *o,p:m,p:p,p* = 1:1:0.25.



I II III

For the fractionation of hormones, three diastereoisomeric systems were chosen:

(*i*) 5β-androstan-3α-ol-17-one
5α-androstan-3α-ol-17-one
(*ii*) 3β-hydroxycholestane
3α-hydroxycholestane
(*iii*) 5β-androstan-3,17-dione
5α-androstan-3,17-dione

The water-*p*-dioxane solvent system was applicable in each case. While the 250-ml. mixing vessel, containing initially 40% dioxane, was used for system (*ii*), a mixing volume of 500 ml. and pure water were used for systems (*i*) and (*iii*).

THE ZIMMERMANN METHOD. The ketonic steroid fractions were analyzed by the colorimetric Zimmermann method (*16*). This method was modified for nonketonic saturated steroids, such as system (*ii*) (*8*). Five milliliters of the fractions were evaporated in 50-ml. test tubes and heated for 30 seconds in boiling water with 1 ml. of a freshlyprepared solution of 1.250 grams of recrystallized 3,5-dinitrobenzoyl chloride in 25 ml. of reagent-grade pyridine. Each mixture was quickly transferred with 25 ml. of benzene to a 60-ml. separatory funnel and washed successively—twice with 10 ml. of 1*N* hydrochloric acid, twice with 10 ml. of 1*N* sodium hydroxide, and twice with water. The benzene was evaporated on a steam bath.

The color was developed by adding 16 drops of technical-grade acetone and 4 drops of a 0.1% ethanolic potassium hydroxide solution. The mixtures were allowed to stand for 5 minutes and then were diluted with ethanol. The absorbance was measured at 550 mμ and plotted against tube number.

Because of the rapid discoloration of the 3,5-dinitrobenzoyl chloride solution, 50 mg. of the acid chloride was added directly to the evaporated steroid fractions, followed by 1 ml. of pyridine. This variation was applied to fractions from the fractional precipitation of 3α-hydroxycholestane.

RESULTS AND DISCUSSION

Fractionation of Polyphenyl Systems. In contrast to the detailed spectrum of benzene, the ultraviolet absorption spectrum of polyphenyls and halogenated polyphenyls consists of broad peaks in the range of 210 to 280 mμ. The difference between the spectra of stereoisomeric polyphenyls often consists only in small wavelength shifts. However, these shifts are generally sufficient to study the degree of separation of mixtures.

The separation of 1,3,5-tri-(3-bromophenyl)benzene from 1,3,5-tri-(4-bromophenyl)benzene and the distribution curves of these compounds are shown in Figure 2. The three fractions of greatest overlap were analyzed from the absorbance values at 260.2 and

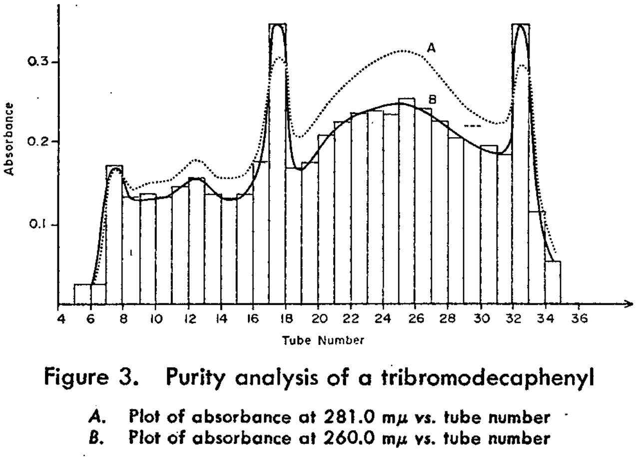


Figure 3. Purity analysis of a tribromodecaphenyl

A. Plot of absorbance at 281.0 mμ vs. tube number
B. Plot of absorbance at 260.0 mμ vs. tube number

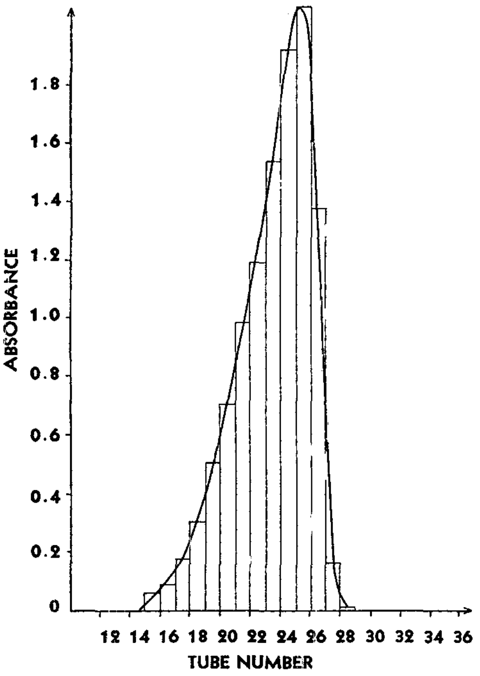


Figure 4. Purity analysis of 1,3-di-(3-bromophenyl)benzene

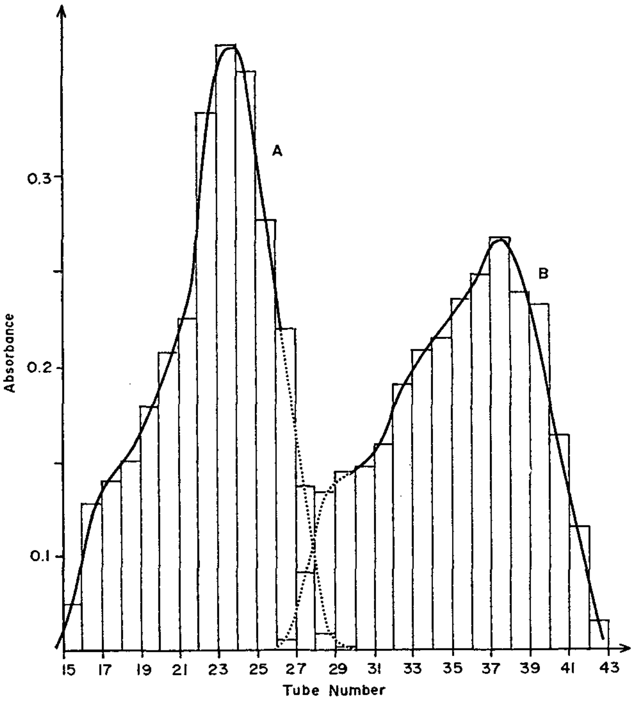


Figure 5. Separation of two quaterphenyls

A. o,p-Quaterphenyl
B. m,p-Quaterphenyl

253.0 mμ using simultaneous equations. The absorptivities of the pure substances at these wavelengths were determined from spectra of the pure compounds to be:

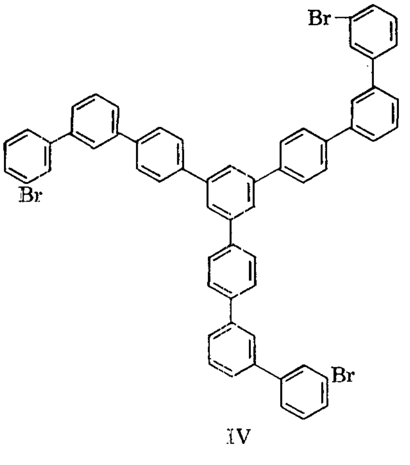
$a_{260.2} = 52,360$ liters/mole-cm.
$a_{253.0} = 61,370$ liters/mole-cm.

for 1,3,5-tri-(3-bromophenyl)benzene and

$a_{260.2} = 77,130$ liters/mole-cm.
$a_{253.0} = 65,310$ liters/mole-cm.

for 1,3,5-tri-(4-bromophenyl)benzene. The calculations were not carried to fractions of smaller overlap, as the error became so large that the calculated concentrations would have been without significance.

The purity analysis of a tribromodecaphenyl showed the presence of four major impurities (see Figure 3). The structure of the tribromodecaphenyl was believed to be:



IV

This structure gives rise to a spectrum with a broad maximum at 281.0 mμ. The impurities were discovered from the spectra of individual fractions and the relative absorbance values at particular wavelengths.

The broad peak of the distribution curve (Figure 3) is due to compound IV, its fractions having the same spectrum as the unfractionated material. The relative height of a peak is not a measure of the quantity of impurity present, since one cannot assume equal absorptivity values for all substances of the mixture. This has been brought out in the change of relative peak heights upon plotting the absorbance at different wavelengths.

The broad peak of the distribution curve was extrapolated to fractions 17 and 32. The absorbance due to compound IV in these fractions was estimated to be equal to one-half of the absorbance of fraction 25, which contained no impurities. By recording the ultraviolet spectra of fractions 17 and 32 against fraction 25 as a reference solution, the absorbance due to compound IV in fractions 17 and 32 was subtracted out. The resulting spectra proved to be identical, having two maxima at 257.4 and 228.4 mμ and were, as such, completely different from previously recorded spectra. Although the structures of the impurities in fractions 17 and 32 are not known, one can assume that both compounds contain the same chromophoric group, giving rise to identical spectra. They must be different compounds because of their differing solubilities.

The purity analysis of 1,3-di-(3-bromophenyl)benzene resulted in a very sharp distribution curve, indicating the absence of impurities (Figure 4). The curve represents the typical distribution of a substance which rapidly establishes equilibrium. A sharp skewed distribution was generally found with amorphous substances, such as the dibromoterphenyl under discussion, which is a viscous liquid.

The distribution curve for 1,3-di-(2-chlorophenyl)-1,3-cyclohexadiene, which is also a liquid and which was fractionated under the same conditions as the dibromoterphenyl, was slightly broader, ranging from fraction 15 to 34, with an initial small shoulder which may have been due to the presence of traces of the monochloro compound, 1-phenyl-3-(2-chlorophenyl)-1,3-cyclohexadiene.

Mixtures of three terphenyls and of some quaterphenyls have been partially separated by adsorption chromatography on various common adsorbants (7). The separation of three isomeric quaterphenyls (I, II, III) was investigated by column fractional precipitation.

From turbidimetric titrations it was evident that the *o,p*-isomer (I) was slightly more soluble than the *m,p*-isomer (II) and that the *p,p*-isomer was very insoluble, in fact so insoluble that no definite turbidimetric end points could be observed from the very dilute solutions which were employed (0.00067 gram/30 ml.). The separation of *o,p*-quaterphenyl from *m,p*-quaterphenyl is shown in Figure 5. The peaks are broad and slightly irregular in their ascending portions. The composition of overlapping fractions was calculated by using absorbance ratios at the two wavelengths of 265 and 248 mμ.

The *p,p*-quaterphenyl was not noticeably eluted when the run was terminated at fraction 51. It could be removed from the column only through prolonged washing with *n*-propanol.

Fractionation of Steroid Systems. The application of column and paper chromatography (*6, 9, 13*) to the separation of synthetic and naturally occurring steroid mixtures has been very successful and contributed much to recent advances in the field of steroid research. During the last three years, with the availability of new column packings, such as methylsilicone gums, polyesters, and fluorinated alkylsilicones (*2, 10, 16*), gas chromatography has become a promising tool not only to separate various families of steroids but also diastereoisomers. Sometimes the separation of steroids is accompanied by decomposition, for example the decomposition of corticosteroids to 17-ketosteroids (*17*).

From gas chromatograms of diastereoisomeric steroids it has been found that the 5β-compound, having the *cis*-configuration, is always eluted ahead of the 5α-compound and that the degree of separation is influenced by the substituent at position C-3 (*10*). While hydroxyl and carbonyl groups in position C-3 affect the resolution equally well, an ester group causes a marked increase in resolution (*10*).

5β-Androstan-3α-ol-17-one has been separated from 5α-androstan-3α-ol-17-one by countercurrent extraction (*13*), by gradient elution from alumina (*11*), by paper chromatography (*12*), and by gas chromatography (*4*). The separation of these isomers by fractional precipitation is shown in Figure 6, and was accomplished with *p*-dioxane–water, water being the nonsolvent. The total sample of 60.0 mg. contained 7.8 mg. of the *cis*-isomer. Because a very small amount of the *cis*-isomer was available, turbidimetric titrations were performed only on the *trans*-isomer. These titrations indicated that the steroid was quite soluble in *p*-dioxane and slightly soluble in water. Consequently, the large 500-ml. mixing vessel was selected and filled with pure water. On the basis of relative areas under the peaks, the first peak was assigned to the *cis*-compound, 5β-androstan-3α-ol-17-one. The *cis*-compound preceding the *trans*-isomer is in agreement with data from gas chromatography.

The colorimetric determination of ketonic steroid fractions by the Zimmermann method proved to be a very sensitive one, the absorptivity being in the range of 10^4 liters/mole-cm. The absorption spectrum of the resulting purple solution was characterized by two absorption peaks at 570.0 and, 510.5 mμ. It was the blue component of the purple color, corresponding to the absorption at 510.5 mμ, which was absent in the blank, but which was also the less stable color, that was employed for the measurements. By controlling the temperature and time of color development, reproducible absorbance values were measured.

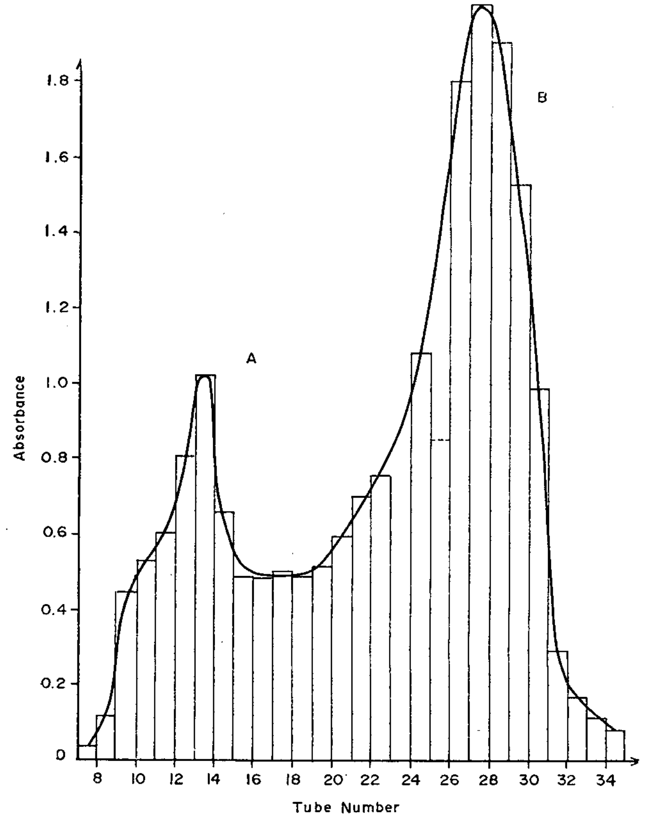


Figure 6. Separation of two ketonic 17-ketosteroids

A. 5β-Androstan-3α-ol-17-one
B. 5α-Androstan-3α-ol-17-one

The separation of diastereoisomeric saturated nonketonic steroids is generally more difficult than the separation of 17-ketosteroids. Clayton (*2*) converted cholestanol and epicholestanol to the corresponding methyl esters and separated them by gas chromatography, using a packing of polyethylene glycol succinate. This method worked equally well for the separation of coprostanol and epicoprostanol. However, only retention times, *r*, relative to cholestane (*r* = 1.00) were reported. It is not clear whether the reported retention times were obtained from chromatograms of single substances or from mixtures:

Compound	*r*
Cholestanol	3.60
Epicholestanol	2.61
Coprostanol	2.36
Epicoprostanol	3.08

Haahti, VandenHeuvel, and Horning (*5*) listed relative retention times for 3α-hydroxycholestane (*r* = 2.42) and 3β-hydroxycholestane (*r* = 2.36). A segmented column packing of silicone polymer SE-30 and ethylene glycol isophthalate was used.

A mixture of approximately 1:1 3β-hydroxycholestane and 3α-hydroxycholestane was resolved into four main peaks by fractional precipitation. To determine the origin of the observed peaks, 3α-hydroxycholestane was fractionated separately. Two broad peaks were obtained in the general region in which the third and fourth peaks of the mixture has appeared. The last two peaks were, therefore, assigned to 3α-hydroxycholestane and the first two to 3β-hydroxycholestane. It is most likely that, in addition to the two cholestane isomers, the mixture contained the corresponding coprostanol isomers, in which the hydrogen atom at position C-5 is *cis* with respect to the methyl group at C-10. All four isomers are products of the reduction of cholesterol to cholestanol (*3*).

While several sensitive colorimetric methods exist for the determination of unsaturated and ketonic steroids, no ready colorimetric method is available for saturated nonketonic steroids. The modified or "reverse" Zimmermann method was used to analyze the fractions from the cholestanol separations. Because each fraction had to be transferred several times and was subjected to six extractions, the errors in absorbance readings were expected to be high. This was confirmed by the large amount of scatter of the points and the large variations in absorbance values of the blank which ranged from 0.05 to 0.30.

A steroid mixture consisting of 5α-androstan-3,17-dione and 5β-androstan-3,17-dione could not be resolved. The fractionation of two mixtures with sample sizes of 33 and 57 mg. resulted in single broad peaks, stretching over tubes No. 10 to 25 and No. 10 to 29, respectively. Gradient elution from alumina also failed to separate this mixture (*11*). Knights and Thomas (*10*) list relative retention times of $r = 4.31$ for the *cis*-isomer and $r = 4.72$ for the *trans*-isomer.

The presence of the carbonyl group in position C-3 introduces a certain amount of strain into the cyclohexane ring, which will also affect the relative position of the hydrogen atom in position C-5 and its interactions with neighboring atoms and bonds. One would expect that the difference in these interactions between the *cis*- and *trans*-isomers, on which basis they can only be separated, to be diminished. Apparently, this difference has become so small that the isomers cannot be separated by fractional precipitation under the conditions employed.

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Radiochemical Determination of Cerium by Solvent Extraction Method

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▶ An efficient radiochemical method for the determination of cerium(III) has been developed based on the principle of the synergic effect in solvent extraction with 2-thenoyltrifluoroacetone and tri-*n*-butyl phosphate. The mixed solvents enhanced the extraction by 10^3-fold over either of the components alone. The extraction procedure provides a clean and efficient separation of radiocerium from other fission products. This method is particularly suitable for the separation of cerium from solutions of fission product mixtures a year or more out of the reactor.

The radiochemical procedures (*2, 3, 5, 10, 11, 13*) for determination of cerium in fission product mixtures are either based on the repeated ceric iodate precipitation method or solvent extraction methods. Separation by the precipitation method (*2*) is tedious and time consuming. A radiochemical method developed by Glendenin (*5*) using 4-methyl-2-pentanone (hexone) requires handling a mixture of an organic solvent and strong nitric acid which can be hazardous. The procedure of Smith and Moore (*13*) for separation of radiocerium by extraction with 2-thenoyltrifluoroacetone (TTA) in xylene from $1N$ sulfuric acid calls for a chemical yield determination by standard methods. Recent work by McCown and Larsen (*11*) showed that cerium is well extracted with di-2-ethyl hexyl phosphoric acid from $10N$ nitric acid solution. In this method a small amount of ruthenium is coextracted with cerium and removed by fuming with perchloric acid. Butler and Ketchen (*3*) developed the method for the separation of multicurie amounts of cerium and yttrium from fission product mixtures which were relatively free of strontium-90, zirconium-95–niobium-95, cesium-137, technetium-99 and, in particular, to ruthenium-106. The most recent work by Marsh and Maeck (*10*) showed that radiocerium extracts as the tetra-*n*-propylammonium nitratocerate ion–association complex into nitroethane from strong nitric acid solution. The formation of solid phase in the liquid-liquid extraction seems to be a disadvantageous step in this method.

The present work was undertaken to develop a solvent extraction method for radiocerium based on the synergic effect in solvent extraction of mixed solvents as predicted by Irving and Edgington (*9*). Healy (*8*) observed that di-, tri-, and tetravalent metal ions showed synergistic extraction. In the present procedure, cerium(III) showed extraction improved by 10^3-fold with mixed TTA and TBP in benzene from nitric acid solutions of pH 2.90. Cerium(IV) was reduced with a drop of hydrogen peroxide and after adjusting the pH of the solution by the addition of $0.02N$ sodium hydroxide, was extracted with equal volumes of $0.2M$ TTA and $0.5M$ TBP (1:1) mixture in benzene. Cerium was back-extracted into an aqueous phase with an aqueous nitric acid solution of pH 0.80. The cerium was obtained in good yield of satisfactory radiochemical purity.

EXPERIMENTAL

Reagents. Tri-*n*-butyl phosphate ($10^{-3}M$ to $1.0M$); 2-thenoyltrifluoroacetone ($10^{-3}M$ to $1.0M$); benzene; dilute and concentrated nitric acid; sodium hydroxide ($0.02N$); hydrogen peroxide; and cerium carrier 5 mg. per

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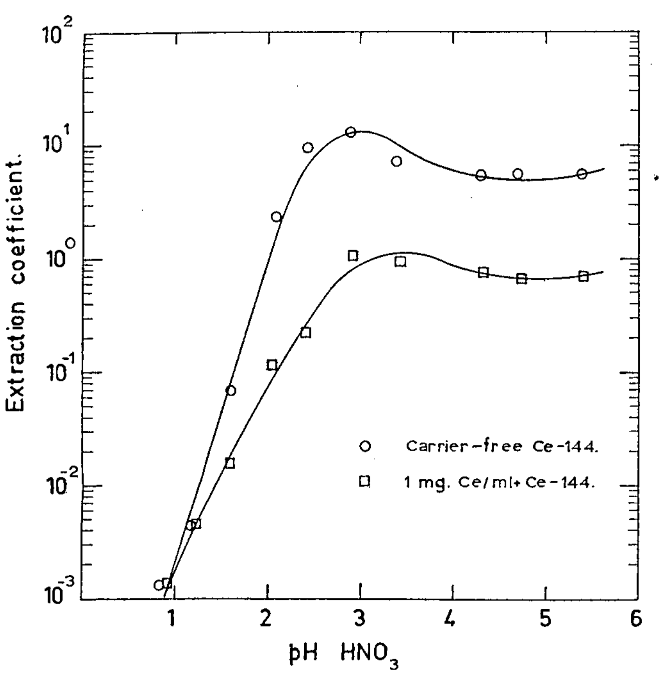

Figure 1. Effect of pH on extraction of cerium by TTA–TBP mixtures (1 to 1 by volume)

ml., standardized by the method of Glendenin (*5*).

Procedure. For extraction studies the reagent grade TBP was purified by distillation (*1*) and TTA was purified by recrystallization in benzene. The synergism in the extraction of cerium (III) was investigated by extracting tracer cerium-144 with varying proportions of TTA and TBP in benzene. The optimum pH of the aqueous solution for maximum extraction coefficient both for carrier-free cerium-144 and with carrier at a concentration of 1 mg. per ml. of cerium was determined with mixed solvents of 0.2*M* TTA and 0.5*M* TBP in benzene. The extraction coefficients were found by counting equal aliquots of organic and aqueous phase with end-window Geiger-Müller counters using 46 mg. per sq. cm. of aluminum absorber.

Recommended Procedure. Pipet an aliquot of the sample (total activity 10^5 to 10^7 c.p.m. β) into a centrifuge tube containing 5 ml. of an aqueous nitric acid solution of pH 2.7 to 3.0. Adjust the final pH to 2.90 by 0.02*N* sodium hydroxide and add 2 drops of 30% hydrogen peroxide. Transfer to a 50-ml. separatory funnel containing 5 ml. of 0.5*M* TBP and 0.2*M* TTA in benzene in the ratio of 1:1 and shake for 15 minutes. Withdraw the aqueous phase and scrub the organic phase twice with 5-ml. portions of nitric acid solution of pH 2.90 together with 2 drops of 30% hydrogen peroxide. Discard the aqueous phase. Back-extract the cerium by shaking the organic phase with 2 ml. of nitric acid solution of pH 0.80 for 2 minutes. Allow praseodymium-144 to grow for 2 hours. Take an aliquot from the aqueous stripped solution and count for beta activity using an absorber of 46 mg. per sq. cm. of aluminum.

RESULTS AND DISCUSSION

Tracer cerium was well extracted between pH 2.60 to 3.40 with the optimum pH at 2.80 to 3.10. The results are shown in Figure 1. The extractions have been studied as a function of time. The maximum extraction was obtained after contacting for 15 minutes. Stripping was completed within 2 minutes after shaking the organic phase with aqueous nitric acid of pH 0.80. The effect of solvent concentrations on the extractability of tracer cerium was determined and the results are shown in Tables I and II. Highest extractions were obtained with 0.5*M* TTA and 1.0*M* TBP at pH 2.90. In the recommended procedure, the concentration of 0.2*M* TTA and 0.5*M* TBP was chosen since a higher concentration is likely to contaminate the product with zirconium-95 and other fission products.

Carrier cerium did not extract as well as the tracer cerium. The low extractability may be due to the dimer and other species (*4*, *6*). Butler (*3*) observed the similar effect with di-2-ethyl hexyl phosphoric acid extraction of cerium.

Separation from the Other Elements. The elements with long-lived radioisotopes formed in appreciable yield in fission, and likely to be extracted with cerium by the mixed solvents TTA and TBP, are yttrium-91, zirconium-95, niobium-95, ruthenium-106, promethium-147, and samarium-151. Other interfering elements present in fission product sources are thorium, uranium, neptunium, and plutonium. The described cerium procedure was tested for

Table I. Extraction Coefficients of Trivalent Cerium-144 Tracer as Function of Varying TTA Concentration at Constant 1.0M TBP and pH 2.90

TTA, *M*	K_d
0.50	10.7
0.20	10.1
0.10	9.8
0.05	4.7
0.01	0.12

Table II. Extraction Coefficients of Trivalent Cerium Tracer-144 as Function of TBP Concentrations at Constant 0.2M TTA and pH 2.90

TBP, *M*	K_d
1.0	10.1
0.50	10.4
0.20	7.9
0.10	5.4
0.01	0.01

Table III. Coseparation of Heavy Elements and Other Fission Products

Element	Per cent extraction	Per cent back-extraction
U^{233}	99.9	...
Np	99.0	...
Pu	99.0	...
Th^{234}	98.9	32.5
Ru^{106}	0.47	0.28
Zr^{95}–Nb^{95}	12.1	0.006

Table IV. Comparison of the Solvent Extraction Method with the Ceric Iodate Precipitation Method

Counts in 1 ml. (β) fission product, × 10^6 c.p.m.	
Precipitation method	Solvent extraction method
1.74 ± 0.02	1.73 ± 0.03
1.75 ± 0.04	1.78 ± 0.05
1.75 ± 0.03	1.76 ± 0.02
1.74 ± 0.03	1.79 ± 0.04
1.75 ± 0.05	1.78 ± 0.03
1.75 ± 0.04	1.79 ± 0.04
1.75 ± 0.06	1.75 ± 0.03
1.75 ± 0.04	1.76 ± 0.06

coseparation of these elements by using the following radioactive tracer, zirconium-95–niobium-95, ruthenium-106, and thorium-234. The distribution data of uranium were taken from the work of Irving (*9*). Neptunium and plutonium as actinides will be extracted by the solvent. The coseparation results are shown in the Table III.

The data indicate that the separation from zirconium, niobium, and ruthenium is satisfactory. Promethium-147 and samarium-151 might extract but the activities will not be detected if an absorber is used. Yttrium-91 does not coextract to any appreciable extent as is evident from the comparison of the procedure with the ceric iodate method (*2*) shown in Table IV. This method is suitable for fission product mixtures obtained as waste from processing plants or irradiated samples from which actinides have been removed by extraction from nitric acid (*7*, *12*).

Ten aliquots of cerium-144 tracer solution containing 3.5×10^5 c.p.m. β were carried through the extraction procedure. The average yield for the 10 determinations was 91.2 ± 0.7% Samples of a one-year-old fission product mixture were analyzed for cerium by the extraction procedure described and by the ceric iodate precipitation method (*2*). The results obtained by the two methods are given in the Table IV and agree to within 2% for every sample. Radiochemical purity of the separated cerium by this procedure was checked by gamma-ray spectrometry. Less than 1% of the activities present were due to fission products other than cerium.

The precision of the method was checked by carrying out 10 replicate analyses on one-year-old fission product mixtures. The relative standard deviation was ±1.2%. Besides speed and simplicity, an added advantage of the procedure is the use of readily available solvents.

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Fast Paper Chromatography of Different Valence States of Mercury and Antimony

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▶ A decrease in the separation time of metals in differing valence states is effected by use of a suitable solvent system. A number of solvent systems are studied for the separation of Hg_2^{+2}–Hg^{+2} and Sb^{+3}–Sb^{+5}. The most selective separation of Hg_2^{+2}–Hg^{+2} is obtained with a mixture of HNO_3–HCl–isopropanol; Sb^{+3}–Sb^{+5} separations are efficient in a mixture of acetic acid–water–ethyl acetate. R_f values are given for ions which are likely to interfere in the procedure, and the phenomenon of double spots is discussed.

The time of separation is a particularly important factor in the paper chromatographic separation of a metal in different valence states. If the separation time is long enough, some interconversion of valence state may occur. Also, one of the valence states—Sb^{+5} in the present case—may be sufficiently reactive to interact slowly with the paper or the solvent system. Several techniques may be utilized to decrease the separation time, including the use of a higher temperature, centrifugal chromatography, or choice of a proper solvent system. A higher temperature may increase the rate of interconversion, as well as decrease the separation time, and centrifugal chromatography requires specialized equipment. Therefore, the choice of a proper solvent system offers the simplest solution to the problem.

The importance of the study of such separations has been discussed previously (*1–3*). A fast solvent system (*3*) was reported for the separation of Fe^{+2} and Fe^{+3}. This paper summarizes recent findings on the separation of different valence states of Hg and Sb. These separations were reported earlier by Bighi (*1*) and Pollard (*2*). We could not reproduce Bighi's separation of Hg_2^{+2} and Hg^{+2}. The separation of Sb^{+3} and Sb^{+5}, as reported by Pollard, requires 1 hour, and Sb^{+5} tails. Neither Bighi nor Pollard mentions the selectivity of the separations. Therefore, we have made a more detailed study and developed faster and more selective solvent systems.

EXPERIMENTAL

Apparatus. Development was performed in 20-X 5-cm. glass jars, using the ascending method. The dimensions of the paper strips were 15 × 4 cm.

Reagents. All results were obtained on Whatman No. 1 paper using reagent grade chemicals. All solvents were purified by distillation.

Mercury Test Solution. Three grams of freshly prepared mercurous nitrate (mercurous nitrate E. Merck, which contains an appreciable quantity of mercuric nitrate, was treated with dilute nitric acid and mercury until colorless crystals of mercurous nitrate appeared) were dissolved in 50 ml. of approximately 3*M* HNO_3. Two grams of mercuric nitrate were boiled with 5 ml. of concd. HNO_3. Spotting was done with 0.1*M* solutions. Ammonia gas and ammonium sulfide were used as detectors.

Antimony Test Solution. The Sb^{+3} solution, 0.2*M*, was prepared as reported earlier (*4*). The 0.2*M* $SbCl_3$ solution in HCl was boiled with $KClO_3$, cooled, and filtered. This was used as the Sb^{+5} solution. Ammonium sulfide and H_2S gas were used as detectors. Rhodamine B was used for the detection of Sb^{+5} only.

RESULTS

To develop suitable methods of separation, a number of pure solvents were examined. The results are summarized in Table I.

Separation of Hg_2^{+2} and Hg^{+2}. The following solvent systems gave fast separations of Hg_2^{+2} and Hg^{+2}: 0.1*M* HCl (S_1); 15% aqueous ammonium acetate–$3M$ HNO_3–$3M$ HCl (6:1:1) v./v. (S_2); $3M$ HNO_3–$3M$ HCl–isobutanol (1:1:10) v./v. (S_3); and $3M$ HNO_3–$3M$ HCl–isopropanol (1:1:10) v./v. (S_4). In all these solvent systems, Hg_2^{+2} has an R_f value of 0.00.

Table I. R_f Values in Various Solvents

S No.	Solvent	R_f values Sb^{+3}	 Sb^{+5}	 Hg^{+2}	 Hg_2^{+2}
1	Formic acid	0.50	0.40	0.80	0.00 & 0.80
2	Acetic acid	0.21–0.53	0.70	0.50	0.00 T.
3	Propionic acid	0.30	0.48	0.00, 0.60	0.00 T.
4	Butyric acid	0.00 T.	0.20	0.00, 0.20	0.00 T.
5	Ethyl acetate	0.00, 0.12	0.00 T. to S.F.	0.00	0.00 T.
6	Propyl acetate	0.00, 0.20	0.00 T. to S.F.	0.00	0.00
7	Methyl alcohol	0.00 T.	0.71	0.00 T.	0.00 T.
8	Isoamyl alcohol	0.00	0.00, 0.90	0.00 T.	0.00 T.
9	Allyl alcohol	0.64	T. down from S.F.	0.64 T. down	0.00
10	Dioxane	0.00 T.	0.3–0.61	0.00 T.	0.00 T.
11	Isopropyl ether	0.00	0.15	0.00	0.00
12	Anisole	0.02	0.05	0.00	0.00
13	Acetone	0.00, 0.20	0.93	0.00 T. to S.F.	0.00
14	Ethyl methyl ketone	0.00, 0.15	1.00	0.00	0.00
15	Acetyl acetone	0.00, 0.20	0.00 T. to S.F.	0.00	0.00
16	Isopropyl alcohol	0.72	0.80	0.00	0.00 T. to S.F.
17	Water	0.00 T.	0.64	0.00, 0.61	0.00 T.

Abbreviations: S. F. = solvent front; T. = tails.

S_1 is the simplest system giving fast separations (R_f Hg^{+2} = 0.81). When the concentration of HCl is gradually varied from $0.1M$ to approximately $3.00M$, the two R_f values as expected remain almost constant. In $3M$ HCl, the Hg_2^{+2} spot is rather elongated. In S_2, Hg^{+2} has an R_f value of 0.84. When the proportions of Hg^{+2} and Hg_2^{+2} in the sample were gradually varied from 4:1 to 4:16, the two R_f values remained constant. When the concentration of the sample applied was gradually altered from 0.2 to $0.01M$, no significant change either in efficiency of separation or in the R_f values was found. After 5 minutes of development the distance between the spot boundaries was 1.9 cm. Of the common cations studied as impurities, only Ag^+, Sn^{+2}, and Bi^{+3} affected the separation. In all other cases, good separations were obtained. Sn^{+2} affects the separation by its reducing action on the Hg_2^{+2}–Hg^{+2} system. In S_3, Cd^{+2} (R_f = 0.30), Bi^{+3} (R_f = 0.30), and As^{+3} (0.40) have R_f values significantly different from those of Hg_2^{+2} and Hg^{+2} (R_f = 0.61). S_4 is even more selective than S_3. Cations having R_f values different from those of Hg_2^{+2} and Hg^{+2} (R_f = 0.66) are As^{+3} (0.43), Sn^{+2} (0.90), Fe^{+3} (0.30), Al^{+3} (0.20), Cr^{+3} (0.30), Mn^{+2} (0.22), Ca^{+2} (0.20), Ba^{+2} (0.20), Sr^{+2} (0.24), and Mg^{+2} (0.22). Cations with R_f values not significantly different from Hg^{+2} are Cd^{+2} (0.63), Bi^{+3} (0.60), and Sb^{+3} (0.61). Cations with R_f values not significantly different from Hg_2^{+2} include Ag^+ (0.00) and Pb^{+2} (0.00 T).

Double Spot Formation. Hg_2^{+2} gives two spots when developed with 15% ammonium acetate solution and 20% ammonium chloride solution. In both cases the R_f values are 0.00 and 0.84. Since mercurous is not known to form any complex, the possibility is that one spot is due to mercurous nitrate while the other spot is due to mercurous acetate (when developed with ammonium acetate) and mercurous chloride (when developed with ammonium chloride). However, when hydrochloric acid is added to ammonium acetate, the double spot formation is prevented because in this case mercurous nitrate is efficiently converted to mercurous chloride.

Separation of Sb^{+3} and Sb^{+5}. A number of developers were tried for this separation—e.g., isopropyl ether, chloroacetic acid, benzyl cyanide, dioxane, ethyl acetate, ethyl propionate, acetic acid, propionic acid, butyric acid, acetone, pyridine, acetyl acetone, cyclohexanone, acetophenone, ethyl methyl ketone, propyl alcohol, isopropyl alcohol, n-butyl alcohol, tertiary butyl alcohol, and anisole. None of these were successful. Separations were obtained, however, by using glacial acetic acid–water–ethyl acetate (1:1:1). This solvent will be called S_5.

Mixtures containing Sb^{+3} and Sb^{+5} in ratios varying from 1:3 to 9:3 were efficiently separated by this method. The R_f of Sb^{+3} fluctuated from 0.65 to 0.71, while Sb^{+5} was always at the solvent front. Almost all important cations were developed with S_5. The cations whose R_f values differ significantly from those of Sb^{+3} and Sb^{+5} are given in Table II.

Of the common cations tried, the following have R_f values close to that of Sb^{+3}. R_f values: Hg^{+2}, 0.76; Cu^{+2}, 0.75; Sn^{+2}, 0.78; Al^{+3}, 0.64; Cr^{+3}, 0.60; and Mn^{+2}, 0.60. None of the cations tried have R_f values close to Sb^{+5}.

Double Spot Formation. Sb^{+3} gave double spots in a number of cases which are shown in Table III.

Table II. Noninterfering Cations

Cation	R_f	Cation	R_f
Ag^+	0.00(tails)	Co^{+2}	0.47
Pb^{+2}	0.40	Ni^{+2}	0.33
Ca^{+2}	0.58	Zn^{+2}	0.54
As^{+3}	0.50	Sr^{+2}	0.55
Fe^{+3}	0.53	Mg^{+2}	0.50

Table III. R_f Values of Double Spots of Sb^{+3}

Developer	R_f Values First spot	 Second spot
Ethyl acetoacetate	0.00	0.14
Benzyl cyanide	0.00	0.12
5% Solution of thiourea in butanol	0.00	0.50
$12M$ Chloroacetic + isopropyl ether (1:1)	0.00	0.20

DISCUSSION

The separation of Hg_2^{+2} and Hg^{+2} proved more difficult than expected. Acetic acid differentiates fairly well between Hg_2^{+2} and Hg^{+2} (Table I). A number of solvent systems containing nitric acid, acetic acid, and water in different ratios were tried. In most of these solvents, Hg_2^{+2} and Hg^{+2} when separate gave different R_f values, but when a mixture of the two cations was used the separation was not distinct. Best results were obtained with S_6—i.e., acetic acid–$3M$ HNO_3–water (3:1:2). If the concentration of nitric acid was less than that in S_6 the mercurous ion was not completely transferred from the point of application, owing to its strong interaction with paper. If, on the other hand, the concentration of nitric acid were increased, the Hg_2^{+2} was transferred completely from the point of application. The solvent system was then so polar, however, that the paper did not function as differentiating medium between the two cations. To achieve fast and efficient separations, precipitation chromatography was tried using S_1 and S_2. These solvents proved to be fast but nonselective. Pb^{+2} and Ag^+ had R_f values close to zero, while all the other common cations had R_f values close to those of Hg^{+2}.

To attain both speed and selectivity, complexation chromatography was superimposed on precipitation chromatography. Methyl alcohol was added to the HCl-HNO_3 system. The result was that mercurous ion began to tail, owing to its strong interaction with methyl alcohol. When methyl alcohol was replaced by isobutyl alcohol, the system became less polar and Hg_2^{+2} gave a compact spot. Three ions gave R_f values significantly different from those of Hg_2^{+2} and Hg^{+2}. With isopropyl alcohol, which is intermediate in polarity between methyl alcohol and isobutyl alcohol, the system became fast, selective, and efficient.

A reference to Table I shows that Sb^{+3} and Sb^{+5} have different R_f values in a number of organic solvents—i.e., acetic, propionic, and butyric acids; dioxane, acetone, ethyl methyl ketone, methyl alcohol, and isopropyl ether. And, therefore, a number of excellent separations are possible with slight modifications in these solvents. All efforts to separate Sb^{+3} and Sb^{+5} using individual organic solvents failed because most of them were not sufficiently polar. Acetic acid gave an elongated spot owing to its low ionization. The addition of water increased the ionization of acetic acid sufficiently to give compact spots. Because of the high polarity of the system, however, ΔR_f was very small. The addition of ethyl acetate decreased the polarity of the system to such an extent that ΔR_f became significant.

It was noticed in this study that the higher valence state almost always had the higher R_f value. This is easily understood because the higher valence state has a greater covalent character and therefore a greater complexing power. Of the solvents we studied only formic acid appears to be an exception to this trend. The R_f of Sb^{+5} in formic acid is smaller than that of Sb^{+3}.

In the homologous series of fatty acids studied, the R_f values decreased with an increase in molecular weight owing to a decrease in the polarity of the system. In this case also formic acid is an exception. The R_f value of Sb^{+5} in formic acid is less than in acetic acid. We are making a more complete study of the behavior of formic acid and the findings will be reported later.

The importance of time of separation in such cases has been emphasized earlier. To incorporate it in paper chromatography we suggest that the time required for a 1-cm. separation between spot boundaries should be mentioned where necessary along with other data. A paper chromatographic separation may be classified as fast, rapid, normal, slow, or extra slow according as this time (t) is 0 to 30 minutes, 30 minutes to 1 hour, 1 to 6 hours, 6 to 24 hours, or more than 24 hours, respectively. Time for the Hg_2^{+2}–Hg^{+2} separation with S_3 and S_4 is 10 minutes, and for the Sb^{+3}–Sb^{+5} separation with S_5 is 20 minutes. Hence, both these separations may be classified as fast.

ACKNOWLEDGMENT

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Separation of Technetium from Mixed Fission Products by Solvent Extraction with Tributyl Phosphate

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▶ A new liquid-liquid separation technique is described for quantitatively extracting technetium-99(VII) into a tributyl phosphate phase from a sulfuric acid solution of mixed fission products. Technetium(VII) distribution ratios are presented as a function of hydrogen ion molarity and tributyl phosphate concentration. Sodium fluoride is used to provide the necessary zirconium-niobium decontamination and a cation exchange column ensures decontamination from metallic ions such as uranium. Decontamination factors for zirconium-niobium and ruthenium are 2 × 10^4 and 1 × 10^4, respectively. Technetium yields of 92% were obtained with a standard deviation of ±0.8%.

TECHNETIUM, the first man-made element (*9*), does not occur naturally (*5*). The most substantial source of this element is irradiated uranium fuel (*1*) which contains the long-lived radioisotope technetium-99. Since these fuel elements are processed chemically for recovery of uranium, plutonium, and some fission products, raffinate solutions containing significant quantities of the pertechnetate ion are available for recovery. Technetium-99 is normally found as the pertechnetate ion in dissolved fuel element solutions and the subsequent raffinate. An analytical method capable of separating pertechnetate from gross quantities of fission products with a minimum elapsed time would be required to support a recovery process.

Analytically, the pertechnetate ion has been separated from most fission products by a variety of methods including precipitation and distillation (*8*), extraction (*10, 12, 13*), and anion exchange resin (*7*). Radioassay, the most sensitive measurement for technetium-99, was used in most methods. This isotope has a low beta energy of 0.29 m.e.v., hence the presence of other radioactive material on the mount would create serious interference. Of all analytical methods reviewed, only the distillation technique provided satisfactory decontamination from radioactive ruthenium isotopes.

Solvent extraction techniques for separating pertechnetate from radioactive wastes have been widely studied. Boyd and Larson (*2*) reviewed pertechnetate separation by 34 organic solvents and determined that tertiary amines or quaternary ammonium salts had the best partition coefficients. Gerlit (*3*), in studying this ion's extraction characteristics into 21 different organic solutions from various acidic, basic, and neutral media, found that tributyl phosphate–sulfuric acid was a potential extraction system. Siddall (*11*) suggested the extraction mechanism of pertechnetate in a 30% tributyl phosphate–nitric acid system using *n*-dodecane as a diluent. Kertes and Beck (*6*) found a different mechanism for the same system using carbon tetrachloride as diluent, and additionally, defined the nitric acid-dibutyl phosphate system. These works lead to the generalization that the pertechnetate ion can be extracted into organic solvents containing oxygen, phosphorus, or nitrogen. Since tributyl phosphate provides excellent decontamination from ruthenium in the Purex process (*4*), potential use as an analytical reagent was investigated.

EXPERIMENTAL

Reagents and Equipment. Potassium pertechnetate (KTc^{99}O$_4$) was obtained from Oak Ridge National Laboratory as a 3.4 grams per liter solution in a 1.0*M* ammonium hydroxide matrix.

Tributyl phosphate (TBP) of C.P. grade was diluted to the desired concentration with a kerosine diluent (Soltrol). All dilutions were prepared on a volume basis. After dilution, TBP degradation products were removed by washing with an equal volume of three per cent sodium carbonate. Prior to use the TBP-kerosine mixture was equilibrated with an equal volume of sulfuric acid at the same molarity to be used in the experiment.

The cation exchange resin used was Dowex-50X8, 100- to 200-mesh, in the hydrogen ion form and of an analytical grade. The resin was gravity loaded in a 5-cm. long, 3-mm. i.d. column until a bed height of 1.5 cm. was attained.

All extractions were performed in flat-bottomed 15-ml. vials on a magnetic stirrer using glass covered stir bars. These contacts were performed at room temperature.

Radioactive sample aliquots were mounted and dried on 1-inch diameter stainless steel dishes, $^1/_8$-inch deep. Beta activities were counted in a gas flow beta proportional counter, and beta energy spectra were obtained using a terphenyl crystal detector and a multichannel analyzer.

Procedure. The sample, calculated to provide a good counting rate, was diluted to 2 ml. with H_2SO_4 and NaF to yield a final concentration of 1*M* H_2SO_4 and 0.025*M* NaF. One drop of 30% H_2O_2 was added to ensure that all technetium was present as pertechnetate, and the solution was stirred for several minutes. Exactly 4 ml. of 45% TBP was then added, and the mixture was stirred at a complete emulsion for 5 minutes. Phases were separated by centrifuging and 3.0 ml. of the organic was transferred to a vial containing 1.5 ml. of 1*M* sulfuric acid–0.025*M* sodium fluoride–0.2*M* H_2O_2 scrub solution. This mixture was stirred for 2 minutes and again centrifuged to separate the phases. One-half milliliter of the organic phase was transferred to a stripping solution consisting of 10 ml. of distilled water. This mixture was stirred at a complete emulsion for 8 minutes, then centrifuged. A half milliliter of the aqueous phase was transferred to a cation exchange column and allowed to flow through it onto a mounting dish. Three 0.2-ml. water washes were also passed through the column and accumulated on the mounting dish. The mount was dried under an infrared lamp, cooled, covered with a thin layer of collodion, and counted in a gas flow beta proportional counter. The analysis was completed within 45 minutes.

RESULTS AND DISCUSSION

Extraction of pertechnetate was first studied as a function of acid molarity with a 45% TBP solvent. Both nitric and sulfuric acid matrices were tried for the aqueous phase. At first, distribution ratios checked neither those available in the literature (*3*, *11*) nor the second equilibration between the pertechnetate laden organic and a virgin acid scrub solution. However, on adding a drop of 30% hydrogen peroxide to the aqueous phase just prior to extraction, distribution ratios consistent with literature were found. Subsequent analyses of plant samples also showed a consistently higher pertechnetate value when the sample was pretreated with hydrogen peroxide.

Pertechnetate distribution ratios in these two acids are presented in Table I. All further work was performed with a 1*M* solution of sulfuric acid which provided a much better distribution ratio than was possible from a nitric acid solution. [Pertechnetate distribution ratios for nitric acid were in good agreement with the work of Kertes and Beck (*6*).] This particular molarity was selected to reduce pertechnetate losses during the organic extraction and stripping cycle. Since the method requires two volumes of organic per volume of aqueous, increased acid concentration would enhance the pertechnetate extraction only slightly (96.4% at 1*M* *vs.* 96.9% at 2*M*), but the acid in the extractant would increase markedly. Presence of excess acid in the solvent would have the negative effect of reducing the stripping efficiency for pertechnetate.

A test to find the minimum equilibration time for the above experiments showed that equilibrium was achieved within 3 minutes.

To find the variation in the pertechnetate distribution ratio as a function of the TBP concentration, a 1*M* sulfuric acid phase was contacted with TBP concentrations between 0 and 60 volume %. Table II contains these distribution ratios.

A TBP concentration of 45 volume % was selected for the analysis. This distribution ratio was very close to the maximum observation and. additionally, the solvent at 45% TBP was less viscous than at 60%, thus permitting better phase separations and more accurate volumetric transfers.

In considering a stripping solution for removing pertechnetate from the organic phase, distilled water was selected. As previously mentioned, sulfuric acid would also be stripped into the water. For an organic to aqueous ratio of 1:20, a *D* of 0.11 was found when the solvent had first been contacted with a 1*M* sulfuric acid solution. Neglecting any variation in acidity due to change of the water volume, the best stripping ratio was calculated to be 1:20. In this case less than 0.5% of the pertechnetate would be retained in the organic phase.

Table I. Distribution of Pertechnetate between Nitric Acid or Sulfuric Acid and 45% TBP as Function of Hydrogen Ion Concentration

Hydrogen ion, moles/l.	Distribution ratio, D^a HNO$_3$	Distribution ratio, D^a H$_2$SO$_4$
0.0 (pH 6.7)	0.0	0.0
0.5	1.36	3.1
1.0	1.16	4.5
1.5	0.91	10.9
2.0	0.65	13.3
3.0	0.30	14.2
4.0	0.09	15.7

[a] Distribution ratio, *D*, is defined as (c./m./ml. in organic phase)/(c./m./ml. in aqueous phase). These data were calculated from duplicate analyses having material balances $\geq 95\%$.

Table II. Distribution of Pertechnetate between 2.0*N* Sulfuric Acid and Solvent as Function of TBP Concentration

TBP concn., vol. %	Distribution ratio, *D*
0	0.0
15	0.66
30	1.47
45	13.3
60	14.8

The cation exchange resin was used to remove radioactive ions such as uranium or plutonium—i.e., decontamination factor was >10^6—which could follow the solvent cycle purification of pertechnetate. Material balance across the exchanger revealed no pertechnetate loss.

Decontamination for pertechnetate from other fission products was very good. The major radioisotopes present in the sample solutions were Zr^{95}-Nb^{95}, Ru$^{103-106}$, Ce^{144}-Pr^{144}, Ce^{141}, Cs^{137}, Sr^{90}-Y^{90}, and Sr^{89}. Of these, the rare earths, cesium, strontium, and yttrium were notably inextractable in TBP. Both zirconium-niobium and ruthenium were extractable to a limited extent. To reduce zirconium-niobium carried by the organic, the aqueous phase for the initial extraction was made 0.025*M* in sodium fluoride. In some highly radioactive samples it was necessary to add a scrub step in which the pertechnetate laden organic was scrubbed with a 1*M* H_2SO_4–0.025*M* NaF–0.2*M* H_2O_2 solution at a 2:1 organic to aqueous ratio. Only one such scrub should be used since there is a pertechnetate loss (*D* = 13.3) of 4% in this step. Decontamination in the extraction step

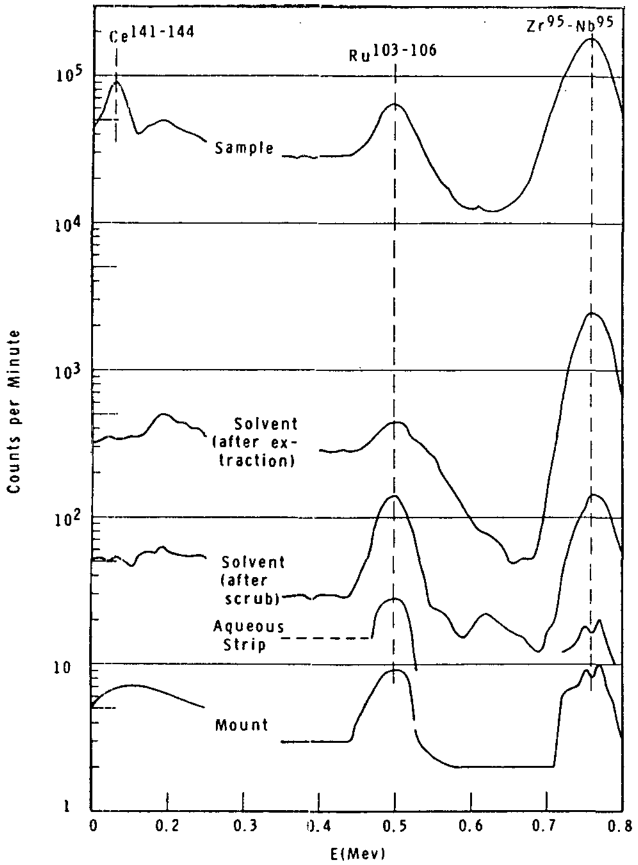

Figure 1. Gamma decontamination profile of pertechnetate analysis

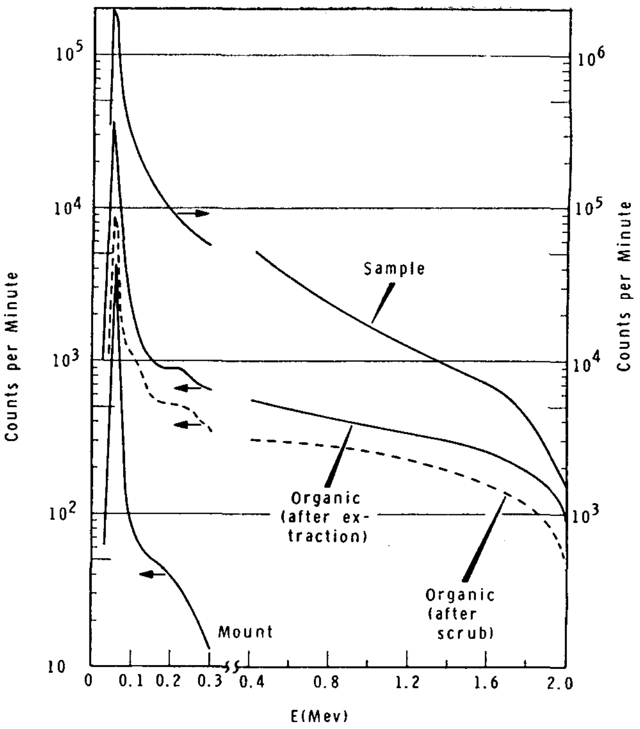

Figure 2. Beta decontamination profile of pertechnetate analysis

was 83 for zirconium-niobium and 311 for ruthenium. Decontamination gained by the scrub was 82 for zirconium-niobium and 6 for the ruthenium. Difference in ruthenium decontamination for the extraction and scrub steps showed it was present in more than one ionic form, one or more of which was not extractable in TBP.

The above decontaminations as well as those for the remaining steps are illustrated in Figure 1. All the gamma spectra have been normalized to represent the quantity of pertechnetate mounted at the end of the analyses. The remainder of the method, consisting of the aqueous strip and the cation exchange purification, yielded decontamination factors of 3 for zirconium-niobium and 6 for ruthenium.

Table III. Determination of Pertechnetate

Analyst	No. of samples	c./m. Tc^{99} taken	Av. % Tc^{99} found
a	6	35,140	92.5
b	4	35,140	91.8
a	4	17,570	91.6
b	2	17,570	92.7

Standard dev.: ±0.8%

The overall decontamination was 2×10^4 for zirconium-niobium and 1×10^4 for ruthenium. These factors are based on actual stream samples, not on prepared tracers which for the most part would not contain the same mixture of ionic species found in plant solutions.

A beta energy profile for the analysis is shown in Figure 2. There is a scale change between 0.3 and 0.4 m.e.v. to distinguish low energy spectra and still present the full spectra obtained (curve shape does not change in the break). As previously mentioned, these spectra were obtained using a terphenyl crystal and a multichannel analyzer. The top curve showing the sample spectra has its ordinate to the right. A high continuum above 0.4 m.e.v. was due to Y^{90} and Pr^{144}. The second and third curves showed the decontamination gained by extraction and scrubbing. In this case, the high energy portion is due to $Ru^{103-106}$. The final spectra demonstrates purity of the mount. Note the maximum energy of 0.30 m.e.v. correlates closely with that of Tc^{99}.

Results of analyses performed on a pertechnetate standard are shown in Table III. The reader should note that all waste solutions of interest originated from fuel elements with at least a 200-day cooling period after irradiation. This analysis would not be specific for Tc^{99} in extremely short cooled fuels which could contain a variety of technetium isotopes depending on fuel type and irradiation history.

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Ion Exchange Chromatography of Amino Acids

A Single Column, High Resolving, Fully Automatic Procedure

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▶ A method for the quantitative determination of 10^{-8} mole of amino acids by ion exchange chromatography is described. A single 0.636- × 125-cm. column packed with 17.5 × 10^{-4} cm. spherical particles is used; operating pressures are approximately 450 p.s.i. The procedure is fully automatic and the time for analysis through to arginine is 21 hours. Increased sensitivity and improved resolution were obtained by optimizing variables related to the ion exchange resin (Dowex 50) column dimensions, and photometer flow cell. 0.01 Micromole (10^{-8} mole) can be determined to within ±5%; the limit of determination is approximately 10^{-10} mole. By zero suppression and range expansion of the strip chart recorder, the analog display of the chromatogram is presented on an expanded scale. An improved resolution of basic amino acids is illustrated. The position of the chromatogram of 186 ninhydrin positive substances of biological interest is indicated. The relationship of the absolute wet density of the resin to cross-linking is examined and the relationship of cross-linking to resolution is given more precise definition than heretofore. The principles involved in optimizing all variables for best performance are fully discussed.

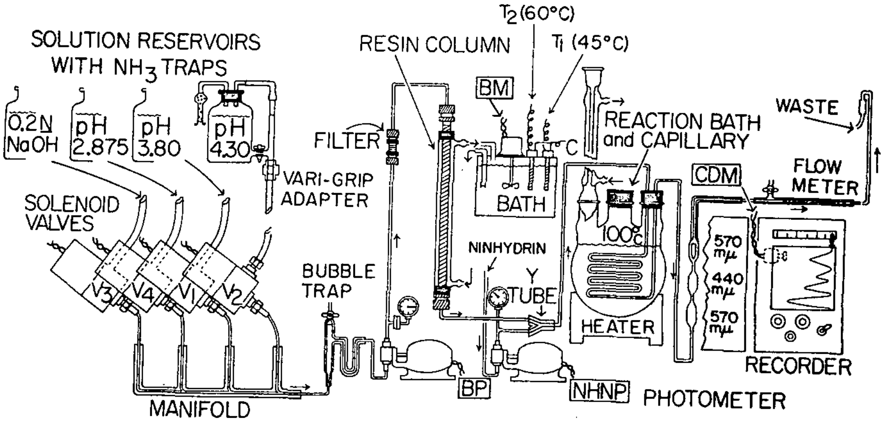

Figure 1. Schematic of apparatus

For description see text

THE OBJECT of this study was to increase the resolving power of current procedures (*7, 17, 20, 23*) for the analysis of amino acids by ion exchange chromatography, and to increase sensitivity so that microgram amounts could be quantitatively determined. These objectives were sought by a thorough application of those principles which have been discussed at theoretical and practical levels (*4, 5, 11, 19*), and by attention to details so that the best performance could be realized. The conditions were imposed that the alteration of existing equipment should be minimal, that a single column would suffice, that the time for a complete analysis of acidic, neutral, and basic amino acids through to arginine plus a regenerating cycle would be no more than 24 hours, that certain critical resolutions—e.g., glutamine from serine—be maintained, and that the procedure be fully automatic. This paper describes the fulfillment of these aims.

With the resolution obtained, analysis of a complex mixture of biological origin —e.g., urine—often indicated 75 to 95 ninhydrin positive components. The sensitivity is such that 0.01 μmole (10^{-8} mole) of most of the compounds (except those with very low ninhydrin color yields) can be determined to within ±5%. The limit of detection is less than 0.0001 μmole (10^{-10} mole).

During the developmental stages, some of the methodology was briefly outlined (*9*); further developments and greater detail are presented here.

EXPERIMENTAL

Apparatus. A bench assembly (*20*) has proved the most satisfactory. A current arrangement is shown in Figure 1. The flow of fluid from each reservoir to the gas capillary manifold (1-mm. i.d.) is controlled by the solenoid valves, V_1, V_2, V_3, V_4 (Model 51P18T23-7-Teflon, Valcor Engineering Corp., Kenilworth, N. J.). The entrance valve ports were provided with $^1/_4$-inch N.P.T. × $^1/_4$-inch tube fittings and the exit ports with reducing adapter bushings plus $^1/_8$-inch N.P.T. × $^1/_{16}$-inch tube fittings; all fittings were Teflon. Because the operating temperature of the continuous duty solenoids was 75° C., the valves were mounted at an angle to allow air, which was evolved from the solution as it passed through, to rise in the input tubes. The valves are controlled by the automatic control unit (see below). A bubble trap (2-ml. volume) between manifold and pump ensured that no stray bubbles were carried to the pump; air which collected was vented through the stopcock. Equally satisfactory was a single degassing unit (*20*) fitted through the large stopper in the central opening of the 3-necked reaction vessel and maintained at 100° C.

The buffer pump (*BP*) fittings, gauge, and isolating diaphragm have been described (*8*). Hastelloy C for the pump liquid end and fittings was mandatory with pH 2.875 buffers. To filter out fragments of pump packing which collected on the resin, a short tube, [0.636 × 5 cm., precision bore, $^1/_4$-inch pipe flanges tooled at both ends (*8*)], was coupled into the line between the pump and column; two Teflon porous disks, $^1/_8$-inch thick (grade 5-55, Fluoro-Plastics, Inc., Philadelphia, Pa.), served as filter (see Figure 1). The volumetric input to the column was 30.0 ml. per hour (94.5 cm./hour, linear flow rate). Operating pressures were approximately 450 p.s.i. with the column at 45° C., 400 p.s.i. at 60° C. During 24 months of continuous use, the pressure slowly rose to 575 p.s.i.; the column was then dismantled and repoured.

Pressure was monitored by means of a potentiometer transducer (not shown in Figure 1)—e.g., Amtex, Model PT-143-A-600B-2M, U. S. Gauge Co., Sellersville, Pa.—coupled to the gauge line. A stable millivolt source to the

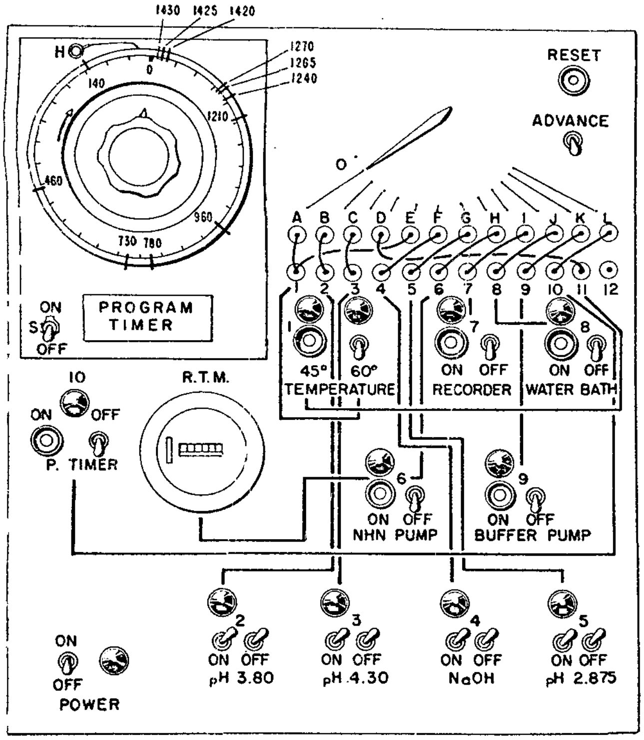

Figure 2. Automatic control unit

Running time meter (R.T.M.) left of center; stepper relay and patch cord connections upper right. See text for details

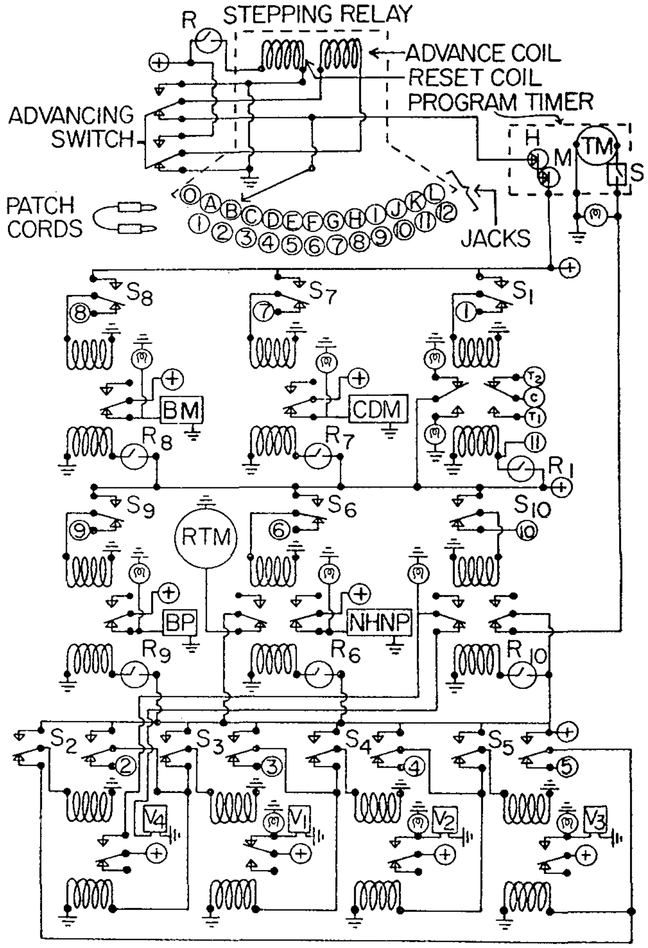

Figure 3. Circuit schematic of automatic control unit

Power connections (110 volts, a.c.) are to + terminals. Other details in text

transducer gave an output that drove a strip chart recorder. Pressure was not recorded all the time, but only when faulty pump action was suspected. This enabled corrective measures to be applied before valuable runs were lost.

The column effluent was joined by the ninhydrin stream at the capillary Y tube (1-mm. i.d.). A standard 3-necked, 2-liter flask, fitted with a condenser, served as 100° C. bath for the Teflon reaction capillary (*20*); a 600-watt heater (Gilmer, Cat. 6130, A. H. Thomas Co., Philadelphia, Pa.) controlled by a variable autotransformer kept the bath at low boil.

The ninhydrin pump (NHNP), gauge and storage reservoirs have been described previously (*20*).

The flow photometer (*20*) (Phoenix Instrument Co., Philadelphia, Pa.) was fitted with a flow cell similar to that of Piez and Morris (*17*) but of modified form (*9*); the light transmitting sections were 4.4-mm. i.d. Fluid entered the flow cell from the bottom and was monitored successively at 570, 440, and 570 mμ; the last section had a sealed-in glass spacer to give an effective fluid depth $^1/_5$ that of the bottom section (*20*).

Connecting fluid lines were as follows: from reservoirs to valves, 0.188 × 0.25 inch Teflon (all Teflon tubing and Teflon capillary was obtained from Pennsylvania Fluorocarbon Co., Philadelphia, Pa.); from valves to manifold to bubble trap (or to degassing unit) to buffer pump, and from ninhydrin reservoir to ninhydrin pump, gauge 19, 0.038 × 0.070 inch Teflon; from buffer pump to chemical gauge diaphragm to filter to column, $^1/_8 \times {}^1/_{16}$ inch flexible Teflon, 2000 p.s.i. test; all other fluid lines, including the reaction capillary, were gauge 22 heavy wall, 0.028 × 0.060 inch, Teflon. Before being placed in service, the reaction capillary was tested for leaks under 200 p.s.i. fluid pressure. Connections to the manifold, bubble trap, degassing unit, Y tube, flow cell, and bubble flow meter, were made by press fit into slightly expanded portions of the glass capillary; the glass was tooled for a length of 2 inches in the flame with steel rods (discarded drills) of increasing diameter to give a tapered section to receive the Teflon capillary. These connections withstood pressures over 50 p.s.i. but were easily dismantled. To extend fluid lines by inserting extra capillary or to join cut lines, butt joints of the Teflon capillary were made by inserting the ends into sleeves of slightly larger diameter Teflon tubing. Joints were wired if necessary.

A 3 point, $4^1/_2$ second pen speed, 6 inches/hour chart speed strip chart recorder (Minneapolis-Honeywell, Philadelphia, Pa., Model 153X72) displayed the chromatogram. Two variations of this model have been used. One was supplied by the manufacturer with continuously variable span, 0–1, 0–21 mv., and continuously variable zero suppression, ±20 mv. max. and the other was a standard 0–10 mv. model modified by substituting 5-ohm, 3-turn Helipots (Beckman Instrument Co.), for the range and span spools of the potentiometer measuring circuit. The pots were mounted on the rear of the chassis door. This span could thus be varied continuously from 0–10 mv., and the range continuously from 0–10 to 9–10 mv. Dial settings for span and range were tabulated, but the recorders were always checked with a standardizing potentiometer after resetting.

Column and Resin. The construction of the column (0.636-cm. i.d.) connecting couplings for pressure work, column jacketing, and temperature control have been described (*8*, *11*). Columns of similar construction and specifications are commercially available from the Technicon Corp., Chauncey, N. Y. For the pump to column connection, $^1/_8 \times {}^1/_{16}$ inch flexible Teflon was substituted for the polyethylene tubing and 3 neoprene O-rings, $^1/_4 \times {}^1/_8$ inch were substituted for the small rubber stopper originally used [See Figure 1, (*8*)]. The O-ring and Teflon washers were prevented from slipping off the Teflon tubing by flaring the end of the latter with a heated tool, (a $^3/_{16}$ inch diameter rod ground to a 75° conical end). The particles for packing the column were separated from −400-mesh resin [Dowex 50 ×8 (The Dow Chemical Co., Midland, Mich.)] by a backwashing technique (*10*). The particles were spherical with a mean diameter of 17.5 microns and 80% of the diameters were within ±2 microns of the mean. The resin had a capacity of 5.1 meq./gram (dry), and a wet density (sodium form) by pycnometer of $\rho = 1.280 \pm 0.002$ [or a tungstate density of $\rho_t = 1.340$ (Appendix and (*21*)]; either density corresponded to a cross-linking of 8.5% (see Figure 6). Column packing with similar physical specifications and of comparable chromatographic performance is currently marketed under the trade name Chromobeads (Technicon).

To pack a column, the resin was slurried in 2 volumes of 0.2*N* NaOH plus Brij 35 and poured to the top. After settling by gravity, the resin was packed under fluid pressure by the pump for 30 minutes. Excess fluid was then aspirated and the process repeated. Several additions of packings were necessary to fill the column to a depth of 125 cm. above the Teflon filter. With use, the column packed further and more resin was added to maintain the 125-cm. level.

Automatic Control Unit. The appearance of this unit is shown in Figure 2, and the circuit in Figure 3. A program timer (Model P5-24, Zenith Electric Co., Chicago, Ill.) controlled the cycle of operation. The time for each event in the cycle was determined by a mechanical trip inserted into the periphery of the dial (Figure 2) which is rotated by a timing motor (*TM*) (Figure 3) once in 24 hours. As a trip is moved past the activating armature of switch *H*, the latter is closed for approximately 7 minutes, but the circuit is completed only when switch *M* (Figure 3) is closed for 2 seconds by a cam which rotates once every 5 minutes. Simultaneous closure of *H* and *M* provides power for 2 seconds, which advances a stepping relay (Type PER, Guardian Electric Co., Chicago, Ill.) to its next consecutive contact and which activates one coil of a latching relay connected to that contact. The wiring of the program timer as supplied, was modified to that shown in Figure 3; the toggle switch *S* was retained for manual control. For greater convenience in reading time, the 0–24 hour scale was replaced with a 0–1440 scale, graduated in 5-minute intervals. (In Figure 2, only the 50-minute graduations are shown). The stepping relay contacts were wired to jacks, *A* to *L*, from which connection was made by patch cords and plugs through jacks *1* to *11* (jack *12* is a spare) to switches (Lev-R, 3003, Switchcraft, Inc., Chicago, Ill.) S_1 to S_{10} which control the upper coils (Figure 3) of the latching relays. For clarity of presentation, these connections have been omitted from Figure 3. The patch cord arrangement provided great flexibility in programming the order of events. Referring to Figure 3 and adopting a descriptive convention that energizing the upper coil of a latching relay throws the relay *SPDT* switch armature up, it follows that power is cut off to the pumps (*BP*, *NHNP*), to the recorder chart drive motor (*CDM*), to the circulating bath motor (*BM*), and to the program timer when the controlling trip and stepper contact is activated. Power to these units may also be cut off manually by switches S_1, S_6–S_{10}, respectively. Dual control provides for manual operation without altering the automatic control settings. Power to the above units is provided by momentarily closing switches R_6–R_{10}, respectively. Contact through jack *1*, changes control from the 45° (T_1–*C*) thermoregulator to the 60° (T_2–*C*) thermoregulator; to return to 45°, R_1 is momentarily closed. Neon pilot lamps identify the condition of any circuit. A running time meter, wired into the ninhydrin pump circuit, enables the time to be easily read during a run.

Relay connections *2*–*5*, controlling the valves, V_1–V_4, need special mention. When the lower coil of relay *2* is energized through jack *2*-S_2 (right) power is simultaneously cut off from V_4, which closes, and applied to the upper coil of relay *3* which opens V_1. Similarly, as V_1 is closed V_2 is opened, as V_2 is closed V_3 is opened, and as V_3 is closed V_4 is opened, thus completing a cycle of 4 solution changes, ending with the starting solution. Beginning with pH 2.87 buffer, the sequence pH 3.80, pH 4.30, 0.2*N* NaOH (wash) and pH 2.87 (conditioning) is readily programmed. The switches S_2–S_5 (referring in Figure 3 to those immediately to the right or left of the symbol), enables the valves to be opened or closed manually. V_4 (pH 2.87 buffer) being last in the sequence, is simultaneously closed when jack *10* is energized to stop the program timer.

Two automatic control units have been in continuous service for 36 months without mechanical or electrical failure. Operation may be automatic and left unattended, or the cycle may be entered at any time for manual operation, and then returned to automatic function. The stepping relay can also be advanced manually by the switch (Lev-R 3006, Switchcraft) as shown; it is returned to 0 by means of a reset switch.

Reagent Solutions. The preparation of buffers is given in Table I; all solutions were made up under nitrogen in a polypropylene vessel and transferred, out of contact with laboratory air to the apparatus reservoir.

The sodium citrate, sodium chloride and sodium acetate (for the ninhydrin reagent) were tested by adjusting nearly saturated solutions of each to pH 11 with sodium hydroxide. If the solution developed color or precipitate on alkalization or contained particulate matter, the reagent was rejected. The solutions were also tested for ammonia by Nesslerization, and if positive that

Table I. The Composition on of Buffers and Method of Preparation

Buffer	Composition	Preparation
pH 2.875	0.205*N* Na, 0.05*M* citrate, 0.3% Brij 35, 0.5% thiodiglycol, 0.1% Versene	0.05 moles $Na_3C_6H_5O_7 \cdot H_2O^a$ + 27.5 ml. 2.00*N* NaOH[a] + 1.0 gram Versene[a] + 25 ml. Brij 35[b] solution + 15 ml. thiodiglycol[c] + 850 ml. low ammonia distilled water[d]. Titrate with 6*N* HCl[e] to pH 2.875 at glass electrode, make to 1 liter and make final adjustment of pH.
pH 3.80	0.200*N* Na, 0.05*M* citrate, 0.3% Brij 35, 0.5% thiodiglycol, 0.1% Versene	Same as pH 2.875 buffer, except 25.0 ml. of 2.00*N* NaOH added. Solution adjusted to pH 3.80.
pH 4.30	0.800*N* Na, 0.05*M* citrate, 0.6*M* NaCl, 0.3% Brij 35, 0.5% thiodiglycol, 0.1% Versene	0.05 moles $Na_3C_6H_5O_7 \cdot H_2O$ + 0.6 moles NaCl[a] + 25 ml. Brij 35 solution, + 5 ml. thiodiglycol + 1 gram Versene + 850 ml. water. Titrate with 6*N* HCl as above, make to 1 liter and make final adjustment of pH.

[a] Analytical reagents, J. T. Baker Chemical Co., Phillipsburg, N. J.

[b] Atlas Chemical Industries, Inc., Wilmington, Del. 200 grams Brij 35 plus 1300 ml. low ammonia water; dispensed from 25 ml. automatic pipet (Kimble Cat. #37077F); provided with ammonia trap.

[c] Pierce Chemical Co., Rockford, Ill.

[d] Laboratory distilled water redistilled in borosilicate all-glass apparatus from 5% sulfuric acid through 4- × 30-cm. reflux column packed with Raschig rings. Distillate collected in borosilicate aspirator bottle (with Teflon stopcock), protected by acid trap. Water dispensed from outlet via Teflon adapter and Teflon tubing.

[e] Reagent grade hydrochloric acid of low ammonia content—e.g., E. I. du Pont de Nemours and Co., Wilmington, Del.—diluted with equal volume of low ammonia distilled water in polypropylene vessel under stream of nitrogen and stored in borosilicate glass bottle protected with ammonia trap. Dispensed via Teflon adapter and capillary.

Table II. Operation Schedule

Time, minutes	Temperature changes
0–140	Column at 45° C.
141	Change to 60° C.
781	Change to 45° C.
981	Change to 60° C.

Time, minutes[a]	Buffer and operational changes
0–460	pH 2.875 buffer
461	Change to pH 3.80 buffer[b]
731	Change to pH 4.30 buffer[c]
1211	Change to column wash[d]
1241	Change to pH 2.875 (regeneration)
1265	Ninhydrin pump off
1270	Recorder off
1420	Circulating bath motor off
1425	Buffer pump off
1430	Program timer plus V_4 solenoid off

[a] 1 minute corresponds to effluent volume from column of 0.5 ml.

[b] Emerges from column at min. 570

[c] Emerges from column at min. 810

[d] 0.2N NaOH +0.3% Brij 35 + 0.1% Versene

particular bottle of reagent was discarded.

Ninhydrin reagent was prepared as described (*20*).

Operational Procedures. The program for analysis is illustrated in Figure 2 and tabulated in Table II.

To commence a run, V_4 (pH 2.875 buffer) is opened by switch *10* (Figure 2); this closes one of two switches in the program timer circuit. The circulating bath motor is started (switch *8*). When temperature regulation is established, the sample is placed on the resin. A sample volume of 0.5 ml. or less is desirable (*11*). Amino acid test mixtures in 0.1N hydrochloric acid were used without further adjustment of pH. Blood serum, plasma, or urine were deproteinized with sulfosalicylic acid (*11*). Other solutions were adjusted to approximately pH 1 with hydrochloric acid.

After the sample has entered the resin, the pump line is coupled to the column and the recorder, buffer pump and program timer are started (switches *7*, *9*, and *S*, respectively). After the pressure has risen to approximately 80% of maximum, the ninhydrin pump is started (switch *6*); 30 minutes later the photometer potentiometers are adjusted. Thereafter, operation is unattended through the ensuing 24 hours.

Integration of Peaks. The area of a peak recorded on the strip chart is determined by the $H \times W$ method of Spackman, Stein, and Moore (*20*). When the span and range of the recorder are equal, display is on charts with ordinates printed in absorbance (log) units,—e.g., chart #9077-N, Minneapolis-Honeywell. When the span is less than the range of the recorder, the printed scale no longer holds and an even-grid paper—e.g., #5839-N, Minneapolis-Honeywell, 20 lines to the inch with every 10th line accented and 11-inch (279.4-mm.) ordinates—may be substituted. Preliminary to calculating, the range and span are read from the dial settings of the recorder or are determined by direct calibration with a potentiometer. For example, if the recorder gives a pen deflection to the extreme right chart line with a 10.0-mv. input signal and to the extreme left chart line with an 8.00-mv. input signal, the recorder is calibrated for a span of 2.00 mv. and the range 8.00 to 10.00 mv. Taking the right chart line as 100% transmittance, the left chart line is therefore at 80% transmittance; that is, of the total range—i.e., from electrical zero to 10.00 mv.—only 20% is displayed on the chart. With an 11-inch (279.4-mm.) chart, electrical zero is therefore situated 279.4/0.20 = 1397 mm. to the left of the 100% transmittance line. The 80% line is therefore (1397 − 279.4) = 1117.6 cm., from electrical zero. In general, a curve whose base is at y mm. and whose peak is at x mm. from the left chart line will have base and peak at (1177.6 + y) and (1117.6 + x mm.), respectively, from electrical zero. Applying Beer's law, net peak height ($C_{\max}$) is therefore given by:

$$\{\log 100 - \log [(1117.6 + y)/(1379) \times 100]\} - [\log 100 - \log (1117.6 + x)/(1379) \times 100)]$$

which reduces to log (1117.6 + y) − log (1117.6 + x); the half height is at $C_{\max}/2$. The position of the half height is on a line drawn z mm. from the left chart line through the peak and parallel to the abscissa; z is given by

$$\text{antilog}\,[\log (1117.6 + x) + C_{\max}/2] - 1117.6$$

Peak width at half height (ΔT) is determined in minutes by dot counting (*20*). The product $\Delta T \cdot C_{\max}$, is a measure of the area of the peak. A calibration constant for each amino acid is determined by analysis of known amounts of each. In practice it is convenient to express these constants as multiplying factors, designated k' and whose units are μmoles per unit area; the relationship of k' to the C constants of Spackman, Stein, and Moore (*20*) is hence of the form $k' \propto 1/C$; in the present work, the relationship for most amino acids was very nearly $k' = 3/C$. The values for k' given in Table III were determined in this laboratory.

Position of Ninhydrin Positive Substances. The position of 135 amino acids and close relatives, 31 dipeptides, 3 tripeptides, 1 tetrapeptide, 1 hexapeptide, 6 amino sugars, 3 amines, 3 aminoalcohols, and 3 other ninhydrin reactive compounds are shown in Figure 4 and listed in Table IV. The position of each compound was established by individual analysis, and its relationship to other compounds by analysis in mixtures. 0.01 Micromole (10^{-8} mole) of each compound was placed on the column. The position, shape, and size of the common amino acids shown in heavy lines are an exact tracing of a single chromatogram to which the other amino acid curves have been added. The shape of the peaks is characteristic; the height of the peaks approximates that seen on analysis, except in crowded regions of the chromatogram, where some peaks were elevated for clarity of presentation. This figure relates the position of less common substances to the more common amino acids and supplements a previous publication from this laboratory (*7*). Although the specific position of an amino acid in the 2-column system of Spackman, Stein, and Moore (*20*) or the 1-column gradient elution system of Piez and Morris (*17*) may be altered relative to its nearest neighbors as compared with its position in the present system, the general region of the chromatogram in which a particular amino acid may be located is similar. Some assistance is therefore given in locating amino acids that have not been positioned by these authors (*17*, *20*) or by Frimpter and Bass (*2*), and Zacharius and Talley (*24*). The possibility of overlap or coincidence of peaks argues strongly for the desirability of confirming, by some independent test—e.g., electrophoresis, paper, thin layer, or gas

Table III. Calibration Coefficients, k'

For multiplying integrated peak areas ($\Delta T \cdot C_{\max}$), to convert to μmoles

Amino acid	. k'
Cysteic acid	0.118
O-PO$_4$-Ethanolamine	0.128
Taurine	0.116
Urea	0.248
4-OH-Proline	0.116
Aspartic acid	0.118
Threonine	0.119
Serine	0.113
Sarcosine	0.290
Glutamic acid	0.120
Proline	0.529
Citrulline	0.114
Glycine	0.123
Alanine	0.117
Glucosamine	0.116
Valine	0.120
Homocitrulline	0.103
Cystine	0.110
Methionine	0.124
Alloisoleucine	0.115
Isoleucine	0.113
Leucine	0.113
Norleucine	0.116
m-Tyrosine	0.146
Tyrosine	0.119
Phenylalanine	0.118
β-Alanine	0.208
β-2-Thienylalanine	0.178
β-Aminoisobutyric acid	0.246
Ethanolamine	ca. 0.17
γ-Aminobutyric acid	0.110
Hydroxylysine	0.103
Ornithine	0.099
Lysine	0.104
Histidine	0.114
1-Methylhistidine	0.118
3-Methylhistidine	0.122
Anserine	0.215
Carnosine	0.147
2-NH$_2$-3-guanidinopropionic acid	0.140
Canavanine	0.118
Tryptophan	0.173
Arginine	0.124

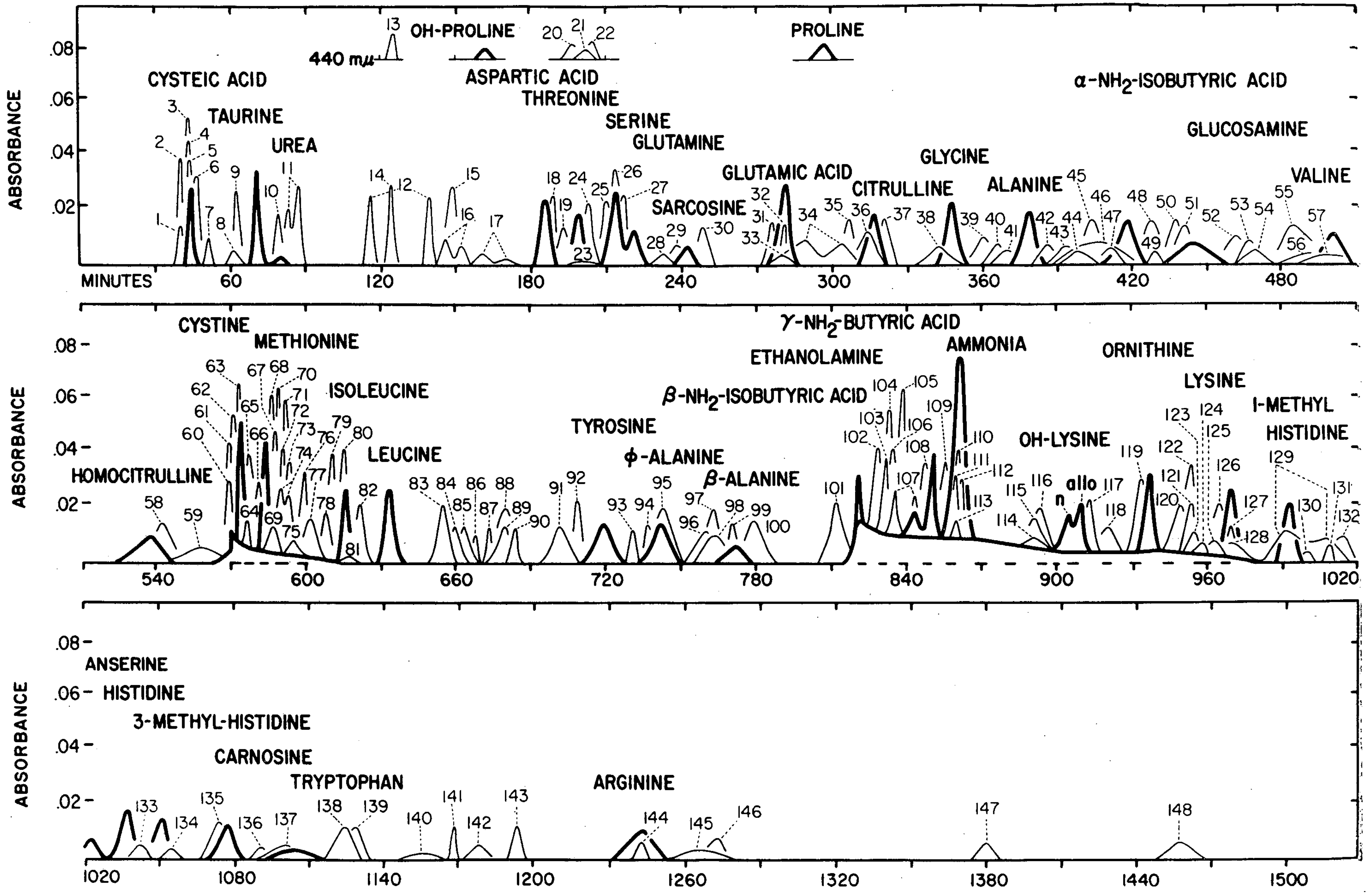

Figure 4. Position of 186 ninhydrin reactive substances on the chromatogram

For identification of numbered peaks (light lines) see Table IV.

Table IV. Ninhydrin Reactive Compounds Corresponding to Numbered Peaks Shown in Figure 4

1. Penicillaminic acid
2. *O*-Phospho-4-hydroxyproline[a]
3. *O*-Phosphoserine
4. *O*-Phosphothreonine
5. Homocysteic acid
6. Cysteine sulfinic acid
7. Glycerylphosphorylaminoethanol[b]
8. Glucosamine-6-phosphate
9. *O*-Phosphoethanolamine
10. Levulinic acid
11. *threo*-β-Hydroxyaspartic acid[c]
12. *erythro*-β-Hydroxyaspartic acid[d]
13. 3-Hydroxyproline[e]
14. Glucosaminic acid
15. *O*-Phosphohydroxylysine[a]
16. Methionine sulfoxides
17. Ethionine sulfoxides
18. Methionine sulfone
19. β-Methylaspartic acid
20. *allo*-3-Hydroxyproline[e]
21. *allo*-4-Hydroxyproline
22. Thioproline
23. 2-Thiolhistidine
24. *allo*-Threonine
25. Ethionine sulfone
26. Asparagine
27. 2-Amino-3-ureidopropionic acid
28. α-Methylserine
29. Muramic acid[f] (3-*O*-Carboxyethyl-D-glucosamine)
30. Homoserine
31. Glutamic acid-γ-methyl ester
32. Glutamic acid-γ-ethyl ester
33. α-Methylglutamic acid
34. Isovalthines[g]
35. *S*-Methylcysteine
36. Felinine[g]
37. Felininic acid[g]
38. Penicillamine
39. β-2-Thienylserine
40. 2-Aminoadipic acid
41. Alanylasparagine
42. *meso*-Lanthionine (peak 1)
43. Formiminoglycine (peak 1)
44. Glycylasparagine
45. *S*-Ethylcysteine
46. Glutathione (oxidized)
47. *meso*-Lanthionine (peak 2)
48. Glycylserine
49. Isoserine
50. Allylglycine
51. α-Amino-*n*-butyric acid
52. *threo*-β-Phenylserine
53. Glutamylglutamic acid[h]
54. Isovaline
55. Manosamine[i]
56. Formiminoglycine (peak 2)
57. Galactosamine
58. Pipecolic acid
59. Glycylglycylglycylglycine
60. *meso*-Cystine
61. Glutamic acid-γ-hydrazide
62. α-Aminopimelic acid
63. Glycylsarcosine[h]
64. Alanylglycylglycine
65. Alanylalanine
66. 3,5-Dinitrotyrosine
67. α-Methylmethionine
68. Phenylglycine
69. Alanine anhydride
70. Djenkolic acid
71. α-Amino-*n*-valeric acid
72. Norvaline
73. Glycylglycine
74. Cystathionine
75. α-Phenyl-α-alanine
76. *allo*-Isoleucine
77. Glycylglycylglycine
78. Glycylalanine
79. Methionine sulfoximine
80. α-ε-Diaminopimelic acid[j]
81. 2-Methylleucine
82. Ethionine
83. Norleucine
84. 3,4-Dihydroxyphenylalanine (DOPA)
85. β-2-Thienylalanine
86. α-Hydroxy-δ-aminovaleric acid[a]
87. 1-Aminocyclopentane-1-carboxylic acid
88. Alanylvaline
89. *meta*-Tyrosine
90. γ-Amino-β-hydroxybutyric acid
91. Glycylvaline
92. *ortho*-Fluorophenylalanine
93. *meta*-Fluorophenylalanine
94. Isoglutamine
95. Alanylmethionine
96. *ortho*-Tyrosine
97. Leucylglycine
98. Leucylglycylglycine
99. *para*-Fluorophenylalanine
100. Alanylnorvaline
101. Glycylnorvaline
102. Alanylleucine
103. Glycylethionine
104. δ-Aminolevulinic acid, methylester
105. Homocystine[k]
106. Glycylleucine
107. Argininosuccinic acid[l]
108. Alanylnorleucine
109. Glycylnorleucine
110. Alaninol (2-Amino-1-propanol)
111. Glycine amide
112. Glycylmethionine
113. β-Amino-*n*-butyric acid
114. Glycyltyrosine
115. Glycamine (methylamine)
116. α-Aminocapyryllic acid
117. Glycylphenylalanine
118. Alanylphenylalanine
119. 2,4-Diaminobutyric acid
120. Alamine (ethylamine)
121. δ-Amino-*n*-valeric acid
122. 2,3-Diaminopropionic acid
123. α-Amino-γ-phenylbutyric acid
124. 2-Amino-1-butanol
125. 5-Hydroxytryptophan
126. Kyneurenine
127. Phenylalanylglycine[h]
128. β-Phenyl-β-alanine
129. 3-Aminotyrosine
130. 6-Hydroxytryptophan
131. δ-Aminolevulinic acid
132. Leucyltyrosine
133. Glycyldehydrophenylalanine[h]
134. 4-Aminophenylglycine
135. Homocarnosine
136. 4-Hydroxytryptophan
137. 3-Iodotyrosine
138. Canavanine
139. 2-Amino-3-guanidinopropionic acid
140. ε-Amino-*n*-caproic acid
141. 2-Amino-4-guanidinobutyric acid
142. 3,5-Dibromotyrosine
143. Leucine amide
144. Homocysteine thiolactone
145. 3,5-Diiodotyrosine
146. 5-Methyltryptophan
147. Glycyltryptophan
148. Homoarginine[m]

[a] Synthesized in this laboratory.

[b] Gift of Erich Baer, University of Toronto, Toronto, Canada.

[c] Major peak; minor peak 10 minutes earlier.

[d] Major peak; minor peak 25 minutes earlier.

[e] Gift of Milan Logan, U. of Cincinnati Coll. of Med., Cincinnati, Ohio.

[f] Gift of Willard Schmidt, Cleveland Metropolitan Hospital, Cleveland, Ohio.

[g] Gift of John Wriston and T. M. Wong, University of Delaware, Newark, Del.

[h] Gift of Joseph Fruton, Yale University, New Haven, Conn.

[i] Gift of Richard Winzler, University of Illinois College of Medicine, Chicago, Ill.

[j] 40% LL + 60% *meso;* geometrical isomers were not resolved.

[k] DL + *meso;* geometrical isomers were not resolved.

[l] Position of major peak; for explanation of associated peaks see Cusworth, D. C., and Westall, R. G. (*1*).

[m] Gift of Theo. Gerritsen, University of Wisconsin University Hospitals, Madison, Wis.

chromatography—the name assigned to any particular peak. This caution is particularly germane when material of biological origin, which has not been previously examined by ion exchange chromatography, is first investigated.

Concerning amino acids emerging after arginine, the positions of homocysteine thiolactone [Zacharius and Talley (*24*)], and homoarginine [Gerritsen *et al.* (*3*)] were confirmed, and other examples are shown. Although the amines and aminoalcohols listed above were eluted, cadaverine, putrescine, tyramine, tryptamine, histamine, or histidyl–histidine were never observed to leave the column, even when analysis was extended to minute 1500 (25 hours).

In the cystine–methionine region (minutes 570–610) the base line is elevated commencing with a small artifactual peak (immediately in front of the cystine peak) which coincides with the exit of the pH 3.80 buffer. A larger and longer base line elevation occurs commencing with the exit of the pH 4.30 buffer (minute 810) and terminating at or near histidine (minute 990, approximately). These irregularities could be, in part at least, due to ammonia which as contaminant of the buffers, would collect on the column during conditioning and development with the pH 2.875 and pH 3.80 buffers. The appearance of the major portion of this ammonia with the pH 4.30 buffer is to be expected because of the decrease in distribution of ammonia between resin and fluid from approximately 10 to 1 to 3 to 1 that occurs when th sodium concentration is increased from 0.2 to 0.8*N*. The temperature changes introduced in the program of operation would also affect the distribution of ammonia, but in lesser degree. However, to date all efforts to eliminate these irregularities have been unsuccessful and all evidence so far obtained, taken together, does not allow the unequivocal conclusion to be drawn that they are solely due to ammonia.

To improve the resolution of the basic amino acids over that previously published for a single column (*7*), examination over a limited range of pH, sodium concentration, and temperature was undertaken. The results are shown in Figure 5.

In every case development was carried through pH 2.875 and 3.80 as for a normal run, and the third buffer was started at minute 780. In Figure 5*A*, the temperature was 60° C., the sodium concentration was 0.80*N*, and the pH was varied. It is apparent that as the pH is lowered the position of each amino acid is changed, but not necessarily in a manner to improve separation. At pH 3.80, the peaks are separated but they are broad so that the excellence of the resolution is more apparent than real and the time necessary for the exit of arginine is excessive. At pH 4.30 resolution is adequate, the peaks are not wide, and arginine is not unduly retarded. In Figure 5*B*, pH and sodium concentration were held constant and at minute 780 the temperature was either continued at 60°, lowered to 50°, or raised to 70° C. The marked temperature dependence of the position of tryptophan is shown; this led to defining more precisely the conditions for its resolution from 3-methylhistidine. In Figure 5*C*, pH and temperature were held constant and the sodium was varied by altering the concentration of sodium chloride in the buffer. Here the positions on the chromatogram of the peaks are shifted, but the relative positions are little altered. Tryptophan is again a marked exception, showing a characteristic independence of sodium concentration (*7*).

In Figure 5*D*, resolution with 0.267*M* citrate is illustrated for comparison with that shown in Figure 5*A*. At pH 5.0, in the high citrate buffer, 1-methylhistidine, 3-methylhistidine, histidine, anserine, carnosine, and tryptophan are grouped together. In the low citrate buffer at the same pH, the grouping of these amino acids is different, and the difference makes possible the resolution of them all at pH 4.30, without undue retardation of the arginine.

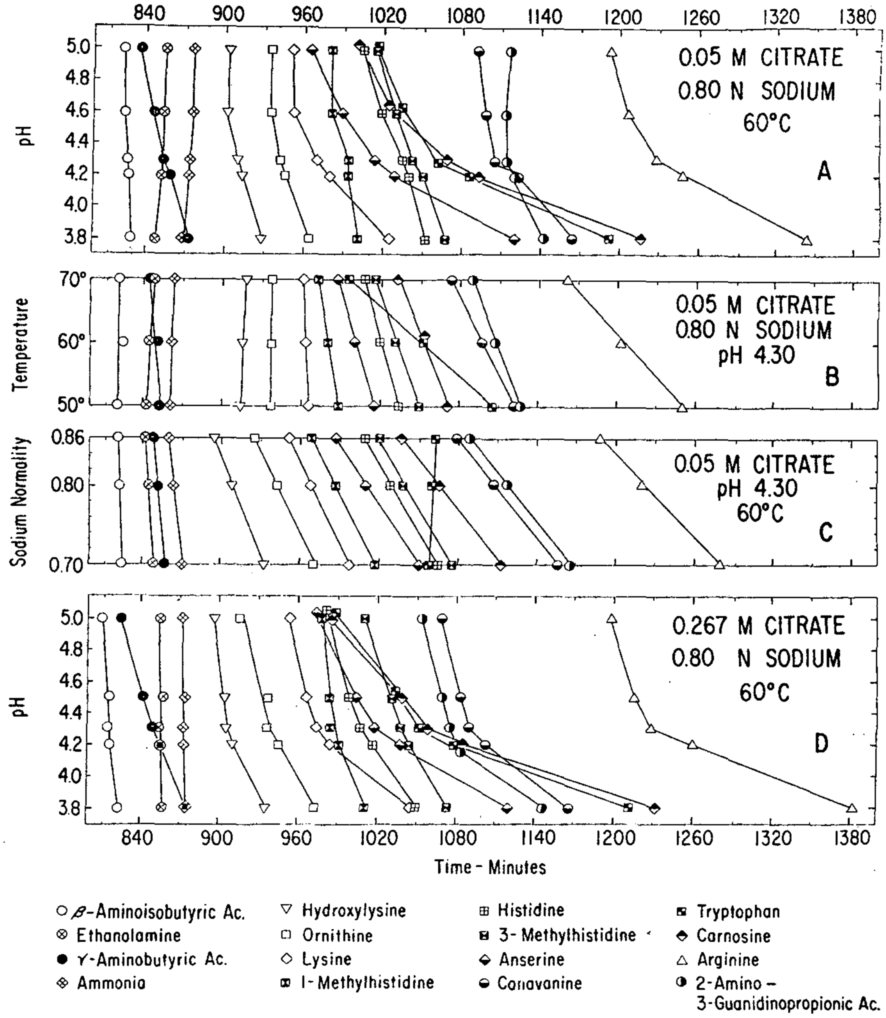

Figure 5. Resolution of basic amino acids as function of pH, temperature, sodium concentration, and buffer composition

DISCUSSION

This discussion is concerned with the gain in sensitivity and resolution that was achieved by attention to details of column design, resin characteristics, photometer flow cell design, and recorder characteristics.

Column Dimensions. Increased sensitivity was obtained by using a smaller diameter column in place of an 0.9-cm. column. Applying Equations 2 and 3 (Appendix A) to each, it can be shown that for the same amount of amino acid and the same volumetric input, peak width for an 0.636-cm. column is reduced by $^1/_2$ (because of reduced cross-sectional area) and increased by $\sqrt{2}$ (because of increased linear flow rate) as compared with the larger column. Combining these results gives a net decrease in peak width of $\sqrt{2/2}$. The quantity $\sqrt{2}$ is approximate, because C_6, though smaller than C_4U_0, is not negligible. The decrease in peak width is accompanied by a corresponding increase in peak height; peak height therefore is increased by approximately $2/\sqrt{2}$, or 1.4. A column of 125 cm. was adequate. As column length is increased, resolution is increased only in proportion to the $^1/_2$ power [Equation 12, (*11*)], while pressure is increased proportionately, so that a longer column gains little in this instance except increased pressure. Since 0.5-ml. sample volumes were to be used, the volume of an 0.636 × 125 cm. column was large enough to prevent peak widening from sample volume size (*11*).

Resin Particle Size. Sensitivity and resolution were both improved by using resin particle diameters as small as possible, consistent with reasonable pressures. The quadratic form of Equation 4 (Appendix A) indicates that for values of $d_p<1$, the rate of decrease of peak width becomes less as d_p approaches zero. Selection of a 17.5-micron particle diameter placed the operating point (for peak width) nearer the origin on the less steep part of the curve. A reduction of particle diameter from 25 to 17.5 microns gives a 20% (approximately) reduction in peak width, or a gain in peak height of 1.2. However, since pressure varies as $1/d_p^2$ (*11*), this change is accompanied by a twofold increase in pressure. Further significant reduction in particle diameter would elevate pressures above the limits of present pump and column design and even then the improvement would hardly merit consideration. Dispersive effects on peak width, due to variation in particle size were minimized by employing a very narrow range of particle diameter.

Resin Density and Cross-Linking. Sensitivity and resolution are dependent on cross-linking as discussed in the following, but the manipulation of cross-linking as a variable is not very satisfactory, partly because of manufacturing convention and partly due to lack of precise knowledge about the actual degree of cross-linking in the finished resin. Industrial practice refers to the proportion of cross-linking agent added in the synthesis of the resin, disregarding the possibility that reaction may be incomplete or that distribution of agent throughout all polymer beads

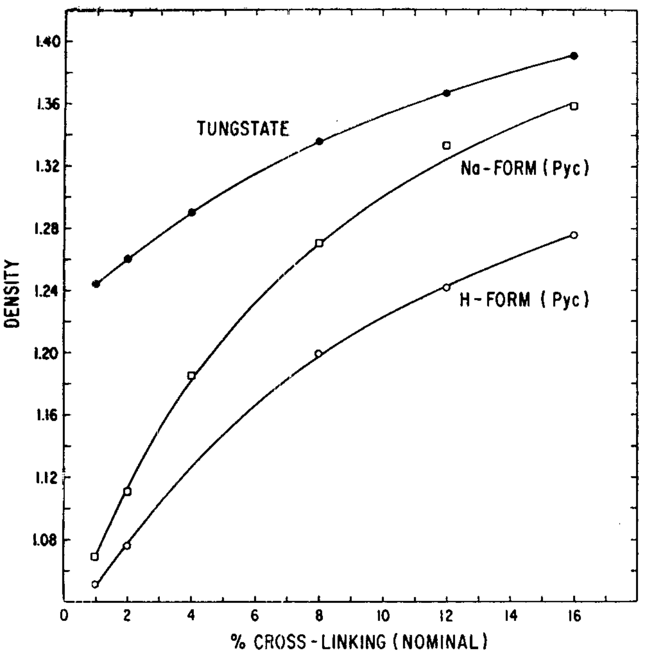

Figure 6. Density determined by sodium tungstate equilibrium position (21) and by standard pycnometric methods (15)

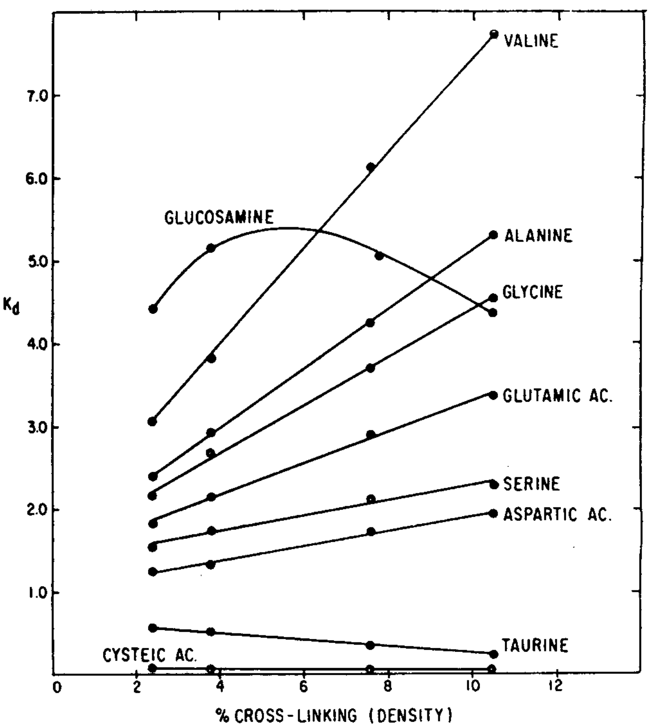

Figure 7. Distribution coefficients, K_d, as a function of cross-linking as determined by density

may not be uniform (*6, 13*). Industrial practice also refers as 8% to resins which may have been made with 7.5 to 8.5% cross-linking agent. The term therefore is nominal, nor can it be verified directly. Wet absolute density (*15*) and water regain (*16*) are parameters that vary with cross-linking but though they may be determined accurately, correlation must still be made with nominal cross-linking which has the inherent uncertainties noted above. This dilemma was resolved here by correlating wet density of a series of resins with their nominal cross-linking and accepting the data as definitive. This seemed justified as the data were consistent and smooth curves could be drawn through the experimental points. The relationship is relative, for an absolute determination of cross-linking, if such were possible, might shift all points relative to the horizontal axis (Figure 6). Bearing in mind these limitations, it was thus possible to assign to a resin a more precise value for its cross-linking by interpolation into this figure. Next, the effect of cross-linking on amino acid distribution coefficients, K_d (see Appendix A for a definition of K_d) was examined, because selectivity coefficients, of which K_d is a special limiting case, had been shown to vary with cross-linking (*18*), and because sensitivity and resolution were known to be functions of K_d [Equations 5 and 6, Appendix A, and Equation 12 (*11*)]. A predictable relationship between K_d and cross-linking would thus make possible the evaluation of the chromatographic suitability of a resin, without trial runs, by a determination of its wet absolute density. This discussion continues therefore with the determination of wet density and distribution coefficients, the way in which these parameters relate to cross-linking, and the relationship of cross-linking to sensitivity and resolution.

A series of resins were first tested for uniformity of density in a tungstate gradient column, [see Appendix C for tungstate density methods based on the work of Suryaram and Walton (*21*)]. The gift by the Dow Chemical Co., Midland, Mich. of these resins for study is gratefully acknowledged. Each resin showed a distribution of particles over a very narrow range of density, indicating excellent uniformity. Some resins have been encountered with a distribution over a wide density range and others with several discrete fractions; the former may represent nonuniformity arising during synthesis, the latter from the mixing of different resin lots. The wet density of the resins was then determined also by a standard pycnometric method (*15*). The results are shown in Figure 6, where density is plotted against nominal cross-linking.

Density varied in a consistent manner and presumably may be considered to be a continuous function of cross-linking. Accepting this data as definitive, it was then found that those resins with demonstrably good chromatographic characteristics for amino acid work had a wet density $\rho = 1.280 \pm 0.002$ equivalent to 8.5% cross-linked. A resin 7.5% cross-linked failed to resolve some amino acids, and a 10% resin, though giving excellent resolution, took too long for the entire chromatogram. On the basis of these findings, it would appear that much amino acid work has probably been done with 8.5% (actual) rather than 8% (nominal) as usually reported.

Using wet density as a criterion of cross-linking, the relationship between amino acid distribution coefficients and cross-linking was next examined, (see Appendix B for a method of determining K_d). The results are shown in Figure 7.

Straight lines were drawn through the points, even though extension of the data over a greater range of cross-linking would likely have shown that the lines had some curvature. This simplification seemed justified by the consistency of the data. It is seen that for the amino acids, K_d increased proportionately to cross-linking and that each of the amino acids exhibited a different slope. Cysteic acid had zero slope, taurine a small negative slope, while glucosamine was nonlinear with negative curvature as shown. It is also evident that as cross-linking increased, the algebraic difference between K_d for any two amino acids that were compared, was also increased. Considering K_d as a linear function of cross-linking, from Equation 5 (Appendix A), it is evident that peak width increases or peak height decreases, as cross-linking is increased. And by applying Equation 6 (Appendix A) to any two amino acids and comparing $\bar{v}$, peak separation is increased as cross-linking is increased, but the time of elution is lengthened because of the larger volume necessary for elution of the peaks. That is, peak height and resolution vary oppositely with cross-linking. With present particle diameters giving nearly minimal peak widths, resolution is therefore largely governed by peak separation, other variables being constant.

Considering the combined effects of cross-linking, pH, sodium concentration, and temperature, for which K_d is a function of each, it is evident that several sets of conditions might possibly be found to accomplish the same ends. For example, specific resolutions obtained on an 8.5% resin may be lost on a 7.5% resin, if the same conditions are used with the latter as with the former. However resolution might be regained, partially if not completely, by adjustment of any or all of pH, sodium concentration, or temperature. Similarly, if the resolution obtained with a 10% resin was excessive and the time of elution too long, appropriate alteration of these variables might recover the desired form of the chromatogram. In some instances it might be necessary to alter column length and flow rate as indicated by Equations 1 and 3 (Appendix A) to obtain a satisfactory result. Evidently optimization involves the selection of conditions that are the best compromise enabling desired ends to be achieved.

With regard to the origin of the resin, it has been the experience here with spherical particles, that if the capacity, wet density, particle diameter, and particle diameter distribution are the same, column and other instrumental variables being constant, the chromatograms obtained are essentially identical regardless of the source or the time of purchase. This has been true at least with Dowex 50 purchased in 1953 and in 1960 and more recently with Chromobeads, Type B, (Technicon).

Photometer Flow Cells. Increased peak height was obtained by using 4.4-mm. flow cells, a somewhat greater depth than that previously reported, but less than the maximum useable (*9*). A 4.4-mm. cell gives a peak height of ×2 over the 2.2 mm. used originally (*20*). Flattened or square cells gave unsymmetrical peaks that trailed, presumably because the radius from axial stream to cell wall was not the same in all directions. Combining the gain in peak height from flow cell design with that obtained from column design (1.4) and from particle size reduction (1.2), a total gain of ×3 over the instrumentation of Spackman, Stein, and Moore (*20*) was thus obtained.

The adoption of tubular flow cells in which the light path and the axis of flow coincide (*14*, *22*) would give a further gain. A currently available commercial photometer with 15-mm. tubular cuvette gave an increased peak height of more than ×3 over the 4.4-mm. flow cell described here. The author acknowledges with thanks the loan of such a photometer from the Technicon Chromatography Corp., for purposes of testing and comparison. However, having in mind minimal alteration of the 3-channel photometer with stacked flow cells (*20*) and realizing that a comparable amplification could be obtained at the recorder, the potentiality of tubular flow cells was reserved for future study.

Recorder. A further increase of peak height was obtained by using recorders with continuously variable span and zero suppression. The ratio (range)/(span) determines the amplification applied to the incoming signal. For example, in the present method, by displaying 2-mv. changes full scale on an 8- to 10-mv. range a factor of ×5 is introduced, equivalent to displaying 0 to 0.0969 absorbance units full scale. Similarly, a 1-mv. span on a range 20- to 21-mv. range, is equivalent to 0 to 0.0213 absorbance units full scale or a factor of ×20. Combining all gain factors, it is evident that peak height can be increased from ×3 to ×30 with very little change in standard apparatus; with tubular cuvettes and a 21-mv. max. recorder, a further amplification of ×7 (approximately) could be realized.

In the present work, a 2-mv. span was selected as it enabled 0.01 μmole of each amino acid to be determined with sufficient precision without too much interference from base line changes. As the amount of many free amino acids in 0.1 ml. of blood serum or plasma from human subjects is, very approximately, 0.01 μmole, it was felt that this limit was not too confining. The determination of one order of magnitude less, though possible, has yet to be achieved in a manner that is satisfactory for a single column operation but this is currently being investigated.

APPENDIX

Appendix A. Equation 10 of Hamilton, Bogue, and Anderson (*11*), expressed in simplified form shows clearly the influence of variable quantities on peak width. Neglecting the last term in D_L of Equation 10, collecting together all quantities that are held constant, noting that for a gauss curve, peak width, W, at the base of the peak is equal to 4σ, and examining one variable at a time, the following relations hold:

$$W = C_1(Z)^{1/2} \tag{1}$$

where Z is column length in cm.

$$W = C_2(A) \tag{2}$$

where A is the column cross-sectional area in cm.²

$$W = \sqrt{C_3 + C_4 . U_o} \tag{3}$$

where U_o is the linear flow rate through the column in cc. per hour—i.e., U_o = (volumetric input)/A.

$$W = \sqrt{C_5 d_p + C_6 d_p^2} \tag{4}$$

where d_p is particle diameter in cm.

$$W = \sqrt{C_7 + C_8 K_{d(a)} + C_9 K_{d\,(a)}^2} \tag{5}$$

where $K_{d(a)}$ is the distribution coefficient of component (a). K_d is a dimensionless number defined as the ratio (moles of amino acid in the resin per cm.³ of column volume)/(moles of amino acid per cm.³ fluid), at equilibrium. K_d is a function of pH, sodium concentration, temperature, cross-linking, degree of sulfonation, and other variables. C_1 to C_9 are constants.

Equation 1 of Hamilton, Bouge, and Anderson (*11*) is:

$$\bar{v} = AZ(K_{d(a)} + F_I) \tag{6}$$

where $\bar{v}$ is the effluent volume from the column at emergence of the peak of component a, whose distribution coefficient is $K_{d(a)}$. F_I is the void volume of the column; it is a dimensionless constant, which, for spherical particles closely approximates 0.40 (*11*).

Appendix B. K_d was calculated by means of Equation 6 from runs on columns packed with different cross-linked resins. In all cases, column development was with citrate buffer, pH 2.95, 0.20N sodium, and at 54° C. The void volume was assumed to be the same in every case—i.e., F_I = 0.40.

A more formal and more general description of K_d is given by Helfferich (*12*).

Appendix C. Tungstate Densities. The following procedures were devised, based on the work of Suryaram and Walton (*21*). A nearly saturated solution of sodium tungstate, sp. gr. = 1.40, was diluted with water to give a series of solutions ranging in specific gravity from 1.200 to 1.400 in steps of 0.01; each solution was checked by weighing and by hydrometer. The cross-linking of any resin was rapidly determined by introducing a knife point into each of a series of small test tubes each containing 1 ml. of one of the tungstate solutions. The tubes were stoppered and set in a draft-free location. After 2 hours, resin that was heavier than the solution was at the bottom of the tube, resin lighter than the solution was at the surface, and resin of the same density was suspended. A resin with particles that were at both the top and bottom of a number of adjacent tubes, had a wide density dispersion or was composed of a number of density fractions. Interpolation gave specific gravities to within 0.005. This test easily distinguished 5, 8, 10, 12, and 16% resins.

For a more precise determination and to better visualize density dispersion, 10 ml. of each solution, starting with the least dense of the range that was to be covered, was carefully layered in a 100-ml. graduated cylinder. Such columns, if handled carefully, changed little for these purposes over a period of several days. A knife point of resin introduced at the top, settled slowly, reaching an equilibrium position in about 12 hours. The cylinders were stored overnight in an incubator and read the next day. With solutions covering a limited range in steps of 0.005, there was little difficulty in reading specific gravities to 0.002. Resins of uniform density occupied a single plane. Nonuniform resins were dispersed throughout a region of the column, or were collected in several discrete planes.

To separate a resin into constituent density fractions, columns were prepared with 500 or 1000 ml. of each solution by layering in a 4-liter graduated cylinder. The cylinder was filled nearly to the top with the least dense solution. 100 to 200 grams of resin could be carefully introduced and after 2 to 3 days, discrete fractions, if present, had separated. Successive layers were removed from the column by aspiration.

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Semiautomatic Machine for Group Separation of Radioelements by Cation Exchange

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▷ A technique for performing a five-group separation of radioelements by cation exchange is described. Suitable counting sources are prepared for each group on a semiautomatic machine. Twelve samples are analyzed simultaneously, and five counting sources for each sample are obtained and counted during an 8-hour day. Total manipulation time for the analyst during this period is about 1.5 hours. Detailed analytical results are presented for two samples: a synthetic mixture of the radioelements Sb^{125}; Zr, Nb^{95}; Cs, Ba^{137}; Co^{60}; Sr, Y^{90}; Ce, Pr^{144}; and a 3.6-year-old mixture of fission products. The radioelements in these two samples report primarily in the following groups: Group I, Sb, Ru, Zr, Nb; Group II, Cs; Group III, Co; Group IV, Sr; and Group V, Y, rare earth elements.

In 1960, a program was started at this laboratory to study the decontamination of radioactive-waste solutions by ion exchange on the natural zeolite clinoptilolite (*2*). It was realized that large numbers of samples would have to be assayed for their radiochemical content, the most important determinations being those for Sr^{90}, Cs^{137}, and the rare earth elements as a group. Since Sr^{90} is a relatively weak β-emitter, such counting techniques as scintillation spectrometry and external β-adsorption would not be applicable, and chemical separations would be required. As a result, work was undertaken to devise a simple and rapid method for the radiochemical determination of the species mentioned above in fission-product mixtures.

A cation-exchange column loading and chromatographic elution scheme was chosen in which the radioelements are divided into five groups. The separation is done on a machine which feeds the samples and elutriants through a battery of columns, the effluents being simultaneously evaporated on counting-source trays. Manipulation is required only at the beginning and end of each fraction. When this method is used in conjunction with an automatic β-counting system, an analyst can separate 12 samples into five fractions and count the fractions in one 8-hour day without difficulty.

EXPERIMENTAL

Apparatus. A schematic diagram of the analyzer appears in Figure 1. The samples and the elutriants are fed to the ion exchange columns from 10-ml. disposable plastic syringes. During the pumping part of the cycle the syringe bodies are clamped in place and the plungers are depressed at a uniform rate by a push bar driven by a 1-r.p.m. motor through a gear train, rack, and pinion arrangement.

The column effluents drip onto heated source trays, on which they are evaporated without boiling. A stream of warm air is blown across each source tray to ensure that the liquid is evaporated as fast as it is pumped. In the chemical scheme all reagents are completely evaporable, so that at the end of each group a set of sources suitable for β-counting is obtained. An adjustable limit switch can be set to stop the machine when sufficient elutriant has been expelled from the syringes.

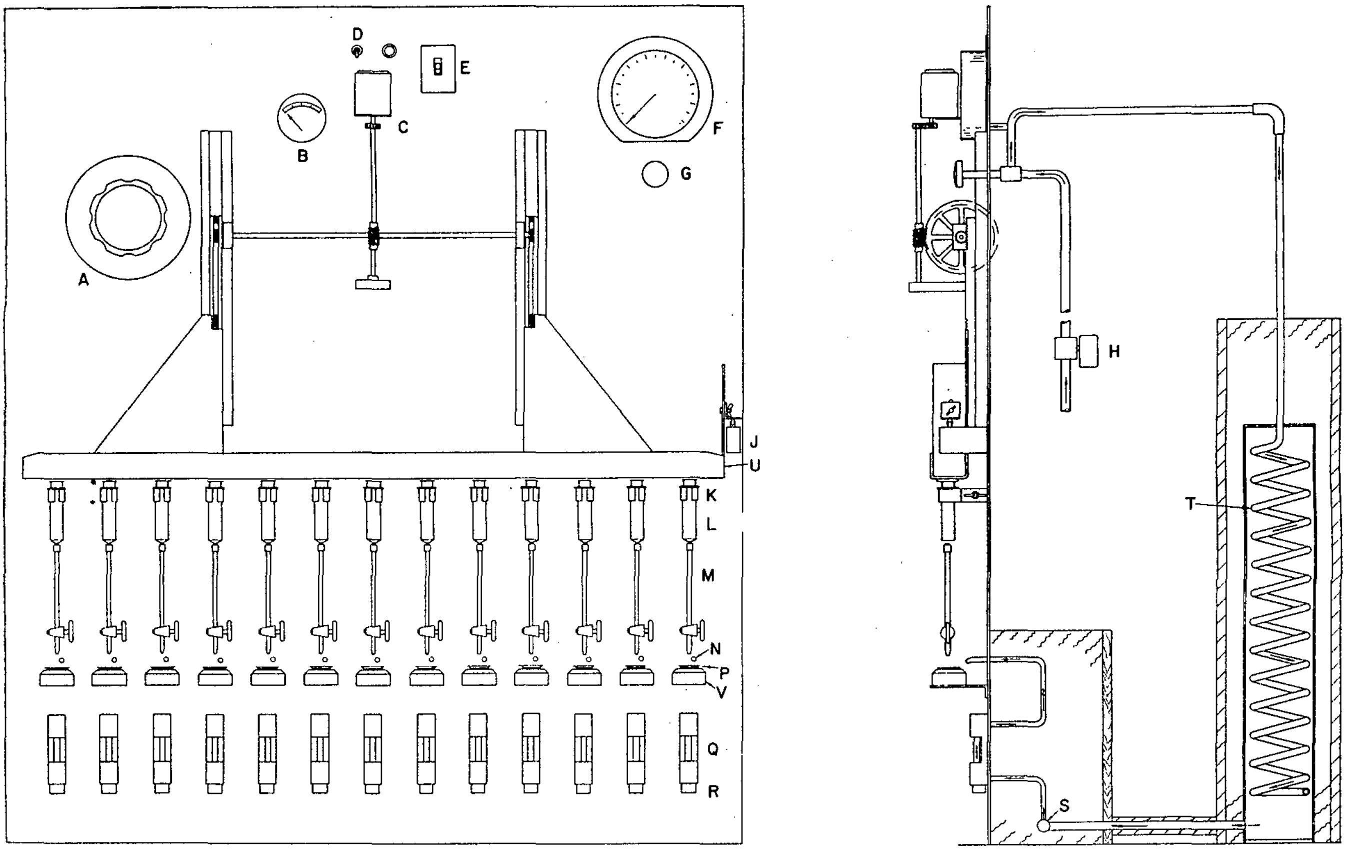

Figure 1. Semiautomatic analyzer

A. Source heaters control
B. Source heaters ammeter
C. Bodine motor
D. Bodine motor switch
E. Main power switch
F. Air supply pressure gauge
G. Air supply pressure-regulating valve
H. Solenoid valve
J. Limit switch
K. Syringe clamp
L. Syringe
M. Ion exchange column
N. Air outlet
P. Source tray
Q. Air rotameter
R. Needle valve
S. Air manifold
T. Air heater
U. Push bar
V. Source heater

A drawing of the syringe, column, and source tray arrangement is given in Figure 2. A loaded syringe is attached to a column by simply pressing it into the Teflon plug at the top of the column. The source trays are made from the alloy Hastelloy C, which is resistant to corrosion by the reagents used.

Some pertinent operating and constructional data for the machine are listed in Table I. The elutriant flow rate was 6.9 ml. per hour for the assembly described. This rate was readily increased by changing spur gears in the gear train and the machine was operated at 9.6 ml. per hour with no apparent effect on the efficiency of the chemical separation. However, at this higher rate it was difficult to evaporate the liquid without occasional spattering, resulting in the loss of material from the source tray. This difficulty was avoided at the lower flow rate.

Mechanical Procedure. At the start of each batch of analyses the glass columns with the stopcocks closed are loaded with fresh, conditioned batches of resin slurried in water. The settled resin-bed height is adjusted to 5 cm. and the remaining space is filled with water.

The samples are loaded into the syringes (see below), which are attached to the columns. In this step and in subsequent syringe loadings the bulk of the trapped air must be removed from the syringe after the liquid is drawn into it. This is achieved by inverting the syringe and tapping the air bubbles to the top, then expelling the air by carefully pushing the plunger.

The push bar is freed from the gear train by disengaging the worm from the worm gear. The push bar is then raised out of the way by turning the worm gear manually.

The assembled syringes and columns are clamped loosely in place and the push bar is lowered manually until it just clears the highest plunger. The push bar is engaged by reversing the above steps. The syringe-column assemblies are then raised until all plungers are in contact with the push bar. They are then clamped firmly.

Table I. Operating and Constructional Data for Semiautomatic Analyzer

Drive motor	Bodine, KYC-22RC
Motor speed	1 r.p.m.
Pinion speed	0.018 r.p.m.
Rack pitch	10 teeth per inch
Syringes	American Hospital Supply, 41775
Syringe bore	1.64 cm.
Pumping rate	6.9 ml. per hour
Columns	0.5 cm. by 5 cm. long, Dowex 50-X4
Source heaters	Fisher Scientific, 11-502-15
Source heater voltage	41 volts
Air heater	610 cm. of coiled stainless steel tubing, 0.635-cm. × 0.075-cm. wall
Air heater voltage	26 volts
Air heater current	28 amperes
Air rotameters	Schutte & Koerting, Fig. 1853
Air flow rate	2 liters per minute
Source trays	Laurentian Metals, A622-9
Source tray material	Hastelloy C

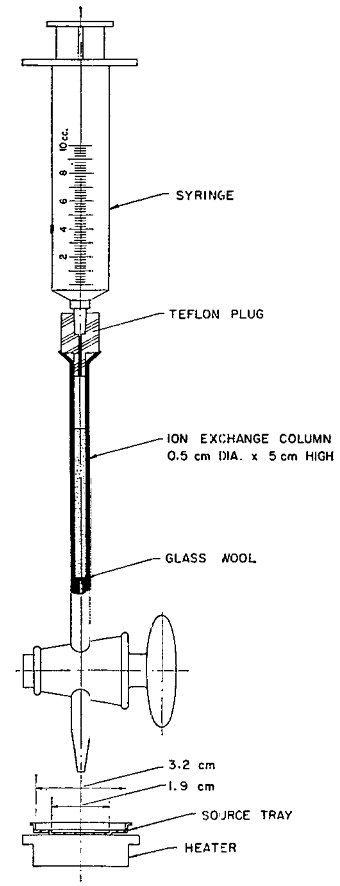

Figure 2. Detailed drawing of syringe-column assembly

Source trays are positioned below the columns. With the motor switch in the off position, the master switch is operated to turn on the air flow, air heater, and source heaters. After a few minutes' warmup the motor switch is turned on and the stopcocks on the bottom of the columns are opened This starts the run.

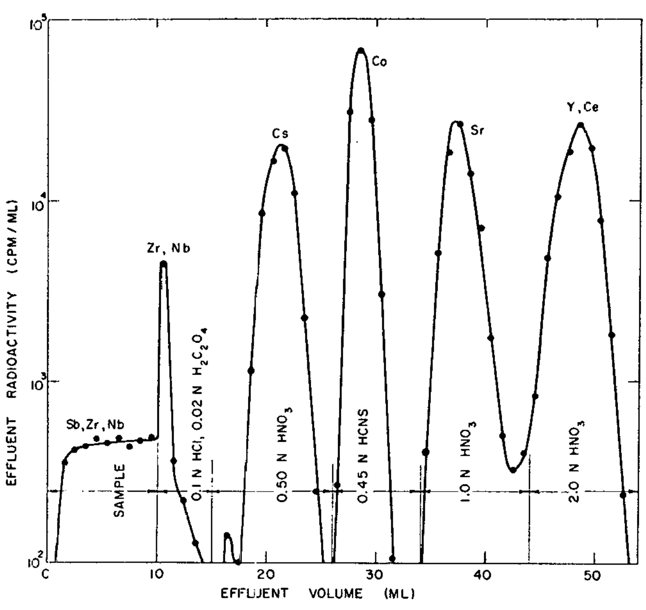

Figure 3. Elution spectrum of synthetic sample

After the syringes are completely emptied, the motor switch is turned off, the column stopcocks are closed, the push bar is raised, and the syringes are removed from the columns and discarded. The first rinse solution may, of course, be put through the same syringes. However, discarding them results in a loss of less than 0.5% of the sample and minimizes cross contamination between the fractions.

The first elutriant is drawn into new syringes and the machine is loaded and started as above. The evaporated residues from the first two pumping cycles form the Group I counting sources.

The next four elutriants are pumped in sequence from the same set of syringes. The evaporated residues from each cycle form the Groups II, III, IV, and V counting sources, respectively.

At the end of the analysis, the resin columns are emptied and washed in preparation for the next run. For best efficiency, it is convenient to have two complete sets of glass resin columns so that a set of freshly loaded columns is available for immediate use.

It is important that the syringes be sufficiently free from air bubbles before attachment to the resin columns, so that no air enters the resin at the end of a pumping cycle. A few tiny bubbles are tolerable and, with a little practice, the analyst has no difficulty in reducing the trapped air to a tolerable amount and recognizing it as such.

Separation Scheme. Many ion exchange methods are reported in the literature for the separation of radioelements into groups (*1*, *3*).

The method used was a modification of that devised by Yamatera and Kubo specifically for the separation of aged fission-product mixtures (*3*). The chief reason for preferring this scheme over others was that all reagents used are completely evaporable. Modifications to the originally described scheme were required to circumvent the following difficulties: Zr and Nb appeared in appreciable amounts in all fractions, and Co appeared in the same fraction as Sr.

Reagents. The resin used was Dowex 50W-X4, 100- to 200-mesh. The as-received resin was pretreated (in a large column) by passing through it 5 column volumes of 1*N* HCl, followed by 5 column volumes of H_2O. It was then ready for use.

The elutriants were prepared from reagent grade chemicals. Their compositions and volumes were as follows: Group I, sample plus 6 ml. of 0.1*N* HCl–0.02*N* $H_2C_2O_4$; Group II, 10 ml. of 0.50*N* HNO_3; Group III, 8 ml. of 0.45*N* HCNS; Group IV, 10 ml. of 1.0*N* HNO_3; Group V, 10 ml. of 2.0*N* HNO_3. The 0.45*N* HCNS was pre-

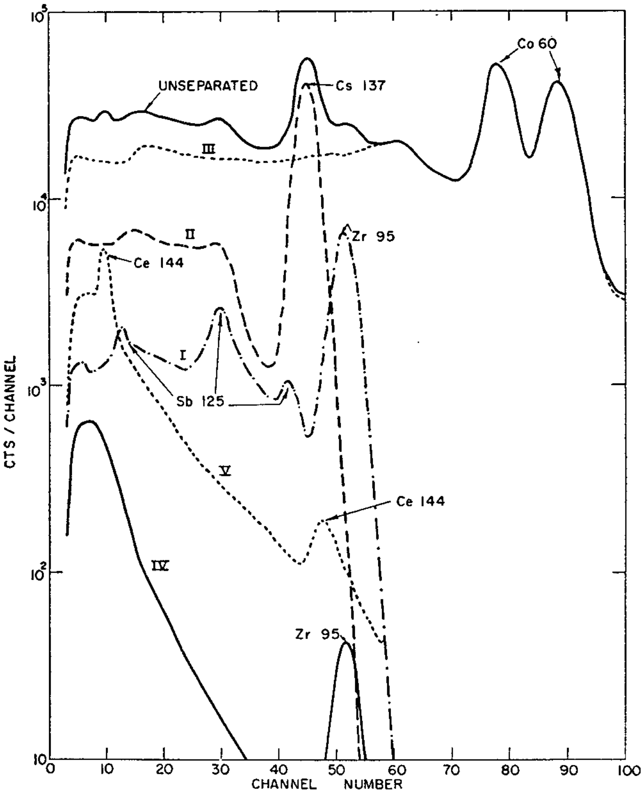

Figure 4. Gamma spectra of separated groups from synthetic sample

NaI crystal 3 × 3 inches
Absorber thickness. 620 mg./sq. cm.
Source distance. 1.5 mm.
Energy scale. ~15 k.e.v./channel
Parameter. Group number

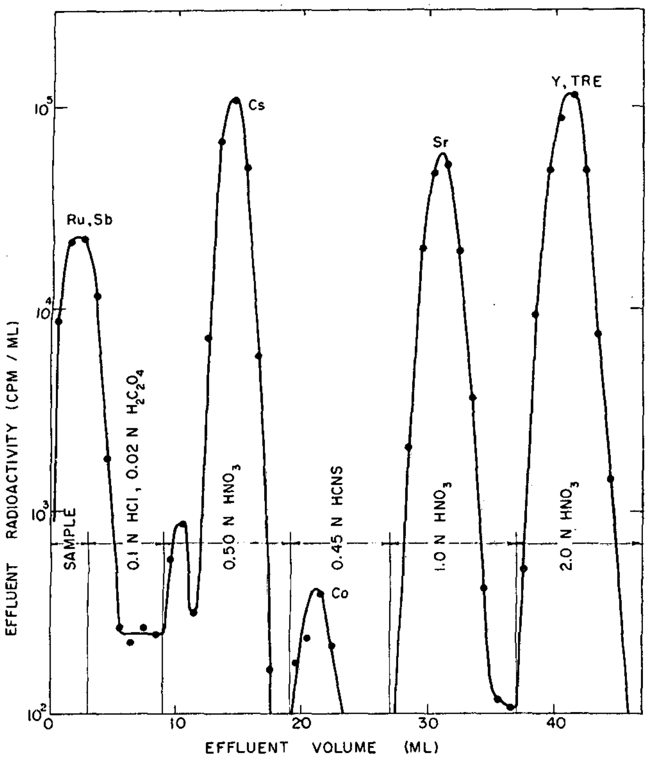

Figure 5. Elution spectrum of fission-product sample

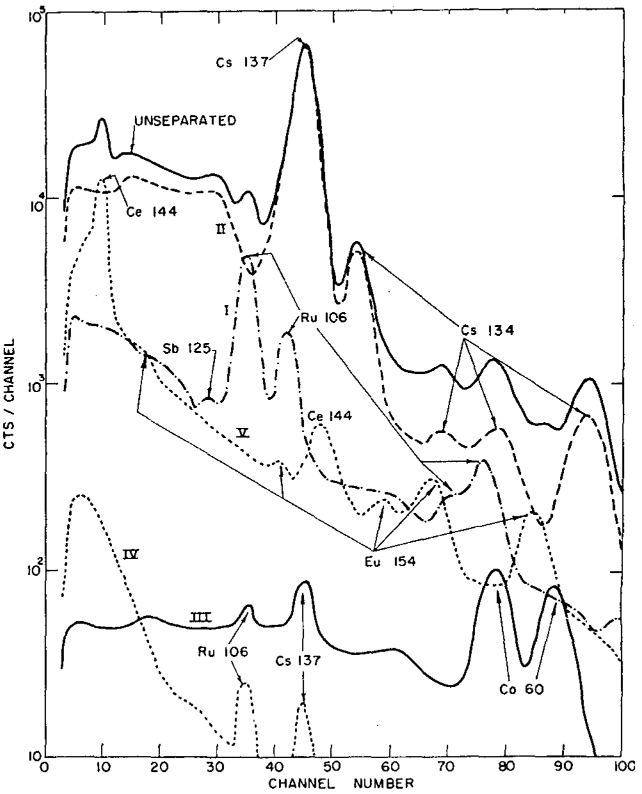

Figure 6. Gamma spectra of separated groups from fission-product sample

NaI crystal. 3 × 3 inches
Absorber thickness. 620 mg./sq. cm.
Source distance. 1.5 mm.
Energy scale. ∽15 k.e.v./channel
Parameter. Group number

pared as follows: One liter of 3.1*M* HCl containing 150 grams of KCNS was extracted with 1 liter of benzene and the aqueous phase discarded. The organic phase was then backwashed with 500 ml. of H_2O. The aqueous phase was separated, titrated for CNS^-, and adjusted to 0.45*N* with H_2O.

Sample Pretreatment. Yamatera and Kubo recommended that the sample for analysis be pretreated by taking it to near dryness with concentrated HCl containing bromine.

The use of concentrated HCl was essential to ensure that the bulk of the Ru was eluted in Group I. Comparative tests on various fission-product mixtures showed, however, that the use of Br_2 caused difficulty with respect to Sb, apparently by colloid formation. Consequently bromine was not used here and the sample pretreatment was as follows:

LOW LEVEL. The sample was evaporated to dryness in a beaker. The residue was dissolved in 2 ml. of 11.6*N* HCl and again taken to dryness. The residue was dissolved in 2 ml. of 0.1*N* HCl–0.02*N* $H_2C_2O_4$, which was drawn into a syringe. One milliliter more of this reagent was used to rinse the beaker and was also drawn into the syringe. The syringe was then attached to the ion exchange column as described above.

HIGH LEVEL. The appropriate aliquot was added to 2 ml. of 11.6*N* HCl and evaporated to dryness. This residue was dissolved in 0.1*N* HCl–0.02*N* $H_2C_2O_4$ as above.

Since the columns used had a total exchange capacity of about 1.3 meq., it was important that the sample aliquot did not have a total cation content (excluding hydrogen ion) approaching this value. Aliquots containing as much as 0.25 meq. were analyzed successfully, however.

RESULTS

A synthetic solution was prepared containing the following radioelements: Sb^{125}; Zr,Nb^{95}; Cs,Ba^{137}; Co^{60}; Sr, Y^{90}; and Ce,Pr^{144}. Two 0.1-ml. aliquots of this solution were applied to two resin columns. For reasons not pertinent to this work, sample pretreatment in this instance consisted simply of diluting each aliquot to 10 ml. with $10^{-3}N$HCl. It is known that Ru would be poorly separated under these circumstances. The elutriation scheme described above was followed.

In one case the column effluent was collected on source trays in 1-ml. fractions. These sources were counted shortly after collection on a thin-window proportional counter with about a 35% geometry factor. The elutriation curve plotted from the counting results is given in Figure 3. In the second case the column effluent was collected on five source trays corresponding to the above groups and each source was counted by scintillation spectrometry using a 100-channel pulse-height analyzer with a gain setting of about 0.015 m.e.v. per channel. The resulting spectra are plotted in Figure 4. The upper curve in Figure 4 was obtained from a source containing 0.1 ml. of the original solution. All sources were counted in the same position and for the same length of time (after corrrection for dead-time loss), so that the lower five curves would be expected to add up to the upper curve.

The β-counting rate of the synthetic solution was known accurately from analysis of the original tracer solutions for Cs^{137}, Co^{60}, and Sr, Y^{90}. The β-counts on Groups II, III, and IV sources from the first experiment were totaled to give a comparison between the above values and those found in the group separation. Group IV sources were counted 3 weeks after separation to allow the Sr, Y^{90} secular equilibrium to be established. These results are given in Table II.

Data similar to those shown in Figures 3 and 4 are given in Figures 5 and 6, in this case for a relatively long-cooled fission product mixture (sepa-

Table II. Comparison between Known and Determined β-Counting Rates

Component	β-Counting rate, c.p.m.[a] Known	β-Counting rate, c.p.m.[a] Determined	Error, %
Cs^{137}	1.57×10^4	1.55×10^4	−1.2
Co^{60}	3.85×10^4	3.97×10^4	+3.0
Sr, Y^{90}	4.51×10^4	4.73×10^4	+4.5

[a] Same counter as used in obtaining data for Figure 3 but in lower position where geometry factor was about 10%.

rated fission products from natural uranium irradiated to 0.7 atom % burnup and cooled for 3.6 years). The samples were pretreated as described above.

The isotopic assignments given to the various photopeaks in Figures 4 and 6 are not strictly correct in every case, since some of the photons identified actually come from short-lived daughter isotopes. The highest energy gamma shown for Ru^{106} and the two highest energy gammas shown for Cs^{134} are "sum" peaks—i.e., they result from two gammas in cascade being captured in the crystal.

The results in Figures 4 and 6 were analyzed to give quantitative information about the disposition throughout the scheme of the various radioelements in the two samples analyzed. The procedure consisted of stripping the various spectra, using standard curves to estimate the amounts of impurity present. In those cases where the stripped spectra did not show a given peak, a statistical estimate was made of the maximum amount that could be present. The results of such an analysis are given below in the order of the elutriant groups.

Group I. Group I contained the bulk of the Ru, Sb, Zr, and Nb. Yamatera and Kubo's scheme was modified by the inclusion of oxalic acid in the Group I reagent. Oxalic acid forms stable, anionic complexes with both Zr and Nb and either prevents or reverses hydrolytic polymerization of both species in the dilute acid medium. It can be inferred from the data shown in Figure 3 that depolymerization of these two species by complexing action is relatively rapid.

There was essentially no contamination of this group by the radioelements that follow in the scheme, except in certain cases by Co, as indicated below. Recoveries were estimated at greater than 98% for Ru, Zr, and Nb. The recovery of Sb could not be estimated with precision from the data given here, but in more sensitive cases it was invariably greater than 95%.

Group II. Cs was eluted in this fraction with a yield of about 99.9%. In Figures 3 and 5 it can been seen that the elution spectra show a double peak in this group. The gamma spectra of the sources under the first peak showed Cs to predominate. No explanation can be offered for this phenonemon. Decontamination factors of the Cs from other species as estimated from Figures 4 and 6 were: Zr-Nb, >100; Ru, >25; Co, >5000; Ce, >60.

The first two and the last numbers quoted are undoubtedly conservative and reflect the lack of sensitivity of the method in this case.

Group III. This group was added to Yamatera and Kubo's scheme to avoid contamination of the Sr fraction by Co. Thiocyanic acid stripped Co from the resin by complexing without any apparent detrimental effect on the subsequent separations. The Co was recovered in high yield (>99.9%) with the following decontamination factors: Ru, ~300; Cs, ~1600; Ce, >1200; Sr, >1000. The last number quoted was evaluated indirectly.

In some of the samples analyzed an appreciable fraction of the Co was eluted in Group I, with the remainder appearing in Group III. Some of the Co in these samples was undoubtedly in the form of a stable anionic complex.

Group IV. Sr was eluted in this group. It can be inferred from Figures 3 and 5 that recovery was high (>99.5%). The Group IV gamma spectra in Figures 4 and 6 show primarily the Sr^{90} bremsstrahlung. In one case there is evidence of slight contamination by Zr, Nb^{95}, while in the other case traces of Ru^{106} and Cs^{137} are present. Decontamination factors estimated from these curves were: Zr-Nb, ∽ 160; Ru, ∽ 300; Co, >4000; Cs, ∽ 3200; Ce, >1200.

In growth and decay of β-activity on Groups IV and V sources indicated that Y^{90}, the 64-hour half-life daughter of Sr^{90}, was mainly eluted in Group V. Since Y^{90} is a β-emitter only, the data in Figures 3 and 5 can give no indication of the completeness of the separation. There is no reason to believe, however, that the decontamination factor of Group IV from Y was any less than the value quoted for Ce—namely, >1200.

Group V. The remaining radioelements in the samples examined—Y and the rare earths—were eluted in Group V. The Group V gamma spectra show no evidence of cross contamination from the other groups, and the decontamination factors for the group as a whole are estimated to be: Zr-Nb, >350; Ru, >30; Cs, >1300; Co, >4000. The recovery of Ce (and, by inference, Y and the remaining rare earth elements) in Group V was essentially quantitative (99.9%).

No radioactivity was found on the columns after the separations.

DISCUSSION

The above data describe the behavior in the scheme of the long-lived radioelements in fission product mixtures and the activation product Co^{60}. The Group I radioelements (Ru, Sb, Zr, Nb) were the least well separated. A recovery of greater than 98% in Group I was found for these elements in the two analyses cited. This figure should be regarded with caution. The aqueous chemistry of all of these elements (especially Ru) is complex and it is possible that their destination in the scheme might depend on the previous history of the sample. It has been the practice in this laboratory to minimize the time lag between evaporation of the sample in concentrated HCl and loading to the column. This was done because of work reported by Minami, Honda, and Sasae [see (*3*)], who found that on dissolving a fission-product sample (in dilute HCl), that had been previously taken to dryness with concentrated HCl, there was a slow ingrowth of cationic Ru.

To test the role of NO$_3^-$ ion in Groups II, III, IV, and V separations, the synthetic sample described above was analyzed, replacing the nitric acid reagents with the same concentrations of hydrochloric acid. When compared with Figure 3 the resulting elution spectrum showed extended trailing edges for both the Cs and Sr peaks and less steep leading edges for the Co and rare earth peaks.

These phenomena resulted in significant cross contamination between Groups II and III and also between Groups IV and V. The latter finding is in agreement with results reported by Yamatera and Kubo (*3*).

A possible explanation of the above results is that both Cs and Sr are complexed to some degree by NO$_3^-$ and Co and the rare earth elements are complexed by Cl$^-$.

The degrees of complexing is relatively weak in all cases.

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Separation of Calcium and Strontium by Liquid Ion Exchange

Determination of Total Radiostrontium in Milk

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▶ A rapid liquid ion exchange method is described for separating quantities of calcium from strontium in dilute acid solution. Separate extractions were made with di-2-ethylhexyl phosphoric acid (HDEHP) dissolved in toluene after stepwise pH adjustments of the aqueous solution. Calcium, strontium, and other cations in 250 ml. of milk were first concentrated in 60 ml. of dilute HCl solution by a batch ion exchange resin technique. After separation of calcium and radiostrontium by the liquid ion exchange method, Sr^{89} and Sr^{90} were stripped from the final organic phase with 3*N* HNO_3 and precipitated from fuming nitric acid. The counting planchet contained only 2% of the calcium and 85 ± 5% of the strontium in the original milk sample. Decontamination factors for Y^{90}, Cs^{137}, K^{40}, La^{140}, and I^{131} were greater than 10^3. Ba,^{140} if present, can be removed by homogeneous precipitation of barium chromate.

A MAJOR PROBLEM in analysis of milk and other biological samples for total radiostrontium is the removal of the large quantities of calcium before counting. While Sr^{90} can be determined by carrier-free separation and counting of the Y^{90} daughter (*2*), Sr^{89} must be counted directly in combination with Sr^{90}. The solid material on the planchet must be small to prevent absorption of the low energy Sr^{90} beta particles during counting.

The classical fuming nitric acid method of Willard and Goodspeed (*8*) is frequently used to separate Sr^{89} and Sr^{90} from calcium. Although this method is rapid and offers good decontamination factors for other radionuclides, it is not convenient for separation of strontium from the large quantities of calcium found in milk. Use of solid cation exchange resins results in good separation, provided that only trace quantities of calcium are present (*6*). Weiss and Shipman (*7*) reported the selective precipitation of strontium as the rhodizonate and this procedure was adapted at the Savannah River Plant for use with several types of biological samples (*1*). However, recovery of strontium from milk by this procedure is low.

Liquid ion exchange is a relatively new technique that shows promise in many chemical separations, and its technology has been summarized in two recent reviews (*3*, *4*). Despite successful use of liquid ion exchange in experimental and pilot plant separations (*9*), a review of extraction procedures (*5*) showed that the method has not been widely exploited for analytical purposes. Coleman, Blake, and Brown (*3*) stressed the potential usefulness of liquid ion exchange in analytical chemistry, indicating that most of the elements might be separated by one or more of 55 anion and cation exchange reagents.

The purpose of this study was to develop a rapid method for separating large amounts of calcium from strontium, to accurately determine total radiostrontium, and to obtain an approximation of strontium-90 content.

EXPERIMENTAL

Tests with Aqueous Solutions. Preliminary experiments were made using dilute aqueous solutions. Fourteen radionuclides at levels of 10^6 d.p.m. or higher were separately prepared in 0.08*N* HCl solution and extracted with 5 and 20% (0.16 and 0.64*M*) di - 2 - ethylhexyl phosphoric acid (HDEHP) dissolved in toluene. Various amounts of NH_4OH (28 to 30% NH_3) were added to the aqueous solutions to attain pH's of 1 to 5 (equilibrium pH) after 1 minute of shaking. Aqueous and organic volumes were 60 ml. Aliquots of one or both phases were counted as the liquid in a gamma well counter or evaporated on stainless steel planchets and counted in a beta counter. A standard pH meter was used.

Of the two exchange media concentrations tested, 20% HDEHP displayed better exchange characteristics. Only Zr–Nb^{95}, Fe^{59}, and Zn^{65} exchanged to the 5% organic phase at pH 1. Results of initial tests with 20% HDEHP are shown in Table I. Greater than 95% Ca^{45} was exchanged at pH 3 compared to 33% Sr^{85}.

The rate of exchange of Sr^{85} is of interest. Tests showed that at pH 5, greater than 99% of the nuclide was exchanged from aqueous solution to the organic phase in a 5-second shaking period. Strontium-85 was also rapidly and quantitatively stripped from 20% HDEHP with an equal volume of 3*N* HNO_3.

Additional tests were made with samples containing Ca^{45} and Sr^{85}. Aqueous-to-organic volume ratios were varied from 1:1 to 1:2 using 5, 20, and 40% HDEHP. Results for 5 and 20% HDEHP are summarized in Table II. Although there was not complete separation of the radionuclides in one extraction, comparisons were made of the amounts of Ca^{45} exchanged with 10 and 15% of the Sr^{85}. The optimum conditions resulted when equivolumes of aqueous and 20% HDEHP solutions were used. Forty per cent HDEHP showed separations similar to those of 20% solution and are not shown. The complete data for the optimum Ca^{45} and Sr^{85} separation are plotted in Figure 1, showing exchange of 94% Ca^{45} and 10% Sr^{85} at equilibrium pH 2.8. These exchanges were reversible on addition of NH_4OH or HCl.

Similar separation was achieved from a sample containing 200 mg. of calcium carrier and Sr^{89} tracer. Calcium was exchanged first at pH 2.8. Strontium-89 was then exchanged to a fresh solution of 20% HDEHP at pH 5. The Sr^{89} was stripped from the organic solution with 3*N* HNO_3, the acid was evaporated, and the residue ws

Table I. Extraction of Radionuclides from Aqueous Solutions

Per cent exchanged to 20% HDEHP phase

Nuclide	Measured equilibrium pH of aqueous solutions			
	pH 1	pH 3	pH 4	pH 5
Ca^{45}	<1	>95	100	100
Sr^{85}	<1	33	90	>99
Cs–Ba^{137}	<1	3	13	37
Ru–Rh^{106}	<1	15	26	65
Ce–Pr^{144}	33	99	>99	>99
Zr–Nb^{95}	72	36	42	75
Nb^{95}	21	...	...	...
I^{131}	11	31	46	46
Co^{60}	<1	46	96	98
Fe^{59}	68	...	...	...
Zn^{65}	17	>99	>99	>99
Cr^{51}	<1	17	33	66
P^{32}	<1	...	...	...
Mn^{54}	<1	96	>99	>96

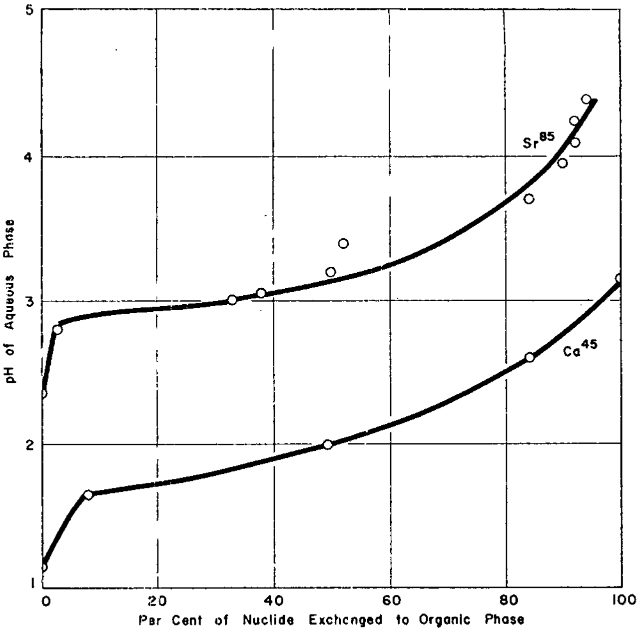

Figure 1. Exchange of Ca^{45} and Sr^{85} from dilute aqueous solution to HDEHP solution

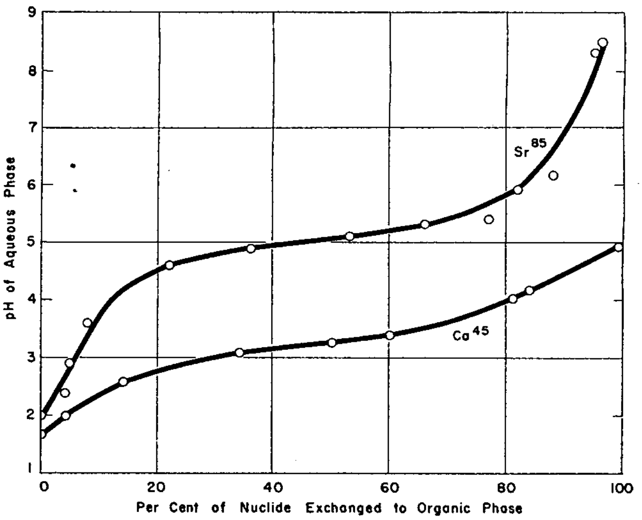

Figure 2. Exchange of Ca^{45} and Sr^{85} from oxidized milk solution to HDEHP solution

mounted on a planchet. The planchet contained 90% of the original Sr^{89} and 10% of the calcium.

Test with Milk Solutions. Tests were made with milk samples spiked with Sr^{85}, Sr^{89}, and Cs–Ba^{137}. The cations in the milk were concentrated by a batch ion exchange resin technique reported previously (*2*). A final dilute HCl solution was prepared containing most of the inorganic cations originally in milk.

Although results for dilute aqueous solutions demonstrated that calcium and strontium can be separated, further experiments were necessary to determine the conditions for separation in milk solutions. A composite solution representing 10 dairy milk samples was exchanged by the procedure used for dilute aqueous solutions. These results are shown in Figure 2. Calcium was not adequately removed from milk solutions at equilibrium pH 2.8. Contrary to the data in Figure 1, however, less than 10% of Sr^{85} tracer was removed by equivolume extraction of milk solution with 20% HDEHP at pH 3.8. Two separations for calcium were necessary. The majority of the calcium was removed in the first exchange from solution adjusted to pH 3.6 to 3.8. The second exchange removed the remaining calcium from the milk solution adjusted to pH 2.6 to 2.8. Radiostrontium was then exchanged from solution at pH 4.8 to 5.0.

Although initial liquid ion exchange separations of calcium and radiostrontium from milk solutions were satisfactory, decontamination factors (D. F.) for Cs^{137} and K^{40} were only 10 and 50, respectively. A fuming nitric acid separation was included in the procedure to improve the D.F.'s.

Procedure for Total Radiostrontium in Milk. Concentrate calcium and strontium from 250 ml. of milk into 60 ml. of 0.08N HCl solution by the batch ion exchange technique (*2*). The pH's in the following steps are those of the solution after extraction. The approximate volumes of concentrated NH$_4$OH required to attain these equilibrium pH's are shown in Table III.

Extract the sample with 60 ml. of 20% HDEHP at pH 3.6 to 3.8, and then with a second 60-ml. portion at pH 2.6 to 2.8, discarding these extracts. Extract with a third 60-ml. portion of HDEHP at pH 4.8 to 5.0.

Strip radiostrontium from the last organic phase with 60 ml. of 3N HNO$_3$. Add 10 mg. of strontium carrier and evaporate the acid to dryness. Dissolve the solids in 1 ml. of distilled water and 2 ml. of 70% HNO$_3$ and transfer to a 100-ml. round-bottomed centrifuge tube with a total volume of 50 ml. of fuming HNO$_3$. Let the sample stand for 15 minutes, with occasional swirling, then centrifuge it to collect the strontium nitrate. Decant the supernate, centrifuge the precipitate with 10 ml. of fuming HNO$_3$ wash, and discard the wash. Dissolve the precipitate in 2 ml. of distilled water and planchet. Count the planchet in a low-background beta counter.

Table III. Calcium and Strontium Exchange

Exchange	Aqueous equilibrium pH	Approximate vol. NH$_4$OH required
First calcium	3.6 to 3.8	1.0 ml.
Second calcium	2.6 to 2.8	0.2 ml.
Radiostrontium	4.8 to 5.0	1.2 ml.

Analyze all samples in duplicate with a third aliquot spiked with Sr–Y^{90} for determination of recovery.

Table II. Separation of Ca^{45} and Sr^{85} in Aqueous Solution

% HDEHP in toluene	Ratio aqueous-to-organic, ml.	Nuclides exchanged to organic phase Sr^{85}	Ca^{45}	pH	Sr^{85}	Ca^{45}	pH
5	60:60	10%	44%	3.5	15%	55%	3.7
20	60:60	10%	94%	2.8	15%	95%	2.9
20	40:80	10%	84%	2.3	15%	85%	2.5

RESULTS AND DISCUSSION

Eight milk samples were spiked with Sr–Y^{90} and analyzed by the total radiostrontium procedure. Sr^{90} recovery was 85% with relative standard deviation of ±5%. The final precipitates weighed 60 mg. compared to 35 mg. found by direct precipitation of strontium carrier. This indicated the presence of only 5 mg. of calcium [weighed as 25 mg. of Ca(NO$_3$)$_2$·4H$_2$O] compared to 300 mg. of calcium in 250 ml. of milk. D.F.'s for Cs^{137}, K^{40}, and Y^{90}—the most likely milk contaminants—were greater than 10^3. Three other nuclides, I^{131}, Ba^{140}, and La^{140}, are possible short-lived milk contaminants immediately following nuclear tests. The D.F.'s for I^{131} and La^{140} are high because of the combined discriminations in the batch resin exchange preparation of milk, the liquid ion exchange separation, and the fuming nitric acid precipitation. A homogeneous precipitation of barium chromate yielded a satisfactory Ba^{140} D.F. (*1*).

The Sr^{89} concentrations are calculated by difference between total radiostrontium and Sr^{90}. An individual sample can be analyzed in 3 hours compared to approximately 8 hours required for the rhodizonate procedure (*1*).

Separate Sr^{90} determinations for 500-ml. samples are still done by extracting and counting the Y^{90} daughter (*2*). The Y^{90} is counted accurately and later recounted to verify purity. Total radiostrontium may also be retained for Y^{90} buildup and then recounted to determine Sr^{90} content, although this procedure involves counting the three-component mixture of Sr^{89}, Sr^{90}, and Y^{90}, which have different counting efficiencies. A rapid approximation of higher levels of Sr^{90} in milk samples may be made by this method. Several Sr^{90} concentrations calculated for milk samples recounted after 3 to 6 days were within 10% of the amount determined by the Y^{90} extraction method. The accurate determination of Sr^{89} will be useful in age-dating of radiostrontium found in milk and will provide a clue to origin—i.e., fallout *vs.* local origin. In addition, the rapidity with which samples can be analyzed for both total radiostrontium and Sr^{90} will be of great value in the event of unusual deposition of radioactive materials in the environs.

The use of the liquid ion exchanger HDEHP for separation of elements other than calcium and strontium was only briefly explored. Tests with a number of radioisotopes (Table I) indicate that such application is feasible.

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Selective Separation of Polycyclic Aromatic Compounds by Countercurrent Distribution with a Solvent System Containing Tetramethyluric Acid

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▶ A solvent system incorporating tetramethyluric acid as a selective complexing agent has been used to separate several polycyclic aromatic hydrocarbons of similar structures. Distribution coefficients measured for other representative polycyclic aromatic hydrocarbons and heterocyclic compounds indicate the general utility of this separation method. Solubilization by the purine is greater for polycyclic compounds with more fused rings and more compact arrangement of these.

SEPARATION of polycyclic aromatic compounds has usually been accomplished by adsorption chromatography on alumina or silicic acid. This resolves a mixture of these compounds into less complex fractions but often fails to give pure individual components. For example, benz(*a*)anthracene and chrysene are not well resolved nor are benzo(*a*)pyrene, benzo(*e*)pyrene, and benzo(*k*)-fluoranthene. Further separations can sometimes be achieved by partition chromatography on paper or by gas-liquid chromatography. The latter methods are usually limited to the use of rather small quantities.

The use of solvent extraction for the purification of polycyclic aromatic compounds has been reported by several workers. Steidle (*14*) utilized nitromethane to extract this type of compound from pentane. More recently, nitromethane has been used for the extraction of polycyclic aromatic hydrocarbons from cyclohexane solutions of cigarette smoke condensates (*9, 11*). Other solvents evaluated by Haenni, Howard, and Joe (*10*) are acetonitrile, dimethylformamide, and methylsulfoxide, which were applied to the removal of polycyclic aromatic compounds from heptane solutions of paraffin wax. These solvents are useful for the separation of unsubstituted polycyclic aromatic hydrocarbons as a class but do not selectively separate individual compounds.

Golumbic (*8*) studied several solvent pairs to obtain a system suitable for the separation of individual polycyclic aromatic compounds by countercurrent distribution. These solvent systems included cyclohexane: 80% ethanol, cyclohexane: 98% acetic acid, benzene: 80% acetic acid, iso-octane: 87% ethanol and *n*-heptane: aniline. He concluded that the first of these was the most selective for isomeric methylnaphthalenes. Relatively little separation was observed for unsubstituted polycyclic hydrocarbons.

In our approach to this problem, we felt that the use of a complexing agent selective for these compounds would be desirable. The solubilization of polycyclic aromatic hydrocarbons in water by purines had been demonstrated by Brock, Druckrey, and Hamperl (*4*). Certain hydrocarbons of this class, including benzo(*a*)pyrene, were solubilized to a greater extent than were others. Weil-Malherbe carried out extensive studies with several purines (*16*). He found that tetramethyluric acid was the most effective of the several purines which he tested for solubilization of benzo(*a*)pyrene. Wanless (*15*) utilized aqueous caffeine solutions to prepare a polycyclic aromatic concentrate from high boiling petroleum products. Solubilities of several carcinogenic aromatic amines were shown by Neish (*13*) to be greatly increased by the addition of caffeine or tetramethyluric acid. Similar findings for the carcinogenic dibenzocarbazoles and dibenzacridines were reported by Booth and Boyland (*2*). Although the purine, tetramethyluric acid, markedly increased the solubility of polycyclic aromatic compounds in water, the amounts of these materials dissolved were still too small to be of practical use in a countercurrent extraction operation. We therefore utilized an aqueous 90% methanol solution containing tetramethyluric acid for the lower phase to give increased solubility for the polycyclic compounds. Cyclohexane was used for the upper phase. By the use of this solvent pair it has been possible by countercurrent distribution to achieve separations of several compounds which are difficult to separate by other means.

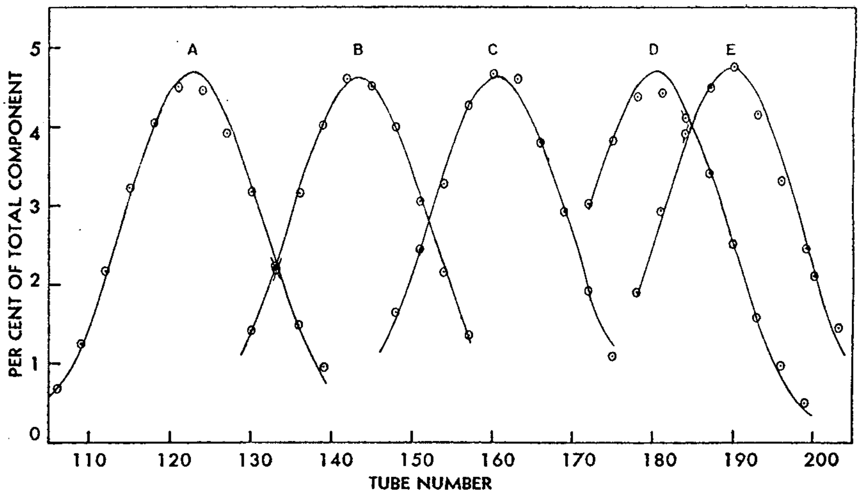

Figure 1. Countercurrent distribution of several polycyclic aromatic compounds between cyclohexane and 0.83% tetramethyluric acid in methanol:water (9:1 v./v.)

A. Benzo(*g,h,i*)perylene
B. Benzo(*e*)pyrene
C. Benzo(*a*)pyrene
D. Chrysene
E. Benz(*a*)anthracene

EXPERIMENTAL

Materials and Apparatus. Preparation of Tetramethyluric Acid. 8-Chlorocaffeine was prepared in 97% yield from caffeine by treatment with chlorine in chloroform solution according to the directions of Fischer and Reese (*6*). The chlorocaffeine (m.p. 191° C.) was converted to 8-methoxycaffeine in 86% yield by refluxing in methanol with sodium methoxide (*5*). Rearrangement to tetramethyluric acid was accomplished by heating 8-methoxycaffeine (m.p. 177–8° C.) for 1 hour at 200–30° C. (*1*). The product crystallized from boiling water upon cooling (m.p. 228.5–9° C.). A yield of 60% was obtained for the synthesis from caffeine. Tetramethyluric acid can be obtained in two crystalline forms, stout heavy blocks or long, fine needles. The former is the more stable and can be obtained upon heating the needles (*12*). Calculated for $C_9H_{12}N_4O_3$: C, 48.21; H, 5.35. Found: C, 48.15; H, 5.17. Ultraviolet absorption maxima at 240 and 294 mμ with log E_{mol} 3.80 and 4.04 (H_2O) were consistent with the spectrum reported by Fromherz and Hartmann (*7*).

Compounds Used. Samples of dibenzofuran, acridine, fluoren-9-one, dibenzothiophene, caffeine, and indole were obtained from Distillation Products Industries, (Rochester, N. Y.). Benz(*c*)acridine, benz(*a*)acridine, benzo(*k*)fluoranthene, and benzo(*e*)pyrene were obtained from Aldrich Chemical Co., Inc. (Milwaukee, Wis.). Anthanthrene, 11 *H*-benzo(*a*)fluorene and dibenzo(*a,l*)pyrene were obtained from L. Light and Co., Ltd. (Colnbrook, Bucks., England). Chrysene, benz(*a*)anthracene, benzo(*a*)pyrene, and pyrene were obtained from Rutgerswerke A.G. (Frankfurt, West Germany). Carbazole was obtained from Matheson, Coleman & Bell (East Rutherford, N. J.). Perylene and benzo(*g,h,i*)perylene were obtained from Bios Laboratories, Inc. (New York, N. Y.). 11*H*-Benzo(*b*)fluorene was obtained from Edcan Laboratories (South Norwalk, Conn.). Fluorene, phenanthrene, dibenz(*a,h*)anthracene, anthracene, and fluoranthene were obtained from commercial sources and were further purified by repeated column chromatography on alumina.

Solvent System for Countercurrent Distributions. Nine grams of tetramethyluric acid was dissolved in one liter of 9:1 (v./v.) methanol:water. This solution was equilibrated with 1 liter of cyclohexane to give 1080 ml. of lower phase and 920 ml. of upper phase. Only a negligibly small portion of the tetramethyluric acid was present in the upper phase. It was calculated that the lower phase contained 0.83% (w./v.). Solvent pairs containing other concentrations of tetramethyluric acid were prepared similarly.

Countercurrent Distribution Apparatus. A 100-tube glass Craig countercurrent distribution apparatus (H. O. Post Scientific Instrument Co., Maspeth, N. Y.) was used for the separation. Each tube contained 10 ml. of each solvent phase.

Procedure for Countercurrent Distribution. Three hundred transfers were performed in the 100-tube Craig countercurrent distribution apparatus by recycling after the 99th transfer. Ten milliliters of each phase of the solvent pair was used in each tube. A mixture of 4.9 mg. of chrysene, benz(*a*)anthracene, benzo(*a*)pyrene, benzo(*e*)pyrene, and benzo(*g,h,i*)perylene was introduced into tube 0 as the sample to be separated. After the final transfer, 5.0-ml. aliquots of upper phase were withdrawn from every third tube for analysis. Each withdrawn aliquot was washed with 5.0 ml. of water and the ultraviolet absorption spectrum was determined. The concentrations of the materials present were calculated from the absorbance at an appropriate maximum. The measured amounts of these materials are plotted as a function of the distribution tube number in Figure 1.

Procedure for Single-Tube Distributions. An amount of the polycyclic compound sufficient to permit accurate absorbance measurements was dissolved in the upper phase of the solvent systems prepared as described above. An aliquot of the upper-phase solution was equilibrated with a measured volume of lower phase by vigorously shaking for about 1 minute. The two phases were allowed to separate and the upper phase was centrifuged to clarify it. Aliquots of the unextracted and extracted upper-phase solutions were then removed, washed once with water, and centrifuged to provide initial and final solutions for ultraviolet absorption measurement. A Perkin-Elmer Model 350 spectrophotometer was utilized for these measurements. Absorption intensities of the two solutions were estimated at an appropriate maximum using base line corrections. The distribution coefficients were calculated using the equation

$$K = \frac{(A_f)}{(A_i - A_f)} \frac{V_l}{V_u} \qquad (1)$$

where

A_f = absorbance of upper phase after equilibration
A_i = absorbance of upper phase before equilibration
V_l = volume of lower phase
V_u = volume of upper phase

Values obtained for a number of polycyclic aromatic compounds in several solvent pairs are presented in Tables I and II.

RESULTS AND DISCUSSION

An advantage of the countercurrent distribution method for separation of mixtures is that quantitative estimates of components can be made on the basis of the binomial distribution if determinations of the quantities in two tubes can be made accurately. This permits the estimation of materials which have not been completely resolved. The degree of resolution required will depend on the reliability of the assay method for each component in the presence of the contaminant. The solvent system described offers a method for the quantitative estimation of a number of individual polycyclic compounds in a mixture without resorting to an excessive number of transfers. A combination of some other fractionation procedure with the countercurrent extraction will separate most of those pairs which are not well resolved by the countercurrent procedure alone. If it is desired to isolate purified components, additional transfers can be utilized to give the required separation.

The distribution coefficients for pyrene and benzo(*a*)pyrene were essentially constant over a wide range in concentrations (Table II). This was confirmed by the close agreement of the measured amounts to the theoretical distributions (Figure 1).

Single-tube distributions indicated that each polycyclic compound tested was more soluble in aqueous methanol containing tetramethyluric acid (TMU) than in the same solvent without this complexing agent. The extent of this increased solubility is given by the ratio of the distribution coefficients in these two systems, K_B/K_A, where K_A is the distribution coefficient in the solvent system containing TMU and K_B is that for the same system without TMU. These values are recorded in Table I. A higher value for this ratio indicates greater solubilization by the purine.

In agreement with the findings of previous workers (*3*), tetramethyluric acid was found to increase the solubility of polycyclic hydrocarbons without regard to their carcinogenic properties. Although only relatively few compounds were examined in the present study, for those polycyclic aromatic hydrocarbons tested compounds with more fused rings were solubilized by the purine to a greater extent. For compounds with the same number of fused rings the TMU-solubilization was greater for the compound with more compact structure. The presence of a partially nonaromatic structure such as is found in fluorene or the benzofluorenes conferred decreased solubilization as compared to the fully aromatic structures such as anthracene or benzanthracene.

Table I. Distribution Coefficients for Several Polycyclic Aromatic Compounds

Compound	Number of fused rings	Distribution coefficient		
		K_A cyclohexane/ 0.83% TMU in 90% methanol	K_B cyclohexane/ 90% methanol	K_B/K_A
Hydrocarbons				
Anthanthrene	6	1.22	5.85	4.80
Dibenzo(*a,l*)pyrene	6	0.99	4.75	4.80
Benzo(*g,h,i*)perylene	6	1.05	4.72	4.50
Perylene	5	1.36	4.51	3.32
Benzo(*e*)pyrene	5	1.36	4.42	3.25
Benzo(*a*)pyrene	5	1.76	4.63	2.63
Benzo(*k*)fluoranthene	5	2.44	5.50	2.25
Dibenz(*a,h*)anthracene	5	2.38	4.70	1.98
Pyrene	4	2.24	4.60	2.05
Fluoranthene	4	1.96	3.90	1.99
Chrysene	4	2.18	4.11	1.88
Benz(*a*)anthracene	4	2.44	4.51	1.85
11*H*-Benzo(*a*)fluorene	4	3.42	5.65	1.65
11*H*-Benzo(*b*)fluorene	4	3.42	5.55	1.62
Phenanthrene	3	2.33	4.10	1.76
Anthracene	3	2.82	4.50	1.60
Fluorene	3	3.86	4.30	1.11
Heterocycles				
Benz(*c*)acridine	4	1.46	2.35	1.61
Benz(*a*)acridine	4	0.31	0.48	1.55
9*H*-Carbazole	3	0.093	0.138	1.48
Dibenzothiophene	3	3.16	4.34	1.37
Dibenzofuran	3	3.06	3.88	1.27
Acridine	3	0.28	0.33	1.18
Fluoren-9-one	3	0.61	0.70	1.15
Indole	2	0.075	0.090	1.20

Table II. Distribution Coefficients for Polycyclic Aromatic Compounds between Cyclohexane and 9:1 Methanol:H_2O Containing Varying Amounts of Tetramethyluric Acid

Polycyclic compound	Concentration of polycyclic compound in cyclohexane before extraction, µg./ml.	Distribution coefficients %TMU in lower phase			
		0.0	0.46	0.83	1.20
Benzo(*a*)pyrene	1.54			1.76	
Benzo(*a*)pyrene	14.7			1.78	
Benzo(*a*)pyrene	146			1.81	
Benzo(*a*)pyrene	2.9		2.52		1.22
Benzo(*a*)pyrene	5.0	4.63			
Pyrene	1.13			2.19	
Pyrene	25.8			2.29	
Pyrene	191			2.29	
Pyrene	38.8	4.55			
Pyrene	2.5		2.98		1.75

For the limited number of heterocyclic compounds tested, those with more fused rings were also solubilized by tetramethyluric acid to a greater extent. The quantitative effects as measured by the ratio K_B/K_A were in the same ranges as were found for the hydrocarbons. Carbazole, with a five-member heterocyclic ring, was solubilized to a greater extent than acridine, with a six-member heterocyclic ring. For the heterocyclics containing the hetero atom in a five-member ring, the order of increased solubilization by the purine was carbazole > dibenzothiophene > dibenzofuran.

A marked effect on the distribution coefficients was noted for different concentrations of tetramethyluric acid in the lower phase. This effect was noted by Weil-Malherbe (*16*) and later confirmed by Boyland and Green (*3*). These workers measured the solubility of polycyclic aromatic compounds in aqueous solutions containing different amounts of purines. They concluded that the solubilities obeyed the relationship

$$k = \frac{C_P{}^f}{C_{HC}} \qquad (2)$$

where

C_P = concentration of purine
C_{HC} = concentration of the test compound
f = a constant with values between 1 and 2, indicative of a reaction involving one to two molecules of purine per complex

For the present study the concentration of the test compound solubilized in the aqueous methanol by the purine is given by the expression

$$C_{HC} = \frac{{}_AC_{HC}}{K_A} - \frac{{}_BC_{HC}}{K_B} \qquad (3)$$

where ${}_AC_{HC}$ and ${}_BC_{HC}$ are the concentrations of the hydrocarbon in the upper phase for the system containing TMU or for the system without TMU, respectively.

When the equilibrium concentrations of hydrocarbon in the upper phases of the two systems are the same, Equation 3 may be simplified as shown in Equation 4. This simplification is generally valid throughout the range of concentrations of hydrocarbon studied, since distribution coefficients were essentially constant throughout this range.

$$C_{HC} = k_1\left(\frac{1}{K_A} - \frac{1}{K_B}\right) \qquad (4)$$

The simplified Expression 4 may then be substituted in Equation 2 to give

$$k_1k = \frac{C_P{}^f}{\frac{1}{K_A} - \frac{1}{K_B}} \qquad (5)$$

The slope of the plot of $\log\left(\frac{1}{K_A} - \frac{1}{K_B}\right)$ *vs.* $\log C_P$ is an estimate of f. For the two compounds studied, pyrene and benzo(*a*)pyrene, the values of this slope calculated for consecutive pairs of points were 1.11, 1.18 and 1.12, 1.47. Weil-Malherbe found values for the exponent, f, either near 1 or near 2 suggesting reactions involving either one molecule or two molecules of purine per complex.

ACKNOWLEDGMENT

The synthesis of tetramethyluric acid used in these studies was performed by Marvin Crutchfield and T. P. Chen. The authors acknowledge the information provided by Charles Keith regarding his preliminary attempts to use purine complexation for the countercurrent separation of closely-related polycyclic aromatic hydrocarbons.

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A General Method for the Chromatographic Separation of Nonionic Surface-Active Agents and Related Materials

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▶ A general chromatographic method has been developed for separating nonionic surface-active agents from each other and from related nonionic materials, using silica gel as adsorbent. The mixture, adsorbed on a silica gel column, is separated by eluting successively with chloroform, 1:99 (v./v.) ethyl ether–chloroform, 1:1 (v./v.) ethyl ether–chloroform, 1:1 (v./v.) acetone–chloroform, 1:19 (v./v.) methanol–chloroform, 1:9 (v./v.) methanol–chloroform, and 1:2 (v./v.) methanol–chloroform. Results obtained with a number of three- and four-component mixtures are described.

COMPOSITIONS CONTAINING nonionic surface-active agents commonly contain more than one nonionic substance. These compositions often contain both water-soluble and oil-soluble surfactants to obtain a better balance of properties, and in addition, usually contain other nonionic, nonsurface-active materials, such as hydrocarbon oils, fatty esters, glycols, and unreacted raw materials from surfactant manufacture. The removal of ionic surface-active agents present in the composition is conveniently accomplished by ion exchange (*1, 7, 8, 10, 12*), but all the nonionic surfactants, together with any nonionic nonsurfactant material, pass unadsorbed through the ion exchanger. The analysis of this mixture of nonionic surfactants and related materials is usually a difficult task and, in spite of its common occurrence, only a few attempts have been made to systematize the analysis of this type of mixture. A liquid-liquid extraction procedure has been suggested (*9*) for separating hexane-soluble nonionics from hexane-insolubles and chromatographic techniques have been suggested for separating mixtures of mono-, di-, and triglycerides (*4–6*) and of fatty acid esters of ethylene glycol and polyoxyethylene glycols (*3*).

This paper describes a general columnar chromatographic method which has been developed to separate the components of mixtures containing various types of nonionic surface-active agents and a number of nonionic nonsurfactant materials commonly encountered with them. A column, rather than a thin-layer or paper, chromatographic technique was used because it permitted convenient qualitative analysis of the eluted fractions by refractive index or melting point, and quantitative measurement by weighing on an analytical balance.

EXPERIMENTAL

Column. A 50-ml. buret (60-cm. × 1-cm. i.d.) with ungreased stopcock.

Procedure. A few milliliters of chloroform were placed in the column, followed by a small plug of glass wool which was tamped in place below the surface of the chloroform with a glass rod, to remove air bubbles. Ten grams of the silica gel (Davison No. 922, through 200-mesh) were slurried with 20 to 30 ml. of chloroform (U.S.P., dried for a few days over anhydrous $CaCl_2$) and poured into the column.

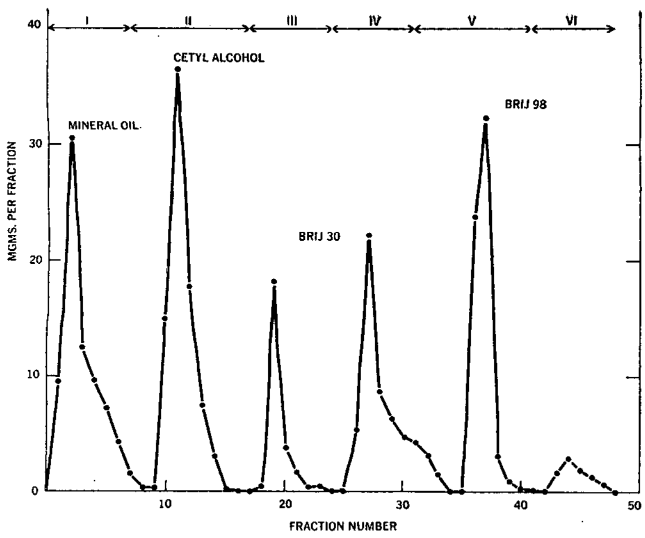

Figure 1. Separation of mixture No. 1

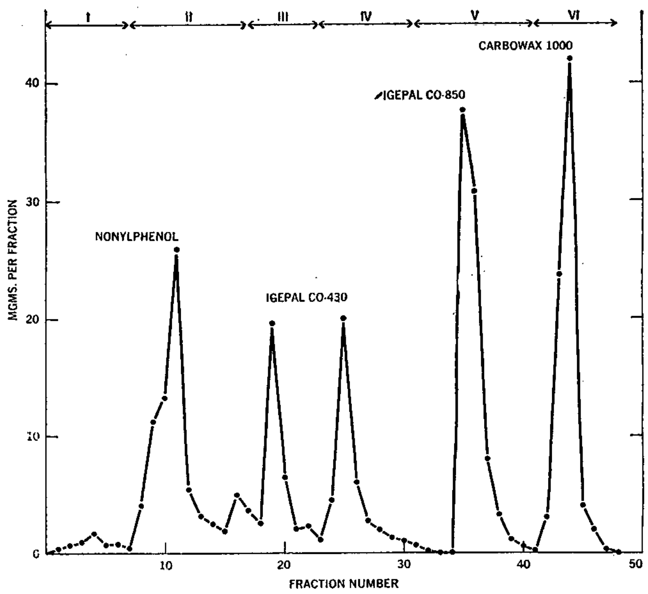

Figure 2. Separation of mixture No. 2

The sample, approximately 300 mg., was added to the column as a solution in chloroform.

The elution program was: chloroform, 70 ml. (I); 1:99 (v./v.) ethyl ether (anhydrous)–chloroform, 100 ml. (II); 1:1 (v./v.) ethyl ether (anhydrous)–chloroform, 70 ml. (III); 1:1 (v./v.) acetone (analytical grade)–chloroform, 80 ml. (IV); 1:19 (v./v.) methanol (U.S.P.)–chloroform, 100 ml. (V); 1:9 (v./v.) methanol (U.S.P.)–chloroform, 70 ml. (VI); 1:2 (v./v.) methanol (U.S.P.)–chloroform, 70 ml. (VII). The 1:19 methanol–chloroform elution was eliminated in most cases where only one highly ethoxylated material (containing > 10 moles of ethylene oxide) was present in the mixture. Ten-milliliter fractions were collected at a flow rate of 1 ml. per minute, the solvents evaporated, and the residues weighed. Refractive indices were taken on all fractions weighing >10 mg. which melted below 40° C.

RESULTS AND DISCUSSION

The results obtained, as shown by the data in Table I and Figures 1 through 5, follow the general rule that the more hydrophilic the material, the greater the polarity of the solvent necessary to elute it. This is consistent with the generally accepted view that the solvent, in order to elute a component adsorbed on a polar surface, must compete successfully for the sites on which the component is adsorbed, thereby displacing it from them. A rough estimate of the hydrophilic-hydrophobic balance in the eluted surfactant molecule can therefore be obtained from the nature of the solvent needed to elute it.

CHLOROFORM elutes only the most nonpolar materials—e.g., mineral oil and methyl laurate. It does not elute even the most hydrophobic surfactants. Triglycerides, such as tristearin, cottonseed oil, or castor oil, are not eluted by this solvent under the conditions used, and this makes possible a convenient separation of hydrocarbon oils from animal and vegetable oils, using chloroform as the eluting solvent.

ETHYL ETHER–CHLOROFORM, *1:99 (v./v.)*, elutes triglycerides (but not castor oil) and long-chain fatty acids and alcohols—e.g., cetyl alcohol, lauric

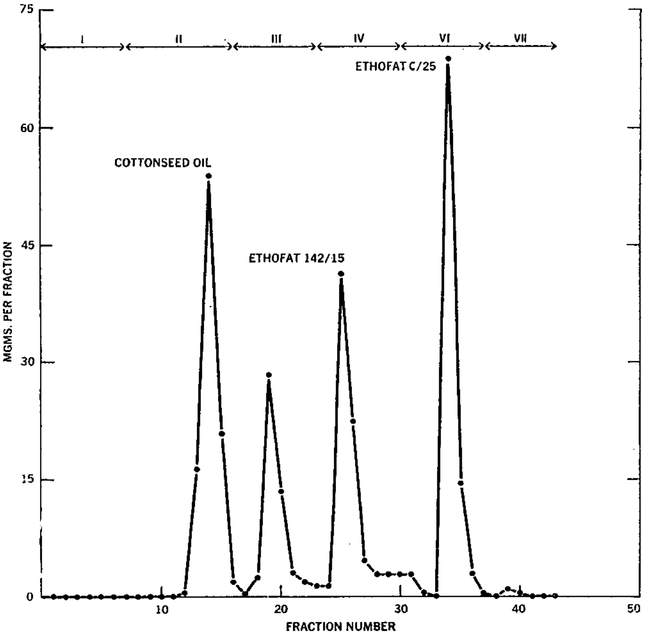

Figure 3. Separation of mixture No. 3

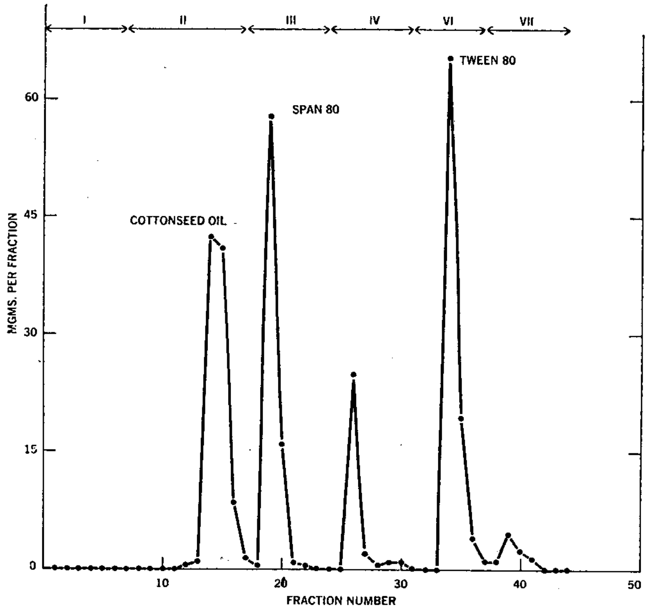

Figure 4. Separation of mixture No. 4

acid, oleic acid. Span 80, a highly hydrophobic surface-active agent (HLB = 4.3), is not eluted by this solvent.

ETHYL ETHER–CHLOROFORM *1:1* (*v./v.*), elutes oil-soluble surfactants, such as ethoxylated fatty acids and alkylphenols with < 4 moles of ethylene oxide and glyceryl monoesters.

ACETONE–CHLOROFORM, *1:1* (*v./v.*), elutes surface-active agents of intermediate polarity—e.g., ethoxylated materials containing no more than about 10 moles of ethylene oxide. This is in accord with the results obtained by Kelly and Greenwald (*2*) on an ethoxylated octylphenol.

METHANOL–CHLOROFORM, *1:19* (*v./v.*) and *1:9* (*v./v.*), elute highly hydrophilic surfactants containing more than 10 moles of ethylene oxide and polyoxyethylene glycols. A separation of these highly hydrophilic surface-active agents from high molecular weight polyoxyethylene glycols may be accomplished by using 1:19 and 1:9 methanol-chloroform solutions consecutively as eluting solvents, because the former does not elute polyoxyethylene glycols of molecular weight 1000 or greater.

METHANOL–CHLOROFORM, *1:2* (*v./v.*), elutes even the most highly hydrophilic substances, such as glycerol and high molecular weight polyoxyethylene glycols.

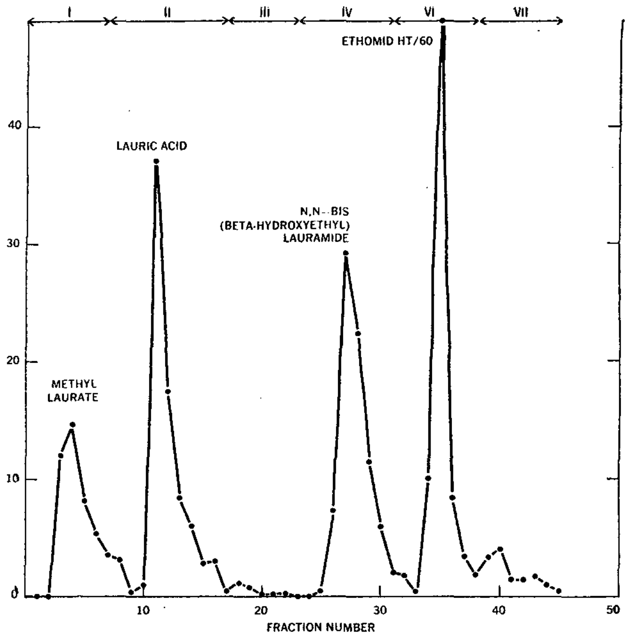

Figure 5. Separation of mixture No. 5

Because almost all the surface-active agents used in these separations were commercial materials, and therefore mixtures of substances with somewhat different hydrophilic – hydrophobic balances, in a number of cases, two different fractions were obtained corresponding to one original material. (Fractions III and IV, Figures 1–4; Fractions V and VI, Figure 1; Fractions VI and VII, Figures 3–5.) The refractive indices and other properties of these fractions indicated that in each case they were indeed fractions of the

Table I. Recovery of Components of Mixtures

Mixture No.	Original components			Recovered material		
	Name and/or structure	Amt., mg.[a]	n_D^{25a}	Eluent	Amt., mg.	n_D^{25}
1	Mineral oil	77.5	1.4778	I	75.8	1.4778
	Cetyl alcohol	78.5	[b]	II	80.1	[b]
	Brij 30-lauryl alcohol + 4 moles EtO	84.1	1.4510	{III IV	25.1 }81.5 56.4	1.4488 1.4538
	Brij 98-oleyl alcohol + 20 moles EtO	71.6	1.4616(33°)	{V VI	60.5 }69.0 8.5	1.4619(33°) ...
2	Nonylphenol	71.7	1.5100	{I II	5.6 }73.5 67.9	... 1.5111
	Igepal CO-430-nonylphenol + 4 moles EtO	80.9	1.4976	{III IV	42.8 }81.6 38.8	1.4998 1.4940
	Igepal CO-850-nonylphenol + 20 moles EtO	78.4	1.4768(33°)	V	81.8	1.4763(33°)
	Polyoxyethylene glycol, mol. wt. 1000	76.9	...	VI	75.1	...
3	Cottonseed oil	97.2	1.4705	{I II	0 }94.0 94.0	... 1.4701
	Ethofat 142/15–oleic acid + 5 moles EtO	131.5	1.4703	{III IV	51.5 }133.5 82.0	1.4698 1.4690
	Ethofat C/25–lauric acid + 15 moles EtO	99.0	1.4612	{VI VII	90.0 }91.5 1.5	1.4620 ...
4	Cottonseed Oil	98.5	1.4705	{I II	0 }95.0 95.0	... 1.4697
	Span 80–sorbitan mono-oleate	101.0	1.4764	{III IV	76.0 }105.5 29.5	1.4741 1.4764
	Tween 80–sorbitan mono-oleate + 20 moles EtO	104.0	1.4720	{VI VII	90.0 }99.5 9.5	1.4722 ...
5	Methyl laurate	64.8	1.4299	I	48.1[c]	1.4303
	Lauric acid	72.8	[c]	II	76.1	[b]
	N,*N*-bis(β-hydroxyethyl)lauramide	78.9	m.p., 48.7°–49.3° C.	IV	80.2	m.p., 48.0°–48.5° C.
	Ethomid HT/60–hydrogenated tallow amide + 50 moles EtO	84.3	[c]	{VI VII	72.7 }86.2 13.5	[b] [b]

[a] Amounts and refractive indices on 100% active basis.
[b] M.p. >40° C.
[c] Loss due to volatilization.

original commercial material and contained no significant amount of the other components of the mixture. For example, in mixture No. 1, Brij 30, a polyoxyethylated lauryl alcohol containing an average of 4 moles of ethylene oxide, was separated into two fractions whose refractive indices, n_D^{25} 1.4488 and n_D^{25} 1.4538, correspond, respectively, to ethoxylated lauryl alcohols containing an average of 3 moles and 5 moles of ethylene oxide (*11*).

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Separation and Determination of Iron(II) and Iron(III) with Anthranilic Acid Using Solvent Extraction and Spectrophotometry

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▶ Iron(III) was extracted as the anthranilate into 1-pentanol in the presence of iron(II). A direct determination of the iron(III) content of the sample was obtained by measuring the absorbance of the pentanol extract. The iron(II) in the aqueous phase was oxidized and extracted as the anthranilate into a solvent mixture. A direct determination of the iron(II) content of the sample was obtained by measuring the absorbance of the nonaqueous phase.

ANTHRANILIC ACID was first prepared by Fritsche in 1841 (*2*) and was proposed as an analytical reagent in 1926 (*1, 8*). Two publications cite the use of anthranilic acid as a reagent for solvent extraction separations (*5, 9*), specifically, for the separation and determination of plutonium by extraction with amyl acetate.

The precipitate formed by iron(III) and anthranilic acid can be extracted into organic liquids such as alcohols, ketones, and ethers. The copper, cobalt, nickel, cadmium, and iron(II) anthranilates could not be extracted. The extracted iron(III) anthranilate in the organic solvent was a red solution with an absorbance proportional to the concentration of iron(III).

EXPERIMENTAL

Reagents. ANTHRANILIC ACID SOLUTION, $0.07M$. Five grams of recrystallized acid were dissolved in approximately 400 ml. of deionized water and 37 ml. of $1N$ sodium hydroxide. pH of the solution was slowly decreased by the addition of $1N$ sulfuric acid until a pH of 4.5 was reached and the solution was diluted to 500 ml. with deionized water. Solutions were stored in brown glass bottles and were discarded as soon as a brownish cast was observed.

STANDARD IRON(III) SOLUTION. Pure iron wire (99.8%) was polished with fine emery cloth, wiped, cleaned in acetone, and weighed. The wire was placed in a 100-ml. beaker and a slight excess of concentrated sulfuric acid was added slowly. Hydrogen peroxide was added to oxidize the iron to the ferric state and the excess was then boiled off. During the heating process, demineralized water was added periodically to maintain the liquid volume. After transfer to a volumetric flask, the solution was diluted to the mark with deionized water.

STANDARD IRON(II) SOLUTION. Various amounts of analytical grade ferrous ammonium sulfate hexahydrate were dissolved in acidified deionized water which had been outgassed with oxygen-free nitrogen. The oxygen was removed from the nitrogen by passing the gas through a basic 15% solution of pyrogallol. Fresh solutions were prepared as soon as a positive test for iron(III) was obtained.

ORGANIC LIQUIDS. All the organic materials used for the extractions were C.P. grade reagents.

AMYL ACETATE, ACETOPHENONE, AND 1-PENTANOL MIXTURE. The mixture was prepared by mixing 130 ml. of amyl acetate, 100 ml. of acetophenone, and 19 ml. of 1-pentanol.

Apparatus. All pH measurements were made with a Beckman Model G pH meter. Quantitative absorbance measurements were made with a Beckman Model DU spectrophotometer, equipped with a Beckman Model 4300 photomultiplier attachment. Corex cells, 1 cm., were used and slit widths of between 0.06 mm. and 0.08 mm. were maintained for all measurements. Other absorbance measurements were made with a Cary recording spectrophotometer, Model 14. An International Centrifuge, 220-ml. capacity, was used to increase the speed of separation of the aqueous and organic phases.

Working Curve. Various amounts of an iron(III) sulfate solution were pipetted into each of several 60-ml. ground glass-stoppered bottles. Sufficient deionized water to bring the volume to 5 ml. was added to each bottle. Twenty-five milliliters of a $0.07M$ solution of anthranilic acid (pH 4.5) were added and then 10 ml. of 1-pentanol were transferred into each of the bottles. The two phases were shaken for 2 minutes and centrifuged. Enough of the organic phase was removed with a medicine dropper to fill the absorbance cell. Absorbance was measured at 475 mμ against a blank prepared by extracting a solution containing 5 ml. of deionized water and 25 ml. of the anthranilic acid solution with 10 ml. of 1-pentanol.

The same procedure was repeated using a mixture of amyl acetate, acetophenone, and 1-pentanol in place of the 1-pentanol. The absorbance of the complex extracted into the mixture was measured at 465 mμ.

Working curve data are given in Table I.

Procedure. The aqueous sample containing iron(III) and iron(II) was outgassed with oxygen-free nitrogen. With a pipet 25 ml. of a $0.07M$ anthranilic acid solution at pH 4.5 were transferred to a 250-ml. ground glass-stoppered bottle containing the sam-

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Table I. Data for Working Curves

Fe(III) added, mg.	Absorbance	Solvent
0.047	0.112	1-Pentanol
0.094	0.221	1-Pentanol
0.188	0.430	1-Pentanol
0.195	0.450	1-Pentanol
0.238	0.650	1-Pentanol
0.385	0.897	1-Pentanol
0.048	0.137	Amyl acetate, acetophenone, and 1-pentanol
0.097	0.270	Amyl acetate, acetophenone, and 1-pentanol
0.146	0.402	Amyl acetate, acetophenone, and 1-pentanol
0.195	0.535	Amyl acetate, acetophenone, and 1-pentanol
0.244	0.673	Amyl acetate, acetophenone, and 1-pentanol
0.292	0.802	Amyl acetate, acetophenone, and 1-pentanol

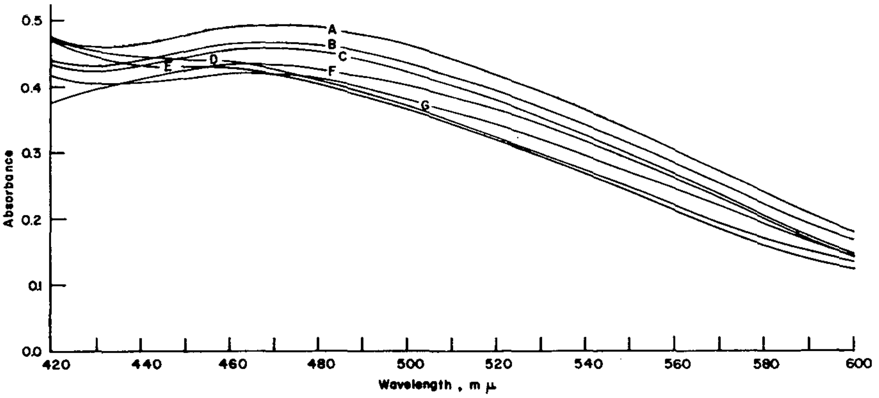


Figure 1. Visible spectra of iron(III) anthranilate extracted into various alcohols

A.	1-Butanol	E.	2-Octanol
B.	1-Pentanol	F.	1-Hexanol
C.	3-Methyl butanol	G.	2-Methyl pentanol
D.	2-Ethyl hexanol		

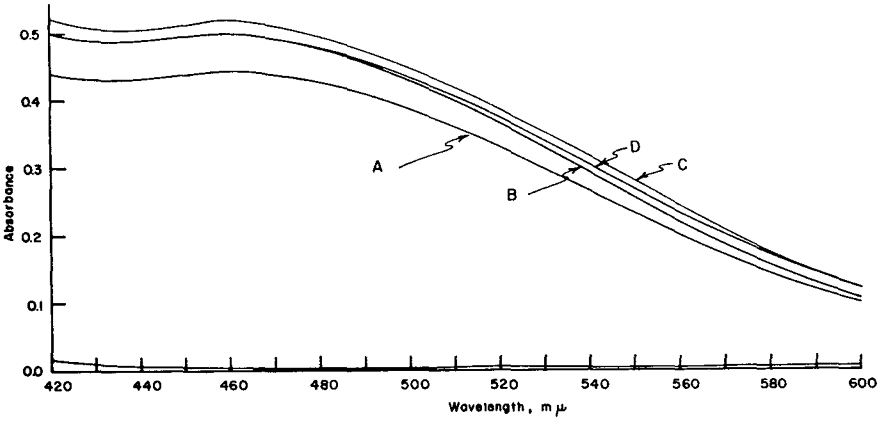


Figure 2. Visible spectra of iron(III) anthranilate extracted into various esters

A.	Isobutyl propionate	C.	n-Amyl acetate
B.	Butyl acetate	D.	Ethyl acetate

ple. The anthranilic acid solution previously had been outgassed with nitrogen. Ten milliliters of 1-pentanol, outgassed with nitrogen, were pipetted into the bottle. The mixture was shaken for 2 minutes and centrifuged. The two phases were separated and the aqueous phase was placed in a 50-ml. beaker. Two milliliters of 30% H_2O_2 were added to the aqueous phase to oxidize the iron(II) to iron(III), and enough 0.5*N* HNO_3 was added to lower the pH to 4.5. The aqueous phase was set aside for 15 minutes to assure complete oxidation of the iron(II), then the solution was saturated with anthranilic acid by the addition of approximately 0.01 gram of the acid. The precipitate was quantitatively transferred from the beaker to a 60-ml. ground glass-stoppered bottle. Ten milliliters of the organic mixture were pipetted into the bottle and the mixture was shaken for 2 minutes and centrifuged.

The absorbance of each of the two organic phases containing the extracted iron(III) anthranilate was measured against reference solutions which were treated in the same manner as the sample. The 1-pentanol solution was measured at 475 mμ and the mixture at 465 mμ.

Several synthetic samples of iron(II) and iron(III) were analyzed and the results are shown in Table II.

DISCUSSION

The visible spectra of iron(III) anthranilate extracted into various organic materials are shown in Figures 1, 2, and 3. The visible spectra of the dissolved iron(III) complex in several solvent mixtures are given in Figure 4. 1-Pentanol and a mixture of amyl acetate, acetophenone, and 1-pentanol had the best separation characteristics—

Table II. Analyses of Iron(III)–Iron(II) Samples

Sample	Iron(II) present, mg.	Iron(III) present, mg.	Iron(II) found, mg.	Iron(III) found, mg.	Absolute error, mg.
1	0.134	...	0.132	...	−0.002
	...	0.195	...	0.194	−0.001
2	0.134	...	0.136	...	0.002
	...	0.390	...	0.390	0.000
3	0.112	...	0.107	...	−0.005
	...	0.195	...	0.194	−0.001
4	12.8	...	...	...	...
	...	0.195	...	0.188	−0.007
5	10.8	...	...	...	...
	...	0.195	...	0.195	0.000
6	0.110	...	0.109	...	−0.001
	...	0.234	...	0.241	−0.002
7	0.084	...	0.078	...	−0.006
	...	0.097	...	0.099	0.002
8	0.252	...	0.245	...	−0.007
	...	0.097	...	0.097	0.000
9	0.050	...	0.050	...	0.002
	...	0.390	...	0.400	0.010
10	0.134	...	0.136	...	0.002
	...	1.85	...	...	...

Table III. Effect of Aqueous Volume on Extraction

Vol. water added, ml.	Total vol., ml.	Fe(III) taken, mg.	Fe(III) found, mg.	Absolute error, mg.	Error, %
0	26	0.195	0.194	−0.001	−0.51
10	36	0.195	0.195	0.000	0.00
20	46	0.195	0.193	−0.002	−0.02
30	56	0.195	0.182	−0.013	−7.14

i.e., low solubility in water, no dispersed water in the organic phase, and quantitative extraction. Even though the complex had a larger maximum absorbance value for a given concentration of iron(III) in the mixture than in 1-pentanol alone, the mixture could not be used for extraction in the presence of iron(II) because it oxidized the iron(II) to iron(III). Therefore, 1-pentanol was used for extractions of iron(III) anthranilate in the presence of iron(II) and the mixture was used for extraction when iron(II) was not present.

Table II shows that iron(III) can be determined in the presence of iron(II) in a quantity 65 times greater than the iron(III) present, and iron(II) can be determined in the presence of iron(III) in a quantity 15 times greater than the iron(II) present. If too large a concentration of iron(II) is present in the solution—i.e., greater than 425 p.p.m.—a negative error in the determination of iron(III) and a positive error in the determination of iron(II) are caused by the occlusion of iron(III) anthranilate with the iron(II) anthranilate precipitate, and consequent incomplete extraction of the iron(III) with the first extraction. Large amounts of iron (III)—i.e., greater than 60 p.p.m.—cause a positive error in the determination of iron(II) because not all the iron(III) anthranilate is removed by the first extraction.

As shown in Figure 5, the pH for maximum precipitation of iron(III) anthranilate occurs at 4.2, whereas the maximum extraction, as shown in Figure 6, occurs in a pH range of 3.7 to 5.0. This can be explained by assuming that the iron(III) anthranilate in solution is in equilibrium with the solid iron(III) anthranilate and that both solid and dissolved iron(III) anthranilate are extracted by the organic solvent.

In the pH range between 3.7 and 5.0, the aqueous phase was tested for the

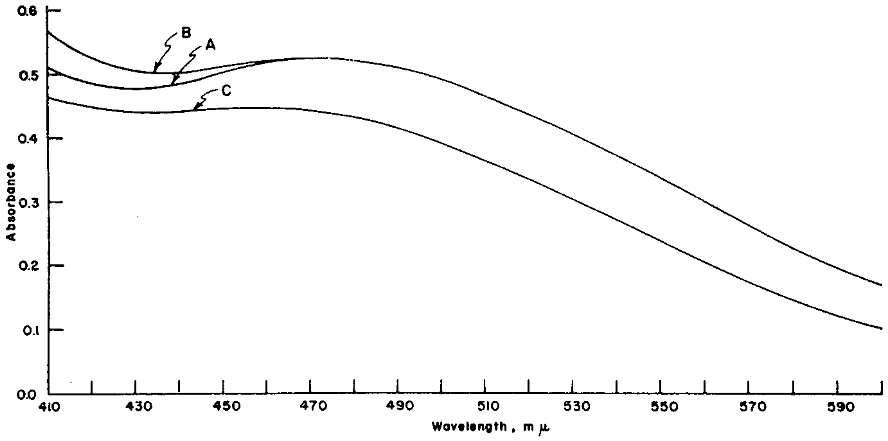


Figure 3. Visible spectra of iron(III) anthranilate extracted into various ketones

A. Acetophenone
B. Propiophenone
C. 2,6-Dimethyl,4-heptanone

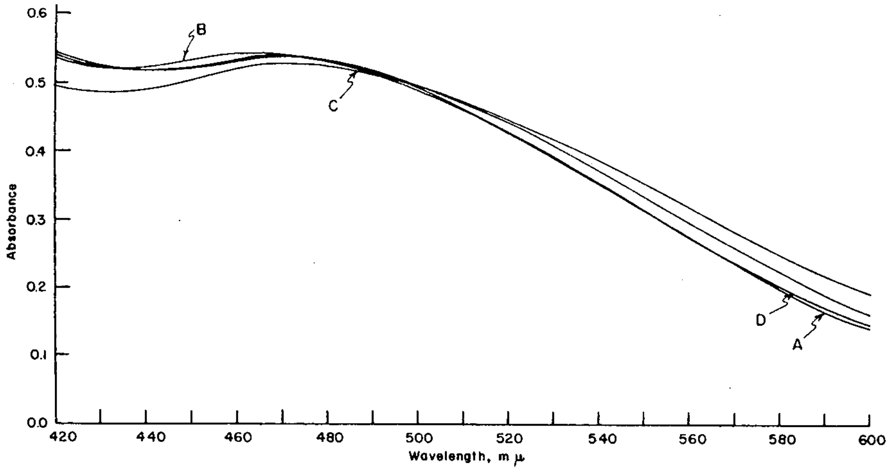


Figure 4. Visible spectra of iron(III) anthranilate extracted into various organic mixtures

A. Amyl acetate and acetophenone (1.5 to 1 by volume)
B. Amyl acetate, acetophenone, and 1-pentanol (1.3 to 1 to 0.05 by volume)
C. 1-Pentanol and acetophenone (3 to 1 by volume)
D. Butyl acetate and acetophenone (5 to 1 by volume)

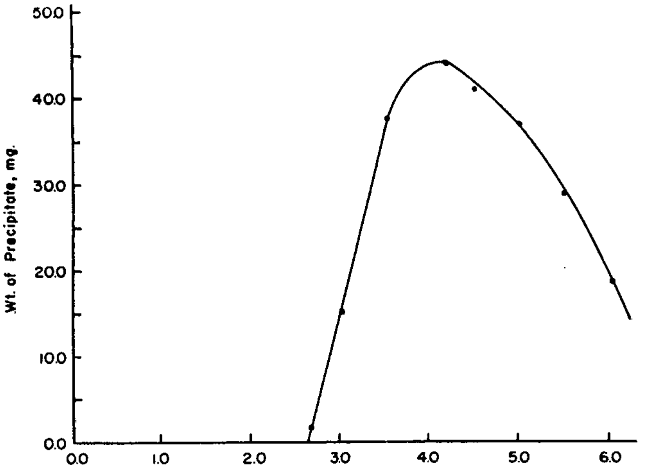


Figure 5. pH-precipitation curve for iron(III) anthranilate

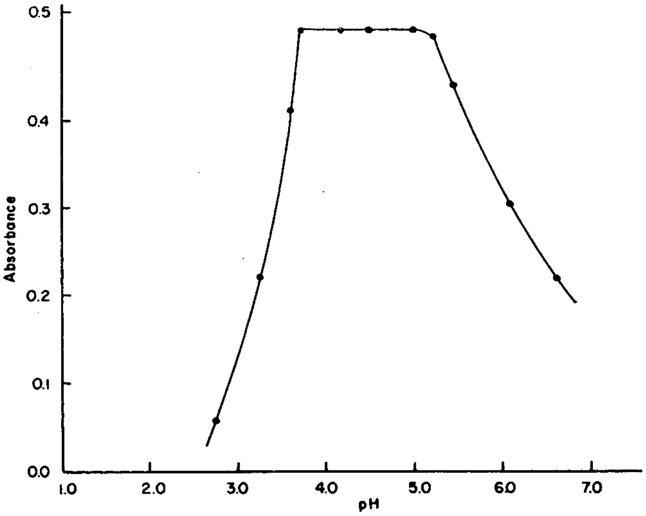


Figure 6. pH-absorbance curve for iron(III) anthranilate extracted into 1-pentanol

presence of iron(III) after the anthranilate was extracted. Several qualitative reagents for iron(III) were used. They included 1,10-phenanthroline dissolved in thioglycolic acid, potassium thiocyanate, and potassium ferrocyanide. All the tests were negative in this pH range. [This is an ideal range for precipitation and extraction of iron(III) anthranilate because it is in a buffer region of the acid and it is not necessary to add an additional buffer to the solution.]

Table IV. Temperature Effect on Absorbance

Temperature, °C.	Absorbance
10	0.530
20	0.540
25	0.540
35	0.542

Table III shows that the volume of the aqueous phase up to 46 ml. has little effect on the amount of iron(III) anthranilate extracted. Therefore, when using 25 ml. of 0.07*M* anthranilic acid solution, the volume of the solution containing the iron could vary from 1 ml. to 20 ml. without affecting the amount of iron(III) anthranilate extracted. The absorbance values were compared with the working curve prepared as described in the experimental section.

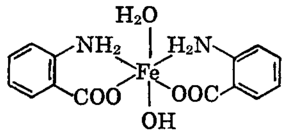
Changes in temperature within 20° to 35° C. do not alter the absorbance of the extracted complex. The effect of change in temperature on the absorbance in shown in Table IV.

The anthranilates of copper, cobalt, and nickel have been isolated and their compositions determined by Funk and Ditt (*3*) and by Ishimaru (*6*). Ishimaru also determined the composition of zinc anthranilate and Kiba and Sato (*7*) determined the composition of the manganese, mercury(II), and lead anthranilates. These divalent metal anthranilates have the formula $M(C_7H_6NO_2)_2$. It is generally accepted that the metal coordinates through the oxygen of the carbonyl group and the nitrogen forming two six-membered rings.

However, no references were found for the composition of the iron(II) anthranilate or the iron(III) anthranilate. Therefore, these compounds were isolated. Both of the salts were precipitated with anthranilic acid at a pH of 4.5, filtered, and dried at 120° C. for one hour. The iron(II) anthranilate was isolated under an atmosphere of nitrogen. Portions of the materials were sent to Galbraith Laboratories for analysis. The results of these analyses are given in Tables V and VI.

The theoretical calculations for Table V are based on the formula, $Fe(C_7H_6NO_2)_2$. The data show that the experimental values agree with the theoretical values calculated on the basis of this formula.

The theoretical values, *A*, in Table VI are calculated from the formula, $Fe(C_7H_6NO_2)_3$. These values are in poor agreement with the experimental values. The *B* set of theoretical values is based on a formula which can be represented by the structure:



in which the iron has a coordination number of six. The theoretical values calculated from this formula are in agreement with the experimental values.

A structure of this type would account for the fact that iron(III) anthranilate can be extracted and the iron(II) and the other divalent metal anthranilates can not be extracted. It can be reasoned that the water molecule is replaced by an organic molecule, thus causing the solution of the precipitate in the organic phase. This hypothesis is supported by the fact that solvents without oxygen do not extract iron(III) anthranilate while solvents containing oxygen in the structure work quite well. Solvents such as chloroform and carbon tetrachloride did not extract the iron(III) anthranilate.

Interferences were studied by the addition of known concentrations of various ions in solutions containing 0.195 mg. of iron(III). Data for the interference studies are found in Table VII. The interfering ion was decreased until a relative error of 2% or less was obtained.

During these studies it was observed that the interference effect could be

Table V. Analysis of Iron(II) Anthranilate

	C, %	H, %	N, %	Fe, %
Theoretical	51.24	3.68	8.54	17.02
Experimental	51.45	3.72	8.64	16.93

Table VI. Analysis of Iron(III) Anthranilate

	C, %	H, %	N, %	Fe, %
Theoretical, *A*	54.32	3.82	9.05	12.03
Theoretical, *B*	46.18	4.16	7.69	15.34
Experimental	46.13	3.89	7.48	15.74

Table VII. Results of Interference Studies

Weight of iron(III) present: 0.195 mg.

Interfering ion	Weight of interfering ion, mg.	Iron(III) found, mg.	Absolute error, mg.	Error, %
Nickel	6.772	0.182	−0.013	6.6
	6.772	0.191	−0.004	2.0
	6.772	0.195	0.000	0.0
Copper	7.124	0.104	−0.091	46.6
	3.562	0.115	−0.080	41.0
	0.677	0.186	−0.009	4.6
	0.388	0.192	−0.003	1.5
	0.194	0.195	0.000	0.0
Cobalt	5.432	0.193	−0.002	1.0
	5.432	0.195	0.000	0.0
Cadmium	13.16	0.185	−0.010	5.1
	6.58	0.187	−0.008	4.1
	3.29	0.194	−0.001	0.51
Zinc	6.608	0.187	−0.008	4.1
	6.608	0.195	0.000	0.0
Chromium	6.50	0.116	−0.079	40.5
	3.75	0.140	−0.055	28.2
	1.87	0.187	−0.008	4.1
	0.94	0.187	−0.008	4.1
	0.094	0.196	0.001	0.51
Mercury	50.0	0.182	−0.013	6.6
	22.5	0.186	−0.009	4.6
	12.5	0.192	−0.003	1.54
Aluminum	8.0	0.177	−0.018	9.2
	2.0	0.195	0.000	0.0
Manganese	3.20	0.196	0.001	0.51
	3.20	0.195	0.000	0.0
Lead	16.1	0.195	0.000	0.0
Chloride	146.	0.191	−0.004	2.0

significantly reduced if the iron(III) anthranilate was extracted immediately after the addition of the anthranilic acid solution. The iron(III) complex forms immediately, and the absorbance was not changed by altering the time between addition of the reagent to the sample and the addition of the extractant. Some of the anthranilates are noticeably slower to form. Thus, immediate extraction reduces the effect of postprecipitation since the iron(III) is precipitated and extracted before the formation of the other anthranilates.

This effect is shown by the data for nickel interference in Table VII. In the first experiment, a period of 10 minutes was allowed before the extraction was performed. The other data for nickel were obtained by extracting immediately after the addition of the reagent. In all subsequent interference studies, extraction was done immediately after addition of the reagent.

In some cases—i.e., copper and chromium—the speed of formation of the iron(III) anthranilate was of no advantage because the interfering ion formed a precipitate as rapidly as the iron(III). These interfering ions must, therefore, be reduced to a point where occlusion of the iron(III) precipitate is minimized.

The proposed method of analysis provides a rapid method for the determination of iron(III) in the presence of iron(II) and iron(II) in the presence of iron(III). Most of the methods for the determination of iron(III) and iron(II) in a sample involve the determination of the iron, usually in the plus two state, a reduction and determination of total iron. An example of this method is the use of 1,10-phenanthroline, as proposed by Harvey, Smart, and Amis (*4*).

The method suggested here eliminates the disadvantage of decreased accuracy inherent in indirect methods. Both the iron(II) and iron(III) are determined directly. Other advantages include the formation of a stable colored complex in the organic phase, a broad working pH range and high sensitivity. The sensitivity is 0.05 p.p.m. iron when the complex is extracted into a mixture of amyl acetate, acetophenone, and 1-pentanol, and 0.07 p.p.m. iron when the complex is extracted into 1-pentanol. This is taken as the concentration of iron(II) or iron(III) in the aqueous phase needed to produce a change in absorbance of 0.005.

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Liquid-Liquid Extraction of Cesium with 2-Thenoyltrifluoroacetone

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▶ A new, rapid, highly selective liquid-liquid extraction method for cesium is described. Recent developments for the inhibition of the hydrolysis of 2-thenoyltrifluoroacetone provide the basis for the quantitative extraction of cesium under a variety of conditions. Small amounts of lithium markedly enhance the extraction of cesium with 2-thenoyltrifluoroacetone. Highest extraction coefficients are obtained in the presence of lithium with 2-thenoyltrifluoroacetone dissolved in solvents containing the nitro group—e.g., 98% for nitromethane and nitrobenzene. Efficient extraction of other alkali elements is possible under certain conditions. The method is applicable for either tracer or macro quantities of cesium. Several practical applications of the method are proposed, both for the analytical chemist and the separations technologist in the purification of cesium and gross removal of fission products.

THE SEPARATIONS CHEMIST is considerably interested in the liquid-liquid extraction of cesium-137 in radioisotopes manufacture, in the decontamination of waste nuclear fuel solutions, and in the analysis of fission product solutions. In general, the alkali metal ions exhibit only a slight tendency toward complex formation. The few extraction methods reported to date are all based on the ion association principle and include the systems, tetraphenylboron (*4*), hexafluorophosphate (*3*), polyiodides (*12*), polybromides (*10*), and phenols (*1*, *5*).

The versatile chelating agent, thenoyltrifluoroacetone (TTA), has been used extensively for the extraction of metal ions from acid solution, particularly by radiochemists (*8*, *9*). Essentially all metal ions of the periodic table form extractable chelates under appropriate conditions with this reagent. The alkali element group is the only one which has not yielded previously. Cesium and other alkali elements have not been extracted before with TTA because of the hydrolysis of the reagent at alkaline pH's. The prevention or inhibition of this alkaline hydrolysis offers an interesting possibility for extending the TTA method to the extraction of the alkali elements. The authors have recently accomplished this and demonstrated the first extraction of cesium and other alkali elements, apparently as chelates, with TTA. This paper describes a variety of conditions for the new separation.

EXPERIMENTAL

Apparatus. Vortex test tube mixer, Model K-500-4, supplied by Scientific Industries, Inc., Springfield, Mass.

NaI(Tl) well-type gamma scintillation counter, 3 × 3 inches.

Beckman pH meter, Model G.

Reagents. Analytical grade reagents were used without further purification.

The 2-thenoyltrifluoroacetone (TTA) (mol. wt. = 222) was supplied by Eastern Chemical Corp., Newark, N. J.

Carrier-free cesium-134 solution, containing about 1.4×10^8 gamma counts per minute per ml., was obtained from ORNL Isotopes Division. One milliliter of this solution was heated to dryness twice with 10 ml. of concentrated nitric acid and once with dis-

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tilled water. The cesium-134 was then taken up in 20 ml. of 1*N* sodium hydroxide, quantitatively transferred to a 250-ml. volumetric flask and made to volume with 1*N* sodium hydroxide. This stock solution, containing 5 × 10^5 gamma counts per minute per ml., was used in the extraction studies.

Procedure. Pipet a suitable aliquot of the cesium tracer solution containing approximately 5 × 10^5 gamma counts per minute per ml. into a 50-ml. centrifuge tube. Add 5 ml. of 1*N* lithium hydroxide and make up to 10 ml. with 2*N* sodium carbonate. Extract for 3 minutes with an equal volume portion of 0.5*M* TTA–nitromethane or 0.5*M* TTA–nitrobenzene, using a Votex mixer or other suitable extraction technique. The final equilibrated pH should be in the pH range 8.7 to 9.0. Centrifuge for several minutes in a clinical centrifuge. Carefully pipet 1-ml. aliquots of the phases into 75-mm. culture tubes. Cork the tubes and count the gamma radioactivity on a well-type scintillation counter. The above procedure gave cesium extractions of 98.0% for both the nitromethane and nitrobenzene systems.

RESULTS AND DISCUSSION

TTA reacts with most metal ions to form unionized chelate compounds soluble in organic solvents and immiscible in water of the form:

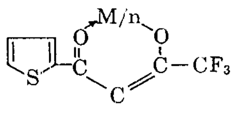


Table I. Cesium-134 Tracer Extracted with 0.25*M* TTA in Hexone [Diluent (1:1) at pH 8.5]

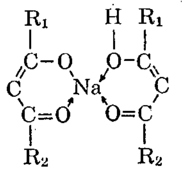
Diluent	Cs^{134} Extd., %
Trichloroethylene	10.3
Chloroform	10.9
Amyl acetate	4.7
2:4 Pentanedione	7.7
2-Butanone	6.6
Diisobutylcarbinol	8.3
Dibutyl carbitol	4.8
Nitromethane	21.6
Xylene	1.0

Table II. Effect of Aqueous Lithium Concentration on Cesium-134 Tracer Extraction at Varying TTA-Hexone Concentrations

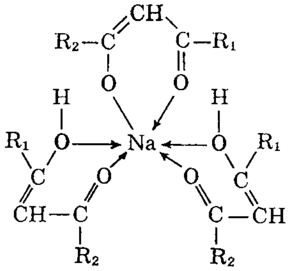
(3-Minute extraction periods. Organic/aqueous vol. = 1)

TTA concn. in hexone, *M*	No lithium pH	No lithium Cs^{134} extd., %	0.3*N* lithium carbonate pH	0.3*N* lithium carbonate Cs^{134} extd., %
0.05	12.1	1.0	12.1	5.0
0.5	9 6	16.5	7.95	56.6
1.0			7.45	71.5
2.0	8.0	65.9	7.4	85.2
2.5	8.1	79.0	7.3	87.1
4.0			7.2	89.9

The alkali elements, however, because of their large size and small charge, form saltlike derivatives of β-diketones, insoluble in hydrocarbon solvents. However, the addition of sufficient neutral molecules to bring the apparent coordination number of the alkali metal ion up to four or six imparts some complex character. Thus, sodium benzoyl acetonate is insoluble in toluene but the 2-hydrate is soluble (*7*). Many of the metallic derivatives which are insoluble in the organic solvents readily dissolve in the presence of excess of the mother substance by forming complexes of the type (*11*):



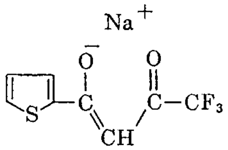
(I)



(II)

These facts suggested the possible enhancement of alkali extractions by a suitable choice of solvent.

Preliminary experiments had clearly indicated that pH values greater than 7 or 8 would be required to attain satisfactory extraction ratios. Now, when TTA is titrated with a base, a curve characteristic of a weak acid of pK_a about 6.2 is obtained (*13*). If the TTA which has been titrated with alkali is back titrated with acid, the curve bears no simple relation to the initial titration curve. The titration proceeds as though a portion of the TTA is converted to strong acid—the case if trifluoroacetic acid is formed by hydrolysis. However, TTA may be dissolved in alkali of pH 12 and quantitatively recovered after 10 minutes (*2*). This anomalous behavior has been explained by the marked difference in behavior of TTA and its monohydrate (*13*). If the hydration of TTA were base catalyzed, one would expect that identical solutions would be obtained on dissolving either TTA or its hydrate in aqueous base. In the latter case, however, an oil separates and only a few per cent of TTA remains unhydrolyzed. The hydrolysis products are acetylthiophene and trifluoroacetic acid. The same treatment of solid anhydrous TTA mentioned above produces essentially complete conversion to the enolate ion:



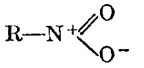
Either TTA or TTA·H_2O can be converted to enolate ion in a buffer of pH 8. But if aqueous TTA solutions are allowed even momentarily to become more basic than pH 9, cleavage of the TTA·H_2O (the principal species) results. The above observations have been confirmed spectroscopically (*2*, *6*).

Because of its structural similarity to TTA, acetylacetone was an obvious solvent but proved unsatisfactory due to the complete miscibility of the two phases under certain conditions. Attempts to use xylene as a solvent for TTA failed because of the rapid hydrolysis of the chelating agent in alkaline solution. Of the solvents tested, hexone appeared at first to be the most promising. Cesium extractions of 4% at pH 7 reaching a maximum of 21% between pH 8.5 to 9.0 with 0.5*M* TTA in methylisobutylketone (hexone) were observed. The results obtained for the different solvents tested are listed in Table I. The pH was adjusted by appropriate additions of 2*N* sodium carbonate. The pH values quoted were the final values determined after equilibration. Equal volume portions (10 ml.) were used for both organic and aqueous phases in 3-minute extraction periods. Increased TTA concentrations proved moderately successful, 2.5*M* TTA giving about 79% cesium extraction. These concentrations are, however, impractical and undesirable in that equivalent alkali concentrations must be added for pH adjustment, thereby increasing the degree of hydrolysis of the TTA. The increased extraction is probably due to the formation of complexes of the types (I) and (II).

At this point it was noted that even small additions of lithium markedly enhanced the extraction of cesium. The effect of lithium addition as saturated lithium carbonate to the aqueous phase is given in Table II. High concentrations of TTA were still, however, required for satisfactory extractions. Further tests with various solvents revealed the dramatic combined effect of lithium and nitromethane.

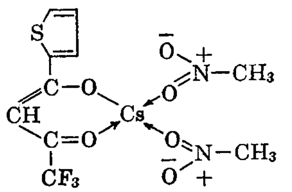
TTA–Nitromethane Extraction System. When an equal volume portion (10 ml.) of 0.5*M* TTA–nitromethane and of aqueous phase 0.3*N* in lithium are used, 98% extraction of the cesium into the organic phase was obtained at pH 8.7 in 3 minutes.

If we consider the organic nitro compounds containing the grouping NO_2, with the N atom attached to a carbon atom, they may be formulated as follows:



$$R{-}N^{+}\begin{smallmatrix}\nearrow O \\ \searrow O^{-}\end{smallmatrix}$$

By analogy to the previously mentioned behavior of alkali β-diketone compounds of forming extractable 4 or 6 coordinated compounds (*4*), it would seem reasonable to postulate that the cesium might be extracted in the form



The initial problem was to determine the interrelation of the four parameter variables, namely, the concentrations of TTA, nitromethane, lithium, and pH. Xylene, added to the organic phase, served as diluent as well as to improve mechanical behavior and to decrease the aqueous solubility of the nitromethane. While cesium extraction coefficients were slightly reduced by the addition of the xylene, they were still high.

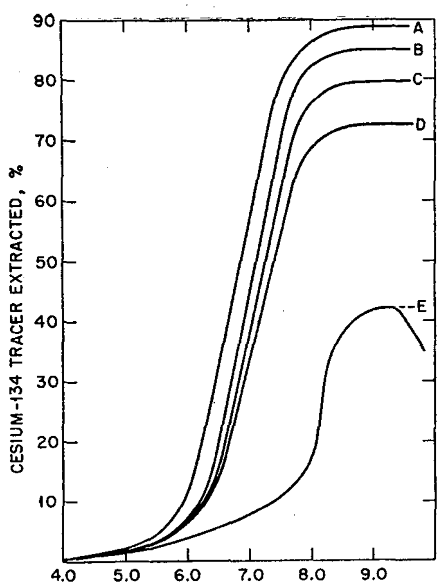


Figure 1. Extraction of cesium-134 tracer as a function of pH

$\frac{\text{Organic}}{\text{aqueous}}$ volume = 1
Organic phase, 0.25*M* TTA-nitromethane-xylene (1:1)
Extraction period, 3 minutes
A. 0.7*M*, 0.5*M*, and 0.3*M* lithium citrate
B. 0.10*M* lithium citrate
C. 0.05*M* lithium citrate
D. 0.025*M* lithium citrate
E. No lithium citrate

In Figure 1 the extraction of cesium-134 as a function of pH is illustrated. The effect of lithium here is twofold; the extractions are greatly enhanced by the addition of the lithium, and also the pH at which equivalent extractions are attained is substantially reduced with increasing lithium concentration.

Table III. Extraction of Cesium-134 Tracer with 0.5M TTA–Nitromethane as a Function of Time

(Organic/aqueous volume = 1)

Time, min.	Cs^{134} extd., % No lithium	Cs^{134} extd., % 0.3*N* lithium carbonate
1	74.4	97.9
5	73.0	98.0
15	70.1	97.3
30	67.9	97.3

Figures 2 and 3 show the variation of the per cent cesium-134 extracted into a nitromethane-xylene (1:1) mixture at varying concentrations of lithium and TTA. The percentage extracted bears a linear dependence to the logarithm of the lithium concentration. Finally, the effect of nitromethane concentration, first with no lithium present and, second, under optimum lithium conditions is shown in Figure 4. Here again the strong influence of lithium upon the extraction is clearly seen.

The addition of lithium has another important effect in that the unfavorable reduction of extraction coefficients due to hydrolysis is apparently inhibited. This effect is shown in Table III where the percentage cesium-134 extraction is shown to decrease with time in the absence of lithium.

In this system, maximum extraction is attained at a pH of 8.7. Care must be taken not to exceed a pH of 9, where visible decomposition of the TTA occurs. The aqueous phase eventually turns dark brown, probably due to the decomposition of some dissolved nitromethane. This may be compared with no visible aqueous reaction in the TTA–nitrobenzene system described below.

Strongly anionic groups, such as chloride, sulfate, and nitrate, depress the extraction of cesium-134 slightly. The effect of these anions is given in Table

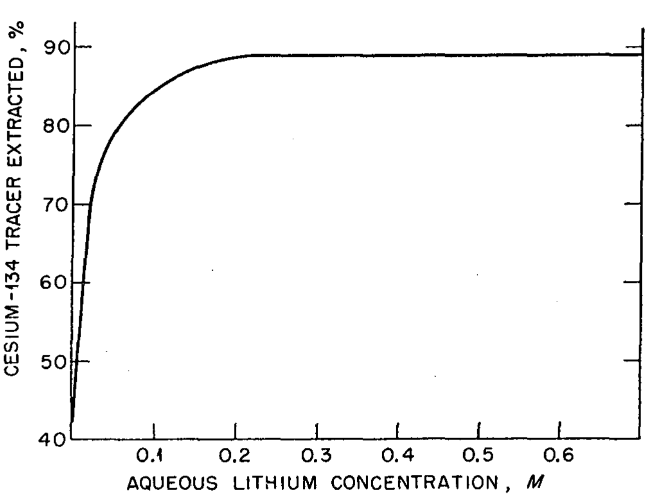


Figure 2. Extraction of cesium-134 tracer as a function of lithium concentration

$\frac{\text{Organic}}{\text{aqueous}}$ volume = 1
Organic phase, 0.25*M* TTA-nitromethane-xylene (1:1)
Extraction period, 3 minutes

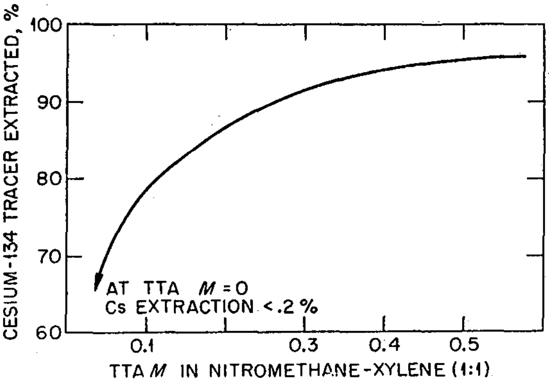


Figure 3. Extraction of cesium-134 tracer as a function of TTA concentration

$\frac{\text{Organic}}{\text{aqueous}}$ volume = 1
Aqueous phase, pH 9; 0.5*M* lithium citrate
Extraction period, 3 minutes

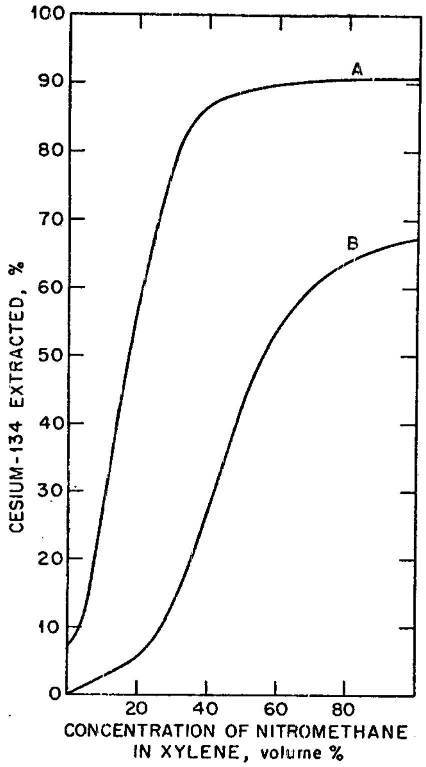


Figure 4. Extraction of cesium-134 tracer as a function of nitromethane concentration

$\frac{\text{Organic}}{\text{aqueous}}$ volume = 1
Aqueous phase, pH 9
Organic phase, 0.25*M* TTA-xylene with varying amounts of nitromethane
Extraction period, 3 minutes
A. 0.5*M* lithium citrate
B. No lithium citrate

IV using the standard procedure described above.

Two successive 3-minute extractions with equal volume portions of 0.5*M* TTA in nitromethane–xylene (4:1) cause 99.5% of the cesium-134 to be extracted from an aqueous phase of pH 8.7. Under these conditions only 22% of the sodium is extracted. With pure nitromethane as solvent for the TTA, the cesium-134 extraction is raised to 98% in the first extraction, but the percentage sodium extracted is increased to 45%. These facts suggest possible means of effecting interesting separations of the alkali elements from one another by varying solvent ratios. Extraction values for the different alkali elements have been determined spectrophotometrically and are shown in Table V. The standard procedure described above was used. The effect of cesium carrier upon the extraction was also tested. No significant deviation from cesium-134 tracer behavior was found at the 1 mg. per ml. cesium level. Interestingly, the extraction coefficients for the alkali metal ions increase regularly with increasing electrode potential (7).

Table IV. Effect of Strong Anionic Groups on Extraction of Cesium-134 Tracer

Anion	Concn., *M*	Cs^{134} extd., %
Control	...	94.8
Chloride	1	89.5
Sulfate	1	89.8
Nitrate	1	87.6

Table V. Extraction of Macro Amounts of the Alkali Elements

Element	Amt. added, mg.	Recovery, %
Lithium	13.6	91.4
Sodium	112.0	44.8
Potassium	10.8	82.1
Rubidium	6.2	92.2
Cesium	7.4	99.5

A brief investigation of nitroethane, nitropropane, and nitrobutane as solvents for TTA gave slightly lower extraction coefficients for cesium.

Use of a strong aqueous complexing agent for most metal ions renders the extraction highly selective for cesium. (Ethylenedinitrilo)tetraacetic acid (EDTA) complexes many metal ions under the conditions recommended for the extraction, thereby preventing their interference either by coextracting or hydrolyzing in the aqueous phase. EDTA does not inhibit the extraction of cesium. Probably weaker complexing agents, such as citrate, tartrate, oxalate, isobutyrate, or lactate, can be used in place of EDTA in certain situations.

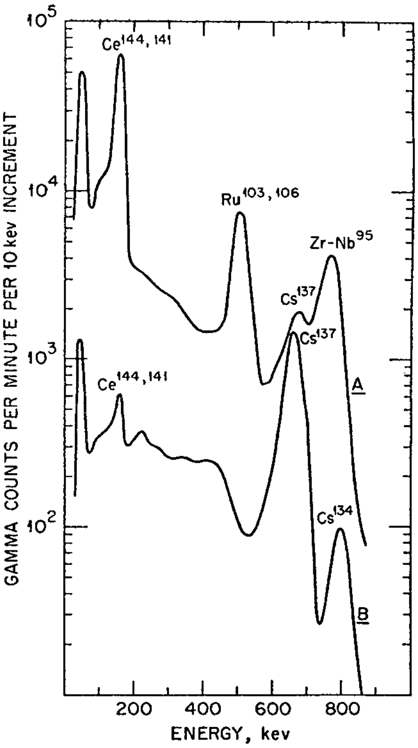


Figure 5. Recovery and decontamination of cesium-134, -137 with 0.5*M* TTA-nitromethane

A. Original gross spectrum
B. Organic phase spectrum

Figure 5 shows typical decontamination data obtained by performing the standard extraction procedure described above with the addition of 5% EDTA.

TTA–Nitrobenzene Extraction System. The high extractability found

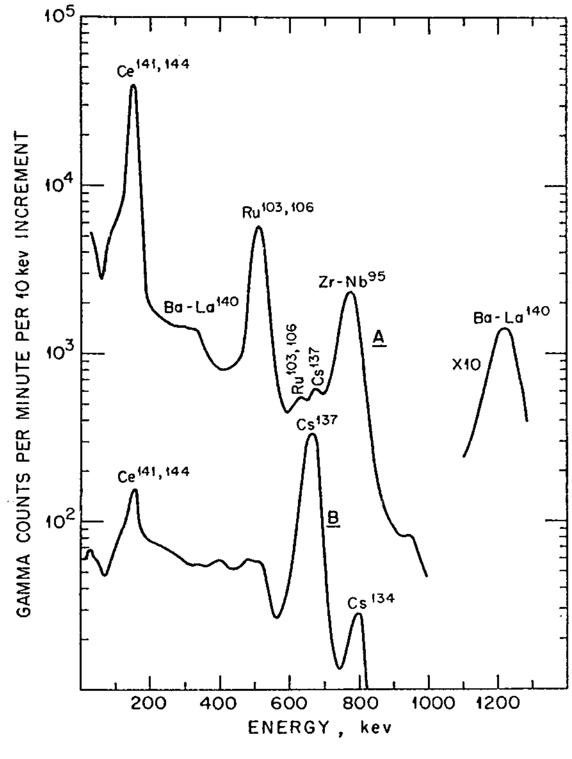


Figure 6. Recovery and decontamination of cesium-134, -137 with 0.5*M* TTA-nitrobenzene

A. Original gross spectrum
B. Organic phase spectrum

for cesium in the TTA–nitromethane system suggested an investigation of the TTA–nitrobenzene system. In the latter system, it was also found possible to extract cesium essentially quantitatively with excellent separation from fission products. The mechanical behavior for nitrobenzene was, however, not as good as for nitromethane. In a typical extraction, the aqueous phase containing the fission product mixture was adjusted to concentrations of 5*M* sodium hydroxide, 0.5*M* lithium hydroxide, and 5% EDTA. This solution was extracted for 3 minutes with an equal volume portion of 0.5*M* TTA–nitrobenzene. After centrifugation for several minutes, the gamma spectrum of each phase was determined and compared with the original gross gamma spectrum. (Sufficient time must be allowed to elapse after the removal of samples, so that the equilibrium between the cesium-137 and its 2.6-minute barium-137 daughter is attained.) Figure 6 shows the excellent cesium recovery and decontamination possible in this system. Interestingly, the peak at 794 k.e.v. was shown to be due to a very slight amount of cesium-134 present. It was completely masked by the high ratio of zirconium–niobium-95 in the original gross spectrum. The decontamination was somewhat better than that found in the 0.5*M* TTA–nitromethane system, especially in regard to ruthenium whose extraction in the nitromethane system was unpredictable (Figures 5 and 6).

Under the described conditions for the 0.5*M* TTA–nitrobenzene system, a slight amount of a fine suspended solid formed in the organic phase. This suspension did not separate on centrifugation but could be readily dissolved by the addition of small amounts of isoamyl alcohol, after separation of the aqueous phase. The addition of isoamyl alcohol prior to the separation of the phases tended to strip the major portion of the cesium back into the aqueous phase. Actually, the fine solid did not contain cesium and was not an interference in radiochemical measurements.

Among the advantages of nitrobenzene over nitromethane as a solvent for TTA are its negligible aqueous solubility and somewhat higher decontamination values. Furthermore, the hydrolysis of the TTA is apparently completely inhibited in the nitrobenzene system, even at concentrations as high as 5*M* with respect to sodium hydroxide. On the other hand, a vigorous reaction is produced in the nitromethane system at sodium hydroxide concentrations in excess of 1*M*. Mixtures of nitrobenzene and nitromethane also proved impractical since the addition of even small amounts of nitromethane to the nitrobenzene system would trigger the rapid decomposition reaction.

In the absence of EDTA the TTA system, under alkaline conditions, offers a possible means for the gross removal of fission products.

The cesium is readily stripped from the organic phase into acid media.

GENERAL APPLICATIONS

The new method described in this paper provides a valuable solvent extraction approach in two categories:

For the Analytical Chemist. Previously, chemists have found it necessary to apply preliminary, tedious, step-wise procedures of removing other elements from the alkali elements. A major advantage of the new method is the possibility of extracting cesium and other alkali elements directly away from many other elements. This simplified rapid separation provides the analytical chemist with a useful tool in their determination or removal when they constitute interferences in other determinations.

The new method is potentially useful for the direct isolation of cesium and other alkali elements in an organic phase for enhanced sensitivity by flame spectrophotometry. The method provides the analytical radiochemist with a simple, highly selective, nongravimetric technique for radiocesium involving only the measurement of the gamma radioactivity in the organic phase. Ready adaptability to remote control work is a distinct advantage over precipitation methods.

For the Separations Technologist. The separations technologist should find profitable applications for this method, both in the manufacture of high specific activity radiocesium and in the decontamination of waste nuclear-fuel solutions prior to disposal. In the former case, the use of complexers such as EDTA to selectively complex metal ions other than cesium is indicated. In the latter case, omission of the aqueous complexing agent provides the desired coextraction of both cesium and other fission products.

While the highest extraction coefficients are found in the nitromethane and nitrobenzene systems, it should be noted that useful extractions of cesium are possible in other systems, such as TTA–hexone. In a several-stage separation, this system may prove more practical.

Among the advantages of TTA over tetraphenylboron are its considerably higher stability and relative inexpensiveness. In addition, the TTA system poses no stripping problems, as one finds with tetraphenylboron, to prevent recycling of the latter reagent.

Another appealing feature of the TTA system is the possibility of applying it in an integrated scheme for multiseparations based on pH control (*8*, *9*). Of considerable advantage is the noncorrosive nature of the described method over systems utilizing iodine, iodide, bromine, bromide, and fluoride.

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Adsorption of the Elements on Inorganic Ion Exchangers from Nitrate Media

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▶The distribution coefficients of 60 metal ions have been measured for four inorganic ion exchangers from nitrate media over the pH range of 1 to 5. The exchangers are hydrous zirconium oxide, zirconium phosphate, zirconium tungstate, and zirconium molybdate. The data are presented as plots of log distribution coefficient vs. pH in a series of four periodic charts. Separations of analytical interest are presented.

The use of inorganic compounds for ion exchange separation has been gaining popularity. The emphasis in research to date has been the synthesis of various inorganic compounds and experiments to characterize the ion exchange mechanisms. The main use has been in the nuclear energy industry for the separation of selected nuclides from dissolved, spent, reactor fuel solutions.

Recently, four inorganic compounds—hydrous zirconium oxide, zirconium molybdate, zirconium phosphate, and zirconium tungstate—have become commercially available for the express use as ion exchangers. This paper presents adsorption data for 60 metal ions for these four exchangers from nitrate media over the pH range of 1 to 5. Potentially useful separations to the analyst and radiochemist are presented.

Compared to the organic ion exchangers, the inorganic exchangers are more stable to high temperature, radiation damage, and oxidation; however, the solubility of the hydrous oxide exchangers becomes appreciable in acidic media and the acid salt types are appreciably soluble in basic media.

Based on the excellent studies of Kraus *et al.* (*5*), Amphlett (*1*), Gal and Gal (*4*), and others (*2, 3*), the adsorption properties of the inorganic exchangers can be summarized as follows.

Hydrous Zirconium Oxide. In acid (at pH levels below the isoelectric point), the positively charged polymer is electrically neutralized by exchangeable anions. The anion exchange characteristics of the material are these. Adsorption of anions decreases with increasing pH; polyvalent anions are more strongly adsorbed than are monovalent anions; negatively charged metal complexes are adsorbed; complexing reactions with the exchanger can cause abnormally high adsorption—i.e., the *D* for fluoride on hydrous zirconium oxide approaches infinity—and the capacity of the exchanger is on the order of 1 to 2 meq. per gram. (The commercial exchanger used in this study has a stated capacity of 1.05 meq. of $Cr_2O_7^{-2}$ per gram at pH 1.)

At pH above the isoelectric point, the hydrous zirconium oxide polymer is negatively charged and is electrically neutralized with exchangeable cations. Its cation exchange characteristics are: Increased pH gives increased cation adsorption; the more strongly adsorbed polyvalent cations are adsorbed at relatively low pH values; and the distribution coefficients for ammonia-transition element complexes are extraordinarily large.

Zirconium Acid Salts. The three exchangers studied—zirconium phosphate, molybdate, and tungstate—are sufficiently acidic to act only as cation exchangers with the following exchange characteristics: (1) With increased pH, adsorption of cations increases; (2) at low pH, the selectivity for alkali metals is noteworthy, characterized by increasing *D* values with increasing number; (3) this high selectivity for the alkalies and also for the alkaline earths is lost at higher pH (with the exchangers in the ammonium form, the alkali metals can be eluted as one group and the alkaline earths as another group); and (4) the capacities of the acid salt exchangers are on the order of 1 meq. per gram. The capacities of the exchangers used in this study

Table I. Exchange Characteristics of Elements

Periodic group	Remarks	Potential analytical usefulness
IA	Low adsorption	
IIA	Low adsorption	Be separated from IA and IIA elements at pH $\geq$ 5
IB, IIB	Adsorption increases with increasing pH, except Ag with high adsorption over pH range 1 to 5	Ag separated from most cations at pH 1
IIIA, IVA (rare earths)	Adsorption increases with increasing pH. Sc and Zr most strongly adsorbed. Zr added from fluoride soln. may be adsorbed as fluoride complex or through heterogeneous exchange reaction with exchanger	Sc separated from IIIA elements at pH 3 to 5
IIIB	Maximum adsorption at pH 3 to 5 except Tl(I) which is low over pH range 1 to 5	Tl(I) separated from IIIB elements at pH 3 to 5
IVB, VB, VIB	Strong adsorption, as anionic species, over pH range 1 to 5 except Pb(II). *D* of Pb(II) maximum at pH 3	Pb separated from IVB, VB, and VIB elements at pH 1
VA	Nb and Ta added from fluoride media. Increasing *D* with increasing pH attributed to increasing stability of fluoride complexes	

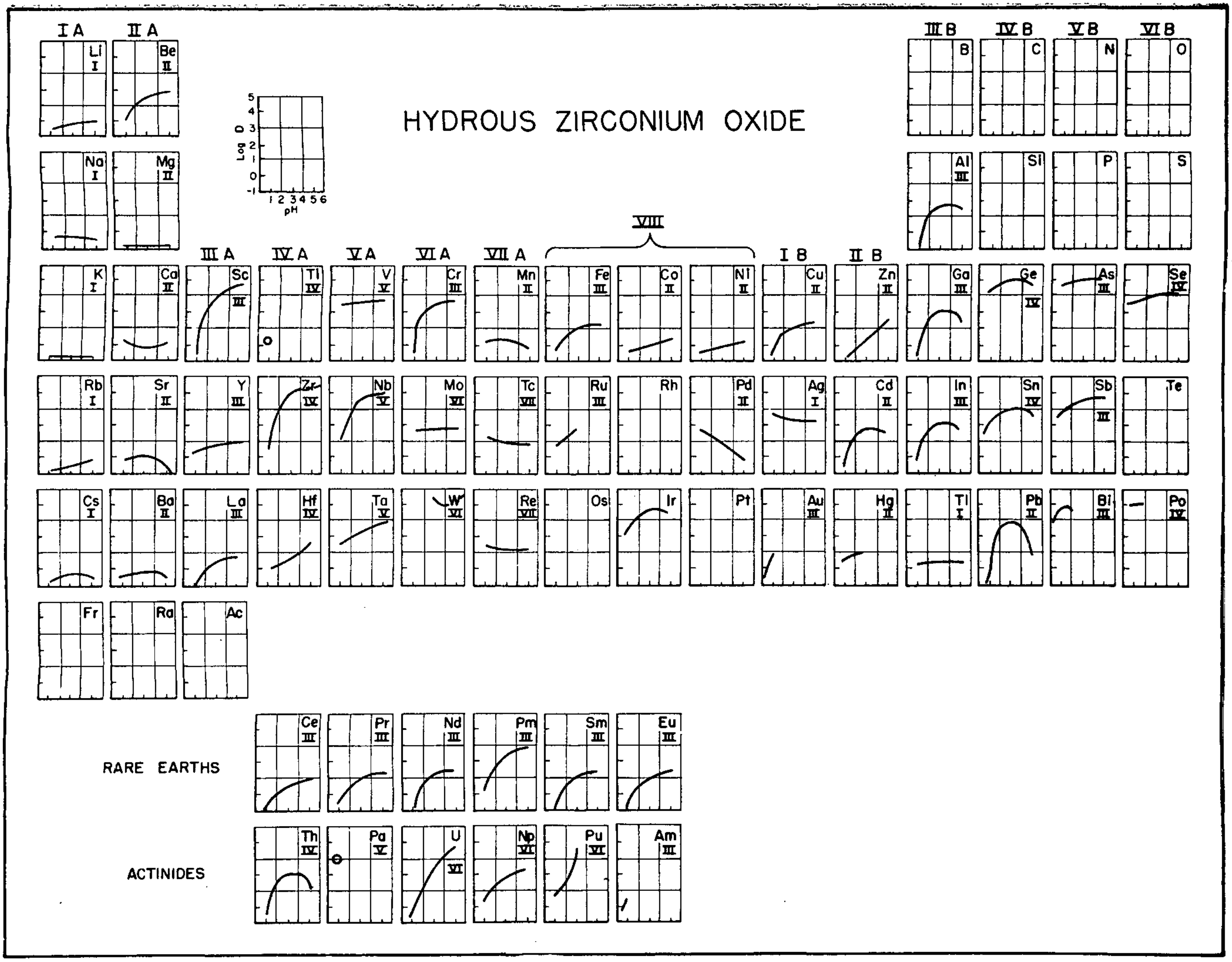

Figure 1. Exchange characteristics of elements on hydrous zirconium oxide

on Hydrous Zirconium Oxide from Nitrate Media

Periodic group	Remarks	Potential analytical usefulness
VIA	*D* of W (as WO_4^{-2}) and Mo (as MoO_4^{-2}) high and independent of pH. Cr (III) adsorption pattern similar to IIIA elements	Cr(III) separated from Fe, Co and Ni
VIIA	Lower *D* for Tc(VII) and Re(VII) than other anions attributed to singly charged species. Adsorption of Mn (II) similar to Group IIA	
VIII	*D* of Co(II) and Ni(II) similar to IIA elements. *D* of Fe(III) and Ru (III) similar to IIIA elements. Decreasing *D* with increasing pH for Pd(II) may be due to formation of nonadsorbing hydrolysis product. Ir(IV), added from chloride media, strongly adsorbed as chloride complex	
Actinides	Decreasing *D* of Th(IV) with increasing pH attributed to formation of nonadsorbing hydrolysis product. *D* values of U, Np, Pu present in +6 oxidation state approach useful high values at pH 5	U separated from Th

(milliequivalents of Cs per gram at pH 4) were:

Zirconium phosphate,	1.05
Zirconium molybdate,	1.12
Zirconium tungstate,	0.77

EXPERIMENTAL

Reagents. The four exchangers (Bio-Rad Laboratories, Richmond, Calif.) of 100 to 200 mesh were used without treatment. Radiotracers either were prepared by neutron irradiation in the Materials Testing Reactor or were purchased from the Isotopes Division of Oak Ridge National Laboratory.

Procedure. To a 50-ml. polyethylene centrifuge tube were added 0.50 gram of one of the four inorganic exchangers and 10 ml. of a 0.005*M* solution of a metal ion and its radiotracer in equilibrium. Oxidation-reduction cycling was used to effect chemical identity for multivalent ions. The metal ion solution had been preadjusted with nitric acid or ammonium hydroxide to pH 1, 3, or 5. A polyethylene stopper was

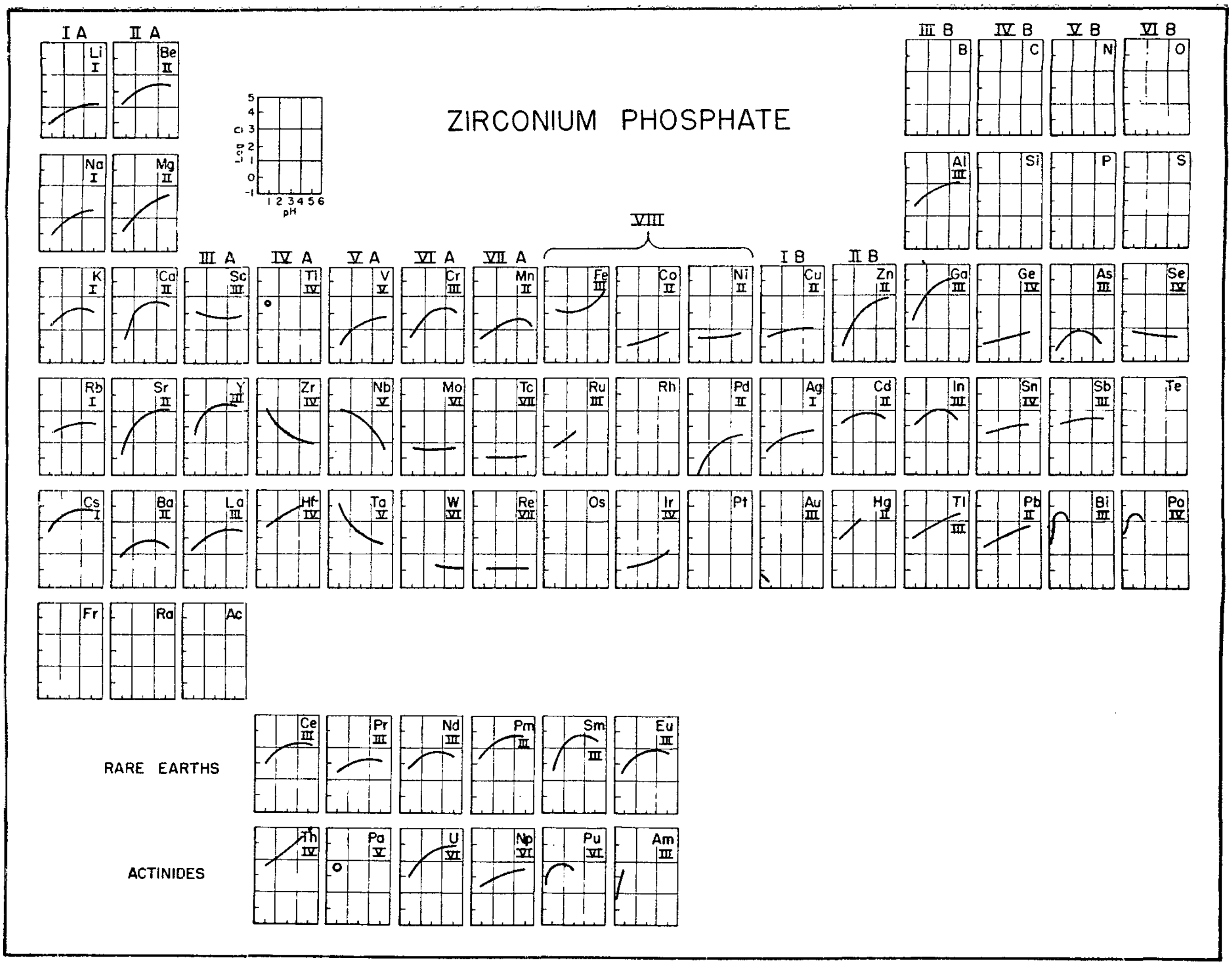

Figure 2. Exchange characteristics of elements on zirconium phosphate

inserted and equilibration was made for 1 hour on a 33-r.p.m. wheel. After centrifugation, 1-ml. aliquots were analyzed. The beta-gamma tracers were counted in a well crystal; the alpha tracers were plated on stainless steel planchets and counted in a zinc sulfide alpha chamber. Suitable radiotracers were not available for several of the elements. Aluminum, beryllium, lead, magnesium, titanium, and vanadium were determined by rotating disk emission spectrography, lithium by flame photometry, and uranium by sodium fluoride pellet fluorophotometry.

The distribution coefficient, D, amount of metal ion adsorbed per gram of exchanger/amount of metal ion per milliliter of contacting solution, for the above conditions, is:

$$D = 20\left(\frac{Cb}{Ca} - 1\right)$$

in which Cb = counts of tracer or amount of metal per milliliter, before equilibration, and Ca = Cb, after equilibration.

Table II. Exchange Characteristics of Elements on

Periodic group	Remarks	Potential analytical usefulness
IA	Order of D is ZP>ZT>ZM.[a] Separation factors differ little for 3 exchangers, best at low pH	Separation of individual alkali metals
IIA	At comparable pH levels, D values are approximately 10 times greater than for IA elements. However, separation factors between members not as high as for Group IA. D of Ba(II) decreases at high pH especially on ZP and ZT exchangers	Ba separated from Sr on ZP at pH 5
IB, IIB	D values generally increase with increasing pH except for Ag on ZT. Au, added from chloride solution, not adsorbed	Ag separated from other members at pH 1 on ZM
IIIA (rare earths)	All members strongly adsorbed. With ZT, D values decrease with increasing pH differing from other two exchangers	Sc separated from other members at pH 1 on ZP
IIIB	D values of all members increase with increasing pH, except Al on ZM	Al separated from other members on ZM at pH 5
IVA	Zr added as fluoride complex. Decreasing D with increasing pH attributed to increasing stability of fluoride complex	
IVB	Increasing D value as atomic number increases as expected for increasing metal character	Separation of members at pH 3 to 5 on ZT

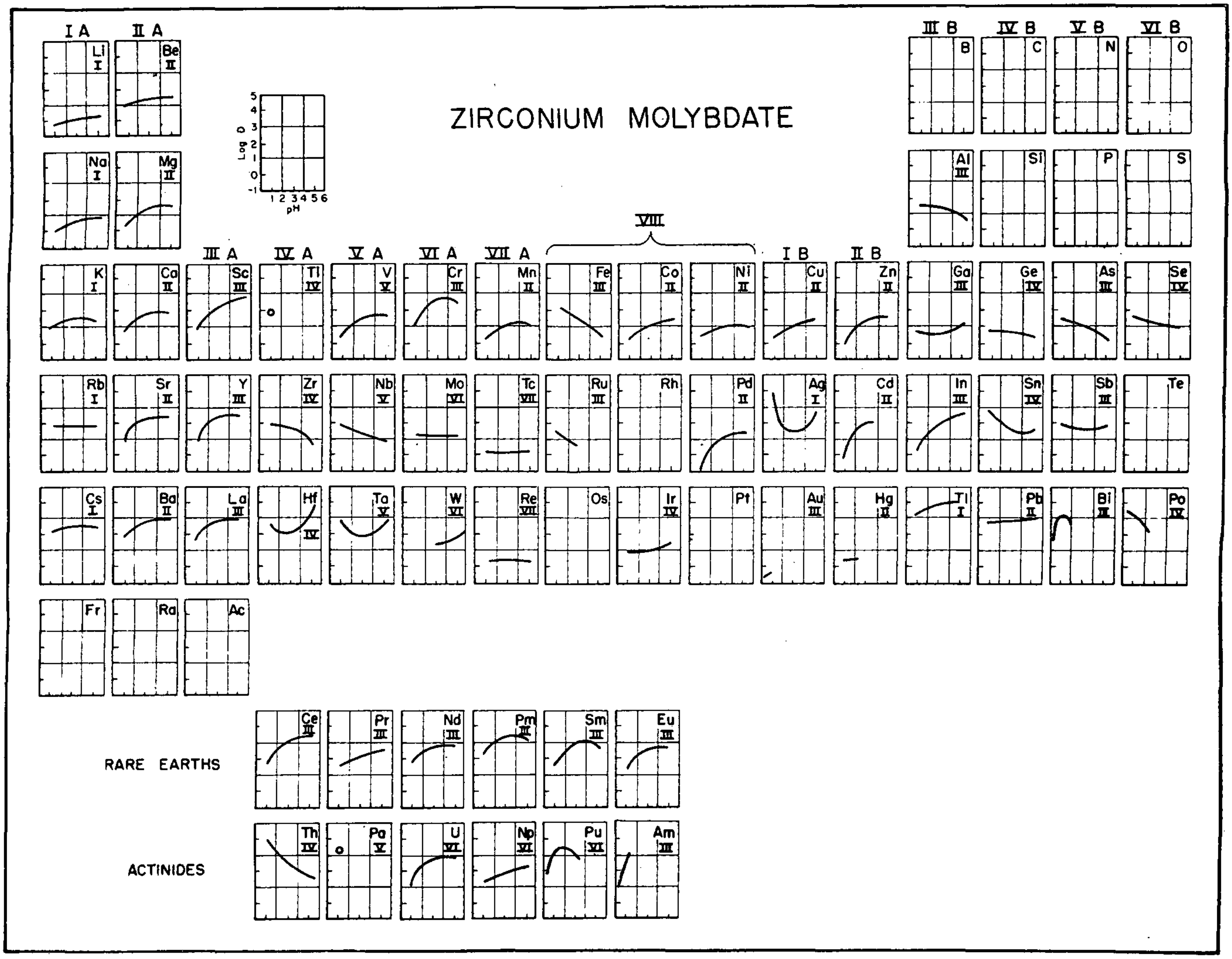

Figure 3. Exchange characteristics of elements on zirconium molybdate

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Zirconium Acid Salt Exchangers from Nitrate Media

Periodic group	Remarks	Potential analytical usefulness
VA	Nb and Ta added from fluoride media. Decreasing *D* values with increasing pH attributed to increasing stability of fluoride complex	
VB, VIB	Increasing *D* values as atomic number increases as expected for increasing metal character. Decreasing *D* value for As with increasing pH attributed to increasing stability of anionic form	Separation of As from Sb on ZP at pH 5. Separate Po from Bi on ZM at pH <1
VIA	Cr(III) adsorption pattern similar to Group IIIA. *D* values of anionic Mo and W are low	Separation of Cr(III) from Fe(III) on ZM at pH 4. Separation of Cr(III) from W and Mo on ZT or ZP at pH 4
VIIA	Positively charged Mn(II) most strongly adsorbed	
VIII	High *D* of Fe on ZP attributed to selective phosphate complexing	Fe separation from Co, Ni, Cu on ZP at pH 5. Ni separation from Pd on ZT at pH <1
Actinides	Decreasing *D* of Pu(VI) with increasing pH attributed to hydrolysis. High *D* of Th and U on ZP attributed to selective phosphate complexing	Th separated from U, Np, Pu on ZM at pH <1

[a] ZP = Zirconium phosphate.
ZT = Zirconium tungstate.
ZM = Zirconium molybdate.

DISCUSSION

The distribution coefficients, as defined under Procedure, for 60 metal ions in nitrate media from pH 1 to 5 are presented in Figures 1 through 4, with one figure for each of the four exchangers. These data represent a total of 720 equilibrations; 4(exchangers) × 3(pH levels of 1, 3, and 5) × 60(metal ions). The pH range of 1 to 5 was selected because below pH 1 the solubility of hydrous zirconium oxide becomes appreciable and above pH 5 hydrolysis reactions of many of the metal ions become competitive.

A nitrate medium was chosen for its minimal complexing action and because nitrate media commonly are encountered in the nuclear energy industry.

Summaries of the exchange characteristics of the elements for the four exchanger materials are presented in Tables I and II. Separations of potential analytical usefulness also are presented in these tables.

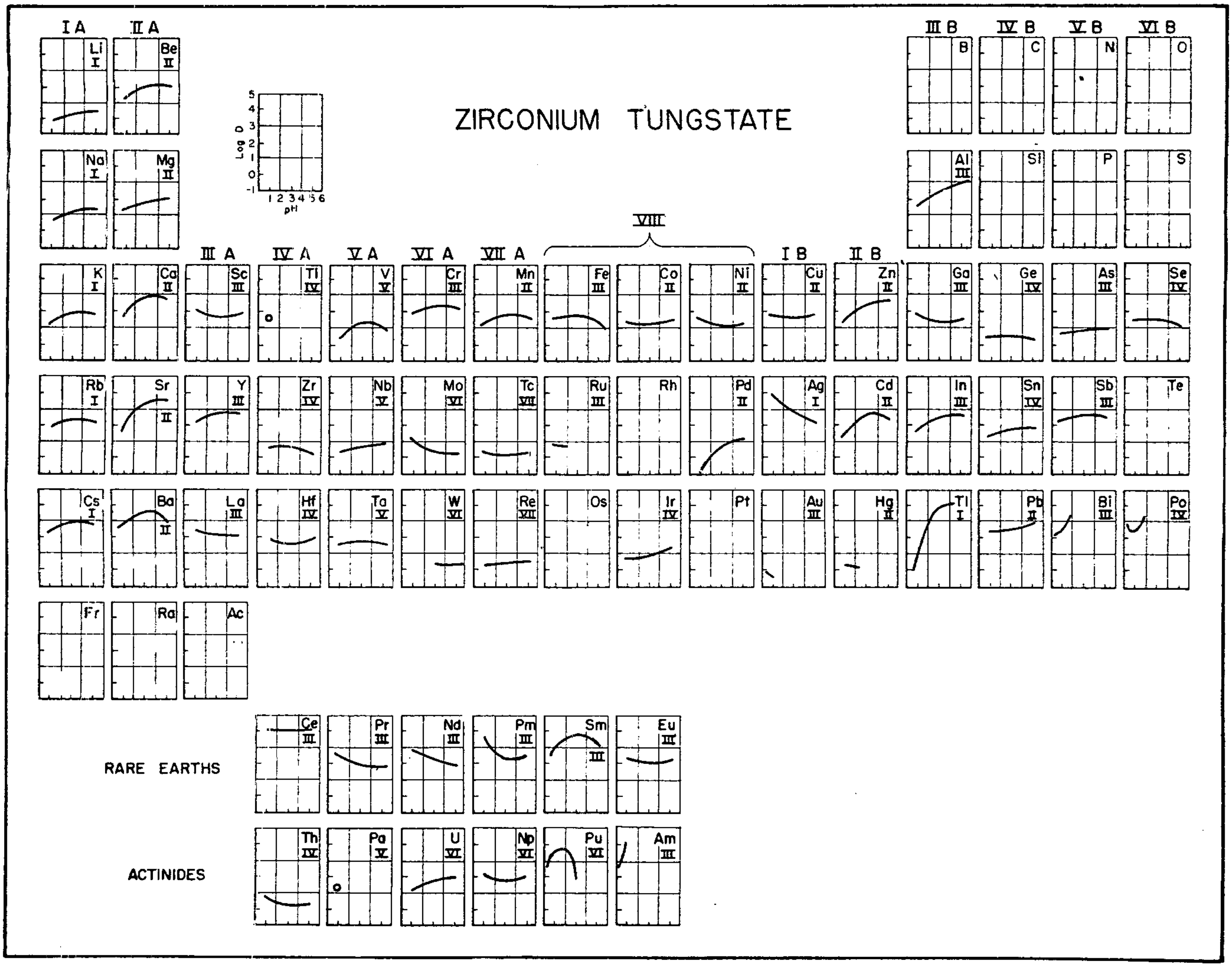

Figure 4. Exchange characteristics of elements on zirconium tungstate

ACKNOWLEDGMENT

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The Application of Stripping Analysis to the Determination of Silver(I) Using Graphite Electrodes

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▶ The extension of stripping analysis to the determination of trace quantities of silver ion has been investigated. The wax-impregnated graphite electrode was applied in these studies instead of the conventional hanging mercury drop electrode, since, in noncomplexing media, silver behaves more nobly than mercury. During the pre-electrolysis step a portion of the silver was electrodeposited on the electrode at constant potential. This step was followed by an anodic linear potential sweep which resulted in the quantitative stripping of the deposited silver from the electrode. The quantity of electricity involved in the anodic peaks is directly proportional to the product of the bulk concentration of silver ion and the pre-electrolysis time over a large range of concentrations. The method was applied to solutions of silver ion as dilute as 4.0 × 10^{-9}M. In addition, the general characteristics of graphite electrodes for further applications to stripping analysis were evaluated.

The technique of stripping analysis is a very sensitive electroanalytical technique. The method consists of two steps. The first is the electrodeposition of a small portion of the unknown electroactive material at a microelectrode under controlled conditions of mass transfer. The second step involves the electrodissolution, or stripping, of the deposit. Analytical correlations are made between the Faradaic signal obtained in the stripping step, the bulk concentration, and the plating time.

The technique has been most useful in the application to the determination of trace quantities of metal ions in solution. In most of these applications the mercury pool electrode (*14*) or hanging drop mercury electrode (*3*, *8*) has been used. These electrodes have been very satisfactory for applications where amalgam formation is involved, provided the stripping step occurs at potentials where the electrode itself is not oxidized.

However, the use of other types of electrodes for stripping analysis has been investigated recently to extend this sensitive method to other electroactive materials. For example, oxidizable electrodes have been applied to the determination of anions which form a deposit upon anodization of the electrode in solution (*1*, *17*). Platinum electrodes have been applied to the determination of substances whose electrodissolution potentials are more anodic than that of mercury, whether this be due to nobility (*10*) or to irreversibility (*12*).

Unfortunately, whenever solid electrodes are used for quantitative work, the usual problems of surface preparation, contamination, and reconditioning become very prominent. It is often difficult to find a means of obtaining reproducible surface conditions for the application of the electrode to a particular system. If a satisfactory electrode conditioning procedure is found, it may be lengthy and inconvenient, and often must precede each run for best reproducibility.

Because of these difficulties, the application of graphite electrodes to electroanalytical chemistry had been investigated previously. The general electrochemical behavior of these electrodes compared favorably to that of platinum and gold (*11*), and refinements in their preparation—e.g., wax impregnation (*4*, *6*)—and application (*5*, *7*) were developed. The outstanding advantage of graphite electrodes, of course, is that a new surface may be exposed for each run, and reproducibility is greatly enhanced by this feature.

This paper describes the application of wax-impregnated graphite electrodes to the determination of silver ion by stripping analysis. The method involved the controlled-potential deposition of metallic silver on the graphite surface from a solution of silver ion. The potential was selected cathodic enough to be in the limiting-current region for the silver reduction, but not so cathodic as to cause significant reduction of the solvent. Stirring and other experimental variables were maintained as constant as possible during this pre-electrolysis step to ensure that a reproducible portion of the silver was deposited during the timed interval.

After the pre-electrolysis step was completed, the deposit was stripped from the electrode by anodic voltammetry with linearly varying potential. One or more oxidation peaks were obtained, and the amount of electricity involved in these peaks gave a measure of the amount of silver deposited and of the bulk concentration. In this manner, it was possible to analyze solutions of silver ion as dilute as 4 × 10^{-9}*M*.

EXPERIMENTAL

Apparatus. The instrument used in this work was the Sargent Model FS Polarograph (E. H. Sargent Co., Chicago, Ill.). This instrument provides linear potential sweeps from 16.7 to 50.0 mv. per second in four equal steps. In addition, the initial potential can be set either by manual adjustment of the slide-wire for the potential scan or by an auxiliary continuously variable low-voltage potential source (+3.0- to −3.0-volt range) incorporated into the instrument.

The working electrode for all experiments was a 12-inch spectroscopic graphite electrode (National Carbon Co., N. Y., No. L4309) prepared for use by immersion in melted paraffin wax (Parowax, U. S. P.) for 60 minutes. With about 4 inches of the electrode left submerged in the liquid, the wax was allowed to cool slowly, and the electrodes were removed when about a 1- to 2-mm. coating had formed about the outside surface. This wax coating acted as an insulation in the voltammetric experiments. The tip was broken off to expose the circular graphite surface, and this was conditioned by sanding, first with medium grade flint sandpaper, and then with fine grade flint sandpaper. The surface was then polished by rubbing on a piece of Whatman No. 1 filter paper on a flat surface until no crevices were plainly visible to the eye. The surface area was approximately 0.32 sq. cm.

The reference electrode was a large saturated calomel electrode connected to the cell through two ultrafine-porosity sintered-glass junctions. Between these two junctions was placed a 2*M* KNO_3 solution. This solution acted as a bridge between the KCl solution of the saturated calomel electrode (S.C.E.) and the silver ion solution in the cell. It was periodically replenished to minimize the chances of cross-contamination by silver and halide ions. The total cell resistance was 300 ohms.

The cell was a 125-ml. capacity poly-

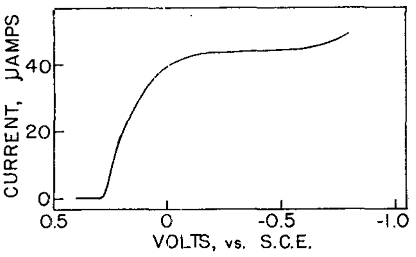

Figure 1. Current-voltage curve for reduction of silver ion in stirred solution $2 \times 10^{-4}M$ Ag^+ in $0.2M$ KNO_3

styrene tumbler on which fit snugly a machined Teflon lid. The lid had various sized holes for the working electrode, salt bridge, and nitrogen inlet (also for a Teflon scoop designed for hanging drops on a hanging drop mercury electrode). In this work the Teflon scoop was used to scrape nitrogen bubbles off the surface of the graphite electrode. To eliminate the photoreduction of silver in these experiments, the cell was shielded from the light by covering it with an opaque material, with provision for periodic observation.

Reproducible stirring was furnished by a Teflon-covered, $^3/_4$-inch magnetic stirring bar and a Sargent synchronous rotator (E. H. Sargent Co., Chicago, Ill.) equipped with a magnet attachment. No attempt was made to control temperature in these experiments.

Materials. All chemicals were reagent grade and were used without further purification. The inert electrolyte for all experiments was $0.2M$ KNO_3. All solutions were prepared in water purified by distillation and passing over a mixed cation-anion exchange resin bed. Silver nitrate was used for stock solutions of silver ion, and these were stored in the dark. Silver solutions as dilute as $10^{-6}M$ could be stored for at least 1 month in preconditioned polyethylene containers without any noticeable changes. Stock solutions were not stored for any longer than 1 month, and solutions of silver ion more dilute than $10^{-6}M$ were prepared as needed. The preparation and use of solutions more dilute than $10^{-6}M$ required the equilibration of all volumetric vessels and the cell assembly with these solutions to minimize concentration changes due to adsorption or desorption at the surfaces.

High-purity nitrogen was bubbled through a gas-washing bottle containing the inert electrolyte solution, and then was dispersed through a coarse-porosity sintered-glass disk in the cell to remove oxygen.

RESULTS AND DISCUSSION

Extensive studies of electrodeposition and stripping of silver deposits at a platinum electrode had been carried out by Rogers and coworkers (*2*, *15*, *16*), and a coulometric method for the determination of silver by electrolytic stripping was developed (*10*). In addition, Lord and Rogers (*11*) have applied the graphite electrode to the voltammetric study of silver reduction.

Thus, the objectives of this study were to investigate the electrochemical behavior of micro deposits of silver at a graphite electrode and to determine whether this electrode could be used for stripping analysis. This involved experiments designed to show that reproducible quantities of silver could be deposited during the pre-electrolysis step, and that the deposit could be removed quantitatively by the subsequent anodic electrolysis (stripping).

Pre-Electrolysis Step. SELECTION OF PRE-ELECTROLYSIS POTENTIAL. To select an electrolysis potential which was sufficiently cathodic to be in the limiting current region of the silver reduction wave and yet not so cathodic as to cause interference from reduction of solvent, current-voltage curves were obtained at the graphite electrode. The conditions were identical to those used later, in the pre-electrolysis procedure—e.g., stirring, electrode, electrolyte, cell, etc. A typical curve is shown in Figure 1.

The effect of dilution on the half-wave potential also was considered in selecting the electrolysis potential. If reversible behavior is assumed for the reaction,

$$Ag^+ + 1e^- \rightleftharpoons Ag$$

and an activity of unity is assigned to the silver deposit, Nernst diffusion theory (*9*) can be used to derive the expression:

$$E_{1/2} = Const. + RT/nF \ln C^*_{Ag^+} \quad (1)$$

where $C^*_{Ag^+}$ is the bulk silver concentration; $E_{1/2}$ is the half-wave potential, and R, T, n, and F have their usual significance. Thus, the half-wave potential is expected to shift approximately 60 mv. cathodic for each 10-fold dilution of silver ion.

At relatively high concentrations ($>10^{-6}M$) the experimental behavior closely followed the above prediction. However, for the more dilute solutions the half-wave potential apparently did not shift cathodic with dilution as rapidly as predicted, since considerably more anodic electrolysis potentials could be used than expected. This behavior is similar to that observed by Rogers and coworkers (*2*, *15*, *16*) who attributed it to activity effects when less than a monolayer was present on the surface. Thus, by trial and error a pre-electrolysis potential of −0.39 volt *vs.* S.C.E. was found to be satisfactory and was used throughout this study.

ELECTRODE PRETREATMENT. The initial preparation of the graphite electrodes was described in the Experimental section. No further pretreatment procedure was followed, except to hold the electrode at a potential of +0.90 volt *vs.* S.C.E. between runs to obtain reproducible stripping results. Furthermore, if the electrodes were not mistreated their characteristics did not deteriorate rapidly with time and use. Numerous determinations were made at the various concentration levels over a period of several weeks without the necessity of renewing the electrode surface. (See Table I.)

Despite the stability of electrode characteristics, the effect of freshly preparing the electrode surface (sanding, polishing, etc.) before each run was investigated also (Table I). The re-

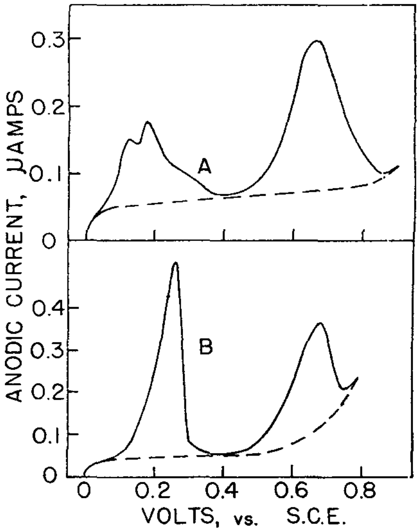

Figure 2. Effect of pretreatment on anodic stripping of silver deposits from graphite electrode

Silver ion concn. was $4.0 \times 10^{-8}M$. Pre-electrolysis time was 15 minutes. Dashed lines are blanks.

A. Without pretreatment.

B. With pretreatment.

Table I. Reproducibility of Stripping Data

Replicate no.	Average quantity of electricity, Q (μcoulombs) A	B	C
1	2.66	2.56	286
2	2.72	2.57	254
3	2.65	2.63	255
4	2.73	2.56	281
5	3.01	2.59	259
6	2.80	2.79	289
Av.	2.71	2.62	271
Rel. std. dev., %	±2.6	±3.4	±6.1

A, B. Stripping analysis results for $4.0 \times 10^{-8}M$ Ag^+ (5-minute pre-electrolysis time). Data in column B were obtained 2 months after those in column A, using same electrode, without resurfacing. The electrode was used many times in between for similar analyses. When not in use, it was stored in deionized water.

C. These data were obtained for a $2.0 \times 10^{-5}M$ Ag^+ solution (1 minute pre-electrolysis), resurfacing the electrode between each run.

producibility was moderately good (±6%) considering the extensive additional operations between runs and the probable variation of the electrode surface with resurfacing. Thus, these experiments showed that the electrode could be useful even in cases where surface deterioration occurred much more rapidly than in this study.

An alternate pretreatment was investigated in an attempt to obtain better-shaped stripping curves at the lower concentrations. The multiple-peaked curves obtained at these concentrations (Figure 2, curve *A*) are not unusual, since similar behavior had been observed by Nicholson (*12*, *13*) in studies of the stripping of micro deposits of nickel from platinum electrodes. In addition, Rogers and coworkers (*2*, *15*, *16*), while studying non-Nernstian behavior of submonolayer silver deposits on a platinum electrode, noticed that a small portion of the silver deposit adhered so strongly to the electrode surface that it could be removed only by anodic electrolysis in cyanide solution. Both investigators concluded that the activities of these deposits could vary with the extent of surface coverage, and this could cause the observed non-Nernstian behavior. Moreover, they concluded that bonding forces between the electrode and the deposit could be stronger than those between the atoms of the deposit, and that some portions of the deposit might be bonded quite firmly to the electrode surface. These phenomena could cause the multiple-peaked stripping curves observed in Nicholson's work and also in this work.

On the assumption that problems similar to those encountered by Rogers and coworkers were causing the anomalous stripping curves at low concentrations in this study, the graphite electrode was pretreated before each of a series of runs by anodic electrolysis in concentrated ammonium hydroxide using 0.1*M* KNO_3 as the supporting electrolyte. The electrolysis was carried out simply by imposing 0.6 volt between the working electrode and a graphite counter electrode for 1 minute.

When electrodes pretreated in this fashion were applied to 4 × $10^{-8}M$ silver solutions, well defined double-peaked stripping curves were obtained. The shapes and sizes of these curves were reproducible when the pretreatment was performed before each run. Figure 2 compares these curves to those obtained without the pretreatment. The total quantity of stripping coulombs is nearly identical in both cases, but residual currents were slightly different.

It was decided not to use this pretreatment in the analytical method, since the overall results were not changed, and the omission of time-consuming steps was highly desirable. However, further studies of the effects of the various experimental parameters on the shape of the stripping curves were carried out. No consistent improvement could be obtained by changing the pre-electrolysis time or potential. Furthermore, the shapes of the curves might vary somewhat as a function of the aging of the electrode (observed, primarily, as a variation in the relative sizes of the various peaks). This property of the system was not critical, however, since the total stripping coulombs always remained approximately constant (within experimental error). Furthermore, the electrode could always be returned to its original state by resurfacing, although this was rarely, if ever, necessary.

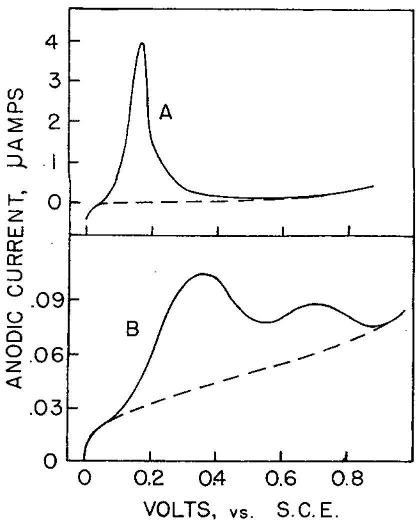

Figure 3. Anodic stripping of silver from graphite electrode

Dashed lines are blanks. Concentrations and pre-electrolysis times were:
A. 2.0 × $10^{-6}M$ Ag^+, 1 minute
B. 4.0 × $10^{-9}M$ Ag^+, 15 minutes

PRE-ELECTROLYSIS PROCEDURE. The cell and electrode assembly were equilibrated with aliquots of the sample solution before a fresh portion of the solution was added for analysis. Dissolved oxygen was removed by bubbling nitrogen through the solution for 10 to 15 minutes. Nitrogen was passed over the surface of the solution during the rest of the experiment, and was bubbled through the solution for 1 minute between replicate runs. The graphite electrode was held at +0.90 volt *vs.* S.C.E. for a total of 2 minutes between runs. Before the pre-electrolysis potential was applied the surface of the electrode was gently scraped with the Teflon scoop to remove the adherent nitrogen bubbles. The electrolysis potential of −0.39 volt *vs.* S.C.E. was then applied to the working electrode for an accurately measured period of time. The length of the pre-electrolysis step varied from 1 to 15 minutes, depending on the bulk concentration. Reproducible stirring effects were obtained by maintaining the cell arrangement and geometry as constant as possible for a series of runs. The pre-electrolysis step was terminated by opening the cell circuit.

Anodic Stripping Step. After completing the pre-electrolysis step, 30 seconds were allowed to pass so that the solution came to rest. The unapplied electrode potential was changed to a value at the foot of the wave; the cell circuit was closed again, and the deposit was removed from the electrode by an anodic potential sweep. The height of the anodic peak(s) obtained is not linearly dependent upon the size of the deposit because of the variation of the activity during the stripping process (*13*). Therefore, the relationship between the number of coulombs of electricity involved in the stripping peak(s), the plating time, and the bulk concentration was investigated. This approach was used successfully for quantitative measurements in previous studies of electrolytic stripping of deposits (*10*, *12*, *13*, *17*). The area under the peak(s) can be determined with a planimeter and readily converted to coulombs.

The selection of a rate of anodic voltage scan was not critical. However, better reproducibility was obtained and no sacrifice of sensitivity resulted when the slower rates of scan were used. Thus, a scan rate of 16.7 mv. per second was used for all runs except at the most

Table II. Anodic Stripping Analysis of Silver Ion[a]

Concn., *C* (moles/liter)	Pre-electrolysis time, *t* (min.)	Average quantity of electricity, *Q* (μcoulombs)	*Q/Ct* × 10^{-6}	Rel. std. dev., %
2.0 × 10^{-5}	1.0	262	13.1	±3.4
2.0 × 10^{-6}	2.0	48.8	12.2	±2.7
2.0 × 10^{-7}	5.0	11.8	11.8	±3.4
4.0 × 10^{-8}	5.0	2.71	13.5	±2.6
4.0 × 10^{-9}	15.0	0.79	13.1	±4.2

[a] Data refer to six replicate determinations at each concentration.

dilute concentrations. At $4 \times 10^{-9}M$ silver ion, the stripping scan rate was increased to 33.3 mv. per second, and sharper peaks were obtained. Thus, sensitivity was increased, and correspondingly shorter electrolysis times could be used.

Typical stripping current-voltage curves are shown in Figure 3. The residual current line was determined by carrying out the pre-electrolysis and stripping procedure on blank solutions. The analytical results are summarized in Table II. The dependence of the stripping coulombs on the product of bulk concentration and electrolysis time is linear over a wide range. The relative error is not as small as would be desired (±4.7%), but this is due probably to the difficulty in preparing and handling the very dilute solutions. The reproducibility, however, is much better, as pointed out in Table II.

The correlation between the total stripping coulombs and that predicted from the convection-controlled cathodic limiting currents is very good. For example, in $2 \times 10^{-5}M$ silver solution, the limiting cathodic current was 4.2 μa. For a 60-second pre-electrolysis, then, a silver deposit corresponding to 252 μcoulombs should be formed; the stripping results show 262 μcoulombs of electricity. This is further evidence that approximately all of the deposited silver is removed by the subsequent stripping procedure.

An entirely unexpected advantage of the graphite electrode found in this study is that the residual currents are not significantly greater than those observed under similar conditions with the hanging drop mercury electrode (*3*). This is despite the fact that the surface area (0.32 sq. cm.) of the graphite electrode used in these studies is about 6 times as large as that of a typical hanging drop electrode (*ca.* 0.05 sq. cm.). Thus, the ratio of the Faradaic signal to the residual signal is greater, and sensitivity is increased by a factor of about 6. This is obvious from the fact that only 15 minutes' plating time was required to obtain the stripping data for $4 \times 10^{-9}M$ silver ion. Moreover, when the pre-electrolysis was carried out for only 5 minutes, sensitivity was not found lacking, but the results were less precise.

Thus, it is reasonable to speculate that this method might be applicable to the determination of silver ion somewhat more dilute than $4 \times 10^{-9}M$, perhaps as low as $10^{-10}M$. No attempts were made to study such dilute solutions in this work, since the preparation and handling of these solutions is considerably more difficult and time consuming.

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Polarographic Determination of Nitrite as 4-Nitroso-2,6-xylenol

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▶ Phenols react with nitrosonium ion under mild conditions to produce the corresponding nitrosophenols. In 5:4:1 (volume ratio) sulfuric acid–water–acetic acid, 2,6-xylenol reacts with nitrite to produce 4-nitroso-2,6-xylenol. The reaction is rapid, reproducible, and nearly quantitative. The product is polarographically reducible with a single diffusion-controlled wave which merges with the anodic dissolution of mercury at 0.0 volt *vs.* the mercury–mercurous sulfate reference electrode. The diffusion current at −0.15 volt *vs.* the reference electrode is linearly concentration dependent in the range 0 to 14 p.p.m. nitrite in the reaction mixture. Determinations may be made directly on the sample within 10 minutes. Side reactions, one of which produces 3,3′,5,5′-tetramethylindophenol limits the yield to approximately 92%. Nitrate constitutes an interference since it forms the corresponding 4-nitro-2,6-xylenol which is also polarographically reducible. However, the appropriate potentials for diffusion current measurement of the nitroso- and nitroxylenols are sufficiently separated that a 100-fold excess of the latter can be tolerated without interference. The overall reproducibility of the method is less than 1% (expressed as relative standard deviation).

NITRATE AND NITRITE react reproducibly with 2,6-xylenol to produce 4-nitro-2,6-xylenol and 4-nitroso-2,6-xylenol, respectively (*1*). These reactions have been utilized in spectrophotometric procedures for nitrate and/or nitrite (*7*) and for polarographic determination of nitrate (*8*). The characteristics of spectrophotometric measurement compared with polarographic measurement are sufficiently different with respect to interferences, simplicity of procedure, and the like that development of a complimentary polarographic procedure for nitrite would be useful.

EXPERIMENTAL

Apparatus and Reagents. Polarographic apparatus and techniques were essentially those previously reported by Hartley and Curran (*8*). Reagents and solutions were prepared according to the directions reported for the spectrophotometric methods (*7*). The Hg/Hg_2SO_4 reference electrode was prepared in the 5:4:1 (v./v.) $H_2SO_4:H_2O:HOAc$ solvent determined to be the optimum composition of reaction solvent. The dropping mercury electrode was of the common stand-tube

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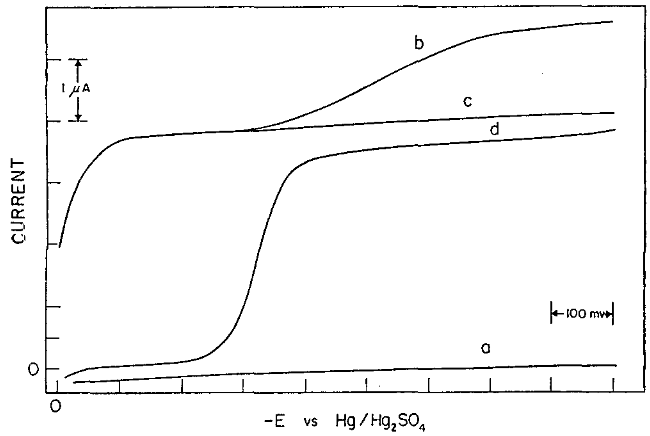


Figure 1. Polarograms of (a) residual current of 5:4:1 v./v. sulfuric acid–water–acetic acid electrolyte, (b) 7.40 × $10^{-4}M$ 4-nitroso-2,6-xylenol in air-saturated solution, (c) 7.40 × $10^{-4}M$ 4-nitroso-2,6-xylenol, and (d) 5.03 × $10^{-4}M$ 4-nitro-2,6-xylenol (concn. based on nitrate taken)

Current axis for curves (b) and (c) has been shifted by 0.50 μa.

leveling bulb construction and had capillary characteristics of $t = 3.180$ seconds at 50-cm. height of mercury and −0.15 volt *vs.* the Hg/Hg_2SO_4 reference electrode and $m = 2.153$ mg. per second.

Procedure. A. PREPARATION OF WORKING CURVE. Prepare aqueous samples of sodium nitrite in the range 5×10^{-4} to $10^{-2}M$. To a 100-ml. electrolysis beaker add 40 ml. of a 5:3 H_2SO_4:H_2O mixture (prepared by adding 5 volumes of concentrated acid to 3 volumes of water). To this solution add, with good stirring, 5.00 ml. of aqueous standard nitrite solution and 5.00 ml. of $0.1M$ 2,6-xylenol in glacial acetic acid in that order. Place the beaker in a water bath maintained at 25° C. and stir until the bath temperature is obtained in the beaker. Record the diffusion current at −0.15 volt *vs.* the Hg/Hg_2SO_4 reference electrode. Record the residual current of a blank solution prepared by destroying the nitrite in an aqueous sample by adding 1.0 gram of sulfamic acid and proceeding as above. The slope and intercept of the working curve are slightly dependent upon reaction temperature, temperature of the polarographic solution, order of reagent addition, and the time sequence of operations. However, for a fixed set of these conditions the curve is linear. If nitrate is present in equivalent or greater amount it is an interference and the procedure given below must be used.

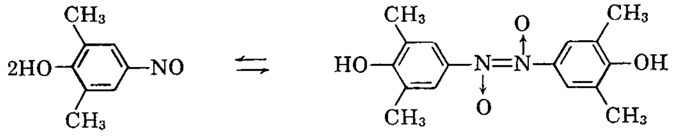
B. PROCEDURE FOR SAMPLE CONTAINING NITRATE. The reaction conditions given above produce the nitrosoxylenol in an amount greater than can be accounted for on the basis of nitrite present if nitrate is also present in the sample. As shown in the text this error can be several-fold if nitrate is in overwhelming excess. If the samples are such that the nitrate concentration can be regarded as constant, the resulting nonlinear working curve can be used provided the nitrate concentration is known. Nitrate may be determined conveniently by the procedure of Hartley and Curran (*8*). Chloride, an interference in the nitration but not the nitrosation reaction, can be removed by the Hg_2SO_4 metathesis procedure given in (*8*).

Divide an aqueous sample into three portions. To one add 1.0 gram of sulfamic acid and proceed with the determination of nitrate. To a second portion add an amount of stock nitrite solution estimated to approximate the nitrite originally present. Determine the diffusion current of this and the third portion according to the procedure given in (A). The original nitrite concentration may be found from the data by the usual calculations of standard addition procedures.

RESULTS AND DISCUSSION

A. Polarographic Characteristics of 4-Nitroso-2,6-Xylenol. The reaction of nitrite with 2,6-xylenol has been shown by Asai (*1*) to yield 4-nitroso-2,6-xylenol as the major product with 3,3′,5,5′ - tetramethyldiphenoquinone and 3,3′,5,5′-tetramethylindophenol as side or consecutive products dependent upon acidity. Polarographic behavior was determined by preparing determinate solutions of the pure compound in the mixed solvent, 5:4:1 H_2SO_4:H_2O:HOAc, which had been determined by Hartley and Asai (*7*) to be optimum with respect to minimal side reactions. The ternary ratios reported throughout this work represent the ratios of original volumes taken and do not refer to the final composition. In this solvent 4-nitroso-2,6-xylenol produces a single diffusion-controlled wave which merges with the anodic dissolution of mercury at 0.0 volt *vs.* the mercurous sulfate reference electrode used throughout this work (Figure 1). The potential of this electrode at 25° C. is +0.450 volt *vs.* the hydrogen electrode when measured in the cell: Pt/H_2 1 atm, 5:4:1 H_2SO_4:H_2O:HOAc, Hg_2SO_4(sat.)/Hg (*10*). Logarithmic analysis of the current-voltage curve in the region of 0.0 volt yields an extrapolated half-wave potential of +0.05 volt. At the rotating platinum electrode (600 r.p.m.) the wave is asymmetric and drawn out over a 200-mv. potential range with a half-wave potential of −0.06 volt. The potential of −0.15 volt was chosen for analysis as the optimum point between the anodic oxidation of mercury and the reduction of oxygen. At this potential the current due to the reduction of oxygen in air-saturated solutions is, for most cases, negligibly small and constant; the half-wave potential for oxygen in this system is −0.60 volt. Hartley and Curran have found the half-wave potential for the 4-nitro-2,6-xylenol in 6:3:1 H_2SO_4:H_2O:HOAc to be −0.27 volt *vs.* the Hg/Hg_2SO_4 electrode prepared in the same solvent; in the present solvent the value is −0.32 volt and the current due to reduction of this compound at −0.15 volt is negligible. All data reported in the present work were obtained in air-saturated solutions.

The diffusion current at −0.15 volt is linearly concentration-dependent up to a concentration of $5 \times 10^{-4}M$ 4-nitroso-2,6-xylenol. A plot of diffusion current (measured at maximum pen deflection with a 1-second recorder) when corrected for a small oxygen contribution in this range has a slope of 5.375 μa. per mM per liter and zero intercept. Above $5 \times 10^{-4}M$ the slope decreases; at $10^{-3}M$ the current is 4% less than expected from the initial linear slope. The decrease is believed to be due to the formation of a dimer (*6*) according to the equation:



The diffusion current constant, $(i_d)_{max}/Cm^{2/3}t^{1/6}$, for 4-nitroso-2,6-xylenol in 6:3:1 H_2SO_4:H_2O:HOAc at −0.60 volt is 1.60. Curran obtained a value of 3.24 for the corresponding nitro compound in the same solvent. Bergman and James (*4*) studies of controlled potential electrolysis of nitro compounds in H_2SO_4 solvent and HOAc solvent identify the process as: $ArNO_2 + 4H^+ + 4e = ArNHOH$. Gowen-

Table I. Effect of Solvent Composition on Nitrosation Yield

Solvent composition (v./v. H_2SO_4:H_2O: HOAc)	Yield[a]	
	Polarographic	Spectrophotometric
6:3:1	83.0	81.9
5:4:1	92.8	82.7
9:9:2	85.7	77.2

[a] Results calcd. on basis of diffusion current constant or molar absorptivity of 4-nitroso-2,6-xylenol in each solvent mixture.

lock and Luttke (*6*) state that the polarographic reduction of nitroso compounds proceeds according to: $ArNO + 2H^+ + 2e = ArNHOH$. The positive half-wave potential of the nitroso compound in the present solvent precludes a test of reversibility, but the ratio of the above diffusion current constants, 2.02, supports an inference of a 4-electron reduction of the nitro compound and a 2-electron reduction of the nitroso compound. The diffusion current constant for the nitroso compound in 5:4:1 solvent is 2.66 at −0.15 volt. If "*n*" is taken to be 2 the simple Ilkovic equation yields a diffusion coefficient of 4.0×10^{-6} cm.^{2} per second. The temperature coefficient of the diffusion current in the range 20° to 40° C. is +2.1% per degree which is slightly less than that reported for the nitro compound (*8*). The difference is believed to be due to the difference in viscosity-temperature behavior between 5:4:1 and 6:3:1 solvents.

B. Reaction of Nitrite with 2,6-Xylenol. Asai has made an extensive study of the reaction of 2,6-xylenol with nitrate and nitrite (*1*). He has shown that the reaction with nitrite yields at least one side product at all acidities. In solutions of low acidity—e.g, 10% aqueous acetic acid—nitrite reacts oxidatively with the reagent to produce 3,3′,5,5′-tetramethyldiphenoquinone in addition to the major product, the 4-nitroso-2,6-xylenol. At higher acidities such as 6:3:1 H_2SO_4:H_2O:HOAc oxidation is minimal but the nitroso compound condenses with excess xylenol to produce the 3,3′,5,5′-tetramethylindophenol. The oxidation reaction leads to loss of nitrite proportional to $(NO_2^-)^{1/2}$ at constant xylenol concentration (*9*). The nitrosation reaction rate is linear with nitrite concentration while the oxidation rate is proportional to $(NO_2^-)^{1/2}$. The rate constant for the oxidation reaction is approximately 10% of that for the nitrosation reaction. Hence the loss of nitrite at constant xylenol concentration is relatively independent of the nitrite concentration and cannot be more than minimized. Asai found the optimum solvent to be 5:4:1.

The relative rates for these two reactions are reflected in Asai's findings that the order of addition of sample and reagent was significant. If nitrite was added to the solution after the addition of the reagent the spectrophotometric results were lower than for the reverse procedure and of poorer precision. Preliminary polarographic results confirmed these observations and all experiments cited in this work were performed by adding the reagent last.

EFFECT OF ACIDITY. Asai's studies of the effect of solvent composition on the rate and yield of the reaction showed that the figure of merit for the solvents H_2SO_4:H_2O:HOAc and H_2SO_4:H_3PO_4:H_2O:HOAc was the acidity as measured by Hammett's acidity function, H_0. When these solvents were composed so as to have the same nitration rates and yields, the acidities as measured with the indicator, 2,4-dinitroaniline, were closely similar.

The nitrosation reaction is affected by acidity in three ways: nitrosation rate which is proportional to $-H_0$; decomposition of nitrite which is maximal in the 5:4:1 solvent (*2*); and loss of product by condensation with excess reagent which is proportional to H_0 (*10*). The yield of nitrosoxylenol was determined polarographically in three solvent mixtures in the region of optimum acidity reported by Hartley and Asai: 6:3:1, 5:4:1, and 9:9:2 H_2SO_4:H_2O:HOAc. The data shown in Table I were obtained according to the directions given in the Procedure section with a constant recording time of 5 minutes after the addition of the reagent. Spectrophotometric results for the same conditions are included from the work of Hartley and Asai for comparison. In agreement with the more extensive studies reported by Asai (*1*) the yield passes through a maximum at about the 5:4:1 ratio solvent. The slightly higher yields found for the polarographic procedure are due in part to a somewhat easier and more precise volumetric technique and to a fundamental difference in the effect of side reactions which is discussed in a later section of this article.

EFFECT OF TEMPERATURE. Asai (*1*) reported an apparent effect of temperature on the nitrosation yield as measured spectrophotometrically. Because the spectrophotometric technique cannot be carried out rapidly enough to follow the reaction in its initial stages it was impossible to determine whether the effect resulted from loss of nitrite or from a change in the reaction per se. The polarographic procedure as presently developed is capable of following the reaction within seconds of addition of reagent and thus allows a test of the alternatives.

Table II. Effect of Temperature on Stability of Nitrite in 5:4:1 H_2SO_4:H_2O:HOAc

T., ° C.	Yield, %[a]	Rel. std. dev.[b]
16.0	89.0	0.32
45.7	89.0	0.26
55.0	88.5	0.32
64.9	87.2	0.37
74.1	83.7	0.60
83.6	79.6	2.54

[a] Based on diffusion current of 4-nitroso-2,6-xylenol equivalent to nitrite added ($2.02 \times 10^{-3}M$).

[b] Calcd. from triplicate measurements.

Forty milliliters of a 5:3 H_2SO_4:H_2O solution was heated to a desired temperature in the range 20° to 40° C. and 5.00 ml. of an aqueous nitrite sample was added. This solution was maintained at the temperature for 1 minute and then cooled rapidly in an ice bath. When the solution temperature reached 25° C., 5.00 ml. of the stock 0.1*M* reagent was added. The solution temperature was readjusted to 25° C. and the diffusion current at −0.15 volt measured after 5 minutes' reaction. The results, given in Table II, are the averages of three or more replicates at each temperature.

The effect of temperature on the reaction itself was determined by a set of experiments in which the solutions were maintained at slightly elevated temperatures until the reaction was complete. Forty-milliliter portions of the 5:3 solvent were heated to a temperature between 10° and 40° C. such that the subsequent addition of aqueous sample would not increase the temperature to more than 45° C. Five milliliters of stock nitrite in water was added to each and the resulting solution cooled to a specified temperature between 20° and 40° C. When the desired temperature had been reached, 5.00 ml. of the reagent solution was added. Since the heat of dilution had been largely dissipated in the initial dilution the addition of the acetic acid did not appreciably change the temperature. After 5 minutes' reaction time the solution was cooled (or heated) to 25° C. and the diffusion current was measured. Triplicate determinations in this manner gave identical average maximum currents at reaction temperatures of 20.0, 30.4, and 40.0° C.

The first two entries of Table II indicate no detectable loss of nitrite provided the temperature of the reaction solution does not exceed 45° C. Since the second set of experiments was so designed that the maximum temperature was always less than this value, the latter results show that the procedure can be carried out at any temperature less than 45° C. without significant loss. The negligible effect of moderate temperatures on the reaction itself indicated that the reaction is not a primary source of low yields and the major effect is decomposition or loss of nitrite before reaction can take place.

EFFECT OF SAMPLE SIZE. The foregoing results are applicable only to samples containing nitrite in sufficiently high concentrations that a tenfold dilution with respect to final composition can be tolerated. In these experiments a 2.02 × $10^{-3}M$ stock solution of nitrite was used. When 5.00 ml. of this solution is added to 40.00 ml. of the 5:3 acid mixture and 5.00 ml. of the reagent solution the final composition is 5:4:1 H_2SO_4:H_2O:HOAc and about 1.8 × $10^{-4}M$ in 4-nitroso-2,6-xylenol which produces a diffusion current of about 1 μa. However, if samples are more dilute in nitrite a large fraction of the water in the final solution must come from the aqueous sample with a resulting increased heat of mixing with a more concentrated acid. As shown in Table II such extremes of temperature resulting from heat of dilution prior to reaction cannot be tolerated. This effect of sample size dictated by concentration is shown in Table III. In these experiments a series of nitrite samples in water was prepared in the concentrations given in column 1. The H_2SO_4:H_2O ratio of the initial solvent was adjusted such that when 5.00 ml. of the reagent and the sample volumes given in column 2 were added the final solution was 50 ml. of 5:4:1 H_2SO_4:H_2O:HOAc. The temperatures in column 3 are those of the solution after addition of the sample to the acid. Four series were run at final concentrations expressed as nitrite of 0.04, 0.20, 0.40, and 0.80 mM.

Table III. Effect of Sample Size on Nitrosation Yield[a]

Nitrite taken (mM)	Sample size (ml.)	Maximum temp. of solution (° C.)	Diffusion current at −0.15 volt (μa.)	Yield, %
0.100	20	94–97	0.181	74.9
0.499	4	31–33	0.187	87.0
1.00	2	27–29	0.186	86.5
2.00	1	26–27	0.188	87.4
3.99	0.5	25–26	0.182	84.7
0.499	20	93–96	0.921	85.7
1.00	10	48–51	0.980	91.2
2.00	5	34–36	0.989	92.0
3.99	2.5	29–30	0.989	92.0
1.00	20	95–97	1.857	86.4
2.00	10	50–51	2.009	93.4
3.99	5	35–37	2.031	94.5
2.00	20	94–98	3.738	89.8
3.99	10	51–52	4.098	98.5

[a] Constant final volume and composition: 50 ml. of 5:4:1 H_2SO_4:H_2O:HOAc.

Table IV. Reproducibility of Nitrosation in Cooled 5:4:1 v./v. H_2SO_4:H_2O:HOAc

Nitrite taken (mM)	Diffusion current[a,d] at −0.15 volt (μa.)	Yield, %[b]	Rel. std. dev.[c]
0.050	0.029 ± 0.001	107.4	1.44
0.100	0.052 ± 0.001	96.3	0.98
0.401	0.197 ± 0.002	92.1	1.02
0.701	0.348 ± 0.002	92.3	0.58
1.00	0.499 ± 0.001	92.8	0.19
2.01	0.943 ± 0.006	87.3	0.64
4.01	1.946 ± 0.014	90.3	0.69
6.02	2.828 ± 0.026	89.3	0.92
8.02	3.912 ± 0.029	93.9	0.74
10.00	4.824 ± 0.025	93.4	0.51

[a] Corrected for zero intercept (0.020 μa.) of i_d (max) *vs.* C curve.
[b] Yields calcd. as in Table I.
[c] Calcd. from triplicate measurements.
[d] Variation in current expressed as average deviation.

Table V. Reproducibility of Nitrosation in Uncooled 5:4:1 v./v. H_2SO_4:H_2O:HOAc

Nitrite taken (mM)	Diffusion current[a,d] at −0.15 volt (μa.)	Yield, %[b]	Rel. std. dev.
0.050	0.021 ± 0.001	77.8	2.22[c]
1.00	0.496 ± 0.001	92.2	0.14
6.02	2.893 ± 0.009	91.3	0.32[c]
8.02	3.824 ± 0.020	91.8	0.53
10.00	4.858 ± 0.022	94.1	0.44

[a] Corrected for zero intercept (0.023 μa.) of i_d (max) *vs.* C curve.
[b] Yields calcd. as in Table I.
[c] Calcd. from duplicate measurements; all others are from triplicate measurements.
[d] Variation in current expressed as average deviation.

The results of these experiments show that if a significant (about 50%) fraction of the water in the final solution can be added prior to adding the sample the effect of heat is removed and yields are significantly higher. The results of experiments 2 to 5 are somewhat low because of difficulties in measuring the small volumes of sample used and the correspondingly low diffusion currents; the precision of these data is, as expected, the least of the set. The yields for the first member of each series are somewhat low because of the method of solution preparation and the method of calculation. In each case the solutions were prepared by addition of known volumes of each solution without regard for final volume. Since these solvents are not ideal the concentration of nitrite is dependent upon solvent composition. The first member of each series is somewhat more dilute than the rest because this nonideality of solution has been removed in succeeding experiments by the prior dilution with water. The yields were calculated using a reference solution of 4-nitroso-2,6-xylenol prepared determinately from the pure compound to correspond to 100% yield based on the nitrite taken. This solution was prepared with 5.00 ml. of aqueous nitrosoxylenol solution, 5.00 ml. of acetic acid and 40.00 ml. of 5:3 H_2SO_4:H_2O. In comparison, the solutions of experiments 2, 8, and 12 are most similar to the reference solution in this respect.

REPRODUCIBILITY OF NITROSATION. When nitrite is determined by this method using the directions given in the Procedure, results typified by those in Table IV are obtained. If the procedure is modified to omit all temperature control prior to final adjustment for polarographic measurement, the results of Table V are typical. A regression analysis of these data yields a "least squares" equation, $i_d = 0.480C + 0.020$ for the first procedure and $i_d = 0.482C + 0.023$ for the second. The residual term in each equation represents the small contribution of oxygen to the residual current at −0.15 volt. The precision of the method (average

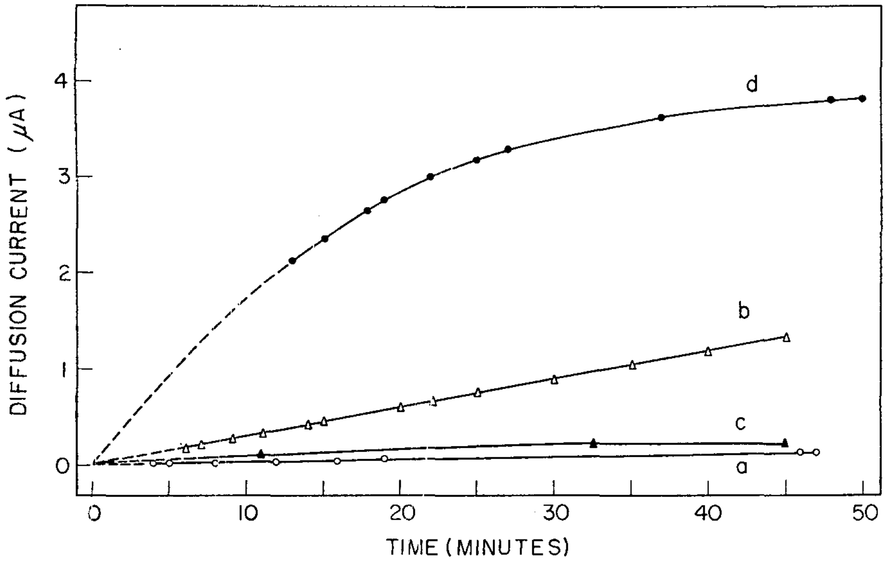


Figure 2. Diffusion current vs. time for nitration in 9:9:2 v./v. H_2SO_4:H_2O:HOAc (O) at −0.15 volt, and (Δ) at −0.60 volt; and in 5:4:1 v./v. H_2SO_4:H_2O:HOAc (▲) at −0.15 volt, and (●) at −0.60 volt

Nitrate concn. is $5.03 \times 10^{-4}M$ in solvent mixture

(relative standard deviation = 0.70) is sufficiently small that the temperature coefficient of the diffusion current must be taken into account. In the 5:4:1 solvent the temperature coefficient is +2.1% per degree in the interval 20° to 30° C. Constant temperature control to ±0.1° C. is desirable.

EFFECT OF NITRATE. It has been shown previously that nitrate reacts with 2,6-xylenol to produce the polarographically (*8*) or spectrophotometrically (*7*) measurable 4-nitro-2,6-xylenol in these mixed acid solvents. The acidity requirements for nitration are greater than for the nitrosation; in the 5:4:1 solvent nitrosation is essentially complete in 2 minutes whereas the nitration reaction is scarcely 5% complete in 5 minutes. Asai (*1*) showed that it was possible to determine both nitrate and nitrite in the same sample simultaneously albeit with less precision than for either separately. In this work Asai observed the curious effect that the presence of small quantities (less than 1% relative to the nitrite) of nitrate improved the yield and precision of the nitrosation.

The possiblity of simultaneous polarographic determination of nitrate and nitrite is attractive because, in contrast to the spectrophotometric characteristics, the polarographic waves for the two products do not overlap. In the 5:4:1 solvent the nitrosoxylenol shows only a diffusion-limited current plateau at 0.0 volt whereas the corresponding nitro compound has a half-wave potential of −0.27 volt *vs.* the Hg/Hg_2SO_4 reference electrode. In solutions containing both compounds it has been found (cf. Figure 1) that for ratios of nitroxylenol to nitrosoxylenol of less than 100:1 the diffusion current at −0.15 volt due to the latter is not affected by the presence of the nitroxylenol.

In the earlier work the ratio of nitrate to nitrite was not investigated beyond approximately equimolar amounts. Since the polarographic characteristics allow a much higher ratio the effect of these higher ratios was studied with the object of developing a method for samples which contain both nitrate and nitrite with the former in much larger concentration.

The magnitude of diffusion currents obtained for nitration in the 5:4:1 solvent is shown in Figure 2. Identical solutions with respect to nitrate were prepared in this solvent and in 9:9:2 solvent, which had been previously shown by Asai to be satisfactory for nitrosation although the rate of reaction was somewhat slower and in which nitration is much slower. Diffusion currents were measured at −0.15 volt, the analytical potential for the nitroso compound and at −0.60 volt, the potential on the diffusion plateau for the nitro compound used previously by Hartley and Curran (*8*). Inspection of the early portions of curves *d* and *b* of Figure 2 indicates that for reaction times of less than 15 minutes (compared to 3–5 minutes needed for nitrosation) the contribution of nitrate is less than 0.1 μa. for $10^{-4}M$ solutions. A nitrite solution of the same concentration yields a diffusion current of 0.96 μa. at this potential.

Preliminary work using the nitrate-nitrite ratios used by Asai (*1*) failed to substantiate the reported favorable effect of added nitrate. Repetition of the spectral investigation using the exact reported procedures and concentrations also revealed no effect other than a deleterious one. We now conclude that the previous observations must have been due to slightly longer cooling periods, necessitated by the additional pipetting, and to an increased manipulative skill since these experiments were carried out near the end of the spectrophotometric investigation.

If the ratio of nitrate to nitrite is increased beyond equimolar proportions, the current at −0.15 volt increases beyond that calculated for the amount of nitrite present. This "exaltation" effect can be as large as 16% of the expected current as shown in Figure 3. In the experiments shown in Figure 3

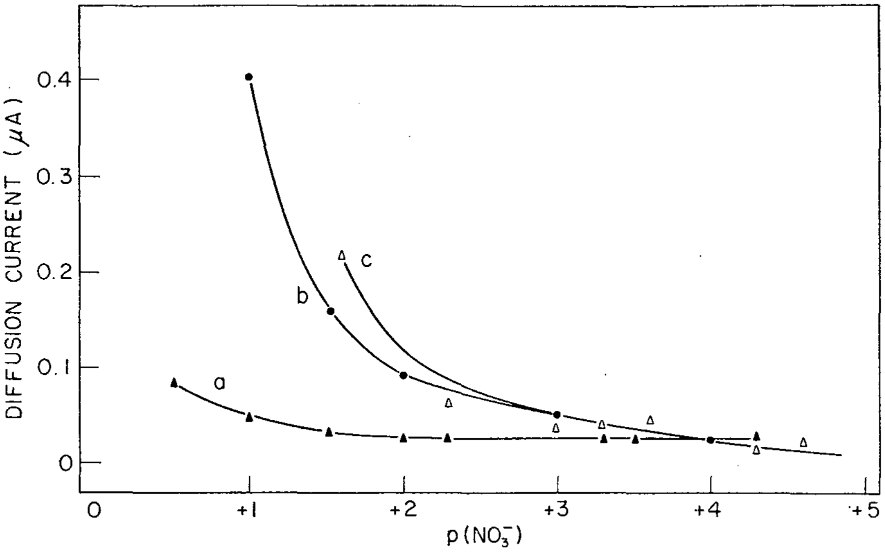


Figure 3. Diffusion current at −0.15 volt vs. p(NO_3^-) for (Δ) pure nitrate samples, (●) $3 \times 10^{-3}M$ nitrite samples, and (▲) $5 \times 10^{-3}M$ nitrite samples

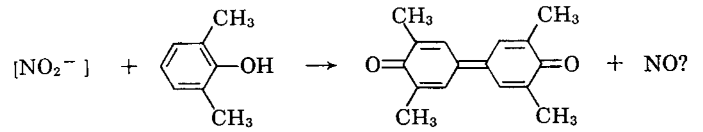
Curves (●) and (▲) are corrected for current of pure nitrite solution a series of nitrate solutions were prepared in the concentration range of 10^{-4} to $10^{-1}M$ and added to the xylenol reagent and the acid solution as for nitrite samples. After 5 minutes' reaction time the current at -0.15 volt was measured to give the lower curve in Figure 3. The data are as expected on the basis of the reaction time-concentration behavior shown in Figure 2. Two additional series containing the same concentrations of nitrate at two levels of added nitrite were examined in the same fashion. The expected diffusion current for the nitrite added was determined from separate experiments in the absence of nitrate. The observed currents for the second and third series were corrected for the expected currents for (a) the nitrite present and (b) the current for the nitrate present. If there were no secondary effect of nitrate on the reaction with nitrite the latter two corrected series should have produced a net zero current. Figure 3 shows that this is hardly the case. We have investigated this reaction kinetically (*9*) and find that the reaction is complex. Initially the reaction of nitrite with the xylenol proceeds as in the absence of nitrate, although at an enhanced rate, accompanied by the formation of the diphenoquinone which in turn decomposes to the diphenol. Kinetically the reaction obeys the rate law: $d(\mathrm{ArNO})/dt = k(\mathrm{NO_2^-})\ (\mathrm{NO_3^-})^{3/2}$ until the nitrite is nearly consumed. Beyond this point the increase in nitroso compound proceeds at a slower rate with the appearance of the nitro compound. The rate of appearance of the latter species is greater than would be estimated from the rate of nitration in pure nitrate solutions. These observations tentatively suggest the formation of a species of +4 nitrogen oxidation state with an equilibrium constant for the formation:

$$\mathrm{HNO_2 + HNO_3 = (N_2O_4) + H_2O}$$

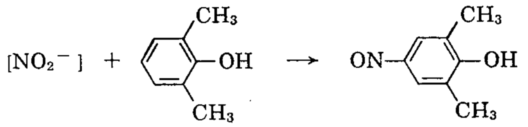
of less than 100; the formalism written in the equation does not imply identification of the exact species.

Since the reaction of nitrite in the presence of nitrate does produce such a large deviation from simple behavior, the estimation of nitrite in mixed samples containing more than equimolar amounts of nitrate is best determined empirically. An estimate can be obtained by a three-step procedure involving prior determination of the nitrate, which can readily be done by the procedure of Hartley and Curran, followed by measurement of the nitrosoxylenol diffusion current using the method described in the Procedure for the sample alone, and for a sample to which has been added a known amount of nitrite. From the three currents, all measured under constant reaction conditions, the nitrite concentration in the original sample may be calculated by the usual methods of standard addition analysis.

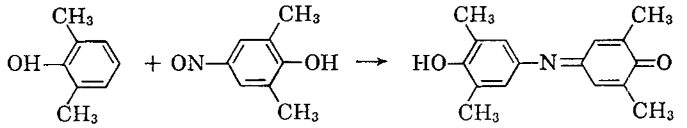
Effect of Side Reactions. Previously Hartley and Asai (*7*) showed that the principal reaction between nitrite and 2,6-xylenol to produce the 4-nitroso-2,6-xylenol is accompanied by either or both of two side reactions:



(1)



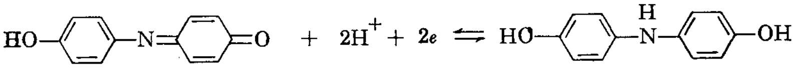
(2a)



(2b)

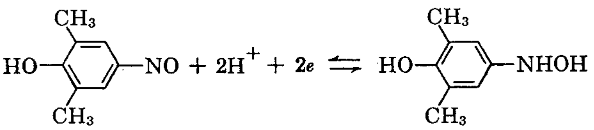
When the reaction is followed by spectrophotometric measurement of the nitroso compound the other products, 3,3′,5,5′ - tetramethyldiphenoquinone (Reaction 1) or 3,3′,5,5′,-tetramethylindophenol (Reaction 2b) decrease the yield of 2a but do not seriously interfere with the spectrophotometric measurement. Reaction 1 occurs only in weakly acid solution—e.g., 10% aqueous acetic acid—while 2b occurs in strongly acid solutions such as the present 5:4:1 $\mathrm{H_2SO_4:H_2O:HOAc}$. The solvent used in the present work was selected such that reaction 2b occurs only slowly. Since reaction 2b can occur only after reaction 2a the apparent yield is dependent upon the time of measurement. Asai showed that the absorbance at 307 mμ of the 4-nitroso-2,6-xylenol passed through a maximum 5 minutes after mixing the reagents. This did not occur when the reaction yield was measured polarographically. If a reaction mixture is prepared and a portion immediately diluted to correspond to the spectrophotometric method conditions, the portion measured polarographically shows a maximum yield approximately 2% higher than the spectrophotometric solution. After 30 minutes the former has suffered less than 1% decrease in diffusion current while the latter has undergone a 14% loss as measured by the absorbance.

When nitrite is allowed to react with 2,6-xylenol under conditions favoring rapid condensation—i.e., 6:3:1 $\mathrm{H_2SO_4:H_2O:HOAc}$—until spectrophotometric examination shows no nitrosoxylenol present, the resulting deep red solution gives a polarogram qualitatively identical to 4-nitroso-2,6-xylenol in the same solvent. Thus the indophenol is polarographically reducible in the same potential region as the nitrosoxylenol. Quantitative evaluation is difficult because both compounds have half-wave potentials positive to the dissolution of mercury; both limiting currents are diffusion-controlled. The indophenols are difficult to obtain in sufficient purity for quantitative determination of the polarographic parameters. Willis (*10*) has shown that the corresponding tetrachloroindophenol when purified to constant molar absorptivity by alumina chromatography yields a polarographic diffusion current constant comparable to that calculated for the tetramethyl compound if it is assumed that the in situ production of the latter is 100% efficient. The indophenols have been shown (*5*) to have reversible redox characteristics in aqueous solution according to the reaction:



(3)

The nitrosoxylenol electrochemical reaction at mercury in these solvents is believed to be:



(4)

by comparison with other nitroso aromatic compounds in similar solvents (*3*) and comparison of the diffusion current constant with that of the corresponding nitro compound (*vide infra*). Comparison of reactions 2a and 4 with reactions 2a, 2b, and 3 shows the reason the polarographic diffusion current suffered such a small loss compared to the spectrophotometric measurement is that the indophenol product essentially replaces the nitroso compound as depolarizer. Willis (*10*) has measured the rate of condensation of 2,6-xylenol with the 4-nitroso-2,6-xylenol by photometric means and found it to be 0.49 liters per mole per minute. When this value is used in connection with accurate current-time measurements the decrease in diffusion current with time can be accounted for solely by differences in diffusion coefficients between the two species.

The diphenoquinone is probably polarographically reducible in these solvents although no polarographic wave is obtained. Spectral measurements indicate that the solubility limit of the compound in these solvents is slightly less than the minimum detectable polarographic limit, $10^{-6}M$. In addition, the quinone is unstable in these acidic solutions and is converted to the reduced form, the diphenol, at a rate approximately equal to the rate of indophenol formation. At the rotating platinum electrode, the compound yields a wave with a half-wave potential of +0.6 volt *vs.* the Hg/Hg_2SO_4 reference electrode. This potential is somewhat lower than that obtainable from potentiometric cell measurements. The potential of the diphenoquinone-diphenol system in 6:3:1 solvent at platinum *vs.* Hg/Hg_2SO_4 reference electrode is approximately +0.8 volt. The system at equilibrium favors the diphenol by a factor of more than 1000:1 This large ratio coupled with the solubility limits of the two components rendered accurate measurement impossible (*9*).

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The Tubular Platinum Electrode

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▶ The tubular platinum electrode offers considerable promise as an analytical tool, because it permits electrochemical measurements to be made on a solution that flows through it. The construction and properties of the TPE are described. It is easy to fabricate and to use. It is stable, has a well defined geometry, and gives reproducible measurements. Solution holdup is low (2–10 μ.). Sensitivity is high, electroactive substances being detectable at concentrations below $10^{-8}M$. The dependence of current upon electrode parameters is derived theoretically and confirmed experimentally.

IN THE EXPANDING area of continuous analysis, there is an increasing need for sensors that will operate continuously in flowing streams. A tubular platinum electrode (TPE) through which the solution flows offers low solution holdup and high sensitivity, and thus appears suited for continuous electrochemical measurements in flowing streams.

The TPE also has promise as a tool in hydrodynamic voltammetry. To date, the best defined methods for performing voltammetric measurements in hydrodynamic systems are based on the rotating wire electrode (*3*, *4*), the rotating disk electrode (*5*), and a conical electrode placed in a flowing stream (*2*). Other hydrodynamic electrodes include a "bypass" electrode (a platinum wire penetrating to the interior wall of a 4-mm. i.d. tube, with an area of 0.3 sq. mm.) (*6*), and a "string" electrode (a rigidly held segment of platinum wire, 0.2 cm. long and 0.02 mm. in diameter, rotated about a center which lies 12 cm. from the wire and on its axis) (*1*).

This paper presents the theory and electrochemical properties of the TPE.

THEORY

Levich (*5*) has given a theoretical analysis of convective diffusion to the surface of a tube where the total flux, J, can be expressed as

$$J = 2.01\ \pi\ CD^{2/3}R^{2/3}X^{2/3}V_0^{1/3} \qquad (1)$$

when conditions of laminar flow exist. Here C is the bulk concentration of substance diffusing to the surface, D is its diffusion coefficient, R is the inside radius of the tube, X is its length, and V_0 is the maximum linear (i.e., axial) velocity of the stream flowing through the tube.

If C is electroactive and if the applied potential is in the diffusion controlled region, the electrolysis current is given by

$$I = nFJ, \text{ or}$$

$$I = 2.01\ nF\ \pi\ CD^{2/3}R^{2/3}X^{2/3}V_0^{1/3} \qquad (2)$$

where n is the number of electrons involved in the electrode reaction and F is the Faraday.

For purposes of measurement, it is convenient to use a volume flow rate, V_f which may be expressed in terms of R and V_0. The velocity profile for a laminar stream flowing through a tube is of parabolic shape, where the linear velocity V at any point r from the center axis of the tube is

$$V = V_0\left(1 - \frac{r^2}{R^2}\right) \qquad (3)$$

The volume flow rate may be found by a simple integration.

$$V_f = \int_0^R 2\pi r V dr \qquad (4)$$

$$V_f = \frac{\pi R^2 V_0}{2} \qquad (5)$$

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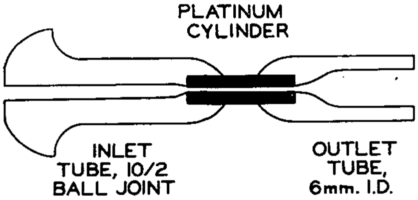

Figure 1. Tubular platinum electrode

Equations 2 and 5 yield I as a function of V_f:

$$I = 5.24 \times 10^5 nCD^{2/3}X^{2/3}V_f^{1/3} \qquad (6)$$

Experimental verification of the current dependence upon the parameters expressed in Equation 6 is given in the following paragraphs.

DESIGN AND CONSTRUCTION OF THE TPE

Figure 1 gives the general form of the TPE. Platinum cylinders were cut from seamless platinum tubing of various diameters (10-mil wall thickness), the ends being finished squarely and smoothly. In sealing the electrode into the soft glass tubing, care was taken to keep the inside of the assembly smooth, and not to have bumps or twists at the glass-to-platinum interfaces; but these efforts were not successful in all cases. Several electrodes with the dimensions shown in Table I were constructed. To lend strength, the inlet and outlet tubes were bridged with a 6-mm. glass rod for all electrodes except the shortest. For the shortest electrode, the platinum tube was completely embedded in the glass, electrical contact being made by a platinum leadout wire wrapped around the electrode before sealing.

CURRENT MEASUREMENTS

The oxidation of $K_4Fe(CN)_6$ in $1M$ KCl was used for all current measurements. A stock solution of $10^{-2}M$ $K_4Fe(CN)_6$ was prepared by dissolving reagent grade $K_4Fe(CN)_6$ in deaerated $1M$ KCl. All other solutions were made by diluting aliquots of the stock solution with deaerated $1M$ KCl. Gravity feed at constant head provided a constant flow of solution through the TPE. The outlet of the TPE dipped into a small beaker to which a saturated calomel electrode was bridged. The flow rate, V_f, was obtained by measuring the time for the overflow of 25 ml. of solution from the beaker. Flow rate measurements were reproducible to about 1%. A constant potential of +0.75 volt *vs.* SCE, well out on the diffusion plateau for the oxidation of $K_4Fe(CN)_6$, was applied to the TPE. Currents were measured with a Sargent Model XXI polarograph. All work was done at room temperature, with no attempts at temperature control.

Dependence of Current upon Flow Rate. Figure 2 is a log-log plot of current *vs.* flow rate at six different concentrations of $K_4Fe(CN)_6$ ranging from 10^{-4} to $10^{-5}M$, for electrode II (R = 0.015 inch, X = 1.006 inches). Below a flow rate of 10 ml. per minute, the plots are good straight lines, with slopes ranging from 0.32 to 0.35 (Table II). The median slope is 0.335, giving almost fortuitously good agreement with the theoretical value of $^1/_3$, according to Equation 6.

At about 10 ml. per minute, a break appears in the plots, and the slopes increase to 0.47–0.48 above 10 ml. per minute. It should be noted that the line segments are definitely straight, indicating an abrupt transition at about 10 ml. per minute. It is probable that this transition represents a change from laminar to turbulent flow in the TPE. For flow rates of 10 ml. per minute, in Figure 2, Reynolds numbers are 200–300, far below the critical values of 2000–7000 that are required for turbulent flow in smooth tubes. However, the edges of the TPE may be quite irregular, and it is known that turbulence appears at protrusions for Reynolds numbers as low as 20–50. At such low Reynolds numbers, the turbulence is local, being rapidly damped out within short distances past the protrusion, and the flow in the TPE may still be largely laminar, accompanied by small turbulent end effects.

At intermediate Reynolds numbers (100–200), the local eddies may break away from the protrusions, and may be propagated downstream, imparting turbulence for large distances beyond the protrusion (*5*). The Reynolds numbers at about 10 ml. per minute are just in this intermediate region, so it seems probable that the breaks in the curves of Figure 2 represent a transition from laminar to turbulent flow. Two observations are in support of this hypothesis. When a kinked platinum wire obstacle was placed close to the inlet end of the TPE, the break in the log I–log V_f plot came at a lower flow rate. Electrodes that were not smoothly constructed showed higher slopes, ranging up to 0.40 instead of the theoretical $^1/_3$.

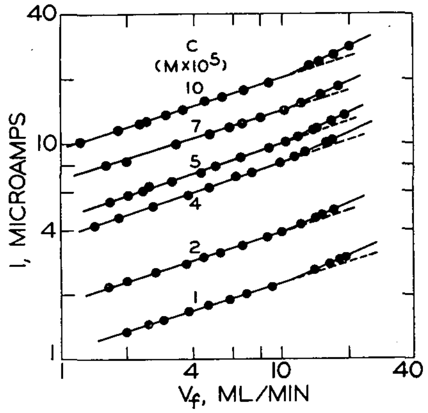

Figure 2. Dependence of current upon flow rate

Electrode II, R = 0.015 inch, X = 1.006 inches

Table I. Dimensions of Tubular Platinum Electrodes

Electrode	Radius (R), inches	Length (X), inches
I	0.020	1.006
II	0.015	1.006
III	0.010	1.006
IV	0.010	0.506
V	0.010	0.266
VI	0.010	0.104

Table II. Slopes of the Log I–Log V_f Plots of Figure 2

(Electrode II, R = 0.015 inch, X = 1.006 inches)

$K_4Fe(CN)_6$ molarity	Slope, ml./min.	
	Below 10	Above 10
10^{-5}	0.32	0.48
2×10^{-5}	0.34	0.48
4×10^{-5}	0.35	0.47
5×10^{-5}	0.34	0.47
7×10^{-5}	0.32	0.47
10×10^{-5}	0.33	0.47
Median	0.335	0.473

Much more data similar to that in Figure 2 was obtained for the other electrodes of Table I. Some of these data are shown in Figure 4. In all cases, the slopes of the log I–log V_f plots at low flow rates were straight lines with slopes ranging from 0.32 to 0.40. A definite correlation between the smoothness of the bore and the value of the slope was observed; electrodes whose inlet tubes were smooth in the region of the platinum gave slopes approaching $^1/_3$, while roughly constructed electrodes gave higher slopes.

Dependence of Current upon Concentration. The data of Figure 2 are used at three flow rates (2.5, 5, and 8 ml. per minute) to give the I *vs.* C plots shown in Figure 3. Examination of Figure 3 reveals two favorable analytical potentialities of the TPE. If other parameters are con-

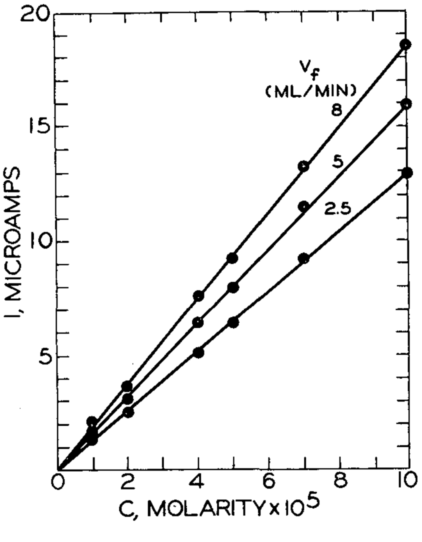

Figure 3. Dependence of current upon concentration

Data from Figure 2

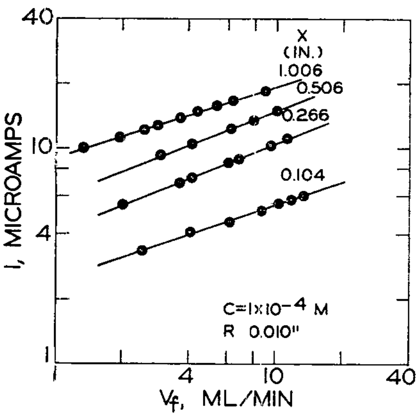

Figure 4. Dependence of current upon electrode length

Electrodes III, IV, V, VI

trolled, the relationship between I and C is linear and reproducible, which means that I may be used as a very convenient measure of C. According to Figure 3, a $10^{-5}M$ $K_4Fe(CN)_6$ solution gives a current of 1 to 2 μa., at moderate flow rates. This current does not show the fluctuations that a dropping mercury electrode or a rotating platinum electrode does. Fluctuations in the TPE current appeared to be much less than 1%, which means that concentrations below $10^{-7}M$ should be detectable (vide infra).

It is of interest that I depends linearly upon C also in the region of high flow rates, above 10 ml. per minute.

Dependence of Current upon Length. Figure 4 is a series of log I–log V_f plots similar to those of Figure 2, but for a set of electrodes of differing lengths. Data were taken from Figure 4 at two flow rates (2.5 and 5 ml. per minute) and plotted on a log-log basis in Figure 5 to show the effect on current of length of the electrode.

The log I–log X plots of Figure 5 are straight lines with slopes of 0.61, in reasonably good agreement with the theoretical value of $^2/_3$, according to Equation 6. Since the exponent of X must be obtained from data from different electrodes, the precision cannot be expected to be as good as the precision with which the exponent of V_f is determined (Figure 2), where all data were taken for a single electrode.

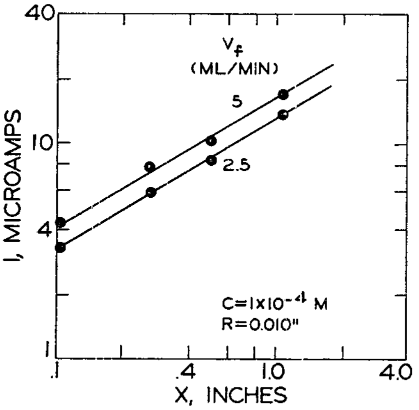

Figure 5. Dependence of current upon electrode length

Data from Figure 4

Dependence of Current upon Radius. Figure 6 is an I vs. C plot for electrodes I, II, and III, of radii 0.010, 0.015, and 0.020 inch, respectively. It may be seen that data for all three electrodes fall upon the same straight line, showing that I is independent of the electrode radius, in accord with Equation 6.

Calculation of *D*. According to Equation 6 the slope of an I vs. C plot permits a calculation of D. The three plots in Figure 3 give 0.75, 0.73, and 0.75 × 10^{-5} sq. cm. per second for flow rates of 2.5, 5, and 8 ml. per minute. The plot of Figure 6 gives 0.70 × 10^{-5} sq. cm. per second, which may be regarded as an average of the data from three 1-inch electrodes of different radii. These values are very consistent, indicating that D is independent of flow rate. The median value of 0.73 × 10^{-5} sq. cm. per second is in good agreement with von Stackelberg's value of 0.63 × 10^{-5} sq. cm. per second (7), indicating that the conditions under which Equation 6 holds may be approached fairly closely with good electrodes.

It would be expected that values of D calculated from TPE data would be high; any turbulence would bring electroactive material to the electrode faster than accounted for by Equation 6, resulting in higher currents and higher apparent values of D. Actually, D-values can also be calculated from data other than that in Figures 3 and 6. Values of D calculated from Figure 5 fall at about 0.9 × 10^{-5} sq. cm. per second, which is in line with the greater turbulence that is suspected to occur in the shorter electrodes used to obtain the data of Figure 5.

Proof of High Sensitivity. The high sensitivity of the TPE was shown by preparing a "blank solution" containing $0.10M$ KCl and $0.10M$ Na_2HPO_4, neutralized to pH 7.5 with NaH_2PO_4. A portion of the blank solution was then made $10^{-3}M$ in $K_3Fe(CN)_6$. Two 100-fold dilutions and a twofold dilution of the $10^{-3}M$ $K_3Fe(CN)_6$ with the blank solution gave a ferricyanide solution that had the same composition as the blank, but in addition contained nominally 5 × $10^{-8}M$ $K_3Fe(CN)_6$.

The blank was pumped with a peristaltic pump (Model 500–1200, Harvard Apparatus Co., Dover, Mass.) through the TPE (0.040-inch i.d., and $^1/_2$ inch long) from a two-way stopcock that permitted alternation between the blank and the 5 × $10^{-8}M$ ferricyanide.

A potential of 80 mv. was applied across the TPE (cathodic) and a saturated calomel electrode. The current through the TPE passed through a 10,000-ohm resistor, and the voltage drop was measured with a microvoltammeter (Model 425A, Hewlett-Packard Co., Palo Alto, Calif.) and recorded (Model G-10, Varian Associates, Palo Alto, Calif.).

Figure 7 is a record of the response when the solution is switched from blank to ferricyanide and back to blank again. The residual current was 0.012 μa, too large to balance out with the recorder zero adjustment, so a bucking voltage was applied to the recorder to bring the blank solution response onto the chart.

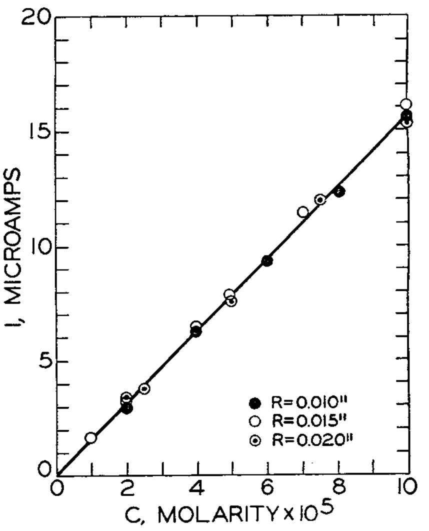

Figure 6. Nondependence of current upon electrode radius

Electrodes I, II, III

The stability of the steady-state currents is such that concentrations well below $10^{-8}M$ $K_3Fe(CN)_6$ should be easily detectable.

It was not possible to dilute $K_4Fe(CN)_6$ solutions. Attempts to do so led to loss of the ferrocyanide at dilutions at about $10^{-6}M$, probably owing to air oxidation. The KCl–Na_2HPO_4 buffer solution was chosen as a supporting electrolyte because of prior experience with the stability of $K_3Fe(CN)_6$ in this medium.

CONCLUSIONS

A principal advantage that accrues to the use of the TPE in a flowing solution is high sensitivity, since transport of

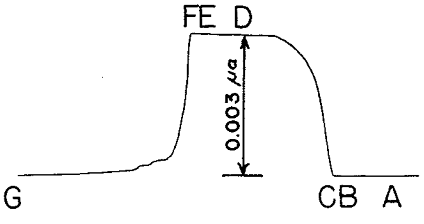

Figure 7. Response of TPE to 5 × $10^{-8}M$ $K_3Fe(CN)_6$

- *A.* Steady-state current with blank solution
- *B.* 5 × $10^{-8}M$ ferricyanide introduced
- *C.* 5 × $10^{-8}M$ ferricyande reaches electrode
- *D.* Steady-state current with 5 × $10^{-8}M$ ferricyanide
- *E.* Blank solution introduced
- *F.* Blank solution reaches electrode
- *G.* Steady-state current with blank solution

TPE, 0.040-inch i.d., and $^1/_2$ inch long; residual current, 0.012 μa.; flow rate, 8.4 ml./min.

electroactive material to the electrode is aided greatly by convection. A typical TPE can detect concentrations of electroactive substances below $10^{-8}M$ in streams of moderate velocity. Other advantages are simplicity of construction and reproducibility of measurements, under turbulent as well as laminar flow conditions. For continuous analysis in flowing streams, the low holdup volume (2–10 μl.) may prove to be a great advantage.

At applied potentials well out into the diffusion limiting region, experimental currents obtained with the TPE are in agreement with theory. No measurements have been made in the potential limiting region, where the dependence of current upon electrode parameters is not yet clear.

ACKNOWLEDGMENT

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Polarography in Fused Alkali Metaphosphates

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▶ Polarograms were obtained with a cell consisting of a platinum microelectrode inserted in a melt contained in a platinum crucible. No reference electrode was employed; the platinum crucible served as a massive and nonpolarizable anode. Electrolyses were carried out in fused $NaPO_3$ at 750° C., or $LiPO_3$-$NaPO_3$ at 730° C. in which a potential span of 0.95 volt was available between the solvent decomposition processes. Twenty oxides and compounds were studied. U_3O_8, CuO, FeO, Fe_2O_3, and V_2O_5 gave redox waves. Silver was the only species that could be reduced to the metal. These data are interpreted with respect to probable electrode reactions.

Fused alkali metaphosphates are well known for their ability to dissolve metal oxides; moreover, they have been used from time to time to "open up" complex minerals to make them water-soluble. The sodium metaphosphate bead test has been used for some time as a means of qualitative analysis in determinative mineralogy, since the fused salt enters into chemical combination with many metal oxides to give characteristic colors (*14*). Little is known concerning the nature of the species present when metal oxides are dissolved in such solvents, although some work is now being done by Soviet electrochemists. Andreeva (*1*) determined the decomposition potentials of a series of metal oxides dissolved in fused sodium metaphosphate and fused sodium pyrophosphate at 1000° C., and Delimarskiĭ and Andreeva (*3*, *4*) used sodium metaphosphate as a solvent in studies of galvanic concentration cells. Delimarskiĭ and Kaptsova (*6*) conducted a polarographic study of solutions of titanium dioxide in molten sodium metaphosphate and found that a two-step reduction wave was obtained. The two steps were ascribed to reduction of titanium(IV) to titanium(III) and thence to the metal. Most work on decomposition potentials involved relatively concentrated solutions of the oxides, however, and no discussion or evidence of the formation of intermediate oxidation states was given by the authors. Preliminary experiments in this laboratory indicated that intermediate oxidation states did exist, and that the electrode reactions of metallic ions in more dilute solutions did not necessarily involve simple deposition of the metal.

The chemistry of alkali metaphosphates is complicated by the fact that the compounds are polymerized in varying degrees (*8*). The molecular formulas of most of the metal metaphosphates have not been determined; therefore, only empirical formulas are used throughout the discussion in this section. The metal oxide, when dissolved in a metaphosphate melt, probably undergoes one of the following types of reactions:

$$M_2O + NaPO_3 \rightarrow NaM_2PO_4 \quad (1)$$

$$MO + NaPO_3 \rightarrow NaMPO_4 \quad (2)$$

$$M_2O_3 + 3\ NaPO_3 \rightarrow 2\ MPO_4 + Na_3PO_4 \quad (3)$$

$$MO_2 + 6\ NaPO_3 \rightarrow 2\ Na_3PO_4 + M(PO_3)_4 \quad (4)$$

Other equations could be written for oxides having different formulas. The reactions are oversimplified, since various complex ions are probably formed. Van Wazer provides ample evidence for the complexity of phosphate systems (*15*). Many reactions are acid-base in nature, as exemplified by the reaction of sulfates in the melt:

$$MSO_4 + NaPO_3 \rightarrow NaMPO_4 + SO_3 \quad (5)$$

The techniques of polarography in molten salts have been amply described and reviewed by several workers (*7*, *10*, *12*) and are not discussed here unless deviations from established practices were made.

The objective of the present study is an evaluation of the use of fused alkali metaphosphates as a solvent in which to conduct electrochemical studies. The polarographic work was undertaken to lay the groundwork for future e.m.f. studies and possible coulometric determination of metals dissolved in the melt.

EXPERIMENTAL

Microelectrodes. Platinum microelectrodes used to obtain the polarograms were constructed by sealing 30-gauge wire in Supremax glass tubing. This tubing is manufactured by the Jenaer Glaswerk Schott of Mainz, West Germany, and was obtained from the Fish-Schurman Corp., New Rochelle, N. Y. It appears to be nearly identical to Corning No. 1720 glass, an aluminosilicate glass used for ignition tubing. Good seals between the glass and the platinum wire were obtained, probably because of the small diameter of the wire used. Two types of electrodes were used, one consisting of a small tip of straight

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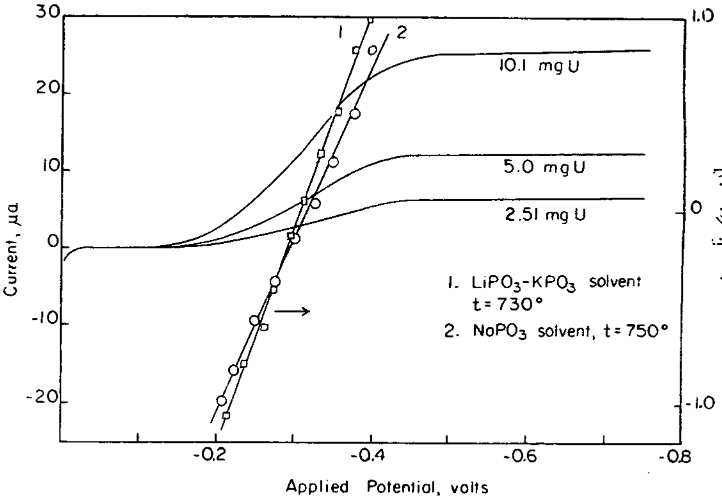

Figure 1. Analysis of polarograms for uranium(VI) in fused alkali metaphosphates

wire protruding from the glass, and the other a small bead of platinum on the end of a 2-mm. length exposed portion of wire. Electrode areas were measured with the use of a microscope and calibrated micrometer eyepiece.

Cell. The polarographic cell consisted of a platinum microelectrode inserted into the melt which was contained in a platinum crucible. No reference electrode was employed; the platinum crucible served as a massive and nonpolarizable anode (*6*), connection to the outside of the cell being made with a piece of 18-gauge platinum wire. The crucible was placed in the bottom of a Vycor container measuring 67 × 260 mm. and was held in place with a ring of ceramic wool. The Vycor container extended about 3 inches above the furnace and terminated with a flat-ground flange. The cover was a borosilicate glass head with three standard-taper joints through which a thermocouple, microelectrode, and platinum lead were placed. One of the joints was fitted with a gas inlet tube, through which an argon flow was maintained to provide an inert atmosphere in the cell. Since all experiments were conducted at temperatures in excess of 700° C. it was necessary to cool the upper portion and cover of the container with two jets of compressed air. Kel-F No. 90 lubricant was used in all glass-ground joints.

Furnaces and Temperature Control. A multiple-unit electrical furnace, 660 watts, manufactured by the Electrical Heating Apparatus Co., was used for all the work, with the exception of the melt purification. The furnace was modified by installing an auxiliary heating coil in the bottom of the cavity. This coil accounted for 10% of the total heating capacity and was regulated by the temperature controller. Voltage to the main coils was adjusted with a variable transformer such that the furnace operated about 10° C. below the desired temperature. The auxiliary coil was operated through another variable transformer which was connected to the controller. This arrangement allowed the temperature to be controlled to within ±1° C. A stainless steel beaker was placed in the cavity to protect the heating cell in the event the cell broke. The beaker was grounded to a water pipe to remove the possibility of any induced voltages being formed within the cell by the furnace coils. Temperature control was provided by a Minneapolis Honeywell potentiometer pyrometer, Model No. 156C16PS-21, from which the temperature could be read directly. A Chromel-Alumel thermocouple was used as the sensing element for the controller as well as for measuring the temperature.

Miscellaneous Equipment. Polarograms were recorded using a Sargent Model 21 polarograph. Scan rates of 50 to 75 mv. per minute were employed. Applied potentials were monitored occasionally with a Gray Model E-3042 potentiometer.

Reagents. Potassium and sodium metaphosphates were prepared by fusing the reagent grade alkali dihydrogen phosphates in platinum dishes and heating at 950° C. for 4 hours.

Lithium metaphosphate was prepared by adding the stoichiometric amount of phosphoric acid to lithium carbonate, evaporating to dryness, and fusing in a platinum dish at a temperature of 950° C.

Anhydrous silver metaphosphate was prepared by precipitating the salt from a solution of lithium metaphosphate, filtering, washing, and fusing in a platinum dish at 950° C.

All metal oxides and salts added to the phosphate melts were of reagent grade quality.

Argon was passed through copper wire and titanium sponge heated at 600° C. to remove any oxygen present. The gas was then dried by passing through columns of magnesium perchlorate.

Purification of Solvents. Basic impurities were removed by adding a few drops of phosphoric acid and igniting the melts at temperatures high enough to drive off water and phosphorus pentoxide. The $LiPO_3$-KPO_3 eutectic (64 mole %–36 mole %), having a melting point of 518° C. (*2*), was used for a solvent in some of the work. The mixture was prepared by melting the required portions in a platinum dish, igniting at 950° C. for a few hours, and pouring out onto a polished nickel slab to solidify. The product was then crushed and stored in screw-capped bottles.

Sodium and lithium metaphosphates form glasses when melted, and if cooled rapidly they do not crystallize; hence the melts are viscous, even at temperatures several hundred degrees above their so-called melting points.

PROCEDURE

Initial work was done using sodium metaphosphate at 750° C., but the $LiPO_3$-KPO_3 eutectic described previously was used at 730° C. during most of the work.

Weighed amounts of the metal oxides or salts were added to weighed amounts of solid solvent and the mixtures fused in a platinum crucible until all the oxide or salt had dissolved. The crucible, with its contents, was then placed in the Vycor container and polarograms were taken at a microelectrode. A weight dilution method was used in some of the work. A prepared solution was poured out onto a polished stainless steel slab in the form of beads which were then weighed and added to the pure solvent as needed.

The microelectrodes were inserted until the glass tip just touched the surface of the melt. At the high temperatures employed some conduction of the glass itself was observed, but such errors were minimized by not inserting the glass into the bulk of the melt. If too rapid scanning rates were used peaks were observed for the reduction of metal ions in the melt; consequently, slow scan rates were employed. Scan rates of 50 mv. per minute were used during most of the work. Some of the work was done in an atmosphere of air, but an inert atmosphere of argon was employed whenever easily oxidized species were being determined.

RESULTS AND DISCUSSION

Limiting Electrode Processes of Melt. The two melts had a potential span of 0.95 ± 0.01 volt between the solvent decomposition processes. When the melts were electrolyzed with two platinum electrodes, the electrode reaction at the anode appeared to be

$$2\ PO_3^- \rightleftarrows {}^1/_2\ O_2 + P_2O_5 + 2e^- \qquad (6)$$

Continuous evolution of gas occurred at current densities above 5 ma. per sq. cm. Identical results were obtained with graphite anodes. The cathodic process was complicated and appeared to involve the reaction

$$4\ PO_3^- + 5\ e^- \rightleftarrows P + 3\ PO_4^{-3} \qquad (7)$$

Reactions yielding products such as phosphite and phosphide ions could

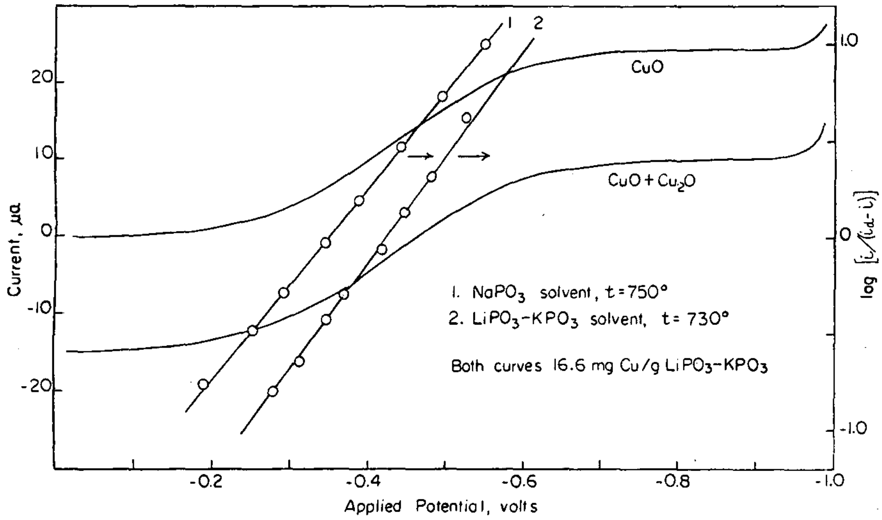

Figure 2. Analysis of polarograms for copper in fused alkali metaphosphates

Table I. Relationship between Limiting Current and Concentration of Uranium(VI)

Concn., C, mg. U/gram $NaPO_3$	i_d (corrected for residual)	i_{d}/C
2.38	6.1	2.56
5.02	13.2	2.63
7.41	19.0	2.56
9.91	25.3	2.55
12.97	34.6	2.66
15.50	41.6	2.68
		Av. 2.61
		Rel. std. dev. ±2.3%

occur, but the only one observed was the evolution of phosphorus, which ignited spontaneously in the air. The white fumes obtained during the burning of the gas bubbles evolved at the cathode were collected, dissolved in water, and gave a positive test for phosphoric acid. Delimarskiĭ and Kaptsova stated that the cathodic reaction was the deposition of alkali metal, but this was not observed, even at current densities as high as 250 ma. per sq. cm. Zinc metal reacted with the melts to produce phosphorus and a solution of the zinc salt. Other active metals exhibited the same behavior, including aluminum, iron, and nickel. Platinum cathodes were severely attacked when phosphorus was evolved, but no discoloration or corrosion was noted during the polarographic work. This could be due to the formation of other products at lower current densities.

In the following discussions the reported half-wave potentials of redox systems were taken from the cathodic current-voltage curves obtained in solutions containing the metal in its highest oxidation state only. When securing anodic or anodic-cathodic curves, the voltage scan was started at an initial potential sufficiently positive to obtain the anodic decomposition of the solvent at the microelectrode. This was necessary since the current-voltage curve, starting at zero volts applied between two platinum electrodes, will originate on the wave itself when both species of a reversible redox couple are present. Thus, for the anodic and composite curves shown in Figures 2, 3, and 4, the point at which the solvent anodic curve originated was taken as an arbitrary "zero applied volts" in order to make the curves and their half-wave potentials directly comparable to those obtained in other solutions. This procedure provided reproducible results in the absence of a true reference electrode. The assumption that the platinum crucible served as a nonpolarizable electrode was justified by the fact that its surface area was at least 1000 times that of the microelectrode.

Uranium. Weighed portions of U_3O_8 were added to both metaphosphate melts and produced a well defined wave which was partially anodic in character. The solidified solution was emerald green, indicating the presence of uranium(IV), but the characteristic yellow-green color of the uranyl species was produced when the melts were heated in the open air and then solidified. The solid exhibited the typical fluorescence of uranyl salts when exposed to ultraviolet light. In another experiment addition of uranyl nitrate to the metaphosphate melts produced reduction waves which were completely cathodic. The nitrate appeared to decompose immediately upon addition. Typical curves are shown in Figure 1. The half-wave potential is −0.3 volt. Plots of applied potential *vs.* log $\frac{i}{i_d - i}$ also shown in the figure, gave least squares slopes of 0.110 and 0.087 in sodium metaphosphate and the $LiPO_3$-KPO_3 eutectic, respectively, compared to theoretical slopes of 0.102 and 0.0995 for a 2-electron electrode reaction. The reduction appeared to be

$$U(VI) + 2\,e^- \rightleftarrows U(IV) \qquad (8)$$

The limiting current was proportional to concentration. Table I shows the relationship of diffusion current as a function of concentration, using a microelectrode of 3.0 sq. mm. area. The reproducibility of the data is better than that usually obtained in fused salts; however, the high viscosity of the melt undoubtedly minimizes the usual large errors due to convection.

Copper. Cupric oxide produced reduction waves in both melts. Addition of cuprous oxide or cuprous chloride caused an anodic wave to appear as shown in Figure 2. The plots of applied potential *vs.* log $\frac{i}{i_d - i}$ had least squares slopes of 0.198 and 0.182 in the sodium metaphosphate and eutectic melts, respectively, compared to theoretical values of 0.203 and 0.199 for a 1-electron reduction process. No deposition of copper metal was observed at the highest concentration

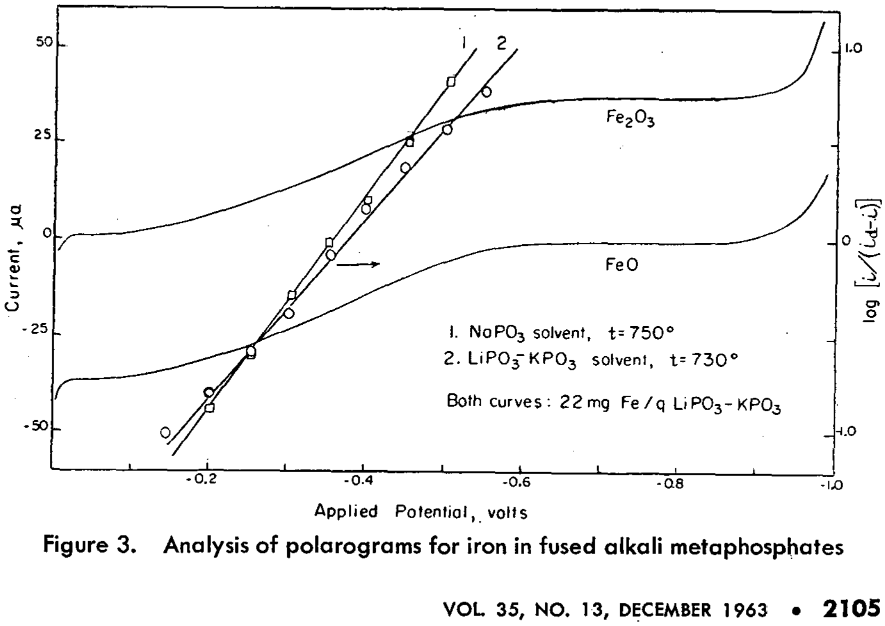

Figure 3. Analysis of polarograms for iron in fused alkali metaphosphates

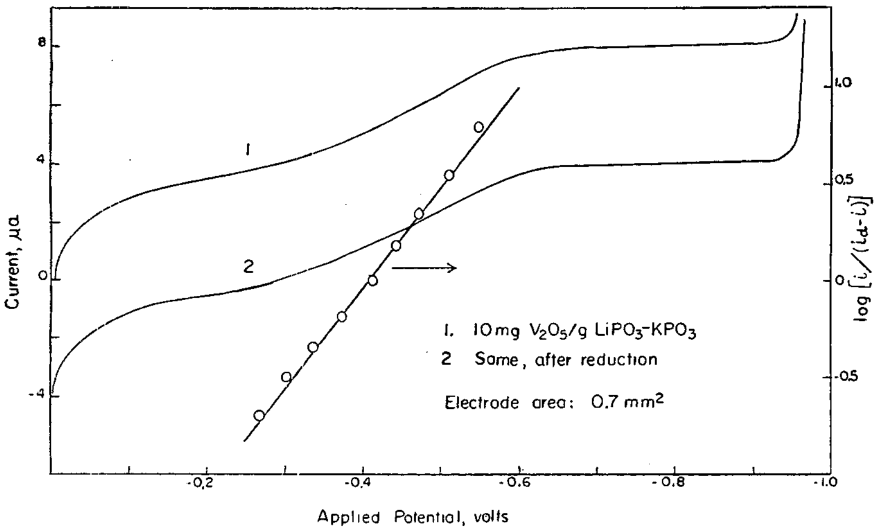

Figure 4. Analysis of polarograms for vanadium in fused alkali metaphosphates

level, 2% copper by weight. The half-wave potential is about −0.4 volt. The heights of the waves appeared to be proportional to concentration.

Iron. Both ferric and ferrous oxides produced waves in the two melts. The waves for ferrous oxide were anodic when care was taken to exclude oxidation in the air. When air was admitted into the cell the waves slowly shifted upward until they were identical to ones produced by ferric oxide. Typical polarograms and the analyses of the waves are shown in Figure 3. The least squares slopes of the plots were 0.179 and 0.216 for waves in sodium metaphosphate and the $LiPO_3$-KPO_3 solvent, respectively, implying a 1-electron electrode reaction. The half-wave potential is about −0.37 volt.

Vanadium. Vanadium(V) oxide was dissolved in a metaphosphate melt by heating in an electric furnace under an atmosphere of air. Curve 1 of Figure 4 shows a polarogram obtained with this solution. The initial anodic wave was largely obscured by the anodic dissolution of the solvent. However, a well defined cathodic wave was observed. On cooling, a yellow glass was formed. When this experiment was repeated, using a Meker burner to heat the melt, a green glass was formed on cooling. The polarogram is shown by curve 2 of Figure 4. Apparently the gases from the burner were able to reduce the vanadium in the melt to a lower valence state. The addition of a small piece of zinc to the original oxidized melt caused a shift of the polarogram from type 1 to 2. The anodic dissolution of the solvent obscured the first wave and made an analysis impossible. The second wave showed a least squares slope of 0.193, corresponding to a 1-electron electrode reaction. The half wave potential was −0.46 volt.

When vanadium(V) oxide was added to the melt it went into solution, accompanied by the evolution of gas. A portion of the oxide decomposed to give vanadium(IV). This was confirmed by allowing the melt to solidify, dissolving a portion in water, and testing for the presence of reduced species. The yellow-orange glass dissolved to form green solutions which contained both vanadyl and vanadate species. Solutions of the reduced melt were cooled, dissolved in water, and found to contain only vanadium(IV). It must be assumed that a small amount of vanadium(III) must have oxidized during solution of the glass in water. Thus the initial wave should be due to the vanadium(V)-(IV) system while the second wave, at half-wave potential −0.46 volt, is due to the vanadium(IV)-(III) system.

Silver. Weighed portions of silver metaphosphate were added to the $LiPO_3$-KPO_3 solvent to give the deposition curves shown in Figure 5. No analysis of the waves could be made, since the upper portion of the curves became obscured by the cathodic decomposition of the solvent. The stability of the silver metal in the melt indicates that a silver–silver metaphosphate reference electrode could be used for future studies in this melt.

Sulfur(VI). Two waves were produced when sodium sulfate was added to the melts. The first wave appeared to be the reduction of sulfate ion to sulfite ion, and the second the reduction of sulfite to sulfur or sulfide ion. Liu (*11*) showed that the electrolysis of sulfate melts produced sulfide ion instead of sulfur, but the poor reproducibility of the second wave in the metaphosphate solvents frustrated any attempts to deduce the nature of the product formed. A few milligrams of silver sulfate were added to another portion of the melt, and polarograms of the kind shown in Figure 6 were recorded. The second wave was well defined and was accompanied by the deposition of a black compound which was soluble only in hot, concentrated nitric acid. Analysis of the waves by means of a plot of E vs. $\log \frac{i}{i_d - i}$ for the first wave and a plot of E vs. $\log (i_d - i)$ for the second wave gave least squares slopes of 0.094 and −0.0328, respectively. The theoretical slopes should be 0.102 and −0.0338 for a 2- and 6-electron process, respectively. The deposit, which was apparently silver sulfide, could be stripped off anodically by reversing the voltage scan at approximately −0.8 volt.

The stripping curve, shown by the dotted line in Figure 5, indicates that the electrode process taking place here is reversible. Two possible reactions yielding silver sulfide are

$$2\,Ag^+ + SO_3^{-2} + 6\,e^- \rightleftarrows Ag_2S + 3\,O^{-2} \qquad (9)$$

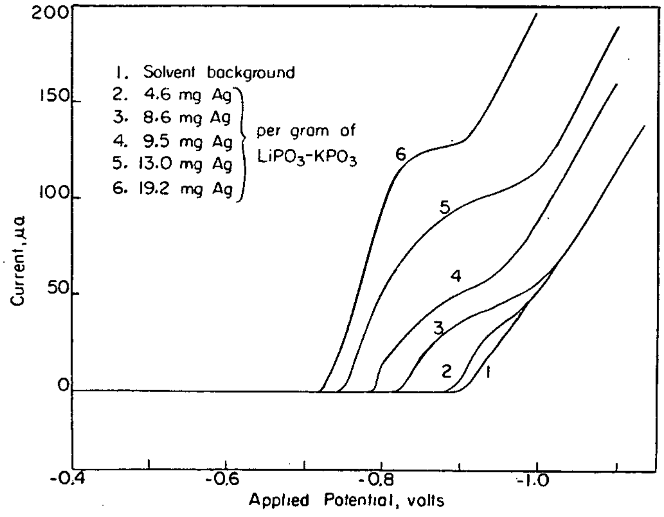

Figure 5. Current-voltage curves for silver in fused $LiPO_3$-KPO_3 eutectic

$$2\ \mathrm{Ag}^+ + \mathrm{SO_4}^{-2} + 8e^- \rightleftarrows \mathrm{Ag_2S} + 4\ \mathrm{O}^{-2} \quad (10)$$

Johnson and Laitinen (*9*) have shown that current-voltage curves of some metal ions in sulfate melts represent the process shown in Equation 10. This process occurs only for those metals which will reduce the sulfate solvent to insoluble sulfides, however. Since silver metal does not reduce sulfate, it appears that silver sulfide will not be formed on an electrode surface until silver metal is first deposited, and sulfide ion is produced at the electrode surface. The shape of the current-voltage curves obtained in the metaphosphate solvent and the slope of −0.0328 obtained by the plot of E *vs.* log $(i_d - i)$ indicate that the second reduction wave is represented by Reaction 9.

Other Metals Studied. Chromium (VI) oxide and potassium chromate reacted vigorously with the melt, with evolution of oxygen to give melts which contained chromium(III). No reduction waves or cathodic currents were observed.

Manganese dioxide reacted vigorously with evolution of oxygen to give dirty-violet solutions which became clear-violet upon cooling. The solid was amethyst colored. No reduction waves were observed, but a steady cathodic current, proportional to the manganese concentration, was assumed to be due to the oxidation of Pt by Mn(III).

Manganese(II) sulfate gave violet solutions when the melt was heated in the air, but the melt became colorless in the reducing flame. The violet solutions appeared to be manganese(III). Red-violet manganese(III) complexes formed in concentrated phosphoric acid have been reported (*13*).

Zinc oxide, lead oxide, nickel oxide, cobalt chloride, zirconium dioxide, niobium pentoxide, and sodium tungstate did not produce noticeable waves or cathodic currents. Not all metals were studied, but it appears that most of them are not deposited as the metal because of their activity in the melt. Undoubtedly many metals could be plated out when present at sufficiently high concentrations, since the decomposition potential of a metal will be shifted to more positive values as the concentration is increased. Copper metal did not react with the solvents, but no deposition of the metal was noted except at relatively high concentration levels. It appears that species forming redox couples are the only ones which provide diffusion-limited current-voltage curves at relatively low concentrations in the metaphosphate melts. The difficulty in obtaining similar waves for the metals may well be caused by a highly stable complex formation which shifts the decomposition potentials of the metals in the negative direction. A

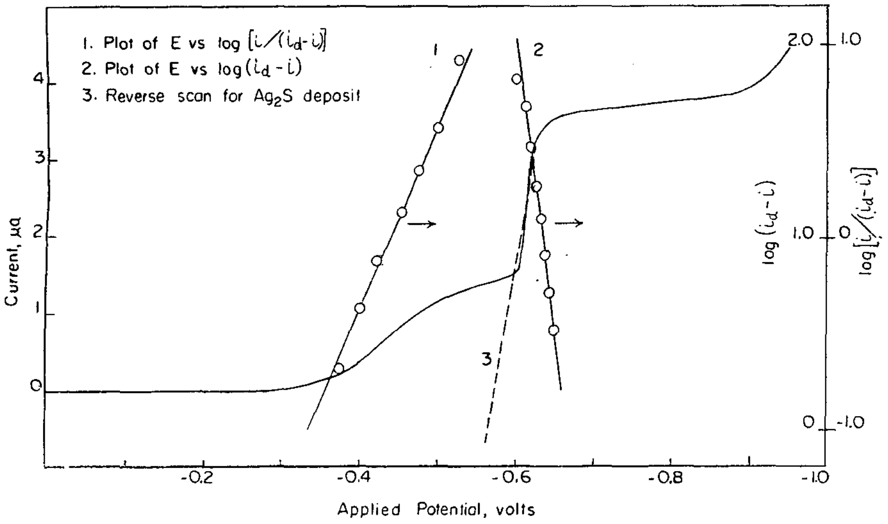

Figure 6. Analysis of polarograms of silver sulfate in fused $NaPO_3$

Table II. Least Squares Slopes Measured from Plots of Log $\frac{i}{i_d - i}$ *vs.* Applied Potential for Polarograms of Metallic Ions and Sulfate in Fused Alkali Metaphosphates

	Observed		Theoretical	
Ion	$NaPO_3$ solvent, 750° C.	$LiPO_3$-KPO_3 solvent, 730° C.	$NaPO_3$, 750° C.	$LiPO_3$-KPO_3, 730° C.
U(VI)	0.110	0.087	0.102	0.0995
Cu(II)	0.198	0.182	0.203	0.199
Fe(III)	0.179	0.216	0.203	0.199
V(IV)	...	0.193	...	0.199
SO_4^{-2}	0.094	0.090	0.102	0.0995
SO_3^{-2}	−0.033[a]	...	0.034	...

[a] From plot of log $(i_d - i)$ *vs.* E.

Table III. Summary of Polarographic Studies of Metallic Ions and Sulfate in Molten $NaPO_3$ and $LiPO_3$-KPO_3 Eutectic at 750° and 730° C.

Compound added to melt[a]	Nature of wave(s)	Number of electrons in electrode reaction	$E_{1/2}$, volt	Color of melt when cold[a]	Remarks[a]
U_3O_8	Anodic-cathodic	2	−0.30	gr.	
$UO_2(NO_3)_2 \cdot 6H_2O$ (d.)	Cathodic	2	−0.30	y.-gr.	Fluorescent under UV
CuO	Cathodic	1	−0.4	blue	
Cu_2O, CuCl	Anodic	1		pale gr.	
Fe_2O_3	Cathodic	1	−0.37	y.	
FeO	Anodic	1		c.	
V_2O_5 (d.)	(1) Cathodic (2) Cathodic	1 1	−0.42	y.-or.	Color green after reduction. V_2O_5 d. only partially to V(IV)
$AgPO_3$	Cathodic	1		c.	Ag metal deposits
MnO_2 (d.)	Continuous cathodic current			v.	Mn(III) formed
Na_2SO_4	(1) Cathodic (2) Cathodic	2 6	−0.45 −0.61	c.	
CrO_3 (d.) K_2CrO_4 (d.)	None			gr.	Cr(III) formed
TiO_2	High residual current only			c.	
$CoCl_2$	None			red	
NiO	None			y.	
PbO	None			c.	
Nb_2O_5	None			c.	
ZrO_2	None			wh.	Oxide appears insoluble
Na_2WO_4	None			c.	

[a] d. -decomposes; gr. -green; y. -yellow; c. -colorless; or. -orange; v. -violet.

survey of the properties of silver compounds shows that most silver salts decompose before melting, but silver metaphosphate solutions in sodium metaphosphate were stable up to, if not above, 950° C. This is probably another example of increased stability due to complex formation. Therefore, the existence of current-voltage waves for redox couples may be due to the fact that the oxidized and reduced species are both complexed to nearly the same degree.

CONCLUSIONS

Polarographic studies of several metal oxides and sulfate ion in fused alkali metaphosphates demonstrated the formation of several stable species which exhibited diffusion-controlled current-voltage curves. The wave heights of the uranium(VI) reduction wave were proportional to the concentration of uranium, thus demonstrating the analytical utility of polarography for the determination of the metal in the melts. The other metallic ions studied gave diffusion currents proportional to concentration, but the relationship was only observed qualitatively. The results obtained from the analyses of the polarograms are summarized in Table II and a summary of all observations is given in Table III.

ACKNOWLEDGMENT

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Polarography of Lanthanum(III), Praseodymium(III), and Ytterbium(III) in Anhydrous Ethylenediamine

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▶Anhydrous conditions are necessary to avoid complicated rare earth polarography. Problems of aqueous systems are reviewed. Anhydrous Yb(III), La(III), and Pr(III) acetates in anhydrous ethylenediamine gave a double irreversible wave for Yb (−0.411, −0.747 volt) and single irreversible waves for La and Pr (−0.565, 0.571 volt). A zinc reference and tetrabutylammonium perchlorate electrolyte were used. Coulometry established 1- and 2-electron steps for Yb and 3 electrons for La and Pr. Metal amalgams were shown by chemical tests. Yb(II) gave no anodic waves, since a transfer rate of 1.15 $\times$ 10^{-9} cm. sec.$^{-1}$ at 0.0 volt caused anodic overvoltages beyond Hg.

The aqueous polarography of the rare earths has been characterized by three types of behavior. The first case, Ce(IV)-Ce(III), has been studied extensively and there is no doubt about the validity of the redox behavior (*5, 13*). The second group includes those ions reduced to the divalent state followed by reduction to the metallic state—i.e., Eu(III), Yb(III), and Sm(III) (*9, 11, 13, 22, 26, 41, 42*). The remaining elements are reported to reduce to the metallic state in a 3-electron process (*10, 13*).

While the 1-electron reduction of Eu(III), Yb(III), and Sm(III) is a valid change, contradictions, lack of information, and recent studies cast doubt upon reduction of the rare earths to the metallic state. Several phenomena produce complicating features. The rare earths are very acidic and react with water to produce free hydronium ions and hydroxy or oxy species whose composition is variant and as yet uncharacterized. The fact that hydronium ion reduction (−1.5 volts *vs.* SCE) follows the first waves of Eu(III) and Yb(III) and precedes the reduction of Sm(III), Sm(II), Eu(II), Yb(II), and the remaining rare earth (III) elements has led some workers to postulate species such as La(II) (*31, 45*). The composition of hydroxy rare earth species varies with pH of solutions and concentration of the rare earths. As a result, reliable correlations between diffusion current and concentration can be obtained only with rigidly defined solution conditions. Therefore, reproducible polarographic analyses of the rare earths [other than Eu(III), Yb(III), and Sm(III)] are difficult to obtain from laboratory to laboratory. Finally, Yb(II) and Sm(II) are capable of being oxidized by hydronium ions, making their polarography potentially unreliable.

Recent studies have indicated that a substance such as La(III) may never really reduce to the amalgam in water. Treindl (*40*) used classical, pulse, and oscillographic polarography in a study of lanthanum chloride in aqueous lithium chloride. He concluded that the lanthanum wave corresponded to the reduction of hydrogen via intermediates such as LaH_3 and $LaHCl_2$. Onstott (*27, 28, 29*) has reported that differential rates of gross electrolysis of rare earths in methanol are due to differences in intermediate hydride species. However, metal amalgams do form in ethanol. Liu, Budov, and Zhdanov (*15*) reported a current efficiency of only 0.1% for preparation of lanthanum amalgams at −2.1 volts. Misumi and Ide (*21*) concluded that Yb(III) waves in unbuffed media are due to hydrogen evolution. Gorokh-

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avakaya (*8*) claims the La(III) reduction wave precedes the hydrogen wave. Flanigan (*6*) has given a critical discussion of these and other peculiarities of rare earth polarography.

A nonaqueous solvent which can solvate rare earth ions to produce solution species of definite and reproducible composition without producing electrochemically active solvolysis by-products should be of interest for the rare earths. Such a solvent, ethylenediamine, has been applied to the polarography of over 30 elements (*2*, *34*–*37*). Only Pr(III) has been studied and reported as not giving a wave (*35*). Bayer (*2*) developed the reversible reference electrode, Zn/Zn-(Hg) (saturated), ZnCl$_2$ (saturated), LiCl (0.25*M*), and achieved good results with the alkali metals in ethylenediamine. Under gross electrolysis conditions, Moeller and coworkers (*23*, *24*, *25*) found nonmetallic deposits on cathodes of platinum and copper from solutions of Nd(III), Sm(III), Gd(III), La(III), and Y(III) in ethylenediamine, pyridine, *N*,*N*′-dimethylformamide, and acetonitrile. Kolthoff and Coetzee (*12*) studied the polarography of seven rare earths in acetonitrile. Neither the solvent nor the rare earth perchlorates were anhydrous and in the case of La(III) an irregular section on the rising portion of the reduction wave was found at a DME. This behavior was attributed to metal ion solvolysis with acetonitrile resulting in an insoluble acetonitrile–metal ion film and hydrogen evolution. Coetzee and Siao (*4*) have reported hydrogen interference when aquated rare earth perchlorates are used in acetone.

Much information about the rare earths in the literature has been obscured because controlled potential coulometry and chemical tests to verify electrode products have been omitted. Thus far, controlled potential coulometry has been used only to verify electrode mechanisms of rare earths in acetonitrile (*12*) and for quantitative determinations of europium and ytterbium in methanol (*43*) and europium in water (*38*).

Lanthanum, praseodymium, and ytterbium embrace the known electrochemical behavior of the rare earths and, since they are also among the least expensive of these elements, they were chosen for the present study.

EXPERIMENTAL

Apparatus. A Sargent XXI polarograph was used for all studies. Capillaries for the DME were constructed from 25-cm. long pieces of tubing that Sargent and Co. uses for its S-29417, 6- to 12-second electrodes. A longer capillary is needed, since low viscosity, lower interfacial tension, and negative potentials cause streaming with ordinary electrodes in ethylenediamine (En). Drop times and rate of flow of mercury were determined for each rare earth studied, since exposure of an electrode at the negative potentials encountered damaged the tip after 15 to 20 polarograms, causing streaming of mercury.

The polarographic cell top in Figure 1 was constructed so that En could be distilled into the assembled cell through *A*, which also guided the DME. Prepurified grade nitrogen was introduced through *B*. The bubbling tube was adjusted vertically by means of a rubber tube slip joint. The reference electrode was contained in *C*, solution contact being provided through a fine, 10-mm. frit. The bottom of *C* was enlarged to 25 mm. to provide a large electrode area. The use of a simple, nonisolated, mercury pool reference electrode proved unsuccessful in this solvent (*2*). Provision was made for a bubbling tube, *D*, or a platinum electrical contact, *E*, to be inserted through *C*-2. The arms, *C*-1 and *C*-3, were used to pass nitrogen over the reference solution and to keep the solvent level in the reference compartment at any desired level. The cell bottom (not shown) was long enough to hold 100 ml. of solution. Resistances were measured with a Serfass conductivity bridge, Model ROM15B1 (Arthur H. Thomas Co.). Accurate potential measurements were made with a Leeds and Northrup 7655 potentiometer. All studies were made at 30° ± 0.02° C. in a water bath. Half-wave potentials were calculated on an IBM 650 computer using a program of McMasters and Schaap (*16*).

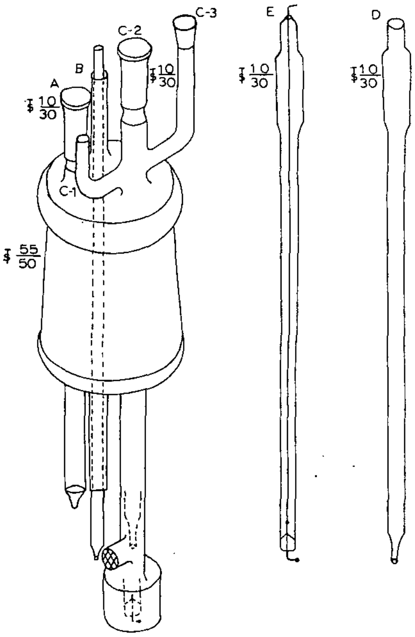

Figure 1. Polarographic cell

Position of *D* and *E* shown by dashes when fitted into C-2

Solutions and Chemicals. Anhydrous En was prepared by refluxing 98% Eastman Organic material over molten sodium for 4 hours under nitrogen, followed by three distillations over sodium and under nitrogen. The third distillate (specific conductance of 1 × 10^{-6} to 2 × 10^{-7} mho cm.$^{-2}$) was refluxed over molten sodium until needed. Then, En was distilled directly into the cell under nitrogen. Such a solvent had an average specific conductance of 5 × 10^{-7} mho cm.$^{-2}$

The supporting electrolyte was anhydrous tetrabutylammonium perchlorate (TBAP) (*12*) and was prepared by neutralizing 50 ml. of 1.0*M* tetrabutylammonium hydroxide (Southwestern Analytical Chemicals) with the stoichiometric amount of dilute perchloric acid. The precipitate was stirred for 15 minutes, filtered, washed with copious amounts of cold water, dried at 100°, and stored in a desiccator. Drying at this temperature and grinding in a mortar at room temperature produced no explosions. However, one should be aware of the general hazard of organic perchlorates. Of the common quaternaries reported for polarography (*39*), TBAP is the easiest to prepare and the most stable in En. The iodides are particularly susceptible to decomposition in En.

Anhydrous rare earth acetates were used because of their favorable solubility in En (*25*), ease of preparation (*18*, *44*), and nonhydroscopicity. Twenty-five grams of 99.99% lanthanum oxide (code 528, Lindsay Chemical) were mixed with a 20% mole excess of glacial acetic acid and 500 ml. of water. The mixture was stirred at 100° C., maintaining constant volume, until all the oxide dissolved. The solution was evaporated to dryness and the resulting cake ground to a powder, dried for 24 hours at 150° C., and stored in a desiccator. Praseodymium and ytterbium acetates were prepared similarly, starting with 99.9% oxides (codes 729.9 and 1202, respectively, Lindsay Chemical).

Hydroxy- and oxoacetates (*1*) were excluded on the basis of acetate analysis (*30*) and EDTA titration of the rare earth content (*3*). At least four determinations were performed for each analysis.

Anhydrous Lanthanum Acetate. Theoretical, 43.95% La, 56.05% acetate; found, 43.85% La, 56.01% acetate, 99.85% total.

Anhydrous Praseodymium Acetate. Theoretical, 44.31% Pr, 55.69% acetate; found, 44.19% Pr, 56.04% acetate, 100.23% total.

Anhydrous Ytterbium Acetate. Theoretical, 49.42% Yb, 50.58% acetate; found, 48.97% Yb, 50.32% acetate, 99.29% total.

The reference electrode was prepared before each polarographic run by pouring 2 grams of saturated zinc amalgam into the bottom of part *C*, Figure 1, followed by 0.2 gram of dried (150°) zinc chloride (J. T. Baker) and 20 ml. of 0.25*M* lithium chloride (J. T. Baker). The two-phase zinc amalgam (about 2%) was prepared by electrolyzing aqueous zinc sulfate at a stirred mercury pool, drying, filtering through a pinhole, and storing under nitrogen. Only a bright shiny material was used. The 0.25*M* lithium chloride solutions were expedited by heating En to 90° C. All

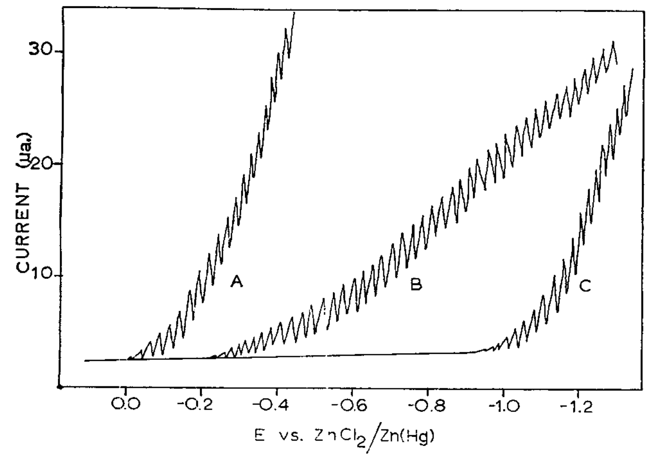

Figure 2. Residual current behavior in ethylenediamine

A. Impure, 0.1*M* quaternary supporting electrolytes
B. Wet solutions of 0.1*M* TBAP in En
C. Pure dry 0.1*M* TBAP in pure, dry En

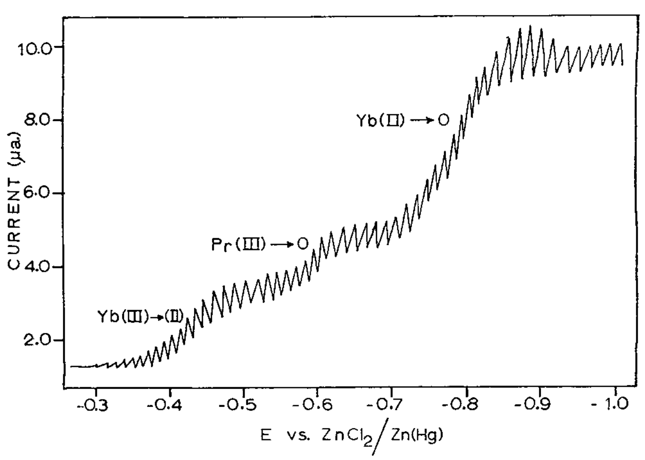

Figure 3. Polarogram of Yb(III) and Pr(III) mixture

Yb(III) = 3.20 × 10^{-3} *M*
Pr(III) = 4.63 × 10^{-4} *M*

potentials in this work are reported against the zinc reference electrode and are designated *E vs.* Zn. Based upon relative values of cesium potentials in water and En (*2*), the estimated potential of this reference is -1.9 volts *vs.* aqueous saturated calomel.

Procedure. The polarographic cell and related parts were thoroughly washed with dilute hydrochloric acid, rinsed, and dried in an oven. The cell was cooled to room temperature by flushing with a heavy stream of nitrogen. About 100 ml. of purified En was distilled into the cell under nitrogen. The reference electrode was simultaneously prepared. A previously weighed capsule of TBAP (3.40 grams) was added to the cell and the assembly transferred to the water bath. The solution was stirred with nitrogen for 15 minutes. All polarograms were run under a nitrogen blanket. If the residual current was less than 1.0 μa. out to -1.0 volt, the solvent was judged satisfactory. A weighed amount of the rare earth acetate was added and dissolution aided by nitrogen bubbling. The resistance of the cell was measured next (usually 12 to 18 kohms). After making the proper polarographic measurements, the volume of the solution in the cell was measured accurately in order to establish the rare earth concentrations. A careful account was kept of movement of En through the reference frit and, since all glass surfaces are uniformly wetted by En, the volume measurement was accurate to 0.5 ml. or less.

The number of electrons for each polarographic step was established by the pilot ion coulometric procedure of Meites and Cover (*19*). Cadmium acetate ($E_{1/2}$, 0.53 volt) was used as the pilot ion and was prepared by drying reagent grade material (J. T. Baker) at 110° C. for 24 hours and then at 170° C. for 24 hours.

The formation of rare earth amalgams was verified by electrolysis at a stationary mercury drop (0.06-sq. cm. area) for 8 to 10 hours at a potential on the diffusion limited plateau corresponding to the reduction of the rare earths to the metallic state. The electrolysis current was followed with the polarograph and decayed exponentially with time (nitrogen bubbling). The hanging drop was carefully removed and washed with distilled water, and any rare earth was stripped out by boiling the drop in concentrated hydrochloric acid for 1 hour. The solution was evaporated to dryness and the residue taken up in 15 ml. of water. The qualitative Alizarin Red S test of Rinehart (*33*) was used to identify each rare earth. Blanks run on nonelectrolyzed mercury drops gave negative tests.

RESULTS AND DISCUSSION

Figure 2 illustrates the residual current behavior when the supporting electrolyte was impure (curve *A*), when either En or TBAP was wet (curve *B*), and when En and TBAP were properly purified (curve *C*). The solutions were extremely sensitive to moisture, oxygen, and carbon dioxide and had to be protected properly. Tetraalkyl and aryl ammonium iodides, bromides, and chlorides never gave satisfactory residual currents in spite of repeated purifications.

Polarographic maxima of the first type were encountered when the rare earth solutions were more than $10^{-3}M$.

Table I. Polarographic Behavior of La(III), Pr(III), and Yb(III) in Ethylenediamine

Rare earth	Concn., mole/l.	i_d (corr.), μa.		i_d/C, μa./mole × 10^4		$E_{1/2}$ (corr.), volt *vs.* Zn		Av. I	Av. $E_{1/2}$, volt *vs.* Zn
La(III)	3.13 × 10^{-4}	0.82		0.262		−0.5585			
(one wave)	5.44 × 10^{-4}	1.40		0.263		−0.581			
	6.75 × 10^{-4}	1.74		0.261		−0.560			
	7.54 × 10^{-4}	1.92		0.255		−0.557			
	9.44 × 10^{-4}	2.46		0.260		Maximum		4.15	−0.565
Pr(III)	3.30 × 10^{-4}	0.90		0.273		−0.574			
(one wave)	5.76 × 10^{-4}	1.56		0.271		−0.566			
	6.24 × 10^{-4}	1.74		0.279		−0.561			
	1.83 × 10^{-3}	4.90		0.268		Maximum			
	2.84 × 10^{-3}	7.40		0.261		Maximum		4.88	−0.571
		1st	2nd	1st	2nd	1st	2nd		
Yb(III)	2.80 × 10^{-4}	0.34	0.69	0.095	0.193	−0.406	−0.763		
(two waves)	6.20 × 10^{-4}	0.60	1.20	0.097	0.193	−0.415	−0.756		
	9.75 × 10^{-4}	0.90	1.92	0.093	0.197	−0.419	−0.756		
	1.29 × 10^{-3}	1.24	2.52	0.096	0.195	−0.412	−0.758		
	2.48 × 10^{-3}	2.50	4.70	0.101	0.189	Maximum		1.51; 3.13	−0.411; −0.747

Table II. Summary of Capillary Conditions for Table I

Wave	Drop time at potential E		Hg flow rate at potential E		Ht. of Hg (corr.), cm.	Capillary length, cm.
	Sec.	Volt	Mg./sec.	Volt		
La(III)	3.3	−0.80	0.368	−0.80	35.3	25
Pr(III)	3.7	−0.82	0.302	−0.82	35.4	25
Yb(III → II)	8.6	−0.52	0.295	−0.52	32.4	28
Yb(II → 0)	6.2	−0.87	0.298	−0.87	32.4	28

Table III. Polarography of Rare Earth Mixtures in Ethylenediamine

Concentration, mole/liter		i_d, R.E. 1, µa.		i_d, R.E. 2, µa.	
R.E. 1	R.E. 2	Exptl.	Calcd.	Exptl.	Calcd.
[a]6.35×10^{-4}, La	1.22×10^{-3}, Pr	1.72	1.71	3.56	3.60
[a]4.15×10^{-4}, La	8.70×10^{-3}, Pr	1.12	1.04	2.64	2.75
[b]4.63×10^{-4}, Pr	3.20×10^{-3}, Yb	1.34	1.33	2.90; 5.88	2.93; 5.85
[b]9.90×10^{-4}, La	2.52×10^{-3}, Yb	2.42	2.39	2.40; 4.71	2.34; 4.55

[a] t and m at −0.80 volt were 3.62 sec. and 0.341 mg. sec.$^{-1}$, respectively.

[b] t and m at −0.56, −0.70, −0.87 volt were 5.7, 4.3, 3.8 sec. and 0.308, 0.317, 0.316 mg. sec.$^{-1}$, respectively.

Table IV. Pilot Ion Coulometry of Rare Earths with Cadmium

Ion concn., mole/l.	E of electrol., volt *vs.* Zn	Electrol. time, hr.	$i_d)^\circ$, µa.	$i_d)^t$, µa.	n for rare earths
Cd^{+2} 1.41×10^{-3}	−0.87	12	4.68	1.68	...
Yb(III) 2.92×10^{-3}	−0.87	12	2.64	1.56	1.09 1st wave
Yb(II) 2.92×10^{-3}	−0.87	12	5.20	2.68	1.89 2nd wave
Cd^{+2} 8.70×10^{-4}	−0.82	4	3.32	2.64	...
Pr(III) 1.57×10^{-3}	−0.82	4	4.24	3.78	2.88
Cd^{+2} 1.61×10^{-3}	−0.82	6	4.90	2.48	...
La(III) 7.74×10^{-4}	−0.82	6	2.00	1.34	2.87

Attempts to eliminate the maxima were abandoned, since successful suppression led to contamination of the solvent to such an extent that well defined polarographic waves were not obtained. Where the situations existed, it was assumed that maxima did not change the diffusion currents. All reduction waves were totally irreversible and had diffusion-controlled limiting currents (i_d *vs.* $\sqrt{h}$ test).

Tables I and II give selected results of individual solutions of La(III), Pr(III), and Yb(III) acetates. Table III shows the results of various mixtures of the ions. Table IV is a summary of the data needed to determine the number of electrons using Equation 1 of Meites and Cover (*19*):

$$\log \frac{i_d)^\circ_{cd}}{i_d)^t_{cd}} = \log \frac{i_d)^\circ_{r.e.}}{i_d)^t_{r.e.}} \times \frac{i_d)^\circ_{cd}\ n_{r.e.}\ C^\circ_{r.e.}}{i_d)^\circ_{r.e.}\ n_{cd}\ C^\circ_{cd}} \quad (1)$$

where $i_d)^\circ$ and $i_d)^t$ are diffusion currents initially and after electrolysis times t, n is the number of electrons, and C° is the initial bulk concentration. Only one reduction wave involving three electrons was found for La(III) and Pr(III). The half-wave potentials are not sufficiently different to distinguish mixtures of the two. However, Table III verifies additive wave heights for admixtures. It is certain that both lanthanum and praseodymium reduce to metal amalgams, since prolonged electrolysis at a stationary mercury drop gave positive chemical tests for both elements in the mercury phase. Polarographic waves due to lyonium ion reduction (−0.25 volt) were not found.

Preliminary studies of Eu(III) acetate produced two, irreversible well defined waves, the first at −0.20 volt and the second at −0.57 volt. Comparing these potentials with the two Yb(III) waves (−0.411 and −0.741 volt), we find markedly different behavior from that reported for acetonitrile (*12*) and water. In all three solvents Eu(III) reduces earlier than Yb(III). However, the second waves for Eu(II) and Yb(II) are at the same potential in acetonitrile and water. Actually, the evidence for the second Eu(II) wave in water has not been satisfactorily presented. In En, under strict anhydrous conditions, the first waves of Eu(III) and Yb(III) are separated by the same amount and are in the same order as their second waves. The second wave of ytterbium leads to amalgam formation, as shown by a positive Alizarin Red S test. In mixtures of Pr(III) and Yb(III), three waves could be observed only when the Yb(III) concentration was five times as large as Pr(III) (Figure 3). At smaller ratios, the Pr(III) wave blended in with the second wave of Yb(II), but wave heights were still additive.

After prolonged reduction of Yb(III) at the DME, no oxidation wave for Yb(II) could be observed, even though the limiting current for Yb(III) decreased. Rate of electron transfer studies (*14*, *20*) established a value of 1.15×10^{-9} cm. sec.$^{-1}$ at 0.0 volt, for the reductive rate and 0.48 for the transfer coefficient, α. Assuming the reverse oxidative rate to be equal to the forward rate and a value of 0.52 for $(1-\alpha)$, the calculated current at the anodic limit of +1.0 volt in En is 0.13 µa. for equal concentrations of Yb(III) and Yb(II). Therefore, no anodic wave can be observed due to such large anodic overvoltages. Gierst (*7*) and Randles (*32*) have reported unusual effects for the anodic polarography of Eu(II). These effects are reflected in values of α close to 1.0 and have been attributed to charge distribution in the Helmholtz layer. Little interest has been paid to hydroxyeuropium species. Where double layer effects are encountered, studies under completely anhydrous conditions should shed some light upon the importance of aqueous reactions such as the electrically neutral Reaction 2 which has been reported (*17*):

$$Eu(OH)^{+2} + e^- \rightarrow Eu^{+2} + OH^- \quad (2)$$

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Anodic Stripping Voltammetry of Gold and Silver with Carbon Paste Electrodes

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▶ Carbon paste electrodes have been applied to the determination of submicrogram quantities of gold and silver using the general technique of anodic stripping voltammetry. The effects of electrode material, electrode area, supporting electrolyte, deposition potential, deposition time, deposition solution volume, stirring rate, and anodic voltage scan rate are discussed. A simple, easily prepared carbon paste electrode provides the determination of as little as 1.0 p.p.b. of gold and 0.25 p.p.b. of silver with approximately 10% relative error. A modified Sargent Model XXI Polarograph was used for these analyses.

ANODIC stripping techniques have been a subject of renewed interest during the past few years, due in part to the remarkable sensitivity that can be obtained with this electroanalytical method. In these analytical applications the metal to be determined is electrodeposited from a dilute solution onto a microelectrode and is then stripped from this electrode by anodic oxidation. The stripping has been carried out using linear voltage scan (*4*), current-step procedures (*7*), and square wave polarography (*3*). Generally some form of a mercury electrode has been used. Platinum and gold electrodes have been employed (*5*) to provide greater sensitivity because of more complete recovery of the deposited metal and at the same time provide a more positive potential range.

One real difficulty with the noble metal electrodes has been the inability to treat them properly, so as to give reproducible results, either qualitative or quantitative. This is due to the oxide films formed in oxidizing and noncomplexing media (*2*) and chloride films formed by platinum in hydrochloric acid solutions (*6*).

This paper reports on the use of a carbon paste electrode (C.P.E.) for use in the anodic stripping voltammetry of the noble metals, gold and silver. Adams (*1*) first reported the use of a C.P.E. for organic polarography in 1958; however, its application to inorganic stripping analysis reported in this paper is new.

The carbon paste electrode has a number of characteristics which make it very good for anodic stripping voltammetry.

It is a relatively inert electrode, whose surface can be easily renewed to give reproducible results and the electrodeposited metal can be completely recovered by anodic stripping. The electrode is almost entirely free of residual currents and has a wide range of anodic potential use.

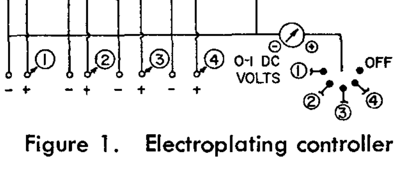

Figure 1. Electroplating controller

EXPERIMENTAL

Apparatus. A Sargent Model XXI Polarograph was used for all anodic stripping analyses. The recorder was modified to give a 2.5-second full-scale pen response and the linear voltage drive was increased for most of the work from 2 to 15 r.p.m. To run more analyses per day, a simple four-position electroplater (Figure 1) was used during the plating cycle. This freed the polarograph for recording the anodic stripping current-voltage curves. The electrolysis cell shown in Figure 2 used a saturated calomel electrode (S.C.E.) as a reference electrode. This cell had a resistance of about 800 ohms with a solution of $0.1N$ hydrochloric acid. A small magnetic stirrer was used to agitate the solutions. The cell was not thermostated for this work.

The various carbon paste electrodes used in this study are shown in Figure 3. All were easily prepared from female glass joints of different sizes, and the area of each size proved to be reproducible from one joint to another. Electrical contact is made to the carbon paste by means of a copper wire sealed to the glass with De Khotinsky cement.

Materials. All chemicals were reagent grade and were used without further purification. Stock solutions of each metal ion were prepared at 10^{-3} and $10^{-5}M$ concentrations, and diluted as necessary, immediately prior to use. All solutions were made with double-deionized water.

A nitrogen gas purge was used to remove oxygen from the solutions before determining silver. Because of the positive reduction potentials used for gold, it was unnecessary to remove the oxygen before gold determinations.

All glass apparatus and Teflon stirrers were cleaned prior to each determination with aqua regia and rinsed with double-deionized water. The tip of the calomel electrode was cleaned with chromate–sulfuric acid cleaning solution and then rinsed with double-deionized water prior to each determination.

The carbon paste was prepared by mixing 50 grams of Acheson's No. 38 graphite powder with 20 ml. of Nujol to obtain a thick uniform paste. If the amount of Nujol was decreased, the sensitivity of the electrode was increased, but the paste became too dry to permit the preparation of reproducible surfaces.

The carbon paste electrode was resurfaced with fresh paste prior to each determination. Usually about 3 days' experience was needed before a technician became proficient enough to prepare electrodes which would give results with only 2 to 3% relative standard deviation.

METHOD

The anodic stripping method involves plating gold and/or silver onto a carbon paste electrode from a stirred solution for a predetermined length of time, usually 15 minutes. The stirring must be reproducible; thus, placement of the electrodes, volume of solution, and stirring rate must be fairly reproducible. The potential of the C.P.E. during the plating cycle was held at a predetermined value, usually +0.1 volt for gold and −0.3 volt *vs.* S.C.E. for silver and mixtures of silver and gold.

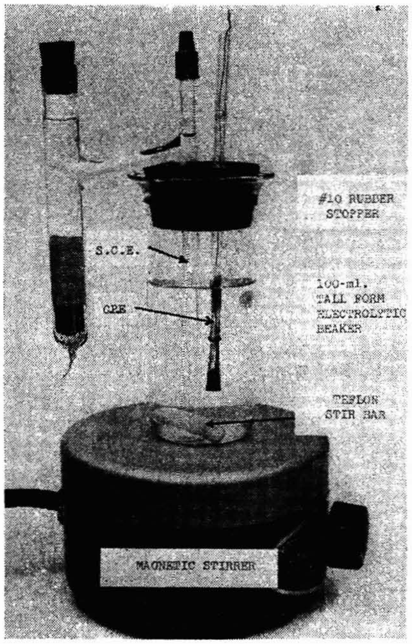

Figure 2. Electrometric cell

After the appropriate electrolysis time had elapsed, the electrodes were disconnected from the electroplater and connected to the polarograph. The C.P.E. was connected to the normal D.M.E. lead and made positive with respect to the S.C.E. After about 20 seconds for the current to decay to a constant value, the anodic stripping wave was recorded by linearly varying the voltage over a range of +0.3 to 1.3 volt *vs.* S.C.E. for gold, −0.3 to +0.7 volt *vs.* S.C.E. for silver, and −0.3 to +1.3 for mixtures of gold and silver. The optimum rate of voltage scan was 1200 mv. per minute.

Typical current-voltage dissolution curves for silver and gold at different concentrations are shown in Figure 4.

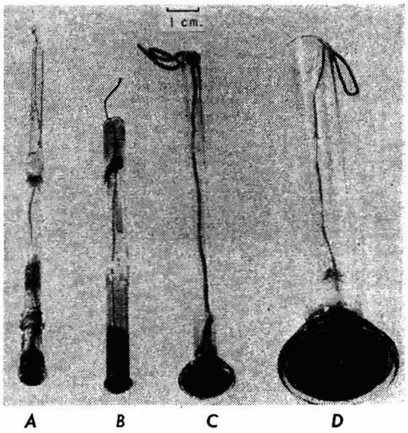

Figure 3. Carbon paste electrodes

A. 0.196 sq. cm.
B. 0.283 sq. cm.
C. 0.502 sq. cm.
D. 1.32 sq. cm.

These determinations were made with a 0.283-sq. cm. C.P.E. from 100 ml. of solution stirred at 1400 r.p.m. and electrolyzed for 15 minutes. The background for each metal ion was determined by anodic stripping after a 15-minute electrolysis of the electrolyte solution. The E_p (peak potential) became slightly more positive with the smaller dissolution waves.

To investigate the variables, precision, and applicability of this method, gold and silver ion concentrations were measured over a range of 5×10^{-7} to $5 \times 10^{-9}M$.

VARIABLES

Besides the electrode material at least seven other variables affect the results of an anodic stripping determination.

Supporting Electrolyte. The potential range for the C.P.E. as well as the plating potential for each metal will differ for each supporting electrolyte. A solution of 0.1*N* nitric acid proved to be the optimum electrolyte for silver, since it gave the greatest plating rate and the most reproducible results. A solution of 0.1*N* hydrochloric acid proved to be the best medium for gold for the same reasons. Nitric acid was used for analysis of mixtures of these metals. The useful potential range of the C.P.E. in either of these two solutions was from approximately −0.4 to +1.25 volt *vs.* S.C.E.

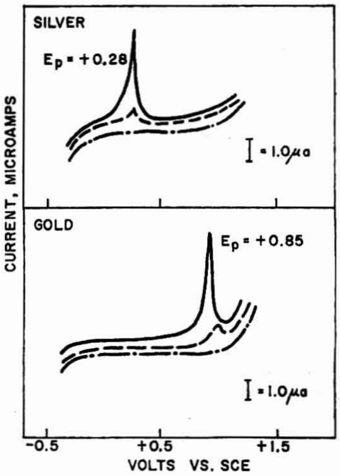

Figure 4. Current-voltage curves for anodic stripping of gold and silver on C.P.E.

—— $10^{-7}M$
- - - $10^{-8}M$
— · — Background

Deposition Potential. A general rule of thumb for anodic stripping voltammetry is to operate the potential of the working electrode during the plating cycle at a value about 0.2 volt more cathodic than the polarographic half-wave potential. Since the reduction half-wave cannot be measured for values below $10^{-6}M$, it is necessary to predict the reduction potential from the Nernst equation. At values of $10^{-6}M$ and lower this prediction may not be strictly obeyed on all electrode surfaces, so it was necessary to determine the optimum plating potential experimentally. In general, the reduction potentials of both silver and gold became more negative as the concentration of the metals decreased.

Figure 5 shows a plot of the plating potential *vs.* the relative amount of metal plated within 30 minutes for solutions of gold and silver at several concentrations. The amount of each metal plated was determined by anodic stripping determinations made on each metal at the various indicated potentials. The amount plated at −0.4 volt *vs.* S.C.E. was taken as 100%.

The conclusions drawn from this figure are that it is possible to plate gold and not silver at the expense of losing some sensitivity for determining gold. Actually in practice we were not able to plate gold without plating some silver. This is possible only by very carefully controlling the cathodic potential, which was not attempted. From the curves

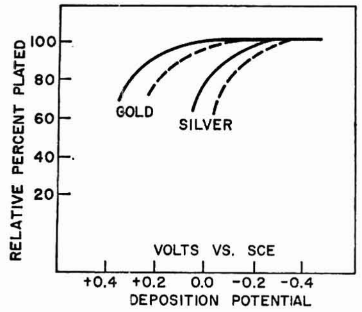

Figure 5. Effect of deposition potential on amount of metal plated

—— $10^{-7}M$
--- $10^{-8}M$

for these metals a value of +0.1 volt was selected for gold and −0.3 volt *vs.* S.C.E. for silver and mixtures of gold and silver as operating plating potentials.

Deposition Time. The amount of metal plated from a solution of a certain concentration is directly proportional to the time of electrolysis, as shown by Figure 6. The amount plated was determined from the coulombs of electricity represented by the area under the anodic stripping peak. The per cent plated was determined by comparing these anodic stripping analysis values to the theoretical amount initially present in the plated solution. These results were obtained from determinations made on $10^{-7}M$ solutions with a 0.5-sq.-cm. area C.P.E. However, these are general curves and they represent the rate of plating for these metals at any concentration onto a carbon paste electrode.

It is obvious from this plot that it is impractical to plate all of the metal in an analytical procedure. For a gold concentration of $10^{-7}M$ a period of 15 minutes is sufficient to plate about 0.1 μg. of gold and this is equivalent to 50 microcoulombs or a flow of 5 μa. for 10 seconds, which can be measured by the apparatus used in this study. The results for anodic voltammetric determination of gold and silver represented by peak height *vs.* concentration for a 15-minute electrolysis time (Table I) show that the peak current is linearly related to the concentration. Generally the peak area in coulombs is used to measure the amount plated, but in these analyses the peak height measurements were just as precise as the area measurements.

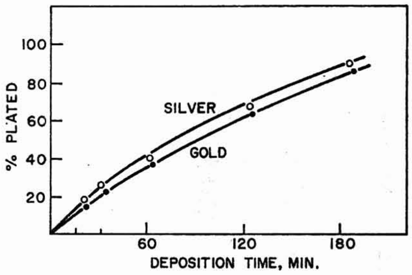

Figure 6. Effect of plating time on amount of metal plated

Deposition Solution Volume. While the rate of plating is proportional to the concentration, it is possible, in the case of extremely long deposition periods, to increase the amount of metal plated by plating from very large volumes of solution. The rate of change of concentration during electrolysis is changed by going to larger volumes, since the ratio of the amount of metal plated out to the amount remaining in solution is smaller. For example, the amount of gold plated from 100 ml. of a $10^{-8}M$ solution of gold during a 1-hour period can be doubled by plating from 1200 ml. of the same solution. Since all of our work was done with at least 100 ml. of solution, the volume required to achieve a worthwhile increase in the amount plated was much larger than practical for general use.

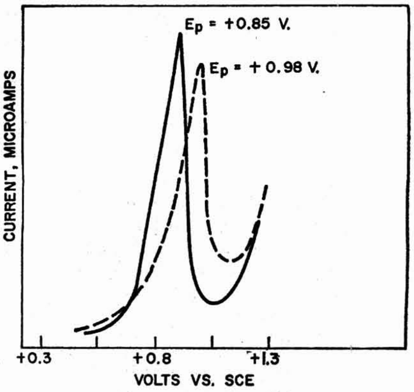

Figure 7. Effect of stirring during anodic stripping of gold

--- Unstirred
—— Stirred

Electrode Area. The larger the area of an electrode the greater was the amount of metal deposited within a given time period. To determine the optimum electrode area for plating and stripping, gold was plated from a 2.5 × $10^{-7}M$ solution onto C.P.E.'s whose apparent surface area varied from 0.196 to 1.32 sq. cm. for 30 minutes at a potential of +0.1 volt *vs.* S.C.E. The amount plated was determined by measuring the anodic stripping peak height. Under these conditions one would expect the ratio of the amount plated (peak height) to the electrode area to be a constant, and the ratio of the residual current during the stripping cycle to the electrode area to be constant. This was only partly true, as shown by the results in Table II. The smallest electrode had a larger plating rate and a greater residual current in proportion to its area. Also, the largest electrode had the lowest plating rate and a high residual current in proportion to its area. Thus it would seem that there is a lower and a higher limit to the electrode area and the optimum area for analysis should be about 0.3 sq. cm.

Stirring Rate. The sensitivity of this method depends on the amount of metal plated, which in turn depends on the diffusion of the metal ion to the cathode during plating. Stirring the solution will increase the rate of diffusion, as shown in Table III. A stroboscope was used to measure revolutions per minute.

Although higher values for the peak height are obtained with each successive increase in the rate, 1400 r.p.m. is the practical limit. Above this value the peak height was not reproducible, because of formation of bubbles in the

Table I. Peak Current as a Function of Concentration

(Electrolysis time, 15 minutes)

Ion	Concn., mole/liter	i_p, μa.
Au^{+3} (0.1M HCl)	5.0 × 10^{-9}	0.12
	1.00 × 10^{-8}	0.21
	5.00 × 10^{-8}	1.34
	1.00 × 10^{-7}	2.78
	2.50 × 10^{-7}	7.00
Ag^+ (0.1M HNO_3)	5.00 × 10^{-9}	0.19
	1.00 × 10^{-8}	0.36
	5.00 × 10^{-8}	1.81
	1.00 × 10^{-7}	3.68
	2.50 × 10^{-7}	9.39

Table II. Effect of Electrode Area on Residual Current and Amount of Gold Found at Constant Plating Time

Electrode area, sq. cm.	Peak height, μa.	Peak height/ electrode area	Residual current/ electrode area
0.196	8.52	43.5	0.68
0.283	10.7	38.1	0.49
0.502	18.2	36.2	0.60
1.32	43.4	32.8	0.64

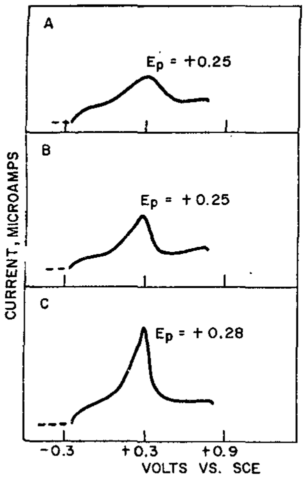

Figure 8. Effect of linear voltage scan rate on anodic stripping of silver

A. 150 mv./min.
B. 562 mv./min.
C. 1200 mv./min.

solution and on the surface of the carbon paste electrode.

Most of the investigations with anodic stripping voltammetry reported in the literature have used quiet solutions during the dissolution cycle, in order to avoid vibrations and obtain the best precision. However, the current-voltage curves shown in Figure 7 indicate that stirring during the anodic stripping cycle with carbon paste electrodes gave greater sensitivity, more symmetrical peaks, and more negative peak potentials. A relative precision of 2 to 3% was maintained in our analyses. The stirring rate used during the dissolution cycle was the same as that used for plating the metal.

Table III. Effect of Stirring Rate on Amount of Gold Plated

Stirring rate, r.p.m.	Peak height, μa.
400	4.5
600	6.2
800	7.5
1200	8.7
1400	9.6
1600	10.2

Voltage Scan Rate. The effect of the voltage scan rate during the stripping cycle is demonstrated by the curves shown in Figure 8. These are the results of a 30-minute plating period from a $10^{-7}M$ silver solution onto a 0.5-sq.-cm. area C.P.E. Not only does the peak height increase with the scan rate but the shape of the peak becomes more symmetrical. While it is probable that greater sensitivity for silver could be achieved with faster linear scan rates, this represents the fastest practical rate for gold, since at faster rates the gold peak cannot be separated from the background.

Calibration. The method was calibrated by plating each metal ion onto a 0.283-sq.-cm. C.P.E. from 100 ml. of a standard solution for 15 minutes. The solution was stirred at 1400 r.p.m. during the plating and stripping cycles. The results of peak height measurement of the anodic wave obtained by stripping the metal at 1200 mv. per minute as compared to the molar concentration are given in Table I.

Precision. The results of anodic stripping analyses on eight separate, but identical samples, run by two operators, using three different electrodes in separate cells, over a period of 2 days are given in Table IV. The precision of the method at this level of concentration is extremely good.

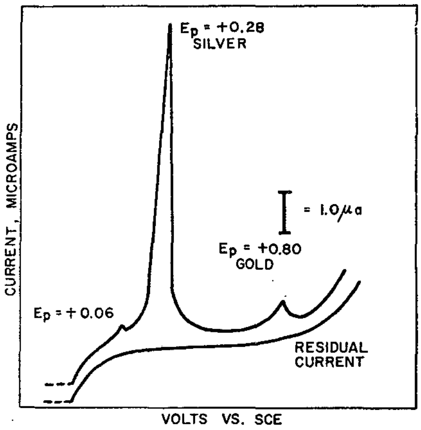

Figure 9. Current-voltage curve for anodic stripping of a mixture of gold and silver

Gold concn. = $1 \times 10^{-8}M$
Silver concn. = $1 \times 10^{-7}M$
Plating time = 15 min.

Table IV. Precision of Anodic Stripping Analysis with Carbon Paste Electrode

(Gold concn. = $2.5 \times 10^{-7}M$)

	CPE-1	CPE-2	CPE-3
No. of detns.	8	8	8
Av. peak height, μa.	11.60	11.44	11.88
Std. dev., μa.	±0.60	±0.37	±0.29
Rel. std. dev., %	±5.17	±3.21	±2.44

Over-all

Av. peak height, μa. = 11.64
Std. dev., μa. = ±0.39
Rel. std. dev., % = ±3.34

Mixed Compounds. The current-voltage curve shown in Figure 9 illustrates the possibility of determining two components simultaneously. The little pip at 0.06 volt *vs.* S.C.E. is due to a trace impurity of copper.

ACKNOWLEDGMENT

I thank Doris L. Carter, who performed the major portion of the analyses reported here and also contributed the idea for using the glass joints as carbon paste electrodes.

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Determination of Some Heavy Metal Ions by Complexometric Titration with Sodium Azide Indicator

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▶ Copper and iron can be determined in acid solution by titration against (ethylenedinitrilo)tetraacetic acid (EDTA) using sodium azide as an indicator. The end point depends upon the difference between the colors of each of the metal azide complexes and the corresponding chelate with EDTA, and upon the fact that the instability constants of the former are greater than that of the latter. Zinc, aluminum, and nickel can also be determined by back-titrating the excess EDTA added to each of these ions against a standard copper solution, using the azide as an indicator.

During the investigations of the orange uranyl (*4*) and green chromium(III) (*5*) complex ions with the azide as a ligand, it was found that small amounts of (ethylenedinitrilo)-tetraacetic acid, EDTA, would reduce the interference of Fe(III) and Cu(II) ions appreciably. The blood-red color of the ferric azide complex and the olive green color of the copper azide ion disappeared completely. These observations suggested the possible use of EDTA as a titrating agent for these ions, using the sodium azide as an indicator. The end point would be dependent upon the difference between the color of the metal azide complex ion and that of the EDTA metal chelate, and also upon the fact that the instability constants of the azide complexes (*1, 2*) are greater than that of the EDTA chelates (*3*). Very few inorganic substances have been used as indicators in complexometric titrations. For example, iodide has been used in the titration of bismuth, and thiocyanate in the titration of iron. Sodium azide, besides being a simple inorganic reagent, has the advantage of offering a buffering medium in presence of suitable amounts of a strong acid such as HCl, and thus the method is comparatively insensitive to variations in the pH values of the solutions.

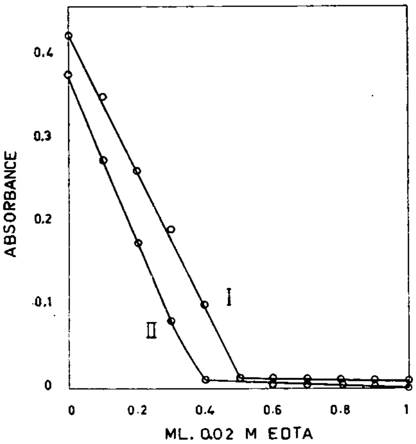

Figure 1. Spectrophotometric titration of copper(II) and iron(III)

I. Cu(II), 0.6354 mg.; wavelength, 400 mμ
II. Fe(III), 0.4468 mg.; wavelength, 450 mμ

In this work, the determination of Cu(II) and Fe(III) in small quantities in solution is presented. Conditions for a visual end point, direct and reverse, against EDTA, together with a spectrophotometric end point, were studied. The possibility of determining metal ions such as Zn, Al, and Ni by back-titrating the excess EDTA with a standard solution in the presence of azide indicator is introduced.

EXPERIMENTAL

Apparatus. A Unicam spectrophotometer, Model SP 500, with 1-cm. glass cells was used. The pH was measured using a Marconi pH meter, type TF 511C.

Reagents. A stock solution of Cu(II), 0.1*M*, was prepared from crystallized copper sulfate. The copper content was determined gravimetrically as the thiocyanate. A stock solution of Fe(III), 0.08*M*, was prepared from AnalaR ferric chloride. The iron content was determined gravimetrically as the oxide. Other concentrations were prepared from these by appropriate dilutions. AnalaR $AlCl_3 \cdot 6H_2O$ was used for the preparation of a solution 0.01*M* in Al(III). The Al(III) content was determined gravimetrically as the oxide. The nickel solution was prepared from $NiCl_2 \cdot 6H_2O$ and analyzed gravimetrically as the dimethylglyoxime complex. AnalaR zinc metal was dissolved in dilute hydrochloric acid and a solution 0.1148*M* in Zn was obtained. This zinc solution was utilized for the standardization of EDTA, using Eriochrome black T as an indicator to prepare a 0.02*M* solution of EDTA (*6*). A 1*M* sodium azide solution was prepared and analyzed by titration against standard silver nitrate, using potassium chromate as an indicator.

Procedure. Copper Determination. Ten milliliters of a 0.01*M* copper sulfate solution were mixed with 25 ml. of 0.01*N* HCl, and 2 ml. of 1*M* sodium azide indicator were added. The EDTA solution was added dropwise to the intense brown copper solution. This brown color is due to the copper azide complex formed in presence of excess azide. The color changed gradually to olive green and suddenly to blue at the equivalence point. The titration was reversible. The blue end point is due to the formation of copper EDTA complex. Back-titrations were also carried out by adding 0.01*M* Cu(II) from a buret to a solution containing 10 ml. of 0.01*M* Cu(II), 2 ml. of azide, 25 ml. of 0.01*N* HCl, and 10 ml. of 0.02*M* EDTA. The blue color of the mixture changed to green at the end point. Spectrophotometric titrations were carried out between EDTA added in 0.05-ml. portions and solutions containing 1 ml. of 0.01*M*

Table I. Results of the Determination of Copper, Iron, Zinc, Aluminum, and Nickel

Element	Taken, mg.	Found, mg.	Relative error, %
Cu(II)	1.271[a]	1.271	0.00
	6.354[a]	6.364	+0.15
	0.635[a]	0.635	0.00
	6.354[b]	6.345	−0.14
	6.354[c]	6.354	0.00
	0.635[d]	0.635	0.00
Fe(III)	2.234[a]	2.198	−1.12
	4.468[a]	4.383	−1.90
	6.702[a]	6.702	0.00
	8.937[b]	8.760	−1.98
	4.468[c]	4.488	+0.45
	0.447[d]	0.447	0.00
Zn(II)	15.018[c]	15.028	+0.06
	15.018[c]	15.018	0.00
	7.509[c]	7.509	0.00
Al(III)	4.046[c]	3.911	−3.33
	4.046[c]	3.992	−1.33
	0.269[c]	0.261	−2.96
Ni(II)	8.804[c]	8.751	−0.60
	8.804[c]	8.810	+0.07
	8.804[c]	8.751	−0.60

[a] Direct titration.
[b] Reverse titration.
[c] Back titration.
[d] Spectrophotometric titration.

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Cu(II) and the same acid and azide mentioned before. Optical measurements were taken at 400 mμ, which is in the vicinity of the wavelength of the maximum absorption of the copper azide complex, 380 mμ.

Iron Determination. A mixture of 5 ml. of 0.008M Fe(III), 2.5 ml. of 0.1N HCl, and 0.2 ml. of 1M azide indicator, diluted to 25 ml. with distilled water, gave an intense red color. It is important to emphasize here that the amount of azide should not exceed the above limit, otherwise the titration mixture would become turbid because of the formation of the basic ferric azide. Even in cases where more acid was added to prevent the formation of the basic salt, increased azide concentration would render the end point obscure. The addition of 0.02M EDTA to the above mixture masks the red color and the end point is reached when the solution turns from orange-red to faint yellow. The pH of these titrations should be below 3. Back-titrations were carried out by adding excess EDTA to the iron solution and back-titration with 0.008M Fe(III). The end point in this case is sharper and is obtained when the solution turns from faint yellow to orange-red. Spectrophotometric titrations were also carried out using 1 ml. of 0.008M Fe(III) and the color was measured at 450 mμ, which is the wavelength of maximum absorption for the ferric azide complex.

Zinc Determination. A known excess of EDTA, 17.5 ml. of 0.02M, was added to 2 ml. of 0.1148M Zn solution, and 2 ml. of 1M sodium azide was added. The mixture was titrated against 0.02M Cu(II) solution. As the copper ion was introduced, it reacted with the EDTA, forming the blue chelate. The blue color increased gradually, and when the end point was reached, the solution turned suddenly to green, the color of the copper azide complex.

Aluminum and Nickel Determination. A mixture containing 15 ml. of 0.01M Al(III), 17.5 ml. of 0.02M EDTA, and 1 ml. of 1M azide indicator, was titrated against 0.02M copper solution. The end point was detected as above. In a similar fashion, the known excess of EDTA added to the nickel solution and sodium azide indicator was back-titrated against the standard copper solution.

RESULTS AND DISCUSSION

The data are depicted in Figure 1 and compared with volumetric results in Table I.

In the determination of copper, changes of pH within a range of 4 to 6 were permissible in both forward and back EDTA titrations. Slight variations in either the acid or the azide concentration were found to have no effect on the sharpness and/or quantity of the titrant at the end point. The zinc titrations could be carried out in solutions of pH ranging from 2 to 5, with no effect on the results. The amount of azide indicator could also be varied widely. In fact, the larger the amount of indicator, the more obvious was the end point. However, it is advisable not to increase the acid concentration, as volatile hydrazoic acid is irritating to the mucous membranes of the nose and eyes.

Attempts were made to determine Pd(II), Pt(IV), Cr(III), and U(VI) by titrating the excess EDTA against copper, but the color produced by these ions with the azide masked the end point.

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Extraction of Submicrogram Amounts of Molybdenum with Cupferron-Chloroform Using Molybdenum-99

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▶ Extraction of Mo at 0.01- to 0.1-μg. levels with cupferron-chloroform has been followed using Mo^{99}. The extraction is most efficient, giving a distribution ratio of over 200 between the chloroform and aqueous phases. Over 90% of Mo can be separated in one extraction when only 0.1 μg. is present in 1 liter of aqueous phase. Phases must be separated within an hour of extraction, otherwise breakdown of cupferron results in return of Mo to the aqueous phase. Bone samples in preparation for extraction can be ashed at temperatures up to 850° C. without loss of Mo.

THE USE OF cupferron (ammonium salt of N-nitrosophenylhydroxylamine) to extract a number of elements, including molybdenum, from acid and neutral solutions into organic phases has been recently reviewed (Morrison, G. H., Freiser, Henry, "Solvent Extraction in Analytical Chemistry," p. 169, 1957, Wiley, New York, N. Y.). In this laboratory, analysis of various materials, including teeth, bone, soft animal tissues, urine, and water, for submicrogram amounts of molybdenum has necessitated a preliminary extraction of molybdenum from acid solutions into an organic phase. This paper reports the use of Mo^{99} to check on the effectiveness of cupferron in complexing molybdenum and facilitating its extraction into a chloroform phase when molybdenum is present in the range 0.013 to 0.13 μg.

EXPERIMENTAL

Apparatus and Materials. CUPFERRON. Freshly prepared 6% aqueous solution of analytical grade cupferron (ammonium salt of N-nitrosophenylhydroxylamine).

Mo^{99}. Solution of sodium molybdate containing 1.3 μg. of molybdenum for each microcurie of Mo^{99}.

CHLOROFORM. C.P.

COUNTING APPARATUS. A Philips (Model PW.4032) Scalar was used with a Geiger-Müller Liquid Counting Tube (20th Century Electronics Type M.6H).

Four solutions were used for these extraction studies: (1) 2N HCl. (2) T550 Solution. Twenty grams of oven-dried human teeth were ashed in a platinum crucible at 550° C. for about 6 hours. The ash was dissolved in concentrated HCl, diluted, and filtered through a No. 542 paper into a 250-ml. flask. The residue and filter paper was re-ashed for 4 hours at 550° C., dissolved in a few milliliters of concentrated HCl and added to the volumetric flask, which was made up to volume. The final solution was approximately 2N in HCl. (3) B550 Solution. Seventeen grams of oven-dried bone (femur of sheep) were ashed at 550° C. for 6 hours, dissolved in concentrated HCl,

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Table I. Extraction of Mo (0.013 and 0.13 μg.) from 2*N* HCl and from Extracts of Teeth and Bone

	0.013 μg. Mo			0.13 μg. Mo		
	Aqueous phase, c.p.m.	Chloroform phase, c.p.m.	Mo extracted, %	Aqueous phase, c.p.m.	Chloroform phase, c.p.m.	Mo extracted, %
2*N* HCl	3	330	87	240	1800	50
T550	0	310	82	91	1900	53
B550	2	240	63	180	1820	51
B650	3	300	79	180	2290	64
Standard (extracted)	0	380	100	56	3560	99
Standard (aqueous)	410	...	...	4060	...	...

Table II. Breakdown of Mo-Cupferron Complex (0.13 μg. Mo)

Postextraction time		Aqueous phase,
h	Min.	c.p.m.
...	11	15
...	35	21
1	..	3
1	25	22
1	55	110
2	25	700
2	45	780
4	35	920
Fresh Cupferron Added		
5	25	28
5	50	27

Table III. Extraction of 0.13 μg. Mo—Phases Separated within 5 Minutes of Extraction

Sample	First extraction $CHCl_3$ phase, c.p.m.	Second extraction $CHCl_3$ phase, c.p.m.	Mo^{99} in first extraction, %
2*N* HCl	3170	27	99
T550	3410	60	98
B550	3310	58	98
B650	3360	56	98

Table IV. Extraction of 0.13 μg. Mo from 1-Liter Water Samples

Sample	First extraction $CHCl_3$ phase, c.p.m.	Second extraction $CHCl_3$ phase, c.p.m.	Mo^{99} in first extraction, %
Distilled water	1790	110	94
City water	1960	45	98

Table V. Effect of Ashing Temperatures on Mo^{99} Content of Bone Ash, c.p.m.

Ashed 550° C.	Ashed 650° C.	Ashed 750° C.	Ashed 850° C.
1010	1010	1040	1040
560	570	570	540
1090	1070	1110	1110

and made up to 250-ml. volume as for T550 solution. (4) B650 Solution. Fifteen grams of oven-dried bone were ashed at 650° C. for 6 hours, dissolved in concentrated HCl, and made up to 250-ml. volume as for T550 solution.

Extraction Procedure. Aliquots (50 ml.) of each of the four solutions were placed in separatory funnels, followed by 1 ml. of 6% cupferron solution and 10 ml. of $CHCl_3$. This was shaken for 1 minute and the two phases were allowed to separate. The $CHCl_3$ phase was drawn off and discarded. This was repeated three times so that the solutions were Mo-free, or nearly so, prior to addition of Mo^{99}. The appropriate amount of Mo^{99} was added plus 1 ml. of 6% cupferron, followed by 11 ml. (to ensure sufficient volume to fill liquid counter) of $CHCl_3$. The separatory funnel was shaken for 1 minute and allowed to stand. Where several extractions were made a further 1 ml. of cupferron and 11 ml. of $CHCl_3$ were added in each case. Where the $CHCl_3$ phase did not separate cleanly, it was transferred to a centrifuge tube and centrifuged. The clear $CHCl_3$ phase was then removed by pipet and placed in the counting tube.

RESULTS

Two standards are included in the results to serve as reference points for extraction efficiency. In the "aqueous" standard, the activities of the two Mo levels used, 0.013 and 0.13 μg. (0.01 and 0.1 μc. Mo^{99}, respectively) were measured in the liquid counter in 11 ml. of H_2O. In the "extracted" standards, the Mo^{99} activities were measured in the $CHCl_3$ phase made up of two successive extractions of the 2*N* HCl aqueous phase to which Mo^{99} had been added. Fresh cupferron reagent was added at each extraction, and the final volume of the $CHCl_3$ phase made up to 11 ml. Prior experiments had shown that no Mo^{99} activity could be detected in a third extraction. The lower activity in the "extracted" standards would appear to reflect the greater beta absorption in the $CHCl_3$ phase. Extraction efficiencies were calculated by expressing the Mo^{99} activity in the $CHCl_3$ phase of a sample, as a percentage of Mo^{99} activity in an "extracted" standard ($CHCl_3$ phase).

The results of extraction of Mo from the four solutions is given in Table I. All count rates have been corrected for background and decay. While transfer of Mo to the $CHCl_3$ phase in one extraction was promising, the results were somewhat variable. To lessen the possibility of contamination, the lower activity aqueous phases were counted first, followed by the $CHCl_3$ phases. There was, therefore, some time elapse between the measurement of activity in aqueous and organic phase of a particular sample. The 0.13-μg. Mo series had the greatest time elapse between extraction and counting, and these show lower extraction efficiencies. This suggested that the Mo-cupferron complex might be unstable and break down with time, the Mo returning to the aqueous phase. The fact that in some samples the sum of activities in the two phases fell short of the known activity —e.g., in T550, 0.13 μg. Mo, 25% of Mo^{99} activity could not be accounted for—supported this possibility.

When a 0.13-μg. level of Mo (0.1 μc. Mo^{99}) and the extraction procedure are used, the activity of the aqueous phase was followed over several hours. After initial separation of the two phases, a sample of the aqueous phase was removed, centrifuged, and counted. Following counting, the sample was returned to the separatory funnel and reshaken for 1 minute. This operation was repeated several times and the post-extraction activities are given in Table II. After 5 hours, when the bulk of the Mo had returned to the aqueous phase, a further 1 ml. of 6% cupferron was added and the operation was repeated. It can be seen that fresh cupferron resulted in extraction of Mo back to the chloroform phase. It appears that the Mo-cupferron complex breaks down rapidly after about 1.5 hours, probably as a result of free cupferron breaking down and falling below a certain minimum concentration.

Extraction of the 0.13-μg. Mo series was repeated and in all cases counts were made within 5 minutes of extraction, the $CHCl_3$ phase only being counted. Results are given in Table III. It would appear that better than 98% of the Mo is removed in the first extraction, indicating a distribution coefficient of over 200.

Extraction of Mo from Water. One liter of water was made approximately 0.25*N* by the addition of 5 ml. of concentrated HCl, 0.13 μg. of Mo was added followed by 5 ml. of 6% cupferron and 25 ml. of $CHCl_3$. Following shaking for 1 minute (not too vigorous, otherwise phases take a long time to separate), the chloroform phase was counted within 5 to 10 minutes. Results for distilled and city water samples are given in Table IV.

Results show that 94 to 98% of the

Mo in the aqueous phase is removed to the chloroform phase in the first extraction. It could well be that the efficiency is even higher, for the aqueous phase still contained fine droplets and surface lenses of $CHCl_3$ difficult to settle out. The activity levels for the second extraction are probably somewhat high on this account.

Ignition Temperatures for Bone Samples. Bone and teeth samples are not easily ashed free of organic matter at temperatures (500° to 550° C.) normally used. Moreover, when the ash is dissolved in acid in preparation for extraction with cupferron a portion of the organic matter appears to dissolve also, imparting a brown color to the solution. Even if residual carbon is re-ashed and dissolved, this does not, of course, prevent organic matter from being taken up when the bulk of the sample is first dissolved in acid. Traces of organic matter prevent clean separation of the chloroform and aqueous phases after shaking and so interfere seriously with Mo extraction. The bone samples (B650) used in the extraction studies were free of this interference because of the higher (650° C.) ashing temperature, and phases separated cleanly and quickly.

Other studies being carried out in this laboratory involved injection of Mo^{99} into rats. When the animals were sacrificed, it was possible to obtain a number of femurs containing quite considerable amounts of Mo^{99}, and as this Mo^{99} had been taken up by metabolic processes it was most likely in a form normal to bone. The femurs were cleaned of soft tissue and three groups of femurs were ashed in platinum basins for 18 hours at 550° C. The three samples were then ground and counted in metal planchets. Four counts were taken for each sample, the planchet being rotated 90° between counts. Mean counts are recorded, but the four separate counts agreed well. After counts were made at 550° C., the samples were returned to the platinum basin and ashed at 650° C. for 2 hours. The samples were transferred to the planchets and recounted. This operation was repeated at temperatures of 750° C. and 850° C. Results are given in Table V, counts being corrected for decay. These showed that at temperatures up to 850° C. no Mo^{99} was lost from the bone samples.

DISCUSSION

Submicrogram determinations may be affected by sample variation in cations and anions apparently without effect at higher microgram levels. Quantitative separation of the elements concerned from the sample matrix minimizes such effects and may be essential for accurate determinations by helping to reproduce standard curve conditions. Extraction of an element, usually in the form of a complex by a solvent immiscible with water, is an especially valuable separation technique, not only because of the selectivity possible with the choice of complexing agents, pH optima, and organic solvents available, but also because the extraction may be repeated several times if the distribution ratio is not particularly favorable.

The Mo-cupferron complex appears to have a distribution coefficient of over 200 between the chloroform and aqueous phases. Cupferron is, therefore, a most effective reagent for complexing Mo in dilute acid solutions, and ensuring its extraction into the $CHCl_3$ phase. The Mo-cupferron residue left after evaporation of $CHCl_3$ is readily digested with HNO_3-$HClO_4$.

Care must be taken that the $CHCl_3$ phase is removed within perhaps 1 hour of extraction, otherwise the Mo-cupferron complex breaks down rapidly with return of Mo to the aqueous phase.

Two or three extractions ensure quantitative removal of Mo, for a single extraction will remove about 95% of Mo from the aqueous phase in amounts of the order of 0.01 μg. In work not reported here, even 0.001-μg. amounts of Mo would also appear to be extracted quantitatively. A similar efficiency can be seen in the extraction of 0.1 μg. of Mo from 1 liter of aqueous phase into 25 ml. of $CHCl_3$.

Ashing of bones or teeth at 650° C. ensures that all organic matter is removed. With material ashed at 550° C. the presence of traces of organic matter inhibits the separation of aqueous and chloroform phases. No losses of Mo^{99} appear to take place when bone is ashed at temperatures up to 850° C.

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Spectrophotometric Estimation of Copper(I) Using Rubeanic Acid

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► **The possibility of the spectrophotometric estimation of copper(I) as a soluble chloro complex of rubeanic acid is discussed. The molecular formula of the complex formed is determined by the method of continuous variation and the probable structure of the complex is indicated. The method compares favorably with similar methods, and is applicable in a concentration range of 2 to 12 p.p.m. of copper(I).**

THE SPECTROPHOTOMETRIC STUDY of copper(I) has been carried out in the past using several organic reagents. To mention but a few instances, copper (I) in ammoniacal solution has been estimated by complexing with 2,2′-dipyridine (*24*), and separately with 2,2′,2″-tripyridine and 2,2′-biquinoline (*20*). Other reagents used for the purpose have been described (*3, 6–9, 19, 21, 22, 27*). Some of these reagents are claimed to be specific for copper(I) with minimum interference from other ions, but their cost and availablity have not always been favorable.

Rubeanic acid (dithiooxamide) can be synthesized easily to a high degree of purity (*14*) and it works well in the spectrophotometric study of copper(I). The suitability of the reagent for the colorimetric estimation of copper(II) has already been established by previous workers (*1, 2, 4, 10, 15, 16, 23*). Its application to the spectrophotometric study of copper(I) has not been investigated. The present investigation shows that the reaction of copper(I) in the form of its chloro complex with rubeanic acid is instantaneous, resulting, therefore, in a rapid method.

EXPERIMENTAL

Reagents. CUPROUS CHLORIDE. Cuprous chloride used during this investigation was prepared in a pure state according to the method given by Palmer (*12*). Commercial samples of cuprous chloride are usually partially oxidized to the cupric state and they have to be freshly reduced by sulfur dioxide and dried before they can be used for such investigations.

RUBEANIC ACID. Three-tenths gram of rubeanic acid was dissolved in 100 ml. of 95 to 96% ethyl alcohol (this strength is not very critical). The solution had a tinge of orange, and 0.5 ± 0.01 ml. was used per estimation.

Buffer. A phosphate buffer was prepared by adding 4 ml. of 1*N* sodium hydroxide to a solution containing 71.6 grams of $Na_2HPO_4 \cdot 12H_2O$ and making up a total volume of 1 liter with distilled water. This buffer showed a pH of 9.4 to 9.5.

Stabilizer. Five milliliters of a 20% aqueous solution of hydroxylamine hydrochloride was added per estimation to stabilize the cuprous complex.

Apparatus. The absorbance measurements were made with the Beckman B spectrophotometer equipped with a set of 4 Beckman borosilicate glass rectangular cells.

Procedure. About 10 mg. of freshly prepared cuprous chloride was accurately weighed and dissolved in 10 ml. of a saturated solution of sodium chloride. From this stock solution different aliquots were pipetted out into standard 25-ml. flasks. One milliliter of phosphate buffer and 5 ml. of 20% hydroxylamine hydrochloride were added to each aliquot, and the volume was made up in each case to the 25-ml. mark with distilled water. Finally, 0.5 ± 0.01 ml. of 0.3% rubeanic acid was added with stirring. The absorbance of each sample was measured with the spectrophotometer at various wavelengths. Reagent blank correction was applied in all cases. Readings obtained indicated that the 400-mμ region showed maximum change in absorbance for unit change in concentration of the copper(I) ion. However, the rubeanic acid reagent also showed appreciable absorbance at this range and therefore the 425-mμ region was the next best for copper(I) estimation, as the absorbance due to the reagent was negligible in this region.

Table I. Quantitative Determination of Copper(I)

Vol. of cuprous chloride, ml.	Wt. of copper in mg./liter, av. of 5 experiments	Std. dev.
0.5	2.638	±0.1339
0.8	4.158	±0.1549
1.0	5.0170	±0.1013
1.5	7.5080	±0.2116
1.7	8.5600	±0.1808
2.0	12.8380	±0.1030

Table II. Interfering Cations and the Colors of Their Respective Rubeanate Complexes

Cations	Color of rubeanate complex
Silver	Yellow, turning to greenish black
Cobalt	Yellow-brown
Bismuth	Tan
Copper(II)	Grass-green
Mercury(I)	Brown turning black
Nickel	Blue-red
Iron(III)	Faint orange
Iron(II)	Dull green
Aluminum	Yellow to greenish black tinge
Palladium	Brown
Platinum	Rose

DISCUSSION

The molar absorptivity values for six samples of the copper(I)–rubeanate complex were 743.0, 749.0, 743.2, 755.3, 776.4, and 745.9 which show that the absorbance obeys Beer's law. A series of quantitative determinations of copper(I) were carried out taking known weights of cuprous chloride in saturated sodium chloride solution, determining their respective absorbances on the spectrophotometer, and referring the values to the working graph. The estimated values were in good agreement with the weighed values. Standard deviations are indicated in Table I.

Cations shown in Table II can interfere with the estimation if present in large amounts. Their concentrations can be reduced considerably by suitable means, such as electrodeposition, before estimating the copper(I) ions. However, if they are present as trace impurities, their interference can be obviated by the addition of suitable buffers and/or sequestering agents (*26, 28*).

Approximate limits of tolerance of some of the diverse ions usually found with copper are shown in Table III. Cations of silver require, for masking, KCN or thiourea which also form complexes with cuprous ions, and hence are not tolerated in this estimation. Silver ions are completely eliminated as insoluble AgCl when the cuprous ions are complexed with saturated sodium chloride solution to give the soluble chloro cuprous complex for the estimation.

Anions such as periodate, nitrite, thiocyanate, and ferricyanide give yellow coloration with the reagent, or react with hydroxylamine hydrochloride. Hence, their presence also is not tolerated.

Table III. Tolerance Limits of Diverse Ions

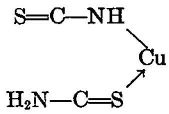
Metal	Added as	Complexing agent	Tolerance limit
Co(II)	$CoCl_2 \cdot 6H_2O$	Malonic acid or EDTA (sodium salt)	20 p.p.m.
Ni(II)	$NiCl_2 \cdot 6H_2O$	Malonic acid or EDTA (sodium salt)	12 p.p.m.
Fe(III)	$FeCl_3 \cdot 6H_2O$	Fluoride or phosphate	1000 p.p.m.
Fe(II)	Fe(II) ammonium sulfate	EDTA (sodium salt)	4 p.p.m.

Though the brown cuprous–rubeanate complex is formed over a wide range of pH (from 4 to 9.5) the color stability was maximum in the alkaline range. Stabilizers are necessary in various colorimetric studies of copper(I) (*5, 13, 18, 24*). In this laboratory Metol (monomethyl *para* amino phenol sulphate), quinol, hydrazine hydrate, and dimethyl formamide were tried as stabilizers without much success. The two former reagents are very susceptible even to small changes in hydrogen ion concentration, and their behavior becomes unpredictable due to rapid tautomeric changes in their constitution. Hydroxylamine hydrochloride was found to be the best stabilizing agent for this estimation; the brown color of the cuprous rubeanate complex was stable for more than 15 minutes in the alkaline medium.

Composition of the Cuprous–Rubeanate Complex. The composition of the complex was determined by the method of continuous variation proposed by Job (*11*) and adopted by Vosburgh and Cooper (*25*). When the different mole ratios of copper(I) to reagent were plotted against their respective absorbances for different wavelengths (Figure 1), it was observed that there was no variation in the location of the maxima, from which it was inferred that only one product was formed, or that the complex was homogenous. The position of maxima of the continuous variation method showed the ratio of copper(I) to reagent in the complex to be 3:2. However, the gravimetric and volumetric analyses of the solid complex indicated the ratio of copper(I) to reagent to be 1:1. Therefore, in the solid state the complex exists in a monomeric state with one ligand per copper(I) atom.

X-ray studies have indicated that the dithiooxamide (rubeanic acid) molecule is planar with a center of symmetry. Barcelo (see *17*) observed that the infrared spectra of the metal complexes of dithiooxamide or *N,N'*, disubstituted dithiooxamide molecule showed strong absorbance of the N—C═S group, but the characteristic absorption of the S—H bond was not found in any of the spectra, which agreed with the formula based on the *trans*-keto form.

Correlating the above two observations, and the results of the gravimetric and volumetric analyses, the structure of copper(I)–rubeanate complex in the solid state may be represented as:



However, the continuous variation method, which is a specific analytical method for trace quantities of very weak complexes, indicated the ratio of copper

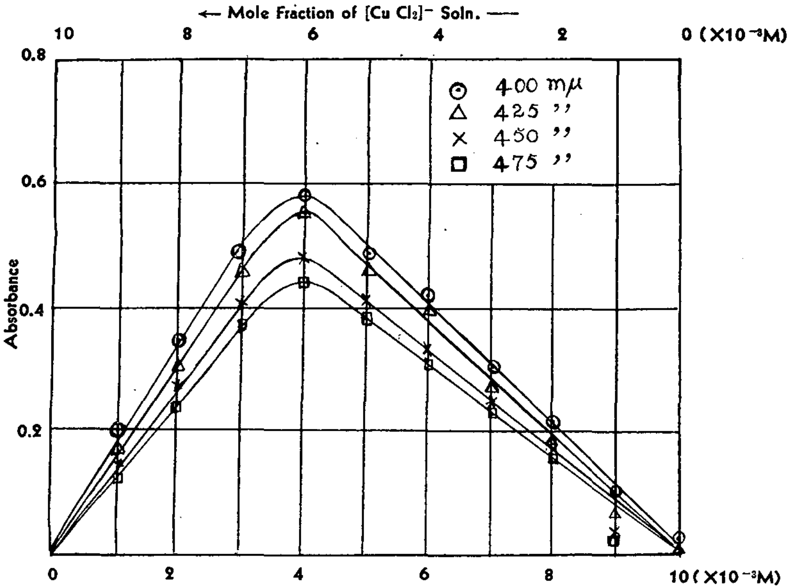
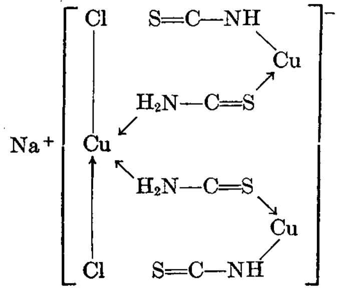


Figure 1. Identification of rubeanic acid–cuprous complex by method of continuous variation

(I) to reagent to be 3:2. Therefore, in solution, in the presence of a high concentration of sodium chloride, there is a possibility of the formation of a polynuclear complex when the two amido groups of two monomeric complexes by their proximity are bridged through a copper(I) atom belonging to the (CuCl$_2$)Na complex. The structural formula of the brown polynuclear complex in solution can be represented as:



Applications. The method is of analytical interest. It does not claim greater advantage than the estimation of copper(II) by the same reagent. However, it can work as an alternate method to the latter. The application of this method is limited by the limited occurrence of copper in its unusual univalency. Although not confirmed, it may be of value in the estimation of copper in cuprite (Cu$_2$O), in the evaluation of cuprous oxide to be used in ceramics, red glaze of porcelain, red glass, and electroplating, and in the estimation of cuprous oxide used in fungicide and in antifouling paints (ship bottom paints). It should also be applicable to the evaluation of cuprous chloride used in catalysis, preservatives, fungicides, and in wet-cell batteries (cuprous chloride and magnesium).

In all the above instances where cuprous oxide or cuprous chloride is handled, the extraction may be done with saturated sodium chloride while bubbling sulfur dioxide through it, when (CuCl$_2$)Na complex is formed. The spectrophotometric method discussed in this paper can then be applied directly after elimination of free sulfur dioxide. The method has not been of quantitative value when the extraction involved acid digestion.

Interferences. The ions that are usually met in association with cuprous oxide or chloride are cobalt, nickel, iron(III), and magnesium. Of these, the last is not an interfering ion. If the interfering ions are present in large proportion, their prior elimination by electrodeposition is necessary. Iron(III) complexes with the phosphate buffer used in the experiment and does not interfere under the conditions of the experiment as has been mentioned earlier. Nickel and cobalt, if present in small amounts, can be sequestered with malonic acid, ethylenediamine (*26*), or EDTA, and thus their interference can be eliminated. However, the addition of these sequestering agents, especially malonic acid, considerably reduces the stability of the cuprous–rubeanate complex. Larger amounts of the hydroxylamine hydrochloride stabilizer (about 15 to 20 ml. per estimation) have to be used when EDTA is used in the experiment, and it is also recommended that a separate working graph be prepared using the conditions adopted for sequestration.

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Indirect Ultraviolet Spectrophotometric Determination of Silicon

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▶ An indirect spectrophotometric method for traces of silicate is based on the ultraviolet absorptivity of molybdate originating from molybdosilicic acid. After extraction of the heteropoly acid with a pentanol–diethyl ether solution, the molybdosilicic acid is stripped with a basic buffer solution. The absorbance of the aqueous molybdate solution is measured at 230 or 210 mμ. Phosphate, arsenate, and iron(III) interfere. The optimum concentration range is 0.06 to 0.5 p.p.m. of silicon when spectrophotometric measurements are made at 230 mμ using 1-cm. cells. The molar absorptivity is 59,000 liters per mole-cm. at 230 mμ.

MOLYBDATE and silicate ions form yellow molybdosilicic acid, which is extractable with certain oxygen-containing organic solvents (*10*). The determination of silicon has been based on the measurement of the color of either the molybdosilicic acid (*2*, *3*, *5*, *6*) or the heteropoly blue of silicon, which is obtained by the selective reduction of the molybdosilicic acid (*1*). The fact that decomposition products of molybdophosphoric acid were used in a sensitive ultraviolet spectrophotometric method for the determination of phosphate (*7*) suggested the desirability of a similar investigation of molybdosilicic acid. The essential steps in the procedure as ultimately developed are: the formation of molybdosilicic acid, the extraction of this heteropoly acid with a pentanol–diethyl ether extractant, the stripping of the molybdosilicic acid with a basic buffer solution, and the measurement of the ultraviolet absorptivity of the molybdate ion.

EXPERIMENTAL

Apparatus. The absorbance measurements were made in 1.000-cm. silica cells using either a Cary 14 recording spectrophotometer or a Beckman DU spectrophotometer equipped with an ultraviolet accessory set.

Solutions. STANDARD SILICATE SOLUTION. Dissolve 3.15 grams of sodium silicate, $Na_2SiO_3 \cdot 9H_2O$, in distilled water and dilute to 500 ml. Standardize this solution gravimetrically. After standardization, use a microburet to transfer sufficient silicate solution to a 500-ml. volumetric flask so that on dilution to the mark the solution contains 0.0100 mg. of silicon per ml.

MOLYBDATE SOLUTION. Dissolve 50.0 grams of ammonium molybdate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, in distilled water and dilute to 500 ml.

BUFFER SOLUTION. Dissolve 53.5 grams of ammonium chloride and 70 ml. of concentrated ammonium hydroxide in distilled water and dilute to 1 liter.

All chemicals were reagent grade and all aqueous solutions were stored in polyethylene bottles to avoid silica contamination.

Recommended General Procedure. Weigh, or measure by volume, a sample containing up to 0.05 mg. of silicon and treat it so that it is present as soluble silicate.

Add 1.0 ml. of 1 to 1 hydrochloric acid and dilute to approximately 45 ml. Add 2.0 ml. of 10% ammonium molybdate. Let stand for 5 minutes, dilute to 50 ml., and let stand again for another 5 minutes. The pH of this solution should be approximately 1.4.

Transfer the solution to a 125-ml. separatory funnel and add 7 ml. of 1 to 1 hydrochloric acid. Rinse the emptied container with 20 ml. of 5 to 1 ethyl ether–1-pentanol extractant mixture, transfer the rinsing to the separatory funnel, and swirl it with the acidified aqueous solution for about 90 seconds. Withdraw the lower aqueous phase into a 125-ml. Erlenmeyer flask and transfer the organic extractant to the original flask for temporary storage. Pour the aqueous phase back into the separatory funnel, rinse the Erlenmeyer flask with another 20 ml. of extractant mixture, and add the rinsings to the aqueous phase in the funnel. Swirl the funnel for about 90 seconds, withdraw and discard the lower aqueous phase, and add to the funnel the first portion of the extractant which had been temporarily stored. Swirl the funnel to collect the water droplets into one globule and discard. Wash the extractant three times with 25-ml. portions of 1 to 10 perchloric acid, first using the first two portions of the acid to rinse out the container that held the first extractant portion, thus assuring complete transfer of the extractant to the funnel. Each washing consists of swirling the funnel for about 45 seconds and discarding the lower phase. Remove excess molybdate by washing the extract with 1 to 10 perchloric acid.

Carefully wash the tip of the funnel with a stream of distilled water to assure complete removal of excess molybdate ions. Transfer 30 ml. of the ammonium chloride–ammonium hydroxide buffered solution to the funnel, swirl for about 1 minute, and withdraw the lower aqueous phase into a 100-ml. volumetric flask. Add another 15 ml. of the ammonium chloride–ammonium hydroxide buffered solution to the funnel, again swirl for 1 minute, and add the lower phase to the flask containing the first portion of the buffered solution. Swirl the flask to collect all of the remaining droplets of water.

Dilute the solution in the 100-ml. volumetric flask to the mark with distilled water and mix well. The pH of the final aqueous solutions should be about 9. Let the solution stand for 30 to 60 minutes and then measure the absorbance at 230 and/or 210 mμ against a reagent blank in matched 1.000-cm. silica cells. Measurement at 230 mμ is recommended because of the high absorbance of the reagent blank solution at 210 mμ. Prepare the reagent blank by following this same procedure, but take distilled water, instead of an aliquot of silicate solution, as a sample. Refer the absorbance values to a standard calibration graph obtained by using standard silicate solutions.

EFFECT OF SOLUTION VARIABLES

Silicon Concentration. Figure 1 shows the ultraviolet absorption spectrum of a solution containing the molybdate resulting from the decomposition of the molybdosilicic acid. Silicate ions at the less than 1-p.p.m. level do not contribute to the ultraviolet absorbance. Conformity to Beer's law was observed for 0.02 to 0.5 p.p.m. of silicon. The optimum concentration range was 0.06 to 0.5 p.p.m. of silicon at 230 mμ and 0.03 to 0.3 p.p.m. at 210 mμ based on Ringbom plots. Figure 2 shows a comparison of sensitivities when absorbance measurements are made at 230 and 210 mμ. This indirect ultraviolet method is 2.5 and 4.5 times as sensitive, when absorbance measurements are made at 230 and 210 mμ, as the heteropoly blue procedure of Boltz and Mellon (*1*).

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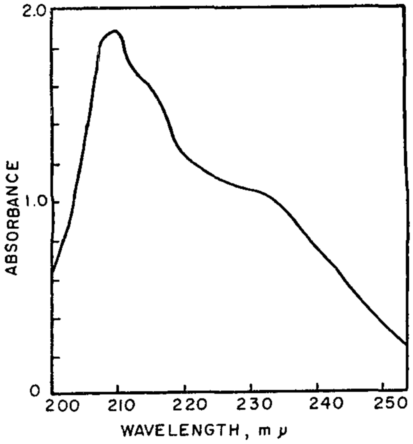

Figure 1. Absorption spectrum of molybdate extract

Buffer extract of molybdosilicic acid (0.5 p.p.m. of Si) vs. reagent blank solution

Acidity Prior to Extraction. A pH of 1 to 5 was found to be satisfactory for the formation of molybdosilicic acid (*8*). Previous investigations have shown that molybdosilicic acid, once formed in weakly acidic solution, is stable in strongly acidified solutions (*9*). The pH at which molybdosilicic acid was formed in this work was about 1.4.

The effect of acidity of the molybdosilicic acid solution on the extent of extraction by the 5 to 1 diethyl ether–1-pentanol extractant was investigated by using 0.1, 0.3, 0.5, 0.7, and 1.0*N* hydrochloric acid solutions. The absorbance readings at 230 mμ for solutions containing 0.4 p.p.m. of silicon at these acidities were 0.155, 0.570, 0.790, 0.790, and 0.790, respectively. Hence, maximum extraction is achieved in the 0.5 to 1.0*N* range, with 0.7*N* being selected for the recommended procedure. The effect of higher acidities was not investigated.

Molybdate Concentration. By using 0.4 p.p.m. of silicon and 1.5, 2.0, and 2.5 ml. of the molybdate solution per 50 ml. of solution, absorbance values of 0.750, 0.755, and 0.755 were obtained, with the same volumes of molybdate solution used in preparing the reference solution. The intermediate volume of 2.0 ml. of molybdate solution was chosen as sufficient.

Table I. Effect of Diethyl Ether–1-Pentanol Ratios on Absorbance of Reagent Blanks

Ratio of diethyl ether–1-pentanol	*A*, at 230 mμ (*vs.* H_2O)
0.10	0.699
0.25	0.597
0.67	0.469
1.00	0.432
1.50	0.367
2.33	0.319
4.00	0.240
5.00	0.135

Extractant. An appropriate extractant should extract the molybdosilicic acid, should not extract the excess molybdate required for complete formation of the molybdosilicic acid, should exhibit a cut-off wavelength below 210 mμ, and should have slight solubility in aqueous buffer solution if exhibiting appreciable absorptivity in the lower ultraviolet region. Wadelin and Mellon found chloroform to be an effective diluent to minimize the extraction of excess molybdate in the extraction of molybdosphosphoric acid with 1-butanol and indicated that 1-pentanol was an effective solvent for molybdosilicic acid, 265 mμ being the shortest usable wavelength (*10*).

After preliminary tests using various solvents and diluents, it was concluded that 1-pentanol was the most efficient solvent for molybdosilicic acid with minimum extraction of the excess molybdate, the addition of diethyl ether as diluent diminished the solubility of the 1-pentanol in the aqueous buffer solution to a tolerable level, and if the ratio of diethyl ether to 1-pentanol exceeded 5 the efficiency of the extraction of molybdosilicic acid decreased. Table I shows the diminution of the absorbance of the reagent blank solution as the relative amount of diethyl ether in the extractant is increased. Thus, the 5 to 1 diethyl ether–1-pentanol mixture was selected as the appropriate extractant.

On the basis of the data cited in Table II, two extractions with 20-ml. portions of the extractant were deemed sufficient to ensure maximum recovery of molybdosilicic acid with a minimum expenditure of time. The results given in Table II are the average value of two experiments.

Stability. The final aqueous buffered solutions were stable for 24 hours, when solutions contained 0.08 and 0.40 p.p.m. of silicon. For both silicon solutions there is a slight increase in absorbance within the first hour, but no change in the 1-hour and 24-hour values. Conformity to Beer's law was observed for a series of solutions allowed to stand for 8 weeks. These findings indicate that the final buffered solution of molybdate should be allowed to stand at least 30 minutes and preferably 60 minutes before absorbance measurements are made.

Diverse Ions. A study was made to determine the permissible amounts of various ions that may be present without interfering with the determination of 0.2 p.p.m. of silicon. No attempt was made to determine the effects of ion concentrations larger than 500 p.p.m., since this concentration is extremely large compared to the concentration of silicon that was present. Errors less than ±3% of the mean transmittance value were considered negligible. The cations tested were added as chlorides, perchlorates, or sulfates and the anions

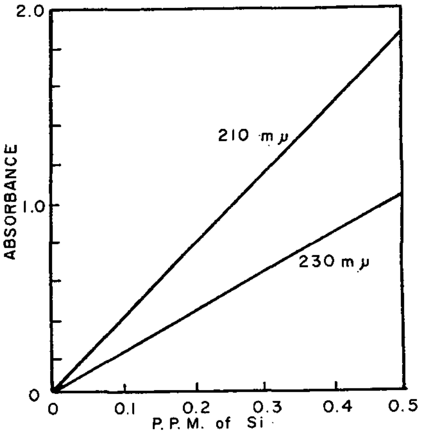

Figure 2. Sensitivity of absorbance measurements at 230 and 210 mμ

Table II. Effect of Extraction Technique

(0.4 p.p.m. Si)

No. of extractions	Ml. of extractant per extraction	Absorbance
1	20	0.475
2	10, 10	0.620
2	20, 20	0.775
3	20, 10, 10	0.800

Table III. Tolerance of Diverse Ions

Ion	Added as	P.p.m. permitted
Ag^{+}	Ag_2SO_4	500
Al^{+3}	$Al(ClO_4)_3$	500
Ca^{+2}	$Ca(ClO_4)_2$	500
Co^{+2}	$Co(ClO_4)_2$	500
Cr^{+3}	$CrCl_3$	500
Cu^{+2}	$Cu(ClO_4)_2$	10
Fe^{+3}	$Fe(NH_4)(SO_4)_2$	1
Mn^{+2}	$MnSO_4$	500
Ni^{+2}	$Ni(ClO_4)_2$	500
Pb^{+2}	$Pb(C_2H_3O_2)_2$	300
Zn^{+2}	$Zn(ClO_4)_2$	500
AsO_2^{-}	$NaAsO_2$	0
AsO_4^{-3}	Na_2HASO_4	0
Br^{-}	$NaBr$	500
$C_2H_3O_2^{-}$	$NaC_2H_3O_2$	250
$C_2O_4^{-2}$	$(NH_4)_2C_2O_4$	500
Cl^{-}	KCl	500
ClO_4^{-}	NH_4ClO_4	500
$Cr_2O_7^{-2}$	$K_2Cr_2O_7$	50
F^{-}	KF	10
MoO_4^{-2}	Na_2MoO_4	200
NO_2^{-}	KNO_2	20
NO_3^{-}	KNO_3	250
PO_4^{-3}	KH_2PO_4	0
SO_4^{-2}	$(NH_4)_2SO_4$	500
VO_3^{-}	NH_4VO_3	250
WO_4^{-2}	Na_2WO_4	100

were added as ammonium; potassium, or sodium salts. Table III summarizes the results of this study.

The interference of phosphate, arsenate, and iron(III) is an especially serious limitation of the method. No attempts were made to remove phosphate or arsenate by a preliminary extraction with ethyl acetate by the DeSesa and Rogers method (*4*), or to circumvent the deleterious effect of iron(III).

Although less than 100 p.p.m. of either the fluoborate or the borate ion exhibits negligible absorbance at 230 mμ, the effect of these ions on the specificity and efficiency of extraction of molybdosilicic acid was not studied. Therefore, their possible deleterious effect on the extraction process would have to be ascertained before this method could be applied to samples in which the solubilization of silicon had been achieved by the fluoride method.

Reproducibility. An indication of the precision of this procedure was ascertained from the results of 12 samples, each containing 0.2 p.p.m. of silicon. These samples gave mean absorbance values of 0.392 and 0.792 at 230 and 210 mμ, respectively. The standard deviations were 0.006 absorbance unit, or a relative standard deviation of 1.5%, at 230 mμ; and 0.012 absorbance unit, or a relative standard deviation of 1.5%, at 210 mμ. In a series of determinations, about 30 minutes is required for each determination.

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Visible Absorption Characteristics of the Bis-(2,9-dimethyl-1,10-phenanthroline)– and Bis-(4,4′,6,6′-tetramethyl-2,2′-bipyridine)–Copper(I) Ions

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▶ Spectrophotometric studies have indicated that the absorbing species in solution for the determination of copper using 2,9-dimethyl-1,10-phenanthroline (dmp) and 4,4′,6,6′-tetramethyl-2,2′-bipyridine (tmb) are the bis complex ions, $[Cu(dmp)_2]^+$ and $[Cu(tmb)_2]^+$, respectively. Solutions of the pure compounds, $[Cu(ligand)_2]X$ where X = Cl, Br, I, NO_3, and ClO_4, have spectral characteristics in agreement with the earlier studies. In general, however, the solutions conform to Beer's law only when a large excess of the ligand is added. Deviations from Beer's law in the absence of excess ligand are attributed to dissociation of the $[Cu(ligand)_2]X$ complexes to the corresponding monochelate species. These ligands coordinate to many transition metals and their apparent specificity for copper in extraction procedures is probably due to complexes of other metals having wavelengths of maximum absorption well removed from the λ_{max} values for the copper complexes, or much lower molar absorptivities.

Reports have been published about the use of 2,9-dimethyl-1,10-phenanthroline (dmp; trivial name, neocuproine) for the determination of copper (*8*, *12*). While the reagent is regarded as specific for copper, it is not well known that the ligand forms complexes with a number of transition metal ions. An analogous reagent, 4,4′,6,6′-tetramethyl-2,2′-bipyridine (tmb), has also been suggested for the determination of copper (*9*). This article describes the absorption behavior of solutions of pure compounds containing the bis(dmp)- and bis(tmb)-copper(I) ions and reports that some complexes may be found that interfere with the determination of copper using these reagents.

EXPERIMENTAL

Apparatus. A Unicam SP500 spectrophotometer was used for the absorption measurements. The wavelength and absorbance scales were checked against standard solutions, data for which are available from the U. S. Department of Commerce (*11*).

Reagents. The compounds CuXtmb, where X = Cl and Br, and $[Cu(tmb)_2]X$, where X = NO_3 and ClO_4, were prepared by reduction of an aqueous solution of the corresponding copper(II) complex with hydrazine sulfate. The copper(II) complexes were isolated by methods similar to those previously described for the analogous dmp complexes (*6*). CuItmb was obtained by the reaction of CuCltmb with excess sodium iodide dissolved in acetone.

The compounds $[Cu(tmb)_2]X.H_2O$, where X = Cl and Br, were prepared by refluxing an ethanol solution of the corresponding mono(tmb) complex and tmb, while $[Cu(tmb)_2]I$ was formed by adding cuprous iodide to an ethanol solution of excess tmb.

The compounds MCl_2tmb, where M = Fe, Co, and Ni, were prepared by addition of tmb to excess metal chloride dissolved in methanol (for $FeCl_2$) or ethanol (for $CoCl_2$ and $NiCl_2$).

All the compounds were analyzed for metal, carbon, hydrogen, and nitrogen. The samples used for the absorption experiments were analytically pure.

The solvents were Baker analyzed reagent chloroform and Analar isoamyl alcohol. The solutions were examined in matched, stoppered 1-cm. cells.

RESULTS AND DISCUSSION

The procedure generally adopted for the determination of copper is to reduce an aqueous solution of copper(II) to copper(I), then add excess neocuproine, and finally extract with isoamyl alcohol (*14*) or chloroform (*5*). The extracts show maximum absorption in the visible at about 455 mμ (molar absorptivity ϵ = 7950 ± 100) and the absorbing species has been determined spectrophotometrically (*14*) to be $[Cu(dmp)_2]^+$.

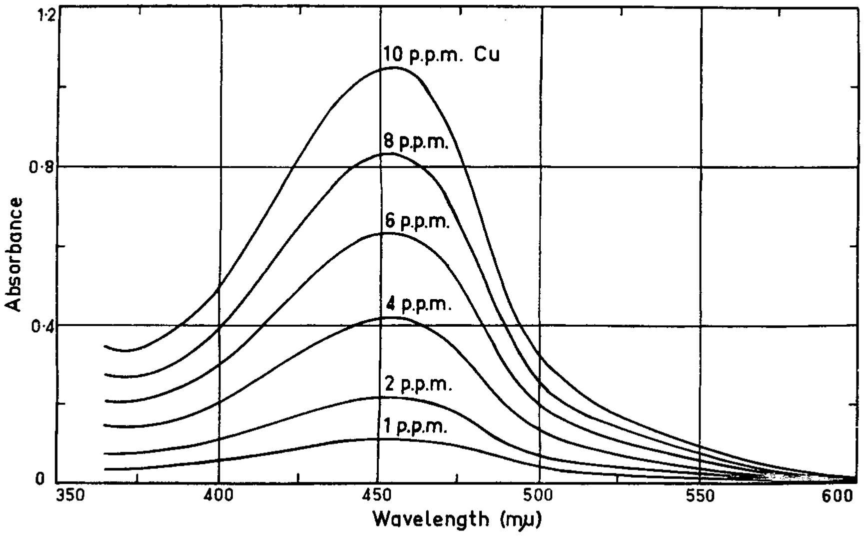

Figure 1. Absorption curves of $[Cu(tmb)_2]NO_3$ in isoamyl alcohol containing excess tmb

Complexes of the type $[Cu(dmp)_2]X$, where X = NO_3, ClO_4, Cl, Br, and I and 2X = SO_4, have been isolated (*6*); these compounds in isoamyl alcohol and chloroform show λ_{max} equal to 454 and 457 mμ, respectively, in agreement with the earlier work (*5, 14*). We found that isoamyl alcohol was the better solvent for the pure compounds, since their solutions conformed to Beer's law and yielded ε values about 7900. The chloroform solutions on the other hand conformed to Beer's law only when the anion was nitrate or perchlorate. The molar absorptivities obtained for chloroform solutions of the halides were very low, decreasing with both age of the solutions and dilution. However, the addition of a sixfold excess of dmp elevated ε to 7900 and the solutions were stable and conformed to Beer's law in the range studied (1 to 12 p.p.m.). The low ε values in the absence of excess dmp were explained (*6*) by the dissociation of the bis complexes to the mono complexes, CuXdmp.

One of the often reported advantages of the use of dmp for copper analysis is its alleged specificity for that metal. This conclusion has been drawn after tests which have shown that addition of ions of other metals such as iron, nickel, mercury, etc., even in large excess to the copper present, causes no apparent interference (*8*). While it is well known that 1,10-phenanthroline readily coordinates to metal ions (*1*)—for example, iron(II) salts yield tris(chelate) complexes which are intensely colored (ferroin reaction)—the coordinating ability of dmp has been little investigated. Dmp when added to solutions of iron(II) salts does not produce intensely colored solutions (*2, 7*) and it has been inferred that this molecule does not form complexes with iron(II). However, mono- and bis(dmp) complexes of iron(II) have been isolated and characterized (*4*).

Dmp coordinates to a variety of metal ions, besides Fe^{+2}, forming both mono and bis complexes. Some of these substances have relatively intense colors and are capable of extraction into organic solvents. The apparent specificity of this reagent for copper is probably the result of other metal complexes having wavelengths of maximum absorption well removed from 455 mμ, with relatively low ε values. For example, chloroform solutions of $CoCl_2$-dmp and $NiBr_2$dmp have λ_{max} (ε) equal to 660 mμ (493) and 514 mμ (215), respectively. Bis(chelate) complexes of these metals have lower absorptivities (about 50) and are unlikely to interfere (*3*).

The analytical method for the determination of copper, using the reagent 4,4′,6,6 - tetramethyl - 2,2′ - bipyridine (tmb), depends on the measurement of absorption at 455 mμ which, according to spectrophotometric studies (*10*), is due to the $[Cu(tmb)_2]^+$ ion. Tmb has a sensitivity toward copper similar to that of dmp, but its usefulness has not been studied to the same extent. Complexes of the type $[Cu(tmb)_2]X$, where X = NO_3, ClO_4, Cl, Br, and I, have been prepared. Table I summarizes the absorption characteristics of these compounds in the solvents chloroform and isoamyl alcohol and the effect of the addition of excess tmb. The chloroform solutions of the halide compounds required the addition of a very large excess of tmb (200 times the molar concentration of the complex) before the molar absorptivities reached the maximum values shown in the table. In all other cases the excess tmb amounted to 12 times the concentration of the complex. Figure 1 illustrates the visible absorption curve of the $[Cu(tmb)_2]^+$ ion at various concentrations.

Tmb behaves like dmp as a reagent for copper(I). Isoamyl alcohol is the better solvent for the pure compounds. Only when excess tmb is present do the solutions in general conform to Beer's law (range studied, 1 to 10 p.p.m.) and produce molar absorptivities approaching the value 6800 reported in the literature (*13*). We have not recorded at any time a value as high as 8100, which was reported by Linnell and Manfredi (*10*). We repeated their extraction experiment and obtained an average value of 6590 in isoamyl alcohol (6510 in $CHCl_3$). The low values for solutions of the halide compounds in the absence of excess tmb are doubtless due to dissociation to the CuXtmb mono complexes. The substances CuXtmb, where X = Cl, Br, and I, have been prepared and characterized. CuCltmb was shown by freezing-point depression measurements to be dimeric in camphor. Low solubilities of the compounds in organic solvents did not permit other determinations to be made. In support of the suggested dissociation

$$2[Cu(tmb)_2]X \rightleftharpoons [CuXtmb]_2 + 2tmb$$

we found that a chloroform solution of the compound $[CuCltmb]_2$ absorbed weakly at 454 mμ but on addition of a large excess of tmb the yellow solution became orange (λ_{max} = 454 mμ) and, assuming complete conversion to the bis complex, yielded an ε value of 5710 at that wavelength. The $CuNO_3$tmb and $CuClO_4$tmb complexes could not be prepared. Attempts to do so always resulted in the production of the bis complexes. This negative result would indicate that NO_3^- and ClO_4^- anions, unlike the halide ions, are reluctant to

Table I. Visible Absorption Characteristics of $[Cu(tmb)_2]X$ Compounds

Compound	Chloroform (λ_{max} = 454 mu) ε	Chloroform (λ_{max} = 454 mu) ε (excess tmb)	Isoamyl alcohol (λ_{max} = 453 mu) ε	Isoamyl alcohol (λ_{max} = 453 mu) ε (excess tmb)
$[Cu(tmb)_2NO_3$	6180	6300	6550	6700
$[Cu(tmb)_2]ClO_4$	5780	6330	6600	6600
$[Cu(tmb)_2]Cl.H_2O$	1100[a]	6000	6180[a]	6600
$[Cu(tmb)_2]Br.H_2O$	1850[a]	6100	5630[a]	6550
$[Cu(tmb)_2]I$	1310[a]	6230	5830[a]	6630

[a] Do not conform to Beer's law.

Table II. Visible Absorption Characteristics of Some tmb-Metal Complexes in Chloroform Solution

Compound	λ_{max}, mμ	ϵ
FeCl$_2$tmb	417, 445	67, 71
CoCl$_2$tmb	573, 653	254, 486
NiCl$_2$tmb	489, 610, 830, 975	144, 12, 32, 75

form covalent bonds with Cu$^+$. If this is so, the normal ϵ values observed for solutions of [Cu(tmb)$_2$]NO$_3$ and [Cu(tmb)$_2$]ClO$_4$ are to be expected, since the equilibrium between the complex and its dissociation products must lie predominantly in favor of the bis complex. In these cases the positions of the equilibria are little changed by the mass action effect of excess tmb (see Table I).

Tmb shows a negative ferroin reaction (*9, 13*) and is reported not to complex with iron(II). Our experiments indicate that complexes with iron(II) are formed. As for the dmp complexes of iron(II), these are pale yellow solids and their solutions are noticeably colored only when concentrated. It appears that tmb is able to complex with the transition metals generally. Table II lists some of the compounds that have been isolated and their spectral characteristics in CHCl$_3$ solution.

The suitability of tmb for the determination of copper in the presence of other transition metals will depend upon the λ_{max} values of their complexes and the intensities of the solutions. Results indicate that other metals will not interfere because of either small ϵ values for the complexes or remoteness of regions of maximum absorption from 454 mμ of the [Cu(tmb)$_2$]$^+$ ion, just as in the case of dmp.

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Purity Examination of Silicon and Germanium Halides by Long-Path Infrared Spectrophotometry

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▶ The infrared absorption spectrum of 10-cm. liquid layers of silicon and germanium tetrahalides is capable of detecting many impurities in the 1- to 200-p.p.m. range. Dissolved gases and volatile compounds, and especially organic contaminants, are seen readily. The technique is also useful for following the progress of purification procedures. Typical long-path spectra of liquid SiCl$_4$, GeCl$_4$, SiBr$_4$, and GeBr$_4$ from 2 to 15 microns are shown, and methods are given for distinguishing impurity absorptions from weak bands of the matrix material. A compilation of common impurities, with absorption maxima, approximate absorptivities, and detection limits, is also presented. Batches of supposedly hyperpure "semiconductor grade" halides from several suppliers show significant differences. Atmospheric moisture introduces hydroxyl groups and such compounds as Si$_2$OCl$_6$ so that exposure is a source of oxygen contamination, even if all the absorbed water reacts with the halide.

THE HALIDES of the Group IV metals are now used extensively for the production of the pure elements by various reduction processes and for the preparation of pure oxides and pigments by hydrolysis or burning. SiCl$_4$, GeCl$_4$, and TiCl$_4$ are particularly important, and the Si and Ge chlorides and bromides are of interest in semiconductor technology not only for production of the bulk metals but for epitaxial deposition from the gas phase.

For all these uses the purity of the halides is of paramount importance, but these compounds are good solvents for many gases and organic materials, and they are powerful dehydrating agents. Hydrolysis by atmospheric moisture contaminates them with hydrogen halides and oxygen-bearing compounds; the latter are particularly unwelcome, since small amounts of oxygen may seriously degrade the physical and electrical properties of Group IV metals.

Little has been published on the detection of impurities in the halides, aside from conventional spectrochemical techniques for foreign metals. It is the purpose of the study reported here to point out the wealth of information obtainable from the infrared absorption spectrum of deep liquid layers of Si and Ge halides. Liquid sample thicknesses of more than 1 mm. are rarely used in infrared work, since most materials would be virtually opaque over most of the 2- to 25-micron analytical range. The Group IV liquid halides, however, are tetrahedral molecules with quite simple spectra; even more important, they contain no light atoms, so that all the fundamental absorptions lie beyond 15 microns. Even the first overtones and simple combinations usually lie beyond 10 microns. Consequently these compounds are quite transparent in the 2- to 10-micron range even in path lengths of many centimeters. Organic compounds and other volatile impurities which do have strong absorptions in this range may then be detected in concentrations as low as 1 p.p.m. Halides from various sources, all represented to be of the highest purity—e.g., "semiconductor grade"—have given spectra showing significant differences. Often the exact impurities may be identified with fair confidence. The success of attempted purification procedures may be followed readily.

Only one compound, titanium tetrachloride, has been examined extensively by the long-liquid-path technique (*4, 15, 24, 32–35*). It is evident from these results, as well as from the present work, that absorption maxima of contaminants ordinarily shift very little upon solution in the halide, generally 0 to 0.1 micron; the same now seems true of the variation

Table I. Fundamental Vibrations of Some Group IV Metal Halides
(Cm.$^{-1}$)

	$SiCl_4$ (*7*)	$SiBr_4$ (*7*)	$GeCl_4$ (*7*)	$GeBr_4$ (*7*)	$TiCl_4$ (*8, 13, 21*)
$\nu_2(e)$	150	90	132	80	120
$\nu_4(f_2)$	221	137	172	112	140
$\nu_1(a)$	424	249	396	235	388
$\nu_3(f_2)$	610	487	453	327	485
ν_3, observed[a]	621	498	460	330	...

[a] For the gas phase. Other data are for the liquid.

in different halide solvents. Thus, many of the existing data on impurity absorptions in $TiCl_4$ are useful for Si and Ge halides.

It is the intention of this report to outline the method of examination and to show typical results, rather than to present complete qualitative and quantitative characterization of impurity spectra. Calibration may be done readily for any impurity considered important, but there are many likely contaminants, usually varying with the history of the halide, the manufacturer, and even the lot number. Furthermore, absorptivities should be determined on the same spectrophotometer to be used in the analysis. Finally, for many purposes almost any impurity is undesirable, and exact identification of an unknown band (which may be laborious) is of only secondary interest.

INTERPRETATION OF SPECTRA

In interpreting long-liquid-path spectra it is considered that the general transmittance level, as well as drifts and minor fluctuations of this level with wavelength, are all without analytical significance. There is a long and uncompensated path of high-refractive-index liquid, bounded by windows of high index and high reflectivity which may not be exactly parallel; some deviation of the beam, and some scattering as well, are likely. A well defined absorption band departing from the neighboring transmittance level is certainly significant, however. The baseline level becomes uncertain as one approaches the region where the Si or Ge halides themselves absorb, since with 10-cm. samples there is strong absorption from the wings of these bands.

The positive identification of an unexpected impurity solely from the infrared spectrum is usually not possible. With some experience and some knowledge of the history of the sample, however, certain recurring absorptions soon take on identity. Two simple techniques which have been very helpful are distillation and deliberate contamination. In the latter the sample, in the cell, is "doped" with a contaminant to observe the exact wavelength of the absorption. A microsyringe is convenient for liquids, and gases are simply bubbled slowly through the cell, in one filler tube and out the other. Minimal exposure to the atmosphere is essential.

With distillation, an absorption band which is more prominent in the first fraction evidently belongs to a more volatile or gaseous impurity; a less volatile impurity concentrates in the boiler residue. A band which is unchanged by distillation probably belongs to the halide itself, although there is always the chance of an impurity with a boiling point too close for significant fractionation.

EXPERIMENTAL

Most of the spectra were obtained on a Beckman IR-4 spectrophotometer; a few were observed also on a Perkin-Elmer 421. Instrument settings and operation were routine. For comparability of spectra the reference beam was attenuated to adjust the transmittance to 90–95% in a region without bands, usually 4.5 to 5 microns. A compensating cell was not required.

The sample path length was 10 cm. throughout. Cells were cut from 30-mm. o.d. borosilicate tubing and provided near each end with a filler tube with overflow bulb. The windows were 1-mm. sheet silver chloride, cemented on with Araldite epoxy resin (Ciba Co.). The usual NaCl or KBr windows contain far too much water for service with the Group IV halides and are quickly coated with insoluble hydrolysis products. Silver chloride was always satisfactory except where a sample contained some strong reducing agent which stained the windows with a silver film. In this case windows of polished high-resistivity germanium were used. Irtran-2 (Eastman Kodak Co.) also serves well.

The still was designed to avoid any joint grease or rubber gaskets, since these are attacked or dissolved by the hot halide, with results evident in the spectrum. The still was flushed continuously with dry nitrogen. The receiver was the cell itself, and provision was made for rejection of a fore-run.

Where as-received electronic grade $SiCl_4$ was further purified the method was essentially that of Clabaugh *et al.* (*4*) followed by the silica gel treatment of Theuerer (*30*). This latter was omitted when the $SiCl_4$ was distilled directly into the absorption cell, since the spectrum showed that silica gel may contribute impurities as well as remove them.

In the figure captions, different code letters indicate different manufacturers.

SPECTRA OF Si AND Ge HALIDES

The infrared spectra of $SiCl_4$ vapor (*28*) and $GeCl_4$ vapor (*17*) have been published. There are no reports on the liquid phase, and no reports of any kind on either $SiBr_4$ or $GeBr_4$. The strongest vapor absorptions of all four compounds have been located in earlier work in these laboratories, and are given in Table I, along with the fundamental vibrations known from Raman spectra (*7*). From the studies cited, it is evident that the $\nu_3(f_2)$ vibration produces by far the strongest infrared absorption, and most of the other strong bands of the spectrum are overtones or combinations of ν_3. The highest frequency absorption ordinarily visible in the vapor is $2\nu_3$, which lies at these wavelengths: $SiCl_4$, 8.2 microns; $SiBr_4$, 10.3; $GeCl_4$, 11.0; $GeBr_4$, 15.2. With a 10-cm. liquid path this absorption governs the useful working limit, since from this region out through lower frequencies the sample is opaque.

Some of the frequencies higher than $2\nu_3$ observed in the long-liquid-path spectra are due not to impurities but to the matrix material itself; these are combination and overtone frequencies too feebly absorbing to be seen under the usual conditions of measurement. The origin of such bands is indicated by two pieces of evidence: their intensity does not change when the sample is distilled; and a single pattern of frequency assignment accounts for them in all of the Si and Ge halides studied, and in $TiCl_4$ also. Table II lists certain combinations, all involving ν_3, calculated from the data of Table I. As shown, in almost every case a persistent absorption band is observed very close to the calculated frequency. Intensities vary, but $\nu_1 + 2\nu_3$ is generally the strongest. In the spectra shown in this report, absorption bands of the matrix material are marked by a heavy dot.

Table II. Absorptions in Long Liquid Paths
(Cm.$^{-1}$)

	$\nu_4 + 2\nu_3$	$2\nu_1 + \nu_3$	$\nu_1 + 2\nu_3$	$3\nu_3$	$2\nu_3$
$SiCl_4$, calcd.	1441	1458	1644	1830	1220
obs.	1452		1643–56	1825	Opaque < 1310
$GeCl_4$, calcd.	1078	1245	1302	1359	906
obs.	1084	1242	1305	1360	Opaque < 980
$SiBr_4$, calcd.	1111	985	1223	1461	974
obs.	[a]	[b]	1224	1463	Opaque < 1140
$GeBr_4$, calcd.	766	797	889	981	654
obs.	766	790	878?	970	Opaque < 700
$TiCl_4$, calcd.	1110	1274	1384	1495	998
obs.[c]	1110	1258	1359	1478	Opaque < 1050

[a] Masked by strong foreign absorption.
[b] The strong $2\nu_3$ absorption interferes.
[c] Refs. (*15*, *24*).

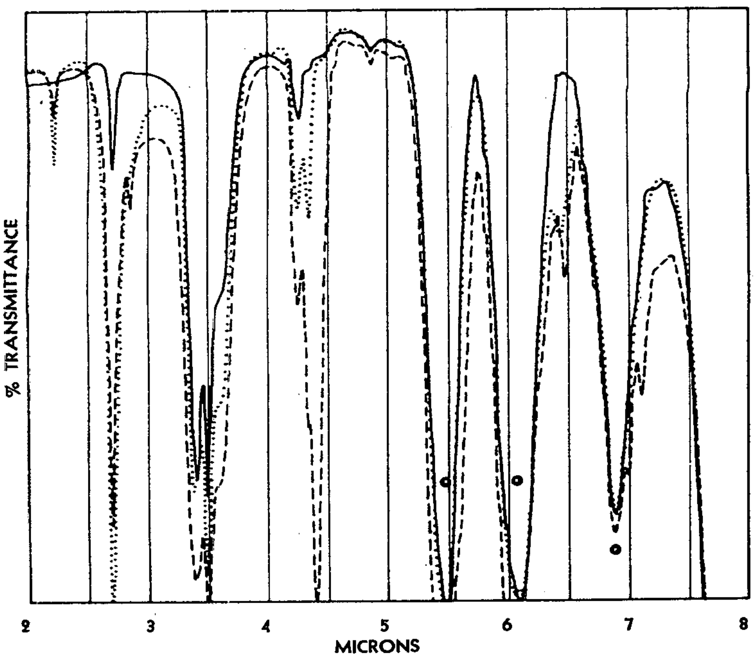

Figure 1. $SiCl_4$, liquid, 10 cm.

– – – Sample M, as received
.... Sample S, as received
—— Sample M, purified and distilled into cell

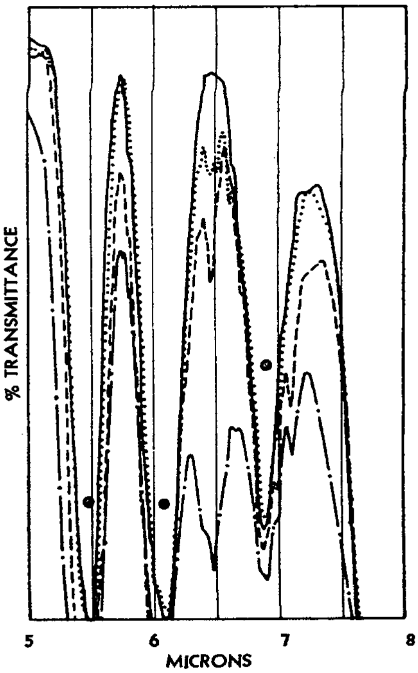

Figure 2. $SiCl_4$, liquid, 10 cm.

– – – Sample M, as received
.... Sample M, purified
—— Sample M, purified and distilled into cell
-.-.-. Sample M plus 0.39% Si_2OCl_6

RESULTS

Figure 1 presents typical long-liquid-path spectra for $SiCl_4$, and is an example of what can be learned about competing samples, all supposedly "pure." Impurities observed, with an order-of-magnitude estimate of concentration in p.p.m., are: HCl, 50–250; CO_2, 5–50; $SiHCl_3$, 1000; CCl_4, 100; Si_2OCl_6, 100–500; and either water or some hydroxyl-containing nonorganic compound.

Absorption maxima (microns) and their assignments follow: 2.22, unknown, but associated with 2.70; 2.70, hydroxyl group, nonhydrogen-bonded—most probably $Si(OH)Cl_3$ from hydrolysis (*2*); 3.39, 3.50, HCl; 4.25, CO_2; 4.35, unknown, has been observed in several halides—strongest in distillation forerun, probably a gas; 4.43, $SiHCl_3$; 6.44, CCl_4; 6.48, Si_2OCl_6—see below; 7.09, unknown.

Bands assigned to $SiCl_4$ itself are included in Table II. It is suspected that the 5.5 and 6.1 micron $SiCl_4$ bands are partially masking the two strong bands of phosgene (5.51, 6.05), a common impurity in samples made by chlorination of the oxide. The 5.5 micron $SiCl_4$ band also is almost certainly masking the 5.43 band of Si_2OCl_6,

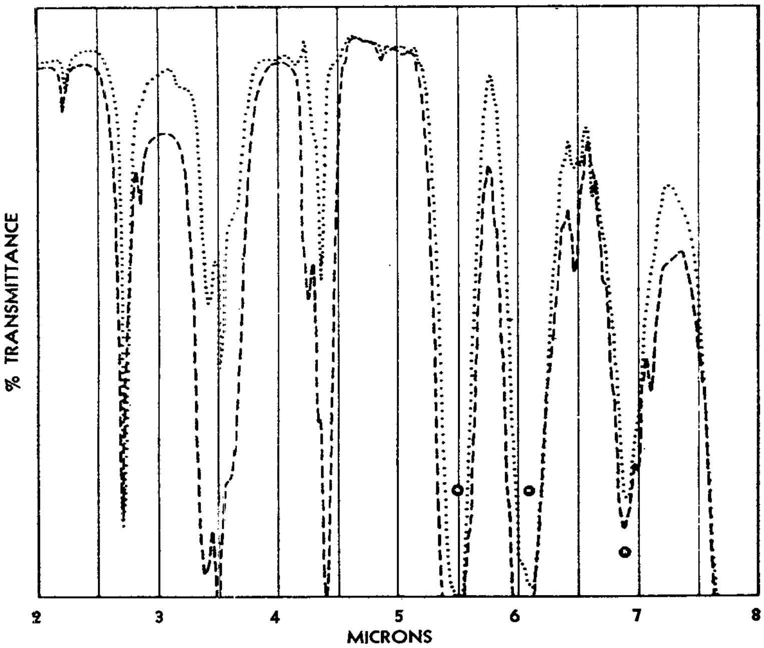

Figure 3. $SiCl_4$, liquid, 10 cm.

– – – Sample M, as received
.... Sample M, purified

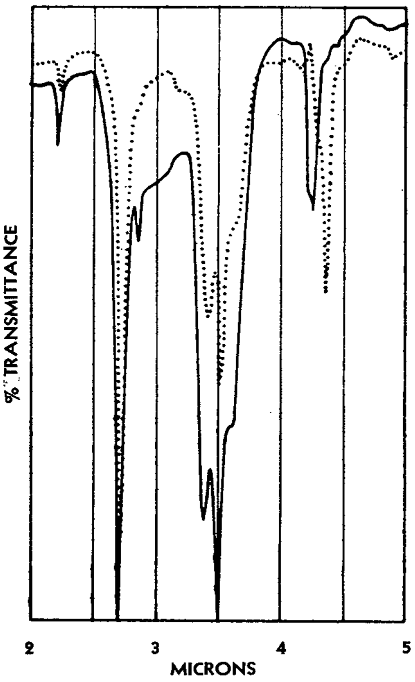

Figure 4. $SiCl_4$, liquid, 10 cm.

.... Sample M, purified
—— Same, after several weeks' storage

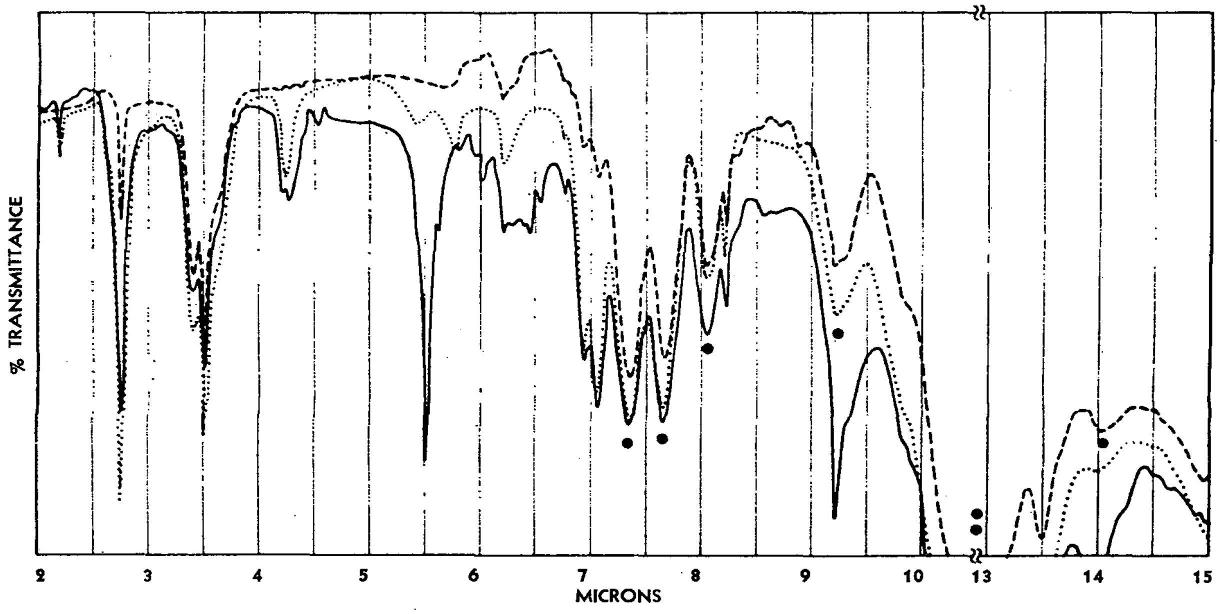

Figure 5. $GeCl_4$, liquid, 10 cm.

—— Sample E, as received
.... Sample S, as received
– – – Sample S, distilled into cell

which is stronger than 6.48. This compound arises from limited hydrolysis:

$$SiCl_4 + H_2O \rightarrow Si(OH)Cl_3 + HCl$$

$$Si(OH)Cl_3 + SiCl_4 \rightarrow Si_2OCl_6 + HCl$$

Hexachlorodisiloxane, Si_2OCl_6, is volatile (b.p. 137° C.) and is a source of oxygen contamination of silicon. To confirm its presence the experiment shown in Figure 2 was carried out: pure Si_2OCl_6 was prepared (*27*) and added to $SiCl_4$. The result confirms the assignment of 6.48-micron absorption to Si_2OCl_6. The purification procedure evidently removes most of it, probably solely because of the distillation step. The sample with no atmospheric exposure had no 6.48-micron absorption.

Figure 3 demonstrates the monitoring of the $SiCl_4$ purification procedure by the infrared method. It is seen that the hydroxyl content and the unknown 4.35-micron absorption are little affected; HCl is much diminished; and virtually complete removal of CO_2, $SiHCl_3$, Si_2OCl_6, and several minor absorptions has occurred.

Figure 4 shows the changes occurring as $SiCl_4$ stands in a closed (but not sealed-off) borosilicate glass container. Hydroxyl, HCl, and CO_2 have all increased. Some 3.0-micron absorption is attributed to hydrogen-bonded hydroxyl; this is seen in all the halides after prolonged exposure, and is characteristically broad and shallow.

Control tests in which traces of liquid and gaseous water were added confirmed the —OH and HCl assignments. Even the minute amount of CO_2 added with the liquid water was visible. No additional absorptions appeared in the 5- to 6-micron range.

Germanium Tetrachloride. Figure 5 shows long-path spectra of "semiconductor grade" $GeCl_4$'s. Again the sharp hydroxyl (2.75 microns), HCl, and CO_2 absorptions are seen. Sample E probably contains phosgene (5.51 microns). There is some structure around 6 microns which could be a trace of water, or some organic compound. Both as-received samples contain some unknown impurity with absorptions at 6.93, 7.07 microns; Ge_2OCl_6 is a possibility. The sharp band at 8.22 microns is weaker in the distilled sample, and is assigned to $SiCl_4$ (or, less probable, $CHCl_3$). The 9.22-micron absorption in sample E is due mostly to an all-but-invisible trace of amorphous silica on the cell windows, since this cell had been used for $SiCl_4$. Windows are easily cleaned by lapping with Linde A or other fine abrasive, however.

Silicon Tetrabromide. Spectra before and after distillation of one sample of $SiBr_4$ are shown in Figure 6. Again the hydroxyl absorption (2.74 microns) is present; 3.81, 3.98 microns

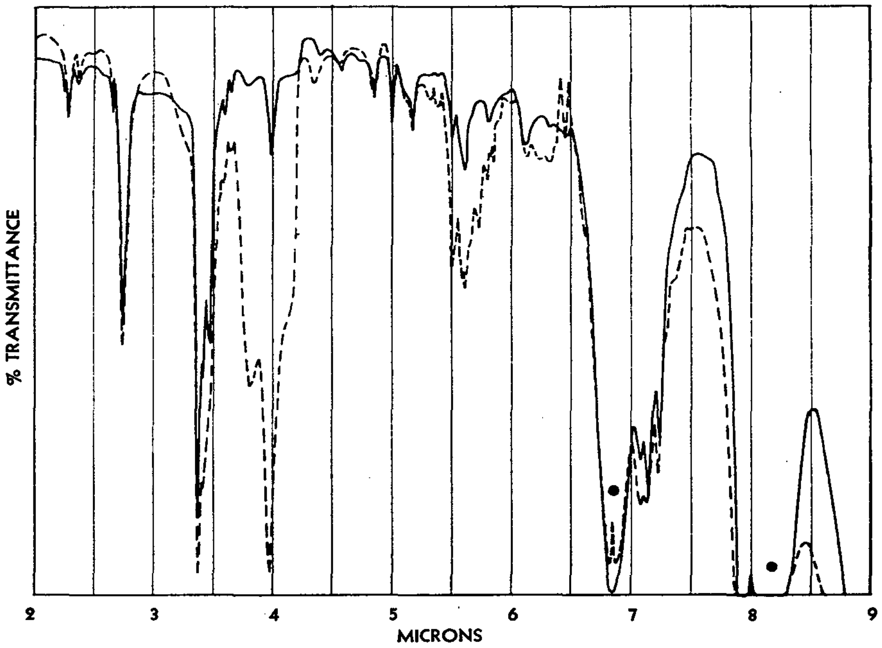

Figure 6. $SiBr_4$, liquid, 10 cm.

– – – Sample A, as received
—— Same, distilled into cell

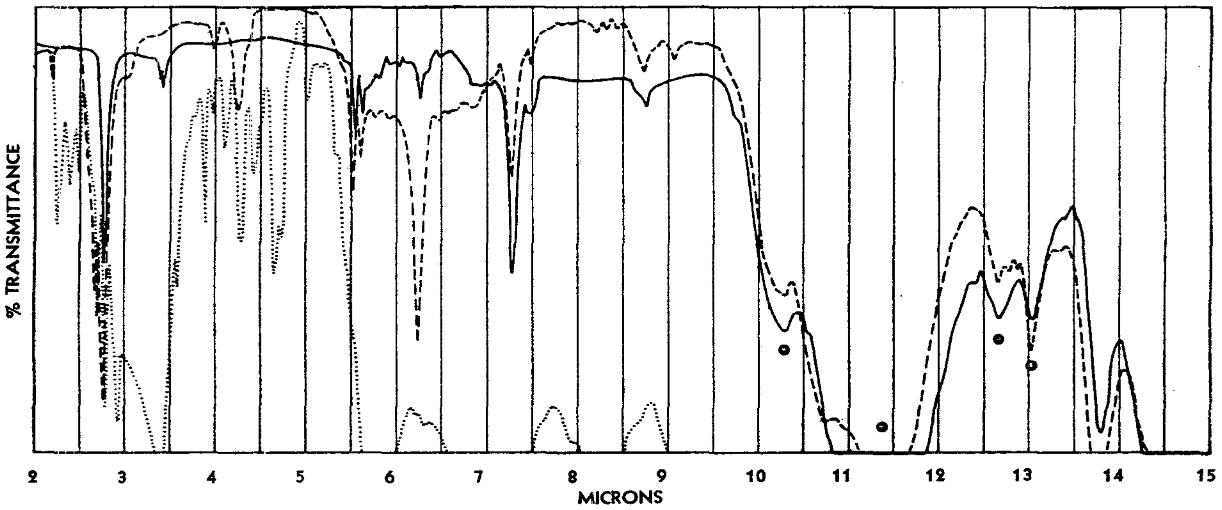

Figure 7. $GeBr_4$, liquid, 10 cm.

— — — Sample E, as received
.... Sample K, as received
—— Sample E, distilled into cell

are HBr. The sample is evidently contaminated by some organic material with the C—H bond (3.37 microns) which is difficult to separate by distillation. Since its spectrum is obviously unusually simple it is suggested that the impurity is bromoform (b.p. $CHBr_3$, 149°C; $SiBr_4$, 153°C.). The absorption at 5.61 microns may be $COBr_2$ (*23*).

Table III. Impurity Absorption Bands in Group IV Metal Halides

(Ten-cm. liquid path)

Impurity	B. P., ° C.	Absorption maximum,[a] μ	Absorptivity, wt. %$^{-1}$ cm.$^{-1}$	Detection limit, p.p.m.	Refs.[b]
—OH	...	2.70–2.80[c]	...	...	Tw
C—H in haloforms	...	3.28–2.35[d]	...	...	(*25*)
HCl	−84	3.53	15	2	Tw; (*4, 15, 24, 32–35*)
		3.41	10	3	
HBr	−67	3.99	...	...	Tw
CO_2	−78	4.27[e]	80	1	Tw; (*4, 15, 24, 32–35*)
P—H	...	(4.3)	...	...	(*1, 18, 19, 22, 26*)
$SiHCl_3$	33	4.43	...	...	Tw; (*10*)
Si—H	...	(4.6)	...	...	(*3, 6, 12, 14, 16, 20, 31*)
Ge—H	...	(4.8)	...	...	(*5, 9, 11, 29*)
COS	−48	4.89	200	0.2	(*24, 32–35*)
$COCl_2$	8	5.51	50	1	(*4, 15, 24, 32–35*)
		6.05	5	10	
$COBr_2$	65	(5.48)	...	...	(*23*)
CCl_3COCl	118	5.54	40	1	(*4, 15, 24, 32–35*)
CCl_4	77	6.44	0.5	100	(*24*)
CS_2	46	6.57	500	0.05	(*24, 32–35*)
SO_2Cl_2	69	7.04	75	2	(*24*)
C_6Cl_6	325	7.69	10	5	(*32–35*)
CH_2Cl_2	40	7.93	20	5	(*24*)
$POCl_3$	105	7.95	80	2	(*24, 32–35*)
		8.21	45	3	
$SOCl_2$	79	8.08	100	0.5	(*24, 32–35*)
		4.06	0.5	25	
$SiCl_4$	57	8.18	0.5	100	(*4, 15, 24, 32–35*)
$CHCl_3$	61	8.24	30	3	(*24*)
Si_2OCl_6	137	8.98	60	1	Tw; (*24*)
		5.43	0.8	40	
		6.49	0.2	150	
SiO_2	...	9.2	...	...	Tw
$VOCl_3$	127	9.66	100	0.5	(*4, 15, 24, 32–35*)
		4.84	0.5	50	

[a] Values in parentheses are for the pure materials; it is not known whether any shift occurs in solution.

[b] Tw = this work (absorptions established by addition of known material to the halides).

[c] The addition of water gives a broad, shallow band near 3μ, attributed to hydrogen-bonded hydroxyl.

[d] For most organic compounds the C—H absorption is usually given as 3.3–3.4μ.

[e] Doublet under high resolution.

Boiling points: $SiCl_4$, 57° C.; $GeCl_4$, 83° C.; $SiBr_4$, 153° C.; $GeBr_4$, 186° C.

Germanium Tetrabromide. $GeBr_4$ spectra are shown in Figure 7. The pure compound has no absorption in the 2- to 10-micron range and deserves consideration as a solvent for infrared work. It is not so sensitive to hydrolysis as the other halides and it is suspected that small amounts of water can exist in equilibrium with it. The broad, shallow 5- to 7-micron absorption in as-received sample E, with a sharp peak at 6.26, is believed to be water. The addition of a drop or two of BBr_3, a potent water-scavenger, removes this absorption, as well as the 2.77-micron hydroxyl band.

There is little HBr. The persistent band at 7.28 microns is presently assigned to $CBr_4(2\nu_3)$, which boils only three degrees higher than $GeBr_4$.

The infrared method reveals that sample K, represented by the manufacturer to be of high purity, is in actuality hopelessly contaminated with organics. There is a heavy 3.35-micron C—H band, many other high-frequency bands, and almost total absorption beyond 5.6 microns. Such a sample would be a poor choice for growing epitaxial germanium.

Addenda. For the convenience of those employing the long-liquid-path infrared absorption technique, a summary of what is presently known about impurity absorptions, qualitatively and quantitatively, is appended in Table III. A complete literature search for the spectra of the compounds listed is incorporated in this compilation. Some of the data are from the $TiCl_4$ studies, believed valid for Si and Ge halides also. Absorptivities should be considered only approximate. References are given throughout. It will be obvious that as much art as science must go into trace analysis by infrared; but even if all the impurities cannot be identified much will be learned about a sample which would not be revealed easily by any other technique.

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Sensitive and Selective Spectrophotometric Reaction for Determination of Trace Amounts of Calcium

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▶ The reagent Calcichrome, cyclo-tris-7-(1-azo-8-hydroxynaphthalene-3,6-disulfonic acid), provides a method for the spectrophotometry of calcium down to the 0.1-p.p.m. level. At pH 12 and 615 mμ the procedure has a molar absorptivity of 7600. The method may be applied in the presence of the other alkaline earths—e.g., 5000 μg. of Ba^{+2}—with some reduction in sensitivity. Several hundred micrograms of metals such as Al, Pb, Zn, Co, Hg, and Cd do not interfere under specified conditions, but Mg^{+2} and PO$_4^{-3}$ interfere. The color system develops within 15 to 20 minutes and maintains an unchanged absorbance for more than 24 hours. The reagent solution is also stable.

ALTHOUGH many methods have been proposed for the spectrophotometric determination of calcium, in general the majority are subject to interference from other ions, and are rather unstable or not very sensitive when compared to spectrophotometric procedures for the determination of other metals. Sandell (*7*) has recently reviewed and discussed these methods. The oxalate procedure is a very indirect one and is subject to rather restrictive conditions. It is dependent on initial complete separation of calcium oxalate with minimum coprecipitation of oxalate ion and it involves a filtration or centrifugation step. The chloroanilate method is less restrictive, but also involves filtration. The most advantageous procedures so far devised appear to be those based on murexide (ammonium purpurate), phthalein complexan (2,6-xylenolphthalein-α,α'-bisiminodiacetic acid), and glyoxal bis(2-hydroxyanil).

The murexide method appears to be applicable in the range 1 to 3 p.p.m. of calcium, but a high concentration of reagent must be used to ensure quantitative formation of the calcium complex. Unfortunately, the reagent is unstable and ca. 50% decomposition occurs over 4 hours at room temperature at the pH of determination. Practically all heavy metals interfere and the tolerance for strontium and barium is 1 and 5 p.p.m., respectively. Magnesium also forms a color and >400 p.p.m. of sulfate interfere. The results obtained by the method appear to be somewhat lacking in reproducibility, though a more detailed and reliable method has been reported (*3*, *5*). The phthalein complexan method is also nonselective and the color system is unstable, so that the absorption must be measured immediately under carefully prescribed conditions.

Undoubtedly the best reagent to date appears to be glyoxal bis(2-hydroxyanil). According to Williams and Wilson (*9*), the method based on this reagent operates in the range 0.5 to 10 μg. per ml. The color system also lacks stability, but when extracted into chloroform it remains unchanged for 15 minutes. Centrifuging of precipitate is required, but removal of liquid is avoided by extraction with chloroform and clarification of the extract by centrifuging. The color of the extract obeys Beer's law in the range up to 10 μg. (0.5 to 10 p.p.m.) and the calibration curve appears to be fairly reproducible, though it must be checked whenever used. The information about interferences is limited. Ten times the concentration of magnesium, or one tenth the amount of strontium or of iron, does not interfere with the determination of ≤ 10 μg. of calcium. Strontium gives a color with the reagent, but the addition of carbonate is apparently successful for amounts ≤ 1 to 10 ratio. Earlier papers on the use of the reagent (*4*) state that in the presence of carbonate ion, cobalt and nickel give red precipitates with the reagent, and

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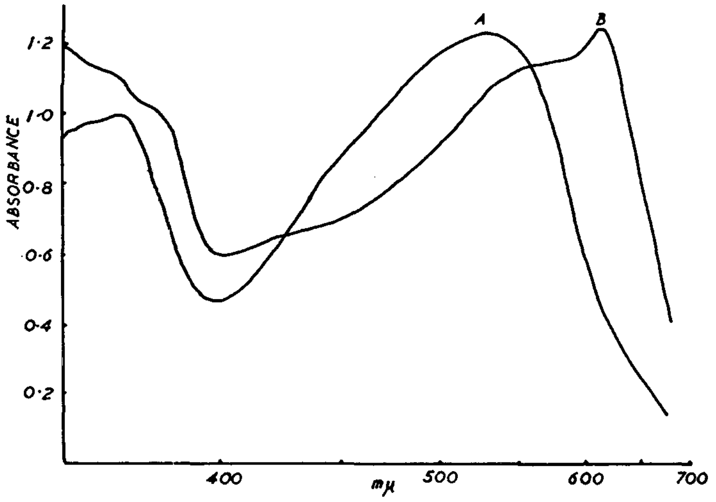


Figure 1. Absorption spectra of Calcichrome and alkaline earth complexes

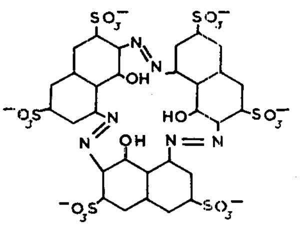
A. Calcium complex. 10 ml. of 10^{-3}M Ca^{+2} + 10 ml. of 10^{-3}M Calcichrome + 10 ml. of pH 12 buffer per 100 ml., 4-cm. cuvettes

B. Calcichrome. Same as A, but without Ca^{+2}

copper and cadmium form colored complexes; only the cadmium complex is extracted into chloroform.

A highly selective colorimetric reagent for calcium, cyclotris-7-(1-azo-8-hydroxynaphthalene-3,6-disulfonic acid) (Calcichrome), has been synthesized and examined by Close and West (*2*). It was reported that, qualitatively at pH 12 to 13, of some 37 cations examined only calcium produced a soluble colored product with the reagent. Magnesium, strontium, and barium did not produce colors. The reagent was used at pH 12 for the complexometric titration of calcium in the presence of soluble barium with *trans*-1,2-diaminocyclohexane-*N*,*N*,*N'*,*N'*-tetraacetic acid as titrant. In addition, it was used as a spot test at a dilution limit of 1 to 1,000,000. The interference of heavy metal hydroxides, which tend to adsorb reagent, was prevented by addition of sulfide ion.

At this time, the exact structure of the reagent is not finally decided, but it is thought to be:



Work is now in progress to establish the exact identity of the molecule by synthetic and degradative techniques, and will be reported in a subsequent communication. The unusual selectivity of this hydroxyazo compound may, according to our present information, be attributed to the cyclic arrangement of the *o*-hydroxyl groups which, with the azo nitrogens, form a clathrate network or chelate cage into which only ions of a certain size may fit. Thus, within the alkaline earth group, while chemical affinity doubtless has its role, there can be little doubt that the decisive factor which allows calcium to react, but not barium and strontium, is the ionic radii of the ions concerned. For $n = 6$ these are Ca^{+2}, 0.99 A.; Sr^{+2}, 1.12 A.; and Ba^{+2}, 1.34 A. Beyond this group, other ions which might fit into the reagent are bound as anionic complexes or are insoluble at pH ≥ 12 (*8*). Several other ions react with the reagent at pH 10, but the reaction is restricted to calcium at pH ≥ 12.

In view of the need for a sensitive and selective reagent capable of producing stable colors with calcium, it was decided to apply the above reagent to its spectrophotometric determination.

EXPERIMENTAL

Reagents. $10^{-3}M$ Calcichrome, 0.5916 gram of the sodium salt per liter of distilled water. The solid dissolves readily with efficient stirring and is stable in solution. The reagent was prepared as described (*2*). It is available commercially from Burdick-Jackson Laboratories, 1953 South Harvey St., Muskegon, Mich.

$10^{-4}M$ Calcium, prepared by dilution of a $10^{-1}M$ calcium solution. [The latter was prepared by dissolving 10.009 grams of $CaCO_3$ (AR) (analytical reagent grade) in the minimum amount of hydrochloric acid (AR) and diluting with distilled water to 1 liter.] This solution contains 4 μg. of Ca^{+2} per ml.

pH 12 Buffer, 3.002 grams of glycine (AR) + 2.34 grams of NaCl (AR) in 500 ml. of water. This solution is then mixed with 3.0 grams of NaOH dissolved in 500 ml. of distilled water. When used as prescribed, this buffer produced color solutions with a final pH of 12.0. It is essential that the pH be maintained at 12 and consequently the buffer and its performance should be checked periodically with a pH meter.

Solutions for Examination of Interferences. Barium, $1.5 \times 10^{-3}M$, prepared from Johnson Matthey Specpure barium carbonate. This solution contains 200 μg. of Ba^{+2} per ml.

Strontium, $2.3 \times 10^{-3}M$, prepared from Specpure strontium carbonate. This solution contains 200 μg. of Sr^{+2} per ml.

Other Cations. $10^{-3}M$ solutions of zinc sulfate, cadmium sulfate, copper sulfate, magnesium sulfate, aluminum sulfate, lead nitrate, mercury(II) nitrate, and cobalt nitrate.

Anion Solutions. potassium cyanide (1%) in water, 1% sodium sulfide in water, etc.

Apparatus. Unicam SP. 600 spectrophotometer and Hilger & Watts Uvispek with 4-cm. cuvettes. The spectra shown in Figures 1 and 2 were measured on a Hilger Ultrascan automatic recording spectrophotometer in 4-cm. cells.

Procedure. CALIBRATION CURVE, 10 to 70 μg. of Ca^{+2}. Aliquots (2.5 to 17.5 ml.) of $10^{-4}M$ calcium solution were pipetted into 100-ml. standard flasks plus 10 ml. of pH 12 buffer solution, followed by 10 ml. of $10^{-3}M$ Calcichrome reagent solution. The contents of the flasks were then diluted to 100 ml. A blank solution containing the standard amounts of buffer and reagent only was prepared simultaneously and all solutions were allowed to stand for 1 hour. The absorption of the blank solution was then measured in turn against zero absorbance set on each of the calcium-containing solutions in 4-cm. cuvettes at 615 mμ in the spectrophotometer. The calibration curve is a straight line from 10 to 70 μg. of Ca^{+2} with the absorbance ranging from 0.075 to 0.535.

CALIBRATION CURVE, 2 to 10 μg. of Ca^{+2}. Aliquots of $10^{-4}M$ calcium solution containing from 2 to 10 μg. of Ca^{+2} were treated in 25-ml. flasks with 2 ml. of pH 12 buffer and 2 ml. of $10^{-3}M$ Calcichrome and diluted to 25 ml. The solutions were then allowed to stand for 1 hour and measured at 615 mμ in 4-cm. cuvettes as described above. The absorbance ranges from 0.075 to 0.290.

DETERMINATION OF UNKNOWN CALCIUM SOLUTIONS. The procedure appropriate to the ranges 2 to 10 or 10 to 70 μg. of calcium is used. When other metals are known to be present, the calibration curve should be drawn up with an approximately equivalent amount of that element present in the blank and calibration solutions. If the metal is one such as Co, Cd, or Cu, which is likely to precipitate out at pH 12, sufficient KCN should be added *before* the buffer and reagent throughout the procedure. It is also appropriate to check the pH of the solutions for rise of pH when appreciable amounts of cyanide are used.

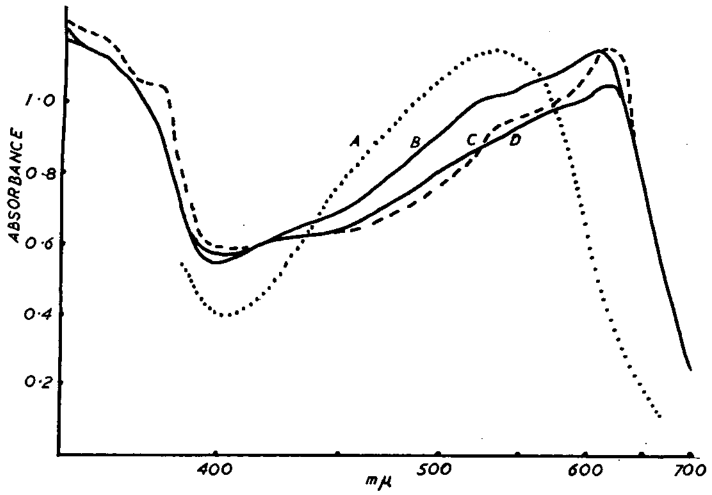


Figure 2. Absorption spectra of Calcichrome and alkaline earth complexes

- *A.* Calcium complex as in Figure 1 *A*
- *B.* Calcichrome + strontium. 2000 μg. of Sr^{+2} + 10 ml. of $10^{-3}M$ Calcichrome + 10 ml. of pH 12 buffer per 100 ml., 4-cm. cuvettes
- *C.* Calcichrome. As in *B*, but without Sr
- *D.* Calcichrome + barium. As in *B*, but with 2000 μg. of Ba^{+2} in place of Sr^{+2}

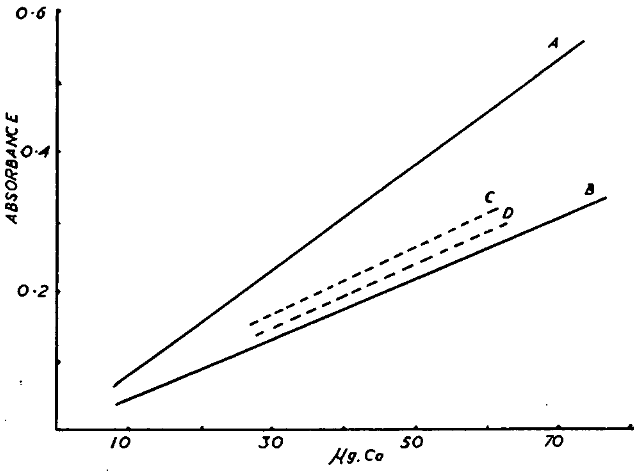


Figure 3. Calibration curves

- *A.* 10 to 70 μg. of Ca^{+2} by recommended procedure at 615 mμ
- *B.* 10 to 70 μg. of Ca^{+2} by recommended procedure at 510 mμ
- *C.* 10 to 70 μg. of Ca^{+2} in presence of 470 μg. of Al^{+3} at 615 mμ (reagent blank)
- *D.* Like C, but measured against reagent blank containing 470 μg. of Al^{+3}

Calibration curves with more positive slope and a wider range may be obtained by using larger amounts of reagent solution—e.g., 15 or 20 ml. of $10^{-3}M$ Calcichrome. The procedure mentioned here is designed for the average small spectrophotometer used in routine laboratories—the Unicam S.P. 600 is sufficiently sensitive to cope with 15 ml. of $10^{-3}M$ Calcichrome per 100 ml., but not with 20 ml.

Where KCN is used, it must be added *before* the buffer and reagent. It is also important to add buffer before reagent when the method is applied generally.

If the spectrophotometer used operates its check control via slit adjustment rather than electronically, a slightly sigmoid-shaped calibration curve may be obtained because of the slightly different width of the spectrum scanned over the range of measurements. We ourselves have not observed this phenomenon.

DISCUSSION AND RESULTS

The optimum pH conditions for applying the reagent to the determination of calcium in pure solution were first established by adding varying amounts of sodium hydroxide to a solution of calcium and the dyestuff and measuring the pH with a pH meter following absorption measurements at the wavelength of maximum absorption. These studies revealed that the maximum sensitivity was obtained at pH 12. When the method was placed on a routine basis subsequently, it was found preferable to replace the sodium hydroxide solution with a glycine–sodium hydroxide buffer at pH 12, since variations in pH obtained with sodium hydroxide alone affected the reproducibility of the method from day to day.

The wavelength of maximum absorption of the calcium complex of the reagent lies at 520 to 530 mμ at pH 12 and at 540 mμ at pH 11, while that of the reagent is at 615 and 610 mμ, respectively. From the data obtained (Figure 1) it is obvious that the formation of the complex may be measured positively at 510 mμ or three to four times more sensitively at 615 mμ by following the decrease in the absorption band due to the reagent. The calcium complex also exhibits an intense peak in the ultraviolet region at 299 mμ (pH 12) or 300 mμ (pH 11), while the reagent shows a strong band at 310 mμ at both pH values. However, no advantage may be gained by measuring the complex in this region because of the high background absorption. To obtain positive readings for the calibration curve at 615 mμ the normal measurement process of spectrophotometry was reversed—i.e., the blank solution was measured against zero absorbance set with the test solution in each case.

The stability of the color system was then examined by preparing a solution of the calcium complex under normal conditions of determination, and measuring its absorption against the reagent solution over a period of several hours, taking due precaution against absorption of carbon dioxide. This study revealed that the color system had developed quantitatively within 15 to 20 minutes and thereafter maintained an unchanged absorption for 24 hours. For the sake of uniformity, the color system was always allowed to stand for 1 hour in all subsequent determinations, although it is apparent that the time factor is not critical. Solutions allowed to stand in open cuvettes absorb atmospheric carbon dioxide and decrease in pH and in absorbance accordingly. This effect becomes apparent with open cuvettes after 15 to 20 minutes.

Initial experiments were carried out with $10^{-3}M$ solutions of calcium and Calcichrome, thus obtaining calibration curves with 1-cm. cuvettes in the range up to 400 μg. of Ca^{+2}. Subsequently, similar experiments were performed with $10^{-4}M$ solutions, thus obtaining straight-line calibration curves with 4-cm. cuvettes in the ranges 2 to 10 and 10 to 70 μg.

To obtain a good positive slope on the calibration curves it is best to employ a large excess of the reagent. Thus for 10 to 70 μg. of calcium (up to 1.75×10^{-3} meq.), 10 ml. of $10^{-3}M$ Calcichrome solution (1×10^{-2} mmole) were used. This produced solutions with absorbance ranging from 0.075 (10 μg.) to 0.535 (70 μg.) when the solution was diluted to 100 ml. and measurements were made in cuvettes at 615 mμ by the process indicated. The molar absorptivity is $\epsilon_{615m\mu} = 7600$, or $\epsilon_{510m\mu} = 4500$. According to the terminology of Sandell (*6*), this represents a sensitivity index of 0.005 μg. of Ca^{+2} per sq. cm. at 615 mμ, or 0.009 μg. of Ca^{+2} per sq. cm. at 510 mμ (Figure 3).

The reagent solution was stable and the calibration curves were reproducible from day to day and with different preparations of reagent solution.

Effect of Foreign Ions. Since it had been established qualitatively (*2*) that a wide range of foreign cations do not form colored products with the reagent or interfere in any way with the calcium chelate except in some cases where precipitation occurs, only a few typical cations were examined in these studies: aluminum, cadmium, cobalt, copper, lead, mercury(II), zinc, barium, strontium, and magnesium. In these experiments with the first-mentioned seven metals, the effect of the addition of 400 to 600 µg. of each was tested on the recovery of 30 to 50 µg. of calcium. Potassium cyanide was added when Cd, Co, Cu, and Hg(II) were examined to prevent their precipitation, but none was necessary where aluminum, zinc, and lead were involved, since these are soluble at pH 12. The results obtained in all cases were almost identical, but the absorbance was lower than the value predicted by the calibration curve. When a blank solution containing the foreign ion, but no calcium, was prepared and employed in the usual way, the straight-line graphs obtained were identical in all cases and passed through the origin (Figure 3). At 615 mµ in the presence of these ions the molar absorptivity was reduced from $\epsilon_{615 m\mu} = 7600$ to 4700.

It was apparent from these results that while none of these ions interfered by producing colored complexes with the reagent, or by preventing color formation with calcium when present in 10- to 20-fold amounts, they did reduce the sensitivity of the color reaction. The curves for calcium obtained in their presence were virtually identical in all cases when measured against a blank containing the same amount of the foreign ion. A similar effect is obtained by adding varying amounts of an indifferent univalent-univalent electrolyte such as sodium chloride.

Figure 4 shows the effect produced by determining 40 µg. of calcium against a reagent blank in the presence of 0 to 100 mg. of sodium chloride in a final volume of 100 ml. This measurement was made at 510 mµ, corresponding to direct formation of the red calcium complex, rather than at 615 mµ as for analytical measurements, and with 15 ml. of $10^{-3}M$ reagent solution instead of the usual 10 ml. The value of $\epsilon_{510 m\mu}$ decreases from 5200 in a medium of ionic strength $\mu \approx 0.01$ to 2800 at $\mu \approx 0.03$. Accordingly, when calcium is to be determined in the presence of any of the above metals (and presumably many others), a roughly similar amount should be incorporated in the blank and standard solutions used to prepare the calibration curve to compensate for this electrolyte effect. As will be seen from Figure 4, the amount used is not very critical. An alternative procedure could probably be devised by incorporating indifferent electrolyte in the buffer or reagent solutions. Finally, where cyanide addition is necessary—e.g., in the presence of Cu, Co, or Cd, where the ions must be held in solution—it should be added before the reagent and buffer.

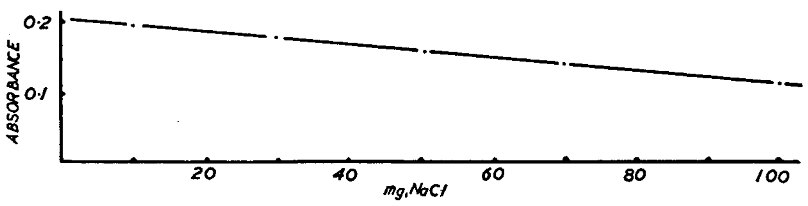


Figure 4. Effect of added electrolyte on sensitivity of color reaction

40 µg. of Ca^{+2} + 15 ml. of 10^{-3} M Calcichrome + 10 ml. of pH 12 buffer + mg. of NaCl per 100 ml., 4-cm. cuvettes at 510 mµ

The effect of barium and strontium is of particular interest, since the determination of trace amounts of calcium in their presence is of the utmost importance. In this instance the amount of these metals added was extended from 200 to 5000 µg. This spectrophotometric study showed that at 615 mµ, and to a lesser extent at 510 mµ, these ions give a slight positive reaction, but the effect of 200, 2000, and 5000 µg. is by no means proportionate. It is indeed doubtful that a colored complex is formed with barium or strontium, and even so, the compounds formed have a near-negligible molar absorptivity at any known wavelength.

Figure 2 shows the absorption spectra of the reagent in the presence of 2000 µg. of Sr and Ba and 400 µg. of calcium. This graph illustrates the nonreactive depressant action of barium and the slight positive effect of strontium when present in large amounts. The over-all effect of the large amount of strontium is to broaden out the reagent band at 530 to 630 mµ. Figure 5 shows the calibration curves obtained for calcium in the range 10 to 70 µg. when 2000 µg. of Ba^{+2} or Sr^{+2} were present, and when measurement was made against a blank containing a similar amount of the appropriate ion. The barium curve is closely similar to that obtained with 400 to 600 µg. of the previous metals, but that for strontium shows some divergence. This is not apparently due to the calcium content of the strontium carbonate used (1 p.p.m. of Ca), since the barium carbonate had a higher calcium content (2 p.p.m. of Ca). However, these graphs show clearly that it is possible to determine calcium in the presence of large amounts of barium or strontium without resort to separation, extraction, or masking.

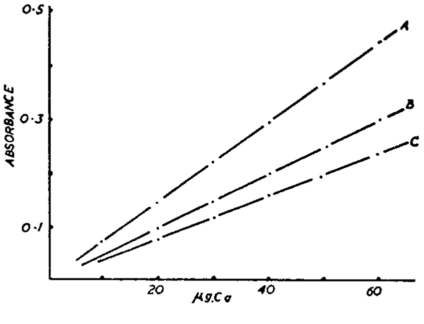


Figure 5. Calibration curves in presence of barium and strontium

A. Calcium by recommended procedure (reagent blank) at 615 mµ at pH 12, 4-cm. cuvettes
B. Like A, in presence of 2000 µg. of Ba^{+2} (reagent + 2000 µg. of Ba^{+2} blank)
C. Like A in presence of 2000 µg. of Sr^{+2} (reagent + 2000 µg. of Sr^{+2} blank)

The addition of large amounts of magnesium is detrimental, however. When magnesium is incorporated in the blank, a straight-line graph through the origin is obtained, but its gradient is low and its analytical value is probably very small. There is little doubt that the magnesium interferes by coprecipitating calcium and probably Calcichrome also. Small amounts of Mg^{+2} ions—e.g., 25 µg.—caused high results, but the correct Ca^{+2} recovery was obtained when similar amounts of Mg^{+2} were incorporated in the blank.

Finally, the effect of a few selected anions was examined. Calibration curves for 10 to 70 µg. of calcium were obtained in the presence of 4800 µg. of SO_4^{-2}, 1300 µg. of S^{-2}, 3300 µg. of F^-, and 5300 µg. of CN^-, as described for the cations. The same electrolyte effect was noted, and when similar amounts were incorporated in the blank solution, the results were virtually identical in all cases, and with those obtained previously with Al, Pb, Cd, Cu, etc. For example, the curve for calcium in the presence of 3300 µg. of F^- is exactly superimposed on the curve obtained with 2000 µg. of Ba^{+2}. Studies with 4700 µg. of PO_4^{-3} yielded considerable interference. A straight-line calibration curve was obtained, but the gradient is too low to be of any analytical significance. Small amounts of phosphate (<100 µg.) cause somewhat low results, which may be acceptable in many cases.

These experiments reveal Calcichrome to be a sensitive and highly selective spectrophotometric reagent for the determination of calcium in the range 2 to 70 µg.—i.e., 0.1 to 4 p.p.m. It is, however, subject to an electrolyte effect which may reduce the sensitivity by 45 to 50%. The only serious interferences encountered were due to magnesium and phosphate. Several hundred fold amounts of other ions may be tolerated, provided account is taken of the electrolyte effect thus introduced when the calibration curves are drawn up. The reagent is stable and the color formation is rapid and is also stable for 24 hours, when protected against atmospheric carbon dioxide.

The calcium complex of the reagent is apparently not a very strong one, because (a) the electrolyte effect is appreciable, (b) it can readily be broken down by EDTA, and (c) a large excess of reagent is necessary to ensure maximum color formation. In the recommended procedure when 10 ml. of $10^{-3}M$ reagent is used $\epsilon_{510m\mu} = 4500$, with 15 ml. of reagent $\epsilon_{510m\mu} = 5200$, and with 20 ml. $\epsilon_{510m\mu} = 5900$. Against the increased sensitivity, however, must be set the high resultant background absorption of such a large excess of reagent. The range of the method may be extended considerably beyond 70 μg. by such methods and can be taken up to 120 μg., but the recommended procedure is felt to be a reasonable compromise between the two considerations involved.

In applying the procedure to the determination of calcium in various substances, the electrolyte level should be kept to a minimum. Thus, for example, calcium in an organic matrix would best be brought into solution by an oxygen flask combustion, as has been demonstrated previously (*1*), rather than by a wet digestion process with subsequent neutralization, etc.

The reagent is applicable in the presence of large amounts of masking agents such as cyanide and fluoride, and consequently should be applicable to much larger amounts of interfering ions than were examined in this study, though large amounts of metals such as iron (III), which are difficult to maintain in solution at pH 12 even with masking agents, would probably have to be separated. When calcium is being determined in the presence of Sr^{+2} and Ba^{+2}, difficulty may arise in obtaining a calcium-free salt for incorporation in the blank and calibration solutions. Under these circumstances, and indeed generally, the method of standard addition of pure calcium to the test solution should prove a successful and convenient alternative, provided that the total calcium still lies within the Beer's law range.

Both murexide ($\epsilon_{506m\mu} \approx 10,000$) and glyoxal bis-(2-hydroxyanil) ($\epsilon_{535m\mu}$ = 15,000 to 16,000) are more sensitive than Calcichrome ($\epsilon_{615m\mu} = 7600$), but both these reagents and their calcium complexes are very unstable. Calcichrome also has a very much higher toleration for strontium and, probably, most other metals.

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Simultaneous Determination of Niobium and Tantalum by Neutron Activation Using Niobium-94*m* and Tantalum-182*m* and Rapid Radiochemical Separations

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▶ The niobium and tantalum content of rocks, graphite, and stainless steel samples has been determined by thermal neutron activation analysis with a 10-minute irradiation of samples in a neutron flux of 10^{12} *n* cm.$^{-2}$ second^{-1}. Niobium and tantalum were separated simultaneously, followed by an extraction of niobium-free tantalum with 1-pentanol (*n*-amyl alcohol). The entire radiochemical separation was completed in less than 10 minutes. The radioactivity and half life of the chemically separated niobium-94*m* and tantalum-182*m* were determined by gamma spectrometry. In practice, microgram amounts of niobium and tantalum can be determined simultaneously in 30 minutes by this method.

THE ELEMENTS tantalum and niobium occur together in nature and have close chemical properties. Thus it is a difficult problem to separate tantalum from niobium quantitatively, and to determine the exact amount of tantalum and niobium in a sample. Among the many methods currently used for the analysis of these elements, the colorimetric procedure appears to be simple, rapid, and fairly sensitive (*3, 16*). However, this method requires effective means of separating niobium and tantalum from interfering iron, titanium, molybdenum, tungsten, and other elements. The clean separation of niobium from tantalum is also difficult and lengthy. Moreover, corrections for reagent blanks become appreciable at low concentrations.

Determination by activation analysis of tantalum in the G-1 and W-1 "standard" rock samples of the U. S. Geological Survey was carried out by Morris and Olya in 1960 (*14*), and Atkins and Smales in 1960 (*1*) using the long-lived tantalum-182. No work, however, has been reported to date on the niobium in this sample or of the use of short-lived tantalum-182*m* in neutron activation analysis.

The simultaneous determination of niobium and tantalum by thermal

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neutron activation of short-lived niobium-94*m* and tantalum-182*m* should give not only a rapid analysis but also a more sensitive detection of these two elements than the colorimetric technique.

EXPERIMENTAL

Apparatus. Samples were irradiated in polyethylene snap-type "rabbits" in the pneumatic tube system of the Ford Nuclear Reactor of the University of Michigan. This system permits irradiations at thermal neutron fluxes of about 10^{12} *n* cm.$^{-2}$ second^{-1} (when the reactor is operating at full power of 1 Mw.) and delivery to a hood in the neighboring Michigan Memorial Phoenix Laboratory within 3 seconds after irradiation. Samples were then processed chemically and were measured by a 3-inch × 3-inch NaI(Tl) crystal coupled with a RIDL 400 channel pulse height analyzer. This equipment has been described in detail elsewhere (*11, 12*).

Burrell mechanical shaker.

Filter paper—glass fiber, Type A, Gelman Instrument Co., Chelsea, Mich.

Reagents. Tantalum foil, 0.00025 inch thick, Ethicon Suture Laboratories, New Brunswick, N. J.

Niobium oxide powder, high purity, Fansteel Metallurgical Corp., North Chicago, Ill.

n-Amyl alcohol, analytical reagent grade, Baker and Adamson.

Potassium bifluoride powder, Merck and Co.

Sodium peroxide, granular, reagent grade, Baker and Adamson.

All other chemicals were of analytical reagent grade.

The niobium-95 radioactive tracer was purchased from Oak Ridge National Laboratory.

Standard solutions of niobium and tantalum were prepared by dissolving a known weight of niobium oxide powder and tantalum foil in 48% reagent grade hydrofluoric acid and diluting with 1% by weight oxalic acid solution to give comparative standard solutions of 70 μg. of niobium per ml. and 98.2 μg. of tantalum per ml. in a final concentration of 1*M* hydrofluoric acid.

Sample Irradiations. U. S. Geological Survey rock sample, G-1 (powdered); National Bureau of Standards, sample No. 446; Spectrographic stainless steel; and Spex Industries Corp., Scotchplains, N. J., spectroscopic graphite powder No. 1002 in which 43 different elements are contained, were used.

The powder samples were weighed and transferred into polyethylene capsules. These capsules were then positioned inside the rabbit and irradiated along with standard gold foil flux monitors.

Procedure. Place a known amount of niobium-95 tracer, 10 mg. of niobium carrier, and an exact amount of tantalum carrier (4.0 mg. in this experiment) in a platinum crucible and evaporate to dryness. Drop the irradiated sample into the crucible and fuse it with 2 to 3 grams of potassium bifluoride for two minutes. Cool the outside of the crucible by dipping into water while manipulating to solidify the melt in a thin layer. Dissolve the melt by adding 3 to 4 ml. of concentrated sulfuric acid and 1 ml. of 48% hydrofluoric acid, and dilute with water to make a total volume of about 20 ml. Transfer to a 50-ml. Boston round bottle containing 10 ml. of *n*-amyl alcohol and shake in a mechanical shaker for 1 minute. Transfer the solution to a 100-ml. separatory funnel, draw off the aqueous layer (*A*) and set aside. Wash the remaining organic layer twice with 10-ml. portions of a solution 1.2*N* in hydrofluoric acid and 2*N* in sulfuric acid and discard the washing. Transfer the organic portion to a 125-ml. wide mouth Erlenmeyer flask and count Ta^{192m} activity in the scintillation spectrometer.

Pour the aqueous solution (*A*) into a 400-ml. beaker containing a boiling solution of 20-ml. of concentrated nitric acid and maintain boiling for two minutes. Add 2 grams of boric acid powder to the boiling mixture. Collect the precipitate on glass fiber filter paper in filter chimney and mount on a counting card for Nb^{94m} activity measurement.

Determine chemical yield of niobium by area under the 0.76-m.e.v. Nb^{95} photopeak. Determine tantalum recovery by reirradiation of the organic tantalum extracts and comparison of resulting tantalum activity with that from a 1-ml. sample of tantalum standard.

For the stainless steel and graphite samples, use a sodium peroxide fusion (*9*) in a zirconium crucible. Dissolve the melt in 15 ml. of water and acidify with 30 ml. of concentrated nitric acid. Heat the solution to boiling and add 0.5 gram of potassium perchlorate powder in small portions, boiling for a few seconds after each addition. Cen-

Table I. Activation Analysis of G-1 Rock Sample for Niobium and Tantalum[a]

Sample G-1 U. S. Geol. Survey	Wt. of sample, gram	Yield of radiochemical separation, %		Level of Nb and Ta in sample, p.p.m.	
		Nb	Ta	Nb	Ta
1	0.2286	21.4	83	31	undetectable[b]
2	0.3238	40.7	61	32	undetectable[b]
3	0.3549	34	62	30	undetectable[b]
4	0.5259	50	67	35	undetectable[b]
5	0.3121	15	54	30	undetectable[b]
6	0.5189	30	60	36	undetectable[b]
			Av. =	32 ± 3[c]	

[a] Value reported in literature is 5 to 8 p.p.m. niobium and 1.33 to 5 p.p.m. tantalum (*5*).
[b] Below 10 p.p.m.
[c] Standard deviation.

Table II. Activation Analysis of NBS-446 Stainless Steel and Graphite Sample for Niobium and Tantalum

Sample Spectrographic Stainless Steel Std., NBS-446	Wt. of sample, gram	Yield of radiochemical separation, %		Level of Nb and Ta in sample, %		Value reported by others, %	
		Nb	Ta	Nb	Ta	Nb	Ta
1	0.0670	16	40	0.52	0.015	0.6	~0.03
2	0.0761	13	40	0.60	0.018		
3	0.05925	4.4	17	0.61	0.012		
			Av.	0.58 ± 0.05	0.015 ± 0.003[a]		
Graphite Std-1002 Synthetic Sample, Spex Ind-Co.			Ta		Ta		Ta
1	0.0647		28		0.010		0.01
2	0.1121		32		0.008		
3	0.0948		25		0.012		
				Av. =	0.01 ± 0.002[a]		

[a] Standard deviation.

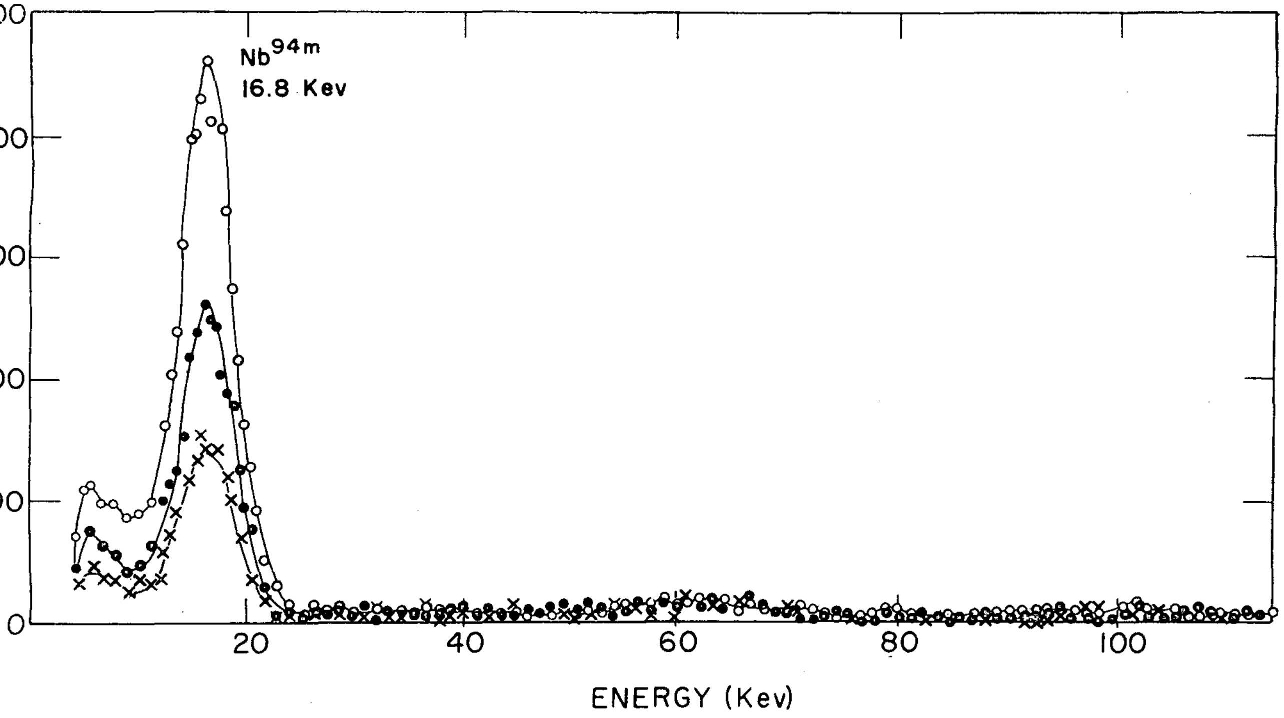

Figure 1. Gamma-ray spectrum of 6.6-minute Nb^{94m} separated from NBS-446 sample

Spectra taken at 5.5-minute intervals

trifuge the niobium and tantalum pentoxide formed, decant the supernate, and dissolve the precipitates in a solution of 1 ml. of 48% hydrofluoric acid and 3 ml. of concentrated sulfuric acid diluted to 20 ml. with water. Extract tantalum with *n*-amyl alcohol. Precipitate the niobium remaining in the aqueous layer by adding concentrated ammonium hydroxide drop by drop to avoid the precipitation of any remaining tungsten (*7*). Collect the precipitates on glass fiber filter paper in filter chimney by rapid suction and count as above.

RESULTS AND DISCUSSION

Results obtained from the activation analysis of the G-1 rock sample are summarized in Table I, the graphite sample and stainless steel sample in Table II, respectively.

In the first three experiments with the G-1 rock sample, the niobium content was four to six times higher than the value reported by the spectrographic method (*5*). The data were reexamined by making three more activation runs but there was little change. Thus it would appear that there is much more niobium in the G-1 standard than the 8 p.p.m. previously reported although some deviation might be attributed to inhomogeneity of the G-1 sample (*5*).

It was not possible to detect tantalum in the G-1 sample although chemical recovery of the tantalum was 60 to 80%. Thus the amount of tantalum in the sample must be less than 10 p.p.m., the lower limit of this method for tantalum analysis. The chemical recovery of niobium throughout the experiment fluctuates greatly since acid hydrolysis of niobium is seldom quantitative. Niobium recovery is particularly poor in the N.B.S. and graphite samples since the acid hydrolysis must be done prior to the tantalum extraction to eliminate excess peroxide from the sodium peroxide fusion.

Spectra obtained from the niobium and tantalum fractions are shown in Figures 1 and 2. The characteristic x-rays at 16.6 and 57.8 k.e.v. for niobium (*2*, *10*) and tantalum (*19*), respectively, were measured for the quantitative determination. The half life of the niobium x-ray peak was found to be 6.6 minutes. The peak corresponding to the 67-k.e.v. gamma-ray from 111-day tantalum-182 formed during the 10-minute irradiation was subtracted from the combined tantalum-182*m*, tantalum-182 peak. The nuclide Ta^{182m} is easily distinguished from Ta^{182} and other nuclides (*2*).

Calibration curves using a known amount of Nb and Ta were made under several conditions of measurement of the Nb^{94m} and Ta^{182m} x-rays. The amount of Nb^{94m} and Ta^{182m} was determined from the area under the 16.6-k.e.v. and 57.8-k.e.v. photopeak, respectively. The curves are straight lines which were then used to evaluate the niobium and tantalum content in the samples. Background and interfering Compton radiations from other activities remaining in the samples were eliminated by extrapolation of the base line from both sides of the photopeak.

Tantalum extraction by *n*-amyl alcohol was adopted to separate niobium, tungsten, and other elements from it. It was found that the extraction of tantalum by *n*-amyl alcohol is not closely dependent on acidity of the solution, nor on the temperature of the solution. Furthermore, the extraction leaves little contamination of other elements, and gives more than 85% extraction of tantalum in a single shaking with 10 ml. of *n*-amyl alcohol. It also permits a fast and clear-cut separation of two immiscible layers immediately after the shaking. Iron (+3), aluminum, beryllium, gallium, indium, and selenium might be possible contaminants (*15*), but no radiochemical interference is found since isotopes of some of these are very short-lived while others have comparatively low cross sections for neutrons.

Tannin precipitation (*4*), 2,6-dimethyl 4-heptanone, and methyl isobutyl ketone (*17*) extractions were tried, but these are all seriously contaminated when applied to low concentration tantalum and niobium samples. The di-isopropyl ketone method (*17*, *18*) was not used because of difficulty in obtaining the solvent and its cost.

Tungsten and titanium are the main contaminants in the niobium hydrolysis

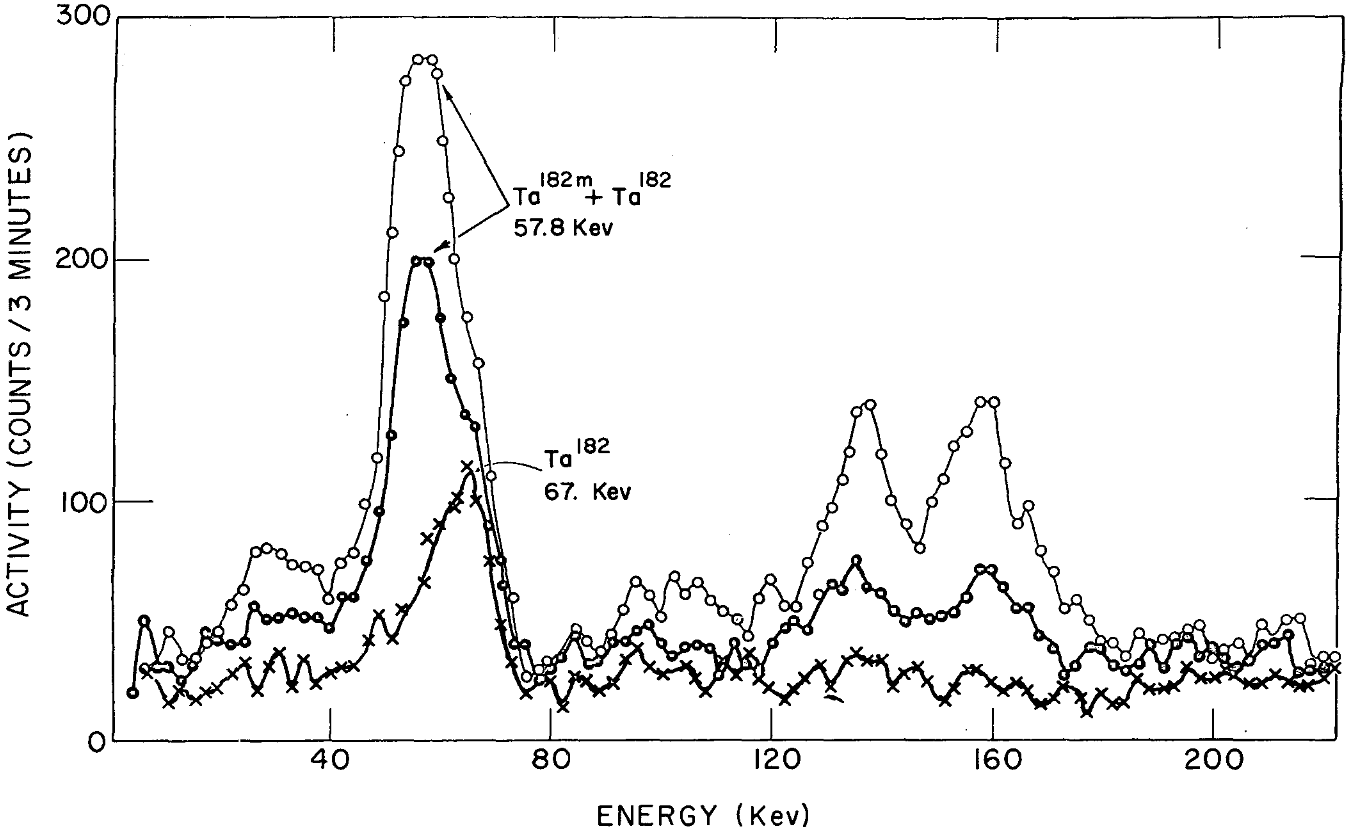

Figure 2. Combined gamma-ray spectrum of 16.5-minute Ta^{182m} plus Ta^{182} separated from graphite sample

Top curve taken immediately after separation; middle curve, 15 minutes later; and bottom curve, 145 minutes after middle curve

step. However, these elements give little interference to the 16.6-k.e.v. Nb^{94m} activity measurement unless present in about tenfold excess.

The reirradiation technique of tantalum extracts for the tantalum yield determination was carefully studied and found accurate, simple, and fast.

The neutron flux was monitored for each irradiation by sending a known weight of gold foil along with each sample and standard since it has been observed that, in the same pneumatic tube during 1-Mw. operation, the flux varies between 1.42 and 1.12 × 10^{12} n. cm.$^{-2}$ second^{-1} (*13*). In this way, all measured activities were normalized to the same specific activity of gold.

Neutron selfshielding effects in general have been well evaluated by Gilat and Gurfinkel (*6*), and Høgdahl (*8*) and amount to no more than 1 to 2% in these samples.

The advantage of this type of analysis for niobium and tantalum is that one sample irradiation gives a single analysis of two elements simultaneously in less than 30 minutes and correction for reagent blank is unnecessary.

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Evaluation of Some Analytical Procedures for Niobium-Base Alloys

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► Analytical data for three commercial niobium-base alloys were used to evaluate conventional chemical and vacuum fusion procedures for carbon, hydrogen, and oxygen. Chemical and optical spectrographic solution procedures developed at this laboratory are discussed regarding the determination of alloying amounts (1 to 25%) of molybdenum, tungsten, titanium, and zirconium. Various chemical, optical, and x-ray spectrographic procedures currently used by industry were compared by round-robin analyses of samples from the three niobium alloys. Examination of the round-robin data enables conclusions to be drawn concerning the elements that will require additional analytical method development.

The investigation of analytical methods for niobium-base alloys, first begun in this laboratory in November 1959, was necessitated by work under a U. S. Air Force contract to develop manufacturing methods for three commercial alloys (F48–General Electric, D31 and D41–Du Pont). The initial literature survey uncovered references to methods (*1, 4*) developed at the Westinghouse Atomic Power Laboratories, mostly dealing with the determination of trace impurities in high purity niobium. Reed (*14*) outlined a procedure for the determination of alloying amounts of molybdenum, titanium, tungsten, and zirconium based on the classical separation method of Powell and Schoeller (*12*). Correspondence with General Electric Co. (*7*) revealed that an ion-exchange technique based on the work of Hague (*5*) could be used to separate titanium, molybdenum, tungsten, and zirconium in a hydrofluoric-hydrochloric acid medium. The Union Carbide Metals Co. (*11*) supplied x-ray methods on the determination of the desired alloying elements. The x-ray method, based on the earlier work of Mitchell (*10*), used chemical dissolution, cupferron and cinchonine precipitation, ignition of the mixed oxides, and fluorescent analyses of a briquet prepared from the ignited residue. In April 1960, Battelle Memorial Institute reported on the determination of oxygen, nitrogen, hydrogen, and carbon in niobium (*9*). The report was essentially a review, but it contained good reference material for application to the interstitial elements.

After the completion of this investigation in July 1961, Elwell and Wood (*3*) briefly outlined chemical methods for the determination of carbon, oxygen, nitrogen, chromium, iron, molybdenum, nickel, silicon, titanium, tungsten, and zirconium. Because of the close similarity of the methods to those for titanium- and zirconium-base alloys, "only brief notes on the methods applied to niobium are recorded."

The literature survey indicated that the determination of the desired metallic elements in the niobium-base alloys was difficult because of their chemical similarity (Table I).

Table II contains a summary of atomic properties of the elements which further emphasizes their similar chemical and physical properties.

These elements are transition elements. Their two outer principal quantum shells are incomplete and they are characterized specifically by their incomplete *d* subshell. The elements are members of transition series corresponding to unfilled 3*d*, 4*d*, and 5*d* orbitals. The transition elements show pronounced resemblance to each other, particularly in their physical properties. Their oxidation states are very numerous and their compounds are often highly colored. Furthermore, coordination compounds are the rule and simple compounds are the exception.

The $(n)d$, $(n + 1)s$, and $(n + 1)p$ orbitals of the transition metals are responsible for the chemical bonding of the various ligands used in compound formation. Ligands such as thiocyanate, peroxide, or fluoride are used in chemical analysis of niobium-base alloys. The principal emission lines shown in Table II result from electronic transitions also involving the $(n)d$, $(n + 1)s$, and $(n + 1)p$ outer orbitals. However, the x-ray lines of columns 6 and 7 result from electronic transitions involving only the innermost or *K*, *L*, and *M* shells of the atom. Many secondary emission lines not shown in Table II can be used for analytical

Table I. Periodic Relationship and Percentage Composition of Alloying Elements in Niobium Alloys

IVA	VA	VIA
22 Ti 47.90 (7-12%)	23 V 50.95	24 Cr 52.01
40 Zr 91.22 (0.5-1.5%)	41 Nb 92.91 (64-80%)	42 Mo 95.95 (5-10%)
72 Hf 178.6	73 Ta 180.88	74 W 183.92 (15-20%)

Table II. Atomic Properties of Alloying Elements in Niobium

Element	Electronic configuration	Atomic size, A.	Ionic size, A.	Principal emission lines, A.	X-ray lines, A. *K*	X-ray lines, A. *L*
Ti	18 $[Ar]3d^2 4s^2$	1.46	0.68(+4)	3349, 3999, 3361 3653, 3234, 3642	$2.748\alpha_1$ $2.514\beta_1$	
Zr	36 $[Kr]4d^2 5s^2$	1.57	0.80(+4)	3392, 3438, 3496 3601, 3557, 3572	$0.784\alpha_1$ $0.700\beta_1$	6.057
Nb	36 $[Kr]4d^4 5s^1$	1.41	0.70(+5)	4059, 4080, 4100 3580, 4124, 4153	$0.745\alpha_1$ $0.664\beta_1$	5.712
Mo	36 $[Kr]4d^5 5s^1$	1.36	0.62(+6)	3798, 3864, 3133 3903, 3170, 3194	$0.708\alpha_1$ $0.631\beta_1$	5.406
W	54 $[Xe]4f^{14} 5d^4 6s^2$	1.37	0.62(+6)	4009, 4074, 4295 2727, 2944, 2947	$0.209\alpha_1$ $0.184\beta_1$	1.480

Table III. Chemical Analysis of D31 Niobium Alloy[a]

(Per cent)

Heat	Location	C	N	Mo	Ti
S98676	Top center	0.11	0.014	9.24	8.07
	Middle center	0.11	0.007	9.32	7.94
	Bottom center	0.088	0.009	10.19	8.08
S98708	Top center	0.090	0.015	9.62	12.10
	Middle center	0.068	0.012	9.55	10.69
	Bottom center	0.057	0.010	9.58	8.43
S98585	Top center	0.077	0.009	10.00	9.47
	Middle center	0.078	0.012	10.08	9.50
	Bottom center	0.081	0.013	9.94	9.83

[a] Underlined values indicate variation.

purposes when the problem of reduced spectral sensitivity or interelement effects occurs. Because of the limited number of x-ray lines available, it is difficult to correct for interelement effects. However, when carefully prepared standards are used, the x-ray fluorescence technique has the advantage of superior reproducibility.

ANALYTICAL DEVELOPMENT WORK

Sampling and Dissolution. Because of the ease of oxidizing or nitriding niobium metal, chips or millings for chemical analysis must be prepared by very careful and slow action of the cutting tool. The precautions that should be used in sampling and dissolving niobium alloys are similar to those described in Codell's book (*2*) on titanium, an element with chemical and physical properties similar to those of niobium. Discolored chips should be particularly guarded against when determining nitrogen by the Kjeldahl procedure, and all chips should be carefully rinsed with chloroform when low amounts ($<0.05\%$) of carbon are determined.

For dissolution by acid attack, niobium is slightly more refractory than titanium and will not dissolve readily in hydrochloric or sulfuric acid without an oxidizing agent. Niobium also hydrolyzes more easily than titanium, so that hydrofluoric acid is necessary to form a complex that is both soluble and stable in strong acid media. Our experience indicates that it is best to use hydrofluoric acid as the principal acid for dissolution, with nitric acid or hydrogen peroxide added dropwise for oxidation purposes. The oxidizing agent greatly accelerates the hydrofluoric acid attack. The recent availability of Teflon plastic beakers has made it possible, after using hydrofluoric acid to dissolve niobium alloys, to use temperatures up to 500° F. Temperatures up to 500° F. are often necessary because acids such as perchloric and sulfuric are used to evaporate hydrofluoric acid completely before the sample is transferred to glassware. The Teflon beakers are placed on asbestos mats which are set on the hot plate to prevent heating above 500° F.

The problem of segregation, which is often due to electrode variation in the melting (VAR) of a niobium-base alloy, can be best illustrated by reference to Table III. Heat S98708 shows pronounced variation in carbon, nitrogen, and titanium.

Carbon. The carbon determination was the simplest of the determinations desired in niobium-base alloys. The procedure involved very little modification of the method used for carbon in steel. It includes the standard oxygen combustion with induction heating, followed by conductometric or gravimetric determination of the evolved carbon dioxide. One deviation from the standard procedure, however, was an increase in the amount of tin flux and the addition of iron to the niobium sample to obtain good combustion.

Oxygen and Hydrogen. The determination of oxygen in niobium did not involve much development work, as it required only adoption of a modified version of the vacuum fusion technique used in steel analysis. In steel analysis, only an iron bath operating at 1700° F. is used. The Battelle report (*9*) indicated that the determination of oxygen in niobium would require a platinum bath or fluxing technique because of the refractory nature of niobium. This procedure has been used at this laboratory for the determination of oxygen in titanium; thus, the determination of oxygen in niobium was not a difficult problem, as was borne out in round-robin testing.

Both the vacuum fusion technique with platinum flux in a graphite crucible and the tin bath technique in a silica crucible were used to determine hydrogen. The tin bath was operated at 1150° C. and the platinum flux at 1950° C.

Nitrogen. To determine nitrogen in niobium alloys, very little modification of the standard Kjeldahl procedure for nitrogen in steel was required. The principal change involves using 48% HF plus dropwise addition of hydrogen peroxide for sample dissolution. After dissolution, hydrofluoric acid and peroxide are eliminated by fuming with perchloric acid. The procedure of Goward and Tretow (*4*) uses hydrofluoric acid and hydrogen peroxide, but does not expel the excess fluoride or peroxide with a higher boiling acid. The present investigators felt that hydrofluoric acid should be expelled to minimize attack on the glassware. Also, the higher temperature reached with perchloric acid would improve the chances of breaking up any refractory nitrides. The procedure of Huber and Chase (*6*) cited by Mallett requires essentially sulfuric acid with additions of hydrofluoric acid and hydrogen peroxide to dissolve the niobium metal. This procedure appears to be satisfactory but may have a slower dissolution rate because hydrofluoric acid is not the principal solvent. The Mallett procedure has the advantage of the use of high boiling acid, which eliminates fluoride and breaks up refractory nitrides. Sulfuric acid was also examined in our investigation to minimize hydrolysis of the niobium and alloying elements during fuming, but some hydrolysis did occur, and equally good data were obtained with perchloric acid. Perchloric acid was preferred because its lower boiling temperature makes it easier to evaporate to fumes in Teflon. Also, we found no safety problem in fuming perchloric acid in Teflon beakers.

Molybdenum. The procedure for molybdenum, an adaptation of the standard thiocyanate photometric method for molybdenum in steel, was the simplest procedure developed for the alloying elements. After dissolution of the sample in nitric and hydrofluoric acids and evaporation to fumes of sulfuric acid, the sample is diluted to volume with a tartaric acid solution to prevent hydrolysis of niobium, tantalum, titanium, and tungsten. An aliquot is removed for color development, and ferric chloride is added to stabilize the molybdenum thiocyanate complex.

Tungsten. Tungsten has proved in our experience to be one of the most difficult alloying elements to determine in niobium alloys. Two methods have been investigated: the anion exchange technique which uses Dowex 1-X8 resin with hydrofluoric-hydrochloric acid mixtures, and a solvent extraction approach with cupferron in chloroform. However, quantitative separations have not been achieved and both techniques are being explored further.

A procedure has been developed which

uses the classical chemical approach recommended by Reed (*14*)—i.e., a modified version of the lengthy and complex classical method of Powell, Schoeller, and Jahn (*12*). The procedure at this laboratory, developed by A. Suk, changes the Reed procedure to an acid dissolution before potassium carbonate fusion to replace the direct fusion following air heating oxidation of the metal. In addition, two potassium carbonate–magnesium sulfate treatments are used to obtain satisfactory results on the gravimetric weighing of the tungstic oxide. Although the procedure provides data suitable for control melting purposes, the method is empirical.

Titanium. A direct color procedure using hydrogen peroxide was developed for titanium. After dissolution in nitric and hydrofluoric acids, the sample is fumed in sulfuric acid. The sulfuric solution is then diluted to volume, using tartaric acid to prevent hydrolysis of niobium and tungsten. An aliquot is removed, hydrogen peroxide added, and the absorbance of the yellow titanium-peroxy complex measured at 410 $m\mu$. Because of some color interference due to molybdenum and niobium peroxy complexes, a correction is made. The correction is usually constant and amounts to only about 10% of the total absorbance. However, caution should be used in applying the background correction technique, as each alloy system will require a different correction because of variable amounts of niobium and molybdenum. The background correction technique in the wet-chemical procedure can be compared with the interelement correction technique of x-ray fluorescence.

Zirconium. A wet-chemical procedure for zirconium was developed using mainly the information from General Electric (*7*). The procedure uses anion exchange from Dowex 1-X8 with 6*N* hydrochloric acid as the eluent. The zirconium is determined by the gravimetric technique, using diammonium hydrogen phosphate as the precipitating reagent. From the recent work of Raber (*13*) at this laboratory, it now appears possible that the separated zirconium can be determined more accurately. Raber's procedure using pyrocatechol violet for the colorimetric estimation of zirconium might improve reproducibility.

Emission Spectrographic Determination of Molybdenum, Titanium, Tungsten, and Zirconium in Niobium Alloys. A spectrographic solution procedure was used to provide rapid, routine, analytical control data on the alloy content of research niobium alloys. The procedure has been used for control analysis for about two years and has been constantly mod-

Table IV. Data Obtained at This Laboratory on F48 Niobium Alloy (Sample 3P)

Method of analysis	C	N	O	H	Mo	W	Zr
Combustion gravimetric	0.056 0.058	—	—	—	—	—	—
Kjeldahl	—	0.0102 0.0090 0.0096	—	—	—	—	—
Platinum flux–vacuum fusion	—	0.0045 0.0057	0.0071	0.0004			
Chemical Mo-SCN color W-oxide gravimetric Zr-ion exchange phosphate	—	—	—	—	4.95 5.01	15.15 15.29	0.75 0.70
Spectrographic spark solution	—	—	—	—	4.99 4.81 5.10 5.05 4.90 5.05 4.97	15.29 15.06 15.17 15.40 15.53 15.18 15.52	0.73 0.68 0.68 0.74 0.70 0.70 0.75
				Av.	4.97	15.31	0.72

Table V. Data Obtained at This Laboratory on D31 Niobium Alloy 98575

Method of analysis	C	N	O	H	Mo	Ti
Combustion gravimetric	0.106 0.107	—	—	—	—	—
Kjeldahl	—	0.0119 0.0128	—	—	—	—
Platinum flux–vacuum fusion	—	0.0060 0.0051	0.030 0.033	0.0017 0.0014	—	—
Chemical Mo-SCN color Ti-H_2O_2 color	—	—	—	—	10.30 10.43 10.42	10.18 10.13 10.12
Spectrographic spark solution	—	—	—	—	10.03 9.91 10.09 9.97 10.15 9.95 10.06 10.11 10.32 10.17	10.21 10.10 9.75 9.98 9.69 10.07 9.87 10.14 10.00 9.87
				Av.	10.08	9.97

Table VI. Data Obtained at This Laboratory on D41 Niobium Alloy 98620

Method of analysis	C	N	O	H	Mo	Ti	W
Combustion gravimetric	0.080 0.086	—	—	—	—	—	—
Kjeldahl	—	0.0088 0.0083	—	—	—	—	—
Platinum flux–vacuum fusion	—	0.0019 0.0022	0.014 0.016	0.0018 0.0014	—	—	—
Chemical Mo-SCN color Ti-H_2O_2 color W-oxide gravimetric	—	—	—	—	6.55 6.60 6.51	9.18 9.12 9.16	20.96 20.84 21.09
Spectrographic spark solution	—	—	—	—	6.55 6.30 6.30 6.30 6.18 6.48 6.48 6.36 6.48 6.36	8.84 9.05 8.94 9.15 8.79 9.06 9.31 9.06 9.06 9.11	21.04 21.04 21.04 21.56 21.56 21.89 21.57 21.74 21.24 21.24
				Av.	6.37	9.04	21.39

ified and improved. It uses the rotating-disk solution technique, which eliminates the need for preanalyzed chemical standards. The technique as applied to iron- and nickel-base alloys has been described by McKaveney and Vassilaros (*8*).

The principal advantage of the rotating disk spectrographic technique is its speed and adaptability for analyzing highly complex alloy matrices. Most of the wet-chemical procedures reviewed so far indicate the difficulty of the analytical problem in effecting separation schemes. With the rotating disk technique, no separations are required in the alloy content ranges examined and rapid analysis is possible. Also, the multiple exposures and analyses necessary to increase accuracy take only minutes.

With niobium-base alloys, the critical step in the analysis is the correct preparation of synthetic standards and their proper dissolution. The preparation involves obtaining high-purity metal powders and weighing them in the proper percentage combinations before dissolution. The solvent mixture chosen is to a 1 to 1 mixture of hydrofluoric and nitric acids. The solvent gives rapid dissolution with nitric acid added for oxidation purposes and with hydrofluoric acid to form acid-soluble anionic complexes. All dissolution and storage of sample solutions are done in Teflon or polyethylene because of the hydrofluoric acid. Glass is used only in the volumetric dilution and mixing of the sample after dissolution.

RESULTS AND DISCUSSION

Many individual samples have been analyzed, and cross checks have been made when possible since the beginning of this laboratory's niobium alloy program early in 1960. Carbon, hydrogen, oxygen, and nitrogen were determined by the procedures in this report at the Central Research Laboratory and at the Midland Works Titanium Laboratory of the Crucible Steel Co. of America. The alloying elements (molybdenum, titanium, tungsten, and zirconium) were analyzed and compared using both wet-chemical and spectrographic spark solution (SSS) procedures. Agreement was usually very good; however, the procedures were subjected to round-robin testing to prove their accuracy.

Table VII. Round-Robin Results for Carbon and Oxygen in Niobium Alloys

Lab	Alloy	C, %	Procedure	O, %	Procedure
1	F48	0.064	Conductometric	0.0026	Pt. flux–vacuum fusion
				0.0038	Pt. bath–vacuum fusion
3	F48	0.070	Gravimetric	0.020	Fe bath–vacuum fusion
				0.0025	Inert gas fusion–cond.
4	F48	0.057	Conductometric	0.0071	Pt. flux–vacuum fusion
5	F48	0.066	Conductometric	0.0030	Pt. flux–vacuum fusion
6	F48	0.080	Gravimetric	0.010	Fe bath–vacuum fusion
1	D31	0.102	Conductometric	0.028	Pt. bath–vacuum fusion
2	D31	0.117	Conductometric	0.031	Pt. bath–vacuum fusion
3	D31	0.110	Gravimetric	0.035	Fe bath–vacuum fusion
				0.032	Inert gas fusion–cond.
4	D31	0.107	Gravimetric	0.032	Pt. flux–vacuum fusion
1	D41	0.087	Conductometric	0.011	Pt. bath–vacuum fusion
2	D41	0.087	Conductometric	0.017	Pt. bath–vacuum fusion
3	D41	0.090	Gravimetric	0.026	Fe bath–vacuum fusion
				0.016	Inert gas fusion–cond.
4	D41	0.083	Gravimetric	0.015	Pt. flux–vacuum fusion

Table VIII. Round-Robin Results for Molybdenum and Zirconium in Niobium Alloys

Lab	Alloy	Mo, %	Procedure	Zr, %	Procedure
1	F48	4.85	Pyrosulfate fusion–x-ray	0.72	Pyrosulfate fusion–x-ray
3	F48	4.90	Sulfide sepn. Zn red. ferric tit.	0.71	Cupferron sepn.–phosphate ppt.
				0.72	Cupferron sepn.–x-ray
4	F48	4.98	SCN direct color	0.72	Ion exchange-phosphate ppt.
		4.97	Spectrographic spark solution	0.72	Spectrographic spark solution
5	F48	4.95	Acid solution–x-ray	1.00	Acid solution–x-ray
6	F48	4.10	X-ray	0.72	X-ray
1	D31	10.16	Pyrosulfate fusion–x-ray		
2	D31	9.97	Ion exchange–oxide gravimetric		
3	D31	10.23	Sulfide sepn. Zn red. ferric tit.		
4	D31	10.38	SCN direct color		
		10.08	Spectrographic spark solution		
1	D41	6.44	Pyrosulfate fusion–x-ray		
2	D41	6.35	Ion exchange–oxide gravimetric		
3	D41	7.05	Sulfide sepn. Zn red. ferric tit.		
4	D41	6.55	SCN direct color		
		6.37	Spectrographic spark solution		

The data from this laboratory for the F48 alloy (Table IV) show that the spectrographic spark solution technique produces data with a relative standard deviation of about 2% for the alloy content. In the tungsten analysis of the F48 alloy in Table IV, the data vary from a high of 15.52% to a low of 15.06%. However, the statistical average of the SSS values compares favorably with the chemical values for all three elements—i.e., molybdenum, tungsten, and zirconium. Thus multiple spectrographic exposures will provide the desirable accuracy. Table V shows the data for the D31 alloy. Table VI shows the results for the D41 alloy.

Table VII lists the round-robin results obtained at several laboratories for carbon and oxygen in the F48, D31, and D41 alloys.

Table VIII lists the round-robin results for molybdenum and zirconium.

The round-robin data in Tables VII (carbon and oxygen) and VIII (molybdenum and zirconium) indicate fair agreement among laboratories for most of the analyses.

Table IX lists the round-robin data for hydrogen and nitrogen in the niobium alloys.

The hydrogen values vary, particularly those on the F48 material. The results on the D31 and D41 are much closer, possibly because there were fewer participants and all laboratories were using almost the same technique. Nevertheless, the scatter of the D31 and D41 data indicates need of additional development work on hydrogen in niobium alloys.

The F48 values for nitrogen show a slightly lower result for Kjeldahl analysis of the solid as compared with the chips; this indicates possible nitriding of the chips during machining. Also, the vacuum fusion procedure for nitrogen produces lower results than the Kjeldahl method for all three alloys. The vacuum fusion data would thus indicate the presence of refractory nitrides not decomposed even by the use of platinum and elevated temperatures. The presence of refractory nitrides might account for some of the scatter among Kjeldahl chemical results, since not all samples were given the same acid dissolution treatment.

Table X lists the round-robin data for titanium and tungsten in the niobium alloys.

The agreement on the D41 data for titanium is very good among all four laboratories using such a diversity of techniques. However, the D31 data are not nearly as good, showing a scatter from high to low of 0.49%. There remains the possibility that despite careful sample selection, some segregation did exist in the D31 sample. This is a distinct possibility because of the known metallurgical behavior of titanium (previously shown in Table III) and the poor reproducibility of duplicate checks at this laboratory.

The tungsten values for the round-robin show the following ranges:

F48	14.30 to 15.96%
D41	20.48 to 21.39%

If the value of 14.3% in the F48 data for Laboratory 6 is omitted, the spread is from 14.8 to 15.96%, which is about 1% absolute. The same spread is also obtained in the D41 results. The determination of tungsten in niobium alloys probably merits further investigation to decrease the absolute spread. Our spectrographic spark solution procedure is thought to be fairly reliable, but our chemical procedure is empirical.

Table XI shows emission spectrographic, semiquantitative results on the tungstic oxide residues obtained by our chemical procedure for the samples shown in Table VI. The values in Table VI of 20.96, 20.84, and 21.09 obtained using our procedure are fairly close to the average of the round robin. However, the oxide residue is obviously contaminated to the extent of perhaps 5% with niobium and molybdenum. Apparently not all the niobium and molybdenum were separated before the final precipitation of tungsten with cinchonine. Through compensating errors, however, the tungsten values agreed with the average. The niobium and molybdenum occluded with the tungsten precipitate appear to compensate for the tungsten lost in the chemical separation of molybdenum and niobium

Table IX. Round-Robin Data for Hydrogen and Nitrogen in Niobium Alloys

Lab	Alloy	H, %	Procedure	N, %	Procedure
1	F48	0.00006	Pt. flux–vac. fusion	0.0046	Pt. flux–vac. fusion
		0.00004	Pt. bath–vac. fusion	0.0038	Pt. bath–vac. fusion
				0.0059	Kjeldahl–solid
				0.0123	Kjeldahl-chips
3	F48	0.0025	Fe bath–vac. fusion	0.010	Kjeldahl-chips
4	F48	0.00040	Pt. flux–vac. fusion	0.0085	Kjeldahl-solid
				0.0096	Kjeldahl-chips
5	F48	<0.00010	Pt. flux–vac. fusion	0.0052	Pt. flux–vac. fusion
6	F48	0.00200	Fe bath–vac. fusion	0.010	Kjeldahl-chips
1	D31	0.00040	Pt. bath–vac. fusion	0.0080	Kjeldahl-chips
2	D31	0.00077	Pt. bath–vac. fusion	0.0091	Kjeldahl-chips
3	D31	0.00060	Pt. bath–vac. fusion	0.0080	Kjeldahl-chips
4	D31	0.00160	Pt. flux–vac. fusion	0.0124	Kjeldahl-chips
				0.0056	Pt. flux–vac. fusion
1	D41	0.00020	Pt. bath–vac. fusion	0.0047	Kjeldahl-chips
2	D41	0.00054	Pt. bath–vac. fusion	0.0035	Kjeldahl-chips
3	D41	0.00040	Pt. bath–vac. fusion	0.0040	Kjeldahl-chips
4	D41	0.00150	Pt. flux–vac. fusion	0.0086	Kjeldahl-chips
				0.0021	Pt. flux–vac. fusion

Table X. Round-Robin Data for Titanium and Tungsten in Niobium Alloys

Lab	Alloy	Ti, %	Procedure	W, %	Procedure
1	F48			15.96	Pyrosulfate fusion–x-ray
2	F48			15.07	Ion exchange–oxide grav.
3	F48			15.59	Cupferron sepn.–oxide grav.
				15.80	Cupferron sepn.–x-ray
4	F48			15.22	$MgSO_4$ sepn. oxide grav.
				15.31	Spectrographic spark soln.
5	F48			14.80	Acid soln.–x-ray
6	F48			14.30	X-ray
1	D31	10.42	Pyrosulfate fusion–x-ray		
2	D31	10.46	Ion exchange–oxide grav.		
3	D31	10.43	Sulfosalicylic acid–direct color		
4	D31	10.14	H_2O_2–direct color		
		9.97	Spectrographic spark soln.		
1	D41	8.87	Pyrosulfate fusion–x-ray	21.07	Pyrosulfate fusion–x-ray
2	D41	9.05	Ion exchange–oxide grav.	20.48	Ion exchange–oxide grav.
3	D41	9.00	Sulfosalicylic acid–direct color	20.77	Cupferron sepn.–oxide grav.
		9.00	Cupferron septn.–x-ray	20.88	Cupferron sepn.–x-ray
4	D41	9.15	H_2O_2–direct color	20.96	$MgSO_4$ sepn.–oxide grav.
		9.04	Spectrographic spark soln.	21.39	Spectrographic spark soln.

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Table XI. Spectrographic Analysis of Tungsten Precipitate

Sample D41 (heat 98620)	Mo	Ti	Cb	W
1	2–5	<0.10	1–2	Major
2	2–5	<0.10	1–2	Major
3	2–5	<0.10	1–2	Major

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Method and Apparatus for Determination of Small Isotopic Oxygen Variations in Beryllium Oxide

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▶ An analytical method has been developed for detecting small changes in the O^{18} abundance of BeO. The method has been tested for 1- to 5-mg. samples of finely ground BeO containing 0.2 to 2.0 atom per cent of the oxygen as O^{18}. A vacuum fusion apparatus using a platinum flux contained in a graphite crucible at 2200° C. was used to release the oxygen in BeO as CO. The resultant CO was analyzed for CO^{18}–CO^{16} ratio with a modified Consolidated 21-620 mass spectrometer. The standard deviation among 19 successive samples was ±.002 when the O^{18} abundance was approximately 0.2%.

FOR AN EXPERIMENT to determine diffusion coefficients for oxygen in beryllium oxide, a method combining mass spectrometer and vacuum fusion techniques was developed for measuring the O^{18}–O^{16} ratio in finely ground BeO. During the experiment, one face of each BeO diffusion block had been plated with BeO^{18} and the O^{18} forced to diffuse into the block by annealing in an argon atmosphere at various elevated temperatures. Thus, changes in the O^{18}–O^{15} ratio of successive serially ground sections could be used to follow the oxygen diffusion into the block. Due to mass spectrometer accuracy problems, all data were referred to the CO^{18} content of a single cylinder of CO run as a standard interspersed every fifth sample within a series. Since only small changes in O^{18} abundance were of interest, no effort was made to determine the absolute O^{18} abundance in the "standard" CO.

Methods for the determination of low concentrations of oxygen in beryllium have been reviewed by Bradshaw (*2*). Of the established procedures such as volatilization, differential solubility, inert gas fusion, radio isotope formation, and isotopic dilution, vacuum fusion seemed most applicable to the present problem of liberating the oxygen in BeO for isotopic oxygen ratio determination. Gregory and Mapper (*5*), using BeO as a standard during the development of a micro method for the determination of total oxygen in beryllium metal, showed that the oxygen in BeO was quantitatively liberated as CO within 4 minutes when reacted with carbon dissolved in a platinum flux at 1900° C. Their recommendation of a maximum Be concentration in the platinum flux of one part in fifty was based on the determination of small amounts of oxygen in beryllium.

Others (*2*) have worked on the problem of quantitative recovery of oxygen from beryllium metal in the range of parts per million to as high as 1%. Brewer (*3*), Evans and Kubaschewski (*4*), and Sloman, Harvey, and Kubaschewski (*7*) in their independent discussions of vacuum fusion fundamentals and thermodynamic considerations generally agree that a mobile liquid bath containing an adequate amount of carbon is a prerequisite for efficient operation of the method. The choice of metals for the bath material is wide, but platinum seems most applicable to the work at hand. Development of the method was simplified because the weight of Be added to the system was quite small and thus did not lead to the gettering problems reported by Gregory and Mapper (*5*). Also, a reasonable assumption could be made that any isotope effect upon the reaction was small and could be neglected.

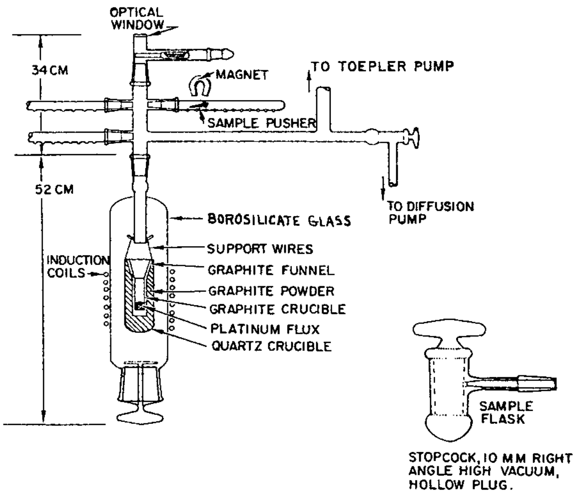

Figure 1. Vacuum fusion apparatus

Therefore, the CO^{18}-CO^{16} ratio would not be influenced by nonquantitative recovery of the CO from the BeO being analyzed.

EXPERIMENTAL

Apparatus. The reaction furnace shown in Figure 1 follows accepted vacuum fusion techniques (*6*). Samples were contained in a series of loading galleys whose total capacity was 30 samples. An external magnet operated the pusher to drop the samples into the crucible. The induction furnace power source was a LePel 2.5-kw. high frequency generator. Maximum temperature attained was 2400° C. as measured with an optical pyrometer. Eighteen grams of Pt in a graphite crucible provided the vacuum fusion matrix. As a purely precautionary measure, the Pt and crucible were changed after every 2 sets of samples.

The vacuum system was standard and consisted of a rotary mechanical forepump, an oil diffusion pump, and a liquid nitrogen-cooled trap. A large capacity Toepler pump was used to transfer the gaseous reaction products into sample vessels for mass spectrometric analysis. The mass spectrometer was a Consolidated 21-620 modified to include a 21-103 type gas inlet system.

Procedure. Samples submitted for analysis were 1- to 5-mg. portions of finely divided BeO which had been serially ground in parallel layers from the face of each diffusion block and collected in carbon tetrachloride. (The collection of cuts from a given block is referred to here as a set.) The BeO suspension was concentrated by centrifuging, the excess liquid removed, and the BeO resuspended in 0.1-ml. carbon tetrachloride. The resultant suspension was transferred to a 2-sq. cm. piece of Pt foil weighing about 0.15 gram. After evaporation of the CCl_4, the edges of the foil were folded over the BeO powder to protect it from mechanical scattering during the loading and dropping processes. The 20 to 25 samples of a given set were loaded into individual depressions in the several sample galleries and the system was evacuated. The system was outgassed at 2400° C. until a pressure of 1×10^{-5} mm. Hg was reached.

With a new crucible and Pt bath, 18 grams of platinum spheres were dropped into the crucible from one of the sample galleries and outgassing continued until a pressure of 1×10^{-6} mm. was reached. If the crucible and bath were not changed, the initial outgassing was continued to a pressure of 1×10^{-6} mm.

After preparatory outgassing was completed, the furnace power was turned off for 5 minutes to allow the temperature of the crucible to drop to about 1550° C. A Pt foil sample container was pushed from its sample gallery depression with an external magnet and its associated internal pusher and dropped into the crucible. With the system still open to the vacuum pumps, the temperature of the crucible was held at 1550° C. for outgassing of the sample until a pressure of 2×10^{-5} mm. was reached. This outgassing operation normally consumed 1 minute from the time of sample drop. The fusion apparatus was isolated from the vacuum system, full furnace power applied and Toepler pumping started to transfer the gaseous reaction products into a small sample container made from a stopcock (Figure 1 insert). In 5 minutes the Pt flux reached 2200° C. and was held at that temperature for an additional 5 minutes. The Toepler pump was then isolated, the gaseous sample container removed, and the fusion apparatus opened to the vacuum pumps for outgassing until a pressure of 2×10^{-5} was reached. At this pressure, the furnace power was turned off. When the flux temperature had fallen to 1550° C., the next sample was dropped and the procedure repeated.

The mass spectrometric analysis consisted of an instrumental blank scan on *m/e* 28, 30, and 32 and multiple scans on the same peaks after the sample had been introduced. The percentage of CO^{18} was based on the 30 to 28 ratio after the contribution of air to the 28 *m/e* had been subtracted based on the increase, if any, in the 32 *m/e*.

Each single sample of a series required an average vacuum fusion time of 30 minutes and mass spectrometer time of 10 minutes.

RESULTS AND DISCUSSION

Table I presents O^{18} abundance in a series of 19 successive BeO samples. The samples were serially ground from a diffusion block in which it was thought that the induced O^{18} enrichment would not penetrate significantly beyond the first two samples. Data from these first two samples were rejected as not representative of the base material because of O^{18} penetration. The mass spectrometer determinations were made within a single day with no non-CO samples interspersed. The relative standard deviation for the determination of the O^{18}-O^{16} ratio based on the series of runs reported in Table I is 1%. However, a graphical analysis of the data indicated a significant decrease in O^{18} abundance with penetration into the block, thus revealing an unexpected and slight depth dependant enrichment. The standard deviation of the data about the regression line is 0.001, almost exactly the standard deviation of the method when replicate tank gas samples were run. This unexpected enrichment does not invalidate these data for if it were ignored, the relative standard deviation would be 1% which is satisfactory for the purpose at hand. A more detailed study of this rapid oxygen diffusion is under way.

An appreciation of mass spectrometer conditioning problems is vital to the success of this technique. The normal problem of sample memory is important but, since only molecule ions are measured, cracking pattern changes have no effect. However, in the mass spectrometer used for this work, a significant change in the observed CO^{18} percentage of the CO gas cylinder used as a standard occurred over a period of 30 days. Two runs were made on each of six days spread over the 30 day period. While each two runs showed the expected precision, the observed CO^{18} content changed from 0.1978% to 0.1883% during the test period. This indicated an unexpected change in the mass discrimination of the instrument and caution is recommended in the case of data taken over a long period of time. The standard deviation for the series was 0.003. When nine samples of standard CO gas were run successively, the standard deviation was reduced to 0.0009 at an average CO^{18} content of 0.1953%. To negate this mass spectrometric deviation, standard CO samples were run with each series and the observed data corrected for any change in the standard CO value.

Table I. O^{18} Abundance in a Set of BeO Samples[a]

Cut No.	CO^{18}, %	Cut No.	CO^{18}, %
21	0.2010	9	0.2036
20	0.2006	8	0.2012
19	0.2005	7	0.2063
18	0.2018	6	0.2061
17	0.2041	5	0.2041
16	0.2035	4	0.2050
15	0.2045	3	0.2064
14	0.2022	2	rejected
13	0.2038	1	rejected
12	0.2001	...	...
11	0.2041	...	...
10	0.2040	...	...

[a] Mass spectrometer No. S 2091–2117.

The possible occurrence of enrichment carry over from one sample to the next one or more succeeding samples was considered and investigated by examination of data from successive samples run in a common platinum matrix. BeO of natural enrichment run immediately after samples of 2% enrichment indicated no detectable carry over from the preceding samples. However, during preliminary testing involving BeO with 10% enrichment, detectable carry over to the succeeding sample did occur. Thus, for these experimental conditions, cross contamination was not observed in the range of 2% or less O^{18}.

Outgassing between successive samples was proved complete (within the error limits of the method) by following the normal sample analysis procedure excluding only the presence of BeO powder in the dropped foil. The gas collected during the time a sample would otherwise be yielding CO gas was less than 0.1% of the CO collected from even the smallest BeO sample and could thus be neglected.

CCl_4 was chosen as the grinding and

collection fluid to minimize exposure of the freshly ground BeO powder to sources of spurious oxygen.

Normal concepts of analytical mass spectrometry would require calibration runs on pure CO^{18} and CO^{16}. However, since the desired end product of the data gathered for this problem was only the change in the CO^{18}–CO^{16} ratio between samples within a set, mass discrimination and calibration inequalities between CO^{16} and CO^{18} were relatively unimportant and were not considered. The calculation of per cent O^{18} is therefore based on the approximations that sensitivities are equal and no fractionation takes place at the molecular leak.

This technique and apparatus were designed solely for the purpose of detecting small changes in O^{18} abundance. However, by employing a suitable standard of known O^{18} abundance, it should be equally applicable to the absolute determination of O^{18} abundance in BeO and other metal oxides which have similar vacuum fusion reactions.

Preparation of diffusion blocks, details of the serial grinding, and the successful reduction of the analytical data to obtain diffusion coefficients for oxygen in BeO are presented in a separate report (*1*).

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A Mass Scale Based on $CH_2 = 14.0000$ for High Resolution Mass Spectrometry of Organic Compounds

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▶ The problems of computing, storing, and retrieving precise masses of the many combinations of elements likely to occur in the mass spectra of organic compounds are considerable. They can be significantly reduced by the adoption of a mass scale in which the mass of the CH$_2$ radical is taken as 14.0000 mass units. The advantage of this scale is that ions differing by one or more CH$_2$ groups have the same mass defect. The precise masses of a series of alkyl naphthalene parent peaks, for example, are 127.9195, 141.9195, 155.9195, etc. Because of the identical mass defects, the similar origin of these peaks is recognizable without reference to tables of masses. Tables of the mass defects for combinations of H, C^{12}, C^{13}, N, O, S^{32}, and S^{34} are presented.

DOUBLE focusing mass spectrometers with resolutions of one part in 5000 or better are now coming into use as analytical instruments. Commercial instruments after the original design by Mattauch and Herzog (*7–9*) have been described by Voorhies and coworkers (*13*) and by Craig and coworkers (*6*). Another instrument based on a design by Nier and Roberts (*4, 10–12*) has been described by Craig and Errock (*5*). Beynon (*1*) has pioneered the application of high resolution mass spectrometers to organic chemistry.

The high resolution of these instruments makes possible precise mass measurement to a few parts in 10^5 or better. This is sufficient to distinguish between ions having the same mass number, but different chemical composition. The precise mass of an ion is the sum of the masses of its constituent elements and the molecular formula can usually be found from the mass determination. This ability to determine the chemical composition of an ion from a measurement of its mass gives mass spectrometry a new dimension.

With the advent of the high resolution mass spectrometer have come the problems of computing, storing, and comparing large amounts of mass data. The masses of the isotopes of most elements are known to very high accuracy, but the number of combinations of these elements in organic chemistry is extremely large. Published tables cover only a fraction of the combinations.

Comprehensive tables of precise masses are required to give the precise masses of ions used for internal calibration standards in high resolution mass spectra and to convert the precise mass measurements made on samples into molecular formulas. Such tables are indispensable for high resolution mass spectrometry.

The mass range to be covered by these tables has no definite limit. High resolution mass spectrometers are certainly capable of working in the range up to 500 mass numbers, and special applications may call for spectra of compounds having masses as high as 1000, or even higher.

The table of precise masses most widely used at present is by Beynon (*1*), which lists, in addition, the relative abundances of isotope peaks. This table, which is based on O^{16} = 16.00000, covers combinations of elements up to a mass number of 250, and is limited to those containing no more than four oxygen atoms, four nitrogen atoms, and a total of no more than six atoms other than carbon and hydrogen. Beynon's table has about 6000 entries. Beynon's table has recently been extended to mass 500 and based on the standard C^{12} = 12.0000 (*2*). The inclusion of sulfur (S^{32} and S^{34} isotopes) and C^{13} would increase the size of the table enormously. Over one and a half million entries would be required to cover the ions up to mass 600.

A mass scale with C^{12}H$_2$ = 14.00000 enables the same data—i.e., up to mass 600—to be expressed in less than 100,000 entries. This reduction is a consequence of the same defect's being repeated at intervals of 14 mass units—i.e., one (CH$_2$) group. Restrictions similar to those imposed by Beynon have been used for these tables. No more than four atoms of oxygen, nitrogen, sulfur or carbon-13 and no more than two atoms of sulfur-34 are allowed in any combination. The total number

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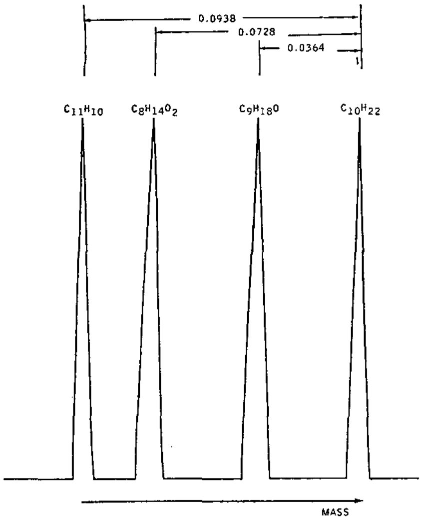

Figure 1. Multiplet at mass 142

of oxygen, nitrogen, and sulfur atoms in any combination has been limited to six.

Carlson *et al.* (*3*) have described a method of interpreting high resolution mass spectra in which the mass differences between an unknown and a reference peak at the same mass number are assigned to differences in molecular formula. The CH_2 mass scale has the advantage of being applicable over a wider mass range and thus is of particular value for instruments with which it is possible to interpolate over a range of mass numbers or to determine exact masses by determining the ratio of the unknown and reference masses.

THE CH_2 MASS SCALE

Masses on the O^{16} scale (atomic mass units) and C^{12} scale (U) can be converted to the CH_2 scale by dividing by 1.0014361 and 1.0011178, respectively. The masses of some of the elemental ions encountered in mass spectrometry are given in Tables I and II.

Table I. Masses of Ions on CH_2 Mass Scale Compared with O^{16} Scale[a]

Element	Ion	Masses O^{16} scale	 $C^{12}H_2$ scale
Hydrogen	$(H^1)^+$	1.0081451	1.006705
	$(H^2)^+$	2.0147406	2.011878
Carbon	$(C^{12})^+$	12.0038156	11.986590
	$(C^{13})^+$	13.0074929	12.98884
Nitrogen	$(N^{14})^+$	14.0075263	13.98751
Oxygen	$(O^{16})^+$	16.0000000	15.97702
Fluorine	$(F^{19})^+$	19.0044429	18.97731
Neon	$(Ne^{20})^+$	19.9987953	19.97012
Phosphorus	$(P^{31})^+$	30.9836126	30.93918
Sulfur	$(S^{32})^+$	31.9822388	31.93631
	$(S^{34})^+$	33.9786635	33.92986
Chlorine	$(Cl^{35})^+$	34.9799720	34.92981
	$(Cl^{37})^+$	36.9776573	36.92463
Argon	$(A^{40})^+$	39.9750926	39.91776
	$(A^{40})^{+2}$	19.9875463	19.95888
Gallium	$(Ga^{69})^+$	68.94757	68.84870
	$(Ga^{71})^+$	70.94737	70.84563
Silicon	$(Si^{28})^+$	27.9456821	27.94569

[a] Although C^{12} has now been adopted as the standard mass unit, most published tables are based on O^{16}.

Table II. Mercury Ions on the CH_2 Mass Scale

Hg^+	Hg^{+2}	Hg^{+3}	Hg^{+4}
197.74572	98.87286	65.91484	49.43643
198.74651	99.37304	66.24869	49.68652
199.74507	99.87254	66.58069	49.93627
200.74591	100.37296	66.91530	50.18648
201.74513	100.87256	67.24838	50.43628
203.74575	101.87287	67.91525	50.93648

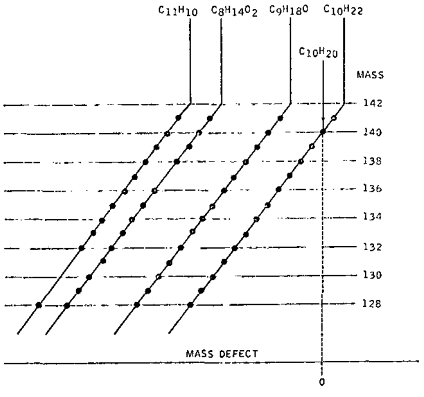

Figure 2. Mass defects *m/e* 128 to *m/e* 142

Masses given in this paper are on the scale $CH_2 = 14.0000$ unless otherwise specified.

The Basis of the CH_2 Mass Scale. The basis of the CH_2 scale is best appreciated by considering a multiplet which could arise at a particular mass. Figure 1 shows such a multiplet at *m/e* 142. These peaks could be the parent ions of methyl naphthalene, a C_8 diketone, a C_9 ketone, and decane. The pattern presented by the peaks will be repeated at *m/e* 156 for compounds one carbon number higher in each series—i.e., for dimethyl naphthalene, a C_9 diketone, a C_{10} ketone, and undecane.

The same pattern will appear at intermediate or lower masses if the same number of protons is stripped from each of the molecules. Thus, at mass 140, we may find the same pattern for ions with the formulas $C_{11}H_8$, $C_8H_{12}O_2$, $C_9H_{16}O$, and $C_{10}H_2O$. The spacings between the peaks are exactly the same as those in the other two cases. The mass defects for ions of these species at different mass numbers vary as the numbers of hydrogen atoms vary, but the differences between the defects for the different species remain constant.

Figure 2 is a representation of the mass defects of ions of the types previously considered containing carbon, hydrogen, and one or two atoms of oxygen. This diagram covers the mass numbers from 128 to 142. It can now be made to cover an infinite number of mass numbers by defining the mass scale as $CH_2 = 14.0000$. Because with $CH_2 = 14.0000$ the defect of the combination $(CH_2)_n$ is always zero—e.g., $C_{10}H_{20} = 140.0000$ and $C_9H_{18} = 126.0000$—the defects for other combinations apply at any carbon number. Figure 3 shows the manner in which the pattern is repeated.

Now that the zero of the mass defect scale has been fixed and with it the units in which the defects are measured, the defects for ions containing say one oxygen atom can be read off. At 140, for example, the $C_9H_{16}O$ ions would have a

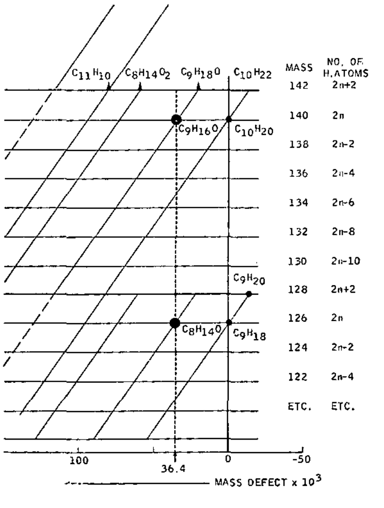

Figure 3. Repetition of defects on CH_2 mass scale

defect of 36.4 × 10^{-3} mass units. At 126, the ions of formula $C_8H_{14}O$ would have the same defect. The difference in composition between the two examples is (CH_2), which has zero defect by definition.

As an alternative to reading a chart, the defects can be found from tables. It is convenient to consider the defect as the sum of contributions from the hydrocarbon ion and from the separation of the peak from the hydrocarbon peak. In the two examples previously considered this doublet separation is 36.4 × 10^{-3} mass units. The hydrocarbon contributions are zero because in both cases the hydrocarbon was of the C_nH_{2n} type.

Figure 4 shows these two contributions in the general case. A is the separation of the peak from the corresponding hydrocarbon, and B is the contribution from the hydrocarbon.

Table III. Comparison of the Masses of Some Organic Ions on the Two Scales

	Mass scale	
	O^{16} = 16.0000	$C^{12}H_2$ = 14.0000
Naphthalenes	128.1036	127.9196
	142.1237	141.9196
	156.1439	155.9196
	170.1640	169.9196
	etc.	etc.
Paraffins	128.1975	128.0134
	142.2176	142.0134
	156.2378	156.0134
	170.2579	170.0134
	etc.	etc.
$C_nH_{2n}O$	128.1611	127.9770
	142.1812	141.9770
	156.2013	155.9770
	170.2214	169.9770
	etc.	etc.

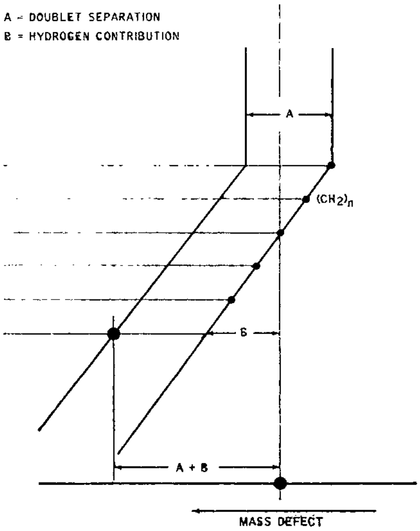

Figure 4. Components of a mass defect

Table IV. Mass Numbers for Different Values of *n* and *m*

	m													
n	2*n* + 2	2*n* + 1	2*n*	2*n* − 1	2*n* − 2	2*n* − 3	2*n* − 4	2*n* − 5	2*n* − 6	2*n* − 7	2*n* − 8	2*n* − 9	2*n* − 10	2*n* − 11
2	30	29	28	27	26	25	24							
3	44	43	42	41	40	39	38	37	36	35	34	33	32	31
4	58	57	56	55	54	53	52	51	50	49	48	47	46	45
5	72	71	70	69	68	67	66	65	64	63	62	61	60	59
6	86	85	84	83	82	81	80	79	78	77	76	75	74	73
7	100	99	98	97	96	95	94	93	92	91	90	89	88	87
8	114	113	112	111	110	109	108	107	106	105	104	103	102	101
9	128	127	126	125	124	123	122	121	120	119	118	117	116	115
10	142	141	140	139	138	137	136	135	134	133	132	131	130	129
11	156	155	154	153	152	151	150	149	148	147	146	145	144	143
12	170	169	168	167	166	165	164	163	162	161	160	159	158	157
13	184	183	182	181	180	179	178	177	176	175	174	173	172	171
14	198	197	196	195	194	193	192	191	190	189	188	187	186	185
15	212	211	210	209	208	207	206	205	204	203	202	201	200	199
16	226	225	224	223	222	221	220	219	218	217	216	215	214	213
17	240	239	238	237	236	235	234	233	232	231	230	229	228	227
18	254	253	252	251	250	249	248	247	246	245	244	243	242	241
19	268	267	266	265	264	263	262	261	260	259	258	257	256	255
20	282	281	280	279	278	277	276	275	274	273	272	271	270	269
21	296	295	294	293	292	291	290	289	288	287	286	285	284	283
22	310	309	308	307	306	305	304	303	302	301	300	299	298	297
23	324	323	322	321	320	319	318	317	316	315	314	313	312	311
24	338	337	336	335	334	333	332	331	330	329	328	327	326	325
25	352	351	350	349	348	347	346	345	344	343	342	341	340	339
26	366	365	364	363	362	361	360	359	358	357	356	355	354	353
27	380	379	378	377	376	375	374	373	372	371	370	369	368	367
28	394	393	392	391	390	389	388	387	386	385	384	383	382	381
29	408	407	406	405	404	403	402	401	400	399	398	397	396	395
30	422	421	420	419	418	417	416	415	414	413	412	411	410	409
31	436	435	434	433	432	431	430	429	428	427	426	425	424	423
32	450	449	448	447	446	445	444	443	442	441	440	439	438	437
33	464	463	462	461	460	459	458	457	456	455	454	453	452	451
34	478	477	476	475	474	473	472	471	470	469	468	467	466	465
35	492	491	490	489	488	487	486	485	484	483	482	481	480	479
36	506	505	504	503	502	501	500	499	498	497	496	495	494	493
37	520	519	518	517	516	515	514	513	512	511	510	509	508	507
38	534	533	532	531	530	529	528	527	526	525	524	523	522	521
39	548	547	546	545	544	543	542	541	540	539	538	537	536	535
40	562	561	560	559	558	557	556	555	554	553	552	551	550	549
41	576	575	574	573	572	571	570	569	568	567	566	565	564	563
42	590	589	588	587	586	585	584	583	582	581	580	579	578	577
43	604	603	602	601	600	599	598	597	596	595	594	593	592	591
44	618	617	616	615	614	613	612	611	610	609	608	607	606	605
45	632	631	630	629	628	627	626	625	624	623	622	621	620	619
46	646	645	644	643	642	641	640	639	638	637	636	635	634	633
47	660	659	658	657	656	655	654	653	652	651	650	649	648	647
48	674	673	672	671	670	669	668	667	666	665	664	663	662	661
49	688	687	686	685	684	683	682	681	680	679	678	677	676	675
50	702	701	700	699	698	697	696	695	694	693	692	691	690	689

Table V. Mass Defects of Hydrocarbon Ions C_nH_{2n+2} to C_nH_{2n-11}

Molecular formula	m													
	$2n + 2$	$2n + 1$	$2n$	$2n - 1$	$2n - 2$	$2n - 3$	$2n - 4$	$2n - 5$	$2n - 6$	$2n - 7$	$2n - 8$	$2n - 9$	$2n - 10$	$2n - 11$
	Mass units × 10^3													
$C^{12}_{n}H_{m}$	−13.4	−6.7	0.0	6.7	13.4	20.1	26.8	33.5	40.2	46.9	53.6	60.3	67.0	73.7
$C^{13}C^{12}_{n-1}H_{m-1}$	−8.9	−2.2	4.5	11.2	17.9	24.6	31.3	38.0	44.7	51.4	58.1	64.8	71.5	78.2
$C^{13}_{2}C^{12}_{n-2}H_{m-2}$	−4.5	2.2	8.9	15.6	22.3	28.9	35.6	42.4	49.1	55.8	62.5	69.2	75.9	82.6
$C^{13}_{3}C^{12}_{n-3}H_{m-3}$	0.0	6.7	13.4	20.1	26.8	33.5	40.2	46.9	53.6	60.3	67.0	73.7	80.4	87.1
$C^{13}_{4}C^{12}_{n-4}H_{m-4}$	4.4	11.1	17.8	24.5	31.2	37.9	44.6	51.3	58.0	64.7	71.4	78.1	84.8	91.5
											$2n + 6$	$2n + 5$	$2n + 4$	$2n + 3$
$C^{13}C^{12}_{n-1}H_{m-1}$														−15.6
$C^{13}_{2}C^{12}_{n-2}H_{m-2}$													−17.9	−11.2
$C^{13}_{3}C^{12}_{n-3}H_{m-3}$												−20.1	−13.4	−6.7
$C^{13}_{4}C^{12}_{n-4}H_{m-4}$											−22.4	−15.7	−9.0	−2.3

Table VI. Mass Defects of Ions $C_{n-1}H_{m-4}O$

Molecular formula	m													
	$2n + 2$	$2n + 1$	$2n$	$2n - 1$	$2n - 2$	$2n - 3$	$2n - 4$	$2n - 5$	$2n - 6$	$2n - 7$	$2n - 8$	$2n - 9$	$2n - 10$	$2n - 11$
	Mass units × 10^3													
$C^{12}_{n-1}H_{m-4}O$	23.0	29.7	36.4	43.1	49.8	56.5	63.2	69.9	76.6	83.3	90.0	96.7	103.4	110.1
$C^{13}C^{12}_{n-2}H_{m-5}O$	27.4	34.1	40.8	46.5	54.2	60.9	67.6	74.3	81.0	87.7	94.4	101.1	107.8	114.5
$C^{13}_{2}C^{12}_{n-3}H_{m-6}O$	31.9	38.5	45.3	52.0	58.7	65.4	72.1	78.8	85.5	92.2	98.9	105.6	112.3	119.0
$C^{13}_{3}C^{12}_{n-4}H_{m-7}O$	36.4	43.1	49.8	56.5	63.2	69.9	76.6	83.3	90.0	96.7	103.4	110.1	116.8	123.5
$C^{13}_{4}C^{12}_{n-5}H_{m-8}O$	40.8	46.5	54.2	60.9	67.6	74.3	81.0	87.7	94.4	101.1	107.8	116.5	121.2	127.9
											$2n + 6$	$2n + 5$	$2n + 4$	$2n + 3$
$C^{13}C^{12}_{n-2}H_{m-5}O$														20.7
$C^{13}_{2}C^{12}_{n-3}H_{m-6}O$													18.5	25.2
$C^{13}_{3}C^{12}_{n-4}H_{m-7}O$												16.3	23.0	29.7
$C^{13}_{4}C^{12}_{n-5}H_{m-8}O$											14.0	20.7	27.4	34.1

Table VII. Contributions to Mass Defects by Elements in Combination with Carbon and Hydrogen[a]

Element	Formula of ion	Defect for $m = 2n$ (millimass units)
Deuterium	$C_nH_{m-2}D$	1.55
Phosphorus	$C_{n-3}H_{m+5}P$	−13.1
Nitrogen	$C_{n-1}H_{m-2}N$	12.5
Oxygen	$C_{n-1}H_{m-4}O$	36.4
Sulfur-32	$C_{n-2}H_{m-8}S^{32}$	90.5
Sulfur-34	$C_{n-2}H_{m-10}S^{34}$	110.3
Fluorine	$C_{n-2}H_{m+5}F$	−37.5
Chlorine-35	$C_{n-3}H_{m+1}Cl^{34}$	23.3
Chlorine-37	$C_{n-3}H_{m-1}Cl^{37}$	41.9
Silicon	C_nH_mSi	54.3

[a] The defects may occur as multiples or combinations of those listed, and may also be combined with the doublet $C^{12} - H_{12}$ = 93.8 millimass units.

Advantages of the CH_2 Mass Scale. The chief advantage of using a mass scale based on CH_2 = 14.0000 is that the number of precise masses to be calculated, stored, and compared with data from a sample is very greatly reduced.

A further advantage of the CH_2 mass scale is that the identification of ions is simplified. As shown in Table III, all ions in a homologous series have the same mass defect. Ions having masses 14 units apart and having similar defects could be assumed to have similar structures and to differ only in the number of CH_2 units. Averaging these defects gives a more accurate value for comparison with the tables.

Still another advantage of the CH_2 scale is that the same defects apply in the higher mass ranges. Above about 500 mass units the defects calculated do not cover all of the possible combinations of atoms, but they do cover a proportion. The proportion covered decreases with increasing mass number, but those combinations not included are unlikely combinations with large numbers of carbon atoms and relatively few hydrogen atoms.

An example of the value of the CH_2 mass scale is given in Table III in which the masses of series of paraffins, alkyl naphthalenes, and aliphatic ketones, which have parent peaks at the same nominal masses, are compared. In these examples the mass defects for all the naphthalenes are the same, and obviously different from those for paraffins or ketones. Ions having one less hydrogen atom than each of the examples given have mass defects which are larger by 0.0067. They therefore give sets of values similar to those presented in Table III.

MASS DEFECT TABLES

It will be appreciated that the data in Table III on naphthalenes, paraffins, and ketones would be better expressed in terms of the defects—i.e., naphthalenes, 0.0804, paraffins, −0.0134, and ketones 0.0230. The negative defect of paraffin parent peaks is an exception to the general rule.

The most useful way of having these data available would be in the form of tables each of which would relate to a particular kind of molecular formula—e.g., the formula C_nH_m, where $(2n + 2) \nless m \nless (2n - 11)$ would cover the parent peaks of all the simpler hydrocarbons including paraffins, olefins, cycloparaffins, akylbenzenes, styrenes, tetralins, and indanes and indenes. For a given mass number, the values of n and m may be obtained from Table IV. The same molecular formula with a lower value of m would cover most cases of ions formed when several hydrogen atoms are stripped off the paraffins, olefins, and cycloparaffins. A paraffin with 14 hydrogen atoms stripped off is indistinguishable from a naphthalene of the same carbon number, and the defects for paraffin ions with between 14 and 28 missing hydrogen atoms may be found, therefore, from the table of defects for the parent and fragment peaks of naphthalenes and other condensed aromatics. To pre-

Table VIII. Mass Defects for Basic Combinations of Carbon, Hydrogen, Sulfur, Nitrogen, and Oxygen

Mass defect × 10^3	Formula
<0.0	
−81.3	$C_{n-2}H_{m+10}N$
−57.4	$C_{n-2}H_{m+3}O$
−45.0	$C_{n-3}H_{m+6}NO$
−32.5	$C_{n-6}H_{m+4}N_2O$
−21.1	$C_{n-3}H_{m+4}O_2$
−20.0	$C_{n-5}H_{m+2}N_3O$
−8.6	$C_{n-4}H_{m+2}NO_2$
−7.5	$C_{n-6}H_mN_4O$
−3.3	$C_{n-3}H_{m+4}S^{32}$
0.0–9.9	
0.0	C_nH_m
3.9	$C_{n-5}H_mN_2O_2$
9.1	$C_{n-4}H_{m+2}S^{32}N$
10.0–19.9	
12.5	$C_{n-1}H_{m-2}N$
15.3	$C_{n-4}H_mO_3$
16.4	$C_{n-6}H_{m-2}N_3O_2$
16.5	$C_{n-3}H_{m+2}S^{34}$
20.0–29.9	
21.6	$C_{n-5}H_mS^{32}N_2$
24.9	$C_{n-2}H_{m-4}N_2$
27.8	$C_{n-5}H_{m-2}NO_3$
28.8	$C_{n-7}H_{m-4}N_4O_2$
29.0	$C_{n-4}H_mS^{34}N$
30.0–39.9	
33.0	$C_{n-4}H_mS^{32}O$
34.1	$C_{n-6}H_{m-2}S^{34}O_2$
36.4	$C_{n-1}H_{m-4}O$
37.5	$C_{n-3}H_{m-6}N_3$
40.0–49.9	
40.2	$C_{n-6}H_{m-4}N_2O_3$
41.5	$C_{n-5}H_{m-2}S^{34}N_2$
45.5	$C_{n-5}H_{m-2}S^{32}NO$
46.6	$C_{n-7}H_{m-4}S^{32}N_4$
48.8	$C_{n-2}H_{m-6}NO$
49.9	$C_{n-4}H_{m-8}N_4$
50.0–59.9	
51.6	$C_{n-5}H_{m-4}O_4$
52.7	$C_{n-7}H_{n-6}N_3O_3$
52.9	$C_{n-4}H_{m-2}S^{34}O$
53.9	$C_{n-6}H_{m-4}S^{34}N_3$
58.0	$C_{n-6}H_{m-4}S^{32}N_2O$
60.0–69.9	
61.3	$C_{n-3}H_{m-8}N_2O$
64.1	$C_{n-6}H_{m-6}NO_4$
65.3	$C_{n-5}H_{m-4}S^{34}NO$
66.4	$C_{n-7}H_{m-6}S^{34}N_4$
69.4	$C_{n-5}H_{m-4}S^{32}O_2$
70.0–79.9	
70.4	$C_{n-7}H_{m-6}S^{32}N_3O$
72.7	$C_{n-2}H_{m-8}O_2$
73.8	$C_{n-4}H_{m-10}N_3O$
76.6	$C_{n-7}H_{m-8}N_2O_4$
77.8	$C_{n-6}H_{m-6}S^{34}N_2O$
80.0–89.9	
81.8	$C_{n-6}H_{m-6}S^{32}NO_2$
82.9	$C_{n-8}H_{m-8}S^{32}N_4O$
85.2	$C_{n-3}H_{m-10}NO_2$
86.3	$C_{n-5}H_{m-12}N_4O$
87.1	$C_{n-5}H_{m-4}S^{32}{}_2$
89.2	$C_{n-5}H_{m-6}S^{34}O_2$
90.0–99.9	
90.3	$C_{n-7}H_{m-8}S^{34}N_3O$
90.5	$C_{n-2}H_{m-8}S^{32}$
93.8	$C_{n+1}H_{m-12}$
94.3	$C_{n-7}H_{m-8}S^{32}N_2O_2$
97.6	$C_{n-4}H_{m-12}N_2O_2$
99.6	$C_{n-6}H_{m-6}S^{32}{}_2N$
100.0–109.9	
101.7	$C_{n-6}H_{m-8}S^{34}NO_2$
102.8	$C_{n-8}H_{m-10}S^{34}N_4O$
103.0	$C_{n-3}H_{m-10}S^{32}N$
105.7	$C_{n-6}H_{m-6}S^{32}O_3$
105.7	$C_{n-8}H_{m-4}S^{34}{}_2O_2$
106.3	$C_nH_{m-14}N$
106.8	$C_{n-8}H_{m-10}S^{32}N_3O_2$
107.0	$C_{n-5}H_{m-6}S^{32}S^{34}$
109.1	$C_{n-3}H_{m-12}O_3$
110.0–119.9	
110.2	$C_{n-5}H_{m-14}N_3O_2$
110.3	$C_{n-2}H_{m-10}S^{34}$
112.1	$C_{n-7}H_{m-8}S^{32}{}_2N_2$
114.2	$C_{n-7}H_{m-10}S^{34}N_2O_2$
115.4	$C_{n-4}H_{m-12}S^{32}N_2$
118.2	$C_{n-7}H_{m-10}S^{32}NO_3$
118.2	$C_{n-9}H_{m-6}S^{34}{}_2NO_2$
118.8	$C_{n-1}H_{m-16}N_2$
119.4	$C_{n-6}H_{m-8}S^{32}S^{34}N$
120.0–129.9	
120.7	$C_{n-10}H_{m-8}S^{34}{}_2N_2O_2$
121.6	$C_{n-4}H_{m-14}NO_3$
122.2	$C_{n-9}H_{m-6}S^{32}S^{34}O_3$
122.6	$C_{n-6}H_{m-16}N_4O_2$
122.8	$C_{n-3}H_{m-12}S^{34}N$
123.5	$C_{n-6}H_{m-8}S^{32}{}_2O$
123.5	$C_{n-8}H_{m-4}S^{32}S^{34}{}_2$
124.5	$C_{n-8}H_{m-10}S^{32}{}_2N_3$
125.6	$C_{n-6}H_{m-10}S^{34}O_3$
126.7	$C_{n-8}H_{m-12}S^{34}N_3O_2$
126.8	$C_{n-5}H_{m-8}S^{34}{}_2$
126.8	$C_{n-3}H_{m-12}S^{32}O$
127.9	$C_{n-5}H_{m-14}S^{32}N_3$
130.0–139.9	
130.1	$C_nH_{m-16}O$
130.7	$C_{n-8}H_{m-12}S^{32}N_2O_3$
131.2	$C_{n-2}H_{m-18}N_3$
131.9	$C_{n-7}H_{m-10}S^{32}S^{34}N_2$
134.0	$C_{n-5}H_{m-16}N_2O_3$
134.7	$C_{n-10}H_{m-8}S^{32}S^{34}NO_3$
135.3	$C_{n-4}H_{m-14}S^{34}N_2$
135.9	$C_{n-9}H_{m-6}S^{32}S^{34}{}_2N$
136.0	$C_{n-7}H_{m-10}S^{32}{}_2NO$
137.0	$C_{n-9}H_{m-12}S^{32}{}_2N_4$
138.1	$C_{n-7}H_{m-12}S^{34}NO_3$
138.7	$C_{n-10}H_{m-8}S^{32}{}_2O_4$
139.3	$C_{n-6}H_{m-10}S^{34}{}_2N$
139.3	$C_{n-4}H_{m-14}S^{32}NO$
140.0–149.9	
140.0	$C_{n-9}H_{m-6}S^{32}{}_2S^{34}O$
140.4	$C_{n-6}H_{m-16}S^{32}N_4$
142.1	$C_{n-17}H_{m-12}S^{32}O_4$
142.1	$C_{n-9}H_{m-8}S^{34}{}_2O_3$
142.6	$C_{n-1}H_{m-18}NO$
143.3	$C_{n-6}H_{m-10}S^{32}S^{34}O$
143.7	$C_{n-3}H_{m-20}N_4$
144.4	$C_{n-8}H_{m-12}S^{32}S^{34}N_3$
145.1	$C_{n-11}H_{m-8}S^{32}{}_3N_2O$
145.4	$C_{n-4}H_{m-16}O_4$
146.5	$C_{n-6}H_{m-18}N_3O_3$
146.7	$C_{n-3}H_{m-14}S^{34}O$
147.7	$C_{n-5}H_{m-16}S^{34}N_3$
148.4	$C_{n-8}H_{m-12}S^{32}{}_2N_2O$
148.4	$C_{n-10}H_{m-8}S^{32}S^{34}{}_2N_2$
150.0–159.9	
150.5	$C_{n-8}H_{m-14}S^{34}N_2O_3$
151.8	$C_{n-5}H_{m-16}S^{32}N_2O$
151.8	$C_{n-7}H_{m-12}S^{34}{}_2N_2$
152.5	$C_{n-10}H_{m-8}S^{32}{}_2S^{34}NO$
154.6	$C_{n-8}H_{m-14}S^{32}NO_4$
154.6	$C_{n-10}H_{m-10}S^{34}{}_2NO_3$
155.1	$C_{n-2}H_{m-20}N_2O$
155.8	$C_{n-7}H_{m-12}S^{32}S^{34}NO$
156.5	$C_{n-10}H_{m-8}S^{32}{}_3O_2$
156.9	$C_{n-9}H_{m-14}S^{32}S^{34}N_4$
157.9	$C_{n-5}H_{m-18}NO_4$
158.6	$C_{n-10}H_{m-10}S^{32}S^{34}O_4$
159.1	$C_{n-4}H_{m-16}S^{34}NO$
159.8	$C_{n-7}H_{m-12}S^{34}{}_2O_2$
159.8	$C_{n-9}H_{m-8}S^{32}S^{34}{}_2O$
160.0–169.9	
160.2	$C_{n-6}H_{m-18}S^{34}N_4$
160.9	$C_{n-9}H_{m-14}S^{32}{}_2N_3O$
160.9	$C_{n-11}H_{m-10}S^{32}S^{34}{}_2N_3$
161.9	$C_{n-7}H_{m-14}S^{34}O_4$
163.2	$C_{n-4}H_{m-16}S^{32}O_2$
163.2	$C_{n-6}H_{m-12}S^{34}{}_2O$
164.2	$C_{n-8}H_{m-14}S^{34}{}_2N_3$
164.3	$C_{n-6}H_{m-18}S^{32}N_3O$
164.9	$C_{n-11}H_{m-10}S^{32}{}_2S^{34}N_2O$
166.5	$C_{n-1}H_{m-20}O_2$
167.6	$C_{n-3}H_{m-22}N_3O$
168.3	$C_{n-8}H_{m-14}S^{32}S^{34}N_2O$
169.0	$C_{n-11}H_{m-10}S^{32}{}_3NO_2$
170.0–179.9	
170.4	$C_{n-6}H_{m-20}N_2O_4$
171.6	$C_{n-5}H_{m-18}S^{34}N_2O$
172.3	$C_{n-8}H_{m-14}S^{32}{}_2NO_2$
172.3	$C_{n-10}H_{m-10}S^{32}S^{34}{}_2NO$
174.2	$C_{n-10}H_{m-8}S^{32}{}_4$
174.4	$C_{n-8}H_{m-16}S^{34}NO_4$
175.6	$C_{n-7}H_{m-14}S^{34}{}_2NO$
175.7	$C_{n-5}H_{m-18}S^{32}NO_2$
176.3	$C_{n-10}H_{m-10}S^{32}{}_2S^{34}O_2$
176.7	$C_{n-9}H_{m-16}S^{34}{}_2N_4$
176.7	$C_{n-7}H_{m-20}S^{32}N_4O$
177.6	$C_{n-7}H_{m-12}S^{32}{}_3$
178.4	$C_{n-10}H_{m-12}S^{34}{}_2O_4$
179.0	$C_{n-2}H_{m-22}NO_2$
179.7	$C_{n-7}H_{m-14}S^{32}S^{34}O_2$
180.0–189.9	
180.0	$C_{n-4}H_{m-24}N_4O$
180.8	$C_{n-9}H_{m-16}S^{32}S^{34}N_3O$
180.9	$C_{n-4}H_{m-16}S^{32}{}_2$
183.0	$C_{n-4}H_{m-18}S^{34}O_2$
184.1	$C_{n-6}H_{m-20}S^{34}N_3O$
184.4	$C_{n-1}H_{m-20}S^{32}$
184.8	$C_{n-11}H_{m-12}S^{32}S^{34}{}_2N_2O$
184.8	$C_{n-9}H_{m-16}S^{32}{}_2N_2O_2$
186.7	$C_{n-11}H_{m-10}S^{32}{}_4N$
187.6	$C_{n+2}H_{m-24}$
188.1	$C_{n-8}H_{m-16}S^{34}{}_2N_2O$
188.1	$C_{n-6}H_{m-20}S^{32}N_2O_2$
188.8	$C_{n-11}H_{m-12}S^{32}{}_2S^{34}NO_2$
190.0–199.9	
190.0	$C_{n-8}H_{m-14}S^{32}{}_3N$
191.5	$C_{n-3}H_{m-24}N_2O_2$
192.2	$C_{n-8}H_{m-16}S^{32}S^{34}NO_2$
192.8	$C_{n-11}H_{m-12}S^{32}{}_3O_3$
193.4	$C_{n-5}H_{m-18}S^{32}{}_2N$
194.1	$C_{n-10}H_{m-10}S^{32}{}_3S^{34}$
195.5	$C_{n-5}H_{m-20}S^{34}NO_2$
196.2	$C_{n-8}H_{m-16}S^{32}{}_2O_3$
196.2	$C_{n-10}H_{m-12}S^{32}S^{34}{}_2O_2$
196.6	$C_{n-7}H_{m-22}S^{34}N_4O$
196.8	$C_{n-2}H_{m-22}S^{32}N$
197.4	$C_{n-7}H_{m-14}S^{32}{}_2S^{34}$
199.2	$C_{n-12}H_{m-12}S^{32}{}_4N_2$
199.5	$C_{n-5}H_{m-20}S^{32}O_3$
199.5	$C_{n-7}H_{m-16}S^{34}{}_2O_2$
200.0–209.9	
200.1	$C_{n+1}H_{m-26}N$
200.6	$C_{n-9}H_{m-18}S^{34}{}_2N_3O$
200.6	$C_{n-7}H_{m-22}S^{32}N_3O_2$
200.8	$C_{n-4}H_{m-18}S^{32}S^{34}$
202.5	$C_{n-9}H_{m-16}S^{32}{}_3N_2$
202.9	$C_{n-2}H_{m-24}O_3$

Table VIII. Mass Defects for Basic Combinations of Carbon, Hydrogen, Sulfur, Nitrogen, and Oxygen *(continued)*

Mass defect × 10³	Formula
204.0	$C_{n-4}H_{m-26}N_3O_2$
204.1	$C_{n-1}H_{m-22}S^{34}$
204.6	$C_{n-9}H_{m-18}S^{32}S^{34}N_2O_2$
205.9	$C_{n-6}H_{m-20}S^{32}_2N_2$
206.5	$C_{n-11}H_{m-12}S^{32}_3S^{34}N$
208.0	$C_{n-6}H_{m-22}S^{34}N_2O_2$
208.7	$C_{n-9}H_{m-18}S^{32}_2NO_3$
208.7	$C_{n-11}H_{m-14}S^{32}S^{34}_2NO_2$
209.2	$C_{n-3}H_{m-24}S^{32}N_2$
209.9	$C_{n-8}H_{m-16}S^{32}_2S^{34}N$
210.0–219.9	
210.6	$C_{n-11}H_{m-12}S^{32}_4O$
212.0	$C_{n-8}H_{m-18}S^{34}_2NO_2$
212.0	$C_{n-6}H_{m-22}S^{32}NO_3$
212.6	$C_nH_{m-28}N_2$
212.7	$C_{n-11}H_{m-14}S^{32}_2S^{34}O_3$
213.3	$C_{n-5}H_{m-20}S^{32}S^{34}N$
213.9	$C_{n-8}H_{m-16}S^{32}_3O$
213.9	$C_{n-10}H_{m-12}S^{32}_2S^{34}_2$
215.0	$C_{n-10}H_{m-18}S^{32}_3N_3$
215.4	$C_{n-3}H_{m-26}NO_3$
216.0	$C_{n-8}H_{m-18}S^{32}S^{34}O_3$
216.4	$C_{n-5}H_{m-28}N_4O_2$
216.6	$C_{n-2}H_{m-24}S^{34}N$
217.3	$C_{n-7}H_{m-16}S^{32}S^{34}_2$
217.3	$C_{n-5}H_{m-20}S^{32}_2O$
218.4	$C_{n-7}H_{m-22}S^{32}_2N_3$
219.0	$C_{n-12}H_{m-14}S^{32}_3S^{34}N_2$
219.4	$C_{n-5}H_{m-22}S^{34}NO_3$
220.0–229.9	
220.5	$C_{n-7}H_{m-24}S^{34}N_3O_2$
220.6	$C_{n-4}H_{m-20}S^{34}_2$
220.6	$C_{n-2}H_{m-24}S^{32}O$
221.7	$C_{n-4}H_{m-26}S^{32}N_3$
222.4	$C_{n-9}H_{m-18}S^{32}_2S^{34}N_2$
223.1	$C_{n-12}H_{m-14}S^{32}_4NO$
224.0	$C_{n+1}H_{m-28}O$
224.5	$C_{n-9}H_{m-20}S^{34}_2N_2O_2$
224.5	$C_{n-7}H_{m-24}S^{32}N_2O_3$
225.0	$C_{n-1}H_{m-30}N_3$
225.7	$C_{n-6}H_{m-22}S^{32}S^{34}N_2$
226.4	$C_{n-11}H_{m-14}S^{32}_2S^{34}_2N$
226.4	$C_{n-9}H_{m-18}S^{32}_3NO$
227.8	$C_{n-4}H_{m-28}N_2O_3$
228.5	$C_{n-9}H_{m-20}S^{32}S^{34}NO_3$
229.1	$C_{n-3}H_{m-26}S^{34}N_2$
229.7	$C_{n-8}H_{m-18}S^{32}S^{34}_2N$
229.8	$C_{n-6}H_{m-22}S^{32}_2NO$
230.0–239.9	
230.4	$C_{n-11}H_{m-14}S^{32}_3S^{34}O$
230.8	$C_{n-8}H_{m-24}S^{32}_2N_4$
231.9	$C_{n-6}H_{m-24}S^{34}NO_3$
232.5	$C_{n-11}H_{m-16}S^{32}S^{34}_2O_3$
232.5	$C_{n-9}H_{m-20}S^{32}_2O_4$
233.1	$C_{n-3}H_{m-26}S^{32}NO$
233.1	$C_{n-5}H_{m-22}S^{34}_2N$
233.8	$C_{n-8}H_{m-18}S^{32}_2S^{34}O$
234.2	$C_{n-5}H_{m-28}S^{32}N_4$
234.9	$C_{n-10}H_{m-20}S^{32}_2S^{34}N_3$
235.9	$C_{n-8}H_{m-20}S^{34}_2O_3$
235.9	$C_{n-6}H_{m-24}S^{32}O_4$
236.5	$C_nH_{m-30}NO$
237.1	$C_{n-5}H_{m-22}S^{32}S^{34}O$
237.5	$C_{n-2}H_{m-32}N_4$
238.2	$C_{n-7}H_{m-24}S^{32}S^{34}N_3$
238.9	$C_{n-10}H_{m-20}S^{32}_3N_2O$
238.9	$C_{n-12}H_{m-16}S^{32}_2S^{34}_2N_2$
239.3	$C_{n-3}H_{m-28}O_4$
240.0–249.9	
240.3	$C_{n-5}H_{m-30}N_3O_3$
240.5	$C_{n-2}H_{m-26}S^{34}O$
241.6	$C_{n-4}H_{m-28}S^{34}N_3$
242.2	$C_{n-9}H_{m-20}S^{32}S^{34}_2N_2$
242.2	$C_{n-7}H_{m-24}S^{32}_2N_2O$
242.9	$C_{n-12}H_{m-16}S^{32}_3S^{34}NO$
244.3	$C_{n-7}H_{m-26}S^{34}N_2O_3$
245.6	$C_{n-4}H_{m-28}S^{32}N_2O$
245.6	$C_{n-6}H_{m-24}S^{34}_2N_2$
246.3	$C_{n-9}H_{m-20}S^{32}_2S^{34}NO$
246.9	$C_{n-12}H_{m-16}S^{32}_4O_2$
248.4	$C_{n-9}H_{m-22}S^{34}_2NO_3$
248.4	$C_{n-7}H_{m-26}S^{32}NO_4$
248.9	$C_{n-1}H_{m-32}N_2O$
249.6	$C_{n-6}H_{m-24}S^{32}S^{34}NO$
250.0–259.9	
250.3	$C_{n-11}H_{m-16}S^{32}_2S^{34}_2O$
250.3	$C_{n-9}H_{m-20}S^{32}_3O_2$
250.7	$C_{n-8}H_{m-26}S^{32}S^{34}N_4$
251.7	$C_{n-4}H_{m-30}NO_4$
252.4	$C_{n-9}H_{m-22}S^{32}S^{34}O_4$
253.0	$C_{n-3}H_{m-28}S^{34}NO$
253.6	$C_{n-8}H_{m-20}S^{32}S^{34}_2O$
253.6	$C_{n-6}H_{m-24}S^{32}_2O_2$
254.0	$C_{n-5}H_{m-30}S^{34}N_4$
254.7	$C_{n-10}H_{m-22}S^{32}S^{34}_2N_3$
254.7	$C_{n-8}H_{m-26}S^{32}_2N_3O$
255.7	$C_{n-6}H_{m-26}S^{34}O_4$
257.0	$C_{n-5}H_{m-24}S^{34}_2O$
257.0	$C_{n-3}H_{m-28}S^{32}O_2$
258.1	$C_{n-5}H_{m-30}S^{32}N_3O$
258.1	$C_{n-7}H_{m-26}S^{34}_2N_3$
258.7	$C_{n-10}H_{m-22}S^{32}_2S^{34}N_2O$
260.0–269.9	
260.3	$C_nH_{m-32}O_2$
261.4	$C_{n-2}H_{m-34}N_3O$
262.1	$C_{n-7}H_{m-26}S^{32}S^{34}N_2O$
262.8	$C_{n-10}H_{m-22}S^{32}_3NO_2$
262.8	$C_{n-12}H_{m-18}S^{32}_2S^{34}_2NO$
264.2	$C_{n-5}H_{m-32}N_2O_4$
265.4	$C_{n-4}H_{m-30}S^{34}N_2O$
266.1	$C_{n-9}H_{m-22}S^{32}S^{34}_2NO$
266.1	$C_{n-7}H_{m-26}S^{32}_2NO_2$
266.8	$C_{n-12}H_{m-18}S^{32}_3S^{34}O_2$
268.0	$C_{n-9}H_{m-20}S^{32}_4$
268.2	$C_{n-7}H_{m-28}S^{34}NO_4$
269.5	$C_{n-6}H_{m-26}S^{34}_2NO$
269.5	$C_{n-4}H_{m-30}S^{32}NO_2$
270.0–279.9	
270.1	$C_{n-9}H_{m-22}S^{32}_2S^{34}O_2$
270.5	$C_{n-8}H_{m-28}S^{34}_2N_4$
270.5	$C_{n-6}H_{m-32}S^{32}N_4O$
271.4	$C_{n-6}H_{m-24}S^{32}_3$
272.2	$C_{n-9}H_{m-24}S^{34}_2O_4$
272.8	$C_{n-1}H_{m-34}NO_2$
273.5	$C_{n-6}H_{m-26}S^{32}S^{34}O_2$
273.8	$C_{n-3}H_{m-36}N_4O$
274.6	$C_{n-8}H_{m-28}S^{32}S^{34}N_3O$
274.7	$C_{n-3}H_{m-28}S^{32}_2$
276.8	$C_{n-3}H_{m-30}S^{34}O_2$
277.9	$C_{n-5}H_{m-32}S^{34}N_3O$
278.1	$C_nH_{m-32}S^{32}$
278.6	$C_{n-10}H_{m-24}S^{32}S^{34}_2N_2O$
278.6	$C_{n-8}H_{m-28}S^{32}_2N_2O_2$
280.0–289.9	
280.5	$C_{n-10}H_{m-22}S^{32}_4N$
281.4	$C_{n+3}H_{m-36}$
281.9	$C_{n-7}H_{m-28}S^{34}_2N_2O$
281.9	$C_{n-5}H_{m-32}S^{32}N_2O_2$
282.6	$C_{n-10}H_{m-24}S^{32}_2S^{34}NO_2$
283.9	$C_{n-7}H_{m-26}S^{32}_3N$
285.3	$C_{n-2}H_{m-36}N_2O_2$
286.0	$C_{n-7}H_{m-28}S^{32}S^{34}NO_2$
286.6	$C_{n-12}H_{m-20}S^{32}_2S^{34}_2O_2$
286.6	$C_{n-10}H_{m-24}S^{32}_3O_3$
287.2	$C_{n-4}H_{m-30}S^{32}_2N$
287.9	$C_{n-9}H_{m-22}S^{32}_3S^{34}$
289.3	$C_{n-4}H_{m-32}S^{34}NO_2$
290.0–299.9	
290.0	$C_{n-9}H_{m-24}S^{32}S^{34}_2O_2$
290.0	$C_{n-7}H_{m-28}S^{32}_2O_3$
290.4	$C_{n-6}H_{m-34}S^{34}N_4O$
290.5	$C_{n-1}H_{m-34}S^{32}N$
291.2	$C_{n-6}H_{m-26}S^{32}_2S^{34}$
293.0	$C_{n-11}H_{m-24}S^{32}_4N_2$
293.3	$C_{n-6}H_{m-28}S^{34}_2O_2$
293.3	$C_{n-4}H_{m-32}S^{32}O_3$
293.9	$C_{n+2}H_{m-38}N$
294.4	$C_{n-8}H_{m-30}S^{34}_2N_3O$
294.4	$C_{n-6}H_{m-34}N_3O_2$
294.6	$C_{n-3}H_{m-30}S^{32}S^{34}$
296.4	$C_{n-8}H_{m-28}S^{32}_3N_2$
296.7	$C_{n-1}H_{m-36}O_3$
297.8	$C_{n-3}H_{m-38}N_3O_2$
297.9	$C_nH_{m-34}S^{34}$
298.4	$C_{n-8}H_{m-30}S^{32}S^{34}N_2O_2$
299.7	$C_{n-5}H_{m-32}S^{32}_2N_2$
300.0–309.9	
300.4	$C_{n-10}H_{m-24}S^{32}_3S^{34}N$
301.8	$C_{n-5}H_{m-34}S^{34}N_2O_2$
302.5	$C_{n-8}H_{m-30}S^{32}_2NO_3$
302.5	$C_{n-10}H_{m-26}S^{32}S^{34}_2NO_2$
303.0	$C_{n-2}H_{m-36}S^{32}N_2$
303.7	$C_{n-7}H_{m-28}S^{32}_2S^{34}N$
304.4	$C_{n-10}H_{m-24}S^{32}_4O$
305.8	$C_{n-7}H_{m-30}S^{34}_2NO_2$
305.8	$C_{n-5}H_{m-34}S^{32}NO_3$
306.4	$C_{n+1}H_{m-40}N_2$
306.5	$C_{n-10}H_{m-26}S^{32}_2S^{34}O_3$
307.0	$C_{n-4}H_{m-32}S^{32}S^{34}N$
307.7	$C_{n-9}H_{m-24}S^{32}_2S^{34}_2$
307.8	$C_{n-7}H_{m-28}S^{32}_3O$
308.8	$C_{n-9}H_{m-30}S^{32}_3N_3$
309.2	$C_{n-2}H_{m-38}NO_3$
309.8	$C_{n-7}H_{m-30}S^{32}S^{34}O_3$
310.0–319.9	
310.3	$C_{n-4}H_{m-40}N_4O_2$
310.4	$C_{n-1}H_{m-36}S^{34}N$
311.1	$C_{n-4}H_{m-32}S^{32}_2O$
311.1	$C_{n-6}H_{m-28}S^{32}S^{34}_2$
312.2	$C_{n-6}H_{m-34}S^{32}_2N_3$
312.8	$C_{n-11}H_{m-26}S^{32}_3S^{34}N_2$
313.2	$C_{n-4}H_{m-34}S^{34}O_3$
314.3	$C_{n-6}H_{m-36}S^{34}N_3O_2$
314.4	$C_{n-3}H_{m-32}S^{34}_2$
314.4	$C_{n-1}H_{m-36}S^{32}O$
315.5	$C_{n-3}H_{m-38}S^{32}N_3$
316.2	$C_{n-8}H_{m-30}S^{32}_2S^{34}N_2$
316.9	$C_{n-11}H_{m-26}S^{32}_4NO$
317.8	$C_{n+2}H_{m-40}O$
318.3	$C_{n-8}H_{m-32}S^{34}_2N_2O_2$
318.3	$C_{n-6}H_{m-36}S^{32}N_2O_3$
318.8	$C_nH_{m-42}N_3$
319.5	$C_{n-5}H_{m-34}S^{32}S^{34}N_2$
320.0–329.9	
320.2	$C_{n-10}H_{m-26}S^{32}_2S^{34}_2N$
320.2	$C_{n-8}H_{m-30}S^{32}_3NO$
321.6	$C_{n-3}H_{m-40}N_2O_3$
322.3	$C_{n-8}H_{m-32}S^{32}S^{34}NO_3$
322.9	$C_{n-2}H_{m-38}S^{34}N_2$
323.6	$C_{n-5}H_{m-34}S^{32}_2NO$
323.6	$C_{n-7}H_{m-30}S^{32}S^{34}_2N$
324.2	$C_{n-10}H_{m-26}S^{32}_3S^{34}O$
324.6	$C_{n-7}H_{m-36}S^{32}_2N_4$
325.7	$C_{n-5}H_{m-36}S^{34}NO_3$
326.3	$C_{n-10}H_{m-28}S^{32}S^{34}_2O_3$
326.4	$C_{n-8}H_{m-32}S^{32}_2O_4$
326.9	$C_{n-4}H_{m-34}S^{34}_2N$
326.9	$C_{n-2}H_{m-38}S^{32}NO$
327.6	$C_{n-7}H_{m-30}S^{32}_2S^{34}O$
328.0	$C_{n-4}H_{m-40}S^{32}N_4$
328.6	$C_{n-9}H_{m-32}S^{32}_2S^{34}N_3$
329.7	$C_{n-5}H_{m-36}S^{32}O_4$
329.7	$C_{n-7}H_{m-32}S^{34}_2O_3$
330.0–339.9	
330.3	$C_{n+1}H_{m-42}NO$
330.9	$C_{n-4}H_{m-34}S^{32}S^{34}O$
331.3	$C_{n-1}H_{m-44}N_4$
332.0	$C_{n-6}H_{m-36}S^{32}S^{34}N_3$
332.7	$C_{n-9}H_{m-32}S^{32}_3N_2O$
332.7	$C_{n-11}H_{m-28}S^{32}_2S^{34}_2N_2$
333.0	$C_{n-2}H_{m-40}O_4$

(continued)

Table VIII. Mass Defects for Basic Combination of Carbon, Hydrogen, Sulfur, Nitrogen, and Oxygen *(continued)*

Mass defect × 10³	Formula
334.1	$C_{n-4}H_{m-42}N_3O_3$
334.3	$C_{n-1}H_{m-38}S^{34}O$
335.4	$C_{n-3}H_{m-40}S^{34}N_3$
336.0	$C_{n-6}H_{m-36}S^{32}{}_2N_2O$
336.0	$C_{n-8}H_{m-32}S^{32}S^{34}{}_2N_2$
336.7	$C_{n-11}H_{m-28}S^{32}{}_3S^{34}NO$
338.3	$C_{n-6}H_{m-38}S^{34}N_2O_3$
339.4	$C_{n-3}H_{m-40}S^{32}N_2O$
339.4	$C_{n-5}H_{m-36}S^{34}{}_2N_2$
340.0–349.9	
340.1	$C_{n-8}H_{m-32}S^{32}{}_2S^{34}NO$
340.7	$C_{n-11}H_{m-28}S^{32}{}_4O_2$
342.2	$C_{n-8}H_{m-34}S^{34}{}_2NO_3$
342.2	$C_{n-6}H_{m-38}S^{32}NO_4$
342.7	$C_nH_{m-44}N_2O$
343.4	$C_{n-5}H_{m-36}S^{32}S^{34}NO$
344.1	$C_{n-10}H_{m-28}S^{32}{}_2S^{34}{}_2O$
344.1	$C_{n-8}H_{m-32}S^{32}{}_3O_2$
344.4	$C_{n-7}H_{m-38}S^{32}S^{34}N_4$
345.5	$C_{n-3}H_{m-42}NO_4$
346.2	$C_{n-8}H_{m-34}S^{32}S^{34}O_4$
346.8	$C_{n-2}H_{m-40}S^{34}NO$
347.4	$C_{n-7}H_{m-32}S^{32}S^{34}{}_2O$
347.4	$C_{n-5}H_{m-36}S^{32}{}_2O_2$
347.8	$C_{n-4}H_{m-42}S^{34}N_4$
348.5	$C_{n-9}H_{m-34}S^{32}S^{34}{}_2N_3$
348.5	$C_{n-7}H_{m-38}S^{32}{}_2N_3O$
349.5	$C_{n-5}H_{m-38}S^{34}O_4$
350.0–359.9	
350.8	$C_{n-4}H_{m-36}S^{34}{}_2O$
350.8	$C_{n-2}H_{m-40}S^{32}O_2$
351.9	$C_{n-6}H_{m-38}S^{34}{}_2N_3$
351.9	$C_{n-4}H_{m-42}S^{32}N_3O$
352.6	$C_{n-9}H_{m-34}S^{32}{}_2S^{34}N_2O$
354.1	$C_{n+1}H_{m-44}O_2$
355.2	$C_{n-1}H_{m-46}N_3O$
355.9	$C_{n-6}H_{m-38}S^{32}S^{34}N_2O$
356.6	$C_{n-9}H_{m-34}S^{32}{}_3NO_2$
356.6	$C_{n-11}H_{m-30}S^{32}{}_2S^{34}{}_2NO$
358.0	$C_{n-4}H_{m-44}N_2O_4$
359.2	$C_{n-3}H_{m-42}S^{34}N_2O$
359.9	$C_{n-8}H_{m-34}S^{32}S^{34}{}_2NO$
359.9	$C_{n-6}H_{m-38}S^{32}{}_2NO_2$
360.0–369.9	
360.6	$C_{n-11}H_{m-30}S^{32}{}_3S^{34}O_2$
361.9	$C_{n-8}H_{m-32}S^{32}{}_4$
362.0	$C_{n-6}H_{m-40}S^{34}NO_4$
363.3	$C_{n-3}H_{m-42}S^{32}NO_2$
363.3	$C_{n-5}H_{m-38}S^{34}{}_2NO$
364.0	$C_{n-8}H_{m-34}S^{32}{}_2S^{34}O_2$
364.3	$C_{n-7}H_{m-40}S^{34}{}_2N_4$
364.4	$C_{n-5}H_{m-44}S^{32}N_4O$
365.2	$C_{n-5}H_{m-36}S^{32}{}_3$
366.1	$C_{n-8}H_{m-36}S^{34}{}_2O_4$
366.6	$C_nH_{m-46}NO_2$
367.3	$C_{n-5}H_{m-38}S^{32}S^{34}O_2$
367.6	$C_{n-2}H_{m-48}N_4O$
368.4	$C_{n-7}H_{m-40}S^{32}S^{34}N_3O$
368.5	$C_{n-2}H_{m-40}S^{32}{}_2$
370.0–379.9	
370.6	$C_{n-2}H_{m-42}S^{34}O_2$
371.7	$C_{n-4}H_{m-44}S^{34}N_3O$
371.9	$C_{n+1}H_{m-44}S^{32}$
372.4	$C_{n-9}H_{m-36}S^{32}S^{34}{}_2N_2O$
372.4	$C_{n-7}H_{m-40}S^{32}{}_2N_2O_2$
374.3	$C_{n-9}H_{m-34}S^{32}{}_4N$
375.2	$C_{n+4}H_{m-48}$
375.7	$C_{n-6}H_{m-40}S^{34}{}_2N_2O$
375.7	$C_{n-4}H_{m-44}S^{32}N_2O_2$
376.4	$C_{n-9}H_{m-36}S^{32}{}_2S^{34}NO_2$
377.7	$C_{n-6}H_{m-38}S^{32}{}_3N$
379.1	$C_{n-1}H_{m-48}N_2O_2$
379.8	$C_{n-6}H_{m-40}S^{32}S^{34}NO_2$
380.0–389.9	
380.4	$C_{n-11}H_{m-32}S^{32}{}_2S^{34}{}_2O_2$
380.5	$C_{n-9}H_{m-36}S^{32}{}_3O_3$
381.0	$C_{n-3}H_{m-42}S^{32}{}_2N$
381.7	$C_{n-8}H_{m-34}S^{32}{}_3S^{34}$
383.1	$C_{n-3}H_{m-44}S^{34}NO_2$
383.8	$C_{n-8}H_{m-36}S^{32}S^{34}{}_2O_2$
383.8	$C_{n-6}H_{m-40}S^{32}{}_2O_3$
384.2	$C_{n-5}H_{m-46}S^{34}N_4O$
384.4	$C_nH_{m-46}S^{32}N$
385.0	$C_{n-5}H_{m-38}S^{32}{}_2S^{34}$
386.8	$C_{n-10}H_{m-36}S^{32}{}_4N_2$
387.1	$C_{n-5}H_{m-40}S^{34}{}_2O_2$
387.1	$C_{n-3}H_{m-44}S^{32}O_3$
387.7	$C_{n+3}H_{m-50}N$
388.2	$C_{n-7}H_{m-42}S^{34}{}_2N_3O$
388.2	$C_{n-5}H_{m-46}S^{32}N_3O_2$
388.4	$C_{n-2}H_{m-42}S^{32}S^{34}$
390.0–399.9	
390.1	$C_{n-7}H_{m-40}S^{32}{}_3N_2$
390.5	$C_nH_{m-48}O_3$
391.6	$C_{n-2}H_{m-50}N_3O_2$
391.7	$C_{n+1}H_{m-46}S^{34}$
392.2	$C_{n-7}H_{m-42}S^{32}S^{34}N_2O_2$
393.5	$C_{n-4}H_{m-44}S^{32}{}_2N_2$
394.2	$C_{n-9}H_{m-36}S^{32}{}_3S^{34}N$
395.6	$C_{n-4}H_{m-46}S^{34}N_2O_2$
396.3	$C_{n-7}H_{m-42}S^{32}{}_2NO_3$
396.3	$C_{n-9}H_{m-38}S^{32}S^{34}{}_2NO_2$
396.8	$C_{n-1}H_{m-48}S^{32}N_2$
397.5	$C_{n-6}H_{m-40}S^{32}{}_2S^{34}N$
398.2	$C_{n-9}H_{m-36}S^{32}{}_4O$
399.6	$C_{n-4}H_{m-46}S^{32}NO_3$
399.6	$C_{n-6}H_{m-42}S^{34}{}_2NO_2$
400.0–409.9	
400.2	$C_{n+1}H_{m-52}N_2$
400.3	$C_{n-9}H_{m-38}S^{32}{}_2S^{34}O_3$
400.9	$C_{n-3}H_{m-44}S^{32}S^{34}N$
401.5	$C_{n-8}H_{m-36}S^{32}{}_2S^{34}{}_2$
401.6	$C_{n-6}H_{m-40}S^{32}{}_3O$
402.6	$C_{n-8}H_{m-42}S^{32}{}_3N_3$
403.0	$C_{n-1}H_{m-50}NO_3$
403.7	$C_{n-6}H_{m-42}S^{32}S^{34}O_3$
404.1	$C_{n-3}H_{m-52}N_4O_2$
404.2	$C_nH_{m-48}S^{34}N$
404.9	$C_{n-3}H_{m-44}S^{32}{}_2O$
404.9	$C_{n-5}H_{m-40}S^{32}S^{34}{}_2$
406.0	$C_{n-5}H_{m-46}S^{32}{}_2N_3$
406.7	$C_{n-10}H_{m-38}S^{32}{}_3S^{34}N_2$
407.0	$C_{n-3}H_{m-46}S^{34}O_3$
408.1	$C_{n-5}H_{m-48}S^{34}N_3O_2$
408.2	$C_{n-2}H_{m-44}S^{34}{}_2$
408.2	$C_nH_{m-48}S^{32}O$
409.3	$C_{n-2}H_{m-50}S^{32}N_3$
410.0–419.9	
410.0	$C_{n-7}H_{m-42}S^{32}{}_2S^{34}N_2$
410.7	$C_{n-10}H_{m-38}S^{32}{}_4NO$
411.6	$C_{n+3}H_{m-52}O$
412.1	$C_{n-5}H_{m-48}S^{32}N_2O_3$
412.1	$C_{n-7}H_{m-44}S^{34}{}_2N_2O_2$
412.6	$C_{n+1}H_{m-54}N_3$
413.3	$C_{n-4}H_{m-46}S^{32}S^{34}N_2$
413.5	$C_{n-4}H_{m-48}S^{32}O_4$
414.0	$C_{n-9}H_{m-38}S^{32}{}_2S^{34}{}_2N$
414.0	$C_{n-7}H_{m-42}S^{32}{}_3NO$
415.4	$C_{n-2}H_{m-52}N_2O_3$
416.1	$C_{n-7}H_{m-44}S^{32}S^{34}NO_3$
416.7	$C_{n-1}H_{m-50}S^{34}N_2$
417.4	$C_{n-6}H_{m-42}S^{32}S^{34}{}_2N$
417.4	$C_{n-4}H_{m-46}S^{32}{}_2NO$
418.1	$C_{n-9}H_{m-38}S^{32}{}_3S^{34}O$
418.4	$C_{n-6}H_{m-48}S^{32}{}_2N_4$
419.5	$C_{n-4}H_{m-48}S^{34}NO_3$
420.0–429.9	
420.2	$C_{n-7}H_{m-44}S^{32}{}_2O_4$
420.2	$C_{n-9}H_{m-40}S^{32}S^{34}{}_2O_3$
420.7	$C_{n-1}H_{m-50}S^{32}NO$
420.7	$C_{n-3}H_{m-46}S^{34}{}_2N$
421.4	$C_{n-6}H_{m-42}S^{32}{}_2S^{34}O$
421.8	$C_{n-3}H_{m-52}S^{32}N_4$
422.5	$C_{n-8}H_{m-44}S^{32}{}_2S^{34}N_3$
423.5	$C_{n-6}H_{m-44}S^{34}{}_2O_3$
424.1	$C_{n+1}H_{m-52}NO$
424.7	$C_{n-3}H_{m-46}S^{32}S^{34}O$
425.1	$C_nH_{m-56}N_4$
425.8	$C_{n-5}H_{m-48}S^{32}S^{34}N_3$
426.5	$C_{n-10}H_{m-40}S^{32}{}_2S^{34}{}_2N_2$
426.5	$C_{n-8}H_{m-44}S^{32}{}_3N_2O$
426.8	$C_{n-1}H_{m-52}O_4$
427.9	$C_{n-3}H_{m-54}N_3O_3$
428.1	$C_nH_{m-50}S^{34}O$
429.2	$C_{n-2}H_{m-52}S^{34}N_3$
429.8	$C_{n-5}H_{m-48}S^{32}{}_2N_2O$
429.8	$C_{n-7}H_{m-44}S^{32}S^{34}{}_2N_2$
430.0–439.9	
430.5	$C_{n-10}H_{m-40}S^{32}{}_3S^{34}NO$
431.9	$C_{n-5}H_{m-50}S^{34}N_2O_3$
433.2	$C_{n-2}H_{m-52}S^{32}N_2O$
433.3	$C_{n-4}H_{m-48}S^{34}{}_2N_2$
433.9	$C_{n-7}H_{m-44}S^{32}{}_2S^{34}NO$
434.6	$C_{n-10}H_{m-40}S^{32}{}_4O_2$
436.0	$C_{n-5}H_{m-50}S^{32}NO_4$
436.0	$C_{n-7}H_{m-46}S^{34}{}_2NO_3$
436.5	$C_{n+1}H_{m-56}N_2O$
437.2	$C_{n-4}H_{m-48}S^{32}S^{34}NO$
437.9	$C_{n-9}H_{m-40}S^{32}{}_2S^{34}{}_2O$
437.9	$C_{n-7}H_{m-44}S^{32}{}_3O_2$
438.2	$C_{n-6}H_{m-50}S^{32}S^{34}N_4$
439.3	$C_{n-2}H_{m-54}NO_4$
440.0–449.9	
440.0	$C_{n-7}H_{m-46}S^{32}S^{34}O_4$
440.6	$C_{n-1}H_{m-52}S^{34}NO$
441.2	$C_{n-4}H_{m-48}S^{32}{}_2O_2$
441.2	$C_{n-6}H_{m-44}S^{32}S^{34}{}_2O$
441.6	$C_{n-3}H_{m-54}S^{34}N_4$
442.3	$C_{n-6}H_{m-50}S^{32}{}_2N_3O$
442.3	$C_{n-8}H_{m-46}S^{32}S^{34}{}_2N_3$
443.3	$C_{n-4}H_{m-50}S^{34}O_4$
444.6	$C_{n-3}H_{m-48}S^{34}{}_2O$
444.6	$C_{n-1}H_{m-52}S^{32}O_2$
445.7	$C_{n-5}H_{m-50}S^{34}{}_2N_3$
445.7	$C_{n-3}H_{m-54}S^{32}N_3O$
446.4	$C_{n-8}H_{m-46}S^{32}{}_2S^{34}N_2O$
447.9	$C_{n+2}H_{m-56}O_2$
449.0	$C_nH_{m-58}N_3O$
449.7	$C_{n-5}H_{m-50}S^{32}S^{34}N_2O$
450.0–459.9	
450.4	$C_{n-8}H_{m-46}S^{32}{}_3NO_2$
450.4	$C_{n-10}H_{m-42}S^{32}{}_2S^{34}{}_2NO$
451.8	$C_{n-3}H_{m-56}N_2O_4$
453.0	$C_{n-2}H_{m-54}S^{34}N_2O$
453.7	$C_{n-5}H_{m-50}S^{32}{}_2NO_2$
453.7	$C_{n-7}H_{m-46}S^{32}S^{34}{}_2NO$
454.4	$C_{n-10}H_{m-42}S^{32}{}_3S^{34}O_2$
455.7	$C_{n-7}H_{m-44}S^{32}{}_4$
455.8	$C_{n-5}H_{m-52}S^{34}NO_4$
457.1	$C_{n-4}H_{m-50}S^{34}{}_2NO$
457.1	$C_{n-2}H_{m-54}S^{32}NO_2$
457.8	$C_{n-7}H_{m-46}S^{32}{}_2S^{34}O_2$
458.1	$C_{n-6}H_{m-52}S^{34}{}_2N_4$
458.2	$C_{n-4}H_{m-56}S^{32}N_4O$
459.0	$C_{n-4}H_{m-48}S^{32}{}_3$
459.9	$C_{n-7}H_{m-48}S^{34}{}_2O_4$
460.0–469.9	
460.4	$C_{n+1}H_{m-58}NO_2$
461.1	$C_{n-4}H_{m-50}S^{32}S^{34}O_2$
461.4	$C_{n-1}H_{m-60}N_4O$
462.2	$C_{n-6}H_{m-52}S^{32}S^{34}N_3O$
462.3	$C_{n-1}H_{m-52}S^{32}{}_2$
464.4	$C_{n-1}H_{m-54}S^{34}O_2$
465.5	$C_{n-3}H_{m-56}S^{34}N_3O$

Table VIII. Mass Defects for Basic Combinations of Carbon, Hydrogen Sulfur, Nitrogen, and Oxygen *(continued)*

Mass defect × 10^3	Formula
465.7	$C_{n+2}H_{m-56}S^{32}$
466.2	$C_{n-6}H_{m-52}S^{32}_{2}N_2O_2$
466.2	$C_{n-8}H_{m-48}S^{32}S^{34}_{2}N_2O$
468.1	$C_{n-8}H_{m-46}S^{32}_{4}N$
469.0	$C_{n+5}H_{m-60}$
469.5	$C_{n-5}H_{m-52}S^{34}_{2}N_2O$
469.5	$C_{n-3}H_{m-56}S^{32}N_2O_2$
470.0–479.9	
470.2	$C_{n-8}H_{m-48}S^{32}_{2}S^{34}NO_2$
471.5	$C_{n-5}H_{m-50}S^{32}_{3}N$
472.9	$C_nH_{m-60}N_2O_2$
473.6	$C_{n-5}H_{m-52}S^{32}S^{34}NO_2$
474.3	$C_{n-8}H_{m-48}S^{32}_{3}O_3$
474.3	$C_{n-10}H_{m-44}S^{32}_{2}S^{34}_{2}O_2$
474.8	$C_{n-2}H_{m-54}S^{32}_{2}N$
475.5	$C_{n-7}H_{m-46}S^{32}_{3}S^{34}$
476.9	$C_{n-2}H_{m-56}S^{34}NO_2$
477.6	$C_{n-5}H_{m-52}S^{32}_{2}O_3$
477.6	$C_{n-7}H_{m-48}S^{32}S^{34}_{2}O_2$
478.0	$C_{n-4}H_{m-58}S^{34}N_4O$
478.2	$C_{n+1}H_{m-58}S^{32}N$
478.8	$C_{n-4}H_{m-50}S^{32}_{2}S^{34}$
480.0–489.9	
480.6	$C_{n-9}H_{m-48}S^{32}_{4}N_2$
480.9	$C_{n-4}H_{m-52}S^{34}_{2}O_2$
480.9	$C_{n-2}H_{m-56}S^{32}O_3$
482.0	$C_{n-4}H_{m-58}S^{32}N_3O_2$
482.2	$C_{n-1}H_{m-54}S^{32}S^{34}$
482.8	$C_{n-6}H_{m-54}S^{34}_{2}N_3O$
483.9	$C_{n-6}H_{m-52}S^{32}_{3}N_2$
484.3	$C_{n+1}H_{m-60}O_3$
485.5	$C_{n+2}H_{m-58}S^{34}$
486.0	$C_{n-6}H_{m-54}S^{32}S^{34}N_2O_2$
487.3	$C_{n-3}H_{m-56}S^{32}_{2}N_2$
488.0	$C_{n-8}H_{m-48}S^{32}_{3}S^{34}N$
489.4	$C_{n-3}H_{m-58}S^{34}N_2O_2$
490.0–499.9	
490.1	$C_{n-6}H_{m-54}S^{32}_{2}NO_3$
490.1	$C_{n-8}H_{m-50}S^{32}S^{34}_{2}NO_2$
490.6	$C_nH_{m-60}S^{32}N_2$
491.3	$C_{n-5}H_{m-52}S^{32}_{2}S^{34}N$
492.0	$C_{n-8}H_{m-48}S^{32}_{4}O$
493.4	$C_{n-5}H_{m-54}S^{34}_{2}NO_2$
493.4	$C_{n-3}H_{m-58}S^{32}NO_3$
494.1	$C_{n-8}H_{m-50}S^{32}_{2}S^{34}O_3$
494.7	$C_{n-2}H_{m-56}S^{32}S^{34}N$
495.3	$C_{n-7}H_{m-48}S^{32}_{2}S^{34}_{2}$
495.4	$C_{n-5}H_{m-52}S^{32}_{3}O$
496.4	$C_{n-7}H_{m-54}S^{32}_{3}N_3$
497.5	$C_{n-6}H_{m-54}S^{32}S^{34}O_3$
498.0	$C_{n+1}H_{m-60}S^{34}N$
498.6	$C_{n-2}H_{m-56}S^{32}_{2}O$
498.7	$C_{n-4}H_{m-52}S^{32}S^{34}_{2}$
499.8	$C_{n-4}H_{m-58}S^{32}_{2}N_3$
500.0–509.9	
500.5	$C_{n-9}H_{m-50}S^{32}_{3}S^{34}N_2$
500.8	$C_{n-2}H_{m-58}S^{34}O_3$
501.9	$C_{n-4}H_{m-60}S^{34}N_3O_2$
502.0	$C_{n-1}H_{m-58}S^{34}_{2}$
502.0	$C_{n+1}H_{m-60}S^{32}O$
503.1	$C_{n-1}H_{m-62}S^{32}N_3$
503.8	$C_{n-6}H_{m-54}S^{32}_{2}S^{34}N_2$
504.5	$C_{n-9}H_{m-50}S^{32}_{4}NO$
505.9	$C_{n-6}H_{m-56}S^{34}_{2}N_2O_2$
505.9	$C_{n-4}H_{m-60}S^{32}N_2O_3$
507.1	$C_{n-3}H_{m-58}S^{32}S^{34}N_2$
507.3	$C_{n-3}H_{m-60}S^{32}O_4$
507.8	$C_{n-6}H_{m-54}S^{32}_{3}NO$
507.8	$C_{n-8}H_{m-50}S^{32}_{2}S^{34}_{2}N$
509.9	$C_{n-6}H_{m-56}S^{32}S^{34}NO_3$
510.0–519.9	
510.5	$C_nH_{m-62}S^{34}N_2$
511.2	$C_{n-5}H_{m-54}S^{32}S^{34}_{2}N$
511.2	$C_{n-3}H_{m-58}S^{32}_{2}NO$
511.9	$C_{n-8}H_{m-50}S^{32}_{3}S^{34}O$
512.2	$C_{n-5}H_{m-60}S^{32}_{2}N_4$
513.3	$C_{n-3}H_{m-60}S^{34}NO_3$
514.0	$C_{n-6}H_{m-56}S^{32}_{2}O_4$
514.0	$C_{n-8}H_{m-52}S^{32}S^{34}_{2}O_3$
514.5	$C_{n-2}H_{m-58}S^{34}_{2}N$
514.5	$C_nH_{m-62}S^{32}NO$
515.2	$C_{n-5}H_{m-54}S^{32}_{2}S^{34}O$
516.3	$C_{n-7}H_{m-56}S^{32}_{2}S^{34}N_3$
517.3	$C_{n-5}H_{m-56}S^{34}_{2}O_3$
518.5	$C_{n-2}H_{m-58}S^{32}S^{34}O$
519.6	$C_{n-4}H_{m-60}S^{32}S^{34}N_3$
520.0–529.9	
520.3	$C_{n-7}H_{m-56}S^{32}_{3}N_2O$
520.3	$C_{n-9}H_{m-52}S^{32}_{2}S^{34}_{2}N_2$
521.9	$C_{n+1}H_{m-62}S^{34}O$
523.6	$C_{n-4}H_{m-60}S^{32}_{2}N_2O$
523.6	$C_{n-6}H_{m-56}S^{32}S^{34}_{2}N_2$
525.3	$C_{n-9}H_{m-52}S^{32}_{3}S^{34}NO$
525.7	$C_{n-4}H_{m-62}S^{34}N_2O_3$
527.0	$C_{n-3}H_{m-60}S^{34}_{2}N_2$
527.7	$C_{n-6}H_{m-56}S^{32}_{2}S^{34}NO$
528.4	$C_{n-9}H_{m-52}S^{32}_{4}O_2$
529.8	$C_{n-6}H_{m-58}S^{34}_{2}NO_3$
529.8	$C_{n-4}H_{m-62}S^{32}NO_4$
530.0–539.9	
531.0	$C_{n-3}H_{m-60}S^{32}S^{34}NO$
531.7	$C_{n-6}H_{m-56}S^{32}_{3}O_2$
531.7	$C_{n-8}H_{m-52}S^{32}_{2}S^{34}_{2}O$
532.0	$C_{n-5}H_{m-62}S^{32}S^{34}N_4$
533.8	$C_{n-6}H_{m-58}S^{32}S^{34}O_4$
535.0	$C_{n-3}H_{m-60}S^{32}_{2}O_2$
535.0	$C_{n-5}H_{m-56}S^{32}S^{34}_{2}O$
536.1	$C_{n-7}H_{m-58}S^{32}S^{34}_{2}N_3$
536.1	$C_{n-5}H_{m-62}S^{32}_{2}N_3O$
537.1	$C_{n-3}H_{m-62}S^{34}O_4$
538.4	$C_{n-2}H_{m-60}S^{34}_{2}O$
539.5	$C_{n-4}H_{m-62}S^{34}_{2}N_3$
540.0–549.9	
540.2	$C_{n-7}H_{m-58}S^{32}_{2}S^{34}N_2O$
543.5	$C_{n-4}H_{m-62}S^{32}S^{34}N_2O$
544.2	$C_{n-7}H_{m-58}S^{32}_{3}NO_2$
544.2	$C_{n-9}H_{m-54}S^{32}_{2}S^{34}_{2}NO$
547.5	$C_{n-6}H_{m-58}S^{32}S^{34}_{2}NO$
547.5	$C_{n-4}H_{m-62}S^{32}_{2}NO_2$
548.3	$C_{n-9}H_{m-54}S^{32}_{3}S^{34}O_2$
549.5	$C_{n-6}H_{m-56}S^{32}_{4}$
550.0–559.9	
550.9	$C_{n-3}H_{m-62}S^{34}_{2}NO$
551.6	$C_{n-6}H_{m-58}S^{32}_{2}S^{34}O_2$
552.8	$C_{n-3}H_{m-60}S^{32}_{3}$
553.7	$C_{n-6}H_{m-60}S^{34}_{2}O_4$
554.9	$C_{n-3}H_{m-62}S^{32}S^{34}O_2$
560.0–569.9	
560.0	$C_{n-7}H_{m-60}S^{32}S^{34}_{2}N_2O$
561.9	$C_{n-7}H_{m-58}S^{32}_{4}N$
564.0	$C_{n-7}H_{m-60}S^{32}_{2}S^{34}NO_2$
565.3	$C_{n-4}H_{m-62}S^{32}_{3}N$
568.1	$C_{n-7}H_{m-60}S^{32}_{3}O_3$
568.1	$C_{n-9}H_{m-56}S^{32}_{2}S^{34}_{2}O_2$
569.3	$C_{n-6}H_{m-58}S^{32}_{3}S^{34}$
570.0–579.9	
571.4	$C_{n-6}H_{m-60}S^{32}S^{34}_{2}O_2$
572.6	$C_{n-3}H_{m-62}S^{32}_{2}S^{34}$
574.4	$C_{n-8}H_{m-60}S^{32}_{4}N_2$
580.0–589.9	
581.8	$C_{n-7}H_{m-60}S^{32}_{3}S^{34}N$
583.9	$C_{n-7}H_{m-62}S^{32}S^{34}_{2}NO_2$
585.8	$C_{n-7}H_{m-60}S^{32}_{4}O$
587.9	$C_{n-7}H_{m-62}S^{32}_{2}S^{34}O_3$
589.1	$C_{n-6}H_{m-60}S^{32}_{2}S^{34}_{2}$
590.0–599.9	
594.3	$C_{n-8}H_{m-62}S^{32}_{3}S^{34}N_2$
598.3	$C_{n-8}H_{m-62}S^{32}_{4}NO$
600.0–609.9	
601.6	$C_{n-7}H_{m-62}S^{32}_{2}S^{34}_{2}N$
605.7	$C_{n-7}H_{m-62}S^{32}_{3}S^{34}O$

serve the values of n and m as those obtained from Table IV, the empirical formula for naphthalenes may be written as $C_{n+1}H_{m-12}$, where $m = 2n + 2$, instead of the usual formula C_nH_{2n-12}. It will be seen from Table IV that for a mass number of 128, $n = 9$ and $m = 2n + 2 = 20$. The formula thus gives the usual formula, $C_{10}H_8$. The number n might be described as the "equivalent carbon number" because it is in fact the number of carbon atoms in the simplest hydrocarbon ion which could be found at a particular mass number.

Ions containing oxygen, nitrogen, or sulfur can have their molecular formulas expressed in a similar manner; for example, the ions containing one oxygen atom and having a nominal mass of 128 would have the empirical formula $C_{n-1}H_{m-4}O$ where m is again equal to $(2n + 2)$.

Peaks from ions containing one C^{13} atom can only be resolved from the normal hydrocarbon peak at the same nominal mass when the mass number is fairly low (below about 50). Further instrumental developments will, however, make it necessary to distinguish between these peaks and precise masses of the ions will be required. Provision has been made for these data in the tables.

Table V gives the mass defects of the ions with the formulas $C_n{}^{12}H_m$, where m lies in the range from $(2n + 2)$ to $(2n - 11)$. Below these are the defects for the first four isotope peaks which could occur at the same mass number.

Substituting an oxygen atom for a carbon and four hydrogen atoms will give a peak at the same mass number, but a mass defect which is larger by 36.4 millimass units, the spacing of the doublet CH_4—O. Thus, for the molecular formula $C_{n-1}H_{m-4}O$ we have a table similar to Table V, but with each entry increased by 36.4 millimass units (Table VI). Similar tables can be prepared for other empirical formulas involving oxygen, nitrogen, sulfur, etc. In practice, Table V is the basic table and the subsidiary tables are prepared

Table IX. Mass Defects of Some Combinations of Elements with Phosphorus[a]

Formula	Mass defect for $m = 2n$ (millimass units)
$C_{n-3}H_{m+5}P$	−13.1
$C_{n-2}H_{m-7}P$	80.7
$C_{n-4}H_{m+3}PN$	−0.6
$C_{n-5}H_{m+1}PN_2$	11.9
$C_{n-6}H_{m-1}PN_3$	24.4
$C_{n-7}H_{m-3}PN_4$	36.9
$C_{n-4}H_{m+1}PO$	23.3
$C_{n-5}H_{m-3}PO_2$	59.7
$C_{n-6}H_{m-7}PO_3$	96.1
$C_{n-7}H_{m-11}PO_4$	132.5
$C_{n-5}H_{m-3}PS^{32}$	77.4
$C_{n-7}H_{m-11}PS^{32}{}_2$	167.9
$C_{n-9}H_{m-19}PS^{32}{}_3$	258.4
$C_{n-11}H_{m-27}PS^{32}{}_4$	348.9
$C_{n-5}H_{m-2}P_2$	66 6
$C_{n-6}H_{m-6}P_2O$	103.0
$C_{n-7}H_{m-10}P_2O_2$	139.4
$C_{n-8}H_{m-4}P_2O_3$	175.8
$C_{n-9}H_{m-18}P_2O_4$	212.2

[a] These mass defects can be added to those for combinations listed in the previous tables.

Table X. Assignment of Masses on the CH_2 Scale

Mass No.	Precise mass (CH_2 = 14.000)	Mass defect (millimass units)	From Table IV		Analysis of defect		Assignment[a]
			n	m	H contribution (Table V) (millimass units)	Residual (millimass units)	
351	350.8402	159.8	25	$2n + 1$	−6.7	166.5	$C_{24}H_{31}O_2$
352	351.8469	153.1	25	$2n + 2$	−13.4	166.5	$C_{24}H_{32}O_2$
353	352.8535	146.5	26	$2n - 11$	73.7	72.8	$C_{24}H_{33}O_2$
354	353.8602	139.8	26	$2n - 10$	67.0	72.8	$C_{24}H_{34}O_2$
365	364.8402	159.8	26	$2n + 1$	−6.7	166.5	$C_{25}H_{33}O_2$
366	365.8469	153.1	26	$2n + 2$	−13.4	166.5	$C_{25}H_{34}O_2$
367	366.8535	146.5	27	$2n - 11$	73.7	72.8	$C_{25}H_{35}O_2$
368	367.8602	139.8	27	$2n - 10$	67.0	72.8	$C_{25}H_{36}O_2$
369	368.8624	137.6	27	$2n - 9$	64.8	72.8	$C^{13}C^{12}{}_{24}H_{36}O_2$
370	369.8647	135.3	27	$2n - 8$	62.5	72.8	$C^{13}{}_2C^{12}{}_{23}H_{36}O_2$
379	378.8402	159.8	27	$2n + 1$	−6.7	166.5	$C_{26}H_{35}O_2$
380	379.8469	153.1	27	$2n + 2$	−13.4	166.5	$C_{26}H_{36}O_2$
381	380.8535	146.5	28	$2n - 11$	73.7	72.8	$C_{26}H_{37}O_2$

[a] Residual defects of 166.5 and 72.8 millimass units correspond to the formulas $C_{n-1}H_{m-20}O_2$ and $C_{n-2}H_{m-8}O_2$, respectively. (See Table VIII.) Hence for $n = 25$ and $m = 2n + 1$, we have $C_{24}H_{31}O_2$, etc.

by adding to each defect a mass defect which is the spacing of the doublet formed by the peak with which we are concerned and the simple hydrocarbon ion.

To avoid listing in full each of the tables of defects, Tables VII, VIII, and IX have been prepared in which the general formula of the combination of atoms and the appropriate doublet spacing have been listed. Table VII lists formulas and defects involving one atom of deuterium, phosphorus, nitrogen, oxygen, sulfur-32, sulfur-34, fluorine, chlorine-35, and chlorine-37. In Table VIII the same information is given for combinations of oxygen, nitrogen, and sulfur with the limitations on the numbers of these atoms which were discussed previously. Table IX lists similar data for a number of combinations involving phosphorus.

The formulas and defects in Tables VII, VIII, and IX may be added together in cases of more complicated formulas.

Application of the Tables. An example of the use of the tables in the interpretation of precise mass measurements is given in Table X. This table shows the initial separation of the precise mass into a mass number with its corresponding n and m values, and a mass defect (mass number − precise mass).

The hydrocarbon ion with the same value of m has a mass defect (found from Table V) which is listed in the sixth column. Subtracting this defect from the observed defect gives a residual corresponding to one of the formulas in Table VIII. This formula is a general molecular formula for the unknown ion. It may be converted into a specific formula by substituting the appropriate values of n and m.

The mass measurements given in Table X are hypothetical and contain no errors in measurement. The calculated residuals and the residuals encountered in actual measurements have been compared in Table XI. The measurements were made with a Consolidated CEC 21-110 spectrometer using photographic detection.

Table XI. Comparison of Residuals with Calculated Residuals from Part of the Mass Spectrum of 4,4-bis-Methylene (2,6 Ditertiarybutyl Phenol)

Mass No.	Mass units × 10³		Assignment
	Residual (found)	Residual (calculated)	
351	159.9	166.5	$C_{24}H_{31}O_2$
352	158.3	166.5	$C_{24}H_{32}O_2$
353	67.5	72.8	$C_{24}H_{33}O_2$
354	72.9	72.8	$C_{24}H_{34}O_2$
365	190.2	166.5	$C_{25}H_{33}O_2$
366	186.9	166.5	$C_{25}H_{34}O_2$
367	84.4	72.8	$C_{25}H_{35}O_2$
368	82.3	72.8	$C_{25}H_{36}O_2$
369	68.3	72.8	$C^{13}C^{12}{}_{24}H_{36}O_2$
370	63.1	72.8	$C^{13}{}_2C^{12}{}_{23}H_{36}O_2$
379	183.4	166.5	$C_{26}H_{35}O_2$
380	173.3	166.5	$C_{26}H_{36}O_2$
381	78.1	72.8	$C_{26}H_{37}O_2$

CONCLUSIONS

The main advantages of the CH_2 scale can be summarized as follows. A reduction by a factor of about 30 times in the amount of data to be calculated, stored, and subsequently compared with precise mass determinations. The mass defects found from high resolution spectra can be compared directly and associated peaks can be recognized immediately.

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A Solid State Digitizer for Mass Spectrometers

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▶ A solid state digitizer is described for collecting data from mass spectrometers. The digitizer accepts ion peaks and acceleration voltage signals, converts the analog signals to digital, and presents the data on punched paper tape and printed output. The punched paper tape may be fed directly into a computer for sample calculations. Minimum detectability is 1 mv. with a background noise level of less than 0.5 mv. Mass number reproducibility is ±0.1 mass numbers from mass 2 to mass 125 and ±0.25 mass numbers from mass 125 to mass 250. Precision of sample calculations is improved by a factor of 4. This is due primarily to elimination of human error. The digitizer is of particular value if mass spectrometers are used to analyze large numbers of samples. On a three-shift operation, payout can be as short as 6 months.

THE NEED for a reliable digital data collection system for mass spectrometers has existed for several years. A large number of control laboratories operate their mass spectrometers on a round-the-clock, 7-day-a-week basis. An operation like this requires at least two people per shift; one to run the samples on the mass spectrometer and one to pick the peaks from the oscillographic chart and to log this data on calculation charts or to mark sense cards for the computer. The justification for having an automatic data collection system is fourfold: a reduction in number of personnel; increased accuracy from the data; elimination of the operational expense of the oscillograph; and an increase in the number of samples which can be run through the mass spectrometer.

An intensive study of available digital data collection systems indicated that the equipment needed was not available on the market and it would be necessary to have the equipment designed for this particular application.

The problem of collecting mass spectrometer data did not appear to be too difficult from an engineering standpoint. The output signals from the mass spectrometer are two analog voltage signals; one represents the amount of ion current produced by a particular mass and the other identifies which mass is being measured. A data collection system must accept these analog signals, convert them to digital form and present them in a format acceptable for calculations.

To describe the problem as clearly as possible, the specifications included photographs of the analog output signals under various operating conditions. The data showed the effect of varying the response and damping circuits on the mass spectrometer amplifier as well as an analysis of the types of noise present. The data also gave the slopes in volts per second of the various peaks encountered from the sharp peaks at low magnetic fields to the broad peaks at high magnetic fields as well as metastable peaks. Photographs of the acceleration voltage at different decay rates were included to illustrate volt-per-second decay rate throughout the decay range and the types of noise that modulated this signal.

A large manufacturer of digital systems accepted the specifications and produced the all solid state digitizer in 7 months.

DESCRIPTION OF SYSTEM

The digitizer accepts the analog signals from the mass spectrometer and converts these signals immediately to digital form. All control functions and subsequent handling of the information is carried out by a unique digital logic system.

Figure 1 is a block diagram of the digitizer and Figure 2 is an illustration of the logic sequence that must be carried out in measuring and identifying each peak.

Assume the digitizer is in logic state I. This means that the output signal from the mass spectrometer is the baseline and the logic circuit is "looking for" a signal that is greater than the signal stored in register (which is zero since each new reading is compared against the last reading). The digital voltmeter is reading the ion input signal at 60-c.p.s. rate. As soon as an ion signal appears, the logic system now has measured and compared a signal that is greater than the previous signal and thus the control logic shifts to state II.

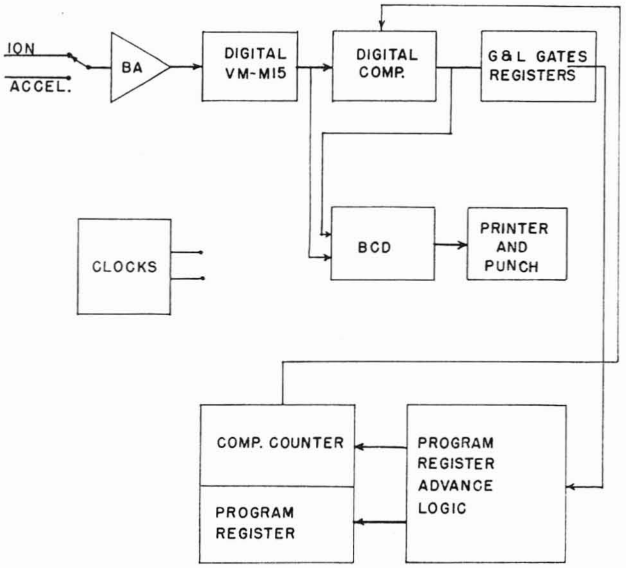

Figure 1. Block diagram of digitizer

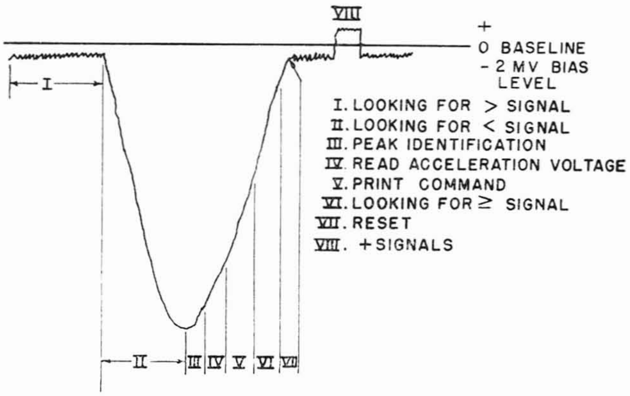

Figure 2. Signal identification—sequence of logic control

Table I. Acceleration Voltage to Mass Number Correlation

Acceleration voltage	Mass number
4.974–4.990	94
4.922–4.937	95
4.871–4.886	96
4.820–4.835	97
4.771–4.786	98
4.723–4.738	99
4.676–4.690	100
4.630–4.644	101
4.585–4.598	102
4.540–4.553	103

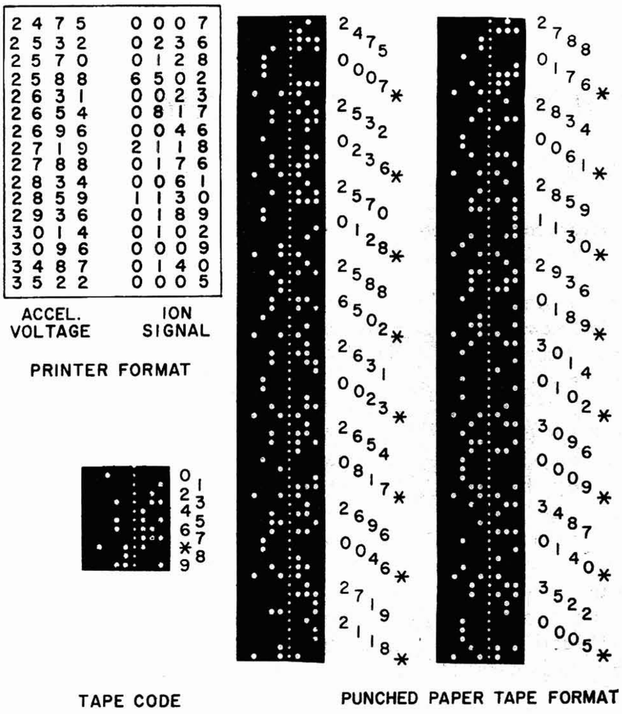

Figure 3. Record format

In state II, the input signal is increasing in amplitude and the logic circuit is "looking for" a reading that is less than the previous reading. Satisfying this criteria identifies the maximum peak amplitude (±1 mv.) and shifts the logic into state III. To prevent the system from falsely identifying spurious noise signals as ion signals, the logic system makes 5 additional comparisons before a decision is made that the maximum signal is a true ion peak. No signal error is encountered since the maximum signal has been held in storage. The 5 consecutive readings actually provide a digital filter that rejects all noise peaks that are less than 80 milliseconds in duration. This particular feature makes the digitizer extremely precise in gathering mass spectrometer data.

After logic state III is satisfied, the control circuit shifts to logic state IV. In logic state IV, the input relay is commanded to switch to the acceleration voltage input and one reading of the acceleration voltage is made and stored in the digital voltmeter. As soon as this reading is complete, the control circuit shifts to logic state V and the stored digital signal is printed and punched out. At the end of the print cycle the control circuit shifts to logic state VI and the input is again measuring the ion peak signal. The ion peak is now decaying in amplitude toward a valley or the baseline. The logic system is "looking for" a reading that is equal to or greater than the previous reading. As soon as the condition is met, the control circuit shifts to logic state VII where all registers are reset and the system shifts to logic state I, reading for another peak.

The above sequence of events occurs within milliseconds, the actual time is determined by the width of the ion peak. Logic state VIII occurs when any ion signal goes positive in polarity. This is an inhibit state and prevents

Table II. Precision Comparison

	CO_2	C_2H_2	CH_4	H_2	CO
Evaluation Sample—Camera					
	4.10	9.13	7.70	51.36	27.77
	4.09	9.13	6.66	51.42	27.71
	4.13	9.18	6.74	51.11	28.00
	4.19	9.34	6.76	50.36	28.38
	4.11	9.22	6.69	50.82	28.18
	3.88	8.78	6.45	53.23	26.69
	3.97	8.92	6.55	52.37	27.70
	3.75	8.52	6.27	55.18	25.67
	4.06	9.19	6.70	51.35	27.79
	4.05	8.99	6.48	52.83	26.88
	4.05	9.09	6.57	52.06	27.29
	4.08	9.08	6.55	51.97	27.28
$\bar{X}$	4.04	9.05	6.59	52.01	27.45
σ	±0.08	±0.21	±0.14	±1.25	±0.71
N	12	12	12	12	12
Evaluation Sample—Digitizer					
	4.06	9.09	6.66	52.45	27.62
	4.07	9.17	6.59	51.84	27.68
	4.08	9.07	6.57	52.57	27.39
	4.07	9.12	6.54	51.98	27.76
	4.07	9.08	6.51	52.34	27.54
	4.11	9.19	6.58	52.27	27 50
	4.08	9.14	6.60	52.30	27.69
	4.07	9.09	6.56	52.53	27.48
	4.33	9.23	6.51	51.60	27.93
$\bar{X}$	4.10	9.13	6.57	52.21	27.62
σ	±0.08	±0.05	±0.04	±0.31	±0.16
N	9	9	9	9	9

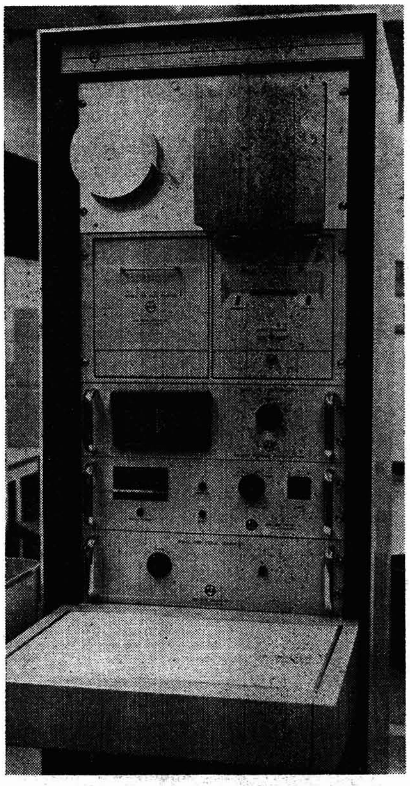

Figure 4. Front view of the solid state digitizer

the digitizer from taking any action on a positive peak.

The entire digitizer is controlled by two clocks, a 60-cycle clock and a 100-kc. clock. The 100-kc. clock controls the digitizing rate, the digital voltmeter, operates the digital comparator, the greater-than and less-than logic gates, and the register. The 60-cycle clock is used to control the logic circuitry of the system and is the fundamental time base for decision making. Due to the widths of the ion peaks (minimum of 70 milliseconds within 2% of the top) no error in measurement occurs. The 60-cycle time base also provides inherent rejection to 60-cycle interference. If desired this clock speed can be increased to several thousands cycles per second.

Installation and Startup. The mass spectrometer digitizer is quite easy to install. There are no modifications to make to the mass spectrometer amplifier. The amplifier output signal and a 0- to 10-volt tap of the acceleration voltage must be supplied to the digitizer input. Both of these signals are filtered by a pi RC filter which reduces the total noise level to less than 1 mv. peak-to-peak.

After the digitizer has been connected and is operating properly, the next step is to determine K factors for each magnet range that will be used. The equation for mass number is: $M/e = \frac{R^2B^2}{2E}$ which can be simplified to $M/e = \frac{K}{E}$ where $K = \frac{R^2B^2}{2}$ and B is fixed for a particular range. Therefore, $K = E \times$ mass number. Since the digitizer always reads only the acceleration voltage from 9.999 down to a lesser value, it is necessary for the operator to indicate which magnetic range is in use. The different K factors can be determined by averaging the K factors from known sample runs. Figure 3 illustrates the output data format and Table I illustrates tabular values of mass numbers *vs.* voltage.

PERFORMANCE

The solid state digitizer has been in use for 11 months. In that time, the operation yielded the following results; per cent onstream, 98; per cent utility, 99+. A labor saving of one operator per shift and a reduction in cost per sample by 57% were realized. The capacity of the lab, in samples per month, increased by 66% to 2000 samples. The estimated annual savings, based upon the results thus far, should be $50,000. As shown by Table II, the precision in analysis was improved by a factor of 4. Mass number reproducibilities were ±0.1 mass number up to mass 125 and ±0.25 mass number up to mass 250.

The instrument has a dynamic range of 10^4. Input signals may vary from 1 to 9999 mv. Minimum detectability is 1 mv., and the system is linear across the complete span. The digitizer, a photograph of which is shown in Figure 4, is unaffected by large voltage swings from the mass spectrometer signals and can accept ranges of over 600%.

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Ultramicrodetermination of Iodine by a Rapid Automatic Reaction-Rate Method

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▶ An automatic spectrophotometric reaction rate method for the ultramicrodetermination of iodide is based on the Sandell-Kolthoff reaction, in which a trace of iodine or iodide acts as catalyst for the reduction of Ce(IV) in the presence of As(III). The time required for the reaction to consume a small fixed amount of ceric ions, and therefore for the absorbance to decrease by a preselected amount (about 0.06 unit), is measured automatically and related directly to the iodide concentration. Ultramicro amounts of iodide in the range of 0.015 to 0.45 μg. were determined with relative errors of about 1 to 2% and measurement times of only about 10 to 100 seconds. The method should be adaptable for the determination of protein-bound iodine (PBI).

The method presented here utilizes the Sandell-Kolthoff reaction (*15*), in which a trace of either iodine or iodide acts as a catalyst for the reduction of Ce(IV) in the presence of As(III). This reaction has been widely investigated because of its great importance for the determination of protein-bound iodine (PBI) in serum (*3*). The overall reaction is summarized in Equation 1.

$$2\ \mathrm{Ce(IV)} + \mathrm{As(III)} \xrightarrow[\text{catalyst}]{\text{iodine}} 2\ \mathrm{Ce(III)} + \mathrm{As(V)} \quad (1)$$

There have been many studies (*11*) to determine the reaction mechanism.

Kolthoff and Sandell developed a quantitative procedure for iodide by measuring the time required to reduce all of the Ce(IV), as indicated by a visual color indicator. It was found that the iodide concentration was inversely proportional to the measured time for total reduction. In subsequent procedures the time for the measurement was decreased by not waiting for complete reduction of cerium(IV). In one basic procedure (*1, 2, 5, 9*) the Ce(IV) remaining after a fixed time (usually 20 minutes) is determined by an absorbance measurement at a selected wavelength characteristic of Ce(IV), usually between 350 and 420 mμ. Since the absorbance changes continuously, it is necessary to read the absorbance value at the exact preset time. A plot of absorbance *vs.* iodide concentration provides a suitable working curve. In another procedure (*7, 8, 10*) the reaction is stopped by adding a reagent that reacts immediately with Ce(IV) to reduce the remaining Ce(IV) quantitatively to Ce(III). One suitable reagent is brucine, which is oxidized to a colored reaction product. A plot of absorbance of the colored reaction product against iodide concentration provides a working curve. In another procedure (*4*) the change of absorbance of Ce(IV) was recorded continuously and the slope of the recorded curve was related to the iodine concentration.

To simplify and automate the iodide determination, a new procedure provides a digital readout value within a few seconds after start of the reaction. For example, extremely low concentrations of iodide in the range of 0.015 to 0.45 μg. per 4.25 ml. of solution were

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determined with relative errors of about 1 to 2% and measurement times of only about 10 to 100 seconds. This method utilizes the type of automatic spectrophotometric reaction-rate system recently used for the determination of glucose (*12*), alcohol (*13*), and amino acids (*14*).

The automatic reaction-rate measurement method was tested for the determination of PBI in digested serum samples, and the results indicated that the procedure should be readily adaptable for this important determination. The method used for releasing the iodine prior to measurement was similar to time-tested procedures; the serum proteins were precipitated with zinc hydroxide and the iodine was determined in the protein precipitate after incineration in the presence of potassium hydroxide. The results obtained were close to the assigned values of commercially available control sera.

GENERAL CONSIDERATIONS

Reaction Mechanism. There is some disagreement about the mechanism of the ceric-arsenite-iodide reaction (*6, 15*). In the absence of chloride ions and at very low iodide concentrations there is no direct proportionality between catalytic activity and iodide concentration. In the presence of chloride, the linear relation is fulfilled and the rate of the reaction is pseudo-first order with respect to iodide. It is assumed that the arsenite is in large excess and the starting concentration of cerium(IV) is the same for each sample. The rate of reduction of ceric ions, and the resulting rate of decrease of absorbance of the solution, are therefore proportional to the iodide concentration.

Interferences. Reducing or oxidizing agents should be eliminated prior to the measurements; silver or mercury in trace quantities which reduce the concentration of the iodide, and osmium and ruthenium, which catalyze the reaction in the same manner as iodide (*15*), interfere seriously. Since the rate of the reaction may be affected by several reaction-stimulating or reaction-inhibiting substances (*17*), the composition of the standard iodide solutions should be similar to that of the samples.

Contamination. Precautions should be taken to avoid contamination of reagents and glassware by iodine and mercury, since they provide the two most serious and frequent sources of error. Iodide adsorbed on glass is not easily removed by rinsing with water. All glassware should be thoroughly cleaned with sulfuric acid-dichromate cleaning solution, followed by repeated rinsings with deionized water. Glassware used for the iodide determination should be reserved for this purpose only. It is advantageous to perform the iodine determination in a separate room away from mercury and iodine compounds to prevent contamination.

All reagent chemicals should be tested for their iodine content and only lots with a minimum iodine content should be used. To keep the blank small, the concentration of reagents should be kept to a minimum for satisfactory reactions. For example, chloride ions should be added by using the purest available source (hydrochloric acid), and only the minimum amount should be added that is necessary for chloride to exert its full accelerating effect on the reduction of ceric.

Optimal Concentration of Reagents. Various initial ceric concentrations can be used. For the procedure described the concentration of ceric in the measured solution is $0.0006N$. With the 16-mm. i.d. cylindrical cell and a wavelength of about 390 $m\mu$ only about 5% of the incident light is transmitted through the cell to the detector. At higher ceric concentrations the rate of reaction is increased but the decrease of transmitted light causes a decrease of signal-to-noise ratio at the output of the photoconductive detector. Although various instrument modifications could improve the magnitude of the output signal for higher concentrations of ceric, it was not considered worthwhile for this application.

The accelerating effect of chloride ions (*15*) on the catalytic reaction for iodide is more pronounced at low iodide concentrations (5 to 25 p.p.b.). It increases with increasing concentration of chloride, as chloride concentration rises from zero to $0.3N$, but further increase of chloride concentration has but slight effect in accelerating the reduction of Ce(IV). Hydrochloric acid is the best source for chloride ions, because other sources often contain cations that inhibit the reaction and are usually more contaminated with iodide than HCl.

The final acidity of the Ce(IV)-arsenite mixture should be larger than $0.5N$ to prevent precipitation of reduced cerium salts. Final acid concentrations up to $2.5N$ have been recommended (*16*). However, such concentrated solutions present mixing and heat problems. The final concentration of sulfuric acid in the proposed method is $0.6N$.

Order of Adding Reagents. In the usual procedures using the Sandell-Kolthoff reaction the ceric solution is added to a solution containing the iodide sample and arsenite reagent. In the rate method described here the arsenite is added to a solution containing iodide sample and ceric reagent. In this way the zero adjustment on the Spectro unit can be made before starting the reaction. Any small change in initial absorbance, A_1 (before the injection of arsenite), is compensated by adjusting the balance control in the Spectro unit. As soon as the arsenite reagent is added, because of dilution, the absorbance changes to a new value, A_2. The instrument measures only the time required for a small preset change in absorbance ($A_3 - A_4$ equal to about 0.06) to occur during the early part of the reaction. It is only the absorbance change ($A_3 - A_4$) and not the absolute values of A_3 and A_4 that are important for the measurement. The time required for the absorbance to reach the value A_3 is not measured and can vary from sample to sample.

Temperature Control. The reaction rate has a large temperature coefficient and therefore the reaction takes place in a thermostated cell at 25° ± 0.1° C. To ensure thermal equilibrium and dissipation of the heat liberated by mixing of the sulfuric acid with the iodide solution, the mixture is stirred for 1 minute prior to starting the reaction by the addition of the arsenite solution.

Premeasurement Times. After initiation of the reaction, a minimum premeasurement time of 5 to 10 seconds is desirable to ensure thorough mixing of the reagents. The premeasurement time is controlled by appropriate setting of the comparator zero adjust. For example, the zero adjust was set at 5.80 to 5.90 for the 5- to 50-p.p.b. iodide range. A higher comparator setting, 6.30 to 6.40, was necessary for the higher concentration range where the reaction is faster. Larger zero adjust settings for the same iodide concentration range result in larger consumption of ceric during the premeasurement time and consequently in longer measurement time, but they do not affect the accuracy and precision of the results. The zero adjust setting may be varied, but that decided upon must be duplicated carefully from sample to sample, to secure starting of measurement time at the same ceric concentration.

Preparation of Standard Curve. Although the rate of the reaction under selected conditions is proportional to the iodide concentration, the standard curves (straight lines) obtained when reciprocals of measurement times are plotted against iodide concentration do not pass through zero because of iodide present as contaminating agent in the reagents used. Therefore, standard curves are rapidly established by using three to four standards which were prepared using the same reagents as for the unknowns.

PBI Determination. The automatic rate method was applied to the determination of PBI. The dry-ashing procedure (*9*) was used for the destruction of protein and the separation of iodide, but the method could readily be adapted to any of the sample preparation procedures now used for PBI determination (*3*).

INSTRUMENTATION

The basic instrumental components are the same as those used for determination of glucose (*12*) and alcohol (*13*). Since in the ceric-arsenite reaction the absorbance of the solution decreases as the reaction proceeds, the leads to the 1.5-volt battery in the bridge circuit which provides the Spectro balance control should be in reverse position to that previously used (*13*). The Sargent Model Q-RR automatic reaction rate adapter is used in combination with the Model Q comparator as control unit. The temperature is controlled at 25° ± 0.1° C.

A narrow third-order transmittance band at 390 mμ is selected by dialing the nominal 575-mμ second-order interference filter on the Spectro unit and inserting a Corning No. 5970 filter in the auxiliary holder.

REAGENTS

All reagents are prepared in deionized water. The iodide solutions are kept in glass-stoppered amber bottles.

Sodium hydroxide, 0.5*N*, 20 grams of NaOH per liter of solution.

Zinc sulfate septahydrate, 10% solution in water. Ten milliliters of this solution are diluted with 50 to 70 ml. of water and titrated with the sodium hydroxide using phenolphthalein as indicator; 10.8 to 11.2 ml. of titrant should be needed.

Potassium hydroxide, 2*N*, 112.2 grams of KOH per liter of solution.

SULFURIC-HYDROCHLORIC ACID REAGENT. Solution A. Concentrated H_2SO_4 (70 ml.) is added to 100 ml. of water in a 1-liter volumetric flask. After the mixture is cooled, 125 ml. of concentrated HCl are added, and the solution is diluted to the mark with water and mixed. This solution is 1.5*N* in HCl and 2.5*N* in H_2SO_4.

Solution B. Solution A is diluted with H_2O (1 to 9).

Sodium arsenite, 0.15*N*. As_2O_3 (7.5 grams) is dissolved in 75 ml. of 1*N* NaOH and diluted to 1 liter with water.

CERIC AMMONIUM SULFATE SOLUTIONS. Solution C, 0.02*N*. Ceric ammonium sulfate, $Ce(SO_4)_2 \cdot 2(NH_4)_2SO_4 \cdot 2H_2O$ (12.65 grams) is dissolved in 800 ml. of water plus 70 ml. of concentrated H_2SO_4. After the mixture has cooled, it is diluted with water to 1 liter and kept in a dark bottle.

Solution D. Twelve milliliters of Solution C are mixed with 70 ml. of water and 6.1 ml. of concentrated H_2SO_4 in a 100-ml. volumetric flask. After the mixture has cooled, 5 ml. of concentrated hydrochloric acid are added, and the solution is diluted to the mark with water and mixed. This solution is prepared fresh every few days and is kept in a polyethylene bottle. It should not be used until 2 hours after its preparation.

IODIDE STANDARDS. Stock Solution I. Potassium iodide (130.8 mg.) dried in desiccator is dissolved in 1 liter of water (1 ml. = 100.0 μg. of iodide).

Stock Solution II. Five milliliters of Stock Solution I are diluted to 1 liter. This solution contains 500 p.p.b. of iodide.

Working standards are prepared from Stock Solution II by appropriate dilution. They should be prepared fresh every month.

Working Standards for PBI. Three, 5, 10, and 15 ml. of Stock Solution II are pipetted into 250-ml. volumetric flasks, and 50 ml. of 2*N* KOH and 25 ml. of Solution A are added to each flask and mixed. After the mixtures have cooled, they are diluted to the mark with water to give standard solutions containing 6, 10, 20, and 30 p.p.b. of iodide; each solution is 0.4*N* in potassium ions, 0.15*N* in HCl, and 0.25*N* in H_2SO_4.

All working standards, the arsenite solution, and Solution D are kept in a water bath at 25° C. until used.

PROCEDURE

Preparation of Equipment. Switch the Spectro-Electro titrator to the Spectro position about 1 hour before the measurements are started. Dial the 575 position of the filter wheel and place the Corning No. 5970 filter in the auxiliary holder; throw the polarity switch to Position 1. (The battery leads are reversed from standard operation, as previously described.) Turn the comparator unit on and the selector switch to PNP ± 0.01 volt.

Measurement Step. PROCEDURE A. For the range 5 to 50 p.p.b. of iodide set the comparator Zero Adjust control at 5.90 (or to different values if necessary). Pipet 1.00 ml. of ceric sulfate solution (Solution D) and 3.00 ml. of sample or standard iodide solution into the reaction cell in the order given. Throw the comparator reagent selector switch to Position 1 to start the stirring and adjust the Spectro balance control (on side of Spectro unit) so that the meter needle is at the center. Set the comparator Zero Adjust at 4.50, and 1 minute after the pipetting of the iodide sample inject (with a 0.25-ml. syringe) 0.25 ml. of the sodium arsenite solution into the cell and press at once the Start button on the Model Q-RR adapter. The analysis is completed automatically and the number on the readout dial is recorded. Between samples empty the cell by inserting an aspirator tube and rinse with water. Repeat the procedure for each analysis.

PROCEDURE B, used for the range 30 to 150 p.p.b. of iodide. Procedure B is similar to Procedure A, except that the Zero Adjust is set first at 6.40; then, after the meter needle is adjusted to zero the Spectro balance control is used at 3.00. Four standard iodide solutions are used to prepare a standard curve.

DETERMINATION OF PB

Preparation of Serum Samples. Pipet 1.00 ml. of serum in a 15- × 125-mm. borosilicate glass tube, add 7 ml. of water, 1 ml. of 10% zinc sulfate, and 1 ml. of 0.5*N* sodium hydroxide and mix thoroughly with a footed glass rod (a separate rod for each tube). Centrifuge for 10 minutes, decant the supernatant liquid, and add 10 ml. of deionized water. Break the precipitate (using the same glass rod) to a uniform fine suspension and centrifuge for 5 minutes. Repeat the washing once more. Dissolve the precipitate in 1.00 ml. of 2*N* potassium hydroxide, place the tubes in a special rack (*9*), and dry overnight at 110°. Transfer the rack with the tubes in a cool muffle oven and raise the temperature gradually over a period of 1 hour until it reaches 600°. Open the door for a few seconds to renew the air in the oven. Maintain the temperature at 600° ± 10° C. for 90 minutes and open the door twice more at half-hour intervals. Remove the rack from the furnace and cover it with aluminum foil. Add 5 ml. of sulfuric-hydrochloric acid reagent (Solution B) to the ash in each tube (add with care to avoid excessive effervescence). By means of a footed glass rod loosen the ashed material attached to the wall and stir the contents of the tube until the reaction appears to be finished. Transfer to a clean tube and centrifuge for 10 minutes to pack the insoluble material. Transfer the supernatant into a small stoppered tube contained in a bath at 25° C. and use a 3.00-ml. aliquot of the supernatant for analysis. Include control sera of known PBI content with each series of unknown samples.

Measurement Step. Apply Procedure A. The iodide standards contain interfering ions in amounts equivalent to those present in the samples.

CALCULATIONS

Standard curves for PBI analysis are made to read directly the micrograms of iodine per 100 ml. of serum. Reciprocal times are plotted *vs.* PBI equivalents of the standards. The PBI equivalents are obtained by dividing the concentration of the standards in parts per billion by 2.

RESULTS AND DISCUSSION

Analysis of aqueous iodide solutions of known concentrations gave the results shown in Table I. The data indicate that ultramicro amounts of iodide in the range of 0.015 to 0.45 μg. in a total volume of 4.25 ml. were determined with precision and relative errors of about 1 to 2%.

Samples containing more than 150 p.p.b. of iodide should be diluted with iodide-free water or they can be analyzed according to Procedure B, but the voltage interval used should be in-

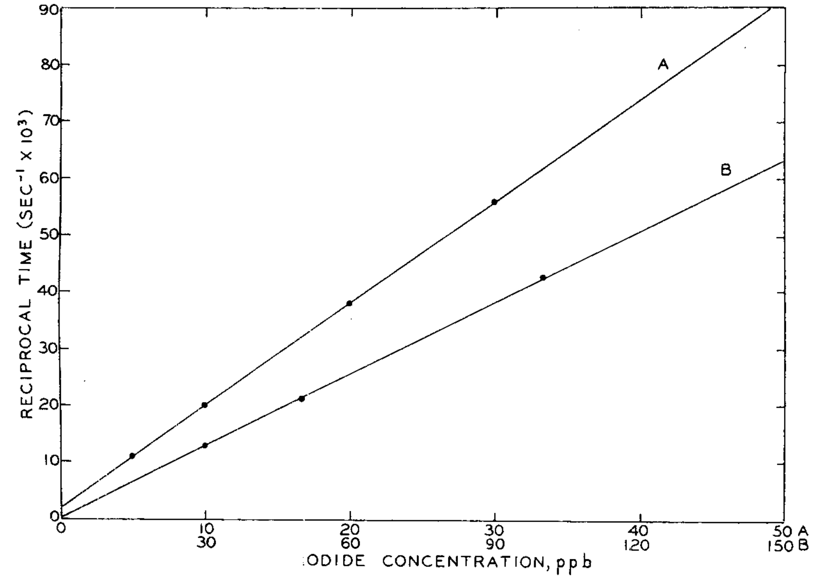

Figure 1. Relation of time to iodide concentration

creased to ensure measurement times longer than 10 seconds.

Standard curves for both the 5- to 50-p.p.b. and 30- to 150-p.p.b. ranges are shown in Figure 1. The straight lines intercept the ordinate because of iodide present as contaminating agent in the reagents used. This contamination was equivalent to about 1 p.p.b. of iodide. Hydrochloric acid is the main source of contamination.

Table I. Automatic Results for Aqueous Iodide Solutions

Reciprocal time, sec.$^{-1}$ × 10^3	(Iodide μg. × 10^2) in 4.25-ml. sample Taken	Found	Relative error[a]
	Range A[b]		
11.0	1.50	1.50	...
10.9	1.50	1.48	−1.3
37.7	6.00	5.97	−0.5
38.0	6.00	6.01	+0.2
93.5	15.0	15.2	+1.3
93.5	15.0	15.2	+1.3
	Range B[b]		
13.1	9.0	9.2	+2.2
12.9	9.0	9.0	...
62.9	45.0	44.8	−0.4
64.1	45.0	45.4	+0.9

[a] From straight-line standard curve.

[b] Ranges A and B run according to Procedures A and B, respectively.

Table II. Results for PBI in Control Sera Using Automatic Reaction-Rate Method

Sample No.	Iodine, μg. per 100 ml. serum Reported[a]	Found
1	3.0	2.9, 3.8
2	3.4	3.7, 3.9
3	4.7	4.2, 4.0
4	4.8	4.9, 4.3
5	6.0	5.9, 5.7
6	6.3	6.2, 6.0
7	10.2	9.7, 9.7

[a] Values of manufacturer, obtained by modified procedure of Barker (*2*).

Determination of PBI. The standard curve used for PBI determination is shown in Figure 2. Curve *A′* was obtained with iodide standards containing interfering ions in amounts equivalent to those present in the PBI samples. The blank is increased to about 4 p.p.b. (corresponding to 2.0 μg. of iodide per 100 ml. of serum), because these standards contained larger amounts of reagents.

For the PBI determination, larger chloride concentrations are used to compensate partially for the inhibiting effect of potassium ions that are present in the final solution. The decrease in the reaction rate caused by the potassium inhibiting ions is larger than indicated by the different slopes of curves *A* and *A′*, because the blank is larger in *A′* than in *A* and the final chloride concentration (accelerating agent) is larger in the standards used for *A′* than in those used for *A*.

An attempt was made to determine the blank for the PBI determination by substituting 1 ml. of iodide-free water for the serum and carrying the blank through the same procedure as the serum samples. However, the results for control sera were always high. Alkaline extraction of the ash with water according to Foss, Hankes, and Van Slyke (*9*) also gave high results. Apparently the pickup of iodine during the incineration was larger for the protein samples than for the aqueous samples. Therefore, it was necessary to use control sera (preferably two) of known PBI content to establish a blank. The difference between the given PBI content of the control sera and the values found from the standard curve was used as a blank, which was substracted from the values found from the standard curve for the unknown serum samples. Table II shows results obtained for seven control sera representing the low, medium, and high ranges of PBI. There is good agreement between duplicate PBI determinations, and the results obtained check closely with the control sera values reported by the manufacturer.

Although the application reported here deals with the determination of protein-bound iodine, the scope of the method is intended to be more general. A basic procedure and general considerations for the determination of iodine are given, so that the method can be adapted to many specific cases—e.g., milk, drinking water, plant material, and salt.

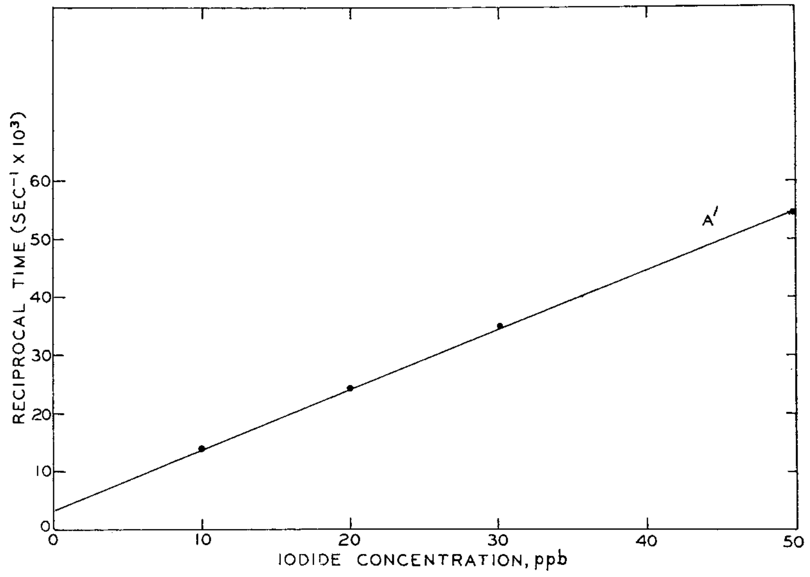

Figure 2. Standard curve for PBI determination

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A Simple Instrument for the Direct Determination of Dielectric Constants of Liquids over a Wide Range of Values

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▶ A simple solid-state instrument is described which will determine the dielectric constants of liquids with values of ϵ ranging up to 35 with an accuracy of better than 1%. The instrument is very stable from the standpoint of drift and reproducibility, and resonance indication is very sensitive. Some typical results are shown.

IN MANY INSTANCES it would be useful and convenient to have available an instrument which would determine simply and directly the dielectric constant of a wide range of organic liquids to an accuracy of better than 1%. In addition, it would be particularly useful if the same instrument could be employed in the determination of dipole moments by accurately measuring the dielectric constants of dilute solutions of polar compounds in nonpolar solutions. An instrument commonly used for dipole moment determination is described by Bender (*1*) and Fischer (*3*), but such instruments are limited in direct determination to liquids whose dielectric constants are less than 7. These instruments involve the conventional capacity-substitution method and use a 6E5 electron-ray tube as resonance detector. Fischer also shows how the range of dielectric constant may be extended with such an instrument on a relative basis by placing liquids of known dielectric constant into the tube around which the inductance of the resonance circuit is wound. When placed in such a tube, liquids with increasing dielectric constant have a mild but increasingly large effect which may be plotted empirically as scale divisions at resonance *vs.* dielectric constant. We have found this relative method based on calibration to be rather unsatisfactory due to the difficulty of obtaining a range of liquids of reasonably well known dielectric constants for calibration and to the lack of reproducibility of results. We have also found that these instruments possess an appreciable amount of drift making it necessary to take measurement of "plates-open" and "plates-closed" capacitances in relatively rapid sequence to minimize the error involved.

The instrument described in this paper also uses the capacity-substitution method but it extends the range of direct determination of dielectric constant to 35. In principle it should be possible to construct an instrument which goes to still higher values, but we have not tried it. We believe this extended range is possible because of the use of a very low supply voltage (6.8 volts) instead of 250 volts, and to the inclusion of a set of relatively cheap fixed precision capacitances as part of the resonance tuning circuit. The solid-state circuit is very stable, it runs cool, and the drift is less than 0.02 μμf. per hour. The low stable voltage is accomplished by means of a Zener diode. Sensitive resonance detection (±0.02 μμf. at resonance) is accomplished by means of a microammeter which gives a very sharp and reproducible signal at resonance.

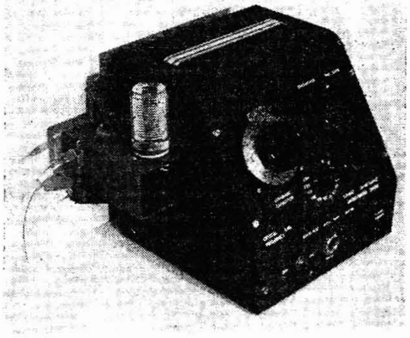

Figure 1. External view of instrument

DESCRIPTION OF INSTRUMENT

Figure 1 gives an external view of the complete instrument. Figure 2 shows the circuit diagram for the power supply. Figure 3 shows the oscillator circuit (at the left), the detector and voltage amplifier circuit (at the right), and the resonance tuning circuit (at the bottom).

In addition to the parts listed under Figures 2 and 3, the following components were also used:

Pilot lamp holder, fuse holder, knobs, 3 universal transistor sockets, octal socket and plug for L_4, vernier dial (0 to 100, 180°), line cord, crystal socket, 2- × 7- × 7-inch chassis and cabinet, and miscellaneous hardware.

For best performance, certain points in construction should be noted. Good results are crucially dependent on the linearity of the variable precision standard capacitor (C_S), and the tolerance on the solid-state silver-porcelain Vitramon capacitors (C_{10-21}). We believe that the quality of the latter capacitors is the limiting factor in the accuracy of the results obtained with this instrument. For reasons of economy we used capacitors with standard RMA values (91, 82, 71, 62, 51, 39 μμf.) instead of those actually shown in Figure 3 (90, 80, 70, 60, 50, 40 μμf.). In the case of the capacitors having values of 90 μμf. or greater, a deviation of as much as 1%

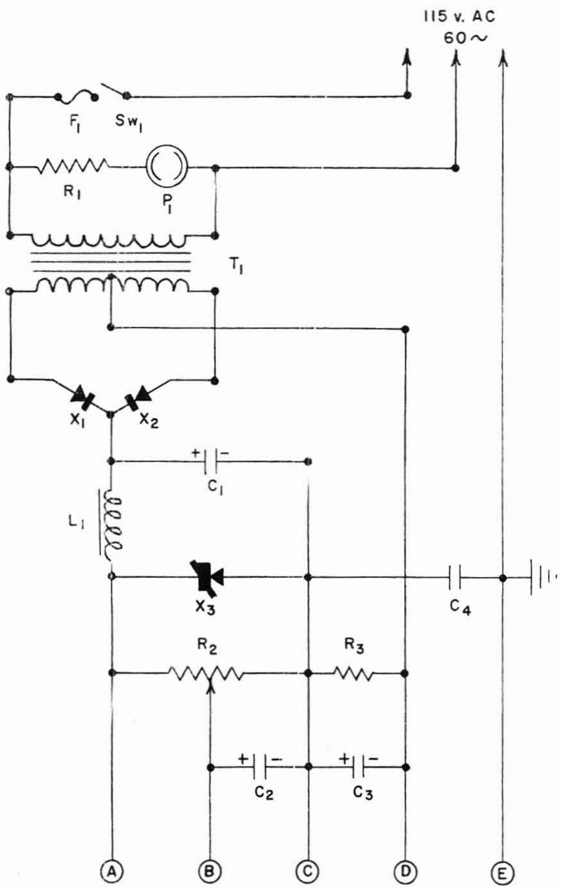

Figure 2. Circuit diagram for power supply. $A = +6.8$ v., $B = +1.0$ v., $C =$ reference ground, $D = -1.0$ v., $E =$ chassis ground

P_1	Ne 51 pilot lamp
F_1	1-amp. 3 AG fuse
R_1	150 K, 1/2-watt resistor
R_2	5 K, wire wound potentiometer
R_3	150-ohm, 1-watt resistor
T_1	12.6-v. C.T., 2-amp., filament transformer, Triad No. F44X
X_1, X_2	Silicon rectifiers, Sarkes M 500
X_3	Zener diode, 6.8-v., 10-watt, Hoffman No. 1N1805
L_1	10-hry., 50-ma., 500-ohm, filter choke, Triad No. C3X
C_1, C_2	1000-μf., 15-v., capacitor, Mallory WP 200
C_3	2000-μf., 15-v., capacitor, Mallory, WP 039
C_4	500-μμf., disk, ceramic capacitor
Sw_1	S.P.S.T. toggle switch

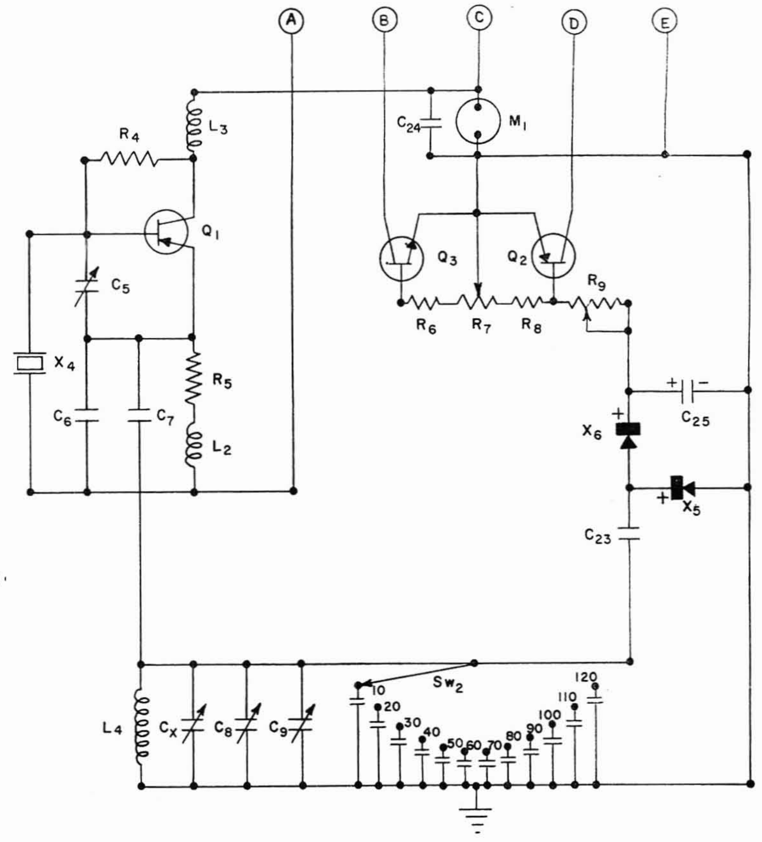

Figure 3. Oscillator circuit (left), detector and voltage amplifier circuit (right), and resonance tuning circuit (bottom); connect to power supply at *A, B, C,* and *D*

Q_1	2N274 transitor
Q_2	2N109 transistor
Q_3	2N647 transistor
R_4	100 K, 1/2-watt resistor
R_5	1 K, 1/2-watt resistor
R_6, R_8	47 K, 1/2-watt resistor
R_7	200 K potentiometer (balance)
R_9	10 M potentiometer (sensitivity)
M_1	0–100 microammeter
X_4	5.0 Mc. crystal
X_5, X_6	CK705, germanium diodes
L_2, L_3	1 mhry., R.F. choke
L_4	16 turns, 1 1/4-inch dia., air wound coil, Air Dux No. 1010T, 10t./inch, 1 5/8 inches long
C_5	10–100 μμf., variable ceramic capacitor, Centralab No. 823-BN
C_6	50 μμf., silver mica capacitor
C_7	240 μμf., tubular ceramic capacitor
C_X	Variable cell capacitor, 10 μμf. (modified by removing all but 2 rotor and 2 stator plates to give a capacitance of about 2.4 μμf. between plates-open and plates-closed positions in air), Johnson No. 160–104
C_8	Variable precision capacitor, 19.6 μμf., Johnson No. 160-110
C_{10-21}	Solid-state silver-porcelain capacitors, Vitramon VY axial radial series with ±1% tolerance, values in μμf.
C_9	Variable capacitor, 10 μμf., Johnson No. 189-4
C_{23}	2000 μμf., tubular ceramic capacitor
C_{24}	100 μμf., silver mica capacitor
C_{25}	2 μf., minature electrolytic capacitor, 15 v.
Sw_2	S.P. 12 position, nonshorting, ceramic rotary switch

may actually introduce a serious error in calculated values of ϵ, especially if in switching from one capacitor to another the deviations work in the opposite direction.

The best capacitor for C_8 was found by trial and error, with linearity being ascertained by means of the instrument itself. To avoid the nonlinear portions that usually lie at the ends of variable linear capacitors, we used the center section only of a 20-μμf. capacitor in order to get a 10-μμf. linear section. To check for linearity we determined the difference in capacitance of C_X at positions *A* and *B* for air, using slightly different settings of C_9 for each determination. Positions *A* and *B* are fixed stops set to permit a reproducible rotation of C_X through an angle of 180°. In this manner different sections of the vernier dial (and therefore different sections of the center section of C_8) were used for tuning. In our instrument there was less than ±0.5% variation from one end of the center section to the other. It is possible to use the manufacturer's rated value of C_8 in determining the capacitance per division of the vernier dial, but by experience we found that we could ascertain a slightly better value from measurements made with purified liquids of known dielectric constants. In our instrument, C_8 showed a capacitance change of 0.196 μμf. per scale division, instead of 0.200; this constant will be referred to as *K*.

Since the transistors Q_2 and Q_3 function as a temperature-compensated voltage amplifier, they should be mounted in close proximity to each other. In order to minimize stray and variable capacitance in connection with the use of the cell, capacitor C_X was rigidly attached to the chassis with attention paid to shielding and short heavy leads. The chassis should be well grounded (E in Figure 2). If inductance L_4 is not mounted externally, close proximity to surrounding metal should be avoided. In order to thermostat the liquid when its dielectric constant was being measured, a simple unshielded water-jacketed cell (8-ml. capacity) was provided for simple insertion and removal.

When construction is complete certain internal tuning adjustments should be made. For this purpose follow the first two steps in the instruction summary and then: (1) adjust R_2 until the potential between B and C is equal to the potential between C and D; this potential should be about 1 volt and the measurement should be made with a VTVM; (2) adjust R_9 until a mid-scale reading is obtained on microammeter M_1; (3) adjust C_5 carefully for nearly maximum deflection on M_1 using slightly *less* capacitance than necessary for the peak, and readjust R_9 if necessary to keep the needle on scale; (4) unplug X_4 and adjust R_7 to zero reading on M_1; and (5) replace X_4 and readjust R_9 for approximately 90 μa. at resonance. Remove X_4 from socket and note that M_1 should fall to zero. Any reading indicates self-oscillation and R_9 should be reduced slightly.

Fine tuning is achieved by finding the smallest value of C_8 which will keep the microammeter indicator needle at a fixed stable position near the right end of the scale. This value is determined by rotating the knob of C_8 from a value which is too high to one which is too low; just at the threshold of resonance the needle will make a small movement to the left, but this movement should not be confused with the large abrupt deflection to the left which corresponds to the establishment of resonance. When near resonance, the needle will oscillate from side to side with a period which depends on the care with which resonance is established. At resonance the period of oscillation is about 10 seconds; at about 1.2 $\mu\mu$f. below the proper resonance point the period is about 4 seconds and the amplitude is much smaller. If the capacitance is more than about 1.2 $\mu\mu$f. too low, the needle simply rests at the left end of the scale.

USE OF INSTRUMENT

This instrument utilizes the capacity-substitution principle described by Bender (*1*) or a standard text (*2*). For a given dielectric in C_X the resonance capacitance (C) of the circuit is given by

$$C = C_{XB} + K(R_8)_B + (C_9)_B + (C_{10-21})_B \quad (1)$$

when the cell capacitor, C_X, is in position B, and by

$$C = C_{XA} + K(R_8)_A + (C_9)_A + (C_{10-21})_A \quad (2)$$

when it is in position A. R refers to the arbitrary dial reading obtained on C_8 at resonance, and K to the conversion factor (vide supra) needed to give capacitance units. Since, for a given liquid, C_9 is never changed, the difference in sample cell capacitance at the positions A and B is given by.

$$C_{XB} - C_{XA} = K[(R_8)_A - (R_8)_B] + [(C_{10-21})_A - (C_{10-21})_B] \quad (3)$$

The dielectric constant of the material (b) in the sample cell is given by

$$\epsilon_b = \frac{[C_{XB} - C_{XA}]_b}{[C_{XB} - C_{XA}]_a} \quad (4)$$

where the subscript a refers to the cell filled with air. When air and nonpolar liquids are used in the sample cell, it is unnecessary to change the position of C_{10-21} as C_X is changed from A to B and the last term in Equation 3 drops out. This, in turn, means that under these circumstances ϵ_b can be given simply by the ratio of the differences in dial readings for K will cancel out.

Instruction Summary. (1) Place the water-jacketed sample vessel around C_X, and swing the vessel support into position under the cell.

(2) Set C_X to position A, set C_8 to a reading of approximately 80, switch fixed-step tuning capacitor (C_{10-21}) to 120 $\mu\mu$f., and then set C_9 to the lowest value that will maintain the microammeter needle at a fixed stable position near the right end of the scale.

Note: With a polar liquid around C_X it will probably be necessary to switch the fixed-step tuning capacitor (C_{10-21}) to a lower value than 120 to compensate for the large capacitance introduced by the polar liquid. If this is done, record $(C_{10-21})_A$.

(3) Carefully rotate C_8 to the position at which the microammeter indicator needle abruptly falls to the left; this is the resonance reading. Return the needle to the right again by rotating C_8 to a higher value, and then repeat the determination of the resonance reading. The resonance reading on the C_8 dial should be reproducible to the nearest 0.1 division (0.02 $\mu\mu$f.).

(4) Set C_X to position B, leaving C_9 and C_{10-21} unchanged. Repeat step (3). Record the new reading as $(C_8)_B$. Observe note in step 2.

(5) Thoroughly clean the water-jacketed vessel and the plates of the variable cell capacitor (C_X) with a pure nonpolar volatile solvent and thoroughly dry them with clean dry air before a new liquid is placed around the plates of C_X.

(6) Calculate ϵ_b, using Equation 4.

Liquids. The liquids listed in Table I were used as obtained or further purified as briefly outlined below; each number corresponds to the liquid with the same number in Table I. Boiling points and indices of refraction were recorded, but are not given here. In some cases additional purification details may be obtained from the references cited.

(1) Phillips Petroleum Co., pure grade, 99 mol % minimum.

(2) Eastman, spectro grade, No. S702.

(3) Baker and Adamson, reagent grade, No. 1554. Refluxed with metallic sodium and fractionally distilled. Method as given in (*6*).

(4) Mallinckrodt, Analytical Reagent, thiophene-free, No. 3856. Shaken with 4 portions of conc'd. H_2SO_4, washed 3 times with H_2O, dried with $CaCl_2$, fractionally distilled, then recrystallized. Method as given in (*7*).

(5) Baker and Adamson, Reagent Grade, No. 1564. Dried overnight with $CaCl_2$ and fractionally distilled with stream of dry N_2.

(6) Eastman, No. 70. Fractionally distilled.

(7) Matheson Co., No. 2464. Washed with dilute NaOH, washed with H_2O, fractionally distilled, dried with $MgSO_4$, filtered, fractionally distilled.

(8) Matheson, Coleman, and Bell, No. 2221. Fractionally distilled, recrystallized, dried with $CaCl_2$, fractionally distilled. Method as given in (*8*).

(9) Brothers Chemical Co., Reagent Grade, No. 3538. Dried with $CaCl_2$, filtered, distilled twice from activated alumina.

(10) Matheson, Coleman, and Bell, No. 5290. Fractionally distilled several times at 25-mm. pressure, dried with P_2O_5, filtered, fractionally distilled at 25-mm. pressure. Method as given in (*4*).

Table I. Values of Dielectric Constant, ϵ, at 25.0° C.

Compound	ϵ, Observed	ϵ, Literature (*5*)	Relative error
(1) *n*-Nonane	1.960	1.965	−0.25
(2) Cyclohexane	2.016	2.015	+0.05
(3) Carbon tetrachloride	2.235	2.228	+0.3
(4) Benzene	2.275	2.274	+0.05
(5) Chloroform	4.78	4.79	−0.2
(6) Chlorobenzene	5.60	5.62	−0.4
(7) 1-Octanol	10.20	10.30	−1.0
(8) Acetophenone	17.50	17.39	+0.6
(9) Acetone	20.97	20.70	+1.3
(10) *O*-Nitrotoluene	26.48	26.65	−0.7
(11) Methanol	33.46	32.63	+2.5
(12) Nitrobenzene	35.10	34.82	+0.8

(11) Baker and Adamson, Reagent Grade, No. 1212. Fractionally distilled from conc'd. H_2SO_4 (15 ml. H_2SO_4 per liter of MeOH), fractionally distilled from alkaline $AgNO_3$ (5 grams/liter), refluxed with freshly ignited CaO, refluxed (900 ml. of it) with Mg methylate (5 grams of Mg per 60 ml. of lime-treated MeOH plus 0.5 gram of I_2), fractionally distilled. Method as given in *(9)*.

(12) Matheson, Coleman, and Bell, No. 5051. Fractionally distilled, steam distilled, dried with $CaCl_2$, recrystallized, dried over P_2O_5, fractionally distilled at 2-mm. pressure. Method as given in *(8)*.

RESULTS

Some values of dielectric constant obtained at 25.0° C. with the instrument and liquids described above are listed in Table I. With one major exception, methanol, the observed values agree with published values to within ±1%; in most cases the agreement is much better. In the cases of worst agreement it is believed that impurities in the compounds are the main source of error, in spite of the reasonable (but not exhaustive) care with which each compound was purified. The other major source of error is believed to be the error involved in the manufacturer's tolerance of the capacitors used in the fixed-step tuning capacitor, C_{10-21}. The tuning indicator is exceptionally sensitive and a given reading is very reproducible, hence these are not considered to be significant sources of error. This instrument has been used very easily by students in the physical chemistry laboratory, and it is simple enough in design so that unusual knowledge of electronics is not required to understand its principle of operation. The students have used this instrument for both dielectric constant and dipole moment determination.

ACKNOWLEDGMENT

We acknowledge with appreciation the assistance which Roger A. Smith rendered in the purification of the liquids used and in some of the test procedures applied to the instrument.

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Determination of Moisture in Ion Exchange Resins by Karl Fischer Reagent

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▶ Application of the Karl Fischer method to determination of moisture associated with ion exchange resins is described. The method of analysis involves direct titration of the resin in either methanol or pyridine solvent. Use of the Fischer reagent overcomes many of the limitations of both oven drying and azeotropic distillation techniques commonly used for detection of moisture in resins.

THE Karl Fischer reagent offers a relatively simple, fast, and reliable chemical means for quantitatively determining moisture present in a wide variety of substances. Since the discovery of the specificity of this reagent for water in the mid-thirties *(1)*, it has proved highly successful for the determination of water associated with either organic or inorganic materials. In addition, the Karl Fischer reagent has served as a useful tool in following the course of chemical reactions in which water appears as a reagent or one of the products formed *(3)*.

With the introduction of macroreticular resins *(4)*, the utilization of ion exchange resins as catalysts or adsorbents in organic systems has received added impetus principally because of the favorable physical and chemical attributes of these exchangers in nonaqueous media. To assure maximum efficiencies of these materials, it has often been necessary to know the moisture levels associated with the resins before utilizing them as either catalysts or adsorbents in moisture sensitive systems. The Karl Fischer reagent has recently been found satisfactory for this purpose. The Karl Fischer reagent is a solution of iodine, sulfur dioxide, and pyridine in methanol, with the strength of any preparation being dependent on the iodine concentration. As the Fischer reagent reacts with water in a particular sample, the red-brown color of iodine is consumed until all the water has been reacted. The reagent thus serves as its own indicator during visual titrations.

EXPERIMENTAL

Apparatus. Though elaborate visual or electrochemical Karl Fischer titration units are available commercially (typical supplier A. H. Thomas Co., Philadelphia, Pa.), the apparatus in its simplest form consists of a buret (5- or 10-ml. capacity), a reservoir for the Fischer reagent, a 125-ml. Erlenmeyer flask, and a magnetic stirrer plus stirring bar. A Drierite tube, placed above the reservoir, serves to protect the reagent from atmospheric moisture contamination. The tip of the buret delivery tube is inserted through a rubber stopper placed into the mouth of the Erlenmeyer flask containing stirring bar plus the contents to be titrated. A small slit, cut into the rubber stopper, facilitates gravity flow of Karl Fischer reagent during titration.

Reagents. Karl Fischer Reagent. May be prepared using the method of Smith, Bryant, and Mitchell *(7)*, or more conveniently, may be purchased as a single stabilized solution (Harleco Chemical Products). The reagent undergoes slow deterioration on standing and therefore should be standardized periodically, preferentially before use.

Sodium Tartrate Dihydrate ($Na_2C_4H_4O_6 \cdot 2H_2O$). Primary water standard (Harleco Chemical Products). Though pure water or a standard solution of water in methanol may be used for standardization purposes *(5)*, sodium tartrate dihydrate is preferred. This

Table I. Amberlyst 15 Moisture Determination

Moisture found, %	
Karl Fischer titration	Zylol distillation
0.9	0.4
0.9	0.8
1.1	1.0
2.7	2.4

stable crystalline hydrate possesses an accurately known water composition (15.66 ± 0.05% H_2O) after 3 hours' drying at 150° C. (*6*).

Methanol. Reagent grade (J. T. Baker Co.).

Pyridine. Reagent grade (J. T. Baker Co.).

Procedure. Into a dry 125-ml. Erlenmeyer flask, pipet 25 ml. of pyridine or methanol. Connect the flask to the tip of a 10-ml. buret (Teflon stopcock equipped) with a rubber stopper. Titrate the contents of the flask with standardized Karl Fischer reagent while the contents of the flask are agitated by means of a magnetic stirrer. Record the milliliters of titrant required to produce a light cherry-red end point which persists in intensity for at least 60 seconds. To the Erlenmeyer flask add an accurately weighed 1 to 5 grams of resin sample, the size of the sample depending on the moisture level expected and on the strength of the Fischer reagent. Resume the titration until the identical end point color observed for the pyridine (or alcohol) blank is attained. Record the total volume of titrant used for the blank plus the sample. The per cent H_2O in the resin sample may then be calculated.

The procedure employed for standardizing the Fischer reagent is the same as the one used for the moisture determination of a resin sample, except that a 0.5- to 1.0-gram sample of $Na_2C_4H_4O_6 \cdot 2H_2O$ is used in place of the resin sample.

RESULTS AND DISCUSSION

In Table I are represented some typical data obtained during moisture analysis of Amberlyst 15, a macroreticular hydrogen-form sulfonic ion exchange resin based on a styrene-divinylbenzene matrix. Results from both the Karl Fischer technique described and zylol azeotropic distillation (*2*) are recorded.

The Karl Fischer technique was used successfully during a drying study for Amberlyst 15 resin to determine the minimum drying time that would be required to dry under vacuo a completely hydrated Amberlyst 15 sample or a sample in toluene containing a relatively low moisture level. Data obtained during the study are tabulated in Table II. The reliability of the Fischer titration values was checked out in several instances by addition of small known quantities of water to the titrated resin samples and then reevaluating the samples for moisture. The added water was accounted for in each case within the reasonable experimental limitations of the method (Table III).

Precision. An indication of the reproducibility of the method may be obtained from an examination of the data in Table IV which records moisture values from a series of independent moisture determinations for an Amberlyst 15 sample made in either methanol or pyridine solvent.

As seen from the data of Table IV, the average deviations differ by less than ±0.75% of the mean values of the individual sets of determinations, with no independent measurement deviating more than ±1.5% from the means. Oven drying of four replicates of the same Amberlyst 15 sample, on the other hand, gave a mean moisture value of 10.25%, with an average deviation from the mean value of ±2%.

The lower moisture values obtained from zylol azeotropic distillation and oven drying of Amberlyst 15 samples are probably attributable to incomplete release of moisture from the internal pores of the macroreticular resins during the distillation and drying processes. Sulfonic resins, especially when in the hydrogen form, are noted for their abilities to hold onto the final residual amounts of moisture rather tenaciously even after prolonged drying. This behavior is apparent from the data of Tables I and II. Inherent disadvantages of both oven drying and azeotropic distillation methods are that they are time consuming and incapable of satisfactorily handling unstable and heat sensitive resins. In addition, the presence of volatiles other than water can lead to serious errors at low moisture levels. Direct Karl Fischer titration of the resin samples should considerably overcome the moisture detection limitations of the customary physical methods at low resin moisture levels. The Fischer reagent appears better suited for determining tightly bound residual moisture which otherwise may escape detection.

Table II. Amberlyst 15 Drying Study

(Drying conditions 100° C. < 1 mm. Hg)

	Moisture, %	
Drying time, hr.	Water wet resin	Toluene wet resin
0	53.30[a]	1.21[a]
1	1.41	0.34
2	0.54	0.21
3	0.29	0.21
4	0.19	0.20
5	0.22	0.19
6	0.16	...
7	0.18	0.14
8	0.10	0.12
9	...	...
10	0.12	0.13
11	0.12	0.13

[a] Weight per cent moisture determined on samples drained of excess solvent (before drying).

Table III. Determination of H_2O Added to Karl Fischer Titrated Amberlyst 15

H_2O added, %	H_2O found by Karl Fischer titration, %
1.25	1.29
5.04	5.12
9.68	9.53
13.08	13.20

Table IV. Amberlyst 15 Moisture Precision Data

Methanol solvent		Pyridine solvent	
Moisture found, %	Deviation	Moisture found, %	Deviation
11.28	0.06	11.06	0.11
11.09	0.13	11.25	0.08
11.29	0.07	11.12	0.05
11.22	0.00	11.24	0.07
11.22 (mean)	±0.07 (av. dev.)	11.17 (mean)	±0.08 (av. dev.)

Though analysis of moisture associated with resins of different structural types was not investigated, no serious interferences should be expected in the case of standard type products available commercially.

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Correction of Luminescence Spectra and Calculation of Quantum Efficiencies using Computer Techniques

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▶ Techniques are described for evaluating the spectral sensitivity of the emission monochromator-phototube system and the spectral energy distribution of the excitation source–monochromator system of an Aminco-Kiers Spectrophosphorimeter, with fluorescence attachment. The treatment and use of sensitivity data for the correction of recorded spectra to units of relative energy or quanta are discussed. For this purpose, a computer program was written to correct the spectra and provide a print-out of the spectrum in units of relative quanta per unit frequency. Provision was made in the computer program for simultaneous integration of the corrected emission spectrum for use in calculation of quantum efficiencies. The quantum efficiencies for several compounds were determined using a comparative method making use of quinine sulfate as the reference standard.

APPLICATION OF LUMINESCENCE TECHNIQUES to analytical, physical, and organic chemical problems has increased recently, particularly now that commercial instruments are being used. The correction of emission spectra and the determination of quantum efficiencies are important in the study of photochemical reactions and of the mechanisms of fluorescence quenching and energy transfer in solution. An understanding of the origin of quenching is necessary when the phenomena of fluorescence or phosphorescence are used for quantitative analytical purposes. The measurement and comparison of quantum efficiencies for fluorescence *vs.* phosphorescence provide information concerning the nature of intersystem crossing (singlet-triplet transitions). Also, increased use of commercial apparatus will result in the publication of numerous luminescence spectra which should be corrected to be of greatest value to other workers.

Parker (*8*), in an excellent review, has recently discussed in detail the correction of fluorescence spectra and the measurement of fluorescence quantum efficiencies. He emphasized the calibration of the fluorescence monochromator-phototube combination, the correction of the emission spectra (to relative quanta per unit frequency interval *vs.* frequency), and the measurement of quantum efficiencies using reference compounds of known quantum efficiency. More recently, Parker (*7*) has discussed the calibration of a fluorescence monochromator in the ultraviolet region and has described the use of a fluorescent screen monitor for this purpose. The comparative method for measuring quantum efficiencies proposed by Bowen (*2*, *3*) has been used with proper modification by Weber and Teale (*12*), and Melhuish (*4*, *5*), as well as by Parker and Rees (*8*).

White, Ho, and Weimer (*13*) stressed the need to calibrate each instrument because sources, gratings, and phototubes perform differently from one spectrophotofluorometer to another. They describe methods for calibrating the Aminco-Bowman Spectrophotofluorometer. The emission monochromator was calibrated over the range 340 to 675 mμ using a previously calibrated Mazda projection bulb. The excitation monochromator (Hanovia xenon arc source) was calibrated by several methods including a chemical actinometer, a phototube of known response, and a thermopile. Although data have been published on the correction of fluorescence spectra, very little effort has been placed on the use of computers to reduce the tedium of such time consuming repetitive calculations.

In the present work, a relatively simple method for the calibration of an Aminco-Kiers Spectrophosphorimeter with fluorescence attachment is described. The emission monochromator (1-P-28 phototube) was calibrated over the entire region from about 220 mμ to 600 mμ using several sources of known spectral energy distribution. Using the calibrated emission monochromator, the spectral energy distribution curve for the excitation monochromator (Osram xenon arc source) was determined. A computer program was written to correct recorded spectra and provide a printout of the corrected spectrum in units of quanta per unit frequency interval *vs.* frequency. The computer program also provides for integration of the spectrum for use in calculating quantum efficiencies.

EXPERIMENTAL

Apparatus. An Aminco-Kiers Spectrophosphorimeter with fluorescence attachment was used throughout this study. The excitation monochromator contained an Osram xenon arc source and a 50- × 50-mm. grating (600 grooves per mm.) blazed for highest efficiency at 300 mμ in the first order. A 1P28 phototube was used with the emission monochromator which was identical to the excitation monochrometer except for a blaze wavelength of 500 mμ. Presentation of spectra from both monochromators was linear in wavelength. The spectral slit widths for these grating monochromators were essentially constant over the wavelength ranges studied.

Absorption spectra were determined with a Cary Model 14M spectrophotometer.

For calibration, a tungsten filament bulb operated at several different controlled voltages was used. Filament temperatures were measured with an optical pyrometer. Assuming black body radiation, the spectral energy distribution was calculated from Wien's law. For the ultraviolet region, a Hanovia quartz mercury-vapor arc lamp (lamp code SH) was uesd. The relative intensities of the numerous lines from this lamp were supplied by the manufacturer.

Spectra were recorded with a Mosely autograf drum-type X-Y recorder.

Corrections of the emission spectra were performed on an IBM 1620 computer.

Chemicals. Quinine sulfate (Matheson Coleman & Bell) was recrystallized twice from hot water acidified with sulfuric acid.

Anthracene (Eastman) was recrystallized from alcohol and the phenol (J. T. Baker, reagent grade), indole (Eastman), and Rhodamine B (Eastman) were used as supplied from the manufacturer.

Temperature was maintained at 25° ± 1° C. for all measurements. Assuming a temperature coefficient of emission intensity of 1 to 2%, variations introduced by temperature fluctuations probably did not exceed variations in instrument operation.

Calibration of Emission Monochromator (1-P-28 Phototube). The miniature tungsten filament lamp was placed inside the cell compartment so that the emission could pass directly through a pair of 0.5-mm. slits into the emission monochromator. The

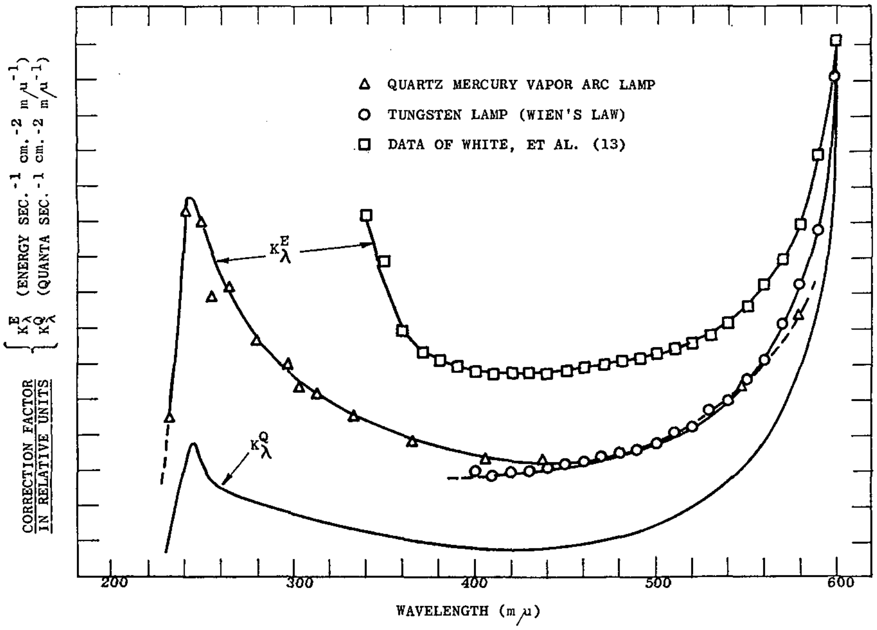

Figure 1. Correction factors for emission monochromator (1-P-28 phototube) in units of energy and quanta per unit wavelength interval

exit slit (photomultiplier slit) was also set at 0.5 mm. The output of the photomultiplier *vs.* wavelength was recorded on the X-Y recorder. From the measured filament temperature, the spectral energy distribution curve was calculated by means of Wien's law.

$$E_\nu = \frac{C_1\lambda^{-5}}{e^{C_2/\lambda T}} \qquad (1)$$

where

λ = wavelength in μ
T = temperature in °K.
C_1 = [5.673 × 10^{-12} watt/(cm.^{2} $K.^{\circ 4}$)] [$(15/\pi^4)C_2^4$]
C_2 = 1.4385 × 10^4 μK.°

As the relative intensity of the emission from the tungsten lamp became small near 400 mμ, the useful spectral range was about 400 to 600 mμ. Beyond 600 mμ, the sensitivity of the 1P28 phototube diminished rapidly, which limited its use to wavelengths below 600 mμ. Correction factors were calculated at 10-mμ intervals by dividing the calculated relative energy by the recorded output of the photomultiplier:

$$K_\lambda^E = E/R \qquad (2)$$

where

E = energy flux per unit wavelength interval
R = recorded response of the monochromator-photomultiplier combination

Thus, multiplication of the recorded intensity at a given wavelength by K_λ^E converts the apparent intensity to a true relative energy. A correction factor for conversion to relative quanta per unit wavelength interval was obtained by multiplying by λ:

$$K_\lambda^Q = K_\lambda^E \cdot \lambda \qquad (3)$$

Presentation of spectra with an abscissa linear in frequency is preferred as the energetic relationship between individual bands (vibrational fine structure) then becomes linear. Therefore, for such presentation a factor must be included to account for the change in wavelength interval with change in frequency interval as shown by Parker and Rees (*8*):

$$\nu = c/\lambda; \; \frac{d\lambda}{d\nu} = -\frac{c}{\nu^2} = -\frac{\lambda^2}{c} \qquad (4)$$

$$\frac{dQ}{d\nu} = \frac{dQ}{d\lambda}\frac{d\lambda}{d\nu} = -\frac{dQ}{d\lambda} \cdot \frac{\lambda^2}{c} \qquad (5)$$

The correction factor then becomes:

$$K_\nu^Q = K_\lambda^E \cdot \lambda^3 \qquad (6)$$

Below 400 mμ, the monochromator-phototube response was determined by use of the Hanovia quartz mercury vapor lamp. The lamp was mounted above the cell compartment and the radiation reflected into the monochromator by placing a sheet of aluminum foil (pure, J. T. Baker) in the cell compartment. No correction was made for a slight variation of reflectivity *vs.* wavelength. The output of the photomultiplier was recorded as in the case of the tungsten lamp. Intensities of the emission bands were supplied by the manufacturer of the mercury vapor arc lamp. Although the relative energy distribution of a line source is expressed in terms of total relative energy emitted by the lines, these energies may be used for this calibration as though they were energies per unit wavelength interval (the spectral slit width of the monochromator; expressed in wavelength units, was constant *vs.* wavelength). Small exit slits (0.5 mm.) and small entrance slits (1 mm.) were used to reduce the intensity of the background emission (continuum) compared to that of the sharp mercury emission lines. In most cases the recorded intensity returned to essentially zero between the sharp emission lines. For the several weaker lines the background (continuum) was accounted for by drawing a base line to determine the peak intensity. The very weakest lines were not used for the calibration. Correction factors, K, were calculated according to the equations given above.

Emission monochromator-phototube correction factors for energy and quanta per unit wavelength interval are plotted in Figure 1. Correction factors for quanta per unit frequency interval are plotted in Figure 2. Data from the tungsten lamp and the mercury vapor arc lamp were normalized at 500 mμ, thus providing a continuous correction curve from about 240 to 600 mμ. Although the actual intensity of the

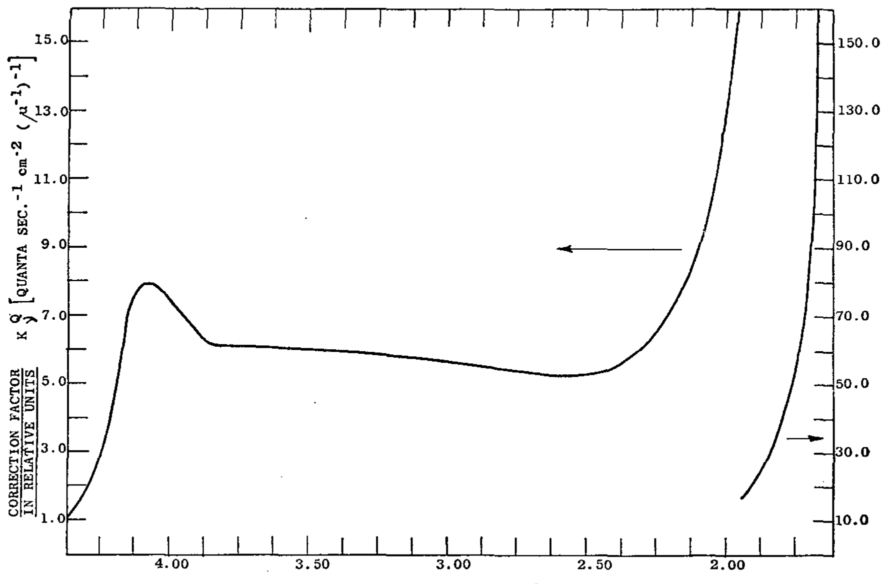

Figure 2. Correction factors for emission monochromator (1-P-28 phototube) in units of quanta per unit frequency interval

individual mercury vapor emission lines varied by several orders of magnitude, the resulting correction factors produced a rather smooth curve. For comparison, the data of White, Ho, and Weimer (*13*) were normalized and included in Figure 1. Their data extend to only 350 mμ at which point a sharp rise is noted. The low intensity from their tungsten lamp at 350 mμ (the Mazda lamp probably had an ordinary glass envelope) was likely the main reason for the sharp cut-off rather than absorption by the envelope of the phototube. The 1-P-28 phototubes are regularly used in spectrophotometers operating in the ultraviolet region of the spectrum and should show a reasonable response between 250 and 350 mμ. A 1-P-28 phototube has an S-5 response showing a maximum near 340 mμ according to manufacturer's data. Above 375 mμ, the data of White, Ho, and Weimer (*13*) agree rather well with the data shown in Figure 1. It is interesting that the response of the monochromator-phototube combination expressed in relative quanta per unit frequency interval approaches linearity between 350 and 500 mμ, the most frequently used region of the spectrum.

Calibration of the Excitation Monochromator (Osram Source). The excitation monochromator was calibrated by making use of the calibrated emission monochromator. Radiation from the excitation monochromator was scattered into the emission monochromator by placing a neutral reflecting or scattering material in the area of the cuvette holder. With narrow exit slits (0.5 mm.) on the emission monochromator the scattered radiation at intervals of 10 mμ was scanned with the emission monochromator and recorded. Several different materials were used to scatter the radiation, viz., a magnesium oxide screen, aluminum foil, quartz wool, and calcium carbonate. The recorded intensities were multiplied by the correction factor, K_λ^E, to obtain the emitted spectral energy distribution curve expressed in terms of energy per unit wavelength interval E_λ^E *vs.* wavelength (see Figure 3). The distribution of radiation from source and monochromator in terms of quanta per unit wavelength interval E_λ^Q was obtained by multiplying by K_λ^Q (or $E_\lambda^Q = E_\lambda^E \cdot \lambda$). Data obtained with the magnesium oxide reflector were chosen without correction for the change in coefficient of reflection with wavelength which varies by only several per cent over the range 250 to 500 mμ (*1, 11*). Although the other scattering materials behaved very much like the magnesium oxide, the magnesium oxide probably represents the best neutral diffuse reflector which is readily available.

The energy (or quanta) distribution curves may be used to correct excitation spectra or correct for differences in quanta of exciting radiation when the wavelengths of excitation are different for the reference standard and the unknown, whose quantum efficiency is being measured.

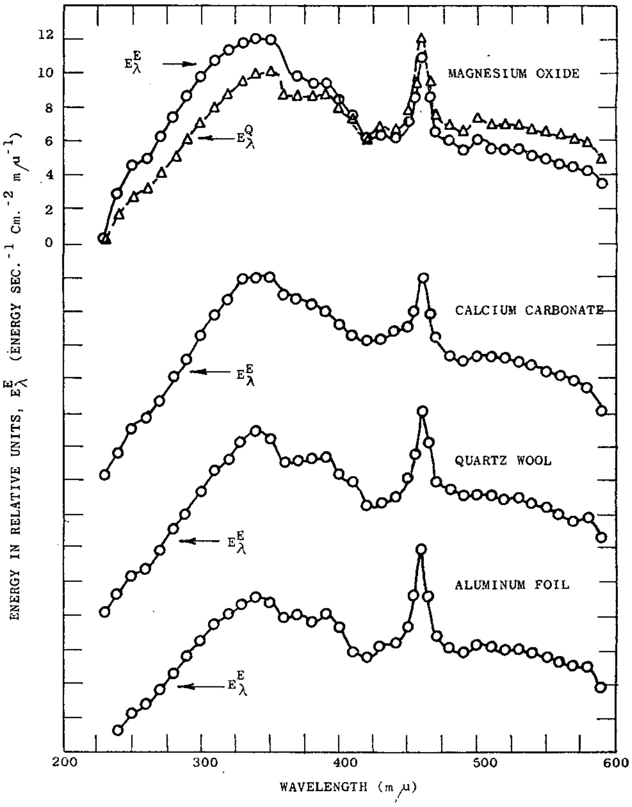

Figure 3. Spectral energy distribution for excitation monochromator (Osram xenon lamp)

Measurement of the Emission Spectrum. All spectra were obtained at solute concentrations representing linear response (emission intensity) *vs.* concentration. Under these conditions the undesirable inner-filter effects are minimized. Inner-filter effects have been thoroughly discussed by Parker and Rees (*9*) in a recent review. For very accurate quantum efficiency measurements, a correction for self-absorption of luminescence must be applied. In the case of certain compounds, this effect may be quite large if sufficiently dilute solutions are not used. In general, however, at very low concentrations the oxygen quenching constant will be much more significant than the self-quenching constant, necessitating the exclusion of oxygen during measurement. Therefore, oxygen was removed by degassing all solutions with high purity dry nitrogen and by continuously flushing the cell compartment with nitrogen.

Computer Processing and Correction of Spectra. Manual calculation of corrected intensities and replotting of the spectra is tedious. Modern computers are ideally suited for such time consuming calculations. A program was written for an IBM 1620 computer to process the spectral data and present the corrected data in a graphical form.

Intensities from the recorded spectra at 5-mμ intervals are punched on IBM cards. For very sharp peaks, shorter wavelength intervals are used. The correction factor K_λ^E at similar intervals of wavelength are also punched on IBM cards. An additional factor, λ^3 (see text above), is calculated for multiplication along with K_λ^E. After applying the correction factors, a parabola is fitted to the three points bracketing the peak to determine the maximum intensity and all of the intensities are normalized with respect to this computed maximum. The reciprocal of each wavelength value is calculated to yield frequency in units of reciprocal microns. The resulting table of normalized, corrected intensities and frequencies is used with a 4-point LaGrangian interpolation subroutine to compute the position for the ordinate character at intervals of 0.01 micron^{-1}. For use in quantum efficiency calculations, the ordinate values are summed to provide integrated area for graphical presentation of the spectrum. A pair of cards is punched for each point. The IBM printer uses the last 60 columns of each card of the pair to give 19 characters of identification and 101 positions for plotting. The first character of each card controls page skipping and space suppression on the printer and the next 19 may be used for identification, sequence, etc., but do not print. Successive pairs of cards represent an increment of the abscissa of 0.01 micron^{-1}. The integrated area (sum of ordinates) and normalization factor are printed last at the lower left hand corner of the spectrum. A suitable grid of scale characters is generated at the start, finish, and at each 10 increments of abscissa to give a finished appearance to the printed spectrum.

The Appendix gives the Fortran

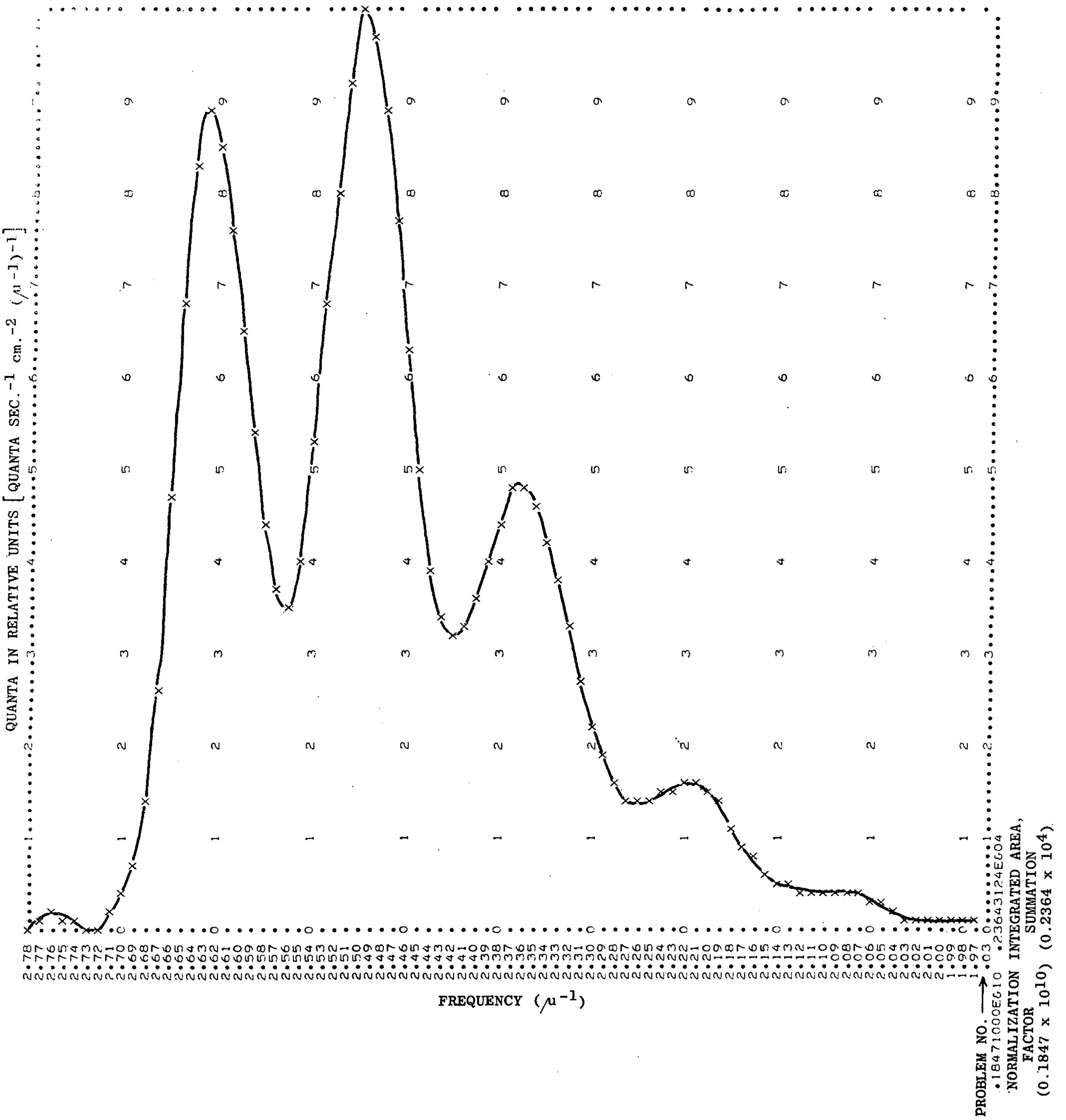

Figure 4. Corrected fluorescence spectrum of anthracene in units of quanta

listing (may be used on other computers) of the program with comments describing the function of each major division. The program uses a special subroutine (available upon request) to punch the cards which list as a graph. An example of a finished plot of the fluorescence spectrum of anthracene is shown in Figure 4.

RESULTS AND DISCUSSION

Quantum Efficiency Calculations. According to Parker and Rees (*8*), the total intensity of luminescence (F^Q for fluorescence intensity in quanta per second) is equal to the intensity of the absorbed energy times the quantum efficiency.

$$F^Q = \phi E^Q(1 - 10^{-\epsilon bc}) \qquad (7)$$

where

ϕ = quantum efficiency = $\dfrac{\text{quanta emitted}}{\text{quanta absorbed}}$
E = intensity of exciting radiation in quanta per second
ϵ = molar absorptivity (liters mole^{-1} cm.$^{-1}$)
b = optical path length in cm.
c = concentration in moles per liter

When the absorbing solute is reduced to low concentrations such that the per cent transmittance is high (region of linear emission intensity *vs.* concn. is when % $T \geqq 95$), Equation 7 reduces to

$$F^Q = \phi E^Q\,(2.3\ \epsilon bc) \qquad (8)$$

When the same instrument and the same exciting wavelength is used the fluorescence intensities (integrated areas expressed in units of quanta) of two different compounds are related as follows:

$$\frac{F_2^Q}{F_1^Q} = \frac{E_2^Q\phi_2\epsilon_2bc_2}{E_1^Q\phi_1\epsilon_1bc_1} = \frac{\phi_2A_2}{\phi_1A_1} \tag{9}$$

where

A = absorbance representative of the solution used for the luminescence intensity measurement

F = area of luminescence spectrum in units of quanta.

However, with the Aminco-Kiers instrument and certain other instruments having a separate excitation monochromator, the wavelength for maximum excitation may be selected for each different compound. In this case, the E^Q terms remain in the equation. If

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Table I. Some Quantum Efficiencies Determined from Computer Processing of Fluorescence Data

(Quinine sulfate used as reference standard, ϕ = 0.55)

Compound	Max. (μ^{-1})	Concn. used (μg./ml.)	Quantum efficiency, ϕ This work[a]	Quantum efficiency, ϕ Literature values	Reference
Anthracene, in ethanol	2.63, 2.50 2.37, 2.22	1.78	0.22	0.28 0.26[b] 0.27[b] 0.27	(*8*) (*6*) (*4*) (*7*)
Rhodamine B, in ethanol	1.75	0.372	0.76	0.69 0.97	(*8*) (*12*)
Phenol, in water	3.34	10.0	0.13	0.22 0.19[c]	(*12*) (*7*)
Indole, in water	2.90	1.00	0.24	0.45	(*12*)
Quinine sulfate, in water	2.14	1.00	...	0.55	

[a] Values not corrected for self-absorption.

[b] The recorded values, uncorrected for self-absorption, were 0.22 (*6*) and 0.228 (*4*).

[c] In ethanol.

APPENDIX I

```
0̄7300 C     SPECTRO-PHOSPHORIMETER PROG. AD-
               JUST, SCALE, PLOT AND INTEGRATE.
0̄7300 C     RCC-HVD-ALS. VERSION 11-19-62.
0̄7300       DIMENSION WAVL (75), CORDG (75), A(3, 4),
               X(4), CRDG(4), D(4)
0̄7300       DIMENSION F(32)
0̄7300 C     READ TABLE OF CORRECTION FACTORS
               (32 ITEMS) AND PROBLEM NO.
0̄7300       DO 5 I = 1, 32
0̄7312     5 READ 11, F(I)
0̄7396   100 BIGRD = 0.0
0̄7420       READ 10, PROBN
0̄7444 C     SET INDEX OF COMPUTED GO TO FOR
               EXIT FROM INTERPOLATION TO 93.
0̄7444       KIF = 1
0̄7468 C     READ DATA, GET CORRECTION FACTOR
               BY INTERPOLATION, SAVE LARGEST
               RDG
0̄7468       DO 20 I = 1, 75
0̄7480       READ 10, WAVL(I), RDG, CORR
0̄7552       IF (WAVL(I) - 999.)23,24,24
0̄7644    23 FREQ = WAVL(I)
0̄7692       IF(FREQ -270.)12,13,13
0̄7760 C     UP TO 270.0 MILLIMICRONS, LINEAR IN-
               TERPOLATION.
0̄7760    12 TEST = (270. - FREQ) /5.0
0̄7808       J2 = TEST
0̄7844       B = J2
0̄7880       J3 = 10 - J2
0̄7916       YRDG = F(J3) + (TEST - B)* (F(J3 - 1) -
               F(J3))
0̄8084       GO TO 93
0̄8092 C     ABOVE 270.0, USE 4-POINT LAGRANGIAN
               INTERPOLATION.
0̄8092    13 TEST = (FREQ -270.)/15.0
0̄8140       J2 = TEST
0̄8176       B = J2
0̄8212       J3 = J2 + 8
0̄8248       IF(TEST - B) 15,16,15
0̄8316    16 YRDG = F(J3 + 2)
0̄8364       GO TO 93
0̄8372    15 IF(J3 - 9)17,17,18
0̄8440    17 TEST = 270.
0̄8464       J3 = 9
0̄8488       GO TO 91
0̄8496    18 IF(J3 - 28)19,19,90
0̄8564    90 J3 = 28
0̄8588       TEST = 570.
0̄8612       GO TO 91
0̄8620    19 TEST = 255. + B* 15.0
0̄8668    91 DO 92 J = 1,4
0̄8680       J4 = J3 + J
0̄8716       CRDG(J) = F(J4)
0̄8788       X(J) = TEST
0̄8836    92 TEST = TEST + 15.0
0̄8908 C     GO TO INTERPOLATION. RETURN TO 93.
0̄8908       GO TO 75
0̄8916 C     MULTIPLY RDG BY CORR. FACTOR AND
               CUBE OF WAVL(I). (NOW IN FREQ)
0̄8916    93 RDG = RDG * YRDG * FREQ * * 3
0̄8952 C     SAVE LARGEST CORRECTED RDG AND
               INDEX RELATED TO IT (1-2).
0̄8952       IF(RDG - BIGRD) 20, 20, 22
0̄9020    22 BIGRD = RDG
0̄9044       J5 = 1 - 2
0̄9080    20 CORDG(I) = RDG
0̄9164 C     DATA ARE ALL IN. SET COMPUTED GO TO
               FOR EXIT INTERPOLATION TO 94.
0̄9164    24 KIF = 2
0̄9188 C     INITIAL AREA UNDER CURVE = 0.0
0̄9188       SUMC = 0.0
0̄9212 C     SET UP MATRIX A FOR SOLUTION OF
               PARABOLA THROUGH 3 POINTS WHICH
0̄9212 C     BRACKET BIGRD, THE MAXIMUM COR-
               RECTED READING.
0̄9212       DO 30 L = 1,3
0̄9224       J1 = J5 + L
0̄9260       A(L,3) = 1000./WAVL(J1)
0̄9344       A(L,2) = A(L,3) * A(L,3)
0̄9452       A(L,4) = CORDG(J1)
0̄9524    30 A(L,1) = 1.0
0̄9608 C     SIMULTANEOUS EQUATION SOLUTION,
               STATEMENTS THROUGH 31. ANS 1 N
               A(L,4)
0̄9608       DO 31 I1 = 1,3
0̄9620       DIAG = A(I1, I1)
0̄9704       DO 32 J = I1,4
0̄9716    32 A(11,J) = A(11,J)/DIAG
0̄9908       K1 = 1
0̄9932    33 IF(K1 - I1)34,35,34
1̄0000    34 FELMT = A(K1,I1)
1̄0084       DO 36 J = I1,4
```

```
1̄0096     36 A(K1,J) = A(K1,J) − FELMT* A(I1, J)
1̄0372     35 K1 = K1 + 1
1̄0408        IF(K1 − 3) 33,33,31
1̄0476     31 CONTINUE
1̄0512 C      COMPUTE MAX. VALUE OF PARABOLA.
               SCALE CORDG(I) TO 100.0 MAX.
1̄0512 C      AND CONVERT WAVL(I) TO UNITS OF FRE-
               QUENCY (RECIPROCAL CM.)
1̄0512         K = I − 1
1̄0548         FREQ = −0.5*A(3,4)/A(2,4)
1̄0608         BIGRD = A(1,4) − 0.25 * A(3,4) * A(3,4)/A(2,4)
1̄0692         CORR = 100.0/BIGRD
1̄0728         DO 25 I = 1,K
1̄0740         CORDG(I) = CORDG(I) * CORR
1̄0824     25 WAVL(I) = 1000./WAVL(I)
1̄0944 C      INITIALIZE PLOTF (GIVE IT PROBLEM NO
               AND (−) SIGN TO INIALIZE CARD
1̄0944 C      NUMBER AND CAUSE FIRST LINE LEGEND
               TO BE PUNCHED).
1̄0944         YRDG = −PROBN
1̄0980         YRDG = PLOTF (FREQ)
1̄1004 C      PICK INIT. FREQ FOR PLOT, AS N*0.02
               JUST EQ. OR MORE THAN WAVL(1).
1̄1004         FREQ = WAVL(1)*50.0
1̄1040         I = FREQ
1̄1076         FREQ = I
1̄1112         FREQ = FREQ*0.02
1̄1148         IF(WAVL(1) − FREQ − 0.01)60,61,61
1̄1228     61 FREQ = FREQ + 0.02
1̄1264 C      FIND 4 BRACKETING VALUES OF WAVL(I).
               PUT THEM, + CORRESPONDING VALUES
1̄1264 C      OF CORDG(I) INTO X AND CRDG AR-
               RAYS USED BY INTERPOLATION R OUT-
               LINE.
1̄1264     60 I = 0
1̄1288     50 I = I + 1
1̄1324        IF(WAVL(I) − 999.0)51,99,99
1̄1416     51 IF(WAVL(I) − FREQ)52,63,50
1̄1508     63 YRDG = CORDG(I)
1̄1556        GO TO 74
1̄1564     52 IF(1 − 3)65,65,68
1̄1632     65 DO 67 J = 1,4
1̄1644        X(J) = WAVL(J)
1̄1716     67 CRDG(J) = CORDG(J)
1̄1824 C      GO TO INTERPOLATION. RETURN TO 94.
1̄1824        GO TO 75
1̄1832     68 IF(I + 1 − K)80,81,81
1̄1912     81 DO 82 J = 1,4
1̄1924        J1 = K − 4 + J
1̄1972        X(J) = WAVL(J1)
1̄2044     82 CRDG(J) = CORDG(J1)
1̄2152        GO TO 75
```

```
1̄2160     80 DO 83 J = 1,4
1̄2172        J1 = I − 3 + J
1̄2220        X(J) = WAVL(J1)
1̄2292     83 CRDG(J) = CORDG(J1)
1̄2400 C      4-POINT LAGRANGIAN INTERPOLATION.
             ARGUMENT IS FREQ, X, CRDG. ANS, YRDG
1̄2400     75 YRDG = 0.0
1̄2424        DO 70 N = 1,4
1̄2436     70 D(N) = FREQ − X(N)
1̄2556        PN = D(1)*D(2)*D(3)*D(4)
1̄2616        DO 71 N = 1,4
1̄2628        DO 72 J = 1,4
1̄2640     72 D(J) = X(N) − X(J)
1̄2784        D(N) = 1.0
1̄2832        PI = D(1)*D(2)*D(3)*D(4)*(FREQ − X(N))
1̄2964        IF(P1)71,73,71
1̄3020     73 YRDG = CRDG(N)
1̄3068        GO TO 74
1̄3076     71 YRDG = YRDG + CRDG(N)*PN/PI
1̄3196 C      END OF INTERPOLATION. ALTERNATE
               EXIT BY COMPUTED GO TO.
1̄3196     74 GO TO (93,94), KIF
1̄3272 C      SOME ANSWERS ARE SLIGHTLY NEGA-
               TIVE. MAKE THEM 0.0
1̄327      94 IF(YRDG) 95,96,96
1̄3328     95 YRDG = 0.0
1̄3352 C      ACCUMULATE AREA UNDER CURVE.
1̄3352     96 SUMC = SUMC + YRDG
1̄3388 C      PLOT THE VALUE OF YRDG VS. FREQ.
1̄3388        YRDG = YRDG
1̄3412        YRDG = PLOTF (FREQ)
1̄3436 C      DECREMENT FREQ. AND RETURN TO 51
               TO PLOT NEXT POINT.
1̄3436        FREQ = FREQ − 0.01
1̄3472        GO TO 51
1̄3480 C      SCALE PROBLEM NO. FOR PLOTTING SUB-
               ROUTINE
1̄3480     99 PROBN = PROBN*0.01
1̄3516 C      GIVE PLOTF SIGNAL (−999.0) TO PUNCH
               FINAL LEGEND AND PROB. NO.
1̄3516        YRDG = −999.0
1̄3552        YRDG = PLOTF (PROBN)
1̄3576 C      PUNCH THE SCALE FACTOR (BIGRD) AND
               AREA (SUMC)
1̄3576        PUNCH 7, BIGRD, SUMC
1̄3612 C      RETURN TO START ANOTHER PROBLEM.
1̄3612        GO TO 100
1̄3620     10 FORMAT (F10.0,F10.1,F10.2)
1̄3652     11 FORMAT (4XF5.2)
1̄3690      7 FORMAT (1H2, 19X, E15.8, E15.8)
1̄3772        END
```

the quantum efficiency of one of the compounds is accurately known, the value for the second compound may be readily calculated.

$$\phi_2 = \phi_1 \cdot \frac{F_2^Q}{F_1^Q} \cdot \frac{A_1}{A_2} \cdot \frac{E_1^Q}{E_2^Q} \qquad (10)$$

Although it is generally held that ϕ is independent of the wavelength of exciting radiation (*10*), Equation 10 permits the use of selected wavelengths for maximum excitation provided that the spectral energy distribution of the excitation monochromator is determined.

Quantum efficiencies for several compounds were calculated by making use of the correction data, the computer program, and the above equations. Quinine sulfate was used as the reference compound throughout. Results are presented in Table I. The values for anthracene and Rhodamine B agree very well with the literature values. The low efficiency for phenol might be a reflection of the effect of pH which determines the decrease in phenol concentration as a result of conversion to the nonfluorescent phenolate ion. However, a more significant factor is self-absorption which is relatively large for anthracene, Rhodamine B, phenol, and indole. As seen in Table I, the values for anthracene, uncorrected for self-absorption, reported by Melhuish (*4*, *6*) agree almost identically with the determined value. Similarly, correction for self-absorption in the case of the other compounds, may have yielded higher values. Fortunately, the quinine sulfate fluorescence spectrum does not overlap its absorption spectrum so that errors introduced by failure to correct for self-absorption are negligible. The extent of overlap of the emission and absorption spectra is an important point in the choice of a quantum efficiency reference standard.

CONCLUSIONS

The purpose of this paper was to present a convenient approach to calibration of luminescence equipment and to show how modern computers can be used for correction of the spectra used in the calculation of quantum efficiencies. Calibration data reported in this paper should not be applied to other instruments. Even though two instruments may be of the same manufacturer and model, the components (source lamp, phototube, electronics, etc.) may not function identically. For this reason, each separate instrument should be calibrated and if a component is changed it should be recalibrated.

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Determination of Propylene in Ethylene-Propylene Copolymers by Infrared Spectrometry

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▶ Pyrolysis of ethylene-propylene copolymers produces derivatives that are rich in unsaturated carbon-carbon functional groups. This unsaturation exhibits strong absorption in the region of infrared light. The ratio of the absorption of the vinyl groups to that of the vinylidene groups varies with the mole fraction of propylene in ethylene-propylene copolymers. Making use of this ratio, an analytical method has been developed for determining propylene in both raw and vulcanized ethylene-propylene copolymers.

Polymers prepared by random copolymerization of ethylene and propylene with Zeigler or Natta catalysts have a wide range of commercial and practical applications (*1*, *7*). To achieve the elastic quality of rubber, the propylene content must be controlled within certain limits (*1*, *13*, *14*, *18*). The work presented in this paper deals with methods for determining the mole fraction of propplene in both raw and vulcanized copolymers.

Several methods have been reported for determining propylene in raw ethylene-propylene copolymers. The basis for calibration of many of these methods is the work published by Natta (*15*), which involves measuring the infrared absorption of the polymer in CCl_4 solutions. The absorption at 7.25 microns, presumably due to methyl vibrations, is related to the propylene concentration in the copolymer. In some cases the dissolution of copolymers with low propylene content or some particular structures is difficult (*5*, *6*). Moreover, Natta's solution method was calibrated against his radiochemical method (*15*), for which the precision of the method was not stated; a considerable amount of scatter is evident in the data presented.

Typical methods that have used Natta's solution procedure (*15*) for calibration are described in publications by Wei (*21*) and Gössl (*8*). These infrared methods avoid the solution problems by employing intensity measurements made on pressed films. The ratio of the absorption at 13.9 microns to that at 8.7 microns is related to the propylene content of the copolymer. Some objections (*3*, *16*) to the use of solid films have been raised because of the effect of crystallinity on the absorption spectra in copolymers with low propylene content. These film methods are reliable only over the range of 30 to 50 mole % propylene.

Bau and Manaresi (*2*) also calibrated a mass spectrometric method by Natta's solution method (*15*). The propylene concentration was reported to be related to a certain ratio of mass peaks measured in the mass spectra of the pyrolysis products of the copolymers. An approximate calibration could be established with homopolymer mixtures of polyethylene and polypropylene.

Drushel and Iddings (*5*, *6*) reported a method calibrated against C^{14}-labeled copolymers. They found that the ratio of absorbances of two bands in the C—H stretching region or two bands in the C—H wagging region could be used to measure the propylene content of pressed films.

In 1953 Harms (*9*) and Kruse and Wallace (*10*) pointed out that most polymers will depolymerize in a rather characteristic fashion on heating, yielding products representative of the polymer. This technique has been used repeatedly as a means for qualitative analysis by infrared spectrometry.

The formation of characteristic pyrolyzates served as the basis for the method presented here. Cross (*4*) indicated that thermal degradation of linear aliphatic polymers produces terminal unsaturated functional groups of the type $RCH{=}CH_2$ and that branched polymers give products consisting mainly of $RR'C{=}CH_2$. In addition, he showed that different types of carbon-carbon double bonds have specific absorption in the infrared region from 1000 cm.$^{-1}$ to that just beyond 700 cm.$^{-1}$

Copolymers of ethylene and propylene are very nearly saturated (*17*). However, on pyrolysis they yield volatile products that are rich in two specific types of unsaturation, namely, vinyl $RCH{=}CH_2$ and vinylidene $RR'C{=}CH_2$ (*20*). The vinyl group absorbs at about 909 cm.$^{-1}$ and the vinylidene at about 889 cm.$^{-1}$ (*4*, *17*). Thermal degradation of diolefin polymers also gives unsaturated products. For example, the absorption measured in a quantitative method for natural rubber determination in natural rubber and SB-R

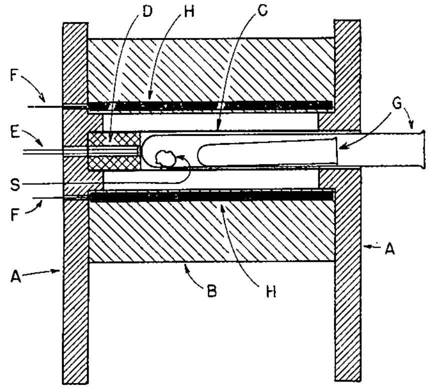

Figure 1. Pyrolysis furnace (20)

A. Transite sheet
B. Furnace from combustion train
C. Stainless steel tube
D. Steel plug with hole for thermocouple
E. Thermocouple
F. Heater leads
G. Test tubes
H. Heater coils
S. Sample
Thermocouple and heater leads connect to an indicator-control device

mixtures (*19*) is probably due to the vinyl groups derived from the butadiene part of the molecule and the vinylidene groups from the methyl branches of the isoprene units.

EXPERIMENTAL

Samples. Five copolymers were used for calibration purposes; they had nominal propylene concentrations of 10, 20, 31, 40, and 50 mole % assigned by the supplier from a materials balance based on the monomers used. Linear polyethylene and isotactic polypropylene (av. mol. wt. = 213,000) served, respectively, as 0 and 100 mole % propylene samples for calibration purposes. The copolymers and the polypropylene were vulcanized according to the recipe given in Table I.

Apparatus. The apparatus used for the pyrolysis is shown in Figure 1 (*19*).

The spectra were scanned using three infrared spectrophotometers, Perkin-Elmer, Models 221, 21, and 137. A simple demountable sodium chloride cell with a 0.025-mm. lead spacer served to contain the liquid pyrolyzate.

Procedure. A sample weighing about 0.25 gram was placed in the pyrolysis vessel, as shown in Figure 1. The mouth of a smaller test tube (10 × 75 mm.) was adjusted to within about 5 cm. of the mouth of the larger test tube (16 × 150 mm.) to aid in directing the distillate along the hot wall of the larger tube and thereby assure maximum contact of the initial pyrolyzate with the hot pyrolysis tube. This technique yielded reproducible pyrolyzates. The assembly was placed in the furnace previously heated to about 450° C. The pyrolyzate condensing at the open end of the test tube was allowed to run into a third test tube. When the pyrolysis was complete (about 3 to 5 minutes), a portion of the pyrolyzate was placed between two NaCl windows having a 0.025-mm. lead spacer to control cell thickness. The cell was then placed in the infrared beam and scanned through the frequency of 950 cm.$^{-1}$ to 850 cm.$^{-1}$ (10.5 microns to 11.8 micons). The instrument controls were set for normal operation with NaCl optics except that the recorder chart speed was increased by a factor of two on the Model 221. These expanded spectra facilitated locating the absorption maxima. The absorbances corrected for background were measured. The ratio, *R*, of the absorbance near 909 cm.$^{-1}$ to that near 889 cm.$^{-1}$ was calculated—i.e., $R = A_{909}$ cm.$^{-1}$/A_{889} cm.$^{-1}$

RESULTS

Figure 2 shows some typical spectra obtained on the pyrolyzates by a Model 221 spectrophotometer in the region of 950 cm.$^{-1}$ to 850 cm.$^{-1}$ The nominal mole % of propylene is indicated above each spectrum. The absorbances of the two bands were corrected for background by drawing a straight line through the points of minimum absorbances—i.e., 950 cm.$^{-1}$ and 850 cm.$^{-1}$

The specimens from each sample were analyzed in a random sequence so that each set contained one specimen from each sample. The values of the ratio, *R*, range from 9.977 to 0.0289, respectively, for 0 to 100 mole % propylene for the raw samples and from 5.440 to 0.0431, respectively, for 10 to 100 mole % propylene for the vulcanized samples. The common logarithm of the ratio, *R*, can be represented by a linear function of the mole % of propylene in the copolymer. The *R* values were multiplied by 100 to avoid negative numbers.

Table I. Vulcanization Recipe[a]

Constituent	Parts by weight
Polymer	100
Phil black O	25
Dicumyl peroxide	2
Sulfur	0.2
Total compound	127.2

[a] Compounds cured 45 minutes at 150° C.

Tables II and III list the results, expressed as common logarithms of 100 *R*, for the raw and the vulcanized samples, respectively. A separate analysis was made for each of these two sets of data, followed by a study of the relationship between the results for raw and vulcanized samples.

ANALYSIS OF RESULTS

A. Precision. For the study of precision it is best to analyze first the data for internal structure, without reference to the propylene values assigned to the samples by their manufacturer. Such an analysis is designed to reveal the reproducibility of the analytical techniques, both within sets and among sets of measurements made either at different times or on different instruments.

The statistical procedure is an extension of the ordinary analysis of variance and involves linear regression techniques (*11, 12*). The data of Tables II and III represent two-way classification—i.e., data arranged according to two criteria

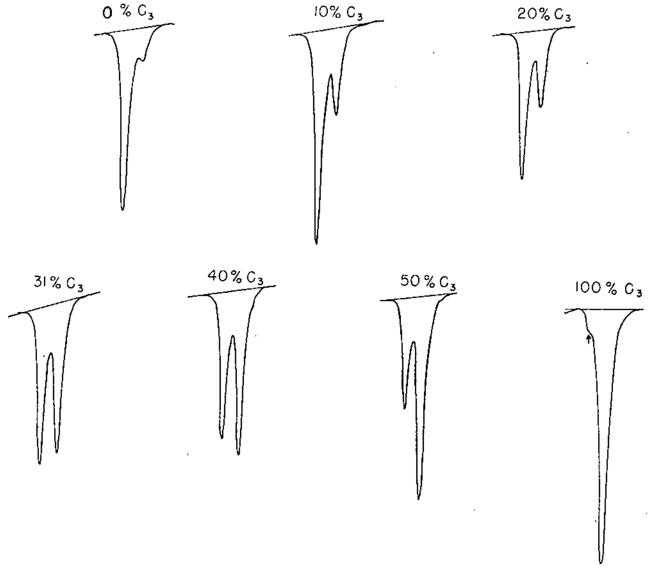

Figure 2. Typical infrared spectra of pyrolyzates obtained from raw ethylene–propylene copolymers

The position of the absorption peaks from left to right are near 909 cm.$^{-1}$ and 889 cm.$^{-1}$, respectively, for each pair and the nominal compositions are indicated in mole % propylene

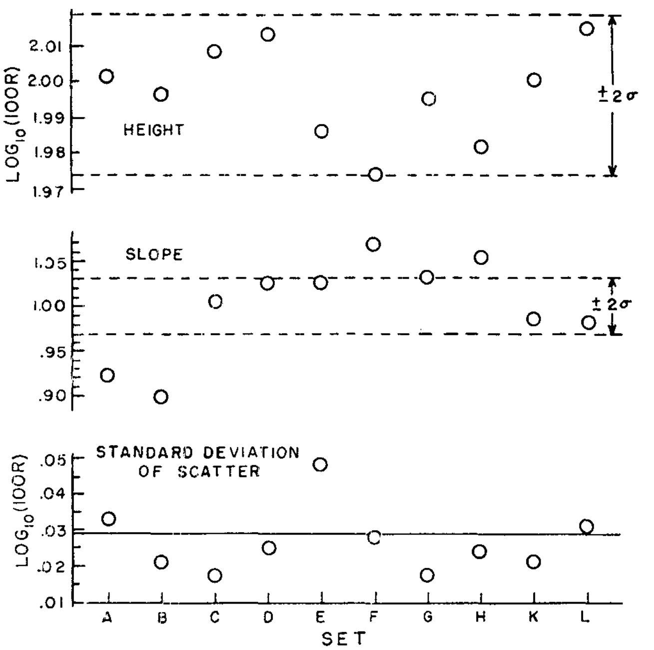

Figure 3. Control chart plot of height, slope, and standard deviation of scatter for the raw samples

The solid line for the standard deviation of scatter data represents the average standard deviation in terms of $\log_{10}(100\ R)$. The dotted line for the slope and height plots represent the 95% confidence interval (±2 σ scatter) for each of these parameters. The set designation is the same as in Table II

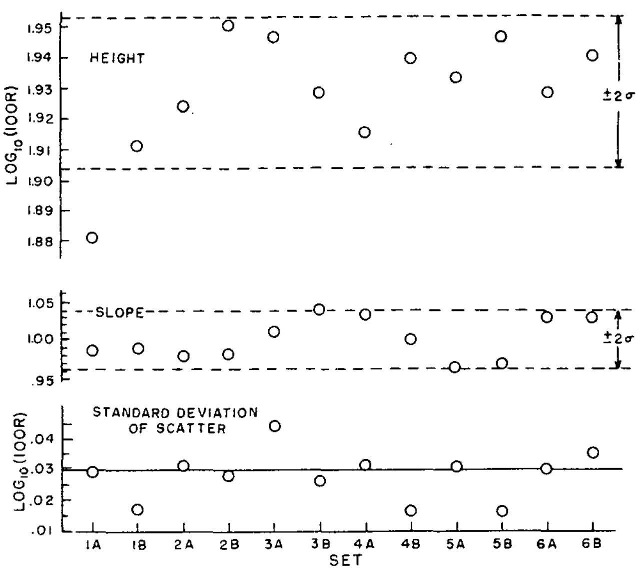

Figure 4. Control chart plot of height, slope, and standard deviation of scatter of the calibration for the vulcanized samples

The solid line for the standard deviation of scatter data represents the average standard deviation in terms of $\log_{10}(100\ R)$. The dotted lines for the slope and height plots represent the 95% confidence interval (±2 σ scatter) for each of these parameters. The set designation is the same as in Table III

Table II. $\text{Log}_{10}(100\ R)$ for Polymer Pyrolyzates (Raw Samples)

Set[a]	$\text{Log}_{10}(100\ R)$ at various propylene concentrations, mole %						
	0	10	20	31	40	50	100
A	2.827	2.584	2.309	2.041	1.931	1.620	0.695
B	2.840	2.525	2.309	2.048	1.896	1.614	0.743
C	2.946	2.604	2.318	2.060	1.940	1.592	0.596
D	2.999	2.587	2.376	2.047	1.908	1.596	0.580
E	2.996	2.552	2.238	2.097	1.886	1.589	0.542
F	2.954	2.568	2.327	2.055	1.896	1.588	0.432
G	2.989	2.562	2.315	2.063	1.902	1.589	0.542
H	2.951	2.578	2.301	2.048	1.916	1.582	0.461
K	2.897	2.567	2.340	2.053	1.933	1.620	0.591
L	2.964	2.561	2.362	2.068	1.904	1.588	0.658
Av.	2.9365	2.5687	2.3193	2.0579	1.9114	1.5979	0.5840

[a] Sets A and B are data obtained on a Model 21, sets C and D on a Model 137, and sets E through L on a Model 221 spectrophotometer.

Table III. $\text{Log}_{10}(100R)$ for Polymer Pyrolyzates (Vulcanized Samples)

Set[a]	$\text{Log}_{10}(100\ R)$ at various propylene concentrations, mole %					
	10	20	31	40	50	100
1A	2.556	2.334	2.125	1.958	1.655	0.659
1B	2.612	2.363	2.136	2.002	1.646	0.705
2A	2.649	2.358	2.168	1.958	1.674	0.742
2B	2.662	2.363	2.170	2.038	1.724	0.744
3A	2.736	2.364	2.144	2.006	1.692	0.733
3B	2.689	2.375	2.143	2.010	1.698	0.652
4A	2.613	2.409	2.153	1.992	1.689	0.635
4B	2.655	2.428	2.140	1.990	1.691	0.729
5A	2.626	2.406	2.120	2.023	1.644	0.780
5B	2.651	2.411	2.130	2.016	1.692	0.779
6A	2.644	2.455	2.114	2.012	1.667	0.678
6B	2.657	2.480	2.136	2.000	1.668	0.697
Av.	2.6458	2.3953	2.1399	2.0002	1.6783	0.7111

[a] The sets marked A are measurements made on a Model 221 Spectrophotometer; the sets marked B are the results obtained on the same samples using a Model 137 Spectrophotometer.

of classification. In this case the criteria are: sample and set of measurements made within a relatively brief time period.

For each of the two tables, averages were calculated for all the results pertaining to any given sample and are shown in the last row of each table. Imagine that the results corresponding to a given set—i.e., a given row in the tables—are plotted against the corresponding average values (last row in Tables II and III). One would expect such a plot to approximate a straight line, passing through the origin, with a slope equal to 45°. Actually, plots obtained in this manner often show systematic departures from this ideal situation. If, for example, a set of measurements made at one time yields consistently higher values than a set carried out at another time, the line corresponding to the first set will be located above that corresponding to the second set. The same situation might occur even more frequently for sets of measurements made on different instruments. The lines corresponding to all the sets would then form a bundle of approximately straight lines, most of which would not pass through the origin. Thus, the lines may have nonzero intercepts. Also, the lines corresponding to different sets vary occasionally in slope, as well as in intercept.

Apart from such systematic differences between sets there is, of course, also a random component of variability, shown by the fact that for any given set the points corresponding to the various samples do not fall exactly on a straight line, but scatter more or less randomly about such a line.

The statistical analysis consistent with this viewpoint yields, for each set, three parameters: the height of the line, the slope of the line, and the measure of scatter about the line. The height of the line is its ordinate at a point representing the grand average of all the values for all samples. The variability of these height values among sets is identical with the familiar main effect of sets in the analysis of variance. The measure of scatter about the line is the familiar standard error of estimate, which is essentially the root-mean-square deviation from the line.

The values of the three parameters are plotted in control-chart fashion in Figures 3 and 4. The charts for height and for slope are provided with two-sigma limits. The interpretation of these lines is based on the fact that if no systematic differences exist among sets, the chance for any one point to fall outside the control limits is only 5%. Thus, if appreciably more than 5% of the points fall outside the control limits, there is evidence that systematic differences exist among sets. The practical importance of this is that in such a case the precision of the method will be poorer on a set-to-set basis than when it is measured within sets. This, in turn, decreases the reliability of determinations involving the use of a calibration line prepared at another time.

The following inferences can be drawn from Figures 3 and 4:

1. The standard deviation of scatter is the same for the raw and for the vulcanized samples. Its average value is 0.0298, in the scale of log (100 R). In order to convert this value to mole % propylene we must first establish the relation between mole % propylene and log (100 R). We will see that this relation is approximately a straight line of slope 0.022. Thus, the standard deviation of scatter, in per cent propylene units, is

Std. dev. =

$$\frac{0.0298}{0.022} = 1.35 \text{ mole \% propylene}$$

For the conditions under which the measurements were made, the precision corresponding to this value is the best that can be obtained by this method.

2. For the raw samples, several points lie outside the control limits for the slope, indicating slight systematic differences among sets. Further calculations show that the contribution of these systematic effects to the over-all variability of the method would be noticeable only at extreme concentrations (close to 0 or 100% propylene).

3. For the vulcanized samples, all but one point lie inside the control limits. The single outlying point is due to Run 1A, in which the measured values tended to be slightly low. Thus, on the whole, the measurements made on the vulcanized samples show little evidence of systematic differences among sets.

B. Calibration. In Figures 5 and 6 the measured values for the raw and the vulcanized samples have been plotted against the values of mole % propylene assigned by the manufacturer. In each case, the measurements are the averages of all the sets—i.e., they are the values shown in the last row of Tables II and III. For both plots straight lines have been fitted to the data by the method of least squares. The equations of these lines are:

For the raw samples:

$$Y_R = 2.759 - 0.02197\ C_3$$

For the vulcanized samples:

$$Y_V = 2.824 - 0.02140\ C_3$$

where C_3 represents mole % propylene (the values assigned by the manufacturer) and Y_R and Y_V represent the measurement log (100 R) for the raw and the vulcanized samples, respectively.

The standard deviations measuring the scatter in these two plots, in units of log (100 R), are:

For the raw samples:

$$s = 0.0407$$

For the vulcanized samples:

$$s = 0.0480$$

Converted to mole % propylene, these values are

$$\frac{0.0407}{0.022} = 1.85 \text{ mole-\% and } \frac{0.0480}{0.022} = 2.18 \text{ mole-\%}$$

Now, each point in the calibration curve is the average of 10 determinations for the raw samples and the average of 12 determinations for the vulcanized samples. From the measure of internal precision, std. dev. = 1.35 mole % propylene, one would expect a scatter in the calibration curves measured by:

Std. dev. =

$$\frac{1.35}{\sqrt{10}} = 0.43 \text{ mole \% propylene}$$

for the raw samples and

Std. dev. =

$$\frac{1.35}{\sqrt{12}} = 0.39 \text{ mole \% propylene}$$

for the vulcanized samples. Actually we obtain 1.85 instead of 0.43 and 2.18 instead of 0.39 as the standard

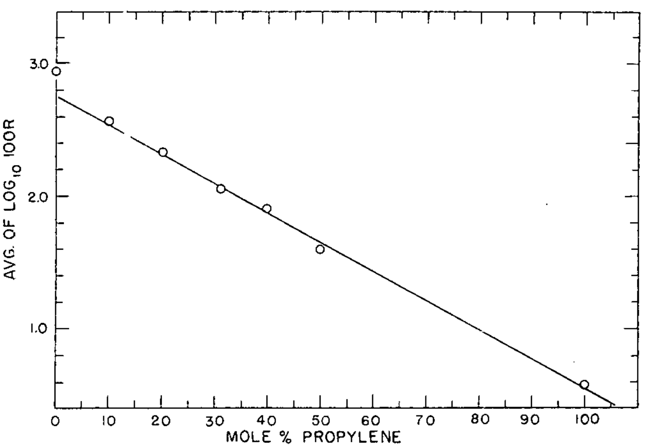

Figure 5. Calibration curve for raw samples

The mole % propylene values are those assigned nominally to samples by the supplier. Each point is the average of 10 determinations. The least square equation for the solid line is:

$$Y_R = \log_{10}(100\ R) = 2.759 - 0.02197\ C_3$$

where C_3 is mole % propylene

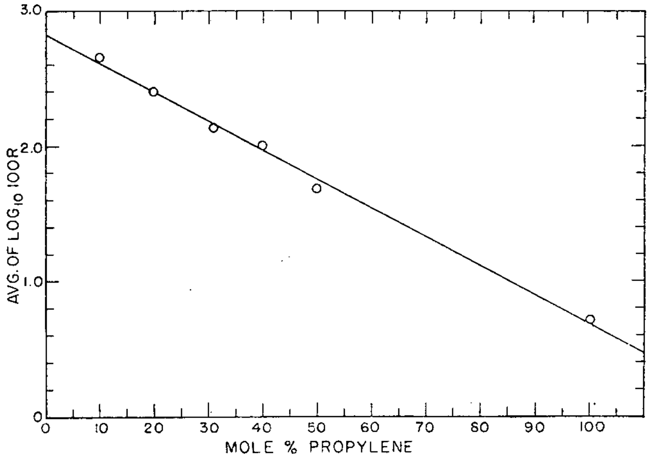

Figure 6. Calibration curve for vulcanized samples

The mole % propylene values are those assigned nominally to the samples by the suppliers. Each point is the average of 12 determinations. The least square equation for the solid line is:

$$Y_V = \log_{10}(100\ R) = 2.824 - 0.02140\ C_3$$

where C_3 is mole % propylene

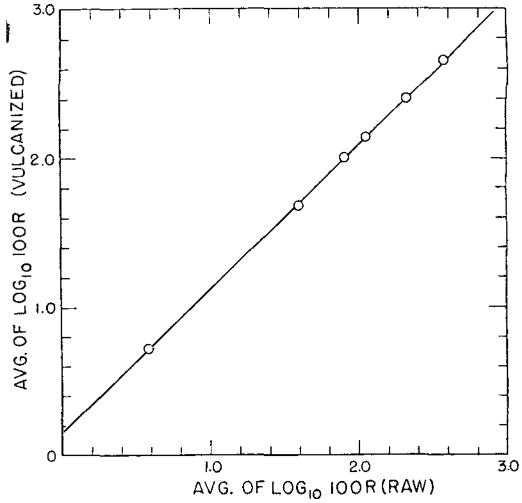

Figure 7. Comparison of results for vulcanized and raw copolymers

The values plotted are the ordinates of Figures 5 and 6. The least-square equation for the solid line is:

$$Y_V = 0.135 + 0.9747\ Y_R$$

deviation of scatter for the calibration curves. The explanation may be found, in part at least, in the observation that points corresponding to the same sample in Figures 5 and 6 show the same pattern of deviation from their respective calibration lines. This suggests the possibility that the assigned values may themselves be in error.

C. Relation between Results for Raw and Vulcanized Samples. Figure 7 is a plot of the measurements on the vulcanized samples *vs.* the corresponding measurements on the raw samples. The straight line shown in this figure was obtained by assuming equal precision for the abscissa and the ordinate. The equation of the line is

$$Y_V = 0.135 + 0.9747\ Y_R$$

The standard deviation of scatter about the line is

$$s = 0.0066$$

This value, converted to per cent propylene units, yields:

$$\text{Std. dev.} = \frac{0.0066}{0.022} = 0.30 \text{ mole \% propylene}$$

This estimate is in agreement with the values 0.43 and 0.39 mole % propylene derived from the precision of the data. This result lends quantitative support to the assumption that the assigned values for the propylene concentrations may be in error.

DISCUSSION

The pyrolysis products collected from the copolymers were mainly liquids and amounted to about 40% of the polymer content of the original sample. However, the polyethylene homopolymer gave a wax solid. The crystalline nature of this pyrolyzate undoubtedly is a major reason for the deviation of R for this sample from the curve obtained using the copolymers and polypropylene. Consequently, the polyethylene R value was not included in calculating the line shown in Figure 5.

The vulcanized samples were pyrolyzed without extractions. Preliminary studies showed no detectable differences between extracted and nonextracted vulcanized samples used in this study. However, in the procedure recommended by Harms (*9*), extraction is suggested for finished commercial samples that may contain volatile plasticizers and/or organic fillers which could yield interfering pyrolysis products.

The random sequence of the analysis within each set ensures that possible drift in the instruments or other causes of gradual or periodic shifts will not introduce systematic errors in the calibration of the procedure. The agreement of the two calibration curves is demonstrated in Figure 7 where the average values of $\log_{10}(100\ R)$ for the raw polymers are plotted against those for the vulcanized polymers. The lower point on the left represents the polymer containing 100 mole % propylene, and the highest point on the right represents the polymer containing 10 mole % propylene. Each of the points falls very nearly on the least-squares straight line because now the assigned concentration uncertainty is avoided. That this line is less than 45° (approximately 44°) is probably due to the vulcanization effectively reducing the availability of propylene units to form vinylidene products. Since the cross-links (*1, 13*) on vulcanization are presumed to occur principally at the tertiary hydrogens in the propylene units, the available propylene concentration would be reduced to a small extent. The deviation is greater at the end representing 100% propylene.

This method is applicable for determining propylene concentration in copolymers that vary over an extremely broad range. To our best knowledge it is the first analytical method that has been suggested for determining propylene concentrations in vulcanized copolymers. The method should be of special usefulness in the analysis of finished materials. Although it is recommended that one should first establish a calibration curve, the equations and method presented here could be used to give fairly accurate estimates of propylene concentrations with double-beam infrared instruments. The formula for calculating the mole % propylene is

$$\%C_3 = K - a \log_{10}(100\ R)$$

where $K = 125.6$ and $a = 45.52$ for the raw samples, and $K = 132.0$ and $a = 46.73$ for the vulcanized samples cured by a recipe similar to the one given in Table I.

ACKNOWLEDGMENT

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Flame Photometric Microdetermination of Boron in Organoboron Compounds

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▶ The flame spectrophotometric method was applied for determination of boron in organoboron compounds to eliminate the ambiguities which are inevitable in determination by other methods. During this investigation, it was observed that boron emission depends upon not only boron content but also the molecular structure or composition of the sample solution. This necessitated the complete decomposition of various boron compounds to boric acid—i.e., the standard substance. The flame spectrophotometric method is characterized by having no ambiguities in determinations and is composed of three steps—decomposition of weighed samples, preparations of standard-added solution and dilute sample solution, and measurement with a flame spectrophotometer. A micro amount of sample (0.5 to 1 mg. as boron) is sufficient for analysis, while the relative error is within 1% of the theory.

MANY analytical methods have been reported for determining boron in organoboron compounds by a technique in which boron is oxidized to boric acid, converted into mannitoboric acid, and titrated as usual. But there remain problems to be solved—for example, danger that a part of the sample may be lost by incomplete digestion or volatilization and troublesome titration in the presence of amine formed during the wet oxidation of compounds containing nitrogen. The purpose of this investigation is to extend the flame photometric method to the determination of boron in organoboron compounds containing nitrogen and to standardize a universal technique which is independent of nitrogen content and involves no ambiguities such as starting and end points of titration.

The concentration-intensity (C-I) curve for boron in boric acid was described as strictly linear up to at least 300 p.p.m. of boron (*3*), and the present author confirmed the linearity up to 500 p.p.m. at 519.5 mμ. When the C-I curve for boric acid was used to convert the flame spectrometer readings into boron concentrations in sample solutions or into boron contents in samples, boron values were too high for most boron-nitrogen compounds, even when analyzed after complete digestion. This was attributed to the effect of amines formed from nitrogen in the compounds, as similar effects of enhancement due to inorganic ions have been reported by Dean and Thompson (*3*).

The mode of interaction between boron emission and amine content was examined with aqueous solutions of boric acid and aniline hydrochloride. In this case, the emission increased continuously but not proportionally with the increment of amine concentration (Figure 1), whereas the C-I curve for a fixed concentration of amine was strikingly linear up to 500 p.p.m. of boron. If this relation between boron emission and amine concentration holds for decomposition products of boron-nitrogen compounds, it apparently indicates that the method of standard addition gives good results. This was the case for some borazine derivatives but not for others, although discrepancies were decreased by adoption of the method of standard addition. Table I presents the results on a number of compounds.

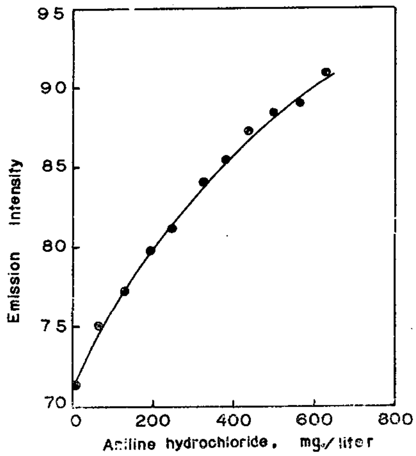

Figure 1. Effect of aniline hydrochloride on boron emission

In boric acid solutions containing 300 p.p.m. of boron

A probable explanation for these discrepancies is that boron emission depends upon the species of atoms or groups attached to the boron atom in the sample solution. If this is true, the use of an oxidizing reagent such as nitric acid may lead to a better agreement, because boron in a sample is gradually converted to boric acid, which is identical to the standard substance. In the experiments with oxidizing acid, a set of values was given as the function of oxidation time for each sample examined. Satisfactory results were obtained after a period of oxidation, the length of which depended upon the species of sample. The procedure was then revised to meet these requirements—i.e., complete digestion.

In preparing the sample solutions, a constant amount of methanol was added to increase the intensity of the boron emission (*3*); methanol is very suitable for this purpose because it is highly miscible with water. Buell (*2*) has recommended cleaner's naphtha and 4-methyl-2-pentanone for this purpose, but as these are insoluble in water they are unsuited for the present investigation. Methanol seems to play an important role also in avoiding the deposition of solid phase during or after the oxidation by dissolving the oxidation products.

EXPERIMENTAL

Apparatus and Instrument Settings. Boron emission was measured with a Type EPU-2A spectrophotometer

Table I. Results by Method of Standard Addition for Boron

Compound	No. of results	Boron found, % Max.	Min.	Av.	Boron theory, %	Av.—theory, %
Phenylboronic anhydride	5	10.89	10.31	10.64	10.41	0.23
B-Trimethyl-*N*-trimethylborazine	2	21.02	21.02	21.02	19.70	1.32
B-Trimethyl-*N*-triphenylborazine	9	10.23	9.67	9.91	9.25	0.66
B-Triethyl-*N*-triphenylborazine	10	8.32	8.00	8.15	8.26	−0.11
B-Tributyl-*N*-triphenylborazine	3	7.10	6.95	7.03	6.80	0.23

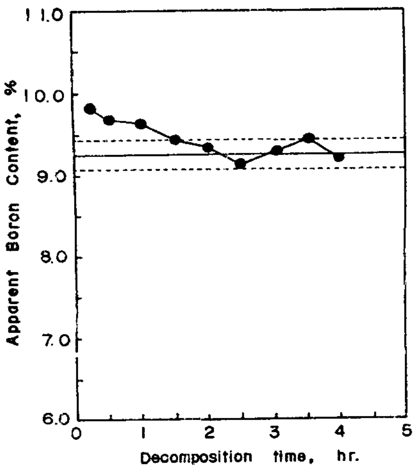

Figure 2. Boron emission of B-trimethyl-N-triphenylborazine as function of decomposition time

Upper and lower dotted lines represent tentatively permitted limits of error (±2% of theory)

with a Type H-2 flame attachment (Hitachi, Ltd., Tokyo), employing oxygen-hydrogen fuel.

The spectrophotometer was adjusted for 300 p.p.m. of boron solution to give a net reading of 80 to 90 in full scale of 110 at 519.5 mμ. Flame height, mirror position, and fuel pressure were adjusted in the usual way (*1*, *5*) to obtain maximum emission.

Slit, mm.	0.035 to 0.045
Oxygen, kg./sq. cm.	1.25
Hydrogen, kg./sq. cm.	0.20

Sample. Samples used in this investigation were synthesized in our laboratory and shown to be of good purity by their physical properties as well as by the determination of carbon, hydrogen, and nitrogen.

Recommended Procedure. A sample containing 0.5 to 1 mg. of boron is weighed into a 4-ml. ampoule, and 0.5 ml. of acetone is added to dissolve the sample. Although the decomposition rate is expected to increase with the solubility in acetone, it is not necessary to dissolve the sample completely. For the sample which is soluble in 17N HNO_3 aqueous solution, the addition of acetone is not necessary. After 1 ml. of 17N HNO_3 aqueous solution is added, the ampoule is sealed and kept in a water bath at 80° C. for a time estimated to be sufficient for complete decomposition, by analogy or by appropriate preliminary work.

After cooling, the ampoule is opened and the content is made up to 2.5 ml. with methanol to give solution I. Two solutions are then prepared by mixing 2 ml. of methanol and 1 ml. of I in each of two 5-ml. measuring flasks. To one flask, 2 ml. of standard solution of boric acid containing 500 p.p.m. of boron and pure water are added to make 5 ml. of solution II. To the other flask is added pure water to make 5 ml. of solution III. A blank solution (IV) is prepared in the same manner as the sample solution. The emissions of solutions II, III, and IV are measured at 519.5 mμ on a spectrophotometer to give the readings of Isa, Is, and Io, respectively, in the following equation, which gives the boron concentration, C, in sample solution III.

$$C = 500 \times Z \times \frac{\text{Isa} - \text{Is}}{\text{Is} - \text{Io}} \text{ (p.p.m.)}$$

where Z is the dilution factor—i.e., 2/5 in this case.

RESULTS

Table II lists the results of determinations of boron in a number of organoboron compounds by this procedure.

Table II. Results for Boron by Recommended Method

Compound	Boron, %		Found − theory	Recovery % of theory	Oxidation time (80° C.), hours
	Found	Theory			
Phenylboronic anhydride	10.45		0.04	100.4	6
	10.21	10.41	−0.20	98.1	5
Trimethylaminoboron trichloride	6.08		−0.06	99.0	2
	6.27	6.14	0.13	102.1	3
Triethylaminoboron trichloride	4.85		−0.10	98.0	2
	4.83	4.95	−0.12	97.6	3
B-Trimethyl-*N*-trimethylborazine	19.45		−0.25	98.7	2
	20.06	19.70	0.36	101.8	2
B-Trimethyl-*N*-triphenylborazine	9.30		0.05	100.5	2
	9.34	9.25	0.09	101.0	2
B-Triethyl-*N*-triphenylborazine	8.05		−0.21	97.5	2
	8.16	8.26	−0.10	98.8	2
B-Triethyl-*N*-tris(*p*-chlorophenyl)-borazine	6.40		−0.14	97.9	4.5
	6.54	6.54	0.00	100.0	4.5
B-Tributyl-*N*-triphenylborazine	6.89		0.09	101.3	2
	6.77	6.80	−0.03	99.6	2
B-Triphenyl-*N*-triphenylborazine	6.21		0.17	102.8–	0.7 (130° C.)
	6.07	6.04	0.03	100.5–	4 (80° C.)
B-Triphenyl-*N*-tris(*p*-*N*,*N*-dimethylaminophenyl)borazine	4.78		−0.09	98.2	3.5
	4.78	4.87	−0.09	98.2	4.5
B-Tris(*p*-*N*,*N*-dimethylaminophenyl)-*N*-triphenylborazine	4.75		−0.12	97.5	3.5
	4.75	4.87	−0.12	97.5	4.5

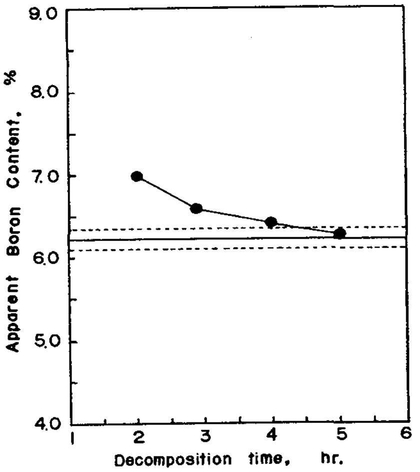

Figure 3. Boron emission of B-trimethyl-N-tris(p-N,N-dimethylaminophenyl) borazine as function of decomposition time

Upper and lower dotted lines represent tentatively permitted limits of error (±2% of theory)

DISCUSSION

Two examples of the dependence of emission on decomposition time are given in Figures 2 and 3, where apparent boron contents in the solid samples are plotted against decomposition time. In this experiment, nitric acid was employed as the oxidizing reagent, and the digestion was carried out in a water bath kept at 80° ± 3° C. Satisfactory results were obtained after a period of oxidation, the length of which depended upon the species of the sample. These preliminary observations led to the above-mentioned recommended procedure.

Most of the samples involved in this investigation could be satisfactorily analyzed by this procedure, but occasionally slight modifications were needed, as described below. When a sample is difficultly soluble in both acetone-acid mixture and 17N HNO_3 at 80° C., it is heated with nitric acid (without acetone) in a sealed ampoule to an elevated temperature—e.g., 130° C.—until all the solid disappears. Then the ampoule is transferred to the water bath at 80° C. to assure complete decomposition.

In a few cases, a rapid depression of emission intensity was observed during the period of measurement, because of vaporization of organic solvent, but this trouble is removed by the use of triethylene glycol dimethyl ether or preferably pure water in place of volatile solvent—i.e., methanol or acetone—in preparation of the sample solutions.

The use of hard glass ampoules is preferable, to avoid an explosion due to the elevated pressure in the ampoule during or after the decomposition. Care must be taken to minimize the loss of solution attached to the cutoff tip of the ampoule on opening. It is desirable to duplicate the determination at different periods of decomposition time to ensure complete decomposition.

Advantages of Method. A small amount of sample containing 0.5 to 1 mg. of boron suffices for analysis—less than in the titrimetric method. The ambiguities in the determination, such as the choice of pH range in the titrimetric method, have been eliminated by converting boron into boric acid, which is identical to the standard substance, so that no calibration is needed against any interfering effect.

In the gravimetric determination of carbon and nitrogen in organoboron compounds low values occasionally were obtained (*4*, *6*), probably because of the formation of boron carbide, boron nitride, or both, in the course of combustion. If boron is determined simultaneously, the value must also be low, because of incomplete digestion. The present method of analysis does not have such handicaps.

While slight modifications are needed in the procedure for preparing sample solutions for a few compounds, the general principle that boron is converted to boric acid is not altered, and naturally the method is applicable to compounds which do not contain nitrogen. The analysis is somewhat time-consuming, because of the comparatively long period for decomposing treatment, but that time can be utilized for other work. The time of manual operation is less than 1 hour for one run.

To exhibit the accuracy and precision of the method, recoveries (per cent of required) are listed in Table II. The mean error is 0.6 (per cent of required) and the relative standard deviation is 0.67% with 22 results in Table II. Thus the method gives satisfactory values for ordinary demands. If a larger amount of sample is available, a higher precision can be obtained by using two or more additions of the standard boron solution.

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Spectrophotometric Determination of Nitrogen in Total Micro-Kjeldahl Digests

Application of Phenol-Hypochlorite Reaction to Microgram Amounts of Ammonia in Total Digest of Biological Material

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▶ Modifications of published methods have permitted the direct estimation of ammonia in total micro-Kjeldahl digests by means of formation of "phenol-indophenol" from ammonia and phenol in the presence of base, sodium hypochlorite, and sodium ferrinitrosopentacyanide ("nitroprusside"). In the reported procedure, 1 to 15 μg. of nitrogen (as ammonia) can be determined. The absorptivity of the colored solutions ranges from 1.1 to 1.4×10^3 cm.-l./gram ($\epsilon =$ ca. $15–20 \times 10^3$) at 630 mμ. Absorptivity is constant with NH_3 concentration from 0.1 μg./ml. to 1.5 μg./ml. Once formed, colored solutions in this range obey the Beer-Lambert law, and those solutions, too dark for direct spectrophotometric observation, may be diluted to 2 to 5 volumes with an appropriate buffer without loss of linearity.

Although there are reports in the literature of the titrimetric or colorimetric determination of nitrogen as ammonia in total micro-Kjeldahl digests (*3*, *8*, *9*), each method has presented some particular difficulty when we have attempted to apply it to biological materials. The problems are principally those of available sample size and a wide variability of nitrogen content. In addition, we required a rapid method (24 hours or less) which did not involve the use of ultramicro equipment and techniques.

Several recent reports (*2*, *4–6*, *11*, *14*, *17*, *19*) have been published in which the formation of "phenol-indophenol" from phenol and ammonia under basic oxidizing conditions has been used to determine ammoniacal nitrogen. The test is extraordinarily sensitive, so that under optimal conditions, amounts of nitrogen (as ammonia) in the microgram range should be determinable. Lubochinsky and Zalta (*11*) described uniquely a system in which the total Kjeldahl digests could be used in this colorimetric procedure. Because we experienced difficulty in repeating this procedure on our Kjeldahl digests, we undertook a more detailed investigation of the color reaction, under the conditions with which we had to contend, namely, the use of a mercuric ion catalyzed Kjeldahl digest, which was adapted from Ogg (*13*) and Steyermark (*18*).

EXPERIMENTAL

Apparatus. A square aluminum block, 8 inches on a side, about 2.5 inches thick, was drilled with 36 evenly spaced holes $^3/_4$ inch in diameter, and 2 inches deep. To the underside of the block was fastened a 1000-watt heating element (Calrod) which was controlled by a variable transformer. Heat loss was cut by surrounding the block with glass wool. A flat pan, filled with washed sand, placed atop a hot plate can also be used, but with less convenience.

Reagents. All water was either distilled or passed over a sulfonic acid ion exchange column.

Zinc dust, low N, finely divided, impalpable powder. Coarser grades (60- to 200-mesh) were not satisfactory.

Sodium Phenolate Reagent. Phenol was distilled from aluminum turnings (*7*) and stored (tightly stoppered) in a refrigerator. Phenol (6.0 grams, 0.059 mole), was placed in a 100-ml. volumetric flask. To it was added about 50 ml. of ice cold water, followed by trisodium phosphate (3.8 grams, 0.01 mole) and sodium hydroxide (2.9 grams, 0.073 mole). The mixture was made up to 100 ml. with water.

Procedure. All standards, samples, and blanks were prepared in triplicate where possible. To ensure parallel treatment of all samples, a single batch of water must be used throughout for washing and dilutions. All glassware must be base rinsed before use.

Samples of homogenates and tissue fractions derived from biological material and, if possible, estimated to contain from 1.0 to 10 μg. of nitrogen (0.07 to 0.7 micromole) were pipetted or weighed into heavy walled "ignition" tubes (18 × 150 mm.). Digestion solution (*18*) (0.50 ml.) was pipetted into each tube, and an alundum or carborundum boiling chip (acid and base washed) was added to each tube. Appropriate reagent blanks and standards were prepared. The tubes were heated to 120° C. in a sand bath or block (as described) until most of the water was driven off. The temperature was then brought as rapidly as possible to 345° to 350° C., and maintained for 1 hour, after which the tubes were removed to a rack to cool. If necessary, additional concentrated sulfuric acid (0.05-ml. volumes) was added with additional heating to complete the digestion.

To each tube was added 1 ml. of water, which was brought to a brief boil over a low flame to wash down the inner walls of the tube. When each tube had again cooled, zinc dust (about 25 to 50 mg.) was added on the tip of a tapered spatula. Immediately after the addition of zinc, each tube was vigorously mixed ("snapped") to break up the "coagulated" mass of zinc dust. Each tube was again heated over a low flame and the contents were boiled vigorously for 20 to 40 seconds. The tubes were allowed to stand at room temperature overnight.

The tubes were then cooled and maintained in a cold water (3° to 5° C.), 0.01% aqueous phenolphthalein (0.1 ml.) was added to each tube, the contents of which were subsequently titrated to a permanent pink with approximately $0.4N$ sodium hydroxide (3.5 to 4.5 ml.). The volume of titrant for each tube was noted, and sufficient water was added to bring the contents of each tube to 6.0 ml.

Into each tube (still in the cold water bath) was pipetted sodium phenolate reagent (1.0 ml.), followed by thorough mixing of the contents of each tube. The pipetting of an aqueous solution of sodium ferrinitrosopentacyanide ($4.2 \times 10^{-4}M$, 1.0 ml.) was also followed by thorough mixing. The final reagent, aqueous sodium hypochlorite solution ($0.015M$, 2.0 ml.), was added in a like manner. The tubes, closed with stoppers, were placed in a covered 40° C. water bath for 30 minutes and were subsequently cooled for about 5 minutes in a cold tap water bath. The precipitates of zinc hydroxide were sedimented by brief centrifugation. The absorbances of the solutions were determined at a wavelength of 630 mμ against a distilled water blank, and the amounts of nitrogen in the unknowns are determined from a calibration curve in the usual way.

RESULTS AND DISCUSSION

Digestion Procedure. Although we did not study the digestion procedure extensively, we did observe low results when either copper or selenium dioxide was used as the catalyst. The relative efficiencies of selenium and mercury have been investigated extensively by Baker (*1*), Steyermark (*18*), and Ogg (*13*), and they have observed the loss of ammonia when selenium was used.

For convenience, we prepared a digestion solution containing sulfuric acid, potassium sulfate, and mercuric sulfate (added as mercuric oxide) and have added in a single aliquot the reagents added separately by Steyermark (*18*). As would be expected, failure to remove the mercuric ion after digestion reduced the absorptivity after color development of the final mixture almost to zero. Neither sodium thiosulfate nor sodium borohydride was as effective in removal of the catalyst as zinc dust, judged by a decreased absorptivity of the final mixture. (Sodium thiosulfate was used as directed by Steyermark (*18*); sodium borohydride was added to the cool diluted digest as 0.1 ml. of 0.4% aqueous solution.)

Because zinc ion is precipitated by base, we investigated its possible interference in the final step of the analytical procedure. Surprisingly, the only difficulty we observed was a result of the effectiveness of zinc ion as a buffer between pH 8 and 9. The indeterminate amount of zinc which enters solution as a result of reaction with the digestion solution required use of an indicator which will permit titration beyond the buffering range of zinc ion but which will not absorb light significantly at 630 mμ. Phenolphthalein and Nile blue A are both effective. The latter, changing from blue to pink, has a somewhat higher pK and, therefore, will allow neutralization to proceed somewhat further than phenolphthalein. Both have been used successfully.

We have not been fully successful so far in decreasing the time required for the removal of mercury. Since mercury metal is attacked by hot $2N$ sulfuric acid (the approximate concentration of the diluted digests), we have added sufficient sodium acetate ($1M$) to neutralize the sulfuric acid before adding zinc. Although the absorptivities of the solution are fairly constant, they are significantly lower than those of solutions which have been allowed to react overnight with zinc.

Color Development. To study the phenol-hypochlorite-ammonia color step by itself, we omitted the digestion step and used 0, 2, 5, and 10 μg. of nitrogen (as ammonium sulfate) to cover a reasonable range of nitrogen concentration. To the standard ammonium sulfate in 7 ml. of aqueous solution was added an appropriate dilution of the reagent under study in 1.0 ml. Except where the other order of reagent addition was purposely changed, the reagents were always added in the order of phenol, ferrinitrosopentacyanide, and hypochlorite. Except when time was the variable, the colors which developed in 60 minutes were estimated at 630 mμ. According to Musso and Beecken (*12*), phenolindophenol in methanolic potassium hydroxide shows four absorption peaks, the largest of which lies at 635 to 640 mμ ($\epsilon = 39.8 \times 10^3$). We have found a very similar maximum (630 to 635 mμ) in aqueous base. (We could not verify the other maxima with our preparation because of interferences by ferrinitrosopentacyanide ion and the large excess of phenol.)

When the phenol concentration was varied between $0.54M$ and $1.8M$, the absorptivity remained constant, but it decreased at concentrations below $0.54M$. The latter figure is equivalent to 5 grams of phenol in 100 ml. of reagent, as described by Lubochinsky and Zalta (*11*); since it is close to the lower limit giving maximal absorptivity, we have increased the concentration to 6 grams per 100 ml. An increase of concentration to $2.7M$ (25 grams/100 ml.) [Russell (*15*)] decreased the absorptivity. Hypochlorite was derived from reagent sodium hypochlorite or Clorox, determined to be 0.754 and $0.777M$, respectively, by iodometric titration. Maximal absorptivity was observed in a concentration range in the reaction

Table I. Absorptivity of Final Solution as Functions of Ferrinitrosopentacyanide Concentration and Time

Development temp. = 40° C.

Ferrinitrosopentacyanide concentration	Absorptivity at stated time (expressed/μg./ml.) minutes					
	10	20	30	60	90	135
$4.2 \times 10^{-4}M$ (1 → 8000)	0.092	0.139	0.146	0.143	0.142	0.141
$5.6 \times 10^{-4}M$ (1 → 6000)	0.102	0.137	0.139	0.138	0.138	0.136
$8.4 \times 10^{-4}M$ (1 → 4000)	0.110	0.135	0.135	0.135	0.134	0.133

mixture of 0.002 to 0.004M. A comparison of Clorox with reagent sodium hypochlorite showed the two to be completely interchangeable.

Varying the concentration of sodium ferrinitropentacyanide in the reaction mixture produced three effects. At the higher concentration ($16.8 \times 10^{-5}M$) the absorbance (and therefore presumably the amount of "phenolindophenol") was smaller than at lower concentration ($4.2 \times 10^{-5}M$). However, the final color was stable for longer time periods at high concentrations than at low, and the final absorptivity was more rapidly attained at the higher concentrations (Table I).

The effect of varying pH (sodium hydroxide concentration) was observed by preparing a phenolate reagent omitting sodium hydroxide (but adding trisodium phosphate). One milliliter was added to each of the standard amounts of nitrogen (as ammonium ion), contained in 1.0 ml. Varying amounts of 0.2N sodium hydroxide were added to the series of tubes; prior to addition of the final two reagents, the volumes of the mixtures were adjusted to 8.0 ml. with water. The results are shown in Table II. There was a definite maximum at pH 11.4 to 11.6. The absorptivity decreased slowly as the pH was increased beyond 11.9. The pH was measured with a type E-2 glass electrode, for which the sodium ion correction was negligible (<0.03).

Optimal Reaction Conditions. Using the optimal reagent concentrations as described above, we have studied the effect of various other factors on the absorptivity. Variation in salt concentration does not appear to have any significant effect on absorptivity. Neither the addition of O to 0.3M potassium sulfate to the final color producing step, nor the addition of chloride or bromide ion (both 0.01M) has a significant effect on the reaction.

The reaction mixtures were prepared on the desk top under ordinary room fluorescent light. The absorptivities of the developed colored solutions after one hour in the dark, one hour on the desk top, and one hour within 5 cm. of two 20-watt daylight fluorescent tubes, were respectively 0.146, 0.133, and 0.135 μg./ml.

Ferrinitrosopentacyanide and phenolate could be interchanged in order of addition with no observed change in absorptivity of the final solution. However, if hypochlorite was added either first or second, the absorptivity was greatly decreased. A delay of 30 minutes after addition of ferrinitrosopentacyanide before addition of hypochlorite had no effect on the final absorptivities. The ferrinitrosopentacyanide solution, itself, was stable at least for 1 hour.

Temperature effects were complex, and an extensive study was beyond the scope of this paper. These effects can be noted: (*a*) when the reactants are warmed to temperatures higher than 20° C.—i.e., low room temperature—before sodium hypochlorite is added, the results are usually erratic, increasing with increasing ambient temperature; (*b*) whether or not the reactants were precooled to 0° to 10° C. during initial mixing, both the rate of color formation and the absorptivity are apparently dependent on the temperature of the reacting mixtures; and (*c*) the stability of the color seems to be dependent on the prior treatment of the reaction mixture. Careful precooling followed by color development at a temperature of 30° to 50° C. seems to enhance the stability of the color. Heating the reactants to temperatures greater than 40° C. leads to greater absorbances but decreased stability. Riley (*14*) has made a similar observation using manganous ion as the catalyst. He decreased the reaction temperature called for by Russell (*15*) from 100° to 70° C. and increased the total time of heating from 10 to 45 minutes. The absorptivity was lower (than at 100° C.) but the stability of the colored product was greater. We noted an anomalous effect at heating to temperatures of 70° C. and greater. This was the fading of the color at high temperatures and its gradual reappearance as the solutions were cooled to room temperature. However, the rate of color reappearance seemed to be temperature controlled, since quick chilling to 0° to 3° C. interfered with maximal color development.

Table II. Absorptivity as Function of pH

pH	Observed absorbance, av. 0 μg.	 5 μg.
10.2	0.064	0.210
10.6	0.171	0.588
10.8	0.254	0.872
11.2	0.232	0.920
11.4	0.269	1.04
11.6	0.276	1.06
11.95	0.269	1.00

Table III. Calibration Curve of Nitrogen (as Ammonium Ion) Using Recommended Method

μg. N taken	λ 630 mμ absorbance (*vs.* H_2O)	Mean
0	0.540, 0.529, 0.495	0.519
1.0	0.612, 0.610, 0.620	0.614
2.0	0.690, 0.730, 0.710	0.710
5.0	1.10, 1.11, 1.10	1.10
10.0	1.77, 1.78, 1.76	1.77
15.0	2.37, 2.32, 2.30	2.33

Absorptivity, calculated by a method of least squares: 0.124/μg./ml.

Apparent zero: 0.48.

Linearity of absorbance with nitrogen (as ammonia) concentration.

Table IV. Recovery of Nitrogen Using Recommended Method

μg. N taken	Absorbance (1 cm.)	Mean	Calcd. from data in Table III
4.3 μg. N as RGG (Sample A)	1.08, 1.05, 1.05, 1.05, 1.05	1.06	4.6 μg. N
5 μg. N as NH_4^+ and 4.3 μg. N as RGG (Sample A)	1.68, 1.69, 1.66, 1.71, 1.60	1.67	9.1 μg. N
5.2 μg. N as RGG (Sample B)	1.15, 1.17, 1.10	1.14	5.3 μg. N

Table V. Estimation of Reliability of Recommended Method

A. Repetitive Determination for Statistical Reliability

μg. N taken	Number of replicates	Absorbance range, ($X_{\min} - X_{\max}$)	Absorbance Av., $\bar{x}$	Variance, $(Xi - X)^2/(n-1)$
0	6	0.340–0.422	0.378	9.28×10^{-4}
5.0 μg. N as NH_4^+	6	1.00–1.13	1.06	13.1×10^{-4}
4.3 μg. N as RGG	6	0.990–1.04	1.01	4.17×10^{-4}
5.0 μg. N as NH_4^+ + 4.3 μg. N as RGG	6	1.52–1.71	1.63	4.06×10^{-4}

B. 95% Confidence Limits for Recommended Method

μg. N Taken	Calcd. from absorbance, μg.	"95% Confidence range," μg.[a]
0	0	
5.0	Ref. standard	4.7–5.3
4.3	4.6	4.4–4.8
9.3	9.2	9.0–9.4

[a] Student "T" distribution = 2.57 at 0.95 for 5 d.f.; $\bar{x} \pm t_{0.05}\,\bar{x}$.

The calibration curve is linear between about 0.05 to 1.5 μg. N/ml. of reactant. Below 0.05 μg., the slope increases to about twice that in the useful range. Above 1 μg. N/ml., the absorbance is too great (about 1.6) for direct observation. The reaction mixture was diluted by $^1/_2$ to $^1/_5$ with a diluent prepared by dilution of phenolate reagent (1 volume of reagent plus 9 volumes of water). Fifteen micrograms of nitrogen in 10 ml. can be diluted with this solution 1 to 10 without loss of linearity. Hence the Beer-Lambert law is obeyed in this range. Above 1.5 μg./ml. the absorptivity decreased to another constant value. In the presence of acetone but absence of ferricyanide, Crowther and Large (*5*) observed that the Beer-Lambert law was obeyed in the range of 0.16 to 3.2 μg./ml.

The variability of the blank absorbance when read against water is evident in Tables II, III, and V. Even with the greatest precautions, ammonia or volatile amines cannot be totally excluded from a laboratory in which microanalysis is but one of several functions. The "absolute" blank absorbance under the best possible conditions seems to be of the order of 0.2. Under conditions in which such a blank is observed, adherence to Beer's law is limited to a range between absorbances of 0.3 to about 2.3—i.e., the absorbances of amounts of nitrogen smaller than 0.1 μg./ml. deviate from Beer's law and the apparent blank has too low an absorbance. However, when "background" nitrogen increases the blank absorbance to 0.3 or higher, this deviation from Beer's law disappears. The blank values given in Table III are extreme examples.

The absorptivity within any series of determinations has been constant. However, it has varied from as low as 1.1/μg./ml./cm. to as high as 1.5/μg./ml./cm. ($\epsilon = 15.4$ to 19.6×10^3). Weller (*19*) reports a slightly higher value ($\epsilon = 20.3 \times 10^3$) than the maximum value we have observed.

The color fades with time, but relatively slowly for the first 30 to 60 minutes. Overnight the color fades to a stable value, perhaps 10 to 15% below the maximum.

Table VI. Analytical Values for Two Representative Compounds

Compound	Taken	Found (av. of 3)
"Tris"	5	4.7
	10	9.5
	5 + 5 μg. N as NH_4^+	9.7
EDTA	5	4.5
	10	9.6

Reproducibility and Accuracy of the Complete Procedure. Ammonium sulfate (ACS, reagent grade) was redried to constant weight and used as the reference standard. Disodium dihydrogen ethylene diaminetetraacetate dihydrate (EDTA), 2-amino-2-hydroxymethyl-1, 3-propandiol ("Tris") (recrystallized from aqueous ethanol), and a sample of purified rabbit gamma globulin (RGG) were used as "unknowns."

In Table III are presented values obtained with the reference standard. In Table IV are data for RGG run concurrently with the reference standard of Table III. An estimate of the reliability of this procedure may be derived from the data in Table V. In Table VI are listed values for "Tris" and "EDTA" determined by this procedure.

Bohnstedt (*2*) has also investigated extensively the effect of variation of reagent concentrations and reaction conditions, especially the influence of reaction rate on the final color. While his conditions did not attain the sensitivity of the procedure described in this paper, his data show reagent concentration dependence and concentration optima much as do ours. The actual values do not correspond because we have worked at different levels of absolute concentrations. However, despite the differences, the absorptivities of the final solutions remain remarkably constant and thus adherence to the Beer-Lambert law is not greatly affected by small variations in reagents or heating temperatures within a run.

While the mechanism of the reaction remains to be elucidated, there are reports that a number of metals capable of existing in at least two oxidation states can successfully catalyze the reaction. These include the following ions: manganous (*2, 8, 14, 15*), argentous (*16*), ferrous (*4*), cuprous (*16*), and ferrinitrosopentacyanide (*11*). We have compared the relative efficiencies in our system only of ferrinitrosopentacyanide and manganous ion and have found the former far more efficient. In his report, Weller found the same effect (*19*). A recent report by Crowther and Large (*5*) mentions acetone as being as effective a catalyst as manganous ion. We have found that, using their conditions, ferrinitrosopentacyanide further increases the absorptivity over that produced by acetone alone.

It is of interest to note that the use of oxidants other than sodium hypochlorite has been reported. Kaplin and Datsko (*10*) report the use of sodium hypobromite and Bolleter *et al.* (*4*) report the use of saturated chlorine water under slightly acid conditions (in half saturated boric acid and 0.8% phenol). In a qualitative study of indophenol formation, Soloway and Santoro (*16*) have used a large series of oxidants including several N-halo compounds, persulfate, and others. Stegemann and Loescheke described the use of chloramine-T in a routine procedure (*17*). Its use at acidic pH is followed by addition of base to develop the chromogen. Weller (*19*) has reported that chloramine-T affords a much lower absorptivity than sodium hypochlorite.

Because of the large number of variables in this reaction, the recommended procedure represents only one set of satisfactory conditions. Further study of reactant concentrations and reaction conditions may well lead to greater sensitivity of the process and greater stability of the final color.

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Sensitive New Methods for Autocatalytic Spectrophotometric Determination of Nitrite through Free-Radical Chromogens

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▶ Many of the methods presented here are more sensitive than any described in the literature. A molar absorptivity of 1,270,000 can be obtained in the 1-methyl-2-quinolone azine procedure, while in most other procedures molar absorptivities average over 200,000. The following reagents are compared: 1-methyl-2 quinolone azine, 3-methyl-2-benzothiazolinone azine, glyoxal bis(N,N-diphenylhydrazone), 3-methyl-2-benzothiazolinone picrylhydrazone, phenothiazine, N,N,N′,N′-tetramethyl-4,4′-diaminostilbene, N,N′ - diphenyl-p - phenylenediamine, N,N,N′,N′-tetramethylbenzidine, N,N,N′,N′-tetramethyl - p - phenylenediamine, and N,N,N′ - trimethyl - p - phenylenediamine. The syntheses of some of the reagents are given. Evidence is presented that free radicals are obtained in all the procedures. The advantages and disadvantages of the methods are discussed. Recommendations are given for application of some of the procedures to the analysis of solutions containing dyes, large amounts of sulfite, or minute amounts of nitrite.

A VERY large number of methods are available for the determination of nitrite ion and its precursors (*14*). In more recent work 52 spectrophotometric methods for the determination of nitrite ion have been critically compared (*15*). In this paper some new methods, which are more sensitive than any available in the literature, are introduced and their advantages and disadvantages are discussed.

EXPERIMENTAL

Reagents and Apparatus. Triphenylamine, 2-chlorophenothiazine, and *N,N,N′,N′*-tetramethylbenzidine were obtained from Aldrich Chemical Co., Milwaukee 10, Wis. The latter two compounds and phenoxazine (K and K Laboratories, Jamaica 33, N. Y.) were crystallized from ethylcyclohexane to a constant melting point. Phenothiazine (Laboratory Services, Inc., Cincinnati 9, Ohio) was crystallized from heptane-ethylcyclohexane to a constant melting point of 183–84° C. *N,N,N′* - Trimethyl - *p* - phenylenediamine dihydrochloride and the *N,N,N′,N′*-tetramethyl analog were obtained from Distillation Products, Rochester 3, N. Y., and K and K Laboratories, respectively. Both compounds were crystallized once out of methanol. *N,N′*-Diphenyl-*p*-phenylenediamine was obtained from the United States Rubber Co., Naugatuck, Conn. Colorless crystals were obtained after two crystallizations from ethylcyclohexane.

Phenoselenazine (*2*), m.p. 193–94° C., and 4,4′-bis(dimethylamino)stilbene (*16, 17*), m.p. 253–54° C., cor., were prepared by literature procedures.

Preparation of 1-Methyl-2-quinolone Azine. Add 2.0 ml. (0.04 mole) of 98% hydrazine hydrate to 100 ml. of a warm aqueous solution of 7.62 grams (0.02 mole) of 2-iodo-1-methylquinolinium methosulfate. [This salt was prepared by warming equivalent amounts of 2-iodoquinoline (Distillation Products) and methyl sulfate at about 80° C. until the liquid solidified (~30 minutes).] Stir for approximately 1 hour. Filter the red-brown precipitate and crystallize from ethylcyclohexane-benzene (2 to 1). The yield is $1^1/_4$ grams (40%) of glistening red plates, m.p. 257–58° C. cor. Lit. m.p. 257°–58° C. (*3*).

Preparation of 3-Methyl-2-benzothiazolinone Azine. Add 2.75 ml. (0.054 mole) of 98% hydrazine hydrate slowly to a hot stirred solution of 36.7 grams (0.1 mole) of 3-methyl-2-methylthiobenzothiazolium *p*-toluenesulfonate (*13*) in 60 ml. of water. Boil the mixture for approximately 5 minutes. Add 28% ammonium hydroxide until the mixture becomes alkaline and then allow the precipitated material to cool. Filter, wash with water, and then crystallize from dimethylformamide. For additional product, add water to the boiling dimethylformamide solution till definite turbidity is obtained. Total yield is 8.6 grams (53%) of pale yellow plates, m.p. 260° C. cor. Lit. m.p. 230° C. (*11*).

Calculated for $C_{16}H_{14}N_4S_2$: C, 58.9; H, 4.30; N, 17.2; S, 19.6. Found: C, 58.9; H, 4.37; N, 17.1; S, 19.6.

Preparation of 3-Methyl-2-benzothiazolinone Picrylhydrazone. Add a solution of 8.4 grams (0.034 mole) of picryl chloride in 95% ethanol to 50 ml. of a hot aqueous solution of 6.45 grams (0.03 mole) of 3-methyl-2-benzothiazolinone hydrazone hydrochloride. Stir the mixture 30 minutes. Crystallize the precipitate from xylene. Eight and eight-tenths grams (75% yield) of glistening black needles, m.p. 216–18° cor., is obtained.

Calculated for $C_{14}H_{10}N_6O_6S$: C, 43.1; H, 2.56; N, 21.5; S, 8.21. Found: C, 43.3; H, 2.74; N, 21.3; S, 8.30.

Preparation of Glyoxal Bis-(1,1-diphenylhydrazone). Add 2 ml. (0.01 mole) of 30% aqueous glyoxal to a hot stirred solution of 4.44 grams (0.02 mole) of 1,1-diphenylhydrazine hydrochloride in 25 ml. of 95% ethanol. During addition keep temperature below 65° C. Extract the collected precipitate with hot 2-methoxyethanol, add charcoal, and filter hot. Add excess water to the filtrate. Refilter and wash residue with aqueous 2-methoxyethanol (1 to 1). Crystallization from heptane-ethylcyclohexane (1 to 1) gives 2 grams (51% yield) of colorless needles, m.p. 202–03° C. cor. Lit. m.p. 207° C. (*19*).

Detection of Nitrite. To 9 drops of reagent solution on a spot plate add 1 drop of aqueous test solution. In the presence of nitrite the following reagents give a blue color: *N,N′*-diphenyl-*p*-phenylenediamine (0.1% in acetic acid containing 20% perchloric acid), 3-methyl-2-benzothiazolinone azine (0.01% in acetic acid containing 10% propionic acid), and *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine dihydrochloride (0.1% in acetic acid). With 3-methyl-2-benzothiazolinone 1-methyl-2-quinolone azine a purple color is obtained after a 5-minute standing period. The detection limit for nitrite with the first-named compound was 0.02 μg.; with the other reagents it was 0.009 μg. Reaction of a drop of reagent solution with a drop of test solution on filter paper increased detection limits by about a factor of 10. The blanks were all colorless.

1-Methyl-2-quinolone Azine Procedures. PROCEDURE A. Dilute 1 ml. of aqueous test solution to 10 ml. with an acetic acid solution containing 0.1% azine. Read at exactly 1 hour at λ_{max} 520 mμ (Figure 1).

Alternatively, 1 hour after the reagent solution had been added, add 1 ml. of 0.1% sulfamic acid (w./v.) in acetic acid containing 25% water (v./v.). The color intensity is stable for at least 1 hour. Read absorbance at λ_{max} 520 mμ within this time.

PROCEDURE B. Add 5 ml. of an aqueous 0.1*N* sodium hydroxide test solution to 5 ml. of 0.1% reagent in acetic acid.

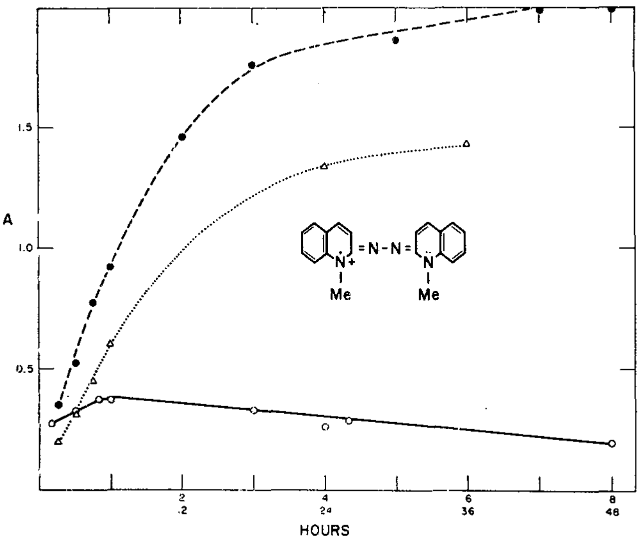


Figure 1. 1-Methyl-2-quinolone azine Procedure A

Change in absorbance at λ_{max} 520 mμ with time after mixing of reagent and test solution

——— 0.23 μg. NO_2^-, 48-hour study
– – – – 0.69 μg. NO_2^-, 8-hour study
. . . . . . 0.345 μg. NO_2^-, 6-hour study

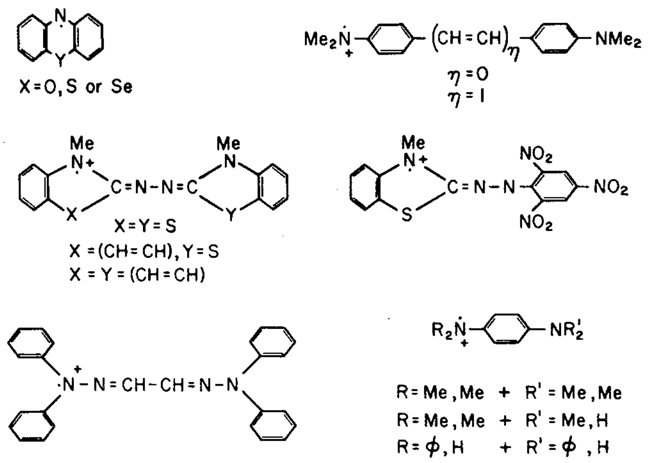


Figure 2. Postulated chromogen structures

In 60 minutes read absorbance at λ_{max} 520 mμ. The spectrophotometric constants obtained in the determination of nitrite with different reagents are compared in Table I.

N,N,N′,N′ - **Tetramethyl - 4,4′-diaminostilbene.** Dilute 1 ml. of aqueous test solution to 10 ml. with a reagent solution containing 0.05% of the stilbene derivative and 20% of 70% aqueous perchloric acid (v./v.) in glacial acetic acid. Read absorbance 65 minutes later at the wavelength maximum.

Phenothiazine. Dilute 1 ml. of aqueous test solution to 10 ml. with 0.1% phenothiazine in distilled glacial acetic acid. Read absorbance exactly 1 hour later at λ_{max} 518 mμ. Phenoxazine, 2-chlorophenothiazine, and phenoselenazine can be substituted for phenothiazine.

N,N′ - **Diphenyl** - *p* - **phenylenediamine.** PROCEDURE A. Dilute 1 ml. of aqueous test solution to 10 ml.

Table I. Comparison of Spectrophotometric Methods for Determination of Nitrite

Reagent	λ_{max}, mμ	Range (A = 0.1 to 2.0), $\epsilon \times 10^{-4}$	Rel. std. dev.	Dil. factor[a]	Sensitivity[b]	Determ. limit,[c] μg.	Proc. time, min.
1-Methyl-2-quinolone azine							
A	520	33–127	4.6	10	33–127	0.14	62
B	520	38–76	2.0	2	190–380	0.12	62
N,N,N′,N′-Tetramethyl-4,4′-diaminostilbene	514	38–72	6.4	10	38–72	0.12	67
Phenoselenazine	517	18–47	5.1	10	18–47	0.26	62
N,N′-Diphenyl-*p*-phenylenediamine							
A	720	9.2–46	11	10	9.2–46	0.50	62
B	705	~15–51	6.3	2	77–255	~0.3	62
N,N,N′,N′-Tetramethyl-*p*-phenylenediamine							
A	614	10–20	5.4	10	10–20	0.45	32
B	614	15–38	7.6	10	15–38	0.30	32
C	612	8.1~16	7.0	1.11	73–144	0.30	22
3-Methyl-2-benzothiazolinone azine	735	13–52	6.5	10	13–52	0.35	62
Glyoxal bis(*N,N*-diphenylhydrazone)	540	10–37	5.5	10	10–37	0.44	7
	1152	7.1–36	6.7	10	7.1–36	0.65	7
Phenothiazine							
A	518	8.4–39	9.5	10	8.4–39	0.55	62
3-Methyl-2-benzothiazolinone picrylhydrazone	406	7.7–32	4.5	10	7.7–32	0.60	32
N,N,N′,N′-Tetramethylbenzidine							
A	473	4.9–20	3.7	10	4.9–20	0.94	2
A	1018	4.9–20	11	10	4.9–20	0.94	2
B	472	7.2–10	3.0	2	36–50	0.64	2
N,N,N′-Trimethyl-*p*-phenylenediamine							
A	578	0.98–1.7	2.7	10	0.98–1.7	4.7	2
B	578	0.77–1.4	4.7	1.11	7–12.7	6.0	2

[a] Essentially final volume/test solution volume.

[b] Sens. $= \frac{\epsilon \times 10^{-3}}{\text{dilution factor}}$.

[c] Total micrograms of nitrite ion in test solution giving absorbance of 0.1 in 1-cm. cell.

with a reagent solution containing 0.05% of the diamine and 20% of 70% aqueous perchloric acid (v./v.) in glacial acetic acid. Read absorbance 60 minutes later at λ_{max} 386 or 720 mμ.

PROCEDURE B. To 5 ml. of aqueous 0.1*N* sodium hydroxide test solution add 5 ml. of an acetic acid solution containing 0.05% of the diamine (w./v.) and 20% of 70% aqueous perchloric acid (v./v.). Read absorbance 60 minutes later at λ_{max} 705 mμ.

***N,N,N',N'* - Tetramethyl - *p*-phenylenediamine.** PROCEDURE A. Dilute 1 ml. of aqueous test solution to 10 ml. with a solution containing 0.1% of the diamine dihydrochloride in glacial acetic acid. Read absorbance 30 minutes later at λ_{max} 614 mμ. The color intensity is stable for at least 60 minutes.

PROCEDURE B. Use the same procedure with a reagent solution containing 0.1% of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride in glacial acetic acid containing 25% water (v./v.).

PROCEDURE C. To 9 ml. of an aqueous 0.1*N* sodium hydroxide solution add 1 ml. of an acetic acid solution containing 0.2% reagent and 10% concentrated hydrochloric acid (v./v.). Read absorbance 20 minutes later at λ_{max} 612 mμ.

3 - Methyl - 2 - benzothiazolinone Azine. Dilute 1 ml. of aqueous test solution to 10 ml. with a glacial acetic acid solution containing 0.1% azine (w./v.) and 10% propionic acid (v./v.). Read absorbance exactly 1 hour later at λ_{max} 735 mμ.

Glyoxal Bis-*N,N*-diphenylhydrazone. Dilute 1 ml. of aqueous test solution to 10 ml. with a glacial acetic acid solution containing 0.01% reagent and 10% of aqueous 70% perchloric acid. Allow solution to stand 5 minutes; read absorbance at 540 mμ.

3 - Methyl - 2 - benzothiazolinone Picrylhydrazone. Dilute 1 ml. of aqueous test solution to 10 ml. with a glacial acetic acid solution containing 0.01% reagent (w./v.) and 20% of 70% aqueous perchloric acid (v./v.). Allow the solution to stand 30 minutes, then read the absorbance at λ_{max} 405 mμ.

***N,N,N',N'* - Tetramethylbenzidine.** PROCEDURE A. Dilute 1 ml. of the aqueous test solution to 10 ml. with a glacial acetic acid solution containing 0.1% of the benzidine derivative. Read absorbance at 473 or 1018 mμ immediately.

PROCEDURE B. Add 5 ml. of an acetic acid solution containing 1% of the benzidine derivative to 5 ml. of an aqueous 0.1*N* sodium hydroxide test solution. Read immediately at 472 or 1018 mμ.

***N,N,N'* - Trimethyl - *p* - phenylenediamine.** PROCEDURE A. Dilute 1 ml. of aqueous test solution to 10 ml. with 0.2% *N,N,N'*-trimethyl-*p*-phenylenediamine in acetic acid. Read absorbance immediately at λ_{max} 578 mμ.

PROCEDURE B. To 9 ml. of aqueous test solution add 1 ml. of 0.2% *N,N,N'*-trimethyl-*p*-phenylenediamine dihydrochloride in glacial acetic acid. Read absorbance immediately at λ_{max} 578 mμ.

Table II. Spectral Evidence for Free-Radical Structure of Chromogens Obtained in Some of the Procedures

Semiquinone	λ_{max}, mμ (relative intensity or *A*)	
	Lit.	Present work
Phenoxazine	522–532 (strong)[a]	530 (0.64)[b]
Phenothiazine	432–440 (strong)[a] 454–460 (very weak) 472–478 (weak) 492–498 (strong) 510–516 (very strong)	440 (1.33) 465 (0.94) 483 (1.11) 502 (1.48) 518 (2.00)
Phenoselenazine	428–438 (strong)[a] 476–486 (very weak) 505–525 (very strong)	432 (1.3) 487s (1.2)[c] 517 (2.45)
N,N,N'-Trimethyl-*p*-phenylenediamine	500s (~0.70)[d] 536 (1.27) 580 (1.29)	500s (0.84)[e] 537 (1.46) 577 (1.52)
N,N,N',N'-Tetramethyl-*p*-phenylenediamine	525s (~0.8)[f] 565 (1.25) 614 (1.25)	525s (0.86)[g] 565 (1.20) 614 (1.20)
N,N,N',N'-Tetramethylbenzidine		425s
		438
		459
	450,[h] 472[i]	473
	790[h]	800s
	900[j]	895
	1010[i]	1018
N,N'-Diphenyl-*p*-phenylenediamine	390 (2.5)[k] 710 (1.37)	386 (0.48)[l] 720 (0.27)

[a] (*7*).
[b] Also weaker band at 406 mμ and inflection at 500 mμ.
[c] s = shoulder.
[d] (*4, 10*). Concn. $10^{-4}M$.
[e] Weak shoulder.
[f] In H_2O $10^{-4}M$ NO_2^- (*1*).
[g] Procedure with 25% water in reagent solution, 4 × $10^{-6}M$ NO_2^-.
[h] In EPA at 70° K. (*7*).
[i] (*18*).
[j] In EPA at 70° K. (*5*).
[k] In acetic acid $10^{-4}M$ (*8*).
[l] $10^{-6}M$ NO_2^-.

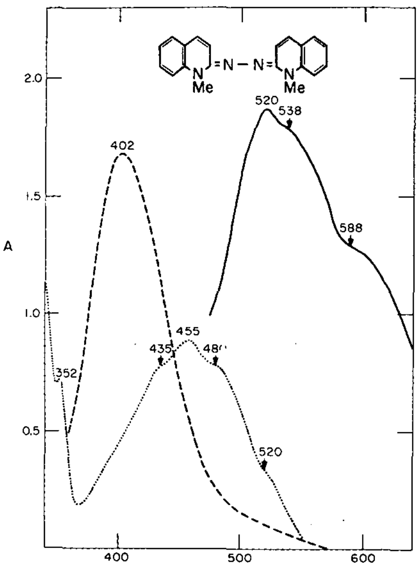


Figure 3. 1-Methyl-2-quinolone azine

Procedure A ——— 0.69 μg. NO_2^-
- - - - - 1 ml. of aqueous solution of 460 μg. NO_2^- diluted to 10 ml. with acetic acid solution of 10^{-4} reagent. Run immediately
. . . . . . 5 × 10^{-5} *M* reagent in ethylcyclohexane or 2-methoxyethanol

MECHANISMS

The structures of the different types of chromogens are shown in Figure 2.

The absorption spectra of the phenoxazine, phenothiazine, and phenoselenazine semiquinone radicals have been reported (*9*). These free radicals were obtained by oxidation of the parent compound with bromine in 80% acetic acid. The absorption spectra were measured with a hand spectroscope. The absorption spectra obtained in the present work in the determination of nitrite with phenoxazine, phenothiazine, and phenoselenazine were closely similar to those reported by the Michaelis group (Table II). Other oxidizing agents gave similar spectral bands but with very much weaker intensities. Even in the attempted preparation of 3*H*-3-phenothiazone from the reaction between phenothiazine and ferric chloride at room temperature, the absorption spectrum of the solution was that of the free radical. When this solution was boiled, the absorption spectrum of 3*H*-phenothiazin-3-one was obtained.

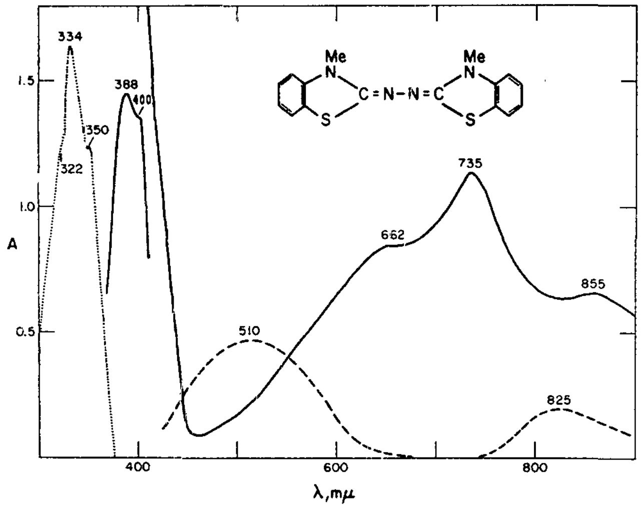


Figure 4. 3-Methyl-2-benzothiazolinone azine

Procedure for nitrite determination ——— 0.92 μg. NO_2^-, λ_{max} 388 mμ, A = 2.45

---- To 1 ml. of aqueous 1% sodium nitrite add 8 ml. of 2 × $10^{-5}M$ reagent in acetic acid followed by 0.5 ml. of 70% aqueous perchloric acid. Read immediately

...... 4 × $10^{-5}M$ reagent in 2-methoxyethanol or acetic acid

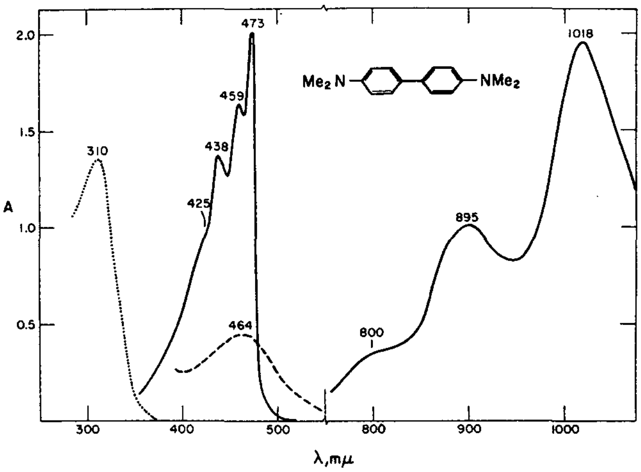


Figure 5. N,N,N′,N′-Tetramethylbenzidine

Procedure A ——— 4.6 μg. NO_2^-

---- Dilute 1 ml. of aqueous solution containing 460 μg. NO_2^- to 10 ml. with $10^{-4}M$ acetic acid solution of reagent

...... 5 × $10^{-5}M$ reagent in 2-methoxyethanol (in acetic acid, λ_{max} 313 mμ, ε = 12,000)

Relatively weaker bands were found at approximately 600, 660, and 750 mμ in the absorption spectra obtained in the determination of nitrite with phenothiazine. Illumination of a solution of phenothiazine in EPA at 90° K. gives a spectrum containing the 532-mμ band of the semiquinone and faint bands at 606 and 654 mμ (*6*). The bands around 400 to 518 mμ are apparently derived from the neutral free radical. The origin of the weak longwavelength bands is uncertain.

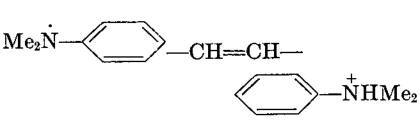
In the references given in Table II evidence has been presented for the free-radical structure of the partially oxidized products (usually obtained by means of bromine or photo-oxidation) of phenoxazine, phenoselenazine, *N,N,N′*-trimethyl-*p*-phenylenediamine, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine, *N,N,N′,N′*-tetramethylbenzidine, and *N,N′*-diphenyl-*p*-phenylenediamine. The reported absorption spectra closely match the spectra obtained when the same reagents are used for the determination of nitrite (Table II).

It is postulated that the remainder of the reagents also form free radicals in the procedures for the determination of nitrite. In all cases small amounts of various types of inorganic oxidizing agents gave exactly the same spectrum as obtained with nitrite.

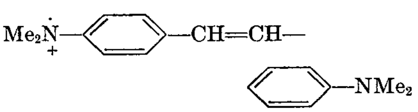
Further evidence for the structure of the chromogens can be deduced from the reaction of five of the reagents with excess nitrite (or bromine) to give an entirely different type of spectrum, which is believed to be derived from dicationic quinonic dyes. For example, under these conditions 1-methyl-2-quinolone azine, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine, 3-methyl-2-benzothiazolinone azine, *N,N,N′,N′*-tetramethylbenzidine, and *N,N′*-diphenyl-*p*-phenylenediamine give bands at 402 (Figure 3), 340, 510, and 825 (Figure 4), 464 (Figure 5), and 510 mμ, respectively. Titration of these solutions with aqueous sodium bisulfite brings back the spectrum of the free radical.

The absorption spectra of the free radicals, many of which are reported for the first time, are very distinctive and reproducible.

With *N,N,N′,N′*-tetramethyl-4,4′-diaminostilbene two types of free-radical spectra are obtained. In acetic-perchloric acid solution a red color, λ_{max} 514 mμ, is obtained, for which the structure



is postulated. This dicationic structure is consistent with the spectra of the reagent in a neutral and acidic solvent (Figure 6). The latter shows a violet shift due to salt formation. In acetic-propionic acid the reaction between nitrite and reagent is characterized by the presence of an initial red color for about 20 seconds, then a blue color (main λ_{max} 600 mμ) for about 20 seconds, followed by decolorization, and then the slow development of a dark green color, λ_{max} 447 and 715 mμ. The latter bands are probably derived from the free radical,



for when enough perchloric acid is added to form a 20% solution, a red color, λ_{max} 514 mμ, is formed with an absorption spectrum closely similar to that of the dicationic free radical.

The phenoxazine and phenoselenazine free-radical spectra obtained in the determination of nitrite resemble that of the phenothiazine free radical (Table II).

The distinctive absorption spectrum of the free radical obtained in the determination of nitrite with glyoxyal bis-(1,1-diphenylhydrazone) is shown in Figure 7. Although the reagent does form a salt in acetic acid–perchloric acid solution (see Figure 7), the free radical is postulated as mainly the simple monocation. The absorption spectra indicate the complexity of this problem, in that the relative intensities of the two bands in the near-infrared region approach unity with a decreasing concentration of nitrite.

The absorption spectrum of 3-methyl-2-benzothiazolinone picrylhydrazone in xylene (λ_{max} 430 mμ) or acetic acid (λ_{max} 425 mμ) closely resembles that of picramide in xylene (λ_{max} 415 mμ). In acetic acid containing 20% perchloric acid (λ_{max} 255, 295 mμ) the absorption spectrum is drastically changed, indicating salt formation. Consequently the spectrum obtained in the nitrite determination (λ_{max} 406 mμ) is probably that of the free-radical cation.

In the determination of nitrite with any of the reagents the formation of the free radical is autocatalytic, as shown by the extremely high molar absorptivities obtained in the procedures. With the type of reagent containing an NH group the mechanism is postulated as taking the course shown in Figure 8. With the other reagents the mechanism

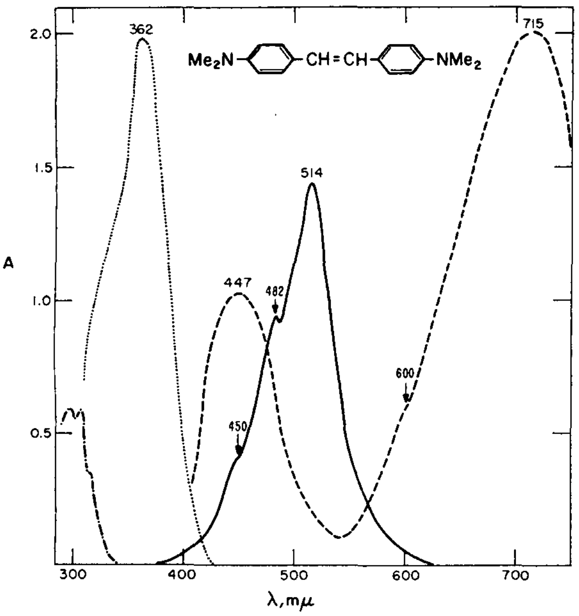


Figure 6. N,N,N′,N′-Tetramethyl-4,4′-diaminostilbene

Recommended procedure ——— 0.92 μg. NO_2^-
- - - - Dilute 1 ml. of aqueous solution of 46 μg. of NO_2^- to 10 ml. with acetic acid solution containing 0.5% reagent and 20% propionic acid (v/v.)
—·—·— 1.88 × $10^{-5}M$ reagent in acetic acid containing 20% of 70% aqueous perchloric acid (v./v.)
...... 5 × $10^{-5}M$ reagent in dioxane

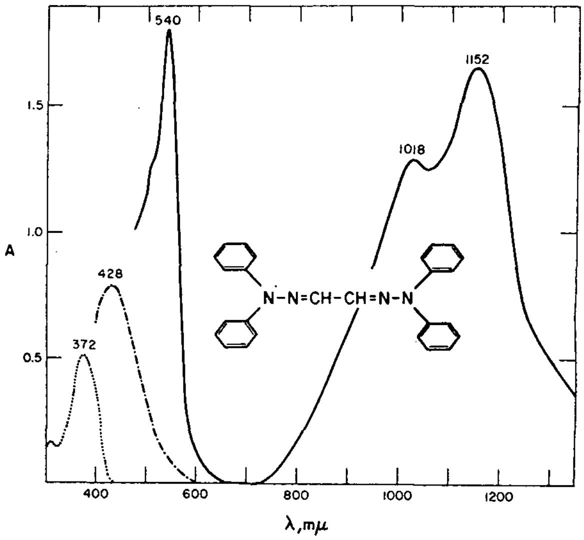


Figure 7. Glyoxal bis(N,N-diphenylhydrazone)

Recommended procedure ——— 2.3 μg. NO_2^-
—·—·— 2.8 × $10^{-5}M$ reagent in acetic acid containing 10% of 70% aqueous perchloric acid (v./v.)
...... $10^{-5}M$ reagent in 2-methoxyethanol

is similar except for the absence of the *N*-nitroso derivative.

EFFECT OF VARIABLES

1-Methyl-2-quinolone Azine. Unless otherwise stated, the following discussion applies to Procedure A. The optimum intensity was obtained with 0.02 to 1% reagent. With 0.1% reagent a better blank and slightly lower intensities were obtained as compared to the 1% reagent. Since the absorbance increased over a fairly long period of time (Figure 1), 1 hour was chosen as a convenient time to make a reading. Figure 1 shows that the sensitivity could be doubled by choosing a longer standing period. If there is a necessity to stabilize the color intensity, sulfamic acid can be added to the solution 1 to 2 hours after mixing (Figure 9).

As in almost all the procedures described in this paper, the slope of the calibration curve decreased with lower concentrations of nitrite. This effect was more drastic in Procedure A. In Procedure B there was a straight-line relation from 0.1 to 1.3 μg. of nitrite. In Figure 3 the absorption spectra of this newly described stable free radical, the starting reagent, and the more completely oxidized quinonic structure are compared.

Nitrates, aldehydes, ketones, alcohols, and hydrocarbons gave negative results. Inorganic oxidizing agents, such as periodate, iodate, permanganate, and chromate, gave positive results in Procedures A and B. An attempt to collect ozone in 0.1*N* sodium hydroxide solution and then analyze by Procedure B gave negative results.

The presence of very large amounts of sulfite interfered with the determination of nitrite. The interference of sulfite was augmented with an increase of water in the final analyzed mixture. In Procedure B, the presence of 50 parts of sulfite to 1 of nitrite decolorized the mixture.

The spectrophotometric constants for all procedures are reported in Table I.

***N*,*N*,*N*′,*N*′-Tetramethyl-4,4′-diaminostilbene.** Optimum intensities were obtained with 0.025 to 0.07% reagent and 20% perchloric acid in the reagent solution. This solution was stable for at least 20 hours. When distilled acetic acid was used to make up the reagent solution, turbidity was found occasionally in the final red solution. Addition of about 1% water to the reagent solution prevented the formation of this turbidity. Once reagent and test solutions were mixed, approximately 60 to 70 minutes were necessary for the development of maximum intensity. After the solution had stood for 24 hours, all peaks between 400 and 800 mμ had disappeared. The concentration-absorbance curve resembled that obtained in the other procedures. The absorption spectra of the reagent and the two types of derived free radicals are shown in Figure 6. In Figure 6 an alternative procedure is given for the determination of nitrite. This method was not explored further.

Exposures of about 10 to 60 minutes to instrumental light at the wavelength maximum accelerated the increase in intensity.

Nitrates, aldehydes, ketones, alcohols, and hydrocarbons gave negative results. Inorganic oxidizing agents gave positive results.

In the presence of sulfite at 400 to 1 (sulfite-to-nitrite ratio), nitrite was determined with no loss in intensity (Figure 10).

Phenothiazine. These procedures were standardized with phenothiazine. The percentage of reagent was not critical in the range of 0.05 to 0.8%. A maximum color intensity was reached at 60 to approximately 105 minutes. The slope of the calibration curve decreased with lower concentrations of nitrite for both the phenothiazine and phenoselenazine methods.

With phenoxazine as the reagent the maximum intensity was reached in 10 minutes; 2.3 μg. of nitrite ion gave a molar absorptivity of 128,000 at λ_{max} 530 mμ. With 2-chlorophenothiazine as the reagent the maximum intensity was reached at 30 to 40 minutes; 2.3 μg. of nitrite ion gave a molar absorptivity of 168,000 at λ_{max} 525 mμ.

Negative results were obtained with sodium nitrate, formaldehyde, potassium iodide, sodium chlorate, and magnesium perchlorate. Ozone gave at most a very weak reaction.

In proportions of at least 2000 to 1,

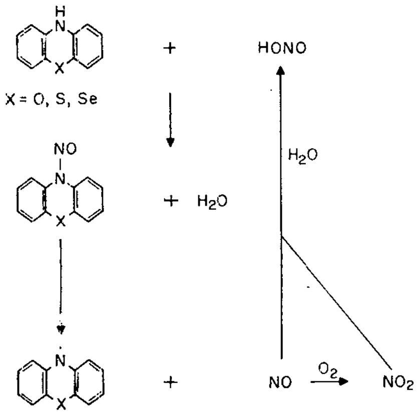


Figure 8. Mechanism for formation of phenothiazine-type free radicals

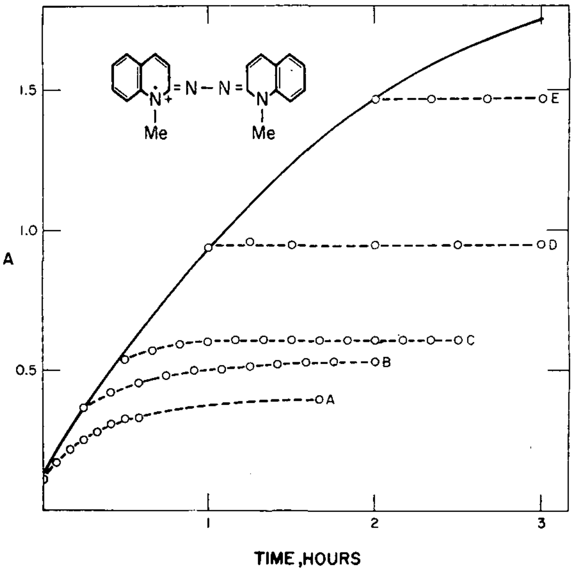


Figure 9. 1-Methyl-2-quinolone azine Procedure A

Change in absorbance with time after mixing in determination of 0.46 μg. NO_2^- at λ_{max} 520 mμ

——— 1 ml. of 0.1% sulfamic acid in 25% aqueous acetic acid (25 ml. of acetic acid diluted to 100 ml. with water) added at:

Minutes after mixing

A.	0	*D.*	60
B.	15	*E.*	120
C.	30		

sulfite had no effect on intensity (Figure 10).

***N,N'* - Diphenyl - *p* - phenylenediamine.** PROCEDURE A. Optimum intensities were obtained from 0.01 to 0.1% reagent, with 0.05% reagent giving slightly higher intensities. The color intensity gradually increased with time and did not reach a maximum in 4 hours. In both procedures the slope of the calibration curve increased with higher concentrations of nitrite (Figure 11). In a procedure similar to Procedure A, except that excess nitrite and a much smaller concentration of reagent are used, a band was obtained at 510 mμ which is probably derived from the quinonic compound.

Nitrates, perchlorates, phenols, alcohols, and hydrocarbon gave negative results. Most inorganic oxidizing agents gave positive results. At 800 to 1, sulfite did not interfere with the determination (Figure 10).

PROCEDURE B. At least 20% of 70% aqueous perchloric acid was required to keep the reagent in solution during the analysis. The highest attainable concentration of reagent was 0.05%. With lower concentrations of reagent,

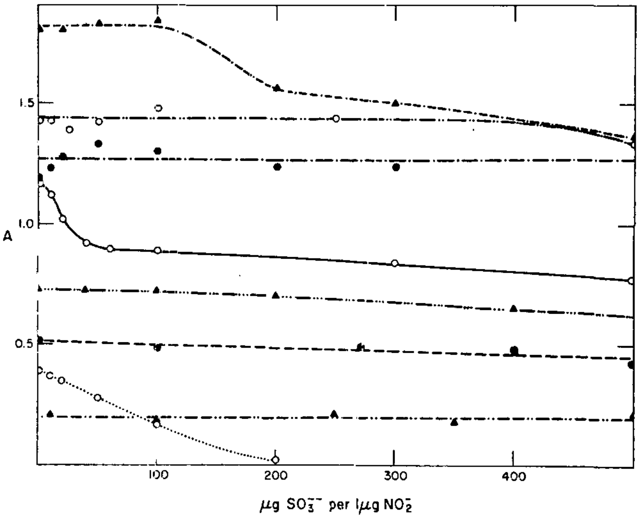


Figure 10. Effect of sulfite on determination of nitrite

—··— 0.92 μg. NO_2^-. *N,N,N',N'*-Tetramethyl-4,4'-diaminostilbene procedure, λ_{max} 514 mμ
—·—· 1.84 μg. NO_2^-. Phenothiazine procedure A, λ_{max} 518 mμ
——— 1.84 μg. NO_2^-. 3-Methyl-2-benzothiazolinone azine procedure, λ_{max} 735 mμ
- - - - 0.72 μg. NO_2^-. *N,N'*-Diphenyl-*p*-phenylenediamine procedure B, λ_{max} 705 mμ;
--·-- 0.50 μg. NO_2^-. Procedure A, λ_{max} 720 mμ
...... 9.25 μg. NO_2^-. Sulfanilic acid plus *N*(1-naphthyl)ethylenediamine procedure (12) λ_{max} 549 mμ
—···— 1.38 μg. NO_2^-. 3-Methyl-2-methylbenzothiazolinone picrylhydrazone λ_{max} 405 mμ
--·---·- 2.3 μg. NO_2^-. Glyoxal bis-(N,N-diphenylhydrazone), λ_{max} 540 mμ

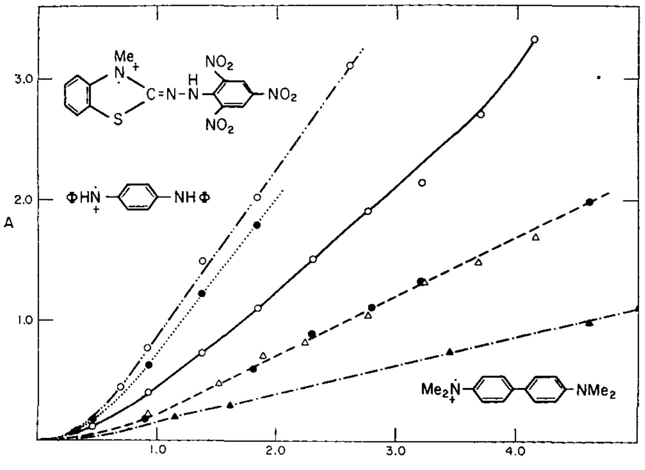


Figure 11. Change in absorbance with concentration of nitrite

...... *N,N'*-Diphenyl-*p*-phenylenediamine Procedure A at 720 mμ
··—··— Procedure B at 705 mμ
——— 3-Methyl-2-benzothiazolinone picrylhydrazone procedure at λ_{max} 406 mμ
● - - - *N,N,N',N'*-Tetramethylbenzidine Procedure A at λ_{max} 473 mμ
△ - - - *N,N,N',N'*-Tetramethylbenzidine Procedure A at λ_{max} 1018 mμ
·—·— Procedure B at λ_{max} 472 mμ

lower intensities were obtained. The reagent solution was stable for at least 8 hours. The blank was colorless to faint blue. As in Procedure A, the color intensity increased with time, so a 1-hour standing period was chosen for the development of color. The concentration-absorbance curve is shown in Figure 11.

Nitrate gave negative results; other inorganic oxidizing agents gave positive results. Sulfite had very little effect (Figure 10); at 2500 to 1 absorbance decreased from 0.52 to 0.40.

***N,N,N',N'* - Tetramethyl - *p*-phenylenediamine.** PROCEDURES A AND B. Increasing the concentration of reagent from 0.005 to 0.2% increased the color intensity. Since 0.2% reagent solution gave a light-bluish blank, 0.1% reagent was chosen. The reagent solution turned light blue after 6 hours. As the amount of distilled water in the reagent solution was increased (from 0 to 25%), the intensity increased. With 50% or greater amounts of distilled water in the reagent, the blank became blue, perhaps because of the presence of minute amounts of ozone in the water. Once the nitrite and reagent solutions were mixed, the color intensity increased rapidly for about $^1/_2$ hour and then much more gradually for the next hour. The slope of the concentration-absorbance curves for the two procedures decreased with lower concentrations of nitrite. In a procedure similar to A except that excess nitrite and a much smaller concentration of reagent are used, a band derived from the quinonic compound was obtained at 340 mμ. The reagent in acetic acid gave no band above 300 mμ.

Nitrate gave negative results; many other types of inorganic oxidizing agents gave positive results. At 1000 to 1, sulfite had no effect on the intensity in Procedure A.

PROCEDURE C. Optimum intensities were obtained with 0.2% reagent and 20 minutes' reaction time. Interferences were the same as for Procedures A and B, except that sulfite had a much more drastic effect: At 60 to 1, the absorbance decreased from 0.75 to 0.08.

3 - Methyl - 2 - benzothiazolinone Azine. Optimum intensities were obtained with 0.005 to 0.02% reagent. Ten per cent propionic acid was necessary to dissolve the reagent. The order of addition of reagents was important, since changes in order caused some differences in intensity. At temperatures higher than 40° F. the intensities decreased. The intensity was also affected by exposure of the solution over a period of minutes to instrumental light at the wavelength maximum. Seven hours after mixing of the reagent and nitrite test solutions, the intensity was still on the increase. The absorbance-concentration curve is similar to that obtained with the other reagents. Addition of 1 ml. of 0.1% sulfamic acid in 25% aqueous acetic acid at 15, 30, 45, 60, and 120 minutes after mixing reagent and test solutions stabilized the intensities for at least 30 minutes.

Common nitrates, iodides, perchlorates, chlorates, aldehydes, ketones, alcohols, hydrocarbons, and selenium dioxide gave negative results. Other inorganic oxidizing agents, especially iodate and periodate, gave positive results. The absorption spectra of the reagent, the free radical obtained in the analytical procedure, and the fully oxidized quinone are shown in Figure 4. The amount of interference of sulfite in the determination of nitrite is shown in Figure 10; at 500 to 1, absorbance dropped from 1.16 to 0.78.

Glyoxal Bis-(*N,N*-diphenylhydrazone). Maximum intensities were obtained with 0.01% of the hydrazone and 10 to 20% perchloric acid in the reagent. The reagent was stable for 1 hour. Maximum intensities were obtained 2 to 6 minutes after mixing the reagent and test solutions. The concentration-absorbance curves obtained with this compound at wavelengths 540 and 1152 have slopes somewhat similar to those of the other reagents. The absorption spectra of the reagent, its salt, and the free radical are shown in Figure 7.

Sodium nitrate, potassium iodide, cupric chloride, iodine, magnesium perchlorate, potassium and ammonium persulfate, selenium dioxide, sodium chlorate, acetone, alcohol, and phenol gave negative results. Oxidizing agents, such as ferric chloride, potassium permanganate, potassium chromate, potassium ferricyanide, potassium iodate, potassium periodate, and bromine gave positive results. At values up to 100 to 1, sulfite did not interfere with the determination of nitrite (Figure 10). At 500 to 1, the absorbance decreased from 1.82 to 1.35.

3 - Methyl - 2 - benzothiazolinone Picrylhydrazone. Optimum intensities were obtained with 0.01 to 0.05% of the hydrazone and 20% of 70% aqueous perchloric acid in the reagent solution. A much paler yellow blank was obtained with 0.01% reagent. The reagent solution was stable for at least 24 hours. The color intensity increased rapidly for the first 30 minutes and then much more slowly for the next 90 minutes. The slope of the calibration curve increases with higher concentrations of nitrite (Figure 11). The reagent has bands at 255 and 295 mμ in acetic acid–perchloric acid, so it does not interfere with the determination of nitrite at 406 mμ.

Negative results were obtained with sodium nitrate, potassium periodate, potassium iodate, cupric chloride, iodine, bromine, potassium persulfate, and magnesium perchlorate. Positive results were obtained with potassium permanganate, potassium chromate, potassium ferricyanide, and ferric chloride. Up to 200 to 1, the interference from sulfite was negligible (Figure 10). At 900 to 1, the absorbance dropped to 0.87 from an initial value of 1.48 (no sulfite).

***N,N,N',N'* - Tetramethylbenzidine.** PROCEDURE A. Optimum intensities were obtained with 0.01 to 0.1% reagent. With the higher percentage the color intensity reached a maximum in 1 to 3 minutes and then faded much more gradually than with the lower percentage. The reagent is stable for about 30 minutes. The slope of the calibration curve decreased with a lower concentration of nitrite (Figure 11).

Nitrite can also be determined in highly colored solutions. For example, it was readily and accurately determined at λ_{max} 1018 mμ in a dark bluish green solution containing the dye *N,N,N',N'* - tetramethyl - 4,4' - diaminoazobenzene.

Negative results were obtained with sodium nitrate, sodium chlorate, magnesium perchlorate, cupric chloride, potassium persulfate (~3000 μg.), and ferric chloride (~3500 μg.). Positive results were obtained with potassium chromate, potassium iodate, potassium permanganate, potassium ferricyanide, and bromine. At 50 to 1, sulfite did not interfere in the determination of nitrite; with much larger amounts of sulfite there was some interference.

PROCEDURE B. Optimum intensities were obtained with 1% reagent. The color intensity was stable for about 5 minutes and then gradually faded. Exposure to instrumental light at the wavelength maximum over a period of minutes accelerated the fading. The slope of the calibration curve decreased with a lower concentration of nitrite (Figure 11). The addition of sulfite interfered more drastically in this procedure by decreasing the intensity and increasing the rate of fading.

***N,N,N'* - Trimethyl - *p* - phenylenediamine.** PROCEDURE A. Optimum results were obtained with 0.05 to 1% reagent. The color intensity was stable for about 7 minutes and then gradually faded. The concentration-absorbance curve was linear from 4.7 to 50 μg. of nitrite; in Procedure B it was linear from 6 to 50 μg. of nitrite.

COMPARISON OF METHODS

Thus far, the highest molar absorptivity obtained in the determination of nitrite was with the 1-methyl-2-quinolone azine Procedure A; with 0.72 μg.

of nitrite a molar absorptivity of 1,270,000 was obtained. If the reaction were allowed to continue for about 7 hours, the molar absorptivity would be approximately doubled. Other methods with molar absorptivities of over 50,000 are the *N,N,N′,N′*-tetramethyl-4,4′-diaminothiobenzophenone (*15*), 1-methyl-2-quinolone azine B, *N,N,N′,N′*-tetramethyl - 4,4′ - diaminostilbene, *N,N′*-diphenyl-*p*-phenylenediamine B, and 3-methyl-2-benzothiazolinone azine procedures. The autocatalytic methods have, on the whole, much higher molar absorptivities than the various nitrosation methods previously described (*15*).

The molar absorptivity obtained in a method can be considered as the potential sensitivity of a method where the test solution volume and the final volume are identical. In most spectral methods of analysis these two volumes are not equal, however. Therefore the sensitivity of an analytical method can be defined as the molar absorptivity multiplied by the fraction of test solution volume in the final analyzed volume. To compare various analytical methods for sensitivity the following arbitrary formula can be used:

$$\text{Sensitivity} = \frac{\epsilon \times 10^{-3}}{\text{dilution factor}}$$

where the dilution factor is final volume/test solution volume. For a method in which Beer's law is complied with, the sensitivity is the same over the entire range of determinable concentrations of analyzed substance. For most of the methods in this paper the slope of the calibration curve, as well as the sensitivity, decreases with lower concentrations of nitrite. The signal obtained on the chart—i.e., the absorbance per equimolar amounts of material in a 1-cm. cell—is relatively greater at higher concentrations of material.

Of all the methods in the literature the 1-methyl-2-quinolone azine Procedure B is the most sensitive. Its sensitivity ranges from 190 at an absorbance of 0.10 to 380 at an absorbance of 2.0. The remainder of the procedures described in this paper range in sensitivity from 0.98 to 255. In the previous paper (*15*) the most sensitive azo dye method reported for the determination of nitrite was the *p*-phenylazoaniline plus 1-naphthylamine procedure, with a sensitivity of 31; the most sensitive diazonium cation method was the chloro-*p*-phenylenediamine procedure, with a sensitivity of 31.6. The new methods with the highest sensitivities reported in that paper are the *N,N,N′,N′* - tetramethyl - 4,4′ - diaminothiobenzophenone, sensitivity 56, and the azulene, sensitivity 37.5.

The determination limit is essentially the number of micrograms in the analyzed test solution giving an absorbance of 0.10 in a 1-cm. cell. In the previous paper the lowest determination limit (0.10 μg.) was reported for the azulene procedure. In the work described in this paper the phenothiazine Procedure B gave a determination limit of 0.07 μg. If all volumes in this method were decreased to 0.4 of the original, the determination limit would drop to 0.028 μg. In a similar fashion determination limits could be decreased in any of the other procedures described in this paper. Except for the *N,N,N′*-trimethyl - *p* - phenylenediamine procedures the determination limits for procedures reported in this paper ranged from 0.07 to 0.94 μg.; for those in the previous paper, limits ranged from 0.1 to 66 μg.

Beer's law was complied with in a very large number of the methods reported in the previous paper; it was not in any of the free-radical methods reported here. Noteworthy is the autocatalytic method for nitrite using the *N,N,N′,N′* - tetramethyl - 4,4′ - diaminothiobenzophenone procedure, because of its straight-line relationship between concentration and absorbance over a wide range of absorbance—from about 0.02 to 12 (*15*). In the analytical procedures increasing the amount of water tended to make the relationship between concentration and absorbance more linear.

On the average better precision was obtained with the nitrosation and diazotization type methods described previously than with the autocatalytic methods.

All the autocatalytic methods involve simple procedures. About one third of the methods require a procedure time of a little over 1 hour; another third require 22 or 32 minutes, and the remainder require 2 or 7 minutes.

These latter procedures take such a short time because the color starts fading within 5 or 6 minutes. In the others the color intensity increases over a more extended period before it starts fading. With some of the procedures the addition of sulfamic acid at an appropriate time stabilizes the color intensity. In respect to color stability, many of the azo dye methods reported in the previous paper (*15*) had color stabilities greater than 1 hour. A few were stable for more than 16 hours.

In most of the autocatalytic methods the presence of large amounts of sulfite caused little, if any, interference; in azo dye methods sulfite and other reducing agents would be expected to interfere. The presence of a larger percentage of water in the autocatalytic methods increased the interfering effect of sulfite. Many types of inorganic oxidizing agents gave the same type of spectra as obtained with nitrite. This kind of phenomenon is absent in the azo dye methods.

RECOMMENDATIONS

The *N,N′*-diphenyl-*p*-phenylenediamine Procedure B is recommended for the determination of nitrogen dioxide in the presence of large amounts of sulfur dioxide. This type of method would be of value in the analysis of source and atmospheric samples containing relatively large concentrations of sulfur dioxide. A few other methods—e.g., *N,N′*-diphenyl-*p*-phenylenediamine Procedure A and phenothiazine Procedure A—are even less affected by sulfite, but are somewhat less sensitive to nitrite. These latter procedures are also less sensitive than the most sensitive azo dye methods.

The 1-methyl-2-quinolone azine Procedure B is recommended for the determination of 0.1 to 1.3 μg. of nitrite (or nitrite precursor) in the absence of inorganic oxidizing agents and high concentrations of sulfite. The intensity is not affected in the presence of a ratio of sulfite to nitrite of about 2; with a ratio of 10 to 1 the intensity is decreased by one third. By use of smaller volumes of test and reagent solutions, 0.04 to 0.52 μg. of nitrite could be determined.

The *N,N,N′,N′*-tetramethylbenzidine procedures are recommended where a quick, simple procedure is desired for the determination of nitrite in the absence of inorganic oxidizing agents and very large amounts of sulfite. Some less sensitive methods with fairly short procedural times have been described (*15*); these methods are relatively insensitive to inorganic oxidizing agents and have much more stable colors.

The *N,N,N′,N′*-tetramethyl-4,4′-diaminothiobenzophenone procedure (*15*) is recommended where it is desirable to have a very large increase in absorbance with a small increase in nitrite concentration.

Where it is desirable to determine nitrite in highly colored solutions, the glyoxal bis(*N,N′*-diphenylhydrazone) procedure at $\lambda_{\max}$ 1152 mμ and the *N,N,N′,N′*-tetramethylbenzidine procedures at $\lambda_{\max}$ 1018 mμ are recommended.

By a slight modification of the procedures, electron paramagnetic resonance spectrometry could be used in the determination of nitrite, nitrite precursors, and some of the inorganic oxidizing agents.

Some of the methods could be modified for use in the determination of oxidants. For example, the 1-methyl-2-quinolone azine reagent solution reacted strongly with ozone. The reaction of the 3-methyl-2-benzothiazolinone azine solution with ozone was fairly strong also. Further work on the reaction of the various reagents with oxidizing agents and with transient free radicals should prove of value.

The relatively stable free radicals introduced in this paper should prove of value in other fields of research.

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Direct Quantitative Isolation of Monocarbonyl Compounds from Fats and Oils

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▶ A quantitative procedure is described for the direct isolation of carbonyl compounds from fats and oils. Carbonyl compounds in the fat are converted to their 2,4-dinitrophenylhydrazones, subsequently freed of fat, and fractionated by adsorption on activated magnesia and partially deactivated alumina. The fat-free monocarbonyl fraction is then separated into classes on magnesia and the members of each class are obtained by column partition chromatography and identified by supplementary techniques. The procedure is ideally suited for small samples of fat, but can be applied to kilogram quantities. Advantages and limitations of the method are discussed.

THIS paper describes a method for the direct quantitative isolation of monocarbonyl compounds from fats and oils. The method was developed in conjunction with a study on off-flavor development in stored whole milk powder.

Carbonyl compounds are usually isolated from autoxidized fats and oils by some form of distillation, although other methods such as extraction of water-soluble derivatives have been reported (*6, 11, 12*). Over 95% of the carbonyl compounds occurring in many fats and oils are nonvolatile when the usual methods of distillation are used (*9, 10*). The nature and role of these nonvolatile carbonyl compounds had not been elucidated at the onset of this work, although it has been suggested that part of them, at least, might be involved in fat oxidation (*2*).

In the proposed method, distillation and extraction techniques are circumvented, and, theoretically, all carbonyl compounds capable of forming a 2,4-dinitrophenylhydrazone under the conditions outlined are isolated lipide-free, regardless of the amount of starting material.

EXPERIMENTAL

Materials. Seasorb 43 (activated magnesia, Fisher Scientific Co., Silver Spring, Md.) is used as received. Hexane (Phillips' high purity grade) and benzene (ACS grade) are rendered carbonyl-free by the method of Schwartz and Parks (*17*). Celite 545 is dried at 150° C. for 24 hours. Nitromethane (Fisher's highest purity) and chloroform (ACS grade) are used.

Scope of Method. The method for isolation of the monocarbonyl fraction from fats and oils may be divided into six steps: (1) reaction of the carbonyl compounds in the fat with 2,4-dinitrophenylhydrazine; (2) adsorption of the resulting derivatives onto activated magnesia while eliminating the bulk of the fat or oil, followed by desorption of the derivatives; (3) fractionation of the derivatives on weak alumina; (4) adsorption of the monocarbonyl derivatives on an anion exchange resin, if necessary; (5) separation of the monocarbonyl derivatives into classes on magnesia; and (6) separation of the members of each class by liquid-liquid partition chromatography.

Step 1. Reaction with 2,4-Dinitrophenylhydrazine. A column of Celite impregnated with dinitrophenylhydrazine, phosphoric acid, and water (reaction column) is prepared as described by Schwartz and Parks (*17*). The column is flushed with 50 ml. of benzene, followed by hexane until a colorless effluent is obtained. The fat or oil is dissolved in hexane and passed over the column.

When the last of the solution has just entered the column, the sides of the tube are washed down with hexane and the washings allowed to enter the column. Fifteen milliliters of hexane are added and permitted to drain into the column by gravity. The column is then flushed with hexane, using N_2 pressure until the effluent emerges colorless or has the same absorptivity (at or near 340 mμ) as the effluent from a blank column, which should always be run simultaneously.

The hexane-fat effluent now contains all of the original lipide, the 2,4-dinitrophenylhydrazine derivatives of the monocarbonyls, semialdehyde, and ketoglycerides (*7, 8*), and other classes of carbonyl compounds whose derivatives are soluble in the fat-hexane solution, a small amount of dinitrophenylhydrazine, and traces of the decomposition products of dinitrophenylhydrazine. Remaining on the column are those carbonyls whose derivatives are insoluble in the fat-hexane solution.

QUANTITATIVE ASPECTS. The quantitative aspects of reaction of carbonyls in fat with dinitrophenylhydrazine on the column were thoroughly studied from two standpoints. First, carbonyl compounds in the form of pure semicarbazone or other suitable derivative were added singly to monocarbonyl-free, butter oil. Schwartz (*15*) had shown that micro amounts of the semicarba-

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zone and some other derivatives could be quantitatively converted to 2,4-dinitrophenylhydrazones by passing a methylene chloride–hexane solution of the derivative over a reaction column. This technique was used here, but monocarbonyl-free butter oil in hexane (25 ml. of a 14.5% solution) was substituted for hexane and the solution was run over the column at a flow rate of 46 ml. per hour. The resulting 2,4-dinitrophenylhydrazones were then isolated using Steps 2 and 3 and recoveries determined spectrophotometrically. Pertinent data are presented in Table I.

Since the results indicated that quantitative or near-quantitative recovery was obtained under a fixed set of conditions, experiments were conducted in order to ascertain whether more flexible conditions could be used without impairing the quantitative aspect. Different levels of a single batch of badly deteriorated butter oil in 50 ml. of hexane were put through Steps 1 through 3 and the isolated monocarbonyl fraction was determined spectrophotometrically. The flow rate of the solution through the column at various fat levels and the amounts of monocarbonyls found are given in Figure 1. Class separation (Step 5) of the derivatives showed that all four classes of monocarbonyls (methyl ketones, saturated aldehydes, 2-enals, and 2,4-dienals) were present in the butter oil. The near-linearity of the plot indicates that the reaction is quantitative over a wide range of fat concentration. The optimum fat concentration to use from the standpoint of throughput of solution per unit of time was calculated to be approximately 20%.

Step 2. Adsorption of Derivatives on Magnesia. Solvents for this and all subsequent steps need not be carbonyl-free.

The concentration of 2,4-dinitrophenylhydrazones in the effluent from Step 1 is estimated approximately by measuring the absorbancy of the solution against hexane at 340 mμ and converting the reading to micromoles, using $E = 22{,}500$.

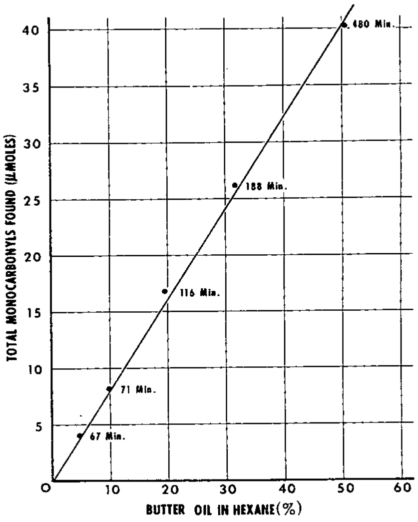

Figure 1. Relationship between fat concentration and yield of 2,4-dinitrophenylhydrazones obtained on reaction column

The effluent from Step 1 is carefully applied to a column of Seasorb 43–Celite 545 (1 to 1) using 1 gram of Seasorb 43 for approximately 5 to 10 μmoles of hydrazone. The column is prepared in the following manner: Equal weights of Seasorb 43 and Celite 545 are slurried in an Erlenmeyer flask with hexane so that all of the material is suspended. The slurry is then poured through a long-stemmed funnel into a chromatographic tube containing a coarse sintered-glass disk or a plug of glass wool at the constricted portion of the tube. The slurry is packed immediately under moderate air pressure (1 to 3 p.s.i.), leaving about 1 cm. of hexane above the surface of the bed. The sides of the tube are washed free of solid before the sample is applied.

The adsorbed hydrazones appear as a deep purple zone. Nitrogen pressure can be used to force the fat solution through the column, so that an unbroken stream of effluent issues from the tube continuously. No loss of hydrazones occurs from this treatment as long as colorless adsorbent can be seen. If a fat containing pigments is being investigated, the effluent may appear yellow but contains no hydrazones.

Table I. Recovery of Carbonyl Compounds from Butter Oil Solution after 2,4-Dinitrophenylhydrazine Reaction on Celite Column

Compound	Amount, μmoles	Recovery, %	Compound	Amount, μmoles	Recovery, %
2-Nonadecanone (as semicarbazone)	0.236 2.63 13.15	103 98 99	Tetradeca-2-enal (as semicarbazone)	0.236 2.366 11.83	104 103 99
2-Octanone (as semicarbazone)	2.20 11.00	101 95	2-Ethyl hex-2-enal (as semicarbazone)	0.196 1.962	105 97
Acetone (as ketal of glycerol)	2.08 10.4 0.208	101 100 96	Octadeca-2,4-dienal (as semicarbazone)	0.209 2.09 6.27	96 95 98
Tridecanal (as semicarbazone)	0.196 1.96	106 103	Deca-2,4-dienal (as semicarbazone)	2.48 12.2	93 97
Heptanal (as oxime)	2.38 23.8	98 96			

After the last of the fat solution has just entered the bed, the sides of the tube are carefully washed down with hexane. The washings are permitted to drain and a small wad of glass wool is placed just above the bed. The column is then washed with hexane until a 25-ml. aliquot of the effluent contains less than 25 mg. of lipide. Elution of the hydrazones is effected with nitromethane-chloroform (1 to 3), the effluent being collected just prior to the breakthrough of the hydrazones. Elution is continued until a colorless or very pale effluent is obtained.

The nitromethane-chloroform effluent now contains all of the monocarbonyls, semialdehyde, and ketoglycerides and probably other classes of monohydrazones. All dicarbonyl bis(2,4-dinitrophenylhydrazones), vicinal and nonvicinal, which may have been soluble in the fat-hexane effluent from Step 1 remain on the magnesia. These are manifested by blue, gray, or violet bands near or at the top of the column (see discussion). The column may have a slight violet tinge covering the area previously occupied by the hydrazones. This is due to slight decomposition of the ketoglyceride fraction. These decomposition products elute slowly with nitromethane-chloroform and account for the slight yellow tinge of the effluent, even though all of the monocarbonyls and ketoglycerides have been eluted long before.

STABILITY AND QUANTITATIVE ASPECTS. The stability and quantitative aspects of Steps 2 and 3 were determined simultaneously on 40 pure 2,4-dinitrophenylhydrazones. Approximately 0.25 μmole of each hydrazone was added singly to a 50% solution of butter oil, olive oil, or corn oil in hexane or to lard in benzene-hexane and the solution was put through Steps 2 and 3. The isolated derivative was then subjected to column partition chromatography in the chloroethanol-hexane system (Step 6) as described by Corbin, Schwartz, and Keeney (*4*). The peak was then collected and its concentration determined spectrophotometrically. Recoveries are given in Table II.

Step 3. Fractionation of Derivatives on Weak Alumina. The nitromethane-chloroform eluate obtained in Step 2 is evaporated on the steam bath under a stream of nitrogen until the odor of nitromethane is absent. The residue is then dissolved in hexane and applied to a column of alumina prepared as described by Schwartz and Parks (*17*). The capacity

Table II. Recovery of 2,4-Dinitrophenylhydrazones from Butter Oil after Steps 2, 3, and 6

Saturated aldehydes		Methyl ketones		2-Enals		2,4-Dienals	
	Recovery, %		Recovery, %		Recovery, %		Recovery, %
C_2	97	C_3	95	C_3	90	C_6	100
C_3	94	C_4	98	C_4	90	C_7	96
C_4	94	C_5	98	C_5	94	C_8	90
C_5	91	C_6	100	C_6	100	C_9	95
C_6	93	C_7	95	C_7	94	C_{11}	97
C_7	98	C_8	99	C_8	95	C_{12}	98
C_8	94	C_9	94	C_9	103		
C_9	99	C_{11}	94	C_{11}	90		
C_{10}	92	C_{13}	97	C_{12}	96		
C_{11}	90			C_{18}	94		
C_{12}	101						
C_{13}	102						
C_{14}	97						
C_{16}	94						
C_{18}	102						

of the alumina for hydrazones varies somewhat with the nature of the small amount of constituents eluted with the hydrazones from the magnesia in Step 2. A good rule to follow is to use 1 gram of alumina for approximately 2 to 3 μmoles present.

When the last of the hexane solution has passed into the column, the sides of the tube are washed down with a few milliliters of hexane, and the monocarbonyl fraction is eluted with a mixture of benzene-hexane (1 to 1). By using 40 model hydrazones it was established that any hydrazone in the four classes studied could be quantitatively eluted from 5 grams of alumina with 50 ml. of a 1 to 1 benzene-hexane mixture, when present at or below 8 μmoles. In practice these conditions usually hold, but in working with the carbonyl fraction isolated from a large volume of fat the ketoglyceride fraction may move too near the tail end of the monocarbonyl fraction, so that sharp fractionation is not realized. When this happens, it is recommended that about the first quarter of the ketoglyceride band be collected with the monocarbonyl fraction, the solvent removed, and the residue then rechromatographed on another column of alumina, in which case sharp fractionation of the monocarbonyl band from the ketoglyceride band is obtained. Alternatively, the column may be eluted with a weaker eluting solvent combination—e.g., benzene-hexane, 1 to 3—until all color is removed below the ketoglyceride band.

Step 4. Adsorption of Monocarbonyl Fraction on Anion Exchanger. Evaporation of the benzene-hexane effluent from Step 3 usually leaves a dry residue or one containing only a few milligrams of fatty impurities. This has been our experience with a number of fats and oils (butter oil, lard, and safflower, olive, and peanut oils) and it has not been necessary to resort to this step. However, in view of the large number of fats and oils that exist in nature, sufficient impurities may accompany the hydrazones to render their subsequent fractionation and identification difficult, or to complicate radioactive tracer work or gas chromatographic analyses of regenerated carbonyls. In these cases, the monocarbonyl fraction is adsorbed onto an anion exchanger in the hydroxyl form precisely as outlined by Schwartz Johnson, and Parks (*16*).

Step 5. Separation of Monocarbonyl Fraction into Classes. The residue from Step 3 or Step 4 (if used) is fractionated into classes, using one of the procedures described by Schwartz, Parks, and Keeney (*18*). The classes obtained are then estimated spectrophotometrically, if desired, using the proper molar extinction coefficient and maxima.

The authors have found it to their advantage when analyzing butter oil to subject the monocarbonyls from Steps 3 and 4 (if used) to partition chromatography (Step 6) prior to running the class separation. Although this involves an extra step (since one or more partition chromatograms will be run following class separation), the classes are separated more cleanly and with more confidence. The reason for this is that butter oil contains a class of naturally occurring compounds, other than the four common classes, which elutes from alumina (Step 3) with the monocarbonyl fraction. This class of constituents is relatively nonpolar, moving with the front on the partition chromatogram, and thus can be removed easily from the more polar hydrazones. The first peak from the partition chromatogram is collected by itself and later subjected to class separation. The remaining bands on the partition column are collected, pooled, and then class separated.

Step 6. Partition Chromatography. The classes obtained in Step 5 are subjected to column partition chromatography by the method of Corbin, Schwartz, and Keeney (*4*). The peak volume gives a good indication of the chain length of a compound, which can be confirmed by such procedures as paper chromatography, cochromatography on partition columns, ultraviolet spectra, and when enough material can be accumulated, melting point and mixed melting point.

DISCUSSION

The procedures have been successfully used in this laboratory in the determination of carbonyl constituents of fats, oils, extracts of cheese, and whole milk powders. A number of different classes of carbonyl-containing compounds have been revealed. Among them are the ketoglycerides, a class of compounds which seem to be present in all fats and oils in various amounts and which will be discussed more fully elsewhere (*8*). The procedure also indicated early in its development that butter oil contained aldehydes bound to glycerol in phosphorus-free, acid-labile compounds; this has since been verified and the complex spectra of the aldehydes have been identified (*11, 13*). There are indications of other classes of naturally occurring carbonyl compounds which have not yet been fully characterized.

The analysis of a fat or oil (or extract) by these procedures may not, of course, reveal the total carbonyl picture. Dicarbonyls, semialdehydes, unesterified keto acids, and other classes of compounds usually are present in autoxidized fats. Although procedures for fractionating these classes have been developed in this laboratory in conjunction with the monocarbonyl analysis, the quantitative aspects of their isolation remain to be verified.

The use of a column of Celite impregnated with 2,4-dinitrophenylhydrazine, acid, and water as a means of forming 2,4-dinitrophenylhydrazones greatly facilitated development of the subsequent steps in the procedure. The column, which was originally developed by Begemann and DeJong (*1*) and modified by Schwartz and Parks (*17*), not only gives quantitative yields of hydrazones, but also gives an acid-free solution of the derivatives, thereby eliminating the need for extraction or some other manipulation. The column procedure affords conversion of extremely low concentrations of carbonyls into the hydrazones, an achievement which apparently is difficult when the reaction is run in true solution (*3*). Thus, it was possible to identify the hydrazones of heptadecanone and octadecylaldehyde by paper chromatography after 2.4 × 10^{-3} μmole (about 0.8 μg.) of the carbonyl had been put through the reaction column in 50 ml. of a 4.3% butter oil solution (in hexane) and the hydrazone subsequently isolated.

It was recognized that direct reaction of a fat with acidic 2,4-dinitrophenylhydrazine might produce carbonyls from hydroperoxides, the structure which would eventually give rise to them naturally. To check this point, methyl linoleate hydroperoxide was put through the procedure. The hydroperoxide had a peroxide value of 4025 and an odor suggesting that carbonyls were present in the original preparation. It was established that the hydroperoxide was altered by passage over the reaction column, the effluent being incapable of oxidizing acidic KI. Analysis of the carbonyls obtained from 7.9 μmoles of the hydroperoxide after passage over the reaction column indicated: 0.03 μmole of ketone, 0.53 μmole of saturated aldehyde, 0.04 μmole of 2-enals, and 0.01 μmole of 2,4-dienal. To ascertain whether these carbonyls were present in the original preparation, the hydroperoxide was adsorbed from a benzene-hexane solution onto a column of Dowex 1-X4 (50- to 100-mesh) in the OH form. This procedure removed approximately 85% of the hydroperoxide, and the neutral effluent when analyzed for carbonyls gave the same data as the original hydroperoxide. It was, therefore, concluded that the original hydroperoxide was contaminated with approximately 7% of monocarbonyls and that no monocarbonyls are produced from methyl linoleate hydroperoxide on contact of this compound with the reaction column.

The acidity of the column (pH about 1.5) is sufficient to hydrolyze quantitatively and form derivatives of the aldehydes present in bound form in plasmalogens and in the phosphorus-free analog present in butter oil and in some other lipide (*5, 11, 13*). The presence of bound aldehydes in fats and oils can easily be established by applying the procedures described here on a fresh product. Although the presence of the bound aldehydes in a fat may complicate interpretation of the results of an analysis, this difficulty may be overcome by applying the procedures described by Parks, Keeney, and Schwartz (*11, 12*).

Activated magnesia has proved to be an excellent adsorbent for 2,4-dinitrophenylhydrazones. Its affinity for 2,4-dinitrophenylhydrazones is presumably due to the ionic species of the *m*-dinitro groups created by the alkaline environment. All monocarbonyl 2,4-dinitrophenylhydrazones turn various shades of red, gray, or purple on contact with this adsorbent. All of the 40 hydrazones studied were stable on magnesia and on alumina. The stability of 2,4-dinitrophenylhydrazones (also 2,4-dinitrophenylosazones) to an alkaline environment has been amply demonstrated (*14, 16, 18*).

The capacity of magnesia for 2,4-dinitrophenylhydrazones depends on factors such as fat concentration, nature of the fat under study, type of class predominantly present—i.e., ketones are held less strongly than saturated aldehydes, etc. (*18*)—and, of course, strength of the adsorbent. Very little variation in the strength of various lots of Seasorb 43 has been observed.

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Critical Study of Different Methods for Determination of Chlorite

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▶ A critical study of methods for the determination of hypochlorite and chlorate with special reference to their behavior for chlorite determination was made and a procedure for the determination of chlorite using ammonium sulfate developed. Two internal indicators for sodium benzene sulfinate–hypochlorite titration are reported.

DURING the study of the kinetics and stability of hypochlorite (*7*), there arose the need for a method for determining chlorite ions in hypochlorite solution. The survey of the literature indicated the availability of many methods (*1–4*) for the determination of hypochlorite and chlorate, but these methods did not describe the fate of chlorite ions during the determination.

Chapin (*4*) employed phenol for the determination of hypobromite and bromite or hypoiodite and iodite in presence of each other and concluded that the latter did not react with phenol and could thus be distinguished from hypohalites. Ackerman (*1*) found that sodium benzene sulfinate reacts quantitatively with hypochlorite. Atkin (*2*) used this reaction for the determination of total halates in sodium hypochlorite solution after the hypochlorite was titrated with sodium benzene sulfinate. Schulek and Endoroi (*11*) used potassium cyanide to distinguish between chlorite and hypochlorite. However, the reagent is not easy to handle and the method cannot be used as a routine procedure.

The method based on phenol, when employed in these laboratories, did not give accurate results for the determination of hypochlorite and chlorite in the presence of each other. Neither Ackerman (*1*) nor Atkin (*2*) described the fate of chlorite ions when sodium benzene sulfinate was used for the determination of hypochlorite and chlorate. A later communication by Farkas and Lewin (*5*) also did not mention the determination of chlorite ions. This led us to the critical study of the above methods for the determination of hypochlorite and chlorate ions with special reference to their behavior for chlorite determination.

We have established that phenol reacts with chlorite in addition to hypochlorite and cannot be used for the determination of either in the presence of the other. Similarly, sodium benzene sulfinate reacts with both hypochlorite and chlorite ions. Ackerman (*1*) and Atkin (*2*) employed starch-iodide paper as an external indicator for sodium benzene sulfinate titrations. In this paper two internal indicators are reported.

In a previous paper (*8*), a method was reported for the simultaneous determination of hypobromite, bromite, and bromate using ammonium sulfate. In the present paper, this reaction of ammonium ion has been used for the determination of chlorite in presence of hypochlorite and chlorate.

EXPERIMENTAL

Materials. All reagents were analytical grade.

Sodium hypochlorite of various strengths was prepared by passing chlorine gas through 0.5*N* sodium hydroxide solution previously cooled to below 0° C. The resulting solution was diluted to pH 11 to 12 and was standardized for its hypochlorite and chlorite contents by arsenious oxide (*6*).

Arsenious oxide (0.1*N* solution) was prepared by dissolving the approximately weighed reagent in 10% sodium hydroxide solution. It was made acid to phenolphthalein. Ten grams of solid sodium bicarbonate was added, and the solution was diluted to 1 liter and standardized with iodine.

Sodium benzene sulfinate (British Drug Houses), 0.1*N* solution, was prepared by weight.

Tartrazine and Bordeaux indicators, 0.2% aqueous solutions, were used.

Potassium iodate (0.1*N* solution) was prepared from the reagent previously dried at 120° C.

Procedure. Potassium iodate was used as the primary standard, and the determination was carried out by the following methods.

I. A known sample of hypochlorite solution was acidified after addition of excess potassium iodide and let stand for 5 minutes before the liberated iodine was titrated against standard thiosulfate. The titer included hypochlorite, and chlorite ions (*6*).

II. A known amount of sodium benzene sulfinate was taken in a 100-ml. conical flask and titrated against standard hypochlorite solution using starch-iodide as an external indicator (*1, 2*). Two similar titrations were carried out using tartrazine and Bordeaux as internal indicators. It was necessary to add about 0.5 gram of sodium bicarbonate and a small amount of potassium bromide to get a distinct end point when Bordeaux or tartrazine was used as internal indicator.

III. A small amount of sodium bicarbonate and potassium bromide was added to a known volume of standard arsenious oxide solution in the flask and was titrated against hypochlorite solution using tartrazine as internal indicator. The titer included only hypochlorite ions (*10*).

IV. A known volume (10 ml.) of hypochlorite solution was taken and an excess (15 ml.) of 20% phenol solution was added to it. The solution was let stand for 5 minutes. An excess of solid potassium iodide and sulfuric acid (4*N*) was added and the solution was further kept for 5 minutes before it was titrated against standard thiosulfate.

V. Ten milliliters of sodium hypochlorite solution was buffered to pH 9.3 by boric acid, followed by an excess of solid potassium bromide. After about 45 to 60 seconds, ammonium sulfate was added with continuous shaking and the solution let stand for 10 minutes before excess potassium iodide and sulfuric acid (4*N*) were added. The liberated iodine was titrated against standard thiosulfate. The titer included only chlorite ions.

Table I. Suitability of Tartrazine and Bordeaux as Internal Indicators for Benzene Sulfinic Acid–Hypochlorite Titration

Hypochlorite and chlorite,^{a} *N*	Strength of hypochlorite solution^b by sodium benzene sulfinate, *N*		
	Starch-iodide paper	Bordeaux	Tartrazine
0.1135	0.1133	0.1135	0.1134
0.1008	0.1000	0.1007	0.1013
0.1005	0.1000	0.1004	0.1010
0.0886	0.0885	0.0885	0.0887
0.0968	0.0955	0.0967	0.0960

^{a} Found by potassium iodide–thiosulfate titration (Method I). The same samples were analyzed for hypochlorite and chlorite (Table II).

^{b} Hypochlorite solution includes hypochlorite and its decomposition products except chlorate (*1, 2*).

RESULTS AND DISCUSSION

The results in Table I indicate that sodium benzene sulfinate reacts with both hypochlorite and chlorite quantitatively and cannot be used for the determination of either in the presence of the other. Two internal indicators—i.e., Bordeaux and tartrazine—give accurate results and are easy to use as compared to starch-iodide as an external indicator.

The determination of chlorite by the arsenite-thiosulfate procedure and the ammonium sulfate method is compared in Table II, which indicates that the ammonium sulfate method gives reliable results for chlorite determination. Hypochlorite ion is converted quantitatively to hypobromite at pH 9 to 9.4 by the addition of potassium bromide and is destroyed by ammonium sulfate (*8*), leaving behind chlorite ions (cf. Table II).

Table II also compares the estimation of chlorite using arsenious oxide and the phenol–potassium iodide–thiosulfate procedure. The results indicate that phenol reacts with both hypochlorite and chlorite, leaving behind a small amount of available chlorine. This is probably due to the fact that when phenol reacts with hypochlorite solution, the substitution goes further to trichlorophenol chloride, $C_6H_2Cl_3OCl$, which may be decomposed by potassium iodide to trichlorophenol and a corresponding amount of iodine is liberated (*9*).

Table II. Determination of Chlorite by Ammonium Sulfate Method

Thiosulfate titer, *N*	Arsenite titer, *N*	Chlorite,^{a} *N*	Chlorite,^{b} *N*	Ions unreacted with phenol,^{c} *N*
0.1135	0.1090	0.0045	0.0047	0.00014
0.1008	0.0968	0.0040	0.0040	0.00044
0.1005	0.0965	0.0040	0.0039	0.00050
0.0886	0.0848	0.0038	0.0037	0.00041
0.0968	0.0928	0.0040	0.0041	0.00030

^{a} Determined by subtracting arsenite from thiosulfate titer.

^{b} Found by ammonium sulfate method (Method V).

^{c} Found by Method IV.

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Determination of Normal Alpha-Olefins by Hydrobromination

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▶ A method is described for the determination of normal alpha-olefins in olefinic mixtures within the C_{12}–C_{18} range. Hydrobromination of the olefin sample in the presence of lauroyl peroxide is utilized to convert normal alpha-olefins to normal primary alkyl bromides which can be separated from other alkyl bromides by 5-A Molecular Sieve pellets. The weight of nonadsorbed bromides is a measure of the impurity in normal alpha-olefins. The method determines normal alpha-olefins in the presence of beta-branched alpha, non-beta-branched alpha, normal and branched internal, and cyclic olefins.

CONSIDERABLE EFFORT and expense have been expended by the petroleum industry to produce high purity normal alpha-olefins. These olefins are particularly valuable in the manufacture of biodegradable detergents. Therefore, the necessity arose for an accurate method for determining the purity of the normal alpha-olefins. Terminal olefins may be analyzed by infrared techniques (*8, 9*), but this approach does not differentiate normal alpha-olefins from alpha-olefins having branching other than on the beta-carbon atom. These non-beta-branched alpha-olefins ($H_2C{=}CHR$, where R is a branched group) could be present with normal alpha-olefins, especially if the alpha-olefins are produced by a wax cracking process.

Thus, a study was initiated to investigate hydrobromination as a tool for the quantitative determination of the nonbranched terminal olefin type. Kharasch (*2–5*) reported that alpha-olefins when reacted in the presence of hydrogen bromide and organic peroxide form only primary alkyl bromides. He worked primarily with lower molecular weight olefins because of a lack of pure high molecular weight alpha-olefins. His limited work with high molecular weight olefins (*5*) resulted in good yields of primary bromides. Therefore, if the reaction were quantitative, the primary bromides resulting from normal alpha-olefins might be separated from the primary bromides formed from branched alpha-olefins, and from the secondary bromides formed from the reaction of normal internal olefins by adsorption on 5-A Molecular Sieves. A measure of the weight of the bromides which were not adsorbed by Molecular Sieves would then be a direct indication of the total impurity in the normal alpha-olefin.

A number of workers (*6, 7, 10*) have used Molecular Sieves (synthetic zeolites) for the separation of normal paraffins from branched paraffins. Barrer (*1*) found that natural zeolites could be used to separate secondary bromides from primary bromides. Working with chabazite, gmelinite, and analcite, he found that primary alkyl bromides are adsorbed by sieves slowly at room temperature, and that secondary alkyl bromides were excluded.

EXPERIMENTAL

Reagents. All chemicals were reagent grade unless otherwise specified. The olefins were obtained from the Phillips Co., American Petroleum Institute, and the Chemical Procurement Laboratories. The anhydrous hydrogen bromide was purchased from the Matheson Co. The 5-A Molecular Sieves pellets, purchased from Linde Co., were dried prior to use by heating for 6 hours at 450° C. under 1 to 5 mm. of Hg pressure, and then cooled under vacuum. The solvent, 2,2,4-trimethylpentane, was obtained from the Phillips Petroleum Co. Prior to use, all normal paraffins were removed from the solvent by adsorption with Molecular Sieves.

Apparatus. The hydrogen bromide reaction flask was a 150-ml. capacity two-necked, round-bottomed flask fitted with a medium porosity fritted disk, 11 mm. in diameter, for hydrogen bromide dispersion. The gas chromatographic apparatus employed for examination of the olefins and alkyl bromides consisted of an F and M Model 300 programmed-temperature chromatograph equipped with a 6-foot, $^1/_4$-inch column packed with 25% SE-31 Silicone Gum on Gas Chrom P.

Procedure. To approximately 0.70 gram of sample in the reaction flask, add 0.05 gram of lauroyl peroxide which has been dissolved in 40 ml. of 2,2,4-trimethylpentane. Immerse the flask in a carbon tetrachloride bath. Add dry ice to the bath until the carbon tetrachloride solidifies, and pass anhydrous hydrogen bromide through the solution at a rate of approximately 25 cc. per minute for 1.25 hours. At that time, remove the bath and continue the hydrogen bromide addition for 0.5 hour. Disconnect the hydrogen bromide source, remove the flask from the reaction assembly, and add 0.5 gram of anhydrous sodium sulfate to remove traces of water if present. Place the flask in a water bath maintained at 10° ± 5° C. Purge the solution with prepurified nitrogen for 25 minutes at a rate of 25 cc. per minute. Decant the contents of the reaction flask into a 250-ml. Erlenmeyer flask fitted with a 24/40 ground glass joint. Rinse the reaction flask with four 7-ml. portions of solvent and add the washings to the Erlenmeyer flask.

Add 5 grams of dried 5-A Molecular Sieve pellets to the Erlenmeyer flask. Stopper the flask, swirl, and allow to stand at room temperature for 25 minutes. Then add an additional 25 grams of sieves and reflux the mixture for 5.5 hours. If the sample contains C_{18} olefin, a reflux time of 6.5 hours is necessary. By means of a fine-fritted Buchner funnel filter the contents of the Erlenmeyer flask under 100 to 200 mm. of Hg pressure. Rinse the flask and the Buchner funnel with five 20-ml. portions of isopentane and collect in the filtering flask. Quantitatively transfer the contents, using isopentane, to a previously weighed 250-ml. Erlenmeyer flask. Place the flask in a water bath, at room temperature, and evaporate the solvent by blowing air over the surface. If the olefin sample is C_{16} or C_{18}, the evaporation time can be shortened by maintaining the water bath at 55° ± 5° C. Obtain the weight of solvent-free residue and calculate the normal alpha-olefin content of the sample.

RESULTS AND DISCUSSION

General Discussion. This method is applicable to olefin fractions within the C_{12} to C_{18} range. The method can be extended to higher molecular weight olefins by extending the reflux time for adsorption of the normal bromides. If the olefin sample contains normal paraffins, they will be adsorbed by the sieves and be calculated as normal alpha-olefins. Branched saturates are not adsorbed and thus are calculated as impurity. The data in Tables I, II, and III, indicate the normal alpha-

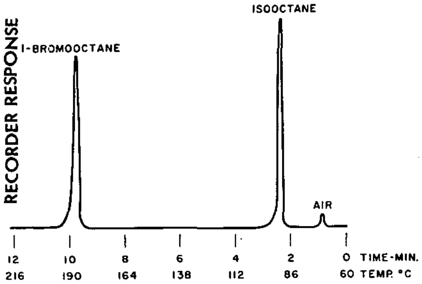

Figure 1. Programmed chromatogram of product of 1-octene and HBr in isooctane

Table I. Reaction Yields of Higher Molecular Weight Olefins with Hydrogen Bromide

Olefin	Sample size, gram	Yield, grams Found	Yield, grams Theory[a]	Mole % reacted
1-Hexadecene	0.7136	1.008	1.021	98.8
1-Tetradecene	0.6735	0.998	1.007	99.1
1-Eicosene	0.8737	1.167	1.174	99.5
	0.8890	1.225	1.196	100.2
11-Tricosene	0.8935	1.137	1.167	97.7

[a] 0.05 Gram added for lauroyl peroxide.

olefin content of an olefin fraction may be determined with a relative error of ±1.3%.

Hydrobromination of Olefins. To determine the completeness of the reaction of hydrogen bromide with olefins, and if primary bromides only are formed from alpha-olefins, the reaction products were examined by gas chromatography. 1-Octene was treated with hydrogen bromide under the reaction conditions specified above and a quantitative yield of 1-bromooctane was obtained. Figure 1 is a chromatogram of the reaction product of 1-octene with hydrogen bromide in isooctane. The presence of any nonreacted 1-octene would have been indicated by a peak at 4.5 minutes. The retention time of 1-bromooctane was verified experimentally. The reaction of hydrogen bromide and 2-octene resulted (Figure 2) in a quantitative yield of 2-bromooctane and 3-bromooctane. Nonreacted 2-octene would have been indicated by a peak at 3.5 minutes. Using the weight of the reaction product as a measure of the completeness of the reaction with higher molecular weight olefins, quantitative yields were also obtained with 1-tetradecene, 1-hexadecene, 1-eicosene, and 11-tricosene. The results are summarized in Table I. In addition, these reaction products of 1-tetradecene, 1-hexadecene, and 11-tricosene were refluxed with Molecular Sieves at 99° C. The reaction products of 1-tetradecene and 1-hexadecene were totally adsorbed indicating the formation of only primary bromides. The 11-tricosene reaction product was not adsorbed, indicating secondary alkyl bromides.

Molecular Sieve Absorption Studies. The adsorption of *n*-bromohexadecane (Table IV) by B-A Molecular Sieves was studied at a ratio of 25 grams of sieves to 1 gram of *n*-bromohexadecane. The results of this study indicated that at the reflux temperature of isooctane, 99° C., the time necessary for complete adsorption of *n*-bromohexadecane was 5.5 hours. The reflux time may be decreased by increasing the ratio of sieves to bromide. For example, at a ratio of 50:1, the time for complete adsorption of *n*-bromohexadecane at 99° C., was <2.5 hours. It is seen from Table II that lauroyl peroxide causes no error in the technique, since it is totally adsorbed by the sieves.

The presence of hydrogen bromide in the solution had an adverse effect on the adsorption of *n*-bromohexadecane, hydrogen bromide behaving similarly to water in deactiviating the sieves. To illustrate, *n*-bromohexadecane was refluxed for 5.5 hours with sieves in the presence of isooctane saturated with hydrogen bromide. The amount of *n*-bromohexadecane adsorbed was 14% compared to 100% in the absence of hydrogen bromide. Two techniques were evaluated to study the removal of hydrogen bromide from the reaction mixture. Initially the reaction mixture was evaporated on a steam bath to a volume of 15 ml. This approach was not suitable for the complete removal of hydrogen bromide. Finally the hydrogen bromide was successfully removed by purging the reaction solution with nitrogen followed by the addition of 5 grams of sieves. Some primary alkyl bromide may be adsorbed at this stage, but this causes no error since the next step involves the removal of primary bromide by the addition of 25 grams of sieves.

The nonadsorption of normal secondary alkyl bromides by 5-A Molecular Sieves was established by experimentation with 2-bromobutane. An isopentane solution containing 2-bromobutane and naphthalene as the internal standard was allowed to stand for 48 hours at room temperature with Molecular Sieves. The isopentane solution was examined chromatographically prior to the addition of sieves and after the

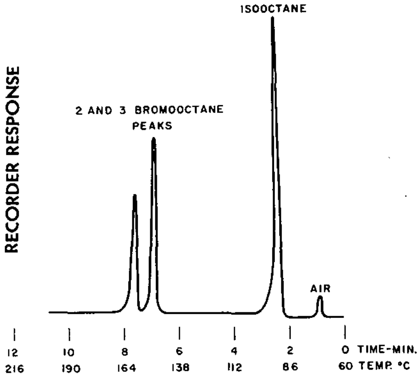

Figure 2. Programmed chromatogram of product of 2-octene and HBr in isooctane

Table II. Examination of Synthetic Olefin Blends

Compound	Mole % normal alpha-olefin Theory	Mole % normal alpha-olefin Found
1-Tetradecene	99.8	100.0
1-Tetradecene + C_{14} impurity[a]	95.0	95.6
1-Tetradecene + C_{14} impurity[a]	91.0	92.0

[a] 89% $R_2C{=}CH_2$ and 11% $R_2C{=}CHR$.

Table III. Hydrobromination and Infrared Analysis of Normal Alpha-Olefins

Carbon range	Mole % normal alpha-olefin Hydrobromination	Mole % normal alpha-olefin Infrared
C_{12}	96.4	96.0
	95.7	
C_{14}	93.6	93.3
	93.1	
C_{12}–C_{16}	96.6	96.9
C_{12}–C_{16}	96.6	96.9
C_{12}–C_{16}	96.9	95.9
C_{12}–C_{16}	93.9	94.8
C_{18}	93.1	93.7
	94.5	

Table IV. Molecular Sieve Adsorption Studies

Compound	Temp., °C.	Time, hours	Weight % adsorbed
n-Bromohexadecane	26	2¼	77.0
n-Bromohexadecane	26	4¼	86.2
n-Bromohexadecane	26	6	88.6
n-Bromohexadecane	99	5½	100.0
Lauroyl peroxide	99	5½	100.0

addition of sieves. These data are shown in Figure 3. It is evident that 2-bromobutane was not adsorbed by 5-A sieves. The nonadsorption of secondary alkyl bromides was further confirmed by the exclusion of the reaction product of 11-tricosene with hydrogen bromide.

Examination of Synthetic Blends. Because of the lack of pure olefins in the C_{14} range, the impurities used to blend with 1-tetradecene were isolated from a commercial C_{14} alpha-olefin. The impurities were separated by contacting the alpha-olefin with 5-A Molecular Sieves for 18 hours at the reflux temperature of 2,3-dimethylbutane. After removal of the solvent, the impurities were blended into API 1-tetradecene. It can be seen from Table II that there is good agreement between the actual normal alpha-olefin content of the sample and the normal alpha-olefin determined by this method.

Hydrobromination Analysis of Alpha-Olefin Samples. Table III is a comparison of the analysis of alpha-olefin samples by the hydrobromination and infrared techniques (*8*). The samples that were analyzed were formed by an ethylene polymerization process. Since the olefins contain little if any non-beta-branched alpha-olefins, there is good agreement between the methods of analysis. In the future, when normal alpha-olefins are prepared by other processed especially wax cracking, and non-beta-branched alpha-olefins are present, the hydrobromination technique may be employed; whereas, the infrared technique will be in error to the extent of non-beta-branched alpha-olefin.

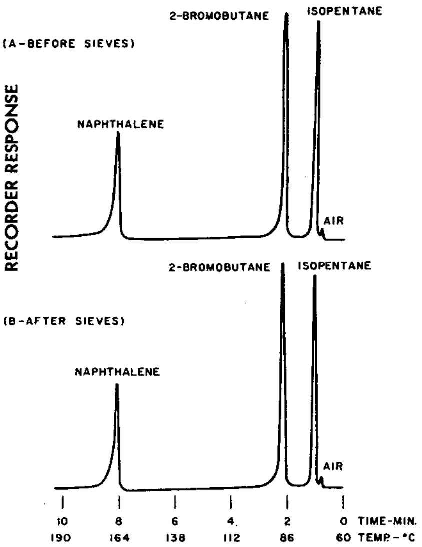

Figure 3. Programmed chromatograms of 2-bromobutane and naphthalene in isopentane

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Reflectance Fluorescence Spectra of Aromatic Compounds in Potassium Bromide Pellets

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▶ The reflectance fluorescence excitation and emission spectra of solid aromatic compounds were measured in potassium bromide pellets in the concentration ranges 0.00002 to 0.2 mmole of compound per gram of potassium bromide. In some instances the fluorescence spectra were identical with those of solution spectra, whereas in others the maxima were shifted to longer wavelengths. For some hydrocarbons the appearance of the spectra was concentration-dependent. The fluorescence excitation and emission maxima of solid charge transfer complexes were also measured in potassium bromide pellets.

THE fluorescence spectra of dilute solutions of aromatic hydrocarbons have in recent years been investigated extensively (*17*) and are often used in analytical work (*18*). However, comparatively few studies have been carried out on the fluorescence characteristics of aromatic hydrocarbons in solid form. The fluorescence of solids has been studied using powdered crystalline materials (*13*, *14*), single crystals (*12*, *15*), microcrystalline suspensions (*6*), and microcrystalline deposits on glass plates (*2*). In the present work the fluorescence of solid aromatic hydrocarbons was investigated in potassium bromide pellets. The pellet technique was first developed for infrared absorption spectrometry and it is now widely used for this purpose. More recently pellets were also used for ultraviolet and visible absorption spectrometry (*10*, *19*).

The reflectance fluorescence method was developed for the examination of weak charge transfer complexes of biological interest. Such application will be described elsewhere. In the course of this work a number of observations were made on the fluorescence of solids and these are examined in this report. The method also offers the obvious advantage that absorption, fluorescence, and infrared spectra can be measured on the same sample.

EXPERIMENTAL

Purification of Aromatic Compounds. All compounds used in this work were purified by one or more of the following procedures: column chromatography, crystallization, and vacuum sublimation. Purity was established by melting points, ultraviolet absorption spectra, and the absence of other fluorescent compounds as shown by thin-layer chromatography on silica gel plates (*1*) (solvent: cyclohexane-benzene, 1:1).

Preparation of Periflanthene [Diindeno(1,2,3-cd:1′,2′,3′-lm)perylene]. This hydrocarbon was prepared by self-condensation of fluoranthene with sodium amide in xylene (*7*). The compound was purified by vacuum sublimation to give red crystals (m. p. > 360° C.).

Anal. Calcd. for $C_{32}H_{16}$: C, 95.97; H, 4.03. Found: C, 96.08; H, 4.03.

1,3,5-Trinitrobenzene. Commercial grade material (K & K Laboratories, Jamaica, N. Y.) was purified by repeated crystallization from 95% ethyl alcohol (m.p. 123–5° C.).

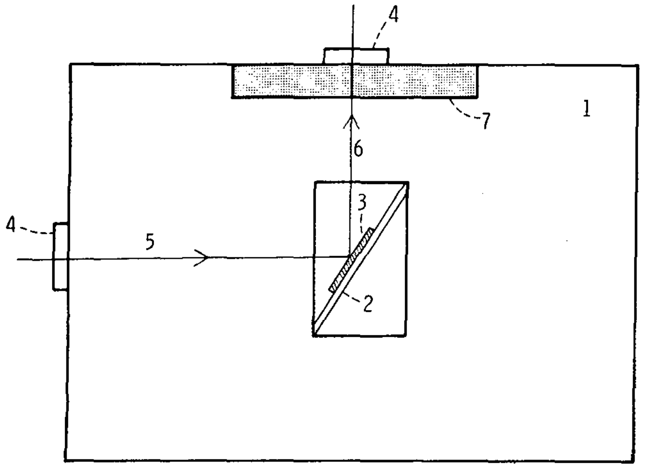

Figure 1. Sample area for measurement of fluorescence of aromatic hydrocarbons in KBr pellets

1. Sample area of Farrand spectrofluorometer
2. Sample holder
3. KBr pellet
4. Slit
5. Monochromatic exciting light
6. Fluorescence emitted
7. Secondary filter

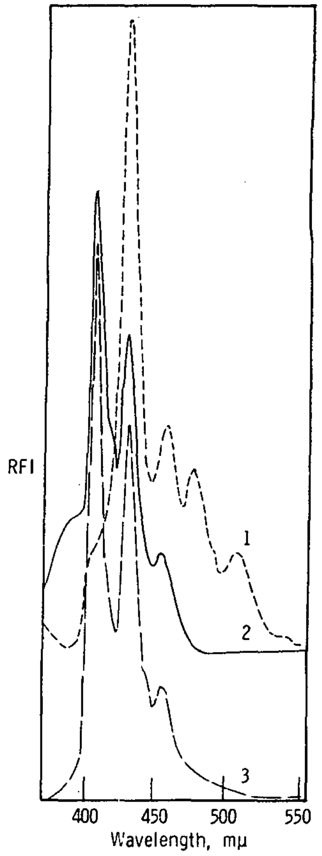

Figure 2. Fluorescence emission spectra of dibenz(*a,h*)-anthracene

1...... 0.2 mmole per gram KBr. Primary filter 7-54. Secondary filter 3-75. Excitation at 300 mμ
2——— Same as 1 but at 0.0002 mmole per gram KBr
3– – – DBA in cyclohexane solution, 0.004 mmole per liter

Solvents. The solvent used in the measurement of solution spectra was spectroscopic grade cyclohexane (Matheson, Coleman and Bell, East Rutherford, N. J.). In the preparation of pellets fluorometric grade benzene (Hartman-Leddon Co., Philadelphia, Pa.) was used.

Potassium bromide. Infrared quality potassium bromide (Harshaw Chemical Co., Cleveland, Ohio) was used for the preparation of pellets.

Fluorescence Instrumentation. A Farrand automatic recording spectrofluorometer (Farrand Optical Co., Inc., New York, N. Y.) equipped with a high intensity 150-watt d.c. xenon arc (Hanovia Lamp Division, Newark, N. J.) was used. Slit widths were adjusted according to requirements. The pellets were examined with proper choices of glass color filters (Corning Glass Works, Corning, N. Y.) and were also compared with spectra obtained without filters. A 1P28 multiplier phototube was used for all measurements. Solution spectra were measured in a quartz fluorescence cell (10 × 10 × 50 mm.). Potassium bromide pellet spectra were measured using potassium bromide disks, 0.7 to 1.0 mm. thick, 13.0 mm. in diameter, which were prepared with an evacuable potassium bromide die. The die is the same as that used for infrared spectrometry (Research and Industrial Instrument Co., London, England). The pellet was placed on a black nonfluorescent metal holder, the same as that used for the examination of paper chromatogram strips (Farrand Optical Co.).

The positioning of the pellet with respect to incident light and fluorescence emitted is shown in Figure 1. The pellet is positioned at an angle of 30° with respect to the incident beam. This angle is preferable to the 45° angle. in that specularly reflected incident light will be greater at the 45° angle. With the arrangement shown here the

Table I. Fluorescence Emission Spectra of Solid Aromatic Compounds

Compound	Concn., mmole/g. KBr	Filters[a] Primary	Secondary	Excitation wavelength, mμ	Emission maxima, mμ	Emission maxima reported, mμ
Naphthalene	0.02–0.2	7-54	0-54	315	340	348[b]
Anthracene	0.05	1-64	3-75	375	425, 447, 475(sh), 515(sh), 550(sh)	427, 445, 470, 505(sh),[c] 448, 475, 535, 575[b]
	0.00002		See text			
Phenanthrene	0.00002–0.2	7-51	1-64	370	390, 405, 430, 455	388, 408, 433, 460,[c] 385, 415, 434, 460, 485[b]
Chrysene	0.00002–0.02	7-54	3-75	300	405, 425, 450	417, 440, 462[b]
Fluorene	0.00002–0.02	7-54	0-51	315	405, 428, 452, 475	410, 430, 465, 491[b]
Pyrene	0.02–0.002	1-64	3-75	370	463, 485(sh)	475[b]
Fluoranthene	0.00002–0.02	1-64	3-75	385	438(sh), 460, 495(sh)	475[b]
Benzo(*a*)pyrene	0.02	7-54	3-75	360	480, 505(sh)	497, 520, 547(sh)[c]
	0.00002	7-54	0-51	360	387, 405, 425, 450, 485(sh)	
Dibenz(*a,h*)anthracene	0.2	7.54	0.52	375	428, 450, 470, 505(sh)	424, 452, 472, 505, 545[c]
	0.00002	See Figure 2				
Perylene	0.0002–0.05	7-59	3-70	375	535(sh), 568	579[b]
Benzo(*g,h,i*)fluoranthene	0.2	1-64	3-75	395	465, 487	...
	0.00002	1-64	3-75	395	425, 452, 465, 487, 520	
Periflanthene[d]	0.001	...	3-69	410	590, 620(sh)	...
Tricycloquinazoline[e]	0.01	7-39	3-72	355	530, 565	...

[a] Color glass filters (Corning Glass Works). Other filter combinations can in some instances be used.

[b] (*12*).

[c] Measurements made on an energy-recording spectrofluorometer (*14*) by D. T. Palumbo, Sylvania Electric Products, Inc., Towanda, Pa.

[d] Synthesis described under Experimental.

[e] Obtained from R. W. Baldwin, Cancer Research Department, The University, Nottingham, England.

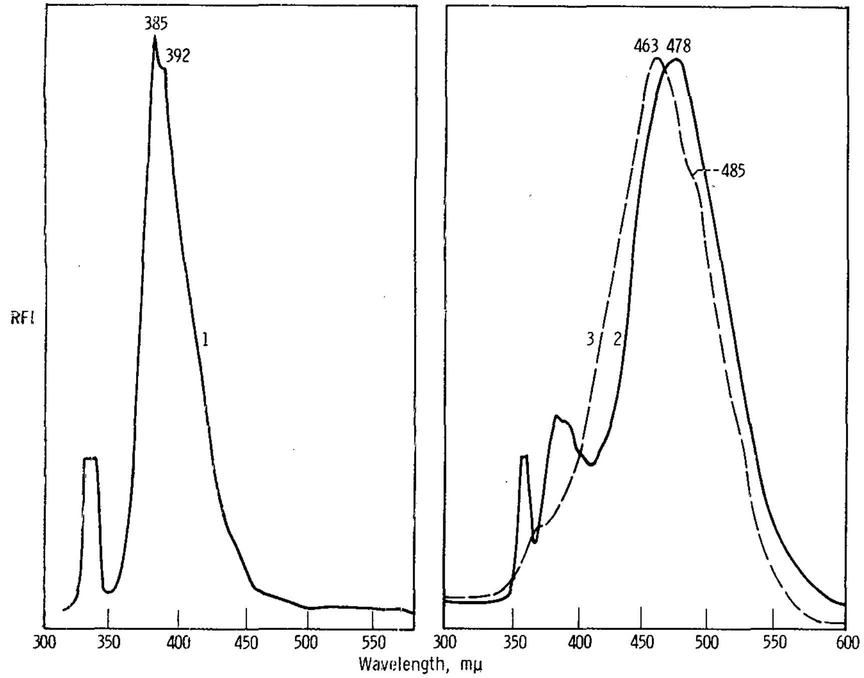

Figure 3. Fluorescence emission spectra of pyrene

1——— 0.2 mmole per liter in cyclohexane
2——— 20 mmoles per liter in cyclohexane
3– – – 0.0025 mmole per gram KBr

incident beam covers the entire area of the pellet. Dibenz(*a,h*)anthracene in cyclohexane and quinine sulfate in water were used to calibrate the spectrofluorometer for intensity (*16*). All spectra were measured at room temperature.

Ultraviolet Absorption Instrumentation. These spectra were obtained with a Beckman DU spectrophotometer equipped with an automatic recording unit (Process and Instruments Co., Brooklyn, N. Y.) using 10 × 10 × 50 mm. quartz cells.

Preparation of Pellets. The pure hydrocarbon or heterocyclic, in weights ranging from 1 to 10 mg., was crushed and mixed in an agar mortar with 200 mg. of potassium bromide. Smaller amounts of hydrocarbons, 1 μg. to 1 mg., were added to 200 mg. of potassium bromide as aliquots of dilute solutions of the hydrocarbons in benzene followed by freeze drying. The pellets were prepared directly from these mixtures. For the preparation of trinitrobenzene complexes the donor and acceptor in equimolar proportions (0.01 mmole of each) were dissolved in benzene (1 ml.) and added to 200 mg. of potassium bromide. Benzene was removed in a high vacuum, the residue was crushed and mixed, and pellets were prepared as described above.

RESULTS AND DISCUSSION

The fluorescence spectra obtained with 13 solid aromatic compounds in potassium bromide pellets are given in Table I.

Concentration Effects and Sensitivity. Within the range of concentration examined the following hydrocarbons did not show changes in fluorescence emission spectra with change in concentration: naphthalene, chrysene, fluorene, fluoranthene, and perylene. However, anthracene, phenanthrene, benzo(*a*)pyrene, dibenz(*a,h*)anthracene, and benzo(*g,h,i*)-fluoranthene exhibit concentration effects similar to the quenching and reabsorption phenomena encountered with solution spectra (*16*). Anthracene shows at 0.00002 mmole per gram of potassium bromide a new peak at 405 mμ, whereas the other peaks shown in the table become weaker peaks or shoulders on the 405-mμ peak.

Bowen and Lawley (*6*) measured the fluorescence of microcrystalline suspensions of anthracene. They found that the shorter wavelength maxima, at 405 and 425 mμ, become more intense as the anthracene particle size decreases; at the smallest particle size examined, the spectrum is similar to that of anthracene in solution. The present findings with anthracene pellets and concentration dependence parallel Bowen and Lawley's (*6*) finding with particle size. Phenanthrene shows the same series of peaks throughout the concentration range examined, but at high dilution (0.00002 mmole per gram of potassium bromide) the 390-mμ peak, which is a shoulder at higher concentrations, becomes the most intense peak. Benzo(*a*)pyrene shows a notable concentration dependence. At high concentration (0.02 mmole per gram of potassium bromide) it shows a single maximum at 480 mμ with a shoulder at 505 mμ. At high dilution the series of shorter wavelength maxima appear. Concentration dependence in the case of dibenz(*a,h*)-anthracene is illustrated in Figure 2.

Concentration effects were not examined for periflanthene and tricycloquinazoline.

The fluorescence of solid hydrocarbons in pellets could be recorded without difficulty in most instances at concentrations as low as 0.00001 mmole per gram of potassium bromide, with the proper choice of filters. The order of fluorescence intensity for anthracene, fluorene, fluoranthene, benzo(*a*)pyrene, chrysene, and dibenz(*a,h*)anthracene, all at concentrations of 0.00002 mmole per gram of potassium bromide, is the same as that of a dilute solution of dibenz(*a,h*) anthracene containing 4 × 10^{-6} mmole per ml. of cyclohexane. Concentration-fluorescence intensity relationships were not examined.

Comparison with Solution Spectra. Naphthalene, pyrene, fluoranthene, and tricycloquinazoline show the same emission spectra in potassium bromide pellets and in solution. The pyrene pellet fluorescence spectrum corresponds to that of a concentrated solution (20 mmoles per liter) of pyrene in cyclohexane (Figure 3). This is expected, since pyrene is known to undergo excited state dimerization in concentrated solution (*11*). Benzo(*a*)-pyrene and dibenz(*a,h*)anthracene show the same maxima in pellet spectra at low concentrations and in solutions in cyclohexane. This identity in solid and solution spectra is shown in Figure 2 for dibenz(*a,h*)anthracene. The solid and solution spectra (*16*) are different for: anthracene, phenanthrene, chrysene, fluorene, perylene, and benzo(*g,h,i*)fluoranthene. In all these cases there is a shift to longer wavelength in going from the solution to the solid spectrum.

The excited state dimerization of pyrene and other hydrocarbons in concentrated solutions was discussed recently by Birks and Christophorou (*3*, *4*). They point out that emissions occurring at longer wavelengths in concentrated solutions and in solids are due to dimer formation.

Excitation Spectra of Solids. Most of the hydrocarbons examined showed useful excitation spectra. The fluorescence excitation and emission spectra of fluorene are given in Figure 4. Fluoranthene, which shows the same emission spectra in the solid and in solution, exhibits a shift to longer wavelength in the excitation spectrum of the solid compared to a solution (Figure 5). However, at low concentration (0.00002 mmole per gram of potassium bromide) the major excitation peak occurs at 355 mμ corresponding to the solution excitation spectrum (curve 2, Figure 5). Benzo(*a*)pyrene shows, at low concentrations, excitation maxima at 365 and

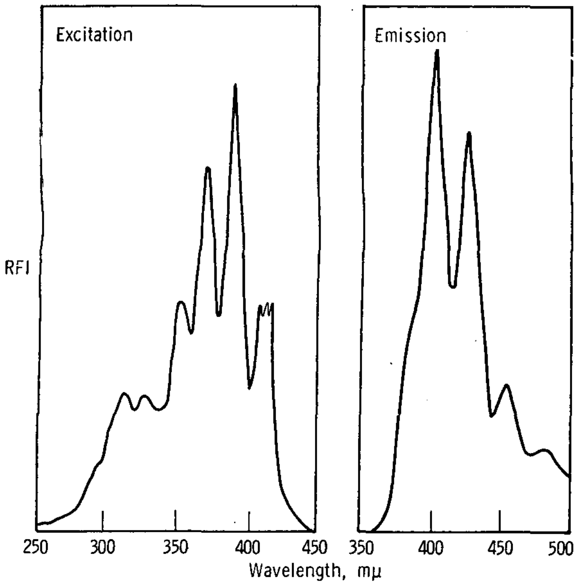

Figure 4. Fluorescence spectra of KBr pellet of fluorene

0.00002 mmole per gram KBr. Primary filter 7-54. Secondary filter 0.51. Excitation with emission at 410 mμ. Emission with excitation at 315 mμ

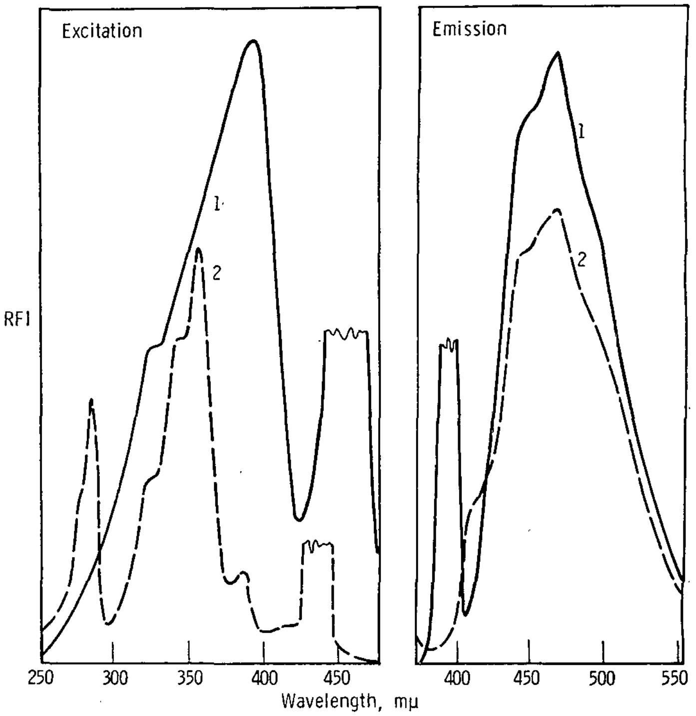

Figure 5. Fluorescence spectra of fluoranthene

1——— 0.01 mmole per gram KBr. Primary filter 1-64. Secondary filter 3-75. Excitation with emission at 460 mμ. Emission with excitation at 385 mμ
2...... 0.07 mmole per liter in cyclohexane. Excitation with emission at 440 mμ. Emission with excitation at 360 mμ

385 mμ which agree with the ultraviolet absorption and fluorescence excitation maxima of dilute solutions. Similarly, dibenz(*a,h*)anthracene shows, at low concentration, excitation maxima at 297 and 325 mμ with a shoulder at 340 mμ corresponding to the excitation and absorption spectra of solutions (*16*).

Single-Crystal and Powder Spectra. Sangster and Irvine (*12*) reported on the fluorescence spectra of a number of aromatic hydrocarbons as single crystals. The values taken from their work, and shown in Table I, were read from their published curves and will therefore be approximate. The general nature of their spectra is in agreement with that found in the present work. The other reported values were measured on an energy-recording spectrofluorometer with powdered materials. The instrument used for this purpose and the method have been described in detail (*14*). These results are in agreement with the measurements made on pellets in the present work with a different instrument. The fluorescence excitation and emission spectra obtained with powdered phenanthrene at room temperature are shown in Figure 6 for comparison with that obtained with the pellet, given in Figure 7.

Schmillen (*13*) and Sponer (*15*) recently reported on the luminescence of solid aromatic hydrocarbons as polycrystalline powders and as single crystals. Energy transfer is of importance in the examination of the fluorescence of mixtures of solids and was examined for chrysene-perylene mixtures (*13*). These and other related

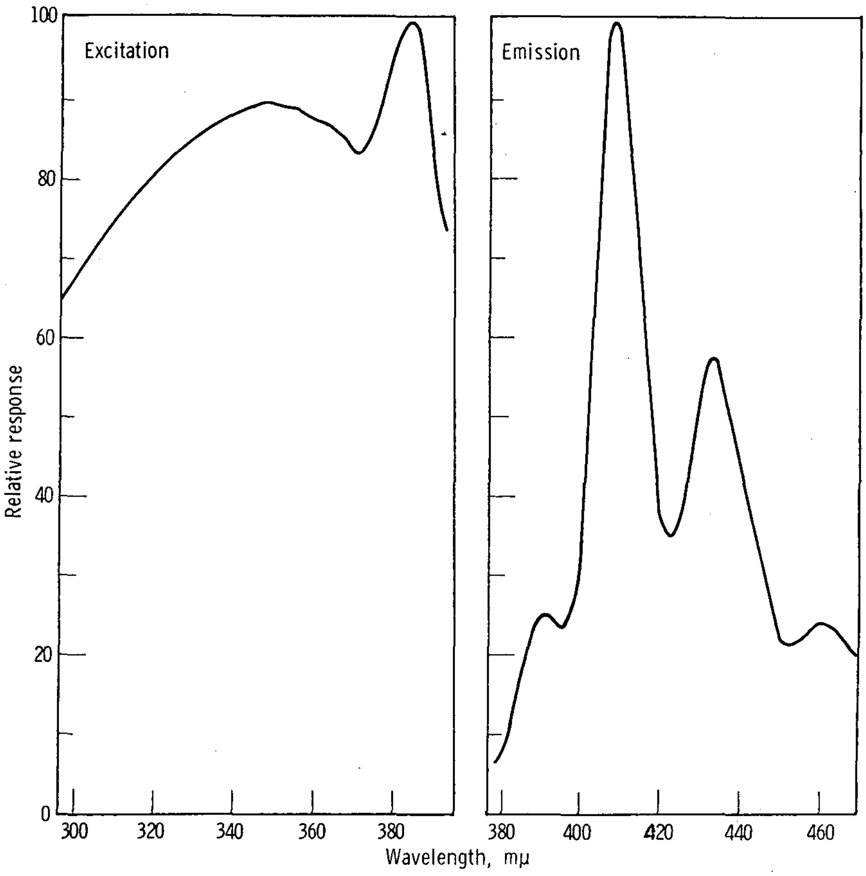

Figure 6. Fluorescence spectra of powdered phenanthrene

Measured with constant energy instrument (Perkin-Elmer Corp.) (9). Emission with excitation at 340 mμ

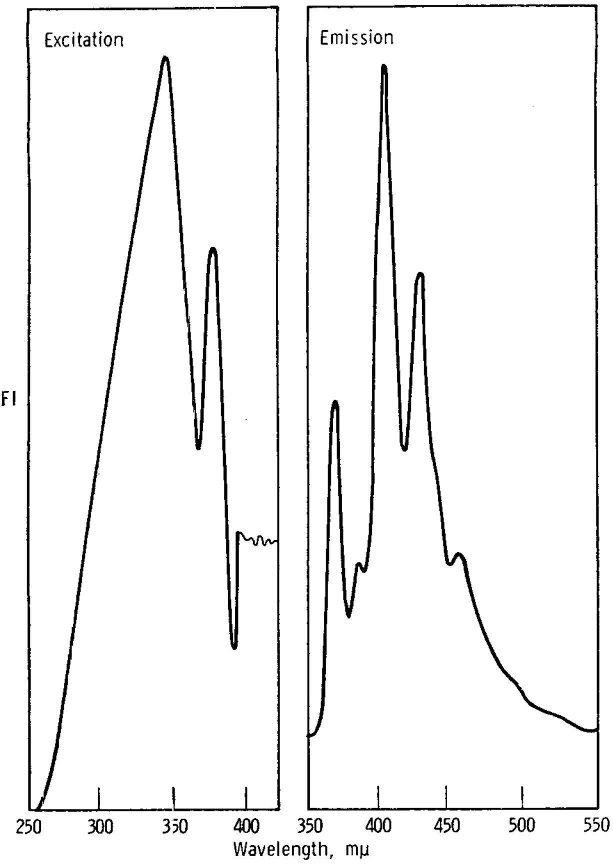

Figure 7. Fluorescence spectra of phenanthrene in potassium bromide pellet

0.02 mmole per gram KBr. Primary filter 1-64. Secondary filter 3-75. Excitation with emission at 410 mμ. Emission with excitation at 370 mμ. Spectrofluorometer (Farrand Optical Co.)

studies indicate the difficulties involved in the measurement of the fluorescence of solids, since minute traces of impurities readily influence the appearance of solid fluorescence spectra. However, with the powerful methods of purification and establishment of purity of materials, such as thin-layer chromatography, this difficulty can be readily overcome.

Fluorescence of Charge Transfer Complexes. Czekalla, Briegleb, and Herre (*8*) have measured the absorption and fluorescence maxima of frozen dilute solutions of the charge transfer complexes formed between aromatic hydrocarbons and several strong acceptors, including 1,3,5-trinitrobenzene. In the present work the reflectance fluorescence excitation and emission maxima of several trinitrobenzene complexes with aromatic hydrocarbons were examined. Some of these maxima are given in Table II together with the reported values (*8, 10*). In cases where reported values are available, they are in good agreement with results obtained on pellets, but there are some shifts in the emission maxima. The pellet method is also expected to be useful in the examination of weak charge transfer complexes, which may not be observed in dilute solutions (*5, 9*).

Table II. Absorption and Fluorescence of Solid Hydrocarbon–1,3,5-Trinitrobenzene Complexes

(Mμ)

	Solutions at −190° C. (*8*)		KBr pellets at room temperature	
Hydrocarbon	Absorption	Emission	Excitation	Emission
Naphthalene	377	513	375	530
Anthracene	463	610	462	570
Phenanthrene	373	521	373	530
Pyrene	422 (10)^a	...	460	560
Fluoranthene	...	...	360	530

^{a} KBr pellet.

ACKNOWLEDGMENT

The authors are indebted to D. T. Palumbo, Sylvania Electric Products, Inc., Towanda, Pa., for kindly examining the fluorescence spectra of several powdered hydrocarbons with a Perkin-Elmer Model No. 195 constant energy spectrofluorometer and to R. W. Baldwin, Cancer Research Department, The University, Nottingham, England, for kindly supplying a sample of tricycloquinazoline. Periflanthene was synthesized by J. A. Bilbao, formerly of these laboratories.

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Oxygen Determination in Rocks, Minerals, and Water by Neutron Activation

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▶ Oxygen in rocks and minerals is determined using the reaction $O^{16}(n,p)N^{16}$ in a 14-m.e.v. neutron flux of 7×10^8 n/cm.^{2}/second, produced by bombardment of a tritiated titanium target by deuteron beam of 500 μa. accelerated at 150 kv. The gamma-ray activity of the 7.4-second nitrogen is counted by a NaI(Tl) scintillation crystal and a multichannel analyzer. Two-gram samples are used. The method permits about 80 analyses per day and is nondestructive. The relative standard deviation achieved on a water standard is 0.39%. Using water and ignited oxides TiO_2 and Al_2O_3 as standards, oxygen was determined in rocks G-1 and W-1. Gamma activities used lie in the region of 6 to 7 m.e.v., whereas most other gamma activities of elements present in these rocks fall in the region of 0 to 3 m.e.v. The short half life of nitrogen-16 requires exact timing and a fast transfer system. In rocks with F and B the reactions $F^{19}(n,\alpha)N^{16}$ and $B^{11}(n,p)Be^{11}$ have to be taken into account.

FAST NEUTRONS have been used for analysis of trace amounts of oxygen, mainly in organic compounds, using the reaction $O^{16}(n,p)N^{16}$ (*3, 9, 10, 12*). Trace amounts of oxygen in inorganic samples have also been determined (*2, 3, 6*). In the cited work (*2, 3*), sensitivity achieved was about 30 p.p.m., and relative standard deviation varied from 10 to 25%. Winchester (*15*) used the reaction $O^{18}(d,2n)F^{18}$ to determine oxygen in quartz, kyanite, and galena, finding interference from Na and other elements.

Because of the relatively tedious nature of all conventional methods for determination of oxygen in organic and inorganic materials, a method was sought that would enable fast instrumental determination of this element in rocks and minerals, that would be compatible to the x-ray emission method presently under development at the Nevada Mining Analytical Laboratory (*13, 14*). In silicate rocks and minerals oxygen is the major component. To be of practical value, oxygen determination in these materials has to be performed with higher precision than previously achieved by similar neutron activation methods.

EXPERIMENTAL

Apparatus. The apparatus consists of a Texas Nuclear neutron generator and control, equipped with the Oak Ridge ion source (*8*). A Radiation Counter Laboratories 256 channel analyzer and a 3- × 3-inch Harshaw Integral Line NaI(Tl) crystal are used for the detection. The spectra are printed out by an IBM typewriter. A fast transfer system, similar to that described by Meinke (*7*), and built in the Oak Ridge Institute of Nuclear Studies is used. Sample transfer time is approximately 0.6 second, at 25-p.s.i. pressure. Extra-thick, 10-curie, 1.3 c. per sq. cm., tritiated titanium target is bombarded by a deuteron beam of 500 μa. accelerated at 150 kv. to produce the reaction $H^3(d,n)He^4$. The initially generated flux of 14-m.e.v. neutrons was approximately 7×10^8 *n* sq. cm./second, with decays of more than two orders of magnitude during use. This neutron flux was measured using the $Si^{28}(n,p)Al^{28}$ reaction and the known cross section for this reaction. The resulting 1.78-

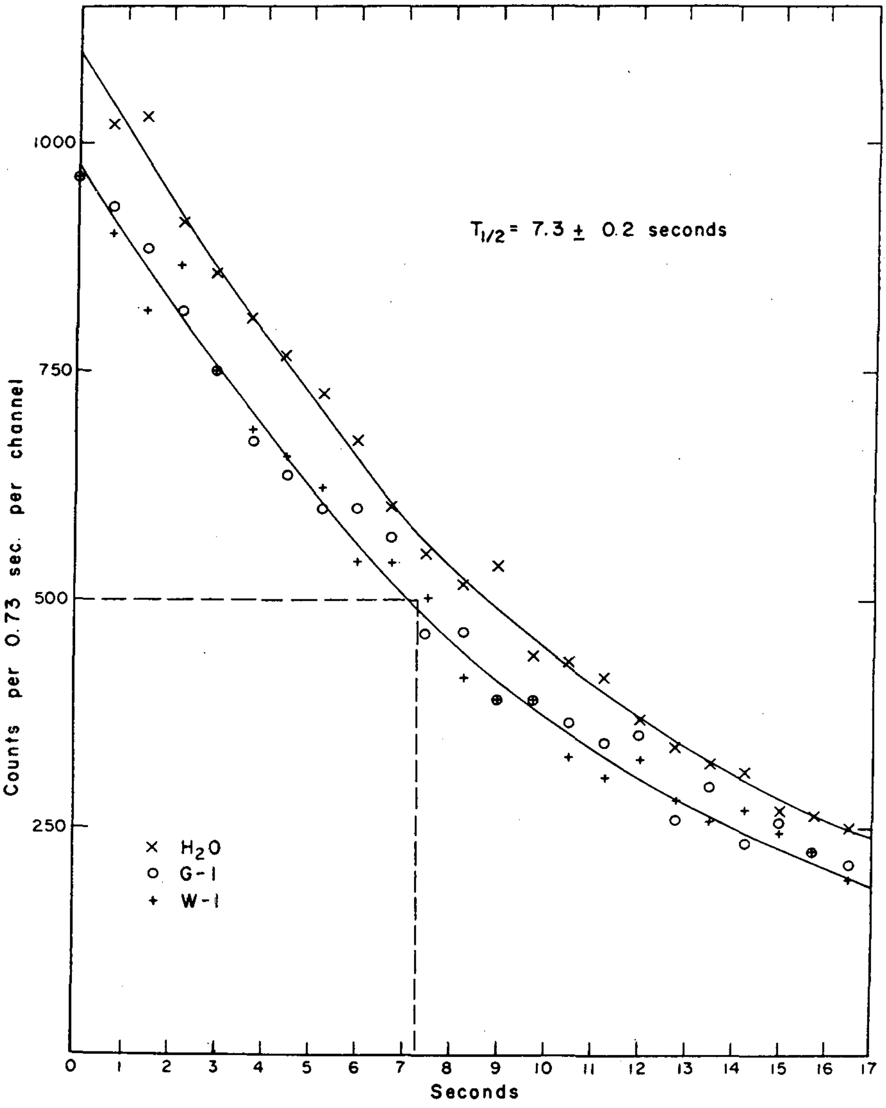

Figure 1. Decay curves for N^{16} in G-1, W-1, and H_2O; 0.73 sec. per channel

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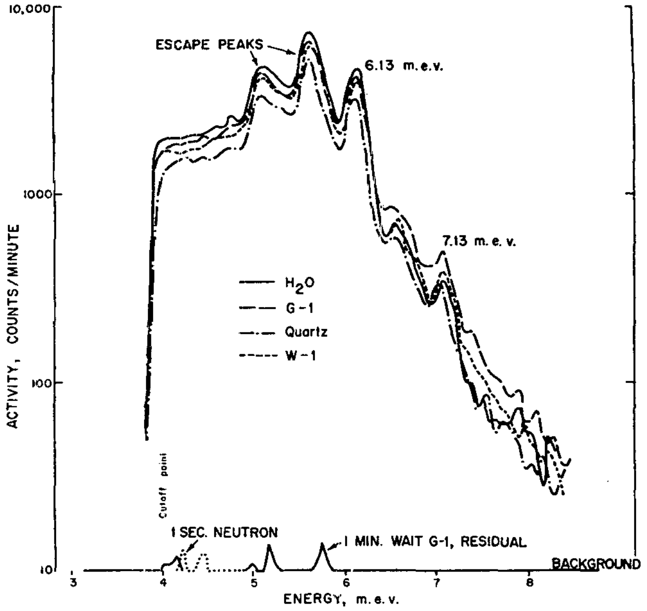

◀ Figure 2. Superimposed N^{16} gamma spectra of water, rocks G-1, W-1, and vein quartz, with residual spectra of same substances

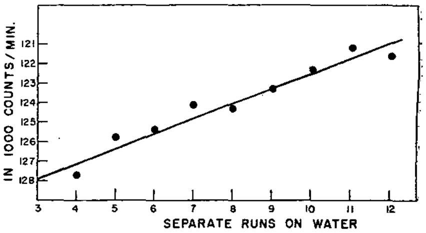

Figure 3. Precision and tritium target decay demonstrated over short period with same water sample

m.e.v. gamma ray was measured with the equipment already described.

Samples. The samples analyzed consisted of U. S. Geological Survey standards Granite G-1 and Diabase W-1 (*11*), distilled water, ignited anhydrous Al_2O_3 and TiO_2 certified by Fisher, and natural quartz. Samples were packed tightly in cylindrical containers 2.8 cm. long and 1.5 cm. in diameter with two circular flanges to reduce friction, and a screw top. Volume of the sample was 1 cu. cm. in all cases. These "rabbits" were machined from Marlex, manufactured by Phillips Petroleum Co. of Bartlesville, Okla. The weight of samples varied from 1.2 to 2.1 grams and the oxygen content from 0.5 to 1.1 grams. As oxygen is the major element in these samples, the contribution of the atmospheric oxygen trapped in the container can be ignored. If this container were filled only with air, the contribution of atmospheric oxygen would be less than 0.3 mg. This is below the detection limit of the present method.

Procedure. The activation procedure consisted of punch-button pneumatic transfer of the sample, 1-minute irradiation and return of the sample, and accumulative counting for 30 seconds. All samples were irradiated four times, alternating each time with the same water standard. This way the activation of each sample was spread over a relatively wide range of target decay of 9 minutes. Intensity ratios were computed on both sides of the sample by comparison to the activity of distilled water. In each case the mean of these two readings was used, and values given represent the mean of four such readings. The empty, air filled, Marlex rabbit showed no detectable activity in the oxygen spectrum region, with the counting apparatus used.

Table I. Precision of Oxygen Determination in Water by Neutron Activation

Counts/min.	Counts corrected for target decay	
127698	124550	Std. dev.
125751	123450	S = 480 counts
125375	123800	
124115	123350	Rel. std. dev.
124321	124250	C = 0.39%
123331	124000	
122316	123800	Oxygen—88.89%
121231	123500	
121559	124650	

RESULTS

Possible interference from other elements in the N^{16} spectrum region from 4 to 8 m.e.v. was investigated in G-1 and W-1 by taking residual spectra after a time lapse of 1, 2, and 3 minutes and comparing them with residual spectra of water. No residual activity was detected. To assert this, half lives of the N^{14} activity in rocks G-1 and W-1 were determined and decay curves compared with that of water. The half life was determined graphically as $T_{1/2} = 7.3 \pm 0.2$ seconds, for both rocks (Figure 1). In addition, the oxygen spectra of water, G-1, W-1, and vein quartz were superimposed on a logarithmic scale to demonstrate their similarity (Figure 2). Background or residual spectrum for G-1, and the background for 1-second counting in neutron flux are also shown on this figure. Discrimination was set at approximately the 4-m.e.v. energy level, or channel 55 of the multichannel analyzer. Under these instrumental conditions and with the 0.6-second delays caused by transfer mechanism, the only interfering reactions are $F^{19}(n, \alpha)N^{16}$, and $B^{11}(n,p)Be^{11}$ (*9, 10, 12*). All other gamma activities lie in the region of 0 to 3.0 m.e.v.

Precision was determined by consecutive runs on distilled water, taking the target decay into account (Table I). From Figure 3 one can see that the target decay can be considered to be linear over a short period [see also Figure 1, in (*10*)]. The relative standard deviation of 0.39% so achieved, is to be regarded as the ultimate with the nonspecialized equipment used. The main difficulty in some runs was the fluctuating accelerator beam current. Microamperage had to be controlled manually and neutron flux variation could not be avoided. Because of this, deviations from the mean on rock and oxide powders varied from 0.0 to 3% of the amount present, and about 10% of all determinations had to be discarded. These fluctuations were measured and recorded by an associated alpha particle counting system.

In this system, alpha particles of approximately 3 m.e.v. energy are counted by a 0.8-mm.^{2} silicon surface barrier detector placed 26 inches from the target. This detector is followed by a Tennelec low-noise preamplifier Model 100A, and a power supply Model 900. The amplified pulses are fed to a Victoreen DD-2 amplifier Model 651A and a single channel analyzer. The amplifier gain is adjusted so that the alpha particle peak is about 50 P.H. units high, and the spectrum integrally counted above 30 P.H. units.

It was found that when the fluctuation occurred during the later stages of the irradiation, even the normalization of results based on the alpha particle count did not produce statistically satisfactory results, sometimes giving deviations of up to 4% from the mean. Some series of runs gave good precision as demonstrated in the case of water. Occasionally, however, unstable periods of runs resulted, and therefore it was necessary to discard some of the results. This difficulty strongly suggests the use of a dual transfer and counting system to enable simultaneous activation of the standard and the unknown.

The rock powders G-1 and W-1, quartz, Al_2O_3, TiO_2, and H_2O were packed into the Marlex containers so as to cover a reasonable range of oxygen content. To avoid dead time corrections and other statistical errors the sam-

Table II. Determination of Oxygen in Rocks G-1 and W-1 and Some Oxides by Neutron Activation

			Oxygen			
No.	Sample	Sample wt., mg.	Theoretical, mg.	Detd., mg.	Theoretical, %	Detd., %
1	H_2O	1250	1111	1110	88.88	88.80
20	G-1	2133	1040	1035	48.76	48.52
34	W-1	2040	915	910	44.85	44.61
6	Quartz	1697	904	885	53.27	52.15
51	Al_2O_3	1708	804	815	47.07	47.72
52	TiO_2	1300	521	520	40.07	40.00

ple weight of rocks G-1 and W-1 was chosen to contain an amount of oxygen comparable with the water sample. A linear calibration curve was obtained on samples of different density over the range from 500 mg. to 1000 mg. of oxygen, based on intensity ratios of sample to water, (Figure 4). Numbers near calibration points indicate number of independent analyses. Milligrams of oxygen are given per 1 cu. cm. of powder. The fact that the samples differ in weight and density indicates that the absorption effect of the high energy gammas in these materials can be ignored. This suggests negligible effects due to the density of the sample in this case. Each analysis is based on approximately 120,000 counts with no detectable background.

The analytical results are compiled in Table II. These results show that with the nonspecialized equipment used it is possible to determine oxygen directly from rock powders with an accuracy of about 5 to 10 p.p.t. The theoretical oxygen content was calculated on the basis of major oxides on as-received basis as given by Stevens, page 78 (*11*). Recently Ingamells and Suhr (*5*) have partly reanalyzed and recalculated the rocks G-1 and W-1, taking into account all trace element data available. As expected, these data when converted to equivalent oxygen, do correspond better with the total oxygen determined by us, giving for G-1 and W-1 48.51 and 44.81% oxygen, respectively. Considering the influence of the adsorbed water on the total oxygen of a rock powder, and the relatively poor precision with present nonspecialized equipment, the difference between these and the neutron activation data has to be considered insignificant.

The cost of each determination in terms of the decaying target was about three dollars. The time required for one determination was 5 to 10 minutes. Improved targets, magnetic beam deflection, and operation at lower yields but with larger crystals can undoubtedly bring the target cost down to 25 to 50 cents per determination.

DISCUSSION AND CONCLUSIONS

The instrumental nondestructive neutron activation analysis of oxygen in rocks, minerals, and oxides is a fast and relatively interference-free analytical method. Because of insignificant interference it is comparable to absolute methods. It makes independent checks of totals of oxides in rock and mineral analyses possible. When precision and accuracy of this method are improved by designing a dual transfer and counting system, the determination of the ferrous/ferric ratio in rocks may become feasible. This will depend, however, on our ability to achieve higher accuracy in the determination of all other constituents. This neutron activation method for oxygen is compatible in speed and precision with the x-ray emission method for other constituents. Currently it appears to be superior in precision and sensitivity to the recently developed oxygen analysis by soft x-rays (*1, 4*) in rocks. The main difficulties of this method result from the short life of nitrogen-16. A fast transfer system and exact timing are therefore required. This is also the main cause for the sensitivity of this method to neutron flux fluctuations.

In geology and petrology the ability of fast total oxygen determination from rocks and minerals makes the comparison of oxygen content of similar rocks at different depths feasible in connection with deep drilling programs. In difficult chemical analyses of meteoritic material, artificial minerals, or glasses, or other complex compounds, the ability to analyze for oxygen nondestructively brings an independent check that should enable the analyst to interpret the results better. In mineralogy and crystallography stoichiometry problems such as the deficiency of sulfur in certain sulfides or silicon in certain silicates and phosphorus in certain phosphates, for example, may be solved by the determination of total oxygen.

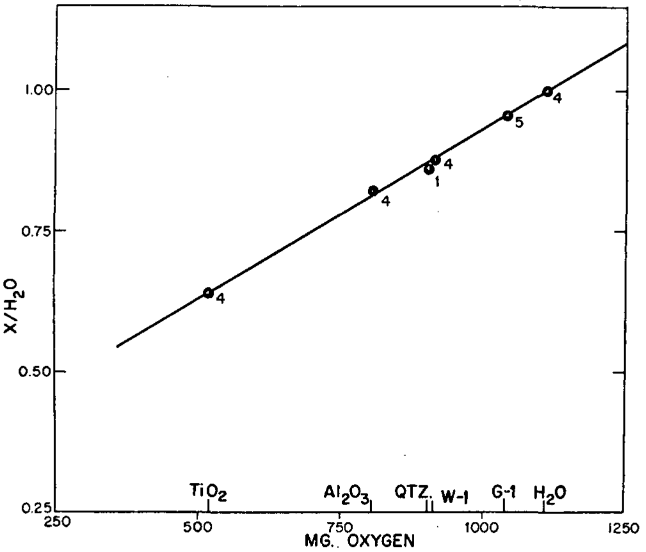

Figure 4. Calibration curve for oxygen in rocks G-1 and W-1 by neutron activation

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Behavior of Demeton in Electron Affinity Detectors

Sir: Electron affinity detectors are being more widely used in gas chromatography for determining pesticides at the submicrogram level. Halogen-containing pesticides have been most commonly determined (*3*, *5*, *16*) because of the high capture cross section of these compounds for electrons; applications of these detectors to some phosphate pesticides have been reported (*1*, *13*, *15*). The behavior of some organic compounds in electron affinity detectors have been studied and the results have been interpreted with regard to relative electron affinities of the compounds (*2*, *9*–*12*). This communication describes the behavior of one phosphate pesticide, demeton (trade name Systox, Chemagro Corp., Kansas City, Mo.), in two electron affinity detectors; the behavior of other phosphate pesticides in several electron affinity detectors will be described in a subsequent paper. Demeton shows an anomalous response in one of the detectors compared to other halogenated and phosphate pesticides that we have studied. Although similar behavior has previously been reported for one or two compounds (*10*), its significance on the design of electron affinity detectors and the interpretation of chromatograms has been neglected.

EXPERIMENTAL

Apparatus. The gas chromatograph was a Micro-Tek Model GC-2500 R, equipped with a Micro-Tek GC 2500-074 electron affinity detector and d.c./pulse variable voltage power supply. The pulse voltage was nominally one microsecond on and 50 microseconds off. The column was 0.6 meter of 6-mm. o.d. borosilicate glass tubing packed with 2% SE-30 and 0.2% Versamid 900 on 100- to 110-mesh Anakrom ABS. Commercial grade nitrogen, purified by passage through a molecular sieve trap, was used as a carrier gas for the d.c. measurements; 90% argon–10% methane (The Matheson Co., Inc.) was used as carrier gas for the pulsed studies.

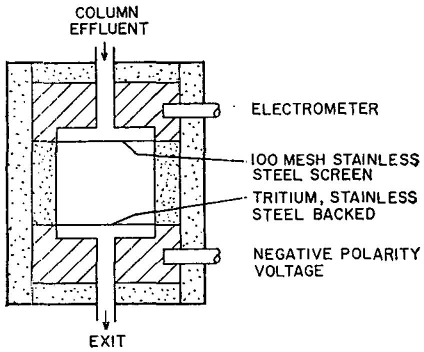

Figure 1. Electron affinity detector

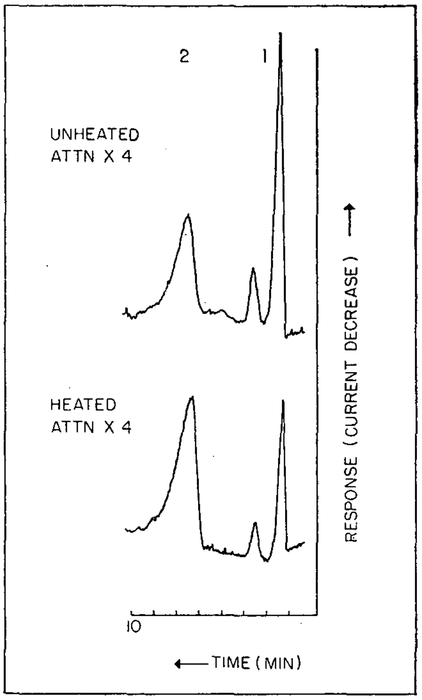

Figure 2. Chromatograms of heated and unheated demeton (Systox)

Sample: 100 ng. in 1 μl. of benzene. Temperature: column, 130° C.; detector, 180° C.; inlet, 190° C. Flow: column, 50 ml./min.; detector, 200 ml./min. Detector: electron affinity, normal pulsed mode; polarizing voltage, 10 volts

1. Thiono isomer
2. Thiolo isomer

Electron Affinity Detector. The detector was of the Lovelock design (parallel plate), as shown in Figure 1. In the normal mode of operation, the column effluent entered the detector through the 100-mesh gauze and flowed past the stainless steel-backed tritium foil to the exit tube. In the reversed mode, the gauze was removed, and the carrier gas flowed in the opposite direction. Hence, the column effluent flowed around the foil into the open chamber and out the tube at the opposite end. In the reversed mode, the detector operated in a similar manner to some commercially available electron affinity detectors; also, the flow is the same as in the Lovelock direct electron mobility detector (*7*).

Demeton. The demeton (Systox) was furnished as analytical standard grade by Chemagro Corp., Kansas City, Mo.; it was used without further purification. Systox is a mixture of two isomers, thiono-Systox [*O,O*-diethyl *O*-2(ethylthio)ethyl phosphorothioate] and thiolo-Systox [*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorothioate]; the ratio of the thiono isomer to the thiolo isomer is usually 60:40. When Systox was analyzed by gas chromatography with electron affinity detection, two major peaks were obtained as expected. To identify the isomer producing each peak, some Systox was heated at 125°C. for $2^{1}/_{2}$ hours to convert the thiono isomer to the thiolo isomer (*4*), and another chromatogram was obtained. Both chromatograms are shown in Figure 2. The peak with the shorter retention time was produced by thiono-Systox; the other peak, by thiolo-Systox.

RESULTS AND DISCUSSION

When the electron affinity detector was operated in the normal mode, Systox yielded a chromatogram with two large peaks for both the d.c. and

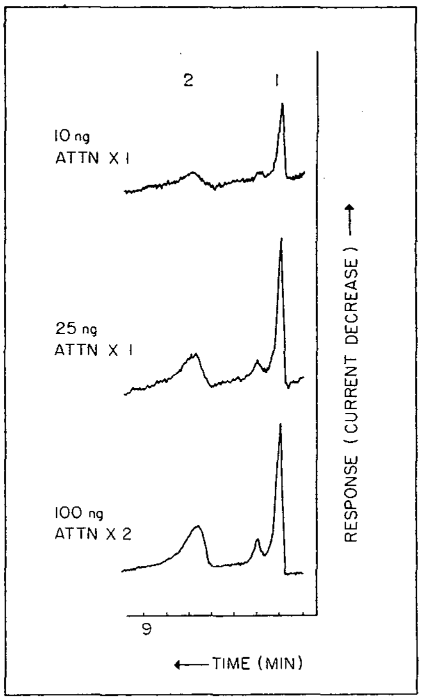

Figure 3. Chromatograms of Systox obtained with detector in normal d.c. mode

Temperature: see Figure 2. Flow: see Figure 2. Detector: electron affinity, normal d.c. mode; polarizing voltage, 20 volts

1. Thiono isomer
2. Thiolo isomer

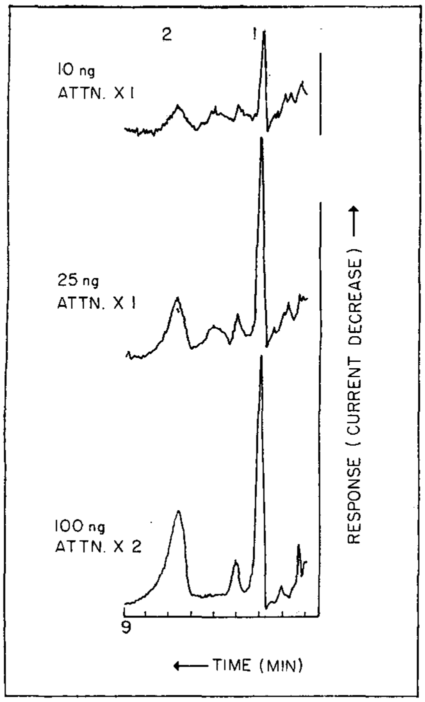

Figure 4. Chromatograms of Systox obtained with detector in normal pulsed mode

Temperature: see Figure 2. Flow: see Figure 2. Detector: electron affinity, normal pulsed mode; polarizing voltage, 10 volts
1. Thiono isomer
2. Thiolo isomer

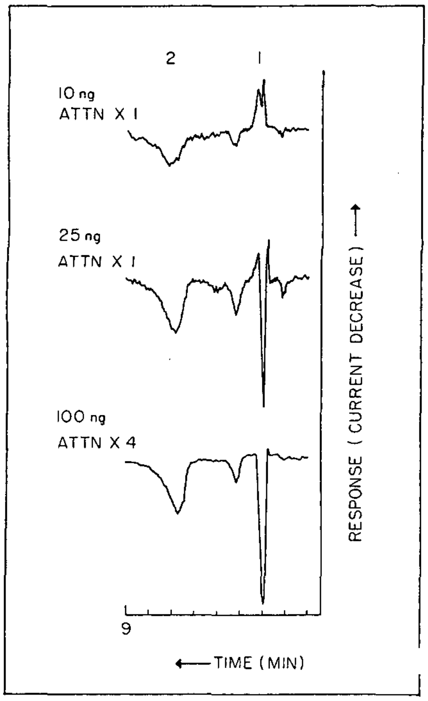

Figure 5. Chromatograms of Systox obtained with detector in reversed d.c. mode

Temperature: see Figure 2. Flow: see Figure 2. Detector: electron affinity, reversed d.c. mode; polarizing voltage, 30 volts
1. Thiono isomer
2. Thiolo isomer

pulsed modes of detection as shown in Figures 3 and 4. A similar detector has been reported not to respond to Systox (*1*). While the signal-to-noise ratio produced by Systox was not as large as for other phosphate pesticides studied, good sensitivity was obtained. Although the small peak following the thiono-Systox peak was not positively identified, it may be produced by the sulfoxide. In all cases the detector was operated at the polarizing voltage which produced the maximum sensitivity on the voltage-response curve. Peak areas for both isomers, as measured with a polar planimeter are given in Table I.

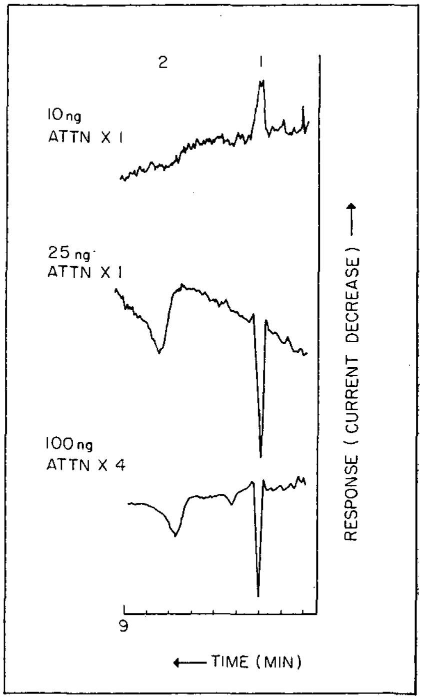

Figure 6. Chromatograms of Systox obtained with detector in reversed pulsed mode

Temperature: see Figure 2. Flow: see Figure 2. Detector: electron affinity, reversed pulsed mode; polarizing voltage, 30 volts
1. Thiono isomer
2. Thiolo isomer

Because of the effect of the carrier gas (*2*), the detector was more sensitive to Systox in the pulsed mode than in the d.c. mode.

When the electron affinity detector was operated in the reversed mode, the thiono and the thiolo isomers produced a signal for both the d.c. and pulsed modes of detection, as shown in Figures 5 and 6, but the thiolo isomer gave an increase in current at all amounts of the isomer. This increase must be produced by ionization instead of electron capture. The response of the detector to the thiono isomer is dependent upon the amount of the isomer in the detector. Electron capture occurs when the amount of isomer is small, while ionization occurs when the amount is large. This reversal of behavior is clearly shown in Figures 5 and 6.

Table I. Response of Electron Affinity Detector to Systox Isomers

Amount, ng.	Peak area (sq. cm.)			
	Pulsed		D.c.	
	Thiono	Thiolo	Thiono	Thiolo
10	10.0	5.4	2.7	2.2
25	23.4	17.6	5.0	3.0
100	49.2	50.4	11.0	9.2

The variation with polarizing voltage in the shape of the peak produced by each isomer when the detector was operated in the reversed mode, is shown in Figure 7. At and above 5 volts thiono-Systox produced a double peak; below 15 volts, electron capture predominates; above 15 volts, ionization predominates. Thiolo-Systox shows capture below 10 volts and ionization above 10 volts.

Normal chromatograms, showing only peaks resulting from electron capture, were obtained for the voltage range up to 60 volts with the reversed mode detector for halogenated compounds

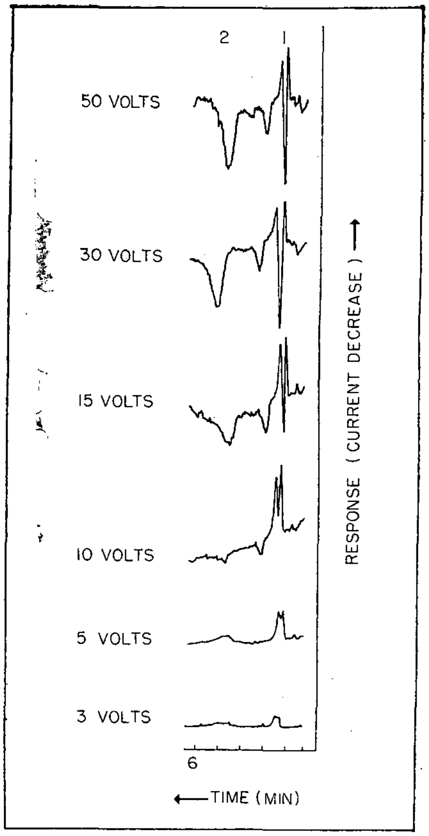

Figure 7. Variation of peak shape with polarizing voltage

Sample: 15 ng. of Systox in 1 μl. of benzene. Temperature: column, 135° C.; detector, 170° C.; inlet, 180° C. Flow: see Figure 2. Detector: electron affinity, reversed d.c. mode
1. Thiono isomer
2. Thiolo isomer

such as carbon tetrachloride and for parathion and methyl parathion.

In gaseous electronic detectors employing radioactive sources to provide a source of free electrons, ionization and electron capture must be competing processes (*6*). The kind of interaction that predominates will be determined by the types and concentration of the compounds present in the gas stream and by the geometry of the detector. Ionization as well as electron capture may be produced at low applied voltages at near-atmospheric pressure with electrons. Otvos and Stevenson (*14*) observed ion production in a number of gaseous hydrocarbons upon irradiation with electrons from carbon-14 and strontium-90 while Lovelock and Lipsky (*10*) observed an increase in current for cyclohexane in an electron affinity detector when radium was used as the source. The latter authors recognized that ionization can occur at higher applied voltages. Low results obtained by Otvos and Stevenson (*14*) for the apparent ionization cross-sections of ammonia and Freon-12 are readily explained by postulating that the electron-capture process was competing with ionization for these compounds so as to produce a lower net relative ion production.

The first peak in Figures 5 and 6 at 25 nanograms of Systox, and the first peak in Figure 7 at voltages above 10 volts show maximum reversal when the amount of the thiono-Systox in the detector is a maximum. This change from electron capture to ionization as the concentration of the active compound in the effluent increases, indicates that both electron capture and ionization can take place in the same detector under the same operating conditions. Detector design is an especially critical variable for those compounds which will both capture and ionize. These studies show that the electron affinity detector designed by Lovelock (*7*), in which electrons move through the detector against the flow of carrier gas, minimizes the ionization phenomenon. The pulsed mode of detector operation (*8*) will not, however, eliminate interferences from ionization in a poorly designed detector.

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Latent Heats of Vaporization from Distillation Rate Data

SIR: The observation that the temperature dependence of physical rate processes follows the same relationship as the temperature dependence of chemical processes (*4*) evolved from a demonstration that chemical kinetic principles apply when physical reactions such as distillation, gaseous effusion, and dialysis occur (*6*). The following discussion shows that, for distillation rate data, a relationship exists between the energy of activation as determined from the Arrhenius equation and the latent heat of vaporization.

The energies of activation were calculated from the slopes of the lines in the plots of log k *vs.* $1/T$. Examples of such plots for benzene and *n*-butanol have been shown (*4*). By using these values for the energies of activation, the Arrhenius equation, $k = Ae^{-E/RT}$, was solved for A. These values for A of the compounds studied were in close numerical agreement. Also, the published values for the latent heats of vaporization, H_v, at the boiling points differed from the calculated energies of activation by a fairly constant value, C. It appears that the constants, A and C, depend on the geometry of the distillation set up and include the work involved in moving the vapor from the surface of the liquid to the receiver. Based on distillation rate constants and on the average values obtained for A and C, heats of vaporization were recalculated using the equation

$$k = Ae^{\frac{-(H_v + C)}{RT}}$$

All these data are presented in Table I.

Table I. Results Obtained

Compound	E (kcal. per mole)	A (mole per min.)	$C = E - H_v$ (kcal. per mole)	H_v (kcal. per mole) calcd. using av. A and C	H_v (kcal. per mole), literature values
Benzene	18.85	22.31	11.40	7.37	7.37[a]
Chloroform	18.18	22.04	11.34	6.89	6.84[a]
Methanol	19.18	22.02	10.69	7.89	8.49[a]
n-Butanol	21.16	22.25	10.73	9.67	10.45[a]
Ethyl acetate	19.19	22.08	11.51	7.84	7.63[b]
Methyl ethyl ketone	18.85	21.90	11.21	7.65	7.64[a]
n-Butylamine	18.75	21.61	11.11	7.78	7.67[c]
Water	21.12	20.77	11.39	10.88	9.73[c]
Av.		21.87	11.18		
Formic acid	20.86	22.47	10.39	9.18	10.47[d]
Acetic acid	21.38	21.98	10.07	10.10	11.31[d]

[a] Ref. (*5*).
[b] Ref. (*3*).
[c] Ref. (*2*).
[d] Ref. (*1*).

Calculations of the latent heats of vaporization for formic and acetic acids presented difficulties because of their associating vapors. The values obtained are based on a volatilization forming the equilibrium vapor and depend on the degree of association under the conditions used. Because of the uncertainties in the degrees of association for these acids, their values for *A* and *C* are not included in the overall averages shown in the table. The latent heats of vaporization calculated for these acids using the average values obtained for *A* and *C* differ somewhat from the literature values, indicating that the degrees of association reported (*1*) do not apply exactly to our experimental conditions.

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Modification of Electron Probe to Detect Carbon

Sir: The electron probe can analyze the atomic contents of a 1-micron cube at the surface of a solid sample (*2*). This is achieved by focusing a 1-micron diameter electron beam on the sample and analyzing the emitted characteristic x-rays in a vacuum x-ray crystal spectrometer. The crystal spacing, *d*, of generally available crystals prevents the detection of elements with an atomic number less than about 11 (sodium) because of the Bragg law: $n\lambda = 2d \sin \theta$.

An Applied Research Laboratory electron probe has been modified to detect carbon (Kα wavelength 44.0 A.) by making two changes: A pseudo crystal with a *d* spacing of $\sim$50 A. has been made of 200 monomolecular layers of barium stearate by the Langmuir-Blodgett technique (*3*). Monomolecular layers of barium stearate are successively picked up from an aqueous substrate. The layers go on with the barium end of the molecule alternating up and then down. This creates a layered structure consisting of planes of barium atoms separated by a distance roughly equal to twice the length of the aliphatic carbon chain. The soap film crystal was laid down on an ARL 4-inch radius crystal backing plate. C. L. Andrews (*1*) constructed a similar crystal for x-ray diffraction work in 1940.

The C Kα pulse-height distribution from the proportional counter for a pure carbon target is shown in Figure 1 and the spectral line profile is shown in Figure 2. The peak-to-background ratio is approximately 25 to 1. The ratio of 25 to 1 gives an overly optimistic estimate for the detection sensitivity of carbon in steel, for example. To give a more practical measure of carbon sensitivity in a suitation where most of the background would be coming from iron as the major component, the following experiment was done:

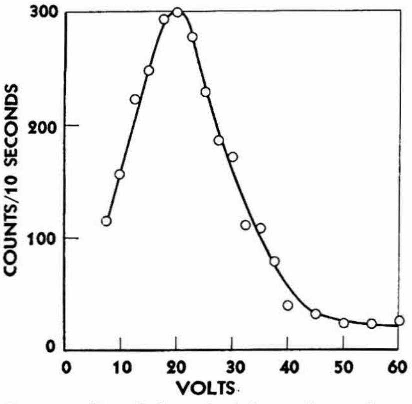

Figure 1. Pulse height of carbon Kα line distribution

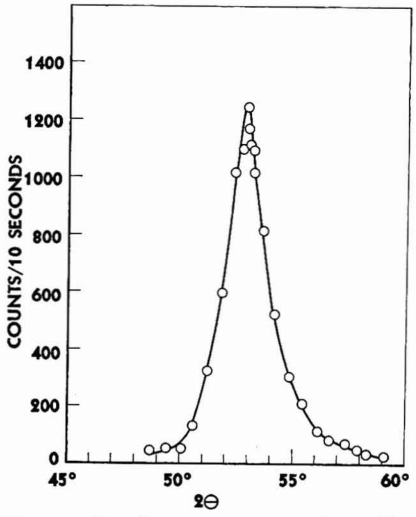

Figure 2. Scan across carbon Kα line

With the x-ray spectrometer set correctly for carbon, the counting rate decreased by a factor of 12 when the target was shifted from carbon to iron.

A sample of cast iron containing flakes of pure carbon was viewed in the scanning mode of operation. Figure 3 is a photograph of the cathode-ray tube which shows bright areas for carbon flakes and dark regions for iron. The snakelike regions of carbon are about 10 microns thick.

The operating conditions were: accelerating voltage of electron beam 6 kv. (higher voltage causes excessive white radiation which in turn causes the soap film crystal to emit secondary x-radiation; electron beam current 0.2 μa.; and electron beam diameter $\sim$4 microns. The proportional counter was filled with P-10 gas (10% methane, 90% argon) at atmospheric pressure. The counter operating voltage was $\sim$1200 volts. The central wire diameter was 0.0015 inch. The counter window was made of two superimposed layers (to avoid pin holes) of collodion (*4*) each 2000 Å. thick. The double window was supported on a nickel mesh 250 lines per inch, 50% transmission.

Figure 3. Scan over cast iron sample with carbon Kα radiation detection

Width of light areas, $\sim$10 microns

ACKNOWLEDGMENT

The help and suggestions of B. L. Henke of the Pomona College physics department are gratefully acknowledged.

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Chronopotentiometric Measurement of Adsorption by Current Reversal

SIR: The determination of the amount of an electroactive species adsorbed at an electrode is of current interest. The chronopotentiometric measurement of the amount of reactant adsorbed was described first by Lorenz (*3*); other work has recently been reviewed (*6*). Generally adsorption of the reactant causes $i_o\tau^{1/2}$ to increase as τ decreases. The extent of adsorption of the product of the electrode reaction can be studied by current reversal chronopotentiometry. Osteryoung (*5*) recently studied the iodide–iodine system by this technique and showed experimentally that when the product of the electrode reaction is adsorbed, the reverse transition time, τ_r, approaches the forward transition time, τ_f, in magnitude. This communication presents a theoretical model for the calculation of the extent of adsorption from current reversal chronopotentiometric data, and gives results for the adsorption of iodine on platinum and leuco-riboflavin on mercury.

Table I. Calculated Values of Γ from Current Reversal Data for Reduction of Riboflavin and Oxidation of Iodide Ion

A. RIBOFLAVIN. The solution contained 0.08m*M* riboflavin and 0.5*M* H_2SO_4–1*M* Na_2SO_4 buffer. The mercury electrode area was 3.08 sq. cm. (*6*)

i_o, ma. per sq. cm.	τ_f, sec.	τ_r, sec.	Γ, × 10^9 mole per sq. cm.
0.0413	40.4	15.8	0.15
0.0561	24.7	9.7	0.13
0.0811	10.9	5.0	0.26
0.114	6.0	2.8	0.22
0.189	3.0	1.6	0.34
0.233	1.8	1.0	0.30
0.316	1.2	0.70	0.36
0.454	0.80	0.50	0.40

$\Gamma_{avg.}$ (excluding first two measurements) is 0.31 ± 0.07 × 10^{-9} mole per sq. cm.

B. IODIDE ION. Data of Osteryoung (*5*). The solution contained 1m*M* iodide and 1*M* H_2SO_4. The platinum electrode had an area of 1.6 sq. cm.

0.038	97	30	...
0.050	48	19	0.18
0.062	28	13	0.50
0.120	6.8	3.6	0.48
0.190	3.0	1.7	0.43
0.250	1.5	0.94	0.41
0.310	1.0	0.71	0.51

$\Gamma_{avg.}$ (excluding first two measurements) is 0.47 ± 0.05 × 10^{-9} mole per sq. cm.

Various theoretical models for the process can be constructed, based upon assumptions concerning the relative formation and electrolysis of the adsorbed and the diffusing species, and different adsorption isotherms. A convenient model, especially useful for reversible electrode reactions, is based upon the concepts of Brdička (*2*). Although the following discussion will consider a reduction followed by a current reversal oxidation, where the reduced species is assumed to be adsorbed, the final results will hold equally well for an initial oxidation followed by a reduction. Brdička (*2*) has postulated that an adsorbed species is in a lower free energy state than a solution species, so that during the initial reduction the reduced species will first form an adsorbed monolayer, and then diffuse into the solution. Upon current reversal, the diffusing species will be oxidized first, and then the adsorbed monolayer. Therefore during the initial reduction, the flux of reduced product into the solution will be zero until time t_a, when the formation of the adsorbed monolayer is completed.

$$t_a = nF\Gamma/i_o \qquad (1)$$

where Γ is the amount of product adsorbed, and i_o is the current density. During the remainder of the forward transition time, t_d, reduced species diffuses into the solution.

$$t_d = \tau_f - nF\Gamma/i_o \qquad (2)$$

On reversal of the current, the diffusing species reacts first, for a time t_d', given by

$$t_d' = t_d/3 = (\tau_f - nF\Gamma/i_o)/3 \qquad (3)$$

The adsorbed layer of reduced species is then oxidized. The current efficiency for this last process is less than 100%, however, because the reduced species in solution continues to diffuse to the electrode surface. The flux of the diffusing reduced species during this oxidation can be calculated by a procedure similar to that of Berzins and Delahay (*1*), and the current density at any time contributing to the oxidation of the diffusing species, i_d, can be determined. The current density contributing to the oxidation of the adsorbed species, i_a, is then

$$i_a = i_o - i_d \qquad (4)$$

The final reversal transition will occur when the adsorbed species is completely oxidized,

$$t_a' = \tau_r - t_d' \qquad (5)$$

An equation for the amount of species adsorbed can be obtained by integration of $i_a dt$ from zero to t_a', which yields

$$nF\Gamma = 2i_o\left[\frac{\tau_r}{\pi}\text{ arc cos}\left(\frac{t_d' - t_a'}{\tau_r}\right) - \frac{2}{\pi}(t_d'\,t_a')^{1/2}\right] - i_o\left[\frac{(t_d + \tau_r)}{\pi} \times \text{arc cos}\left(\frac{t_d + t_d' - t_a'}{t_d + \tau_r}\right) - \frac{2}{\pi}\{(t_d + t_d')\,t_a'\}^{1/2}\right] \qquad (6)$$

Calculation of Γ using the experimental values of i_o, τ_f, and τ_r, involves simultaneous solution of Equations 2, 3, 5, and 6.

A computer program was written which utilizes an iterative method for calculating Γ when different values of i_o, τ_f, and τ_r are read in. Using this program with current reversal chronopotentiometric data obtained during the reduction of riboflavin and the oxidation of iodide (*5*), Γ-values for adsorption of leuco-riboflavin and iodine were calculated (Table I). Data at long transition times are relatively insensitive to small amounts of adsorbed species, and were not included in the averages. The value of Γ for leuco-riboflavin is in good agreement with the value computed on the basis of molecular models, 0.2 × 10^{-9} mole per sq. cm. (*6*). The value for the adsorption of iodine, based on the data of Osteryoung (*5*), agrees with that found by Lorenz and Mühlberg (*4*), 0.5 × 10^{-9} mole per sq. cm., from the direct chronopotentiometric reduction of I_3^-. Using the atomic radius of iodine, 1.35 A. and assuming the effective area of an iodine molecule to be 14.6 sq. A. a monolayer containing a maximum of 1.1 × 10^{-9} mole per sq. cm. is calculated.

This model will hold rigorously only when a polarographic pre-wave is observed. When oxidation of the adsorbed product occurs simultaneously with oxidation of the diffusing species, calculations based on this model will yield a value of Γ which is somewhat low. As in all cases of chronopotentiometric measurements of adsorption, the value of Γ obtained must be regarded as an estimate. A subsequent communication will further discuss the model presented here, as well as other models for current reversal chronopotentiometry.

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Solvents for Phosphorimetry

Sir: The development of phosphorimetry as a means of analysis is highly dependent on the availability of solvents which form clear rigid glasses rather than cracked glasses or snows when cooled down to liquid nitrogen temperature. Phosphorimetry as a means of chemical analysis was introduced in 1957 by Keirs, Britt, and Wentworth (*1*). In 1962 a review by Parker and Hatchard (*2*) appeared. In 1963, Winefordner and Latz (*3*) analyzed small concentrations of aspirin in blood with negligible interference and indicated that phosphorimetry could be applied to the quantitative analysis of trace concentrations of drugs in biological fluids. For the method of phosphorimetry to be used, suitable solvents must be readily available. Therefore, in this brief communication a large number of solvents which had been previously described in the literature were studied in order to determine the number and kinds of solvents which will form clear rigid glasses at liquid nitrogen temperature.

In Tables I and II a large number of solvents and solvent mixtures, which had previously been purified by distillation, column chromatography over alumina, etc., are listed according to type and behavior on cooling down to

Table I. Behavior of Solvents upon Rapid Cooling with Liquid Nitrogen

Kind of solvent	Form of media at 77° K.	Frequency of forming cracks or snow	Possibility for use
Hydrocarbons			
Pentane (tech.)	Clear glass	0 out of 10	Excellent
Petroleum ether	Clear glass	1 out of 10	Good
Heptane	Clear glass	9 out of 10	Not usable
Toluene	Clear glass	10 out of 10	Not usable
Methyl cyclohexane	Clear glass	9 out of 10	Not usable
Iso-octane	Snow	10 out of 10	Not usable
Cyclohexane	Snow	10 out of 10	Not usable
Benzene	Snow	10 out of 10	Not usable
m-Xylene	Snow	10 out of 10	Not usable
Hexane	Snow	10 out of 10	Not usable
Bases and *N*-containing Compounds			
Triethylamine	Clear glass	5 out of 10	Poor
Triethanolamine	Clear glass	10 out of 10	Not usable
Dimethyl formamide	Glass or snow	10 out of 10	Not usable
Isopropylamine	Clear glass	10 out of 10	Not usable
Pyridine	Snow	10 out of 10	Not usable
Diethylamine	Snow	10 out of 10	Not usable
Dipropylamine	Snow	10 out of 10	Not usable
Formamide	Snow	10 out of 10	Not usable
N,N-dimethylformamide	Snow	10 out of 10	Not usable
Acetonitrile	Snow	10 out of 10	Not usable
Ethers			
Diethyl ether	Clear glass	1 out of 10	Good
Di-*n*-propyl ether	Clear glass	9 out of 10	Not usable
Di-*n*-butyl ether	Clear glass	9 out of 10	Not usable
Methyl cellosolve	Clear glass	10 out of 10	Not usable
Ethyl cellosolve	Clear glass	10 out of 10	Not usable
Butyl cellosolve	Clear glass	10 out of 10	Not usable
Dimethoxymethane	Snow	10 out of 10	Not usable
Ethyl cellosolve acetate	Snow	10 out of 10	Not usable
Di-isopropyl ether	Snow	10 out of 10	Not usable
1,4-Dioxane	Snow	10 out of 10	Not usable
Alkyl halides			
Carbon tetrachloride	Snow	10 out of 10	Not usable
Chloroform	Snow	10 out of 10	Not usable
Bromoform	Snow	10 out of 10	Not usable
Dichloromethane	Snow	10 out of 10	Not usable
Tetrachloroethylene	Snow	10 out of 10	Not usable
1-Bromopropane	Snow	10 out of 10	Not usable
2-Chloropropane	Snow	10 out of 10	Not usable
1,2-Dibromoethylene	Snow	10 out of 10	Not usable
2-Bromobutane	Cloudy glass	2 out of 10	Good
2-Bromopentane	Snow	10 out of 10	Not usable
Alcohols			
Methanol	Clear glass	10 out of 10	Not usable
Ethanol	Clear glass	1 out of 10	Good
n-Propanol	Clear glass	2 out of 10	Good
Isopropanol	Clear glass	9 out of 10	Not usable
n-Butanol	Clear glass	4 out of 10	Poor
Isobutanol	Clear glass	10 out of 10	Not usable
4-Chloro-1-butanol	Clear glass	10 out of 10	Not usable
Isoamyl alcohol	Clear glass	8 out of 10	Poor
Glycerol	Clear glass	10 out of 10	Not usable
Ethylene glycol	Snow	10 out of 10	Not usable
Miscellaneous			
Acetone	Snow	10 out of 10	Not usable
Methyl isobutyl ketone	Snow	10 out of 10	Not usable
Acetic acid	Snow	10 out of 10	Not usable
Formic acid	Snow	10 out of 10	Not usable
Perfluorocarbon oil (Kel F)	Snow	10 out of 10	Not usable

Table II. Behavior of Solvent Mixtures upon Rapid Cooling with Liquid Nitrogen

Mixture	Volume ratio	Form of media at 77° K.	Frequency of forming cracks or snow	Possibility for use
1,4-Dioxane: diethyl ether	4 to 1	Snow	10 out of 10	Not usable
1,4-Dioxane: heptane	4 to 1	Snow	10 out of 10	Not usable
Ethanol: methanol	5 to 1 to 9 to 1	Clear glass	2 out of 10 to 1 out of 10	Good
Ethanol: glycerol	11 to 1	Clear glass	1 out of 10	Good
Carbon tetrachloride: pet. ether	1 to 1 to 1 to 2	Snow to glass	10 out of 10 to 8 out of 10	Not usable
Ethanol (96%): diethyl ether	2 to 1	Clear glass	3 out of 10	Good
Ethanol: concentrated HCl	19 to 1	Clear glass	3 out of 10	Good
Ethanol: water	<20 to 1	Clear glass	3 out of 10	Good
Ethanol: water	10 to 1	Snow	10 out of 10	Not usable
Ethanol: NH_3 (28% aqueous)	<20 to 1	Clear glass	3 out of 10	Good
Ethanol: NaOH[a]	<20 to 1	Clear glass	3 out of 10	Good
Ethanol: methanol: diethyl ether	8 to 2 to 1	Clear glass	1 out of 10	Good
EPA (diethyl ether: isopentane: ethanol)	5 to 5 to 2	Clear glass	0 out of 10	Excellent
EPA: chloroform	12 to 1	Clear glass	0 out of 10	Excellent
Triethylamine: diethyl ether, *n*-pentane	2 to 5 to 5	Clear glass	0 out of 10	Excellent
Diethyl ether: ethanol: NH_3 (28% aqueous)	10 to 9 to 1	Clear glass	1 out of 10	Good
Dimethyl formamide: ethanol: *n*-pentane: diethyl ether	1 to 16 to 4 to 2	Clear glass	4 out of 10	Poor
Ethanol: diethyl ether, *n*-pentane: HCl (conc.)	1 to 1 to 1 to 0.1 *M* HCl	Cloudy glass	1 out of 10	Poor
Methyl cellosolve: *n*-pentane	2 to 1	Clear glass	10 out of 10	Not usable
Methyl cellosolve: ethanol	1 to 1	Clear glass	10 out of 10	Not usable
Methyl cellosolve: triethylamine	2 to 1	Clear glass	10 out of 10	Not usable
Methyl cellosolve: water	4 to 1	Clear glass	10 out of 10	Not usable
Ethyl cellosolve: *n*-butanol: *n*-pentane	1 to 2 to 10	Clear glass	2 out of 10	Good
n-Pentane: *n*-heptane	1 to 1	Clear glass	0 out of 10	Excellent
Methyl cyclohexane: *n*-pentane	4 to 1 to 3 to 2	Clear glass	0 out of 10	Excellent
n-Propyl ether: *n*-pentane	2 to 1	Clear glass	1 out of 10	Good
Methanol saturated with KI		Snow	10 out of 10	Not usable

[a] 0.5% aqueous solution by weight of NaOH.

liquid nitrogen temperature. The results were obtained by taking the designated solvent, placing it in a quartz sample tube as previously described (*3*) and cooling the tube in a clear Dewar flask containing liquid nitrogen. The cooling process is observed and the final media are described in Tables I and II as being a clear glass, a cracked glass, or a snow. Also the frequency of cracking or forming a snow is given. No results on phosphorescence background for the solvents are given because the background is highly dependent on trace impurities which do not affect the type of rigid media formed at liquid nitrogen temperature. A solvent which forms a rigid clear glass most of the time may have to be specially purified for use in analysis or in many cases can be obtained commercially (*e.g.*, the fluorescence grade solvents of Hartman-Leddon Co., Philadelphia 43, Pa.).

It must be particularly stressed that if the sample tube is dirty or contains scratches, the solvent will generally crack on cooling. Also if the sample tube is struck against the side of the Dewar flask during cooling, the vibration many result in cracking. Just as in the process of precipitation where the availability of nucleation sites results in growth of the crystals, the availability of small dust particles or scratches apparently results in sites for cracking.

From the results given in Table I, it can be seen that there are relatively few pure solvents which form clear rigid glasses the majority of the time. Only pentane (technical grade) and petroleum ether (30° to 60° C.) are good hydrocarbon solvents. The only basic solvent which can be used at all is triethylamine. Of the ethers only diethyl ether can be used with good results. Of the alkyl halides only 2-bromobutane can be used. Both ethanol and *n*-propanol form clear rigid glasses most of the time. However, *n*-butanol will occasionally form an uncracked glass. No ketones or acids were found to form clear and uncracked glasses. From the results in Table II it can be seen that a number of mixtures form clear glasses without cracks or snow the majority of the time. As long as the amount of water in ethanol is kept below approximately 5%, ethanol with most any other component forms a good media for analysis To prepare any of the ethanol solutions it is best to start with absolute ethanol and dilute. Of course, EPA (*1*, *3*) is one of the best mixtures. In addition, mixtures containing *n*-pentane are usually excellent solvents for phosphorimetry. The solvents listed in Tables I and II represent solvents used for phosphorimetric studies found in the literature over the past 50 years. As is evident in the Tables, only relatively few of the solvents are of any use for phosphorimetric analysis.

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Application of Electron Spin Resonance to the Determination of Hydroperoxides

SIR: Electron spin resonance (ESR) is extensively used in the studies of free radicals as well as in the studies of paramagnetic salts. However, there are few examples in which ESR has significant importance as an analytical technique.

The experimental result, which is reported in this paper, will find an application to the determination of hydroperoxides in a solution.

The solutions of 1,1-diphenyl-2-picryl hydrazyl (DPPH) in benzene and, in the case of *tert*-butyl hydroperoxide (BHPO) in several other solvents, were prepared. To these solutions, BHPO (containing 10% of *tert*-butyl alcohol), cumene hydroperoxide (CHPO), peracetic acid (PAA) or *meta*-chloroperbenzoic acid (CPBA) was added and after a definite interval of time the ESR spectra were observed. The ESR measurements were made on an X-band spectrometer at room temperature and the spectra were recorded as the second derivatives. The solvents used were reagent grade and were not treated to remove any dissolved oxygen.

EFFECT OF ADDITION OF PAA OR CPBA

The semilogarithmic plots of the concentration of DPPH, after the hydroperoxides are added, *vs.* time are shown in Figure 1. The curve obtained after the addition of CPBA shows that the DPPH is decomposed exponentially with respect to time. In the case of PAA, the curve deviates from the straight line. This may be attributed to the gradual decomposition of PAA in the solution. From the experimental relation for the decay of the concentration of DPPH, it can be concluded that the hydroperoxides are not themselves consumed in the decomposition. During the reaction the ESR spectra observed are always the same as that of a solution of DPPH in benzene to which no hydroperoxides are added. However, when a large excess (beyond saturation) of one of these hydroperoxides is added to a benzene solution, the spectrum observed 10 hours after the addition of the hydroperoxide consisted of three lines.

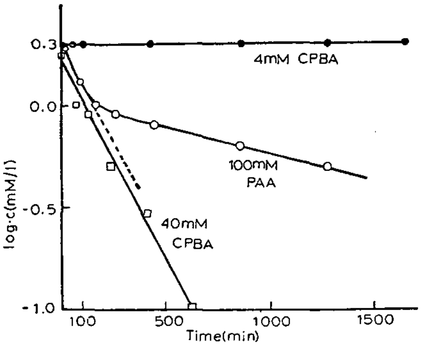

Figure 1. Semilogarithmic plot of concentration of DPPH in benzene after hydroperoxide is added, *vs.* time

CPBA: metachloro perbenzoic acid. PAA: peracetic acid. Initial concentration of DPPH is 2 mmoles per 1 as shown in figure

EFFECT OF CUMENE HYDROPEROXIDE

When CHPO is added to a solution of DPPH in benzene, both decay and transformation of the ESR spectrum take place even when the concentration of CHPO is small. The original five spectral lines caused by DPPH are gradually replaced by a triplet. When the concentration of CHPO is 4 mmoles per liter, a considerable amount of the triplet lines is observed, as shown in Figure 2. As seen in Figure 2, the chemical species giving rise to the triplet is not perfectly stable. When different solvents are used, the rates of conversion of the original quintet into the triplet are different, as shown in Table I. In Table I, the original concentration of the solution of DPPH is 2 mmoles per liter except in the solution of DPPH in cyclohexane, where the solubility of DPPH is about 0.5 mmole per liter at 20° C.

EFFECT OF *tert*-BUTYL HYDROPEROXIDE

When BHPO is added to a solution of DPPH in benzene an effect similar to that observed by CHPO is found. However, the reaction of BHPO with DPPH is faster than that of CHPO with DPPH. The decay and transformation of DPPH is, as is shown in Figure 2 and Table I, not complete. Therefore, the spectrum obtained when the reaction is still taking place is a hybrid of the quintet and the triplet. The slight difference in g-factor between the triplet and the quintet makes this hybrid spectrum asymmetric. The concentration of the transformed free radical *R* (giving rise to the triplet) can be estimated by an analysis of this spectrum. The relation between the concentration of added BHPO and the percentage of decomposed and transformed DPPH, is shown in Figure 3. It can be seen that the rate of decomposition of DPPH shows a greater dependence on the concentration of BHPO than the rate of the transformation of DPPH.

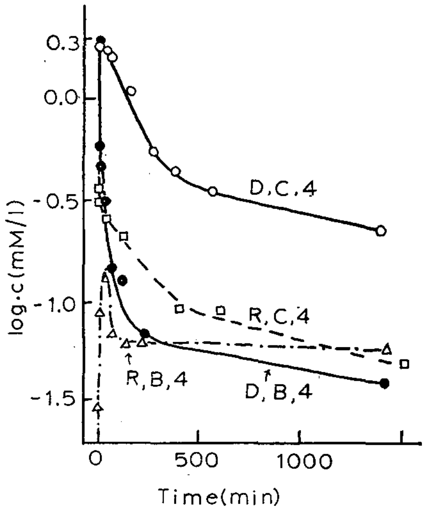

Figure 2. Semilogarithmic plot of concentration of DPPH and *R* in benzene after peroxide is added *vs.* time

○ or *D,C, 4*: DPPH concentration after addition of CHPO (cumene hydroperoxide, 4 mmoles per liter) ☐ or *R,C,4*: *R* concentration after addition of CHPO (4 mmoles per liter) ● or *D,B,4*: DPPH concentration after addition of BHPO (*tert*-butyl hydroperoxide, 4 mmoles per liter). △ or *R,B,4*: *R* concentration after addition of BHPO (4 mmoles per liter)

Table I. The Percentage Conversion of Quintet into Triplet after 10 Minutes

Solvents	Benzene	Toluene	Cyclohexane	CCl_4
Hydroperoxides, mmole/l.				
40 CHPO	13	14	37	22
10 BHPO	16	12	40	9

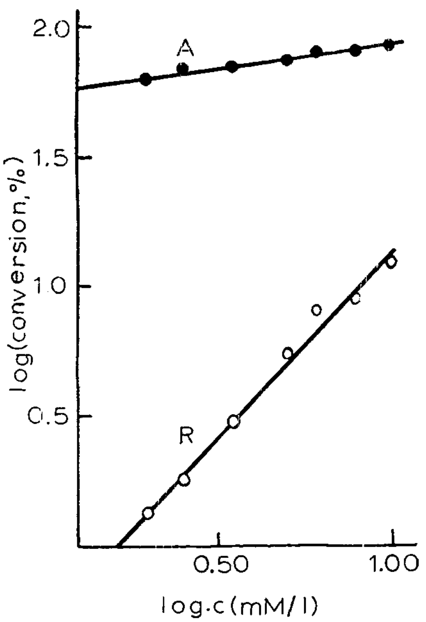

Figure 3. Logarithmic plot of percentage conversion of DPPH to A and R at 10 minutes after addition of BHPO *vs.* concentration of BHPO (initial concentration of DPPH in benzene 2 mmoles per liter)

The intensities of the ESR spectra of the solutions of DPPH, at various concentrations and before and after the addition of BHPO, are compared when the concentration of BHPO is the same in each case—i.e., 2.5 moles per liter. It is shown in Figure 4.

A solution of DPPH exhibits an exchange interaction phenomenon when the concentration is greater than some value between 2 and 9 mmoles per liter (*1*). However, it can be seen from Figure 4 that when the concentration of DPPH is 30 mmoles per liter, the addition of BHPO decreases the intensity of the ESR spectrum to less than 1/100 and it is this decrease in concentration which may account for the fact that an exchange interaction for the species *R* is not observed.

RATE CONSTANTS FOR BOTH REACTIONS

On the basis of these results, the reaction between DPPH and hydroperoxides will be formulated as:

$$\text{DPPH} + 0.2\ \text{HPO}_2 \rightarrow X \rightarrow A + 0.2\ \text{HPO}_2{:}k_1 \quad (1)$$

$$\text{DPPH} + 1.5\ \text{HPO}_2 \rightarrow R{:}k_2 \quad (2)$$

where, HPO_2 denotes the hydroperoxide, X denotes some decomposition intermediate, and A denotes the final nonparamagnetic species, and k_1 and k_2 are rate constants, k_1 being the overall rate constant for the reaction,

$$\text{DPPH} \rightarrow A \quad (3)$$

The proportions of HPO_2 appearing in Equations 1 and 2 were determined from the slopes of the curves in Figure 3.

The values of k_1 for various hydroperoxides were estimated from the initial slopes of the curves in Figure 1 and Figure 2. The initial velocities for the reaction 1 and 2 will be,

$$\frac{d\,(\text{DPPH})}{dt} = -k_1\,(\text{DPPH})_0\,(\text{HPO}_2)_0^{0.2} \quad (1')$$

$$\frac{d\,(\text{DPPH})}{dt} = -k_2\,(\text{DPPH})_0^{1.5} \quad (2')$$

If the ratio of initial rates of disappearance of DPPH in reaction 1 and 2 is approximated by the ratio, $1/r$, of the amount of DPPH which disappeared in reactions 1 and 2 after the reaction for the first 10 minutes, then

$$r = \frac{k_2}{k_1}\,(\text{HPO}_2)_0^{1.3} \quad (4)$$

Thus k_2 can be estimated from k_1 and r. The values of k_1 and k_2 derived in this manner are listed in Table II.

In the earlier communication (*2*) the authors stated that the transformation of DPPH occurred only in the presence of tertiary hydroperoxides. This has been shown incorrect, though the rate of transformation of DPPH in the presence of per acids is very small.

Table II. The Values k_1 and k_2 for Various Hydroperoxides

Hydroperoxides	CPBA	PAA	BHPO	CHPO
k_1	2.1×10^{-3}	1.8×10^{-3}	4.7×10^{-2}	4.0×10^{-3}
k_2	less than 10^{-7}		1.6×10^{-4}	5.8×10^{-6}

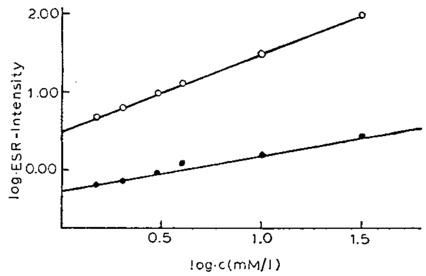

Figure 4. Logarithmic plot of ESR intensity *vs.* concentration of DPPH in benzene

○: When hydroperoxide is not added. ●: When BHPO is added to 2.5 mmoles per liter

ANALYTICAL APPLICATION

The typical technique for the determination of hydroperoxides is iodometry. Iodometry, however, does not determine different types of hydroperoxides separately from their mixture. In some instances it will be desirable to know the amount of a particular type of hydroperoxide in a mixture of many different hydroperoxides such as primary, secondary, tertiary, etc., as the reactivity of a hydroperoxide depends greatly on its structure. The different reaction rate constants presented here will be useful in such cases, although no stoichiometric relation between DPPH and hydroperoxides has been found.

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CORRESPONDENCE

Liquid Chromatography with Operating Conditions Analogous to Those of Gas Chromatography

SIR: Under a title similar to the above, Karr, Childers, and Warner recently described a successful liquid chromatographic (LC) system based on considerations of gas chromatography (GC) (*7*). The former was made analogous to the latter through the use of long narrow tubing, column packing that can be used indefinitely, a single carrier fluid, automatic recording, and reproducible retention volumes. It is the purpose of this note to add that a full analogy must also involve considerations of column efficiency. (A distinction should perhaps be made between analogous systems, a concept applying essentially to the quoted work, and analogous operating conditions as discussed primarily here.) Factors affecting column efficiency have been extensively investigated in gas chromatography (*3*), and their application has led to considerable advancements in improving resolution, analysis speed, etc. (*1*). The lessons learned in this area of gas chromatography can also be applied to liquid chromatography through the use of a reduced plate height equation. This assertion is based on the recent development of a universal equation, applicable to nearly all chromatographic systems (*5*). This equation will be used here to obtain the parameters for liquid chromatography which are analogous to those which lead to optimum operation in gas chromatography.

The measure of column efficiency in a chromatographic column is the plate height H. This quantity may be obtained from an elution diagram by the equation $H = L\tau^2/t^2$, where L is the column length, τ is the standard deviation of the Gaussian or near-Gaussian zone profile and t is the retention time. If the conditions in two chromatographic systems are truly analogous then the ratio

$$h = H/d_p \qquad (1)$$

will be the same in the two systems. The average particle diameter is denoted by d_p. The quantity h will be called the reduced plate height (*4, 5*). One finds that h will be the same in two systems providing comparable conditions exist in both the mobile phase and in the stationary phase. Analogous mobile-phase conditions will exist if the reduced flow velocity

$$\nu = d_p v/D_m \qquad (2)$$

is the same in both systems (*4, 5*). The quantity v is the mean cross-sectional flow velocity and D_m the diffusion coefficient of the component in the mobile phase. Analogous stationary phase conditions will exist (ignoring longitudinal diffusion in the stationary phase) if the ratio $C_s v/d_p$ is held the same (*4*). This dimensionless quantity may be written as $\Omega\nu$ where Ω is the reduced mass transfer coefficient for the stationary phase

$$\Omega = C_s D_m/d_p^2 \qquad (3)$$

Values of C_s, the mass transfer coefficient for the stationary phase, have been determined from gas chromatographic data (*2, 9*). (In gas liquid chromatography, C_s is usually written as C_l.)

The foregoing parameters form a basis for the reduced plate height equation which may be written in approximate form as (*5*)

$$h = \frac{2\gamma}{\nu} + \Omega\nu + \sum \frac{1}{1/2\lambda_i + 1/\omega_i\nu} \qquad (4)$$

Workers in gas chromatography will recognize the longitudinal diffusion term with the obstruction factor γ and the inverse dependence on velocity; the liquid mass transfer term, proportional to velocity; and the coupling expression which contains the parameter λ_i of eddy diffusion and ω_i of gas phase mass transfer. These constants, γ, λ_i, and ω_i, are structural factors and should be essentially the same in any chromatographic column. If this is so, and if ν and Ω are designed to be equal in the two systems, then the above equation shows that h will also be the same, as postulated previously.

It is instructive to discuss the numerical magnitude of the parameters; ν and Ω, which will give analogous performance in the two columns. Since the diffusion coefficient, D_m, is approximately 10^5 times larger in gases than liquids, and since the particle diameter, d_p, is usually the same order of magnitude, then the flow velocity v must be $\sim 10^5$ times slower to make LC conditions analogous to those in GC. It has long been known that an H (or h) *vs.* v (or ν) plot shows a minimum at some particular velocity and that this corresponds to the maximum resolution of the column (*1*). This optimum velocity is about 10 cm. per second in GC. Thus an analogous LC column (with similar d_p) would have an optimum velocity of $\sim 10^{-4}$ cm. per second. This low velocity would seriously hinder any attempt to obtain a rapid analysis. [Experimental values, (*6, 10, 11*) confirm this prediction in a general way, although occasional values higher than this indicate that the operation is not entirely analogous.]

If the parameter Ω is to be the same in LC as in GC, then the C_s value for LC must be $\sim 10^5$ times larger than in GC for a similar value of d_p. Since C_s values in efficient GC columns are $\sim 10^{-3}$ second, the corresponding LC value is $\sim 10^2$ second.

The numerical values just given for analogous parameters depend on the simplifying assumption that d_p values are about the same. This is not necessary to keep the dynamic processes analogous and certain advantages may be found in reducing d_p in LC. The reason for this is that the length of column and thus resolution is often limited by the pressure drop which can be used to force the fluid through the column. If d_p is the same in the two systems and v is 10^5 times slower in LC; then, since viscosity, η, is only about 10^2 times larger in liquids than gases, the pressure drop in the analogous liquid system will be 10^{-3} times that in the gas system. One can then use smaller particles, a change that will give a higher optimum velocity, and a column which will provide the same resolution in a shorter length. The details of this argument are too long to reproduce here, but, assuming the same ratio as stated above for diffusivities and viscosities, and assuming that the two systems are operated with the same pressure drop, then for the fastest analysis the particle diameter for LC will be about $^1/_{30}$ of that for the analogous GC system (this number is a result of d_p being proportional to $\sqrt{\eta D_m}$). This will increase the optimum velocity by a factor of 30 over that previously assumed. At the same time the column length required for the same separation will be reduced by ~ 30. Thus the analysis speed will be approximately 1000 times faster than previously indicated, and will be only 100 times slower than an analogous GC separation—i.e., analysis time will be proportional to viscosity. There are some considerations which might improve this factor even further—e.g., the high pressure drops that might be encountered will increase the value of D_m for GC, and high pressures will be more feasible in LC because of the reduced danger of explosion. Difficulties encountered in using very fine packing will naturally require attention. Considerations similar to these—i.e., increasing analysis speed with a limited pressure drop—have been discussed for GC by Knox (*8*).

The foregoing treatment has indicated the magnitude of the parameters needed to make LC fully analogous to GC. It is found that such analogous conditions put LC at a distinct disadvantage; the time required for analysis is increased by a factor of from 10^2 to 10^5. It is thus natural to inquire if analogous conditions are really the most desirable conditions. We have indicated, for instance, that the mass transfer coefficient for the stationary phase, C_s, should be considerably larger in LC than in GC for analogous conditions. In GC this term is generally large enough to be very significant in limiting resolution and analysis speed. It would be equally harmful in LC under analogous conditions. It is probable that C_s can be reduced well below its GC analog in most LC systems; in some cases the actual value of C_s should be comparable, which means that the reduced transfer parameter, Ω, is $\sim 10^5$ times less in LC than analogous conditions would demand. If C_s can thus be rendered negligible in LC, higher reduced velocities are probably feasible without the severe loss of resolution expected for such an increase in GC. This would improve analysis speed in LC considerably.

The brief treatment given here indicates that column parameters can undoubtedly be found that will make column performance in LC fully analogous to those in GC. This path may be profitable in acquiring all possible advantages of the GC technique. However, some of the disadvantages of LC should be avoided by the intentional departure from the GC analog.

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Direct Coulometric Titrations with Hypobromite Ion

Sir: The coulometric titration of ammonia with electrolytically generated hypobromite ion developed by Arcand and Swift (*1*) has been very useful. Recently, an extension of the method to organic nitrogen compounds was made (*2*).

The technique used by Arcand and Swift involved generation of BrO^- in basic solution until beyond the titration end point, acidification of the solution, and determination of the excess bromine either by a cuprous ion back-titration or a further generation of bromine with an amperometric titration plot. A simple titration to a fixed current level in basic solution could not be made because the indicator electrode currents were not sufficiently stable. Both of the methods for end point detection developed by Arcand and Swift suffer from two major faults: an extra step, and possible source of error, is added to the method and the oxidized species may react with the bromine in acid solution. The latter item, in particular, is important since it limits the applicability of the method. Therefore, a study was made to see if the end point detection might be simplified.

Table I. Direct Coulometric Titration of 10.2 Micromoles of Ammonium Chloride with Hypobromite Ion[a]

Run	Micromoles Br consumed	Micromoles NH_4Cl found
1	30.9	10.3
2	30.9	10.3
3	30.9	10.3
4	30.9	10.3
5	30.7	10.2
6	30.7	10.2
7	31.0	10.3
8	31.9	10.6
9	30.9	10.3
10[b]	31.1	10.4
	Mean	10.3
	σ	±0.11

[a] Titrations carried out at 19.3 ma. current.

[b] Titration carried out at 9.65 ma. current.

EXPERIMENTAL

Reagents. A buffer containing 10 grams of sodium tetraborate decahydrate and 500 grams of potassium bromide per liter was used for all titrations. All other chemicals were reagent or U.S.P. grade and were used without further purification.

Apparatus. Two different coulometers were used for the study. One consisted of a Sargent Constant Current Power Supply (No. 30974) and a Simpson 50-μa. meter with a 1.5-volt battery and variable resistor for the indicator system. The other coulometer used a Regatron Constant Current Power Supply, Model C 612-A (Electronic Measurements Co., Inc.), Model S-10 Precision Timer (Standard Electric Time Co.) and a 10-0-10 μa. meter (Assembly Products, Inc.) with a 1.5-volt battery and variable resistor for the indicator system. A platinum anode and isolated platinum cathode (Leeds and Northrup Co.) and two platinum wire detecting electrodes comprised the titration cell assembly. A

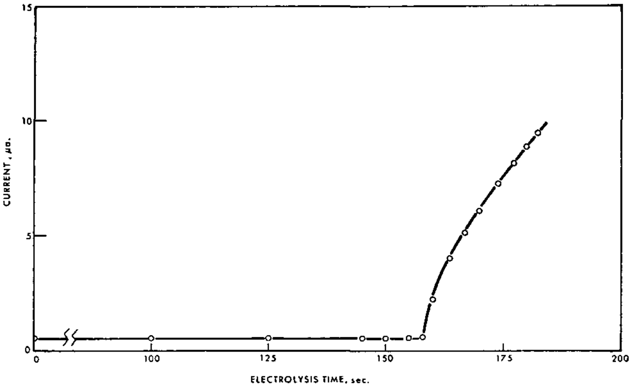

Figure 1. Representative plot for direct coulometric titration of ammonia with hypobromite ion

small voltage (0.15 volt) was impressed across the detecting electrodes.

Procedure. A suitable-sized sample was transferred to a 100-ml. beaker, 10 ml. of buffer solution were added, and the solution was diluted to 50 ml. with distilled water. The pH at final dilution was 8.9. A stirrer and the electrode assembly were dipped into the solution and the initial current in the detection system recorded. Bromine (hypobromite) was generated and the current in the detection system recorded at suitable electrolysis intervals. The current-electrolysis time data were then plotted and an extrapolation to the end point was made. A blank titration was carried out in the same manner and the time subtracted from the sample titration. Calculations based on Faraday's law were then made to obtain the amount of reactive species in the cell.

RESULTS AND DISCUSSION

Although the indicator currents in basic solution were not stable enough for titration to a fixed current level, it was thought that a plot of the indicator current as a function of electrolysis time might be suitable for analytical purposes.

Figure 1 shows a typical curve for the titration of ammonium chloride. The current prior to the end point of the titration was essentially constant and then increased in a nonlinear fashion after the end point. However, an extrapolation to the end point was easily and reproducibly made. Since the current during the titration was constant, it was necessary to record only one or, at most, two points prior to the end point. A blank titration was carried out in the same manner and the time was subtracted from the titration of the unknown. At a generation current of 19.3 ma., the blank titration was 3.5 seconds with the sample titration ca. 158 seconds.

Table I lists the data for 10 determinations of ammonium chloride. The standard deviation for the 10 analyses was ±0.11 with a mean of 10.3 micromoles of ammonium chloride. The experimentally-found mean deviated from the true value by 0.1 micromole.

Table II shows the results for various concentrations of sulfamic acid. Included in the data are two sets of replicate analyses. In one set of replicate analyses, the standard deviation was ±0.04 with a mean of 5.01 micromoles and in the other it was ±0.09 with a mean of 10.63 micromoles. The former mean deviated from the true value by 0.03 micromoles and the latter by 0.03 micromoles also.

Table II. Direct Coulometric Titration of Sulfamic Acid with Hypobromite Ion

Micromoles NH_3SO_4 added	Generating current, ma.	Micromoles NH_3SO_4 found
4.98	9.65	5.03
4.98	9.65	5.06
4.98	9.65	5.00
4.98	9.65	4.97
4.98	9.65	4.97
10.66	22.0	10.55
10.66	22.0	10.75
10.66	22.0	10.58
10.66	22.0	10.58
10.66	22.0	10.71
14.22	19.3	14.18
10.66	19.3	10.68
7.11	19.3	7.48
3.56	19.3	3.80

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Coulometric Titration of Ammonia with Hypobromite Using Direct Amperometric End Point Detection

SIR: Willard and Cake (*5*) and Kolthoff and Stenger (*2*) have developed volumetric methods for the determination of ammonia by oxidation to nitrogen with hypobromite. Since then Kolthoff, Stricks, and Morren (*3*) have described an amperometric end point detection in the titration of 6×10^{-4} to $4 \times 10^{-6}M$ ammonia in sodium bicarbonate solution. They employed a rotating platinum wire electrode at +0.2 volt. *vs.* S.C.E. as the indicating electrode. Laitinen and Woerner (*4*) employed a similar technique in titrating ammonia with hypochlorite.

Arcand and Swift (*1*) developed a coulometric titration of 14 to 230 μg. of ammonia using electrogenerated bromine in a supporting electrolyte of pH 8.5. In alkaline medium, bromine disproportionates to bromide and hypobromite ions. Two platinum foil indicator electrodes with 150 mv. impressed between them were used for the amperometric end point. Arcand and Swift found that the indicator current resulting from a given quantity of bromine in alkaline solution was not sufficiently reproducible for use as a means of determining the end point. Therefore, they used the current in the alkaline solution only to indicate when a slight excess of bromine had been generated. Then an indirect method was employed to find the end point; the solution was acidified with perchloric acid and additional bromine was generated at 1-second intervals. The current readings were plotted against the generation time and a straight line resulted. A blank was run in the same manner and an arbitrary current reading in the acid solution was chosen as the end point current.

The authors have experienced difficulty in trying to employ the method of Arcand and Swift in the titration of ammonia solutions obtained from biological samples. We found that the indirect end point determination was not reproducible. The current readings due to the excess bromine in acid solution appeared to be very dependent on the amount of excess bromine generated before the solution was acidified. In a blank showing a difference of only 1.7 seconds to generate excess bromine at 4.825 ma. before acidification, the generation-time readings differed by 7 seconds to reach the same current reading in acid solution. Also, a given current reading depended upon strict reproduction of the stirring rate and volume.

The authors observed the same difficulty experienced by Arcand and Swift in trying to generate to an arbitrary current value directly in alkaline solution. However, in preliminary titrations we noted that the generation time needed for the current readings to increase in alkaline solution appeared to correspond closely to the end point value and was reproducible. Therefore, a direct, conventional end point detection was investigated. This method has been employed to determine as little as 1.4 μg. of ammonia.

EXPERIMENTAL

Reagent grade chemicals were used without further purification. Solutions of ammonium sulfate were prepared by dissolving Mallinckrodt "Analytical Reagent" ammonium sulfate in an appropriate volume of water. The ammonium sulfate had previously been dried at 110° for 2 hours. The stock buffer solution employed was prepared by dissolving 38.1 grams of sodium tetraborate decahydrate in 1 liter of water and adding 72% perchloric acid to adjust the pH to 8.6.

The coulometric titrations were made with a Sargent Coulometric Current Source, Model IV, at a generating current of 19.30 or 4.825 ma. The generating anode and cathode were platinum foils of 2 sq. cm. and 0.8 sq. cm., respectively. The cathode was isolated from the test solution by placing it in a glass tube fitted with a sintered-glass frit end and containing the stock buffer solution as catholyte. The indicating electrodes were two platinum foils (2 sq. cm.) with 150 mv. impressed between them. The impressed potential was supplied by a Sargent Model XV Polarograph. All amperometric measurements were made by recording the current on this instrument either manually or automatically.

Table I. Manual Titrations

NH_3, μg.		Error,	
Taken	Found	μg.	ma.
354.3	353.4[a]	−0.9	19.30
144.3	144.1	−0.2	4.825
35.43	35.46	+0.03	4.825
35.43	35.75	+0.32	4.825
35.43	35.57	+0.14	4.825
35.43	35.17	−0.26	4.825
35.43	35.40	−0.03	4.825
Ave.	35.47	+0.04	
34.78	34.78[a]	0	4.825
34.78	34.69[a]	−0.09	4.825
34.78	34.72[a]	−0.06	4.825
Ave.	34.73	−0.05	
28.86	27.95	−0.91	19.30
28.86	28.64	−0.22	4.825
Ave.	28.30	−0.56	
14.43	14.09	−0.34	19.30
7.09	7.29	+0.20	4.825
7.09	7.15	+0.06	4.825
Ave.	7.22	+0.13	
6.38	6.46[a]	+0.08	4.825
6.38	6.46[a]	+0.08	4.825
Ave.	6.46	+0.08	4.825
2.89	3.12	+0.23	4.825
2.89	3.14	+0.25	4.825
2.89	3.26	+0.37	4.825
2.89	3.14	+0.25	4.825
Ave.	3.17	+0.28	
1.44	1.76	+0.32	4.825

[a] Samples added to a pretitrated solution as described in text.

Table II. Automatic Titrations

NH_3, μg.		Error,	
Taken	Found	μg.	ma.
354.3	353.4[a]	−0.9	19.30
354.3	354.1	−0.2	19.30
354.3	354.5[a]	+0.2	19.30
Ave.	354.0	−0.3	
34.78	34.04[a]	+0.06	4.825
34.78	34.97[a]	+0.19	4.825
34.78	34.84[a]	+0.06	4.825
34.78	34.66[a]	−0.12	4.825
34.78	34.90[a]	+0.12	4.825
Ave.	34.84	+0.06	
28.86	29.30	+0.44	4.825
6.38	6.38[a]	0	4.825
6.38	6.38[a]	0	4.825
6.38	6.28[a]	−0.10	4.825
6.38	6.48[a]	+0.10	4.825
Ave.	6.38	0	4.825
3.19	3.29[a]	+0.10	4.825
3.19	3.17[a]	−0.02	4.825
3.19	3.14[a]	−0.05	4.825
Ave.	3.20	+0.01	
2.89	3.17	+0.28	4.825

[a] Samples added to pretitrated solution as described in text.

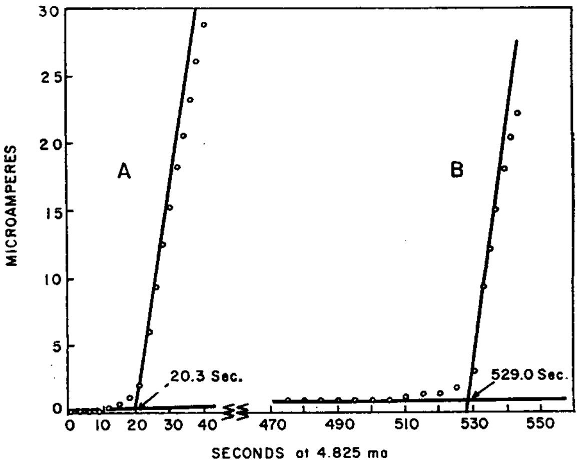

Figure 1. Coulometric titration of ammonia

A. Blank: 8.00 ml. of stock buffer solution, 6.00 ml. of 5*M* NaBr, and 16.00 ml. of water. Bromine generated at 4.825 ma.

B. Test solution: 8.00 ml of stock buffer solution, 6.00 ml. of 5*M* NaBr, 11.00 ml. of water, and 5.00 ml. of 8.47 × $10^{-4}M$ ammonium sulfate. Bromine generated at 4.825 ma.

During generation and current measurement, the solution was stirred with a magnetic stirring bar. The titration vessel was either a 250-ml. beaker or a 50-ml. weighing bottle.

Procedure. In titrations at 4.825 ma., 8.0 ml. of stock buffer solution and 6.0 ml. of 5*M* sodium bromide were added to a 50-ml. weighing bottle. Standard ammonium sulfate solution was pipetted into the bottle and water was added to bring the volume to 30.0 ml. In titrations at 19.30 ma., either the above procedure or 15.0 ml. of buffer and 15.0 ml. of 5*M* sodium bromide were added to a 250-ml. beaker. Standard ammonium sulfate solution was pipetted into the beaker and water was added to make a total volume of 75.0 ml.

Manual plots were obtained by generating bromine at 10-second intervals until a slight rise in the current readings indicated the approach of the end point. The current rise was then recorded at 1-second intervals until sufficient points were obtained to determine the end point break. Automatic plots were obtained by allowing the chart to record the current continuously until a sharp rise in current indicated the end point. The end point break was obtained by extrapolating the straight lines in a manner analogous to that employed in the manual plots (See Figure 1). The divisions on the chart paper were calibrated in terms of seconds. There was always a slight initial rise in current before the levelling-off observed by Arcand and Swift.

RESULTS AND DISCUSSION

A typical blank and test solution curve is shown in Figure 1 with current readings being obtained manually. In the 30-ml. volume, a large blank reading equivalent to about 2.9 μg. of ammonia was found. The major portion of the blank impurities appeared to be in the sodium bromide solution since a decrease in the buffer concentration by a factor of ten decreased the blank reading by only 13%. Arcand and Swift observed a blank reading of about one-third this value.

The reaction at the indicator electrodes is sufficiently rapid to allow current to be recorded automatically on the polarograph, thus decreasing the titration time considerably.

An added advantage of direct end point detection is that the blank can be pretitrated to a sufficient rise in current to extrapolate the end point, and the excess hypobromite noted. At this point sample can be added directly and titrated by adding the quantity of excess hypobromite initially generated to the end point value. In this manner a series of samples can be titrated in succession in the same generating solution. The disadvantage of a large blank and the pipetting errors involved in reproducing a reagent blank are also eliminated. In applying this method the end point value of the first sample following pretitration of the blank was usually in error while subsequent samples were satisfactory. Therefore, the procedure followed was to pretitrate the blank to a slight excess and then add an ammonia sample of approximately the same size as the expected test sample and pretitrate it, noting the excess of hypobromite generated beyond the end point. Then the sample of a series of samples was added and titrated as above.

In these pretitration procedures, a large excess of hypobromite should not be generated before adding the sample, as this may lead to a positive error (*1*).

In the manual titrations, variations of stirring rate and total volume should have no effect on the end point break, although the generating reagents should be pipetted to reproduce the blank impurities. In applying the automatic plot, however, these should be reproduced fairly closely with a volume duplication of ±1 ml. appearing to be satisfactory. For a series of titrations in the same solution, satisfactory end points are obtained if the stirring rate is increased in approximately the same proportion as the volume increase when a new sample is added.

In these titrations the recommended pH should be adhered to within ±0.1 pH unit as discussed by Arcand and Swift (*1*).

In any event where small ammonia samples are titrated with hypobromite, which is a strong oxidant readily reduced by many different impurities, the analyst should always run a blank to determine the titration error due to impurities.

The data of this investigation are shown in Tables I and II. A series of samples ranging from 354.3 to 1.44 μg. of ammonia were titrated either manually or automatically using both the direct and pretitration methods. Automatic titration appears to give results slightly superior to the manual titrations.

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Infrared Analysis of Alpha-Olefins

SIR: The olefin group type analysis previously reported (*2*) has been in use in this laboratory for a number of years. Recently a need arose for the accurate quantitative determination of olefin group types in normal α-olefin rich mixtures. It was particularly desirable to achieve the highest possible accuracy in the α-olefin determination. Since the required accuracy was beyond the limits of the group type analysis, it was necessary to introduce a number of modifications in the procedure to obtain a satisfactory analysis. The purpose of this communication is to report these modifications.

Simple averaging of the absorptivities of the characteristic α-olefin band (11.0 microns) to obtain a group type value results in errors too large for the present requirements. This is illustrated in Table I. For example, the absorptivity of hexene-1 deviates from the average by 14% and decene-1 by 5.5%. Thus, it is necessary to calculate a statistically weighted absorptivity to be used with each sample. Accordingly, the following procedure was developed.

The absorbances for all the normal α-olefins to be included in the analysis were obtained at all the analytical wavelengths—e.g., 10.35, 11.00, 11.25, 12.03, 12.15, 12.33, 12.50, and 14.42 microns, on a Perkin-Elmer Model 21 infrared spectrophotometer. A 0.15-mm. cell was used and dilutions were made in carbon disulfide when necessary. The absorptivity of each of the pure α-olefins was calculated at their group absorption wavelength, 11.00 microns. The calibration compounds were high-purity American Petroleum Institute samples. In the present case, we included only the even-numbered α-olefins from C_6 through C_{20}. Octadecene-1 and eicosene-1 were not available and their data were determined by extrapolation of the data for the lower molecular weight compounds.

When the absorptivities of pure α-olefins are used and the carbon number distribution of the sample is obtained from a high temperature programmed gas chromatographic analysis, the statistical absorptivity at 11.00 microns is calculated for the particular sample under analysis. The absorbance of the sample at 11.00 microns divided by its statistical absorptivity yields the concentration of α-olefins in the sample (in moles per liter). To determine the other olefin groups which are present as impurities in the α-olefin sample, the statistical absorbances are calculated for the sample at all the analytical wavelengths assuming the sample contains 100% normal α-olefins. The statistically calculated absorbances are then substracted from the respective observed absorbances of the sample. These difference absorbances

Table I. Absorptivities of α-Olefins at 11.0 Microns

α-Olefin	Absorptivity, liter mole^{-1} cm.$^{-1}$	% Dev. from av.
Hexene-1	138	−14
Heptene-1	150	−6.2
Octene-1	158	−1.4
Nonene-1	156	−2.6
Decene-1	169	+5.5
Undecene-1	170	+6.2
Dodecene-1	166	+3.7
Tridecene-1	165	+3.1
Tetradecene-1	164	+2.4
Pentadecene-1	165	+3.1
Hexadecene-1	160	+0.1
	Av. 160.1	

Table II. Matrix Used in Group-Type Olefin Analysis

(Absorptivities in liter mole^{-1} cm.$^{-1}$)

λ, Microns/ olefin group	Trans $RHC{=}CHR$	Vinyl $RHC{=}CH_2$	Vinylidene $R'R''C{=}CH_2R$	Trisubstituted $R'R''C{=}CHR$	Cis $RHC{=}CHR$
10.35	147.2	5.59	1.65	4.19	6.09
11.00	4.20	160.4	8.29	2.23	4.71
11.25	4.46	9.45	184.7	2.53	3.01
Av. of 4 12.0 to 12.5	1.01	1.88	2.43	15.7	2.39
14.42	0.24	1.35	0.41	0.31	24.8

Table III. Synthetic Blend

Olefin Type	Concentration (moles/liter)	
	Known	Determined
Trans (*trans*-heptene-3)	0.075	0.04
Vinyl (octene-1)	6.134	6.15
Vinylidene (2-methylbutene-1)	0.045	0.02
Trisubstituted (2-methylbutene-2)	0.106	0.09
Cis(*cis*-heptene-2)	0.078	0.13
Total	6.438	6.43

represent the departure of the sample from the assumed 100% α-olefin. When these difference absorbances are applied to the olefin group type absorptivity matrix (*2*) shown in Table II, the concentrations of the various impurity olefin types are obtained. The difference absorbance at 11.00 microns will, of course, be a negative number if the sample is less than 100% pure α-olefin, but the negative sign should be carried along in the matrix calculations; a negative value will result representing the departure of the α-olefin concentration in the sample from the assumed 100% α-olefin. This value could be algebraically added to the total theoretical olefin concentration in the sample to obtain the α-olefin concentration. However, the concentration for the α-olefins determined by this procedure is not sufficiently accurate since the group type absorptivity is used.

The mean error of this analysis when applied to an aromatic free material that contains 80% or more α-olefins is believed to be ±1% for the α-olefin value and ±1.5% for the other olefin groups. This is indicated by the analysis of a synthetic blend shown in Table III where all of the mean errors are less than 1%. In a blend of four α-olefin compounds, the error in the total vinyl concentration was 0.13%. In some cases, diolefins and cyclomono-olefins will interfere if present in quantities greater than 3%. The diolefin and or cyclomono-olefin content may be determined by a low-voltage mass spectrometric method (*1*).

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Modified Thermal Conductivity Detector for Capillary Columns

Sir: Although Golay (*2*) initially used a thermal conductivity detector for his investigations with capillary columns, these columns have been used almost exclusively with ionization detectors. Recently, Schwartz (*4*) and also Camin (*1*) reported using primarily 0.02-inch or larger i.d. capillary columns with thermal conductivity detectors.

At this laboratory, a gas chromatograph equipped with a modified thermal conductivity detector has been used for some time with 0.01-inch i.d. capillary columns.

EXPERIMENTAL

Apparatus. The gas chromatograph was fabricated along conventional lines using essentially standard components. The detector was the Gow-Mac Micro-Cell Model JDC-015. A 2-mv. Brown recorder was used. A sample stream splitter after the design of Halasz (*3*) was included in the inlet system. The splitter was fabricated from a Swagelok heat exchanger tee and stainless steel tubing. The split ratio was varied with a Nupro micrometering valve. Both detector and capillary column were housed in a temperature-controlled air bath suitable for operation up to 125° C. The significant feature of the apparatus was the modification made in the sample cavity of the detector. Figure 1 shows this modification in detail. The modification consisted of positioning a 2-inch length of stainless steel, 0.010-inch i.d., tubing up through the sample cavity orifice with the end of the tubing as close as possible to the thermistor bead itself. This distance was approximately 1 mm. The tubing was then silver-soldered in this position. The capillary column was then connected to the other end of the tubing.

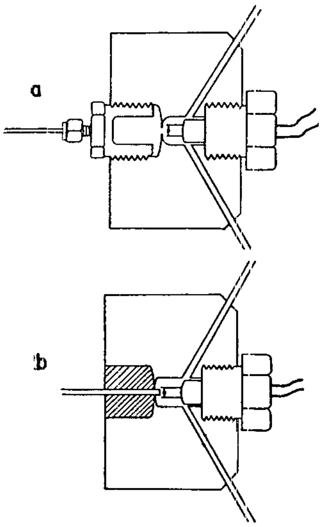

Figure 1. Modification of sample cavity in cell

a. Before modification
b. After modification

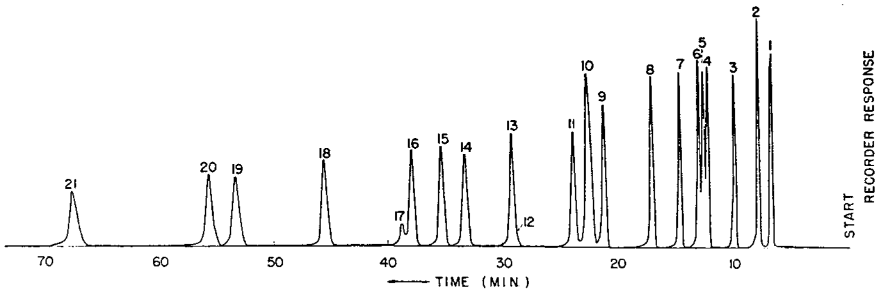

Figure 2. Chromatogram of C_5 through C_7 blend on modified cell

Column, 190-ft. × 0.010 in. stainless steel capillary coated with squalane; 25° C.; helium 1.7 ml./min. sample size, 1 µl. 100:1 split

Components

1. Isopentane
2. *n*-Pentane
3. 2,2-Dimethylbutane
4. Cyclopentane
5. 2,3-Dimethylbutane
6. 2-Methylpentane
7. 3-Methylpentane
8. *n*-Hexane
9. 2,2-Dimethylpentane and methylcyclopentane
10. Benzene and 2,4-dimethylpentane
11. 2,2,3-Trimethylbutane
12. 3,3-Dimethylpentane
13. Cyclohexane
14. 2,3-Dimethylpentane
15. 3-Methylhexane
16. 1,*trans*-3-Dimethylcyclopentane
17. 3-Ethylpentane and 1,*trans*-2-dimethylcyclopentane
18. *n*-Heptane
19. 1,*cis*-2-Dimethylcyclopentane
20. Methylcyclohexane
21. Toluene

Table I. Analysis of C_5–C_7 Blend

Components	Wt. % Known	Wt. % Found	Deviation
Isopentane	4.2	3.8	−0.4
n-Pentane	4.3	4.8	+0.5
2,2-Dimethylbutane	4.5	4.9	+0.4
Cyclopentane	5.2	4.9	−0.3
2,3-Dimethylbutane	4.6	5.1	+0.5
2-Methylpentane	4.5	5.4	+0.9
3-Methylpentane	4.6	5.4	+0.8
n-Hexane	4.5	5.4	+0.9
2,2-Dimethylpentane Methylcyclopentane	5.6	5.9	+0.3
Benzene 2,4-Dimethylpentane	10.6	9.6	−1.0
2,2,3-Trimethylbutane	4.7	4.9	+0.2
3,3-Dimethylpentane	0.5	0.5	0
Cyclohexane	5.3	5.3	0
2,3-Dimethylpentane	4.8	4.6	−0.2
3-Methylhexane	4.7	5.0	+0.3
1, *trans*-3-Dimethylcyclopentane	5.1	4.8	−0.3
3-Ethylpentane 1, *trans*-2-Dimethylcyclopentane	1.0	1.0	0
n-Heptane	4.7	4.9	+0.2
1, *cis*-2-Dimethylcyclopentane	5.3	4.6	−0.7
Methylcyclohexane	5.4	4.7	−0.7
Toluene	5.9	4.5	−1.4

RESULTS AND DISCUSSION

The thermal conductivity detector was first evaluated without any modification using a 200-foot by 0.01-inch i.d. squalane coated capillary column. The sensitivity of the detector was more than adequate; however, the peak shapes and resolution were very poor compared to typical results obtained on ionization detector instruments. Since the operating conditions on the capillary column were very similar to those employed when used with ionization detectors, it was concluded that the poor peak shapes and poor resolution were caused by excessive dead volume in the detector block from the column exit to the orifice leading into the sample cavity and also by excessive diffusion in the sample cavity itself. Therefore, the detector was modified as described above and shown in Figure 1. A typical chromatogram from the modified detector is shown in Figure 2 of a synthetic blend run on a 190-foot by 0.01-inch i.d. squalane coated stainless steel capillary column. The sample size was 1 μl. and split 100:1, and the carrier gas flow was 1.7 ml. per minute. Thus, using operating conditions normally employed with 0.01-inch i.d. capillary columns, the modified detector furnished curves comparable to those obtained with ionization detectors.

The analysis of the blend shown in Figure 2 is given in Table I. These results were obtained using a planimeter to measure the areas and were normalized directly using no calibration response factors.

The modified thermal conductivity detector instrument has been used routinely at this laboratory for the analysis of hydrocarbons in the C_8 and lighter range. The range and the performance can be improved by making similar modifications with the more recently developed Gow-Mac microvolume cells. Although the modified thermal conductivity detector is not as sensitive as the ionization detectors, it does permit the convenient and inexpensive conversion of existing thermal conductivity instruments for capillary columns.

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Fluorometric Determination of Selenium in Plants and Animals with 3,3′-Diaminobenzidine

Caution Notice

SIR: Benzidine is known to be carcinogenic. Please be advised that 3,3′-diaminobenzidine, recommended as an analytical reagent in a recent issue of your journal (*1*), is also suspect as a carcinogen. We therefore recommend that your readers be informed that the compound should be handled with extreme caution.

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Equations for the Average Solvent Composition and the Interstitial Column Volume in Column Fractional Precipitation Methods

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The separation of substances with similar physical and chemical properties, such as the fractionation of polymers into homogeneous molecular-weight fractions, remains a challenging problem. One method that has proved successful in the fractionation of industrial polymers is the column fractional precipitation technique of Baker and Williams (*2*). This is an equilibrium method in which the polymer, contained in a column and supported by an inert material such as glass beads, is subjected to simultaneous temperature and solvent gradients. A linear temperature gradient along the length of the column is achieved by jacketing the column with an aluminum block, heating the upper end of the block with Nichrome wire, circulating a cooling liquid through the lower part of the block, and insulating the entire column well. A constant-volume mixing vessel delivers solvent to the column at a rate of approximately 6 ml. per hour and contains initially a high percentage of nonsolvent, in which the polymer is only very slightly soluble. The percentage of a second solvent of higher elution power or "good" solvent increases exponentially with the total volume delivered to the column. At a particular solvent composition, the most soluble molecular-weight species of the polymer, which is precipitated nonselectively on the column support, dissolves and moves down the column to a lower temperature region, where it is again precipitated. It is redissolved by solvent of a higher elution power and is carried down the column in a series of precipitation and extraction steps. The fractions emerge from the column as saturated solutions and are generally collected with a constant-volume fraction collector. It, therefore, becomes necessary to know the average solvent composition of each collected fraction.

Equations are developed to predict the average solvent composition of these fractions on the basis of the exponential mixing function (*1*, *3*) and to determine the interstitial column volume.

EXPERIMENTAL

Apparatus. The fractional precipitation apparatus, shown in Figure 1, was built in this laboratory and was similar in design and dimensions to the one described by Baker and Williams (*2*).

The mode of solvent addition to the mixing vessel differed in many respects from that previously described (*2*). While the nonsolvent which was placed initially in the mixing vessel was either the pure solvent or a solvent mixture, the good solvent in every case constituted a single solvent which was distilled directly into the mixing vessel. A 250-ml. boiling flask and a 35 cm. long, water-cooled, Friedrichs condenser were used. The drip-tip joint of the condenser allowed the solvent to flow into the funnel of the distillate collector, which was placed between the boiling flask and the condenser. The outward-leading funnel was connected to the head of the mixing vessel by Teflon tubing, $^5/_{16}$-inch i.d. The solvent was distilled at such a rate that the funnel was kept constantly full, the excess solvent returning to the boiling flask.

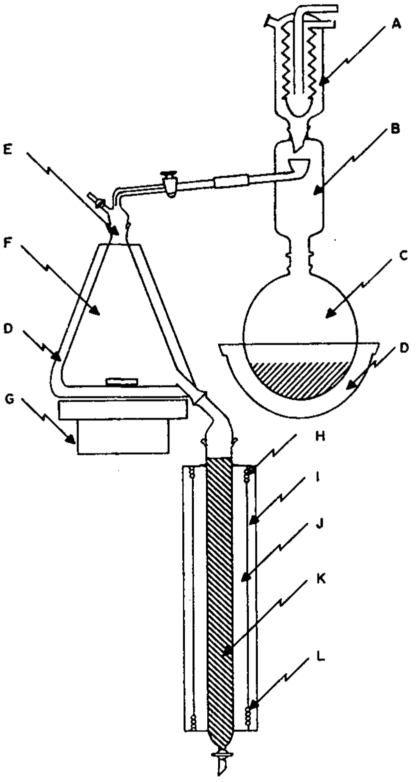

Figure 1. Fractional precipitation apparatus

- A. Condenser
- B. Distillate collector
- C. Boiling flask
- D. Heating mantle
- E. Mixing vessel head
- F. Mixing vessel
- G. Magnetic stirrer
- H. Heating wire
- I. Insulation
- J. Aluminum Block
- K. Micro glass bead packing
- L. Cooling coils

The head of the mixing vessel consisted of a 27 ₮ male joint, the upper end of which was fused to a short section of capillary tubing. The solvent flow was controlled by a stopcock, which was inserted between the capillary tubing and the Teflon connector. During the early experiments the mixing vessel had to be filled through its lower outlet by applying suction to the top of the distillate collector. The filling process was facilitated when a microstopcock was added to the head to provide an outlet to the atmosphere. In addition, this microstopcock permitted gas to be removed from the head where it may have collected during an experimental run.

Ten-milliliter fractions were collected in test tubes with a Rinco Automatic Fraction Collector.

Procedure. The solvent composition of the emerging fractions was measured using a cyclohexane–chloroform solvent system. The column was washed with 250 ml. of Spectroanalyzed cyclohexane (Fisher Scientific Co.). The same solvent was placed in the 250-ml. mixing vessel. Purified chloroform (*4*) was distilled into the mixing vessel. The column was kept at room temperature without a temperature gradient, and about 20 ml. of the solvent were left above the packing. Thirty-five fractions were collected with a 10-ml. siphon at a rate of approximately one fraction per hour. The tubes were stoppered with rubber stoppers. Aliquot portions were transferred to volumetric flasks immediately after completion of the run and diluted with Spectrograde cyclohexane.

Spectrophotometric absorption curves were recorded with a Beckman IR 4 spectrophotometer. The path length was 0.15 mm., and the reference medium was air. Per cent transmittance values at 13.1 microns were read off the chart, converted first to absorbance values, and then to volume per cent of chloroform in cyclohexane. A volume per cent of chloroform in cyclohexane calibration curve was prepared under identical conditions from chloroform–cyclohexane solutions of known compositions.

The 10-ml. siphon was calibrated by employing conditions of an actual run.

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The siphon was wetted with *p*-dioxane and then filled with the same solvent from a 50-ml. buret. Near the point where the siphon began to operate, the solvent flow from the buret was lowered to an approximate rate of 10 ml. per hour and the flow was not interrupted until the siphon was almost empty. The volume of the siphon was read from the buret.

The 250-ml. mixing vessel was calibrated by delivering five 50-ml. portions of *p*-dioxane with a pipet to the mixing vessel. After closing the vessel with the mixing head, it was completely filled from a 50-ml. buret through the upper connecting head, allowing air to escape through the microstopcock.

CALCULATIONS

The mixing function for the case of a constant-volume mixing vessel has been demonstrated to be (*1*, *3*):

$$\ln \frac{C_2 - C_1}{C_2 - C} = \frac{v}{V} \qquad (1)$$

where

C_2 = volume per cent of good solvent added to mixing vessel
C_1 = volume per cent of good solvent initially in mixing vessel
C = volume per cent of good solvent in effluent
v = total volume of effluent
V = volume of mixing vessel.

The concentration of good solvent in the effluent at any particular effluent volume is

$$C = C_2 - e^{\left(a - \frac{v}{V}\right)} \qquad (2)$$

where $a = \ln(C_2 - C_1)$, a constant.

The average concentration of good solvent, $\bar{C}$, for an effluent fraction, $v_2 - v_1$, is

$$\bar{C} = \frac{\int C(v)dv}{\int dv}$$

$$\bar{C} = C_2 + \frac{V\left[e^{\left(a - \frac{v_2}{V}\right)} - e^{\left(a - \frac{v_1}{V}\right)}\right]}{v_2 - v_1} \qquad (3)$$

The effluent volume, v, should be measured from the point where the first infinitesimally small change in solvent composition occurs in the effluent (breakthrough point). The effluent volume from the beginning of the run to this point corresponds to the interstitial column volume. Since this interstitial volume is generally not known, some means has to be found to determine the breakthrough point.

Theoretically, v can be calculated from Equation 3 by measuring the average concentration, $\bar{C}$, of any fraction over a known range, $v_2 - v_1$. If v_1 is then expressed as

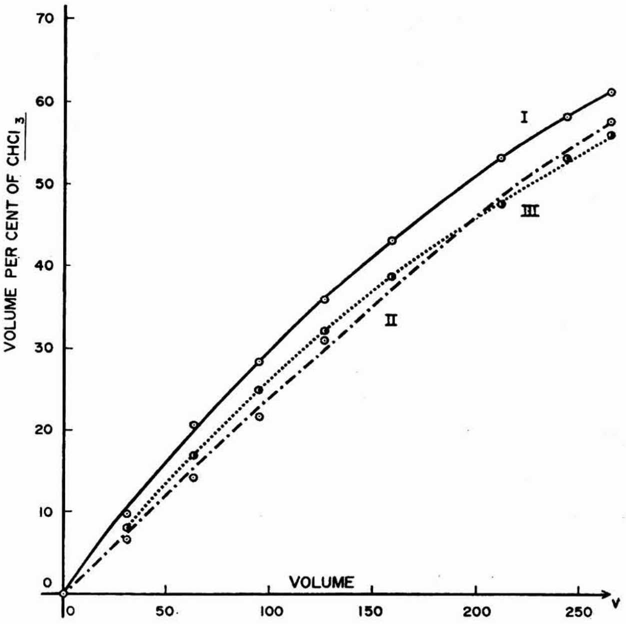
$$v_1 = v_2 - (v_2 - v_1)$$

v_2 can be obtained directly.

If the column is initially filled with pure nonsolvent, the breakthrough point corresponds to the point where the first infinitesimally small fraction of good solvent leaves the column. However, the first fraction which indicates the presence of good solvent already represents the average concentration over a small range in solvent composition. The breakthrough point, v_0, can be calculated by measuring the volume per cent of good solvent, $\bar{C}_0$, in the first fraction

Figure 2. Solvent gradient

Curve *I*: Theoretical curve
Curve *II*: Experimental curve
Curve *III*: Corrected experimental curve



$$\bar{C}_o = \frac{C(v_2' - v_o) + O(v_o - v_1')}{v_2' - v_1'} \qquad (4)$$

where C = the average concentration of good solvent over the volume range, $v_2' - v_0$.

$$\bar{C}_o(v_2' - v_1') = C_2(v_2' - v_o) + Ve^a\left[e^{\left(-\frac{v_2'}{V}\right)} - e^{\left(-\frac{v_o}{V}\right)}\right] \qquad (5)$$

If $v_o = 0$, then

$$v_2' = \frac{\bar{C}_o(v_2' - v_1')}{C_1} \qquad (6)$$

For the case that the mixing vessel contains initially only nonsolvent ($C_1 = 0$), v_2' from Equation 6 becomes infinite. A more reasonable expression is obtained for the limiting case of $C_1 = 0$, if the exponential terms in Equation 5 are expanded to the quadratic terms.

$$v_2' = \left[\frac{2V\bar{C}_o\,(v_2' - v_1')}{C_2}\right]^{1/2} \qquad (7)$$

DISCUSSION

The cyclohexane–chloroform system was used to compare theoretical solvent composition values of fractions with observed values. The amount of chloroform in a fraction was determined from infrared absorption measurements, using the C–Cl stretching band at 13.1 microns. Cyclohexane was completely transparent at this wavelength. The calibration curve followed Beer's law. The interstitial column volume was calculated to be 81.40 ml.

The observed chloroform concentrations were low compared to the theoretical values, although the general shape of the exponential curve was followed (Figure 2). This indicated that mixing occurred not only in the mixing vessel but also in the column, the mixing volume being much larger than the measured volume of 270 ml. By arbitrarily selecting a point on the experimental curve, such as the point corresponding to fraction No. 31 with 53.5 volume per cent of chloroform, and recalculating the theoretical mixing volume, a corrected volume of 310 ml. was obtained. Using this new volume to recalculate all theoretical values, a new curve was obtained, which coincided much more closely with the experimental curve. The fact that the two curves were not parallel indicated that the additional mixing volume could not simply be added to the measured mixing volume.

The difference between the measured and calculated mixing volumes was much larger than could be explained by assuming mixing to have occurred in the liquid above the packing. Mixing must also have occurred within the packing and was probably caused by diffusion, especially in view of the slow solvent flow.

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Desalting by High Voltage Electrophoresis

Hsiu-Ying T. Yang and M. R. Shetlar, Department of Biochemistry, University of Oklahoma School of Medicine, Oklahoma City, Okla.

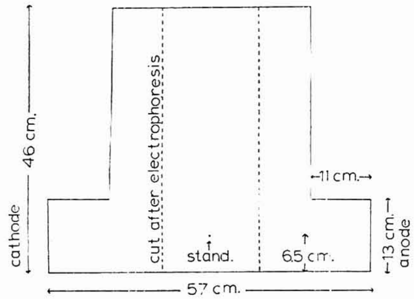

Figure 1. Preparation of paper for desalting by high voltage electrophoresis

Sample is applied at site indicated. Extensions to right and left dip into anode and cathode chambers, respectively

IN THE PAPER CHROMATOGRAPHY of sugars, interfering salts are formed by hydrolysis and subsequent neutralization. To obtain salt-free samples, several techniques have been used: electrolytic desalting, ion exchange resin, and organic solvent extraction (*1*). However, these techniques require relatively large volumes of solution, and therefore are not convenient for microquantities of material. Recently we have found that desalting can be carried out by high voltage electrophoresis directly on a sample which has been previously spotted on the paper. A buffer containing only volatile constituents is used. This method allows the desalting of small volumes of solutions (5 to 25 μl.) and does not require reconcentration of the desalted sample as in other procedures. In this method, the inorganic ions are rapidly removed and neutral sugars are left at the position near the origin. Amino acids, amino sugars, and sugar acids migrate in the electrical field, but are also satisfactorily desalted.

EXPERIMENTAL

For the Servonuclear Model ET48 high voltage electrophoresis equipment, a sheet of filter paper is cut to the size and dimensions shown in Figure 1. The sample to be desalted is spotted on the filter paper, and electrophoresis is carried out with a buffer of pH 3.8, pyridine–acetic acid–water (1:10:89), at 3000 volts for 35 to 40 minutes. The paper is then dried carefully with a hair dryer to remove the buffer and is cut for chromatography as indicated in Figure 1. The standards may then be applied on the paper, and chromatography carried out with the solvent flowing at right angle to the electrophoretic run.

Figure 2 shows the results with a sample desalted by this procedure followed by descending chromatography. The original sample of 25 μl. contained 20 μg. of galactose and 30 μg. of *N*-acetylglucosamine in 0.125*M* tris buffer and 2.5*M* NaCl. The spots were developed by benzidine reagent after paper chromatography (solvent system, butanol–acetic acid–water, 4:1:5). Without preliminary desalting, no discrete spots were detected.

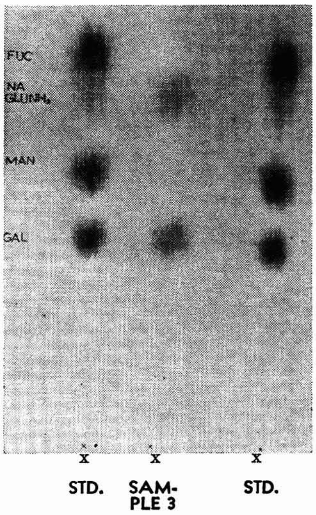

Figure 2. Chromatography of sugars with butanol–acetic acid–water mixture (4:1:5 by volume) after desalting by high voltage electrophoresis

Sugars are indicated by GAL, galactose; MAN, mannose; NAGLUNH$_2$, *N*-acetylglucosamine; FUC, fucose. Sample 3 is an enzymatic hydrolysate of a glycoprotein preparation

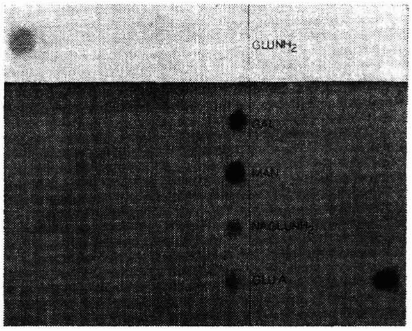

Figure 3. High voltage electrophoresis of sugars with pyridine–acetic acid–water mixture (1:10:89 by volume)

Sugars are GAL, galactose; MAN, mannose; NAGLUNH$_2$, N-acetylglucosamine; FUC, fucose. Glucuronic acid preparation (GLUA) yields two spots: lactone at origin and free acid moving toward anode

When the sample was spotted equidistant from both electrodes, no appreciable movement of the sugar occurs. When the sample was spotted at the position near the anode, the neutral sugars moved slightly toward the cathode by electro-osmosis flow. However, this movement did not influence the result of chromatography. When only neutral sugars were involved, several samples were desalted on the same strip by this technique. Both standard and samples were applied on the paper before electrophoresis.

Under the conditions given above, amino sugars moved toward the cathode and free sugar acids toward the anode (Figure 3). Dicarboxylic amino acids moved as anions; other amino acids moved as cations. Preparations containing these compounds were satisfactorily desalted.

LITERATURE CITED

(1) Smith, I., "Chromatographic and Electrophoretic Techniques," Vol. 1, p. 40, Interscience, New York, 1960.

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ANALYTICAL CHEMISTRY

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Abbreviation used: col., column; corr., correction; corresp., correspondence; rev., review; sci. commun., scientific communication; symp., symposium. Numbers followed by the letter "A" refer to pages in the advertising section; those followed by the letter "R" refer to pages in the April review issue.

SUBJECT INDEX

Abbreviation used: col., column; corr., correction; corresp., correspondence; rev., review; sci. commun., scientific communication; symp., symposium. Numbers followed by the letter "A" refer to pages in the advertising section; those followed by the letter "R" refer to pages in the April review issue.

Spectrometry Nomenclature

We have compiled the following list of terms, their definitions, and abbreviations, which occur most frequently in papers on spectrometry. The list indicates our preferred usages in an attempt to obtain some consistency in a field where much discrepancy exists. Sources used in this compilation were: ASTM Committee E-13 on Absorption Spectroscopy (1959 revision of tentative definitions); H. K. Hughes *et al.* [ANAL. CHEM. 24, 1349 (1952)]; and *Chemical Abstracts*. Our advisory board members considered the terms and the following list includes their suggestions.

Absorbance, A. (Not optical density, absorbancy, or extinction.) Logarithm to the base 10 of the reciprocal of the transmittance $A = \log_{10}(1/T)$.

Absorptivity, a. (Not k.) (Not absorbancy index, specific extinction, or extinction coefficient.) Absorbance divided by the product of the concentration of the substance (in g./l.) and the sample path length (in cm.),

$$a = \frac{A}{bc}$$

Absorptivity, Molar, ϵ. (Not molar absorbancy index, molar extinction coefficient, or molar absorption coefficient.) Product of the absorptivity, a, and the molecular weight of the substance.

Angstrom, A. (Not Å.) Unit of length equal to 1/6438.4696 of wavelength of red line of Cd. For practical purposes, it is considered equal to 10^{-8} cm.

Beer's Law. (Representing Beer-Lambert law.) Absorptivity of a substance is a constant with respect to changes in concentration.

Concentration, c. Quantity of the substance contained in a unit quantity of sample. (In absorption spectrometry it is usually expressed in grams or moles per liter.)

Frequency. Number of cycles per unit time.

Infrared. The region of the electromagnetic spectrum extending from approximately 0.78 to 300 microns.

Micron, μ. Unit of length equal to 10^{-6} meter.

Millimicron, mμ. Unit of length equal to one thousandth of a micron. Almost but not exactly equal to 10 angstroms.

Sample Path Length, b. (Not l or d.) Internal cell or sample length, usually given in centimeters.

Spectrograph. Instrument with an entrance slit and dispersing device that uses photography to obtain a record of spectral range. The radiant power passing through the optical system is integrated over time, and the quantity recorded is a function of radiant energy.

Spectrometer, Optical. Instrument with an entrance slit, a dispersing device, and with one or more exit slits, with which measurements are made at selected wavelengths within the spectral range, or by scanning over the range. The quantity detected is a function of radiant power.

Spectrometry. Branch of physical science treating the measurement of spectra.

Spectrophotometer. Spectrometer with associated equipment, so that it furnishes the ratio, or a function of the ratio, of the radiant power of two beams as a function of spectral wavelength. These two beams may be separated in time, space, or both.

Transmittance, T. (Not transmittancy or transmission.) The ratio of the radiant power transmitted by a sample to the radiant power incident on the sample.

Ultraviolet. The region of the electromagnetic spectrum from approximately 10 to 380 mμ. The term without further qualification usually refers to the region from 200 to 380 mμ.

Visible. Pertaining to radiant energy in the electromagnetic spectral range visible to the human eye (approximately 380 to 780 mμ).

Wavelength. (One word.) The distance, measured along the line of propagation, between two points that are in phase on adjacent waves—units A., mμ, and μ.

Wavenumber. (One word.) Number of waves per unit length. The usual unit of wavenumber is the reciprocal centimeter, cm.$^{-1}$ In terms of this unit, the wavenumber is the reciprocal of the wavelength when the latter is in centimeters in vacuo.

Guide for Measures of Precision and Accuracy

It is important to know what an author means when he writes of precision and accuracy. The following definitions are applicable when one operator provides a series of test results and no prior information on the method is used. They are endorsed by ANALYTICAL CHEMISTRY—after careful study by members of its Advisory Board and on advice of qualified statisticians—and suggested when results reported are suitable for statistical treatment (based on 5 or more determinations).

Series. A number of test results which possess common properties that identify them uniquely.

Mean. The sum of a series of test results divided by the number in the series. Arithmetic mean is understood.

Precision Data. Measurements which relate to the variation among the test results themselves—i.e., the scatter or dispersion of a series of test results, without assumption of any prior information. The following measures apply:

VARIANCE. The sum of squares of deviations of the test results from the mean of the series after divisions by one less than the total number of test results.

STANDARD DEVIATION. The square root of the variance.

RELATIVE STANDARD DEVIATION. The standard deviation of a series of test results as a percentage of the mean of this series. This term is preferred over "coefficient of variation."

RANGE. The difference in magnitude between the highest test result and the lowest test result in a series.

Accuracy Data. Measurements which relate to the difference between the average test results and the true result when the latter is known or assumed. The following measures apply:

MEAN ERROR. The average difference with regard to sign of the test results from the true results. Also equal to the difference between the average of a series of test results and the true result.

RELATIVE ERROR. The mean error of a series of test results as a percentage of the true result.

MANUSCRIPT REQUIREMENTS

The following guide is published by the Editors of ANALYTICAL CHEMISTRY to aid authors in writing, and editors and reviewers in expediting review and publication of manuscripts.

SCOPE. Articles should describe significant developments in qualitative or quantitative analysis or instrumentation in either theoretical or applied aspects. They are intended to meet needs of those engaged in theoretical and fundamental analytical research and those who are developing practical analytical methods, procedures, and techniques. Articles of a theoretical nature which hold promise of future analytical applications, even though not directly related to analysis at the moment, are included. Papers involving spectrometric data should offer a new or modified approach to analysis in a particular field, not just extend the existing library of data.

In addition to full-length articles, *Scientific Communications, Correspondence,* and *Aids for the Analyst* are also published. *Scientific Communications* should be brief disclosures of new analytical developments. They may be offered to establish priority with the full article to follow or as reports of work which the authors do not plan to continue. *Correspondence* should describe modifications or extensions of previously published work or discuss published articles. It may also include exchanges of views between author and reader. *Aids for the Analyst* should be brief descriptions of novel apparatus or techniques, requiring real ingenuity on the part of the author, which offer definite advantages over similar ones already available.

ABSTRACT. Use no more than 150 words to summarize results as specifically as possible and to indicate what is new, different, and significant. No abstracts are used for *Scientific Communications, Correspondence,* and *Aids.*

TITLE. Use specific and informative titles. Indicate, where applicable, compound or element determined, method, and special reagents—e.g., "Spectrophotometric Determination of Thallium in Zinc and Cadmium with Rhodamine B." Do not use series designations.

AUTHORSHIP. Given name and initial of second name are generally adequate for correct identification. (Write out first name if there is no middle initial.) Omit professional and official titles here, as well as in the Acknowledgment. Give complete mailing address for place where work was done. Add current address of each author, if different, as footnote on title page.

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Write for the specialist. Do not include information and details or techniques which should be common knowledge to him. With this in mind we suggest:

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Experimental

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Results, Discussions, and Conclusions. Be complete and relevant but concise. Omit calculations which are well known to the specialist. Use Conclusions only when necessary for interpretation and not to summarize information already given.

Graphs and Tables. Do not use graphs or tables which duplicate each other or material already in the text. Omit straight-line calibration curves; give information in tabular form or in a sentence or two in the text.

Organization. Indicate the breakdown among and within sections with center heads and side heads. Results and Discussion typically follow Experimental Section. Keep all information pertinent to a particular section within that section—e.g., do not present results in the Experimental Section. Avoid repetition. Do not use footnotes; include the information at an appropriate place in the text.

NOMENCLATURE. Follow nomenclature style of *Chemical Abstracts;* avoid trivial names. Well known symbols and formulas may be used (write out in title and abstract) if no ambiguity is likely. Define trade names and abbreviations at point of first use. Trade names should be capitalized.

Use consistent units of measurement (preferably metric) and give dimensions for all terms. If nomenclature is specialized, as in mathematical and engineering reports, include a Nomenclature section at end of paper, giving definitions and dimensions for all terms. Write out names of Greek letters and other special symbols in margin of manuscript at point of first use.

Write all equations and formulas clearly (type if possible) and number equations consecutively. Place superscripts and subscripts accurately; indicate capital letters and distinguish between characters which are alike on the keyboard—e.g., one and the letter "EL," zero and the letter "OH." Avoid superscripts which may be confused with exponents.

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(1) Adams, J. A. S., Maeck, W. J., Anal. Chem. **26,** 207 (1954).

(2) American Instrument Co., Silver Spring, Md., *Bull.* **2778H** (1956).

(3) Arthur, Paul, Donahoo, W. P., *U. S. At. Energy Comm. Rept.* **CCC-1024-TR-221** (1957).

(4) Bellamy, L. J., "Infrared Spectra of Complex Molecules," 2nd ed., p. 20, Methuen and Co., London, 1958.

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(6) Thurston, J. T. (to American Cyanamid Co.), U. S. Patent **2,525,247** (Oct. 10, 1950).

(7) Zemany, P. D., General Electric Co., Schenectady, N. Y., private communication, 1960.

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Submit original drawings (or sharp glossy prints) of graphs and diagrams prepared on tracing cloth or plain paper. All lines, lettering, and numbering should be sharp and unbroken. If coordinate paper is used, use blue cross-hatch lines as no other color will "screen out." Typed lettering does not reproduce well; use black India ink and a lettering set for all letters, numbers, and symbols. On 8- × 10-inch copy, lettering should be at least $^1/_8$ inch high and 0.017 inch thick—for example, with a Leroy lettering set, use template 120 C and pen No. 0. Lettering on copy of other sizes should be in proportion. Label ordinates and abscissas of graphs along the axes and outside the graph proper.

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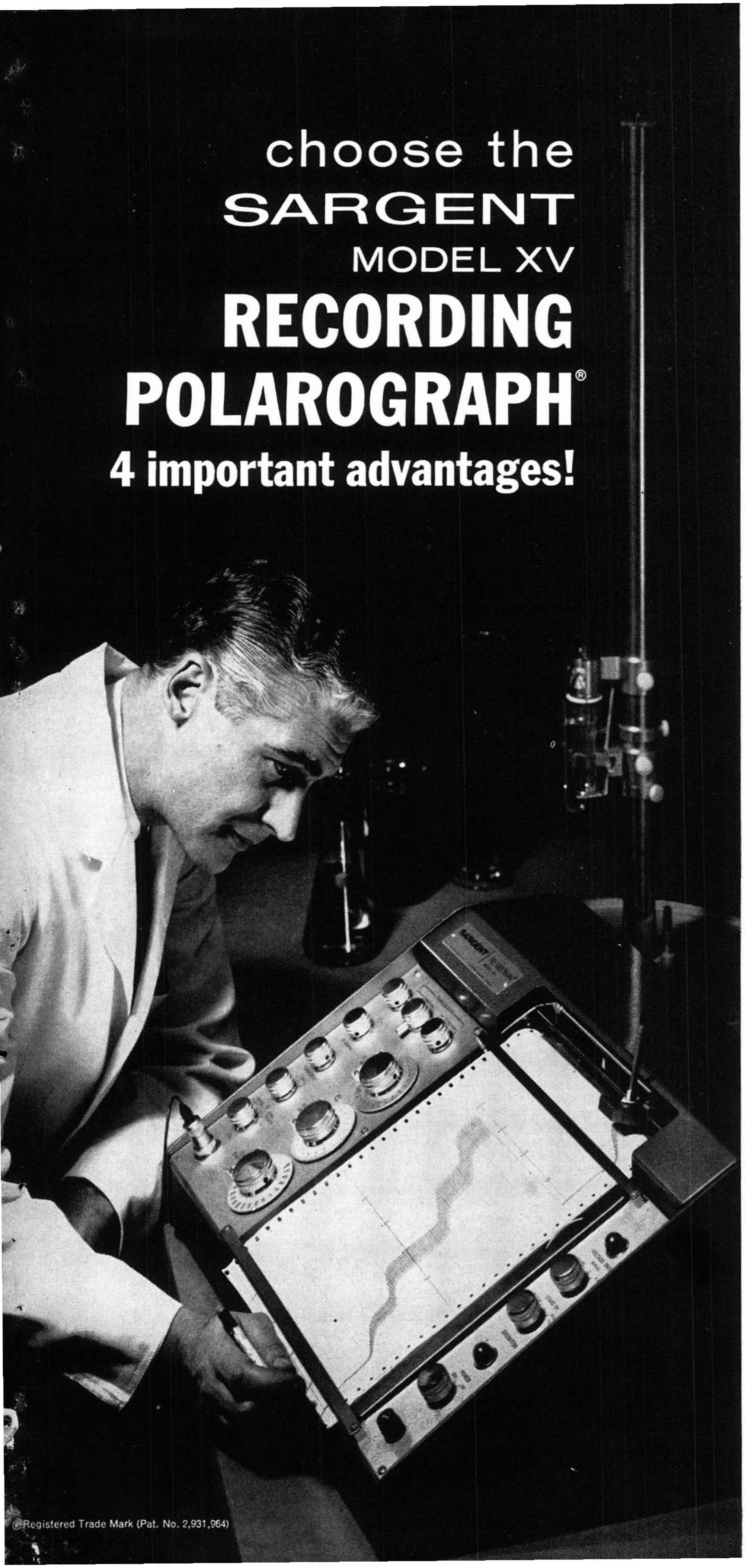

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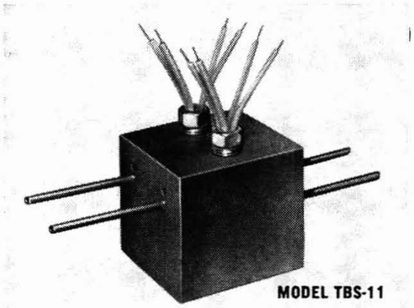
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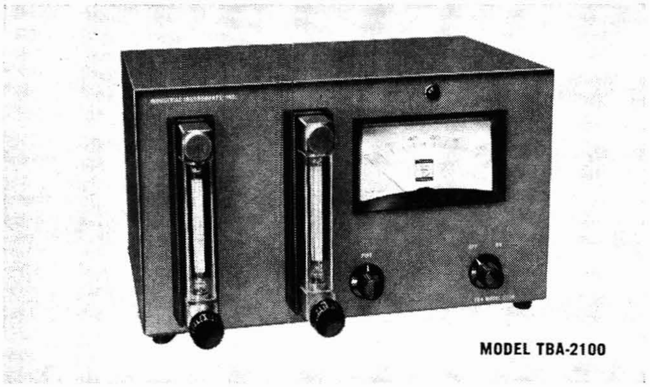
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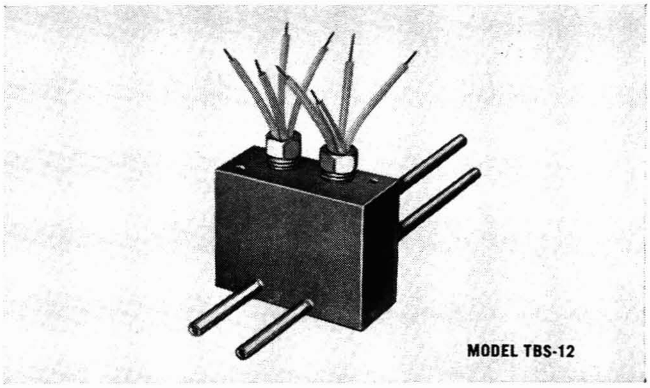
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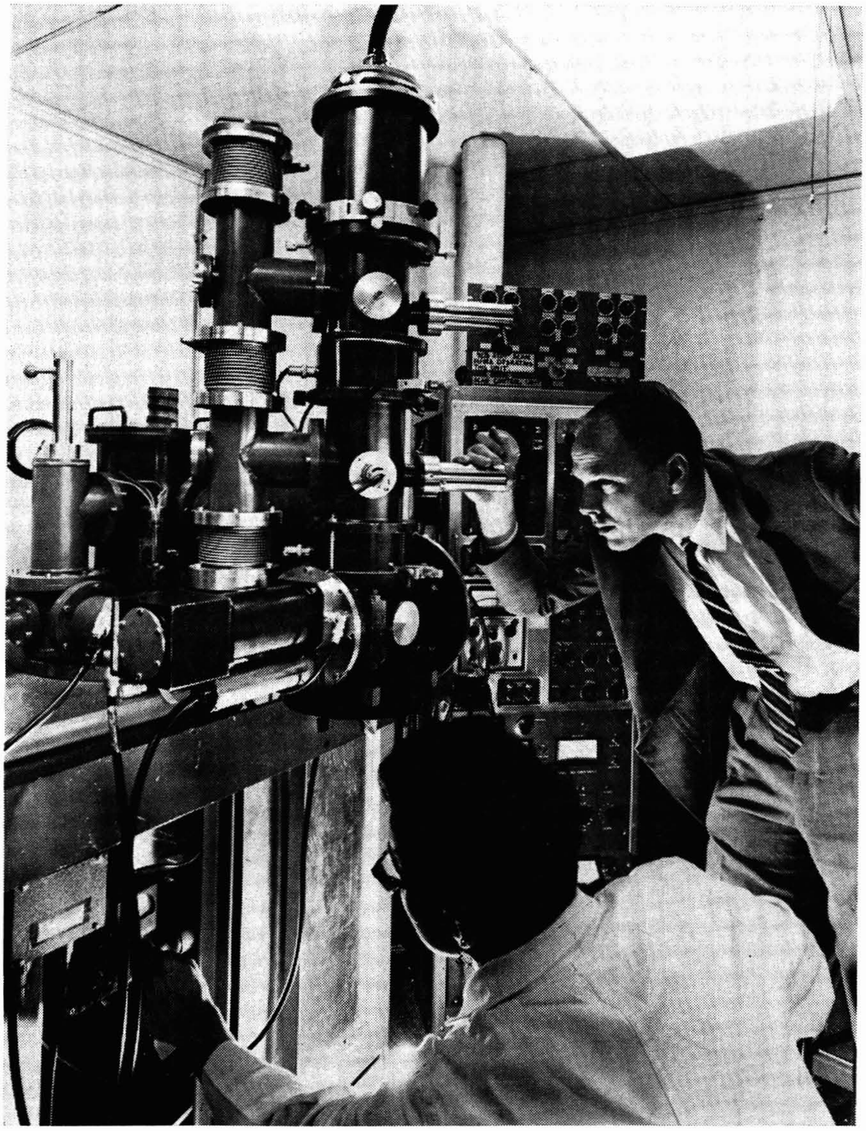

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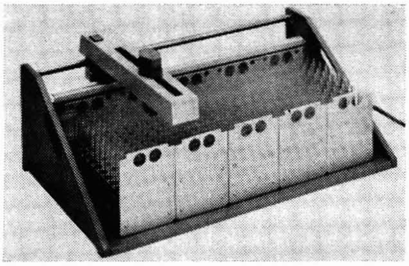
Radioassay Electroscope

The Model RE-1050 radioassay electroscope can be used for tracer studies, radioassays, and for teaching the basic fundamentals of nuclear science on all school levels. The instrument measures the number of ionizing events per unit of time from any radioactive source. It is essentially an ion chamber with a quartz-fiber voltmeter as the indicating element. A fiber image drifts across the eye-piece scale in direct proportion to the radiation in the sample. Functioning independently of outside light and power sources, it is charged by a transistorized power supply operating on one standard "D" battery. Atomic Accessories Inc., 811 West Merrick Road, Valley Stream, N. Y. **402**



Light in weight and easily carried, the Model 132 fraction collector holds 250 tubes. The system automatically collects effluent from chromatographic columns on a timed flow, drop counting, or volumetric basis. Controls are housed in a separate unit. Spinco Division of Beckman Instruments, Inc., Palo Alto, Calif. **403**

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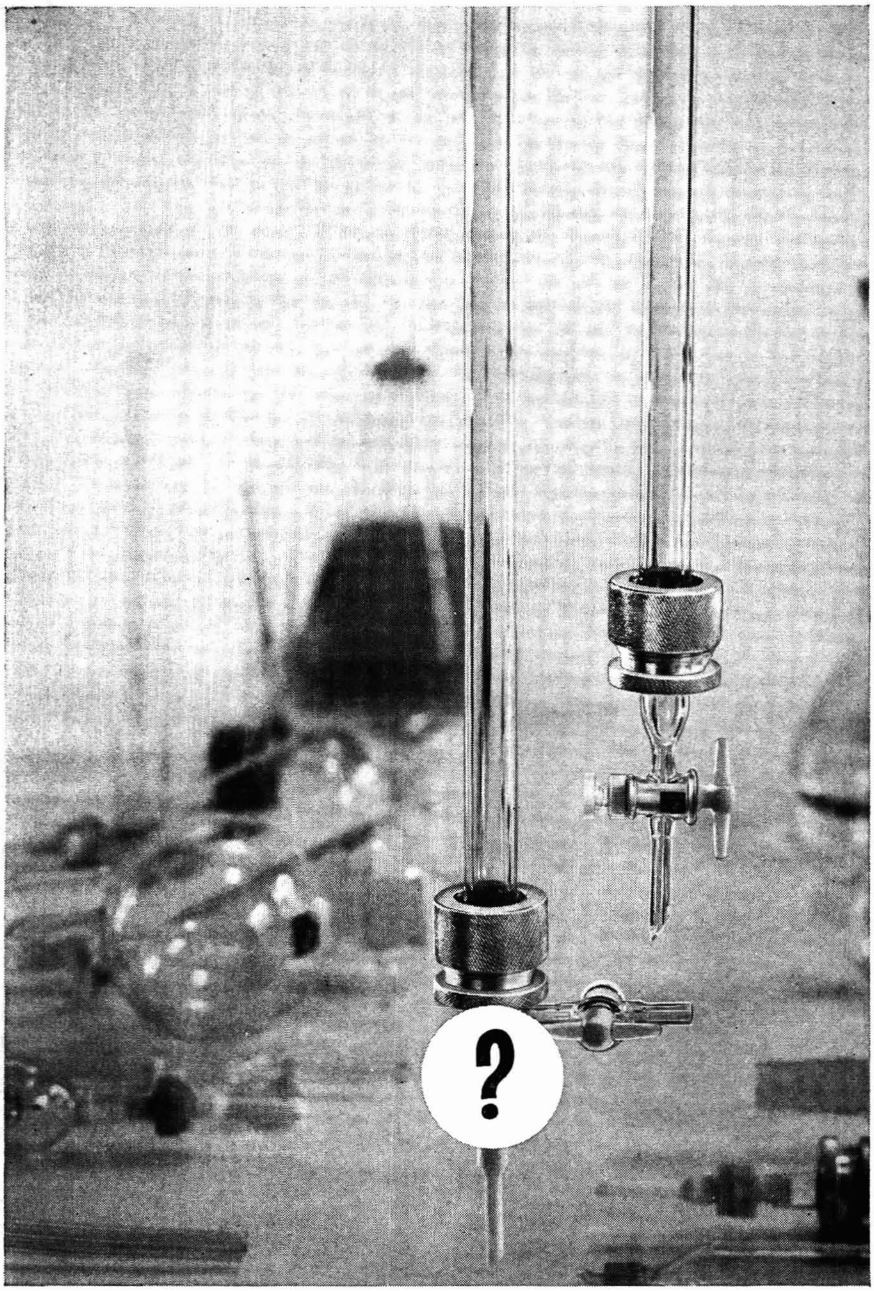

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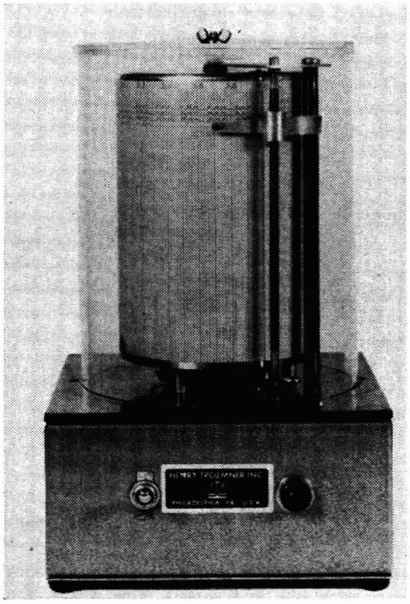
The Series FH-300 fume hoods are specifically designed for air conditioned laboratories and "breathe" with the laboratory area in which they are installed. The hood draws only 50% of its air from the room itself, the balance being ducted in from the outside. Thus, complete exhaustion of undesirable fumes is effected while the load on the air conditioning system is reduced by half. Laboratory Furniture Co., Mineola, N. Y. **405**



The drum-and-stylus recorder, Series SP, indicates "on" and "off" conditions of any piece of laboratory apparatus, enabling the analyst to determine how long a particular operation takes to perform, and also elapsed time between operating cycles, down to a half second. It is also used for charting activity of animals during experiments. Henry Troemner, Inc., 22nd and Master Sts., Philadelphia 21, Pa. **406**

Automatic Analyzer

A newly-developed automatic analyzer for continuously monitoring major atmospheric pollutants has been perfected by Kem-Tech Laboratories, Inc., Baton Rouge, La. Precision Scientific Development will manufacture and sell the analyzer. The analyzer employs wet chemical systems which permit determination of atmospheric concentrations of nitrogen dioxide, sulfur dioxide, and formaldehyde in the part-per-hundred million range on a continuous basis. Precision Scientific Development Co., 3737 W. Cortland St., Chicago 47, Ill. **407**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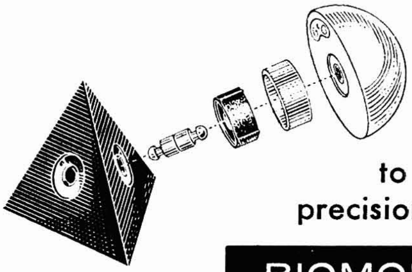

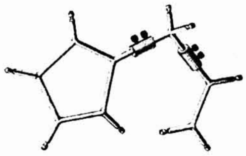

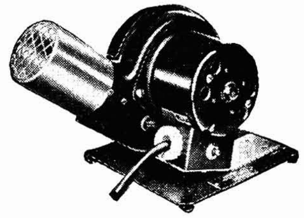

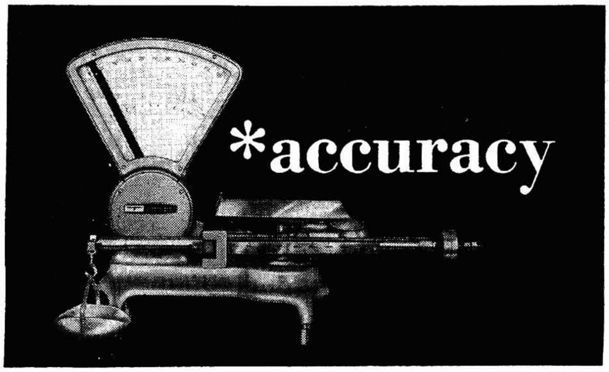
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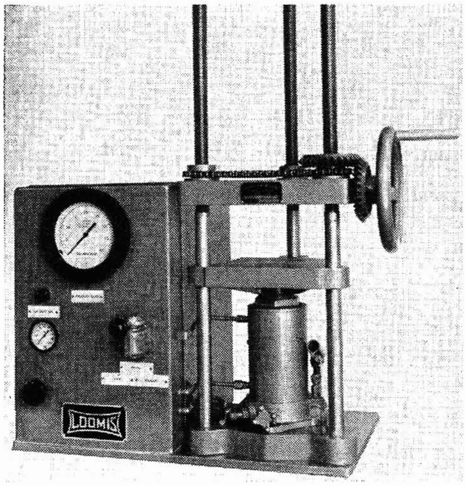

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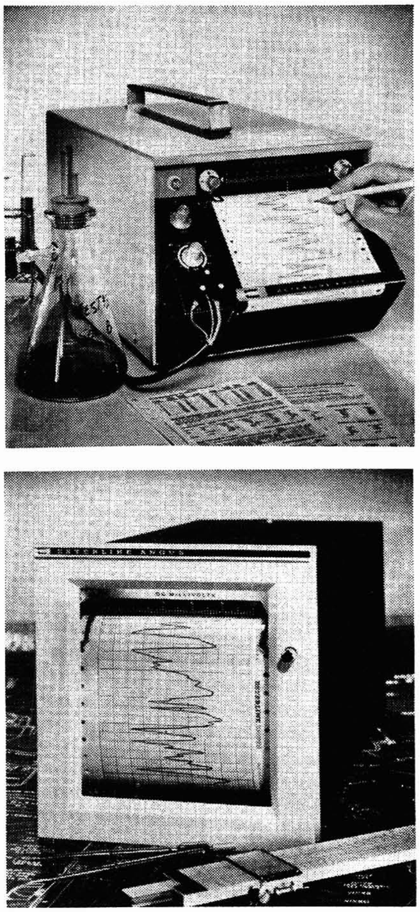


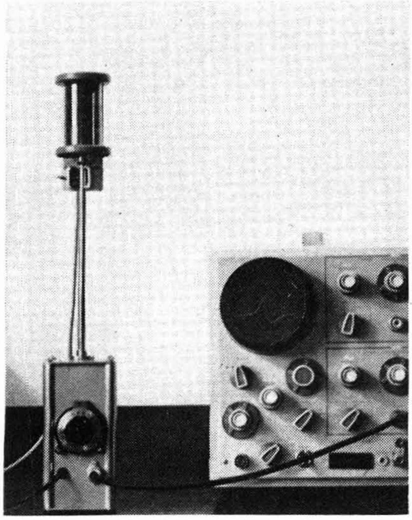
A new line of instruments features the SPEEDServo whose response speed is ⅛ second full scale. Also included is the GRAPHLine, Series "S", which includes rectilinear single-channel and rectilinear two-channel permanent-magnet moving-coil recorders, plus inkless and ink-type event recorders. All instruments are available in either portable or flush models. The portable model shown at top has a large, sloped writing surface and the flush model case shown has an 8″ × 8″ front. Both models accommodate a full-size six-inch chart driven from both sides. Esterline Angus Instrument Co., P. O. Box 596, Indianapolis 6, Ind. **408**

Thermoelectric Immersion Cooler

This thermal pump is 12 inches long, $2^3/_4$ inches wide, and $1^1/_8$ inches thick. It can be used to cool a beaker of chemicals, or, when used with a thermoregulator, it can hold a constant temperature bath below the laboratory ambient temperature. A 25-amp. d.c. power supply is available for energizing the cooler. A 12-page booklet describes the operation of this equipment. Whirlpool Corp., Benton Harbor, Mich. **409**

Durable Glass Pipets

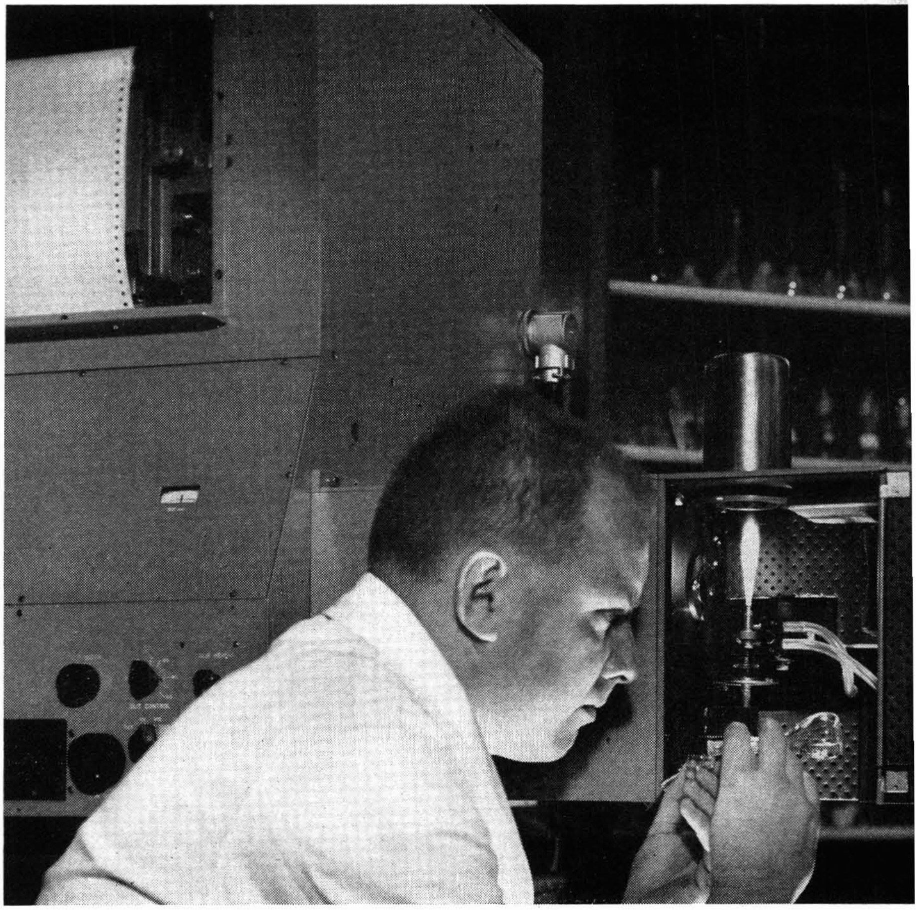
New serological pipets that offer maximum tip protection and surface durability are available at no increase in cost. The pipet, SAFE-GARD, is made of specially formulated borosilicate glass. This glass, N-51A, also minimizes clouding and fogging and resists abrasion and scratching. These pipets are available in nine styles and 53 sizes. Owens-Illinois Glass Co., Toledo 1, Ohio. **410**

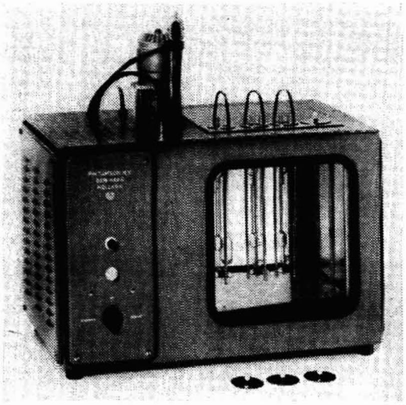


The Scientifica electron spin resonance kit is designed for comparison with an X band spectrometer. The apparatus will provide a useful instrument for research workers or serve as an introduction to electron spin resonance for science students. The instruction manual describes how fundamental constants such as the g value, gyromagnetic ratio, e/m, can be measured to a high degree of accuracy. Use of the apparatus will also demonstrate the significance of magnetic field homogeneity, marginal oscillation, frequency, and magnetic field dependence of the signal, relaxation processes, etc. Scientifica, 148 St. Dunstan's Ave., Acton, London W.3, England. **411**

Syringe Pumps

Motor driven syringe pumps for the laboratory offer a variety of discrete flow rates, ranging from 0.11 cc. per day to 29.5 cc. per minute. One model handles syringes up to 10 cc. in capacity and another model holds up to 100 cc.-capacity syringes. Continuously variable speed models include a separate control and cover a range of 40 to 1 in the smaller size, and 48 to 1 in the larger. Sage Instruments, Inc., 2 Spring St., White Plains, N. Y. **412**

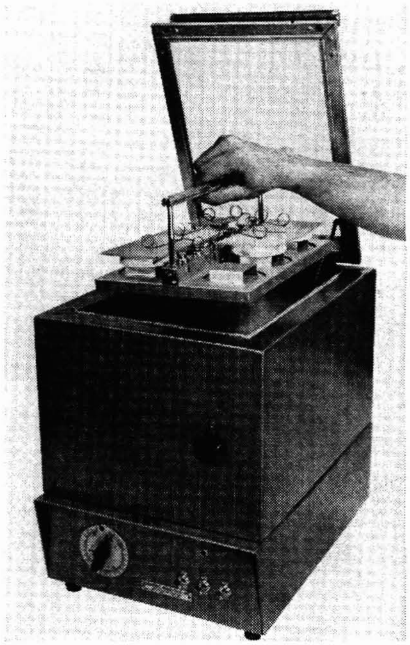




The Model TV-40 viscometer bath offers close temperature control over the range 0° to 230° C. With water as bath medium, maximum temperature variation in the entire bath is only ±0.005° C. With oils as media, maximum variation is only ±0.01° C. Two immersion heaters are used: a quartz control heater and a stainless-steel-jacketed booster heater. Once the contact thermometer of the bath's thermoregulator has been set to give the desired temperature, no other adjustment is required. A cooling coil with inlet and outlet connections to a refrigerant circulating system is also provided. Below room temperature, the control heater operates to offset the coolant's effect. This equipment is manufactured by P. M. Tamson of the Netherlands for Fisher Scientific Co., 300 Fisher Bldg., Pittsburgh 19, Pa. **413**

Differential Thermal Analysis and Thermal Gravimetric Attachment

The differential thermal analysis apparatus offers automatic control. Results are recorded on graph paper in the X-Y recorder. The furnace operates to 1800° C., and can be equipped for operation in a vacuum, under pressure, and in atmospheres with compatible gases. The thermal gravimetric attachment operates on the null point system, using a transducer sensitive to 0.00025 mm. at the balance. Variations in the instruments can be made to meet specific test conditions. Harrop Precision Furnace Co., 3470 East Fifth Ave., Columbus, Ohio. **414**



The diffusion oven Model OD-370 offers gentle swirling mixing at controlled temperatures for Conway dishes, Erlenmeyer flasks, and beakers. Alcohol and ammonia microdiffusion procedures as well as urea, amines, and chlorides by Conway methods can be performed. The oven may also be used for measurement of oxidation rates, tissue culture growths, Mohr chloride and acetone determinations. Scientific Industries, Inc., 220-05 97 Ave., Queens Village, N. Y. 11429. **415**

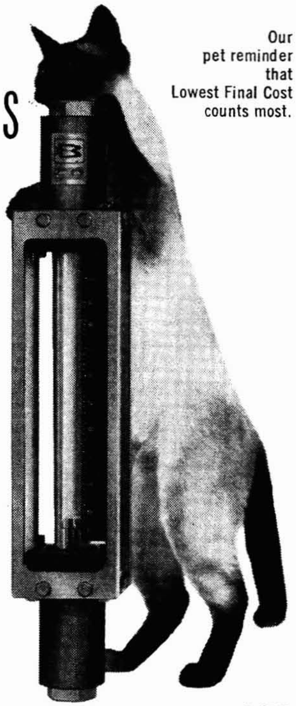
Water Analysis Equipment

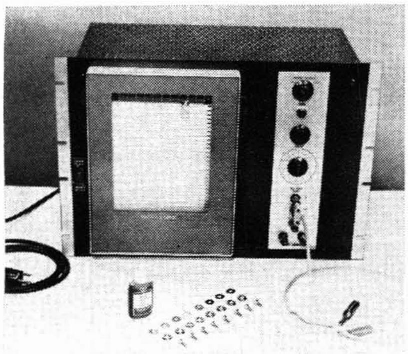
Three types of water analysis labs contain all the material needed for testing water quality. The basic furniture consists of a 5-foot wide sink and a 4-foot wide, 3-drawer cabinet. In addition, each kit contains an analytical balance, filter pump, glassware washer, 4-place vacuum manifold, magnetic mixer, bottles, burets, cylinders, flasks, funnels, thermometer, and supports plus the necessary accessories and reagents to meet the buyer's special needs.

Lab A for well-water supplies includes material necessary for coliform bacteria and 4 standard chlorine tests.

Lab B for surface-water supplies contains the material necessary for coliform bacteria, chlorine, alkalinity, CO_2, fluorides, pH, surfactants, and turbidity tests.

Lab C for water-softening plants contains material necessary for all the tests of Lab B plus hardness, phosphates, saturation, and stability, and sulfates. Fisher Scientific Co., 300 Fisher Bldg., Pittsburgh 19, Pa. **416**

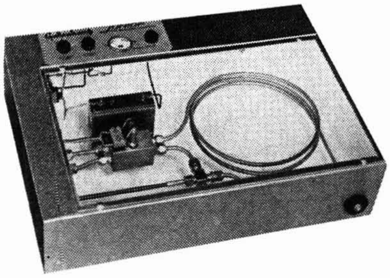




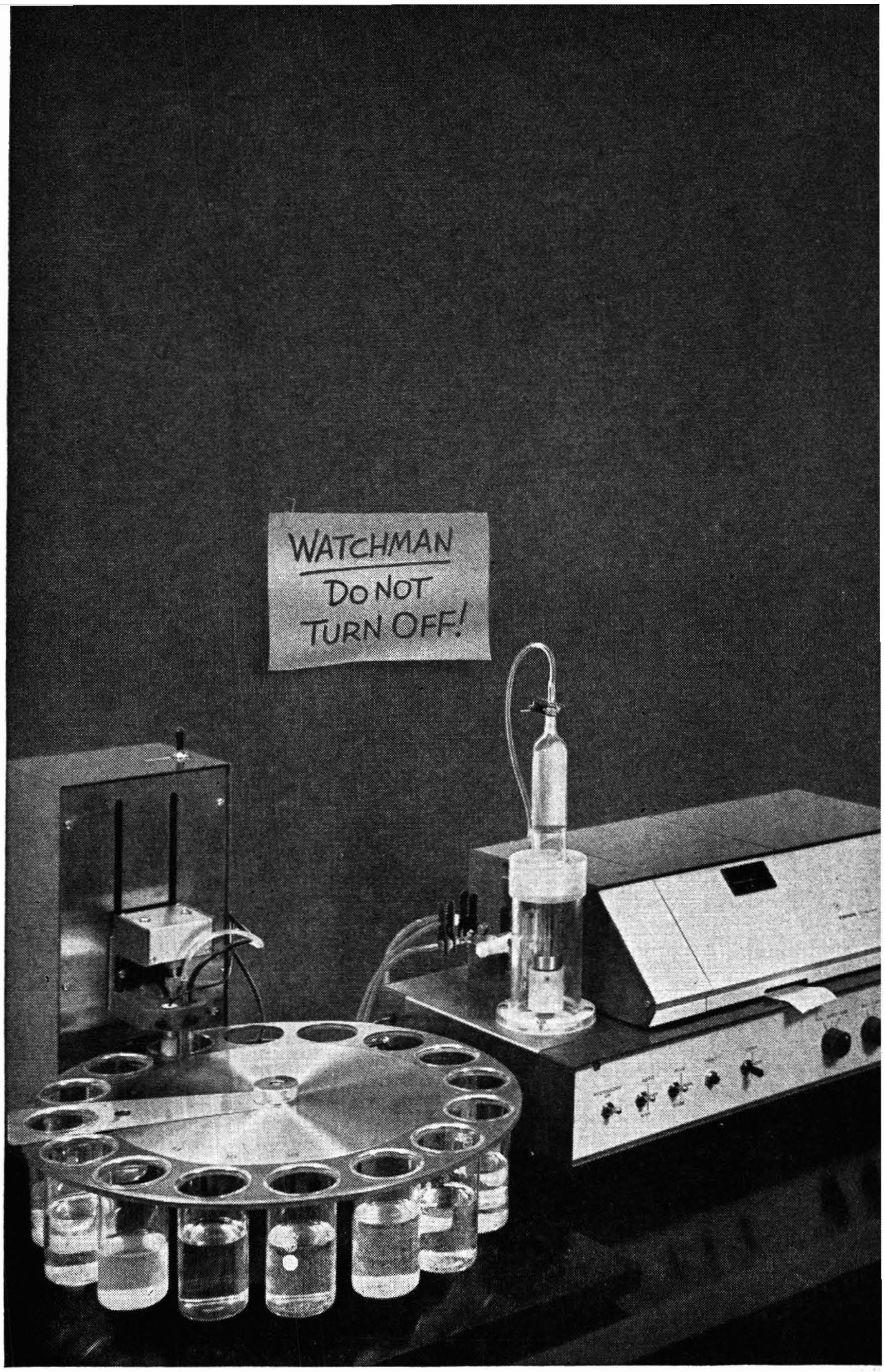
A rack mounted model of the V.O.M.-5 strip chart recorder is now available. Design of a special mounting panel with slotted edges permits the recorder to fit into the standard 19″ rack. As a result, the V.O.M.-5 can be used as an integrated component of a larger instrument panel. Operation of the new model is identical with the standard B&L recorder, except that input signal terminals are provided for both front and rear connection. The rear area of the instrument has a protective cover. Bausch & Lomb Inc., Rochester 2, N. Y. **417**

Economical Gas Chromatograph

The Chromagas C. G. 1 has a flame ionization detector, temperature programming, and independent detection and oven heating. It weighs only 18 kg. Both preparative and analytical columns are available. Temperature is variable from 50° to 320° C. Laboratoire E. B. Profit, 54, rue des Alouettes, Paries 19^{e}, France. **418**



The Junior gas chromatograph can be used to analyze gas and liquids with boiling points up to 80° C. The thermistor katharometer is powered by a dry battery. Peaks may be plotted by using a galvanometer, or by using a 6-inch strip chart recorder. The detection limit of the katharometer is about 10^{-7} gram. This gas chromatograph was designed for teaching purposes but it can also serve some industrial needs. A. Gallenkamp & Co., Ltd., Technico House, Christopher St., London, E. C. 2, England. **419**



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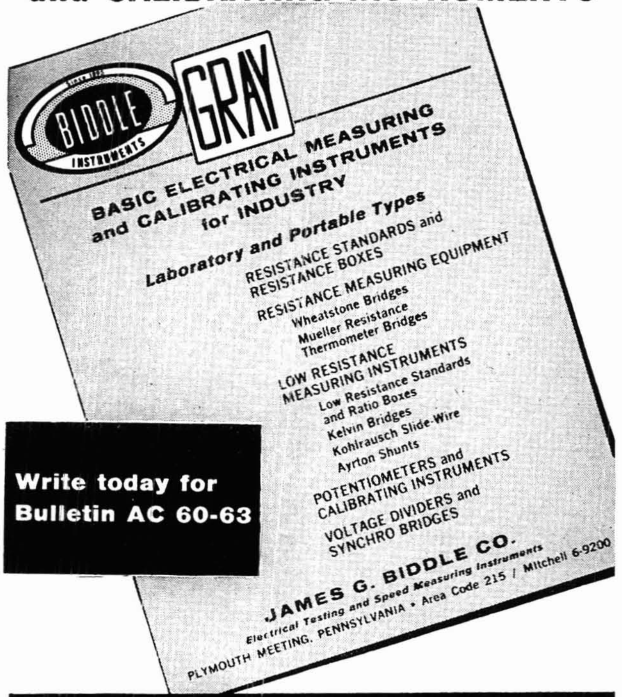

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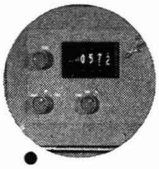

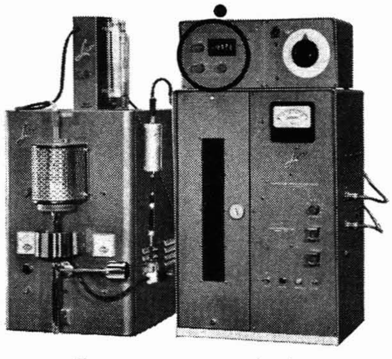

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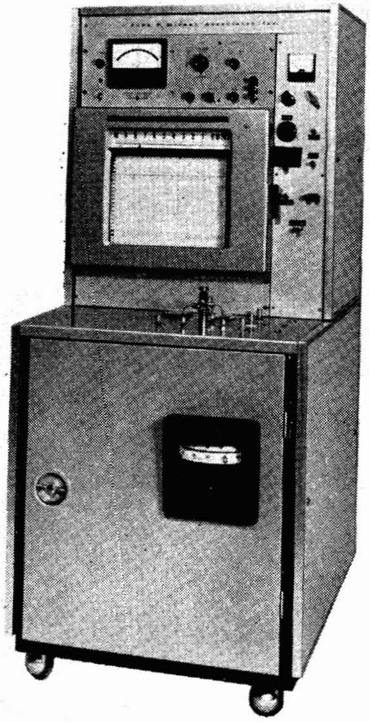
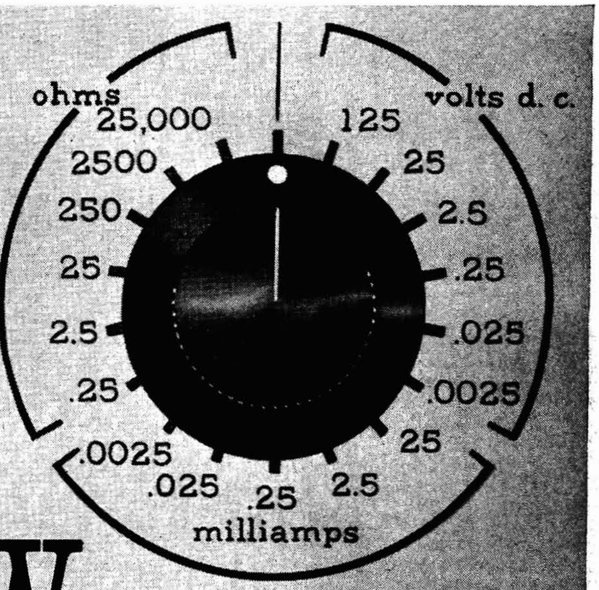
Electron Microscope Image Intensifier

Developed by Hitachi in Japan, a new image intensifier amplifies the image brightness of an electron microscope by 1000 times. The attachment provides more sensitive detection of electrons and electronic amplification of the image, which is presented on a standard television receiver. The system consists of the electron microscope, special pick-up system, electronic control system, and television picture monitor. The pick-up is mounted under the normal viewing screen of the electron microscope in vacuum. This unit includes an image converter and scanning device. The system works on the principle of electron bombardment induced conductivity. It is the relationship between the conduction current gain *vs.* the current density of the electron beams that permits the image intensifier to turn a faint image into a bright one. Perkin-Elmer Corp., Main Ave., Norwalk, Conn. **420**



Gas chromatographs are available, custom designed to meet individual requirements. By providing a thermal conductivity microcell, preamplifier, and sensitive recorder, the instrument can analyze impurities in gases in the p.p.m.-range. The preamplifier is continuously variable from 1 to 100,000. The unit can include flame detectors, pyrolyzers, and other accessory equipment. John P. Mickel Assoc., Inc., 158 S. Rochester Rd., Clawson, Mich. **421**



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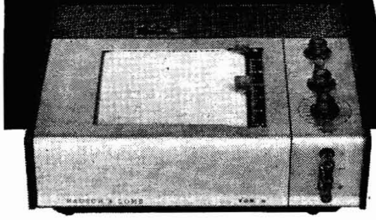

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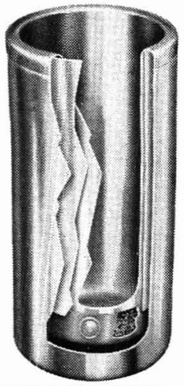

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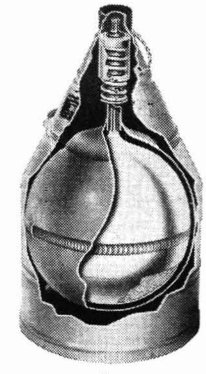

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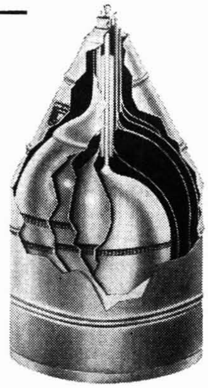

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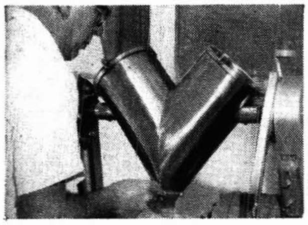

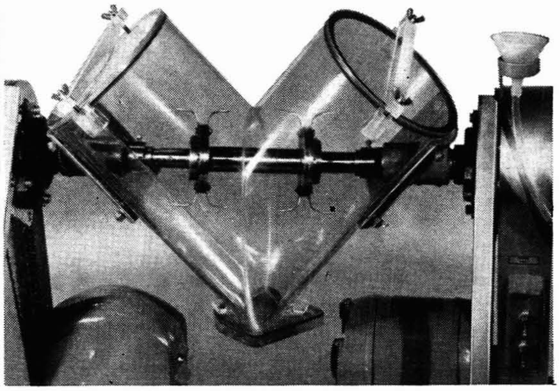

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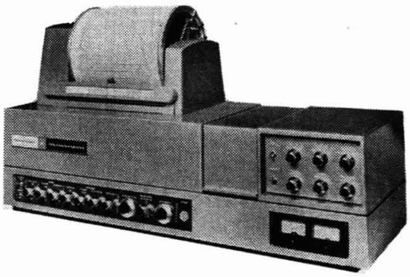
Microporous porcelain filter crucibles are available in four controlled-porosity designations and four cubical sizes. Applications range from procedures where large particle sizes are encountered and rapid filtration is advantageous, to the filtration of crystalline precipitates and fine grain materials. They are particularly useful in quantitative analyses. Information concerning manufacturing techniques, testing procedures, properties, and care and cleaning of these crucibles is available. Selas Corp. of Amercia, Dresher, Pa. **422**



The Model 521 infrared spectrophotometer uses a single high-resolution filter-grating interchange to give an unusually broad infrared spectrum. The spectrum from 4000 to 250 cm.$^{-1}$, 2.5 to 40 microns, is presented on one continuous chart record. With linear wavenumber presentation, the spectral record is uninterrupted even at points where automatic grating and filter switchovers occur. Other automatic features include gain control, speed suppression, and slit programming. A wide range of standard accessories extends the usefulness of this equipment. The interchange unit is compatible, with minor modification, with earlier company Models 421 and 221 spectrophotometers, and is available separately. Perkin-Elmer Corp., Norwalk, Conn. **423**

Beam Attenuator

The AT-02 attenuator can be used with most double beam spectrophotometers. It has two intermeshing combs which can be gradually introduced into the radiation path. The combs are inside a stainless steel assembly which fits directly into the instrument cell guide. Setting of the combs, controlled by a knurled wheel, can be fixed with a locking device at any attenuation level. For simultaneous solvent compensation and attenuation, a cell slide is provided to hold a matched or variable path length cell. Limit Research Corp., P. O. Box 852, Darien, Conn. **424**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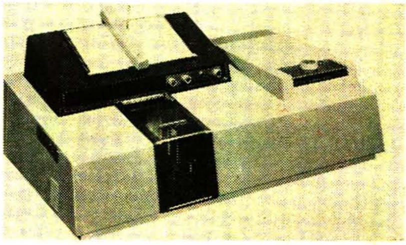
High Vacuum Manometer

An all-glass inclined type manometer provides continuous, direct indication of absolute pressure from 50 to 1000 microns Hg. The instrument has a 10-inch scale with 10-micron graduations, about $^1/_8$ inch apart, and is accurate to within 2%. The manometer operates on the principal of head difference in liquid level. The manometer is evacuated to 10 microns or less and then attached to the inlet connection, and a three-way valve turned to apply inlet pressure. Fluid level in the manometer well rises because inlet pressure is greater than reference pressure. Since the reference pressure is near zero, inlet absolute pressure is measured directly. Schutte and Koerting Co., Cornwells Heights, Bucks Co., Pa. **426**

X-Ray Analysis Equipment

A new x-ray diffraction and emission equipment system is available which is less than one fourth the size of conventional equipment. A mobile, tilt-table cart is supplied with the SPG 5. While the equipment is designed for use with the company's XRD-5 and XRD-6 spectrochemical analysis systems, it is compatible with most x-ray and neutron systems commercially available.

The main chassis of the unit houses up to five separate modular components in two decks. These include a high-voltage power supply, a combination single-channel pulse height selector and linear amplifier, and a ratemeter on the lower deck; plus a scaler-timer and, if required, a second high voltage supply on the top deck. The assembly weighs 60 pounds and measures $14^1/_2$ inches wide by $18^3/_4$ inches high by $21^3/_4$ inches deep. General Electric Co., X-Ray Dept., 4855 West Electric Ave., Milwaukee 1, Wis. **427**



The SP. 200 infrared spectrophotometer has a variable slit program, or program energy control. This maintains the pattern of the slit program, while permitting general increases in the energy level. The instrument also features a large flat bed recorder, a Golay detector, and unitized plug-in electronic chassis. A full range of accessories is available. Unicam Instruments, Ltd., Cambridge, England. **428**

Crystal Orienter

Crystal orientation studies may now be carried out by using high-speed fluoroscopy instead of "trial and error" radiography. The crystal orienter includes a fluoroscopic image-intensifying tube with a nine-inch-diameter window of beryllium. Beryllium transmits the soft x-rays necessary for producing the Laue pattern. Remote controls permit the worker to rotate and otherwise manipulate the crystal so that the desired Laue pattern can be "dialed in." Changing spot patterns are viewed continuously on the image intensifier as the crystal is manipulated. Picker X-Ray Corp., 1275 Mamaroneck Ave., White Plains, N. Y. **429**

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(Act of October 23, 1962; Section 4369, Title 39, United States Code)

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3. FREQUENCY OF ISSUE: Monthly with "Review Issue" in April (13 issues)
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5. LOCATION OF THE HEADQUARTERS OR GENERAL BUSINESS OFFICES OF THE PUBLISHERS *(Not printers)*: 1155 Sixteenth Street, N. W., Washington, D. C. 20036

6. NAMES AND ADDRESSES OF PUBLISHER, EDITOR, AND MANAGING EDITOR

PUBLISHER *(Name and address)*: American Chemical Society, 1155 16th Street, N. W., Washington, D. C. 20036
EDITOR *(Name and address)*: Lawrence T. Hallett, 1155 16th Street, N. W., Washington, D. C. 20036
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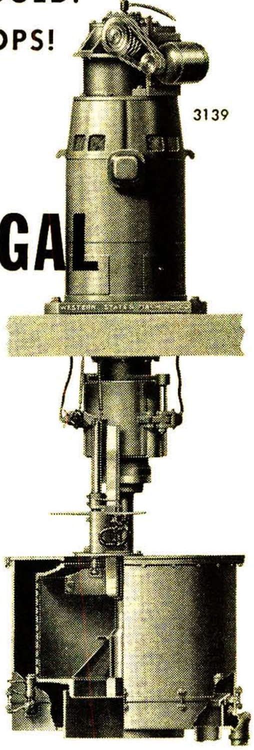
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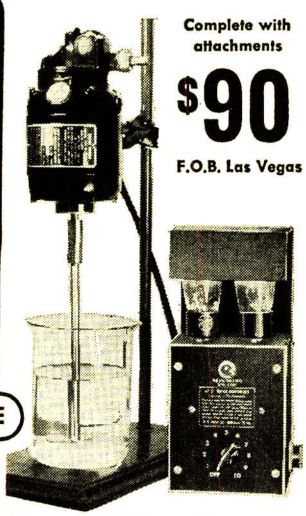

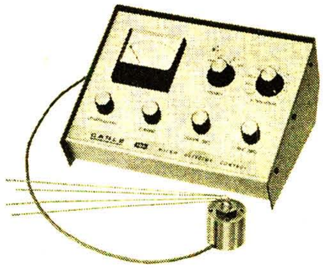

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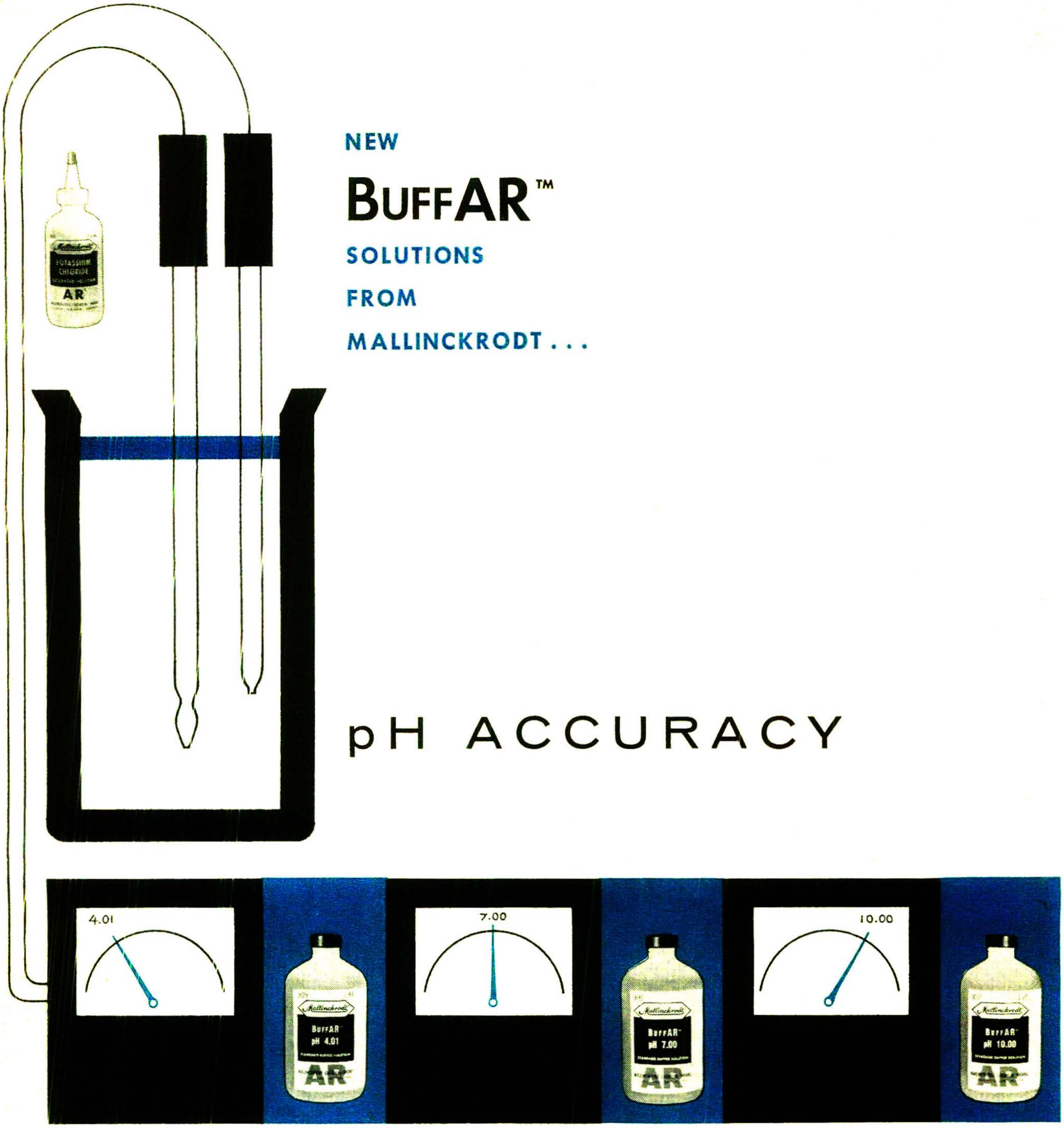

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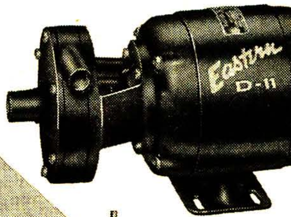

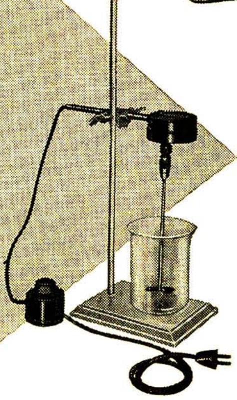

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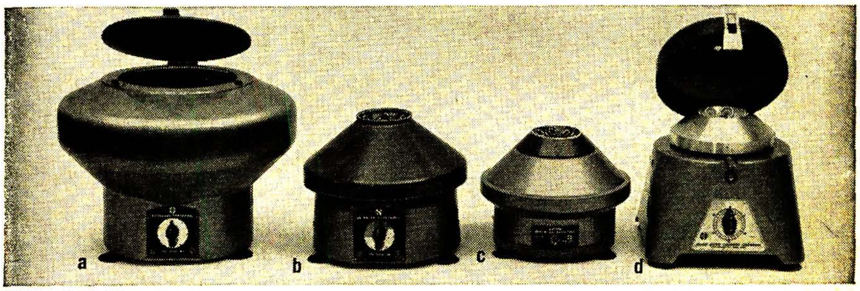

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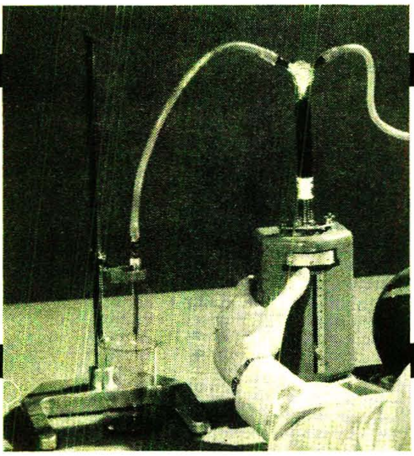

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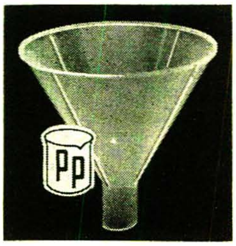

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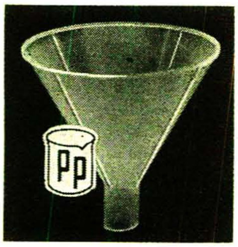

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Supplement No. 1 describes the new materials and services available. Lachat Chemicals, Inc., 10540 S. Western Ave., Chicago 43, Ill. **502**

Atomic Energy Commission Withdraws Distribution of Radioiodine

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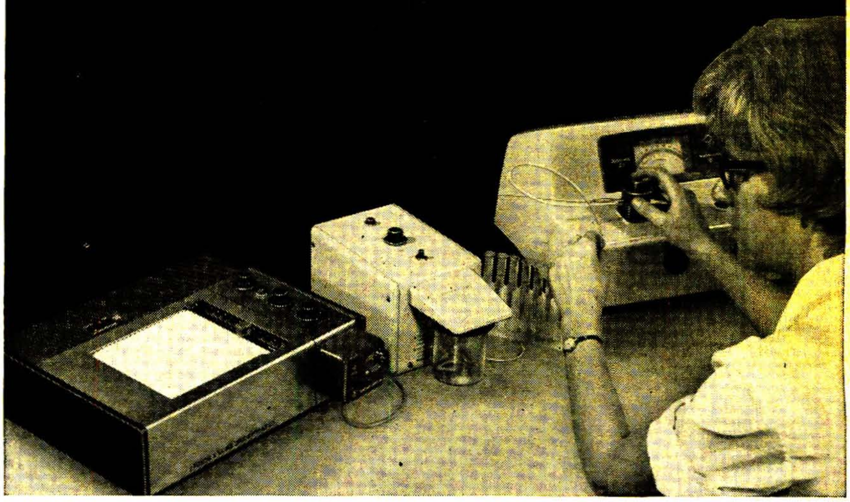
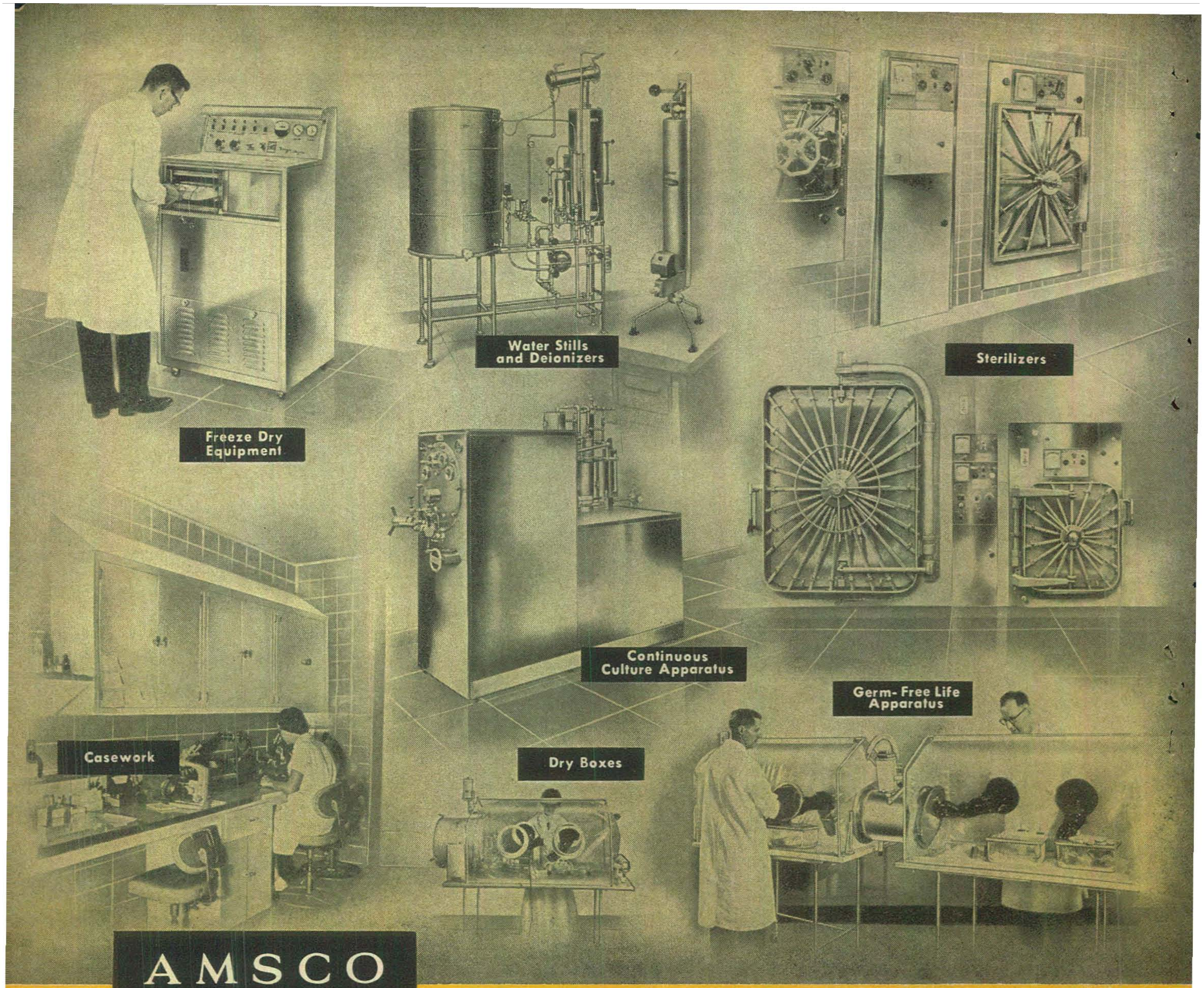
This material is supplied in distilled water in rubber-capped bottles. Quantities of 5 mc. cost $105 and 25 mc., $420. Nuclear-Chicago Corp., 359 East Howard Ave., Des Plaines, Ill. **504**

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Laboratory Glassware. A 360-page illustrated laboratory glassware catalog lists full technical information and prices on all Pyrex brand, Vycor brand, and Corning brand lab glassware. It also sets out in tabular form the properties of 32 selected commercial glasses. Corning Glass Works, Laboratory Glassware Department, Corning, N. Y. **601**

Hydrocarbons and Petro-Sulfur Compounds. A 20-page booklet (No. 14) lists new prices for hydrocarbons and petro-sulfur compounds. Besides cataloging 37 new products, the booklet explains the new terms for Phillips customers on returnable gas cylinders. Phillips Petroleum Co., Bartlesville, Okla. **602**

Laboratory Equipment. A 12-page bulletin discusses some of the new laboratory equipment available. Included in the equipment described are syringes, electrodes, freeze dryers, metallographs, balances, clamps, and pH meters. Chicago Apparatus Co., 1735 North Ashland Ave., Chicago, Ill. 60622. **603**

Differential Pressure Gages. An 8-page bulletin describes Dwyer Series 2000 Magnehelic Gages. These are diaphragm actuated gages for indicating differential pressures in air or non-corrosive gases in 30 standard ranges from 0 to 0.5″ water up to 0 to 5 p.s.i. F. W. Dwyer Manufacturing Co., P. O. Box 373, Michigan City, Ind. **604**

Radioactivity Counting Systems. Two 16-page bulletins describe the Widebeta and Lowbeta II radioactivity counting systems. The bulletins detail complete system descriptions, comparison of counting systems, applications, and comprehensive specifications. The concept of background, its origin, reduction, and reproducibility are treated in a special section. Scientific and Process Instruments Division, Beckman Instruments, Inc., 2500 Harbor Blvd., Fullerton, Calif. **605**

Radioactivity Monitoring. A 4-page bulletin describes how the radioactivity of a sample material eluted from a liquid-partition chromatographic column can be continuously monitored and charted during a routine analytical run on the automatic amino acid analyzer. Beckman Instruments, Inc., Spinco Division, Palo Alto, Calif. **606**

Fraction Collector. A 4-page brochure discusses the Model 132 fraction collector. The 250 test tubes of the Model 132 remain stationary while the delivery head indexes over them, filling each row automatically in convenient front-to-back order. Beckman Instruments, Inc., Spinco Division, Palo Alto, Calif. **607**

Thin Layer Radiochromatography. A 4-page bulletin, No. 16, summarizes the use of radioisotopes with thin layer chromatography. It describes the characteristics of the method and presents detailed preparative and radioactivity detection techniques. Also included are details on the analytical applications of the method and a reference bibliography. Nuclear-Chicago Corp., 4505 West North Ave., Melrose Park, Ill. 60160. **608**

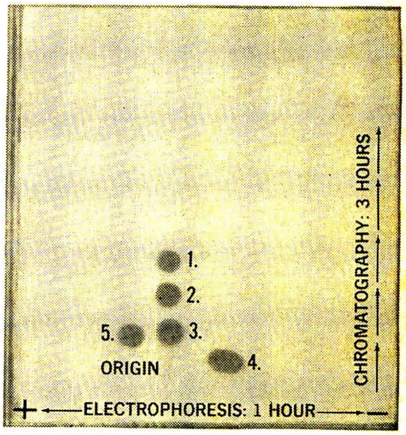
Enzometer. A 2-page bulletin describes the Norcross Enzometer, a completely automatic system controlling enzyme starch conversion to viscosity. Norcross Corp., 247 Newtonville Ave., Newton, Mass. 02158. **609**

Liquid Scintillation Spectrometers. An 8-page bulletin discusses the 3000 and 4000 Series Tri-Carb liquid scintillation spectrometers. The bulletin is illustrated with photographs of the basic equipment, components, controls, and printout units. A schematic diagram of components and basic circuitry is included. Packard Instrument Co., Box 428, LaGrange, Ill. **610**

Mass Spectrum Digitizer. A 4-page brochure describes the mass spectrum digitizer, Model VR16-MSD, an instrument for automatic digital readout and recording of mass spectrometer data. The brochure includes specifications based on actual application, operating principles, applications information, and a discussion of accuracy. Adage, Inc., 292 Main St., Cambridge 42, Mass. **611**

High Vacuum Pumping System. A 4-page bulletin discusses the KPW-2 high vacuum pumping system. Among the components described are: roughing-backing valve, diffusion pump, main valve, vacuum gage, dual-coolant baffle, and mechanical pump. Kinney Vacuum Division of the New York Air Brake Co., 3529 Washington St., Boston 30, Mass. **612**

Multichannel Analyzers. A 16-page bulletin provides complete descriptions and specifications on Packard Models



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15 and 16 400-channel analyzers. Included are descriptions of operating modes of the analyzers, with typical oscilloscope traces; an explanation of analyzer operations at various voltage/channel combinations, together with schematic diagrams; and methods of automatic programming. Packard Instrument Co., Box 428, LaGrange, Ill. **613**

Porcelain Filter Media and Apparatus. A 24-page catalog entitled "Micro-Porous Porcelain Filter Media and Apparatus" describes porous porcelain filter media and apparatus available for a variety of analytical determinations and industrial filtrations. Selas Corp. of America, Dresher, Pa. **614**

Standard Fabricated Quartzware. A 4-page brochure on standard fabricated quartzware lists standard taper joints, ball and socket joints, special O-ring joints, graded seals, flasks, and test tubes. Berkeley Glasslab, 1717 Fifth St., Berkeley 10, Calif. **615**

Paper Electrophoresis Unit. A 2-page brochure describes a high voltage paper electrophoresis unit, fully self-contained in one console. Unit specifications are given and pictures are shown of results, after 45 minutes operation, using high voltage for separation of amino acids. International Sales Associates, U. S. Route 130 North, Burlington, N. J. **616**

X-Y Recorders. An 8-page brochure discusses the Model HR-95 and HR-97 X-Y recorders. Also described is a line of converters and accessories for the recorders. A general description of the recorders, detailed descriptions and pictures of the recorders' features, complete specifications for the recorders' performance, and typical performance curves are given. Houston Instrument Corp., 4950 Terminal Ave., Bellaire 101, Texas. **617**

High Vacuum Pumps. A 6-page brochure describes four laboratory-size high vacuum pumps manufactured by E. Leybold's Nachfolger of West Germany. Curves of pumping speed and pump-down times of single- and two-stage pumps are shown. LaPine Scientific Co., 6001 South Knox Ave., Chicago 29, Ill. **618**

Chromatography Supplies. A 16-page catalog lists gas chromatography, thin layer chromatography, and column chromatography supplies and high purity lipid reference standards. Applied Science Laboratories, Inc., P. O. Box 140, North Barnard St., State College, Pa. **619**

Log Voltmeter-Converter. An 8-page brochure contains technical descriptions, schematic specifications, and applications of wide range Model HLVC-150 Log Voltmeter-Converter. Houston Instrument Corp., 4950 Terminal Ave., Bellaire 101, Texas. **620**

Freezing Point Calibration Standards. A 4-page catalog, FPS 500, describes freezing point calibration standards which utilize fixed freezing point of pure metal to provide discrete temperature for calibration of temperature probes. Temptron Division of Consolidated Controls Corp., 7030 Darby Ave., Reseda, Calif. **621**

Laboratory Equipment and Chemicals. A 1088-page catalog, "Selected Laboratory Equipment & Chemicals 63/64," features the latest equipment, apparatus, furniture, supplies, and general aids. Also included is a section devoted to a comprehensive listing of the most commonly used reagents, biological chemicals, spectro-solvents, chemicals for chromatography, stains, dehydrated culture media, etc. Schaar Scientific Co., 7300 W. Montrose Ave., Chicago, Ill. 60634. **622**

High Vacuum Pumps. A 16-page catalog (No. 10) illustrates and describes the full line of Nevaco high vacuum pumps. The catalog covers performance, installation, and technical data for laboratory and field application. Nelson Vacuum Pump Co., 2133 Fourth St., Berkeley, Calif. 94710. **623**

Soft X-Ray Measurements. A set of data sheets and actual graphs summarize soft x-ray measurements originating from the K and L shells of low atomic number nuclides. Nuclear Measurements Corp., 2460 N. Arlington, Indianapolis, Ind. 46218. **624**

Fast Coincidence Spectrometry. A 4-page brochure discusses a fast coincidence spectrometry system. The "fast" configuration is possible because of the design of the N-685 jitter-free pulse height analyzer. Hamner Electronics Co., P. O. Box 531, Princeton, N. J. **625**

Laboratory Glassware. A 28-page catalog lists a variety of laboratory glassware. Included are pipets, centrifuge tubes, diagnostic tubes, test tubes, and automatic pipets. Rochester Scientific Co., 623 Norton St., Rochester 21, N. Y. **626**

Parr Oxygen Bombs. A 12-page illustrated bulletin discribes ten different bombs for calorimetry and oxygen combustion tests. Bombs for both routine and research purposes are covered, including styles for microcombustion, explosive tests, fluorine combustion, and

rotating bomb calorimetry, some with platinum liners. Parr Instrument Co., 211 Fifty-third St., Moline, Ill. **627**

Indicator Chart. The chart, "pH Ranges and Color Changes of Eastman Indicators" contains four new items—a total of 61. Distillation Products Industries Division of Eastman Kodak Co., 343 State St., Rochester 4, N. Y. **628**

Reprint List. A revised reprint list contains information on materials available describing the techniques of vacuum weighing, thermogravimetry, sorption measurements, contamination analysis, ultramicro weighing, and thin film deposition monitoring. Cahn Instrument Co., 15505 Minnesota Ave., Paramount, Calif. **629**

Laboratory Supplies. A 24-page catalog describes micrometric controlled monometers, McLeod gages, syringes, burets, vacuum and pressure gages. RGI, Inc., Box 732, Vineland, N. J. **630**

Recorders. A 16-page catalog illustrates and describes the Series "S" recorders. Heading the line is the Speed-Servo, a recorder with $^1/_8$-second response. Esterline Angus Instrument Co., Inc., P. O. Box 596, Indianapolis 6, Ind. **631**

Dead Weight Testers. A 2-page data sheet includes details on dead weight testers and gages in the 5- to 500- and 50- to 10,000-p.s.i. ranges. Chandler Engineering Co., 7707 East 38th St., Tulsa, Okla. **632**

Laboratory Working Surfaces. A 24-page book describes materials, dimensions, and recommended uses for scientific laboratory working surfaces. Detailed instructions are given for cleaning and maintaining the materials. Hamilton Manufacturing Co., Two Rivers, Wis. **633**

Company Periodicals

Requests for copies of the following publications, catalogs, etc., should be sent directly to the address shown. Business or professional letterheads are requested.

What's New for the Laboratory. No. 51, Fall, 1963. Approximately 36 new items are illustrated and described in this 16-page issue. Among them are: a Teflon-sealed stirrer that can be used under vacuum and with highly corrosive chemicals, a line of reagent bottles with high vacuum seal joints, and a new densitometer for thin layer chromatography. Scientific Glass Apparatus Co., Bloomfield, N. J.

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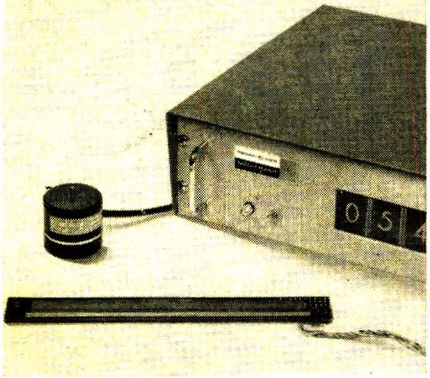

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MANUFACTURERS' LITERATURE

Instrument News. Vol. 14, No. 3&4, Spring/Summer 1963. This 12-page issue discusses the cryostat accessory for the Model 350 spectrophotometer, the Model 810 and Model 820 gas chromatographs, the Model 139 spectrophotometer and accessories, electrophoresis apparatus, the KBr disk technique, new infrared cells, and the Model 801 chromatograph for steroids. The Perkin-Elmer Corp., Norwalk, Conn.

Lablog. No. 2-63 (Summer). This 8-page issue includes descriptions of the Autoset spectrophotometer, a student microscope, a new balance, and a Fiberglas fume hood. Will Scientific, Inc., Box 1050, Rochester 3, N. Y.

Research Notes. Fall, 1963. This 8-page issue includes a discussion of two-channel gas chromatography and alkyl lead analysis by electron capture. Wilkens Instrument & Research, Inc., P. O. Box 313, Walnut Creek, Calif.

Philips Defense and Space Laboratory Bulletin, Vol. I, No. 1. This 4-page issue describes the development of a space power supply to withstand the extreme environments of launch, spaceflight, and touchdown. Philips Defense and Sapce Laboratory, 900 South Columbus Ave., Mount Vernon, N. Y. 10550.

Penn-Chem News. Vol. 1, No. 1. This 8-page issue of a new external house organ describes a variety of laboratory equipment. Penn-Chem Corp., Lancaster, Pa.

Apparatus Review. Autumn, 1963. This 16-page issue features a densitometer for thin layer chromatography, liquid CO_2 freeze dryers, a vacuum oven with wrap-around heating elements on four sides, an oxygen meter, a new type of standard taper joint clamp, a magnetic stirrer that illuminates vessel contents, and a 70-inch wide Fiberglas fume hood for radioactive work. La-Pine Scientific Co., 6001 South Knox Ave., Chicago 29, Ill.

Atomlight. No. 31. This 12-page issue discusses the microchemical identification of steroids from biological media and automatic counting of radioactivity on two-dimensional paper chromatograms. New England Nuclear Corp., 575 Albany St., Boston 18, Mass.

Jena Review. No. 3/63. This issue discusses the Universal spectrophotometer's use for fluorescence measurements of macromolecular solutions. VEB Carl Zeiss JENA, Jena, Carl-Zeiss, Strasse 1, German Democratic Republic.

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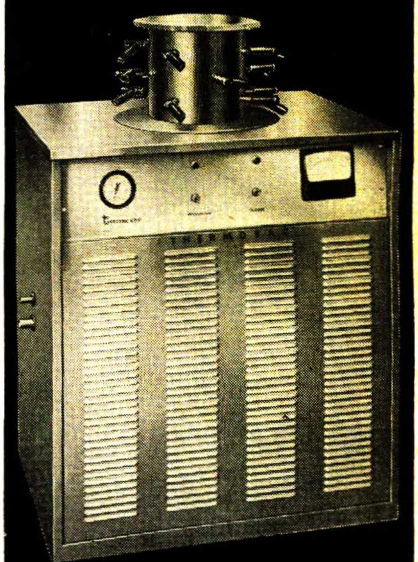

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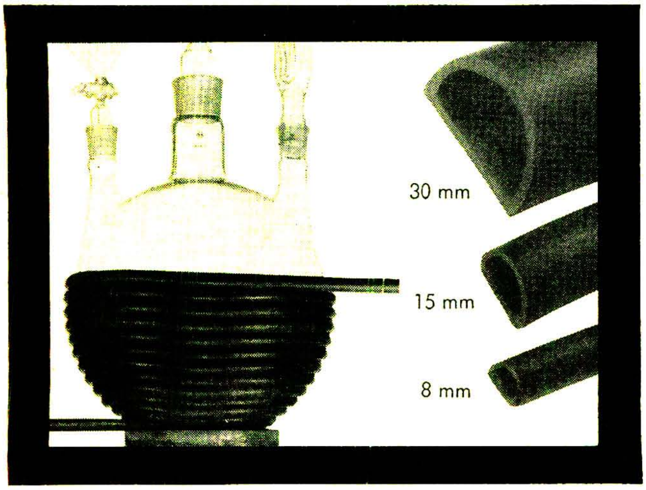

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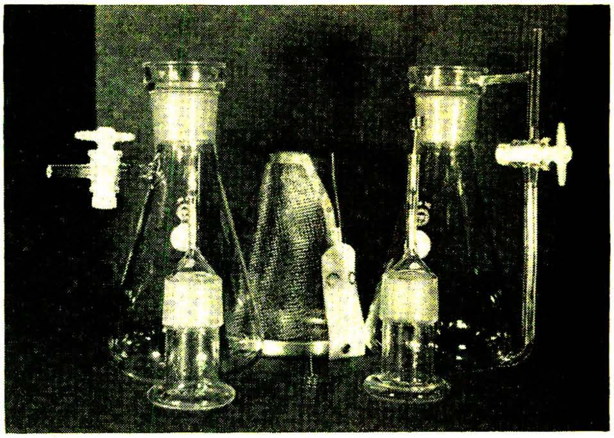

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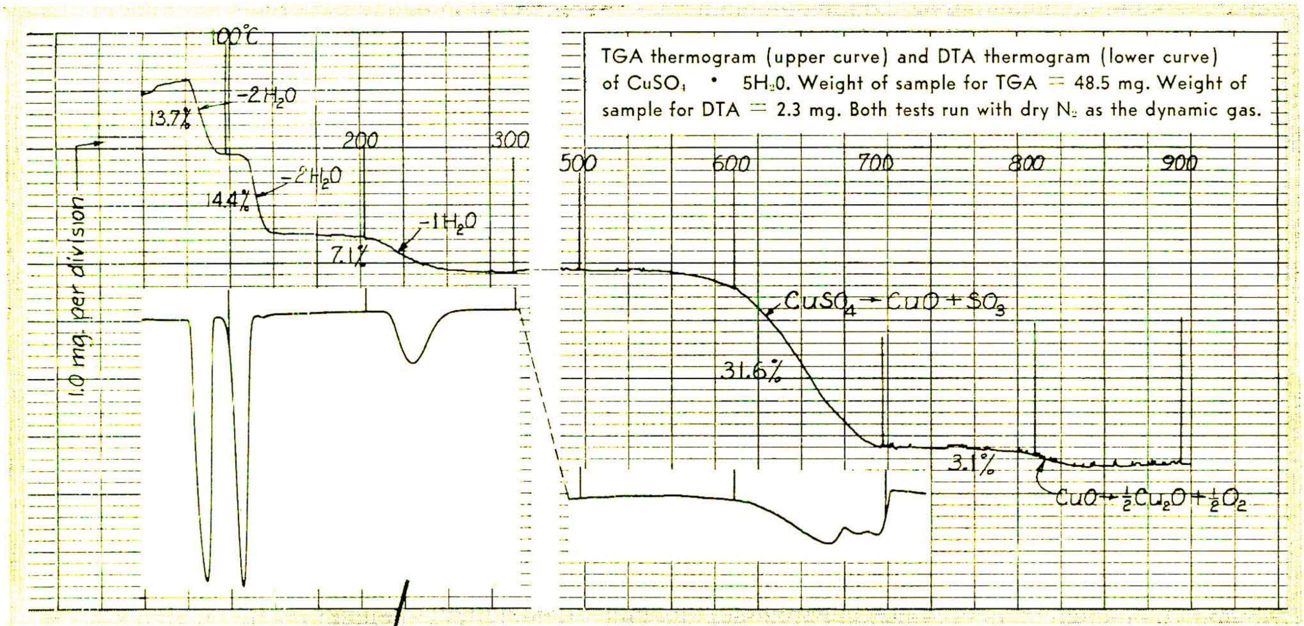

TGA thermogram (upper curve) and DTA thermogram (lower curve) of $CuSO_4 \cdot 5H_2O$. Weight of sample for TGA = 48.5 mg. Weight of sample for DTA = 2.3 mg. Both tests run with dry N_2 as the dynamic gas.

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Adsorbents. 16-page booklet of chromatographic adsorbent. **134**

Amplifiers. Data sheet on new lock-in unit for obscured signals. **38**

Analyzers. Bulletin on new electron probe microanalyzer. **41**

Analyzers. Bulletin details line of multichannel units. **71**

Analyzers. Automatically does five amino acid chromatograms in 24 hours. **166**

Analyzers. Specs on multichannel unit and power supply. **80**

Analyzers. DTA and TGA unit to 1200° C. **36**

Analyzers. Recording polarographic unit. **176**

Analyzers. 100-channel pulse height unit has visual single-channel window. **167**

Bags. Data sheet on collodion bags offered. **2**

Balances. New top-loading unit has tare indicator. **60**

Balances. Info on line of balances and weights. **17**

Balances. New 1000 gram unit has no-knife edge construction. **169**

Balances. Unit has solid titanium beam/jeweled mechanism. **30**

Balances. Info on line of weights and balances. **70**

Balances. Spectroscopist's balance for IR, UV, NMR samples. **121**

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Cells. Info on new IR sampling cells. **114**

Centrifuges. Data file on preparative unit with large rotor chamber. **138**

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Chips, Boiling. Info on micro-porous boiling chips. **185**

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Chromatographs. New gas units have dynathermal control. **157**

Chromatographs. Details on dual column modular gas unit. **81**

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Chromatographs. Bulletin on gas systems and components. **22**

Chromatographs. Gas unit has proportional temp. controller-programmer. **139**

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Classifiers. Info on micro-particle classifier. **126**

Comparators. "Modern pH and Chlorine Control" book offered. **93**

Controllers. Temperature controller for bomb calorimeters. **72**

Counters. 30 sample automatic beta counting system. **195**

Counters. Digital counter for direct reading of volume dispensed. **44**

Counters. Data file on beta counting systems and detectors. **115**

Counters. Transistorized integrated counting systems. **158**

Crystals. Copy of "Polished Optics" booklet offered. **46**

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Densitometers. New recording unit for TLC. **76**

Desiccants. 100-page book on dessicant for solids, liquids, gases. **45**

Dispensers. Plastic unit for repetitive dispensing. **152**

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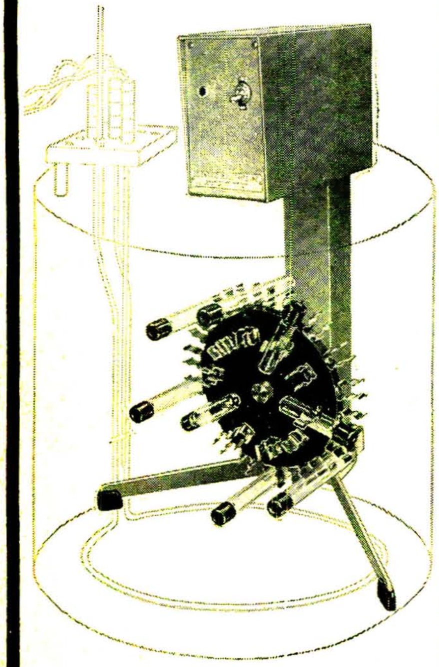

Product Capsules

Distillers. All quartz automatic unit for single, continuous operation. **188**

Dry Boxes. Book on safety enclosures offered. **117**

Dryers. Info on laboratory glassware dryer. **28**

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Electrodes. Data and samples on line of stock and custom electrodes. **3**

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Filters. Application manuals on line of filters offered. **62**

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Galvanometers. Bulletin details galvanometer for pressure measurement. **168**

Gases. 24-page one-source rare gas booklet offered. **58**

Gels. Info on liquid scintillators. **69**

Generators. Neutron generator yields 2.5 × 10^{14} n/sec. **16**

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G.C. Accessories. Details on syringe holders, peakometers. **173**

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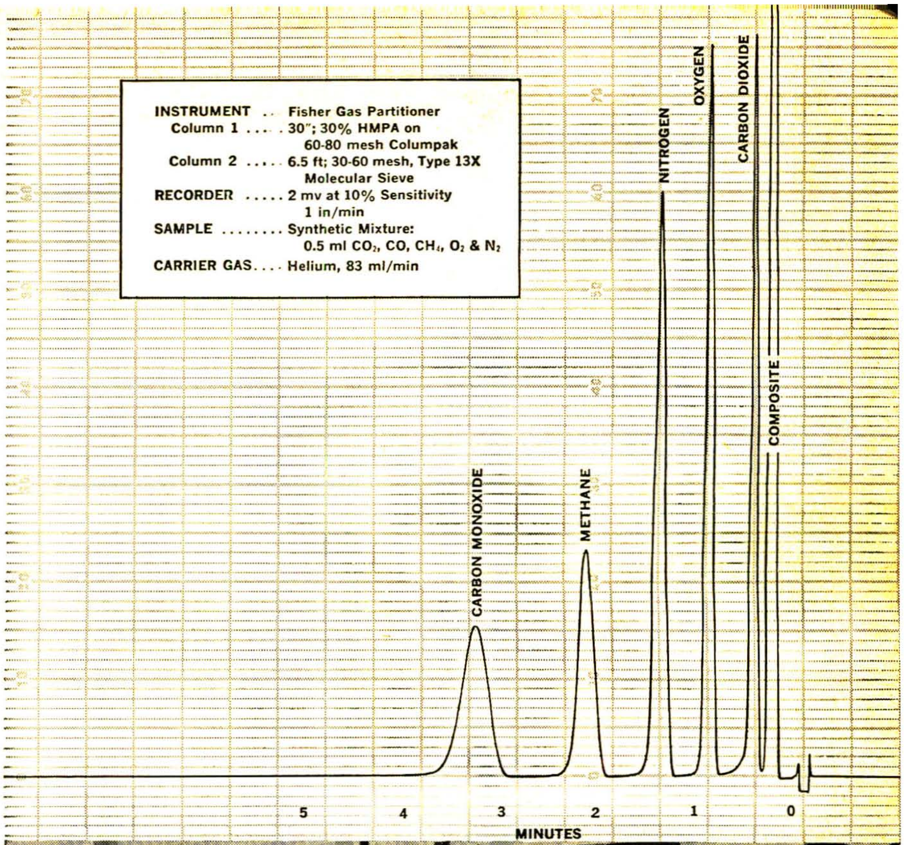

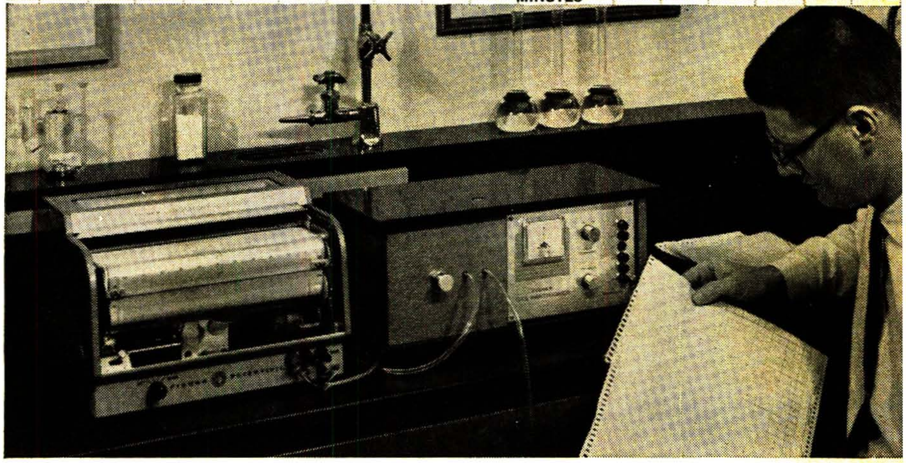

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Photometers. Light scattering unit for molecular weight measurement. **178**

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Recorders. Integrating unit for gas chromatography. **154**

Recorders. Portable unit has 14 chart speeds. **39**

Recorders. Info on unit with adjustable paper speeds. **92**

Recorders. Unit has 25,000 ohms source. **64**

Recorders. Line of 38 models of X-Y units. **163**

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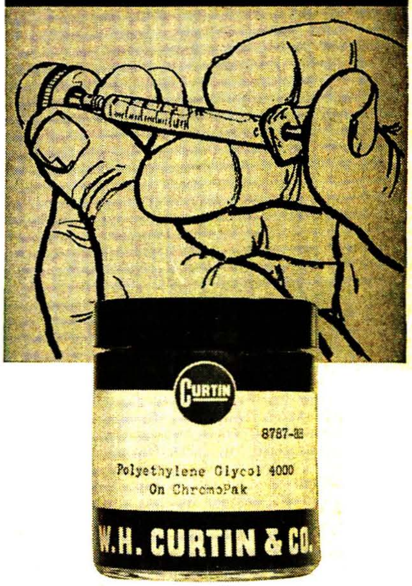

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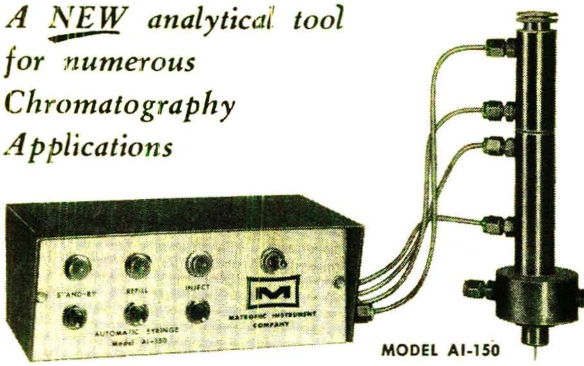

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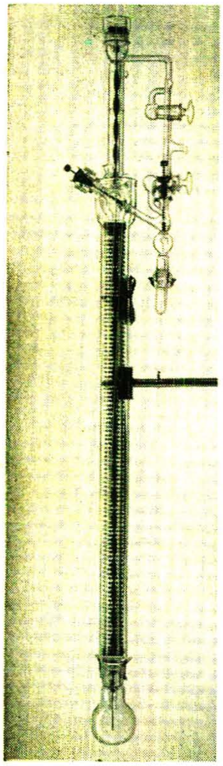

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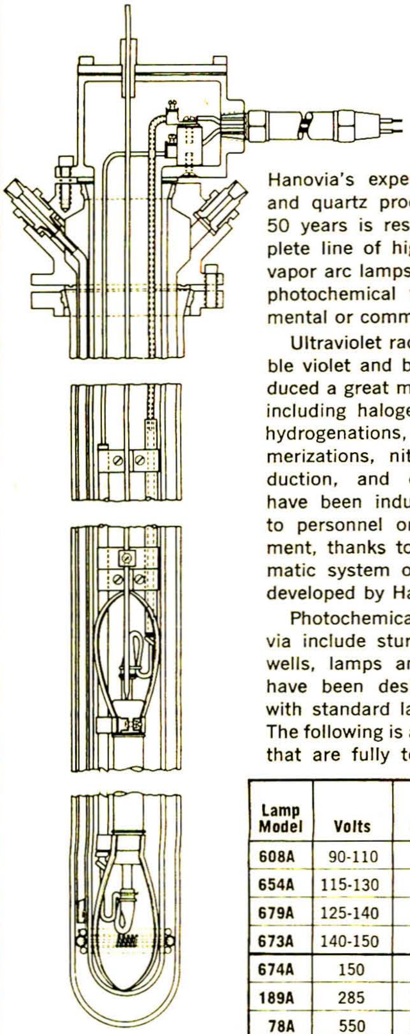

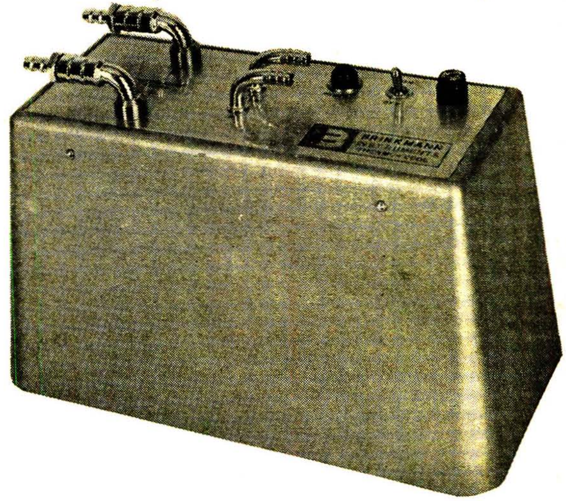
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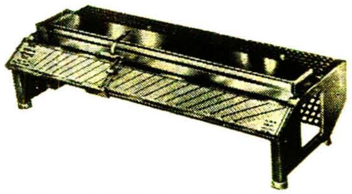

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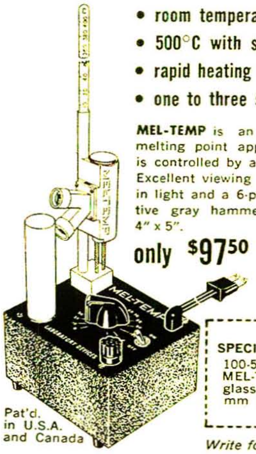

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Colorimetric Determination of Large Amounts of Iron Using the Oxidized Ferrous-Phenantholine Complex
Harold L. Watts

A Microreactor Gas Chromatographic Method for the Identification of Polymeric Materials
B. C. Cox and Bryan Ellis

Spot Tests for Aromatic and α,β-Unsaturated Aldehydes
Fritz Feigl and Ester Libergott

Use of *p*-Phenylazobenzoyl Chloride for Chromatographic Analysis of Fatty Alcohols
Ira Katz and Mark Keeney

Chemical Microscopy of the Platinum Metals: Reactions with 4-Bromoisoquinoline and 4-(*p*-Nitrobenzyl) Pyridine
Harold F. Schaeffer

Determination of Primary Alcohol Groups in Polyglycols Using Trityl Chloride
J. G. Hendrickson

Chelometric Determination of Aluminum
K. E. Burke and C. Manning Davis

Inorganic Salts for Gas-Solid Chromatography
J. A. Favre and L. R. Kallenbach

Spectrophotometric Determination of Dithiocarbamate Residues on Food Crops
T. E. Cullen

Spectrophotometric Determination of Iron and Aluminum in Silicone Polymers
Shizuo Fujiwara and Hisatake Narasaki

Determination of *p*-Chloroacetanilide in Phenacetin: A Polarographic Method
Richard Jones and B. C. Page

Determination of Total Sulfur in Benzene
K. H. V. French

Spectrophotometric Determination of Trace Concentrations of Carbonyl Compounds
D. E. Jordan and F. C. Veatch

Head Space Techniques in Quantitative Gas Chromatographic Analysis of Volatile Components in Aqueous Solutions
R. E. Kepner, H. Maarse, and J. Strating

Adaption of a Galvanic Cell for Microanalysis of Oxygen
Harry Lipner, L. R. Witherspoon, and V. C. Champeaux

Gas Chromatographic Determination of Microgram Amounts of Carbon in Sodium Metal
T. G. Mungall, J. H. Mitchen, and D. E. Johnson

Apparent Conformational Changes of Liquid Phases in Gas Liquid Chromatography
Chiadao Chen and Dione Gacke

Resolution of Neomycin and Catenulin Antibiotic Complexes by Ion Exchange Resin Chromatography
Hubert Maehr and C. P. Schaffner

Routine Separation and Determination of Total Saturated Hydrocarbons in Heavy Petroleum Samples by Adsorption Chromatography
L. P. Snyder and W. F. Roth

Determination of Organic Peroxides by Iodine Liberation Procedures
R. D. Mair and A. J. Graupner

Prediction of Response Factors for Thermal Conductivity Detectors
B. D. Smith and W. W. Bowden

Accurate Multicomponent Analyses by Gas Chromatography
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Extraction of Titanium with Dioctyl Methylenebisphosphonic Acid and Direct Spectrophotometric Determination of Titanium in the Organic Phase
Henrika Gorican and Drago Grdenic

Synergistic Effects in Analyses Based on Differential Reaction Rates
Sidney Siggia and J. G. Hanna

Use of Differential Reaction Rates to Analyze Mixtures of Organic Materials Containing the Same Functional Group. Application to Amides and Nitriles
Sidney Sigga, J. G. Hanna, and N. M. Serencha

Use of Combination Columns In Gas Liquid Chromatography
G. P. Hildebrand and C. N. Reilley

Determination of Solubility of Several Phosphine Oxides in Aqueous Solutions Using a New Spectrophotometric Procedure
J. W. O'Laughlin, F. W. Sealock, and C. V. Banks

Amperometric Titration of Uranium(IV) with Cerium(IV) Utilizing the Pyrolytic Graphite Electrode
H. E. Zittel and F. J. Miller

Integrated Absorption Standardization for the Infrared Determination of Ester Content in Oxidized Polyethylene
J. N. Lomonte

Determination of Methyl Ketones and Acetaldehyde by Titration with Hypobromite Using Bordeaux Indicator
M. H. Hashmi and A. A. Ayaz

A Solid State Potentiostat for Controlled Potential Electrolysis
Frederick Lindstrom and J. B. Davis

Multiple Reflection Cells for Internal Reflection Spectroscopy
N. J. Harrick

Coulometric Titration of Total Arsenic and Arsenic(III) in Glasses
W. M. Wise and J. P. Williams

Mass Spectra of Ethyl *N*-Phenylcarbamate and Ethyl *N*-Ethylcarbamate
C. P. Lewis

Determination of Hydroxyethyl Group in Hydroxyethyl Starch by Pyrolysis—Gas Chromatography Technique
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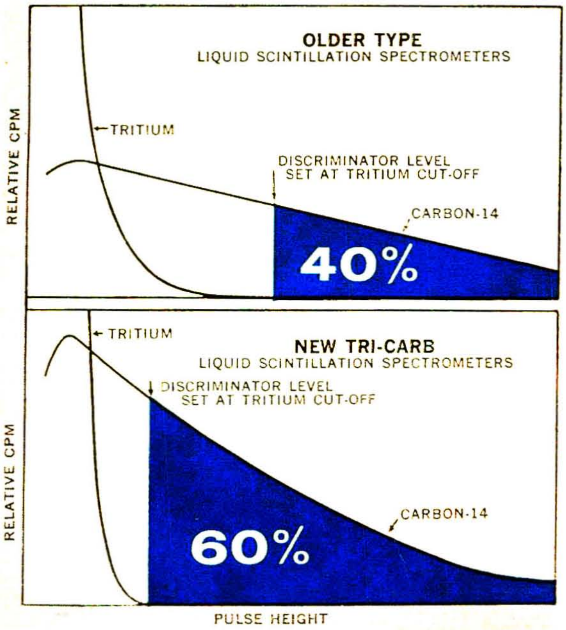

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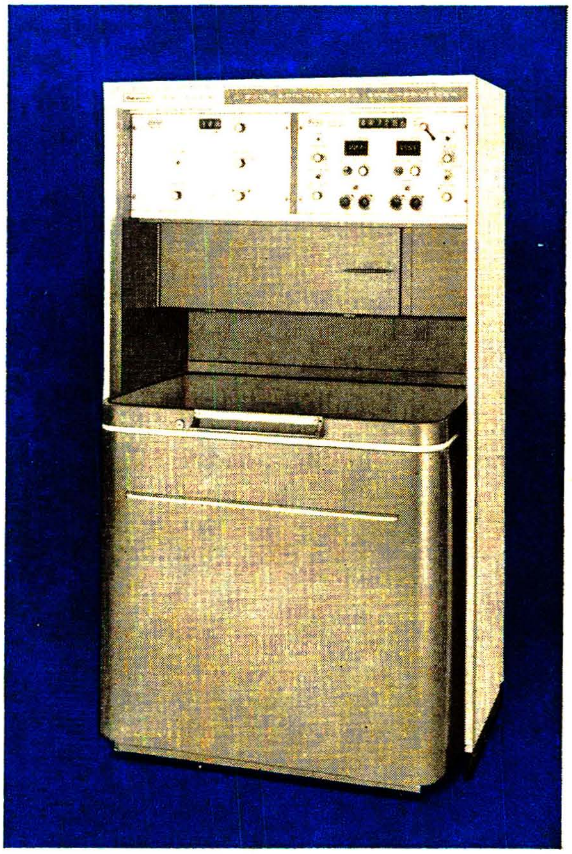

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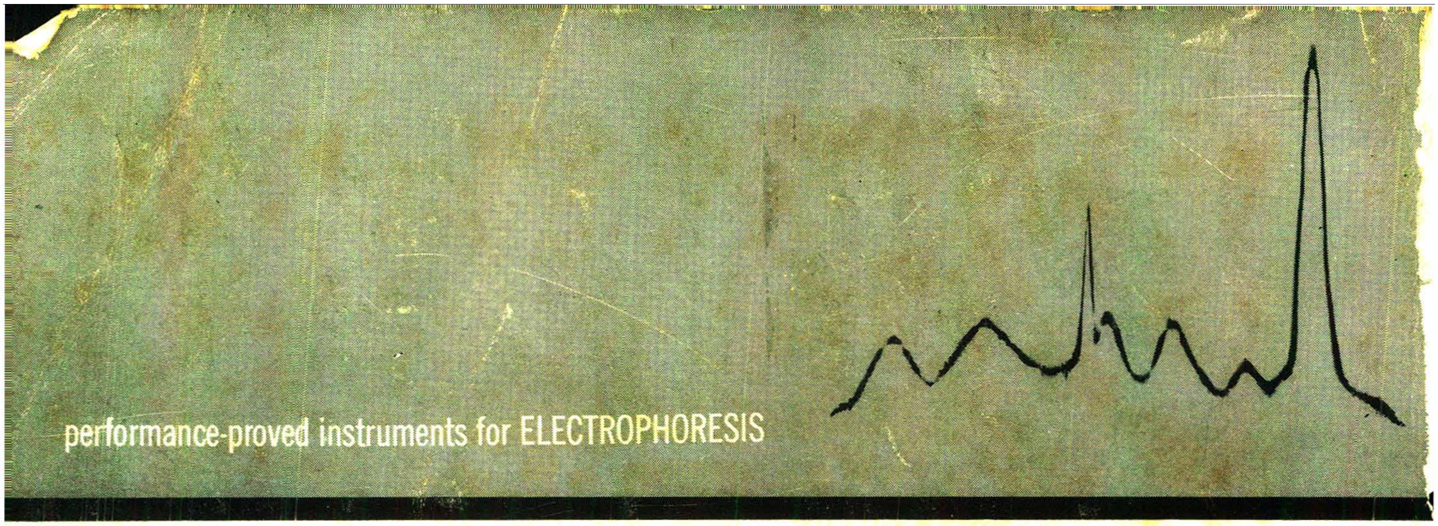
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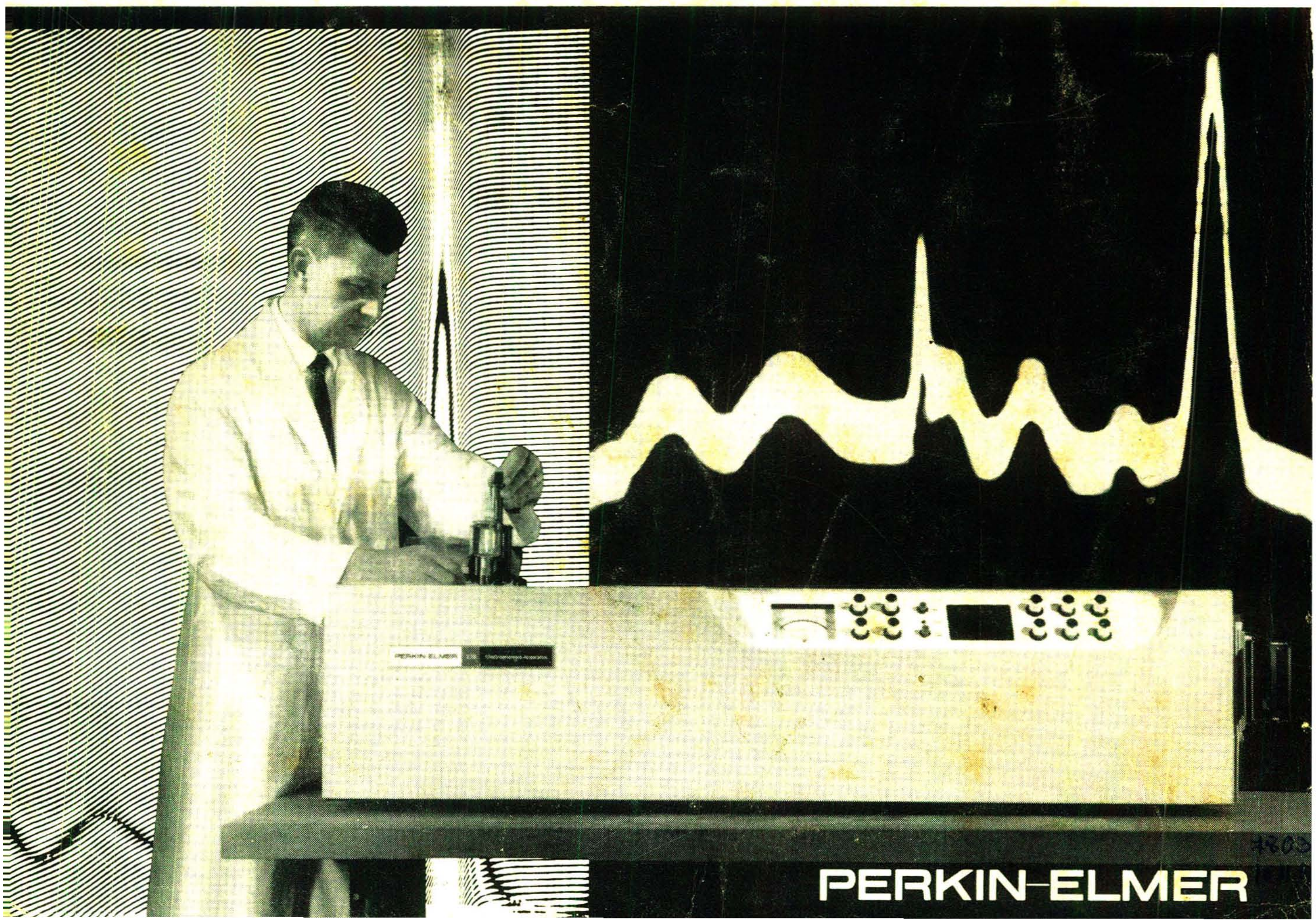
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