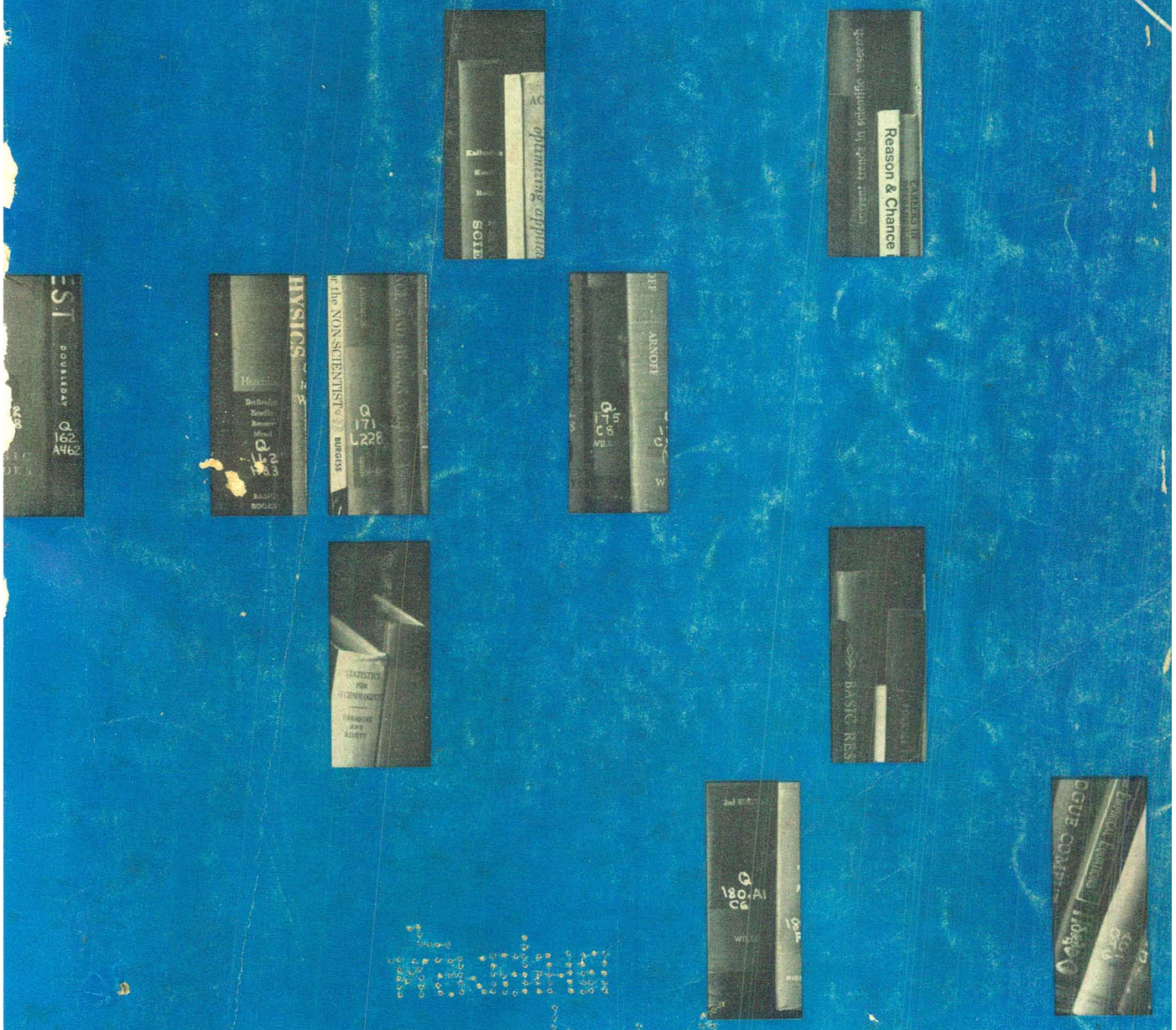
OCTOBER 1963

ANALYTICAL CHEMISTRY



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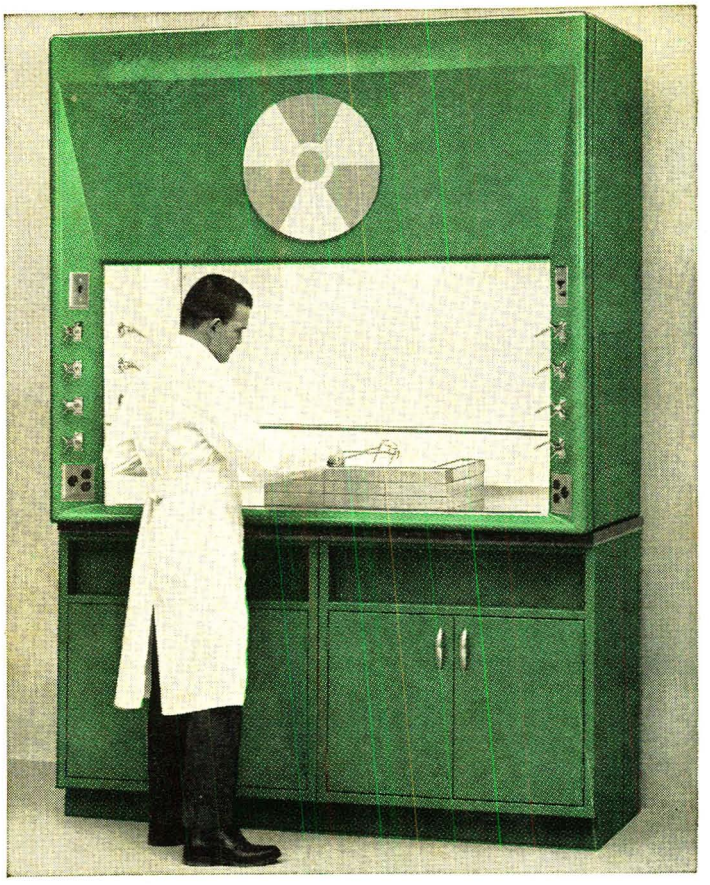

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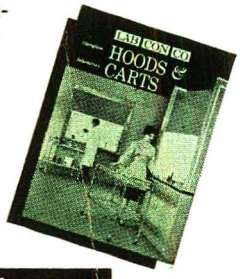

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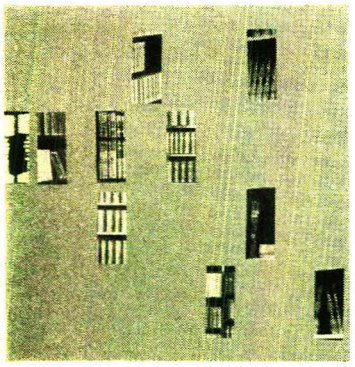

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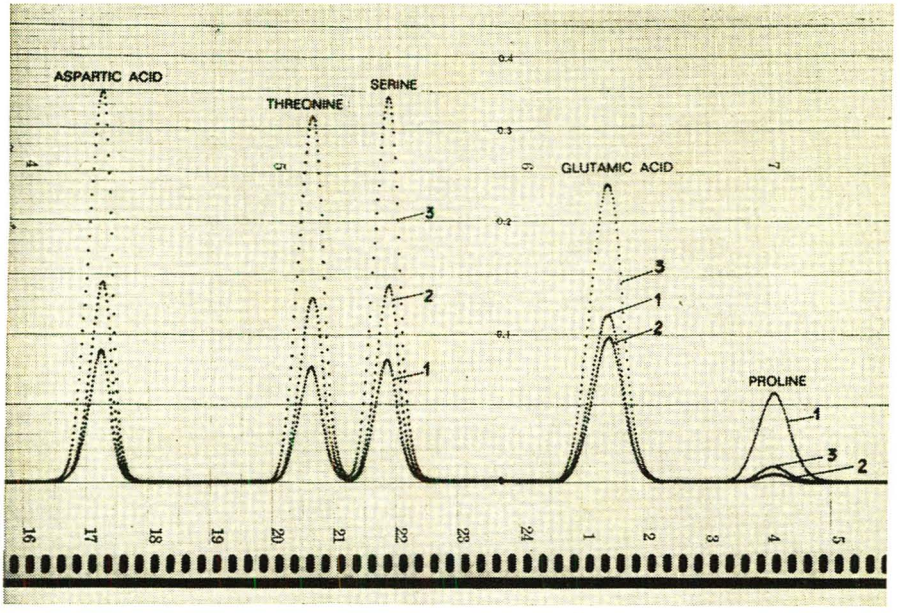
LKB 5900A
MULTICHANNEL ABSORPTIOMETER



Part of the record from an automatic amino-acid analysis according to Moore and Stein. Effluent analyzed with LKB 5900A Multichannel Absorptiometer and LKB 6550A Philips Automatic Multichannel Recorder at 1) 4400 Å (2mm optical path length), 2) 5700 Å (0.7 mm), and 3) 5700 Å (2 mm).

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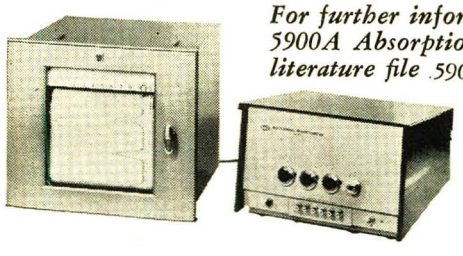
High volumetric resolving power is achieved because of small cuvette volumes and streamlined cuvette construction which prevents blurring of front or zones.

Typical applications include assaying effluent from chromatographic columns (e.g. during amino acid analysis), checking impurity of flowing industrial liquids, and direct measurement of coloured substances such as hemoglobin, carotenoids, flavonoid compounds, chloroplast pigments



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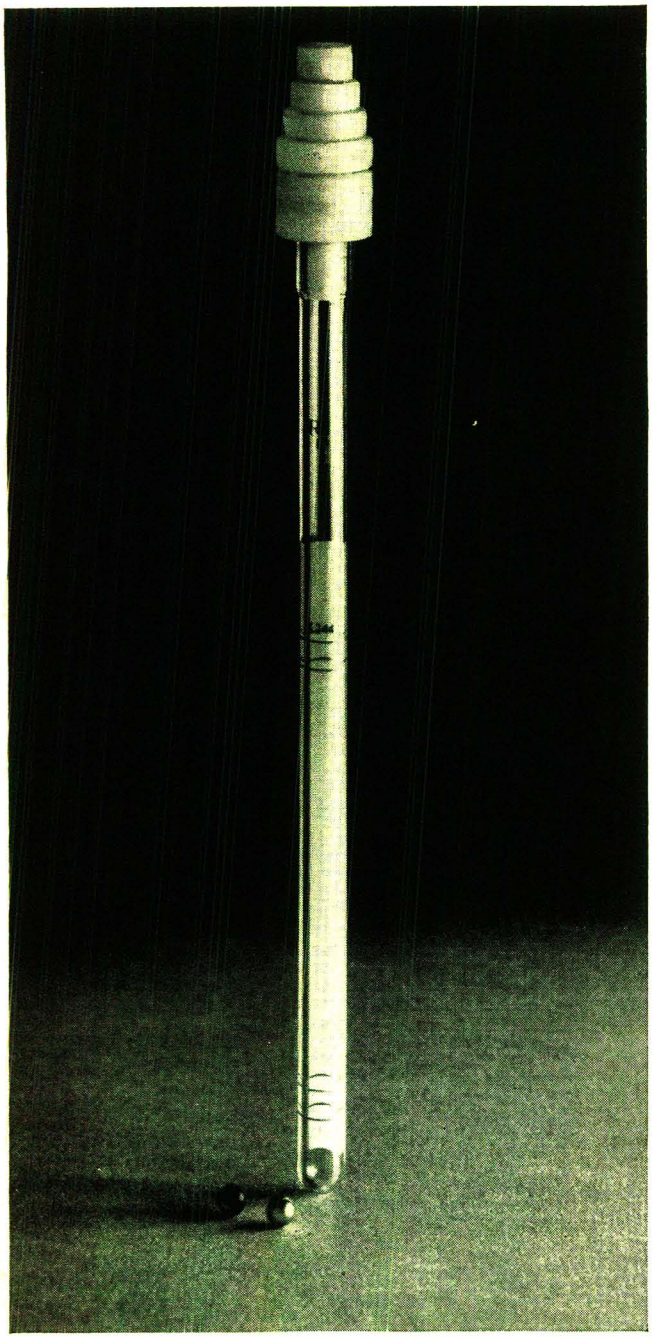

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AC BRIEFS

Highlights of the scientific and technical articles in this issue

The Role of Argon Metastable Atoms in the Ionization of Organic Molecules

A microionization chamber of coaxial design was used as a detection system in a gas chromatographic system to study the mechanisms by which argon carrier gas provides high sensitivities to organic molecules with ionization potential below 11.6 e.v.

M. M. SHAHIN and S. R. LIPSKY, Department of Internal Medicine, Yale University School of Medicine, New Haven 11, Conn.

Anal. Chem. 35, 1562 (1963)

Determination of Partition Coefficients at Infinite Dilution by the Gas Chromatographic Analysis of the Vapor above Dilute Solutions

Partition coefficients from water at 25° C. for acetone and the normal alcohols (one to four carbon atoms) and from squalane at 80° C. for normal paraffins (five to nine carbon atoms) have been determined. The results obtained by gas chromatographic analysis of the vapor above dilute solutions agree with those obtained by other methods.

M. G. BURNETT, Low Temperature Research Station, Downing St., Cambridge, England

Anal. Chem. 35, 1567 (1963)

Absolute Calibration of Gas Chromatographic Apparatus from Ion-Chamber Measurements

The fact that the absolute amount of activity measured by a suitable ion chamber is independent of the molecule to which the unstable isotope is attached, provides a calibration method unaffected by most gas chromatograph variables. The proposed method gives an over-all accuracy within 1%.

D. C. NELSON and D. L. PAULL, Applied Physics Corp., 2724 South Peck Road, Monrovia, Calif.

Anal. Chem. 35, 1571 (1963)

Performance of an Instrument for Simultaneous Gas Chromatographic and Radioactivity Analysis

The ion chamber for the radioactivity measurement is located in the same temperature-controlled oven as the normal chromatography detector. Data are given for programmed and isothermal column operation and specific applications are described.

D. C. NELSON, P. C. RESSLER, Jr., and R. C. HAWES, Applied Physics Corp., Monrovia, Calif.

Anal. Chem. 35, 1575 (1963)

Gas Chromatography of Trimethylsilyl Derivatives of Compounds Related to Chloramphenicol

While several of these compounds are eluted in approximately the order of their molecular weights, changes in substituents sometimes causes variations in retention times not explained by molecular weight. Possible explanations for variations in retention times are offered.

PAUL D. SHAW, Department of Plant Pathology, University of Illinois, Urbana, Ill.

Anal. Chem. 35, 1580 (1963)

Tetracyanoquinodimethanide as a Color Reagent for Inorganic Thin Layer Chromatography

Alkali, alkaline earth, and some mono- and divalent post-transition metals ions were separated using silica gel adsorbent in thin layer chromatography. R_f values for two solvent systems are given: 5% glacial acetic acid in ethanol and 5% 2*N* HCl in *tert*-butanol.

LEONARD F. DRUDING, Department of Chemistry, Rutgers, The State University, Newark 2, N. J.

Anal. Chem. 35, 1582 (1963)

Gas Liquid Chromatography of Phenanthrenes. Use in Identification of the Components of Mixtures of Alkyl Phenanthrenes

The relative retention times of alkylphenanthrenes determined on the nonpolar liquid phase SE-30 and on the polar liquid phase QF-1-0065 have been factored to permit the prediction of relative retention times on these phases. The data can be used to help identify phenanthrenes.

A. J. SOLO and S. W. PELLETIER, The Rockefeller Institute, New York 21, N. Y., School of Pharmacy, State University of New York at Buffalo, Buffalo 14, N. Y., and University of Georgia, Athens, Ga.

Anal. Chem. 35, 1584 (1963)

Argentation in Countercurrent Distribution to Separate Isologues and Geometric Isomers of Fatty Acid Esters

Mixtures of fatty acid methyl esters were fractionated by countercurrent distribution between hexane and 0.2*N* $AgNO_3$ in 90% methanol. *Cis* and *trans* monoenoic esters were separated.

C. R. SCHOLFIELD, E. P. JONES, R. O. BUTTERFIELD, and H. J. DUTTON, Northern Regional Research Laboratory, Peoria, Ill.

Anal. Chem. 35, 1588 (1963)

A New Specific Color Reaction of Formic Acid

The violet complex formed with anisole-formic acid mixture and lead nitrate was studied. It appears that the reaction can be used for the detection of formic acid as well as nitrate ions. A tentative mechanism for the color reaction is proposed and discussed.

MOHSIN QURESHI, WAQIF HUSAIN, and JAGDISH P. RAWAT, Prince of Wales Chemical Laboratories, Aligarh Muslim University, Aligarh, India

Anal. Chem. 35, 1592 (1963)

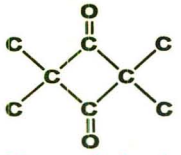
Detection of Metal Chelates in Gas Liquid Chromatography by Electron Capture

Trace quantities of Cr(III) and Al(III) acetylacetonates, trifluoroacetylacetonates, and hexafluoroacetylacetonates are detected. Instrument conditions are described and sensitivity limits given. The eluents, nitrogen and argon, are compared.

WILLIAM D. ROSS, Dayton Laboratory, Monsanto Research Corp., Dayton, Ohio

Anal. Chem. 35, 1596 (1963)

Eastman Briefs
FOR OCTOBER



Tetramethyl-1,3-cyclobutanedione

Form	*solid*
Melting point	*115-116°C.*
Boiling point, 760 mm.	*159°C.*
Purity	*99.5%*

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Eastman Chemical Products, Inc.
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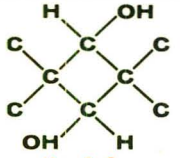
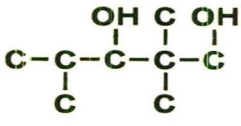


2,2,4,4-Tetramethyl-1,3-cyclobutanediol

Form	*white solid*
Melting point	*124-135°C.*
Boiling range, 760 mm.	*220-225°C.*
Isomer composition (approx.)	*50% cis, 50% trans*

The symmetry and high degree of substitution of this lineal descendant of B14 impart unusual thermal and hydrolytic stability to its diesters. They could be of more than passing interest as plasticizers and lubricants.

Eastman Chemical Products, Inc.
Kingsport, Tennessee **B15**



2,2,4-Trimethyl-1,3-pentanediol

Form	*white solid*
Melting point	*49-52°C.*
Molten color, APHA, ppm.	*15*
Purity	*98%*

A sterically hindered glycol for the manufacture of unsaturated polyester resins with good storage stability, unique cure properties, and excellent dimensional stability.

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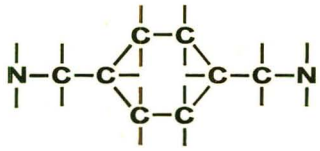
Kingsport, Tennessee **B28**

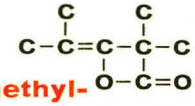


1,4-Cyclohexanebis (methylamine)

Form	*clear liquid*
Isomer content	*40% cis, 60% trans*
Boiling range, 760 mm.	*239-244°C.*
Purity	*99%*

This diamine is versatile: it mixes with water and organic solvents; it reacts with atmospheric CO_2 to form a carbonate insoluble in the parent amine; it should also interest urethane chemists.

Eastman Chemical Products, Inc.
Kingsport, Tennessee **B31**



2,2,4-Trimethyl-3-hydroxy-3-pentenoic acid,β-lactone

Form	*liquid*
Specific gravity, 20/4°C.	*0.946*
Boiling point, 730 mm.	*170°C.*

Thiol esters are obtained in the base- or acid-catalyzed reaction of this lactone with mercaptans. Reaction in the presence of a free radical catalyst produces intermediates which decarboxylate to give alkyl (1-isopropyl-2-methyl-propenyl) sulfides.

Eastman Chemical Products, Inc.
Kingsport, Tennessee **B42**

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Formal Oxidation-Reduction Potentials and Indicator Characteristics of Some Cyanide and 2,2′-Bipyridine Complexes of Iron, Ruthenium, and Osmium

Formal oxidation-reduction potentials for $[Fe(bipy)_2(CN)_2]$, $[Ru(bipy)_2(CN)_2]$, $[Os(bipy)_2(CN)_2]$, $[Fe(bipy)_3]SO_4$, $[Ru(bipy)_3]SO_4$, and $[Os(bipy)_3]SO_4$ in sulfuric acid solutions ranging in concentration from 0.01 to 12*M* have been measured.

ALFRED A. SCHILT, Department of Chemistry, Northern Illinois University, DeKalb, Ill.

Anal. Chem. 35, 1599 (1963)

Chronopotentiometric Oxidation of Hydrazine at a Platinum Electrode

The effect of electrode pretreatment on the anodic oxidation of hydrazine in sulfuric acid solutions was investigated. A variation of the chronopotentiometric constant, $i\tau^{1/2}/C$, with current density and concentration was found.

ALLEN J. BARD, Department of Chemistry, The University of Texas, Austin 12, Texas

Anal. Chem. 35, 1602 (1963)

Atomization Efficiency of Total Consumption Atomizer-Burners in Flame Photometry

The method is based on the measurement of the absorption of radiation from a sharp-line zinc source by zinc atoms in a flame of known composition and temperature. By calculation, the efficiency of sample introduction can be found. This efficiency is studied as a function of sample solvent, of height of region measured above the tip of inner cone of flame, and of flow rate of solution.

J. D. WINEFORDNER, C. T. MANSFIELD, and T. J. VICKERS, Department of Chemistry, University of Florida, Gainesville, Fla.

Anal. Chem. 35, 1607 (1963)

Temperatures of Some Typical Flames Used in Flame Photometry

Flame temperatures of H_2/O_2 and C_2H_2/O_2 flames are measured as a function of solution flow rate (via total consumption atomizer-burners) into the flame for a variety of flame compositions and heights in the flame. Empirical equations are determined which can be used to calculate the flame temperature of given flame compositions at a given height above the inner cone.

J. D. WINEFORDNER, C. T. MANSFIELD, and T. J. VICKERS, Department of Chemistry, University of Florida, Gainesville, Fla.

Anal. Chem. 35, 1611 (1963)

Spectrophotometric Determination of Uranium with 4-(2-Pyridylazo)resorcinol

Uranium forms a 1:1 complex with 4-(2-pyridylazo)resorcinol, which has a molar absorptivity of 38,700 at 530 mμ. A preliminary separation of uranium from gross amounts of impurities is accomplished by a rapid chelating-resin ion exchange procedure. A mixed complexing solution is used which masks most metals including thorium.

T. M. FLORENCE and YVONNE FARRAR, Australian Atomic Energy Commission Research Establishment, Lucas Heights, N.S.W., Australia

Anal. Chem. 35, 1613 (1963)

Characterization of Alkyl and Aryl Halides by 2,4-Dinitrophenylhydrazones of Aldehydes from Reaction of Their Grignard Reagents with Dimethylformamide

The basic reaction, formylation of the Grignard reagent with dimethylformamide is carried out in a test tube in 1 hour. A number of compounds, not before characterized this way, are reported. Reactions were not obtained with allylic halides, some tertiary halides, and halides with electron-attracting substituents.

JACOB G. SHAREFKIN and ALEX FORSCHIRM, Brooklyn College of the City University of New York, Brooklyn 10, N. Y.

Anal. Chem. 35, 1616 (1963)

Determination of Beryllium by the Photoneutron Method

An apparatus designed and constructed for the determination of beryllium by the photoneutron method uses large $B^{10}F_3$ neutron counter tubes. Sample solutions containing as little as 60 μg. of Be per ml., or 1.5 mg. of total beryllium, can be analyzed with a relative standard deviation of ±1%.

GERALD GOLDSTEIN, Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Anal. Chem. 35, 1620 (1963)

Neutron Flux Monitoring for Activation Analysis of Oxygen

Oxygen in a piece of Lucite located near the neutron source and oxygen in the sample are activated simultaneously and counted simultaneously. The analytical result is independent of variations in neutron flux, durations of irradiation, sample transfer to counter, and counting periods. Relative standard deviations of 1% are achieved.

J. T. GILMORE and D. E. HULL, California Research Corp., Richmond, Calif.

Anal. Chem. 35, 1623 (1963)

A Spectrophotometric Study of the Complexes Formed between Uranyl and Thiosulfate Ions in Water and Water–Ethanol Solvents

The method of continuous variations at 25° C. was used to prove the existence of the complex $UO_2S_2O_3$ in dilute aqueous solutions and in solutions of 30-, 60-, and 90-volume per cent ethanol. The complex $(UO_2)_3(S_2O_3)_2^{+2}$ was found in more concentrated aqueous solutions. Equilibrium constants for the complexes were determined.

SHARON LYNN MELTON and EDWARD S. AMIS, Chemistry Department, University of Arkansas, Fayetteville, Ark.

Anal. Chem. 35, 1626 (1963)

Precipitation of Zinc and Cadmium 8-Quinolinates from Homogeneous Solution

Precipitates of excellent physical characteristics were obtained by an ammonia volatilization technique. An approximate formation constant for this complex has been calculated.

F. H. FIRSCHING and J. G. BREWER, Department of Chemistry, University of Georgia, Athens, Ga.

Anal. Chem. 35, 1630 (1963)

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Determination of Four Closely Related Triaryl-s-Triazines by Infrared Spectrometry

Well-resolved out-of-plane carbon-hydrogen bending vibrations located between 750 and 825 $cm.^{-1}$ are intense enough to permit quantitative determination of each component in mixtures of all four triazines.

R. DONALD SPENCER and BEVERLY H. BEGGS, Mellon Institute, Pittsburgh 13, Pa.

Anal. Chem. 35, 1633 (1963)

Temperature Programmed Capillary Columns in Gas Chromatography

A modified commercially available gas chromatograph was used for the analysis of dilute hydrocarbon gas mixtures (99.8% N_2). For qualitative and quantitative analyses, the precision of the retention volume and peak area measurements were determined for the method.

DAVID J. McEWEN, General Motors Research Laboratories, Warren, Mich.

Anal. Chem. 35, 1636 (1963)

A Gas-Density Detector for Gas Chromatography

Equations were developed to show the interrelationship of change in density, flow, and electrical response in gas-density detectors for gas chromatography. A simplified gas-density detector was developed and used to analyze complex mixtures of aromatics, saturates, and chlorinated and oxygenated compounds.

A. G. NERHEIM, Research and Development Department, American Oil Co., Whiting, Ind.

Anal. Chem. 35, 1640 (1963)

Preparation of Methyl Esters for Gas Liquid Chromatography of Acids by Pyrolysis of Tetramethylammonium Salts

The tetramethylammonium salts are prepared either by titrating the acids with tetramethylammonium hydroxide or by ion exchange on an anion exchange resin. The salts are converted to the corresponding methyl esters in high yield when injected into a gas chromatographic unit at a vaporizer temperature of 330° to 365° C.

ERNEST W. ROBB and JOHN J. WESTBROOK III, Philip Morris Research Center, Richmond, Va.

Anal. Chem. 35, 1644 (1963)

Gas Chromatographic Determination of Free Toluene Diisocyanate in Adducts with Trimethylolpropane

Conditions for the method were chosen to minimize the thermal dissociation of the adduct. When compared with a wet chemical method, the described method gave greater precision and accuracy in the range reported.

N. R. NEUBAUER, G. R. SKRECKOSKI, R. G. WHITE, and A. J. KANE, National Aniline Division, Allied Chemical Corp., Buffalo, N. Y.

Anal. Chem. 35, 1647 (1963)

Chromatographic Liquid Leak Detection

Gas chromatographic techniques were used to detect the leaking of volatile silicone fluid from sealed containers. The technique used can be applied in preference to the more common helium and Radiflo leak detectors in many cases.

ISHMAEL ORTEGA and J. D. CYRUS, Sandia Corp., Sandia Base, Albuquerque, N. M.

Anal. Chem. 35, 1649 (1963)

Gas Chromatographic Analysis of Chlorophenol Mixtures

Complete resolution of eight chlorophenols and phenol can be obtained using one column and no sample pretreatment. A phosphoric acid additive and a thermally stable substrate are used.

R. H. KOLLOFF, L. J. BREUKLANDER, and L. B. BARKLEY, Monsanto Chemical Co., 800 North Lindbergh Blvd., St. Louis 66, Mo.

Anal. Chem. 35, 1651 (1963)

Gas Chromatographic Determination of Nitrogen in Refractory Materials

The method uses a conventional induction furnace for combustion of the material in oxygen at about 1500° C. The liberated nitrogen gas, collected in a modified Hempel buret, is determined by gas chromatography. The procedure has been used for nitrogen in AlN, BN, CrN, Si_3N_4, TiN, ZrN, AlN·SiC, and B_6Si samples.

ROBERT J. HYNEK and JOSEPH A. NELEN, Allis-Chalmers Research Laboratories, P. O. Box 512, Milwaukee 1, Wis.

Anal. Chem. 35, 1655 (1963)

Gas Chromatographic Technique for Determination of Hydridic and Active Hydrogen in Organic Materials. Microanalysis of Borane Compounds and Alcohols

The hydrogen gas was evolved from borane compounds by reaction with a protonic agent and from compounds containing active hydrogen by reaction with lithium aluminum hydride. The generated hydrogen gas was determined in nitrogen carrier gas stream by thermoconductivity detection. Sample sizes were in the milligram range.

IHOR LYSYJ and R. C. GREENOUGH, Rocketdyne Division of North American Aviation, Inc., Canoga Park, Calif.

Anal. Chem. 35, 1657 (1963)

The Brown Front in Paper Chromatography

The brown front in filter paper chromatography and elution, apparently caused by modification of the paper at the wet-dry interface, has been studied. The brown residue is made up of water-soluble organic material and salts, which can modify the chromatographic behavior of sugar acids.

ROBERT A. SCHWANE, and GEORGE N. KOWKABANY, The Catholic University of America, Washington 17, D. C.

Anal. Chem. 35, 1660 (1963)

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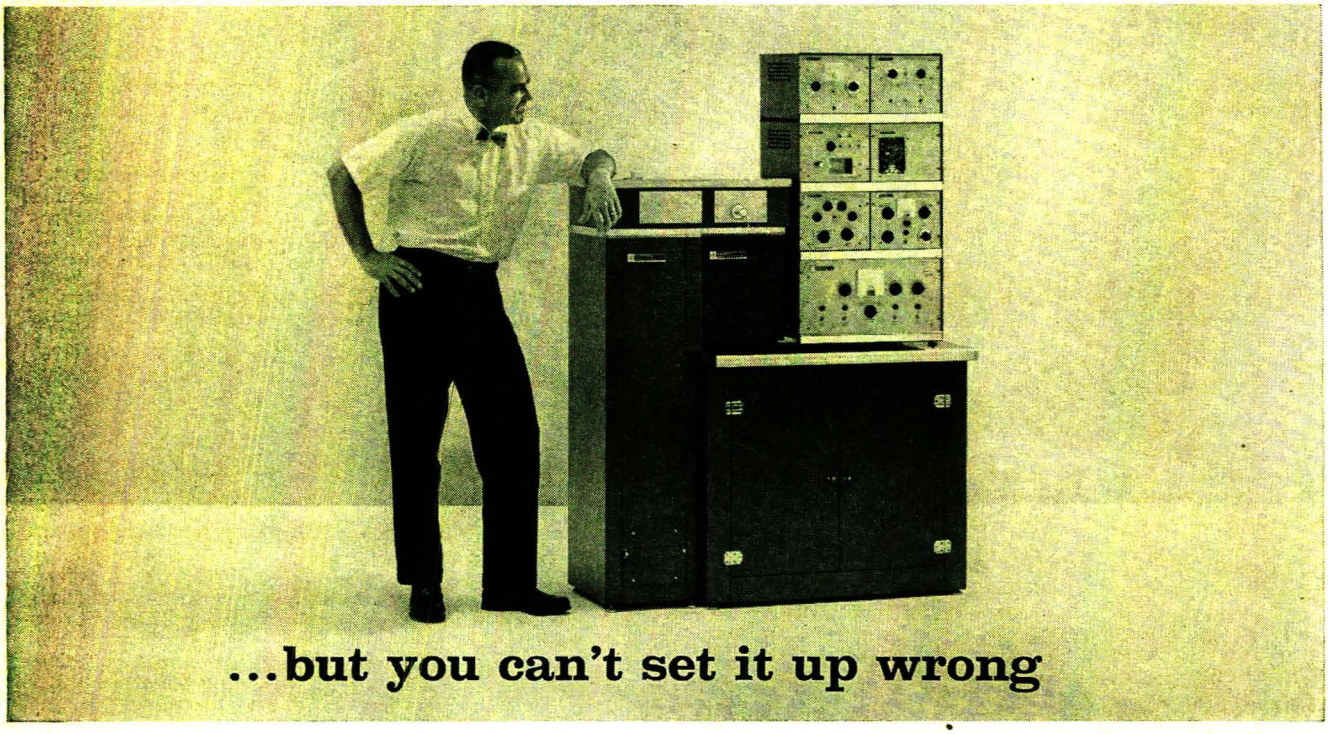

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Determination of Nicotinic Acid in Coffee by Paper Chromatography

A mild acid treatment of the coffee solution or suspension was followed by neutralization and decolorization with basic lead carbonate. After filtration, the supernatant was resolved by paper chromatography. The nicotinic acid spots were revealed by a cyanogen bromide–benzidine reagent.

HELGA BÖDDEKER and A. R. MISHKIN, Westreco, Inc., Marysville, Ohio

Anal. Chem. 35, 1662 (1963)

Attenuated Total Reflectance vs. Transmission Infrared Spectrometry in the Quantitative Evaluation of Paint Vehicles

Attenuated total reflectance is shown to be preferable to transmission spectrometry for the quantitative evaluation of paint vehicles by infrared analyses. Knowledge of the thickness of thin films which is essential for quantitative transmission infrared spectrometry is not necessary in attenuated total reflectance techniques.

RAYMOND J. McGOWAN, Physics and Mathematics Division, U. S. Naval Civil Engineering Laboratory, Port Hueneme, Calif.

Anal. Chem. 35, 1664 (1963)

Attenuated Total Reflectance Infrared Analysis of Aqueous Solutions

The attenuated total reflectance technique is used for the qualitative and quantitative infrared analysis of aqueous solutions. Spectra are obtained throughout the rock salt region except in the immediate vicinity of the 3300 $cm.^{-1}$ water band. Spectra data are given and advantages and limitations of the method are discussed.

BERNARD KATLAFSKY and ROBERT E. KELLER, Research Department, Organic Chemicals Division, Monsanto Chemical Co., St. Louis 77, Mo.

Anal. Chem. 35, 1665 (1963)

Determination of Plutonium in Biological Material by Solvent Extraction with Primary Amines

Concentrated urine, or a solution of bone ash is made at least 1M in H_2SO_4, and plutonium is extracted with a mixture of C_{18} to C_{23} highly branched primary amines in xylene. Plutonium extracted with 8M HCl is measured by alpha counting.

F. W. BRUENGER, B. J. STOVER, and D. R. ATHERTON, Division of Radiobiology, Department of Anatomy, University of Utah College of Medicine, Salt Lake City 12, Utah

Anal. Chem. 35, 1671 (1963)

Amperometric Titration of Plutonium(VI) with Iron(II)

The plutonium is oxidized to the sexivalent state in a sulfuric acid medium with argentic oxide, the excess oxidant destroyed by heating, and the plutonium titrated by the addition of standard iron(II) sulfate from special weight burets. Precision data are given for the method.

C. A. SEILS, Jr., R. J. MEYER, and R. P. LARSEN, Chemical Engineering Division, Argonne National Laboratory, Argonne, Ill.

Anal. Chem. 35, 1673 (1963)

Direct Mass Spectrometric Analysis of Petroleum Samples Boiling in the Kerosine Range. Heavy Reformates and Hydrodealkylated Products

Nine compound types, including eight hydrocarbons and alkyl benzothiophenes, are reported. The method requires no separation of the sample prior to analysis and is carried out in less than 2 man-hours per sample.

L. R. SNYDER, H. E. HOWARD, and W. C. FERGUSON, Union Research Center, Union Oil Co. of California, Brea, Calif.

Anal. Chem. 35, 1676 (1963)

Ion Exchange Separation of Brominated Salicylanilides and Hydroxybenzoic Acids

After adsorption on Dowex 2-X8, acetate form, mixtures of brominated salicylanilides or of benzoic acid and hydroxybenzoic acid isomers are selectively desorbed with acetic acid–methanol mixtures. Concentrations are determined by ultraviolet spectrophotometry.

NORMAN E. SKELLY and WARREN B. CRUMMETT, Special Services Laboratory, The Dow Chemical Co., Midland, Mich.

Anal. Chem. 35, 1680 (1963)

Quantitative Analysis of Aspirin, Phenacetin, and Caffeine Mixtures by Nuclear Magnetic Resonance Spectrometry

Several known mixtures and some commercial preparations have been analyzed. The average deviations from the correct results were: aspirin, 1.1%; phenacetin, 2.2%; and caffeine, 3.2%. Twenty minutes is required for the analysis.

DONALD P. HOLLIS, Spectroscopy Applications Laboratories, Instrument Division, Varian Associates, 611 Hansen Way, Palo Alto, Calif.

Anal. Chem. 35, 1682 (1963)

Spectrophotometric Determination of Vanadium in High Purity Aluminum

The vanadium–phosphotungstate colored complex is extracted from aluminum powder with n-hexanol. Vanadium is determined at 436 mμ. The procedure requires only 25 minutes after the complex is formed; however, the total time is dependent on the rate at which aluminum powder can be dissolved and the excess acid removed.

DONALD G. BIECHLER, DONALD E. JORDAN, and W. D. LESLIE, Research and Development Department, Continental Oil Co., Ponca City, Okla.

Anal. Chem. 35, 1685 (1963)

Fluorometric Determination of Selenium in Plants and Animals with 3,3-Diaminobenzidine

Two methods of oxidizing samples (Parr bomb and Schöniger flask), new and/or improved have been studied intensively. The combustion techniques, measured by 13 determinations with each technique on the same ground sample of alfalfa, gave for the Parr bomb an average of 1.14 p.p.m. of Se with a standard deviation of ± 0.019. The Schöniger flask gave an average of 1.17 p.p.m. of Se with a standard deviation of ± 0.012.

W. B. DYE, ERICH BRETTHAUER, H. J. SEIM, and CLIFTON BLINCOE, University of Nevada, University Station, Reno, Nevada

Anal. Chem. 35, 1687 (1963)

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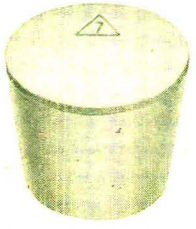

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Determination of Water and Alkali Metal Oxide in Alkali Metal Salts of Selenium and Tellurium by Karl Fischer Titration

Typical data on the determinations of alkali metal oxide and/or water in sodium selenite, sodium selenate, potassium tellurite, and disodium tetrahydrogen tellurate are presented. The results are compared with weight loss determinations by thermal drying and with selenium or tellurium by potentiometric titration.

PETER TAO-I CHIANG and W. CHARLES COOPER, Noranda Research Centre, Pointe Claire, Quebec, Canada

Anal. Chem. 35, 1693 (1963)

Determination of Phosphorus in Iron and Steel with Quinoline Molybdate

This improved method applies to phosphorus in all classes of iron and steel. Tests on NBS samples and current alloy types of iron and steel have shown reproducibilities of ±0.001, ±0.002, ±0.01, and ±0.03 for phosphorus levels of 0.01, 0.1, 0.5, and 1.5%, respectively.

ARTHUR LENCH, Australian Defence Scientific Service, Department of Supply, Defence Standards Laboratories, Melbourne, Australia

Anal. Chem. 35, 1695 (1963)

Application of the Pararosaniline–Formaldehyde Spectrophotometric Method to the Determination of Sulfur in Blister and Refined Copper

Factors affecting the combustion, furnace temperature, use of fluxes, and combustion accelerators and retarders were studied. The devised procedure permits accurate determination of sulfur in copper in the range 2 to 500 p.p.m. Standard deviation at 10-p.p.m. level is less than ±1, and at the 100-p.p.m. level less than ±4.

SILVIO BARABAS and JOSEPH KAMINSKI, Canadian Copper Refiners Ltd., Montreal East, Quebec, Canada

Anal. Chem. 35, 1702 (1963)

Flame Photometric Determination of Manganese in Copper and Steel Alloys

Manganese is extracted with 2-thenoyltrifluoroacetone (TTA). Complexing agents are used to prevent extraction of other ions. Triplicate samples of alloys containing from 0.02 to 1.00% (0.05 to 0.50 mg.) manganese were analyzed within 45 minutes with a standard deviation of ±0.01.

DOLORES A. JOHNSON and PETER F. LOTT, St. John's University, Jamaica 32, N. Y.

Anal. Chem. 35, 1705 (1963)

A Continuous Electrolytic Analyzer for Acidic or Basic Components of Process Gas Streams

The analysis proceeds by scrubbing the gas stream with an aqueous salt solution and titrating the dissolved species with electrolytically generated hydronium or hydroxide ions. The apparatus was used successfully in monitoring levels of HCl content from 0.1 to over 300 p.p.m. by volume.

R. L. BURNETT and R. F. KLAVER, California Research Corp., Richmond, Calif.

Anal. Chem. 35, 1709 (1963)

A Rapid Radiochemical Procedure for Tin

The described method gives a tin chemical yield from 15% for an antimony decontamination factor of 2×10^4 to 60% for an antimony decontamination factor of 10^3. The time required for separation of tin from other fission product elements is about 10 seconds.

ALLEN E. GREENDALE and DANIEL L. LOVE, U. S. Naval Radiological Defense Laboratory, San Francisco 24, Calif.

Anal. Chem. 35, 1712 (1963)

Automatic, Amperometric, Cupferron Titration of Zirconium in Highly Radioactive Solutions

A stirred mercury pool is the indicating electrode which senses the point where cupferron comes into excess, and a platinum wire is used for the reference. Precision of 1% relative standard deviation is obtainable for benchtop analysis and 1.5% for titrations in a hot cell.

HISASHI KUBOTA and J. G. SURAK, Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Anal. Chem. 35, 1715 (1963)

Hydrogen Cell Assembly for Standard Electromotive Force Measurements

A hydrogen cell assembly for the calibration of buffer solution, glass electrodes, and reference electrodes has been designed which permits such calibrations to be made in 20 to 30 minutes. Maximum standard deviation is ±0.6 mv. (±0.01 pH).

PETER G. SIBBALD and GEORGE MATSUYAMA, Beckman Instruments, Inc., Scientific and Process Instruments Division, Fullerton, Calif.

Anal. Chem. 35, 1718 (1963)

Sensitive Method for the Determination of Submicrogram Quantities of Manganese and Its Application to Human Serum

The method is based on the oxidation of malachite green with periodate in the presence of manganese. The first order reaction is catalyzed by manganese. The method was applied to human serum. The normal level of serum manganese was found to be 0.36 to 0.90 mμg./ml.

ALBERTO A. FERNANDEZ, CHARLES SOBEL, and S. L. JACOBS, Bio-Science Laboratories, 12330 Santa Monica Blvd., Los Angeles 25, Calif.

Anal. Chem. 35, 1721 (1963)

Fast Qualitative and Quantitative Microanalysis of Plasticizers in Plastics by Gas Liquid Chromatography

Heavy esters such as dioctyl phthalate or dioctyl adipate and related compounds are analyzed on gas chromatographic columns at 200° to 240° C. using SE 30 or poly(neopentylglycol adipate) on glass beads. A special technique of mild pyrolysis is used.

JAVIER ZULAICA and GEORGES GUIOCHON, Ecole Polytechnique, Laboratoire du Professeur L. Jacque, 17, Rue Descartes, Paris (5e), France

Anal. Chem. 35, 1724 (1963)

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LEE CAHN and HAROLD SCHULTZ, Cahn Instrument Co., Paramount, Calif.

Anal. Chem. 35, 1729 (1963)

Spectrophotometric Determination of Bromide Ion in Water

The determination is applied to concentrations of bromide ion in the range of 0.02 to 1.20 p.p.m. Reactions proceed in solutions buffered to pH 5.6 between 23° and 26° C., and the concentration of bromide ion is measured as a function of the ammonia lost because of oxidation by hypobromite to nitrogen.

FRED ZITOMER and JACK L. LAMBERT, Department of Chemistry, Kansas State University, Manhattan, Kan.

Anal. Chem. 35, 1731 (1963)

Determination of Lithium in Aluminum by Flame Photometry. Utilization of Organic Solvents for Submilligram Amounts

Microgram levels of lithium can be determined without separation. The method uses the addition of isopropyl alcohol and acetone to acid solutions containing lithium and aluminum. Background corrections, gas pressures, and solvent addition techniques are described.

W. E. PILGRIM and W. R. FORD, Reynolds Metals Co., Metallurgical Research Division, Richmond, Va.

Anal. Chem. 35, 1735 (1963)

Improved Spectrophotometric Method for the Determination of Small Amounts of Chloroform

The method requires the addition of pyridine to a chloroform solution in the presence of sodium hydroxide. The absorbance is measured at 366 mμ. Accuracy of the method is 5% and 0.2 p.p.m. of chloroform can be detected.

MARIANNA MANTEL, MAGDA MOLCO, and MARIANNA STILLER, Israel Atomic Energy Commission, Rehovoth, Israel

Anal. Chem. 35, 1737 (1963)

Spectrophotometric Determination of Acetylenic Compounds as Mercuric Acetate Complexes

The method is based on the formation of mercuric acetate addition products of the acetylenic compounds and measurement of the ultraviolet absorption of these addition compounds. The method is specific for acetylenic compounds and is sensitive to low ranges of concentration.

SIDNEY SIGGIA and C. R. STAHL, General Aniline & Film Corp., Central Research Laboratory, Easton, Pa.

Anal. Chem. 35, 1740 (1963)

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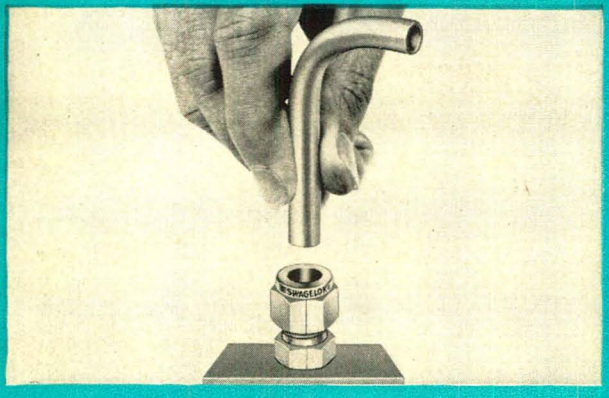

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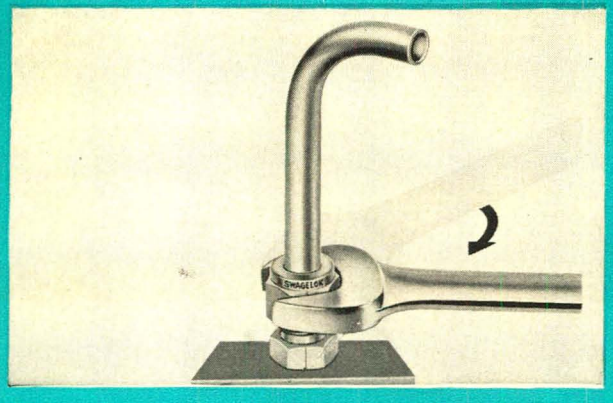

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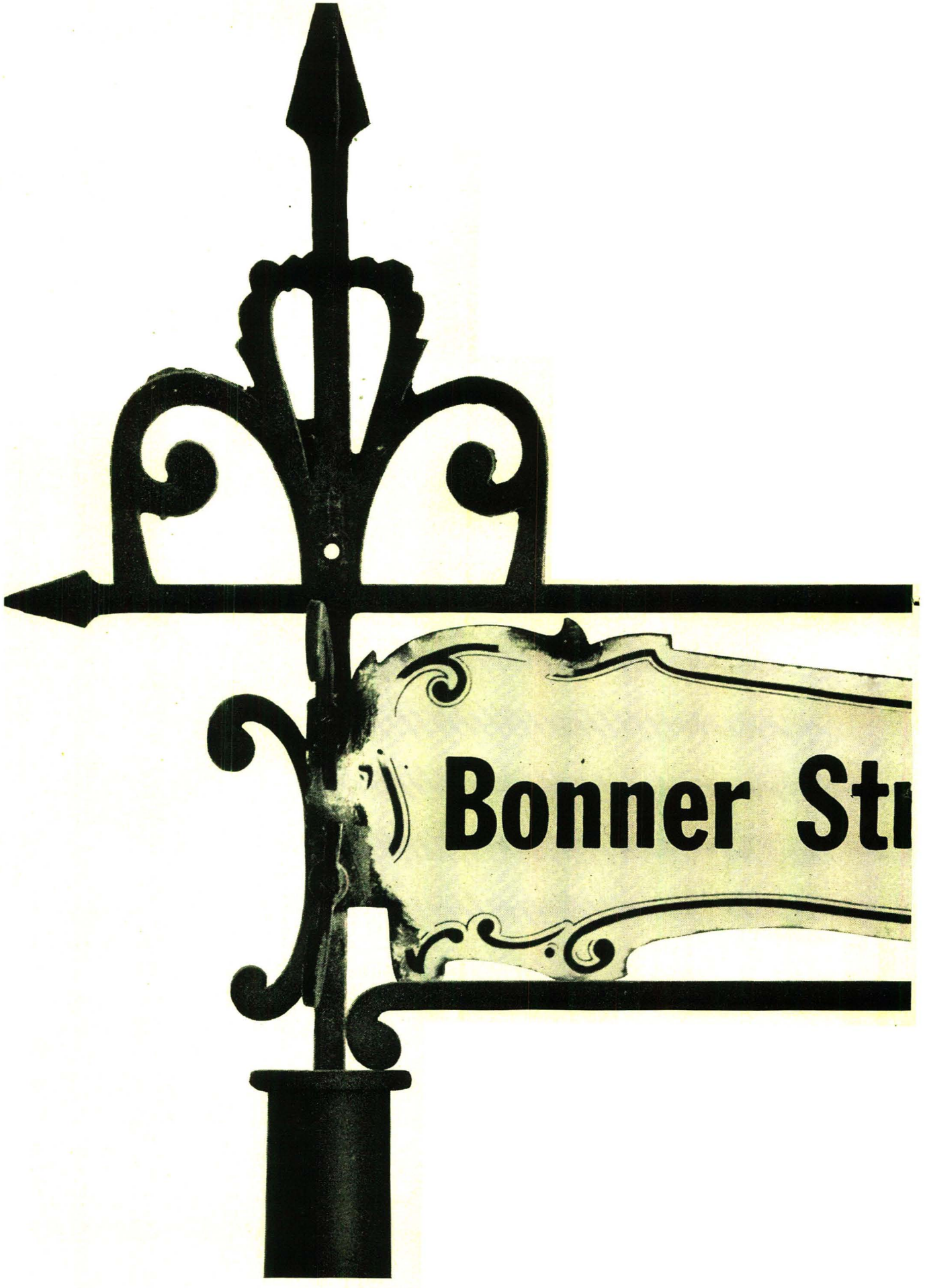
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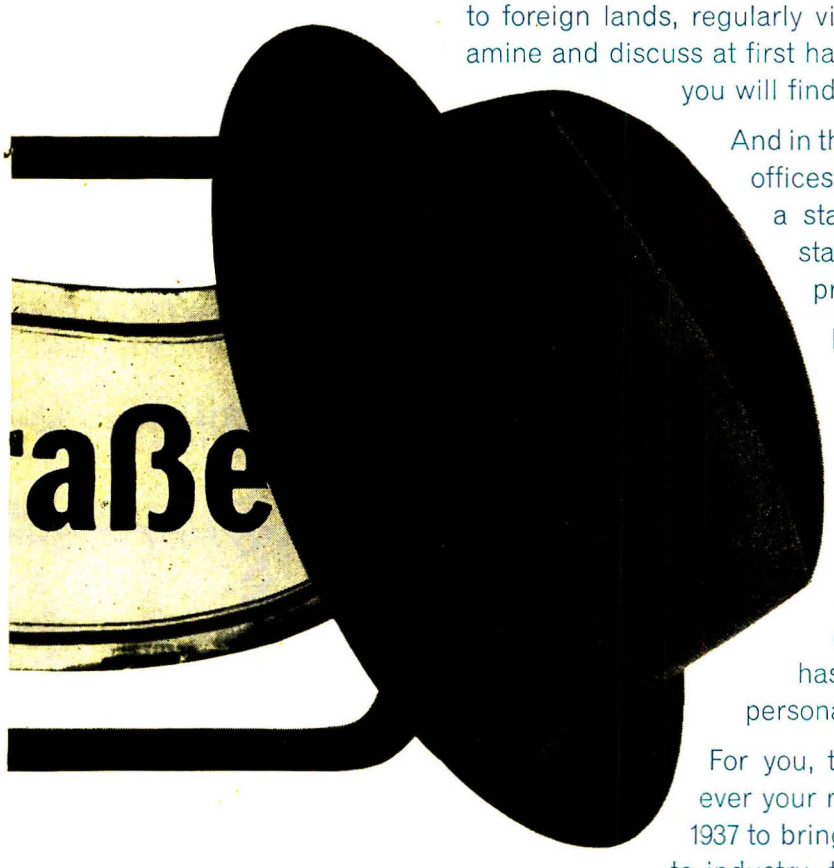

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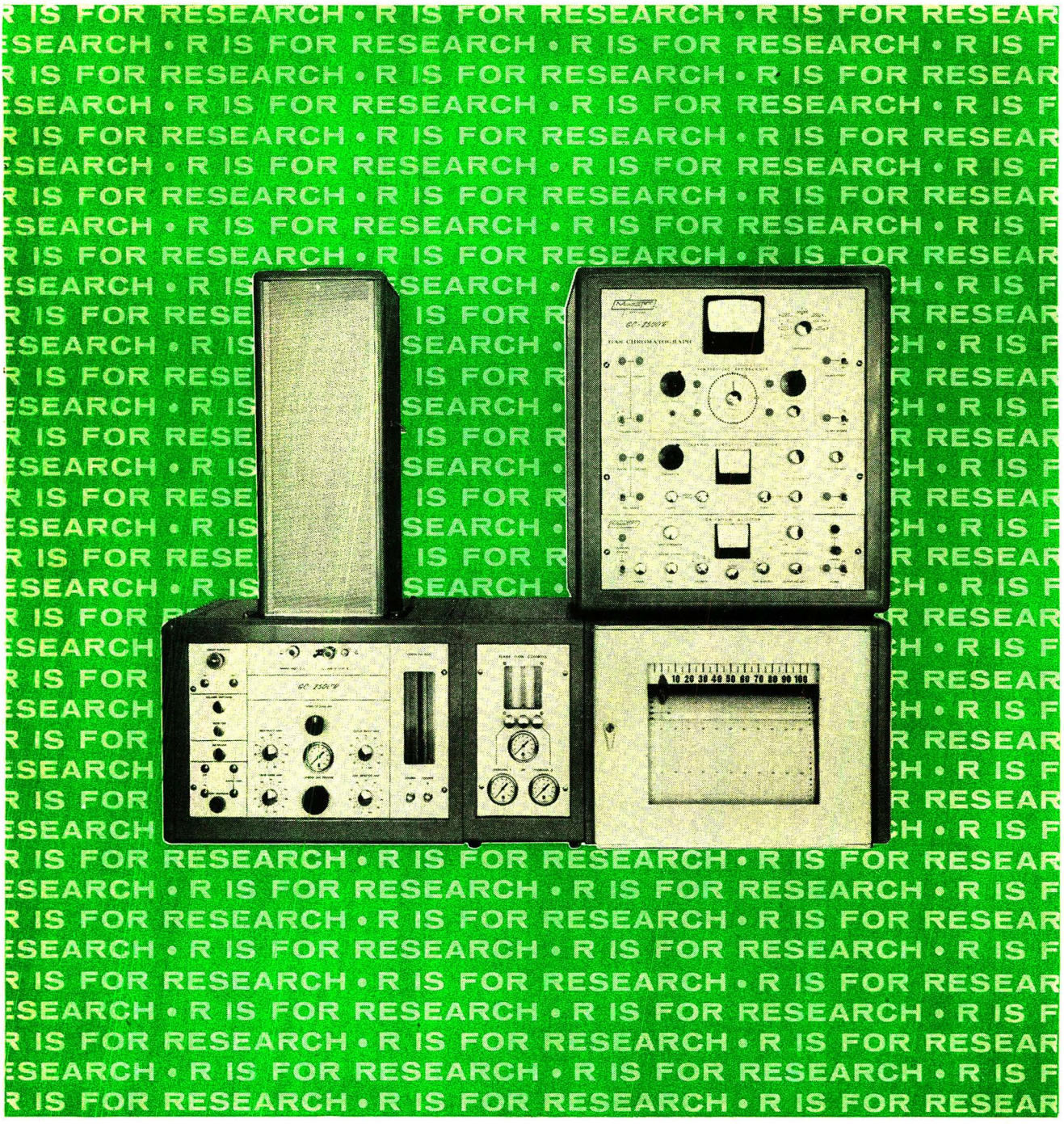

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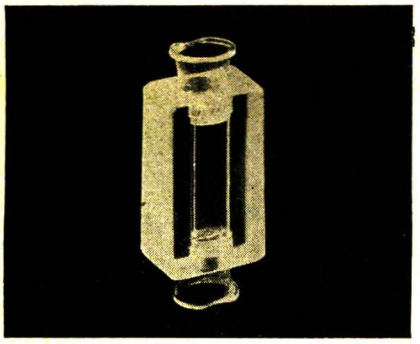

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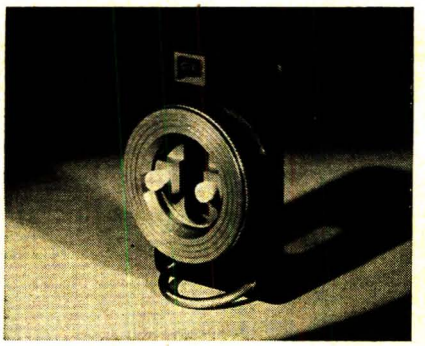

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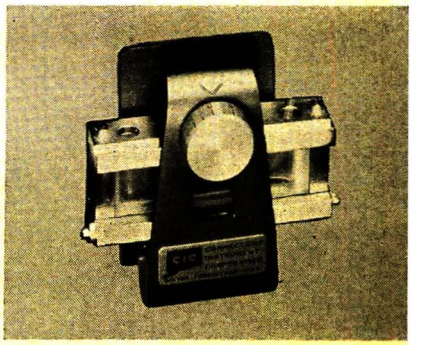

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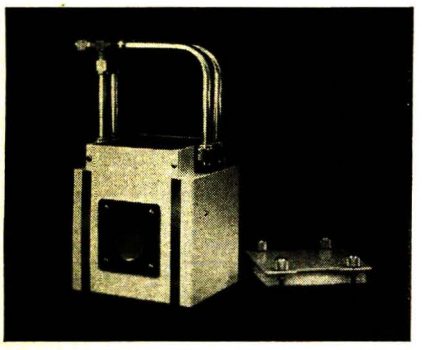

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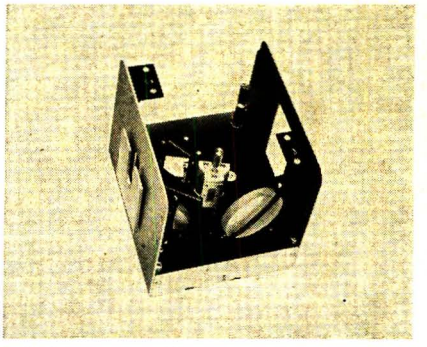

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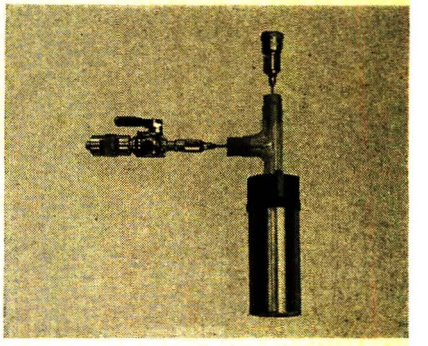

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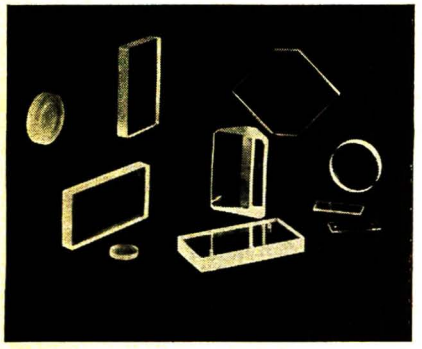

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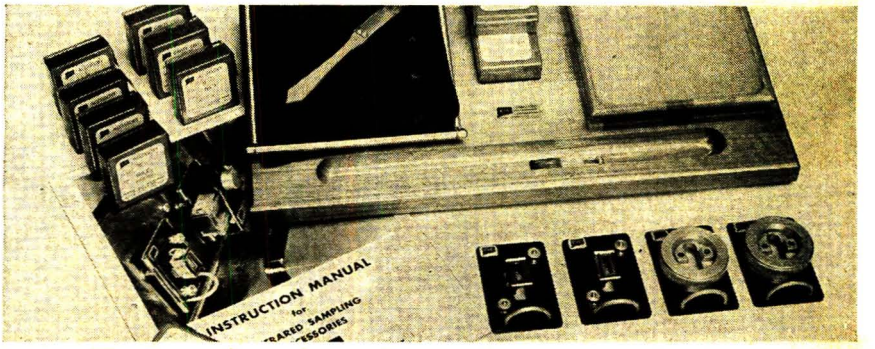

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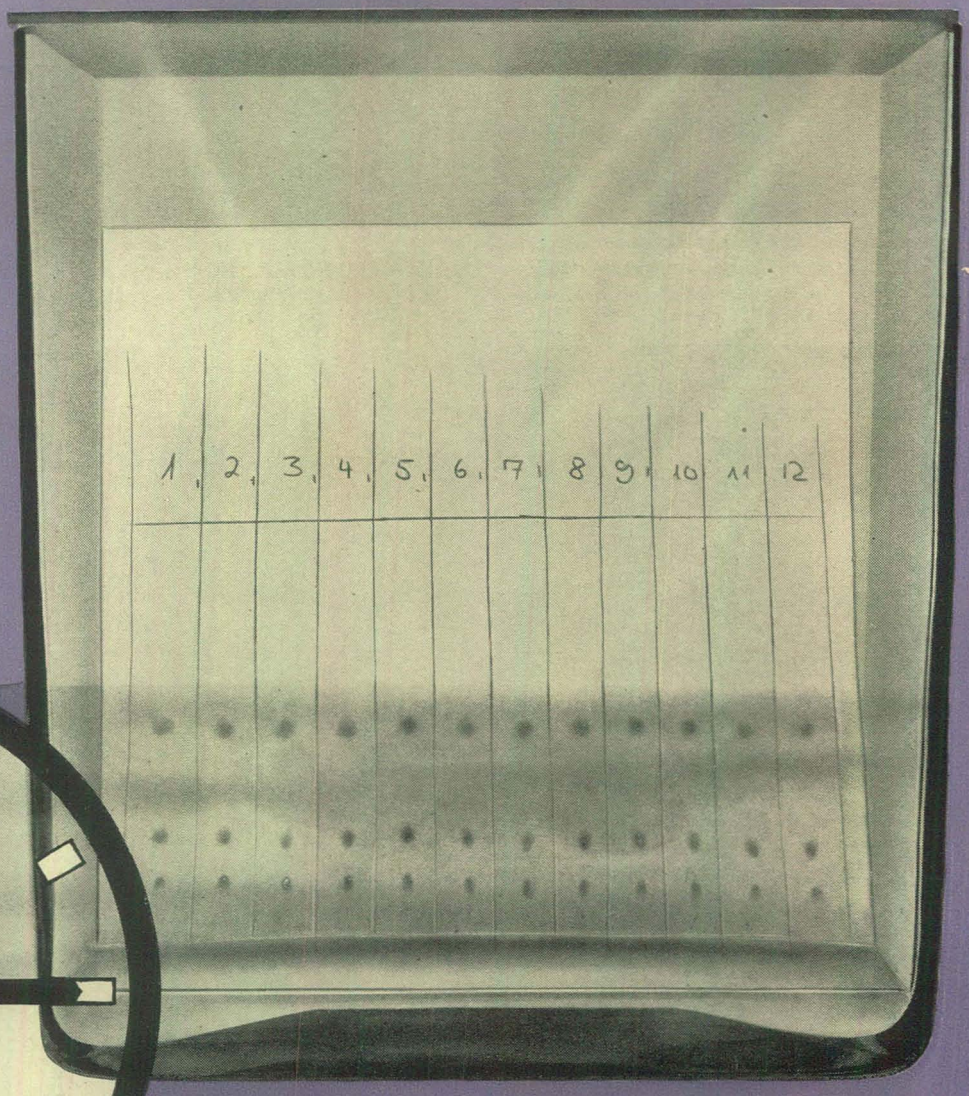

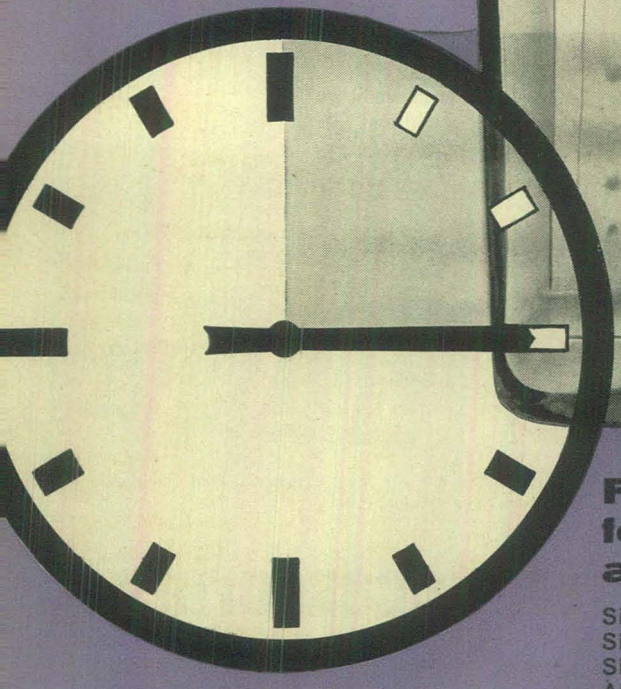

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Centers for Storage and Retrieval of Scientific Information in the U. S.

SCIENTISTS AND ENGINEERS today face a serious problem in trying to keep up with the technical literature. The enormous increase in output of scientific information in the last decade has put an overwhelming burden on the individual technical man to keep up to date in his field.

Every day the working scientist or engineer also finds it harder to locate the specific information he needs when he needs it. More important, when he does find it, the information is often incomplete, the data from separate sources may be incompatible and, unless he is a specialist in the field, he may find it impossible to judge its reliability and authority.

In discussing the science communication problem, Lord Todd, President of the International Union of Pure and Applied Chemistry, recently said, "The enormous and ever-increasing number of these (original papers) has swollen our traditional journals out of all recognition, has led to the appearance of more and more new journals, and is gradually overwhelming their publishers as it has already overwhelmed their readers. The great abstract journals—*Chemical Abstracts, Chemisches Zentralblatt,* and the *Referativny Jurnal*—have struggled manfully with the problem of providing chemists with a précis of original papers, but it is getting beyond their power."

In the United States, the general problem of the transfer of scientific and technical information has been studied by a number of groups, including the President's Science Advisory Committee, the Federal Council on Science and Technology, the Senate Committee on Government Operations, the House of Representatives Committee on Science and Astronautics, the National Science Foundation, and the National Academy of Sciences–National Research Council. In addition, many professional societies such as the American Chemical Society, the American Institute of Chemical Engineers, the American Physical Society, the American Society for Testing and Materials, and the American Society for Metals are working on information transfer problems in specific areas.

To combat the information problem a large number of manual and mechanical retrieval devices have been developed: simple manual devices (edge-notched cards and peekaboo cards), equipment in which images of the document and indexing information are together (Rapid Selector, Filmsort, Minicard, etc.), equipment containing document images and using a separate index (Verac, Microcite), punched cards and sorters, and a host of electronic computers developed by many different companies.

However, as one prominent scientific information specialist sees it [Y. S. Touloukian, *Intern. J. Heat Mass Transfer.* **6,** 301 (1963)], "Our technical know-how and capabilities are fully adequate to solve the document retrieval problem adequately from the machine and systems design standpoint. The greatest bottleneck which confronts us in the solution of scientific information problems is the lack of qualified scientific and technical people interested in working in this area. The primary reason for this lack of personnel is not one of the need for more extensive training, but rather the stigma associated with such an activity as a second grade activity not par with the conduct of original research."

As an outgrowth of the increased concern for more efficient communication of information to the working scientist, a large number of centers for storage and retrieval of scientific information have been established in the United States in the last ten years. Many are sponsored by the Federal Government; others are supported by universities, research institutions, professional societies, or private industry. Many of the larger centers cover a broad area of information and mainly deal with document retrieval; some deal only with scientific and technical data, while others specialize in fairly narrow subject areas such as metallurgy. The aims of this report are (1) to acquaint the reader with several outstanding scientific information centers in the United States and (2) to inform him of the storage and retrieval services offered by these centers that may be helpful in solving his individual information problems.

Scientific Information Storage and Retrieval Centers

Defense Documentation Center (DDC). One of the largest centers in the United States for storage and retrieval of scientific information is the Defense Department's Defense Documentation Center for Scientific and Technical Information, formerly called the Armed Services Technical Information Agency (ASTIA). DDC has recently moved its headquarters from Arlington Hall Station, Arlington, Va., to Cameron Station, Alexandria, Va. The move will essentially double the Center's floor space and put all the Center's facilities on one floor and enable it to better serve its customers who last year sent in more than 1 million requests for documents.

The change in name to DDC reflects the greatly increased responsibilities given the Center by the Department of Defense (DOD). Most important, the name change provides an opportunity for a new approach in the handling of scientific and technical documents in the DOD.

DDC has five new objectives important to the nation's 400,000 defense related engineers and scientists that it serves: (1) Acquisition

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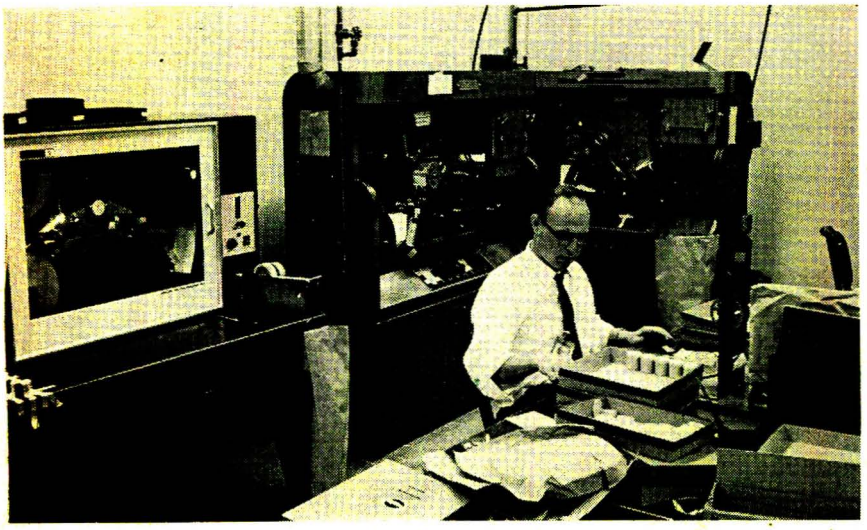

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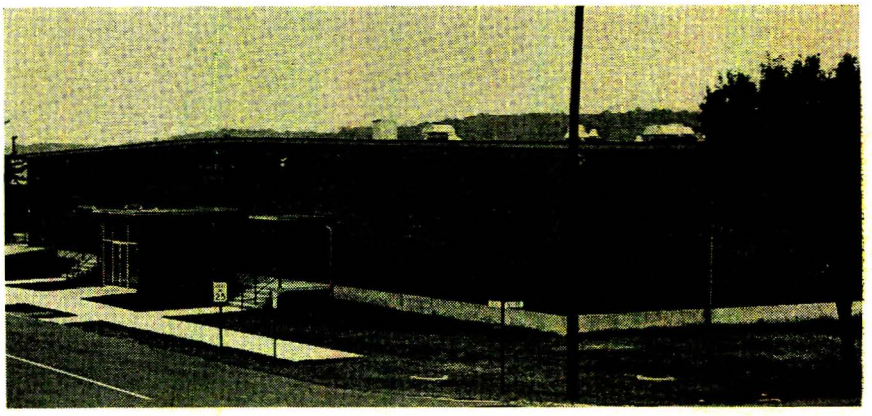


The Defense Documentation Center (DDC) for Scientific and Technical Information recently occupied new quarters at Cameron Station, Alexandria, Va. Army, Navy, Air Force, and contractor engineers and scientists make use of DDC's reference service

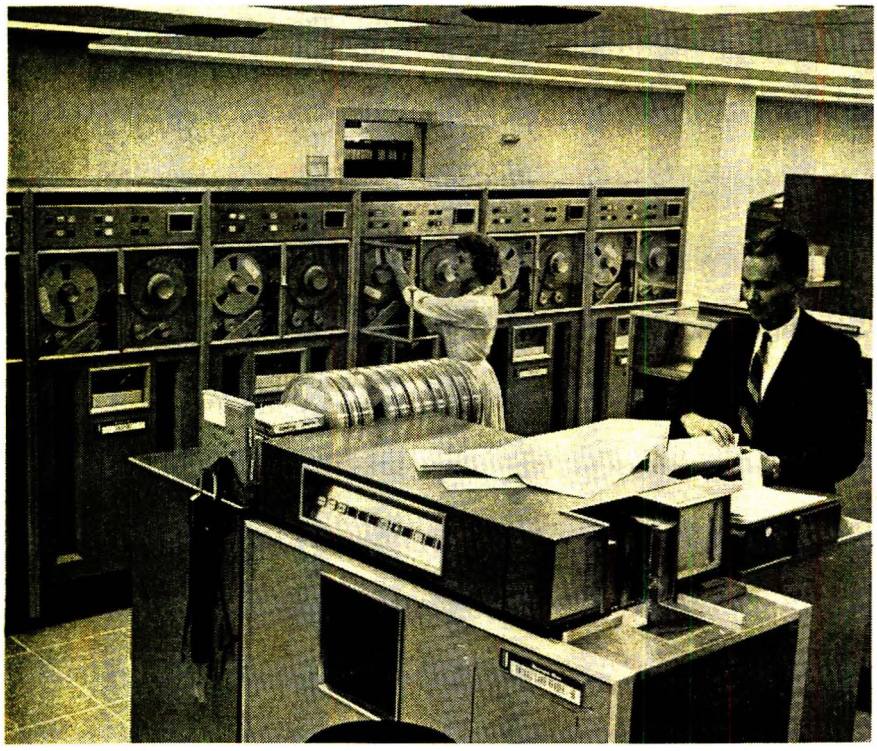
of all DOD scientific and technical documents, except small amounts in highly specialized fields, (2) Prompt and well-indexed announcements of newly acquired DOD scientific and technical information documents, (3) Timely dissemination of scientific and technical documents to the DOD community, (4) Maintenance of a clearing-house, in the form of an index and current research, development, test, and, evaluation programs, (5) Establishment of a centralized directory and referral service on available DOD-sponsored scientific and technical information activities.

The first requirement of a scientific and technical information system is to acquire within the limits of practicability all the documents of value to scientists and engineers within its area of responsibility whether it be mission-oriented, as serving the DOD or NASA's space mission, or subject-oriented such as the Atomic Energy Commission (AEC) in nuclear matters or the Army Plastics Technical Evaluation Center. Unless this comprehensive acquisition can be obtained, no engineer or scientist can be sure that the Documentation Center or Specialized Information and Evaluation Center will have the one document which contains the best information on the subject of his inquiry.

No one knows how many documents this will total. In the Department of Defense, DDC's best estimates indicate an immediate annual increase from the present 50,000 to at least 80,000.

DDC plans to speed document distribution by prepublication announcement which will, in effect, establish information of current work being performed in the laboratory or in the operational field efforts. Also, the Center is establishing a format and standards which will speed up the cataloging, indexing, and retrieval of a document when it reaches the Documentation Center.

Unless all engineers and scientists talk the same language, the efficient interchange of scientific and technical information, and particularly that based upon machine retrieval, will not be possible. Consequently, DDC is maintaining and improving a working vocabulary of terms for use in the processing of scientific and technical documents. Working with nearly one hundred organizations from the services, industry, and education, DDC has created a master thesaurus. This assignment is continually changing as science and



Tapes are checked on one of two servo tape units, which are part of the Univac Solid State 90 computers used by DDC. Automatic data processing equipment is used by the Center for document inventory control and need-to-know screening of requests for classified material, for making subject searches of the several hundred thousand documents in the collection, and for management information in the defense research, development, test, and engineering programs. Scheduled for operation in January 1964 is a third generation Univac 1107 thin-film memory computer that provides immediate access to 500 million alpha numeric characters on drums

technology progress and leave behind the words of outdated achievements and coin new words to describe their latest advance.

DDC has the responsibility of rapidly notifying DOD specialized information and evaluation centers of the availability of documents by maintaining profiles of their needs, supplying documents to them, receiving their documents for secondary distribution, maintaining a directory of all these centers and up-to-date information on their subject areas, and providing a referral service on the capabilities. Currently DOD supports directly or indirectly about fifty such centers.

DDC cooperates in the effective interchange of scientific and technical documents with other government documentation services and transmitting such documents to the federal agency designated by law or otherwise responsible for the dissemination of scientific and technical documents to the general public. With foreign governments, DDC works toward the objective of coordination of techniques with their documentation centers and standardizing and improving bibliographic techniques in accordance with established procedures.

Most important is the value to a sponsoring DOD activity such as the AEC of making full use of DDC services before authorizing an in-house project, task, or program, or before contracting outsiders. This is the critical point at which unnecessary duplication can be prevented before any time is lost even considering proposals. DDC will provide the means whereby the sponsored military activity can request and receive timely documents and information on what has been done, what is being done, and what other engineers and scientists are involved.

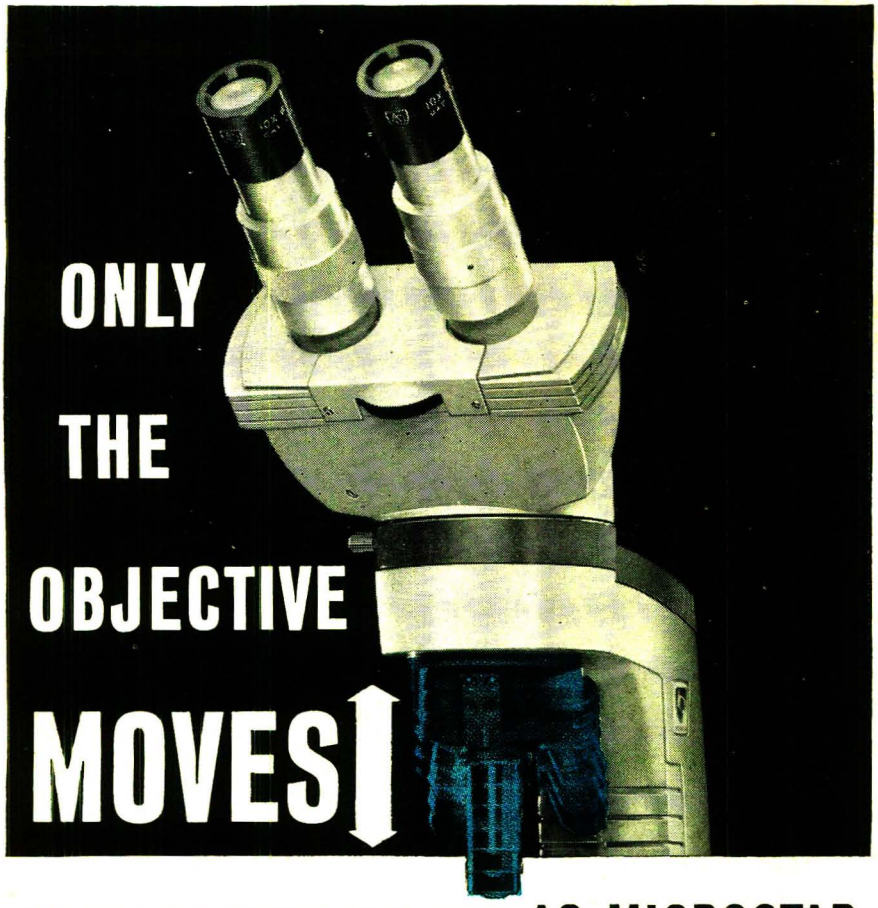
DDC publishes the well known Technical Abstract Bulletin (TAB). This semimonthly publication announces the acquisitions of DDC and is distributed to DOD activities, their contractors, and authorized federal agencies. All reports announced in TAB are available without charge for use in support of active military projects. Reports announced in the portion printed on white paper are those which may also be made available for sale to the general public through the Office of Technical Services (OTS), U. S. Department of Commerce.

The other announcement part of TAB, which is printed on buff paper, lists unclassified citations for the classified reports and those unclassified reports which, because of proprietary or other reproduction limitations cannot be made available for sale through OTS. Both parts of TAB are divided into 33 general subject areas as shown in Table I.

A large amount of automatic data processing equipment is used by DDC for document inventory control and need-to-know screening of requests for classified material, for making subject searches of the several hundred thousand documents in the collection, and for management information in the defense research, development, test, and engineering programs.

In the DDC photo facility, diazo microfilm copiers turn out one-thousand-foot rolls of 35-millimeter film by the hundreds to meet the needs of DDC users, to stock its Technical Operations Divisions in the field, and to provide microfilm copies of unclassified documents to various users. To convert from microfilm back to document size, DDC has one of the largest single installations of Xerox Copyflo printers in the country.

NASA's Scientific and Technical Information Facility. An information center widely used by scientists and engineers engaged in the nation's space program is being operated by the Office of Scientific and Technical Information of the



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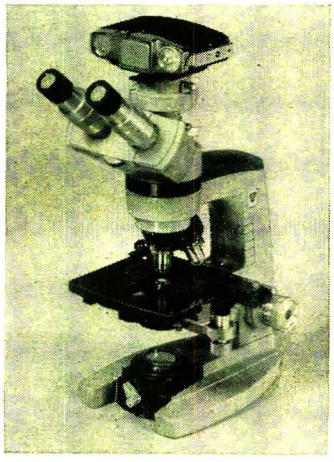

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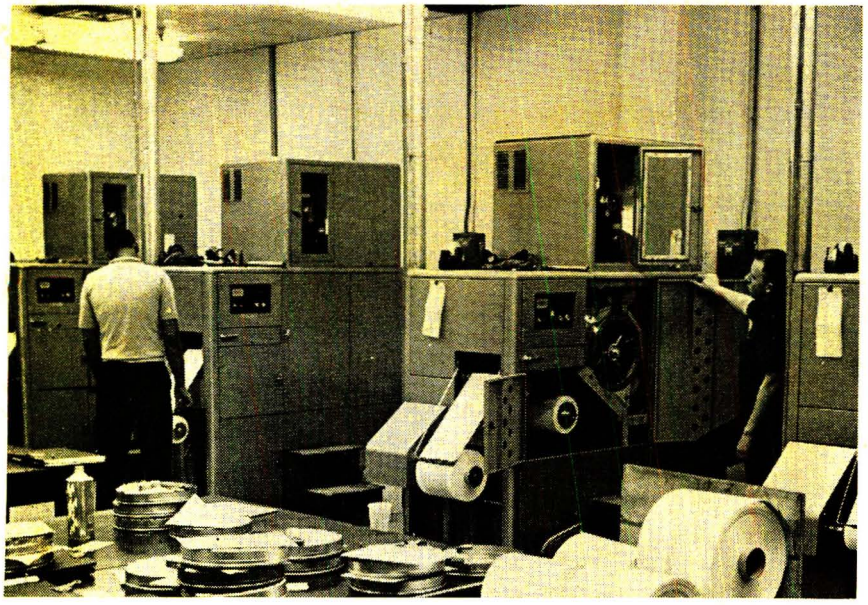
Table I. Subject Areas Covered in DDC's Technical Abstract Bulletin

Aircraft & Flight Equipment
Astronomy, Geophysics, & Geography
Chemical Warfare Equipment & Materials
Chemistry
Communications
Detection
Electrical Equipment
Electronics & Electronic Equipment
Fluid Mechanics
Fuels & Combustion
Ground Transportation Equipment
Guided Missiles
Installations & Construction
Materials (Non-Metallic)
Mathematics
Medical Sciences
Metallurgy
Military Sciences & Operations
Navigation
Nuclear Physics & Nuclear Chemistry
Nuclear Propulsion
Ordnance
Personnel & Training
Photography & Other Reproduction Processes
Physics
Production & Management
Propulsion Systems
Psychology & Human Engineering
Quartermaster Equipment & Supplies
Research & Research Equipment
Ships & Marine Equipment
Miscellaneous Arts & Sciences
Transportation

National Aeronautics and Space Administration (NASA). Its principal objectives are rapid announcement and dissemination of new scientific information of value to the many participants in the national space program. In order to achieve these objectives, it has established the Scientific and Technical Information Facility. The Facility consists of an organization of information specialists, employing advanced techniques and machines to acquire and organize information for prompt announcement, dissemination, storage, and efficient retrieval.

The Facility receives reports and papers from all segments of the scientific community, including NASA laboratories, contractors, universities, and other government and industrial research organizations.

Reports are cataloged at an early point in the operations. From the reports a group of specialists prepare the abstracts which serve



Machines at DDC shown are part of one of the largest single installations of Xerox Copyflo printers in the country. Documents preserved on microfilm are copied onto paper rolls at the rate of twenty feet of paper per minute. The rolls are then cut into page size and assembled as individual documents for release to the Army, Navy, Air Force, and their contractors working in research, development, tests, or evaluation projects

to inform the scientific community of the new information received. The same group of specialists also analyzes each report to extract as many as twenty to thirty subject retrieval terms for the published indexes and for machine access.

After the reports have been analyzed, they are microfilmed and made into a 5- by 8-inch microform from which many duplicates can be manufactured and disseminated to other NASA Centers.

In order to make the information received known to individuals and organizations participating in NASA programs, a biweekly announcement journal is prepared and disseminated. This journal, called the Scientific and Technical Aerospace Reports (STAR) contains abstracts of all items received within the five-to-six week interval prior to cover date and is provided with a set of computer compiled indexes. In addition, these indexes are compiled into quarterly, semiannual, and annual printed indexes.

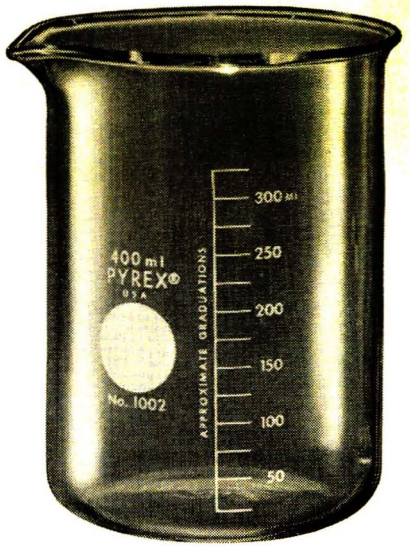
STAR consists of two major sections. The first section contains a complete bibliographic citation, including a unique accession number, and an informative abstract for each report announced.

The second section consists of a subject index, a corporate source index, a personal author index, a report number index, and accession number list for the items in each issue.

In the first section, the abstracts are prepared by photocomposition techniques in order to provide a high-quality readable information medium for informing the user of new developments of potential interest. The abstracts are grouped by some thirty major subject categories for effective assimilation of the new information.

The indexes in the second section are all compiled by computer in final camera-ready form in the course of the daily operations of the Facility. Each issue, then, contains indexes to its own contents for immediate detailed search.

Atomic Energy Commission (AEC). The Office of Technical Information of the U. S. Atomic Energy Commission (AEC) in Washington, D. C., is operating an information center which collects U. S. and foreign research reports, journal articles, patents, books, and translations on nuclear science and technology. The collection is used in the production of abstracts for,



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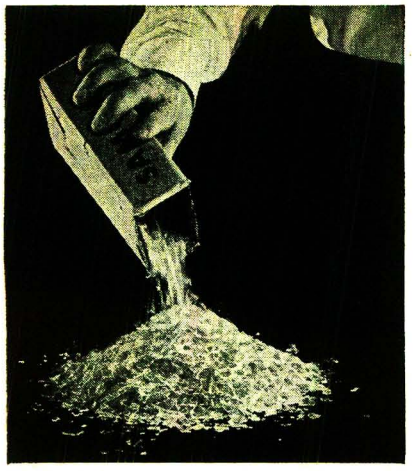

How We Were Embarrassed To Find We Had Overlooked The Real Importance Of A Product We've Been Making For Ten Years! Occasionally a company will develop a product that gets pushed aside because it's introduced with other products more glamorous—which get all the attention from the company's sales and advertising people. Such a product is usually dropped, eventually, because no one wants to bother with it. ■ We were considering a suggestion to drop our Piccolastic® Resin series a few months ago for the same reason. We checked into production records, though, and found to our amazement (and embarrassment) that we're selling millions of pounds of this resin without benefit of advertising, adequate technical data, or the slightest sales emphasis! ■ We checked into the applications our customers have for these resins and found many cases where the properties were uniquely adapted... where no other resin would do. The wide scope of these applications amazed us—from adhesives to electrical insulation. We apparently failed to realize just how versatile pure low molecular-weight styrene resin could be. ■ But we're mending our ways—fast. We are preparing a new Piccolastic catalog and making it the most complete set of data in the Picco library. Chances are you've never evaluated Piccolastic Resins yourself. They're tough, flexible, completely inert, widely compatible and light in color. Don't be embarrassed to find you have overlooked the importance of Piccolastic Resins too. Mail the coupon below for your free copy of the new Piccolastic Resins catalog. ■

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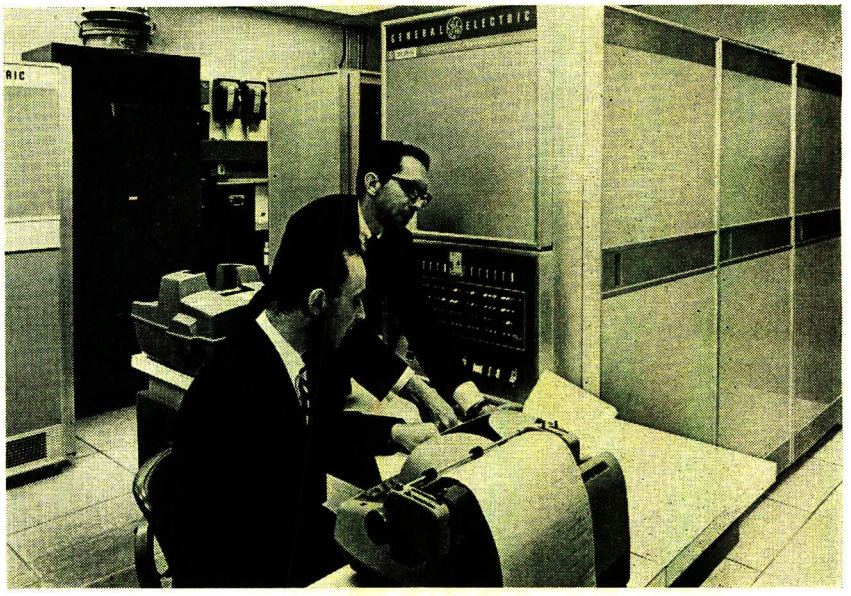

Preliminary search results produced by the GE 225 computer are examined by scientists at Western Reserve University's Center for Documentation and Communication Research. The Center's complete installation valued at approximately $300,000 includes the central processing unit, six high-speed tape-handling units, a high-speed printout, both paper tape and magnetic tape input, and complete keypunch and tabulating department

and indexes, to *Nuclear Science Abstracts* (*NSA*), AEC's semimonthly abstract journal. The Center serves the worldwide scientific community, including scientists and engineers engaged in basic and applied research, librarians, and documentalists.

The AEC's Office of Technical Information also publishes *Technical Progress Reviews,* quarterly; *Abstracts of Classified Reports,* semimonthly; *Classified Reports for Civilian Applications,* monthly; and *Abstracts of Weapon Test Reports,* quarterly.

Western Reserve University Center for Documentation and Communication Research. The Western Reserve University (WRU) Center for Documentation and Communication Research in Cleveland, Ohio, established eight years ago has done outstanding research in information retrieval by applying mathematics and computer technology to library science.

Several systems have been developed by the WRU Center for the magnetic-tape storage of indexes to vast quantities of literature which may be "searched" or scanned rapidly by means of an electronic computer.

In 1960 the WRU Center undertook studies for the American Society for Metals on the storage and retrieval of information related to metallurgy and established the Metals Documentation Service.

The Metals Documentation Service collected books, periodicals, external reports, and patents related to the subject. The subject content of the documents was analyzed, and conventional and "telegraphic" abstracts were prepared. After being edited and checked for quality control, the material was keypunched and verified. Abstracts were encoded automatically, and cards were converted to magnetic tape.

In searching the metallurgy information, analysis was done by subject-matter specialists; programming, by nonsubject-matter specialists. The search request was encoded, structured, and run on the computer. Each "hit" was evaluated by referring to information given in the "telegraphic" abstract, the conventional abstract, and when necessary the full document. Conventional abstracts considered to be suitable answers were pulled from the master file, reproduced, and mailed to the requester. The Metals Documentation Center served industry, government, colleges and universities, and profes-

sional societies, on a subscription basis.

Early in 1963 the operation of the Metals Documentation Service was transferred from the WRU Center to the American Society for Metals (ASM) at Novelty, Ohio. ASM has assumed responsibility for materials acquisition and selection, abstracting, editing, coding, question programming, evaluation, and various related clerical tasks. The WRU Center for Documentation and Communication Research with its tabulating and computer facilities will continue to act in a service capacity in processing searches.

The WRU Center has received an award of $138,000 from the National Institutes of Health to establish a "Facility for Health Sciences Documentation Research." The Center will apply its techniques to the language of medicine, and the new facility will include a laboratory equipped to compare and evaluate nonconventional library systems. The project will absorb and expand present medical documentation studies with the cooperation of the WRU School of Medicine faculty. It marks a change in direction of the WRU Documentation Center toward greatly expanded activity in the health sciences which is expected to represent one half of the Center's program by 1967.

Chemical Abstracts Service. The information storage and retrieval research performed by the American Chemical Society's Chemical Abstracts Service (CAS) is well known by scientists in this country. CAS at Columbus, Ohio, has been working for several years on storage and machine retrieval of chemical information with support from the National Science Foundation. For this work a modified IBM 1401 computer which has a 1403 printout in 120 characters has been installed and put into operation. It uses the CAS Character Set (Model 1) in which uppercase and lowercase letters; normal, subscript, and superscript numerals; and some Greek and italic letters are available, together with a wide range of conventional scientific signs such as brackets, parentheses, plus, degree, and equals. Any character or group of characters can be underlined. This

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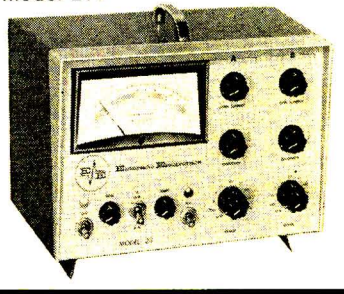

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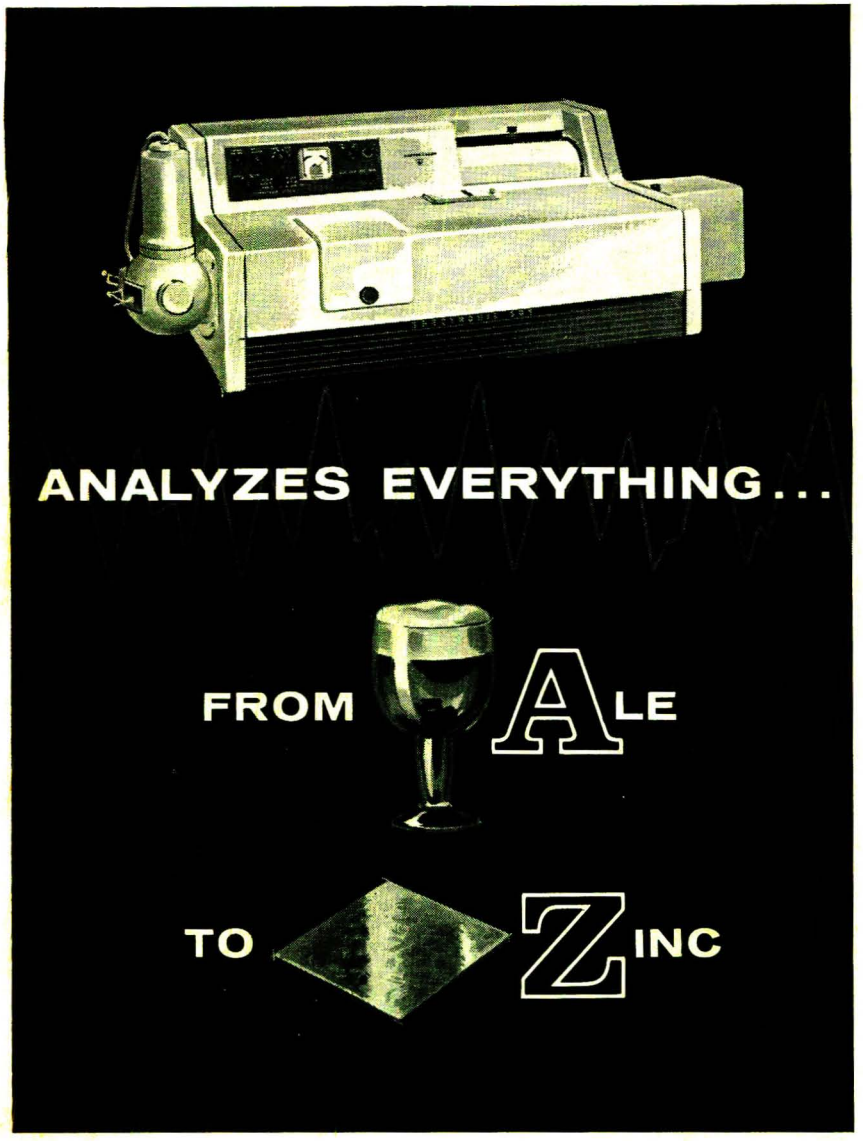

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computer has been programmed with the algorithms for detecting any given substructure in a molecule.

With the computer, CAS is studying the utilization of labor at a lower level of sophistication for the entry of notations from the supercollective index to the computer tapes. The supercollective file represents a "pre-editing" phase used prior to transfer to tape in order to decrease machine time used in searching.

Using the IBM computer CAS started publishing *Chemical Titles* in 1961. Now in its third year, this journal lists the latest titles received at CAS according to the Keyword-in-Context system and provides a quick approach to current chemical literature.

Now in the testing stage is a new journal, *Chemical-Biological Activities*, also prepared with Chemical Abstracts' IBM 1401 computer. An experimental issue of the journal was published in November 1962. *Chemical-Biological Activities* is an alerting system to guide the user quickly to recent literature dealing with the interrelation of chemical structure and biological activity. Relevant information, extracted from a range of the more significant primary journals in this field, is prepared for input by a group of document analysts. Each compound is defined in terms of its molecular formula, cipher (according to the official system of the International Union of Pure and Applied Chemistry), name, and CA Registry number. The information is intended to be indicative rather than specific, and includes the information from the primary publication given in Table II.

CAS plans to publish *Chemical-Biological Activities* every 14 days. It is expected that the price will be between $350 and $650 yearly, depending on the circulation that is attained.

Science Information Exchange. The Science Information Exchange of The Smithsonian Institution in Washington, D. C., now serves the entire scientific community. Originally established in 1950 to help federal research directors and administrators quickly exchange up-to-date information on their current

research activities, the Exchange currently has a staff of more than 30 scientists and specialists in life and physical sciences who review, classify, and index the resumes of more than 50,000 projects that are annually registered in the Exchange.

The Exchange receives resumes of current research projects on a voluntary basis from all available sources. Notice of new work is received long before it may appear in normal publication channels, and any research scientist or engineer, who is associated with a research institution, foundation, or laboratory may request and receive, without charge, up-to-date information on who is currently working on a specified topic, problem, or project.

At present, the Exchange collection is fairly comprehensive in the life sciences including almost 90% of all the basic and applied research sponsored or conducted by the federal agencies. In addition, more than 100 nongovernment foundations, universities, and state and city governments actively cooperate in furnishing records of their programs, and an annual growth rate of about 20% is being maintained.

Registration of basic and applied research in physical sciences began this year and is now being developed as fast as current research records can be identified and secured. In such areas as chemistry, materials, electronics, and earth sciences, useful information can be obtained already.

Table II. Information Given in Chemical–Biological Activities

Comparative activity
Type of drug
Action shown
Organ or isolated organ concerned
Disease or condition, and where applicable the pathogen giving rise to it
Operations
The stage of development, sex, and identity of the animal subject
The host, where subject is parasitic
The mode of administration
Side-effects and general comments
Metabolic products and the use of concomitant modifiers
The LD_{50}

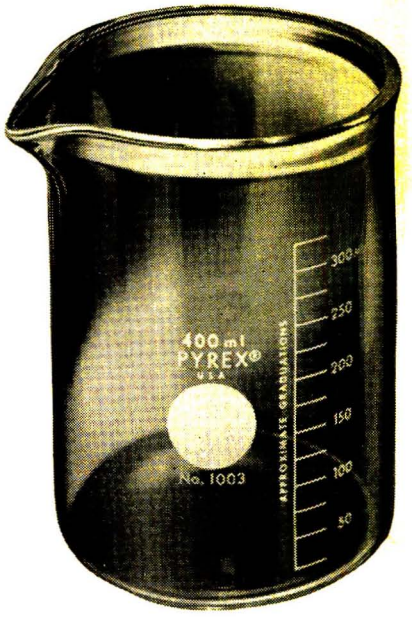
Data Storage and Retrieval Centers

Based upon recommendations of its Committee on Scientific Information, the Federal Council for Science and Technology is establishing a National Standard Reference Data System (NSRDS). This national effort will be administered by the National Bureau of Standards, which is already compiling standard data. The system integrates to a single point of responsibility the present data-compiling activities of the NBS, Department of Defense, AEC, NASA, the National Science Foundation, and several other agencies.

Standard reference data are defined by NBS as critically evaluated data on the physical and chemical properties of materials, authoritatively documented as to reliability, accuracy, and source. The average lag in time, however, between publication of the data in a journal and its ultimate appearance in a compilation is estimated to be about ten years, leaving about 2,000,000 papers in the backlog of unevaluated data sources. No single institution, relying solely on its own, in-house resources, could hope to solve the problem. This backlog of unevaluated data must be brought under control. And a sound, comprehensive experimental program must be a part of any program to compile and evaluate the existing data in the literature.

What is needed, then, is a comprehensive program which will result in; (1) the accumulation, in all fields of the physical sciences, of those quantitative data which have been determined by careful evaluation to be useful to the nation's scientists and engineers; (2) the critical evaluation of both the significance and reliability of the accumulated data; (3) creation of new knowledge in the form of correlations between existing data, interpolations and extrapolations to obtain new data, and determinations of the specific areas where further work is required.

The NSRDS Program. As envisioned by NBS the program will consist of three parts: an input from scientists in many different locations, a central source of the evaluated data, and an output system geared to the needs of the



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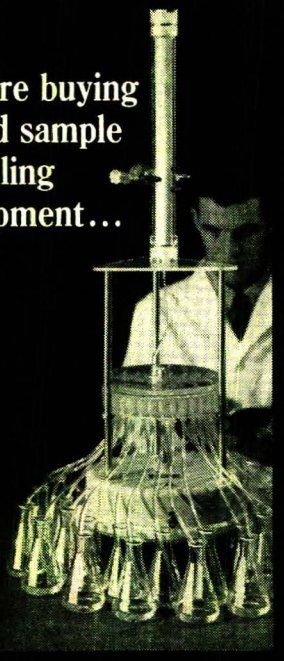

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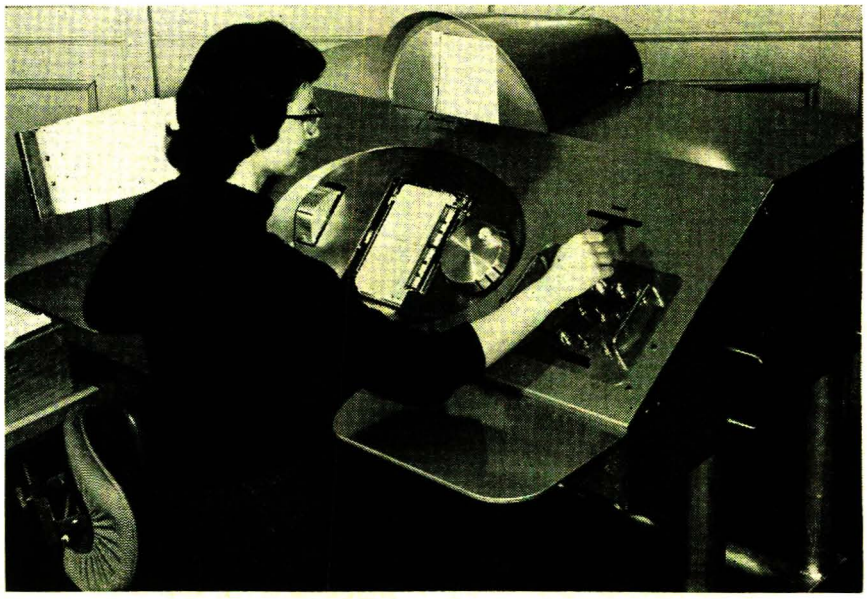


The microcite searching instrument at the National Bureau of Standards enables the operator to search a set of 18,000 document abstracts at one time. The instrument is used with a special punched-card peekaboo technique

nation's scientists and engineers.

Input. The input will come from scientists who are comprehensively reviewing the literature in their fields of specialization and critically evaluating the data for ultimate inclusion in the storehouse of standard reference data. These scientists may be in universities or in industrial or government laboratories. Some will be at NBS, others will be under contract to the Bureau. They will work singly or in small groups oriented to the traditional scientific disciplines.

At the same time other scientists will be engaged in experimentally determining the standard reference data that do not exist in the literature. Again, these scientists may be at the National Bureau of Standards, or they may be performing their work under contracts from the Bureau. Clearly, the interplay between the two groups must be close and continuous.

Central Core. The central core will consist of a Standard Reference Data Center at NBS where the evaluated data will be located, in punched cards, on magnetic tape, in notebooks, in many other forms, all mechanized for storage and retrieval. A review and control office will label the incoming data as to relative quality and reliability. The Center will classify the data into as many major and minor categories as are required by the needs of the data users.

Output. The output will take the form of a series of services aimed at different technical levels and tailored to the needs of various segments of industry. In general, it will be oriented toward the application of the data, rather than toward a field of science. The output services will include: (1) *Periodical Service* designed to keep the user up to date on new data acquisitions in the Center. It will provide information on the data available in the Center (but will not provide the data itself) by means of a monthly newsletter and by annual and semiannual reviews of data acquisitions; (2) *Subscription Service* in which the user pays to receive all available data on a specific subject on a continuing basis. These data packages will be designed to meet the needs of specific industries, industry groups, or Government research and development programs; (3) *Referral Service* which will handle narrow, one-time requests for data by referral to the files of the Standard Reference Data Center. In general, this service will take care of needs that are not met by the other output services; (4) *Correlation and Prediction Service* for computing values in areas where some data exist, but where requests come in for specific

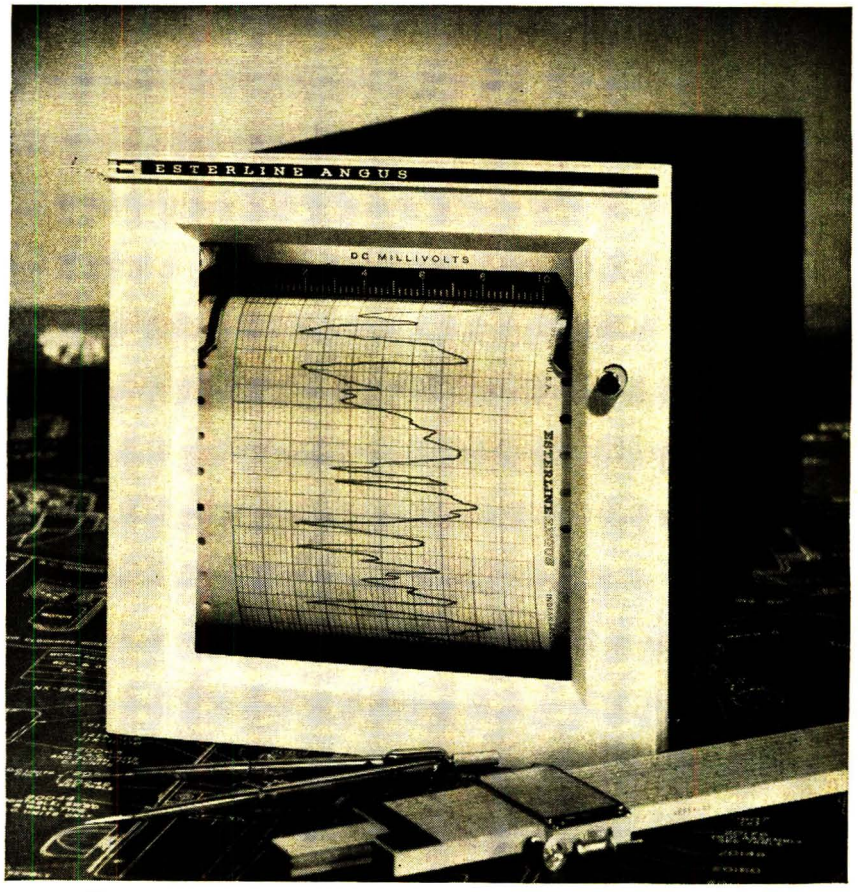
information not contained in the center. Values will be computed by making use of correlations based on molecular structure and the properties of related compounds; (5) *Mathematical and Statistical Service* which will offer mathematical and computer techniques to customers for evaluating new data for subsequent inclusion in the files of the SRD Center or for individual use. It will also provide techniques to assist in the Prediction and Correlation Service; (6) *Aperiodical Products* including tabulations, review monographs, review papers, computer card decks, and computer tapes. These will constitute the formal end products of the Center; (7) *Summary Reviews* to provide a rapid assessment of the state-of-the-art in fields where there are few data but which must suddenly be explored because of scientific breakthroughs or crash programs.

It is estimated that an ultimate level of perhaps $10 million per year will be required to operate the program. The fiscal 1963 budget of NBS includes $810,000 for data compilation activities, all of which are now being conducted in house. NBS proposes that this figure be increased to $2,280,000 in 1964 to provide support for the new program, and to $4,087,000 in 1965.

Office of Critical Tables. Among the groups who will play a prominent role in the operation of the NSRDS is the Office of Critical Tables (OCT), located in the American Chemical Society Building, Washington, D. C.

Supported by the National Academy of Sciences–National Research Council, OCT has the following aims: to survey the needs for critical tables of numerical data, to stimulate and coordinate the preparation of critical tables, to develop standards for the presentation of numerical data, and to provide a central index of critical numerical data. Necessary first steps in the achievement of these aims are the identification of all continuing projects now in operation that compile and publish numerical data and an analysis of the quality of data being compiled by these projects.

Accordingly, OCT has issued a directory of projects that compile and publish the numerical data of



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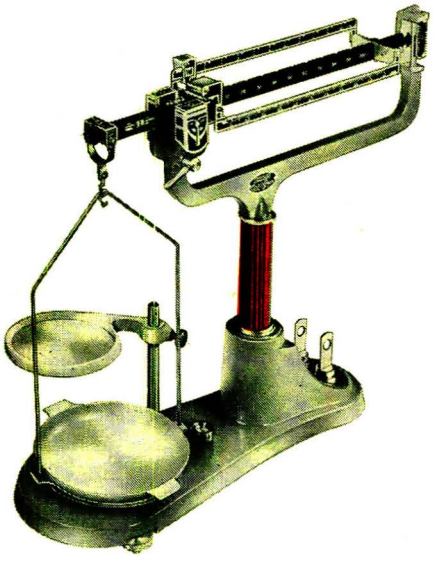

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science and technology on a continuing basis. (Directory of Continuing Numerical Data Projects, Publication 837, National Academy of Sciences–National Research Council, Washington, D. C., 1961.)

The general categories of projects in this directory are as follows: (1) Physicochemical Projects (of which the greatest number deal with chemical thermodynamic properties), (2) Crystallographic and Mineralogic Projects, (3) Nuclear Physics Projects, (4) Thermophysical Projects (thermodynamic and transport properties involved in physical change), (5) Spectroscopic Projects, and (6) Comprehensive Projects. Spectroscopic Projects are subdivided into those that deal with atomic, infrared, electronic, and other types of spectra.

The Office of Critical Tables is currently developing a central index which will show the specific contents of all of those publications that meet quality standards. The Office has recently issued an index that is limited to the contents of six important physicochemical and thermodynamic compilations. The six compilations chosen were representative examples of critically evaluated data compilations of good quality.

The OCT Consolidated Index has four principal purposes. First, it will lead the user to publications of reliable, selected property values for well characterized substances. Second, gaps in knowledge and undesirable overlap of projects are revealed. Third, a codified central index to numerical data will provide a basis for varied mechanical search and storage systems. Fourth, when it can be brought to a state of currency for all available publications of good quality in the physical sciences and engineering, such an index will give a measure of coherence to the continuing data projects that serve science by producing tables that contain critically evaluated data.

For the near future, the OCT effort will be (1) to keep the index current for fields already covered, and (2) to incorporate the contents of other available compilations in fields such as nuclear science, spectroscopy, and crystallography. For

the long term, the contents of new critical compilations must be included as they become productive parts of the total compilation effort. The ultimate desires are a prompt evaluation of all numerical property values appearing in the primary literature, their incorporation in critical compilations, and their indexing in the Consolidated Index.

Centers for Specific Subject Areas

Purdue University Center. Purdue University's Thermophysical Properties Research Center (TPRC) is one of a large number of scientific information centers that concentrate on a specialized subject area. Started in January 1957, with the financial support of a number of industrial and governmental organizations, TPRC separated from the University's School of Mechanical Engineering in November 1962, and was established as a new unit of the Schools of Engineering.

In the summer of 1963, TPRC was relocated in its new building at McClure Park, the University's industrial research park, one and a half miles north of the campus. Costing about $500,000, fully equipped, the new building provides three times the space TPRC formerly occupied, allowing room for needed expansion. The new building increases the total research area being used by TPRC to approximately 18,000 square feet.

Objectives of TPRC are "to become a world center for research and the collection, analysis, correlation, and dissemination of thermophysical properties information and, as such, to serve education, science, and technology through a better knowledge of this area."

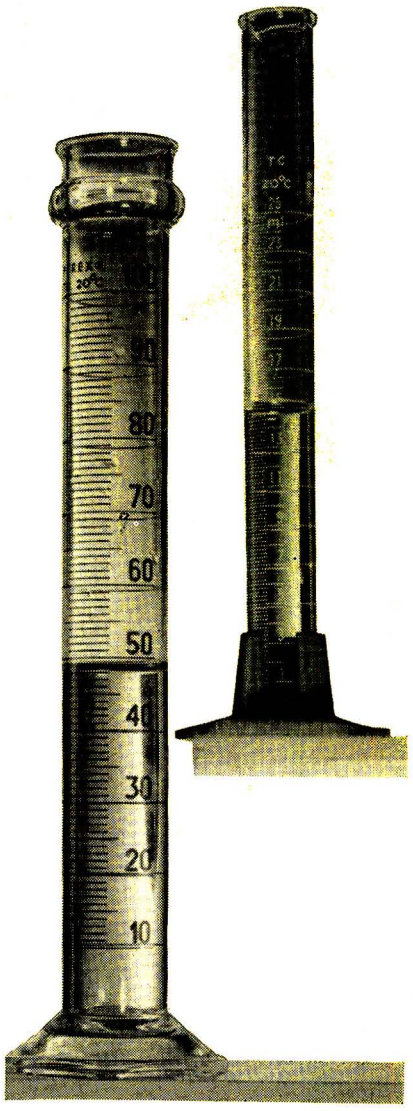
The Center's three major areas of activity are:

- Scientific Documentation
- Critical Tables of Properties
- Experimental and Theoretical Research

TPRC's activities in the scientific documentation area became well known through the publication in December 1960 of Volume I of the "Retrieval Guide to Thermophysical Properties Research Literature." One of the main aims of the Retrieval Guide is to reach the ultimate in comprehensiveness of cov-

Table III. Thermophysical Properties Covered by Purdue's Thermophysical Properties Research Center in Its Retrieval Guide

Property Group	Number of Properties	Property Names
1	2	Thermal conductivity, accommodation coefficient
2	1	Specific heat at constant pressure
3	2	Viscosity, fluidity
4	4	Emissivity, absorptivity, reflectivity, transmissivity (total and spectral)
5	2	Diffusion coefficient, permeability
6	1	Thermal diffusivity
7	1	Prandtl number
8	3	Coefficient of thermal expansion (linear, superficial, cubical)
9	1	Surface tension
10	1	Thermal diffusion coefficient
11	1	Specific heat at constant volume
12	3	Thermoelectric constants (Seebeck, Peltier, Thomson coefficient)
13	2	Electrical resistance, ionic mobility
14	2	Solubility, miscibility
15	1	Velocity of sound
16	8	Temperatures and heats of sublimation, fusion, vaporization, transition
17	1	Vapor pressure
18	7	Critical constants, equation of state data, virial coefficient, compressibility, Joule-Thomson coefficient
19	3	Enthalpy, internal energy, entropy
20	1	Density



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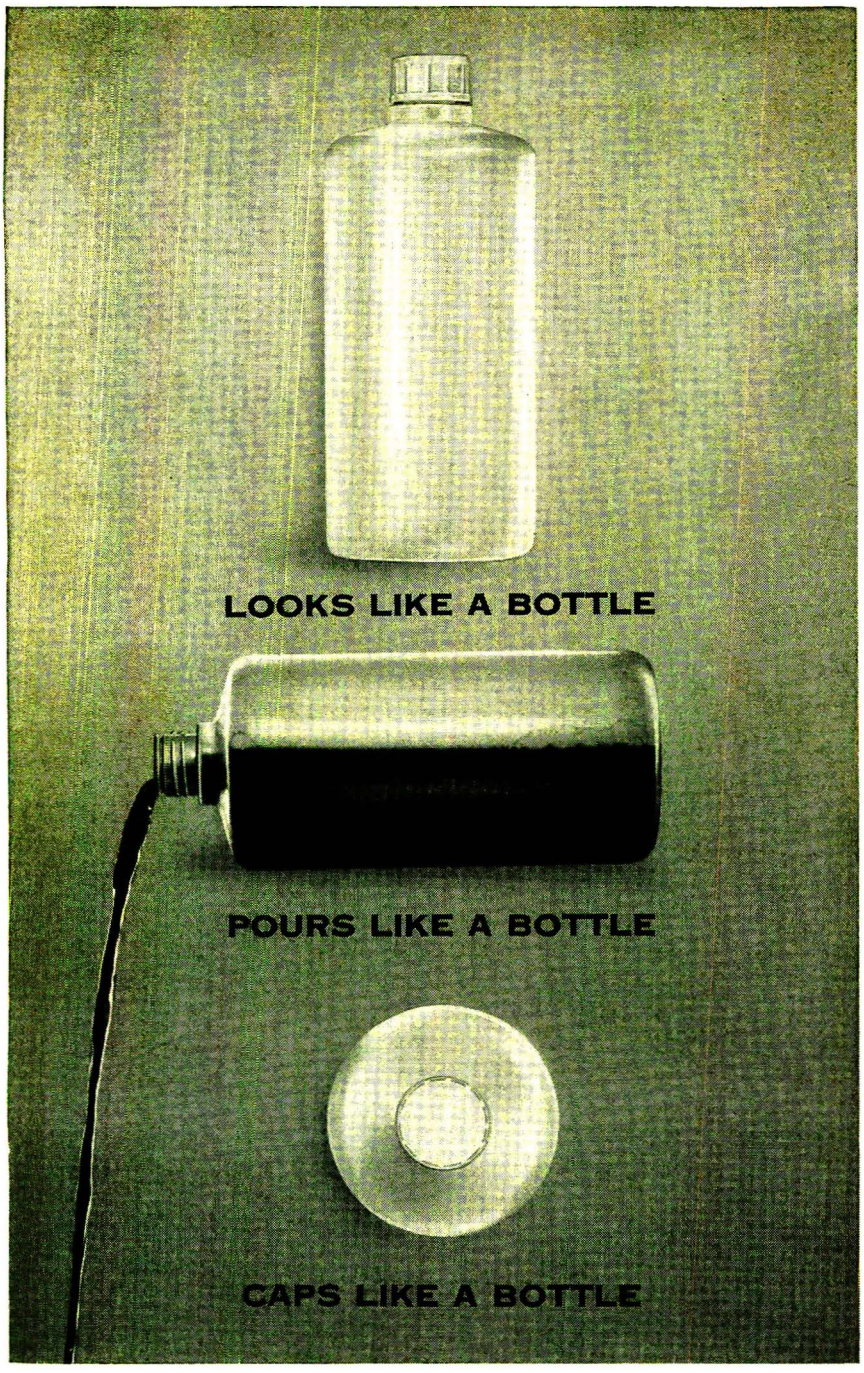

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erage of thermophysical properties information. Volume II of the Retrieval, which was published early in 1963, brought the total coverage of this work to 20,000 research papers covering the world literature contributed by approximately 19,940 authors published in over 2420 journals. Nearly 30,000 substances were reported in some 81,300 reference entries, covering the initial thirteen of a contemplated forty-seven thermophysical properties. The coverage includes the open literature, government reports, academic dissertations, and, to a limited degree, reports of private and industrial organizations.

Subsequent volumes of the Retrieval Guide will be published approximately every eighteen months, each volume adding to the previous ones an additional 10,000 or more research papers on thermophysical properties. With the publication of Volume III, it is anticipated that nearly all of the world literature of the first seven groups of thirteen properties, covering all matter, will have been reported in highly organized form for all literature back to the year 1920. The properties presently covered and those contemplated beginning with Volume IV, are shown in Table III.

TPRC also publishes each year "Masters Theses in the Pure and Applied Sciences," which represents the most complete listing of Masters' Theses of over 180 United States colleges and universities.

In the area of critical tables of properties TPRC's aim is to bring about critical evaluation, analysis, correlation, as well as new calculations of data on a continuing basis. TPRC's activities in this area (1) ensure the preservation of the results of basic and applied research, (2) shorten the time for research results in finding their place in design, (3) establish a "feedback" into basic science, and (4) provide the key ingredient in avoiding costly mistakes and duplications in useless hardware development.

TPRC is working on three data books. They are: Data Book, Volume 1: Metallic Elements and their Alloys (gaseous, liquid, and solid states);

Data Book, Volume 2: Nonmetallic Elements and their Com-

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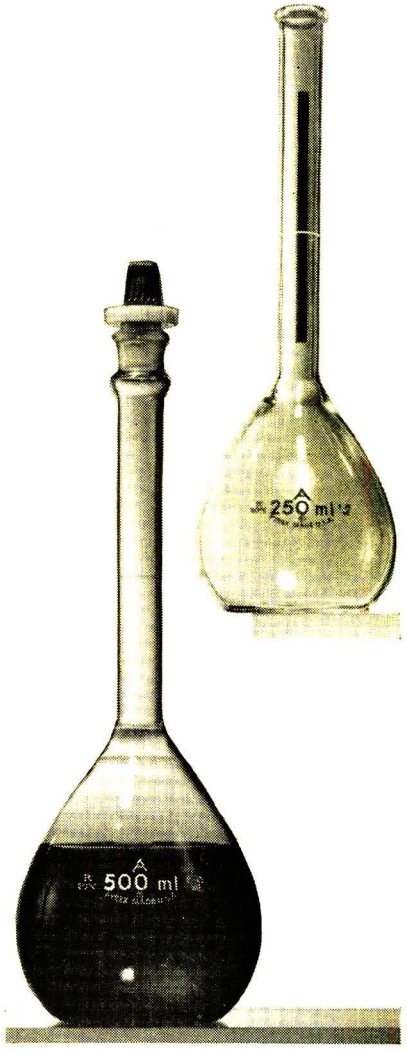

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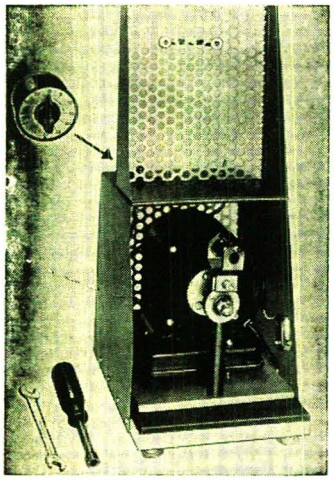

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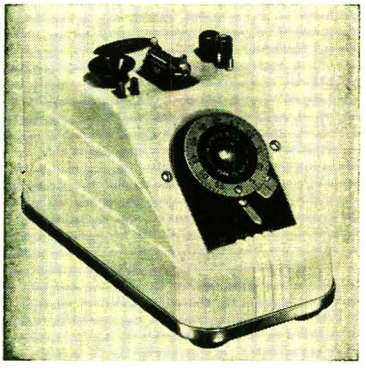
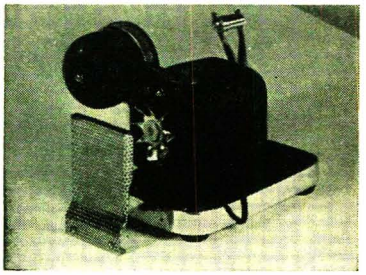
Stainless Steel Vials 6 ml or 10 ml and Rod Pestles or Ball Pestles. Tungsten Carbide 6 ml and 25 ml Vials and Rod or Ball Pestle.

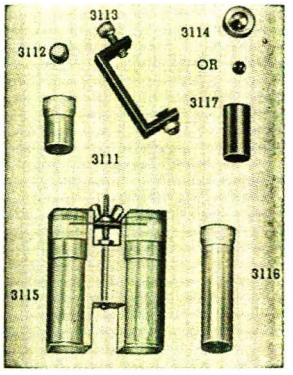
For literature and details write to:



5A—Wonder Electric Mortar—the action is reciprocating in the form of a figure 8 swung through a 6 1/2° arc at 3200 RPM, the ball pestle then strikes the end of the vial some 2000 times in ten seconds. Can be run continuously for 5 minutes (and this depends on the load) with a rest period between the 5 minutes to cool the motor completely



3A—Model with Fan and 1 Hour Timer—same type of motor and action as 5A Model but this 3A can be run for 20–30 minutes with cooling off periods between to cool the motor. $96.50 115V-60c. FOB factory.



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3113 Adapter, for 1/2" dia. x 1" long vials.
3111 Vial, 1/2" x 1" polystyrene with cap.
3112 Ball-pestle, clear plexiglas, 3/4" dia.
3115 Adapter for 1/2" dia. x 2" long vials.
3116 Vial, 1/2" x 2" polystyrene with cap.
3113 Adapter for 1/2" dia. x 1" long vials.
3114 Vial, stainless steel 1/2" x 1" with ball-pestle 1/4" dia.
3117 Vial, tool steel (hardened) 1/2" x 1" with steel ball-pestle 1/4" dia.
3118 Vial, agate 9/14" dia. x 15/16" long 2 ml capacity with agate ball-pestle 1/4" dia. (Requires 3115 adapter.)

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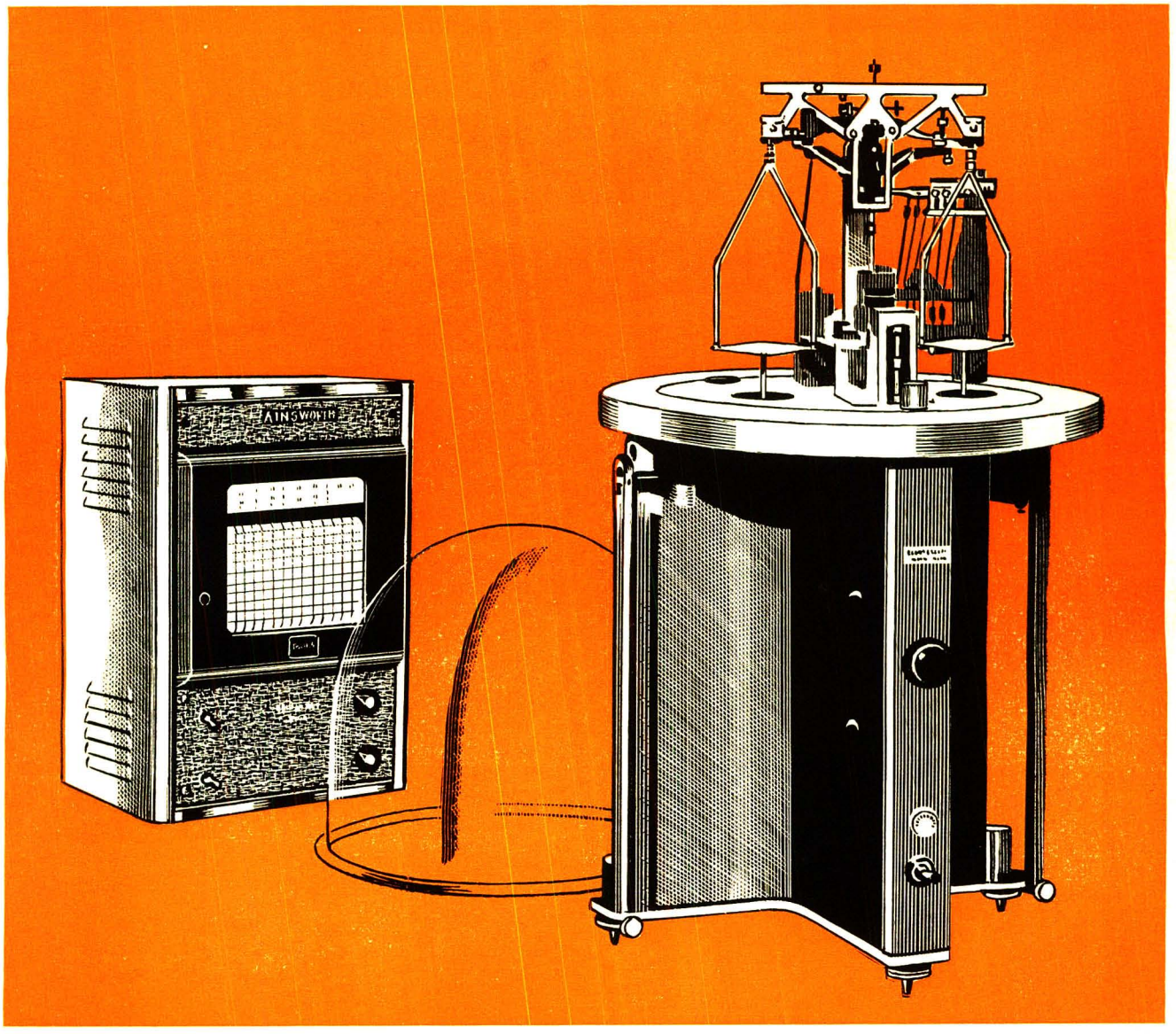

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A discussion of OPIUM and EUROPIUM and hybridized orbitals

which merely reviews well known material

but which seems exciting enough to the type of personnel employed at writing advertising copy for large corporations

The core of the message, to which all the rest is but elaboration, is simply that

DPi

DISTILLATION PRODUCTS INDUSTRIES, Rochester 3, N. Y. (Division of Eastman Kodak Company) stands ready, willing, able, and anxious to supply professionally prepared persons with any of a vast array of compounds, as detailed in "Eastman Organic Chemicals List No. 43," obtainable on request.

And now for the opium. Many years ago it was a practice, when investigating the possibility that a victim had been drugged, to test the contents of suspicious vials with Marme's Reagent. This threw down a yellowish-white precipitate with salts of alkaloids.

Papaver somniferum, the opium poppy

Opium was known to be rich in alkaloids. They were defined as organic substances of alkaline nature. As time went on, fences were erected against this sweeping generality. Not mere alkalinity *per se* but precipitation by certain inorganic "alkaloidal reagents" was required to qualify as an alkaloid. (Concepts can shrink by swallowing their tails.) One such was apparently devised by somebody named Marme from KI and CdI_2. We doubt he knew why a precipitate formed by this concoction correlates with the dream world. (Neither do we, but we have to appear more knowledgeable.)

When a request for Marme's Reagent was received recently, we consulted an old chemical dictionary to find out what it was. Then we put a 1963 orientation on the matter.

In 1963 chemists can't afford to make their own reagents unless they happen to be in the reagents business, which we are. So we made Marme's Reagent, but we don't think of it that way. We offer it as a dry compound named *Potassium Iodocadmate* (EASTMAN 8814). We think of it not as $CdI_2 \cdot 2KI$ but as $K_2^+[CdI_4]^=$. We see that cadmium ion with its four hybridized orbitals (from the 5s and the three 5p orbitals) coordinating to the four iodides, and we see that $[CdI_4]^=$ ion forming its precipitate in response to the electron-hunger of the heterocyclic nitrogen that gives the "alkaloids" their alkalinity. We look around a little and note that while Marme's name is little heard any more, his ion is still talked about for identifying amines, imines, and heterocyclic N's in general, judging from an excellent review of chelatometric quantitative organic analysis in *Chimie Analytique*, *43*, 449 (1961). We also note that EASTMAN Organic Chemical No. 8814 is not very organic, but it does not upset us.

Wherever we look we see those hybridized orbitals that explain coordination chemistry that underlies almost all colorimetric analytical methods that people still ask us in ever-increasing numbers to help them out with, fortunately.

"Would your firm be interested in adding zinc dibenzyldithiocarbamate, in a grade suitable for use as an analytical reagent, to your list of organic chemicals?" asked a recent letter from an ASTM committee member. This salt provides the alternative method for copper determination in rubber, he went on, and while it is faster, more sensitive, and less subject to interference than the referee method, it remains the alternative method because in the form found on the industrial chemical market it leads to high blanks and slow extraction of copper. Of course we are interested and indeed offer it in List No. 43 as *Dibenzyldithiocarbamic Acid Zinc Salt* (EASTMAN 7308) instead of under "Z," which may be a pity. The reagent for the referee method happens to be *Diethyldithiocarbamic Acid Sodium Salt*, which also happens to be an EASTMAN Organic Chemical, No. 2596.

Having mentioned zinc and cadmium compounds as reagents, we can reverse the theme and offer a reagent *for* zinc and cadmium: *N-8-Quinolyl-p-toluenesulfonamide* (EASTMAN 8882). U.S.S.R. Patent No. 102,029 tells about the fluorescent chelate formed at pH 8.0 - 8.3 and how sensitive the method is and all that, but there is a lot more to be said these days about fluorescent chelates than that they are useful for the determination of some metal or other.

Much, much more than we have space or inclination for. Let us merely record that

1) it has not escaped the notice of the Kodak Research Laboratories (which are strongly optically oriented) that the rare earth ions in general and Eu^{+3} in particular, exhibit very sharp fluorescence lines because the 4f subshell involved is well shielded by the 5s and 5p subshells

2) this fluorescence leaves the ion in a state from which it drops so quickly to ground state that the jump-terminating level remains thinly populated

3) here is the kind of setup wherein the stimulated emission of laser action can compete successfully against the spontaneous transitions of fluorescence and absorption

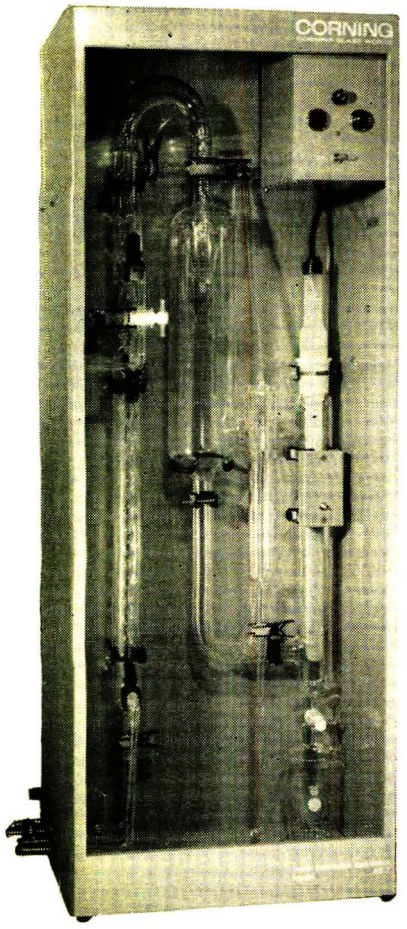
4) the lifetime of the upper state is not too short to achieve a good inversion and not too long to retain the inversion while waiting for action to commence

5) chelating the rare earth ions shields them from influences that might prematurely discharge the inversion

6) the ligand of the chelate absorbs over a broad u-v band and passes the energy along from its own metastable triplet states to excite the 4f levels of the rare earth ion

7) *Tris(1-phenyl-1, 3-butanediono) europium* is now EASTMAN 8989, *Tris[4,4,4-trifluoro-1-(2-thienyl)-1,3-butanediono]europium* is EASTMAN 8990, and *Tris(1-phenyl-1,3-butanediono)cerium* is EASTMAN 8629

and 8) anybody finding himself in a state of personal excitation over any ligand-lanthanide combination (except Pm) has but to name it and he may find we have a little on hand.



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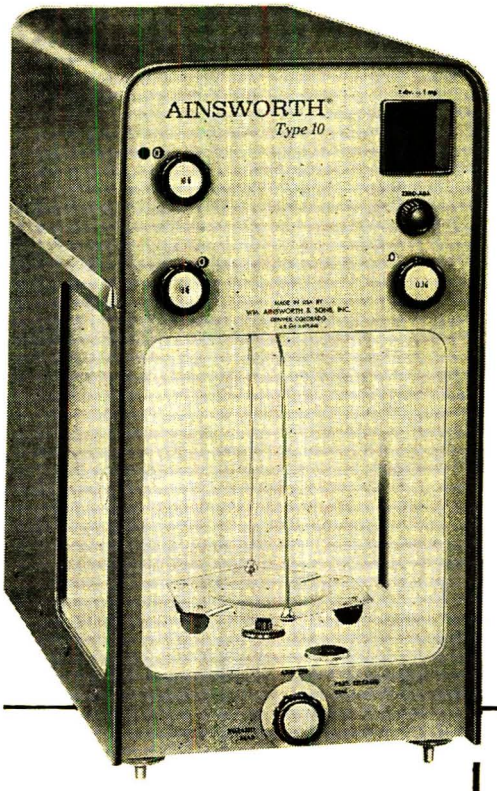

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REPRODUCIBILITY	±0.03 mg.	±0.01 mg.

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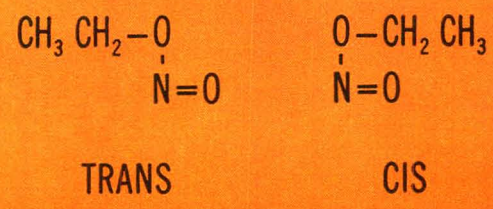
NO. 7 A-60 AT WORK SERIES

Example #5 of this series illustrated the value of high temperature operation of the A-60 Spectrometer in the study of polymer chemistry. Low temperature operation is also desirable in many cases, particularly where hindered internal molecular motions are of interest.

ETHYL NITRITE



TRANS CIS

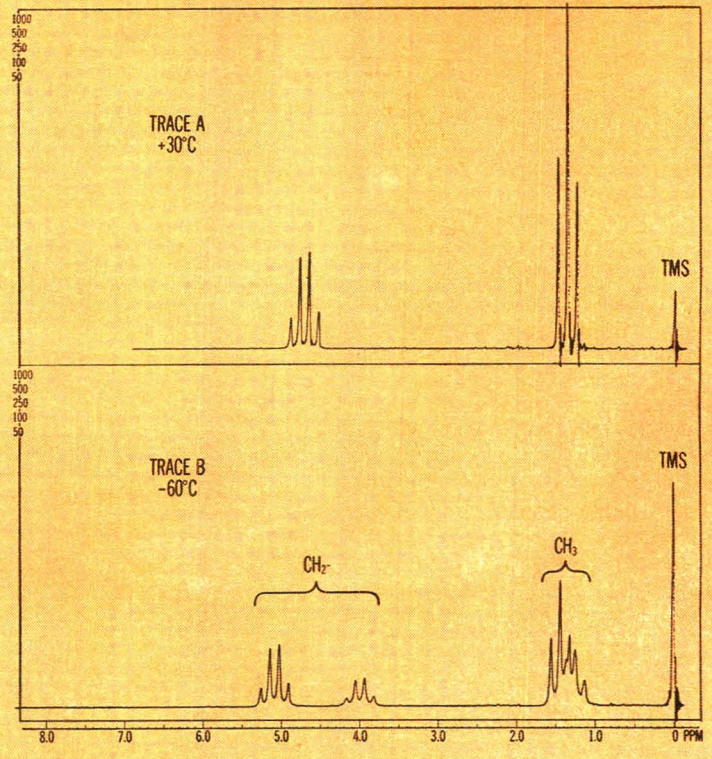
A typical example of the use of NMR in the study of hindered rotation is given by the case of the alkyl nitrites which may exist in two forms, **cis** and **trans**, as shown in the figure for ethyl nitrite. At room temperature the number of interconversions per second taking place between the two forms is large compared to the chemical shift expressed in cycles per second, between the ethyl groups of each form. Under this condition only a single typical ethyl resonance is observed as shown in trace A.

NMR IN THE WORLD OF CHEMISTRY

As the temperature is lowered, however, striking changes in the spectrum occur, as the interconversion rate decreases to the point that the spectra of the two forms are individually observable as shown in trace B which was taken at −60°C. Here we observe two methylene resonances at $\delta=4.0$ and $\delta=5.1$ and two methyl resonances at $\delta=1.2$ and $\delta=1.5$, corresponding to the two forms. At intermediate temperatures the two spectra broaden and merge together. A careful study of the line width and frequency separation of the two spectra as a function of the temperature permits the potential energy barrier to the interconversion to be determined.[1] Other examples of hindered rotation are found in the study of amides, nitrosamines, aldoximes and substituted ethanes.[2]

1. L. H. Piette and W. A. Anderson, J. Chem. Phys. **30**, 899, (1959).

2. J. A. Pople, W. G. Schneider and H. J. Bernstein, High Resolution Nuclear Magnetic Resonance, Chapter 13, McGraw Hill (1959).



Accessories such as the Model V-6040 Variable Temperature Controller (−60 to +200°C) used for experiments like the one above make the Varian A-60 Spectrometer useful for a wide range of research as well as routine analytical applications.

This versatility, coupled with the fact that the A-60 is fast, non-destructive, and simple to operate, make it a practical instrument for use in universities, government and industrial plants and laboratories. For more information, please write the Instrument Division. In Europe, contact Varian A. G., Zug, Switzerland.

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DEAE-Sephadex a major development in Ion Exchangers

Introduction of ionic groups into SEPHADEX makes possible ion exchange materials with high capacity and low nonspecific adsorption. SEPHADEX, a hydrophilic insoluble substance, is made by cross-linking the polysaccharide dextran. Both dextran and SEPHADEX were developed by Pharmacia in Uppsala, Sweden.

DEAE-SEPHADEX, containing the active radical diethylaminoethyl, is the first commercially available ion exchanger derived from dextran. DEAE-SEPHADEX is anionic, medium basic, and has a strong affinity for water. The swelling is proportionate to the degree of cross-linkage, the counterions, and ionic composition of the surrounding medium.

DEAE-SEPHADEX has many unique characteristics. Its stability in many mediums – water, weak acids, saline and alkaline solutions – offers many application opportunities. The gel is affected only by strong acids at elevated temperatures and strong oxidizing agents. DEAE-SEPHADEX is especially suitable for labile compounds.

DEAE-SEPHADEX is available in two types with different porosities: A-25 and A-50. A-25 is highly cross-linked and has a high capacity for molecules smaller than 10,000 in molecular weight. Ionic groups in A-50 are accessible to much larger molecules. A-50 has far greater binding capacity than A-25 for the larger molecules. Each has a total exchange capacity 3-4 meq/g.

Illustration of Relative Capacities for Various Proteins

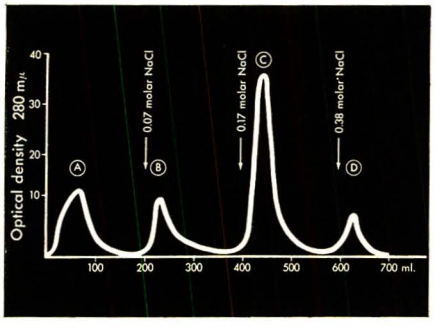
PROTEINS	DEAE-SEPHADEX	
	A-25	A-50
Pepsin at pH 3.6	0.2 g./g.	1.6 g./g.
Amylase at pH 8.3	0.3 g./g.	2.4 g./g.
Ovalbumin at pH 7.6	0.2 g./g.	1.2 g./g.
Hemoglobin at pH 8.8	0.4 g./g.	1.4 g./g.

APPLICATION

DEAE-SEPHADEX may be used in column and batch operations. Chromatographic fractionations are preferably made with Medium and Fine grades. The Coarse grade is more suitable for separations in batches. The particle size of these grades are Coarse (approx. 50-140 mesh), Medium (approx. 100-270 mesh), and Fine (approx. 200-400 mesh).

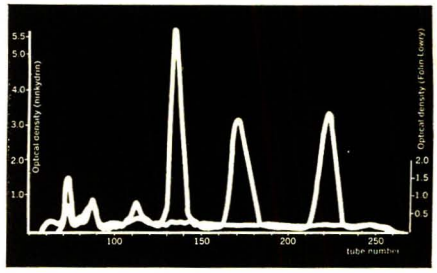
Purification, separation, and fractionation in organic and biochemical research and production processes achieve substantial yields with selection of the proper DEAE-SEPHADEX. A variety of problems in protein chemistry can be solved, e.g., purification of enzymes. Easy fractionations and high yields of serum proteins are possible with DEAE-SEPHADEX A-50. This is illustrated by Figure I.

Separation of Normal Human Serum
Fig. I



Nucleic acids, nucleotides, and a wide range of oligonucleotides can be readily bound and separated by fractional elution. Separation of polypeptide mixtures is possible by fractionating through ion exchange and ion exclusion. This is exemplified by Figure II.

Fractionation of Pituitary Hormones
Fig. II



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For separation and fractionation of complex organic substances and protein groups, Pharmacia has developed two new cationic SEPHADEX compounds which are dextran derivatives. Sulfoethyl is the active group for SE-SEPHADEX and is strongly acidic. The active radical for the weakly acidic CM-SEPHADEX is carboxymethyl.

SE-SEPHADEX and CM-SEPHADEX are prepared in two forms. C-25, which is ideal for separating low molecular weight complex organic substances, and C-50, which has proved very satisfactory for purification of proteins, enzymes, and related nitrogenous compounds.

SE-SEPHADEX and CM-SEPHADEX cover a broad range in total exchange capacity, 2-2.5 meq/g and 4-5 meq/g respectively. Both products are available in the following sieve fractions: Coarse (approx. 50-140 mesh), Medium (approx. 100-270 mesh), and Fine (approx. 200-400 mesh).

For complete information including latest developments on SEPHADEX *and* ION EXCHANGERS, *send the coupon with your letterhead.*

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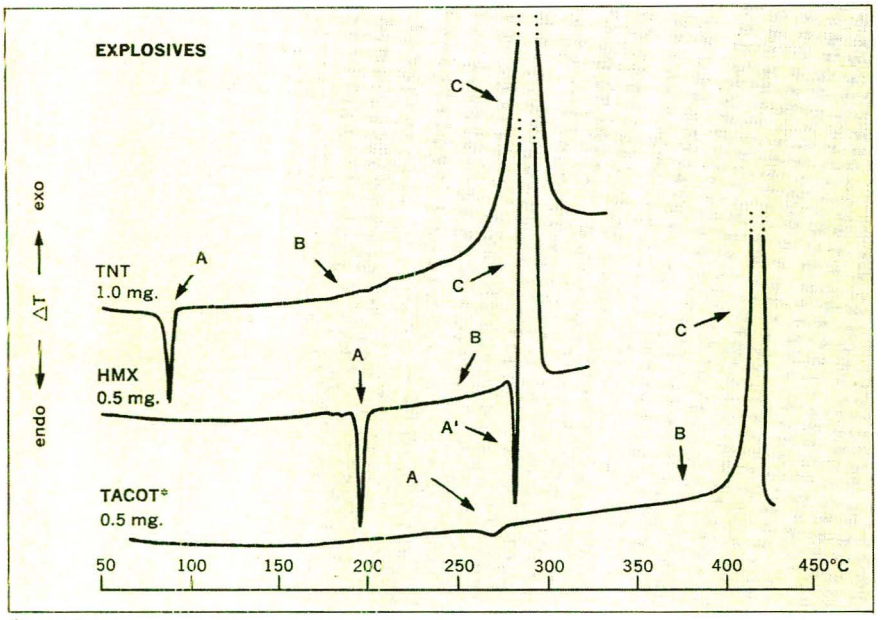
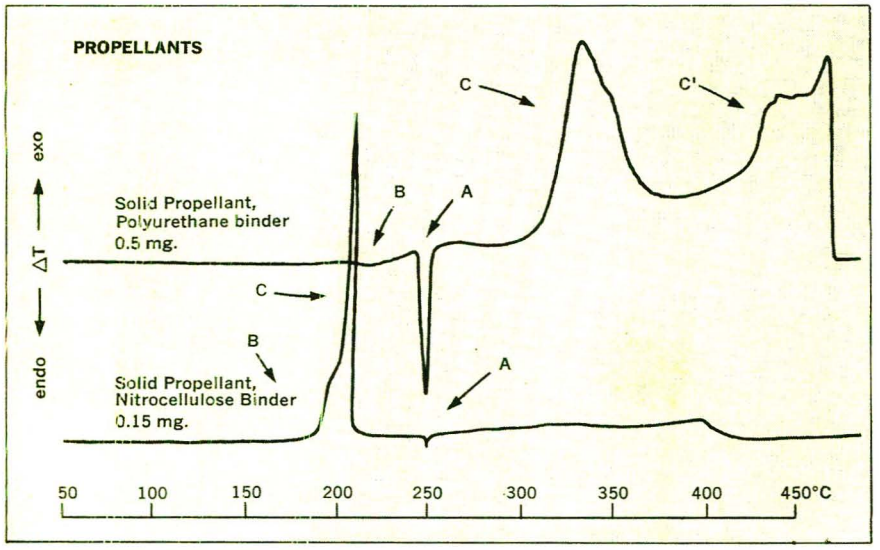
Thermograms like these can increase safety in the handling of high-energy materials.

(And you can obtain this information more safely, too!)



*Du Pont's trademark for its temperature-resistant high explosive



These thermograms of typical explosives and propellants were made quickly and safely using the Du Pont 900 Differential Thermal Analyzer. They provide a wealth of information useful in the manufacture, storage and application of high-energy materials. For example:

The endotherms marked "A" in the traces, indicate either crystalline phase transitions or melting. These are important in determining optimum processing conditions.

The exothermic departure from the base line, marked "B", is caused by the onset of thermal decomposition. Knowledge of the temperature at which this occurs is vital for determining suitable conditions for safe handling.

"C" is the temperature of runaway decomposition. This is important in determining the upper limit of stability.

Other Information. The thermograms offer other useful information, too. The temperature and shape of the endotherms associated with first order transitions reflect the purity of the materials. Lowering of decomposition temperatures warns of potential hazards that may have developed because of the presence of sensitizing impurities.

In addition, the onset of exothermic decomposition indicates a temperature range to be avoided when quantities of the material larger than the sample are processed. This is because limited heat transfer in massive sections may permit self-heating to a temperature high enough to cause runaway decomposition.

And thermograms can be made right in the laboratory, because the high sensitivity and resolution of the Du Pont 900 DTA permits the use of micro samples...one milligram or less in most cases.

For more information about application of the Du Pont 900 Differential Thermal Analyzer, write to: E. I. du Pont de Nemours & Co. (Inc.), Instrument Products Division, 2533 Nemours Building, AC103, Wilmington 98, Delaware.

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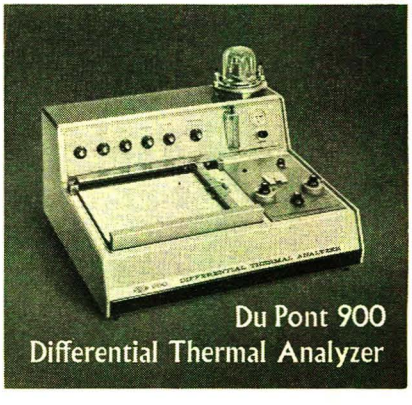
Thermal Penetrometer that measures changes in mechanical properties as a function of temperature.

Thermogravimetric Analyzer measures and records changes in sample weight as a function of temperature.

High, Low and Intermediate Temperature Cells that extend the range of the 900 DTA from −150 to +1200°C.

Visual Cell that permits observation of a sample during differential thermal analysis.

PATENT APPLIED FOR



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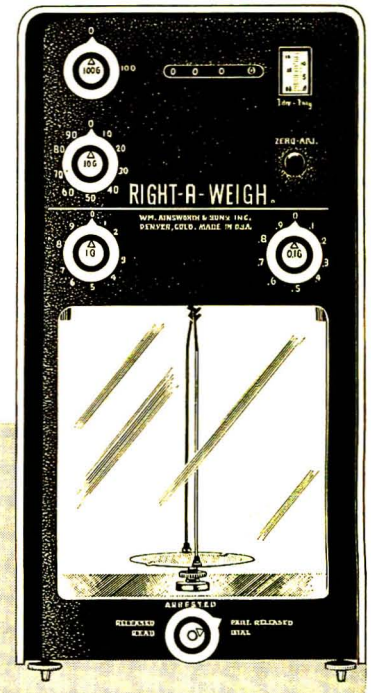

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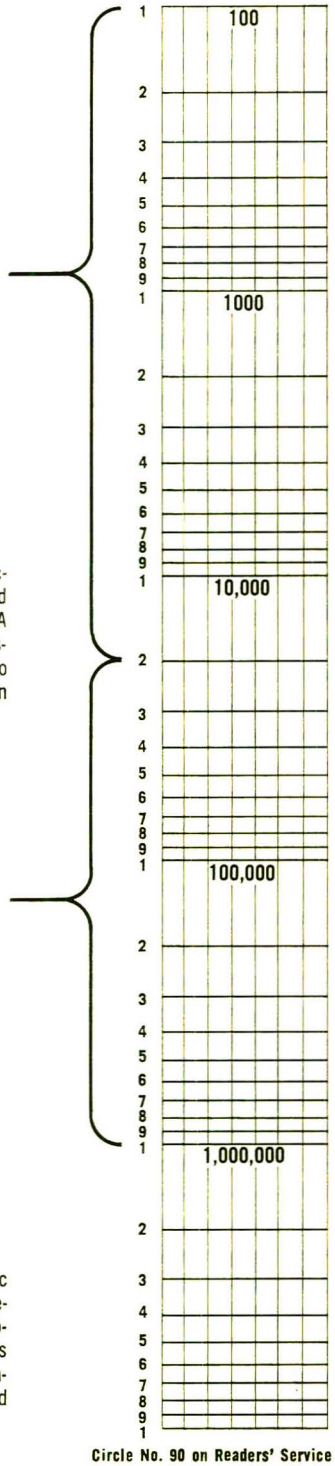

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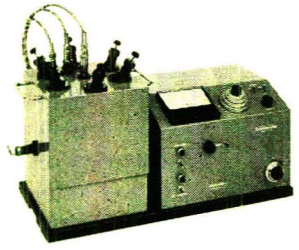

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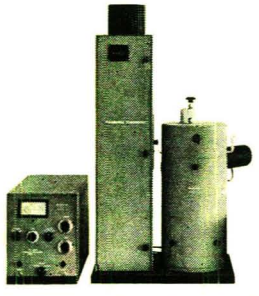

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Fisher, Labline, Sargent Winners Announced

THE WINNERS OF THE ANNUAL FISHER, LABLINE, AND SARGENT AWARDS were announced at the 145th ACS National Meeting in New York City just before ANALYTICAL CHEMISTRY went to press. The recipients and their biographies appear below.

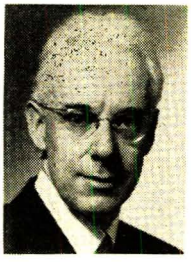
John Mitchell, Jr., of the Du Pont Co. is the winner of the Fisher Award in Analytical Chemistry, sponsored by the Fisher Scientific Co. Mr. Mitchell was born in Catonsville, Md., in 1913. He received a B.E. degree in gas engineering from Johns Hopkins University in 1935 and an M.S. degree in organic chemistry from the University of Delaware in 1945. After working for the Consolidated Edison Co. of New York City, he joined the Chemical Division of Du Pont's Ammonia Department at the Experimental Station, Wilmington, Del. He became a research supervisor in 1945. After consolidation of the company's Ammonia and Plastics Departments in 1950 to form the Polychemicals Department (renamed Plastics Department in 1962), Mr. Mitchell was made senior supervisor in charge of the Research and Development Division's Analysis Group, a position that he continues to hold.

Mr. Mitchell's research in the Analysis Group led to methods for the quantitative determination of functional groups, to analytical applications of optical crystallography, and to new applications of the Karl Fischer reagent for the titrimetric determination of water. With W. M. D. Bryant and D. M. Smith, he developed an entirely new area for functional group analysis—quantitative reactions involving the absorption or formation of water, which could be determined by titration with Karl Fischer reagent. Among the procedures developed were those for alcoholic hydroxyl, carboxyl, aminoalcohols, anhydrides, nitriles, carbonyl compounds, amines, esters, and peroxides. Discussions of the nature of the reagent determination of water in the presence of organic and inorganic compounds and functional group analyses were given in the book, D. M. Smith, which was published by Interscience in 1948 (the second edition is in preparation).

Mr. Mitchell is author or co-author of over 41 scientific papers. He served as editor and has contributed to three chapters of the four-volume work, "Organic Analysis." He has also written chapters for the Encyclopedia of Chemical Technology, the Encyclopedia of Chemistry, and the Treatise on Analytical Chemistry.

Mr. Mitchell was chairman of the analytical sub-group of the Delaware Section of the ACS. He served on the Advisory Board of Analytical Chemistry from 1957 to 1960 and was chairman of the Analytical Division of the ACS in 1962.

Dr. Stanford Moore and Dr. William H. Stein, The Rockefeller Institute, New York City, have been selected as joint recipients of the 1964 ACS Award in Chromatography and Electrophoresis sponsored by Labline Instruments, Inc. Dr. Moore and Dr. Stein have made significant contributions to the chromatographic separation of amino acids, peptides, and proteins. This work culminated in the establishment of the structure of ribonuclease, the first enzyme for which a chemical structure can be written. In addition, their work on chromatography has provided the basis for the exact and automatic analysis of the amino acids composition of proteins and other natural substances. They are co-authors of over twenty scientific publications.

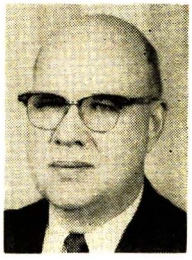


Dr. Stanford Moore was born in 1913 in Chicago, Ill. He received an A.B. from Vanderbilt University in 1935 and the Ph.D. in organic chemistry from the University of Wisconsin in 1938. He joined the Rockefeller Institute as an assistant in 1939 and became a member and professor of biochemistry in 1952. He was a visiting professor at Brussels University in 1950–51 and a visiting investigator at Cambridge University, England, in 1951. He received an honorary M.D. from Brussels in 1954.

Dr. William H. Stein was born in 1911 in New York City.

He received a B.S. from Harvard University in 1933 and the Ph.D. in biochemistry from Columbia University in 1938. He joined the Rockefeller Institute as an assistant chemist in 1939 and became a member and professor in 1952. Dr. Stein was a fellow of the American Swiss Foundation in 1956 and a visiting professor at the University of Chicago in 1960.

Robert H. Cherry of Leeds and Northrup Co., North Wales, Pa., is the winner of the 1964 ACS Award in Chemical Instrumentation sponsored by E. H. Sargent & Co. Mr. Cherry has made important advances in three distinct branches of analytical instrumentation—electroanalysis, fluorescence photometry, and thermometric techniques of gas analysis.



Mr. Cherry's major research has been thermometric techniques of gas analysis. He worked out details of a highly precise thermal conductivity system for measuring dew point and also developed a convenient method of measuring relative humidity. In addition to this, he played a major role in applying thermal conductivity principles to the development of a precision recording barometer and a carbon monoxide detector. A most noteworthy accomplishment in this field was his work with G. M. Foley on the development of the thermomagnetic oxygen gas analyzer, which led to the development of a successful commercial instrument.

Mr. Cherry played a significant role in the origination, improvement, and application of pH instrumentation, especially in the crucial formative years when this field was just beginning to expand rapidly. He devised and developed the circuits for three Leeds & Northrup instruments, the 7660, 7661, and 7673. He also designed and built the prototype of the portable direct-reading 7662 pH meter. This instrument was produced for more than ten

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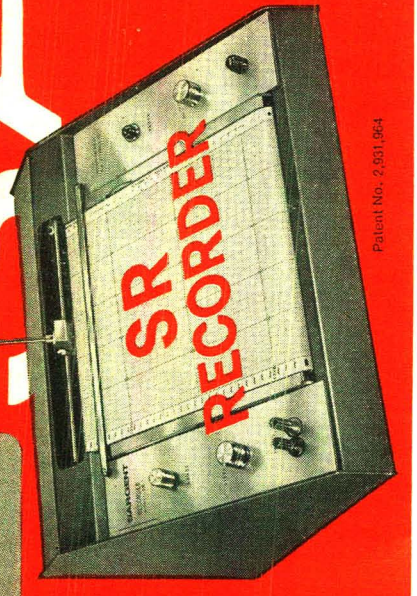

Patent No. 2,931,964

years and many remain in use today. Mr. Cherry also made important contributions to the development of recording systems for polarographic analysis.

In the field of fluorescence photometry Mr. Cherry's work demonstrated for the first time that fluorescence photometry could be successfully applied in practical equipment to obtain reliable, continuous, and specific measurement of organic substances in air at extremely low concentration levels. He played a prominent role in the development of a nerve gas analyzer-alarm and the development of an accurate, stable, and reliable instrument capable of detecting and measuring traces of nerve gases far below toxic levels.

Mr. Cherry was born in Singapore, Straits Settlement, in 1907. He came to the United States at the age of seventeen and completed the senior year of high school at Langhorne, Pa. He received the B.S. degree in Electrochemical Engineering from Pennsylvania State University in 1929. He then joined the Leeds and Northrup Company as a research assistant in the Electrical Section of the Research Department, and in 1931, tranferred into the Chemical Section. In 1951 he was made head of the Chemical Section. He became manager of the Research Division in 1956 and manager of the Development Division one year later. In 1961 Mr. Cherry was made Director of the Research and Development Department, a position he holds at the present time.

Society for Applied Spectroscopy National Meeting

The Second National Meeting of the Society for Applied Spectroscopy will be held from October 14 to 18 at the El Cortez Hotel, San Diego, Calif.

In addition to contributed papers and invited speakers, there will be an exhibition of equipment including products from over 30 companies.

The scientific program is given below. Further information is available from Earl P. Wadsworth, Jr., Program Chairman, San Diego State College, San Diego, Calif.

PROGRAM

Monday Morning, October 14

GAMMA-RAY AND NUCLEAR PARTICLE SPECTROMETRY

J. F. Cosgrove, Presiding

8:50 **Introductory Remarks.** V. P. Guinn, General Atomic Division, General Dynamics Corp., San Diego, Calif.

9:00 **Semiconductors as Detectors of Charged Particles, X-Rays and Gamma-Rays.** C. Borkowski, Oak Ridge National Laboratory, Oak Ridge, Tenn.

10:00 **The Cerenkov Counter in the Determination of Oxygen.** H. R. Lukens, Jr., and J. E. Lasch, General Atomic Division, General Dynamics Corp., San Diego, Calif.

10:45 **Magnetic Charged Particle Analyser.** H. Bichsel, Physics Department, University of Southern California, Los Angeles, Calif.

11:15 **A Study of Flux Monitoring for Instrumental Neutron Activation Analysis.** F. A. Iddings, Esso Research Laboratories, Baton Rouge, La.

MASS SPECTROMETRY

J. H. Norman, Presiding

9:30 **Competitive Processes for the Formation of Troyplium Ion in Electron Impact Studies of Toluene.** J. G. Burr and R. A. Meyer, North American Aviation Science Center, Canoga Park, Calif.

10:00 **Accuracy in Spark Mass Spectrometry.** C. M. Judson and R. F. Howard, Consolidated Electrodynamics Corp., Pasadena, Calif.

10:45 **Electron Collisions with Atoms and Molecules.** W. Fite, General Atomic Division, General Dynamics Corp., San Diego, Calif.

Monday Afternoon, October 14

GAMMA-RAY AND NUCLEAR PARTICLE SPECTROMETRY

C. Borkowski, Presiding

1:30 **The Present State of Gamma-Ray Scintillation Spectrometry.** R. Heath, Phillips Petroleum Co., Idaho Falls, Idaho.

2:30 **The Use of Gamma-Ray Spectrometry in the Study of Fission Product Release from Nuclear Fuels.** D. R. Lofing, E. E. Anderson, and L. R. Zumwalt, General Atomic Division, General Dynamics Corp., San Diego, Calif.

3:15 **Nondestructive Analysis Using a Research Reactor.** W. Meinke, Department of Chemistry, University of Michigan, Ann Arbor, Mich.

3:45—**The Appropriate Roles of Small Accelerators and Nuclear Reactors and of Instrumental and Radiochemical Techniques in Neutron Activation Analysis.** V. P. Guinn, General Dynamics Corp., General Atomic Division, San Diego, Calif.

MASS SPECTROMETRY–COLLISION PROCESSES

R. T. Brackman, Presiding

1:30 **Problems and Trends in the Mass Spectrometric Study of Reactions between Gaseous Ions and Molecules.**

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D. Stevenson, Shell Development Co., Emeryville, Calif.

2:45 **Ion Cyclotron Resonance in Weakly Ionized Gases.** D. Malone, Cornell Aeronautical Laboratory, Buffalo, N. Y.

3:30 **Aston Bands and Ion Collision Physics.** J. W. McGowan, General Atomic Division, General Dynamics Corp., San Diego, Calif.

Tuesday Morning, October 15

MASS SPECTROMETRY-HIGH TEMPERATURE CHEMISTRY

L. S. Theard, Presiding

9:00 **Thermodynamics and Kinetics of Vaporization Processes Studied by Mass Spectrometry.** W. A. Chupka and J. Berkowitz, Argonne National Laboratory, Argonne, Ill.

10:00 **Mass Spectrometric Study of the Vaporization of Mixed Oxides of Arsenic and Antimony.** J. H. Norman and H. G. Staley, General Atomic Division, General Dynamics Corp., San Diego, Calif.

10:45 **Mass Spectrometric Study of the BeO–BeF_2 System.** J. Efimenko, National Bureau of Standards, Washington, D. C.

11:15 **Mass Spectrometric Study of Strontium Oxide Vaporization.** H. G. Staley and J. H. Norman, General Atomic Division, General Dynamics Corp., San Diego, Calif.

GAMMA-RAY AND NUCLEAR PARTICLE SPECTROMETRY

R. Heath, Presiding

9:00 **The Use of Computers in the Analysis of Gamma-Ray Spectrum Data.** J. Trombka, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, Calif.

10:00 **Computer Analysis of Complex Gamma-Ray Spectra.** A. K. Perkons, R. L. Hummel, and R. E. Jervis, Department of Chem., Eng. and Applied Chem., Univ. of Toronto, and Attorney-General's Laboratory, Toronto, Canada.

10:45 **A Computer Program for Rapid Optimization of Experimental Conditions for Activation Analysis.** T. L. Isenhour and G. H. Morrison, Department of Chemistry, Cornell University, Ithaca, N. Y.

11:45 **Practical Computer Routines for Neutron Activation Analysis.** D. E. Hull and J. T. Gilmore, California Research Corp., Richmond Laboratory, Richmond, Calif.

BIOCHEMISTRY AND MOLECULAR BIOLOGY

M. Kamen, Presiding

9:00 **The Theory of Fluorescence Polarization and Its Application to Biochemical Studies.** G. Weber, Biochemistry Division, Department of Chemistry and Chemical Engineering, University of Illinois, Urbana, Ill.

10:00 **A Polarization Fluorometer and Its Use.** N. S. Bromberg, A. Pesce, and N. O. Kaplan, Graduate Dept. of Biochemistry, Brandeis University, Waltham, Mass.

10:45 **Near Infrared Absorption Spectra of Native and Chemically Modified Wool and Related Structures.**

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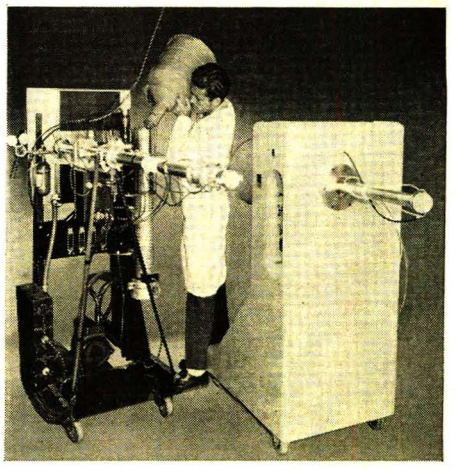

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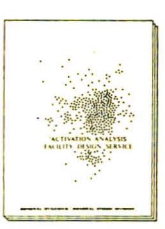

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G. F. Bailey, Western Regional Research Laboratory, U.S.D.A., Albany, Calif.

11:45 **On the Ultraviolet Spectrophotometry for Measuring Proteins.** S. Gabay and D. E. Jordan, Biochemical Research Laboratory, Veterans Administration Hospital, Brockton, Mass., and Department of Biochemistry, Boston University School of Medicine, Boston, Mass.

GEOCHEMISTRY AND GEOPHYSICS

E. Goldberg, Presiding

9.00 **The Identification of Organized Elements in Meteorites.** B. Nagy, Fordham University, New York, N. Y., and University of Calif., La Jolla, Calif.

10:00 **The Application of a Nuclear Magnetic Resonance Spectrometer for Quantitative In-Situ Measurements of Brine in Sea Ice.** C. Richardson, U. S. Navy Electronics Laboratory, San Diego, Calif., and E. E. Keller, General Dynamics/Convair, San Diego, Calif.

10:45 **Abundances of Na, Sc, Mn, Cr, Fe, and Co in Stony Meteorites Via Instrumental Neutron Activation Analysis.** R. A. Schmitt, General Atomic Division, General Dynamics Corp., San Diego, Calif., and G. G. Goles, Department of Chemistry and Earth Sciences, University of California, La Jolla, Calif.

11:15 **Electron Microprobe Analysis of Some Minerals in Stony Meteorites.** K. Keil and K. Fredriksson, Institute for the Study of Matter and Scripps Institution of Oceanography, University of California, La Jolla, Calif.

Tuesday Afternoon, October 15

GAMMA-RAY AND NUCLEAR PARTICLE SPECTROMETRY

J. Trombka, Presiding

1:30 **Computer Correction for Shifts in Gain and Baseline in Gamma Scintillation Pulse Height Spectra.** D. F. Covell, U. S. Naval Radiological Defense Laboratory, San Francisco, Calif.

2:00 **Application of a Rapid Print-Out System for Analyzer Dead-Time Correction.** E. Steele, L. Fite, R. Wainerdi, and W. Wilkins, Texas A. and M. University, College Station, Texas.

2:30 **Some New Techniques in Beta- and Gamma-Ray Spectroscopy.** J. A. Eisele, J. F. McClary, E. R. Zamecki, and T. H. Adensam, Texas A. and M. University, College Station, Texas.

3:15 **Instrumental Activation Analysis for Identification of Forensic Specimens.** R. E. Jervis, Dept. of Chem., Eng., and Applied Chem., University of Toronto, Toronto, Canada.

3:45 **New Applications of Neutron Activation Analysis to Criminalistics.** R. R. Ruch, S. B. Madigan, and V. P. Guinn, General Atomic Division, General Dynamics Corp., San Diego, Calif.

BIOCHEMISTRY AND MOLECULAR BIOLOGY–N.M.R. AND E.P.R. APPLICATIONS

L. Joseph, Presiding

1:30 **Some Applications of Nuclear Magnetic Resonance Spectroscopy to Biochemical Problems.** M. Cohn, Johnson Foundation for Medical Phys-

ics, University of Pennsylvania Medical School, Philadelphia, Pa.

2:30 **EPR Study of the Chlorpromazine Free Radical during Enzymic Oxidation.** L. H. Piette, I. Yamazaki, and G. Bulow, Varian Associates, Palo Alto, Calif.

3:15 **Application of EPR to the Study of Some Transition—Metal Complexes in Biological Systems.** J. J. Windle, Western Regional Research Laboratory, U.S.D.A., Albany, Calif.

3:45 **An NMR Study of the Effect of Configuration on the Proton Spin Coupling Constants in Some Five-Membered Rings in Relation to the Stereo-chemistry of the Betamethylaspartase Reaction.** R. E. Lundin, Western Regional Research Laboratory, U.S.D.A., Albany, Calif., H. J. Bright, University of Pennsylvania Medical School, Dept. of Biochemistry, Philadelphia, Pa., L. L. Ingraham, Dept. of Biochemistry and Biophysics, University of California, Davis, Calif.

APPLICATIONS OF X-RAY AND MICROPROBE ANALYSIS TO GEOCHEMISTRY AND GEOPHYSICS

R. Gastil, Presiding

1:30 **X-Ray Fluorescence Analysis of the Light Elements in Rocks and Minerals.** I. Adler, H. J. Rose, Jr., and F. J. Flanagan, United States Department of the Interior, Geological Survey, Washington, D. C.

2:30 **The Preparation of Silicate Rocks and Minerals for Light Element Analyses by X-Ray Spectrography.** A. K. Baird and E. E. Welday, Department of Geology, Pomona College, Claremont, Calif.

3:15 **The Use of a High-Speed Computer in Light Element Analysis by X-Ray Fluorescence.** D. B. McIntyre, Department of Geology, Pomona College, Claremont, Calif.

3:45 **Electron Microanalysis of Metallic Meteorites.** R. E. Ogilvie, Metallurgy Department, Massachusetts Institute of Technology, Cambridge, Mass.

Wednesday Morning, October 16

GAMMA-RAY AND NUCLEAR PARTICLE SPECTROMETRY

A. Metzger, Presiding

9:00 **Applied Nuclear Geophysics.** R. L. Caldwell, Nuclear Physics and Well Logging Research, Socony Mobil Oil Co., Inc., Dallas, Texas.

10:00 **Fast Neutron Techniques in Coal Analysis.** T. C. Martin, S. C. Mathur, J. T. Prud'homme, Texas Nuclear Corp., Austin, Texas.

10:45 **Photoactivation Analysis.** H. R. Lukens, Jr., General Atomic Division General Dynamics Corp., San Diego, Calif.

11:45 **An Investigation into the Utilization of Metastable Isomers of Stable Isotopes in Activation Analysis.** H. H. Kramer and W. H. Wahl, Union Carbide Nuclear Research Center, Division of Union Carbide Corp., Tuxedo, N. Y.

NUCLEAR MAGNETIC RESONANCE AND ELECTRON PARAMAGNETIC RESONANCE

W. G. Clark, Presiding

9:00 **Some Recent Advances in Carbon-**

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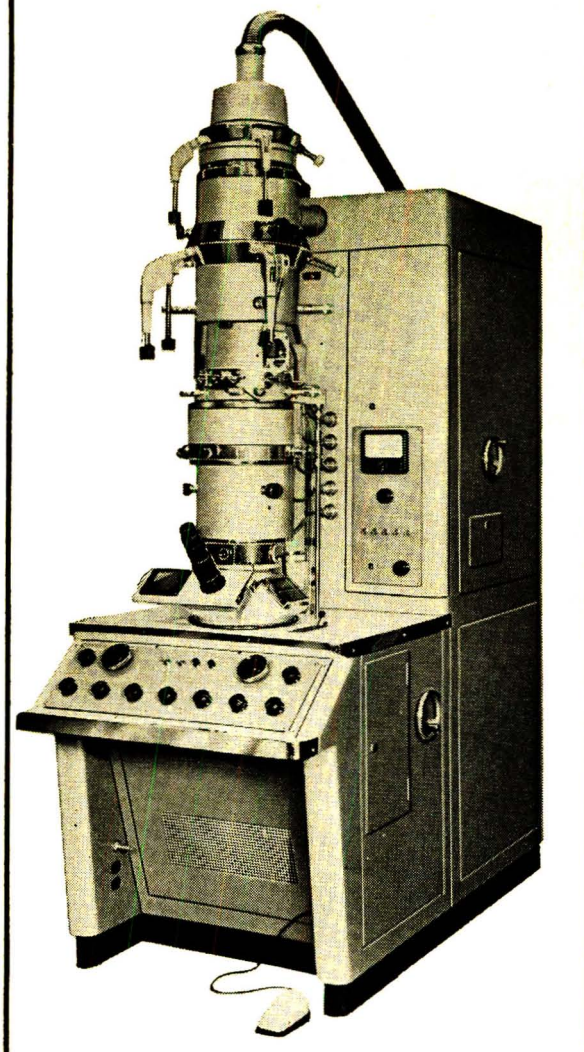

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NEWS

13 **Magnetic Resonance.** D. Grant, Chemistry Department, University of Utah, Salt Lake City, Utah.

10:15 **High Pressure EPR Spectroscopy: Emphasis—Paramagnetic Ions in Solids.** R. Sands, Physics Department, University of Michigan, Ann Arbor, Mich.

X-RAY

V. Macres, Presiding

9:00 **X-Ray Fluorescence Analysis for the Light Elements Sodium through Boron.** B. Henke, Department of Physics, Pomona College, Claremont, Calif.

10:00 **The Experimental Determination of the $M_{II, III}$ Soft X-Ray Emission Spectra of Nickel, Copper, and Zinc and Their Alloys.** B. J. Thompson, Physics Department, Leeds University, England, and Technical Operations, Burlington, Mass.

10:45 **The Determination of Microgram Quantities of Chloride in High-Purity Titanium by X-Ray Spectrochemical Analysis.** J. S. Rudolph and R. J. Nadalin, Westinghouse Electric Corp. Research Laboratories, Pittsburgh, Pa.

11:15 **The Determination of Trace Elements in Solid Hydrocarbons and Related Materials by X-Ray Fluorescence Spectroscopy.** T. C. Yao and R. E. Harrison, Shell Development Co., Emeryville, Calif.

11:45 **An X-Ray Diffractometer for Diffractometry and/or Spectroscopy.** W. Ashby, V. E. Buhrke, G. V. Patser, Picker Analytical Laboratory, San Francisco, Calif.

Wednesday Afternoon, October 16

GAMMA-RAY AND NUCLEAR PARTICLE SPECTROMETRY

R. Caldwell, Presiding

1:30 **Structural Chemical Applications of Neutron Diffraction.** J. Ibers, Department of Chemistry, Brookhaven National Laboratory, Associated Universities, Inc., Upton, Long Island, N. Y.

2:30 **He^3 Activation Analysis: Oxygen: Carbon: Nitrogen and Other Elements.** S. S. Markowitz and J. D. Mahoney, Lawrence Radiation Laboratory and Department of Chemistry, University of California, Berkeley, Calif.

3:15 **Use of Radio Chemistry and Mass Spectrometry for fuel Depletion Studies.** J. E. Hudgens, Westinghouse Electric Corp., Bettis Plant, Pittsburgh, Pa.

3:45 **Utilization of Reactor Fast Neutrons for Activation Analysis.** H. P. Yule, H. R. Lukens, Jr., and V. P. Guinn, General Atomic Division, General Dynamics Corp., San Diego, Calif.

NUCLEAR MAGNETIC RESONANCE AND ELECTRON PARAMAGNETIC RESONANCE

K. Vos, Presiding

1:30 **Nuclear Magnetic Resonance Study of Keto-Enol Tautomerism in Beta-Dicarbonyls.** M. T. Rogers and J. Burdett, Department of Chemistry, Michigan State University, East Lansing, Mich.

2:00 **An NMR Study of Competitive π Bonding in Ni(II) Chelates.** D. R.

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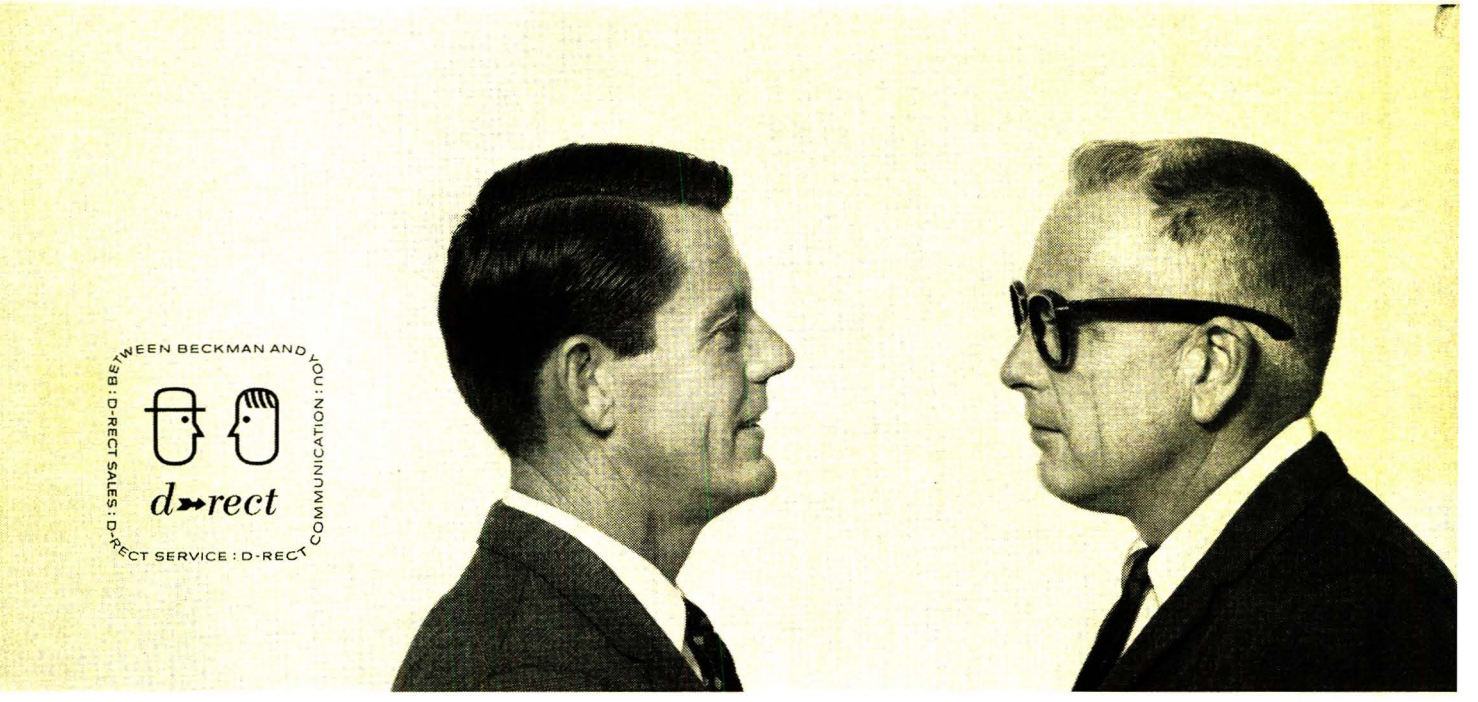

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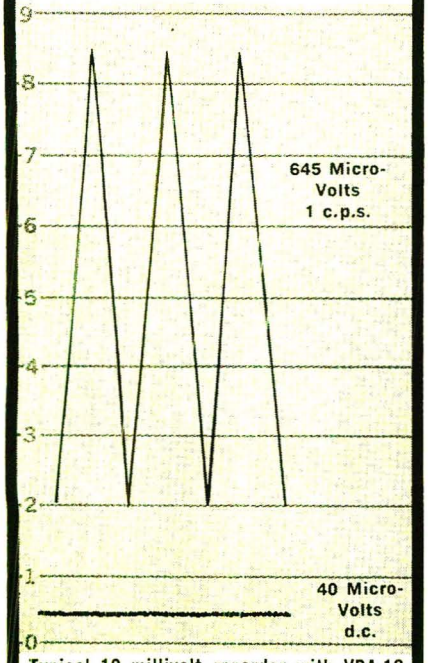

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2:30 **Tautomerism—Exchange in B_3H_7: NMe_3 and B_3H_7: THF.** M. A. Ring, Present Address—Chemistry Dept., San Diego State College, San Diego, Calif., R. C. Greenough, and E. F. Witucki, Rocketdyne, Division of North American Aviation, Inc., Canoga Park, Calif.

ELECTRON PROBE AND SECONDARY ION EMISSION ANALYSIS

K. Fredriksson, Presiding

1:30 **New Developments in Microanalysis.** R. Castaing, and G. Slodzian, Department of Solid State Physics, University of Paris, Paris, France.

2:30 **Electron Probe Analysis of Minerals.** I. Adler, United States Department of the Interior, Geological Survey, Washington, D. C.

3:15 **New Techniques in Electron Probe Analysis.** D. Wittry, Department of Electrical Engineering, University of Southern California, Los Angeles, Calif.

4:15 **Quantitative Phase Determination in Polyphase Mixtures by X-Ray Diffraction.** G. O. Arrhenius, E. Bonatti, and R. Fitzgerald, Scripps Institute of Oceanography and Institute for the Study of Matter, University of California, La Jolla, Calif.

4:45 **Interelement Effects in the Analysis of Nickel–Iron Thin Films.** J. C. Lloyd, Components Division, International Business Machines Corp., Poughkeepsie, N. Y.

Thursday Morning, October 17

GAMMA-RAY AND NUCLEAR PARTICLE SPECTROMETRY

R. Jensen, Presiding

9:00 **Applications of X-Ray, Gamma-Ray, and Nuclear Particle Spectroscopy to Lunar and Planetary Compositional Analysis.** A. Metzger, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, Calif.

10:00 **Some Applications of Thermal Neutron Capture Gamma-Ray Analysis.** R. C. Greenwood and J. H. Reed, Physics Division, IIT Research Institute, Chicago, Ill.

10:45 **Chemical Analysis Using Miniature Nuclear Accelerators and Inelastic Neutron Scattering Techniques.** J. A. Waggoner, University of California Lawrence Radiation Laboratory, Livermore, Calif., and E. F. Martina and C. D. Schrader, Aerospace Corp., El Segundo, Calif.

11:15 **Gamma-Ray Spectrometry of Terrestrial Radioactivity.** B. Zemel, Shell Development Co., Exploration and Production Research Division, Houston, Texas.

ELECTRON PROBE ANALYSIS AND X-RAY

I. Adler, Presiding

9:00 **Application of Kossel Technique to Metallurgical Problems.** R. E. Ogilvie, Department of Metallurgy, Massachusetts Institute of Technology, Cambridge, Mass.

10:00 **Fine Structure in Superconducting and Other Alloys, Studied by Electron Microprobe Analysis.** C. J. Raub, C. A. Anderson, G. O. Arrhenius, and B. T. Matthias, Institute for the Study of Matter and Scripps Institution of Oceanography, University of California, La Jolla, Calif.

10:45 **Absorption and Fluorescent Effects in Inclined Targets in the Electron Microprobe.** V. G. Macres and W. D. Donnelly, Department of Materials Science, Stanford University, Stanford, Calif.

11:15 **X-Ray Diffraction and Microfluorescence Investigations of Hyaloclastic Aggregates.** G. O. Arrhenius, C. A. Andersen, E. Bonatti, and N. Kharkar, Scripps Institution of Oceanography and Institute for the Study of Matter, University of California, La Jolla, Calif.

11:45 **Design Considerations and Analytic Capabilities of an X-Ray Emission Spectrograph for Lunar Surface Analysis.** A. E. Metzger and R. A. Shields, Space Sciences Division, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, Calif., and H. W. Schnopper, Physics Department, Cornell University, Ithaca, N. Y.

ATOMIC EMISSION AND ABSORPTION

C. Chaney, Presiding

9:00 **Recent Developments in Flame Atomic Absorption and Emission Spectroscopy.** V. Fassel, Chemistry Department and Institute for Atomic Research, Iowa State University, Ames, Iowa.

10:00 **An Arc Device for Spectral Excitation in Controlled Atmospheres.** B. F. Scribner and M. Margoshes, National Bureau of Standards, Washington, D. C.

10:45 **Spectral Radiation Measurements of the Earth and Its Atmosphere from Onboard the X-15 Aircraft.** L. C. Block, H. E. Band, and E. K. Dana, Air Force Cambridge Research Laboratories, Laurence C. Hanscom Field, Bedford, Mass.

11:15 **A Study of the Spark Spectra of Selenium.** A. M. Crooker and S. George, Department of Physics, University of British Columbia, Vancouver, Canada.

Thursday Afternoon, October 17

GAMMA-RAY AND NUCLEAR PARTICLE SPECTROMETRY

R. Schmidt, Presiding

1:30 **Industrial Applications of Activation Analysis.** P. R. Gray and L. M. Cartwright, Phillips Petroleum Co., Bartlesville, Okla.

2:00 **The Role of Instrumental Activation Analysis in a Diversified Chemical Research and Development Laboratory.** R. A. Johnson, Shell Development Co., Emeryville, Calif.

2:30 **Fast Neutron Activation vs. Vacuum Fusion Analysis for Oxygen in Metals.** F. Burns, Watertown Arsenal, Watertown, Mass.

3:15 **Silicon, Aluminum, and Silicon/Aluminum Analyses by Neutron Activation and Gamma-Ray Spectrometry.** W. E. Mott and D. F. Rhodes, Gulf Research and Development Co., Pittsburgh, Pa.

3:45 **Reactor Pulsing in Activation Analysis.** H. R. Lukens, Jr., H. P.

Yule, and V. P. Guinn, General Atomic Division, General Dynamics Corp., San Diego, Calif.

ASTROPHYSICAL

C. M. Huffer, Presiding

1:30 **Spectroscopic Observations of Faint Galaxies and Stars.** J. Oke, Mount Wilson and Palomar Observatories, Carnegie Institute of Washington, D. C., and California Institute of Technology, Pasadena, Calif.

2:45 **The Current Status of Far Ultraviolet Stellar and Nebular Spectroscopy.** J. Kupperian, Goddard Space Flight Center, Greenbelt, Md.

3:45 **Space Instruments for the Measurement of the Ultraviolet Spectra of Planetary Atmospheres.** D. Margetts, E. F. Mackey, and C. A. Barth, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, Calif.

INFRARED AND OPTICAL-MOLECULAR STRUCTURE

W. Ware, Presiding

1:30 **The Vibrational Spectra of Excited Electronic States.** R. Hexter, Mellon Institute, Pittsburgh, Pa.

2:30 **Spectrophotometric Studies of Molecules in Their Triplet States.** M. W. Windsor and D. Weber, Quantum Chemistry Department, Space Technology Laboratories, Inc., Redondo Beach, Calif.

3:15 **Spectral Line Splitting in Europium Chelates.** M. L. Bhaumik, Electro-Optical Systems, Inc., Pasadena, Calif.

3:45 **The Luminescence of Transition Metal Complexes.** L. S. Forster, University of Arizona, Tucson, Ariz.

4:15 **Long-Lived Luminescence from Organic Materials.** H. R. Lukens, Jr., General Atomic Division, General Dynamics Corp., San Diego, Calif.

4:45 **Optical Measurements of Radiation Damage Processes on a Fast Time Scale.** J. F. Bryant, D. M. J. Compton, and J. Kirkbride, General Atomic Division, General Dynamics Corp., San Diego, Calif.

Friday Morning, October 18

ATOMIC EMISSION AND ABSORPTION

ASTM-E2 Discussion Period on Unsolved Problems in Spectrochemical Analysis

B. Buell, Presiding

9:00 **A High Resolution Scanning System for the Optical Study of Laser Crystals.** J. K. Powers and B. W. Harned, Philco Scientific Laboratory, Blue Bell, Pa.

9:30 **Instrumental Parameters in Atomic Absorption.** H. L. Kahn, The Perkin-Elmer Corp., Norwalk, Conn.

10:15 **Unsolved Problems in Spectrochemical Analysis.** R. Barras, Atlantic Refining Co., Philadelphia, Pa.

11:15 **Discussion Period—Unsolved Problems in Spectrochemical Analysis—ASTM Committee E-2;** Discussion headed by R. Barras.

INFRARED AND OPTICAL-STRUCTURE

W. Jones, Presiding

9:00 **The Evaluation of Transferable**

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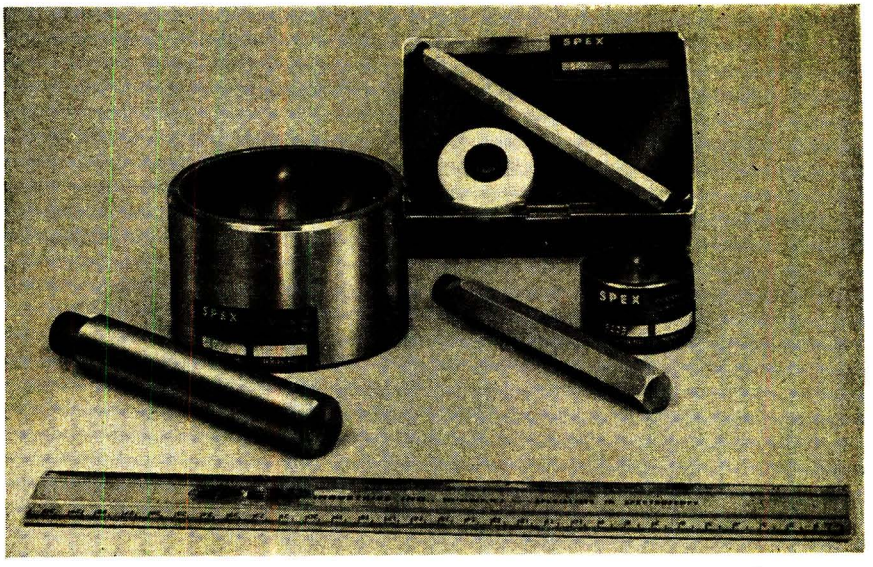

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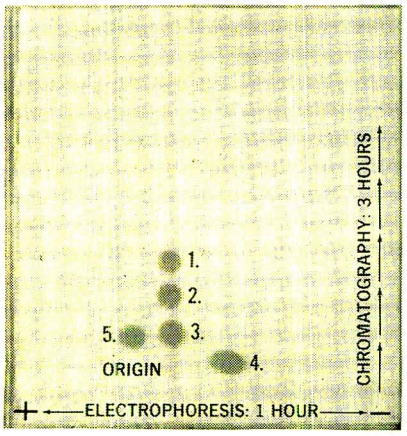

Two-dimensional separation of 5 amino acids on silica gel, first by electrophoresis, then by chromatography: 1. Arginine 2. Glutamic acid 3. Valine 4. Histidine 5. Leucine

Thin-layer electrophoresis is a new analytical technique which combines with thin-layer chromatography to give you separations that you can't get by using both techniques separately. Scientists experimenting with TLE report instances of unexpectedly high mobilities as well as greater speed, sharper resolution, increased sensitivity, wider range of sample size, better reproducibility, and other advantages. □ RSCo is the only domestic company which makes complete equipment for TLE, and our dealers now have it in stock. If you already use RSCo electrophoresis or other flatbed equipment, you can adapt it to TLE with our assembly at a cost of only $162.

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Force Constants. J. Schachtschneider, Shell Development Co., Emeryville, Calif.

10:00 **Group Theoretical Vibrational Analysis of Impurities in Solids.** L. A. Veguilla-Berdecia and G. C. Turrell, Department of Chemistry, Howard University, Washington, D. C.

10:45 **Optical Spectra and Energy Levels of Trivalent Thulium.** K. E. Lawson, Sandia Corp., Albuquerque, N. M.

11:15 **The Infrared Spectrum of B_2F_4.** J. N. Gayles and J. Self, Department of Chemistry, Oregon State University, Corvallis, Ore.

11:45 **Far Infrared Studies on Barbiturates.** W. F. Ulrich and J. E. Stewart, Beckman Instruments, Inc., Fullerton, Calif.

Friday Afternoon, October 18

ATOMIC EMISSION

J. Guffy, Presiding

1:30 **Calculation and Measurement of the Radial Distribution of Line Broadening in a Cesium Plasma.** D. L. Tipton and R. T. Schneider, Allison Division, General Motors Corp., Indianapolis, Ind.

2:00 **Stabilized Plasma Jet for Spectroscopic Excitation.** M. Yamamoto, Central Research Laboratory, Hitachi, Ltd., Kokubunji, Tokyo, Japan. Paper to be presented by C. Chaney, General Atomic Division, General Dynamics Corp., San Diego, Calif.

2:45 **An Investigation into the Analysis of Nitrogen for Hydrogen by Optical Spectroscopy.** G. W. Dickinson and G. V. Wheeler, Phillips Petroleum Company, Atomic Energy Division, Idaho Falls, Idaho.

3:15 **Design Aspects of a New Compact Spectrographic Laboratory.** W. G. Shequen, H. Neuhaus, R. L. Gordon, and A. J. Hartwick, Applied Research Laboratories, Glendale, Calif.

3:45 **The Detection and Estimation of Some Trace Elements by D.C. Arc Excitation Using the Vacuum Cup Technique.** E. Bevege, General Atomic Division, General Dynamics Corp., San Diego, Calif. and R. Gallion, Ultra Carbon Corp., Upland, Calif.

INFRARED-SOLID STATE

W. F. Ulrich, Presiding

1:30 **Absolute Intensities of Infrared Absorption Bands in Molecular Crystals.** W. Person, Chemistry Department, State University of Iowa, Iowa City, Iowa.

2:30 **Vibrational Spectrum of Single Crystals of Sodium Azide.** J. I. Bryant, Basic Research Group, U. S. Army Engineer Research and Development Laboratories, Fort Belvoir, Va.

3:15 **Infrared Spectrum of Potassium Nitrate in Phases I, II, and III.** R. K Khanna, J. Lingscheid, and J. C. Decius, Department of Chemistry, Oregon State University, Corvallis, Ore.

3:45 **Infrared Microsampling Using Microspecular Reflectance.** H. J. Sloane, T. Johns, and W. F. Ulrich, Beckman Instruments, Inc., Fullerton, Calif.

4:15 **Crystal Structure and Spectroscopic Study of the Potassium TCNQ Ion-Radical Salt.** G. R. Anderson and C. J. Fritchie, Jr., Central Research Dept., E. I. du Pont de Nemours and Co., Wilmington, Del.

The Robert A. Welch Foundation Analytical Chemistry Meeting

The Robert A. Welch Foundation Conference on Modern Developments in Analytical Chemistry will be held from November 18 to 20 at the Rice Hotel, Houston, Texas. This conference is open to the general scientific public but advance registration is desirable. More information and applications forms are available from W. O. Milligan, Director of Research, The Robert A. Welch Foundation, 2010 Bank of the Southwest Bldg., Houston 2, Texas.

The conference will consist of invited speakers and discussions led by prominent specialists. The following topics and speakers are on the program: Isotopes in Analysis, Henry Eyring; Polarography, Paul Delahay; Activation Analysis, W. W. Meinke; Microanalysis, Oliver Lowry, Nuclear Magnetic and Paramagnetic Resonance, H. S. Gutowsky; and Mass Spectrographic Analysis, Klaus Biemann. The discussion leaders include, F. Bloch, G. E. Boyd, D. J. Fisher, J. L. Franklin, David Glick, V. P. Guinn, D. N. Hume, I. M. Kolthoff, J. N. Shoolery, D. P. Stevenson, A. D. Suttle, Jr., and C. L. Wilson. An informal dinner will be held on Tuesday Evening, November 19, at the Grand Ballroom of the Rice Hotel. The address of the evening will be given by Dr. Philip Abelson. He will provide "A Critical Appraisal of Government Research Policy."

NMR/EPR Workshop

The Seventh Annual Nuclear Magnetic Resonance and Electron Paramagnetic Resonance Workshop will be held by Varian Associates in Palo Alto, Calif., the week of November 11 to 15. Separate and concurrent sessions will be conducted on NMR and EPR. Registration and program information may be obtained from Varian Associates, Instrument Division, 611 Hansen Way, Palo Alto, Calif.

1965 SAC Conference

The Society for Analytical Chemistry will hold a five-day conference on all aspects of analysis in July 1965 at Nottingham University. The program will consist of original papers. More information is available by writing the Society for Analytical Chemistry, 14 Belgrave Square, London, S. W. 1, England.

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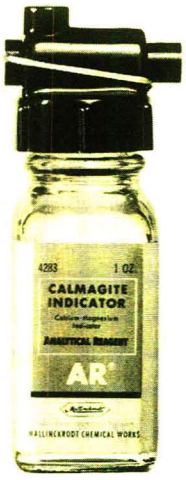

This companion product to HYDROXY NAPHTHOL BLUE AR determines calcium plus magnesium. It has these important advantages over Eriochrome Black T (commonly used in this type of disodium EDTA titrations): sharper, more easily recognized end point; stable and convenient form.

The following procedure is used to determine magnesium: Adjust an aliquot of the sample solution to pH 12-12.5 with KOH solution and titrate with disodium EDTA using HYDROXY NAPHTHOL BLUE AR indicator. Adjust a second aliquot of the same sample solution to pH 10 with NH_4Cl-NH_4OH buffer solution and titrate with disodium EDTA using CALMAGITE indicator. The end-point color change is from wine-red to blue. The amount of magnesium present is obtained from the difference of the two determinations.

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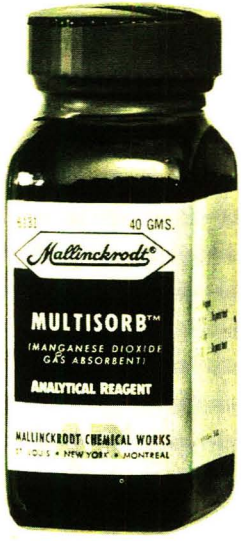

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Rare Earth Conference

The Fourth Rare Earth Research Conference will be held April 22 to 25, 1964, at the Camelback Inn, Phoenix, Ariz. The meeting will be devoted to the physics, chemistry, metallurgy, and ceramics of these elements, their compounds and alloys. Attendance is limited to those actively engaged in research in the areas covered by the meeting.

Abstracts of proposed papers should be received by November 4, 1963. Requests for further information should be addressed to Dr. LeRoy Eyring, Dept. of Chemistry, Arizona State University, Tempe, Ariz.

International Mass Spectrometry Conference

The American Society for Testing and Materials, Committee E-14 on Mass Spectrometry has announced its participation jointly with the G. A. M. S. and the Hydrocarbon Research Group of an International Conference on Mass Spectrometry. This meeting will be held September 14 to 18, 1964, at Paris, France. Deadline for abstracts is November 30, 1963. Further information is available by writing to the Secretariat du G. A. M. S., 1, rue Gaston Boissier, Paris XV, France.

Vacuum Microbalance Techniques Conference

The fourth informal conference on vacuum microbalance techniques will be held May 7 and 8, 1964, at the Mellon Institute, 4400 Fifth Ave., Pittsburgh, Pa. Papers are invited on the following topics: theory, new types of balances, difficulties, accessory equipment, other microweighing methods, and applications. Titles and abstracts should be submitted by March 26 to Mr. F. A. Brassart, Westinghouse Research and Development Center, Beulah Rd., Churchill Borough, Pittsburgh 35, Pa.

Testing Conference of TAPPI

The 14th Testing Conference of the Technical Association of the Pulp and Paper Industry (TAPPI) will be held November 12 to 14 at the Fontainebleau Motor Hotel Inn in New Orleans, La. Nineteen papers will be presented dealing with new paper testing methods, an improved instrument for meas-

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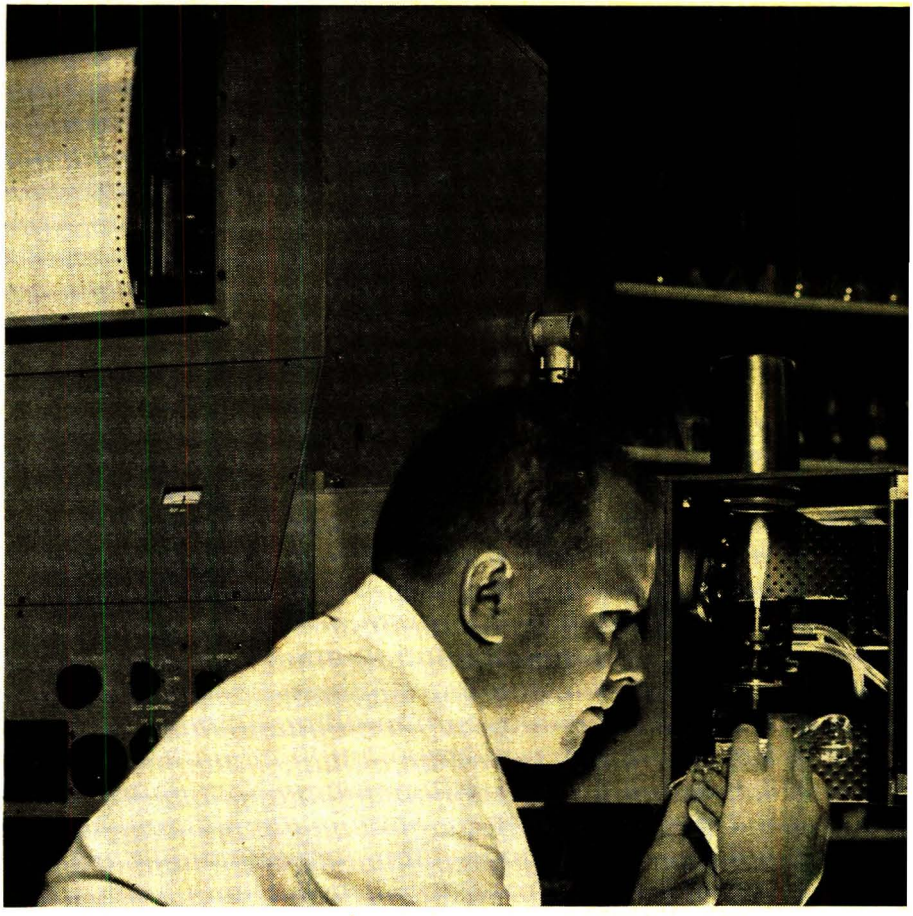

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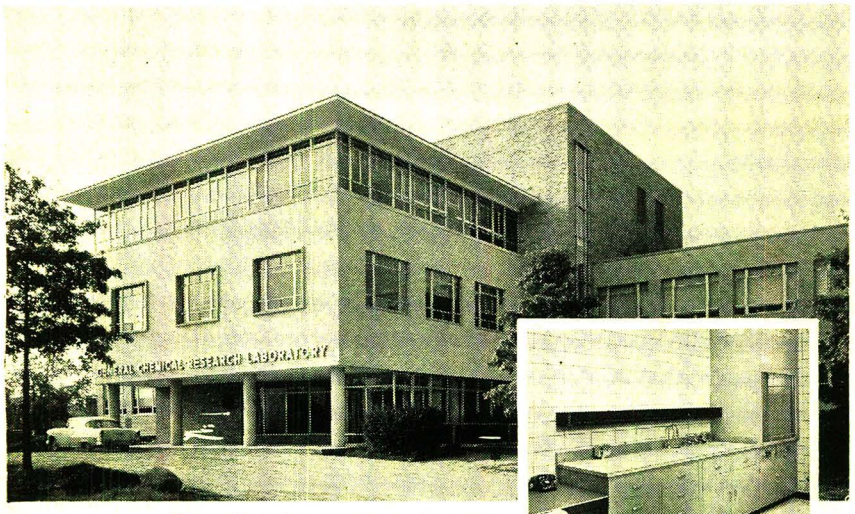

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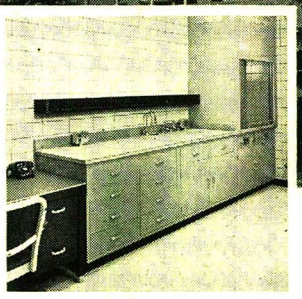
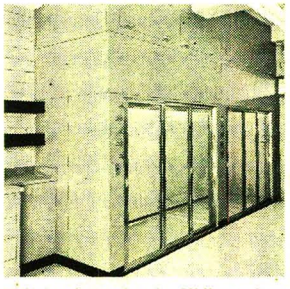
General Chemical Division of Allied Chemical Corp. has established new centralized research facilities in Morristown, New Jersey. The extensive laboratories are equipped with Conflex laboratory furniture throughout. Blickman specialized hoods for handling hazardous materials are also part of the installation. To learn more about versatile Conflex equipment and how well it can serve your laboratory, request literature or ask for a representative to give you engineering assistance.



Counter assembly with fume hood at right. Drawers and cupboards are interchangeable after installation.



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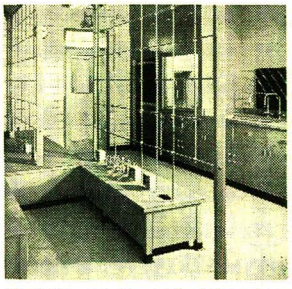

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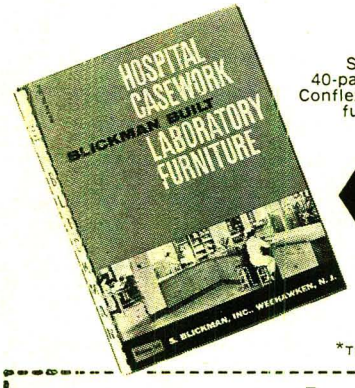

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uring adhesive bonding, linerboard testing at the mill, and a field comparison of paper sacks and polyethylene bags, and other subjects. More information is available from M. A. Burnston, TAPPI, 360 Lexington Ave., New York, N. Y., 10017.

Tracer Methodology

The Eighth Annual Symposium on Advances in Tracer Methodology will be held at the Blackstone Hotel, Chicago, Ill., Friday, November 8, 1963. These symposia are sponsored by New England Nuclear Corp. and Packard Instrument Co. to provide a forum for the free exchange of information on new and recent developments in the use of radioactive tracers. The program will include talks on analytical techniques, biosynthetic methods, and other applications. Registration and admission are free. Further information is available from Paul A. McNulty, New England Nuclear Corp., 575 Albany St., Boston 18, Mass.

Two Programs in Gas Chromatography

An advanced research conference and a short course in gas chromatography are offered by the University of California, Los Angeles, in February 1964. The two programs are independent but both are jointly sponsored by the Dept. of Chemistry, Engineering Extension, and Physical Sciences Extension, with Dr. Robert L. Pecsock as the Coordinator.

The research conference, February 3 and 4, will present prominent gas chromatographers who will discuss "Progress in Gas Chromatography," emphasizing their own work. The fee for this conference is $25.

An intensive three-day course, "Fundamental Principles of Gas Chromatography," February 5 to 7, is offered industrial personnel and workers in academic and government laboratories. The approach will be nonmathematical, stressing theory only to the extent necessary to understand the practical aspects and to obtain optimum results.

In addition to lectures the course will include laboratory demonstrations by several chromatograph manufacturers. The fee for this course is $100.

Additional information and application blanks may be obtained from H. L. Tallman, Physical Sciences Extension, Room 6532 Engineering Bldg., University of California, Los Angeles 24, Calif.

CALENDAR OF EVENTS

Oct. 7 to 10	**National Institutes of Health 13th Annual Instrument Symposium and Research Equipment Exhibit.** NIH, Bethesda 14, Md. *Contact:* Dr. Fred Alt, National Institutes of Health, Bethesda 14, Md. Page 47 A, Sept.
Oct. 8 to 10	**Seventh Conference on Analytical Chemistry in Nuclear Technology,** Sponsor: Oak Ridge National Laboratory. Conference subject: Analytical Instrumentation. *Contact:* C. D. Susano, Oak Ridge National Laboratory, P. O. Box X, Oak Ridge, Tenn. Page 76 A, Sept.
Oct. 14 to 17	**77th Annual Meeting of the Association of Official Agricultural Chemists.** Shoreham Hotel, Washington, D. C. *Contact:* Mr. Luther G. Ensminger, Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington 44, D. C. Page 41 A, July.
Oct. 14 to 18	**Second National Meeting of the Society for Applied Spectroscopy.** San Diego, Calif. *Contact:* Earl P. Wadsworth, Jr., San Diego State College, San Diego 15, Calif. Page 56 A, Oct.
Oct. 16 to 18	**Tenth National Vacuum Symposium.** Statler Hilton Hotel, Boston, Mass. Sponsor: American Vacuum Society. *Contact:* G. H. Bancroft, 398 Kilbourn Rd., Rochester 18, N. Y. Page 56 A, Apr.
Oct. 21 to 23	**Eleventh Detroit Anachem Conference.** McGregor Memorial Conference Center, Wayne State University, Detroit, Mich. *Contact:* Mr. C. M. Gambrill, Ethyl Corp., 1600 West 8 Mile Rd., Detroit 20, Mich. Page 71 A, Sept.
Oct. 23 to 25	**Optical Society of America Meeting.** Sherman House, Chicago, Ill. *Contact:* Exec. Sec., Optical Society of America, 1155 Sixteenth St., N. W., Washington 6, D. C.
Oct. 30 to Nov. 1	**National Convention of the American Association of Textile Chemists and Colorists.** Statler Hilton Hotel, Boston, Mass. *Contact:* Stanley M. Suchecki, P. O. Box 685, Ridgefield, Conn.

Coming Events

Nov. 6 to 8—Pittsburgh Diffraction Conference. Mellon Institute, Pittsburgh, Pa. *Contact:* W. M. Biagas, Crucible Steel Co., P. O. Box 7257, Pittsburgh 13, Pa. Page 42 A, July.

Nov. 7 to 8—Seventh Annual Technical Conference of the Chemical Div. of the American Society for Quality Control. St. Paul, Minn. *Contact:* Bruce A. Drew, The Pillsbury Co., 311 Second St., S.E., Minneapolis 14, Minn. Page 60 A, Aug.

Nov. 12 to 14—Fourteenth Testing Conference of the Technical Association of the Pulp & Paper Industry (TAPPI). Fountainebleau Motor Hotel Inn, New Orleans, La. *Contact:* M. A. Burnston, TAPPI, 360 Lexington Ave., New York 17, N. Y.

Nov. 13 to 15—Eastern Analytical Symposium. Statler Hilton Hotel, New York City. *Contact:* James F. Cosgrove, Gen. Telephone & Electronics Labs, Bayside 60, N. Y. Page 56 A, Aug.

Nov. 14 to 16—ACS Southeastern Regional Meeting. Charlotte, N. C. *Contact:* V. S. Salvin, Celanese Fibers Co., P. O. Box 1414, Charlotte, N. C. Page 41 A, July.

Nov. 18 to 20—Conference on Modern Developments in Analytical Chemistry. The Rice Hotel, Houston, Texas. Sponsor: The Robert A. Welch Foundation. *Contact:* W. O. Milligan, Director of Research, The Robert A. Welch Foundation, 20th Floor, Bank of the Southwest Bldg., Houston 2, Texas. Page 64 A, Oct.

Nov. 18 to 21—American Nuclear Society Winter Meeting. New York City. Includes AtomFair and 11th Hot Laboratory and Equipment Conference. *Contact:* American Nuclear Society, 244 E. Ogden Ave., Hinsdale, Ill. Page 51 A, June.

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

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.

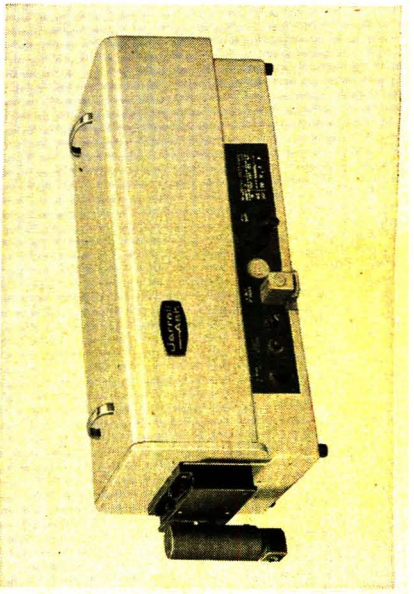
Feb. 27 to 29—Fifth Omnibus Conference on Experimental Aspects of NMR Spectroscopy. Mellon Institute, Pittsburgh, Pa. *Contact:* Mellon Institute, 4400 Fifth Ave., Pittsburgh, Pa.

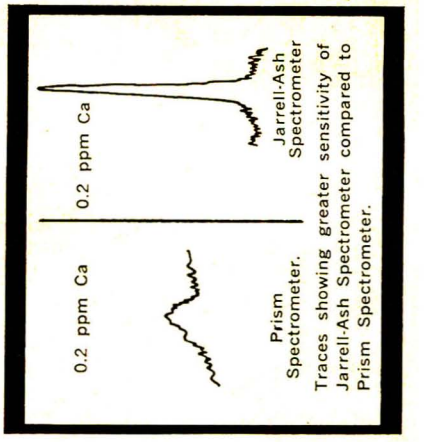
Mar. 2 to 6—Fifteenth Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. Penn-Sheraton Hotel, Pittsburgh, Pa. *Contact:* Rudolph B. Fricioni, Allegheny Ludlum Steel Corp., Corporate Quality Control Laboratory, Research Center, Brackenridge, Pa. Page 81 A, Sept.

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, Houston, Texas. Page 81 A, Sept.

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.





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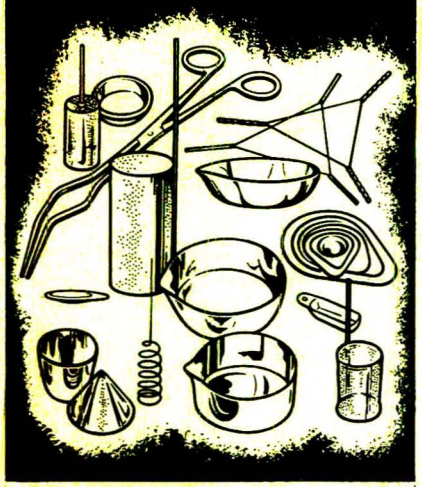

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Chromatography Course

A comprehensive course in gas and thin-layer chromatography will be conducted in Stratford, Conn., during the week of Oct. 7. This is the 2nd annual chromatography course sponsored by Analytical Engineering Laboratories, Inc. (Analabs). Three days will be devoted to gas chromatography and two days to thin-layer chromatography. Designed for both experts and relative beginners, the course will include lectures and round-table discussions. For more information, write to Analytical Engineering Labs., Inc., P. O. Box 5215, Hamden 18, Conn.

Mass Spectrometer Symposium

The 5th Annual Bendix Time-of-Flight Mass Spectrometer Symposium will be held at the Carrousel Inn, near Cincinnati, Ohio, from September 30 to October 4. Guest researchers will lecture on their work with the time-of-flight spectrometer in organic structure, gas chromatography, Knudsen cell effusions, and fast reactions. Seminars on operation, application, and maintenance will be conducted by Bendix personnel.

Although the symposium will be of special interest to users of the company's instruments, anyone wishing to learn more about time-of-flight mass spectrometry is invited. A fee of $10 per day is charged. Additional information is available from D. C. Damoth, The Bendix Corp., 3625 Hauck Rd., Cincinnati 41, Ohio.

Journal of the Association of Public Analysts

A new journal, published quarterly by the Association of Public Analysts, London, S.E.1, has been announced. The publication will include analytical and technical contributions, correspondence, general items of interest, legal notes and reports of cases of special importance, and, from time to time, the official views of the Association. Analysts, consultants, chemists in the food and drug industries, and others interested in various aspects of the work of the public analyst are encouraged to use the journal for the exchange of ideas.

The annual subscription rate is 30/-d. Requests to receive this journal should be sent with remittance to Hon. Treasurer of the Association, Mr. R. C. Spalding, M.A., F.R.I.C., Public Analyst's Laboratory, County Hall, Maidstone, Kent, England.

Industry Items

Beckman Instruments, Inc., has expanded its direct marketing organization to include France and the Republic of South Africa. The new sales organization will be headquartered in Paris and will consist of more than 100 technical marketing and support personnel.

Beckman Instruments, Inc., has acquired **Sharp Laboratories, Inc.,** of La Jolla, Calif. The Sharp company adds new capabilities in nuclear instrumentation to Beckman's existing lines of analytical and control instruments of scientific, industrial, and medical use.

The Brown Instrument Co. will henceforth be known as the Philadelphia division of **Honeywell's** Industrial Products Group. Honeywell acquired the Brown Instrument Co. in 1934.

The Chemical Rubber Co. has opened a new supply center at 9974 Springfield Pike, Cincinnati, Ohio. The facility will stock over 20,000 different laboratory items for same-day deliveries to area customers.

Connecticut Instrument Co. of Wilton, Conn., is now the Instrument Division of **Barnes Engineering Co.,** 30 Commerce Rd., Stamford, Conn.

General Atomic Division of the **General Dynamics Corp.,** P. O. Box 608, San Diego 12, Calif., has recently installed two complete neutron generator facilities to aid in the "Participant Program in Activation Analysis." This program permits work by scientists on special problems of their own using the company's activation analysis facilities. Effective Sept. 1, the charge for this program is $500 for a one-week period.

The Greiner Scientific Corp. and **Palo Laboratory Supplies, Inc.,** have merged. All equipment and personnel have been brought together at the Greiner Bldg., 22 North Moore St., New York City.

Ionics, Inc., Cambridge, Mass., has announced the establishment of an environmental radiochemical analyses service. The company will analyze solid and liquid samples for environmental radiochemical contaminants—radium, thorium, etc.

The **Jarrell-Ash Co.,** 26 Farwell St., Newtonville 60, Mass., has added the new Laser Microprobe developed by the company to its range of analytical services. The Analytical Services Laboratory facilities include x-ray dif-

fraction, spectrographic, fluorometric, atomic absorption, chromatographic, and chemical analysis. The microprobe permits spectrographic analysis of precise micro-areas of any sample.

The Materials Analysis Co., 81 Encina Ave., Palo Alto, Calif., manufacturer of the Model 400 electron microprobe analyzer, has expanded its facilities to include additional space for production and development work.

The Matheson Co., Inc., East Rutherford, N. J. has acquired the Harshaw Scientific Div. of the **Harshaw Chemical Co.,** Cleveland, and the **Chicago Apparatus Co.,** Chicago. A nationwide sales organization has been established as a wholly owned subsidiary called the **Matheson Scientific Co.**

Packard Bell Computer and **Computer Products, Inc.,** have reached an agreement to undertake joint design, development, manufacturing, and marketing of large hybrid computing systems which involve both analog and digital computers. Packard Bell Computer, a division of **Packard Bell Electronics,** presently located in Los Angeles, is building new headquarters in Santa Ana, Calif.

Research, Inc., Minneapolis, has formed two new divisions and expanded plant site facilities. The newly formed operations are the Materials Test Systems Division (MTS) and the Controls Division. The Controls Division will concentrate on instruments and closed-loop programming, temperature, and current control. The MTS Division will focus on closed-loop electro-hydraulic systems and components for material testing, structures testing, fatigue studies, and hydrotest or rapid pressurization testing.

Resources Research, Inc., Washington, D. C., has become a subsidiary of **Hazleton Laboratories, Inc.,** of Falls Church, Va.

Scientific Kit Co., P. O. Box 244, Washington, Pa., announces that it will distribute the products of **Gas Chromatography, Ltd.,** of England in the United States and Canada. Scientific Kit will act as sole agent and sell through established laboratory supply dealers.

Texas Instruments, Inc., will build an electrical and thermostatic controls plant in Ontario, Canada. Texas Instruments has maintained sales and service offices in Canada for many years, but this will be the first manufacturing operation located there.

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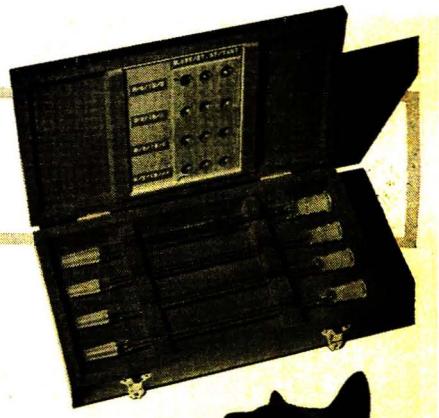

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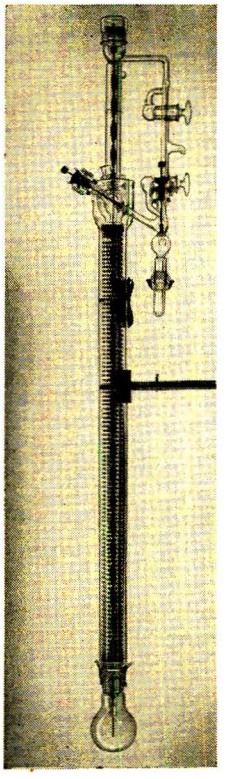

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The columns contain spiral screen bands having 300 teeth per inch contacting the walls of the still. This construction provides violent agitation of the reflux liquid and gives intimate contact of descending and ascending vapors. The reflux ratio of the still is controlled by means of an adjustable needle valve eliminating the possibility of leakage.

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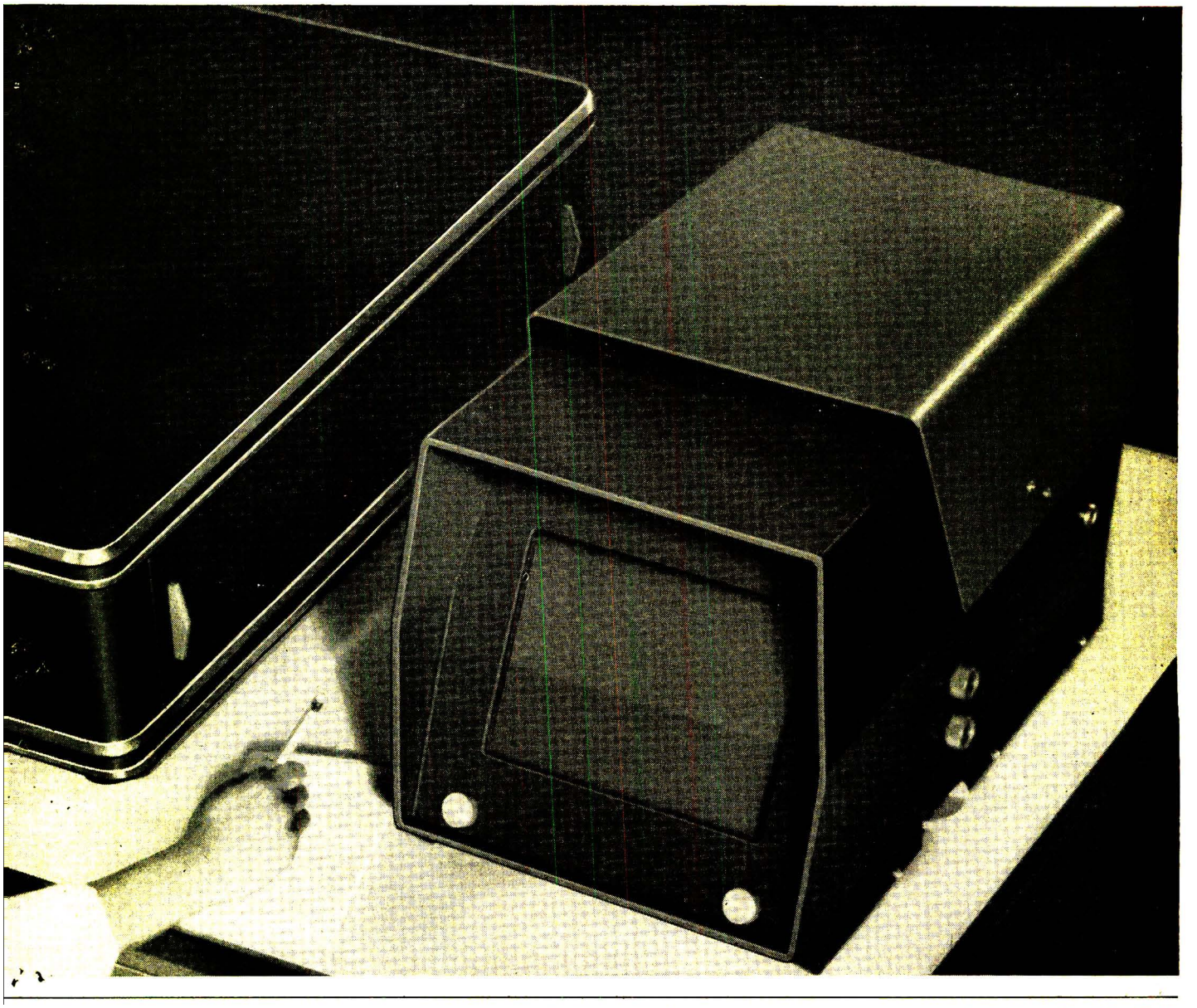

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It incorporates a modified Wadsworth mount with a focal length of 0.855 meters. Special design provides a dispersion of 6.08 A/mm in the ultraviolet region. Resolving power is attained through the use of a Bausch & Lomb Certified Precision Grating of 960 lines/mm giving wavelength coverage of 4500-9300A in the first order and 2250-4650A in the second order. Viewer-comparator is equipped with a master film of the more important spectrum lines of common elements.

Optics are factory calibrated and no adjustments are necessary. Temperature variation from 65° to 95°F can be tolerated without any detectable change in focus. All units are totally enclosed for safety and are equipped with safety interlock switches. The complete instrument fits easily on the top of an ordinary desk.

It will pay you to investigate the ARL Spectrographic Analyzer. Demonstrations may be arranged in your laboratory. For information or brochure, write to Applied Research Laboratories, 20200 West Outer Drive, Dearborn 8, Michigan or contact your nearest Applied Research Laboratories sales office.

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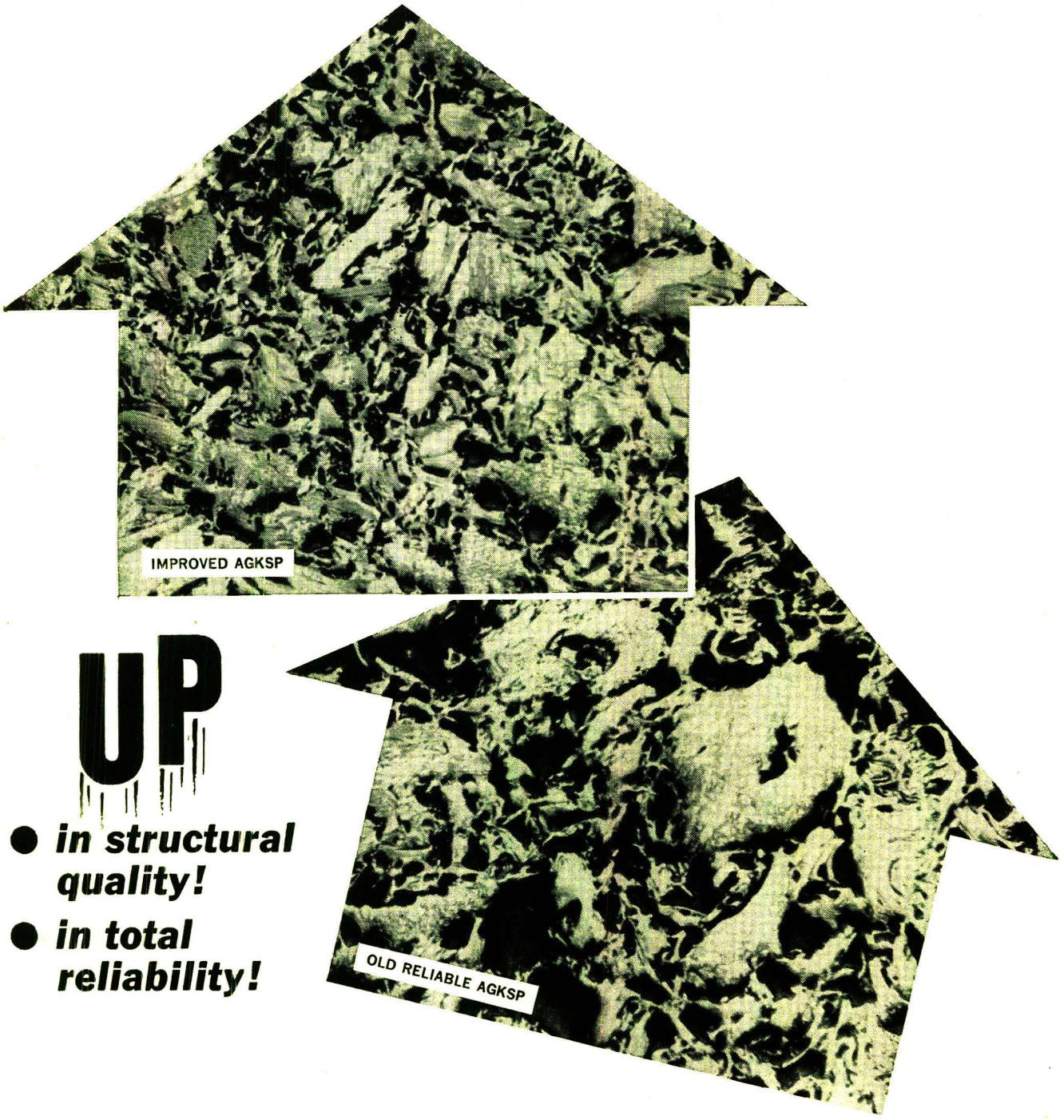

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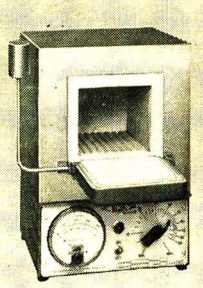

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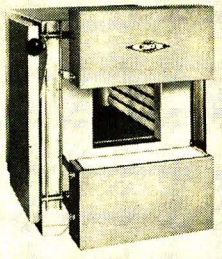

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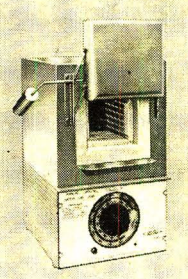

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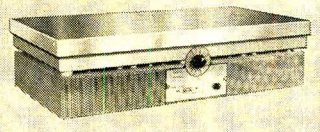

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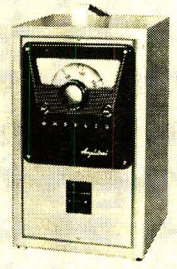

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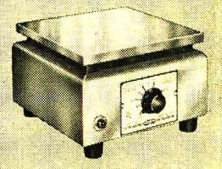

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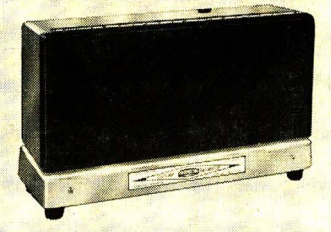

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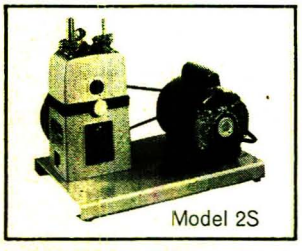
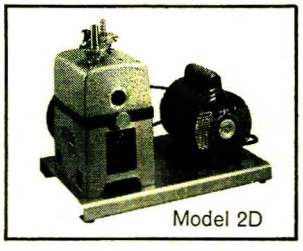
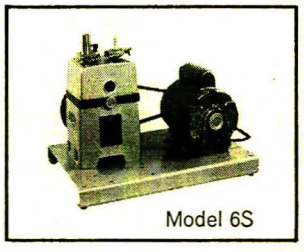
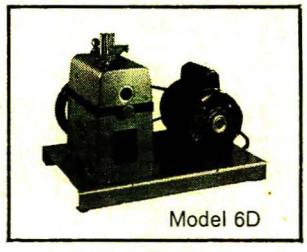

Model 2S


Model 2D


Model 6S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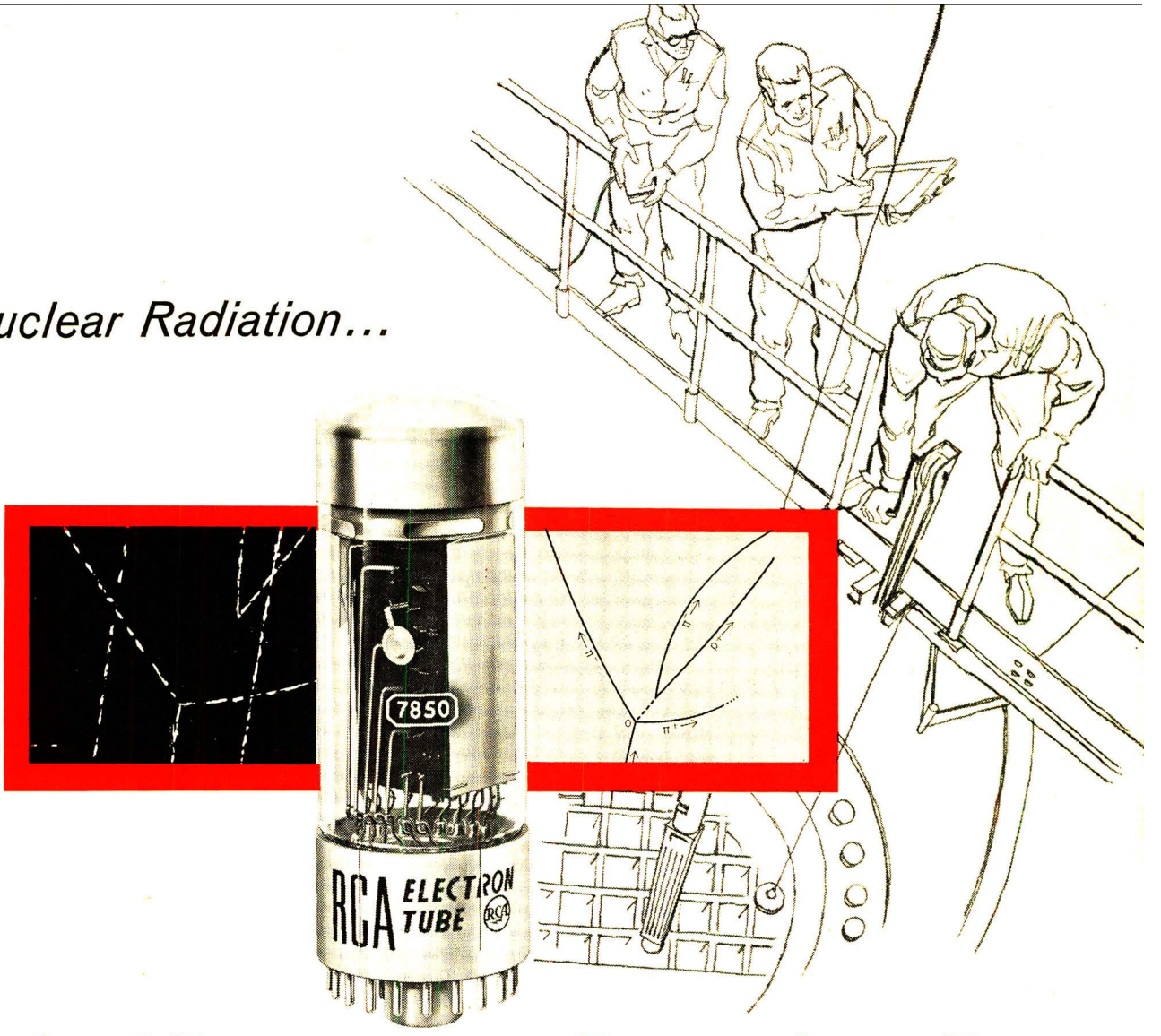
Nuclear Radiation...



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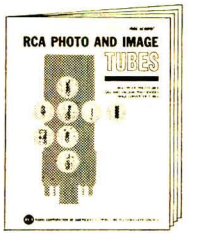

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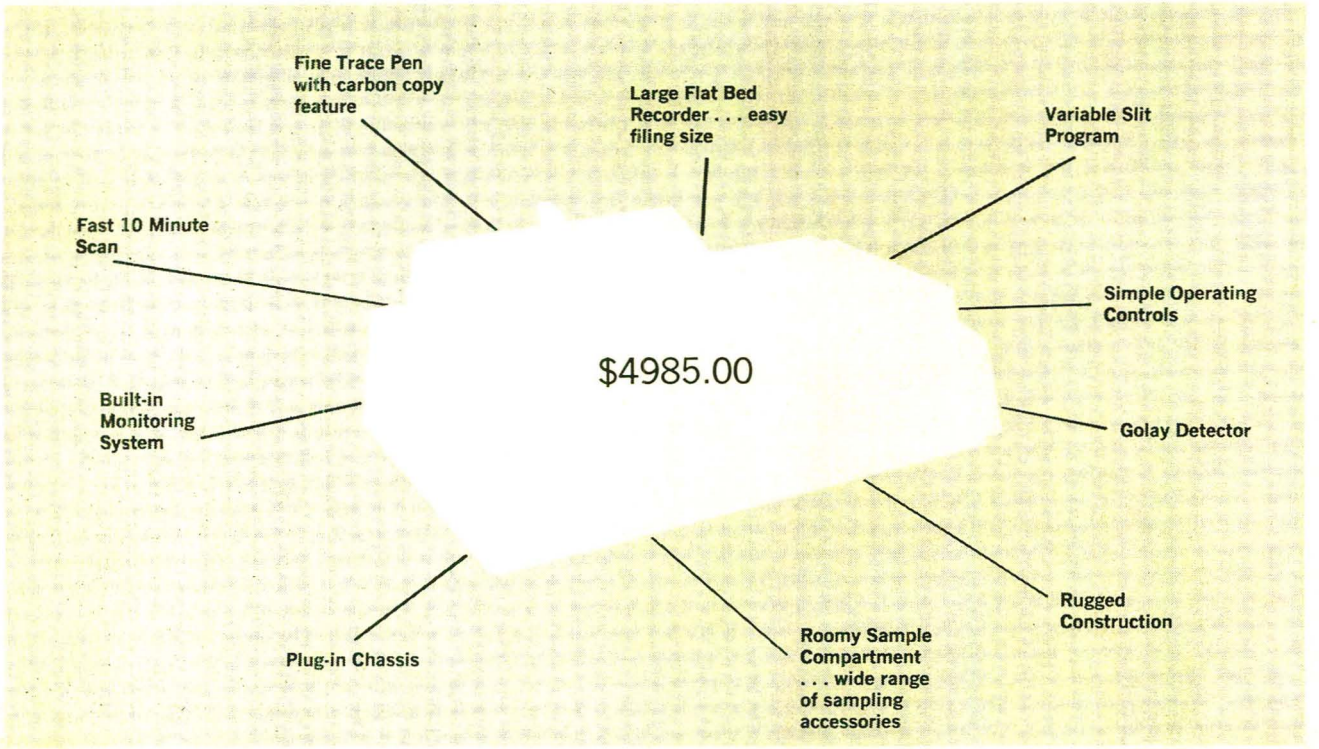

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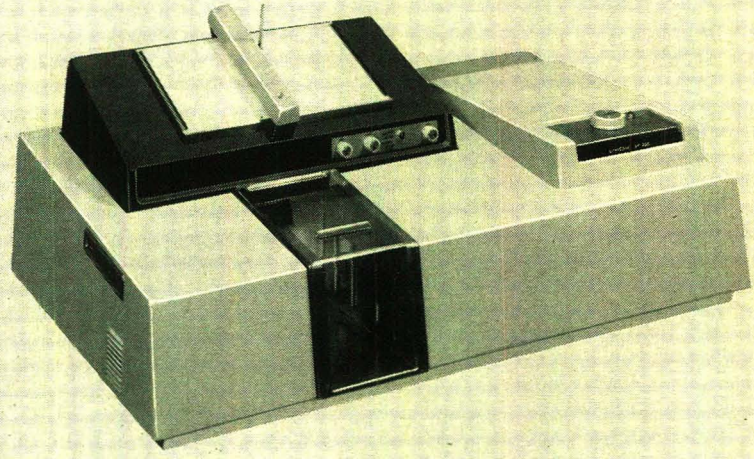

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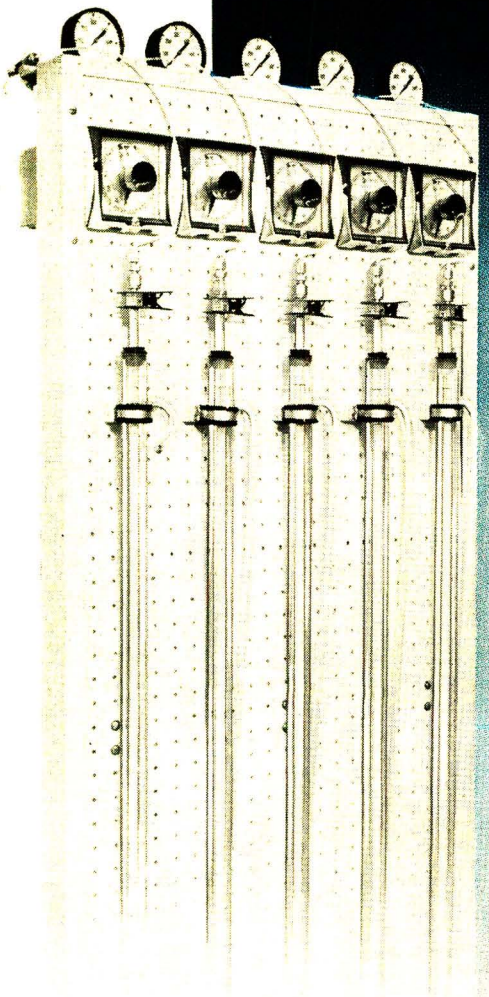

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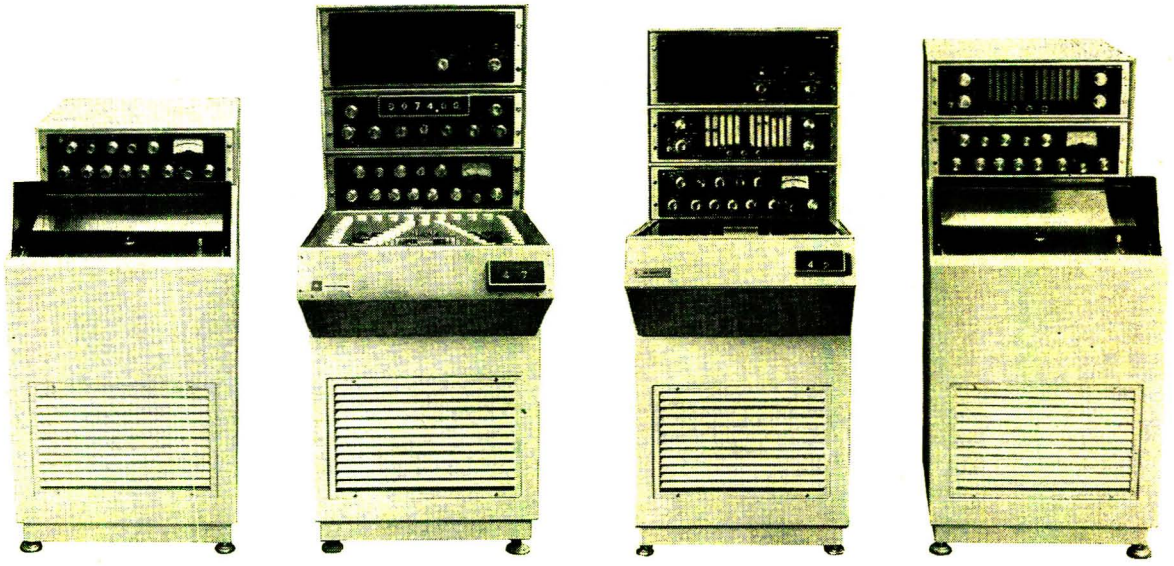

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Methods of Biochemical Analysis. *Vol. II. David Glick, editor. ix + 442 pages. Interscience Publishers, Inc., 440 Park Ave., South, New York 16, N. Y. 1963. $14.50.*

Reviewed by John C. Wriston, Jr., Associate Professor, Department of Chemistry, University of Delaware, Newark, Del.

The latest volume in this annual series contains eight chapters: Analysis of Biological Materials by Atomic Absorption Spectroscopy, by J. B. Willis; Separation and Determination of Steroids by Gas Chromatography, by E. C. Horning, W. J. A. Vanden Heuvel, and B. G. Creech; Advances in Direct Scanning of Paper Chromatograms for Quantitative Estimations, by I. E. Bush; Automated Methods for Determinations of Enzyme Activity, by Morton K. Schwartz and Oscar Bodansky; Estimation of Magnitudes of Alternative Metabolic Pathways, by Irwin J. Kopin; Determination of Coenzyme Q (Ubiquinone) by F. L. Crane and R. A. Dilley; The Measurement of Carbonic Anhydrase Activity, by Robert P. Davis; and Polarographic Analysis of Proteins, Amino Acids, and Other Compounds by Means of the Brdička Reaction, by Otto H. Müller. The reviews seem to fulfill the editor's intention of providing a discussion of background and previous work, a critical evaluation of the various approaches, and a presentation of the procedural details of the method or methods recommended by the author. The chapter by Horning *et al.*, on the gas chromatography of steroids (with thin-film columns) seems particularly timely and well organized. The review by Müller, on the polarographic analysis of proteins and other materials by means of the Brdička reaction, while presenting a wealth of information, seems to suffer from the burden of having to deal with earlier claims in the literature with regard to the application of the reaction. It would appear that a considerable amount of experience with polarography would be needed before the methods described could be applied.

Electronic Circuits and Instrumentation Systems. *Jack J. Studer. xiii + 423 pages. John Wiley and Sons, Inc., 440 Park Ave., South, New York 16, N. Y. 1963. $10.75.*

Reviewed by D. J. Fisher, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Studer teaches a one-semester electronic instrumentation course for nonelectrical-engineering majors for which this book is the text. A judicious selection and organization of topics and a clear, logical treatment that omits no details essential for attaining basic understandings of instrumentation circuits and systems are decisive. Appropriately, Studer covers circuit analysis, electronics, and systems, in that order. This scope is enormous for a one-semester course. Though time is severely limited, superficial treatment which would only waste time and create misconceptions must be avoided. This reviewer believes that important topics that cannot be discussed in a short course should at least be mentioned with definitive references to avoid oversimplification and to encourage additional work by those having time and motivation. Examples of topics that this reviewer believes should have at least been mentioned in this book include: chopper-stabilized amplifiers; operational amplifiers and systems; analysis of system stability; regulated power supplies; field-effect transistors; velocity servos; synchros; and simple systems such as potentiostats or those for conductivity, polarography, constant-current, or controlled-potential coulometric titration. This book might be expected to prepare one to select a system or circuit, (but not to design complex circuits), to predict or to recognize artifacts in data due to electronic shortcomings, and to at least work efficiently with design experts to select, modify, or produce instruments that will provide adequate data for a job. Although omission of certain topics is unfortunate, this book does provide a good basis for using many circuits and systems with insight.

This book has a very conversational style which increases intelligibility and which might facilitate its use for self-teaching. Questions often introduce and emphasize major points; answers are usually clear. There are many illustrations and solved examples. Prior knowledge of calculus and physics—e.g., Ohm's law—is required and assumed. Laboratory experiments, operating instructions, or construction diagrams are not included. The lack of laboratory experiments could be unfortunate; again, time is very short. Appendixes cover Algebra of Complex Numbers and Fourier's Series; for most chapters there is a bibliography of recommended reading and a set of problems.

Part One. A good foundation for circuit analysis is provided starting with sinecosine equations and phasor diagrams but emphasizing the use of complex-number equations. Techniques are included of simplifying the analysis of linear circuits by network reduction and by use of Thévenin's theorem and superposition. Bode diagrams to represent the frequency response of two-port circuits to sinusoidal signals are discussed in some detail and are also used extensively in Part Two and in Part Three. The use of Fourier analysis is shown for predicting required response. The response of linear one- and two-port circuits to sinusoidal signals and of circuits to d.c. voltage steps and square wave signals is described, respectively, in three chapters. The use of operational mathematics for circuit analysis is not mentioned. The advisability of describing this technique is currently controversial among several teaching similar courses. This reviewer agrees that a foundation must be provided prior to the introduction of operational circuit analysis to establish significance and guide its use. Is there time in a one-semester course to provide both a sound understanding of and a fast and powerful method of circuit analysis? Since this subject is not discussed, it certainly should be mentioned with references.

Part Two. Operation of vacuum and gas tubes and semiconductors are explained in detail by physical principles. Their circuits are analyzed by graphic and by small-signal equivalent circuit methods. Gain, frequency response, gain-bandwidth product, distortion and noise are comprehensively discussed for vacuum tube and transistor amplifiers. Other topics include feedback (and oscillators), rectifiers (and filtering principles), oscilloscopes, vacuum-tube voltmeters and principles of amplitude modulation and demodulation. Discussions involving junction transistor principles and circuits and amplitude modulation and demodulation are very good and are unusually complete for a nonelectrical-engineering course.

Part Three. Surveys are given of general characteristics of electronic instrumentation systems and of selected systems including those using strain gages, recording potentiometers, and those for nuclear magnetic resonance spectroscopy and infrared absorption spectrophotometry.

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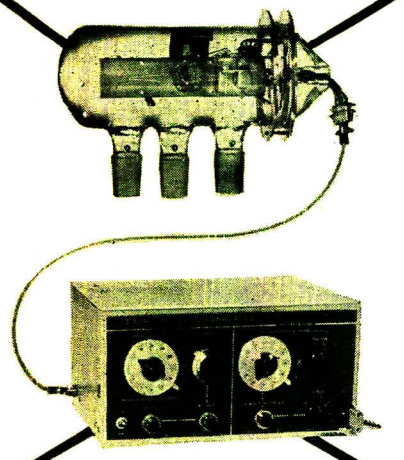

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In conclusion, it is recommended that anyone teaching or planning to teach a course of this sort or wishing to study this subject should consider this book. As is often the case in relatively new teaching fields, no one book alone is sufficient, but this book contains several excellent sections.

Gas Chromatography. *J. H. Purnell. vii + 441 pages. John Wiley and Sons, Inc., 605 3rd Ave., New York 16, N. Y. 1963. $12.*

Reviewed by W. E. Harris, Department of Chemistry, University of Alberta, Edmonton, Alta.

The author states in the preface, "It is significant that almost all major technical advances of recent years have stemmed from theoretical speculation . . . . .therefore the most profitable use of gas chromatography already demands a reasonable understanding of the basis for the choice of experimental conditions and equipment. In recognition of this situation this book contains a substantial account of both theoretical views current at this time and the efforts made to test and profit by them." The author fulfills his stated objectives very well. In a general way this book has the same scope as other recent books such as those by Littlewood and Dal Nogare and Juvet.

In the first section physical chemical background is outlined. Topics such as the partition coefficient, solubilities of gases in liquids, vaporization of liquids, diffusion, and flow of gases are covered in a competent manner.

A second major section of the book deals with theoretical background specifically desirable for an understanding of the gas chromatographic process. The author presents both a well integrated discussion of theory and a review of the experimental tests of the theories presented. In this section an excellent treatment is given of the problems involved in establishing optimum conditions for fast analysis.

The last section is concerned with practice. The wide experience of the author is quite evident and any potential practicing chromatographer could read this section with profit. There are sections dealing with size grading of the solid supports, sample introduction, analytical separations, preparative and continuous chromatography, and measurement of physical chemical quantities. There is also an extensive section on detectors. Special analytical techniques involving such matters as gas-solid chromatography, pre-column abstractors, temperature programming, and multiple columns are described but the treatment here does not seem up to the standards of the other sections of the book.

The theoretical portions of the book are rigorous, so rigorous that they can probably be read easily only by physical chemists. More ordinary mortals can nevertheless profit from these sections without necessarily following all of the arguments. The difficulty of following these parts is compounded by the unfortunate omission of a glossary of symbols at the beginning or end of the book.

The illustrations are well chosen and the documentation from the original literature to 1961 is good. Inevitably there are some errors but they are mostly of a minor nature. This book is an important contribution to the consolidation of the information on gas chromatography which is increasing at a prodigious rate.

Papers on Industrial Water and Industrial Waste Water-STP 337. *72 + vi pages. American Society for Testing and Materials, 1916 Race St., Philadelphia 3, Pa.*

The introductory author asks several questions relative to the meaning of water quality. The first paper discusses setting up an organized laboratory, while another paper describes current testing methods and equipment used for water for steam generation. Methods used to accumulate data necessary to formulate a set of regulations governing the discharge of heated water into streams is discussed in the third paper. In the fourth paper, the meaning of well known methods of analysis for cyanogen compounds in the evaluation of treatment requirements and results of treatment of industrial wastes are considered, while the following paper proposes a gas chromatography technique for water plant operation. The final two papers present a reappraisal of the phenol–disulfonic acid method for the determination of nitrate, and a description of continuous automatic boiler water silica analysis.

Experimental Gas Chromatography. *James M. Miller. v + 103 pages. Gow-Mac Instrument Co., 100 Kings Rd., Madison, N. J. 1963. Paperbound. $4.*

This laboratory manual contains the information necessary for a beginner to build and use a simple gas chromatograph. The company, Gow-Mac, markets a gas chromatography kit which contains most of the parts necessary for construction of a simple inexpensive instrument.

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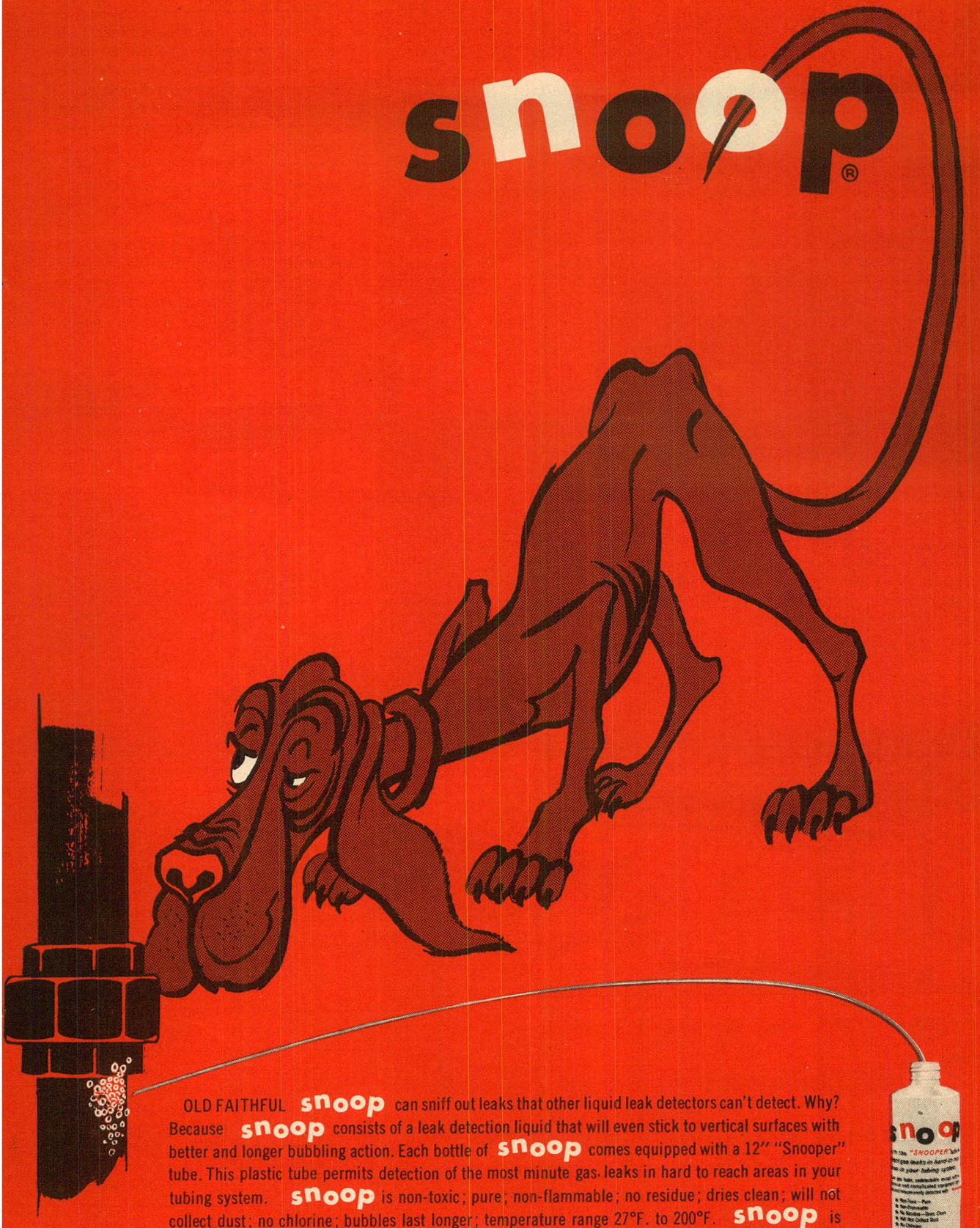

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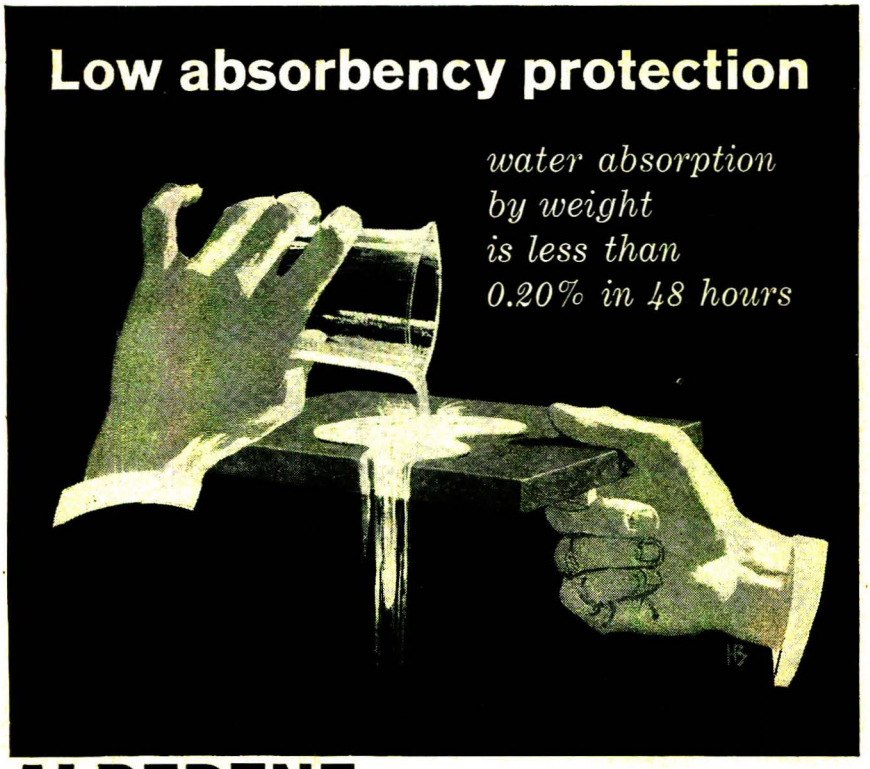

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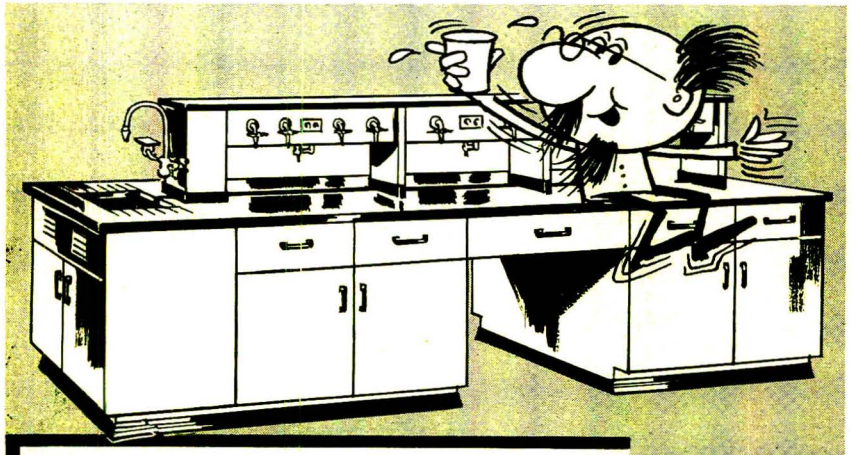

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The appendixes list suppliers of gas chromatography equipment, gas chromatography books, instructions for cleaning the thermal conductivity cell, and instructions for filament replacement.

Production and Use of Short-Lived Radioisotopes from Reactors. *Vols. I, II. International Atomic Energy Agency. National Agency for International Publications, Inc., 317 East 34th St., New York 16, N. Y. 1963. Vol. I, 433 pages, $8.50. Vol. II, 272 pages, $5.50.*

The two volumes contain the proceedings of a seminar held in Vienna, November 5 to 9, 1962. More than 150 participants from 29 countries are represented in these volumes. Of the more than 50 papers in the two volumes, 32 are in English, 16 in French, 1 in Russian, and 1 in Spanish. Non-English papers include summaries in English. Volume I covers the production, separation, and handling of radioisotopes and their application in industry, hydrology, and metallurgy. Volume II discusses activation analysis and applications in medicine and biology. Particular attention is paid to neutron activation analysis.

Molecular Stills. *P. Ridgway Watt. xi + 351 pages. Reinhold Publishing Corp., 430 Park Ave., New York 22, N. Y. 1963. $15.*

This book is a compilation of molecular distillation techniques and includes apparatus from both foreign and domestic sources. The principal aim of the book is to provide a practical guide for design, construction, and use of molecular stills encompassing both research and fractionating stills and industrial distillation equipment.

The theory of molecular distillation is covered as far as is necessary to show the operating principles and to give an appreciation of design requirements. However, the greatest emphasis is placed on the equipment itself. The chapter headings are: Historical, Principles of Molecular Distillation, Degassing, Pot Stills, Rotary Stills,

Falling-Film Stills, Wiped-Film Stills, and Centrifugal Stills.

The appendixes contain 46 pages of comments on vacuum system design and a short discussion on continuous *vs.* batch processing. There is a "Materials Handled Supplement" with recent references to materials handled in molecular stills. Finally, there are general subject, materials, and author indexes.

U. S. Government Publications

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

Radiobiological Dosimetry: Recommendations of International Commission on Radiological Units and Measurements. *National Bureau of Standards Handbook 88. April 1963. 26 pages. 25 cents.*

Handbook 88 is ICRU Report 10e, the latest in a series of publications presenting the 1962 recommendations of the International Commission on Radiological Units and Measurements (ICRU). This report deals with radiobiological dosimetry and considers methods of improving the accuracy and comparability of absorbed dose measurements. It is a handbook for the experimental radiologist and emphasizes the importance of planning experimental work to make dosimetry easier and more accurate.

Refractive Indices and Densities of Aqueous Solutions of Invert Sugar. *C. F. Snyder and A. T. Hattenburg. National Bureau of Standards Monograph 64. June 1963. 6 pages. 15 cents.*

This publication presents the refractive indices and densities of aqueous solutions of invert sugar determined at the Bureau. Data are given for concentrations up to 82% of invert sugar by weight, at temperatures of 15°, 20°, 25°, and 30° C. Five-decimal tables give the refractive indices and densities of invert sugar solutions containing 1 to 85% sugar for each percentage of invert sugar at the four temperatures.

Standard X-ray Diffraction Powder Patterns. *H. E. Swanson, M. C. Morris, Roger Stinchfield, and E. H. Evans. National Bureau of Standards Monograph 25. May 1963. 46 pages. 35 cents.*

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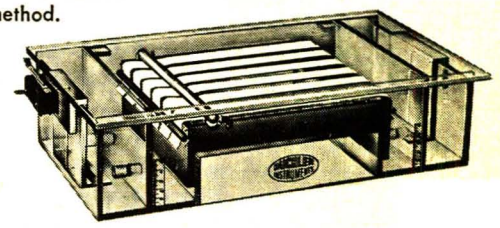

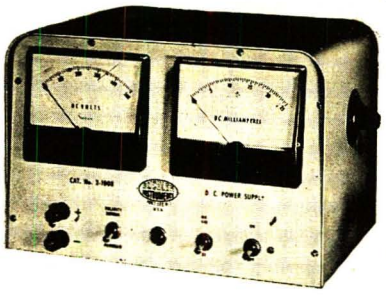

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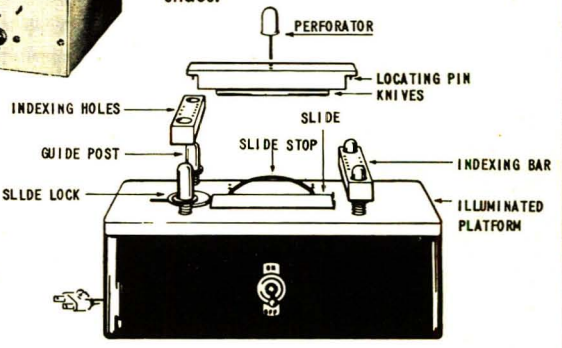

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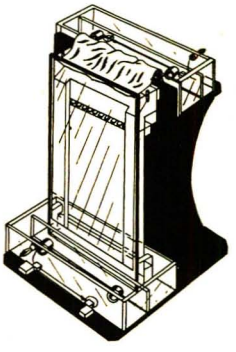

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By R. Stock and C. B. F. Rice

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

Automatic Mass Spectrometer for Monitoring Uranium Isotope Concentrations. *A. Langdon et al. Union Carbide Nuclear Co. for the U. S. Atomic Energy Commission. January 1963. 17 pages. Order K 1445. 50 cents.*

Two instruments were designed. Each instrument is fully automatic and capable of making a uranium isotope determination every two and one half minutes. The results are recorded directly in weight per cent U^{235}. These low-cost monitoring spectrometers were operated reliably for more than seven years.

Development of Krypton-85 as a Universal Tracer. *D. Chleck et al. Parametrics, Inc., for the U. S. Atomic Energy Commission. March 1962. 126 pages. Order NYO 9720. $2.50.*

Krypton-85 is used for corrosion and wear, and chemical aging studies. Two methods are given for incorporating krypton-85 into a variety of solid materials. In the first method the krypton is ionized and then impelled into the solid under the influence of a potential drop. In the second method, the solid is placed in contact with the gas at high temperature and pressure; diffusion of the gas into the solid takes place. The surface location of the radioisotope combined with the temperature dependent loss makes possible the measurement of surface and interfacial temperatures.

The Effects of Ion Hydration in Solvent Extraction and Ion Exchange. *D. C. Whitney. Lawrence Radiation Laboratory for the U. S. Atomic Energy Commission. October 1962. 186 pages. Order UCRL 10505. $2.75.*

The experimental data given in this report deal with processes involving the transfer of either ions or dipolar molecules from an aqueous phase to one that is less aqueous in terms of its physical and chemical properties, within a system where the two phases are readily distinguishable. The following factors were found to be important in these processes: water-water, ion-water, and ion-ion interactions in the aqueous and resin phases, and dipole-water, dipole-ion, and dipole-dipole interactions in the organic solvents phase.

This work is aimed at developing a comprehensive theory of ion exchange and solvent extraction.

Evaluation of Diethers as Uranium Extractants. *J. M. Googin. Union Carbide Nuclear Co. for the U. S. Atomic Energy Commission. January 1963. 28 pages. Order Y-1408. 75 cents.*

The three dialkoxypentanes, 1,5-dimethoxypentane, 1,5-diethoxypentane, and 1-ethoxy-5-ethoxypentane, can be used for uranium extraction from impure solutions. The highest uranium distribution coefficient is found with 1,5-dimethoxypentane, while the lowest is found with 1,5-diethoxypentane. The extraction power on an ether is shown to be a function of its carbon-to-oxygen ratio and its dipole moment.

Thermal Analysis and Gradient Quenching Apparatus and Techniques for the Investigation of Fused Salt Phase Equilibria. *H. A. Friedman et al. Union Carbide Corp. for the U. S. Atomic Energy Commission. 32 pages. Order ORNL 3373. 75 cents.*

Apparatus and methods were devised for determination of high temperature equilibrium phase relationships in condensed systems of molten salts. Emphasis is given experimental techniques required for investigation of nonvolatile hydroscopic fluorides. Measurements are made of the thermal effects occurring on melting and freezing polycomponent mixtures, and others in which unequivocal identification of solid phases formed during crystallization is obtained.

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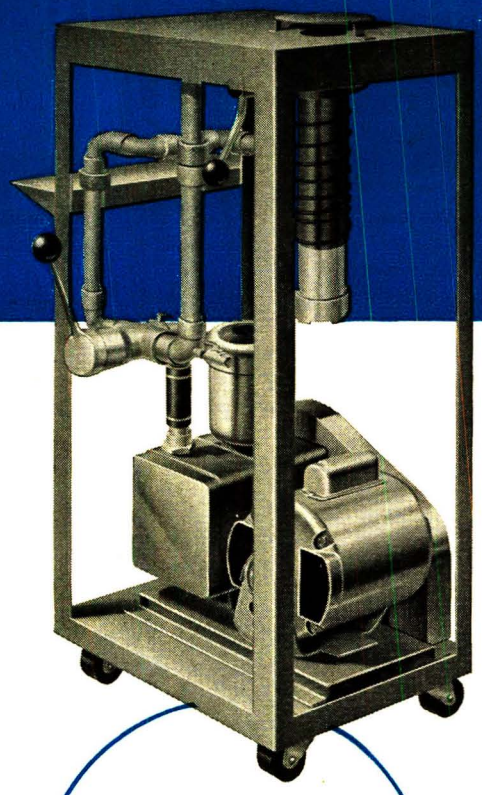

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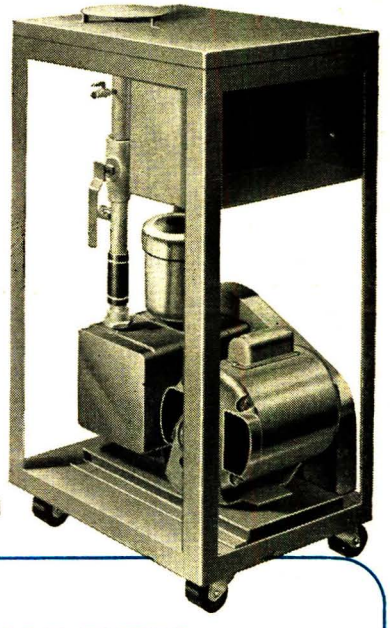

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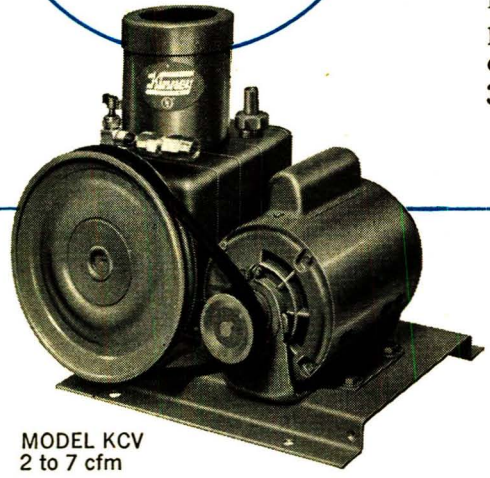

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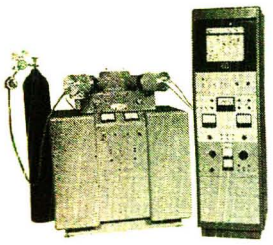

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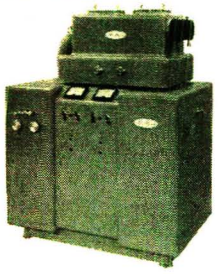

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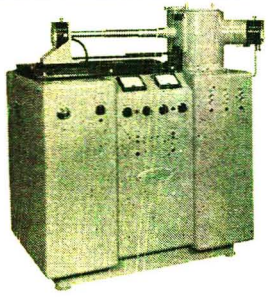

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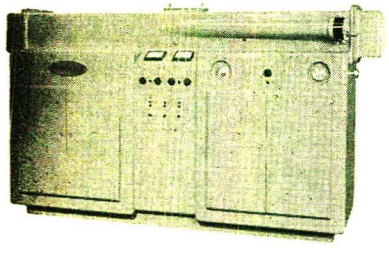

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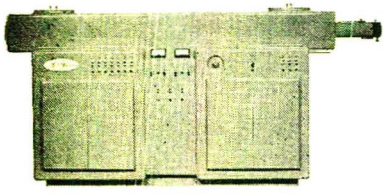

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Many major advantages never before available *in combination* in a single instrument are now encompassed in the new McPherson Vacuum U. V. Scanning Monochromator, Model 225.

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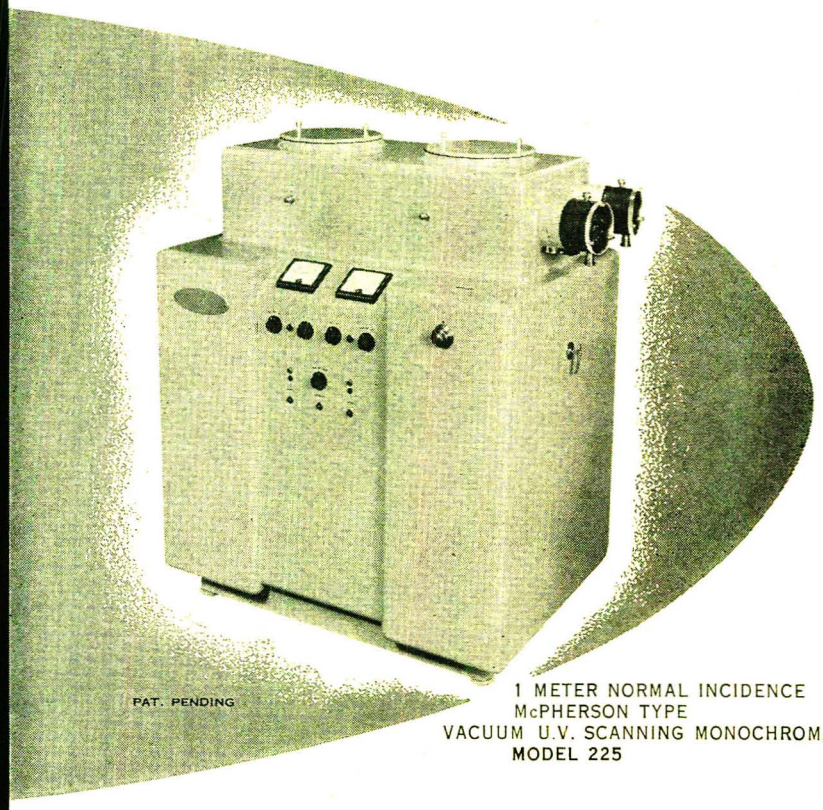

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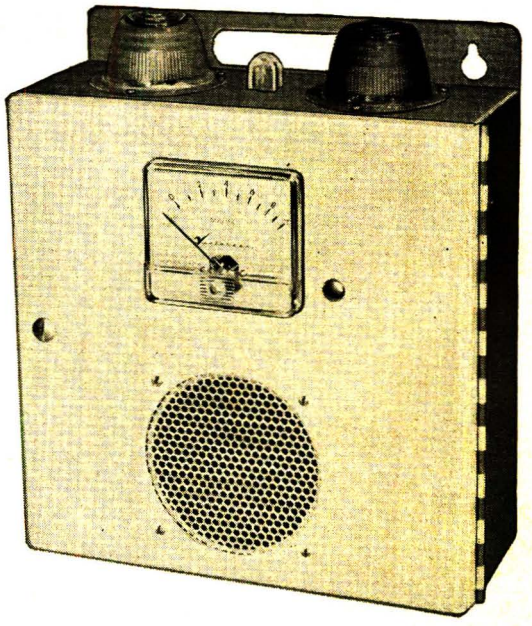
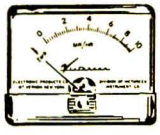
gives dependable, **continuous monitoring to comply with** *tighter AEC standards*

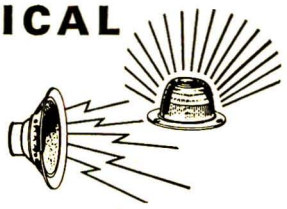


PRACTICAL

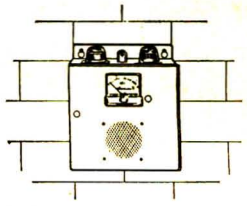


Indicates beta-gamma — full scale ranges of 0-10 or 0-50 mr/hr.

Internally adjustable alarm point, from 20 to 100% full scale, prevents unauthorized tampering.



Presettable visual and audible alarm system.

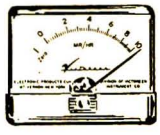


Easily installed. Can be mounted to wall, used on table top or transported to any location.

FAIL-SAFE

White pilot light shows if A-C power is On.

Amber failure light indicates when instrument is not continuously reading built-in check source.



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Bright flashing red alarm light.

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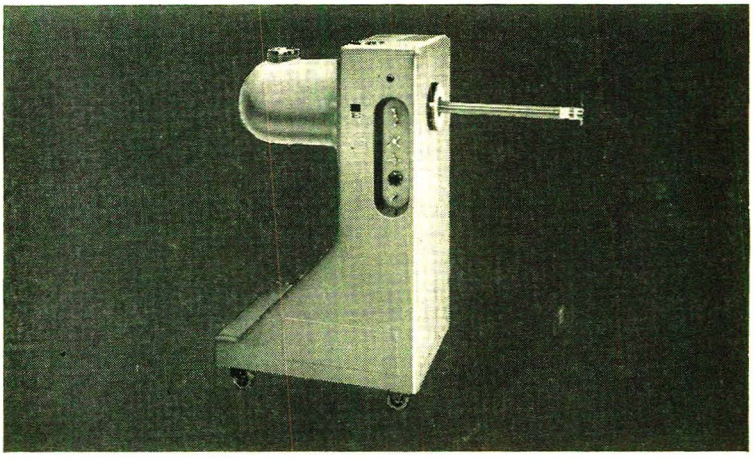
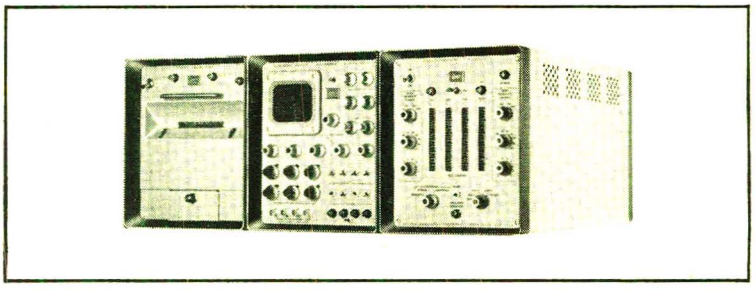
Activation and Analysis from TMC

the activation . . . The Ellison Activatron-110. This 150 KV neutron generator is a simply designed unit that will produce up to 14 MEV neutrons from a Deuteron-Tritium reaction. Its size, mobility and high yield — more than 10^{10} n/sec — make it an excellent choice for the laboratories of radiochemists, geochemists, criminologists, metallurgists and other scientists who need efficient activation analysis in a manageable, moderately priced setup. The Activatron has a unique ion source that takes its DC operating voltage from the 150 KV accelerating voltage. No electronic circuits or isolation transformers are needed. The main advantage of this design is that both the accelerating tube and the source are immersed in an oil tank; the outside case is at ground potential for the complete safety of the operators.

Complete specifications and prices are contained in our bulletin describing the Activatron-110 and the 400 series analyzers (below).





and the analysis . . . The portable 400 series 400-channel pulse analyzer handles the accumulation and storage of the gamma ray pulses from the sample activated by the Activatron-110. It will store data in memory groups of 400 channels (1 group); 200 channels (2 groups); 100 channels (4 groups). Data can also be transferred from one half of the memory to the other, or from one quarter to another within the same half. Data can be examined while it is being accumulated on the cathode ray tube display in the analyzer. For analog readout, paper tape punch, reader, IBM typewriter and digital printers may be used.

accessories The Model 522 pushbutton Resolver/Integrator functions as either a resolver or an integrator and does not need intermediate taping equipment. As a resolver it makes data available from a quarter or a half of the analyzer memory and adds it to or subtracts it from data in the adjacent quarter or half. This enables the operator to remove reference peaks from a complex spectrum and leave only the unknown elements. As an integrator it integrates memory-stored information in any band of channels from 0 to 399 in one operation and a choice of two modes — Normal (summation and total in the last channel); Subtotal (running subtotal).

The Model 500P High Speed Printer prints out channel number and data on 3-inch wide paper tape at 5 lines per second. It has a built-in high voltage supply that will power up to four detectors.

Your nearest TMC Sales-engineering office can provide you with complete specifications and price or you can write the main office for a copy of the latest activation analysis bulletin. Technical Measurement Corporation, 441 Washington Avenue, North Haven, Connecticut. Tel. 239-2501.

DOMESTIC: Gardena, Calif.; San Mateo, Calif.; La Grange, Ill.; Silver Spring, Md.; Stoneham, Mass.; White Plains, N. Y.; Oak Ridge, Tenn.; Dallas, Texas.
IN CANADA: Allan Crawford Associates, Ltd., 4 Finch Avenue, W., Willowdale, Ontario, Canada.
IN EUROPE: Technical Measurement Corporation, GmbH, Mainzer Landstrasse 51, Frankfurt/Main, Germany.
IN JAPAN: Nichimen Company, Ltd., Muromachi, Nihonbashi, Chuo-Ku, Central P.O. Box #1136, Tokyo, Japan.

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2810*	15 ml, 15 mm x 119 mm	29.70	28.05	26.40
1650	50 ml, 29 mm x 104 mm	31.50	29.75	28.00
2809*	50 ml, 29 mm x 133 mm	36.00	34.00	32.00

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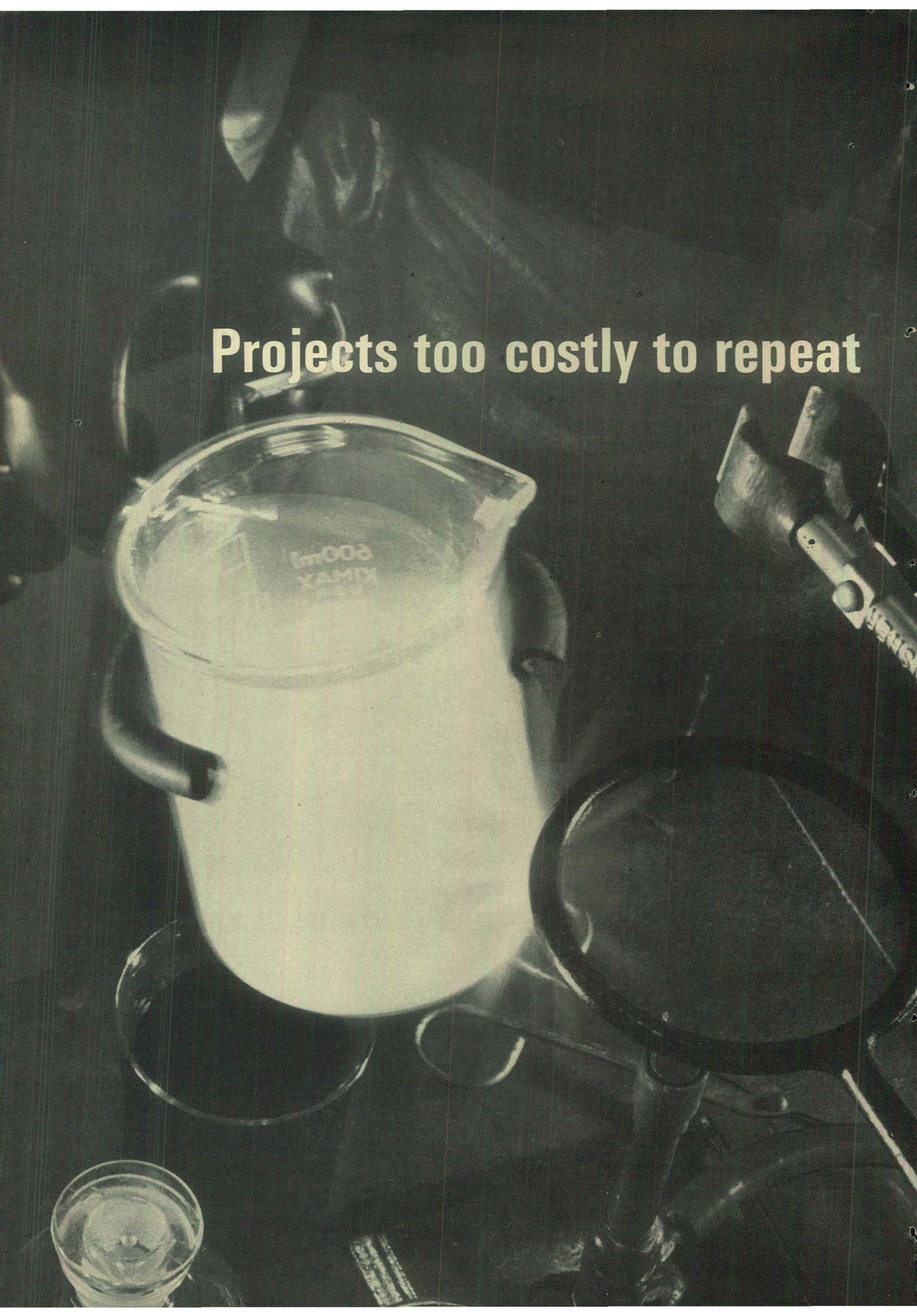
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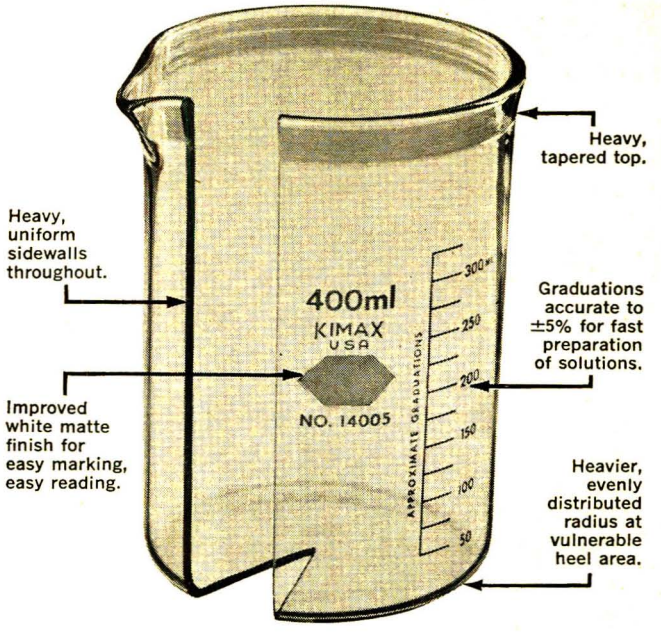

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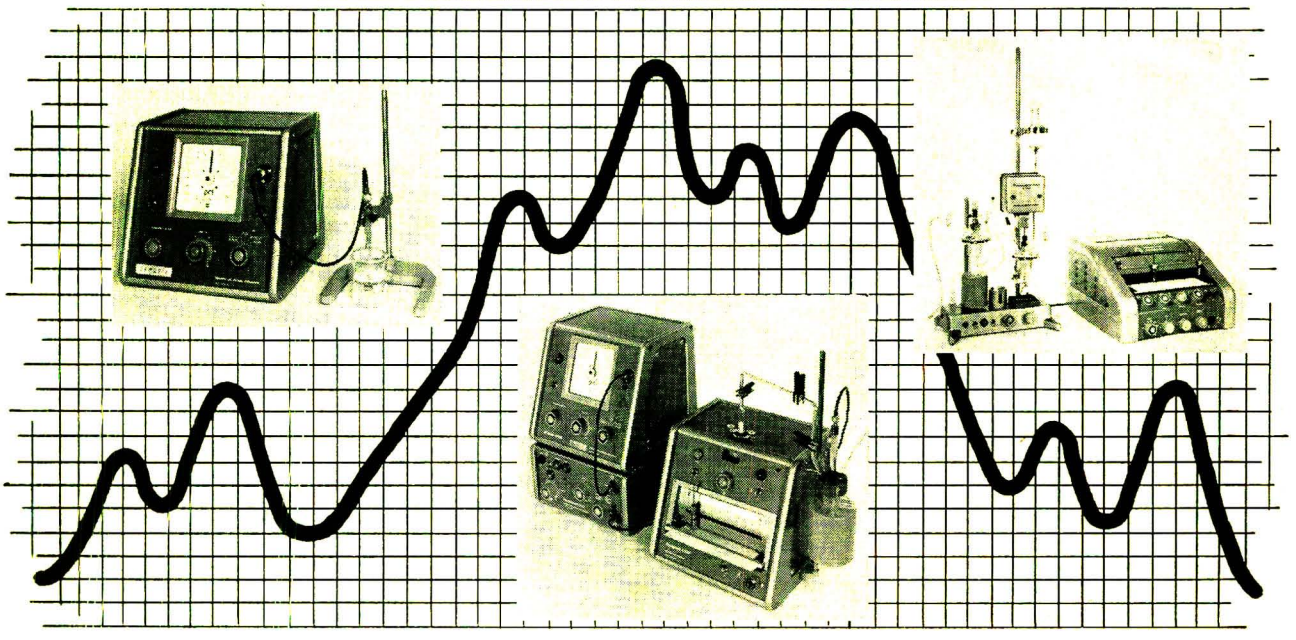

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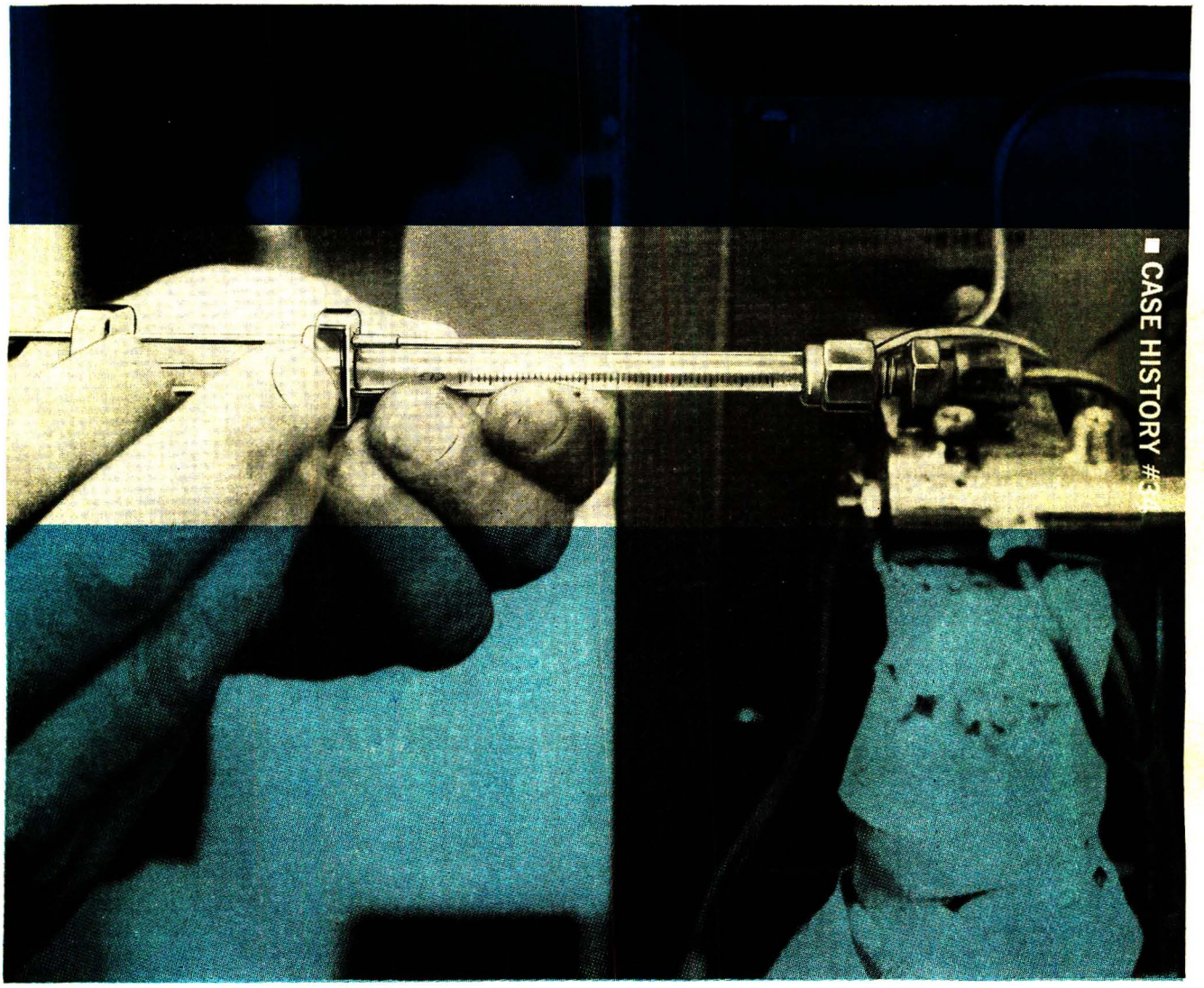

REPRODUCIBILITY AIDS FORMALDEHYDE ANALYSIS

The difficult task of analyzing the important industrial chemical, Formaldehyde, has been accomplished by Chemist Karl J. Bombaugh, of the R & D Division of the Spencer Chemical Company, Kansas City. Bombaugh reports (1) that a Hamilton Microliter syringe with a Chaney Adaptor was used because the method required reproducible injections of the actual sample and calibrating solution. First the calibrating solution which did not contain formaldehyde was used as a secondary external standard because of its stability. That solution was then calibrated using the cumbersome formaldehyde-containing solution, permitting preparation of a reference solution that could be used in a chromatograph at any time as an external standard—provided that it and the sample could be injected reproducibly. Since the errors in the "primary calibration" and in the "use calibration" are additive, extreme accuracy was required from the injection device. However, no attempt was made to determine the absolute accuracy of the syringe, "Because," Bombaugh states, "we place higher confidence in the accuracy of the syringe than in our ability to calibrate it by any simple procedure." (1) Bombaugh, K., Bull, W., **Ana. Chem., 34,** 1237, (1962). **Hamilton manufactures a complete line of precision syringes from a capacity of 0.5 μl to 500 μl and other related chromatograph equipment.**

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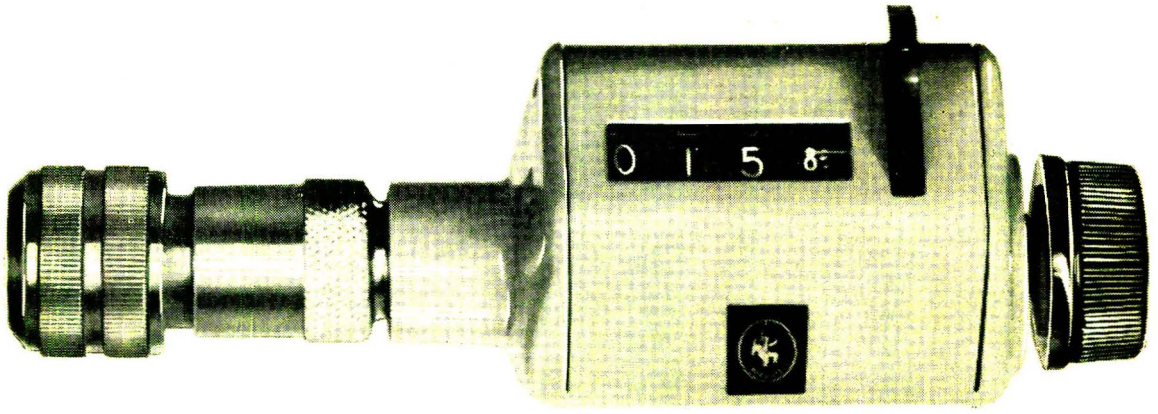

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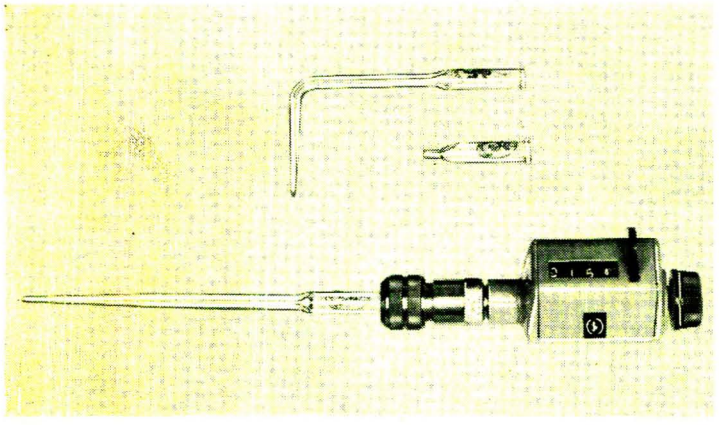

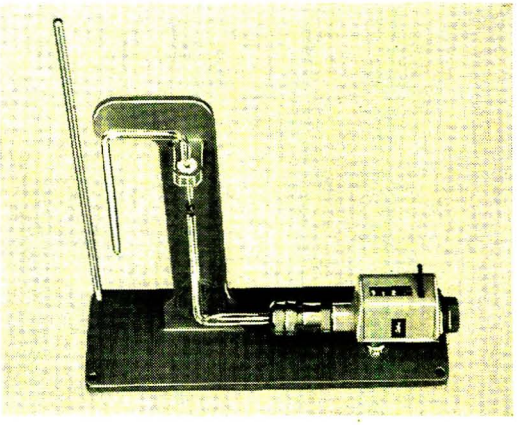

No Calculations—new digital counter provides quick, accurate *direct reading* of volume dispensed. *Sensitivity: .01%! Accuracy: up to 0.1% of dispensed volume!* You can perform repeated titrations with a single filling. ■ **Other Digi-Pet Features** —unique push-button action lets you dispense and reset *with one hand*. Counter is designed for easy grip and comfortable hand operation. Comes complete with adapters for clamp mounting. Pipet models are available with interchangeable tips: straight, luer or right angle bend. Buret models for ultramicro work come complete with mercury and metal stand. Digi-Pet is available in 0.01 ml, 0.1 ml, 1.0 ml and 10.0 ml sizes—all at amazingly modest prices: from **$139.50** each.

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the EDITOR'S column

L.T. Hallett, Editor

IN A PROGRAM sponsored by the Analytical Division of the ACS in honor of the 25th Anniversary of the Student Affiliates at the ACS National Meeting in Los Angeles last April, several undergraduate students were given a chance to present some of their work and then to hear some well known chemists talk of their analytical specialties. Many chemistry professors throughout the country are now introducing undergraduate students to research quite early in their educational programs. One such professor is Dr. Louis Gordon of Case Institute of Technology.

During the past few years, Dr. Gordon has utilized a portion of CH 24, Analytical Chemistry II, to introduce the sophomore chemistry majors to research. Analytical Chemistry, I and II, is a two-semester 8-credit course at Case. The latter half of the laboratory of Analytical Chemistry II is devoted to research activities and the last three weeks of class are used for seminar presentations.

Most of the research problems originate from sources within the research activities of Dr. Gordon's group, although the student is permitted the option of presenting his own research problem and, in a few rare cases where a reasonable idea is presented, the student is allowed to pursue it. Problems have included (1) the precipitation of metal 8-hydroxyquinolates from homogeneous solution with 8-acetoxyquinoline and (2) the infrared analytical applications to the metal 8-hydroxyquinolates.

When the sophomore is ready to begin research, the instructor discusses a number of research problems with him. After a selection is made, the instructor outlines further the background of the research and

(*Continued on page 103 A*)

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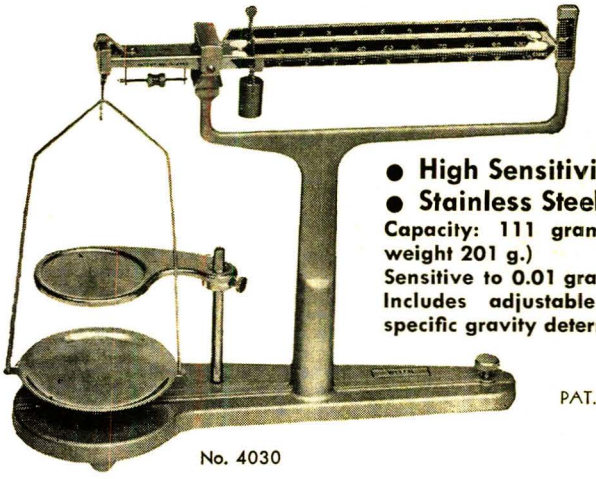

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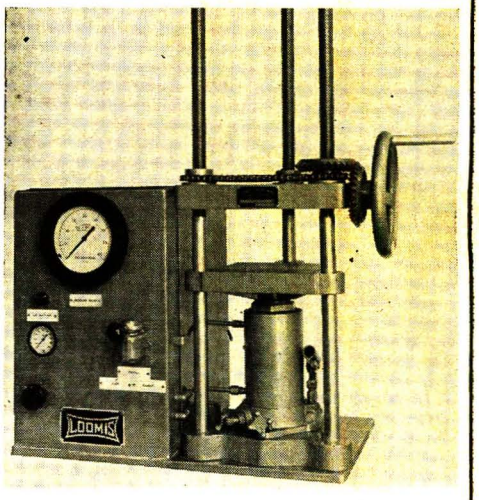

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Accessories for temperature, pressure, extrusion testing units, etc. are available for all models.

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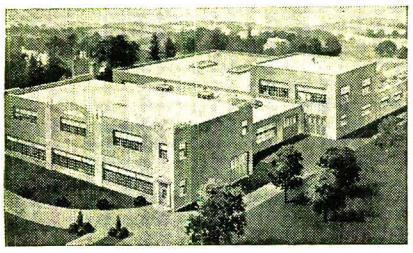

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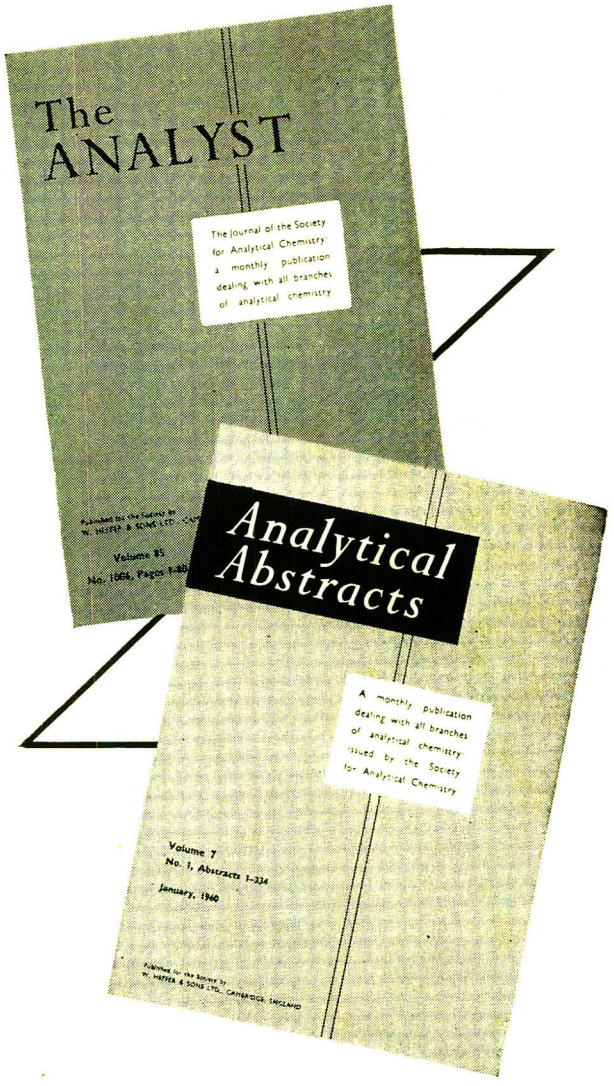

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PRACTICE

ture programmer (LP) modules. With the LP, oven cool-down is automatic but door-close is not. A manual switch operates the door at any time.

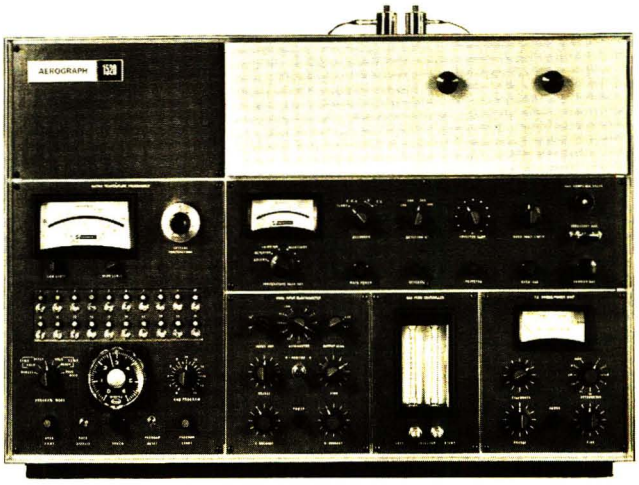
The 1520 is a more deluxe version of the 202. It contains both the dual flame and thermal conductivity detectors. The detector choice is made by re-connecting the downstream ends of the column. The exclusive Aerograph matrix temperature programmer is standard. This deluxe programmer not only gives complete flexibility in pre-setting temperature programming rates but automatically closes the oven door after cool-down and re-establishes the start temperature.

The injectors are independently heated. Temperature readout on an accurate, compensated pyrometer reads column oven, detector oven, injector, collector and auxiliary equipment plug. An automatic upper oven temperature limit control on all Moduline instruments protects columns and equipment from accidental overheating.

At lower right is shown a typical temperature program profile. The program rate may be caused to increase at any time during analysis or may be pre-set before the analysis. The programming rate is established by the resistance value of the matrix plugs . . . the time of change by their position on the matrix board.

At lower left is an interior view of the 1520 showing cabinet air cooling and modules. The 202 is identical to the "A" portion. Modules in this case would be either electrometer or bridge • manual programmer or linear programmer.

The 1520



FEATURES

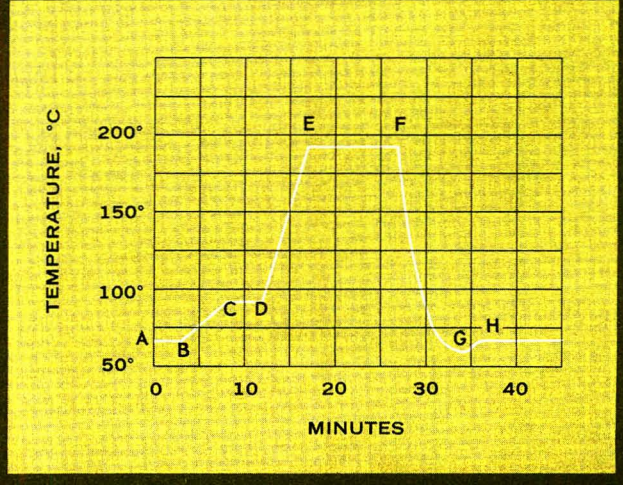
- Dual-column with both flame and T.C. detectors
- Matrix board temperature programmer
- Automatic oven cool-down and re-set to start temperature.
- Both dual input electrometer and bridge modules
- On-column or flash vaporized injection
- Separately heated detector oven

ORDER:

Aerograph 1520—dual column gas chromatograph with both dual flame and T.C. detectors. Flow controllers, electrometer, bridge/power supply and matrix temperature programming modules are standard.

MATRIX TEMPERATURE PROGRAMMER

A	Pre-set temperature—70°C.
A-B	Isothermal operation—@ 70°C for 3 min.
B-C	Linear programming—@ 5°C/min for 5 min.
C-D	Isothermal operation—@ 95°C for 4 min.
D-E	Linear programming—@ 20°C/min for 5 min.
E-F	Isothermal operation—@ 195°C for 10 min.
F-G	Door opens and oven cools down to 60°C in 7 minutes pre-set by timer.
G-H	Door closes and oven heats to pre-set temperature—70°C.

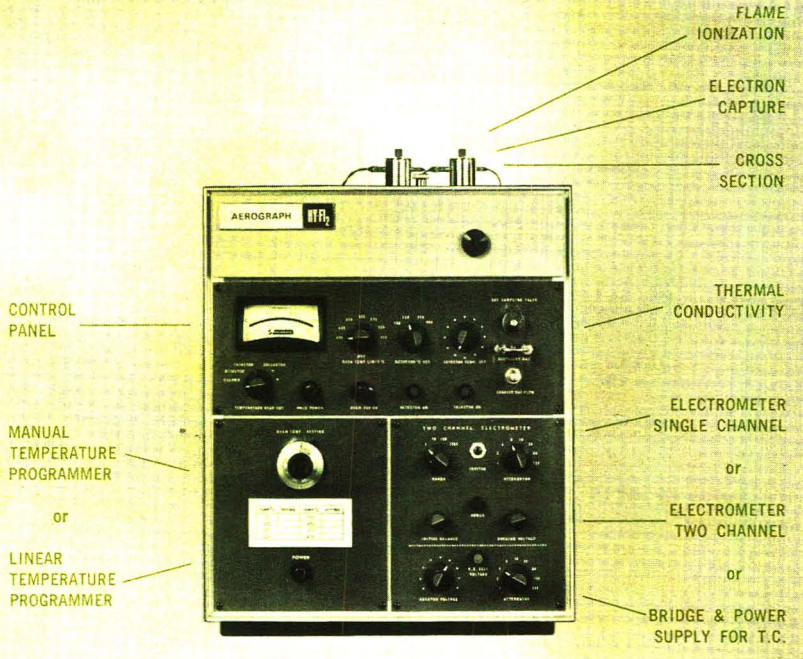


The Hy-FI_2

The Hy-FI_2, a big brother to the popular Hy-FI 600-C, is a single column instrument which adapts perfectly to the three common ionization detectors and to the four-filament Thermal Conductivity detector.

Hy-FI_2 Dual Channel

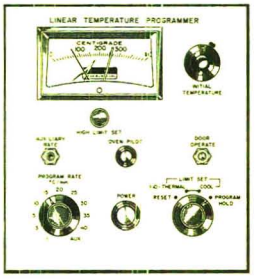
A further interesting option incorporates both flame and electron capture detectors. With this combination the column effluent is divided by a stream splitter to feed both detectors. The signals from both detectors are amplified by a two-channel electrometer which in turn operates a two-pen recorder. A striking trace of peppermint oil was made with this equipment. Many peaks were exhibited by electron capture which were not detected by the flame because of their low concentration. Please write for details.



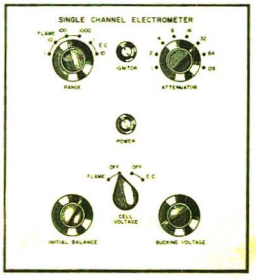
BONUS FEATURES

- Separate detector oven, heats to 400°C.
- 2″ injector, stainless, separately heated.
- Variable power and thermocouple plug for auxiliary injection equipment.
- Stainless column oven, heats 40°C/min.
- Sample collection—capillary, with Fiame Det.
- Carrier gas flow controller with long, tapered needle valve.

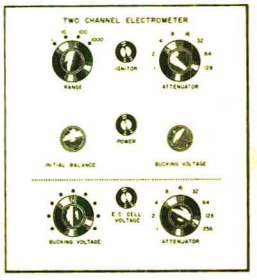
Manual Temperature Programmer — This unique controller supplies constant power to the column oven, by a solid-state electronic, adjustable power controller (APC) and multi-turn pot.



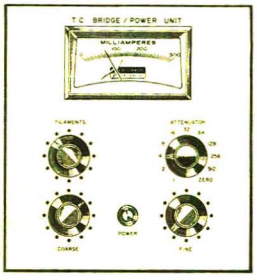
Linear Temperature Programmer—Tnis programmer is completely electronic with no gears, motor or clutch. It programs at 10 precise rates. A matrix plug permits any additional rates.



Single Channel Electrometer — This high impedance electrometer used with single ionization detectors features 100% feedback, decade and binary attenuation and buckout control.



Two Channel Electrometer—This electrometer, with flame and electron capture combination, is actually two electrometers with one common power supply. It features 100% feedback and buckout on each channel.



Bridge-Power Supply — This 12 or 20 volt solid-state bridge and power supply module is used in conjunction with 4-filament T.C. detectors. It is interchangeable in the Hy-FI_2, 202 or 1520.

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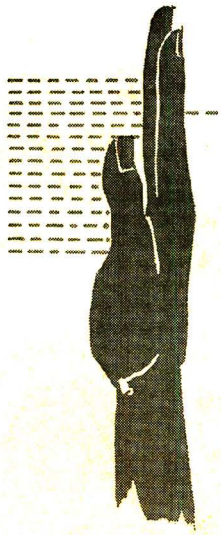
if current papers are available, he gives them to the student. He also conveys to the student the view that there is one major difference between the research problem and the conventional laboratory work. For example, in the laboratory he is assigned a proven method of analysis with specific directions written in the imperative mood, with all necessary chemicals and solutions provided on the side-shelves, with equipment set up ready to function, and with a laboratory assistant supervising the entire operation. In contrast to this, because he is assigned only a research idea, he must consequently conduct himself in his approach to the problem as if he were a graduate student—i.e., he must make a literature search, obtain and/or build instrumentation, find chemicals, and seek advice both from the faculty and other students, particularly graduate students.

Finally, at the end of the semester, seminars are held during which the students report on their research efforts. A sufficient number of class periods is reserved, and each student is allowed a total of ten minutes (8 minutes for presentation and 2 minutes for questions). Each student must submit to the instructor in advance an abstract of his talk.

The student is also required to submit a written report of his research work. He is advised to examine current journals in order to determine the style in which to prepare his report.

It is Dr. Gordon's general impression that the early introduction of the student to research in this manner represents a markedly advantageous addition to the undergraduate curriculum. It provides a stimulus which motivates the student as does no other educational technique. Further, the proviso of both written and oral reports develops communication skills at an appropriate educational stage.

In several cases where the students have elected to continue their research either during the summer or during the regular academic year, publications have resulted.



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residual stress
low angle scattering
retained austenite
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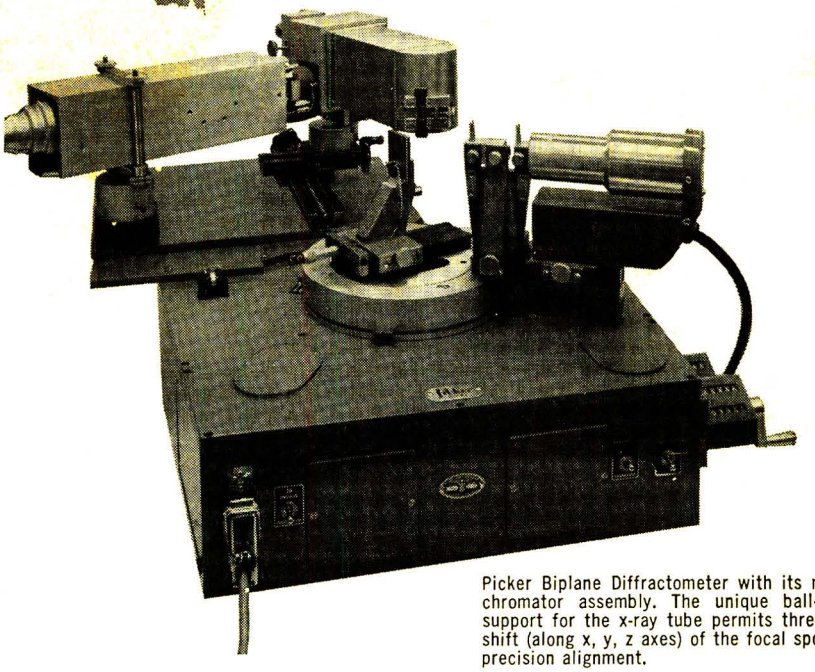

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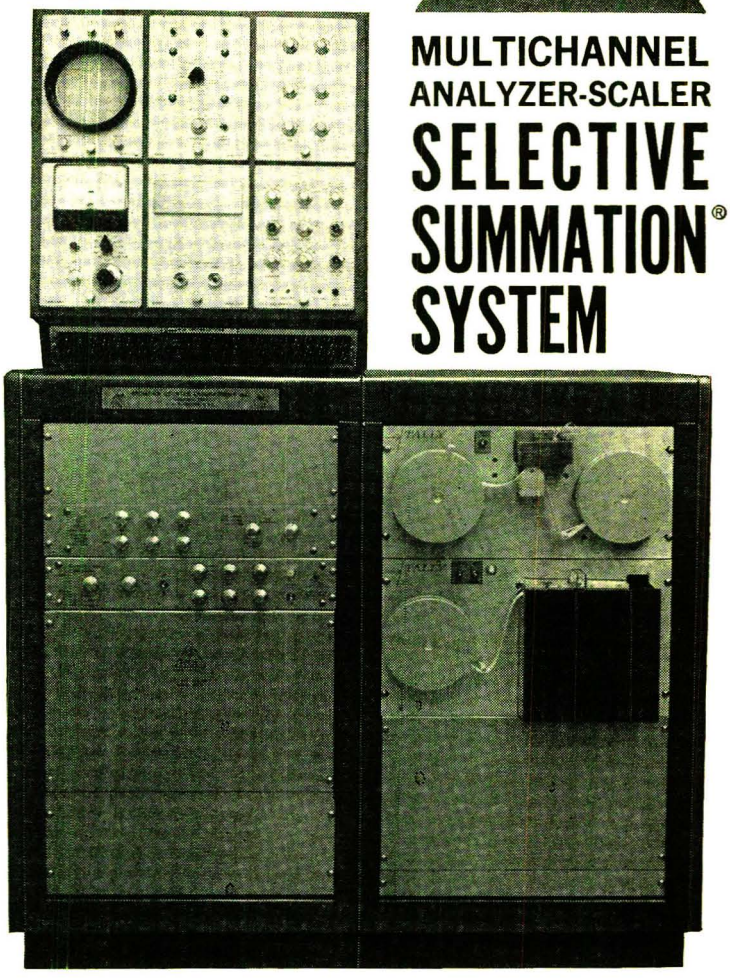

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For complete Model 303 specifications write to Instrument Division, Perkin-Elmer Corporation, 751 Main Avenue, Norwalk, Connecticut.

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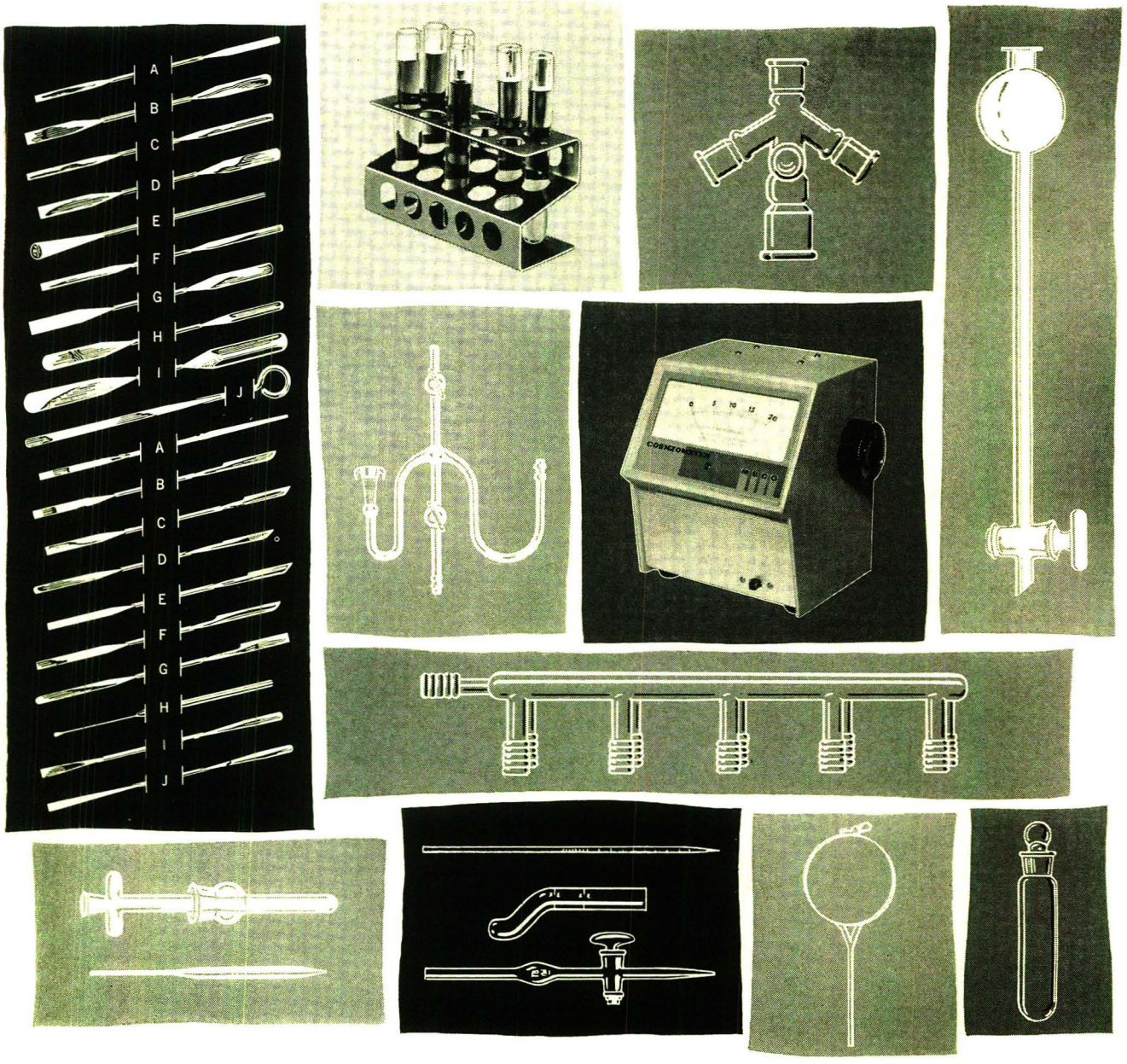

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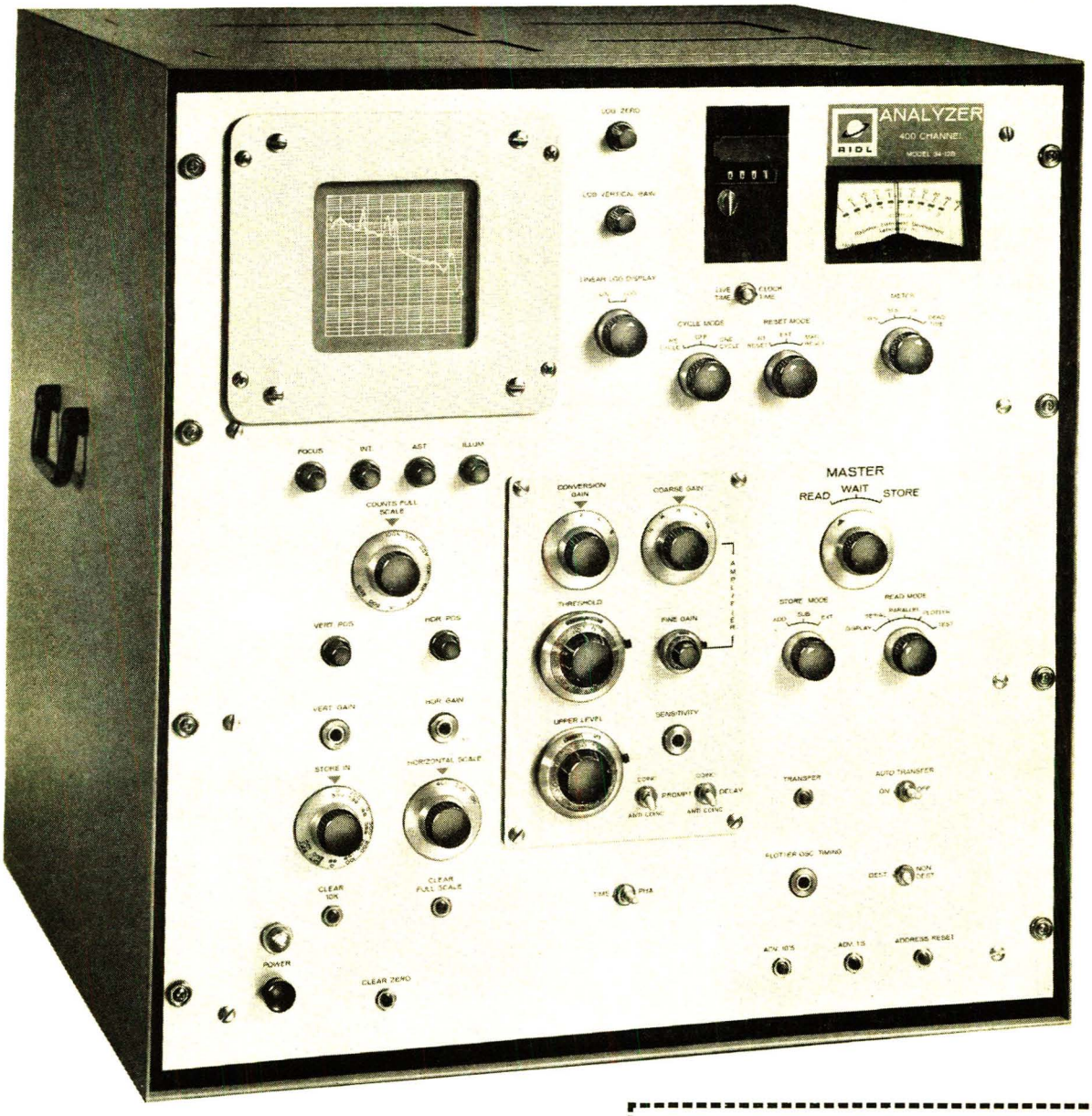

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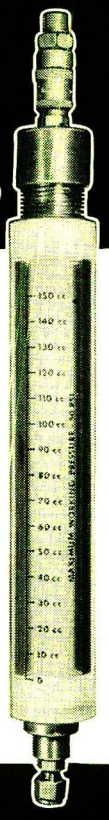

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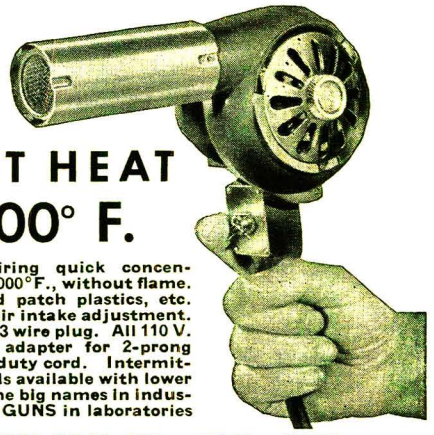

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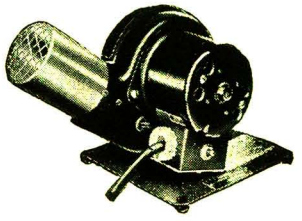

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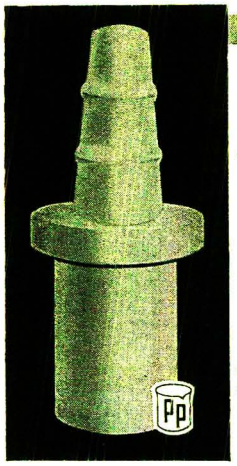

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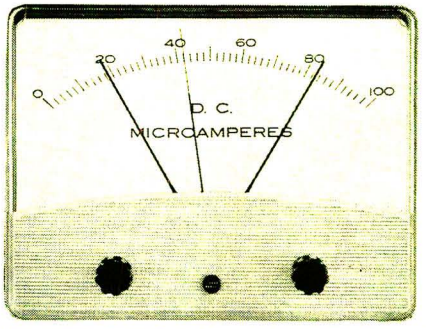

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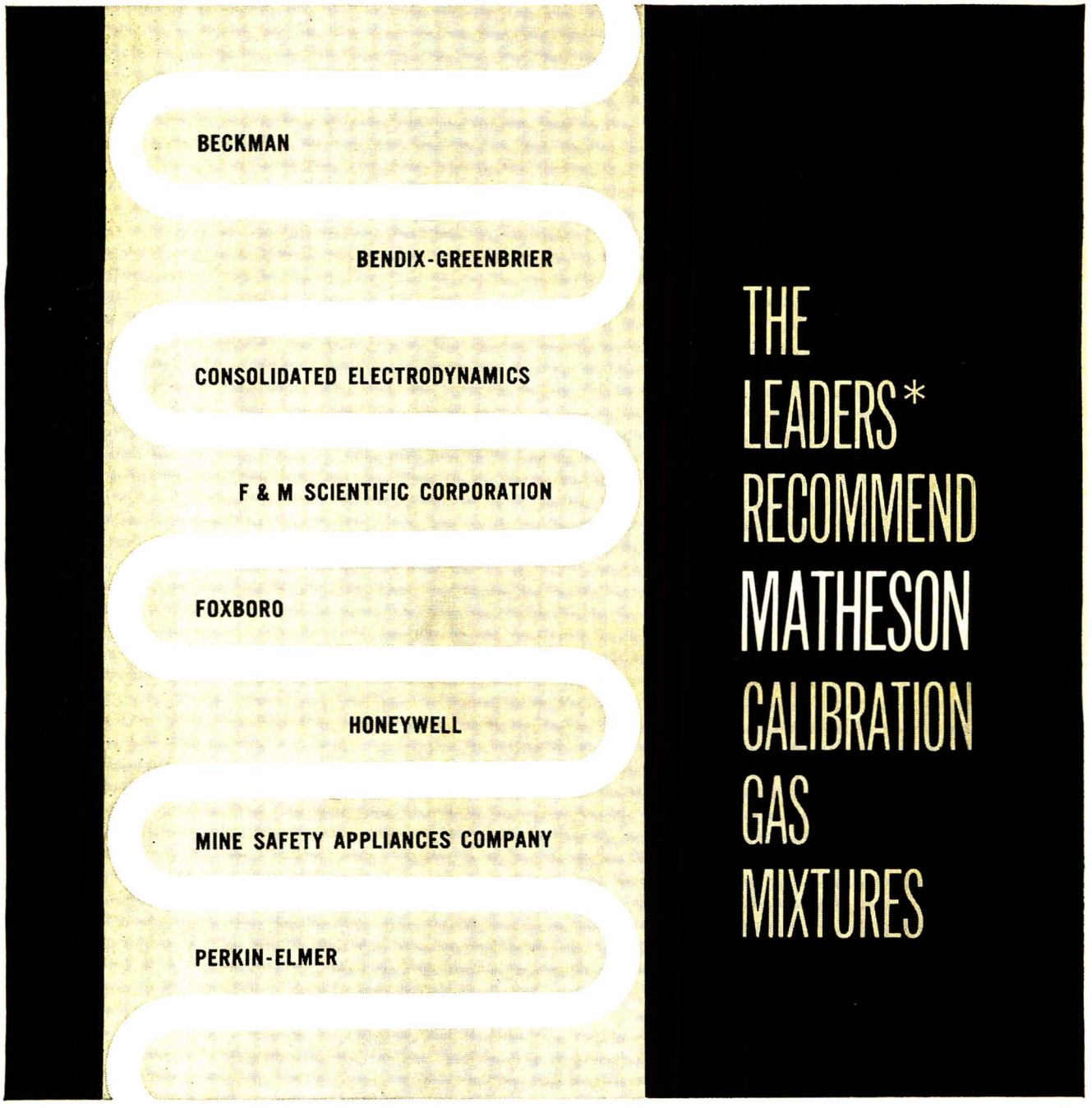

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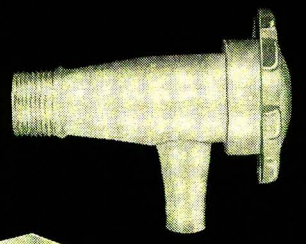

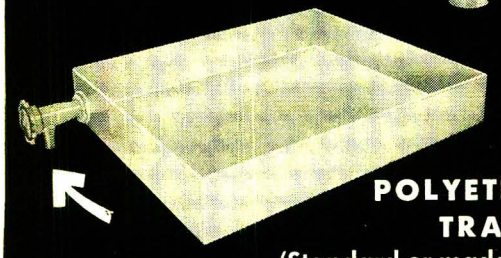

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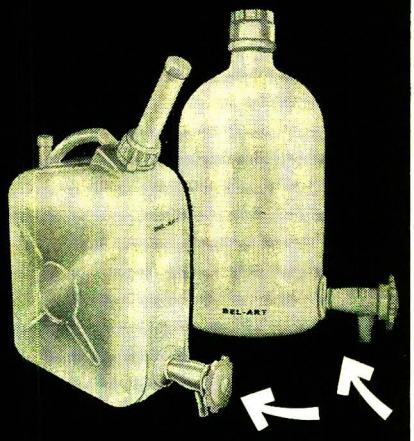

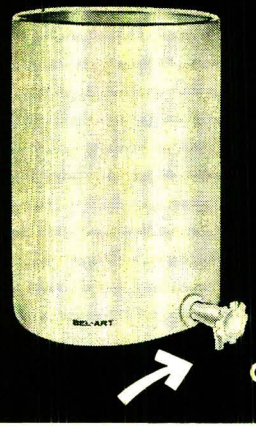

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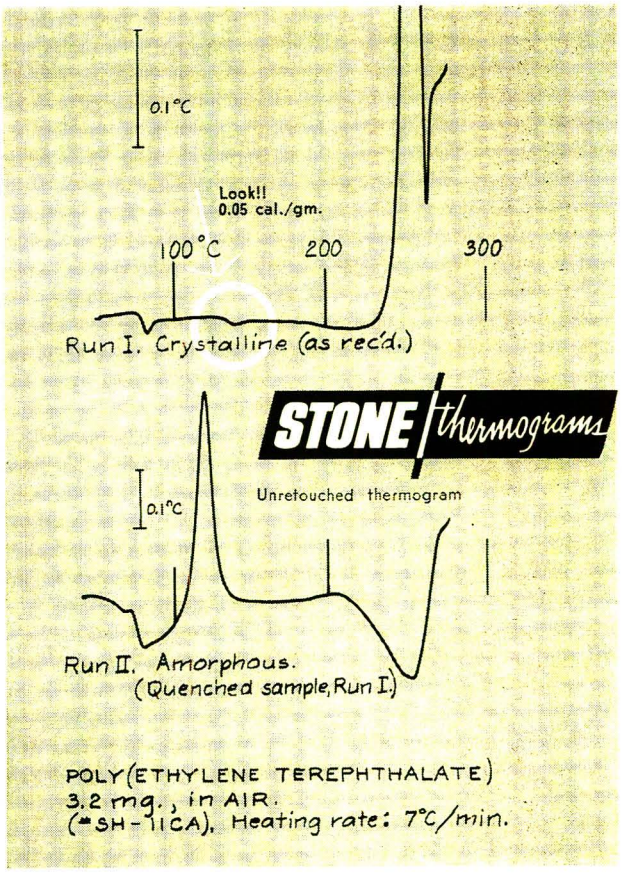

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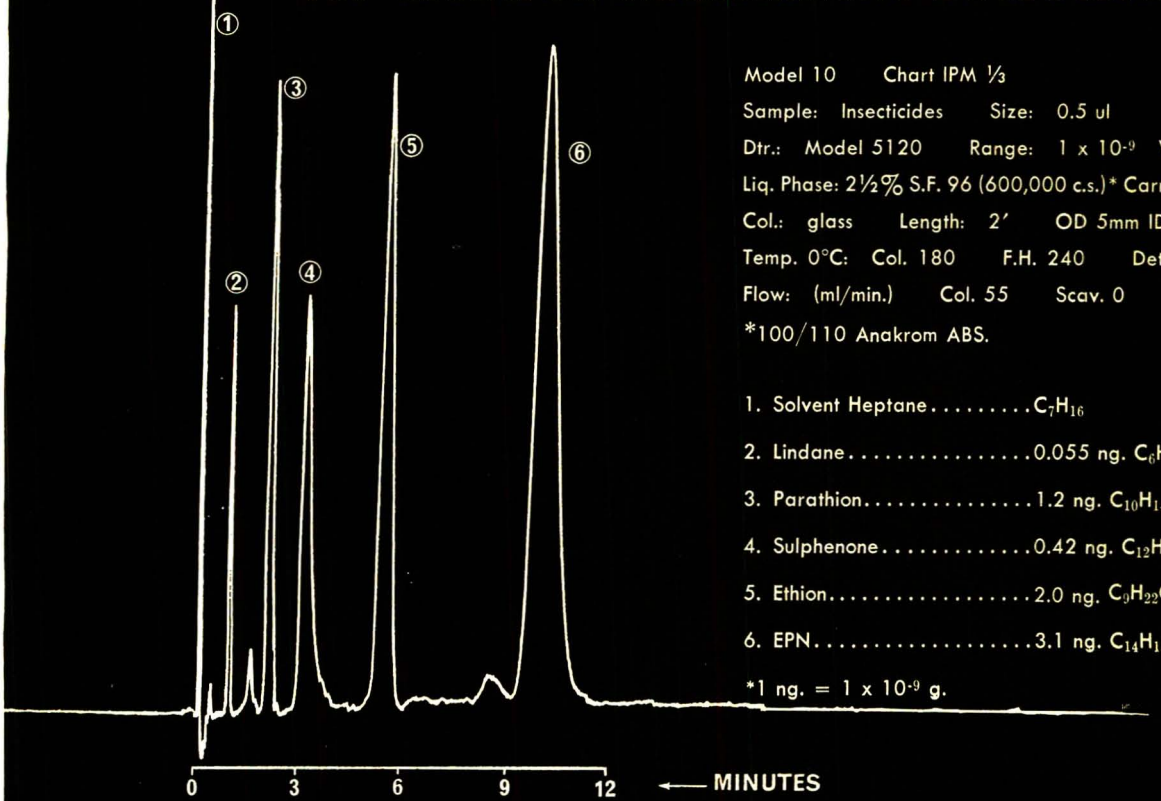
In Gas Chromatography—Specify Barber-Colman for Electron Attachment Detectors

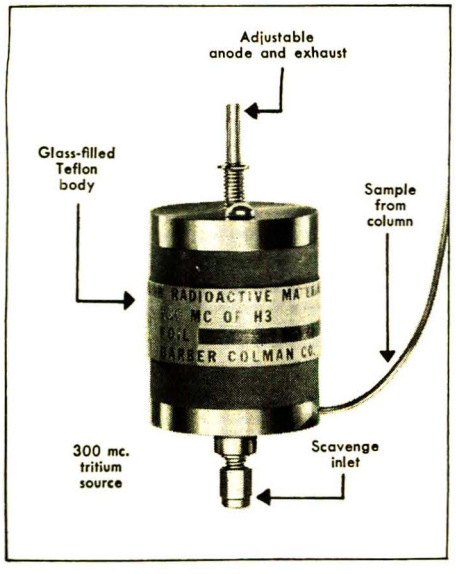


Model 10 Chart IPM ⅓
Sample: Insecticides Size: 0.5 ul
Dtr.: Model 5120 Range: 1 x 10^{-9} Voltage 40
Liq. Phase: 2½% S.F. 96 (600,000 c.s.)* Carrier 10 psi N_2
Col.: glass Length: 2′ OD 5mm ID 3mm
Temp. 0°C: Col. 180 F.H. 240 Detr. 180
Flow: (ml/min.) Col. 55 Scav. 0 Split 0
*100/110 Anakrom ABS.

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.

for Pesticides and other compounds of high Electron Affinity



You have more advantages with the Barber-Colman Detectors:

BROAD RANGE — Not only analyzes chlorinated and thiophosphate pesticides but can also be used for other halogenated compounds, nitriles, nitrates, organo-metallics, conjugated carbonyls, steroid and amino acid derivatives.

FLEXIBILITY — Electrode spacing in the Barber-Colman design is adjustable from 1 to 25 mm. You have optimal control of attachment phenomenon. It is usable with either packed or capillary* columns.
*Golay patent 2920478

HIGH SENSITIVITY — 1 centimeter inside diameter permits source strength of 300 millicuries of tritium, providing detection of picogram amounts (10^{-15} mole/sec. or better in many cases).

UNIFORM FIELD STRENGTH — parallel plate electrode design insures uniform fields.

The Barber-Colman Electron Attachment Detector can be supplied as a standard item.
Specify the Electron Attachment Detector on your Barber-Colman Chromatograph or secure it in kit form for field conversion.

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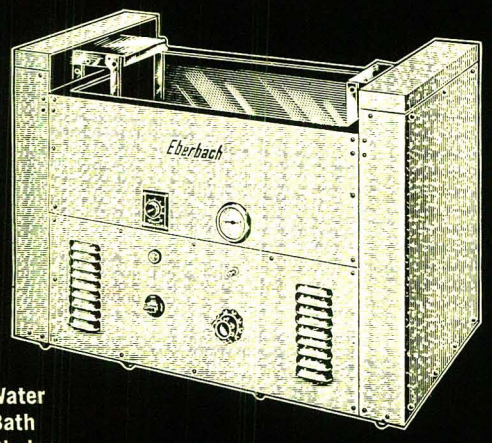

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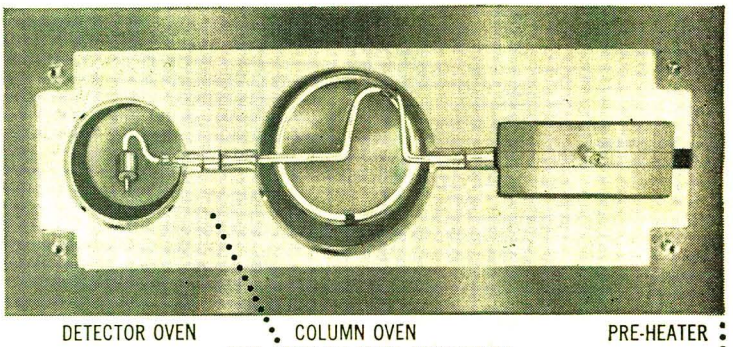

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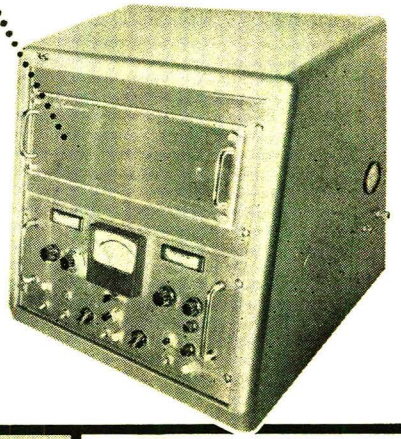

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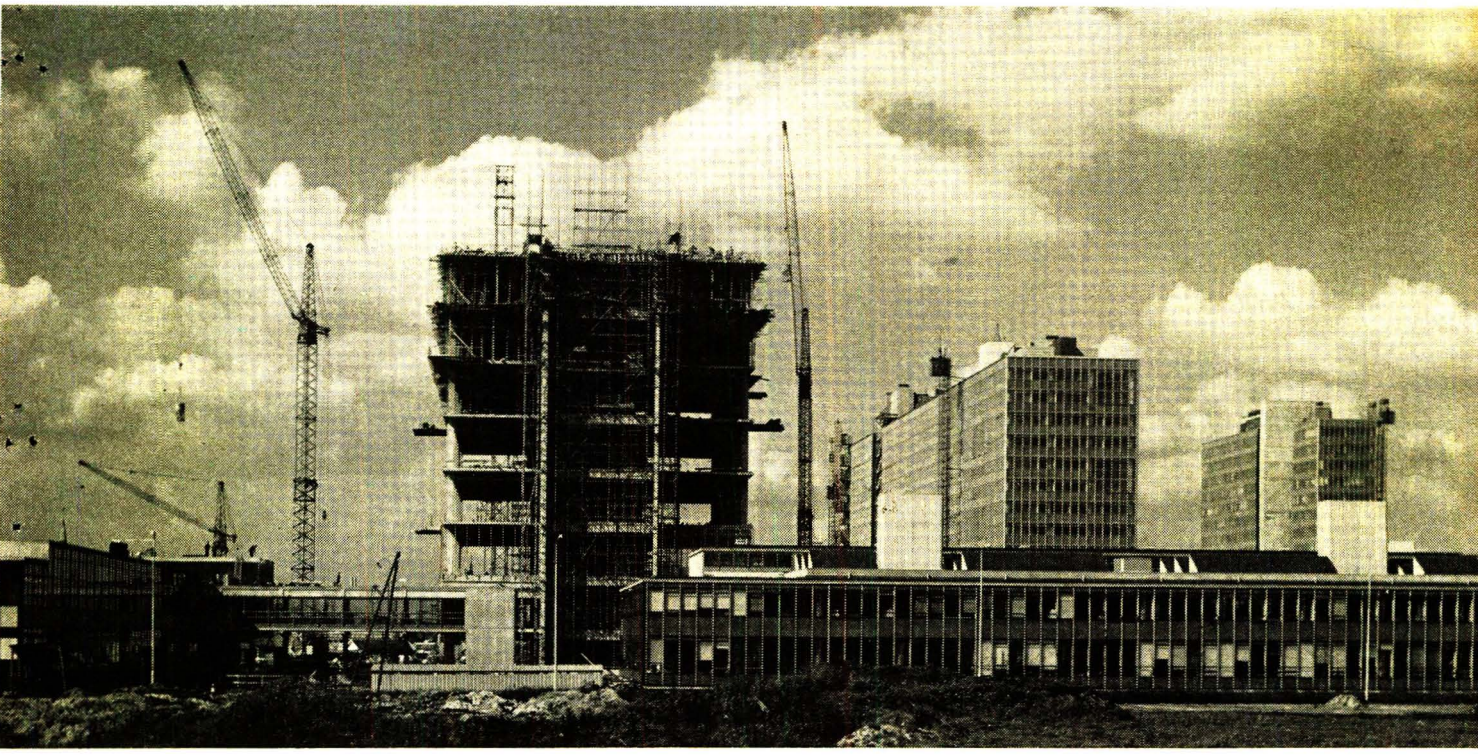
University in the Netherlands Has Modern Instrumental Analysis Division



Contemporary architectural design is a distinctive feature of the Technical University at Eindhoven. Glass walls, used throughout, ensure maximum light. The University was founded in 1956. In 1966 the erection of 13 buildings will complete the first stage of the building program. The three major areas of engineering, chemical, mechanical, and electrical, are taught. The Chemical Engineering Department's 500 students occupy five buildings: an 80,000 sq. ft. freshmen chemistry building; a 130,000 sq. ft. main chemistry building housing organic, inorganic, and physical chemistry and instrumental analysis divisions; two 60,000 sq. ft. buildings, one for unit operations and process engineering, and one for chemical engineering and reactor design; and a building for high pressure reaction studies. There is a central auditorium/lecture hall capable of seating 1200 people as well as 12 smaller lecture theatres throughout the campus. The University expects over 3000 students to attend by 1968. A novel feature of the design is the series of connecting "air bridges" between the buildings. These not only serve as convenient walkways during inclement weather, but, more important, equipment can be moved from one area to another without the necessity of negotiating steps

THE PROGRESSIVE and imaginative attitude toward higher technical education in the Netherlands is no better exemplified than by the Instrumental Analysis Division of the Technical University's Chemical Engineering Department in Eindhoven. A wide range of up-to-date equipment coupled with a dynamic program of undergraduate and graduate study in modern surroundings make the work there of the highest quality.

The "Technische Hogeschool" at Eindhoven is one of ten institutes for higher education in Holland and comes under the university system of the Netherlands. Founded as recently as 1956, the first phase of the building program that began in 1957 will be complete by 1966. Originally designed for 1000 students in chemical, mechanical, and electrical engineering, the university is expected to exceed 3000 students by 1968. The chemical engineering department now has 500 students but will probably have 800 by 1970.

The Instrumental Analysis division under the direction of Prof. A. I. M. Keulemans is one of eight occupying the five buildings of the Chemical Engineering Department. These buildings cost over one third of the $30 million spent on the campus to date. Equipping the Instrumental Analysis division alone has cost over $500 thousand. The total capital laid aside for instruments and equipment in the Chemical

Gas chromatographic investigations are carried out in this laboratory, one of many for graduate students in instrumental analysis. Here specially designed instruments permit more accurate measurements than are often possible using commercially available apparatus. Graduate courses place considerable emphasis on sponsoring inventiveness and adaptability in the students. There are 20 students working toward their doctorate in chemical engineering, seven of whom will take their doctorate in instrumental analysis by next year

Engineering Department during 1957 to 1965 is about $3.3 million.

Prof. Keulemans, who was appointed to his present position after nearly 25 years with Royal Dutch Shell, has been given every opportunity of equipping his division with the most up-to-date analytical tools available. These include an extensive range of instruments for gas chromatographic analysis, a mass spectrometer, two Zeiss spectrophotometers, two automatic ultraviolet spectrophotometers and five automatic infrared spectrophotometers, a nuclear magnetic resonance instrument with permanent magnet, and an instrument for atomic absorption studies. There is also an extensive variety of apparatus for analytical distillation, counter-current extraction, and electroanalysis.

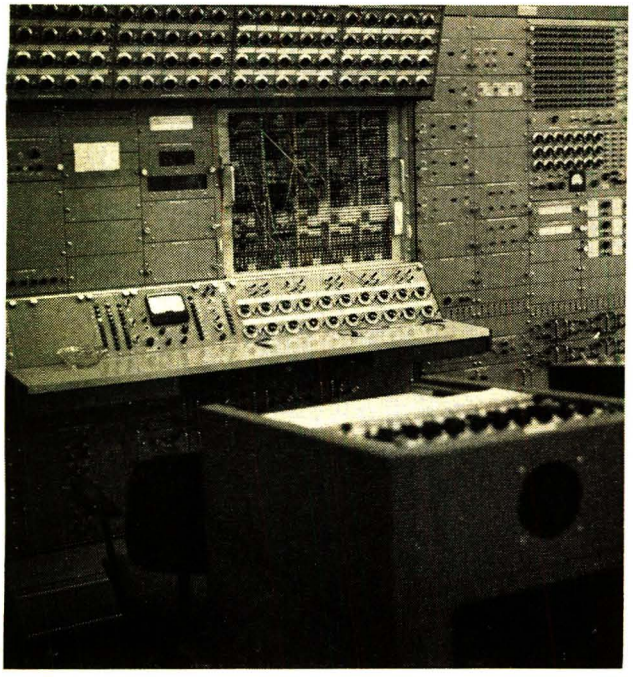
In addition, the Instrumental Analysis Division uses the services of the Chemical Engineering Department's up-to-date instrument and glass-blowing shops. The staff and students have designed and built special equipment for analytical studies. These include ultra high efficient stills for improved analytical rectification, gas chromatography detectors, devices for preparing gas streams of extremely low concentrations of organic vapors used in the calibration of such detectors and in studying performance characteristics of organic compounds. A new detector for mass spectrometry is currently under construction.

The improvements in analytical tools should provide an important stimulus to the development of physico-chemical theories. It is now possible to detail the products of chemical reactions which previously could not be studied with such a high degree of accuracy. Studies currently under way are the detailed investigation of elimination reactions such as pyrolysis of acetates and the catalytic dehydration of alcohols.

Prof. Keulemans points out that students at all levels, from undergraduates to Ph.D. candidates, have the opportunity to use the best analytical tools available. And they are encouraged and expected to design their own equipment to fill specific needs.

Research is carried out mainly in the field of natural products such as petroleum fractions and flavors. The work generally falls into two categories. One is the study and improvement of physical methods of separation involving all phases of chromatography and analytical distillation. The other is concerned with structure determination and identification.

A long term research program that Prof. Keulemans initiated is the accumulation of precise gas chromatography profiles for a wide range of organic compounds. The work is still in its initial stage of checking gas chromatography characteristics of these classes of compounds which differ in their functional group. For instance alkanes, olefins, ketones, esters, ethers, etc. are being investigated. He esti-



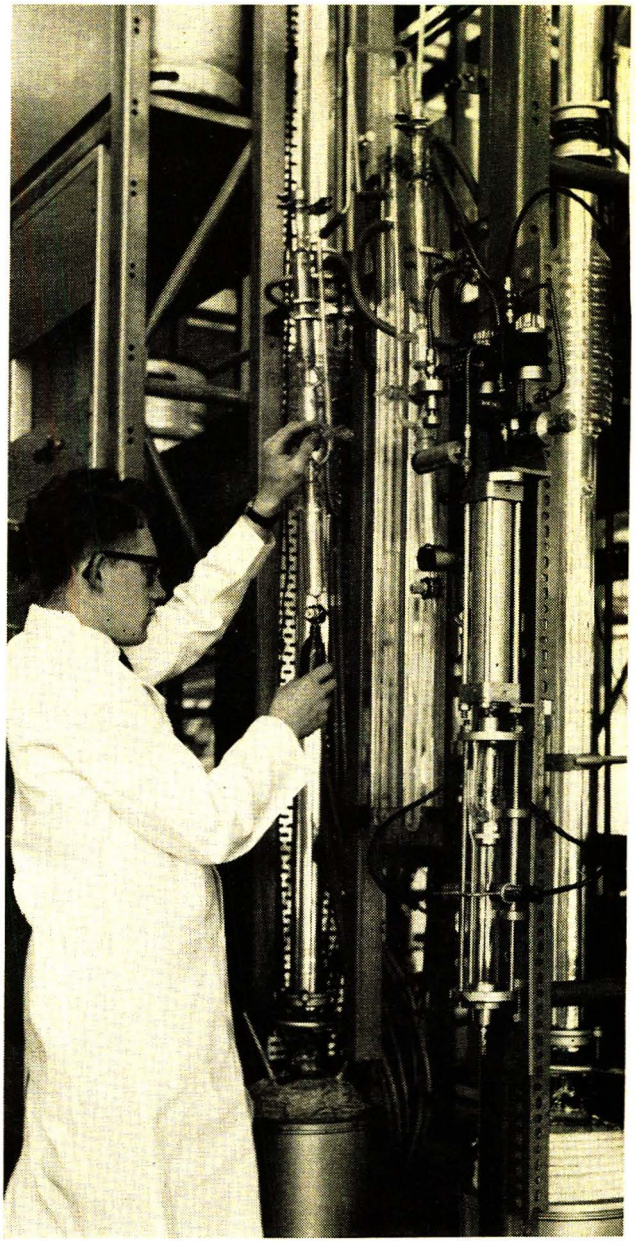
Both an analog and a digital computer are in use at the University at Eindhoven. These permit rapid and accurate analyses of statistical measurements and by simulating reaction equations, aid in the interpretation of chemical processes

mates it will take about two or three years to complete a good starting catalog before being able to launch into a compound-by-compound study of the various classes of compounds. His aim is to have published a series of gas chromatographic charts, issued periodically, to aid in the rapid and accurate identification of all known substances that lend themselves to this type of analysis. There are two full-time staff members working on this project. Students during their regular course work also carry out gas chromatographic analysis on the compounds so that each is subjected to no less than ten analyses.

Prof. Keulemans also sees a great need to build up a good catalog of infrared spectra data for organic compounds based on modern improved techniques and standards. This involves the design of equipment to get complete separation of compounds and their recovery in a pure state. Eventually he hopes to prepare comprehensive charts for each compound showing characteristic gas chromatographic profiles as well as IR, UV, NMR, and mass spectral characteristics.

Prof. Keulemans' ambitions may seem extraordinarily high. But in a country such as the Netherlands where there is such a remarkable drive toward high educational and technical standards, and where the current education budget is higher than any other, including that of defense, the chances of his achieving his goals are not remote.

(*Continued on page 118 A*)



Distillation columns in the Pilot Operations section of Technical University's Chemical Engineering Department are over 18 feet high and are serviced at the top by means of platforms. Here distillation studies are conducted with major emphasis on improvement in separation techniques. One simple but effective development is the wrapping of the columns with aluminum foil to ensure maximum retention of heat throughout their length. The foil wrappings have slits to allow viewing during distillation. Instrumental control is an important aspect of the distillation units and serves to give students a thorough groundwork in modern industrial distillation procedures

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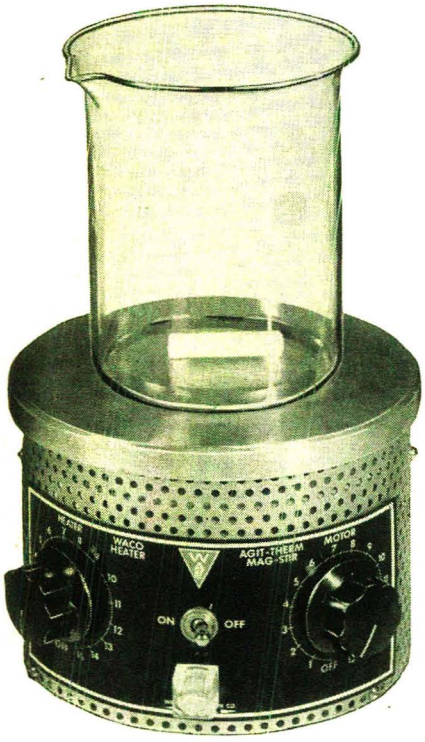

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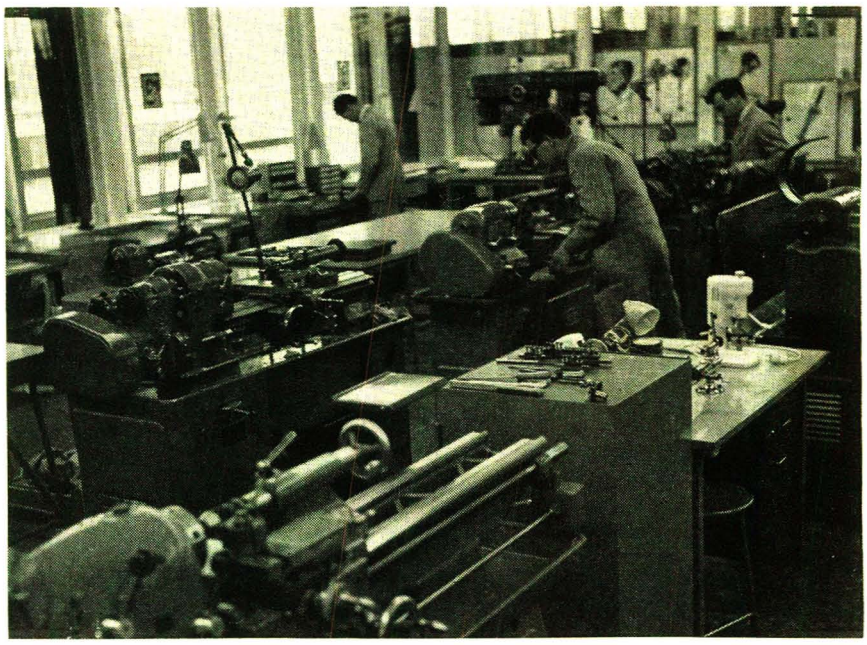
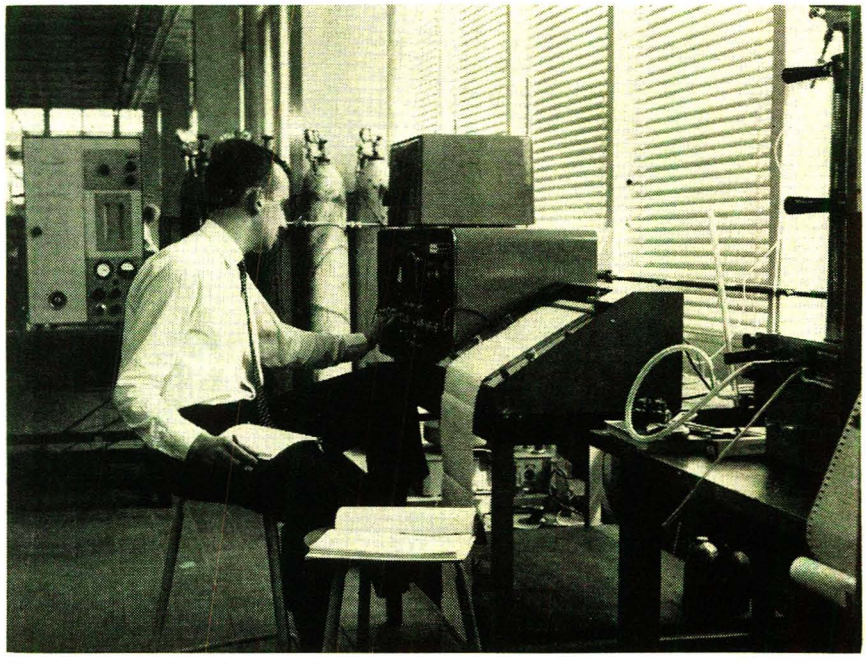


Ten professional technicians work in the University's first-rate instrument shop. Lathes, cutting tools, presses, and other equipment of the very latest design allow the production of precision equipment. Here, instruments are designed and made by top flight technicians for carrying out special analytical procedures. No cost has been spared in equipping and staffing this section of the department's service unit



The accumulation of reliable gas chromatographic data for a wide range of chemicals is one of the special projects of the Instrumental Analysis division. Two specialists are engaged full time on the project and graduate students also make gas chromatograph measurements which serve to double-check the accuracy of the results as well as giving them practice in use of the equipment. During the first year of this program over 40 simple basic compounds have been analyzed to establish the optimum conditions of temperature, pressure, flow, etc. This work will continue during the next two years during which compounds representing alkenes, olefins, ketones, alcohols, esters, ethers, and aromatics will be studied. The ultimate goal is to issue an accurate gas chromatographic dictionary of compounds

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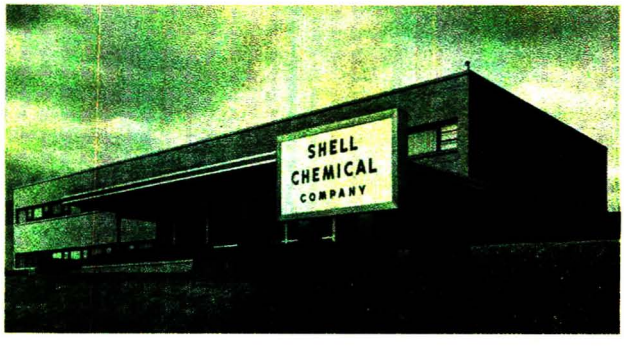

Entrance to Technical Service Laboratory, Shell Chemical Company, Union, New Jersey. In illustration that appears at right, product application chemist filters decolorizing charcoal from a solvent solution, using S&S No. 589 White Ribbon Filter Papers. Photographs courtesy of Shell Chemical Company.

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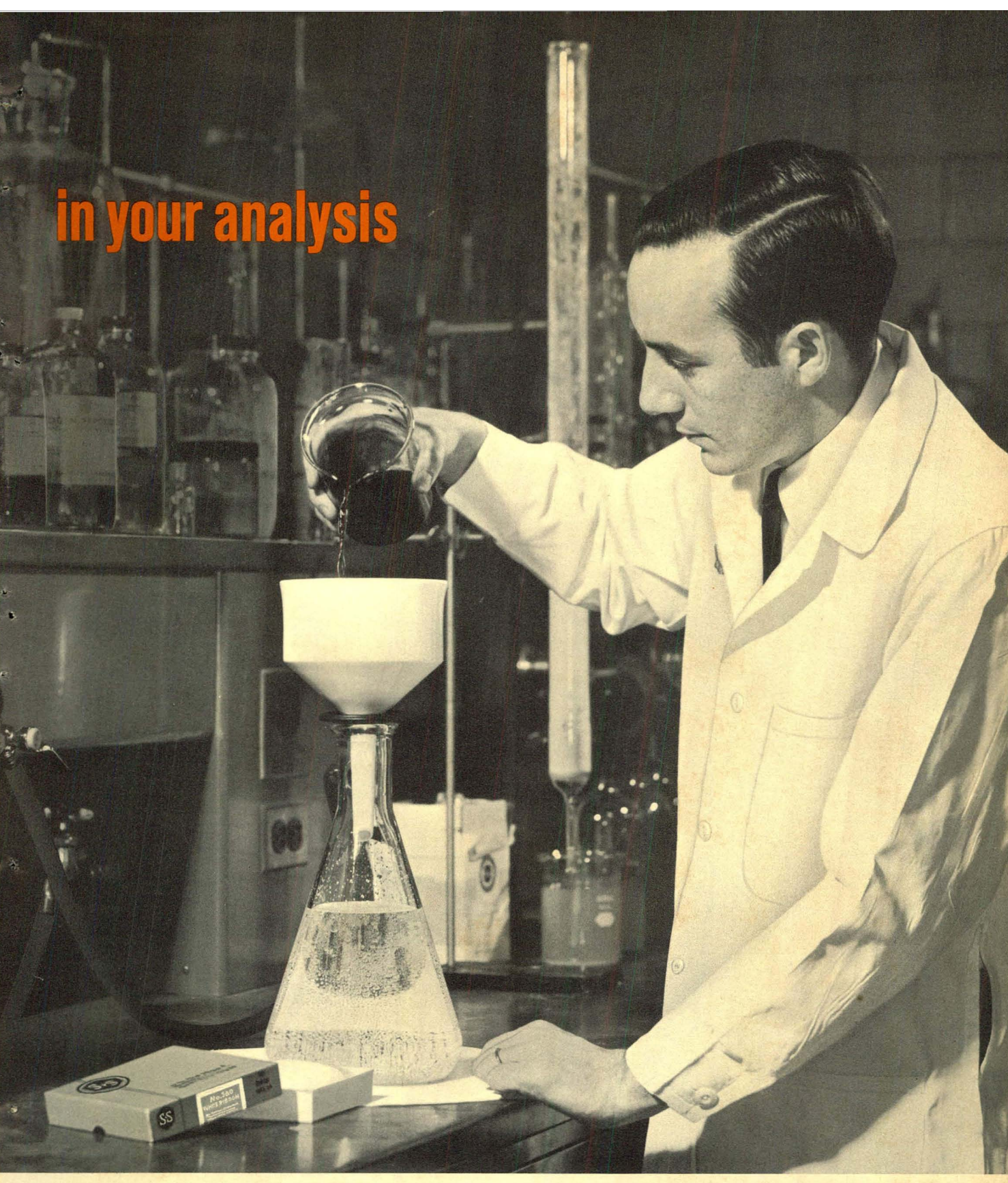

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gas chromatographic determination of combustion products for fast, accurate and reliable results

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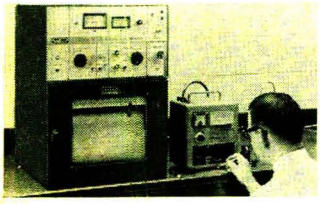
price . . . approximately $4,000 including balance and recorder.

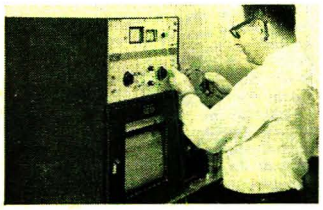
The Model 180 can handle solid or liquid materials and gas samples can also be analyzed with only minor instrument modifications. In addition, sulfur, halogens and nitriles will not adversely affect the accuracy of the analytical results.

For full information on the Model 180, write for Bulletin 1800. F & M Scientific Corporation, Route 41 and Starr Road, Avondale, Penna., 215-COlony 8-2281. *European subsidiary: F & M Scientific Europa N. V., Leidsestraat 67, Amsterdam, The Netherlands.*

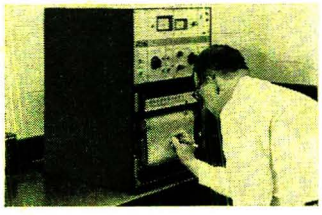
The Model 180 greatly simplifies the analytical procedure for the microdetermination of C, H and N. Here's how:



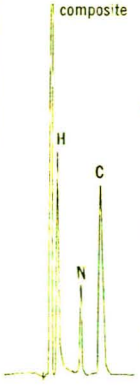
Using a low-capacity micro balance, the analyst weighs a 0.2 to 3 mg sample (solid or liquid) . . .



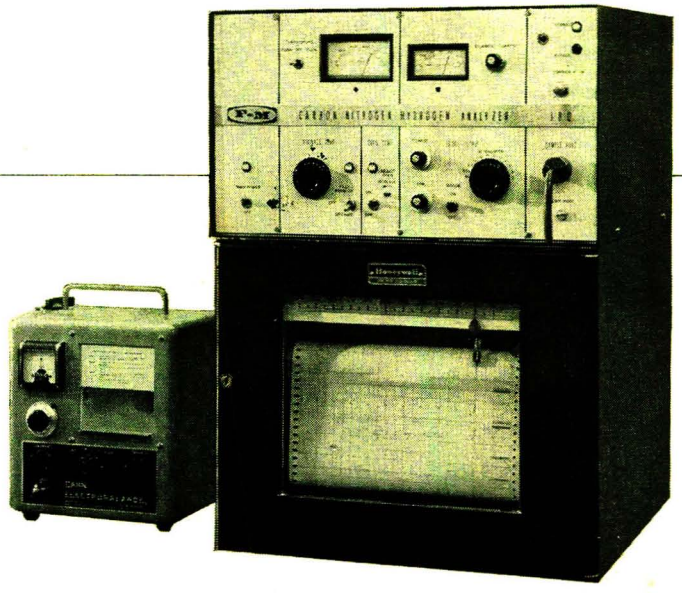
injects the sample into the instrument, purges the furnace by flipping a switch, further inserts the sample into the furnace. After 4 minutes . . .



the analyst introduces the products of combustion into the dual-column, dual-detector gas chromatograph, again by operating a switch. Soon, individual peaks appear on the recorder chart.



■ The first peak is the composite; the second is proportional to the amount of hydrogen in the sample; the third, to the amount of nitrogen; and the last to the amount of carbon. In each case, peak height readout yields quantitative results comparable to those obtained by classical methods.



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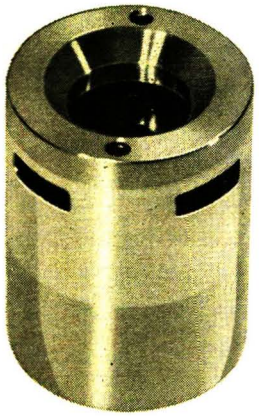

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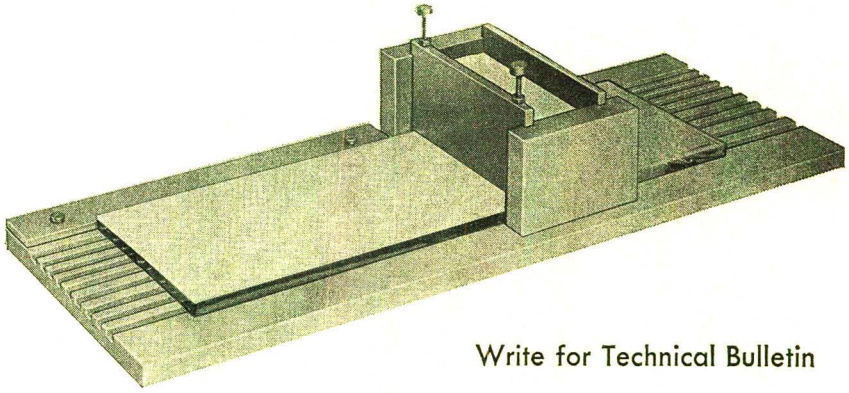

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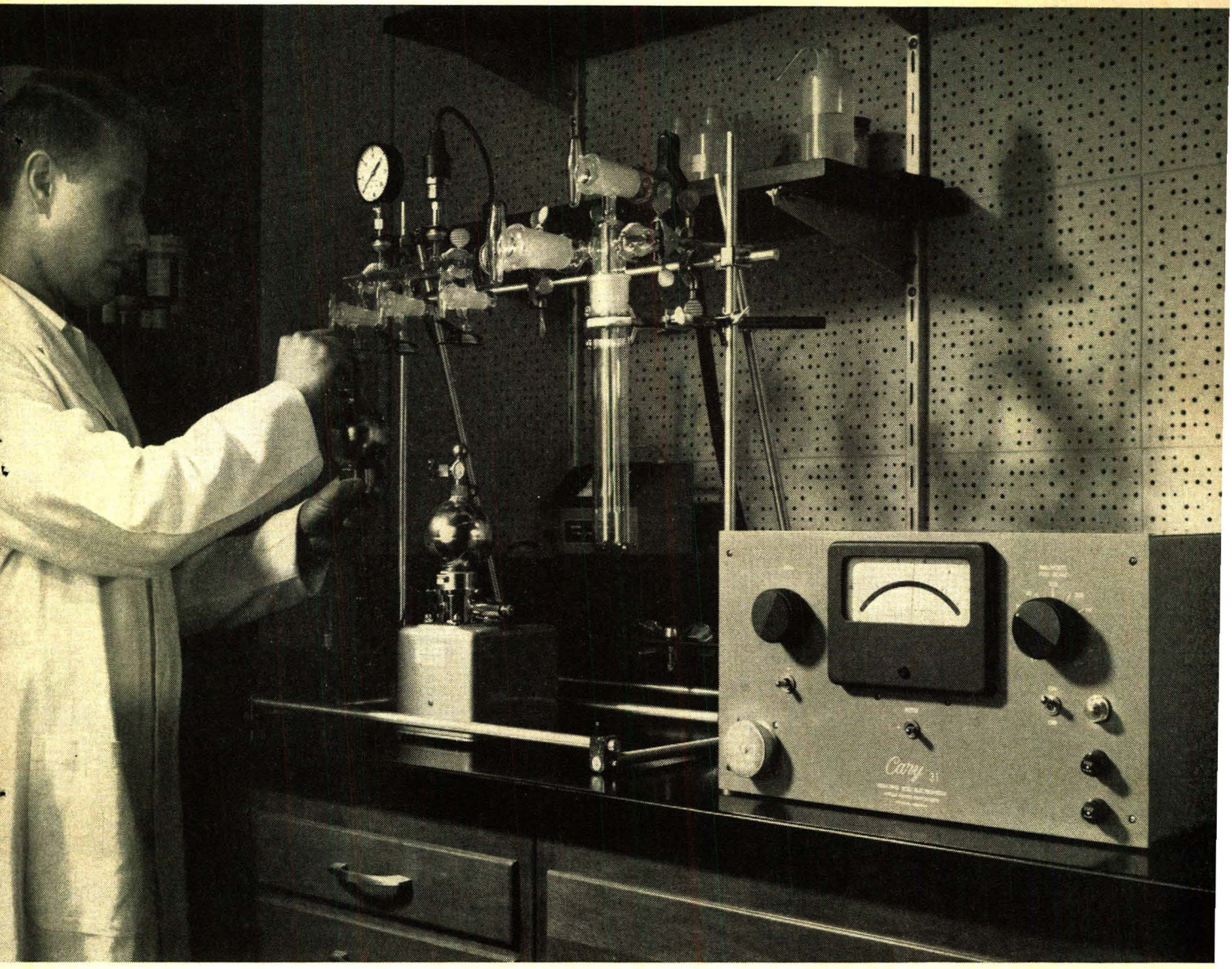

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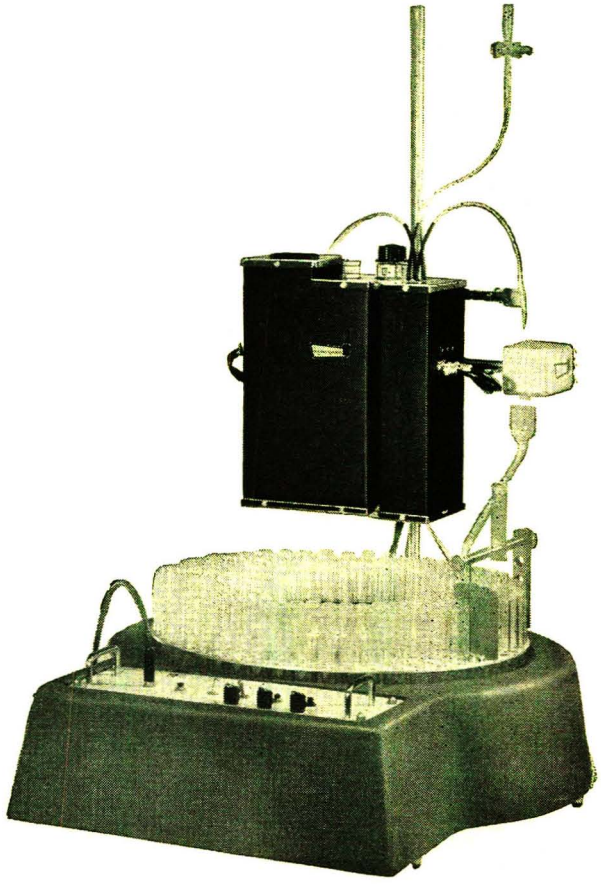

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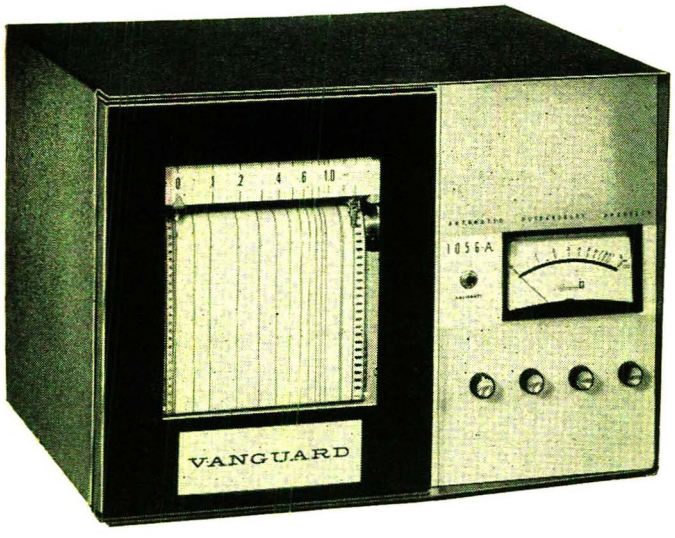

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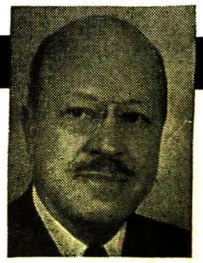

by Ralph H. Müller

Some Russian Instrumentation Developments

THE JOURNAL, *Instrument Construction*, is, we assume, an adequately descriptive translation for the Russian journal *Priborostroenie*. It affords an English translation from the January 1959 issue onwards. It is printed and published for the Department of Scientific and Industrial Research by Messrs. Taylor and Francis, Ltd., of Red Lion Court, Fleet St., London, E. C. 4. More enlightening than its title may be the list of sections, which we find in the contents of No. 2 for February 1963. These deal with Theory and Design, Automatic Control Equipment, Instrument Production Technology, Information and Exchange of Experience, and Reviews and Abstracts.

NICKEL PLATING

Three items which happened to interest us dealt, in one case (p. 19), with hard bright nickel plating using oxalate electrolytes. Hitherto, in the nickel plating of instrument parts, nickel has been deposited from free nickel electrodes and not from electrodes containing complex compounds of nickel. The coats plated in the conventional processes were not very hard and thermal treatment did not increase their hardness. These coats could not be made bright without the use of special organic additives, which complicated the composition of the electrolyte. As carefully worked out by V. N. Brusentsova and D. V. Pletnev, the optimum composition of oxalate nickel baths was established as 140 g./l. of nickel sulfate, 300 g./l. of ammonium oxalate, 15 g./l. of sodium or ammonium chloride. Plating was done at a current density of 10–20 amp./dm^2, the pH at 7.8 to 8.2, and optimum temperature of 78° to 82° C. Periodically, the electrolyte was "neutralized" to pH 8 with ammonium hydroxide.

Heating the plated parts to 300° C. increases the hardness still further and it is then almost equal to that of chromium plate. Chemical analysis of the deposits revealed a constant 0.38% of carbon in the nickel coat and this increases the hardness during the heat treatment. Carbon content is increased by an increase in the quantity of oxalate radical and decreased by increases of pH and temperature or decrease of the current density.

The rate of deposition of nickel in oxalate baths is ten times greater than the deposition of chromium. Both the current output and the dispersion are greater in the oxalate-nickel baths than in chromium plating baths.

TRANSISTORIZED PHOTO-RELAY

A second development (p. 28) deals with a transistorized photo-relay with temperature compensation. Three transistors are used in a simple circuit powered from a stepdown transformer connected to the line. A diode rectifier and hum filter using a 10 mf. 50 v. electrolytic capacitor provides d.c. potentials. The first stage acts as a preamplifier for the photocell current in the common emitter configuration. The light cell is of the photoconductive type. This stage also acts as an impedance matching element between the high resistance photocell and the low resistance input to the second transistor. The third transistor acts as a power amplifier with a relay connected to the collector. An MKU-48 relay, as used by authors P. M. Vaisburd and F. G. Vinengauz, requires 30 ma. for pull-up, which seems to us to be unnecessarily insensitive to be used in a circuit which otherwise has very low power consumption. The amplifier, without compensation, exhibits a 10 ma. increase in output current for a temperature rise of 30° C. (20–50°) whereas with temperature compensation, the output seems to be completely independent of ambient temperature from 20° to some 65°. The nature of the temperature compensation is not explained but, presumably, depends upon temperature coefficients of resistance of opposite sign between photoconductor and the transistors and their associated resistor elements.

It might be mentioned, in passing, that photoconductors are available in a great variety of sizes—some of them almost microscopic, so that optical arrangements can be extremely simple and utilize such things as vitreous or plastic "light-pipes."

TRANSFORMING TEMPERATURE INTO A FREQUENCY SIGNAL

An interesting description of an electro-acoustic unit for transforming temperature into a frequency signal is given by V. V. Kovalevskaya and V. K. Potapkin. The device utilizes the well known principle of acoustic feedback or "ring-around" in which the sound output of a receiver is fed into a transmitter, the output of which, after unit gain amplification, is fed back to the receiver again. Such a system will "ring" at a frequency depending upon the velocity of sound in the medium between receiver and transmitter. A

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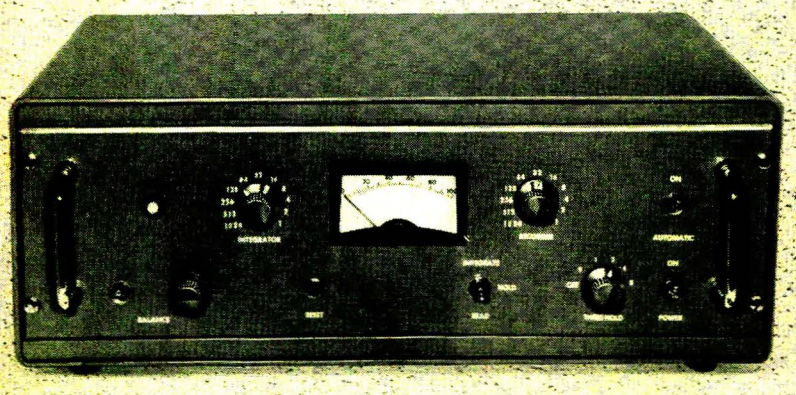

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tube, sealed at the ends with transmitter and receiver, will resonate at a frequency $f_0 = nc/2l$ where c is the velocity of sound, l is the length of the pipe, and $n = 1, 2, 3, \ldots$. The velocity of sound in an ideal gas is: $c = \sqrt{(\gamma RT/m)}$ where γ is the ratio of the specific heats, R is the molar gas constant, T is the absolute temperature, and m is the molecular weight. If this is substituted in the preceding equation, we have:

$$f_0 = (1/2l)\sqrt{(\gamma RT/m)}$$

hence the resonant frequency is proportional to $\sqrt{T}$ and is a function of the parameters of the gas.

The authors have given details of a transducer of this type for the measurement of temperature. In their case, the sensitivity attained (depending upon the length of the resonator) was 1 to 9 cycles per second for ° C.

Actually, the general technique of such resonators, particularly when used with modern electronic counting techniques, can attain high precision and be employed, as the equations indicate or imply, for such things as specific heats, the analysis of gases, liquids, or solids, or for determining elastic constants, Young's modulus, etc. Frequency measurements are easily made to 1 part in 10^8 or better and over a wide range of counting intervals.

Once more we mention the fact that during World War I the Germans were analyzing nitrogen–hydrogen mixtures for use in the Haber nitrogen fixation process by blowing them through an organ pipe. For a pipe of fixed dimensions, the pitch or frequency is a function of the average molecular weight of the gas mixture. The speed of sound in hydrogen is 1260 meters/second and that of nitrogen 337.5 m./sec. In the absence of fancy electronics at that time and to avoid the need for analysts with a sense of absolute pitch, a motor driven siren was used to match the tone to zero beat.

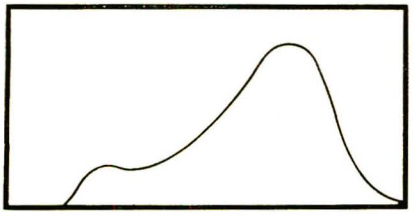
TRANSLATIONS OF RUSSIAN INSTRUMENTATION LITERATURE

Another convenient source of current information on Soviet developments in instrumentation, measurement techniques, and automatic control is *Measurement Techniques*, a translation of *Izmeritel'naya Tekhnika*, a publication of the Academy of Sciences of the U.S.S.R. It is published and distributed at nominal subscription rates under a grant in aid to the Instrument Society of America from the National Science Foundation.

The Analytical Ultracentrifuge... *a review of some exciting new measurements*

The Analytical Ultracentrifuge has come a long way from its early days of simply photographing molecules as they sediment in high force fields. It now provides many of the highly sophisticated measurements needed in such rapidly advancing disciplines as biochemistry, biophysics, genetics and polymer chemistry. Three measurement areas are particularly active.

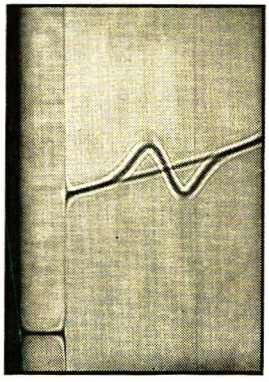
Interacting Systems The analysis of systems containing interacting components is the focus of considerable theoretical interest. An important contribution has been Gilbert's theory for reversibly interacting systems involving a single component. Systems of two components which react to form a complex also have been studied in detail. Bethune and



Monomer-Trimer Equilibrium Forms Two Boundaries

Kegeles have applied a computer to analyze these systems as well as systems involving polymerization. Townend, Timasheff and co-workers have studied molecules which associate in aggregates as large as pentamers, and dissociate into sub-units. Others have examined isomerizing systems in which molecular interactions occur at speeds comparable to the time of separation of the molecular species. Both sedimentation and electrophoresis have provided important measurements in these studies.

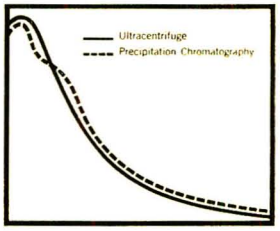
Density Gradients Now established as a powerful and sensitive method to study nucleic acids, equilibrium sedimentation in a density gradient is rapidly finding other uses. Ifft and Vinograd have



Schlieren Pattern of BMA at Sedimentation Equilibrium in a Density Gradient

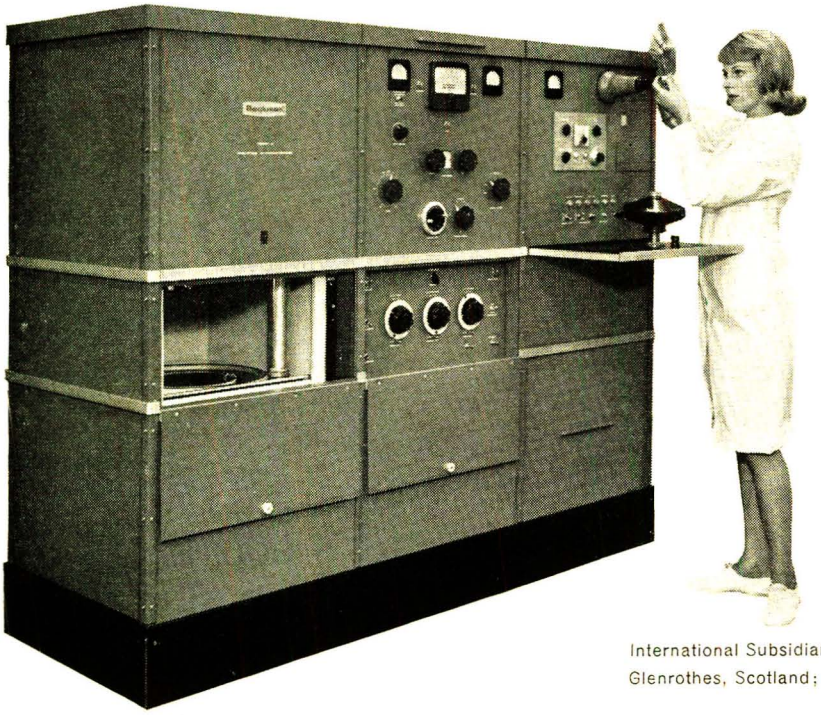
used density gradients to calculate molecular weights for solvated macromolecules, and have studied in detail the behavior of a protein of known molecular weight in a density gradient. Hu, Bock and Halvorson through use of stable isotopes have distinguished between newly synthesized and pre-existing proteins in a cell-free system. Wales has used density gradients of organic solvents to study extremely small quantities of various synthetic polymers, and Hermans has used density gradients to analyze for molecular weight distribution and density distribution of polymers. Both the analytical ultracentrifuge with ultraviolet and schlieren optics, and the preparative unit with swinging bucket rotors are widely used in density gradient centrifugation.

Synthetic Polymers The two density gradient studies noted above are only part of the recent surge of research using the ultracentrifuge to study synthetic polymers in organic solvents. Important papers have been published by investigators at the National Bureau of Standards,



Comparison of Data for Molecular Weight Distribution of Polystyrene

Esso, Shell Development, Dow, and Chemstrand. Their work covers linear polyethylene, polystyrene, Hevea rubber, and cis-1, 4-polyisoprene. A particularly significant example is the study by Wales and Rehfeld showing excellent results in measuring molecular weight distributions from sedimentation velocity data, and demonstrating clearly that their method did not require calibration with fractions of known molecular weight.



Such developments as these, together with up-to-date information on advances in the instrumentation itself, are reported regularly in our publication "Fractions" which is sent to owners of ultracentrifuges, electrophoresis-diffusion instruments, amino acid analyzers, and other Beckman biochemical instruments. If you would like a copy of "Fractions", we would be happy to send one to you. Please write Beckman Instruments, Inc., Spinco Division, Stanford Industrial Park, Palo Alto 7. Calif.

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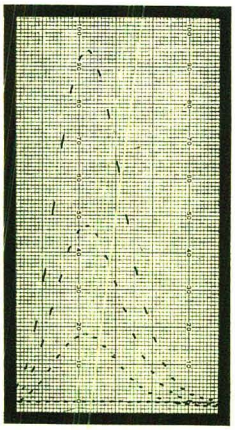
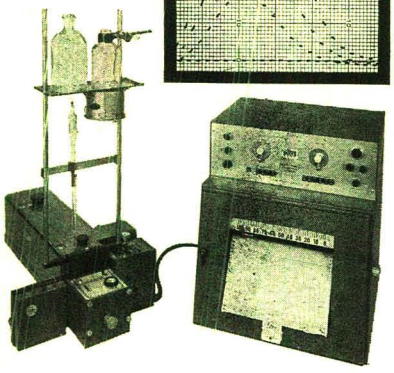


Chart illustrates elution of AMP from Dowex-1 column with formic acid. Quantitative record with zero baseline comparison was established by passing the eluent first through a 10 mm path length cell, then the column and subsequently 2, 5 and 10 mm cells. Gilford flow-through cells, automatic sample positioning and blank compensation were employed.



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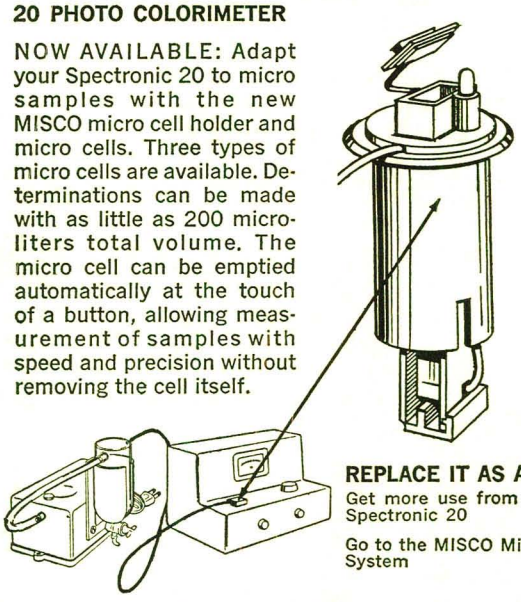

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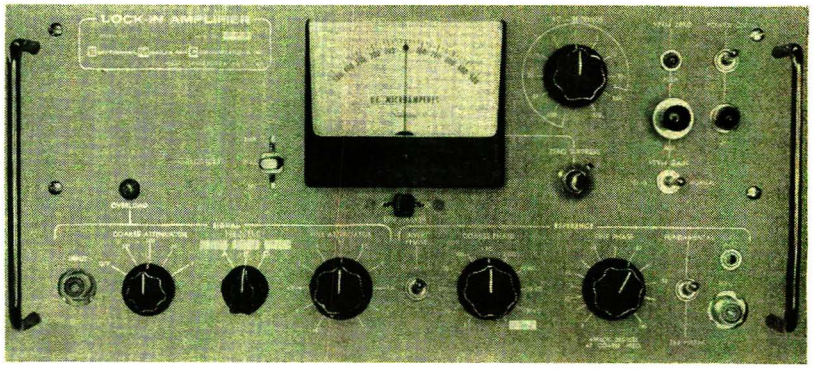

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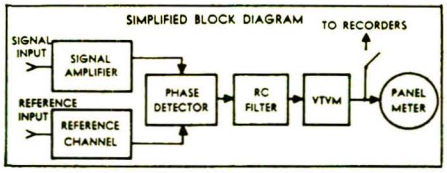

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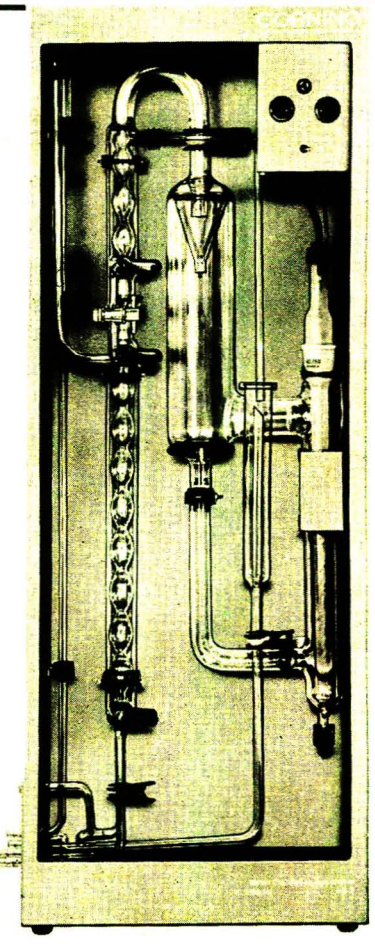

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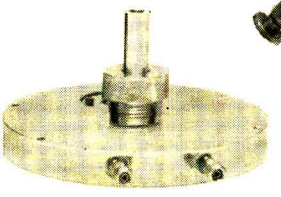

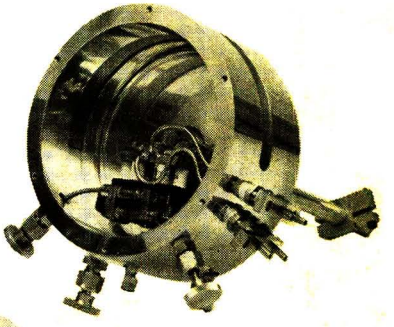

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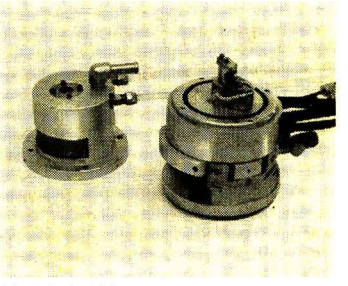

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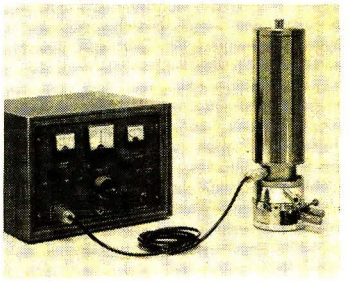

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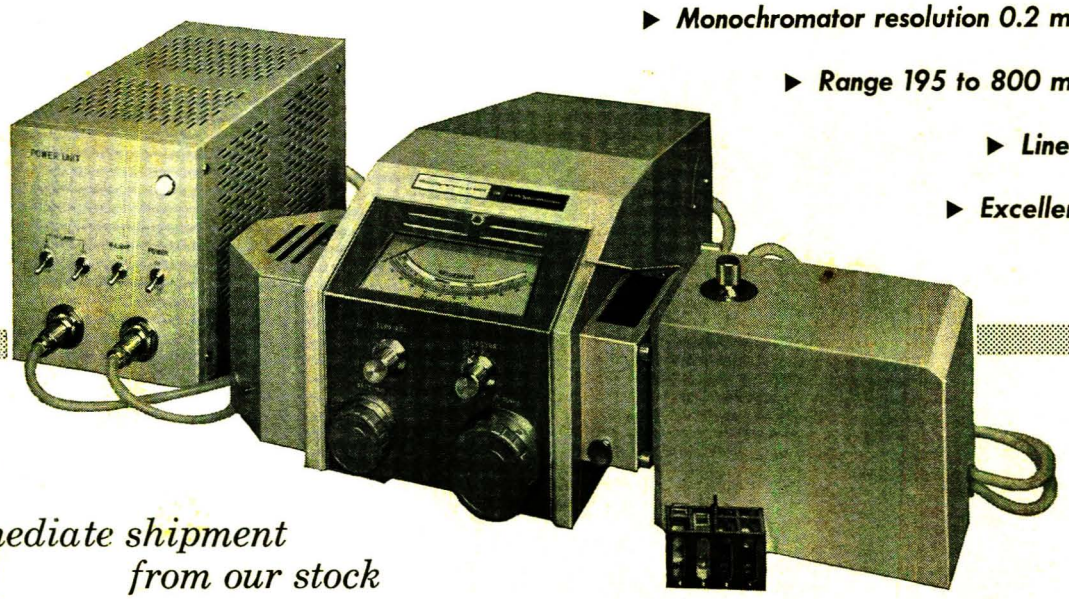

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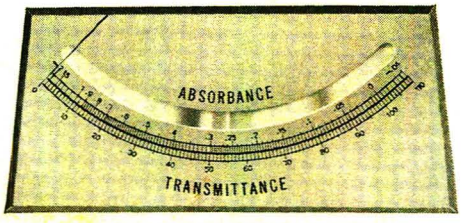

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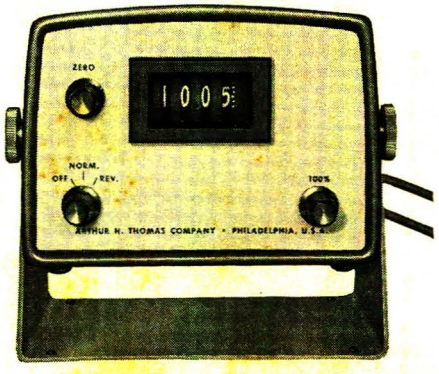

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Professors Move Away from Specialization

WE CONTINUE to hear discussions of curriculum changes in general and the difficulties encountered by analytical chemists in adapting to—often it's outright opposition to—change.

It is encouraging, however, to hear a constructive note as analytical professors try to create new analytical courses to make analysis a more dynamic subject.

The classical approach of the nineteen hundreds was modified by the emphasis on instruments. We are now in a further change where the emphasis is not on the role of instruments but on the use of diverse methods in the solution of analytical problems. Modern instruments in themselves and the role of the specialist are not as important as they were when instruments were constructed in the laboratory.

This change has now been felt in the teaching of analytical chemistry. Professors of analytical chemistry must now try to master and conduct research in several fields. This emphasizes the use of various techniques in problem solving, and it is refreshing to report that professors of analytical chemistry are beginning to teach courses with this new concept in mind.

Papers in several fields are now being submitted by professors who, up until recently, have limited their research to one narrow specialty. This trend, we feel, will do much to help analytical chemistry maintain its position as a major subject in our colleges and universities. It will also turn out graduates who are useful problem solvers rather than narrow specialists.

The Role of Argon Metastable Atoms in the Ionization of Organic Molecules

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▷ A microionization chamber of coaxial design, previously described as a detection system for gas chromatography, has been used to investigate the mechanisms by which argon carrier gas provides high sensitivities to organic molecules with ionization potential below 11.6 e.v. It is shown that for vapor concentrations (ethylene) up to 3 to 4 p.p. 10^4 in the carrier gas the Penning Effect is the sole reaction mechanism for the response of the detector. Beyond this concentration, the collisions of the secondary electrons with sample molecules become important and the response is either increased or decreased depending on the ionization potential of the test molecule and the field strength which determines the electron energy. The decrease is affected by quenching of the electron energy, while the increase is brought about by ion multiplication of the test molecule. Molecules of *I.P.* >11.6 e.v. have been used to further demonstrate these latter processes. The detailed analysis of the mechanisms has also revealed conditions under which such an ionization system could operate. Vapor concentrations as high as 5 to 10% of the carrier gas may exist without any overloading phenomena, thus extending the range of this detector by two orders of magnitude beyond that of conventional argon detectors.

One of the outstanding developments in the field of gas chromatography in recent years has been the introduction of sensitive ionization devices which have radically simplified the detection of minute quantities of organic compounds. Among the systems widely in use today is the argon detector which was initially introduced by Lovelock, James, and Piper (*8*, *9*) and later developed (*7*) as a sensitive detector for almost all organic molecules. Until recently this device had certain relative limitations which involved lack of response to inorganic vapors and fixed gases and to those few organic molecules whose ionization potential (*I.P.*) are greater than 11.6 e.v., and overloading phenomenon which is usually observed when the sample concentration exceeds 1 p.p. 10^3 of the carrier gas.

The first of these deficiencies has now been removed (*4*, *12*) by the design of a new ionization system which provides the highest known response of any detector for fixed gases. In a later study (*5*), it was found that for the analysis of permanent gases the mechanism responsible for the operation of this particular detector system is different from that which operates for organic compounds. For the latter, detection with this device appears to be dependent upon a mode of operation similar to that noted for the conventional argon detectors in that both argon carrier gas and high field strengths are prerequisite to their operation.

From the original work on argon detectors (*8*) it has been maintained that because only organic molecules of *I.P.* below 11.6 e.v. could be detected with such high sensitivity, then the dominant reaction responsible for the operation of these devices must be due to the "Penning Effect." In this reaction the ionization of the sample molecules is brought about not by direct electron impact but through collisions with energetic metastable argon atoms. Recently, however, the predominance of this mechanism has been questioned (*13*). It has been further suggested (*13*) that in addition to the Penning Effect, direct ionization of the sample molecules by energetic electrons may contribute to the high sensitivity of these detectors. It is the purpose of this paper to report on an investigation which was subsequently carried out to study the details of the mechanisms of ionization, particularly as applicable to the new and more versatile detector, and also to present data on the conditions which delay overloading phenomena when large samples are eluted from the column.

The availability of Kr and Xe as carrier gases also made it possible to observe distinct changes in response patterns of certain compounds. Since the metastable energies of Kr and Xe (8.3 e.v. and 9.9 e.v., respectively) are considerably lower in energy than those for argon (11.6 e.v.), it was hoped that the extent of the contribution of the various mechanisms to the response of the organic compounds could be clarified by the comparison of their respective performances.

EXPERIMENTAL

The ionization detector used in this work has been previously described (*4*, *12*). This system which is basically an ionization chamber of coaxial design with 1-mm. electrode spacing, uses a

Table I. Applied Voltage Required for On-set of High Sensitivity Region of Detector for Various Carrier Gases and Test Samples[a]

		Sample gases[b]												
Carrier gas	Energy of metastable states in e.v.	Xylene *I.P.* 8.9 e.v.	Toluene *I.P.* 9.2 e.v.	Butadiene *I.P.* 9.2 e.v.	Benzene *I.P.* 9.4 e.v.	Butene-1 *I.P.* 9.7 e.v.	Ethylene *I.P.* 10.6 e.v.	Acetylene *I.P.* 11.4 e.v.	Ethane *I.P.* 11.6 e.v.	Xenon *I.P.* 12.1 e.v.	Krypton *I.P.* 13.1 e.v.	Methane *I.P.* 13.1 e.v.	Hydrogen *I.P.* 15.4 e.v.
Argon	11.5, 11.7			35 volts	35 volts		35 volts	35 volts	45 volts	80 volts	130 volts	220 volts	90 volts
Krypton	9.9, 10.5			35 volts	35 volts	35 volts	90 volts	230 volts	>400 volts				
Xenon	8.3, 9.4	110 volts	160 volts				>>300 volts						

[a] Concn. of H_2 and CH_4 maintained at 1 p.p. 10^3. All others were approximately between 1 and 3 p.p. 10^4.
[b] Ionization potentials were taken from reference (*11*).

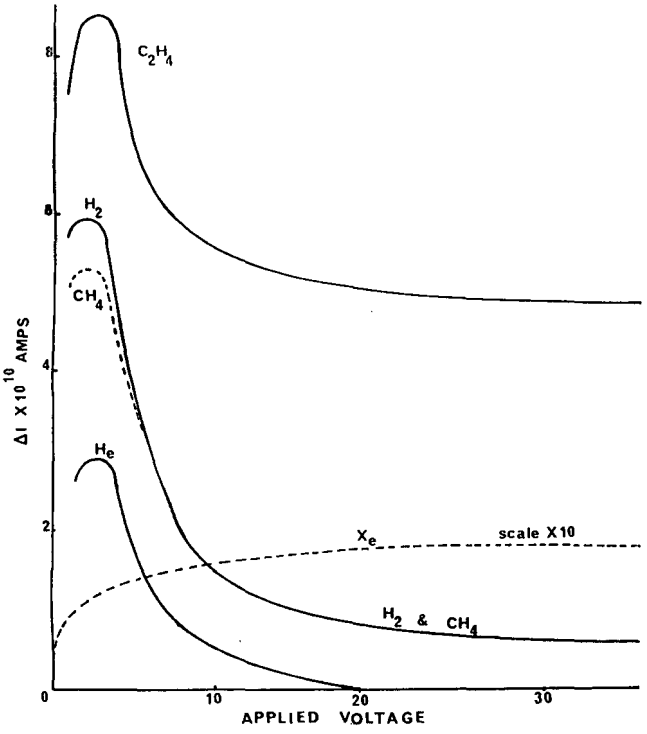

Figure 1. Comparative response of detector to various gases at different voltages

Concns. of C_2H_4, H_2, CH_4, and He are accurately maintained at 1 p.p. 10^3; that of Xe is approximately 1 p.p. 10^3. Argon carrier gas (25 ml./min.). Temp. 165° C.

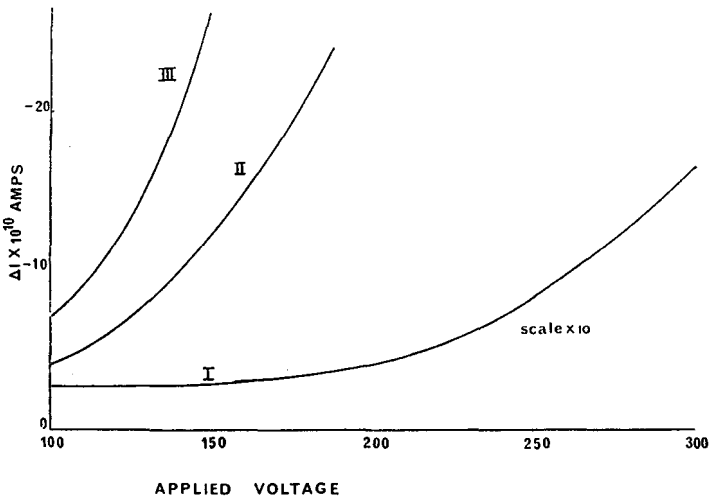

Figure 2. Response of detector to ethylene at 1 p.p. 10^3 concn. at various voltages, using pure argon(III), argon + 0.27% hydrogen(II), and argon + 2.85% methane(I) as carrier gases (25 ml./min.)

200-mc. tritiated titanium foil as the source of radiation. The total volume of the detector is 280 μl.

The ancillary apparatus used for this work was a conventional gas chromatographic unit (Model 10, Barber-Colman Co., Rockford, Ill.) equipped with a high sensitivity electrometer (Model E-302, Gyra Co., Chicago, Ill.) which was operated at ranges of 3×10^{-10} to 1×10^{-11} amp. A Keithley Model 240 variable high voltage supply was used as the precise source of voltage.

Three different chromatographic columns were used in the course of this study. They were: (1) an 8-ft. × 1/8-inch i.d. column packed with Linde Molecular Sieve 5A, 60 to 80 mesh, and maintained at 100° C.; (2) a 4-ft. × 1/8-inch i.d. column packed with silica gel, 40 to 60 mesh, and maintained at 100° C.; and (3) a 4-ft. × 1/8-inch i.d. column packed with 15% ethylene glycol succinate on 80 to 90 mesh Anakrom ABS (Analabs, Hamden, Conn.), and maintained at 165° C. Reproducible microliter quantities of sample gases were injected into the column through a silicone rubber stopper by means of a gastight, precision calibrated microsyringe (No. 263 M.L.S. Starrett Co., Athol, Mass.). Liquids were introduced by means of a 10-μl. Hamilton syringe. High purity krypton and xenon used as carrier gases were supplied by the Linde Co. (The Rare Gas Division, New York, N. Y.). Mixtures of argon with known quantities of impurity used in this work were made up by Matheson Co. (East Rutherford, N. J.) and subsequently analyzed by mass spectrometry. In experiments where a precise level of contamination of the carrier gas by the test molecule was required, a secondary flow containing the contaminant was allowed to mix with the effluent stream emerging from the column. The combined flow was then allowed to enter the detector.

RESULTS AND DISCUSSION

Using argon as a carrier gas a number of representative test gases were analyzed at a constant concentration in an effort to compare their relative responses in the detector at different field strengths. Figure 1 depicts the response patterns of concentration of 1 p.p. 10^3 of ethylene, methane, hydrogen, helium, and xenon at various voltages. The application of the low-voltage (0 to 2.5 volts) region of this ionization system for the analysis of diatomic and certain polyatomic gases has been already described (*4*, *12*), and its mechanism of operation has been attributed to the changes in the drift velocity of the electrons in the system (*5*). After the maximum, where this effect has diminished and saturation current is reached (10 volts), the effective response of the system to ethylene still appears to be significantly different from that of hydrogen and methane. Thus, at approximately 30 volts (field strength 330 volts per cm.) the sensitivity of the detector to ethylene is about 8 times greater than that of hydrogen and methane for the same level of concentration. This phenomenon which is characteristic of all molecules with *I.P.* <11.6 e.v., has long been ascribed to the effect of metastable argon atoms (Penning reaction) which have been formed in the system as a result of the action of primary radiation (*2*).

Production of Metastable Atoms by Energetic Secondary Electrons. As the increase in applied potential is continued beyond 30 volts, the response of ethylene, and in general all molecules of *I.P.* <11.6 e.v., appears to rise sharply at a particular potential, *Em*, which appears to be independent of individual *I.P.* (Table I). In contrast, the response of the detector to molecules of *I.P.* >11.6 e.v. was unaffected at this voltage.

The on-set of this high sensitivity region can be explained by either or both of the two following mechanisms: ionization of sample molecules by electrons which have now gained sufficient energy from the field, and a two-step mechanism including the initial excitation of argon atoms and their subsequent transfer of energy to the test molecules via Penning reaction. That the contribution of the first mechanism is negligible to the overall sensitivity at this concentration is seen from Table I, where the responses of compounds differing in *I.P.* by as much as 3 e.v. but still below 11.6 e.v., appear to rise at about the same voltage.

A similar effect was noted (Table I) when krypton with its lowest metastable energy at 9.9 e.v., was used as a carrier gas. Thus for butadiene and butene-1, which have *I.P.*'s below 9.9 e.v., the voltage required for the on-set of the increased sensitivity region is identical, while for ethylene with an *I.P.* of 10.6 e.v., almost equal to the energy of the second Kr metastable (10.5 e.v.), it has more than doubled. For acetylene (*I.P.* = 11.4 e.v.), the required potential is

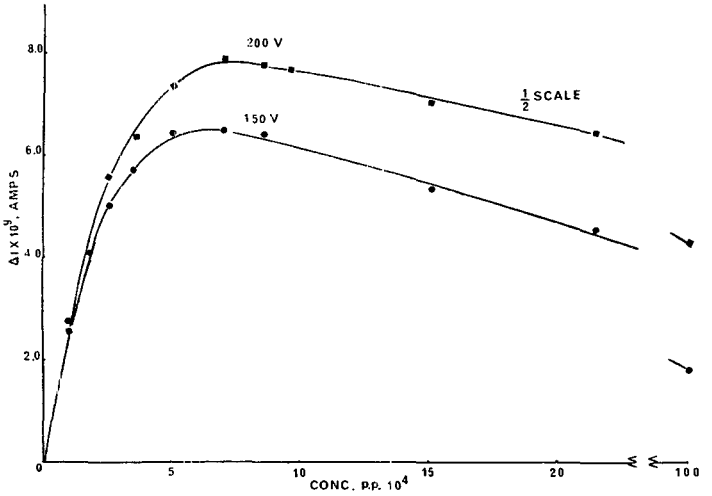

Figure 3. Response of detector to various concns. of ethylene at 150- and 200-volt applied potential

Temp. 165° C. Argon carrier gas (25 ml./min.)

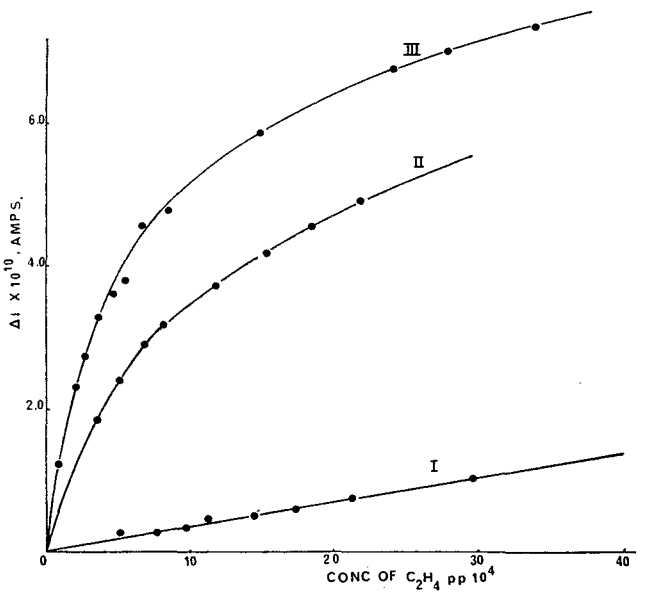

Figure 4. Response of detector to various concns. of ethylene at 165° C. and 30 volts using pure argon(III), argon + 0.27% hydrogen(II), and argon + 2.85% methane(I) as carrier gases

more than eight times as much, while for ethane (*I.P.* = 11.6 e.v.) even at 4400 volts per cm., the region of increased sensitivity had yet to be reached.

For xenon as carrier, with its first metastable state at a low level of 8.3 e.v., no organic molecules with ionization potentials lower than this value were tested because very few are available. The results obtained when xenon carrier gas was used in the analysis of various test samples of higher *I.P.*, however, showed a close correlation between the on-set of the high sensitivity region and their respective ionization potential similar to that noted for krypton. Because of the absence of the Penning Effect the relative response of the detector to those compounds of *I.P.* between the metastable energies of xenon and krypton and 11.6 e.v., was less when these gases were used as carriers than in argon.

Thus it is apparent that where the *I.P.* of the test gas is less than the excitation potential of the carrier, *Em* corresponds to a field strength at which a significant increase in the number of the electrons capable of exciting the carrier gas takes place. This raises the concentration of the metastable species which in turn causes an observable increase in the level of ionization of the test sample. At the concentrations used in these experiments (see Table I) any direct ionization of such a test sample by energetic electrons would appear negligible at this particular voltage. On the other hand, when test molecules of ionization potentials greater than the metastable energies of these carrier gases are used the values of *Em* represent the potentials at which direct ionization of the test gas by energetic electrons becomes feasible.

Mechanisms of Operation of Detector at High Voltages (Molecules of *I.P.* <11.6 e.v.). Using ethylene as an example, Figure 2, curve III, shows that as the applied voltage on the detector is further increased (150 to 200 volts), the sensitivity of the detector to organic molecules continues to increase, possibly because of an increase in the metastable concentration. This results in an extremely high response which is characteristic of this class of argon detectors (*8*). Curves I and II of this graph further demonstrate that the presence of impurities in the carrier gas substantially reduce the observed response.

The performance of the detector to various concentrations of ethylene at these high voltages is also noted in Figure 3 which indicates both the range of linearity and the upper limit of concentration beyond which the response is actually reduced. The effect at high concentrations—e.g., 10 to 20 p.p. 10^4—produces the familiar double peaks which are correctly attributed to overloading (*1*).

In an effort to study the mechanism responsible for the operation of the detector at these voltages in more detail, it was convenient to test the applicability of the Penning reaction to this system at these high voltages and to

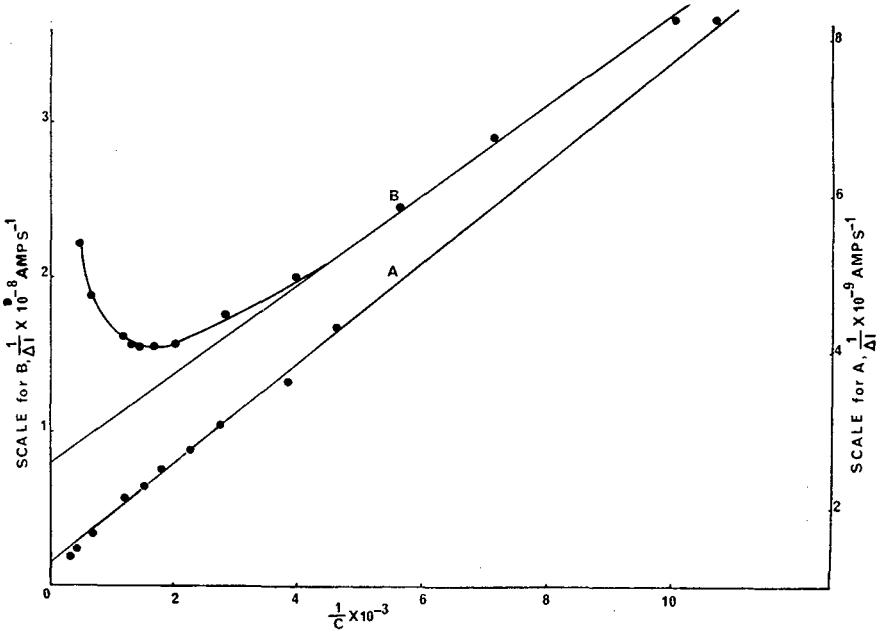

Figure 5. Inverse of detector response against reciprocal of ethylene concentration

Curve A, at 30 volts. Curve B, at 150 volts. Temp. 165° C. Argon carrier gas

determine whether any deviations due to secondary effects occur. The Penning Effect has been recognized (*2*, *3*), though not proven, to be of major importance in systems where the effects of ionizing radiation on rare gases are studied. Moreover, evidence was presented in the previous section which indicated that the on-set of the high sensitivity region applicable to organic molecules is due to this reaction rather than to direct ionization of the test molecules by energetic electrons at the sample concentrations used. Jesse and Sadauski (*2*), in their study of the Penning Effect in helium, successfully applied the following equation which describes the increased level of ionization (Δn_i) in a system of rare gas atoms containing small concentrations (c) of impurity gas—i.e.,

$$\frac{1}{\Delta n_i} = \frac{1}{k} + \frac{k_d}{k.k_i} \cdot \frac{1}{c} \qquad (1)$$

where k, k_i, and k_d are the rate constants for the production of the metastable atoms, ionization of the contaminant molecule by the latter, and the destruction of the metastable atoms in pure rare gas, respectively. Although the behavior of the metastable species in its parent gas is now more fully understood (*6*), this equation still describes the basic phenomena observed, and any refinements in reaction schemes (*13*) would only alter the description of the constants used in this equation which is unnecessary for the present study.

To demonstrate the general application of Equation 1 to this study and to provide additional reference data, the results obtained for ethylene at an applied potential below *Em*—e.g., at 30 volts, Figure 4, curve III—where the metastable atoms are known to be formed from the action of primary radiation, were replotted according to this equation. These results appear on Figure 5, curve *A*. The linearity of this plot demonstrates that Equation 1 is strictly followed as anticipated since the Penning reaction is the sole mechanism for the response to ethylene at this voltage (*2*). The ratio of the intercept to the slope of this line $\left(\frac{k_i}{k_d}\right)$ was found to be 2.0×10^3 which is of the correct order of magnitude for these processes.

Taking the results obtained at 150 volts (Figure 3) as an example of the behavior of the system at applied potentials beyond *Em* and replotting them according to Equation 1, a somewhat more complex form is noted as observed on curve *B* of Figure 5. The linear portion of this curve demonstrates that Equation 1 is applicable up to sample concentrations of 3 to 4 p.p. 10^4 of the carrier gas. Furthermore, that the response of ethylene within this concentration range is entirely due to the Penning Effect is indicated by the

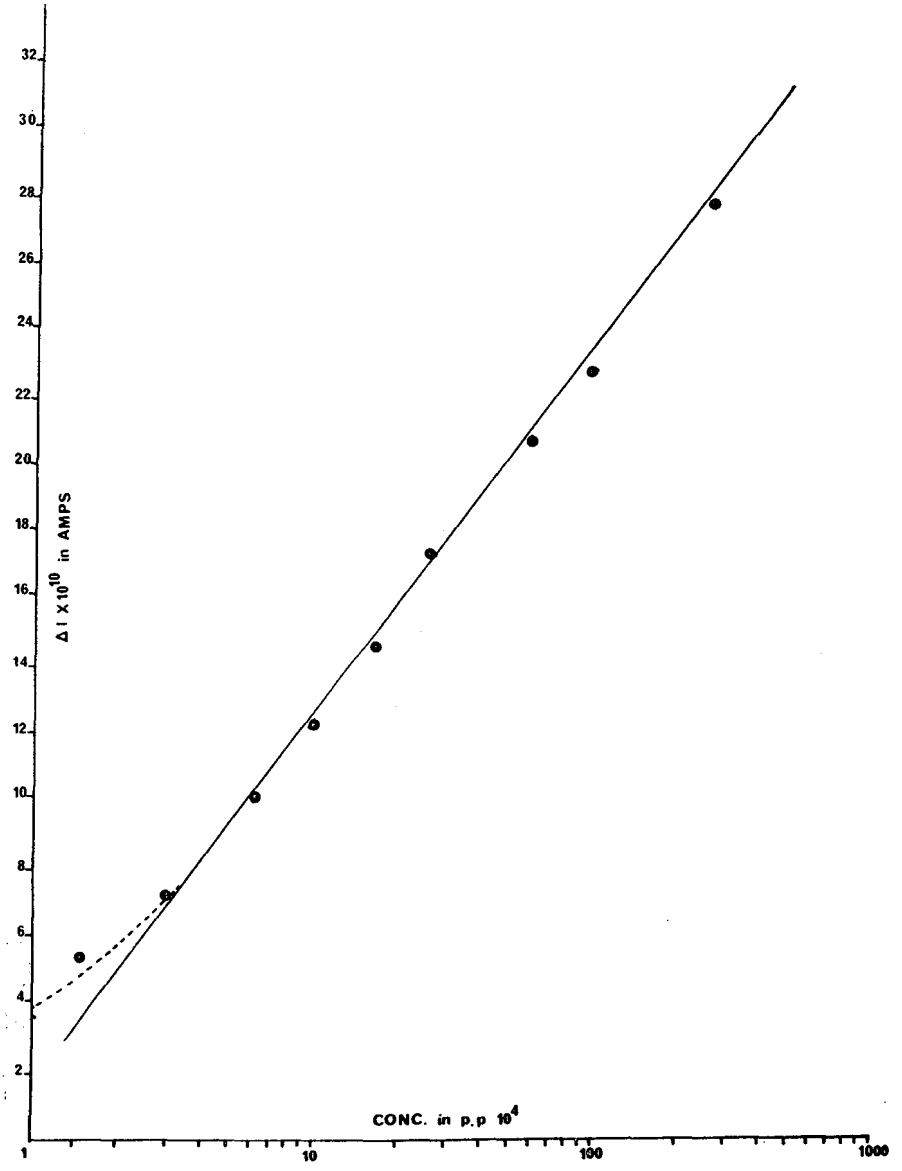

Figure 6. Response of detector at 30 volts to various concentrations of ethylene

Temp. 165° C. Argon carrier gas

value for $\frac{k_i}{k_d}$ obtained for the linear portion of this curve. This value is 2.8×10^3, which within experimental error, is close to that found for curve *A*. Thus, it would appear that at these concentrations part of the secondary electrons which have gained sufficient energy from the field by collision produce excited metastable argon atoms which in turn account for the high response to ethylene. Curve *B*, however, indicates that a mechanism which acts to reduce the concentration of the metastable species begins to operate in the system when the proportion of ethylene exceeds 3 to 4 p.p. 10^4 of the carrier gas. Undoubtedly the quenching by ethylene of the energetic electrons which otherwise would produce excited argon atoms, is responsible for this phenomenon. Such a process would be expected since electrons undergo many collisions with the gas atoms and molecules during the time interval necessary to acquire excitation energy. Moreover when the concentration of the sample molecules is sufficiently high, the probability of inelastic collisions is also increased. As a result the electronic energy is lost to the sample molecules without ionization. The results obtained at 200 volts (Figure 3) can be explained in a similar fashion.

As the applied voltage was increased to 250 volts (2800 volts per cm.) along with an increase in the concentration of C_2H_4, the response of the detector appeared to rise exponentially producing an effect similar to that reported by Lovelock for the conventional argon detector (*8*). From the discussion presented here it is apparent that at and above such an applied potential, the energy of the secondary electrons

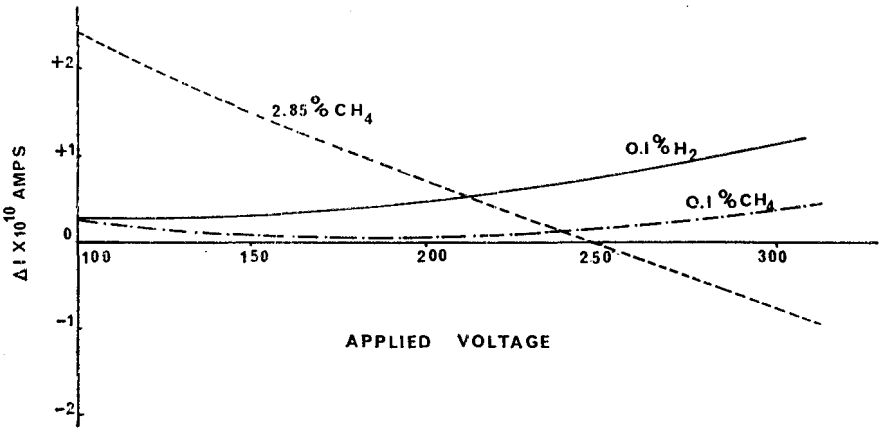

Figure 7. Response of detector at various applied voltages to H_2 and CH_4, at different level of concentrations

Temp. 165° C. Argon carrier gas

acquired within one mean free path of the sample molecules now exceeds the ionization potential of ethylene. Thus when the concentration of the latter is above 3 to 4 p.p. 10^4 of the carrier gas, and the probabilities of energy transfer processes—i.e., ionization by the metastable atom and by electron impact—become comparable, then part of the response elicited from the detector would arise from the direct ionization of sample molecules by energetic electrons. The exact proportion of the contribution of the two mechanisms, however, will acutely depend on the magnitude of the cross sections for various processes involved.

In ionization systems where the geometry of the detector produces a nonuniform electric field—e.g., conventional argon detectors— the concurrence of the various mechanisms stated above becomes obvious. Inherent in the design of the conventional argon cells (*7*) is a region near the inlet of relatively high sample concentration. In this area the possible contribution of direct ionization by energetic electrons is increased even though the overall concentration of sample within the detector may not yet have approached the maximum limit for maintaining linear response by way of metastable reactions only.

Extension of Detector Response to Higher Vapor Concentrations. From the discussion in the previous section it is clear that in order to extend the range of such an argon detector to higher concentrations of organic vapor, the system should be operated under conditions where most metastable atoms are derived from excitation via primary electrons and none or few metastables are produced through excitation by secondary electrons which have gained their energy from the applied field. Under such circumstances quenching of electron energy through frequent collisions with sample molecules would have little effect on the rate of formation of metastable argon atoms. This requirement is fulfilled when the applied voltage is below *Em*, the field necessary for the on-set of the increased sensitivity region, which for this source strength was 35 volts. At such a voltage the minimum level of detection of C_2H_4 as extrapolated was 5 p.p. 10^7 of the carrier gas. Under these circumstances the sensitivity of the device to very small samples is sacrificed to some extent. Figure 6 shows the performance of the detector at 30 volts (330 volts per cm.). It is observed that the practical range of the system is extended by at least two orders of magnitude when compared to the high voltage region. It is obvious that the plot will eventually deviate from the straight line, showing a reduced response at higher sample concentrations, since the rate of metastable formations by the primary radiation will be reduced as argon concentration is decreased in the system. Therefore, the useful limit of the operation of the detector under these conditions will probably be between 5 and 10% of sample concentration (*10*) which is more than the range encountered in practice.

Performance of Detector at High Voltage (Molecules of *I.P.* >11.6 e.v.). The use of ionization chambers at high applied potentials with argon as carrier gas offers little promise as a sensitive detector for those few molecules which have *I.P.* >11.6 e.v. These molecules will, in general, effectively quench metastable argon atoms through destructive but nonionizing collisions, and their sensitivity pattern will exhibit the effect of other processes such as direct ionization by energetic electrons and quenching of the latter before they have sufficient energy for ionization. The interdependence of these processes, however, will not permit the response elicited from the detector to be a simple linear function of the sample concentration. Figure 7 shows the response patterns of H_2 (0.1%) and methane (0.1 and 2.85% for applied potentials between 100 to 300 volts). Examination of the curves for methane shows the complex dependence of the detector response to sample concentration at various voltages. Although the response of methane at high concentrations is negative beyond 2800 volts per cm. and up to 5500 volts per cm. as tested, the response for the lower concentration of methane appears to rise continuously. Apparently this may be attributed to a larger contribution of direct ionization of sample to the flow of current, over the quenching effect of these molecules. Even at such low concentrations a simple interpretation of the results is not adequate, as shown by the much lower applied potential (1100 volts per cm.) required for the on-set of ion multiplication region for an equivalent concentration of hydrogen. A comparison of these values is given in Table I, with the ionization potential of the test molecule for a number of gases. Hydrogen with a much higher ionization potential than Xe appears to require almost the same potential for the on-set of the multiplication region. No simple explanation of this phenomenon is available at this time.

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Determination of Partition Coefficients at Infinite Dilution by the Gas Chromatographic Analysis of the Vapor above Dilute Solutions

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▶ **A method is described for measuring gas liquid partition coefficients at infinite dilution for volatile and involatile solvents. It depends on measuring the rate of removal of a solute as an entraining gas is passed through a very dilute solution. Partition coefficients from water at 25° C. for acetone and the normal alcohols (one to four carbon atoms) and from squalane at 80° C. for normal paraffins (five to nine carbon atoms) have been determined; the results are in good agreement with those found by other methods.**

GAS LIQUID chromatography has frequently been used to determine partition coefficients at infinite dilution by the method first described by Porter, Deal, and Stross (*15*), which depends on the measurement of specific retention volumes. In those cases where independent determinations by other methods (*1*, *3*, *11–13*, *15*) have been made, there is reasonable agreement between the two sets of data. On the other hand, in some systems, particularly those involving volatile solvents, or solutes and solvents of widely differing polarity, the chromatographic method is hard to apply (*13*), and the results in some cases are of doubtful validity (*13*, *14*). A further method has therefore been studied, based on the measurement of the rate of removal of a solute from a dilute solution by a gas stream which is analyzed periodically by gas chromatography. This method avoids the difficulties of the conventional static measurements (*4*, *6*, *9*, *11*, *12*, *17*) and the assumptions needed to convert chromatographic specific retention volumes into partition coefficients (*15*).

THEORETICAL

In principle, an entrainment method such as that described by Shaw and Butler (*17*) could be applied to very dilute solutions, the entraining gas being analyzed chromatographically using a sensitive detector. However, to avoid the difficulties (*5*) of the absolute calibration of such detectors for each solute used, a slightly different approach was adopted. The entraining gas was passed through a quantity of solution which was sufficiently small to ensure that the concentration of the solute decreased at a measurable rate. The detector was calibrated initially using gas samples saturated by solutions of known concentration, so that the results of the gas analysis gave the corresponding solute concentrations. The removal of the solute from the solution then followed a simple kinetic law, the rate constant of which involved the partition coefficient as the single unknown parameter.

The kinetic law for the removal of the solute from solution may be derived from the definition of the partition coefficient, K, defined by the relation

$$K = \frac{\text{mass of solute/unit volume of solvent}}{\text{mass of solute/unit volume of gas}}$$

The mass of solute per unit volume of solvent at any point in the experiment may be expressed as follows. Let v ml. of entraining gas be passed through m' grams of solute dissolved in m'' grams of solvent, the volume of the gas being corrected to 273.2° K. and measured after saturation by the solution. The solution is made sufficiently dilute for the vapor pressure of the solvent in the solution to be very close to that of the pure solvent (p'' mm. Hg). If the vapor is assumed to be an ideal gas, then

$$-\frac{dm''}{dv} = \frac{M''p''}{2.24 \times 10^4 \times 760} \qquad (1)$$

where M'' is the molecular weight of the solvent.

The mass of solvent, after v ml. of gas passed, is therefore $(m''_o - 5.88 \times 10^{-8} M''p''v)$, where m''_o is the initial mass of solvent present. In cases where the solvent is sufficiently volatile for the perfect gas assumption to introduce serious error, dm''/dv can be determined by weighing the sample before and after each experiment. If dm''/dv is found to have a value α and the density of the solvent is ρ, the concentration of solute in the solvent at this stage will be $m'\rho/(m''_o - \alpha v)$ grams of solute per ml. of solvent. This form of the expression for the mass of solute per unit volume of solvent is used in the subsequent calculation. The mass of solute per unit volume of gas measured at T, the temperature of the system, is given by $(273.2/T) \times (dm'/dv)$. On substituting these two expressions in the definition of K, the partition coefficient, and rearranging, the loss of solute from the sample is found to be given by Equation 2.

$$-\frac{dm'}{dv} = \frac{m'\rho T}{K\ 273.2\ (m''_o - \alpha v)} \qquad (2)$$

Equation 2 is used to interpret the experimental results in one of two integrated forms, depending on whether the solvent is volatile or involatile.

Equation 3 is used for volatile solvents where p'' and α are large.

$$\log_e (m'/m'_o) = (\rho T/K\ \alpha\ 273.2) \log_e (1 - \alpha v/m''_o) \qquad (3)$$

Equation 4 is used for involatile solvents where p'' is negligible.

$$\log_e (m'/m'_o) = -T\rho v/Km''_o\ 273.2 \qquad (4)$$

The values of m' during an experiment can be calculated from the calibration relating the peak height, h, to the concentration, m'/m''. In general, $m'/m'' = f(h)$, the form of the function $f(h)$ being deduced by plotting h_o, the extrapolated peak height at zero time, against m'_o/m''_o. This procedure can be made very accurate by carrying out a separate series of experiments in which m''_o is made sufficiently large to ensure that $-dm'/dv$ is small and, in some cases where K is large, extrapolation becomes unnecessary.

Consequently by plotting $\log_{10} m'/m'_o$ against either $\log_{10}(1 - \alpha v/m''_o)$ or v/m''_o, K can be found from the slopes, $\rho T/K\alpha\ 273.2$ or $1.59 \times 10^{-3}\ \rho T/K$.

In practice, errors can be introduced by the effect of the dead space, v_d above the sample. If mixing does not occur in the dead space, account need be taken only of the fact that the first v_d ml. of gas to leave the apparatus will not have passed through the sample. However, if mixing does occur, the concentration of the entraining gas at any point in the experiment will

Table I. Partition Coefficients from Water

(T = 25° C.)

Solute	$10^4\ m'_o/m''_o$ (approx.)	$10^{-3}K$	$\sigma\ (10^{-3}K)$
Acetone	0.3	0.682	0.012
Methanol	3.0	5.58	0.20
Ethanol	3.0	5.32	0.15
1-Propanol	1.0–2.0	4.00	0.10
1-Butanol	0.2–2.0	3.39	0.09

Table II. Partition Coefficients from Squalane

(T = 80° C.)

Solute	$10^6 m'_o/m''_o$ (approx.)	K	$\sigma\ (K)$
Pentane	4	27.4	0.6
Hexane	12	64.4	1.3
Heptane	22–120	148	3
Octane	2–25	335	7
Nonane	6–130	701	14

be altered by being mixed with the gas already in the dead space. The case of complete mixing can be dealt with theoretically. Assuming, for simplicity of analysis, that the solvent is involatile—i.e., $\alpha \sim 0$—then:

$$dg'/dv = (m'\rho/m''_o K - g'/v_d)T/273.2 \quad (5)$$

where g' is the mass of solute vapor in the dead space after v ml. of gas measured at 273.2° K. has passed. The integrated form of this equation, after substitution for m' using Equation 4, is Equation 6.

$$\frac{g'}{v_d} = \frac{m_o'\rho \exp - (T\rho v/Km''_o\ 273.2)}{m''_o K}\left(\frac{1 - \exp - (Tv/v_d\ 273.2)\ (1 - \rho v_d/Km''_o)}{1 - \rho v_d/Km''_o}\right) \quad (6)$$

If $\rho v_d/Km''_0$ is always arranged to be less than 0.02 and all readings before Tv/v_d 273.2 = 4 are neglected, the extrapolated value of g'/v_d at time zero will be within 2% of the equilibrium vapor concentration of the solute above the solution of concentration m'_o/m''_o. Under these conditions the experimental value of the partition coefficient should be unaffected by the dead space error.

The error calculated from Equation 6 is an upper limit and since mixing in the dead space is likely to be slight, the error will, in practice, probably be much less.

EXPERIMENTAL

Chemicals. Squalane, polyethylene glycol 400, analytical reagent grade acetone, and 1-butanol were obtained from British Drug Houses, Ltd. 1-Propanol was obtained from the same source, as a laboratory grade reagent. After redistillation, a fraction boiling at 97° C. was collected which, when analyzed by gas chromatography, was found to contain less than 2% impurity. Absolute ethanol and analytical reagent grade methanol were supplied by Burrough's, Ltd. Pentane was obtained from B. Newton Maine, Ltd., and hexane, heptane, and octane from L. Light and Co., Ltd., all being specified to contain 99% hydrocarbon. Nonane, specified to be of 95% purity, was also obtained from L. Light and Co., Ltd. The hydrocarbons all gave a single peak on chromatographic analysis and were used as supplied. Aqueous solutions were made up with laboratory-distilled water.

Apparatus. The solution samples were held in cells of the types shown in Figure 1. The maximum dead space error for these cells, when used with the solvents indicated, calculated from Equation 6 was always negligible. The wire gauze screen shown in Figure 1 was necessary to prevent the sample foaming and being lost in the form of spray carried out of the cell by the entraining gas. In the experiments with squalane the screen was unnecessary, since this liquid showed little tendency to foam.

The temperature of the cells was controlled by a Techne thermostat and bath, the thermostat being sensitive to a change in temperature of about 0.05° C. The temperatures were measured correct to ±0.02° C. at 25° C. and ± 0.1° C. at 80° C.

Hydrogen was used as the entraining gas and the volume passed at any time during an experiment was calculated from the time interval and the gas flow rate, which was measured by a 50-ml. bubble flowmeter. The total volume of hydrogen passed during a single experiment was about 4500 ml. for water solutions and between 1200 and 3000 ml. for squalane solutions, depending in the latter case on the hydrocarbon solute.

Gas sampling was generally performed using a conventional O-ring, sealed, push-pull sampling valve with a 5-ml. sampling loop. The sampling system was heated where necessary to about 100° C. to prevent the loss of sample by adsorption on surfaces, particularly those of the silicone rubber O-rings. This effect became noticeable during experiments involving octane and nonane. Syringe injection was used in some of the earlier experiments, but no variation in the experimental results occurred when the more convenient sampling valve system was adopted, apart from a marked decrease in the random error of the observations.

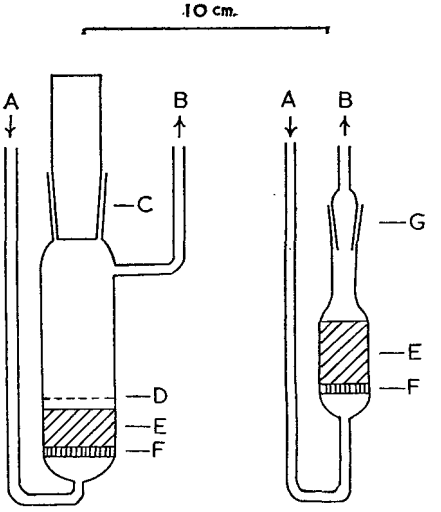

Figure 1. Entraining cells

Left. Cell used for aqueous solutions
Right. Cell used for squalane solutions
A. Hydrogen gas supply inlet
B. Sampling valve and flowmeter outlet
C. B 24 cone and stopper
D. Wire gauze screen
E. Solution sample
F. Glass sinter, average porosity 20 to 30 microns
G. B 7 joint

Analyses were carried out using a hydrogen flame ionization detector of the type described by Purnell (*16*) combined with one of two columns:

A column, 35 cm. long and of 0.45-cm. i.d., packed with 15% squalane on 80- to 100-mesh Celite.

A column, 45 cm. long and of 0.4-cm. i.d., packed with 2.6% polyethylene glycol 400 on 80- to 100-mesh Celite.

Procedure. Each experiment was started from the time when a measured quantity, varying between 2 and 7 grams of samples of known composition, was placed in the cell. One flow rate measurement was made after each analysis and the sample loop was flushed with about 50 ml. of the entraining gas from the cell before sampling. The flow rates varied between 60 and 120 ml. per minute. Column 1 was used at 25° C. for acetone, methanol, and ethanol and at 50° C. for 1-propanol, 1-butanol, pentane, hexane, and heptane. Column 2 was used at 25° C. for octane and at 50° C. for nonane. The choice of analytical column was decided by the necessity to analyze samples quickly, the analysis times ranging from 0.5 to 4 minutes.

The peak height–solution concentration calibration plots mentioned earlier were determined from initial peak heights and concentrations and $\log_{10} m'/m'_o$ was plotted, calculated from the measured peak heights using the calibration curves.

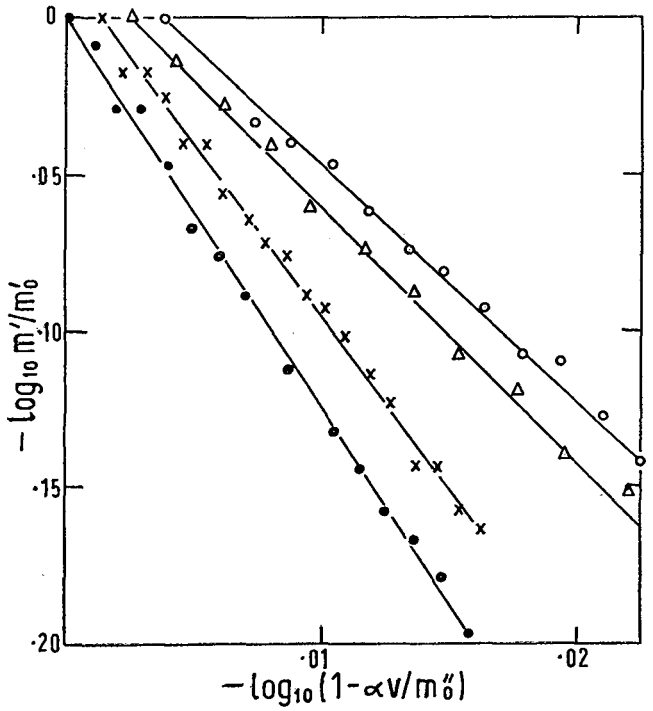

Figure 2. Plots of $\log_{10} m'/m'_0$ against $\log_{10}(1 - \alpha v/m'')$ for aqueous solutions

	m''_0 gm., g.	$10^4 m'_0/m''_0$
○ Methanol	4.00	3.05
△ Ethanol	3.97	2.69
× 1-Propanol	4.99	9.72
● 1-Butanol	5.00	1.21

Origins of experiments with ethanol, 1-propanol, and 1-butanol displaced along abcissa to increase clarity of figure

RESULTS

The method has been applied to two solvents, water and squalane. No evidence was found that deviation from Henry's law occurred at the concentrations used in this work (Tables I and II) for any of the systems studied.

Calibration experiments using large samples of aqueous solutions, designed to ensure that dm'/dv was small, gave the same chromatographic gas analysis when the gas flow was changed from 18 to 180 ml. per minute. Thus it seems likely that gas passed into a solution through a glass sinter of porosity 20 to 30 microns is saturated over the range of flow rates used (60 to 120 ml. per minute).

No chance of inhomogeneity occurs when the cells shown in Figure 1 are used, because of the stirring action of the entering gas. The pressure of the gas bubbles in the solution is always within 3 mm. of atmospheric pressure, because of the small depth of the solution.

Water (T = 25° C.). The results of plotting $\log_{10} m'/m'_0$ against $\log_{10}(1 - \alpha v/m''_0)$ are illustrated in Figure 2. The scatter of the points on such plots suggests that the random error of each observation is generally 1% or less. In other groups of experiments the concentration was allowed to change in the course of a run by a factor of 2 and, in the case of butanol, the initial concentration used was varied between experiments by a factor of 10. The mean values of the partition coefficients found from these experiments are given in Table I.

Squalane (T = 80° C.). The results of plotting $\log_{10} m'/m'_0$ against v/m''_0 are illustrated in Figure 3. As before, the random error of the observations is generally about 1% or less.

A group of experiments was performed using solutions containing the C_5, C_6, and C_7 hydrocarbons, so that their partition coefficients could be found simultaneously. The results of these experiments did not vary significantly from those in which a single solute was used.

The range of initial concentrations used is shown in Table II; the change in concentration observed during a run varied from 50% for C_9 up to a factor of 100 for C_5.

DISCUSSION

In Tables III and IV, the results of this work are given, expressed as specific retention volumes calculated using the recommended definitions (*2*). They are compared with independent estimates calculated from data taken from

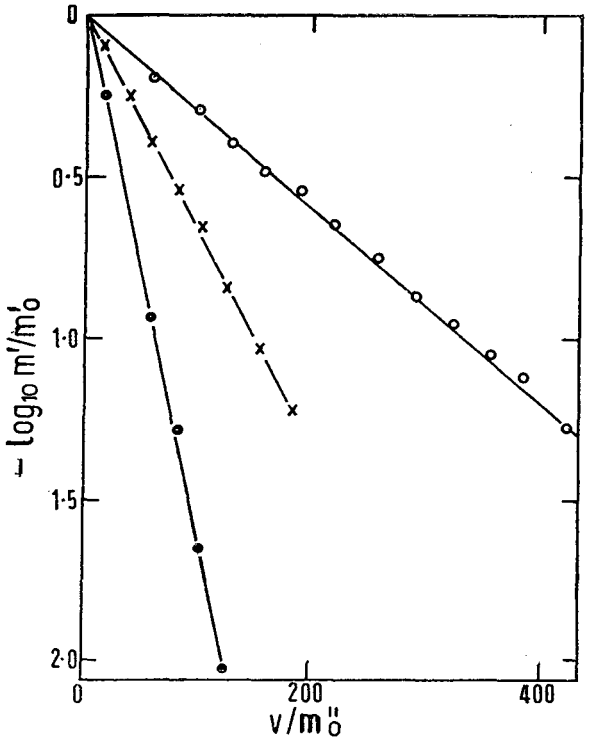

Figure 3. Plots of $\log_{10} m'/m'_0$ against v/m''_0 for squalane solutions

	m''_0 g.	$10^6 m'_0/m''_0$
● Pentane	7.05	3.77
× Hexane	7.05	12.0
○ Heptane	5.22	118

Table III. Comparison of the Results with Previous Determinations

Solvent, water (T = 25° C.)

Specific retention volume, V_g

Solute	This work	(4)	(6)	(9, 17)
Acetone	625	644		
Methanol	5110		4950	
Ethanol	4870			4600
1-Propanol	3660		3480	
1-Butanol	3100		2920	

Table IV. Comparison of Results with Previous Determinations

Solvent, squalane (T = 80° C.)

Specific retention volume, V_g

Solute	This work	(10)	(8)	(13)	(1)	Mean of cols. 3 to 6	(7)
Pentane	27.3	24	24.7	25.1	26.7	25.1	...
Hexane	64.1	62.5	61.7	60.7	62.8	61.9	71.6
Heptane	148	145	144	145	146	145	159
Octane	334	330	334	339	...	334	365
Nonane	698	750	772	...	...	761	811

the sources referred to at the head of each column.

The error due to the extrapolation required to give the figures in columns 3, 4, and 5 of Table III is generally ±3%, but rises to about ±5% for 1-butanol. Columns 4 and 5 are based on the activity coefficients at infinite dilution given by Butler, Thomson, and Maclennan (*6*) but column 3 has been calculated from the original vapor pressure–concentration data (*4*). All these studies were carried out using an entrainment method similar to that described by Shaw and Butler (*17*).

The relative standard deviation of the results shown in columns 3 to 6 cannot be accurately calculated from so few observations, but it appears to be about 2%, roughly equal to that of the observations in column 2. Results obtained using capillary columns (*7*) (column 8) disagree markedly with this work and that done with packed columns (*1, 7, 8, 10, 13*). However, the unusual difficulty of determining specific retention volumes with a capillary column probably accounts for the variation.

The data presented in Tables III and IV are generally in good agreement, although the specific retention volume for pentane in column 2 (Table IV) is considerably higher than the mean value given in column 7 (Table IV). When the results are plotted as $\log_{10} V_g$ against the carbon number, the former result falls on the line given by the C_6—C_9 hydrocarbons and may therefore be preferred.

The unique behavior of water as a solvent is shown by the fact that the retention volumes of the members of the alcohol series fall slightly as the number of carbon atoms in the molecule increases. This may be compared with the usual behavior within a homologous series exemplified by the data given in Table IV, which shows a logarithmic increase of retention volume with carbon number.

The range of retention volumes measured in this work is as great as that which may conveniently be measured by the conventional chromatographic method. Furthermore, it appears to be practicable to extend this range up to 10^4 ml. and down to 1 ml. by suitably adjusting m''_g and dv/dt. The method has the further advantage of being equally applicable to volatile and involatile solvents. It is quicker and may be used with much lower solute concentrations than the classical static (*12*) and entrainment (*17*) methods and is without the difficulties and errors involved in measuring partition coefficients by conventional gas liquid chromatography.

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Absolute Calibration of Gas Chromatographic Apparatus from Ion-Chamber Measurements

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▶ **When an ion chamber is connected in series with any gas chromatograph, a given amount of radioactivity in a fraction results in the same ion-chamber signal regardless of the molecule to which the unstable isotope is attached. The effect of various peak shapes on this signal is treated theoretically and compared to experimental results. A simple generalized method using a radioactive "spike" is proposed, by which overall accuracy within 1 % can be attained, independent of column, mass detector, and other operating conditions.**

IT HAS become more apparent since the inception of gas chromatography that measurement of the radioactivity of a separated fraction in addition to the mass measurement can be a valuable analysis technique, particularly in the biological sciences where tracer work is an extremely important and well established part of research. At first, chromatograph fractions were collected for subsequent activity determinations (*2, 5, 7, 10*). More recently, activities have been measured in the flowing gas phase as the fractions leave the mass detector of the gas chromatograph (*1, 4, 6, 8, 9, 11, 12*). The latter method, using an ion chamber as the detector, was used for the data presented here.

Advantage may be taken of the fact that the absolute amount of activity measured by a suitable ion-chamber technique is independent of any characteristics of the molecule of which the unstable isotope is a part, to provide a calibration method independent of most of the usual variables in gas chromatographic work: peak position, temperature, pressure, etc. Thus two molecules with completely different characteristics, such as CO_2 and cholesterol, will result in the same activity measurement if the absolute amount of C^{14} in the two samples is the same.

In this work the transient behavior of the ion-chamber detector was investigated theoretically, and the predictions were verified experimentally. By making the radioactivity measurement in the manner described, three important advantages can be achieved. First, a single standard compound of known activity can be used for chamber calibration and the resultant stable calibration data applied to any other compound of unknown activity. Once established, these calibration data can be used indefinitely. Second, the analysis technique is independent of all of the gas chromatograph instrument variables except the flow rate through the ion chamber, which is easily controlled. Third, the accompanying mass record provides information leading to the specific activity of the fraction, without requiring the usual control sample.

THEORETICAL CONSIDERATIONS

In considering the current produced in an ion chamber, it is best to consider first a static condition. If the chamber contains some suitable ionizable gas and some active compound, the theoretically possible current is given by Equation 1a:

$$I = \frac{BNeS}{w} \qquad (1a)$$

where I is current in amperes, N is the number of disintegrations per second occurring from the sample, S is the average energy per disintegration for the unstable isotope in the sample in electron volts per disintegration, w is the number of electron volts required to produce an electron pair in the ionizable gas being used, e is the number of coulombs per electron, and B is the chamber efficiency. Experimentally, e, S, w, and B are constants for a given unstable isotope, diluent gas, and chamber.

S = 45,000 average e.v. per disintegration for C^{14} or 5700 average e.v. per disintegration for H^3
w = 26.4 e.v. per ion pair for argon
e = 1.59×10^{-19} coulomb per electron
B = 0.4 for 275-cc. spherical ion chamber for C^{14}

The ubiquity of the carbon atom in compounds of interest, the stability of C^{14}-labeled compounds, and the ready availability of carbon-14 in conveniently used intermediate compounds make it the most widely used isotope. Argon is chosen as a diluent gas because of its relatively low ionization potential and relatively high density.

For 1 mμc. of C^{14} activity, which is our detection limit (3 to 1 signal to peak-to-peak noise ratio), the current produced in argon is:

$$I = B \times 10^{-14} \text{ ampere}$$

Equation 1a can be rewritten as 1b:

$$N = EM \qquad (1b)$$

where E is in volts and M is a constant equal to:

$$M = \frac{w}{BSeR} \qquad (1c)$$

and R is the value of the input resistor to the electrometer (normally 10^{12} ohms). Equation 1c is rigorous for a static condition—that is, when all of the active molecules in a fraction are in the chamber at once. The active fraction entering a spherical chamber mixes completely with that already present, so that immediately after entry exhaust of the active fraction begins, and the signal E_t at any time t after $t = 0$ in Figure 1 is given by Equation 2a:

$$E_t = E_o \exp\left(\frac{-ft}{v}\right) \qquad (2a)$$

where f is the flow rate in cubic centimeters per minute, and v is the volume of the chamber in cubic centimeters. Thus if the chamber volume is decreased, or the flow rate is increased, less time is required to reach E_t. This relation assumes that the electrometer response time can itself be neglected, a valid assumption under usual conditions. However, Figure 1 assumes a "plug sample." Of course, this is an

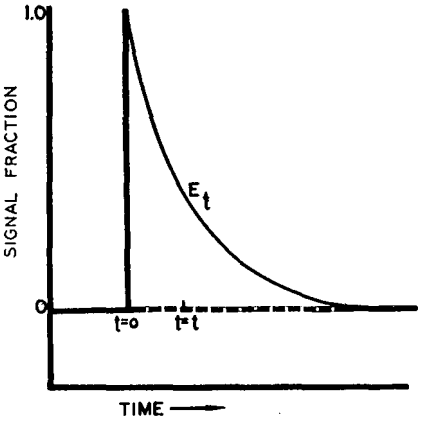

Figure 1. Hypothetical ion-chamber response to plug input where $E_t = E_o \exp(-ft/v)$

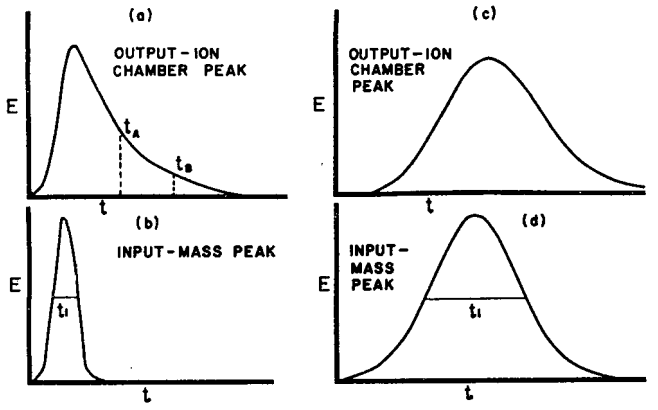

Figure 2. Input-output profiles for narrow, *b*, and broader, *d*, mass peaks

a is output corresponding to *b*, *c* corresponding to *d*

idealization. Depending on the sample, column, etc., it may be a sharp peak providing an ion-chamber record closely approximating Figure 1, or a peak of substantial width.

If the peak width is due mostly to diffusion spreading, we can assume a Gaussian shape, as in Figure 2, *b* and *d*. Figure 2, *a* and *c* shows how the chamber output might respond to inputs like *b* and *d*. With a sharp input peak, relatively few active molecules leave the chamber before all enter, as in *a*. As the input peak gets broader and/or as the diluent flow is increased, the ion-chamber output curve begins to follow the shape of the input, as in *c*. Thus, Equation 1b cannot be applied with an input peak of finite width. However, it is not difficult in practice to correct back to the plug input by considering what effect moving a Gaussian shape through a system with an exponential decay has on the peak height observed for the exponentially decaying system. Non-Gaussian shapes will be considered also.

A transient electrical signal is modified by passing through an electrical filter. Thermal or chemical inputs are modified in analogous ways by linear processes. The principal mathematical tool for studying such processes is called convolution, and depends upon linear superposition of the effects of a series of short input pulses of varying amplitude into which the input waveform may be divided (*3*). For the problem at hand, the input was considered to be a Gaussian pulse with a width at half height of t_1 minutes. The system weighting function is determined from Equation 2a. The convolution was solved by the use of an RCA 301 computer. The computer program employed uses a finite series of points (200) to describe the input and produces the convolution as a finite sum. Figure 3 includes examples of ion-chamber output curves from the computer data. The curves reflect the effect of Kt_1 on the peak height of the output, where K is the ratio f/v. As Kt_1 gets larger, the peak height goes down and the shape approaches that of the input. Figure 4 shows a plot of output peak height to input integral *vs.* Kt_1, over a wide range of the variables. This curve may be used as the correction curve for the experimental data, the correction factor being designated D. The numerical values used in constructing Figure 4 are given in Table I. Knowing D we can extend Equation 1b to apply to all Gaussian-shaped inputs.

$$N = EMD \qquad (3a)$$

If we choose some readily available standard of known activity with a Gaussian-shaped chromatograph peak as the input, Equation 3b results:

$$N_s = E_s M D_s \qquad (3b)$$

where N_s is disintegrations per second of the standard, E_s is the observed peak height in volts, and D_s is the correction factor under the instrument conditions

Table I. Numerical Values from Computer Used in Constructing Figure 5

D	Kt_1	D	Kt_1
1.0325	0.0235	1.6793	0.7042
1.0595	0.0469	2.1053	1.1737
1.1338	0.1174	2.6455	1.7606
1.2484	0.2347	3.1969	2.3474
1.4660	0.4695	5.4945	4.6950

used. Dividing 3a by 3b and solving for N:

$$N = \frac{E}{E_s} \times \frac{D}{D_s} \times N_s \qquad (3c)$$

This is the final form of the equation one would use for quantitative analysis of the radioactivity of an unknown.

Non-Gaussian inputs were treated in the same manner by the computer program, since the input can be arbitrary. The shape resulting from a convolution of a Gaussian pulse with an exponential was used as the shape of the input, since this closely resembles observed tailing mass peaks. To characterize them in some way easily measurable, P was defined as the ratio of the time from the peak to 5% of the peak height on the trailing edge to the time from the peak to 5% of the peak height on the leading edge. Thus the symmetrical peak has a P value of 1. The resultant correction curves, D_P, are plotted in Figure 5 for various values of P from 1.0 to 4.7 and for all values of Kt_1 likely to be found in practice. As one would expect, tailing lowers the activity peak height, requiring a large correction factor, D_P. To check the validity of the correction curves for non-Gaussian input peaks, we ran samples of phenol at different Kt_1 values. Phenol gives strongly asymmetric curves on the columns employed. It soon became apparent that thorough experimental verification would comprise another report.

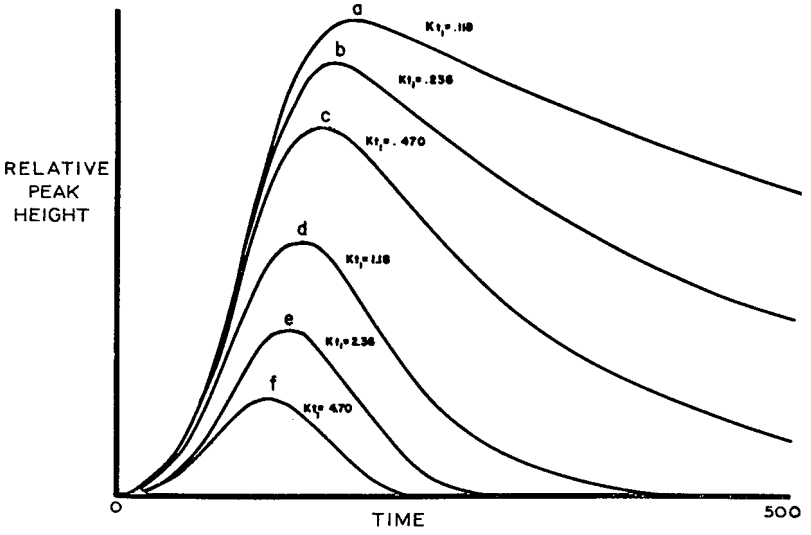

Figure 3. Output profiles at various Kt_1 values from computer data for mass peak of constant t_1

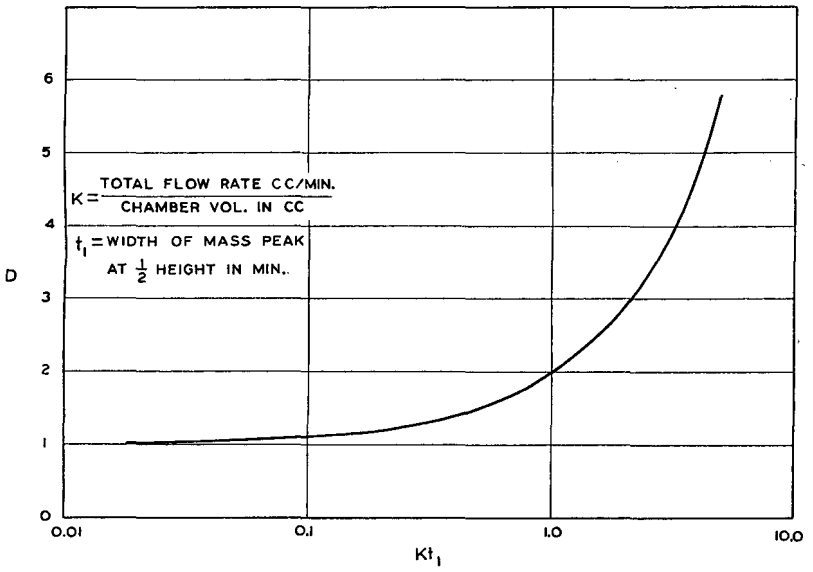

Figure 4. Correction curve for Gaussian input peaks showing correction factor *D* vs. Kt_1

However, significant deviations from the correction curve for Gaussian inputs ($P = 1.0$) do not occur until the peaks are strongly asymmetrical ($P = 1.44$). In examining a mass peak of this shape a working chromatographer would be very dissatisfied and undoubtedly look for a better column. With the growing improvement in columns for polar compounds, any curve other than the one shown in Figure 5 for Gaussian inputs will seldom be required.

EXPERIMENTAL

Apparatus. The apparatus has been described (*6*). The instrument is a Cary-Loenco Model 70. The flow system [Figure 1, (*6*)] is not unusual, except that after the sample passes through the mass detector it is mixed with argon, following the principle first described by Cacace (*1*), and passes into a 275-cc. ion chamber. The radioactive compounds, usually containing carbon-14 and sometimes tritium emit soft beta particles. The resulting ionization is measured in a flow-type ionization chamber adapted for sensitive measurements over a wide range of temperature. The ion current is measured with a Cary Model 31 vibrating reed electrometer and displayed on the 0- to 25-mv., 1-second pen-speed channel of a dual-channel recorder. The electrometer is critically damped, providing a response speed of 1.2 seconds for 98.8% response with a 10^{12}-ohm input resistor. The other recording channel receives the output of the mass detector. This system is sensitive to less than 1 mμc. of activity (37 d.p.s.).

Samples. To check the validity of Equation 3c under actual experimental conditions, four C^{14}-labeled compounds were used: benzene, toluene, hexadecane, and octadecane. Each was tested under different Kt_1 conditions, varying both K (flow rate through chamber) and t_1 (width of mass peak).

Sample Injection. A 4-μl. sample was injected for all determinations, using the same Hamilton 5-μl. syringe each time.

Measurement of *K*. K is the ratio f/v, the total flow rate through the chamber divided by the chamber volume. Although this ratio can be determined by measuring the flow at the exhaust of the ion chamber, correcting it back to the temperature of the ion chamber, and dividing by the volume, these steps involve several obvious uncertainties. Calculating K from an ion-chamber record of a sharp incoming peak is more accurate. For example, if curve *b* of Figure 2 represents a mass peak and curve *a* the output peak, we know that after t_A a negligible amount of sample is entering the chamber and that E is varying as $E_o \exp(-Kt)$. Thus by using the E values at t_A and t_B we can calculate K. For an average, several points after t_A were taken. It is not necessary to know the activity of the sample. Any active sample will do, or even the unknown itself, as long as the mass peak is narrow enough to permit some reasonable time between t_A and t_B. Obviously, it would not be possible to measure K from curves like those of Figure 2, *c* or *d*. The K thus determined will be constant for a given flow through the chamber, and can be reproduced by restoring the flow rate at the same detector operating temperature without having to recalculate K.

Measurement of t_1. Because the measurement of t_1, the width of the mass peak at half height, is critical, measurement on a steel scale, to 0.003 inch, was routinely employed, since an error of 1% in t_1 results in a like error in N. The fastest peak encountered in this work was 0.09 minute (5 seconds), requiring a chart speed of 3 inches per minute for 1% accuracy.

Sample Activities. Since an independent check of the method was desired, a known amount of each of the compounds was counted with a liquid scintillation counter. Since each sample contained only one of the four active compounds, the scintillation counter values could be used to check the results obtained on the gas chromatograph. Three samples of each compound were counted.

RESULTS

Table I is an example of the raw recorder data for benzene and subsequent calculations leading to the corrected

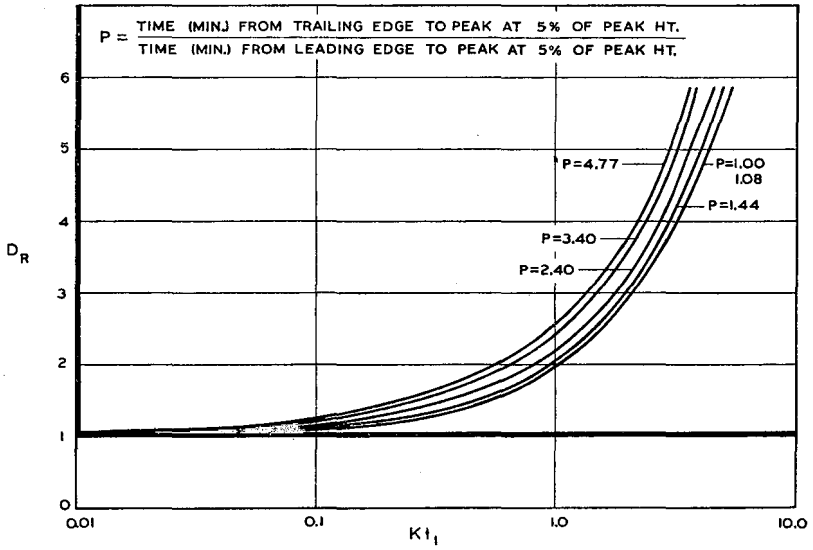

Figure 5. Correction curves for Gaussian ($P = 1.0$) and non-Gaussian input peaks showing D_R vs. Kt_1

activity peak heights. Because sample injection nonreproducibility was troublesome, many samples were run each time K, t_1, or the sample was changed. For example, in the case of benzene the mass peak area of 17 samples was averaged (changing K or t_1 will not change this area). Each activity peak height was further corrected to this average area by multiplying the corrected height by the ratio of the average mass peak area to the mass peak area corresponding to that particular sample. This final value is indicated in the last column of Table II.

The other three samples were treated in the same manner. Table III summarizes the results for all four compounds. Thus K was varied from as low as 2.3 up to 8, and t_1 varied from 0.090 to 1 minute, the D values ranging from 1.2 to 8. In practice, K will usually be constant at about 5, resulting in a range of Kt_1 values of from 0.3 to 3 for the usual chromatograph peaks.

The errors which limit the accuracy of this method accrue from two general sources. The first is the activity peak height measurement, which is a function of sample activity, injection reproducibility, and the linearity and reproducibility of the ion chamber and electrometer. The second is the correction itself, which is a function of how accurately K and t_1 are known and how well the assumptions are met on which the calculations are based. We are primarily interested in the latter errors and the validity of the method itself.

If Figure 4 is plotted on linear paper, the result is nearly a straight line whose slope varies between 0.90 and 1.15. This means that a 1% error in Kt_1 results in a 1% error in D and final N. Thus both K and t_1 should be known to within 1%. K can be determined to this accuracy easily by averaging many observations. The time spent in making several estimations is not excessive, since once calculated, the K value can be used until operating conditions are changed. However, t_1 will differ from fraction to fraction. Since 0.003 inch is a practical limit in measuring a distance on chart paper, we can calculate the minimum required chart speed to permit the measurement of t_1 to within 1%. For a 1-second peak width, the chart speed should be about 10 inches per minute. It is improbable, especially for the type of samples used in radioactivity work, that a peak faster than 6 seconds will be encountered, and most will be longer. For a 6-second peak a chart speed of 3 inches per minute is required. Broader peaks can be recorded at proportionately slower chart speeds.

Standard deviations were calculated according to the methods of analysis of variance. S_k indicates the standard deviations resulting from sample injection, mass peak area estimation, and electrometer uncertainty, and refers to deviations within a given set of K and t_1 values; thus, it does not test the correction method itself. S_o is an indication of deviations among sets of data of different K and/or t_1 values. The square root of the variance for each compound type was calculated, where the variance is:

Table II. Evaluation of Corrected Activity Peak Height

Det. temp., ° C.	220
Det. curr., ma.	75
Inlet temp., ° C.	110
Carrier flow, cc./min.	90
Column	4-foot, 1/4-inch SE 30

4-μl. benzene injections

Column temp., ° C.	Total flow rate, cc./min.	Mass pk. ht.	Mass, t_1, min.	Activity pk. ht. × 300	K	Kt_1	D (corr.)	Act. ht.	Area avg. pk. ht.
76	933	54.7 × 128	0.140	54.6	5.57	0.780	1.74	96.0	97.3
		63.0 × 128	0.140	63.8	5.57	0.780	1.74	111.9	97.2
		54.8 × 128	0.140	53.4	5.57	0.780	1.74	96.1	97.2
		56.0 × 128	0.140	57.0	5.57	0.780	1.74	100.0	99.0
		62.3 × 128	0.140	64.4	5.57	0.780	1.74	113.0	99.0
		56.0 × 128	0.140	55.5	5.57	0.780	1.74	97.3	96.3
76	375	59.4 × 128	0.140	74.3	2.38	0.333	1.34	99.3	98.3
		59.3 × 128	0.146	78.1	2.38	0.347	1.35	105.3	100.2
		57.1 × 128	0.146	75.4	2.38	0.347	1.35	101.6	100.4
		58.0 × 128	0.146	74.4	2.38	0.347	1.35	100.2	97.5
		54.1 × 128	0.146	73.6	2.38	0.347	1.35	99.2	103.5
		56.0 × 128	0.146	73.9	2.38	0.347	1.35	99.6	100.3
76	682	60.2 × 128	0.143	70.9	4.18	0.598	1.59	111.4	100.8
		54.2 × 128	0.140	60.8	4.18	0.585	1.59	95.9	97.8
		55.3 × 128	0.143	62.8	4.18	0.598	1.59	99.5	98.0
		57.8 × 128	0.143	67.2	4.18	0.598	1.59	106.5	100.3
		54.6 × 128	0.143	62.0	4.18	0.598	1.59	98.3	98.2

Table III. Summary of Correction Technique Results for Gaussian Input Peaks

Compound	No. of detns.	K	t_1	D	Corr. pk. ht.	Av. dev.
Toluene	4	5.56	0.562	3.97	99.7	0.8
	9	5.82	0.326	2.79	99.7	0.7
	2	2.68	0.489	2.26	101.4	0.4
	3	7.95	0.146	2.14	100.0	1.1
	6	5.82	0.187	2.05	99.2	1.1
	3	4.55	0.146	1.65	100.3	0.1
	5	4.08	0.109	1.44	99.4	1.5
	3	2.91	0.146	1.42	100.7	0.6
	3	2.92	0.112	1.32	98.6	0.2
	3	2.68	0.090	1.35	99.6	0.8
	41			Mean	99.9	
				Std. dev., S_k		1.2
				Std. dev., S_o	1.3	
Benzene	6	5.57	0.140	1.76	97.7	0.9
	6	4.18	0.143	1.59	99.0	1.2
	5	2.38	0.146	1.35	99.3	1.1
	17			Mean	98.6	
				Std. dev., S_k		1.2
				Std. dev., S_o	2.0	
Hexadecane	1	6.72	1.072	8.00	132.3	...
	3	6.72	0.364	3.34	128.8	1.9
	4	5.17	0.364	2.79	131.3	1.0
	2	3.40	0.364	2.20	130.9	1.0
	10			Mean	130.8	
				Std. dev., S_k		1.8
				Std. dev., S_o	2.2	
Octadecane	4	5.43	0.525	3.71	93.6	1.0
	6	5.43	0.227	2.18	94.9	1.2
	4	5.43	0.210	2.11	92.3	0.3
	7	5.43	0.115	1.61	93.1	1.1
	21			Mean	93.5	
				Std. dev., S_k		1.4
				Std. dev., S_o	2.5	

$$S_m^2 = S_o^2 - S_k^2$$

The resulting values of S_m are: toluene 0.5, benzene 1.45, hexadecane 1.27, and octadecane 2.07. Their weighted average is 1.25%, which estimates the error inherent in the method plus other uncontrolled variables. Hence, the method is as good as the ability to sample in gas chromatography, and can doubtless be improved by study of technique and further replication of samples.

Table IV shows the correlation among the four compounds from the scintillation counter data, arbitrarily using toluene as the standard. The deviations again show a relative error of around 1%.

Table IV. Correlation among Compounds

Compound	mv.	mv./μl.	mμc./μl.[a]	mμc./μl.[b]	Dev.
Toluene[c]	299.7	74.9	22.7	22.7	...
Benzene	295.8	74.0	22.4	22.6	0.2
Hexadecane	391.8	98.0	29.7	29.6	0.1
Octadecane	280.5	70.1	21.2	21.4	0.2

[a] Calculated from ion chamber results using toluene as standard.
[b] Measured with scintillation counter.
[c] Std.

ACKNOWLEDGMENT

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Performance of an Instrument for Simultaneous Gas Chromatographic and Radioactivity Analysis

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▶ **An instrument for simultaneous gas chromatography and radioactivity analysis is described. The ion chamber used for the radioactivity measurement is contained in the same temperature-controlled oven as the normal chromatography detector, and can be heated to over 300° C. Data are given for both programmed and isothermal column operation at high and low temperatures. Specific applications are described. Sensitivity, linearity of response, response times, and resolution are discussed for the entire system.**

Not too long after gas chromatography in the conventional sense was well under way in the United States, articles began to appear on the detection of the radioactivity of labeled fractions in addition to the mass detection of the separated components. Two general methods of measuring the activity of a separated component have been used. One is to collect the sample on a coated solid scintillator or in solution, or as the pure material, and then measure the radioactivity with a counter (*3, 6, 7, 10*). The second technique, used more often recently, is to measure the activity of the fraction in the flowing gas phase as it leaves the mass detector.

As early as 1953, Kokes, Tobin, and Emmett (*8*) passed active fractions containing soft beta emitters by a Geiger counter with a thin window. In 1956 Evans and Willard (*4*) measured hard β and γ particles in a similar manner. However, it was soon realized that the window materials of the detectors make for poor efficiency of measurement for soft beta emitters such as C^{14} and H^3, the most active isotopes. Wolfgang and Rowland (*14*), Wolfgang and Mackay (*13*), and James and Piper (*5*) have investigated proportional counters, with and without windows.

Dobbs (*2*), Cacace (*1*), and Karmen and Winkelman (*7*) have used an ion chamber in series with the GC detector. Although slightly higher sensitivity can be attained by proportional counting, the ion-chamber technique offers the advantages of being simple, easy to install, stable, resistant to poisoning effects (the most serious deficiency of the proportional technique), sensitive to soft beta decay, reproducible, and usable at least to detector temperatures up to 300° C. Samples may be trapped as they emerge from the ion chamber.

EXPERIMENTAL

Gas Chromatograph. The gas chromatograph was a Cary-Loenco Model 70. A Minneapolis-Honeywell dual channel recorder was used. The gas chromatography channel is 0- to 1-mv., 1-second response for full scale, and the radioactivity channel is 0- to 25-mv. full scale regardless of the electrometer range, 1-second response. The pens are physically separated by a little more than one chart division to keep from colliding. Figure 1 shows the flow diagram for the entire assembly. Separate temperature control is provided for the inlets, detector compartment, and column compartment, where either programmed or isothermal work can be done. All three can be operated to temperatures over 300° C. The detector compartment will accept thermal conductivity, ionization, and radioactivity detectors. After the mass detectors, the reference stream ex-

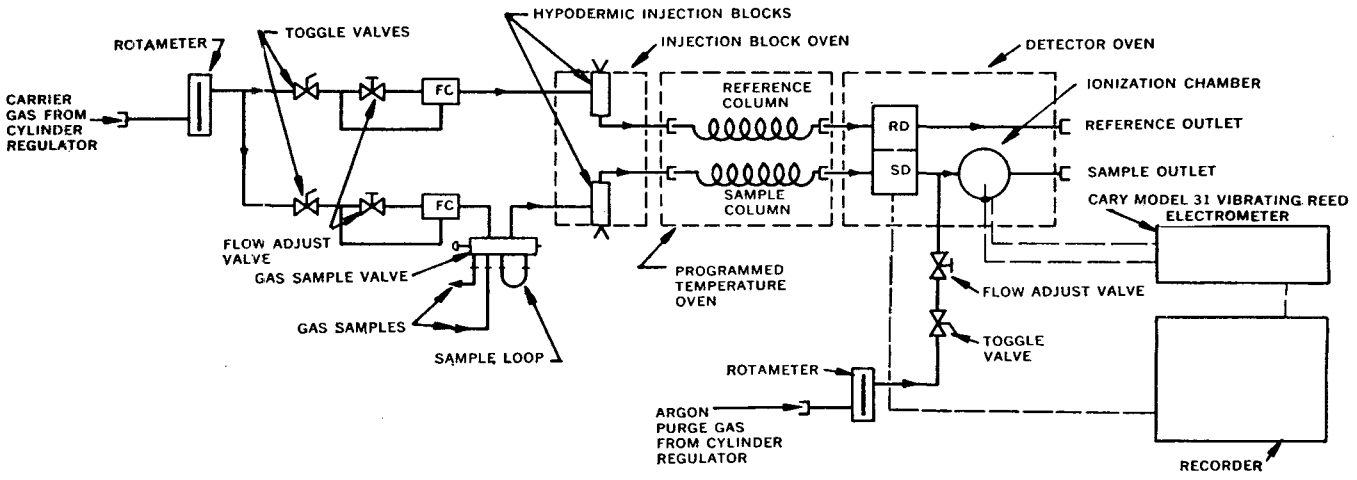

Figure 1. Flow diagram of gas chromatography–radioactivity analysis system

RD. Thermal conductivity reference detector cells
SD. Thermal conductivity sense detector cells
FC. Flow controller

hausts and the sample stream is mixed with the diluent gas at a tee, whence it passes into the ion chamber.

Radioactivity Detector. Activity was measured with an ion chamber of 275-cc. volume, shown in Figure 2. As Cacace (*1*) pointed out, the chamber may be large for higher ionization efficiency without loss of response speed

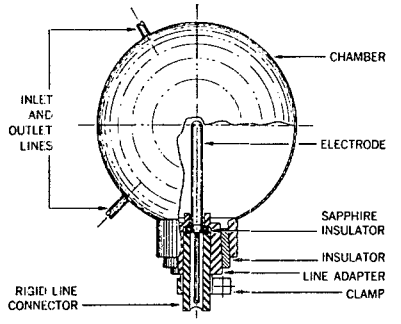

Figure 2. Cutaway of high-temperature ionization chamber construction

if a diluent gas is employed. However, smaller chambers have been used (*2*). The electrometer used is a Cary Model 31 vibrating reed instrument. The full-scale range normally used is 100 mv. when using a 10^{12} ohm resistor, or 10^{-13} ampere. The electrometer is preferably critically damped, providing a 1.2-second full-scale response speed (98.7% of full scale). The base line noise under these conditions is about 0.5%, and does not change with chamber temperatures at least up to 300° C. We prefer the high resistance leak method of measurement, which yields peaks similar to mass peaks. The rate of charge method (*6*), which offers slightly higher sensitivity and results in an integral presentation of charge, is much less convenient to interpret and requires frequent resetting, and the record may run off scale just at the most interesting point.

The chamber wall is negatively charged with respect to the electrometer ground and probe with a dry B battery. The probe is attached to a rigid-line connector leading directly to the input of the vibrating reed amplifier. The probe and conductor are both insulated with sapphire. Surrounding the upper end of the stainless steel rigid line is a conical support for the chamber. Between this support and the stainless steel chamber walls is a conically shaped ceramic insulator. The chamber must be electrically insulated from the rest of the instrument by inserting in the entrance line to and exit line from the chamber a glass to Kovar connector. The instrument connected in this manner can easily detect 10^{-15} ampere.

Flow measurements were made with soap bubble burets connected to the exhaust of the instrument.

Hamilton syringes were used for sample introduction.

The labeled compounds were obtained for the most part from Chemtrac Corp., Cambridge, Mass. The activities of the samples were verified with a liquid scintillation counter through the courtesy of J. Meade, University of California at Los Angeles.

RESULTS

Response Times. With the usual 275-cc. chamber, normal carrier flow rates used in columns of 1/4-inch or smaller diameter would not sweep the sample through the ion chamber fast enough to permit resolution of

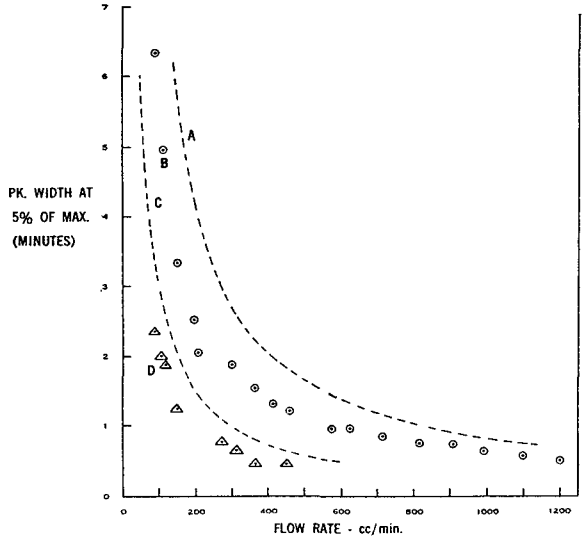

Figure 3. Flow rate *vs.* time to decay to 5% of peak height

A. Theoretical curve from $\exp(-ft/v)$ for 275-cc. chamber
C. Theoretical curve from $\exp(-ft/v)$ for 100-cc. chamber
○ Experimental points for 275-cc. chamber
△ Experimental points for 100-cc. chamber

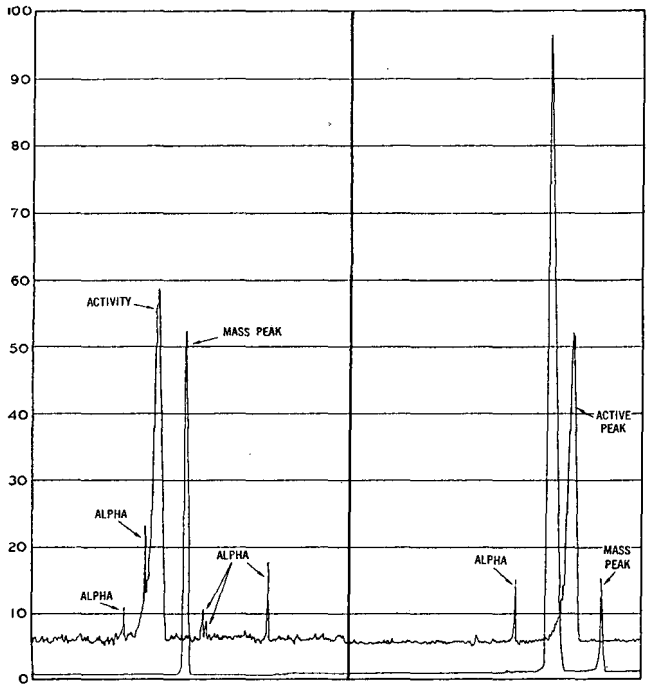

Figure 4. Chromatograms illustrating alpha event occurrence

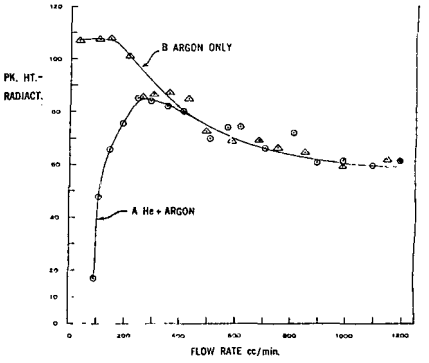

Figure 5. Radioactivity peak height vs. flow rate through 275-cc. chamber using argon only and helium-argon mixture

adjacent peaks. Helium is not the best gas for ion-pair formation, because of its relatively high ionization energy (*9*). Argon is added as a diluent gas.

Assuming a "plug" sample and perfect mixing in the ion chamber, response would follow Equation 1:

$$S = S_o \exp(-ft/v) \qquad (1)$$

where S is the signal remaining t minutes after the peak (S_o), f is the flow rate in cubic centimeters per minute, and v is chamber volume in cubic centimeters. Figure 3 shows the peak width at various chamber diluent flow rates, using for width the time at 5% of full peak height. The dashed lines are the theoretical curves predicted by Equation 1. The circled points were found experimentally.

The shape of the theoretical curve, A, is followed, but points B show shorter times than predicted. Essentially the same results were obtained by reversing input and exit lines, or using a 100-cc. ion chamber (C and D). The difference is attributed to imperfect mixing. This effect does not affect efficiency and allows a lower diluent flow rate to be used. From Figure 3 it is apparent that flows of 600 to 900 cc. per minute are adequate for the 275-cc. chamber. We operated at 900 cc. per minute normally to narrow the base of the peak as much as possible.

The front of a peak entering the chamber can be sharp if the sample

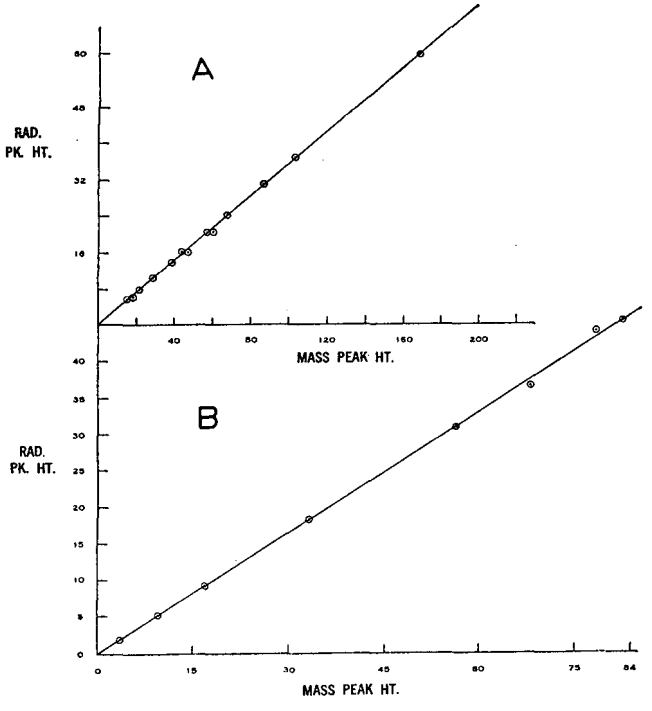

Figure 6. Radioactivity peak height vs. mass peak height for linearity of response

A. C^{14}-labeled octadecane, column temp. 220° C. Av. dev. from linearity 2.1%
B. C^{14}-labeled benzene, column temp. 70° C. Av. dev. from linearity 1.4%

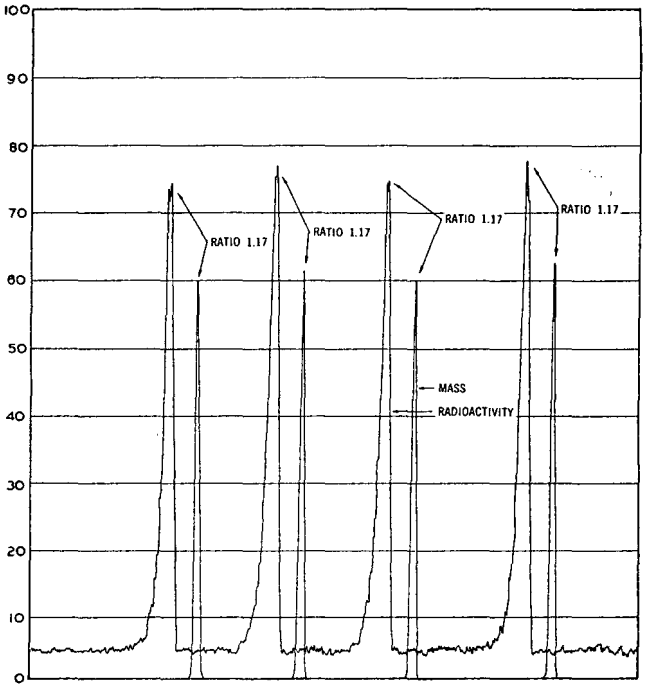

Figure 7. Reproducibility of repeated injections of C^{14}-labeled benzene

Column temp. 70° C.

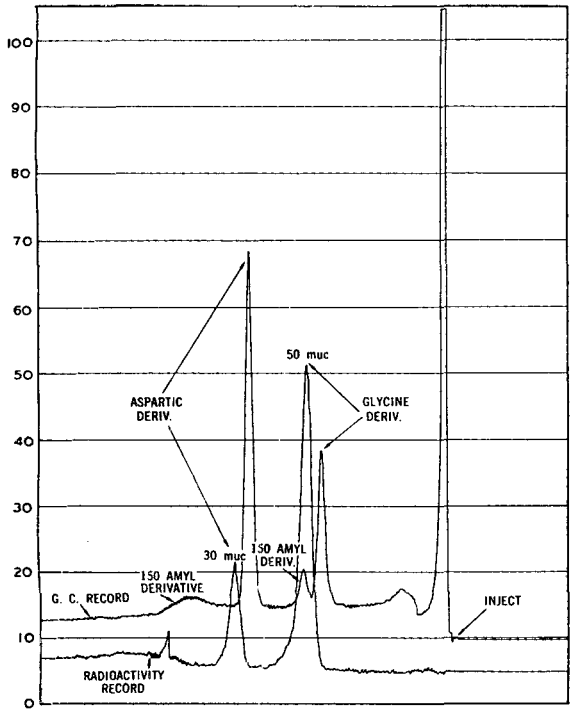

Figure 8. Separation of C^{14}-labeled glycine and aspartic acid derivatives

On 3-foot, 1/4-inch diameter Carbowax 20,000 on acid-washed Chromosorb columns using temperature programming at detector temperature of 250° C.

comes through the column without much diffusion spreading or "tailing." Thus the response speed is controlled in the limit by the electrical response of the electrometer. The electrometer is critically damped for a full scale response of 1.2 seconds, which is the speed normally encountered when the conventional 1-second full scale GC recorder is used.

Alpha-Particle Interference. Another important reason for fast electrometer response is ease of discrimination against ionization caused by alpha particles originating from traces of radioactive contaminants in the metal of the chamber and associated parts, within the vibrating reed itself, and from radon in the air within the connecting line from chamber to input. Figure 4 shows a chromatogram with alpha events occurring during the run. The peak always has the characteristic very sharp front. Because the electrometer is critically damped, the pen returns to the base line almost immediately, causing little difficulty even when occurring on a peak.

Stability. The stability of the ion-chamber system is determined by the electrometer amplifier stability, insulation leakage and strain currents, and any background current that may build up in the ion chamber. Since the ion chamber can be kept as hot as necessary, long-term background due to condensation or adsorption is minimized. The short circuit stability of the electrometer is less than 0.1 mv. per day. Essentially no base line drift has been observed during any part of the work with the detector at low or high temperatures, using the high resistance leak method.

Sensitivity. The sensitivity of the radiation detection in terms of peak height above the base line for a given amount of injected activity is a function of type of radioactive emission (C^{14}, H^3, etc.), chamber volume, background level and noise, diluent gas, carrier and diluent gas flow rates, and peak width of fraction entering chamber.

C^{14} and H^3, both of which emit soft beta particles, are used almost exclusively for radiation studies. C^{14} disintegrations have an average energy of 45,000 e.v. per disintegration and H^3 has 5700 (*11*). Their short-path beta emission can be efficiently measured in a relatively small chamber. A 275-cc. chamber provides a suitable compromise between efficiency and volume for C^{14} (*12*). In this work an efficiency of about 33% was realized for C^{14} using a sample of very small retention volume, so that it enters the chamber in a "plug" relative to the size of the chamber. A 100-cc. chamber was tested under equivalent flow conditions and found to be 21% efficient for C^{14}.

Tritium can be measured with about 75% efficiency in a 275-cc. ion chamber. However, for a given activity the signal

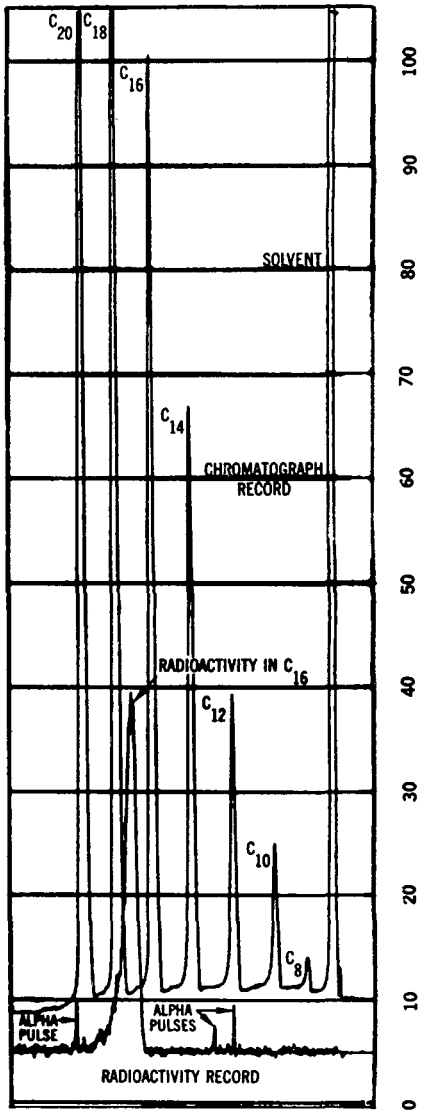

Figure 9. Mixture of methyl esters of saturated fatty acids with C^{14}-tagged palmitate having activity of 10 mμc. μl.

is about one-third of that for C^{14} because of tritium's much lower energy per disintegration.

We find that 0.3 mμc. (about 13 d.p.s.) will provide a signal to peak-to-peak noise ratio of 1 when the electrometer is critically damped. Peak-to-peak noise is averaged from intervals ten times the period of the amplifier. This noise level amounts to 3×10^{-16} ampere.

In addition to being a function of the diluent gas use, the peak height is a function of the flow rate of the diluent gas. Figure 5 is a plot of total flow rate (carrier plus diluent) *vs.* the peak height of the activity for 4-μl. injections of C^{14}-labeled benzene. Curve *A* near 100 cc. per minute refers to pure helium. As argon is added, the peak height increases until the flow reaches about 250 cc. per minute, total. At this point the ion-pair formation is entirely to argon-beta collision, since further increase in argon flow does not increase

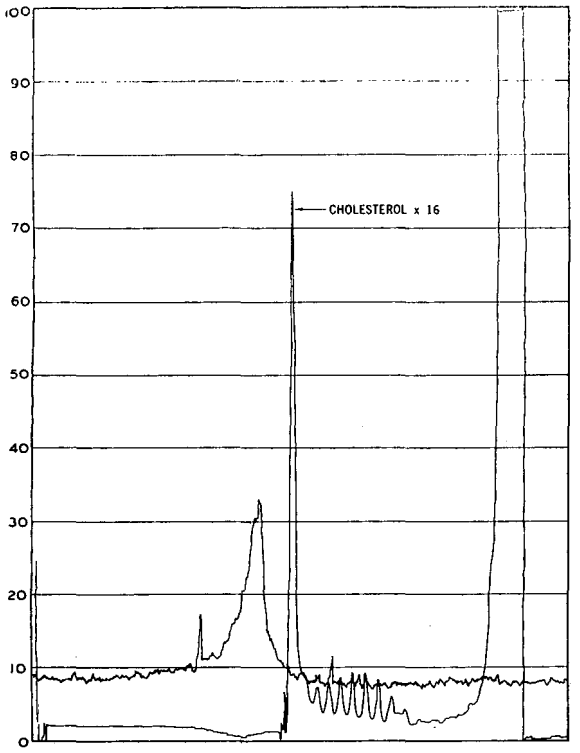

Figure 10. C^{14}-labeled cholesterol on 4-foot, 1/4-inch diameter SE 30 columns

Temperature programming with detector temperature at 295° C.

the peak height. Curve *B* was plotted from results obtained using argon only as both carrier and purge gas. Peak heights decrease from above 250 cc. per minute flow because the benzene peak has a finite width and some leaves the chamber before all has entered at the faster flow rates. The point scatter is reproducible and is thought to be due to slight changes in flow pattern when the flow rate is changed by large amounts. In practice, the instrument is operated at a constant flow rate of about 900 cc. per minute.

Reproducibility and Linearity. C^{14}-labeled benzene and octadecane samples were used to check linearity and reproducibility, the former for a low retention volume, and the latter for a larger retention volume. Figure 6 illustrates the results of these data, showing an average deviation of the points from the least squares calculated slope of 1.4 and 2.1%. To eliminate sample injection errors the activity peak heights were corrected to a given mass peak height. The higher per cent error in the case of octadecane is probably due to slight changes in retention volume at the higher column temperature.

Reproducibility of a given amount of activity is illustrated by Figure 7. The ratio of activity peak height to mass peak height is 1.17 in all four cases.

Sample Applications. Since much of the interest in this type of detection is in the medical field, several examples were developed for this area.

Amino Acids. Figure 8 is a chromatogram of C^{14}-labeled derivatives of aspartic acid and glycine. These were measured on 8-foot, 1/4-inch diameter stainless steel columns filled with 1% Carbowax on acid-washed Chromosorb.

Fatty Acids. Figure 9 shows a mixture of fatty acid esters. The C_{16} component was labeled, the radioactivity peak corresponding to 10 mμc. This chromatogram was produced on 2-foot, 1/4-inch diameter stainless steel columns filled with SE 30 on hexamethyl disilazane treated Chromosorb. The column temperature was programmed from 150° to 290° C. at 12° C. per minute. The detector temperature was 250° C.

Steroids. Figure 10 is a chromatogram of desmosterol and cholesterol and unidentified impurities. Cholesterol was the only labeled compound. The column temperature was programmed from 250° to 350° C. using 4-foot, 1/4-inch diameter stainless steel columns filled with SE 30 on hexamethyl disilazane–treated Chromosorb. The detector temperature was 295° C.

CONCLUSIONS

Similar work can be performed using any gas chromatograph with a separate oven and flow system for the ion chamber connected to the exhaust of the sample side of the thermal conductivity detector, or from a suitable stream splitter when a hydrogen flame ionization detector is used.

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Gas Chromatography of Trimethylsilyl Derivatives of Compounds Related to Chloramphenicol

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▷ **A method is described for the preparation and gas chromatography of the trimethylsilyl derivatives of some compounds related to chloramphenicol. Several of these compounds are eluted in approximately the order of their molecular weights. Changes in substituents, in certain cases, however, cause variations in retention time which cannot be explained on a molecular weight basis. The apparent molar response of the hydrogen flame detector to the phenylserinol derivatives is increased by the presence of an acyl group on the side chain but is not affected by changes in structure of the side chain.**

During studies on the biosynthesis of chloramphenicol by *Streptomyces venezuelae* and of other naturally occurring nitro compounds, it became necessary to separate chloramphenicol from structurally related compounds and to determine it quantitatively in the presence of these related materials. Paper chromatographic methods have been described for the separation of various aromatic nitro compounds (*2*, *8*), and if individually present, chloramphenicol and certain related compounds can be determined quantitatively by biological assay. However, these techniques do not lend themselves readily to the quantitative estimation of a mixture of such compounds usually present in an *S. venezuelae* growth medium or a cell-free extract.

Recently, several reports have described the separation of phenols (*5*), carbohydrates (*1*, *3*), and sterols (*6*, *10*, *12*) by vapor phase chromatography as trimethylsilyl ethers. Since the conversion of a hydroxyl group to its trimethylsilyl ether has the effect of increasing the volatility and decreasing the polarity of the compound bearing the hydroxyl group, such derivatives might be useful in the vapor phase chromatography of the relatively nonvolatile chloramphenicol.

EXPERIMENTAL

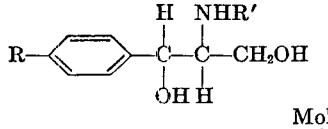
Materials. Trimethylchlorosilane and hexamethyldisilazane were obtained from Applied Science Laboratories, State College, Pa. Pyridine was dried over potassium hydroxide pellets before use. All compounds used in these studies except *N*-acetyl-*p*-nitrophenylserinol were gifts of Parke, Davis and Co., Ann Arbor, Mich. This compound was prepared by the method of Rebstock (*7*). The structures of these compounds are shown in Table I. They are derivatives of the parent compound, 1-phenyl-2-amino-1,3-propanediol (compound 1), which has been given the trivial name, "phenylserinol." All are of the D-*threo*- configuration except compounds 1, 10, 11, and 13, which are DL-. The antibiotic, chloramphenicol, is compound 12. The identities of these compounds were confirmed by comparison of their melting points with the values reported in the literature (*7*).

Chromatograms were run on an F & M Model 609 gas chromatograph with a hydrogen flame detector and using a 6-foot coiled column with an internal diameter of $^3/_{16}$ inch. The column was packed with 1.5 % SE-30 silicone on 100- to 120-mesh Gas Chrom P (Applied Science Laboratories). The support had been pretreated with dimethyldichlorosilane before use (*4*).

Methods. The trimethylsilyl derivatives were prepared by the method of Bentley *et al.* (*1*). In a typical experiment, about 10 mg. each of compounds 3, 4, and 12 were dissolved in 2.0 ml. of pyridine. Aliquots (0.01 to 0.5 ml.) were transferred to 1-ml. volumetric flasks and treated with 0.05 ml. each of trimethylchlorosilane and hexamethyldisilazane. After standing for 10 minutes at room temperature, the mixtures were diluted to 1.0 ml. with pyridine and the tubes stored in a desiccator until used. Pyridine was chosen as solvent because of the high solubility of the phenylserinol derivatives and the short time required for formation of the trimethylsilyl derivatives.

For the chromatography of the synthetic mixture shown in Figure 1, trimethylsilyl derivatives were prepared from solutions containing about 4 mg.

Table I. Derivatives of *threo*-1-Phenyl-2-amino-1,3-propanediol



Compound	R	R'	Molecular weights
1	H	H	167
2	NH_2	H	182
3	NO_2	H	212
4	NO_2	CH_3CO	254
5	NO_2	OHCCO	268
6	NO_2	CH_2FCO	272
7	NO_2	$(CH_3)_2CHCO$	282
8	NO_2	CH_2ClCO	289
9	NO_2	CHF_2CO	290
10	CH_3O	$CHCl_2CO$	308
11	NO_2	CF_3CO	308
12	NO_2	$CHCl_2CO$	323
13	NO_2	CH_2BrCO	333
14	CH_3SO_2	$CHCl_2CO$	356
15	NO_2	CCl_3CO	358
16	NO_2	CHBrClCO	368
17	NO_2	$CHBr_2CO$	412
18	NO_2	CBr_3CO	491

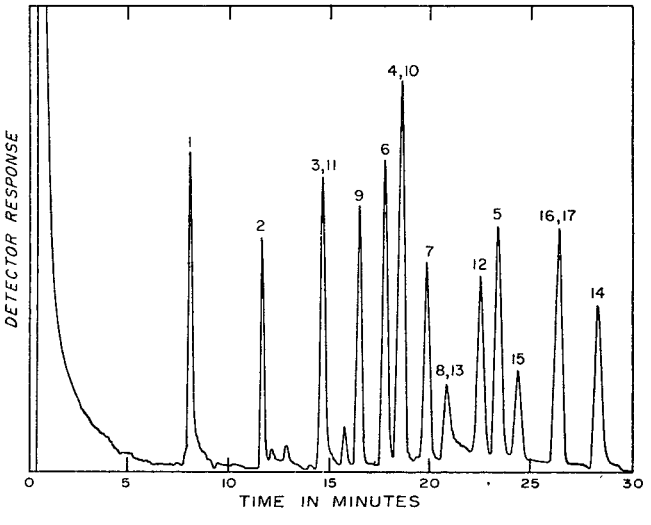


Figure 1. Gas chromatography of phenylserinol derivatives

Range 100
Attenuation 32

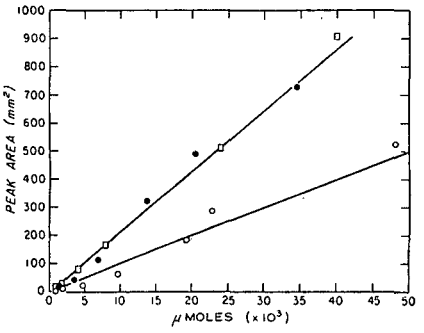


Figure 2. Variations of peak area with sample size of phenylserinol derivatives

○ p-Nitrophenylserinol
● Chloramphenicol
□ N-Acetyl-p-nitrophenylserinol

per ml. of each component. Volume of the injected sample was 4 μl. The flash heater was maintained at 312° C. and the detector at 280° C. The gas flow rates (ml. per minute) at the start of each chromatogram were: helium, 93; hydrogen, 45; oxygen, 333. The column temperature was programmed from 110° C. at 6.4° C. per minute for 12 minutes, then at 2.3° C. per minute to 260° C. Each component of the mixture was first separately chromatographed under these same conditions. When two materials appeared to have the same retention time, they were also chromatographed together to confirm their identification. For the quantitative estimation of each component of a mixture of compounds 3, 4, and 12, the flash heater was held at 275° C. and the detector at 245° C., and the initial gas flow rates (ml. per minute) were: helium, 85; hydrogen, 44; oxygen, 297. The column temperature was programmed from 140° to 220° C. at a rate of 6.4° C. per minute, and then the column was allowed to operate isothermally for 5 minutes. Peak areas were measured by triangulation. The areas given in Figure 2 were computed on the basis of zero attenuation, and the molar response is the area in square millimeters divided by the micromoles of compound injected.

RESULTS AND DISCUSSION

Seventeen derivatives of phenylserinol were separable into 13 peaks, as shown in Figure 1. The numbers identifying each peak refer to the compounds in Table I. In four cases, individual members of a pair could not be separated —3 and 11, 4 and 10, 8 and 13, and 16 and 17.

Nine of the phenylserinol derivatives are eluted in approximately the order of their molecular weights (Figure 3). Deviations from linearity, especially in the first few compounds eluted, are a result of changing the rate of temperature programming after 12 minutes. Of the remaining eight compounds examined, only those having fluorine in the acyl group formed any sort of pattern; these three (compounds 6, 9, and 11) are eluted in the reverse of the expected order. The remaining five compounds are randomly distributed; the monobromoacetyl derivative has a retention time shorter than expected on the basis of its molecular weight, whereas the bromochloroacetyl and glyoxalyl derivatives have longer retention times. Attempts to detect the trimethylsilyl derivative of the tribromoacetyl derivative were unsuccessful. In this case steric hindrance by the bulky bromine atoms may have prevented the formation of the trimethylsilyl derivative.

The effects on retention time of substitution in the position para- to the serinol side chain are variable. The substitution of an amino group (compound 2) or a nitro group (compound 3) for the para-hydrogen atom of phenylserinol (compound 1) causes no change other than that attributable to an increase in molecular weight. The exchange of the nitro group of chloramphenicol (compound 12) for a methoxyl group (compound 10) causes a decrease in retention time greater than that due to a decreased molecular weight. The substitution of a methanesulfonyl group (compound 14) increases retention time.

A series of closely related compounds would be expected to be eluted from the relatively nonselective SE-30 column in order of decreasing volatility. It was reported by Luukkainen *et al.* (*6*) that in the case of sterol trimethylsilyl ethers, the elution pattern followed approximately the order of molecular weights. However, variations in the molecular weights of the trimethylsilyl phenylserinol derivatives did not always lead to predictable changes in relative retention times. These results suggest that the volatility of these compounds is not necessarily directly related to their molecular weights.

Variability is particularly apparent in the derivatives having fluorine in their acyl groups. The retention times of these compounds decrease progressively as fluorine is substituted for hydrogen in the acetyl group of *N*-acetyl-*p*-nitrophenylserinol. The decreased retention time of the trifluoroacetyl derivative in relation to *p*-nitrophenylserinol and *N*-acetyl-*p*-nitrophenylserinol is not surprising in view of the greater volatility of trifluoroacetic acid and its derivatives. For example, cholestanyl trifluoroacetate is eluted from an SE-30 column before cholestanol and cholestanyl acetate (*11*). Similar experiments with sterol mono- and difluoroacetates are not available for comparison, however.

Figure 2 shows that gas chromatography can serve as a quantitative method for the determination of phenylserinol derivatives. These compounds shown were chosen because they often occur together in our enzymatic reaction mixtures. They were chromatographed as mixtures. For these compounds there is a linear relationship between concentration and peak area. However, the slope of the line for the unacetylated compound is about one half that of the acetyl derivative and chloramphenicol. Some of the other available derivatives were also examined, and as can be seen in Table II, most of these compounds give the same response as the acetyl derivative within experimental error. Unfortunately, some of the derivatives contained impurities and were not available in sufficient quantities for purification, so their molar responses could not be determined.

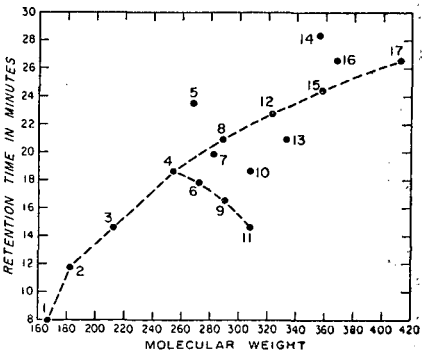


Figure 3. Relationship of retention time to molecular weight of phenylserinol derivatives

The low response of compounds 3, 8, and 15 probably cannot be due to the presence of impurities, since they give single peaks on the gas chromatograph, and their melting points agree closely with those reported in the literature (*7*). It was observed that phenylserinol (compound 1) and *p*-aminophenylserinol (compound 2), both of which have free amino groups, also appear to give a lower molar response than the acyl derivatives. Unfortunately, these two compounds contained volatile impurities, so an exact comparison could not be made. However, if it is assumed

Table II. Response of Hydrogen Flame Detector to Derivatives of *threo*-1-Phenyl-2-amino-1,3-propanediol

Compound	Relative molar response	Compound	Relative molar response
3	0.46	10	0.96
4	1.00	11	1.05
5	0.93	12	1.12
6	1.06	14	0.96
7	0.94	15	0.66
8	0.71	17	0.98
9	1.02		

$$\text{Relative molar response} = \frac{\text{molar response of compound}}{\text{molar response of compound 4}}$$

that the molar response of the impurities is no greater than that of the phenylserinol derivatives and if they have approximately the same molecular weights, the relative molar response for phenylserinol is 0.57 and for *p*-aminophenylserinol 0.50. Since both of these compounds have free amino groups potentially able to form silyl amines (analogous to hexamethyldisilazane), the mole fraction of silicon may be important in determining the response of the detector to these compounds. An alternative and perhaps more likely explanation is that the amino groups of the phenylserinol derivatives are not silylated, and the trimethylsilyl ethers may be partially lost by adsorption onto the column or injection port. Also it is possible that because of a charge on some of the amino groups, the silylation reaction was incomplete and these salts were nonvolatile. Sweeley has reported that charged groups will not react with silanes (*9*).

ACKNOWLEDGMENT

The gifts of the compounds used in these studies by Harry M. Crooks, Jr., Parke, Davis and Co., and the helpful discussions with C. C. Sweeley on certain phases of the work are gratefully acknowledged.

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Tetracyanoquinodimethanide as a Color Reagent for Inorganic Thin Layer Chromatography

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▶ **The separation of the alkali, alkaline earth, and some mono- and divalent post-transition metal ions by thin layer chromatography using silica gel adsorbent is reported. Two solvent systems, 5% glacial acetic acid in ethanol, and 5% 2*N* HCl in *t*-butanol were used, with the former providing excellent resolution of the alkali metals while the latter is more effective for the remaining ions. The R_f values for all ions in each solvent are reported. The use of tetracyanoquinodimethanide ($TCNQ^-$) as a color reagent for these ions was investigated and found particularly sensitive for the detection of monovalent ions.**

WHILE MANY paper chromatographic separations of inorganic ions have been reported over the past years, comparatively few have been reported using the thin layer technique (*5–8*). This new method of separation shares the advantage of applicability to microamounts and offers greater speed and convenience than paper chromatographic methods.

Separation and identification of the alkali and alkaline earth metal ions was of immediate interest. Seiler and Rothweiler (*6*) used 1% glacial acetic acid in absolute ethanol to separate Li^+, Na^+, Ki^+, and Mg^{+2} on a plate coated with silica gel. With their system, they were able to achieve a maximum R_f of about 0.4 for Li^+, with lesser values for the other ions. A solvent system that would produce a greater degree of separation was desired; this study was extended to include all of the alkali and alkaline earth metal ions.

The second part of the research was devoted to the investigation of a more sensitive means for detecting and identifying the various ions of these two families. In the past, violuric acid was used for both paper chromatography, and in Seiler's work. This process can be time consuming, since the chromatograms must be heated for at least 30 minutes at 120° C. to develop the color. Recent work by Acker and Hertler (*1*) and Melby *et al.* (*4*) on cyanocarbons has shown that tetracyanoquinodimethan (TCNQ) can easily be reduced to an anion radical ($TCNQ^-$), and that this anion forms insoluble salts with many mteal cations, including the alkali metal ions (*4*). Both the anion, $TCNQ^-$, and the salts are intensely colored. From these intense colors, it appears that $TCNQ^-$ may be a more sensitive reagent for the identification of the alkaline earth ions. Also investigated were some mono- and divalent post-transition metal ions whose chemical behavior with $TCNQ^-$ suggested inclusion.

As tetracyanoquinodimethan itself appears to be a stronger π-acid than tetracyanoethylene (TCNE) (*4*), this suggests the use of TCNQ as a color reagent in place of TCNE for the detection of aromatics (*9*).

EXPERIMENTAL

Preparation of Silica Gel. One drawback of all commercial adsorbents, including those without added binder, is the presence of large quantities of cations, especially those of sodium, magnesium, calcium, and iron. It is necessary to remove these by washing the silica gel with acid and distilled water (*8*).

Two hundred and fifty grams of silica gel, (Kieselgel without binder, Arthur H. Thomas Co. or Silica Gel H, Brinkmann Instrument Co.) were stirred with 500 ml. of 1:1 (v./v.) HCl–water for 2 hours, then filtered and washed with 200 ml. of acid, 700 ml. of distilled water, and finally 200 ml. of benzene. The deionized silica gel was dried at 110° C. This silica gel was applied directly, instead of rice starch being added as a binder. The resulting plates were quite fragile, and extreme care had to be exercised in spraying color reagent on the plates.

Preparation of Plates. Glass plates, 200 × 200 mm., were coated with the deionized silica gel by spraying on a slurry of the adsorbent (*2*). Silica gel, 12 grams (enough for three plates), was mixed with 40 ml. of water. This was poured into an atomizer attached to the laboratory air line. One slow pass with the spray produced a plate thickness of about 250 to 300 microns. The plates were activated by drying at 110° C. for at least 1 hour.

Development of Chromatogram. Each of the metal ions was originally present as either the nitrate or chloride salts. Two microliters of a 0.1*M*

Table I. R_f Values of Group I and Group II Ions

Ion	R_f 5% HAc in ethanol	R_f 5% 2N HCl *tert*-butanol	Color Violuric acid	Color Li(TCNQ)	Sensitivity of $TCNQ^-$, μg.
Li	0.98	...	yellow	...	...
Na	0.89	0.39	orange	bright blue	0.5
K	0.60	0.20	red	bright blue	0.5
Rb	0.53	0.17	red-violet	blue	2
Cs	0.38	0.10	red-violet	gray	2
Be	0.99	1.00[a]	yellow-green	yellow	20
Mg	0.95	0.96[a]	yellow	olive green	20
Ca	0.94	0.93[a]	orange	gray-green	20
Sr	0.93	0.15	red-pink	pale green	50
Ba	0.90	0.00	pink	blue	50
Cu(II)	0.80[c]	0.95	...	green	25
Ag	0.89[c]	0.38[c]	...	bright blue	0.1
Zn	0.90	1.00[c]	...	blue	10
Cd	1.00	1.00	...	blue	10
Hg(I)	0.98[b]	0.00	...	bright yellow	10
Hg(II)	1.00	0.97	...	bright yellow	10
Tl(I)	0.30[c]	0.08	red	gray	40
Pb(II)	0.90	0.02	orange	blue	75

[a] Very narrow band.
[b] Spot remained at 0.00.
[c] Comet or long trail.

aqueous solution of each ion was applied to the plate. For mixtures, the concentration was about 0.05*M* for each ion.

After trying a variety of solvent combinations, two were selected for the complete set of elements. Five per cent glacial acetic acid in ethanol (95%) gave the best separation of the alkali metals, but for the remaining elements rather high R_f values were obtained. The second solvent used was 5% 2*N* HCl in *tert*-butanol. Development time for the solvent to travel 100 mm. was about 1 hour for the ethanol solvent, and 2 hours for the butanol solvent.

Color Reagent. A 1% solution of violuric acid in 1:1 (v./v.) ethanol–water was sprayed on the developed plate and dried at 120° C. for at least 30 minutes to bring out the colors.

Alternatively, a 0.5% solution of $NH_4(TCHQ)$ or Li(TCNQ) in 1:1 ethanol–water was used. The tetracyanoquinodimethanides were prepared by the procedure of Melby *et al.* (*4*). The solution must be prepared immediately before use, as reaction with water slowly converts the green $TCNQ^-$ to yellow-brown H_2TCNQ (*p*-phenylenedimalononitrile). Since the presence of acid greatly increases the rate of this reaction, the plates were either air-dried at 80° C. or were subjected lightly to NH_3 vapor before the $TCNQ^-$ was applied. The colors due to individual ions appeared immediately.

RESULTS AND DISCUSSION

Table I lists the R_f values for each solvent, the colors obtained with both violuric acid and $TCNQ^-$, and the estimated sensitivity of $TCNQ^-$.

Initially, ammonium tetracyanoquinodimethanide was used as the color reagent but when this was found inadequate for the detection of lithium, the more easily prepared lithium salt was used. For the alkali metals, with the exception of lithium, $TCNQ^-$ appears to be more sensitive than violuric acid, especially for detecting sodium and potassium. It is slightly less sensitive than violuric acid for the detection of the alkaline earths. Of the post-transition metal ions, notable is the sensitivity of $TCNQ^-$ toward silver and thallium(I) ions, whose charge and ionic radii are close to sodium and potassium. While the colors appear immediately upon contact of the reagent with the ions, the bright green background of unreacted $TCNQ^-$ often obscures some of the colors. After about 15 minutes, the acidity of the silica gel changes the background color to yellow-brown, causing the colors of the metal ions to stand out more distinctly.

The 5% glacial acetic acid in ethanol proved to be an excellent solvent for the resolution of the alkali metals. Mixtures of the five ions could readily be separated into five distinct bands. An unusual effect noticed in the separation of mixtures of these ions was an apparent decrease of the R_f values for the heavier ions. Table II shows the comparison between R_f values when the ions were run alone and when run in mixtures. The distinct ion separation and this depression of R_f values for Rb^+ and Cs^+ seem to suggest a possible displacement mechanism between protons on the silica gel, and the various metal ions.

Table II. Comparison of R_f Values for Alkali Metal Ions Run Individually and in Mixtures

Ion	R_f Indv.	R_f Mixt. of alkalis
Li	0.98	0.98
Na	0.89	0.89
K	0.60	0.60
Rb	0.53	0.27
Cs	0.38	0.15

For the remaining elements, the relatively high R_f values for most of the ions, as well as the spot length precluded any good analytical separation with the ethanol solvent. When mixtures of the alkaline earths were run, a depressing effect on the R_f values of Sr and Ba was noted, similar to the decrease noted with the heavier alkali metal ions.

A 5% solution of 2*N* HCl in *tert*-butanol was used for the separation of the remaining elements. In spite of the similarity among R_f values for the first three alkaline earths, the bands were narrow and sharp enough to allow easy detection of each ion when mixtures were used.

Observations concerning silver ions in the butanol solvent show a maximum R_f of 0.38, but the bulk of the silver had not moved, and a long trail was evident. This effect was probably due to limited complexation of the silver with chloride ion. Other evidence of the effect of complexation was apparent in the comparison of zinc and cadmium. The latter moves with the solvent front in a narrow band, while the former is spread out. Comparison of $K_{inst.}$ for the chloride complexes (*3*) shows that cadmium forms stronger complexes with chloride than does zinc; thus, there would be fewer cadmium than zinc species (probably only $CdCl_4^{-2}$) to interact with adsorbent.

Evidence of a displacement mechanism or ion exchange equilibrium functioning between cationic species may be seen by the high R_f values, in both of the solvents, for those substances which can either form uncharged or anionic species—e.g., $HgAc_2$, $AgCl_2^-$—soluble in the solvent. Low R_f values are obtained when the ion either exists as a cationic species, or is precipitated by the solvent, as are mercury(I), thallium(I), and lead in the butanol–HCl solvent.

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Gas Liquid Chromatography of Phenanthrenes

Use in Identification of the Components of Mixtures of Alkyl Phenanthrenes

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▷ The relative retention times of 60 alkyl phenanthrenes have been determined on a variety of columns. The relative retention times determined on the nonpolar liquid phase SE-30 and on the polar liquid phase QF-1-0065 have been factored in a manner which permits the prediction of relative retention times of alkyl phenanthrenes on these phases. The data presented facilitate the identification of small amounts of phenanthrenes obtained from the degradation of natural products.

THOUGH drastic dehydrogenation has long been recognized as an important approach to the elucidation of the skeletal structures of natural products (*8*), the method has remained subject to extreme experimental difficulties arising both from low yields and from the complexity of the mixtures usually produced. In a recent communication (*9*), we presented data indicating the possibility of separating and identifying typical components of such mixtures by gas liquid chromatography (*1, 2, 7*). We have concentrated our study on the alkyl phenanthrenes because of the importance of these compounds to work on the di- and higher terpenoids and because successful application of the method to phenanthrenes makes obvious its utility in simpler series.

The identification of new alkyl phenanthrenes, such as obtained from the dehydrogenation of natural products, has in the past presented a difficult problem, particularly when, as often happens, too little material is isolated to permit structure deteminations by chemical degradation. Infrared and ultraviolet spectral studies can limit the number of structures which need be considered, at least by showing the presence or absence of certain nuclear-substitution patterns (*6*). Nuclear magnetic resonance spectrometry can also be of great help, especially with respect to determining the number and nature of the alkyl substituents. The problem could be further simplified if the relative retention times of unknown or unavailable phenanthrenes could be predicted. The treatment outlined in this paper permits the prediction of relative retention times of alkyl phenanthrenes on two phases.

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EXPERIMENTAL

Equipment. All runs were made on either an Aerograph A-110-C equipped with a thermal conductivity detector or an Aerograph A-600-B equipped with a flame ionization detector. A Brown-Honeywell 1-mv. full scale recorder was used with both instruments. All columns used were obtained from the Wilkens Instrument and Research Corp., Walnut Creek, Calif.

Materials. The phenanthrenes studied were obtained from the following sources:

F. A. L. Anet, University of California at Los Angeles. 9-*n*-Butylphenanthrene, 9-isobutylphenanthrene.

P. M. B. Bavin, Smith Kline and French Laboratories, Welwyn Garden City, Hertfordshire, England. 4-Methylphenanthrene, 4,5-methylenephenanthrene (ex Leights), 3,6-dimethylphenanthrene, 9,10-dimethylphenanthrene (ex Bradsher), 3-ethylphenanthrene, 9-*n*-propylphenanthrene, 9,10-diethylphenanthrene, 9-isobutylphenanthrene, 9 - *tert* - amylphenanthrene, 9-cyclopentylphenanthrene, 9-benzylphenanthrene, 9,10 - dehydrophenanthrene.

C. K. Bradsher, Department of Chemistry, Duke University. 9-Methylphenanthrene, 9,10-dimethylphenanthrene, 9-ethylphenanthrene, 9 - methyl - 10-ethylphenanthrene, 9 - isopropylphenanthrene, 9-*n*-butylphenanthrene, 9-ethyl-10-*n*-propylphenanthrene, 9-methyl-10-*n*-butylphenanthrene, 9-ethyl-10-phenylphenanthene.

R. C. Cambie, University of Auckland, New Zealand. 1,2,8-Trimethylphenanthrene.

E. Chandross, Bell Telephone Laboratories, Murray Hill, N. J. 1,7-Dimethylphenanthrene, 1,4,7 - trimethylphenanthrene.

C. Djerassi, Department of Chemistry, Stanford University. 4-Ethylphenanthrene.

O. E. Edwards, Division of Pure Chemistry, National Research Council, Ottawa, Canada. 1,6,7-Trimethylphenanthrene.

L. F. Fieser, Department of Chemistry, Harvard University. 2,3-Dimethylphenanthrene, 2-*tert*-butylphenanthrene, 3-*tert*-butylphenanthrene.

W. J. Gensler, Department of Chemistry, Boston University. 1,2,7,8-Tetramethylphenanthrene, 1,7,8,9-tetramethylphenanthrene.

E. B. Hershberg, Schering Corp., Bloomfield, N. J. 1-Ethyl-2,8-dimethylphenanthrene.

W. A. Jacobs, Rockefeller Institute. 1-Methylphenanthrene, 1,6-dimethylphenanthrene, 1,7-dimethylphenanthrene, 1-methyl-7-isopropylphenanthrene, fluorene, 1'-methyl-1,2 - cyclopentanophenanthrene, 1,2,8-trimethylphenanthrene (ex N. L. Drake).

F. E. King, T. J. King, and J. W. W. Morgan, Forest Products Research Laboratory, Prince Risborough, Aylesbury, Bucks, England. 1,8-Dimethylphenanthrene, 1,3,8 - trimethylphenanthrene, 1,4,8 - trimethylphenanthrene, 2,4,8-trimethylphenanthrene, 1-methyl-8-ethylphenanthrene.

B. J. Mair, Petroleum Research Laboratory, Carnegie Institute of Technology. 3 - Methylphenanthrene, 1,8 - dimethylphenanthrene.

E. Mossetig, N.I.A.M.D., National Institutes of Health, Bethesda, Md. 2-Ethylphenanthrene, 9-ethylphenanthrene.

M. S. Newman, Department of Chemistry, Ohio State University. 4,5-Dimethylphenanthrene, 2,7 - dimethylphenanthrene.

E. Ochiai and T. Okomoto, Faculty of Pharmaceutical Sciences, University of Tokyo. 1,9-Dimethylphenanthrene, 1,3-dimethylphenanthrene, 1,6,7 - trimethylphenanthrene, 1 - methyl - 8 - ethylphenanthrene, 2 - isopropylphenanthrene, 1,3-dimethyl-7-ethylphenanthrene, 1,3,9-trimethyl - 7 - ethylphenanthrene, 1,2-dimethyl-6-isopropylphenanthrene, 1,3-dimethyl-7,9-diethylphenanthrene, 1,3,9-trimethyl-7-isopropylphenanthrene.

F. Piozzi, Politecnico di Milano. 1-Methylphenanthrene, 2-methylphenanthrene, 3-methylphenanthrene, 2-ethylphenanthrene, 1 - methyl - 7 - ethylphenanthrene.

D. L. Turner, Jefferson Medical College, Philadelphia. 1,2-Dimethylphenanthrene.

W. B. Whalley, School of Pharmacy, University of London. 1,6,10-Trimethylphenanthrene.

S. W. Pelletier. 1,6-Dimethylphenanthrene, 1-methyl-6-ethylphenanthrene, 1-methyl-6-isopropylphenanthrene.

Eastman Kodak Co. Phenanthrene.

With the exception of several of the compounds of highest molecular weight (which appear to be over 70% pure and

Table I. Relative Retention Times of Alkyl Phenanthrenes

	SE-30[a]	Calcd. SE-30	QF-1[b]	Calcd. QF-1	Dow 11[c]	Dow 710[d]	APL[e]	NPGS[f]	NPGS[g]
Phenanthrene	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
9,10-Dihydrophenanthrene	0.756		0.553						
1-Methylphenanthrene	1.52	1.51	1.50	1.50	1.44	1.37	1.58	1.46	1.51
2-Methylphenanthrene	1.40	1.40	1.43	1.43	1.38			1.33	1.35
3-Methylphenanthrene	1.37	1.37	1.39	1.40	1.33			1.30	1.33
4-Methylphenanthrene	1.48	1.44	1.39	1.27					1.45
9-Methylphenanthrene	1.48	1.48	1.48	1.48					1.50
4,5-Methylenephenanthrene	1.48		1.40						
1,2-Dimethylphenanthrene	2.36	2.38	2.42	2.43					
1,3-Dimethylphenanthrene	2.05	1.98	2.02	1.96				1.78	
1,6-Dimethylphenanthrene	2.09	2.08	2.08	2.09					
1,7-Dimethylphenanthrene	2.13	2.12	2.14	2.13	1.98	1.72	2.31	1.93	2.05
1,8-Dimethylphenanthrene	2.28	2.28	2.19	2.17					2.29
1,9-Dimethylphenanthrene	2.14	2.18	2.10	2.11					
2,3-Dimethylphenanthrene	2.17	2.16	2.29	2.26					
2,7-Dimethylphenanthrene	1.97	1.97	1.96	1.97					
3,6-Dimethylphenanthrene	1.88	1.89	1.94	1.95					1.29
4,5-Dimethylphenanthrene	1.57		1.14						1.29
9,10-Dimethylphenanthrene	2.50	2.46	2.48	2.48					2.69
2-Ethylphenanthrene	1.89	1.87	1.80	1.81	1.76			1.65	1.73
3-Ethylphenanthrene	1.78	1.78	1.69	1.71					1.63
4-Ethylphenanthrene	1.73	1.73	1.49	1.49					1.55
9-Ethylphenanthrene	1.86	1.86	1.75	1.73					1.75
1,2,8-Trimethylphenanthrene	3.57	3.59	3.60	3.52					
1,3,8-Trimethylphenanthrene	3.06	2.99	2.88	2.85					
1,4,7-Trimethylphenanthrene	3.05	3.05	2.72	2.72	2.76	2.30	3.33		
1,4,8-Trimethylphenanthrene	3.19	3.28	2.60	2.77					
1,5,7-Trimethylphenanthrene	3.01	3.05	2.67	2.72					
1,6,7-Trimethylphenanthrene	3.27	3.27	3.41	3.39					
1,6,10-Trimethylphenanthrene	3.45	3.44	3.37	3.51					
1-Methyl-6-ethylphenanthrene	2.69	2.69	2.59	2.56	2.50	1.98	2.77	2.26	
1-Methyl-7-ethylphenanthrene	2.83	2.83	2.72	2.70	2.59			2.37	2.59
1-Methyl-8-ethylphenanthrene	2.81	2.87	2.56	2.47					2.69
9-Methyl-10-ethylphenanthrene	2.82	2.75	2.67	2.66					2.83
2-Isopropylphenanthrene	2.25	2.23	2.05	2.05				1.77	
9-Isopropylphenanthrene	2.14	2.14	1.90	1.90					1.89
9-*n*-Propylphenanthrene	2.33	2.33	2.09	2.09					
1,2,7,8-Tetramethylphenanthrene	5.44[h]	5.65	5.46	5.55					
1,7,8,9-Tetramethylphenanthrene	5.13[h]	5.43	4.99	5.22					
1-Ethyl-2,8-dimethylphenanthrene	4.11	4.03	3.61	3.67					
1,3-Dimethyl-7-ethylphenanthrene	3.80	3.71	3.66	3.55				3.03	
9,10-Diethylphenanthrene	3.23	3.18	2.85	2.83					2.92
1-Methyl-6-isopropylphenanthrene	3.06	3.11	2.79	2.77	2.79	2.14	3.10	2.36	
1-Methyl-7-isopropylphenanthrene	3.33	3.37	3.06	3.07	3.05	2.31	3.49	2.58	2.87
1'-Methyl-1,2-cyclopentanophenanthrene	3.95		3.71		3.59	2.98	4.43	3.56	
2-*tert*-Butylphenanthrene	2.75	2.75	2.54	2.54					
3-*tert*-Butylphenanthrene	2.45	2.45	2.26	2.26					
9-*n*-Butylphenanthrene	3.19	3.18	2.63	2.65					
9-Isobutylphenanthrene	2.55	2.55	2.23	2.23					
1,3,9-Trimethyl-7-ethylphenanthrene	5.24	5.00	4.85	4.62					
1,2-Dimethyl-6-isopropylphenanthrene	4.90	4.89	4.45	4.50					
1,3-Dimethyl-7-isopropylphenanthrene	4.62	4.43	4.15	4.03	4.24		4.46	3.28	
9-Methyl-10-*n*-butylphenanthrene	4.70	4.71	4.12	4.08					
9-Ethyl-10-*n*-propylphenanthrene	3.95	3.99	3.40	3.42					
9-*tert*-Amylphenanthrene	3.39	3.39	2.64	2.64					
9-Cyclopentylphenanthrene	5.76	5.76	5.15	5.15					
1,3-Dimethyl-7,9-diethylphenanthrene	6.02	6.28	5.31	5.41					
1,3,9-Trimethyl-7-isopropylphenanthrene	6.01	5.97	5.34	5.25				3.95	
9-Benzylphenanthrene	9.35	9.35	8.05	8.05					
9-Ethyl-10-phenylphenanthrene	9.12		5.88						
Fluorene	0.55		0.46		0.56	0.56	0.48	0.43	0.42

[a] 10 foot × 1/8 inch o.d. stainless steel column packed with 20% SE-30 silicone on 60/80 mesh Chromosorb W, T = 240° C.; P = 42 lb. N_2; flame ionization detector.
[b] 10 foot × 1/8 inch o.d. stainless steel column packed with 20% QF-1-0065 fluorosilicone on 60/80 mesh Chromosorb W, T = 215° C.; P = 52 lb. N_2; flame ionization detector.
[c] 10 foot × 1/4 inch o.d. stainless steel column packed with 25% Dow-11 silicone on 35/80 mesh Chromosorb, T = 235° C.; P = 16 lb. He; thermal conductivity detector.
[d] 10 foot × 1/4 inch o.d. stainless steel column packed with 25% Dow-710 phenyl silicone on 35/80 mesh Chromosorb, T = 294.5° C.; P = 16 lb. He; thermal conductivity detector.
[e] 5 foot × 1/4 inch o.d. stainless steel column packed with 25% Apiezon L on 35/80 mesh Chromosorb, T = 235.5° C.; P = 17.3 lb. He; thermal conductivity detector.
[f] 5 foot × 1/4 inch stainless steel column packed with 20% neopentyl glycol succinate on 60/80 mesh firebrick, T = 230° C.; P = 24 lb. He; thermal conductivity detector.
[g] 5 foot × 1/8 inch stainless steel column packed with 20% neopentyl glycol succinate on 60/80 mesh firebrick, T = 225° C.; P = 48 lb. N_2; flame ionization detector.
[h] Data obtained by Rajindra Aneja.

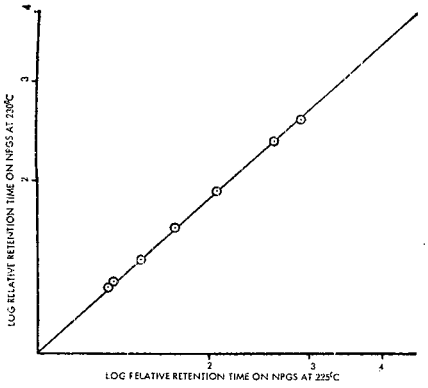

Figure 1. Log relative retention time determined on NPGS

Compounds arranged in order of increasing retention times
- Phenanthrene (at origin)
- 3-Methyl-
- 2-Methyl-
- 1-Methyl-
- 2-Ethyl-
- 1,7-Dimethyl-
- 1-Methyl-7-ethyl-
- 1-Methyl-7-isopropyl-

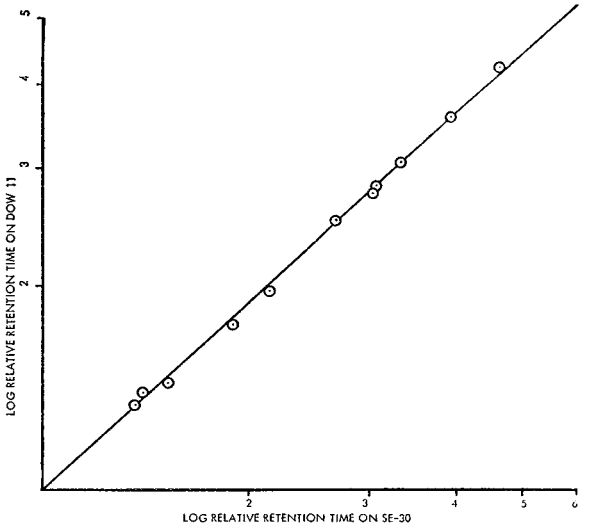

Figure 2. Log relative retention time on SE-30 and Dow 11

Compounds arranged in order of increasing retention times
- Phenanthrene (at origin)
- 3-Methyl-
- 2-Methyl-
- 1-Methyl-
- 2-Ethyl-
- 1,7-Dimethyl-
- 1-Methyl-6-ethyl-
- 1,4,7-Trimethyl-
- 1-Methyl-6-isopropyl-
- 1-Methyl-7-isopropyl-
- 1-Methyl-1,2-cyclopentano-
- 1,3-Dimethyl-7-isopropyl-

were not available in sufficient quantities for further purification), all samples used for the determination of relative retention times were over 90% pure as judged from their behavior on GLC over SE-30 and QF-1-0065 phase using a flame ionization detector.

Determination of Relative Retention Times. Before being used, columns were preconditioned by being heated overnight, in a slow stream of nitrogen or helium, to a temperature at least 50° C. above that employed for the determination of relative retention times. All determinations were made by injecting a solution of fluorene, phenanthrene, and alkylphenanthrene in xylene or pyridine into a superheated block connected to the appropriate column. In runs made on the Aerograph A-110-C equipment, retention times were measured as the difference in time between the emergence of an air peak and the peak in question. In runs on the A-600-B equipment, the point of initial emergence of the solvent peak was taken as reference. The conditions employed for the determination of relative retention times of the phenanthrenes shown in Table I are listed in the footnotes to this table.

Table II. Log Factors for Calculating Log_{10} Relative Retention Time of Alkyl Phenanthrenes

	SE-30 Nuclear position					QF-1-0065 Nuclear position				
Group	1(8)	2(7)	3(6)	4(5)	9(10)	1(8)	2(7)	3(6)	4(5)	9(10)
Methyl	0.179	0.147	0.138	0.158	0.170	0.175	0.154	0.145	0.105	0.169
Ethyl	0.279	0.272	0.250	0.238	0.269	0.231	0.257	0.233	0.173	0.237
Isopropyl		0.349	0.313		0.330		0.312	0.268		0.279
n-Propyl					0.367					0.320
tert-Butyl		0.439	0.389				0.405	0.354		
Isobutyl					0.407					0.349
n-Butyl					0.503					0.424
tert-Amyl					0.530					0.422
Cyclopentyl					0.760					0.712
Benzyl					0.971					0.906

To determine log_{10} relative retention time of an alkyl phenanthrene, add to sum of the appropriate factors any of following correction terms which may be pertinent:

On SE-30		On QF-1	
Vicinal di Me	+0.050	Vicinal di Me	+0.056
1,10-di Me	+0.050	1,10-di Me	+0.056
8,9-di Me	+0.050	8,9-di Me	+0.056
Vicinal Me-Et (or larger)	0	Vicinal Me-Et (or larger)	+0.018
Vicinal di Et (or larger)	−0.035	Vicinal di Et (or larger)	−0.023
1,3-dialkyl	−0.020	1,3-dialkyl	−0.027
6,8-dialkyl	−0.020	6,8-dialkyl	−0.027
1,9-dialkyl	−0.020	1,9-dialkyl	−0.027
8,10-dialkyl	−0.020	8,10-dialkyl	−0.027
2,10-dialkyl	−0.020	2,10-dialkyl	−0.027
7,9-dialkyl	−0.020	7,9-dialkyl	−0.027
		1,8-dialkyl	−0.013
		2,7-dialkyl	−0.013

Calculation of Relative Retention Times. The three examples below illustrate the use of the log factors and corrective terms in Table II.

1,3,9-Trimethyl-7-ethylphenanthrene on SE-30 column. From Table II, the appropriate log factors and corrective terms are added: 0.179 (1-Me) + 0.138 (3-Me) + 0.170 (9-Me) + 0.272 (7-Et) − 0.020 (1,3-dialkyl) − 0.020 (1,9-dialkyl) − 0.020 (7,9-dialkyl) = 0.699. Antilog of 0.699 = 5.00. The experimentally observed value is 5.24.

1,7,8,9-Tetramethylphenanthrene on QF-1-0065. From Table II: 0.175 (1-Me) + 0.154 (7-Me) + 0.17: (8-Me) + 0.169 (9-Me) + 0.056 (7,8-di Me) + 0.056 (8,9-di Me) − 0.027 (1,9-dialkyl) − 0.027 (7,9-dialkyl) − 0.013 (1,8-dialkyl) = 0.718. Antilog 0.718 = 5.22. The experimentally observed value is 4.99.

1,2,7,8 - Tetramethylphenanthrene on QF-1-0065 column. From Table II, the appropriate log factors and corrective terms are added: 0.175 (1-Me) + 0.154 (2-Me) + 0.154 (7-Me) + 0.175 (8-Me) + 0.056 (1,2-di Me) + 0.056 (7,8-di Me) − 0.013 (1,8-dialkyl) − 0.013 (2,7-dialkyl) = 0.744. The anti-

log of 0.744 = 5.55. The experimentally observed value is 5.46.

DISCUSSION AND RESULTS

Our study has now been extended to include a much larger number of phenanthrenes than previously reported (*9*). This extension was made possible, in part, by employing a very sensitive flame ionization detector which enabled us to study submilligram quantities of phenanthrenes. Full realization of the potentialities of the detector requires the use of a liquid phase of high thermal stability in order to minimize background noise caused by column bleed. To this end, where the flame ionization detector was utilized, Dow-Corning QF-1-0065 (a fluorinated silicone) was employed as polar phase and G.E. SE-30 (a methyl silicone) as nonpolar phase (*10*).

Table I lists, for each of the columns employed, the retention times of the various compounds relative to that of phenanthrene. All relative retention times reported represent the results of at least two determinations. The values for the various runs on a given column have a maximum standard deviation of ±2.0%.

If, at least, two of the phenanthrenes of Table I are available, the relative retention times reported may be extrapolated, as shown in Figures 1 and 2, to accommodate determinations made under similar, but nonidentical conditions. Figure 1 is a log-log plot of relative retentions times as determined on 5-foot (neopentylgycol succinate) columns, in one case a 1/8 inch o.d. column at 225° C. and in the other a 1/4 inch o.d. column at 230° C. Figure 2 is a log-log plot of relative retention times as determined at 240° C. on a 10 foot × 1/8 inch o.d. column containing SE-30 methyl silicone *vs.* relative retention times determined at 235° C. on a 10 foot × 1/4 inch o.d. column containing Dow 11 methyl silicone as the liquid phase.

Mixtures of phenanthrenes, such as would result from a typical dehydrogenation, can often be easily fractionated by gas liquid chromatography. In our hands optimum results were obtained by the use of loads of less than 50 mg. on 1/4 inch o.d. columns. Resolution generally increased as the load decreased. Preparative runs were therefore made by repeatedly injecting small samples and collecting the various fractions. Corresponding fractions were then combined. [Instruments which perform these operations automatically (Aerograph Model 700 and F & M Model 770) have recently been introduced.] Fractions isolated in this way may often be tentatively identified by determining their relative retention times under standard conditions on several different liquid phases. If sufficient material is available, identification may be confirmed by spectral studies.

A plot of log relative retention time of a homologous series of organic compounds *vs.* carbon number gives a straight line (*4*). Homologous series differing in amount or position of chain branching or unsaturation may be plotted against carbon number as a set of parallel lines (*4*). Alternatively, the lines may be combined by projecting the log relative retention times of one series of compounds onto the line determined by a second series. Points so projected will be displaced along the carbon-number axis by a distance equal in magnitude but opposite in direction to the distance which separated the intercepts of the original lines with this axis. The latter concept was used successfully in our previous paper (*9*), in which log relative retention times for several families of phenanthrenes were found to give linear plots against "degree of alkyl substitution"—a factor determined solely by the nature and extent of alkyl substitution.

In attempting to apply this method to the increased number of phenanthrenes included in the present study, we found that only those series of phenanthrenes in which the substituent being varied is located as position 2 (7) or 3 (6) would give linear plots against a common set of substituent constants.

This deviation is probably caused mainly by steric interaction. The hindrance encountered by groups at the 4 (5) position is obvious. Groups at 1 (8) undergo a perinaphthalene-type interaction with substituents (H or alkyl) at 10 (9) and vice versa. We therefore set out to test the more general hypothesis, that the contribution of a substituent to the retention time of a phenanthrene is a constant, the value of which is dependent both on the nature of the substituent and on the position of substitution (*3*).

The log relative retention times of the methylphenanthrenes were factored to determine the contribution made by this substituent at the various positions on the nucleus. The log relative retention times of the majority of the methylphenanthrenes could readily be fitted to the common sets of factors shown in Table II. The exceptions fell into several easily recognized classes. Vicinal or 1,10-(8,9-) dimethylphenanthrenes have abnormally long retention times, but the deviation is constant for all cases studied. On a QF-1 column, 1,8- and 2,7-dimethyl substitution resulted in smaller than normal contributions, again by a constant amount. On either QF-1 or SE-30 columns dimethyl substitution at positions 1 and 3 (6 and 8), 1 and 9 (8 and 10), or 2 and 10 (7 and 9) requires substraction of a constant factor. From the limited data available the log relative retention times of phenanthrenes containing substituents larger than methyl seem best to be approximated by the procedure described above, except that special correction factors, given in Table II, must be employed for vicinal interactions involving these substituents. Not unexpectedly, the extreme hindrance (*5*) encountered by substituents in the 4 (5) position leads to irregular behavior. The values in Table II for 4 (5) substituents should therefore be used with caution. The use of the log factors and corrective terms appearing in Table II is illustrated in the Experimental Section by the calculation of relative retention times for three phenanthrenes.

Columns 2 and 4 of Table I give the relative retention times of the phenanthrenes, on SE-30 and QF-1 as calculated from the factors in Table II. With the exception of the 4-substituted phenanthrenes (for the reason mentioned above), the calculated relative retention times of all phenanthrenes fall within 6% of the measured values, thus demonstrating the validity of this general approach. These data should facilitate the rapid identification of small amounts of phenanthrenes obtained from the degradation of natural products.

ACKNOWLEDGMENT

We express grateful appreciation to the investigators listed in Table I, who made available samples of substituted phenanthrenes. We thank Rajindra Aneja for obtaining the data for 1,2,7,8- and 1,7,8,9-tetramethylphenanthrene on the SE-30 column.

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Argentation in Countercurrent Distribution to Separate Isologues and Geometric Isomers of Fatty Acid Esters

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▷ **Mixtures of fatty acid methyl esters were fractionated by countercurrent distribution between hexane and 0.2N silver nitrate in 90% methanol. *Cis* and *trans* monoenoic esters were separated. With polyenoic esters, separation depends upon both configuration and number of carbon atoms between double bonds.**

SEVERAL PROCEDURES have been used for separation of *cis* and *trans* isomers of fatty acids. The most common is crystallization for the isolation of elaidic acid and of geometric isomers of polyunsaturated acids. Urea adducts have also been used (*19*). On an analytical scale, methyl esters of geometric isomers can be separated by capillary column gas chromatography (*10, 11*). Methyl esters have also been separated after reaction with a limited amount of mercuric acetate. *Cis* isomers react more rapidly than *trans* with mercuric acetate, and unreacted *trans* esters can be separated from the adduct by countercurrent distribution (CCD) (*8*).

Lucas, Moore, and Pressman (*12*) pointed out that compounds with *cis* double bonds form complexes with silver ion to a greater extent than do their *trans* isomers. In 1952, Nichols (*13*) applied this information to a study of methyl oleate and methyl elaidate. He suggested that these could be separated by a 300 transfer countercurrent distribution using 0.2*N* silver nitrate in 90% methanol as a lower phase with isooctane as an upper phase. We have confirmed this separation, but used either petroleum ether or hexane instead of isooctane as the upper phase, and have extended the procedure to some isomers of methyl linoleate (*6*). This paper describes these separations, as well as applications to other methyl ester mixtures and to triglycerides.

EXPERIMENTAL

Countercurrent Distribution Procedure. All distributions were carried out in a 200-tube automatic CCD apparatus in which each tube contained 40 ml. of the lower solvent layer. Ten-milliliter portions of upper layer were used.

The distributions were made according to the single-withdrawal procedure (*10*) in which each portion of upper layer passes completely through the instrument and is withdrawn from the last tube into a fraction collector. The solution from two transfers was collected in one fraction, and the weight of material in this fraction was plotted against the transfer number at which it emerged from the instrument.

The amount of material in each fraction was also measured by passing the solution through a recording differential refractometer, which plotted the change in refractive index between the sample solution and pure upper layer solvent. This measurement avoided the labor of evaporating and weighing many individual fractions. In recycle operation the fractions were automatically pumped back into the first tube of the CCD instrument. In this operation the refractometer provided information about the location of bands and the degree of separation being achieved while the distribution was still in progress. Combination of fractions was based upon the shape of the CCD curve and analysis of individual samples. Combined fractions were ordinarily distilled under vacuum before analysis.

Analysis for *trans* Double Bonds. Infrared analyses for *trans* double bonds were made by measurement of carbon disulfide solutions in a 0.5-mm. cell. Transmission at the 10.4-micron band was measured from a base line drawn tangent to the adsorption curve at approximately 9.5 and 10.7 microns. The percentage of *trans* bonds was calculated on the basis of methyl elaidate as 100%.

Gas Chromatograms. The gas chromatograms in Table I were run on a 120- × 0.5-cm. column packed with 10% polyvinyl acetate on Chromosorb W at 170° C. and with a gas flow of 60 ml. per minute of argon. A radium D ionization detector was used.

Capillary column chromatograms were run on a 200-foot × 0.01-inch capillary coated with Apiezon L with a flow rate of 1.5 ml. per minute of argon through the column. A radium D ionization detector was used.

Methyl Oleate–Methyl Elaidate. A mixture of methyl oleate and methyl elaidate containing 49.5% *trans* esters by infrared analysis was used. Fifteen milliliters of this mixture were recycled to 700 transfers and then collected in the fraction collector to 1050 transfers.

Selenium-Isomerized Methyl Linoleate. Methyl linoleate was isomerized by heating under nitrogen at 190° C. for 7 hours with 0.5% of selenium. The product contained 60% *trans* esters by infrared measurement. Twenty milliliters of this material were recycled once through the CCD apparatus before being run to the fraction collector. Fractions corresponding to transfers 586 through 602 were combined to give 0.72 gram of a *trans,trans* material (*trans, trans* Run No. 1). Fractions 650 to 700 were combined to give 2.20 grams of *cis,-trans* and *trans,cis* esters (mono *trans* Run No. 1).

In a similar experiment 20 ml. of selenium-isomerized methyl linoleate containing 90% *trans* esters was recycled twice before collection of the fractions. From this distribution 3.35 grams of *trans,trans* ester (*trans,trans* Run No. 2) and 4.50 grams of mono *trans* esters (mono *trans* Run No. 2) were recovered. Gas chromatograms of the samples were run on an Apiezon L, 200-foot capillary column at 200° C.

Fractions from Run No. 2 were oxidized by a permanganate-periodate procedure. The dibasic acids produced were analyzed by gas chromatography of their methyl esters.

Alkali - Isomerized Linoleate. Methyl linoleate was isomerized by heating with potassium hydroxide in ethylene glycol at 200° C. The isomerized acids were recovered and re-esterified with methanol using sulfuric acid as catalyst. Twenty milliliters of the ester ($\lambda^{Isooctane}_{max}$ 232 mμ a = 49.5), were distributed in the CCD instrument.

In another experiment 30 ml. of alkali-isomerized methyl linoleate ($\lambda^{Isooctane}_{max}$ 233 mμ a = 87.7), was recycled once through the CCD instrument before collection in the fraction collector. Fractions in the mono *trans* band were combined and distilled to yield 11.16 grams of ester ($\lambda^{Isooctane}_{max}$ 232 mμ a = 90.4).

Methyl 9,15-Linoleate. Linolenic acid was reduced with hydrazine to an iodine value of 128 and separated into triene, diene, monoene, and saturated esters by CCD between acetonitrile and petroleum ether (*16*). The diene fraction (16.4 grams) was separated, using the silver-nitrate solvent system, into two chief fractions. Capillary gas chromatograms on Apiezon L indicated that the first fraction was 9,12- and 12,15-linoleate and the second was 9,15-linoleate.

Linseed Oil Methyl Esters. Methyl esters were prepared from linseed oil by transesterification with sodium methoxide catalyst. Ten milliliters

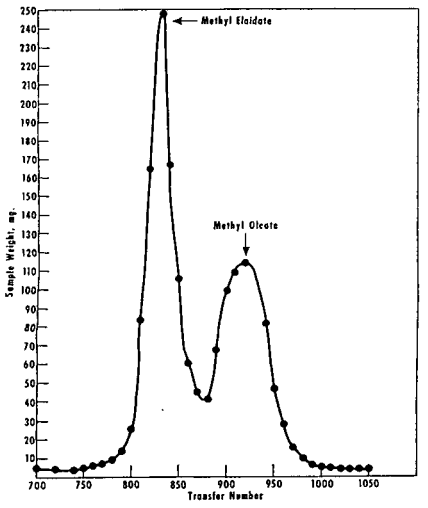

Figure 1. Countercurrent distribution of methyl elaidate and methyl oleate

of the esters were fractionated by CCD with the silver-nitrate system.

Linseed Oil. Twelve milliliters of linseed oil were distributed in the silver-nitrate solvent system. Transfers 370 through 460 were combined to yield 1.67 grams; transfers 295 through 330 yielded 1.10 grams. Gas chromatography of methyl esters showed the first fraction to be trilinolenin. The second fraction was calculated to contain 72% dilinolenolinolein, 14% dilinolenoolein, and 14% linolenodilinolein.

RESULTS AND DISCUSSION

Separation by CCD depends upon differences in partition coefficients of the substances being fractionated. Since no silver ion is present in the upper solvent phase, these differences depend largely upon the tendency of the substances to complex with silver ion and remain in the lower solvent phase:

$$\begin{array}{l} RCH{=}CHR' \text{ (upper phase)} \\ \quad \Updownarrow \\ RCH{=}CHR' \rightleftarrows RCH{=}CHR', \\ \qquad\qquad AgNO_3 \text{ complex (lower phase)} \end{array}$$

The term "argentation" describes the formation of the loose pi-bonded complex between the double bonds of the esters and silver ion.

The *cis* and *trans* isomers are separated because the equilibrium between free and complexed esters in the lower phase is more to the right for the *cis* form. Isologous esters that increase in degree of unsaturation, such as oleate, linoleate, and linolenate, can also be separated because of increasing argentation. Polyunsaturated esters are separated too according to the relative positions of the double bonds. When the double bonds are separated by several methylene groups, there is increased argentation and a greater tendency for the compound to remain in the lower phase than when the double bonds are separated by only one methylene group.

The single-withdrawal procedure (*4*) was used in which the sample is recovered free of silver in the upper phase. The methanolic-silver nitrate lower phase was left in the instrument for several runs. Possibly because of changes in the lower phase, changes in room temperature, or other causes, partition coefficients of an ester varied more than 15% in different runs. For this reason no values for partition coefficients are included.

Figure 1 illustrates the separation of methyl oleate and methyl elaidate—the esters studied by Nichols. Good separation was obtained by recycling the esters twice through a 200-tube CCD instrument. Based on the curve and infrared analyses of individual fractions, transfers 800 through 844 were combined to yield 3.62 grams of methyl elaidate. Infrared analysis indicated 99.8% purity for this material. Gas chromatography indicated at least 97% purity.

Figure 2 illustrates the separation of isomers from selenium-isomerized methyl linoleate, which was recycled through the CCD instrument. In Table I where analyses of combined fractions from the *trans,trans* and *cis,-trans*, *trans,cis* regions hereafter referred to as mono *trans* are given, this experiment is designated as Run 1. Fractions from a similar run recycled twice are shown as Run 2. The mono *trans* esters are rather pure. Gas chromatograms on both packed and capillary columns show only the expected components. Absorptivity in the conjugated diene region is low. Although it is generally considered that the adsorption at 10.4 microns caused by *trans* bonds, is the same as for elaidate, we had only about 85% as much adsorption.

In the *trans,trans* samples both gas chromatograms and ultraviolet adsorption indicate the presence of some conjugated esters as impurities. When the infrared adsorption is corrected for these impurities, the esters are about 166% *trans* as elaidate or twice that of the mono *trans* esters.

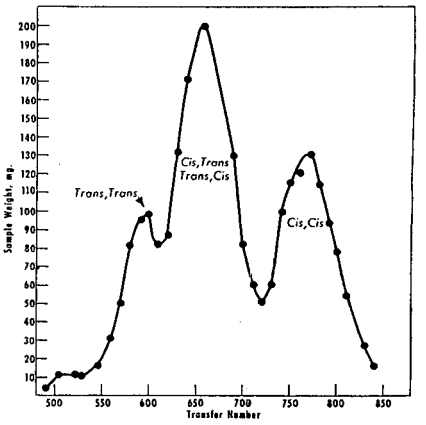

Figure 2. Countercurrent distribution of selenium-isomerized methyl linoleate

Run 1

It has been generally assumed that the absorption caused by *trans* bonds in *trans,trans* linoleic acid (linolelaidic acid) is twice that of elaidic acid, but little quantitative data have been published. Jackson and his coworkers (*7*) state adsorption for methyl linolelaidate is very nearly twice that of methyl elaidate. Ahlers (*1*) and his associates state the value for linolelaidic acid is approximately twice that for elaidic acid and the value for linolenelaidic acid is approximately three times that for elaidic acid. However, his coefficients, in agreement with our data, seem to indicate that although the adsorption per *trans* double bond is additive, it is less for polyunsaturated acids than for elaidic acid. In general, the environment of a functional group determines its physical characteristics so that the immediate environment of the double bond will be important. The homodiene system of the 9,12-octadecadienoic acids can exist in two conformers with respect to the C-11 methylene group (neglecting the dissimilar nature of alkyl substituents at C-9 and C-13). One form, the *gauche* (a doubly degenerate) is less stable than the *trans* staggered form which will thus represent the principal conformer (with respect to the C-11 methylene group). The infrared band at 968$^{cm.-1}$ (10.3 microns) represents the out-of-plane

Table I. Analyses of Fractions from Selenium-Isomerized Methyl Linoleate

Fraction	Gas chromatogram	*Trans* esters as methyl elaidate, %	Absorptivity isooctane 231 mμ	N_D^{30}
Mono *trans* Run no. 1	1 Component	84.8	1.9	...
Mono *trans* Run no. 2	1 Component	85.8	0.6	1.45713
Trans, trans Run no. 1	85% in main component	142.7	13.0	...
Trans, trans Run no. 2	94% in main component	155.9	5.7	1.45703

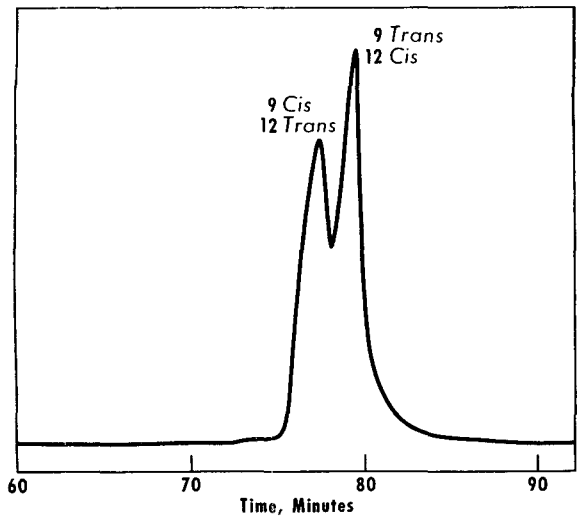

Figure 3. Gas chromatogram (Run 2) of mono *trans* fraction from selenium-isomerized methyl linoleate on Apiezon L, 200-ft. capillary column at 200° C.

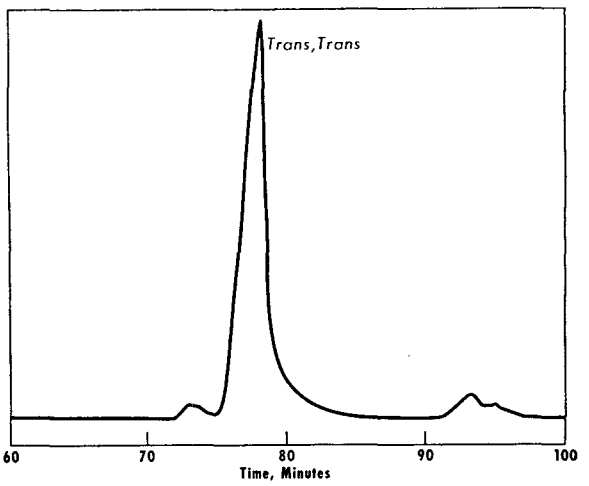

Figure 4. Gas chromatogram (Run 2) of *trans,trans* fraction from selenium-isomerized methyl linoleate on Apiezon L, 200-ft. capillary column at 200° C.

symmetric bending frequency of the protons on a *trans* double bond. Since the principal conformer at C-11 is *trans* staggered, the 9 and 12 double bonds will lie in the same plane. This means that the transition dipole of the double bond will tend to polarize the adjacent double bond and thus decrease it own magnitude since the induced polarized dipole will be antiparallel to it. Since the absorption intensity is proportional to the integral of the transition dipole, there will be a net decrease. This suggests that with two or more methylene groups between the double bonds, the absorption intensity will be additive because the polarization effect is an inverse function of the distance between them (*18*). Thus, the lower adsorption caused by *trans* double bonds in linolelaidic acids is explicable.

Gas chromatograms of fractions from Run 2 on an Apiezon L capillary column are shown in Figures 3 and 4. The two peaks in the mono *trans* sample (Figure 3) are believed to be 9-*cis*,12-*trans*, and 9-*trans*,12-*cis* corresponding to their identification by Litchfield, Isbell, and Reiser (*11*). The *trans,trans* sample (Figure 4) has only one main component with smaller amounts of conjugated impurities.

In Run 2 the position of the double bond nearest the carboxyl was determined by permanganate–periodate oxidation and analysis of the resulting dibasic acids (*9*). Results in Table II show that although there was some movement, about 90% of the double bonds remained in their original positions.

Figure 5 illustrates the separation of alkali-isomerized methyl linoleate with no recycling. Unconjugated esters are separated from conjugated; and *trans,-trans* conjugated from mono *trans* conjugated. In another experiment in which the sample was recycled once to obtain better separations a capillary gas chromatogram (Figure 6) of the supposed *cis,trans* and *trans,cis* fraction shows the presence of other components. Comparison with GLC curves for dehydrated castor oil esters on the same column as well as the work of Body and Shorland (*2*), indicated the isomers shown in Figure 6. Two explanations are suggested for the presence of these unexpected isomers. First, other isomers besides the 9-*cis*,11-*trans* and 10-*trans*,12-*cis* linoleates suggested by Nichols, Herb, and Riemenschneider (*14*) are formed by alkali isomerization and are not separated by CCD. Secondly, the unexpected esters were formed by isomerization during processing of the fractions. It is known

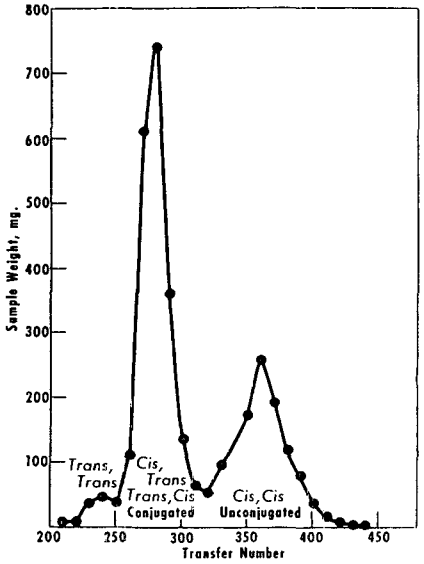

Figure 5. Countercurrent distribution of alkali-isomerized methyl linoleate

Table II. Per Cent Dibasic Acids from Fractions of Selenium-Isomerized Methyl Linoleate of Run 2 after Oxidative Cleavage

Fraction	Per Cent Dibasic Acids with 6 to 14 Carbon Atoms								
	6	7	8	9	10	11	12	13	14
Mono *trans*	0.4	0.9	5.3	86.6	4.6	0.7	0.3	...	1.2
Trans,trans	0.6	0.7	5.7	91.9	...	...	...	...	1.1

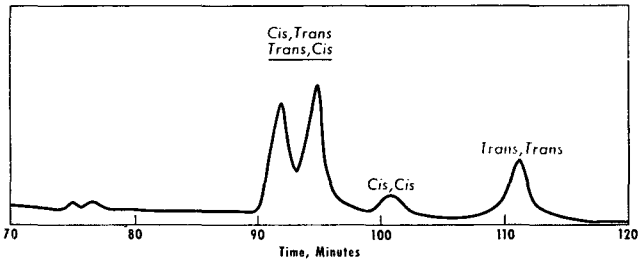

Figure 6. Gas chromatogram of fraction of alkali-isomerized methyl linoleate on Apiezon L, 200-ft. capillary column at 200° C.

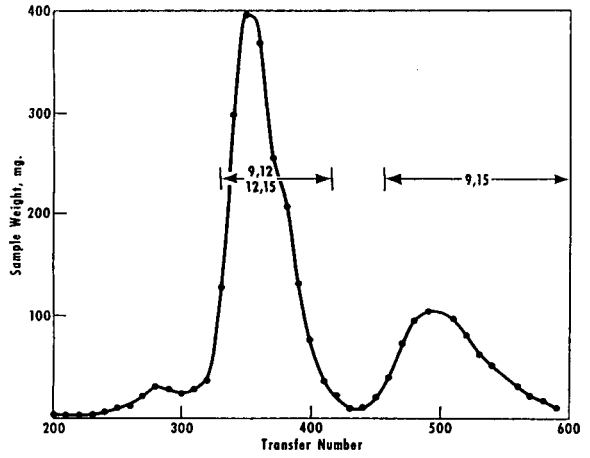

Figure 7. Countercurrent distribution of mixture of 9,12,- 12,15-, and 9,15-methyl linoleates

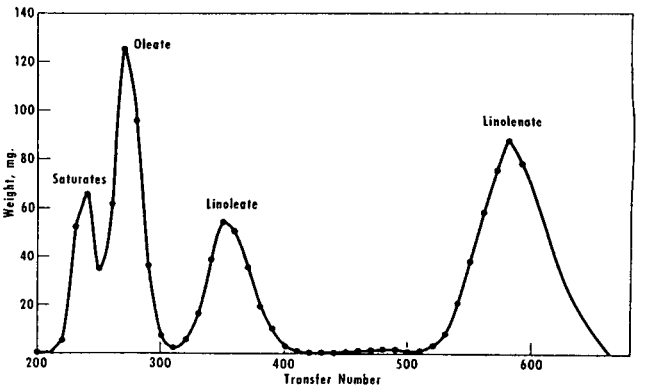

Figure 8. Countercurrent distribution of linseed oil methyl esters

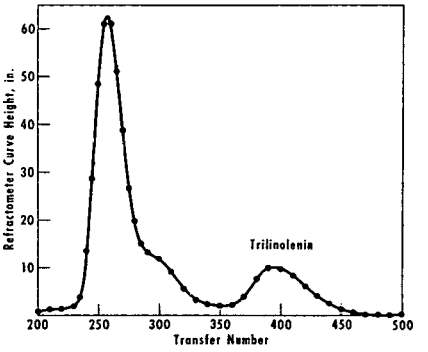

Figure 9. Countercurrent distribution of linseed oil

that light may cause *cis,trans* isomerization of conjugated esters (*3*).

In the distributions described, separations depend upon the geometric configuration of the double bonds. In polyunsaturated esters, separation also depends upon the number of methylene groups between the double bonds. When the double bonds are separated by more than one methylene group, the silver complex forms to a greater extent. This fact is illustrated in Figure 7 where methyl 9,15-linoleate is well separated from 9,12- and methyl 12,15-linoleates. A similar distribution curve was obtained for the diene fraction from catalytically hydrogenated methyl linolenate (*15*).

The silver nitrate-containing solvent system can be used for separations, depending upon the number of double bonds in the molecule, but for such separations other solvent systems are preferable. Figure 8 illustrates separation of linseed oil methyl esters. The separation is good, but acetonitrile and hexane would ordinarily be used as the solvent system (*17*). With the silver nitrate system, linolenate is extremely well separated from linoleate. This separation may result because methyl linolenate, in addition to having a total of three double bonds, has two of its double bonds widely separated, as in *methyl* 9,15-linoleate, and in a more favorable position for argentation. Also, because separation depends largely upon silver complexing, methyl palmitate will be found with stearate in the saturates fraction rather than with oleate as it is when the acetonitrile system is used.

The silver nitrate system may even be used for triglycerides, as illustrated by the fractionation of linseed oil in Figure 9. Furfural, nitroethane (50/50), and petroleum ether would be a preferable solvent system here (*5*), but the separation of trilinolenin illustrates the selectivity that can be achieved with the silver nitrate system.

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A New Specific Color Reaction of Formic Acid

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▷ A new specific color reaction for formic acid has been developed. Spectrophotometric studies have been performed to determine the effect of temperature, lead nitrate concentration, and time on the violet-colored complex. A tentative mechanism for the color reaction is proposed and discussed.

DURING investigations (*5*) of the paper chromatography of Sn^{+2} and Sb^{+3} in these laboratories, it was noticed that an anisole–formic acid mixture gives a violet color with some nitrates. No reference to this reaction could be found in the literature, and since it could be used for the detection and determination of formic acid, it was decided to investigate this reaction further. It has been found that, of the common organic substances, only formic acid gives a violet color with anisole and lead nitrate, and this reaction can be used as a specific test for nitrite and in the absence of nitrite as a test for nitrate.

EXPERIMENTAL

Reagents and Apparatus. Formic acid (Merck, Darmstadt), anisole (W. J. Bush & Co. Ltd., London), and lead nitrate (Merck) were used. All other chemicals were of reagent grade. Spectrophotometric work was done on the Bausch & Lomb Spectronic 20. Usually the nitrates were dissolved in water, but when there was danger of hydrolysis they were dissolved in dilute HCl or H_2SO_4. HNO_3 was avoided because it may complicate the interpretation.

Procedure. To about 0.5 ml. of the organic liquid add an equal volume of anisole. Add a drop of 0.1*M* aqueous lead nitrate solution, and observe after 2 minutes. A violet color shows the presence of formic acid.

Studies of Violet Complex. The violet complex has the spectrum given in Figure 1. As the maxima occur at 575 and 775 mμ, 575 mμ was chosen for spectrophotometric work on this complex. Curves of time *vs.* absorbance are given in Figure 2. After the formation of the violet complex, the reaction mixture was left for 24 hours at room temperature. Curves of absorbance *vs.* time for this extended period (Figure 3) show that maximum absorbance is obtained in about 2½ hours. To study the effect of temperature on this reac-

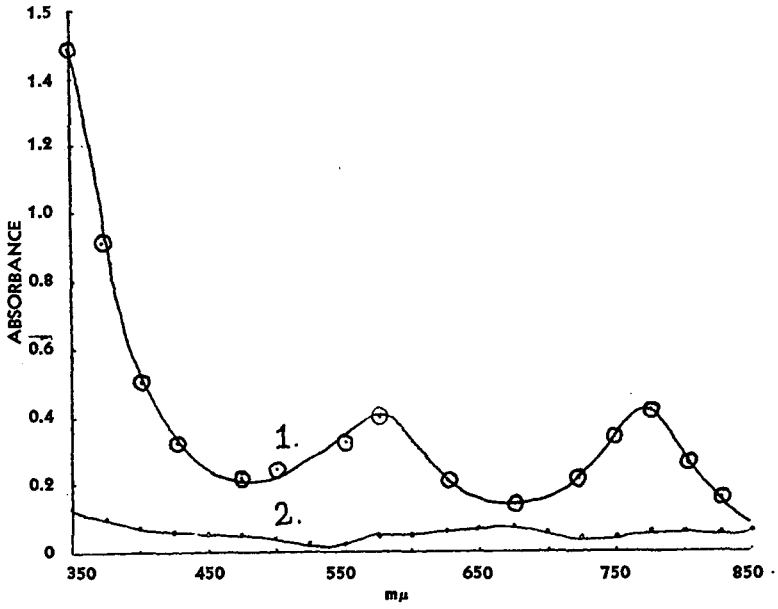


Figure 1. Spectra of violet complex with distilled water

1. Complex
2. Anisole–formic acid (1 to 1)

Table I. Organic Substances Tested with Anisole and Lead Nitrate

Class of compound	Individual organic compounds
Alcohols	Methyl, ethyl, *n*-butyl, isopropyl, *tert*-butyl, benzyl, *tert*-amyl, glycerol
Aldehydes	Formaldehyde, acetaldehyde, crotonaldehyde, benzaldehyde, chloral
Ketones	Acetone, acetophenone, methyl ethyl ketone, cyclohexanone
Acids	
Pure liquids	Acetic, lactic, propionic, butyric, isovaleric, oleic, thioglycollic
Pure solids	Adipic, oxalic, tartaric, citric, succinic, cinnamic, benzoic, salicylic, stearic, malonic, gallic, *o*-nitrobenzoic, *p*-nitrobenzoic, *o*-amidobenzoic, carbolic
Saturated alcoholic solutions	Oxalic, tartaric, succinic, citric, lauric, stearic, malic, gallic
Aqueous solutions	Tartaric, malonic, chloroacetic
Ethers	Diethyl, isopropyl, diphenyl
Esters	Ethyl acetate, ethylacetoacetate, methyl acetate, ethyl cyanoacetate
Halogen compounds	Chloroform, carbon tetrachloride
Hydrocarbons	Benzene, petroleum ether
Nitrogen-containing compounds	Pyridine, nitrobenzene
Solids	Urea
Saturated alcoholic solutions	Urea, acetamide
Anhydrides	Acetic anhydride
Carbohydrates (solids)	Glucose, fructose

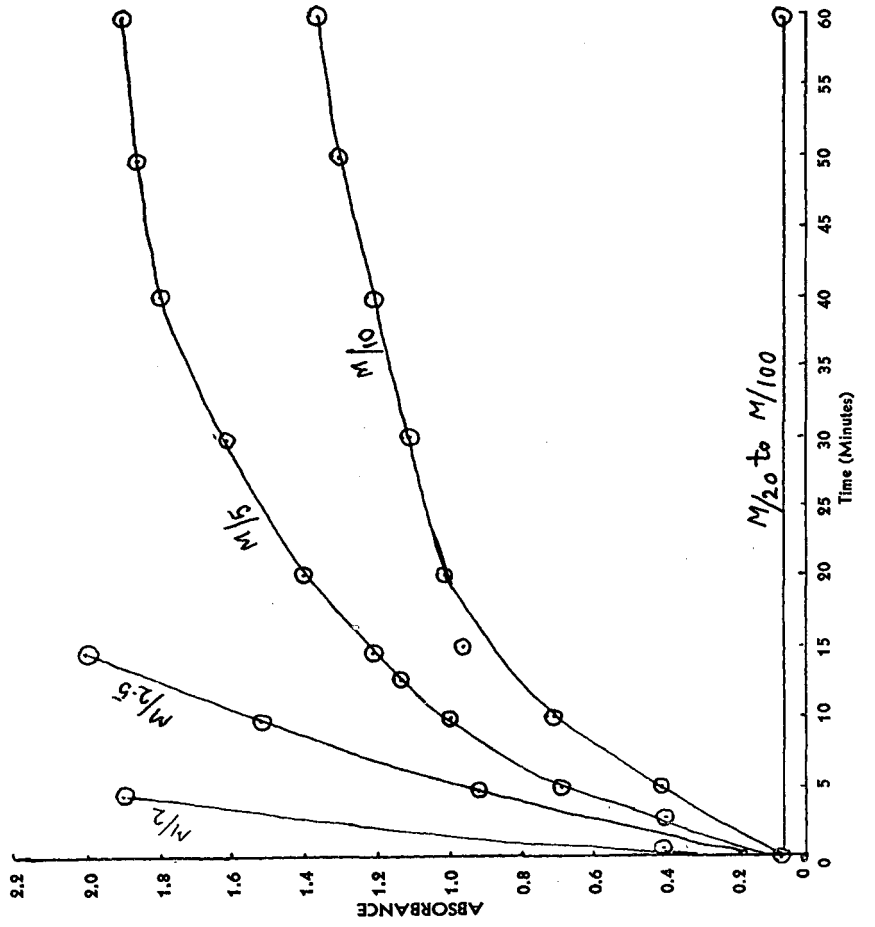


Figure 2. Curves of time *vs.* absorbance

Different lead nitrate concentrations, 575 mμ, distilled water

tion, curves of absorbance *vs.* temperature were obtained (Figure 4). The results show that the stability of the complex is strongly temperature-dependent and the optimum absorbance is obtained at 0° C.

Specificity of Color Reaction. Formic Acid. To test the specificity of the reaction for formic acid, anisole was added to organic liquids in the ratio of 1 to 1. In the case of solid substances a small quantity (approximately 0.2 gram) was added to anisole, and in a few cases saturated solutions of solid substances in alcohol were added to anisole in the ratio of 1 to 1 by volume. A drop of 0.1*M* aqueous lead nitrate was finally added in each case. No color was obtained even in 24 hours with the compounds listed in Table I.

Anisole. To test the specificity of the color reaction for anisole a number of organic substances were used in place of anisole (Table II). No color was obtained in 24 hours.

Table II. Organic Compounds Tested with Formic Acid and 0.1*M* Lead Nitrate

Class of compound	Individual organic compounds
Ethers	Diphenyl, isopropyl, diethyl
Alcohols	Methyl, isoamyl, *n*-butyl, *n*-propyl
Hydrocarbons	Petroleum ether, benzene
Ketone	Acetone
Ester	Ethyl acetate
Chlorine compounds	Chloroform

Table III. Color Reaction of Various Anions with Formic Acid and Anisole

Anions used	Color obtained
Fluoride	Colorless
Chloride	
Bromide	
Carbonate	
Sulfate	
Sulfite	
Oxalate	
Hydrogen phosphate (HPO_4^{-2})	
Thiosulfate	
Borate	Very light yellow
Acetate	Colorless
Nitrite	Violet

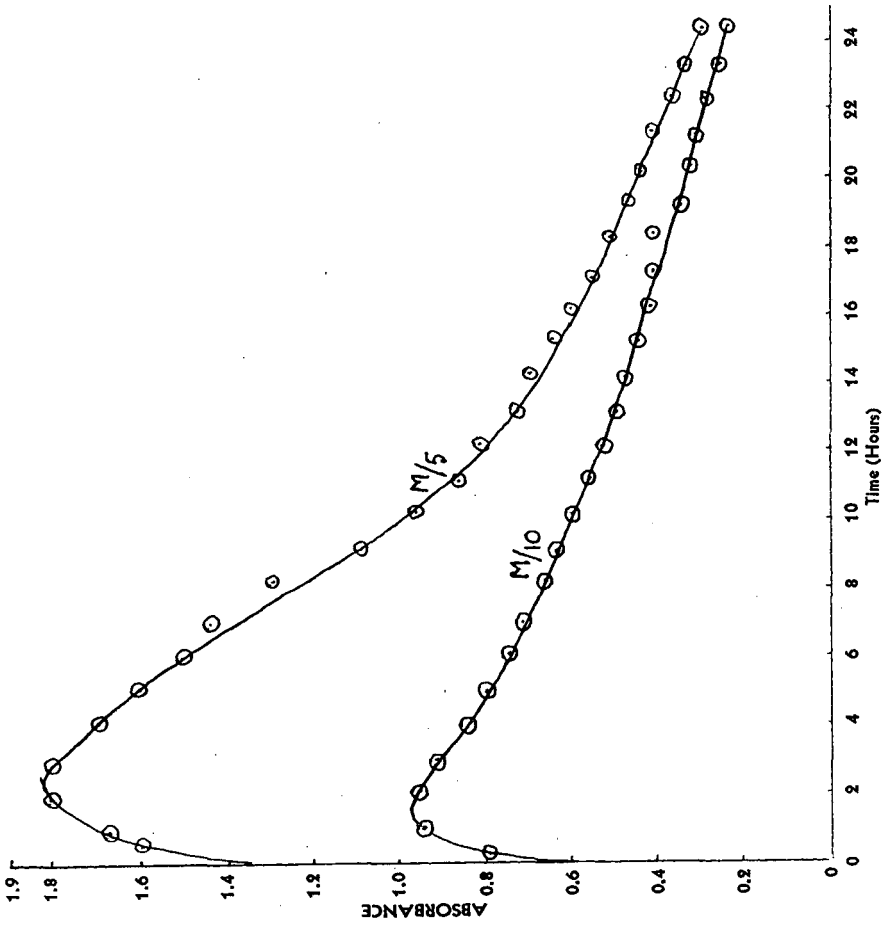


Figure 3. Curves of time *vs.* absorbance

24 hours, 575 mμ

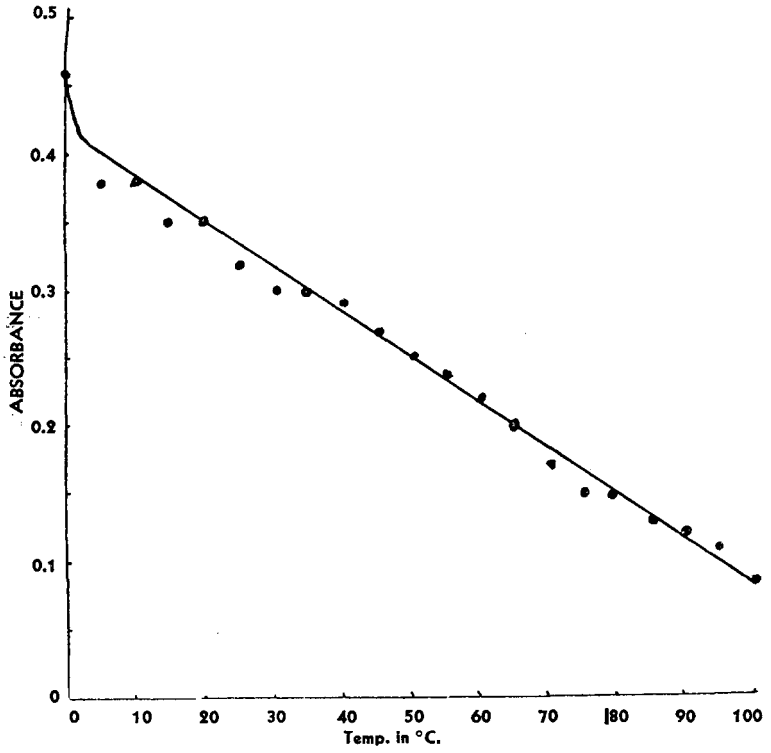


Figure 4. Curves of temperature *vs.* absorbance of complex

575 mμ

NITRITE AND NITRATE. Aqueous solutions of sodium or potassium salts (0.1*N*) of a number of anions were used in place of nitrate. Only nitrite gave a violet color (Table III).

Color Reaction with Different Cations. One drop of 0.1*M* nitrate was added to 1 ml. of a 1 to 1 anisole–formic acid mixture. The color of the solution was noted initially and at specified intervals (Table IV).

Proposed Mechanism of Color Reaction. The course of this reaction may be divided into three steps:

A. Reaction of lead nitrate with formic acid.
B. Reaction of anisole with the products of Reaction A.
C. Role of Pb^{+2} and other polyvalent cations.

A. REACTION OF LEAD NITRATE WITH FORMIC ACID. Lead nitrate is sometimes used as a mild oxidizing agent in organic chemistry—i.e., for the preparation of benzaldehyde from benzyl chloride—but the mechanism has not been clarified (*3*). A search of the literature was unsuccessful. However, since formic acid is a reducing agent and lead nitrate is an oxidizing agent, it is reasonable to suppose that the reaction takes place as follows:

$$Pb(NO_3)_2 + 2HCOOH \rightarrow Pb(HCOO)_2 + 2HNO_3 \quad (1)$$

$$HCOOH + HNO_3 \rightarrow HNO_2 + CO_2 + H_2O \quad (2)$$

Support for this comes from two sources:

When solid lead nitrate is treated with formic acid in the cold, evolution of carbon dioxide is noticed and this is confirmed by passing the gas through lime water, which turns milky.

Lead nitrate–formic acid mixture gave a positive Griess test (*2*) for nitrous acid. A much weaker test was given by a mixture of lead nitrate and acetic acid and a negative test by a mixture of formic acid and acetic acid. That nitrous acid is formed by the lead nitrate–formic acid reaction was confirmed by the fact that on adding solid potassium iodide and sulfuric acid to this mixture violet fumes were obtained. Violet fumes were not obtained when formic acid was replaced by acetic acid.

B. INTERACTION OF PRODUCTS OF REACTION A WITH ANISOLE. Nitrous acid formed in Reaction A reacts with anisole to form nitrosoanisole. To test whether nitration takes place prior to nitrosation, *p*-nitroanisole was treated with lead formate and formic acid; anisole, formic acid, and lead acetate; and glacial acetic acid and sodium nitrite. In no case was any change in color noticed. This can be explained on the basis of the inactivity of nitroanisole resulting from the electron-withdrawing property of the nitro group.

Support for this comes from the work of Bunton *et al.* (*1*) and Westheimer (*8*). Bunton found that nitrosophenols are intermediates in the nitration of phenol to nitrophenols. Westheimer (*8*) and coworkers found that anisole reacts rapidly with nitric acid containing a little nitrous acid. However, with nitric acid containing urea (to remove nitrous acid) no reaction occurred during many hours. Both anisole and nitrosoanisole give the same products in roughly the same proportions. This strongly indicates that nitrosoanisole is an intermediate in the nitration of anisole. Nitroanisole and nitrophenols were the main products in the nitrous acid–catalyzed nitration of anisole.

Table IV. Progress of Color Reaction with Different Cations at Specified Intervals

0.5 ml. FA + 0.5 ml. AL + NS	Color					
	Initial	2 min.	5 min.	30 min.	6 hr.	24 hr.
Hg_2^{+2}	...	V	V	DV	DV	DB
Ag^+	...	...	...	...	...	...
Cd^{+2}	...	L green	LV	LV	V	DV
Al^{+3}	...	...	...	...	...	...
Mn^{+2}	...	LGV	V	V	V	R
Ba^{+2}	...	...	...	...	V	V
Sr^{+2}	...	...	...	...	V	V
Ca^{+2}	...	...	...	...	LV	LB
Mg^{+2}	...	...	...	...	LV	LB
NH_4^+	...	...	...	...	...	...
Na^+	...	...	...	...	...	...
K^+	...	...	...	...	...	...
Ni^{+2}	...	...	...	...	V	B
Hg^{+2}	...	...	...	LV	LV	LV
Cr^{+3}	...	...	...	...	...	LV
Fe^{+3}	...	Y	V	V	DV	DB
Co^{+2}	...	...	...	...	V	B
Cu^{+2}	...	...	...	...	V	B
Bi^{+3} (H_2SO_4)	...	V	V	V	V	B
Pb^{+2}	...	V	V	DV	DV	DB
Bi^{+3} (HCl)	...	...	...	...	D	B

FA. Formic acid. AL. Anisole. NS. One drop of 0.1*M* nitrate solution. ---. Colorless. V. Violet. L. Light. D. Deep. B. Brown. Y. Yellow. G. Greenish. R. Red.

With 40% nitric acid the main products were nitrophenols. However, with more concentrated nitric acid—i.e., 60%—nitroanisoles were the main products. We have quoted at length from the work of Westheimer, as it substantiates the prior nitrosation of anisole and is necessary for our arguments.

In the present case, since lead nitrate reacts with formic acid, we have a mixture of nitrous and nitric acids. Therefore nitrous acid–catalyzed nitration takes place. Since, however, a very small quantity of lead nitrate is used, the concentration of nitric acid is small. Hence nitroanisoles are produced in a very small quantity. The main products in this case are possibly nitrosoanisole and dinitrophenol. The presence of dinitrophenol was proved by adding sodium hydroxide to a lead nitrate–anisole–formic acid solution; a yellow solution was obtained.

It is clear that there are two possibilities: Nitrosoanisole gives a colored complex with lead ions, or it condenses with 2,4-dinitrophenol or with another molecule of anisole to give an indophenol type of compound.

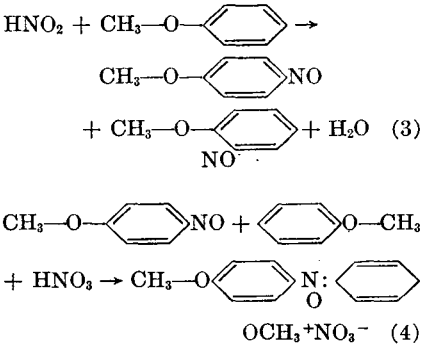
Indophenols are known to be formed by the condensation of *p*-nitrosophenol with phenol ethers in which the *para* position is free, in the presence of 70% sulfuric acid as condensing agent (*7*). It therefore appears that the formation of violet color involves either the formation of an indophenol type of compound or complexing by lead ions with an anisole derivative. The latter alternative is strengthened by the fact that a number of divalent cations, and some trivalent cations, gave a similar result (Table IV), but monovalent cations—i.e., sodium, potassium, ammonium, and silver—gave a negative test, as they lack complexing properties.

It is unlikely that polyvalent cations catalyze the formation of the colored product, because in the absence of Pb^{+2}, for example, no color could be detected even after 96 hours of reaction time. It is possible that cations like sodium, potassium, magnesium, calcium, and aluminum do not give a color reaction even in 30 minutes because of the inert gas structure of the cations concerned, which weakens their complexing power.

C. Role of Lead Ions. Aromatic nitroso compounds are known to produce colored products with such cations as Fe^{+3} and Zr^{+4} (*4*, *9*). It therefore appears possible that lead ions and other similar cations form a colored complex with a nitroso derivative of anisole. Westheimer (*10*) and coworkers have shown that mercuric nitrate catalyzes the nitrosation of benzene. A similar role may be played by lead nitrate in this reaction.

RESULTS AND DISCUSSION

The formation of a violet color with formic acid in the presence of anisole and lead nitrate appears to be specific for formic acid only. Out of 40 organic compounds having different functional groups, no positive test could be obtained in any case. Even reducing substances like formaldehyde, glucose, acetaldehyde, and tartaric acid did not give a positive test. After the formation of nitrous acid according to Equation 2, the course of the reaction may be tentatively proposed as follows:



(3)

(4)

Phenol ethers are known to form on nitration intensely colored compounds from which crystalline compounds can be obtained by the addition of perchloric acid (*6*). In the present case it is possible that the perchlorate ion is replaced by nitrate ion, as shown in Equation 4. It seems plausible that this test is specific for formic acid, because formic acid plays three different roles at the same time: it reacts as an acid, thus releasing nitric acid according to Equation 1; it acts as a reducing agent according to Equation 2; and it acts as a condensing agent for the nitroso derivative of anisole. Thus when anisole is treated with lead nitrate in the presence of acetic acid, the reaction does not occur, for the simple reason that acetic acid does not reduce nitric acid to nitrous acid. When lead nitrate is replaced by lead acetate, the reaction does not occur because the nitrate ion which is responsible for the formation of nitrous acid is not present. This reaction can therefore be used for the detection of formic acid as well as nitrate ions. In the absence of nitrite ions, the test is specific for nitrate ion also. Further work is in progress regarding the nature of the colored substance and the role of the cations.

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Detection of Metal Chelates in Gas Liquid Chromatography by Electron Capture

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▶ An electron capture detector is capable of detecting trace quantities of chromium(III) and aluminum(III) acetylacetonates, trifluoroacetylacetonates, and hexafluoroacetylacetonates. Instrument conditions for maximum sensitivity are defined. Limits of detection are: 3.3×10^{-11} gram of Cr hexafluoroacetylacetonate and 4.8×10^{-10} gram of Al hexafluoroacetylacetonate. Fluorine - containing chelates have higher electron affinity than nonfluorinated compounds, the degree of affinity increasing with degree of fluorination. Chromium chelates show higher electron affinity than those of aluminum. A mixture of Al hexafluoroacetylacetonate and Cr hexafluoroacetylacetonate has been rapidly and efficiently separated by gas liquid chromatography.

GAS CHROMATOGRAPHY in the past has been used primarily for the analysis of organic compounds. It has now been extended to inorganic and organometallic compounds. In the recent literature, the analysis of metal chelates is prominent. For example, Floutz (*3*) investigated 11 metal acetylacetonates by gas liquid chromatography (GLC) but successfully eluted only Al, Be, and Cr acetylacetonates. Bierman and Gesser (*1*) established gas chromatographic conditions for resolving Be and Al acetylacetonates. Recently Brandt and Heveran (*2*) have studied the analysis of chromium by gas chromatography.

The major problem involved in analyzing metal chelates by gas chromatography arises from their relatively low volatility. As a result, the temperature required to vaporize and elute them causes thermal degradation of all but the most stable chelates. However, Sievers, Ponder, Morris, and Moshier (*7–10*) have found that chelates of the fluorine-containing ligands, trifluoro- and hexafluoroacetylacetonates, have significantly higher vapor pressures, hence require lower injection port and column temperatures for elution. This discovery has increased substantially the number of metal chelates separable by gas liquid chromatography.

Fluorinated metal acetylacetonates have the added advantage of high electron affinity, which permits extremely small concentrations to be detected. Lovelock (*5*, *6*) has described the affinity of halogenated compounds for low energy electrons such as are found in the electron capture detector.

This investigation was initiated to determine the sensitivity of the electron capture detector for metal chelates containing fluorinated and nonfluorinated ligands. The study comprised a comparison of the sensitivity of the electron capture detector for Cr(III) and Al(III) acetylacetonate, trifluoroacetylacetonate, and hexafluoroacetylacetonate and the influence of two eluents (nitrogen and argon) on sensitivity.

EXPERIMENTAL

A Barber-Colman Model 20 gas chromatograph equipped with an ionization detector (diode type), cell Model A-4150 was used in the investigation. (Model A-5120 gave comparable results.) Electron capture was achieved by reducing the cell potential, using the variable autotransformer of the power supply to control the primary voltage. The cell potential was reduced to less than 200 volts. The only modification needed was one to provide a range change in the existing cell potential voltmeter. This modification permitted more accurate voltage readings in the 0- to 200-volt range.

Compounds Investigated. Six metal chelates were investigated. They include: Cr(III) acetylacetonate [$Cr(acac)_3$]; Cr(III) trifluoroacetylacetonate [$Cr(tfa)_3$]; Cr(III) hexafluoroacetylacetonate [$Cr(hfa)_3$]; Al (III) acetylacetonate [$Al(acac)_3$]; Al(III) trifluoroacetylacetonate [$Al(tfa)_3$]; and Al(III) hexafluoroacetylacetonate [$Al(hfa)_3$].

Selection of Solvents. Solvents for these compounds were selected based on the solubility of the compounds and elution time of the solvent as compared with elution time of the compound being analyzed, to assure no interference by the solvent.

The electron affinity of the solvent or impurities in the solvent may determine whether the solvent will interfere with detection of the compound. It was therefore important to select a solvent with little or no electron affinity and one which does not contain electron-capturing impurities. Benzene proved suitable for all compounds investigated except $Cr(hfa)_3$ and $Al(hfa)_3$. The latter two were eluted so rapidly that a solvent (toluene), which was eluted after these compounds, was used.

Preparation of Samples. Samples of the six compounds were prepared by accurately weighing 10- to 15-mg. quantities in a 5.0-ml. volumetric flask, and making up to volume with solvent. From this initial solution, three consecutive 1:25 dilutions were made.

The samples were injected into the column via a Hamilton 10-μl. syringe. The sample sizes varied from 0.2 to 10.0 μl.

Column and Instrument Conditions. The columns used were $^1/_8$-inch o.d. stainless steel packed with 20% by weight of Dow Corning Silicone Fluid 710R on Gas Chrom Z. For the very volatile (hence fast-eluting) hexafluorinated compounds, an 11-foot column was used. The other samples were analyzed on a 2-foot column.

Each of the six compounds required unique instrument conditions to obtain the elution times and symmetry of elution peaks that would allow the ultimate signal intensity by the particular compound. Peak sharpness was critical since the response was measured by peak heights. The respective volatilities and elution properties of the compounds determined the column flow rates, injection port temperatures, and column temperatures to be used. Table I summarizes the instrument conditions used for the evaluation of the six compounds listed above. Data in Table II were collected using both argon and prepurified nitrogen eluents.

The gain setting of 1000 and positive polarity were used on the electrometer. The maximum recorder noise was always less than 1 mm. (24 cm. full scale, 0 to 5.0 mv.) in height. Table II lists the peak heights of the lowest detected concentrations of the compounds evaluated and the signal to noise ratios based on 1 mm. of noise.

RESULTS AND DISCUSSION

Al and Cr acetylacetonates and their trifluorinated and hexafluorinated analogs were investigated to establish the instrument conditions for ultimate signal-to-noise ratios. Table II compares the detector sensitivity for these compounds under the instrument conditions indicated in Table I.

To determine the optimum instrument operating conditions, column and injection port temperatures, injection technique, column conditioning, cell

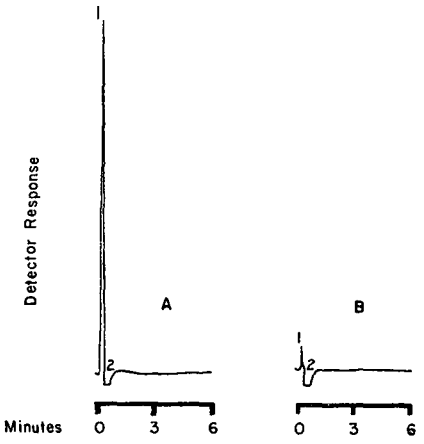

Figure 1. Chromatograms of *A*: 1.2×10^{-7} gram $Al(hfa)_3$ and *B*: 3.0×10^{-8} gram $Al(hfa)_3$

1. $Al(hfa)_3$
2. Toluene

voltage, column inlet pressure, and scavenge flow rate were considered. The cell voltage, scavenge flow, and column flow rates all were found to have critical and perhaps interdependent influences on the sensitivity of the detector for these samples.

Effect of Column Temperature. The relatively high thermal stability of these particular metal acetylacetonates permitted the use of higher column temperatures than normally used for other metal chelates. As a result, the eluted peaks were sharp, symmetrical, and fast-eluting (less than 5 minutes). The sharpness of peaks permitted measurement of signal intensity by comparing peak heights, measured from the base line to the apexes. No evidence of extraneous peaks due to thermal degradation was found. Column temperature was held constant after satisfactory peaks were attained.

Effect of Injection Port Temperature. Again the thermal stability of these chelates prevented this instrument condition from being too critical. The temperature was maintained high enough to permit rapid and total vaporization, to aid in the elution of sharp, symmetrical peaks. Injection port temperature was held constant during the evaluation at 165° C.

Sample Injection Technique. The method of sample delivery was critical in achieving reproducible peak sizes. It was necessary to hold the syringe needle in the injection port septum for 20 seconds to obtain reproducible peak heights. If this is not done, the entire sample is not volatilized.

Column Conditioning It was essential to condition the column to obtain maximum sensitivity. Successive injections of the compound under investigation were made until maximum and reproducible peak heights were obtained. At least six 1-μl. samples containing in the range of 10^{-3} gram per ml. of the compound being measured were necessary to condition the column adequately.

Determination of Optimum Cell Voltage. With all other instrument conditions static, the cell voltage was varied by 5-volt increments from 5 to 50 volts for each sample, until the voltage for highest response was found. This was considered the optimum voltage.

Determination of Optimum Column Inlet Pressures and Scavenge Flow Rates. Changes in inlet pressure, hence changes in column flow rates, influence detector sensitivity. The range of pressures for maximum sensitivity was 20 to 50 p.s.i.g.

Scavenge flow rates influence cell sensitivity, the optimum flow being different for each compound. The optimum flows ranged from 75 to 300 ml. per minute.

Table I tabulates the optimum instrument conditions found in this investigation for the detection of each compound, using argon and nitrogen as eluents. Each compound, because of different volatilities and different electron affinities, required a different set of instrument conditions. The conditions were determined to attain the sharpest and most symmetrical peak. The detection limits were determined using the best instrument conditions found to date.

Figures 1 and 2 are the actual curves obtained for solutions of $Al(hfa)_3$ and $Cr(hfa)_3$. Figures 1*A* and 2*A* illustrate the peak symmetry and response to 1.2×10^{-7} gram of $Al(hfa)_3$ and 5.2×10^{-8} gram of $Cr(hfa)_3$, respectively, and Figures 1B and 2B the curves obtained for the lowest detectable concentration.

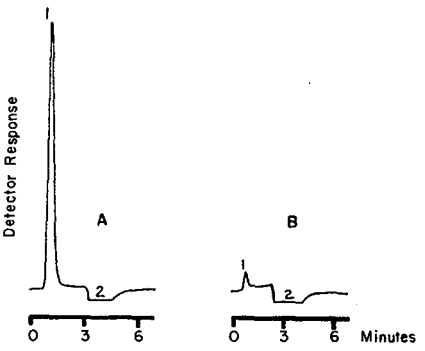

Figure 2. Chromatograms of *A*: 5.2×10^{-8} gram $Cr(hfa)_3$ and *B*: 8.3×10^{-10} gram $Cr(hfa)_3$

1. $Cr(hfa)_3$
2. Toluene

Chromatograms in Figure 1*A* and *B* were obtained at 95° C. column temperature at an inlet pressure of 50 p.s.i.g., scavenge flow of 300 ml. per minute, and cell voltage of 15 volts. The curves in Figure 2*A* and 2*B* were obtained at 95° C. column temperature, an inlet pressure of 20 p.s.i.g., scavenge flow of 200 ml. per minute, and cell voltage of 20 volts. Argon was used as an eluent in the analyses shown in both figures. It is interesting to note that the hexafluoroacetylacetonate complexes are so volatile that they are eluted more rapidly than toluene.

Effect of Fluorination. Lovelock has reported that the halogens capture electrons readily to form stable negative ions, and several electron volts of energy are released by the reaction (*5*).

The data of Table II indicate that $Cr(acac)_3$ and $Al(acac)_3$ have relative electron affinities as indicated by the lower limit of the amount of sample detected (8.8×10^{-8} gram and 5.1×10^{-5} gram, respectively). However, by fluorinating the ligands of these compounds,

Table I. Instrument Conditions Used in Investigation

Compound	Inlet pressure (p.s.i.g.)	Scavenge flow (ml./min.)	Cell voltage	Column temp. (° C.)
		Nitrogen eluent		
$Cr(acac)_3$	50	100	25	215
$Cr(tfa)_3$	24	200	20	162
$Cr(hfa)_3$	40	200	15	90
$Al(acac)_3$	50	300	40	185
$Al(tfa)_3$	24	250	30	162
$Al(hfa)_3$	40	200	15	90
		Argon eluent		
$Cr(acac)_3$	20	240	20	215
$Cr(tfa)_3$	22	75	20	175
$Cr(hfa)_3$	20	200	20	95
$Al(acac)_3$	20	100	16	185
$Al(tfa)_3$	22	200	25	160
$Al(hfa)_3$	50	300	15	95

Table II. Lower Detection Limits Determined for Al and Cr Chelates

Compound	Sample size (μl.)	Sample concn. (g./ml.)	Peak height (mm.)	Signal/noise ratio	Amt. sample detected (grams)	Amt. metal detected (moles)
			Nitrogen eluent			
$Cr(acac)_3$	5.0	1.76×10^{-6}	10	10/1	8.8×10^{-8}	2.5×10^{-10}
$Cr(tfa)_3$	1.0	8.96×10^{-8}	4	4/1	9.0×10^{-11}	1.8×10^{-13}
$Cr(hfa)_3$	0.4	8.32×10^{-8}	7	7/1	3.3×10^{-11}	4.9×10^{-14}
$Al(acac)_3$	5.0	1.02×10^{-2}	17	17/1	5.1×10^{-5}	1.6×10^{-7}
$Al(tfa)_3$	1.0	2.68×10^{-6}	2	2/1	2.7×10^{-9}	5.9×10^{-12}
$Al(hfa)_3$	1.0	4.8×10^{-7}	2	2/1	4.8×10^{-10}	7.4×10^{-13}
			Argon eluent			
$Cr(acac)_3$	10.0	1.76×10^{-5}	5	5/1	1.8×10^{-7}	5.0×10^{-10}
$Cr(tfa)_3$	0.1	2.24×10^{-6}	5	5/1	2.2×10^{-10}	4.4×10^{-13}
$Cr(hfa)_3$	0.4	2.08×10^{-6}	5	5/1	8.3×10^{-10}	1.2×10^{-12}
$Al(acac)_3$	0.5	2.1×10^{-2}	10	10/1	1.1×10^{-5}	3.2×10^{-8}
$Al(tfa)_3$	0.2	6.7×10^{-5}	9	9/1	1.3×10^{-8}	2.8×10^{-11}
$Al(hfa)_3$	1.0	4.8×10^{-6}	8	8/1	4.8×10^{-9}	7.4×10^{-12}

their electron affinity is increased considerably [$Cr(tfa)_3$ — 9.0×10^{-11} gram, $Al(tfa)_3$ — 2.7×10^{-9} gram]. Also the increase in response may be due in part to improved chromatographic properties of the fluorinated compounds. However, an effort was made to find the optimum conditions for each compound. The chelates of hexafluoro ligands appear to have higher electron affinity than those of the trifluoro ligands [$Cr(hfa)_3$ — 3.3×10^{-11} gram, $Al(hfa)_3$ — 4.8×10^{-13} gram], the only exception being the higher affinity of $Cr(tfa)_3$ than $Cr(hfa)_6$ when eluted with argon.

Effect of Metallic Ion on Electron Affinity. The influence of the metallic ion of the chelate on the electron affinity appears to be significant. The chelates of Cr(III) exhibit greater electron affinity than the corresponding chelates of Al(III), as shown in Table II.

Effect of Eluent on Electron Affinity. The eluent is an important factor in determining the highest sensitivity of the electron capture detector. Knox (4) has stated that when argon is used as the carrier gas, the average electron energy is high since the collisions of electrons with argon are in general highly elastic. When nitrogen or hydrogen is used, the electron energy is lower.

In agreement with this postulate, our data (Table II) indicate that higher sensitivity is achieved in nitrogen than in an argon environment for both the Al and Cr chelates.

Separation of Al(III) and Cr(III) Hexafluoroacetylacetonate. Figure 3 illustrates the elution peaks of $Al(hfa)_3$ and $Cr(hfa)_3$ (peaks 1 and 2, respectively) which were resolved in 3 minutes. Peak 3 is an electron-capturing impurity in the toluene solvent. The toluene has no electron affinity and does not cause any detector response in a nitrogen eluent. The elution time of toluene was 6 minutes. This was determined when this same mix was analyzed in argon and a negative peak resulted for toluene.

This chromatogram was obtained at a column temperature of 65° C., an inlet pressure of 30 p.s.i.g., a cell voltage of 15, and a scavenge flow of 200 ml. per minute.

CONCLUSION

This investigation has shown that the Sievers-Moshier method of gas chromatography of fluorine-containing metal chelates is a highly promising approach to trace as well as conventional analysis of metals. This conclusion is based on the following:

The electron capture detector is capable of detecting quantities of metals as small as 2×10^{-12} gram.

Because of the increased volatilities of the fluorinated chelates, the elution system temperatures required should be within a range where many of the chelates are thermally stable.

The increase in electron affinity of the fluorinated compounds permits the use of much smaller sample sizes, with resultant increase in efficiency of the chromatographic system.

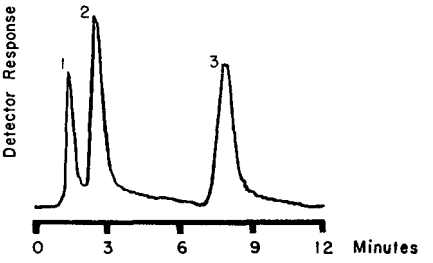

Figure 3. Chromatogram of mixture of $Al(hfa)_3$ and $Cr(hfa)_3$

1. $Al(hfa)_3$
2. $Cr(hfa)_3$
3. Impurity in solvent

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Formal Oxidation-Reduction Potentials and Indicator Characteristics of Some Cyanide and 2,2′-Bipyridine Complexes of Iron, Ruthenium, and Osmium

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▷ Formal oxidation-reduction potentials for $[Fe(bipy)_2(CN)_2]$, $[Ru(bipy)_2(CN)_2]$, $[Os(bipy)_2(CN)_2]$, $[Fe(bipy)_3]SO_4$, $[Ru(bipy)_3]SO_4$, and $[Os(bipy)_3]SO_4$ in sulfuric acid solutions ranging in concentration from 0.01 to 12*M* have been measured. Data for $[Ru(bipy)_2(CN_2)]$ in nitric acid solutions were also obtained. The neutral mixed ligand complexes all exhibit increasing formal potentials with increasing acid concentration; formal potentials of the simple tris complexes decrease markedly with increasing acid concentrations. Explanations are proposed for the observed trends. With the exception of $[Ru(bipy)_2(CN)_2]$, which behaves irreversibly on oxidation and reduction, the complexes show promise as indicators for a variety of oxidation-reduction titrations. By proper selection of complex and acid concentration, indication of redox potential in the range of 0.37 to 1.27 volts is possible.

VARIOUS METAL DERIVATIVES of 1,10-phenanthroline, 2,2′-bipyridine, and related compounds have been proposed and demonstrated to be advantageous as high potential, reversible indicators in oxidation-reduction titrations. The best known and most widely used of these is the tris-(1,10-phenanthroline)-iron(II) complex ion, commonly referred to as ferroin. The chemical literature on these various compounds and their applications, published prior to January 1, 1954, has been admirably reviewed by Brandt, Dwyer, and Gyarfas (*2*). Only one paper has appeared since 1953 that deals with oxidation-reduction characteristics of complexes of the ferroin type; in this, Dwyer and Gyarfas report formal potentials for the terpyridine complexes of iron, ruthenium, and osmium systems (*7*). At least two novel applications of ferroin type indicators have been described since the review article appeared. One involves the use of nitroferroin in conjunction with a slight excess of bromine as an indicator for the argentometric determination of bromide (*9*). Another demonstrates the applicability of ferroin as an indicator for the cerimetric determination of hydroquinone in nonaqueous media, specifically acetonitrile and acetonitrile–acetic acid mixtures (*10*).

Recently a number of new complexes of mixed ligand type involving cyanide, aromatic diimines, and the iron group metal ions have been synthesized that may prove to be suitable as oxidation-reduction indicators (*12, 13*). An investigation of two of these, the neutral dicyano - bis - (1,10 - phenanthroline)-iron(II) complex and the analogous 2,2′-bipyridine derivative, revealed that they exhibit rather remarkable characteristics as indicators in comparison to the tris complexes (*11*). One objective of the present work is to extend the study to include the analogous neutral mixed ligand complexes of ruthenium(II) and osmium(II). Since comparisons are desired and the data available for the corresponding tris 2,2′-bipyridine complexes are somewhat meager, further measurements on the oxidation-reduction characteristics of the tris complexes over a broader range of acid concentrations are also of interest.

EXPERIMENTAL

Preparation of Neutral Complexes. Gram quantities of the neutral dicyano - bis - (2,2′ - bipyridine) - metal (II) complexes were prepared following procedures previously described in the literature (*12, 13*). In all cases, analysis by potentiometric titration with standard cerium(IV) sulfate indicated purities of 98% or greater for the preparations.

Preparation of $[Fe(bipy)_3]SO_4 \cdot 2H_2O$. The solution obtained by adding 1.390 grams of $FeSO_4 \cdot 7H_2O$ (0.05 mole) and 2.343 grams of 2,2′-bipyridine (0.15 mole) to 25 ml. of distilled water was saturated with SO_2 and then evaporated to dryness. The resulting dark red solid, after recrystallization from 95% ethanol, was dried in vacuum over magnesium perchlorate. A purity of 99.9% for the product was found by potentiometric titration with standard cerium (IV) sulfate.

Preparation of $[Ru(bipy)_3]SO_4 \cdot 2H_2O$. The cyanide salt of this complex is obtainable as a by-product in the preparation of the neutral dicyano-bis - (2,2′ - bipyridine) - ruthenium(II) complex (*13*). It can also be readily obtained following the procedure of Dwyer (*5*) if sodium cyanide is used in place of sodium iodide as the precipitating agent. The sulfate salt was obtained by dissolving 3 grams of the cyanide salt in 15 ml. of 1*M* H_2SO_4, heating to drive off hydrogen cyanide, and cooling to induce crystallization. The orange-red crystalline product was collected by suction filtration, washed with acetone, and dried over magnesium perchlorate. Potentiometric titration with standard cerium(IV) sulfate indicated a purity of 99%.

Preparation of $[Os(bipy)_3]SO_4 \cdot 5H_2O$. The iodide salt of this complex can be obtained as a by-product in the preparation of the neutral dicyano-bis - (2,2′ - bipyridine) - osmium(II) complex (*13*), or it may be prepared more directly as described by Burstall, Dwyer, and Gyarfas (*3*). The sulfate form was prepared by metathesis of the iodide salt with an equimolar amount of silver sulfate. Silver iodide, after prolonged boiling to promote coagulation, was filtered from the reaction mixture and the sulfate salt was obtained from the filtrate by evaporation. The product was dried at room temperature over magnesium perchlorate. A purity of 98% was observed for the product on potentiometric titration with standard cerium(IV) sulfate.

Analysis of Complexes. Water of hydration was determined by loss in weight on drying at 196° C. in vacuo over magnesium perchlorate. In the case of tris-(2,2′-bipyridine)-iron(II) sulfate, bipyridine is also lost under these conditions; hence water was not determined. Purity of the various complexes was ascertained in conjunction with the potentiometric titrations carried out to determine formal redox potentials. Since the dicyano-bis-(2,2′-bipyridine) - ruthenium(II) complex could not be so titrated, its purity was established by spectrophotometric comparison with a sample available from earlier work that had been subjected to elemental analysis (*13*). Failure to observe absolute purity is believed to be a result of slightly indefinite water content rather than the presence of any objectionable contaminating substances. Note that the measurements of formal potentials are not subject to errors due to impurities or weighing of

Table I. Formal Redox Potentials at 25° C. in H_2SO_4 Solutions[a]

Complex	Formal concentration of H_2SO_4 moles/liter									
	0.01	0.05	0.11	0.50	1.0	2.0	4.0	6.0	10	12
$[Fe(bipy)_3]SO_4$	1.069	...	1.062	...	1.026	1.00	0.95	0.88	0.8	...
$[Fe(bipy)_2(CN)_2]$	0.781	0.777	0.776	0.776	...	0.79[b]	0.79[b]	0.82[b]	0.90[b]	0.98[b]
$[Ru(bipy)_3]SO_4$	1.274	1.273	1.262	...	1.236	1.20	1.15	1.09	0.88	0.76
$[Ru(bipy)_2(CN)_2]$	1.12	1.11	1.10	1.12	...	1.13	1.16	1.22	1.26	1.30
$[Os(bipy)_3]SO_4$	0.858	...	0.841	...	0.803	0.777	0.723	0.63	0.44	0.37
$[Os(bipy)_2(CN)_2]$	0.778	0.783	0.791	0.804	0.810	0.836	0.89	0.95	1.01	1.02

[a] Potentials are expressed in volts *vs.* the standard hydrogen electrode potential. Each value is the average of at least two determinations.
[b] Taken from reference (*11*).

Table II. Formal Redox Potentials for $[Ru(bipy)_2(CN)_2]$ in Nitric Acid Solutions at 25° C.

Acid concentration, moles/liter	E°′ *vs.* standard H_2 electrode, volts
1.0	1.10
2.0	1.13
4.0	1.14
5.0	1.18
8.0	1.24
16.0	1.40

samples since the titration curves indicate the actual quantities of complex taken.

Reagents. Standard Cerium(IV) Sulfate Solution. A solution 0.05*M* in cerium(IV) and approximately 0.9*M* in H_2SO_4 was prepared using ceric hydroxide (G. Frederick Smith Chemical Co.) and the procedure described by Diehl and Smith (*4*). Arsenious oxide was employed for standardization.

Standard Cerium(IV) Nitrate. A solution 0.05*M* in cerium(IV) was prepared by dissolving the requisite amount of $(NH_4)_2Ce(NO_3)_6$ in 1 liter of 1*M* HNO_3. Arsenious oxide was used for standardization.

Reagent grade chemicals were employed for all tests and measurements. Solutions of acids were prepared in known concentrations, accurate to within 1 to 3%, by appropriate dilution of the concentrated acids, assayed by the manufacturers. The uranium(IV) solution was prepared by reducing a uranyl sulfate solution with a Jones reductor, then shaking it in air to oxidize any uranium(III) to the tetravalent state.

Apparatus. A Beckman Model G pH Meter equipped with a saturated calomel electrode and a platinum electrode was employed for the potentiometric titrations. Stirring was done with a magnetic stirrer; a 10-ml. microburet, graduated in 0.05-ml. divisions, was used.

Procedures. Determination of Formal Redox Potentials. Weighed amounts (10 to 100 mg.) of the solid complexes were dissolved in known concentrations of acid solution (25 to 100 ml.) and titrated potentiometrically with standard cerium(IV) solution. Temperature was maintained at 25° ± 0.5° C. by a water bath. The formal potential was taken as the potential of the platinum electrode at the halfway point in the titration as measured from the potentiometric end point rather than from the calculated theoretical equivalence point. In certain instances, potentiometric titrations were not practical. To determine formal potentials in such cases, a calculated amount of cerium(IV) (sufficient to oxidize exactly one half of the weighed complex) was added all at once to the acid solution of the complex and an immediate measurement of potential was made. This technique was necessary in the case of $[Ru(bipy)_2(CN)_2]$ for all acid concentrations since potentials decreased appreciably with time. It was also employed for $[Ru(bipy)_3]SO_4$ for acid concentrations approximately 1*M* or less since poorly defined inflection points are observed in the titration curves.

A procedural modification was also necessary for measurement of formal potentials of $[Fe(bipy)_3]SO_4$ in acid concentrations greater than 0.1*M* since the complex undergoes dissociation at an appreciable rate in strong acid solutions. The rate of dissociation increases with increasing acid concentration. To minimize, as well as correct for, the effect of dissociation on measurements of formal potential, the calculated amount of cerium(IV) sulfate was added to the acid solution prior to addition of the complex. After the weighed complex and the prepared solution were mixed, potential measurements were made at 30-second intervals. The rate of potential decrease thus estimated was employed to calculate the redox potential at time of mixing. Dissolution was essentially complete within a few seconds after mixing. Initially, the rate of potential decrease in 10*M* H_2SO_4 was approximately 50 mv. per minute, and in 1*M* H_2SO_4 it was 2 mv. per minute. Since measurements could be made within 30 seconds after mixing, corrections to zero time were relatively small in all cases.

Indicator Properties. The behavior of complex after successive, repetitive treatments with cerium(IV) sulfate and various common reducing agents was examined. Solutions of the complex in 2*M* H_2SO_4 (1 to 3 mg. of complex in 20 to 30 ml. of acid) were treated first with a slight excess of cerium(IV) sulfate, then with a slight excess of reducing agent. To test for reversibility of the color changes, the procedure was repeated at least 10 times using the same solution.

RESULTS AND DISCUSSION

Formal Potentials. The results obtained for sulfuric acid solutions of the complexes when cerium(IV) sulfate was used as the oxidant are compiled in Table I. In all cases in which titrations could be performed to evaluate formal potentials it was evident that cerium(IV) sulfate served as an effective oxidant: well defined potentiometric end points were observed, and the formal potentials exhibited by the cerium(IV)–cerium(III) couple ranged from 1.40 to 1.45 volts. Only in the case of $[Ru(bipy)_2(CN)_2]$, where rapidly changing electrode potentials precluded potentiometric titration, was there any doubt that cerium(IV) sulfate would react quantitatively with this more difficulty oxidizable complex. On the basis of observed formal potentials, cerium(IV) sulfate should be an effective oxidant for the ruthenium(II) complex. To test the validity of this contention, measurements were also performed in nitric acid solutions with the more potent cerium(IV) nitrate oxidant which exhibits formal potentials that range from 1.56 to 1.62 volts in 1 to 8*M* nitric acid solutions. The results are given in Table II. The use of the stronger oxidant and nitric acid solutions gives essentially the same results as when the formal potentials for $[Ru(bipy)_2(CN)_2]$ are measured in the sulfuric acid system.

Smith and Richter report formal potentials of 0.97 and 0.92 volt for $[Fe(bipy)_3]SO_4$ in 1 and 4*M* sulfuric acid, respectively (*14*). These values are lower than the corresponding ones reported here, perhaps because the necessity of minimizing or correcting for the effect of dissociation was overlooked. Similarly low values were obtained initially in the present study, until it was recognized that the rates of dissociation of the iron(II) and (III) species were appreciable in acid solutions of one molar or greater concentration and that time was thus an important variable to take into consideration.

Formal potentials for $[Ru(bipy)_3](NO_3)_2$ at 0° C. in various concentrations of nitric acid have been determined by Dwyer: 1.303, 1.288, 1.279, 1.270, 1.257, 1.240, and 1.222 volts for 0.002, 0.1, 0.3, 0.5, 1.0, 2.0, and 3.0 molar nitric acid, respectively (*5*). Considering the differences in temperatures and acids used in this and the present study, surprisingly minor differences are shown by the two sets of data. The pronounced decrease in formal potentials with increasing acid concentration, especially above 4*M*, is notable. A similar trend is evident for both $[Fe(bipy)_3]SO_4$ and $[Os(bipy)_3]SO_4$.

Dwyer and coworkers have measured formal potentials for $[Os(bipy)_3](ClO_4)_2$ in concentrations of perchloric acid up to 5*M* and observed values which decreased from 0.877 volt in neutral solution to 0.727 volt in 5*M* acid (*6*). When their results are compared with those in Table I, it is evident that nearly identical formal potentials are exhibited in sulfuric and perchloric acids of the same normalities, at least up to a concentration of 2*N*. Continuing close agreement at higher concentrations of course is not expected since HSO_4^- is only a moderately strong acid.

The influence of acid concentration on the formal potentials of the tris 2,2′-bipyridine complexes of iron, ruthenium, and osmium is most striking. The present investigation reveals that the decreasing trends in potential continue at even more pronounced rates as the concentration of acid is increased beyond the moderate levels previously investigated. The decreases in formal potentials are such that very extensive and stable ion-pair formation between the complex cations and acid anions appears to be indicated. Any attempt at quantitative correlation of formal potentials with one or more solution variables, however, is quite impractical, at least for the concentrated solutions of this study. The difficulty stems from lack of reliable values for liquid junction potentials and activity coefficients, both of which vary with electrolyte concentration. Even under more amenable and nearly ideal conditions, Barnes, Dwyer, and Gyarfas found that the Debye-Huckel limiting law did not accurately predict experimental values obtained for the $[Os(bipy)_3](ClO_4)_3/[Os(bipy)_3](ClO_4)_2$ system in various dilute electrolyte solutions (*1*). They suggested that the nature of the ligand–metal bonds may also change with ionic environment.

The trends in formal potentials with increasing acid concentration exhibited by the mixed ligand complexes of this study are just the opposite of those displayed by the simple tris complexes. Previous studies have revealed that the mixed ligand complexes of iron(II), ruthenium(II), and osmium(II) possess appreciable dibasic character and that each of the bound cyanide groups can be protonated (*13*). The corresponding iron(III) complex was found to be an extremely weak base; presumably, so also should be the analogous ruthenium (III) and osmium(III) mixed ligand complexes. With this information available, the influence of acid concentration on formal potentials is readily understood. As the acid concentration is increased, the extent of protonation of the metal(II) complexes increases; the reduced species take on greater positive charge; and the oxidation becomes increasingly more difficult. The oxidation process requires more positive potentials since removal of electrons from the positively charged species is more difficult than from the uncharged species. Also the process necessitates loss of protons to the medium, since the metal(III) complexes are too weakly basic to retain the protons formerly held by the metal(II) species. Thus, increased acidity, in discouraging loss of protons as well as loss of electrons, gives rise to more positive formal potentials.

Unfortunately, quantitative treatment of the results to determine the number of protons involved in the oxidation process and to evaluate basicity constants for the uncharged mixed ligand complexes proved unreliable. Liquid junction potentials and activity coefficients can neither be neglected nor assumed to be essentially constant. It is possible only to draw certain qualitative or semiquantitative conclusions from the data obtained. First, it is apparent from the changes in potentials that the metal(II) mixed ligand complexes are considerably stronger bases than the corresponding metal(III) complexes. Also, the order of increasing basicity in aqueous solution are $[Fe(bipy)_2(CN)_2] < [Ru(bipy)_2(CN)_2] < [Os(bipy)_2(CN)_2]$, as evidenced by the approximate acid concentration in which the formal potentials of each complex begins to show an appreciable increase [the sulfuric acid concentrations at such changes are 5, 3, and 1.5*M*, respectively, for the iron(II), ruthenium(II), and osmium(II) complexes]. Spectrophotometric measurements indicated this same trend in basicities for the complexes in glacial acetic acid (*13*).

Indicator Characteristics. Observations made in evaluating behavior and indicator properties of each complex in various simulated oxidation-reduction titrations are reported in Table III. One of the six proved quite unsatisfactory as an indicator: $[Ru(bipy)_2(CN)_2]$ does not exhibit reversible color changes on oxidation and reduction. The original yellow color of the ruthenium(II) complex in strong acid, once changed by an oxidant, cannot be restored by a reductant. Instead, an orange-brown color is produced. It appears likely that oxidation of the ruthenium(II) complex is accompanied by loss of coordinated cyanide, and that some ruthenium(IV) as well as ruthenium(III) species may be produced.

As a consequence of their dibasic character, the uncharged mixed ligand complexes exhibit colors in solution that are influenced by acid concentration. The colors of the oxidized complexes are

Table III. Indicator Characteristics in 2*M* H_2SO_4

Complex	Color change on oxidation	Effect of various reductants on oxidized complex[a]							
		$FeSO_4$	Hydroquinone	H_3AsO_3	$H_2C_2O_4$	$NaHSO_3$	H_2O_2	$K_4[Fe(CN)_6]$	$UOSO_4$
$[Fe(bipy)_3]SO_4$	Red → pale blue	+	+	O	O	+	+	+	+
$[Fe(bipy)_2(CN)_2]$	Orange → pale violet	+	+	O	O	S	O	+	S
$[Ru(bipy)_3]SO_4$	Orange → green	+	+	S	S	+	+	+	+
$[Ru(bipy)_2(CN)_2]$	Yellow → pale green → dark green → orange brown[b]	−	−	−	−	−	−	−	−
$[Os(bipy)_3]SO_4$	Green → pale pink	+	+	O	O	S	O	+	S
$[Os(bipy)_2(CN)_2]$	Orange → pale violet	+	+	S	O	S	O	+	+

[a] Meaning of symbols: + = rapid and reversible color changes on alternately adding excess reductant and excess ceric sulfate; S = slow and reversible color changes; O = no color change; and − = irreversible color changes.
[b] Colors observed on adding increasing amounts of ceric sulfate.

not similarly affected. Changes in colors and changes in formal potentials are concomitant as acid concentration is changed. In dilute acid solutions the colors of $[Fe(bipy)_2(CN)_2]$, $[Ru(bipy)_2(CN)_2]$, and $[Os(bipy)_2(CN)_2]$ are red, yellow-orange, and red-brown, respectively; in concentrated acid solutions, the colors in the same order are yellow, pale yellow, and orange. Regardless of acid concentration, the color changes that accompany oxidation are distinct and discernible.

All of the reducing agents selected for the present study can be successfully titrated with ceric salts using ferroin as the indicator (*8*). A catalyst, usually osmic acid or iodine monochloride, is necessary to provide rapid reaction and indicator response in certain of these titrations. Presumably, use of suitable catalysts should also make practical the use of the complexes of this study as indicators in the same titrations—e.g., arsenic(III) or oxalic acid *vs.* cerium(IV).

With the exception of $[Ru(bipy)_2(CN)_2]$, all the complexes that were studied exhibit properties suitable for their application as indicators. They should be appropriate in a great variety of oxidation-reduction titrations. Judicious selection of both indicator and acid concentration can provide redox potential indication over a broad range of 0.37 to 1.27 volts. Particularly inviting is the choice of the mixed ligand complexes of iron(II) and osmium (II), both of which exhibit increasing formal potentials with increasing acid concentration. Since certain titrants—e.g., vanadium(V) and chromium(VI)—also increase in oxidation strength with increasing acid concentration, use of an indicator which behaves similarly might eliminate the necessity of careful adjustment of acid concentration to ensure coincidence of indicator end point with the titration equivalence point.

Solution stability is favorable for all the complexes except $[Fe(bipy)_3]SO_4$ which dissociates rapidly into colorless species in concentrated solutions of strong acids. Intense absorption characteristics enable use of small quantities of the complexes; hence indicator titration blanks are minimal or negligible.

ACKNOWLEDGMENT

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Chronopotentiometric Oxidation of Hydrazine at a Platinum Electrode

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▶ **The effect of electrode pretreatment on the anodic oxidation of hydrazine in sulfuric acid solutions was investigated. The reaction occurred with the smallest activation overpotential at a platinized electrode. An increase of $i\tau^{1/2}/C$ with increasing i and decreasing C, over a concentration range of 0.5 to 25mM hydrazine, was observed, and several possible mechanisms for this variation, involving adsorption of hydrazine, bubble formation, occurrence of an intermediate chemical reaction, and occurrence of catalytic decomposition of hydrazine at the electrode surface, are considered.**

THE ELECTROOXIDATION OF HYDRAZINE is interesting both for its possible analytical applications and because hydrazine has been suggested as a possible fuel cell component. Recently Karp and Meites (*12*) examined the oxidation of hydrazine by voltammetry at mercury and platinum electrodes. They suggested that the oxidation proceeded more rapidly at oxide-coated than at reduced-platinum electrodes. Anson and King (*2*), however, have demonstrated that increases in reversibility sometimes ascribed to oxide films may, in fact, be due to platinization of the electrode surface. The present study lends support to this idea, and examines the effect of various electrode pretreatments on the anodic oxidation of hydrazine. A variation of the chronopotentiometric constant, $i\tau^{1/2}/C$, with current density and concentration was also found, and some speculations concerning the source of this variation are considered.

EXPERIMENTAL

The experimental apparatus and procedures have been described (*6*). The working electrode was a plane shielded platinum disk, oriented horizontally for upward diffusion. The reference electrode was a mercury–mercurous sulfate electrode in contact with a saturated potassium sulfate solution (S.M.S.E.) with a potential of 0.40 volt *vs.* S.C.E. The reference electrode was positioned so that the tip of its salt bridge was very close to the surface of the platinum working electrode. All potentials given are referred to a S.M.S.E. The chronopotentiometric cell was thermostated at 25.0° C. The hydrazine solutions were prepared from reagent grade hydrazine sulfate, $N_2H_4 \cdot H_2SO_4$. Although acidic aqueous solutions of hydrazine are stable to air oxidation and autodecomposition (*12*), test solutions were usually freshly prepared.

RESULTS AND DISCUSSION

Effect of Electrode Pretreatment. The anodic oxidation of hydrazine occurs in acidic solution by the reaction

$$N_2H_5^+ - 4e \rightarrow N_2 + 5H^+ \qquad (1)$$

Equation 1 represents only the overall reaction, and it is not meant to describe the detailed steps in the reaction. The potentials at which this oxidation occurs depend strongly upon the previous history of the platinum anode, and chronopotentiometric waves occurring at potentials separated by as much as 0.5 volt are observed. A chronopotentiogram of the anodic oxidation of hydrazine at an electrode which has been preoxidized by anodization in a $1M$ H_2SO_4 solution is shown in Figure 1. Also shown in Figure 1 are anodic

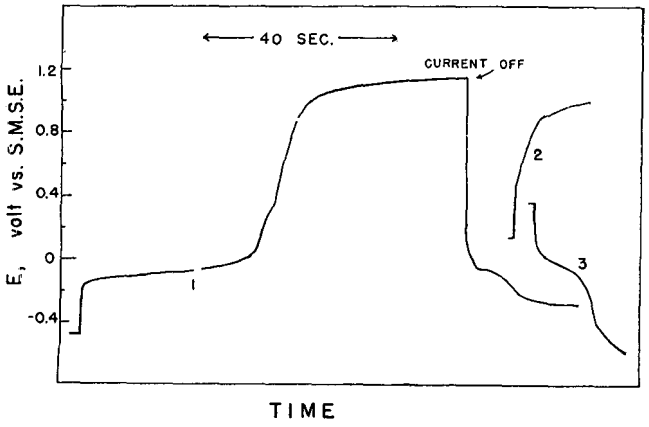

Figure 1. Chronopotentiograms for oxidation of hydrazine and for formation and reduction of PtO

1. 5mM hydrazine, 0.1M H_2SO_4 at preoxidized platinum electrode at a current density of 1.172 ma. per sq. cm.
2, 3. 1M H_2SO_4, current density of 85 μa. per sq. cm.

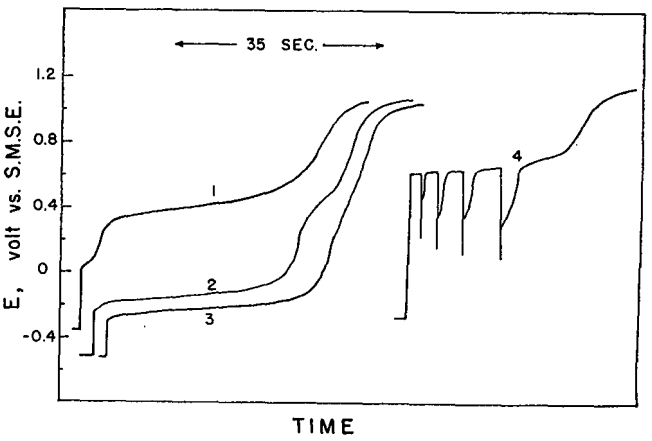

Figure 2. Chronopotentiograms for oxidation of hydrazine at activated and deactivated electrodes

Solution contained 5mM hydrazine and 0.1M H_2SO_4; current density was 1.17 ma. per sq. cm.

1. After several hours immersion in test solution
2. Repeat after 1
3. After a.c. activation
4. After treatment with aqua regia

and cathodic scans in 1M H_2SO_4 alone, at a lower current density, showing the formation and reduction of the platinum oxide film. (Throughout the remainder of this paper, this film will be termed PtO only for convenience and should not be taken to mean this particular oxide rather than another or perhaps chemisorbed oxygen.) Therefore, at a preoxidized electrode, oxidation of hydrazine occurs, followed by formation of PtO starting at about 0.4 volt and finally by evolution of oxygen. The ill defined transition corresponding to PtO formation is prolonged by continued diffusion of hydrazine to the electrode. When the current is turned off, the potential rapidly falls to that characteristic of PtO reduction, and a transition is found corresponding to the chemical reduction of PtO by hydrazine diffusing toward the electrode (Figure 1). Apparently, hydrazine rapidly reduces PtO, and a preoxidized electrode when immersed in a solution containing hydrazine is rapidly converted to one with a freshly reduced platinum surface.

That PtO is rapidly reduced by hydrazine is also shown by the following experiment. When a platinum electrode is anodized in a 1M H_2SO_4 solution, removed, washed, replaced, and cathodized, the characteristic PtO reduction wave is found (curve 3, Figure 1). If the electrode is briefly immersed in a hydrazine solution after the anodization, and then washed and replaced, the PtO reduction wave is no longer seen, and upon cathodization, the potential immediately drops to that characteristic of hydrogen ion reduction; the small hydrogen ion reduction prewave found with a platinized electrode by Anson (*1*) is also observed. Therefore, at a freshly reduced platinum surface [a surface presumably containing a layer of finely divided platinum (*2*)], called henceforth "an activated surface," hydrazine is oxidized at a potential of −0.1 to −0.2 volt *vs.* S.M.S.E.

At an electrode which has remained immersed in the test solution for long times, or has been treated with aqua regia, the oxidation of hydrazine occurs at a more positive potential, about 0.5 volt, and the chronopotentiograms are sometimes characterized by sharp oscillations in potential (curves 1 and 4, Figure 2). Repeating the oxidation after the electrode has been allowed to evolve oxygen for a brief time and after the solution has been stirred and allowed to come to rest, gives an oxidation scan characteristic of an activated surface (curve 2), although the transition at 0.5 volt is somewhat longer than that found with a preoxidized electrode. If the electrode is removed from this solution, stripped of any finely divided platinum on its surface with aqua regia, and then subjected to a treatment which will be termed "a.c. activation," the most reversible behavior is obtained (curve 3). The a.c. activation consisted of connecting the working electrode and an auxiliary electrode to a source of d.c. biased 60-cycle a.c. (through an autotransformer and stepdown transformer) so that the working electrode potential is rapidly cycled between about −0.4 and 0.95 volt *vs.* S.M.S.E. This treatment results in the repeated formation and reduction of PtO, and forms a lightly platinized, active surface (*2*). Oxidation of hydrazine at a heavily platinized electrode (formed by cathodic reduction in a H_2PtCl_6 solution at 20 ma. for 200 seconds) occurred at a potential of about −0.4 volt, but the potential-time curve was very drawn out, with several small humps, and no clear transition could be obtained.

Precathodization of the platinum electrode—with or without finely divided platinum on its surface, either potentiostatically in 1M H_2SO_4 or at a constant current in the test solution—at potentials where hydrogen ion reduction occurs, leads to behavior characteristic of a deactivated surface (such as that found after long immersions in the test solution or aqua regia treatment) (curve 1 in Figure 3). Repeated trials (curves 2, 3, and 4), after oxidation of the electrode has occurred, indicate partial activation of the surface.

Mechanism of Pretreatment Effects. The following mechanism for the electrode reaction is consistent with the above results. The platinum electrode may be in either an activated or deactivated state, depending upon its previous treatment. The activated state has a surface of freshly prepared, finely divided platinum, which may be produced by reduction of PtO, platinization, or a.c. activation, and upon which the direct oxidation of hydrazine, Equation 1, occurs at −0.1 to −0.2 volt. The deactivated state is characterized by an electrode which has been allowed to remain in contact with the hydrazine solution for long periods of time, which has been treated with aqua regia, or which has been used to evolve hydrogen. Presumably in this case the finely divided platinum on the surface of the electrode is either missing or is poisoned by adsorption of impurities at the active sites or by recrystallization of the surface. The oxidation of hydrazine on a deactivated surface occurs at the same potential as that for PtO formation, and since hydrazine rapidly reduces PtO, the reaction may occur as follows.

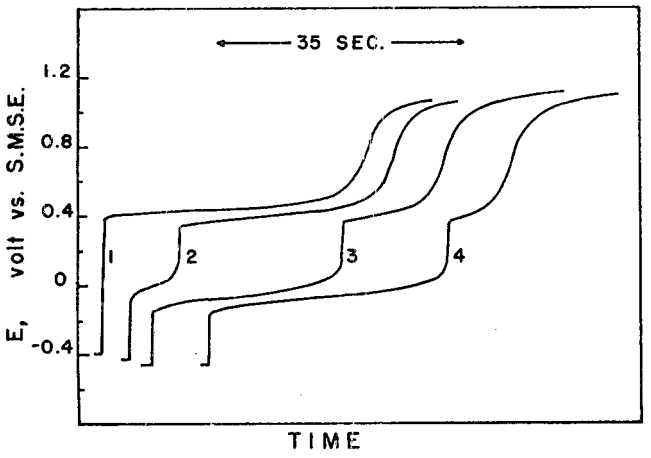

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Figure 3. Effect of precathodization on chronopotentiograms for oxidation of hydrazine

Solution contained 5mM hydrazine and 0.1M H_2SO_4; current density was 1.17 ma. per sq. cm.

1. After potentiostatic cathodization of electrode in 1M H_2SO_4 at −0.8 volt for about 2 minutes
2, 3, 4. Consecutive trials following 1

$$2(Pt + H_2O - 2e \rightarrow PtO + 2H^+) \quad (2)$$

$$2PtO + N_2H_6^+ \rightarrow 2Pt^* + N_2 + 2H_2O + H^+ \quad (3)$$

The reduction of PtO by hydrazine (reaction 3) may occur either directly or by an electrochemical reaction such as that postulated by Breiter (*8*) for the reaction of PtO and hydrogen. In the course of these reactions, activated platinum (represented as Pt*) is formed, and active sites are produced at the electrode. On repeated scans—e.g., curves 2, 3, and 4 in Figure 3—hydrazine will be oxidized first at the active sites; when these are used up, for example by adsorption of products on the sites or coverage of the sites with gaseous nitrogen, the potential shifts sharply to that characteristic of the deactivated surface. Alternately, the shift in potential may be caused by the complete occurrence of concentration polarization at the active sites. The overall transition time is about the same, except for the small contribution from PtO formation, at activated and deactivated electrodes.

The oscillations in potential sometimes seen at a deactivated electrode have also been found during the oxidation of hydrogen (*3, 14*) and methanol and formaldehyde (*9*). An explanation similar to that given by Sawyer and Seo (*14*) can be proposed, namely, that oxidation occurs at the deactivated surface, and in the course of reactions 2 and 3 forms active sites. The potential then drops to that for oxidation of hydrazine at an activated surface, but when the few active sites are covered with nitrogen or adsorbed intermediates, the potential again rises to that characteristic of oxidation at a deactivated electrode, forming more active sites, etc. This explanation conforms better to experimental observations than the one which ascribes the oscillations to gas evolution at the electrode. The oscillations when viewed on an oscilloscope are each shaped like an individual chronopotentiogram, and their sequence is usually quite regular and involves longer and longer times within each oscillation until the transition occurs. The regularity of the oscillations and the absence of oscillations in many other electrode reactions in which a gas is evolved argues against the gas evolution mechanism. On the other hand, if the activation-deactivation mechanism is correct, it is difficult to explain why oscillations are not always obtained at a deactivated electrode, and why an intermediate steady potential is not obtained. Similar oscillations are also found during the electrooxidation of some substituted hydrazines and will be the subject of further investigation.

It may be fruitful to speculate further on the nature of the active sites. The familiar concept in heterogeneous catalysis, that of active sites at edges and corners of crystals, and at grain boundaries, cracks, dislocations, and other imperfections in crystals, was first suggested by Taylor (*10*). Preferential adsorption of products, intermediates, or other substances is known to block these active sites. To test the effect of this type of poisoning of the surface, the experiments yielding the results shown in Figure 4 were undertaken. The electrode was given the a.c. activation treatment, and the activated electrode oxidation wave of hydrazine (curve 1) was obtained. The electrode was removed from the cell, immersed for about 15 seconds in a dilute acidified potassium iodide solution, washed well with water, and returned to the cell. The resulting chronopotentiogram (curve 2) was now characteristic of a deactivated surface. The electrode could be returned to its activated state by an a.c. activation treatment. When the electrode was treated with several other substances known to poison a platinum catalyst for other heterogeneous reactions, such as hydrogen sulfide and arsenic(III), only slight changes in the behavior of an activated electrode were observed. Iodide ion may preferentially adsorb and block the active sites, or it may alter the nature of the surface by, for example, promoting

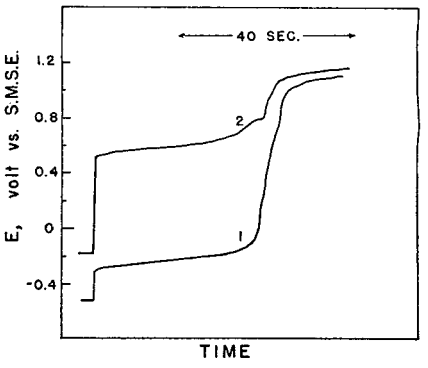

Figure 4. Effect of iodide ion on chronopotentiogram for oxidation of hydrazine

Solution contained 5mM hydrazine and 0.1M H_2SO_4; the current density was 1.18 ma. per sq. cm.

1. After a.c. activation
2. After the electrode was immersed for about 15 seconds in an acidified potassium iodide solution, washed, and returned to the test solution

Table I. Variation of $i\tau^{1/2}/C$ with τ for Various Concentrations of Hydrazine

Solution was 1.44*M* H_2SO_4. Platinum electrode had projected area of 1.0 sq. cm.

C, m*M*	*i*, ma. per sq. cm.	τ, sec.	$i\tau^{1/2}/C$, ma.-sec$^{1/2}$ sq. cm.-m*M*
25	2.131	127.0	0.96
	2.963	67.0	0.97
	4.732	28.32	1.01
	6.490	16.68	1.06
	7.540	12.94	1.08
10	0.9002	149.7	1.10
	1.174	90.4	1.12
	2.244	27.7	1.18
	3.537	12.44	1.25
5	0.5175	134.9	1.20
	0.8663	50.2	1.23
	1.175	27.8	1.24
	1.488	17.91	1.26
	1.808	12.4	1.27
2.11	0.2266	141.7	1.28
	0.2826	92.8	1.29
	0.4235	41.9	1.30
	0.7167	15.26	1.33
0.98	0.1252	106.4	1.32
	0.1615	65.8	1.34
	0.2104	39.2	1.34
	0.3581	14.76	1.40
0.50	0.05505	125.9	1.24
	0.09382	40.6	1.20
	0.1620	16.51	1.32

the crystallization of the freshly deposited platinum into a more ordered, less active configuration. Deactivation of the electrode by treatment with aqua regia probably involves removal of the finely divided platinum from the surface. Long standing in the test solution might involve adsorption of impurities and products of the electrode reaction. Deactivation of the electrode upon cathodization may arise from the destruction of active sites by hydrogen (*11, 14*).

In conclusion, the results presented here confirm Anson's observations (*1, 2*) that electrode reactions tend to be more reversible on a platinum electrode containing a freshly prepared layer of finely divided platinum. The conclusion of Karp and Meites (*12*), that oxidation of hydrazine occurs much more rapidly on an oxide-coated electrode, has not been confirmed. The half-wave potential obtained by these authors for an oxide-coated rotating platinum electrode is several tenths of a volt more positive than that obtained for the activated electrode in these studies. The current increase during the controlled potential oxidation of hydrazine at a prereduced electrode [Figure 2 in (*12*)] may simply involve the activation of the electrode surface by a mechanism corresponding to Equations 2 and 3, rather than an increase in reaction rate due to oxide film formation.

Variation of $i\tau^{1/2}/C$. In all further experiments, before a series of trials, the electrode was treated with aqua regia to remove any old finely divided platinum from its surface, and then it was given an a.c. activation treatment for 90 seconds to form an activated electrode with a reproducible surface area. This type of pretreatment is also useful for several other applications of a platinum electrode in chronopotentiometry. Chronopotentiograms were carried to oxygen evolution in every trial, so that the electrode was maintained in an activated state throughout a series of experiments. Data for the oxidation of 0.5 to 25mM hydrazine in a 1.44M H_2SO_4 solution at various current densities are given in Table I. Each entry represents the average of at least two trials at the same current. Note that the chronopotentiometric constant, $i\tau^{1/2}/C$, increases with increasing current density, and decreases with increasing concentration of hydrazine. Several alternate mechanisms can be suggested to account for this variation of $i\tau^{1/2}/C$. These are discussed below.

Adsorption Mechanism. When only charging of the double-layer, oxide-film formation, and adsorption of the electroactive species complicate the diffusion controlled electrode reaction, $i\tau^{1/2}/C$ will increase with increasing i. A previously proposed model for correcting for these effects yielded the following equation (*5*).

$$\frac{i\tau^{1/2}}{C} = \frac{nFD^{1/2}\pi^{1/2}}{2} + \frac{B}{C\tau^{1/2}} \qquad (4)$$

where B, the correction factor, is a function of double-layer capacity, of the amount of electricity involved in formation of PtO, and of the amount of electroactive substance adsorbed. When $i\tau^{1/2}/C$ is plotted against $\tau^{-1/2}$, a series of straight lines is obtained. The slopes and intercepts of these lines, and the standard deviations of each, computed by a linear least squares treatment of the data using a computer program designed for determining the extent of adsorption (*15*), are given in Table II. For the oxidation of hydrazine under these conditions, especially at higher concentrations of hydrazine, contributions to B from charging of the double layer and from electrode oxidation will be negligible. Assuming that adsorption of hydrazine is the only complicating factor, and using the model previously proposed (*15*), values of Γ, the amount of adsorption of hydrazine in moles per sq. cm., can be calculated (Table II). Two factors argue against these computed values of Γ actually representing the amount of hydrazine adsorbed. First, according to the model employed, the intercepts of the lines should be constant and equal to $^1/_2nFD^{1/2}\pi^{1/2}$. In this case, the intercepts decrease with increasing hydrazine concentration. Using the value for the diffusion coefficient found in 1M NaOH (*12*), 1.35×10^{-5} sq. cm. per second, and correcting for the difference in viscosity between 1M NaOH and 1.44M H_2SO_4, we calculate a D of 1.47×10^{-5} sq. cm. per second for hydrazine in our supporting electrolyte (assuming the additional protonation of the molecule does not decrease D appreciably), and a theoretical $^1/_2nFD^{1/2}\pi^{1/2}$ of 1.31 ma.-second$^{1/2}$ per sq. cm.-mM. This value is larger than all of the values found here, and corresponds almost exactly to an extrapolation of the observed intercepts to a zero concentration of hydrazine (excluding the less accurate value for 0.5mM hydrazine). A second argument against an adsorption mechanism is the very large extent of adsorption computed. Construction of various modes of orientation of the molecule on the electrode surface indicates that a monolayer of adsorbed hydrazine should involve less than 0.5×10^{-8} mole per sq. cm. of hydrazine. Therefore the calculated values of Γ, for concentrations of hydrazine greater than 5mM, represent adsorption of multilayers, with the Γ at 25mM, hydrazine representing at least 8 layers of hydrazine. Although this extent of adsorption is possible—e.g., by hydrogen bonding of additional layers of hydrazine molecules—it seems unlikely.

Table II. Slopes and Intercepts of $i\tau^{1/2}/C$ vs. $\tau^{-1/2}$ Plots and Apparent Extent of Adsorption of Hydrazine

Experimental conditions as in Table I. Values of Γ calculated assuming only adsorption contributes to B.

C, mM	Intercept[a]	Slope $\times$ C(or B), mcoulombs/sq. cm.	$\Gamma \times 10^8$ moles/sq. cm.
25	0.89 ± 0.01[b]	0.68 ± 0.06	4.4 ± 0.4
10	1.041 ± 0.002	0.73 ± 0.01	1.89 ± 0.02
5	1.174 ± 0.004	0.35 ± 0.02	0.46 ± 0.02
2.11	1.259 ± 0.004	0.26 ± 0.02	0.14 ± 0.01
0.98	1.268 ± 0.008	0.51 ± 0.04	0.13 ± 0.01
0.50	1.16 ± 0.08	0.6 ± 0.5	0.07 ± 0.06

[a] Theoretically constant for only the cases of adsorption, double layer charging, or oxide film formation effecting the diffusion controlled reaction, and equal to $nFD^{1/2}\pi^{1/2}/2$.
[b] Variations represent the standard deviations.

Bubble Mechanism. Since nitrogen gas is produced during the electrode reaction, bubbles of gas accumulating during the electrolysis possibly block portions of the electrode. The effect of this type of blockage, effectively and continually increasing the current density during the electrolysis, was calculated using a simple model of bubble formation and assuming maximum possible coverage per bubble—i.e., that the effective blocking area of each bubble is its cross-sectional area—for various numbers of bubbles. The effect indeed produces an increasing $i\tau^{1/2}/C$ with increasing i, but the overall effect becomes smaller at higher concentrations of the electroactive substance, and should be negligible for 25mM hydrazine solutions at short transition times. Details of this work will be presented elsewhere, but we conclude that this effect is not a major contribution to the variation of $i\tau^{1/2}/C$ in this case.

Competing Intermediate Chemical Reaction. The variation may be caused by a competing chemical reaction involved in the overall electrode reaction. Most types of complicating chemical reactions, such as slow preceding reactions producing the electroactive species, or catalytic reactions regenerating the electroactive species, lead to a decrease of $i\tau^{1/2}/C$

with increasing i. However, consider a reaction scheme such as

$$O - n_1e \rightarrow R \quad (6)$$

$$R \rightleftarrows R' \quad (7)$$

$$R' - n_2e \rightarrow X \quad (8)$$

$$R' \xrightarrow{k} Y \quad (9a)$$

or

$$2R' \xrightarrow{k} Y \quad (9b)$$

where O is the original electroactive species, R is the product of the primary electrode reaction, and X and Y are not electroactive. Although this scheme has not been treated mathematically for chronopotentiometry, the overall behavior should qualitatively involve increases of $i\tau^{1/2}/C$ with increasing i. At very short times the effect of the competing chemical reaction, 9, becomes negligible, and $i\tau^{1/2}/C$ will essentially equal $^1/_2(n_1 + n_2)FD^{1/2}\pi^{1/2}$. At very long times, reaction 9 becomes important, effectively consuming all of R' in the limit, and $i\tau^{1/2}/C$ becomes equal to $^1/_2n_1FD^{1/2}\pi^{1/2}$. Therefore, in an intermediate time range, depending upon the magnitude of k, $i\tau^{1/2}/C$ will increase with increasing i. If the competing reaction is a second order reaction, such as 9b, the effect will become larger at higher concentrations of O leading to a decrease in $i\tau^{1/2}/C$ with increasing C. The proposed mechanism of the anodic oxidation of hydrazine in acidic solutions (*12*) [written in a somewhat condensed form (*7*)] is similar to (but more complicated than) the scheme presented above.

$$N_2H_4 - 2e \rightarrow N_2H_3^+ + H^+ \quad (10)$$

$$N_2H_3^+ \rightleftarrows N_2H_2 + H^+ \quad (11)$$

$$N_2H_2 - 2e \rightarrow N_2 + 2H^+ \quad (12)$$

$$2N_2H_2 \xrightarrow{k} NH_3 + HN_3 \quad (13)$$

$$HN_3 - e \rightarrow H^+ + {}^3/_2\,N_2 \quad (14)$$

The existence of the additional reaction, 14, and the proposed electroactivity of $N_2H_3^+$ itself (*12*) make the exact theoretical treatment of this scheme difficult. While this type of reaction mechanism would give the $i\tau^{1/2}/C$ behavior found here, it is unlikely that this is the sole contribution to the variation. Although conditions existing at the electrode are somewhat different in chronopotentiometry and controlled potential coulometry, results obtained with the latter technique (*12*) indicated that the effect of reaction 13 should be negligible in a 1.44M H_2SO_4 solution, a fact which originally suggested the choice of this medium for the chronopotentiometric experiments. A more detailed study of the mechanism of the anodic oxidation of hydrazine is currently being undertaken.

Table III. Calculated and Experimental Values of $i\tau^{1/2}/C$ Assuming the Catalytic Decomposition Mechanism

Experimental conditions as in Table I. Calculations performed by assuming
$i_o = nFk\,C_{N_2H_4}{}^2$
$K = nFD^{1/2}\pi^{1/2}/2 = 1.31$ ma.-sec.$^{1/2}$/sq. cm.-mM
$k = 2.5 \times 10^{-11}$ mole/sq. cm.-sec.

C, mM	i_o, ma. per sq. cm.	i, ma. per sq. cm.	$i\tau^{1/2}/C$, ma.-sec.$^{1/2}$/sq. cm.-mM Calcd. assuming t_1 = 60 sec.	Calcd. assuming t_1 = 120 sec.	Experimental
25	0.625	2.131	0.97	0.92	0.96
		2.963	1.02	0.97	0.97
		4.732	1.07	1.01	1.01
		6.490	1.09	1.02	1.06
		7.540	1.10	1.02	1.08
10	0.100	0.9002	1.16	1.14	1.10
		1.174	1.18	1.16	1.12
		2.244	1.22	1.19	1.18
		3.537	1.23	1.20	1.25
5	0.025	0.5175	1.24	1.23	1.20
		0.8663	1.26	1.24	1.23
		1.175	1.26	1.25	1.24
		1.488	1.27	1.25	1.26
		1.808	1.27	1.25	1.27
2.11	0.00445	0.2266	1.28	1.28	1.28
		0.2826	1.28	1.28	1.29
		0.4235	1.29	1.28	1.30
		0.7167	1.29	1.29	1.33

For concentrations of 0.98 and 0.5mM hydrazine, corresponding to i_0's of 0.00096 and 0.00025 ma. per sq. cm., respectively, the theoretical $i\tau^{1/2}/C$ calculates as 1.30 for all values of i.

Catalytic Decomposition of Hydrazine. Finally the variation in $i\tau^{1/2}/C$ may be caused by the catalytic decomposition of hydrazine. Although acidic aqueous solutions of hydrazine are stable, hydrazine sulfate is readily decomposed on contact with active platinum (*4*) in accordance with the equation (in acidic solutions).

$$3N_2H_5^+ + H^+ \rightarrow N_2 + 4NH_4^+ \quad (15)$$

Hydrazine is not decomposed on smooth or deactivated platinum. Since the activated platinum electrode contains a layer of finely divided platinum on its surface, reaction 15 must occur before, and simultaneously with, the electrode reaction. The effect of this heterogeneous chemical reaction is to decrease the concentration of hydrazine in the vicinity of the electrode before electrolysis, during the waiting period of 1 to 2 minutes after stirring, when the solution is allowed to come to rest. As a simple model of this effect, we can consider the heterogeneous chemical reaction to be equivalent to a small constant current density, i_o, flowing continually at the electrode surface. After the solution has been stirred before a trial, i_o flows until time t_1, when the actual electrolysis current, i, is switched on. The fictitious current density, i_o, is assumed to be related to the rate constant of reaction 15 and the concentration of hydrazine at the electrode surface, $C_{N_2H_4}$ ($x = 0$).

$$i_o = nFk\,C_{N_2H_4}(x = 0) \quad (16a)$$

or

$$i_o = nFk\,C_{N_2H_4}{}^2(x = 0) \quad (16b)$$

The simplifying assumption of a constant i_o implies that reaction 15 is slow, so that the concentration of hydrazine at the electrode surface is not significantly depleted before the electrolysis, and it neglects the variation of i_o during the electrolysis, as $C_{N_2H_4}$ ($x = 0$) goes to zero. A model with a varying i_o could readily be treated by the method proposed below, but would yield a more complicated final expression. The quantitative results of the proposed model are obtained using the treatment of chronopotentiometry with step functional changes in current (*13*, *16*), and they lead to the equation

$$\frac{\pi^{1/2}nFD^{1/2}C}{2} = i_o(t_1 + \tau)^{1/2} + i\tau^{1/2} \quad (17)$$

where τ is the observed transition time, measured from t_1. Writing $\pi^{1/2}nFD^{1/2}/2 = K$, and assuming that $i_o < i$ and that $i_o t_1^{1/2} < KC$, the solution of reaction 17 is

$$\tau^{1/2} = \frac{iKC - i_o[(i^2 - i_o^2)t_1 + K^2C^2]^{1/2}}{(i^2 - i_o^2)} \quad (18)$$

Values of $\tau^{1/2}$ and $i\tau^{1/2}/C$ were calculated, using Equation 18, assuming K = 1.31 ma.-sec$^{1/2}$/mM-sq. cm. and various values for t_1 and i_o, for each experimental i and C. The best fit to the experimental data for a t_1 of 60 to 120 seconds (the usual waiting period between stirring the solution after a trial) was obtained for a second order dependence of i_o on C (Equation 16b) and a k of about 2.5×10^{-11} mole per sq. cm.-sec. The agreement between

calculated and experimental values of $i_\tau t^{1/2}/C$ in Table III is quite good, considering the approximate nature of the theoretical model and the actual experimental variability in t_1. Moreover k depends upon the condition of the electrode surface, and decreases to zero as the electrode becomes deactivated. Note that the effective currents involved in the catalytic decomposition would not alter the total bulk concentration of hydrazine appreciably. For example, even in the worst case, where i_o is 0.6 ma. in 25mM hydrazine, the bulk concentration would only decrease about 0.2% per hour. The formation of various intermediates and products during the catalytic decomposition, and the accumulation of these on the electrode surface may account for the loss of activity of the electrode upon standing in the hydrazine solution.

Clearly the relatively small amount of data and the present unavailability of other supporting experiments do not allow a satisfactory choice to be made from among the possible mechanisms proposed. Probably all of the perturbing effects, adsorption, bubble formation, a secondary intermediate chemical reaction, and catalytic decomposition, occur to some extent. Because the relative contribution of these effects is not currently understood, no attempt was made in this study to interpret the experimental potential-time curves.

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Atomization Efficiency of Total Consumption Atomizer-Burners in Flame Photometry

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▶ **A simple method of measuring the efficiency of sample introduction into H_2/O_2 and C_2H_2/O_2 flames when using total consumption atomizer-burners is described. The method is based on the measurement of the absorption of radiation from a sharp-line zinc source by zinc atoms in a flame of known composition and temperature. Using a simple calculation, the efficiency of sample introduction can be found. The efficiency of sample introduction by total consumption atomizer-burners into a number of H_2/O_2 and C_2H_2/O_2 flames is studied as a function of sample solvent, of height of region measured above the tip of inner cone of flame, and of flow rate of solution into the given flame. The efficiency of sample introduction into H_2/O_2 and C_2H_2/O_2 flames decreases with increasing solution flow rate, increases with flame height for most H_2/O_2 flames (the opposite effect is observed in several C_2H_2/O_2 flames), and increases as the concentration of methanol increases in a methanol–water mixture.**

IN FLAME EMISSION and absorption spectrometry, an important parameter is the efficiency of the sample introduction process. When a sample solution is introduced into a flame in flame photometry, a series of processes occurs involving: mass transfer of the sample solution containing a solvated salt from a cuvette to the flame which results in large drops, dispersion of the large drops into small droplets, evaporation of the solvent from the salt to produce a mist of salt particles, and dissociation of the salt into atoms. This dissociation depends on the nature of the salt, the flame composition, and the flame temperature. Although each of the individual processes has been previously studied (*4*, *7*, *8*), no studies involving the over-all efficiency of sample introduction have been performed. To the analyst, the efficiency of sample introduction is generally of much more importance than any of the individual processes.

Because the final step of sample introduction, the dissociation of the salt into atoms, depends to a large extent on the type of salt present, the efficiency of sample introduction, δ, will be defined as follows. For any given flame composition and temperature and for any flame region, the efficiency is the ratio of the concentration of unsolvated salt experimentally present in the flame—whether in atomic, molecular, or ionic forms—to the concentration of unsolvated salts which would be present if the processes of droplet dispersion and solvent evaporation were 100% efficient. In this paper a salt, zinc chloride, in aqueous and nonaqueous solution which exists predominantly in the atomic form in most flames, is introduced into a variety of flames, and the absorption of resonance radiation by the zinc vapor is used to measure the efficiencies of sample introduction as a function of a number of experimental conditions.

The factors which influence the rate at which a solution flows into a flame when using total consumption atomizer-burners have been studied by Winefordner and Latz (*10*). The processes of dispersion of large drops into small droplets, particularly with regard to the factors controlling the size of the droplets formed, have been reviewed by Dean (*3*) and Herrmann and Alkemade (*7*). Dean and Carnes (*4*) have recently studied the size of droplets formed when using total consumption atomizer-burners. The process of evaporation of solvent from the solvated salt particles has been reviewed by Herrmann and Alkemade (*7*).

THEORY

The efficiency of sample introduction, δ, has been defined above. The measurement of salt molecule concentration in a flame is not practical because of experimental difficulties and changes of the degree of dissociation of the salt with flame temperature. The simplest method is to introduce into the given flame a salt which is essentially completely dissociated for all flame compositions and temperatures of interest. In addition, the dissociated metal atoms must not ionize appreciably at the flame temperature studied, and a sharp line resonance lamp of the particular metal must be readily available. A salt which essentially fulfills these criteria is zinc chloride. Zinc salts, according to Clinton (*2*), are completely dissociated even in cool coal-gas flames. Zinc atoms even at temperatures up to 4000° K., and at low concentrations should undergo negligible ionization because of the high first ionization constant of zinc—i.e., 9.3 e.v. (*7*). Therefore, $ZnCl_2$ was used in all efficiency measurements in this paper.

The method of calculating the efficiency of sample introduction is as follows. A zinc chloride solution is introduced into a flame, in which the composition is known, at a flow rate of ϕ, in cc. per minute, and the absorbance, A_ϕ, is measured at a certain height above the tip of the inner cone of the flame. Similarly, the same zinc chloride solution is introduced into the flame at such a low flow rate, ϕ_{min}, in cc. per minute, that the processes of dispersion and evaporation are approximately complete, and the absorbance, A_{min}, is measured. The efficiency of sample introduction, δ, can then be calculated from Equation 1.

$$\delta = \frac{A\phi}{A_{min}\frac{\phi}{\phi_{min}}} \qquad (1)$$

In all of the measurements performed in this paper ϕ_{min} was 0.10 cc. per minute, and so Equation 1 can be rewritten as

$$\delta = \frac{0.1A\phi}{\phi A_{min}} \qquad (2)$$

EXPERIMENTAL

Equipment and Solutions. A Jarrell-Ash Ebert scanning monochromator (Jarrell-Ash Co., Newtonville 60, Mass.), with two 25-micron fixed slits and with the manufacturer's support base and optical bench, was used for all measurements. A zinc hollow-cathode discharge tube (Micro-Tek Instruments, Inc., Baton Rouge, La.), powered by a well-regulated d.c. power supply (0 to 600 volts, 0- to 30-ma. range) was mounted on a rod, placed in a rider base on the optical bench, and optically aligned. One spherical quartz lens of 100-mm. focal length was placed between the zinc tube and the flame, and a second similar lens was mounted between the flame and the spectrometer entrance slit. The first lens and zinc source were so mounted that a circular cathode image about 0.5 cm. in diameter was focused on the center of the flame. The other lens was adjusted until the zinc radiation from the discharge tube fully illuminated the entrance slit. A flat black metal plate was placed in front of the spectrometer entrance slit as a shutter. A 1P28 photomultiplier tube was placed in the Jarrell-Ash vertical detector housing, and the photomultiplier socket was rewired to adapt to the Model ph-200 Eldorado universal photomultiplier photometer circuit (Eldorado Electronics Co., Berkeley, Calif.). A 10-mv. strip chart recorder was connected to the output of the photometer circuit. A variety of Beckman total consumption atomizer-burners (No. 4050 small-bore capillary, No. 4020 medium-bore capillary, and No. 4060 large-bore capillary with either the H_2 or C_2H_2 burner jackets) could be positioned in front of the entrance slit of the monochromator. The burners were mounted on a rod held in a rider base on the optical bench. The rod was marked in increments of 0.5 cm. so that the burner could be accurately raised or lowered in such a manner as to study various regions of the flame. The oxygen flow rate (ϕ_{O_2}) and the hydrogen or acetylene flow rates (ϕ_{H_2} or $\phi_{C_2H_2}$) were controlled by single-stage regulators on the tanks and by the Beckman pressure regulator for the Beckman DU flame photometer. Between the Beckman pressure regulator unit and the atomizer-burner gas connections, flow meters (4A rotameter tubes, Ace Glass, Inc., Vineland, N. J.) were placed to read accurately the flow rates of the O_2 and of H_2 or C_2H_2 in cc. per minute.

The flow rate of the solution (ϕ_{soln}) into the desired flame was controlled by application of pressure to the solution contained in a 50-ml. flask with a side arm. Pressure was applied from a nitrogen tank. Fine control of the flow rate was achieved by inclusion of a needle valve in the line prior to the solution. To obtain low flow rates of solution at high flow rates of aspirating gas, it was necessary to place a screw clamp on the line between the solution in the flask and the atomizer capillary. This method of solution flow rate control was similar to that used by Foster and Hume (*5*). The solution flow rate was measured by placing an Ace 1A rotameter tube between the capillary and the solution flask. All flow rates could be read with an error of less than 5% after calibration according to the method described by the manufacturer.

A solution containing 50.0 p.p.m. of zinc was prepared for use in all absorption measurements.

Procedure. The zinc hollow-cathode discharge tube was turned on by increasing the voltage on the power supply to about 300 volts where the lamp started and then adjusting the voltage until the current remained at 7.0 ma. The current was readjusted to 7.0 ma. every 10 minutes, and usually three readjustments were required for the lamp current to completely stabilize at this value. The detector circuit and recorder were turned on and adjusted to the desired sensitivity. The spectrometer wavelength was adjusted to the peak wavelength of the 2139-A. zinc line, and the wavelength control was then locked into position. The flame composition was adjusted to any desired value. The shutter was placed in front of the spectrometer entrance slit, and the recorder was adjusted to zero. The shutter was opened, the recorder was adjusted to nearly full-scale, and the recorder deflection (I^0) was measured. The zinc solution of 50.0 p.p.m. was then introduced into the flame at a flow rate of 0.10 cc. per minute, and the recorder deflection (I_{min}) was read. Each set of measurements was done in duplicate. From each set of values of I^0 and I_{min} an absorbance, A_{min}, was calculated, and the average value of A_{min} was calculated. The same procedure as described above was repeated for solution flow rates greater than 0.10 cc. per minute, and the average absorbance was calculated for a given solution flow rate ϕ. The efficiency of sample introduction, δ, for various solution flow rates was calculated using Equation 2.

RESULTS AND DISCUSSION

The validity of Equations 1 and 2 depends upon the width of the zinc resonance line (2139 A.) from the zinc hollow-cathode tube being appreciably narrower than the zinc absorption line due to the hot zinc atoms in the given flame (*9*). The emission line from the zinc source acts as a slit width for the absorption line. If the emission line is too wide, then the measured per cent transmittance—i.e., $(I/I^0) \times 100\%$—will be too large. The effect of slit width on absorption lines has been discussed by Broderson (*1*). Because the emission line width of spectral lines from hollow cathode tubes depends on the tube current, an experimental study was performed in which values of per cent transmittance were measured at various tube currents (4 to 20 ma.) at a certain solution flow rate and at a single flame composition and temperature. For currents above 10 ma., the emission line width must be about the same or greater than the absorption line width because the per cent transmittance values increased quite rapidly. For currents below 10 ma., the per cent transmittance approached a constant value. All currents of 8 ma. and below gave the same values, and so the 7 ma. current was used in all efficiency studies.

As previously discussed, Clinton (*2*) noted that zinc compounds even in cool flames were completely dissociated. If this is true, then variation of the zinc concentration should not result in a

measurable change in δ. This was experimentally verified over the concentration range of 10 to 200 p.p.m. zinc. The 50.0-p.p.m. zinc solution was chosen for all experimental efficiency studies because appreciable absorption occurred at 0.1 cc. per minute and yet was still measurable at 3 cc. per minute.

The diameter of the beam of radiation from the resonance source which passes through the flame gases is 0.5 cm., and the average width of flame gases through which the beam passes is about 0.7 cm. Therefore, in measuring A_ϕ and A_{min}, only a portion of the total atoms introduced per unit time are actually measured. By a series of measurements of absorbance from a beam of radiation 0.1 cm. in diameter, the distribution of absorbance as a function of distance, x, from the vertical center of the flame was determined experimentally for several flame heights, compositions, and solution flow rates. These measurements and the results obtained were quite similar to those determined by Gibson, Grossman, and Cooke (*6*). When radiation from a narrow line source is passed through a flame, the distribution of absorbance, A_x, as a function of distance x from the vertical center of the flame follows approximately a Gaussian distribution. By experimental determination of a number of A_x *vs.* x curves for a number of flame conditions, it was found that the following empirical equation gave an accurate means of correcting the measured efficiencies for the change in width of the flame gases with change in solution flow rate

$$\delta_{corr} = \delta_{meas} \frac{\left\{1.06 - 0.36\frac{\Delta x}{s}\right\}_{\phi_{min}}}{\left\{1.06 - 0.36\frac{\Delta x}{s}\right\}_{\phi}} \quad (3)$$

where δ_{corr} is the efficiency corrected for flame width, δ_{meas} is the efficiency measured according to the method described in the experimental section, Δx is the half maximum width in cm. of the A_x *vs.* x distribution, and s is the width of the beam of resonance radiation in centimeters passing through the flame gases. The upper term in brackets is evaluated for the minimum solution flow rate, ϕ_{min}, and the lower term in brackets is evaluated for the desired solution flow rate, ϕ. The value of δ_{corr} differs significantly from δ_{meas} only for very large, aqueous-solution flow rates and for most organic-solution flow rates. If the change in width of the flame gases is not greater than 2-fold when the solution flow rate is increased from ϕ_{min} to ϕ, then Δx in the above equation can be replaced with the value $W/2$ where W is the width of the flame gases in centimeters. Because the relative standard deviation of measuring efficiencies is $\pm 8\%$, this approximation does not introduce significant error. The above equation for δ_{corr} can be derived by considering the distribution of absorbance with distance in the same general manner as absorbance with frequency interval for atomic absorption lines (*1*). This derivation and the discussion of the resulting equation will be presented in a later paper.

The validity of Equations 1 and 2 depends on the use of a minimum flow rate, ϕ_{min}, which is sufficiently small that dispersion and evaporation are essentially complete. A series of efficiency measurements at solution flow rates of 0.15 cc. per minute gave the same results. Therefore, all solution flow rates of 0.15 cc. per minute and lower at all flame heights gave 100% efficiency of sample introduction. As can be seen from Table I, organic solution flow rates significantly greater than 0.15 cc. per minute resulted in 100% efficiency. However, the minimum solution flow rate used in all measurements was arbitrarily taken as 0.10 cc. per minute.

It was not necessary to make any correction for reflection losses of the zinc resonance radiation due to water droplets in the flame. This could cause an error in the values of δ because the I^0 readings were made with no solution flowing into the flame whereas all other readings were made with a certain zinc-solution flow rate. If appreciable, this could result in erroneously-high δ values. It was experimentally shown that the apparent absorbance due to reflection losses was only 0.008 at 2 cc.

Table I. Efficiency of Introduction of Methanol–Water Solutions into H_2/O_2 Flames[a]

ϕ_{O_2} = 2500 cc. per minute
ϕ_{O_2}/ϕ_{H_2} = 0.5
Medium-bore atomizer

Methanol, %	h, cm.	Efficiency, δ				
		ϕ_{soln} = 0.1	ϕ_{soln} = 0.2	ϕ_{soln} = 0.5	ϕ_{soln} = 1.0	ϕ_{soln} = 2.0
20	0.5	1.00	0.93	0.87	0.83	0.60
50	0.5	1.00	1.00	0.94	0.85	0.64
80	0.5	1.00	1.00	1.00	0.86	0.67
20	1.5	1.00	0.93	0.81	0.60	0.55
50	1.5	1.00	1.00	1.00	0.95	0.84
80	1.5	1.00	1.00	1.00	1.00	0.98

[a] Key to symbols used:

ϕ_{O_2} = flow rate of oxygen
ϕ_{O_2}/ϕ_{H_2} (or $\phi_{O_2}/\phi_{C_2H_2}$) = ratio of O_2 to fuel flow rates
h = height of region measured above inner cone of flame
ϕ_{soln} = aqueous-solution flow rate, cc. per minute
δ = efficiency of sample introduction

Table II. Efficiency of Introduction of Aqueous Solutions into H_2/O_2 Flames

Small-bore atomizer

ϕ_{O_2}	ϕ_{O_2}/ϕ_{H_2}	h, cm.	Efficiency, δ				
			ϕ_{soln} = 0.1	ϕ_{soln} = 0.5	ϕ_{soln} = 1.0	ϕ_{soln} = 2.0	ϕ_{soln} = 3.0
2000	0.5	0.5	1.00	0.82	0.61	0.43	0.39
2000	0.5	1.0	1.00	0.81	0.77	0.55	0.45
2000	0.5	1.5	1.00	0.98	0.84	0.55	0.48
2500	0.5	0.5	1.00	0.78	0.60	0.48	0.43
2500	0.5	1.5	1.00	0.74	0.56	0.36	0.31
3000	0.5	0.5	1.00	0.76	0.55	0.45	0.44
3000	0.5	1.0	1.00	0.71	0.61	0.57	0.54
3000	0.5	1.5	1.00	0.73	...	0.48	0.44
3500	0.5	0.5	1.00	0.88	0.74	0.60	0.57
3500	0.5	1.0	1.00	0.84	0.79	0.72	0.58
3500	0.5	1.5	1.00	0.97	0.81	0.65	0.59
2500	0.2	1.5	1.00	0.80	0.60	0.50	0.47
2500	0.4	1.5	1.00	0.93	0.67	0.47	0.45
2500	0.6	1.5	1.00	0.74	0.60	0.45	0.36
2500[a]	0.5	0.5	1.00	0.94	0.74	0.55	0.47
2500[a]	0.5	1.0	1.00	0.93	0.78	0.54	0.44
2500[a]	0.5	1.5	1.00	0.69	0.62	0.51	0.44
3000[a]	0.5	0.5	1.00	0.46	0.42	0.33	0.31
3000[a]	0.5	1.0	1.00	0.63	0.55	0.41	0.36
3000[a]	0.5	1.5	1.00	0.75	0.61	0.53	0 49

[a] Medium-bore atomizer.

Table III. Efficiency of Introduction of Aqueous Solutions into C_2H_2/O_2 Flames

Medium-bore atomizer[a]

ϕ_{O_2}	$\phi_{O_2}/\phi_{C_2H_2}$	h, cm.	Efficiency, δ $\phi_{soln} = 0.1$	$\phi_{soln} = 0.5$	$\phi_{soln} = 1.0$	$\phi_{soln} = 2.0$
2000	1.5	0.5	1.00	0.78	0.61	0.45
2000	1.5	1.0	1.00	0.68	0.49	0.31
2000	1.5	1.5	1.00	0.56	0.37	0.21
2500	1.5	0.5	1.00	0.86	0.63	0.44
2500	1.5	1.0	1.00	0.62	0.46	0.28
2500	1.5	1.5	1.00	0.65	0.45	0.26
3000	1.5	0.5	1.00	0.64	0.47	0.30
3000	1.5	1.0	1.00	0.57	0.43	0.27
3000	1.5	1.5	1.00	0.63	0.47	0.28
4000	2.0	1.0	1.00	0.87	0.75	0.60
3500	2.5	1.0	1.00	0.84	0.68	0.47
4000	2.5	1.0	1.00	0.87	0.73	0.51
4500	2.5	1.0	1.00	0.89	0.77	0.53
4000	3.0	1.0	1.00	0.74	0.55	0.44

[a] Data obtained with different medium-bore atomizer than that used for data in first table.

per minute and only 0.020 at 3 cc. per minute. Both of these values are negligible in comparison with the absorbance readings due to zinc absorption at these same flow rates.

In Tables II and III, the sample introduction efficiency values, δ, are given for various flame compositions as a function of aqueous solution flow rate. The flame size remained approximately constant (as determined by a series of photographs for various solution flow rates) for all solution flow rates in Tables II and III, and so the correction for change in flame width was unnecessary.

In Table I, the sample introduction efficiencies, δ, are given as a function of flow rate of several methanol–water mixtures into H_2/O_2 flames. All efficiencies were corrected for the change in width of flame gases with solution flow rate. Similar data were obtained for C_2H_2/O_2 flames under similar experimental conditions.

Several interesting conclusions can be drawn from the results given in Tables II and III. An increase in solution flow rate always results in a less efficient sample introduction, and the rate of change of efficiency with flow rate of solution is always greatest at low flow rates. It is also interesting to note that the efficiency of most H_2/O_2 flames increases with flame height at any given solution flow rate, whereas the efficiency of the C_2H_2/O_2 flames and several of the H_2/O_2 flames, in which the flow rate of oxygen is large, decreases with flame height at any given solution flow rate. The increased efficiency of the H_2/O_2 flames with height is probably due to the additional time allowed for the processes of dispersion and evaporation. The data in Tables II and III are insufficient to permit a discussion of the reasons for the decrease in efficiencies with height for C_2H_2/O_2 flames as well as the few H_2/O_2 flames mentioned above.

From the data in Table I it is evident that the sample introduction for all methanol–water mixtures is more efficient than for aqueous solutions under similar conditions. The decrease of efficiencies with solution flow rate is much less rapid than with aqueous solution under similar conditions. The increase of efficiency with flame height is also quite pronounced when using methanol–water mixtures as compared to aqueous solutions. Gibson, Grossman, and Cooke (*6*) have already explained the increase in emission for several elements introduced into a flame via organic solvents as being due essentially to the increased sample introduction efficiency and the increase in flame temperature. The increase in absorption of radiation in atomic absorption spectrometry when using a flame cell should be due primarily to the increase in sample introduction efficiency. This should particularly be the case when using total consumption atomizer-burners. Efficiency data and flame temperature measurements for other solvent systems may enable the experimenter to calculate the approximate increase in emission or absorption for a given element in a given solvent system.

The bore size of the capillary of the atomizer also seems to have an effect on efficiency. For the same flame composition and solution flow rate, the medium-bore atomizer gives more efficient sample introduction than the small-bore atomizer. As previously noted (*10*), the geometry of total consumption atomizer-burners varies from one to another, which could possibly account for the differences noted.

The precision of the measurements performed in this paper depends on several factors. The relative standard deviation of setting and determining the oxygen and fuel flow rates and all solution flow rates greater than 0.5 cc. per minute was about ±5%. Unfortunately, the relative standard deviation in measuring the 0.10 cc. per minute solution flow rate was ±10%. The relative standard deviation in measuring the intensities of the zinc resonance radiation with no zinc in the flame (I^0) and with zinc in the flame (I) was about ±1%. By using the 50.0-p.p.m. zinc solution for all measurements in this paper, the relative standard deviation in log (I^0/I) did not exceed ±10% for all solution flow rates at all flame heights. Therefore, the relative standard deviation in all efficiency measurements and in all solution flow rate measurements was about ±10%. The relative errors in the same measurements was also about 10%.

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Temperatures of Some Typical Flames Used in Flame Photometry

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▶ **Flame temperatures of H_2/O_2 and C_2H_2/O_2 flames, in which aqueous solution is introduced via total consumption atomizer-burners, are measured as a function of solution flow rate into the flame for a variety of flame compositions and heights in the flame. A simple method of flame temperature measurement, based on the measurement of the ratio of intensities of two iron lines (Fe 3737.13 A. and Fe 3734.87 A.), is used to measure the flame temperatures. From the measured temperatures, empirical equations are determined which can be used to calculate the flame temperature of a given flame composition at a given height above the inner cone. The use of the derived equations and the effect of flame composition, solution flow rate, and flame height on flame temperature of H_2/O_2 and C_2H_2/O_2 flames are discussed.**

THE OUTER CONE of a flame can be considered to be approximately in thermal equilibrium (*1*), and the most important parameter describing a thermal source is its temperature, T. The intensity of a spectral line of a flame source containing gaseous atoms is exponentially dependent upon the temperature of the flame (Boltzmann factor). The absorption of radiation by the same flame is nearly independent of the flame temperature (*9*). However, because the sample is usually introduced into the flame as a salt solution, the flame temperature also influences the processes of dispersion of introduced salt solution into droplets, evaporation of solvent to produce a salt mist, dissociation of the salt into atoms, association of the atoms in concern with flame gas products, and ionization of the atoms. For these reasons, it is important that the approximate temperature of any given flame be known when either absorption or emission measurements are to be made.

Most flame emission and absorption measurements are performed with H_2/O_2 or C_2H_2/O_2 flames. Therefore, flame temperatures of H_2/O_2 and C_2H_2/O_2 flames are measured as a function of flame gas composition, solution flow rate, efficiency of sample introduction, and height above the inner cone of the flame. From the experimental measurements, empirical equations for the flame temperature of a number of flame gas compositions are determined as a function of the above factors. The measurement method employed in this paper is based on the Ornstein two-line method (*3*), and is similar to the method described by Broida and Shuler (*6*). This method involves the measurement of the intensity of two iron lines, Fe 3737.13 A. and Fe 3734.87 A., from which the temperature can be found by a simple calculation. Other methods for the measurement of flame temperatures have been fully described by Gaydon and Wolfhard (*8*) and Broida (*4*), and so will not be discussed here. For flame temperatures above 2800° K., the two-line method is the simplest and most rapid method, and gives good precision. Flame photometry involves a study of the electronic deactivation of excited atoms or the electronic activation of atoms from lower to higher electronic states, and so a method which measures an electronic temperature is desired. Temperatures measured using the two-line method are electronic temperatures.

EXPERIMENTAL

Equipment and Solutions. The same experimental equipment as previously described (*10*) was used for all temperature measurements. However, in this case no condensing lenses were used. The flame was placed 2 cm. from the spectrometer entrance slit. Between the entrance slit and the flame (1 cm. from each), a flat black baffle with an opening 0.5 cm. by 0.5 cm. was centered and permanently positioned on the same rider base as the burner.

A stock solution of ferric chloride containing 500 p.p.m. of iron was prepared for use in all temperature measurements.

Procedure. To make a temperature measurement, the flow rate of iron solution (ϕ_{H_2O}) into the flame, the flow rates of oxygen (ϕ_{O_2}) and of hydrogen (ϕ_{H_2}) or acetylene ($\phi_{C_2H_2}$) were adjusted to any desired value, and the values were recorded. The two iron lines were scanned manually, and the sensitivity of the photometer was adjusted so that the Fe 3737.13-A. line was about 80 to 90% of full scale. Then the two iron lines were scanned automatically at the rate of 2 A. per minute, and the output signal was recorded. This process was repeated at least twice for each flame temperature measurement, and the heights of the two peaks were measured. The ratio of the intensities was determined, and the flame temperature, T, was calculated using the two-line method (*3*). The intensity of a spectral line emitted by atoms in a flame in thermal equilibrium is given by Broida and Shuler (*6*). The temperature, T, can be found by taking the ratio of the intensities and solving for T.

With the experimental setup used, it was found that for a given set of conditions the relative standard deviation of the intensity ratio was ±5% even if the absolute intensity of the two iron lines varied. The relative standard deviation of all temperature measurements was about ±10%. Minor fluctuations in solution flow rate may cause appreciable changes in intensity but only small changes in temperature, T.

The proper choice of a line pair for application of the Ornstein two-line method to the measurement of flame temperatures is governed by a number of criteria. The two lines must be negligibly self-absorbed or the self-absorption must be known and approximately the same for both lines under the same conditions. Crosswhite (*7*) has indicated the two lines in concern show negligible self-absorption. Broida and Lalos (*5*) have discussed the selection of spectral lines with respect to the value of the transition probabilities and statistical weights. A number of instrumental factors also limit the choice of a line pair. The lines must be close enough together that the two lines can be scanned in a relatively short time and at such a slow speed that the recorder responds accurately to both lines. Also, the two lines must be close enough together that the photocathode has essentially the same response to both lines or else the spectral response must be corrected for. Finally, the two lines must be of sufficient intensity as to be accurately measurable at the sensitivity setting used. Using the experimental setup described, the two iron lines chosen are nearly ideal. Numerous other iron lines for which spectral data are readily available (*7*) can be used. For example, when using the two-line method with the Beckman DU monochromator or with similar

Table I. Evaluation of Constants in Empirical Equation for Determination of Temperatures of H_2/O_2 Flames

Flame conditions		Constants			
ϕ_{O_2}/ϕ_{H_2}	ϕ_{O_2}, cc. per min.	T_{tip}, ° K.	a	b	c
0.2	2500	2700	0.05	0.00	40
0.4	2500	2750	0.07	0.025	40
0.5	2000–3500	2750	0.07	0.025	50
0.6	2500	2630	0.09	0.05	40

Table II. Evaluation of Constants in Empirical Equation for Determination of Temperatures of C_2H_2/O_2 Flames

Flame conditions		Constants				
$\phi_{O_2}/\phi_{C_2H_2}$	ϕ_{O_2}, cc. per min.	T_{tip}, ° K.	a	b	c	f
1.0	2500	3050	5	10	70	0.04
1.5	2000–3000	3050	5	10	70	0.05
2.0	4000	3050	5	10	70	0.08
2.5	3000–4500	3050	5	10	70	0.08
3.0	4000	3050	7	20	70	0.08

monochromators, the separation of the lines would have to be greater for accurate measurement of the line intensities.

RESULTS AND DISCUSSION

The two lines used in the temperature measurements were thoroughly checked for self-absorption at various concentrations of iron. No measurable self-absorption was found for concentrations of iron of 2000 p.p.m. or below.

Using the two-line method, flame temperatures were measured as a function of aqueous solution flow rate for a large number of H_2/O_2 and C_2H_2/O_2 flames at various ratios of flow rate of oxygen to fuel, at various absolute values of ϕ_{H_2} or $\phi_{C_2H_2}$ and ϕ_{O_2}, and at various heights, h, in centimeters above the inner cone of the flame. The average result of duplicate measurements of flame temperatures was plotted *vs.* solution flow rate for certain values of ϕ_{O_2}/ϕ_{H_2} or $\phi_{O_2}/\phi_{C_2H_2}$, for certain absolute values of ϕ_{O_2}, and for various values of h.

It is, however, unnecessary to give the measured data in numerous graphs because the following empirical equation was found to give H_2/O_2 flame temperatures with mean errors from the measured values of less than 50° K.

$$T_{H_2/O_2} = \frac{T_{tip} - ch^2}{1 + a(\chi\delta) + b(\chi\delta)^2} \text{ ° K.} \quad (1)$$

The symbols a, b, and c are constants and have been empirically evaluated from the measured temperature *vs.* solution flow rate curves for a variety of flame conditions. The values of a, b, c, and T_{tip} (the temperature of H_2/O_2 flames at the tip of the inner cone) for several fuel-rich, for a stoichiometric, and for one oxygen-rich flame are given in Table I. The term χ is the solution flow rate expressed as number of moles of solution per mole of oxygen gas introduced into the flame, and δ is the efficiency of sample introduction as defined in a previous paper (*10*).

The empirical equation listed below was found to give C_2H_2/O_2 flame temperatures with mean errors from the measured values of less than 50° K.

$$T_{C_2H_2/O_2} = \frac{T_{tip} - ch^2}{1 + f\log_e[1 + a(\chi\delta) + b(\chi\delta)^2]} \text{ ° K.} \quad (2)$$

The symbols a, b, c, and f are constants which have been empirically determined for the flame conditions listed in Table II. The term T_{tip} is the flame temperature of a C_2H_2/O_2 flame of given composition at the tip of the inner cone. The terms χ and δ have the same meaning as given above, and $\log_e$ is the natural logarithm of the term in brackets.

To determine a flame temperature of a given H_2/O_2 or C_2H_2/O_2 flame the constants are evaluated from Table I or II, respectively, the value of h is approximated for the flame in concern, the value of δ is found from the data given in a previous paper (*10*), and χ is calculated from the following equation which can be readily derived from a dimensional analysis. The solution flow

$$\chi = 1.25 \times 10^3 \frac{\phi_{H_2O}}{\phi_{O_2}} \quad (3)$$

rate, ϕ_{H_2O}, and the oxygen flow rate, ϕ_{O_2}, are in cc. per minute. The flames studied and listed in Tables I and II were considered to be the most important flames used when employing total consumption atomizer-burners in analytical emission or absorption flame photometry. In the event an analyst wishes to know the temperature of a flame whose composition is not listed in Tables I or II, an approximate flame temperature can still be found as long as the ratio of O_2 to fuel is in the ranges listed in Table I (0.2 to 0.6) or in Table II (1.0 to 3.0). In this case, the value of δ is estimated from data previously given (*10*), and T_{tip}, a, b, c, and f (for C_2H_2/O_2 flames) are estimated from data in Tables I or II. Because the values of δ, a, b, c, f, and T_{tip} do not differ greatly even when the ratio of O_2 to fuel is varied, estimates of flame temperatures with mean errors less than 100° K. should be readily possible. The authors have had excellent success with such approximations on flames other than those listed in Tables I and II.

The denominator of the above empirical equation for a H_2/O_2 flame is quite similar to the denominator of the theoretically derived equation given by Baker and Vallee (*2*) for a stoichiometric H_2/O_2 flame. The denominator of the empirical equation for a C_2H_2/O_2 flame is similar to the denominator of the theoretically derived equation given by Baker and Vallee (*2*) for a stoichiometric $(CN)_2/O_2$ flame. The numerator of the empirically derived expressions differs from that predicted by Broida and Lalos (*5*). They found that the flame temperature of a stoichiometric H_2/O_2 flame and of a stoichiometric C_2H_2/O_2 flame decreased linearly with height above the inner cone. They used a slit 1 mm. high by 0.01 mm. wide and so measured an adiabatic temperature—i.e., the flame temperature over the height and width of the slit was approximately a constant value. In this paper, a baffle 5 mm. high by 5 mm. wide was used to approximate actual flame photometric conditions. When using such a baffle, the flame temperature within the baffle will certainly vary both vertically and laterally as pointed out by Broida and Lalos (*5*), and so an average flame temperature rather than a true adiabatic temperature is measured. The use of an outer flame sheath would minimize the lateral variation and to some extent the vertical. However, most total consumption burners are used for analytical purposes without an outer flame shield, and so the measurements in this paper were made without the use of one. It was empirically determined that the average flame temperature measured by the two-line method dropped with the square of the height above the inner cone. The use of an outer flame shield and/or the use of a much smaller baffle probably would result in the linear decrease of temperature with height above the inner cone. In most H_2/O_2 flames used the inner cone tip was about 0.5 cm. above the burner tip, and in most C_2H_2/O_2 flames used the inner cone tip was about 1 cm. above the burner tip.

The values of the tip temperature,

T_{tip}, were found by extrapolation to zero height. The values found compare quite favorably with similar values given by Broida and Lalos (*5*).

The empirical equations gave accurate values of temperature when using small, medium, or large bore capillary atomizers. The differences in flame temperature resulting from different size atomizers can be attributed primarily to the difference in efficiency of introduction of sample into the flame—i.e., differences in the dispersion of droplets and rates of evaporation of water. The use of the δ factor seems to fully account for these processes.

A number of interesting conclusions result from observation of the empirical equations for the flame temperatures of H_2/O_2 and C_2H_2/O_2 flames. In all cases the flame temperature decreased with solution flow rate. However, this decrease is not nearly so rapid as theoretically predicted by Baker and Vallee (*2*). This is a result of several factors. In the relationship derived by Baker and Vallee it was assumed that sample introduction was 100% efficient which is not experimentally valid. In addition, in this paper an average flame temperature is measured rather than a single flame temperature of a small portion of gases. These factors would tend to moderate the change in temperature with flow rate of solution. The variation of flame temperature over the region of measurement results in a system which deviates slightly from being a true adiabatic system. However, the number of excited atoms to the number of ground state atoms per cc. of flame gases in each portion of the flame as well as in the region of observation can still be represented by a Boltzmann factor whose value is determined by a local temperature in the former and by an average temperature in the latter.

From the empirical equations, it can be shown that in most cases the flame temperature of H_2/O_2 flames decrease by about 100° K. when the solution flow rate is increased from 0.5 to 3.0 cc. per minute. A similar change in solution flow rate for an C_2H_2/O_2 flame gives a decrease of about 200° K. It should be pointed out that the temperature above the inner cone of fuel rich flames is essentially the same as for stoichiometric flames (see Tables I and II). This was also observed by Broida and Lalos (*5*). This phenomenon is probably a result of entrainment of air from the atmosphere.

Although few measurements as a function of solution flow rate have been made using total consumption atomizer-burners, the flame temperatures measured by the two-line method in this paper compare favorably with flame temperatures measured by other methods (line reversal and rotational methods). Insufficient data are usually given in other papers to allow direct comparison of flame temperatures with ones calculated from the above equations.

Using the empirical equations given in this paper approximate electronic temperatures of almost any H_2/O_2 or C_2H_2/O_2 flame can be determined. This should allow the analyst the opportunity of selecting any desired flame temperature and flame condition. The selection of flame temperature is particularly important when ionization and compound formation are important processes in the flame.

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Spectrophotometric Determination of Uranium with 4-(2-Pyridylazo)resorcinol

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▶ **A highly sensitive and selective method is described for the spectrophotometric determination of uranium. The chromogenic reagent is 4-(2-pyridylazo)resorcinol (PAR), and the determination is carried out at pH 8.0 in the presence of a mixed complexing solution containing (1,2-cyclohexylenedinitrilo)tetraacetic acid, sulfosalicylate, and fluoride. Most metals, including thorium, are masked by this complexing solution. Uranium forms a 1:1 complex with PAR, which has a molar absorptivity of 38,700 at 530 mμ. A preliminary separation of uranium from gross amounts of impurities may be accomplished by a rapid chelating-resin ion exchange procedure.**

ALTHOUGH NUMEROUS reagents have been proposed for the spectrophotometric determination of uranium, none of the methods at present available is completely satisfactory. Many of the more sensitive chromogenic agents such as dibenzoylmethane, oxine, and arsenazo are nonselective, while thiocyanate and hydrogen peroxide, which are quite selective, are relatively insensitive (*9*). Thorium, in particular, is a serious interference in most of these methods.

The pyridine–azo dyes 1-(2-pyridylazo)-2-naphthol (PAN) (*2*, *7*) and 4-(2-pyridylazo)resorcinol (PAR) (*1*, *8*) are among the most sensitive uranium reagents yet investigated. The molar absorptivity of the PAN–uranyl complex is 23,000 (560 mμ) and that of the PAR complex, 39,000 (530 mμ). A definite advantage of PAR is that both the free dye and the uranyl complex are water-soluble. Cheng has shown recently (*3*) that the selectivity of both these reagents toward uranium can be greatly improved by the use of (1,2-cyclohexylenedinitrilo)tetraacetic acid (CyDTA) as masking agent.

This paper presents a detailed investigation of the use of PAR for the spectrophotometric determination of uranium. Full sensitivity of the reagent has been retained, but the method has been made highly selective by including a mixed complexing solution containing CyDTA, fluoride, and sulfosalicylate. An important advantage is that a 50-

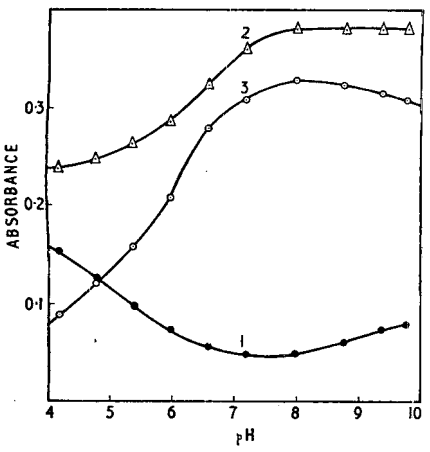


Figure 1. Effect of pH

1. Reagent blank vs. water
2. Uranium (103 μg.) plus PAR vs. water
3. Uranium (103 μg.) plus PAR vs. reagent blank

Each solution contained 5 ml. of complexing solution and 2.00 ml. of 0.20% PAR per 50 ml., measured at 530 mμ

fold excess of thorium causes only a slight error. A simple and rapid chelating-resin ion exchange procedure is described for the preliminary separation of uranium from samples containing gross amounts of impurities.

EXPERIMENTAL

Apparatus. A Hilger Uvispek spectrophotometer with 1-cm. matched quartz cells was used for all absorbance measurements.

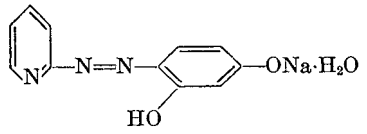
Reagents. CyDTA was obtained as the pure free acid from Fluka Ltd., Chemische Fabrik, Buchs (Switzerland).

The complexing solution was prepared by suspending 50 grams of CyDTA, 5 grams of sodium fluoride, and 130 grams of sulfosalicylic acid in 500 ml. of water, neutralizing to pH 8 with sodium hydroxide, and diluting to 1 liter.

A 1*M* triethanolamine buffer was prepared by dissolving 149 grams of reagent-grade triethanolamine in 800 ml. of water, neutralizing to pH 8.0 with hydrochloric acid, then diluting to 1 liter.

A standard 0.05*M* uranyl perchlorate stock solution was made by dissolving high-purity U_3O_8 in excess perchloric acid. This stock solution was standardized volumetrically, and appropriate dilutions made for the spectrophotometric work.

The laboratory grade of 4-(2-pyridylazo)resorcinol, sodium salt (The British Drug Houses Ltd., Poole, England) was purified by two crystallizations from 50% ethanol, followed by drying to constant weight over silica gel. Titanous chloride titrations gave a purity of 100 ± 2%, and elemental analysis indicated a similar result.



Required: C = 51.7%, H = 3.92%, N = 16.5%, Na = 9.01%

Found: C = 51.9%, H = 4.02%, N = 16.4%, Na = 8.7%

Dowex A-1 chelating resin, 50- to 100-mesh, was supplied by Dow Chemical International Ltd., Mich.

Recommended Procedure. Direct Spectrophotometric Determination. Pipet an aliquot of the neutral or faintly-acid sample solution, containing less than 300 μg. of uranium, into a 50-ml. volumetric flask. Add 5.0 ml. of complexing solution, 5.0 ml. of triethanolamine buffer, and 2.00 ml. of 0.20% aqueous PAR. Mix and dilute to volume. Measure the absorbance in 1-cm. cells against a reagent blank at 530 mμ. If appreciable amounts of iron are present (more than 200 μg.), the absorbance should be measured within 10 to 15 minutes.

Ion Exchange Separation. Condition a 2-ml. column of Dowex A-1 chelating resin by passing 20 ml. of 1*M* NaCl, followed by 20 ml. of water. Add 2 ml. of 5% CyDTA to the sample aliquot, and adjust the acidity to pH 3.0 with HCl. Pass the sample solution through the resin column (1 ml. per minute) and wash the column with 20 ml. of water (pH 3.0). Elute the uranium with 20 ml. of 5*M* HCl. Add 1 ml. of 72% $HClO_4$ to the eluent and evaporate to dryness under an infrared lamp. Complete the analysis as described under Spectrophotometric Determination.

Table I. Effect of Some Common Complexons on the Uranyl–PAR Complex

Complexon	Depression of color, uranyl–PAR complex, %[a]
(1,2-Cyclohexylenedinitrilo) tetraacetic acid (CyDTA)	0.5
(Ethylenedinitrilo)tetraacetic acid (EDTA)	43
Nitrilotriacetic acid (NTA)	47
[Ethylene-bis-(oxyethylene-nitrilo)]tetraacetic acid (EGTA)	57
Diethylenetriaminepentaacetic acid (DTPA)	49

[a] Each solution contained 105 μg. of U and 2 ml. of 5% complexon (Na salt) per 50 ml., pH 8.0.

RESULTS AND DISCUSSION

Effect of Complexing Agents, pH, and Temperature. The effect of some common complexons on the uranyl–PAR complex is shown in Table I. Although CyDTA forms metal complexes which are generally more stable than the corresponding (ethylenedinitrilo)tetraacetic acid (EDTA) complexes, the uranyl ion apparently reacts weakly, or very slowly, with CyDTA. Addition of fluoride to the complexing solution greatly increases the tolerance of the method toward thorium, while sulfosalicylate prevents the precipitation of beryllium, and aids in the complexing of aluminum. Figure 1 shows the influence of pH on the absorbance of the PAR complex. A pH of 8.0 was chosen as the optimum value.

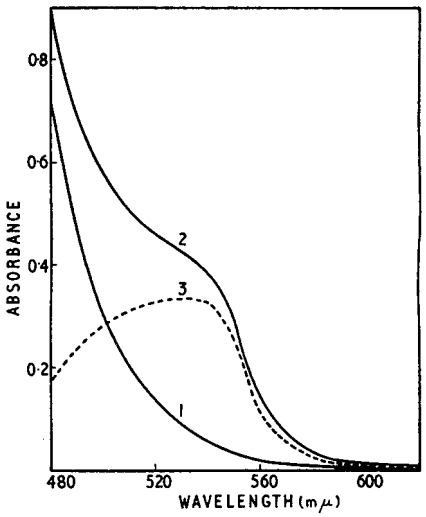


Figure 2. Wavelength–absorbance curves

1. Reagent blank vs. water
2. Uranium (103 μg.) plus PAR vs. water
3. Uranium (103 μg). plus PAR vs. reagent blank

Each solution contained 5 ml. of complexing solution and 2.00 ml. of 0.20% PAR per 50 ml., pH 8.0

Change in temperature had little effect on the absorbance values. A temperature coefficient of −0.05% per ° C. was found in the range 20° to 45° C.

Adherence to Beer's Law, Stability, and Reproducibility. The molar absorptivity of the PAR–uranyl complex in a triethanolamine buffer of pH 8.0 is 38,700 at 530 mμ. This corresponds to 0.0062 μg. of uranium per cm.^{2} on the Sandell scale, and is very similar to the sensitivity reported by Pollard, Hanson, and Geary (*8*). Beer's law is obeyed to a concentration of at least 7 μg. of uranium per ml. in the final solution.

In the presence of the complexing mixture, the absorbance of a standard uranium–PAR solution showed only a gradual 3% decrease over 20 hours at 25° C. Without the complexing agents, a drop in absorbance of 3% was noted in the first hour, with a further decrease of 7% over 20 hours. The PAR reagent is quite sensitive to oxidation, and traces of uncomplexed ferric ion were found to destroy the color slowly. This could be the reason for the greater stability in the presence of the complexing agents.

To test the reproducibility of the

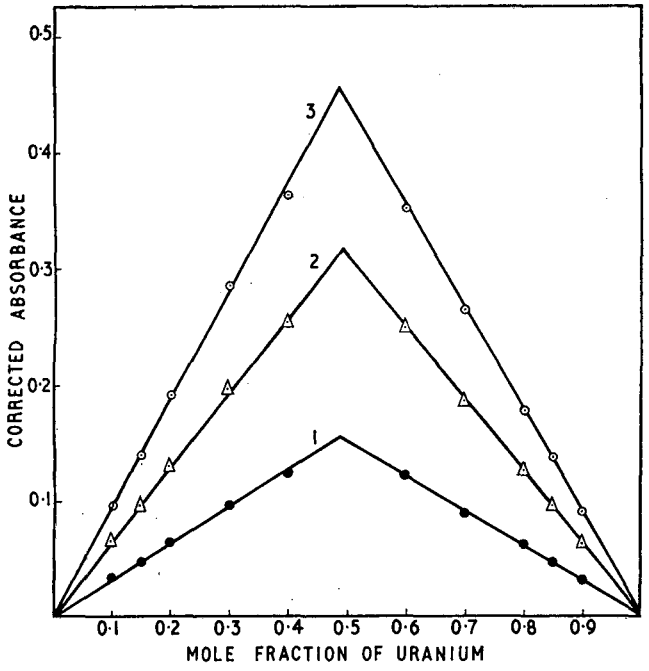


Figure 3. Composition of complex by continuous variations method

1. 560 mμ
2. 550 mμ
3. 530 mμ

Each solution (pH 8.0) contained 5 ml. of complexing solution

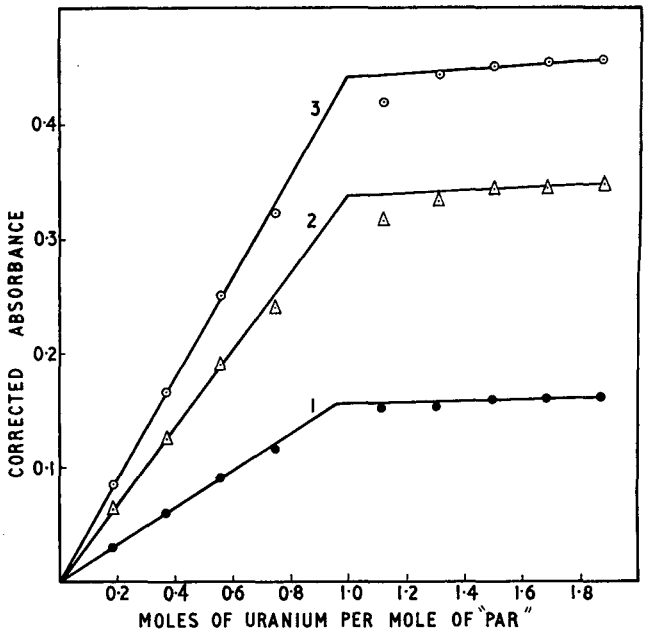


Figure 4. Composition of complex by mole ratio method

1. 560 mμ
2. 550 mμ
3. 540 mμ

Each solution (pH 8.0) contained 5 ml. of complexing solution

method under ideal conditions, 10 repeat determinations were carried out on aliquots of the standard uranyl perchlorate solution (100 μg. of U). The results showed a relative standard deviation of ±0.3%.

Wavelength–Absorbance Curves, and Composition of the Complex. Wavelength–absorbance curves for PAR and its uranyl complex are shown in Figure 2. The composition of the complex was investigated by spectrophotometric and potentiometric techniques. The continuous variations and mole ratio methods (Figures 3 and 4) indicated that the 1:1 complex was the predominating species absorbing at the wavelengths used. This is in agreement with Busev and Ivanov (*1*), but Geary, Nickless, and Pollard (*6*) claim a 2:1 PAR–uranyl complex is formed at pH 8.

Table II. Study of Interferences in Direct PAR Method

Foreign element	Amount added, mg./50 ml.	Relative error, %[a]	Foreign element	Amount added, mg./50 ml.	Relative error, %[a]
Al	1.0	+1	Ni	1.0	−1
Al	5.0	+6	Pb	1.0	0
Be	1.0	+1	Sb(III)	1.0	0
Be	10.0	+6	Sn(II)	1.0	+3
Bi(III)	1.0	+1	Th	1.0	0
Ca	1.0	0	Th	5.0	+3
Cd	1.0	0	Ti(IV)	1.0	−2[c]
Ce(III)	1.0	0	V(IV)	1.0	+6
Co(II)	1.0	−1	V(V)	1.0	>+100
Cr(III)	1.0	+6	W(VI)	1.0	0
Cr(III)	5.0	+23	Zn	1.0	+1
Cr(VI)	1.0	+2	Zr	1.0	+12
Cu(II)	1.0	+2	Zr	2.0	+1[d]
Fe(III)	1.0	+11	ClO_4^-	800	0
Fe(III)	2.0	+3[b]	CO_3^{-2}	1.0	0
Hg(II)	1.0	0	CO_3^{-2}	50	−6
Li	1.0	0	NO_3^-	2000	0
Mg	1.0	+1	PO_4^{-3}	1.0	0
Mn(II)	1.0	0	PO_4^{-3}	50	−10
Mo(VI)	1.0	+1	SiO_3^{-2}	3.0	−10
NH_3	1.0	0	SiO_3^{-2}	6.0	−20
Nd	1.0	−1	SO_4^{-2}	800	0

[a] Each solution contained 103 μg. of U and 5 ml. of complexing solution per 50 ml., pH 8.0. Absorbance values measured after 0.5 hour.
[b] Absorbance measured within 10 minutes of mixing.
[c] Solution slightly hazy.
[d] Plus 2 ml. of 2% *meso*-tartaric acid.

Table III. Separation of Uranium by Chelating Resin

Foreign element added (5 mg.)	Uranium found, PAR method after separation[a]	Recovery, %
None	103	98
Al	104	99
Fe(III)	103	98
Zr	102[b]	97
Cr(III)	105	100
SiO_3^{-2}	104	99
Cu(II)	104	99
Ti(IV)	97[c]	92
V(IV)	105	100
V(V)	380	...
PO_4^{-3} (100 mg.)	93	89
NaCl (1 gram)	105	100

[a] Procedure described under Ion Exchange Separation used.
[b] No *meso*-tartaric acid added to spectrophotometric solution.
[c] Spectrophotometric solution hazy.

Potentiometric investigations were hindered by the formation of a precipitate when mixtures of uranium and PAR were titrated with alkali. The precipitate appeared in the pH range pH 4 to 8, even in solutions where the total dye concentration was as low as $1 \times 10^{-4}M$. For this reason reliable $\bar{n}$ values could not be obtained from the titration data.

Study of Interferences, and Ion Exchange Separation. The effect of foreign elements on the direct determination of uranium with PAR is shown in Table II. The only serious interferences are vanadium(V),

Table IV. Determination of Uranium in Diverse Materials

Sample	Uranium Found, PAR method, %		Uranium Present,[b] %
UO_2–ThO_2–BeO[d]	0.30	0.30[a]	0.30
U–Al alloy	25.9	25.6[a]	25.6
Uranium ore 1	16.1	16.2[a]	15.9
Uranium ore 2	14.8	14.7[a]	14.4
Uranium ore 3	8.42	8.48[a]	8.50
Uranium ore 4	0.044	0.043[c]	0.046
Uranium ore 5	0.026	0.028[c]	0.025
Uranium ore 6	0.016	0.016[c]	0.014

[a] Direct spectrophotometric method.
[b] Determined by a.c. polarography, (*5*).
[c] Samples decomposed with $HClO_4$ + H_2F_2, and uranium separated by chelating resin. Vanadium reduced with ascorbic acid before spectrophotometric determination.
[d] Fuel element pellet.

zirconium, iron, chromium(III), and silicon. Zirconium may be effectively masked with *meso*-tartaric acid, and iron can be tolerated in reasonable amounts if absorbance measurements are made within 10 minutes of mixing. Vanadium may be reduced to the tetravalent state with ascorbic acid.

Dowex Al chelating resin is capable of removing trace metals from solutions of high salt concentration (*4*). In the presence of CyDTA at pH 3, uranium is still quantitatively adsorbed by the resin, but most other elements pass into the effluent (Table III). Using this technique, uranium can be separated, if necessary, from many of the elements which interfere in the direct method (Table II). Vanadium(V), however, is also retained on the column, and leads to high results.

Applications. In Table IV the PAR method has been applied to the determination of uranium in some materials commonly encountered in nuclear technology, and the results compared with those obtained by a highly precise a.c. polarographic method (*5*). In the authors' laboratories, a wide range of chemical engineering and processing samples are now analysed on a routine basis using the PAR spectrophotometric procedure. The high sensitivity of the PAR method often allows uranium to be determined without a preliminary separation. This has resulted in a considerable saving of analytical time.

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Characterization of Alkyl and Aryl Halides by 2,4-Dinitrophenylhydrazones of Aldehydes from Reaction of Their Grignard Reagents with Dimethylformamide

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▷ A wide variety of aliphatic and aromatic halogen compounds have been characterized by forming aldehydes having one additional carbon atom, which aldehydes are then converted to their 2,4-dinitrophenylhydrazones. The latter were purified by recrystallization from different solvents and their melting points determined and compared with literature values. The basic reaction, formylation of the Grignard reagent with dimethylformamide, is carried out in a test tube in 1 hour and does not require elaborate equipment. A number of compounds, not before characterized in this way, are reported. Reaction was not obtained with allylic halides, some tertiary halides, and halides with electron-attracting substituents.

FINAL IDENTIFICATION of a compound in qualitative organic analysis requires the preparation of one or more solid derivatives that are rapidly prepared and purified, melt between 50° and 250° C., and are obtained in good yield with a minimum of side reactions (*14*). The derivatives now available are limited to either aliphatic or aromatic monohalogen compounds and a general reaction for both types using common reagents and simple equipment would be advantageous.

Primary and secondary aliphatic and alicyclic halides have been characterized as their mercuric halides, *N*-alylphthalimides, anilides, toluidides, naphthalides, and α-naphthyl ethers, but these methods are too specific and also are accompanied by experimental difficulties. More recently, thiourea has been reacted with alkyl bromides and iodides to give *S*-alkylthiouronium salts that are precipitated as their picrates. Ethylene thiourea has been employed to form *S* - alkyl - 2 - mercapto - 4,5 - dihydroglyoxalinium salts that are identified by their melting points and those of their picrates, as well as volumetric determination of their equivalent weights and those of their free bases formed by reaction with aqueous ammonia (*5*). The carboxylate anion of methyl fluorene-9-carboxylic acid, readily generated from its methyl ester, reacts rapidly with alkyl halides to give good yields of 9-alkyl derivatives that have sharp melting points (*1*).

Derivatives of aryl halides are more limited inasmuch as they are prepared by electrophilic ring substitution to form nitro or sulfamido compounds. Chloro- or bromo-substituted benzenes having alkyl side chains are generally oxidized to corresponding benzoic acid derivatives. The greater reactivity of iodobenzene has made possible the formation of polyvalent iodine derivatives that are aryl iododichlorides (*12*), and also iodoxy and iodoso compounds as well as iodoso diacetates (*13*).

The Grignard reaction to characterize

alkyl halides by formation of an aldehyde was applied by Fales (*6*), who added Grignard reagents to the double bond of 6-methyl-3-tolyl-3,4-dihydroquinoline. The adduct gave aldehydes with one more carbon than the alkyl halide and the carbonyl compounds were characterized as their dinitrophenylhydrazones. Methods of converting alkyl halides to aldehydes with a Grignard reagent reviewed by Smith and Nichols (*15*) include the action of excess ethyl formate (*7*), ethyl *ortho*-formate (*2, 16, 17*), ethoxymethyleneaniline (*11*), carbon disulfide and semicarbazide (*18–21*), and Bouveault's use of disubstituted formamides (*4*).

Although the Bouveault reaction (*3*) for forming aldehydes from the Grignard reaction with dialkylformamides is quite complex, it presented the opportunity to use inexpensive, commercially available, and relatively-pure dimethylformamide. The method also seemed to be applicable to both aliphatic and aromatic halogen compounds, and the aldehydes thus produced should be readily identified since their 2,4-dinitrophenylhydrazones are described in the literature.

$$RX + Mg \xrightarrow{ROR} RMgX \xrightarrow{HCON(CH_3)_2} RCH(OMgX)-N(CH_3)_2$$

$$RCH(OMgX)-N(CH_3)_2 + H_2NNH-C_6H_3(NO_2)-NO_2 \longrightarrow RCH{=}NNH-C_6H_3(NO_2)-NO_2 + H_2O$$

The reducing action of the Grignard reagent may also convert the dimethylformamide to a tertiary amine, but this product does not react with the reagent and is easily separated.

$$2RMgX + HCO-N(CH_3)_2 \rightarrow R_2CH-N(CH_3)_2 + MgO + MgX_2$$

Other side reactions may result from the presence of another functional group in the halogen compound which also reacts with the Grignard reagent. Allylic compounds also present problems because of their rapid coupling reaction to form diallyls when the Grignard reagent is formed. The allylic Grignard reagents are usually prepared under special conditions that involve a large excess of magnesium, dilute solution, slow halide addition, and long reaction time in an inert atmosphere. The preparation of benzyl magnesium halides is usually accomplished with freshly distilled benzyl halides.

Another limitation of the Grignard reagent is the inertness of fluorine compounds and vinyl-type compounds with the chlorine atom on an unsaturated carbon atom. However, chlorobenzene does react if tetrahydrofuran is used as a solvent in place of ether and the selective use of solvents makes it possible to prepare a Grignard reagent with a bromine or iodine substituent on the ring while a second chlorine substituent does not react.

EXPERIMENTAL

Reagents. Halogen Compounds. All halogen compounds were Eastman Grade (highest purity) of Distillation Product Industries. They were used without further purification except as noted in the experimental section.

Magnesium. The magnesium turnings were from Dow Chemical and were listed at 99.9% pure. They were stored in a tightly-sealed bottle and used without further treatment.

Ether. Anhydrous, ACS grade was stored over calcium hydride in an amber bottle that was fitted with a drying tube containing Drierite.

Tetrahydrofuran. This solvent was regular commercial grade from E. I. du Pont de Nemours & Co., and was used without further purification. As with the ether, the residue was discarded and fresh material was used when the volume had decreased by one half.

Dimethylformamide. Merck reagent grade was used without further purification.

2,4-Dinitrophenylhydrazine Solution. (0.05*M* in 2*M* hydrochloric acid in methanol). This was prepared by dissolving 10 grams of Eastman grade 2,4-dinitrophenylhydrazine in 850 ml. of methanol, adding 170 ml. of concentrated hydrochloric acid with stirring, and filtering the solution.

Apparatus. Most of the reactions were carried out in a 25- × 150-mm. borosilicate glass test tube in which was inserted a water-cooled, 6-inch, semimicro finger condenser. In some cases, the bulb of a micro thermometer was kept in the reaction mixture and a 5-ml. hypodermic syringe used for slow addition of the solution of halide in ether. For allyl and cinnamyl halides, the syringe was replaced by a Teflon needle valve body to which was attached a hypodermic needle and a piece of glass tubing that permitted automatic dropwise delivery of the solution of halide in ether over a long time period. A combination magnetic stirrer and hot plate was used for simultaneous heating and stirring.

Formation of the 2,4-dinitrophenylhydrazones was carried out in a 250-ml. Erlenmeyer flask and their recrystallizations in a small test tube.

Melting points were determined in open capillary tubes in a Thomas-Hoover Uni-Melt apparatus and all melting points are corrected values.

Procedure. The object of this work was to devise a simple and rapid method that would yield sufficient solid derivatives for purification by crystallization. The methods outlined below do not necessarily represent optimal conditions for maximum yield.

Procedure *A*, Total Halide Addition in Ether. Into a clean 25- × 150-mm. borosilicate glass test tube that was thoroughly dried was placed 0.243 gram (0.1 gram atom) of magnesium turnings and 10 ml. of ether. A solution of 0.01 mole of the halide in 10 ml. of ether (0.1 mole) was added to the ether, together with a crystal of iodine. Crushing the magnesium with a flame-dried glass rod generally induced reaction. Gentle heating was used when crushing did not initiate reaction. The test tube was stoppered with a clean, dry, water-cooled semimicro finger condenser and the reaction was allowed to proceed until the vigorous boiling subsided. If considerable magnesium still remained, heating was continued until most of the magnesium reacted.

After the reaction mixture had cooled to room temperature, 0.8 ml. (0.1 mole) of dimethylformamide was added slowly with stirring. This initiated a very vigorous reaction and, in most cases, produced a gelatinous mass. This mass was transferred to a 250-ml. Erlenmeyer flask containing 200 ml. of the 2,4-dinitrophenylhydrazine solution. Precipitation of the phenylhydrazone usually occurred immediately. This solution was filtered, the precipitate washed and dried in a desiccator at room temperature, and then weighed. A portion was recrystallized three times from suitable solvents and the melting point determined.

Procedure *B*, Total Halide Addition in Tetrahydrofuran. This was identical with Procedure *A* except that tetrahydrofuran was used instead of ether as the solvent.

Procedure *C*, Slow Halide Addition in Tetrahydrofuran. This procedure was preferred where equipment and time permitted and because it generally produced better yields and reaction with some halides which were inert in Procedures *A* and *B*.

A 25- × 150-mm. borosilicate test tube was carefully flame dried, and cooled. In it was placed 0.243 gram (0.01 gram atom) of magnesium, a crystal of iodine, and a Teflon-coated magnetic stirring ball. The tube was clamped so that the bottom was about 1/4 inch from the top of a magnetic stirrer–hot plate. Into the neck of the tube was inserted a rubber stopper having a micro thermometer whose bulb was immersed in the reaction mass, a semimicro cold finger condenser, and a hypodermic syringe. A solution of 0.01 mole of the halide in 8.1 ml. (0.1 mole) of tetrahydrofuran was drawn into the syringe. (In some experiments the molar ratio of tetrahydrofuran to halide was 2 to 1). The stirrer was started, a few drops of ethyl bromide added, and about 5 ml. of the halide solution delivered. Reaction generally started immediately with the temperature rising to about 70° C. The remaining halide solution was added over a period of about 1/2 hour, after which reaction was allowed to continue for an additional 30 to 45 minutes. The reaction tube was then cooled to about

Table I. Data Obtained from Reactions of Aliphatic Halogen Compounds

Halide	Grignard product	2,4-Dinitrophenylhydrazone M.P., ° C.	Yield, %	Procedure	Lit. m.p. ° C.
1-Iodoethane	Propanal	149	32	A	155
1-Iodopropane	Propanal	124	61.5	A	123
1-Chlorobutane	Pentanal	107–9	20.3	A	106
1-Bromobutane	Pentanal	107–8	30	A	106
1-Iodobutane	Pentanal	102–3	10	A	106
2-Chlorobutane	2-Methylbutanal	129–30	35.5	C[e]	129–30
2-Chlorobutane	No reaction	...	...	A	...
2-Bromobutane	2-Methylbutanal	129–30	17	A	129–30
2-Iodobutane	Halide decomposed	...	...	...	...
1-Chloropentane	Hexanal	105–6	8.6	A	106
2-Chloropentane	2-Methylpentanal	112–14	32	C[e]	[a]
2-Chloropentane	No reaction	...	...	A	...
1-Bromohexane	Heptanal	103–4	25	A	106
2-Bromohexane	2-Methylhexanal	122–6	70	A	[b]
3-Bromohexane	2-Ethylpentanal	124–5	18.1	C	[c]
3-Bromohexane	2-Ethylpentanal	124–5	7.2	A	..
1-Chloroheptane	Octanal	106	5.5	A	106
1-Bromoheptane	Octanal	106	56	A	106
1-Iodoheptane	Octanal	103	21	A	106
1-Chlorodecane	Undecanal	106	51.5	A	106
1-Bromodecane	Undecanal	106	41.8	A	106
1-Iododecane	Undecanal	106	35	A	106
Allyl chloride[f]	No reaction	...	...	...	...
1-Chlorooctadecane	Nonadecanal	104–6	55	A	[d]
Isobutyl chloride	3-Methylbutanal	122–3	9	C	123
Isobutyl chloride	No reaction	...	...	A	...
tert-Butyl chloride	2,2-Dimethylpropanal	209	18.4	C	210
tert-Butyl bromide	No reaction	...	...	B and C	...
iso-Amyl chloride	4-Methylpentanal	91	7.5	A	99

[a] No literature value given. Calculated for $C_{12}H_{16}N_4O_4$: C, 51.40; H, 5.71; N, 20.0; Found: C, 52.04; H, 5.91; N, 18.6.
[b] No literature value given. Calculated for $C_{13}H_{18}N_2O_4$: C, 53.10; H, 6.12; N, 19.0; Found: C, 53.36; H, 6.24; N, 17.3.
[c] No literature value given. Calculated for $C_{13}H_{17}N_4O_4$: C, 53.10; H, 6.12; N, 19.0; Found: C, 53.14; H, 6.37; N, 18.15.
[d] No literature value given. Calculated for $C_{25}H_{42}N_4O_4$: C, 64.90: H, 9.10; N, 12.1; Found: C, 66.39; H, 9.52; N, 11.0.
[e] Ratio of moles of tetrahydrofuran to RX is 2 to 1.
[f] Freshly distilled from calcium sulfate prior to use. Boiling point 45° C.

Table II. Data Obtained from Reactions of Alicyclic and Side Chain Halogen Compounds

Halide	Grignard product	2,4-Dinitrophenylhydrazone M.P., ° C.	Yield, %	Procedure	Lit. m.p.,[a] ° C.
Bromocyclopentane	Cyclopentanal	162–4	31.5	A	160–2
Chlorocyclopentane	Cyclopentanal	162–3	25.5	A	160–2
Bromocyclohexane	Cyclohexanal	174	30	A	172
Chlorocyclohexane	Cyclohexanal	173–4	243	A	172
(2-Chloroethyl)-benzene	3-Phenyl-1-propanal	154	20	C	149–50
(2-Chloroethyl)-benzene	No reaction	...	...	B	...
(2-Chloroethyl)-benzene	No reaction	...	...	A	...
(2-Bromoethyl)-benzene	3-Phenyl-1-propanal	154	31.5	A	149–50
Chlorophenetole	No reaction	...	...	C	...
Benzyl chloride	Phenylacetaldehyde	120–3	20.3	C[a]	121
o-Chloro-*p*-xylene	*o*-Tolylacetaldehyde	156–7	28.4	C	...
Cinammyl chloride	No reaction	...	...	C	...
1,5-Dichloropentane	Azelaicdicarboxaldehyde	132–5	5.5	C	[b]
(1-Bromoethyl)-benzene	No reaction	...	...	A and C	...

[a] Distilled just prior to use. Boiling point 66–70° C. at 43 to 46 mm. Hg.
[b] No literature value given. Calculated for $C_{19}H_{20}N_8O_8$: C, 46.70; H, 4.10; N, 23.0; Found: C, 45.75; H, 4.64; N, 21.3.

50° C. and a solution of 0.77 ml. (0.01 mole) of dimethylformamide in 0.8 ml. (0.1 mole) of tetrahydrofuran was slowly added. Reaction was very vigorous and the temperature rose immediately to 70° C. After addition was completed and the reaction had subsided, the reaction mass was transferred to 200 ml. of 2,4-dinitrophenylhydrazine solution in a 250-ml. Erlenmeyer flask. Precipitation of the hydrazone usually occurred immediately and purification was effected by Procedure *A* above.

Gilman Color Test for Formation of Grignard Reagent. About 1 ml. of the reaction mixture from formation of the Grignard reagent was added to an equal volume of a 1% solution of Michler's ketone in dry benzene and the reaction product was then hydrolyzed by slow addition of 1.0 ml. of water. This was followed by addition of several drops of a 0.2% solution of iodine in glacial acetic acid, which gave a characteristic green-blue color as a sign of positive reaction (*8*).

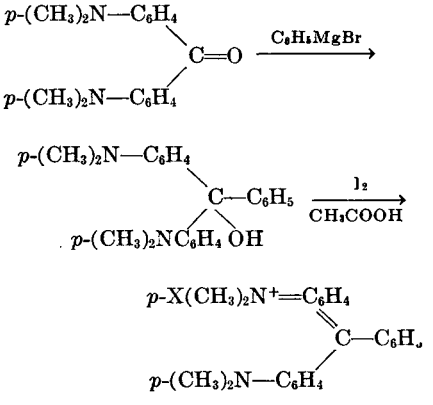
The data obtained are summarized in Tables I–III, which list the various aliphatic, alicyclic, aromatic side chain and nuclear substituted halogen compounds used, the aldehydes formed as intermediates, the percentage yields of their 2,4-dinitrophenylhydrazones, and their observed melting points, as well as the values in the literature.

DISCUSSION

Examination of Tables I–III shows that characterization of different types of organic halides by synthesizing aldehyde 2,4-dinitrophenylhydrazones was widely applicable but that it was subject to some drawbacks.

No reaction occurred with some compounds, even with the preferred Procedure *C* (*10*).

Reaction in Tables I–III refers to the nonformation of the Grignard reagent which was determined by the Gilman color test (*9*), and not the reaction with dimethylformamide or the conversion of aldehyde to dinitrophenylhydrazone.



With radicals other than phenyl in the Grignard reagent, the color in the Gil-

man test was due to the corresponding di- or triphenylmethane dye.

Halogen compounds that did not react are believed to have a halogen atom that was either inert, too reactive, sterically hindered, or possessed another reactive functional group.

In some cases, it was possible to bring about reaction by using tetrahydrofuran as the solvent. The higher boiling point and greater basic strength of tetrahydrofuran than that of diethyl ether may allow it to solvate and remove the Grignard reagent from the magnesium surface. The better solvent properties and higher boiling point of tetrahydrofuran also provided more favorable conditions for the Grignard reaction. The aliphatic compounds 2-chlorobutane, 2-chloropentane and isobutyl chloride did not react in ether but satisfactory results were obtained in tetrahydrofuran. Similarly, 3-bromohexane gave yields of 7.2% in ether and 18.1% in tetrahydrofuran.

The aromatic halogen compound chlorobenzene and the chlorotoluenes, which were unreactive in ether, gave good yields in tetrahydrofuran. This was also found with (2-chloroethyl)-benzene, which was inert when reaction was carried out by Procedures *A* and *B* but gave a 20% yield with Procedure *C*. This also indicated the desirability, where practicable, of slow addition of the solution of halide in ether to the magnesium.

The use of tetrahydrofuran as solvent did not induce successful reaction with inactive halides. Both fluorine and chlorine substituents on the benzene ring also produced negative results and no reaction was obtained with *o*-, *m*-, or *p*-fluorochlorobenzene or *o*- or *p*-trifluoromethylchlorobenzene. Fluorobromobenzene did react, but the reaction of trifluoromethylbromobenzene with magnesium followed by carbonation to the trifluoromethyl benzoic acid has been reported (*10*). 1-Chloronaphthalene was similarly inert, even though 2-chloronaphthalene and 1-bromonaphthalene reacted normally.

Allyl and cinammyl halides did not yield products with the procedures given above and the high reactivity of the halogen in these compounds may have produced coupling products. When these halides were freshly distilled, added slowly in very dilute solution to excess magnesium, and allowed to react over a long period of time, no reaction was obtained. Benzyl chloride was freshly distilled prior to use to obtain reaction.

Benzhydryl chloride did not yield a dinitrophenylhydrazone by any of these procedures and this was attributed to the high reactivity and side reactions of the allylic system, while the inability to produce a derivative with 1-chloronaphthalene was ascribed to the inertness of a halogen atom on an unsaturated carbon atom.

The failure to produce suitable derivatives by these reactions with the nitrochlorobenzenes may have resulted from reaction of the nitro group with the Grignard reagent or an electronic effect like the strong electron-withdrawing trifluoromethyl substituent in trifluoromethylchlorobenzene.

$$C_6H_5NO_2 + 4C_6H_5MgBr \rightarrow (C_6H_5)_2NH + (C_6H_5)_2 + C_6H_5OH$$

The failure to obtain a Grignard reagent with β-chlorophenetole was investigated by Grignard, who found that this halogen compound gave chiefly ethylene and the halomagnesium salt of phenol (*11*).

Excellent results were obtained in characterizing the several monohaloxylenes.

Aldehydes characterized for the first time as their 2,4-dinitrophenylhydrazones were 2-methylpentanal, 2-methylhexanal, 2-ethylpentanal, nonadecanal, azelaicdicarboxaldehyde, and *p*-ethoxybenzaldehyde.

Table III. Data Obtained from Reactions of Aromatic Halogen Compounds

Halogen compound	Grignard product	2,4-Dinitrophenylhydrazone M.P., °C.	Yield, %	Procedure	Lit. m.p., °C.
Bromobenzene	Benzaldehyde	234–5	58	B	237
Chlorobenzene	Benzaldehyde	238	79	C	237
Chlorobenzene	No reaction	...	...	A and B	...
Iodobenzene	Benzaldehyde	235–7	64	A	237
o-Chlorotoluene	*o*-Methylbenzaldehyde	192–4	28.5	C	193–4
o-Chlorotoluene	No reaction	...	...	A and B	...
m-Chlorotoluene	*m*-Methylbenzaldehyde	210–12	49	C	211
m-Chlorotoluene	No reaction	...	...	A	...
p-Chlorotoluene	*p*-Methylbenzaldehyde	239	19.5	C	235
p-Chlorotoluene	No reaction	...	...	A and B	...
m-Bromotoluene	*m*-Methylbenzaldehyde	208–10	35.5	A	211
m-Bromotoluene	*m*-Methylbenzaldehyde	208–10	53	C	211
p-Bromotoluene	*p*-Methylbenzaldehyde	230–2	30	B	235
p-Bromotoluene	*p*-Methylbenzaldehyde	236–8	28	A	235
o-Iodotoluene	*o*-Methylbenzaldehyde	195–6	49	C	193–4
4-Bromo-*o*-xylene	3,4-Dimethylbenzaldehyde	230–1	46.8	C	231
4-Iodo-*m*-xylene	2,4-Dimethylbenzaldehyde	220–2	51.6	C	221
2-Bromo-*m*-xylene	2,6-Dimethylbenzaldehyde	252	42	C	252
5-Bromo-*m*-xylene	3,5-Dimethylbenzaldehyde	243	48	C	244
2-Iodo-*m*-xylene	2,6-Dimethylbenzaldehyde	252–3	38.5	C	252
2-Bromomesitylene	2,4,6-Trimethylbenzaldehyde	251–2	52.5	C	250–1
p-Bromoanisole	*p*-Methoxybenzaldehyde	251–2	29	B	253
p-Bromoanisole	*p*-Methoxybenzaldehyde	250–2	40	A	253
p-Bromophenetole	*p*-Ethoxybenzaldehyde	233–5	24.5	A	
p-Iodoanisole	*p*-Methoxybenzaldehyde	252–3	28	C	253
1-Bromonaphthalene	1-Naphthaldehyde	259–61	71	A	254–5
2-Bromonaphthalene	2-Naphthaldehyde	271	65.4	A	271
1-Chloronaphthalene	No reaction	...	...	A and C	...
2-Chloronaphthalene	2-Naphthaldehyde	267–8	15.2	C	271
p-Chlorobromobenzene	*p*-Chlorobenzaldehyde	264–6	65	A	264–70
p-Fluorobromobenzene	*p*-Fluorobenzaldehyde	274–6	52.5	C	278
1-Chloro-2-fluorobenzene	No reaction	...	...	C	...
o-Chlorobenzotrifluoride	No reaction	...	...	C	...
m-Chlorobenzotrifluoride	No reaction	...	...	C	...
p-Chlorobenzotrifluoride	No reaction	...	...	C	...
p-Dibromobenzene	Terephthaldehyde	325	62.5	C	...
2,4-Dibromoanisole	4-Methoxyisophthaldehyde	300	57.4	C	...
m-Dibromobenzene	Isophthaldehyde	325	60	C	...
p-Nitrochlorobenzene	No reaction	...	...	A and C	...
1-Chloro-2-nitrobenzene	No reaction	...	...	...	...

[a] No literature value reported. Calculated for $C_{15}H_{14}N_4O_5$: C, 54.45; H, 4.24; Found: C, 54.48; H, 4.60.

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Determination of Beryllium by the Photoneutron Method

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▷ **Apparatus was designed and constructed for the determination of beryllium by the photoneutron method which can be employed in any analytical laboratory without hazard or difficulty. Because large $B^{10}F_3$ neutron counter tubes (2-inch) are used, the neutron detection efficiency of the instrument is sufficiently great so that a gamma source activity of only ~300 mc. of Sb^{124} is necessary for most samples. With this source activity sample solutions containing as little as 60 μg. of Be per ml., or 1.5 mg. of total beryllium, can be analyzed with a relative standard deviation of ±1%. Interference caused by elements which absorb neutrons can be eliminated by the cadmium shield technique. Deuterium is an interference if present in a 25-fold excess over beryllium. Results are shown for the determination of beryllium in several types of materials.**

SINCE the photoneutron method for the determination of beryllium was first introduced (*8, 9*), it has been extensively utilized to detect and measure beryllium in geological samples (*1–5, 17, 18, 20*), ore processing samples (*15*), and even in beryllium metal (*19*). Portable instruments, which have revolutionized beryllium prospecting methods, have been designed (*1, 2, 4, 10, 12, 14, 17, 18, 20*) and are commercially available.

In most respects the photoneutron reaction provides an ideal analytical method; it is rapid, nondestructive, and virtually free of interferences, and is far more versatile and reliable than the chemical methods used for beryllium analysis. This method is, therefore, potentially of great value as a laboratory technique because it is capable of determining beryllium over a wide concentration range and in a large variety of materials without prior separation of the beryllium. Instruments for applying the photoneutron method in the laboratory have been described by British (*13, 15*), Russian (*14*), French (*6*), and Italian (*7*) workers, but these have the disadvantages that large samples are required and a gamma source of high activity (1 to 1.5 curies) is necessary for adequate sensitivity. Consequently, work was undertaken to design and construct an instrument suitable for use in the average analytical laboratory. The primary objective was to devise an instrument which is simple to construct and operate, can be used for both solid and liquid samples, and provides adequate sensitivity with minimum radiation hazard. In addition, for our purposes, a certain amount of portability was desirable—i.e., when the source is removed, the instrument can be transported from place to place.

Excellent discussions of the principle of the photoneutron method and the considerations involved in designing an instrument are available (*2, 6, 11, 12, 14*) and will not be repeated here.

EXPERIMENTAL

Apparatus. A schematic diagram of the apparatus is shown in Figure 1, and details of the sample stage and container are presented in Figure 2. The electrical connections to the BF_3 tubes are connected by a single concentric cable to the high-voltage supply and pre-amplifier. Associated electronic equipment consisting of a high-voltage power supply (NJE Corp., Model S-324), linear amplifier (Atomic Instrument Co., Model 218), scaler (Atomic Instrument Co., Model 101B), and timer are placed in a 4-foot rack. Four inches of lead shielding was stacked around the apparatus which was placed on a table in a corner of the laboratory, and the table area was roped off.

Reagents. Standard beryllium solution, 10.0 mg. per ml., was prepared by dissolving 5.00 grams of beryllium metal in dilute HCl, then diluting the solution to 500 ml. with 10% HCl. Other solutions were prepared by appropriate dilution of the stock solution.

Procedure. INITIAL ADJUSTMENT. With the Sb^{124} source in place, set the band width at 0.5 megacycle, high voltage at 1600 volts, amplifier coarse gain to 16, fine gain to 1, pulse height selector at maximum, and count 1-minute backgrounds at decreasing pulse height discriminator settings. Stop when the counting rate becomes greater than 100 c.p.m. Then increase the high voltage by 100 volts and repeat, until 2000 volts is reached. Insert a sample in position (2 mg. of Be per ml. in 5-ml. cell) by loading the sample on the sample stage and slipping the stage over the source rod by means of 2-foot tongs, and obtain counting rates at the same settings as the backgrounds. From this data, instrument settings can be chosen at which the net sample counting rate is at a maximum and is independent, or almost independent, of high voltage and pulse height discriminator settings.

If there is any doubt that the initial gain settings are the best, repeat the adjustment procedure at other settings.

This adjustment should be repeated once a month.

CALIBRATION. Obtain net counting rates for standard beryllium solutions as follows. In the 5-ml. cells count standards ranging from 1.0 to 3.0 mg. per ml.; in the 25-ml. cells, 0.3 to 1.0 mg. per ml. standards; and in the 50-ml. cells, 0.1 to 0.5 mg. per ml. standards. Collect at least 10,000 counts in each case. Calculate an average calibration factor for each cell (c.p.m. per mg. per ml.).

SAMPLE ANALYSIS. Pipet a 5-, 25-, or 50-ml. portion of a sample solution containing between 0.1 and 3 mg. of beryllium per ml. into the ap-

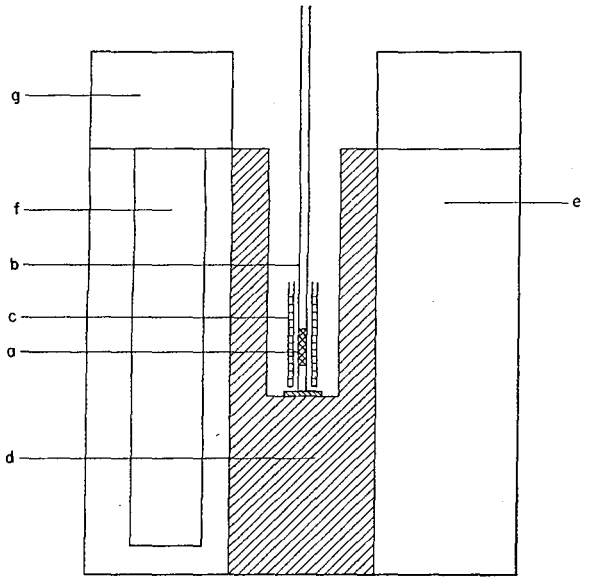

Figure 1. Schematic diagram of apparatus

a. Sb^{124} source, 40 mg. of 98% enriched Sb^{123} sealed in a quartz tube and packed in a $1\frac{1}{2} \times 0.25$-inch screw-cap aluminum can
b. Source holder, $\frac{5}{16}$ inch o.d. × 11-inch brass tube fixed to a circular base plate $1\frac{3}{4}$ inch diameter × $\frac{1}{8}$ inch thick
c. Sample container
d. Lead cylinder, 4 inches in diameter × 12 inches in height over-all, with a well 2 inches in diameter × 7 inches deep.
e. Moderator, 12 × 12-inch polyethylene cylinder with a central hole 4 inches in diameter, and 5 equally spaced 2-inch holes drilled at a radius of $3\frac{1}{2}$ inches.
f. $B^{10}F_3$ neutron counter tube. 5 tubes, 2 inches in diameter × 12 inches over-all length, 40-cm. pressure. Reuter-Stokes RSN-28A
g. Desiccated space containing BF_3 tube electical connections

propriate cell. Load the sample in the apparatus and count until at least 10,000 counts are collected. Calculate counts per minute and with the calibration factors determine milligrams of Be per milliliter.

RESULTS AND DISCUSSION

Evaluation of Apparatus. Although this apparatus, as well as other instruments designed for use in the laboratory, is fundamentally the same as Gaudin and Pannell's original (*9*), several improvements have been incorporated.

The use of enriched Sb^{123} as the source, as suggested by Milner, Edwards, and Henry (*16*), rather than natural antimony has several advantages. Thermal neutron activation of natural antimony produces high activities of Sb^{122} (Table I) which takes no part in photoneutron generation. Besides increasing the radiation hazard, the additional gamma flux can cause instrumental difficulties. Only a small weight of enriched Sb^{123} is necessary to produce sufficient Sb^{124} activity; 40 mg. of 98% enriched Sb^{123} irradiated for 14 days in the Oak Ridge Research Reactor produced about 400 mc. of Sb^{124}.

Polyethylene is used as the moderator rather than paraffin because of its better physical properties.

Increased sensitivity was achieved by the use of 2-inch BF_3 tubes instead of the 1-inch tubes normally employed. The larger tubes are about 5 times more efficient than the smaller. In tests with a calibrated Am^{241}-Be neutron source (4.5-m.e.v. average energy), the efficiency of the apparatus was 5.1%. However, the background is also greater, 40 c.p.m. compared to ~20 c.p.m. for 1-inch tubes, and the 2-inch tubes are somewhat more sensitive to gamma radiation. Because of the effect of gamma radiation on the tubes, a high voltage plateau may not be found when the instrumental operating conditions are evaluated (*11*, *14*). Figure 3 shows typical data which were obtained in the initial adjustment procedure with a beryllium solution in place. Most regulated high voltage supplies are sufficiently stable so that there is no drift in the period of time required for calibration and sample analysis.

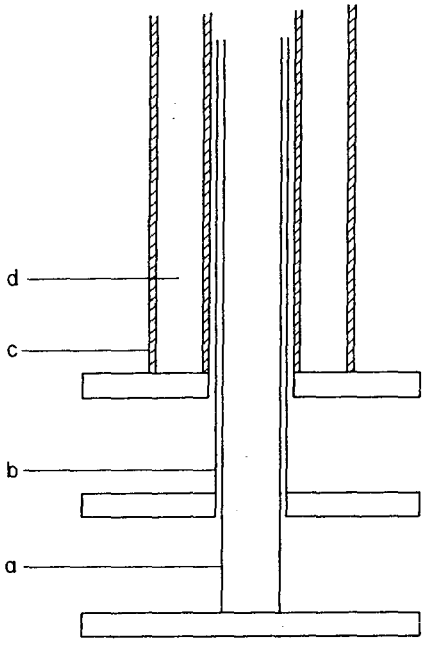

Figure 2. Sample stage and sample container

a. Source holder
b. Sample stage. Brass tube, $\frac{3}{8}$ inch o.d. × 14 inches in height fixed to a circular $1\frac{3}{4}$-inch diameter base plate having a central hole $\frac{3}{8}$ inches in diameter
c. Sample container, fused silica. Base plate $1\frac{3}{4}$ inches in diameter × $\frac{1}{8}$ inch thick having a central hole 1 cm. in diameter. Inner tube 10 mm. i.d. × 13 mm. o.d. × 3-inch height. Outer tube 3-inch height × 16, 25, or 35 mm. i.d. for capacities of 5, 25, and 50 ml., respectively
d. Sample

Table I. Calculated Activities[a] of Sb^{122} and Sb^{124} in Natural Antimony and 98% Enriched Sb^{123} after 14-Day Irradiation

Cooling time, days	Sb^{124}, mc.	Sb^{122}, mc. Natural Sb	Sb^{122}, mc. 98% Sb^{123}
0	315	4220	61
7	290	747	11
14	268	131	1.9

[a] Does not include resonance activation.

Table II. Approximate Radiation Level at Various Positions with a 300–400 mc. Source

Position	mr./hr.
Under table	20
Surface of Pb barrier	<10
Top of Pb barrier	100
Top of instrument	400
Top of source holder	2000

Radiation Hazard. Table II shows survey meter readings at various points around the installation. Some hazard is associated with the beamed gamma flux from the top of the source holder, but this is easily remedied with a 6-inch piece of lead rod. The operator is exposed to 100 mr. per hour on the hands and 50 mr. per hour at head level in changing samples, but the total exposure time is small, certainly less than 1 hour per week. In transferring a fresh source from the carrier to the source holder, precautions must be taken since the source activity is about 20 r. per hour at 1 foot. The transfer can usually be made in a few seconds with 3-foot tongs.

Sensitivity and Precision. The data in Table III were calculated from calibration data obtained with a source activity of 315 mc. and a background of 40 c.p.m. Sensitivity and precision depend only on counting

Table III. Sensitivity of Photoneutron Apparatus

Sample cell, ml.	K[a]	Sensitivity, mg. Be/ml.[b] 30 min.	60 min.	Equiv. to bkgd.
5	2.72	0.58	0.35	0.05
25	10.2	0.16	0.09	0.01
50	16.6	0.10	0.06	0.008

[a] C.p.m. per mg. Be/ml. per mc.
[b] Minimum amount which can be determined with a relative standard deviation of ±1% in the counting time indicated.

Table IV. Effect of Cadmium on the Determination of Beryllium

Ratio, Cd/Be	Relative error, % 5-ml.	25-ml.	50-ml. cell
60	+0.5	−2.3 (−0.9)[a]	−4.1 (−0.8)
120	+0.7	−3.7 (+0.6)	−5.2 (−0.6)
190	−2.0	−4.1 (+0.4)	−5.4 (−2.1)

[a] Values in parentheses obtained with cadmium shield in place.

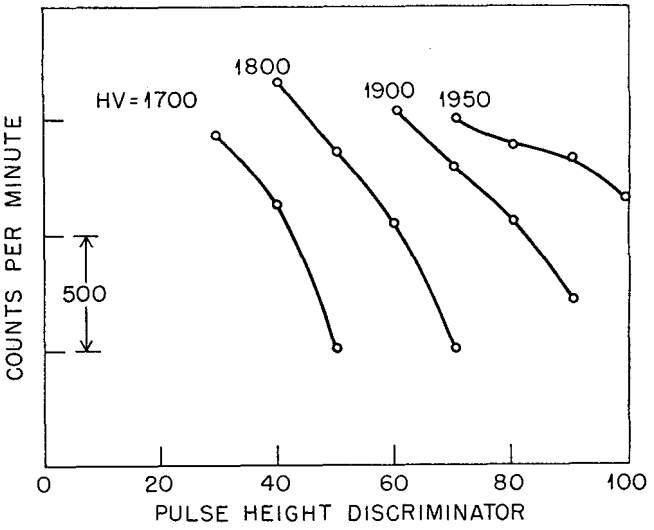

Figure 3. Count rate as function of high voltage and pulse height discriminator settings

Table V. Photoneutrons from Deuterium

Sample cell, ml.	C.p.m. per ml. of D_2O	Grams D_2O equiv. 1 mg. Be
5	75.8	2.4
25	57.8	2.3
50	46.0	2.4

Table VI. Application to Samples

Sample composition	Be, % Photo-neutron	Other method
$LiF-BeF_2-ZrF_4$	6.94, 6.90	6.91
$LiF-BeF_2-ZrF_4-ThF_4$	6.66, 6.63	6.87
BeO-MgO	19.2, 19.4	19.4, 19.5
$BeO-MgO-ThO_2$	30.4, 30.0	30.1
Be-Cu alloy	2.04, 2.06	...

statistics and can be recalculated for other source activities by using the K value. A minimum of about 1.5 mg. of beryllium is necessary under these conditions, but this can be decreased by using a source of higher activity, accepting a larger standard deviation, counting for a longer time, or using a smaller capacity cell.

The most sensitive instrument previously designed used 12 1-inch BF_3 tubes and gave a count rate of 400 c.p.m. per mg. of Be per curie (*11*). The same factor for this instrument is 544 c.p.m. using the 5-ml. sample cell and should be about 700 c.p.m. for a 1-ml. cell.

Effect of Other Elements. Potential interferences fall into two classes: elements having high thermal or epithermal neutron cross sections which would tend to absorb the photoneutrons, and elements having a high cross section for the (γ,n) reaction and might also release photoneutrons. Table IV shows the effect of an element with a high thermal-neutron cross section, cadmium (σ = 2500 barns), on the determination of beryllium. In these tests 20,000 counts were collected for both standard and sample, and so the relative standard deviation of the difference is ±1.0%, and therefore, a relative error of ±2.0% is significant at the 95% confidence level. The larger the volume of solution, the greater the interference because water moderates the photoneutrons which are originally 20-k.e.v. average energy. Results in parentheses were obtained with a 0.1-inch cadmium shield in place between the sample and the BF_3 tubes to screen out thermal neutrons (*9, 13, 16*). There is a loss in counting rate of about 15% with the shield in place, but the error caused by neutron absorbers is completely eliminated. Actually, only a few elements (Cd, Sm, Eu, Gd) have sufficiently high cross sections to interfere. Elements which were tested in a 20:1 ratio to Be and did not interfere are Li, K, Cu, Zn, Ba, Al, Zr, Sn, Pb, Bi, Fe, Co, Ni, Th, U, and B to the limit of solubility of H_3BO_3 in acid solution.

Only one isotope besides Be^9 is capable of undergoing a (γ,n) reaction with Sb^{124} gamma irradiation and that is deuterium. Samples of D_2O were placed in the apparatus, and the results are presented in Table V. A 24:1 weight ratio of D_2O to Be would only cause a 1% relative error in the determination of Be. These results do indicate, however, that the photoneutron method may be used to determine deuterium. With this apparatus, the sensitivity for D_2O in H_2O, for example (60-minute count, ±1% relative standard deviation, 50-ml. cell), is ~10% v./v., and by using a gamma source which has a greater flux at >2.2 m.e.v. (Co^{56}, for example), sensitivity could probably be increased by two orders of magnitude.

Application to Samples. The apparatus described has been used to determine beryllium in various types of samples as shown in Table VI, compared with results obtained by colorimetric methods after appropriate separations. In all cases the sample was first put into solution by the most convenient method and a portion of the solution analyzed directly without any separations by the recommended procedure. Counting times required were not greater than 10 minutes with a 300-mc. source. The relative standard deviation of duplicates was ±1.1%, slightly smaller than the theoretical value based on counting statistics.

CONCLUSIONS

As a laboratory method, the photoneutron method has the advantages of rapidity, versatility, and high precision compared to most chemical methods for beryllium analysis. Chemical separations are rarely necessary. Some colorimetric and fluorimetric methods, however, are more sensitive than the photoneutron method as described here. It is probably not practical in an ordinary laboratory to increase the sensitivity of the photoneutron method by using more than 500 mc. of Sb^{124}. The decay of the source can also be a drawback if beryllium determinations are only performed at irregular intervals.

Some mention should be made of the cost of this apparatus. Materials, including electronics and enriched Sb^{123}, are about $3000. Irradiation charges are $150 for 14 days, and for most purposes this 400-mc. source is useful for about 2 half-lives (120 days). Sb^{124} sources are also commercially available.

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Neutron Flux Monitoring for Activation Analysis of Oxygen

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▶ **A neutron flux monitor has been constructed which gives improved accuracy and speed in the activation analysis for oxygen. Oxygen in a piece of Lucite located near the neutron source and oxygen in the sample are activated simultaneously. Following irradiation, activities in this Lucite monitor and in the sample are counted simultaneously. The simultaneous irradiation and counting of monitor and sample make the analytical result independent of variations in neutron flux, durations of irradiation, sample transfer to counter, and counting periods. Monitoring accuracy is limited primarily by counting statistics, and relative standard deviations of 1% are achieved. For routine analysis, a sequence timer controls irradiation, sample transfer, and counting in a 45-second cycle, leaving only sample weighing and calculation for the complete analysis.**

A NUMBER of laboratories now employ neutron activation for a rapid, nondestructive analysis of total oxygen from part-per-million to per cent concentrations. In most procedures, accelerator sources provide 14-m.e.v. neutrons to induce the reaction

$$O^{16} + n \longrightarrow H^1 + N^{16}$$
$$N^{16} \xrightarrow{\text{beta decay with 6 to 7 m.e.v. gamma rays; half life, 7.36 seconds}} O^{16} \qquad (1)$$

Gamma rays from the decay of N^{16} nuclei are counted and compared with the N^{16} counts from a standard to give the weight of oxygen in the sample.

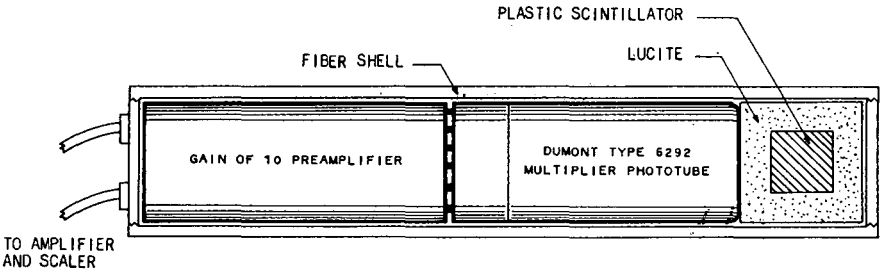

Figure 1. Monitor detector with preamplifier

$$\text{Wt. O in sample} = \frac{N^{16}\text{ counts in sample}}{N^{16}\text{ counts in standard}} \times \text{wt. O in standard} \qquad (2)$$

The sample and standard are normally irradiated and counted individually, and N^{16} counts from each must be corrected to identical conditions of irradiation. This correction requires monitoring of the neutron flux in each case and is usually accomplished by recording the counts from a neutron detector. Both BF_3 counters (*2*, *5*, *7*) and plastic scintillators to detect recoil protons (*3*) have been used as neutron detectors. As an additional measure of the neutron output, Veal and Cook (*7*) counted associated alpha particles from the $H^3(H^2, n)He^4$ reaction for producing neutrons. Steele and Meinke (*6*) employed an activation monitoring technique in which a Geiger tube detected N^{16} activity in cooling water flowing from the accelerator target.

A major problem in flux monitoring for oxygen analysis stems from variations in flux coupled with decay of N^{16} nuclei during the irradiation. Neutron output may vary several per cent because of fluctuations in deuteron beam current and shifting of the beam over portions of the target containing different concentrations of tritium. Since typical irradiations range from 10 to 30 seconds, significant decay of 7.36-second N^{16} occurs before the sample is counted. Where varying neutron output causes the rate of formation of N^{16} to fluctuate, the N^{16} decay rate at the end of irradiation is no longer proportional to the integrated neutron flux, even though the irradiation time may be kept constant.

SIMULTANEOUS ACTIVATION MONITOR

Our initial approach to the problem of monitoring a varying flux was based on simultaneous activation of the sample (or standard) and a small piece of oxygen-containing plastic. As the neutron output fluctuated, the rates of N^{16} formation and decay changed in exact proportion in the sample and plastic monitor so that the ratio

$$\frac{N^{16}\text{ count rate in sample at end of irradiation}}{N^{16}\text{ count rate in monitor at end of irradiation}}$$

was independent of changes in neutron flux during irradiation.

In most cases the monitor was a Lucite cap which fitted the sample bottle and was carried through the pneumatic transfer tube with it. Consecutive 15-second counts were made of the sample and monitor taking care to start and stop the counting periods according to a precise schedule. After subtracting backgrounds from each count, the weight of oxygen in the sample was obtained from a modified form of Equation 2.

A second monitor design is also based on the simultaneous activation of an oxygen-containing plastic but contains features which improve the sensitivity and accuracy of analysis and reduce analysis time. Construction and operation of the new monitor differ from the old in that:

The new monitor is fixed in position near the neutron generator during both irradiation and counting periods.

N^{16} beta rays, rather than gamma rays, are counted.

Sample and monitor activities are counted simultaneously instead of consecutively.

The third factor brings a considerable increase in flexibility of operation. As in the earlier monitor, simultaneous activation of sample and monitor ensures that the ratio of their N^{16} count rates at the end of irradiation will be independent of varations in neutron flux. By counting sample and monitor simultaneously, the ratio will also be independent of the times at which the counts are started and the duration of count. The need for precise timing is thus eliminated, and variations in sample transfer time have no effect on the result. Besides reducing over-all counting time for the analysis, prompt counting of the decaying monitor activity results in accumulation of more counts and lowers the statistical uncertainty of the ratio.

In-place activation monitors based on short-lived isotopes have been used previously to record flux levels of pulsed-neutron sources. Ruby and Rechen (*4*) counted gamma rays of 0.82 second Pb^{207m} from reactions of 14-m.e.v. neutrons on natural lead, and Byerly (*1*) described a counter to record the beta decay of N^{16} from the O^{16} (n, p) reaction in water. In our design (Figure 1), the oxygen to be activated is contained in a Lucite mantle surrounding the detecting element, a 1-inch diameter by 1-inch high cylinder of Nuclear Enterprises Type NE 102 plastic scintillator. Energies of beta-ray groups from N^{16} extend to 10.3 m.e.v. or a range in Lucite of 2.2 inches so that counts are recorded even by some of those beta rays originating in the outermost portion of the Lucite mantle. The scintillator is shielded from beta-emitting activities of the phototube glass or the fiber shell by the Lucite mantle. A measurement of the decay of the monitor activity shows that it follows the 7.36-second half life of N^{16} over three decades to background. Detector efficiency for gamma rays (from activated components of the monitor or nearby neutron generator) is low enough that interference from gamma-ray pulse pile up is not observed.

The monitor is fixed in position with the scintillator element about 8 inches from the neutron generator target. Signal and power supply cables connect

$$\text{Wt. O in sample} = \frac{\left(\frac{N^{16}\text{ counts in sample}}{N^{16}\text{ counts in monitor}}\right)_{\text{unknown}}}{\left(\frac{N^{16}\text{ counts in sample}}{N^{16}\text{ counts in monitor}}\right)_{\text{standard}}} \times \text{wt. O in standard} \qquad (3)$$

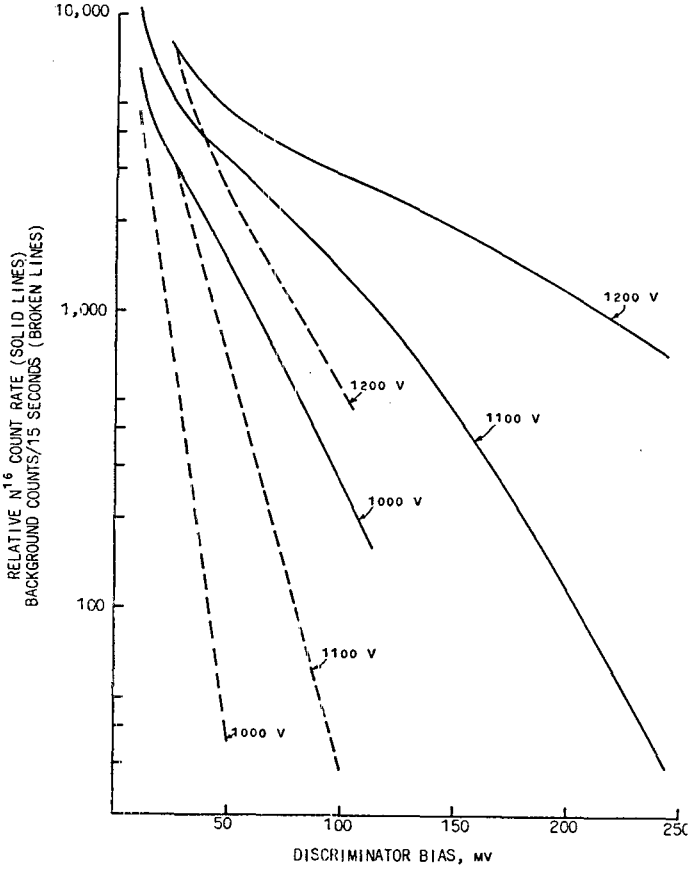

Figure 2. Discriminator bias curves

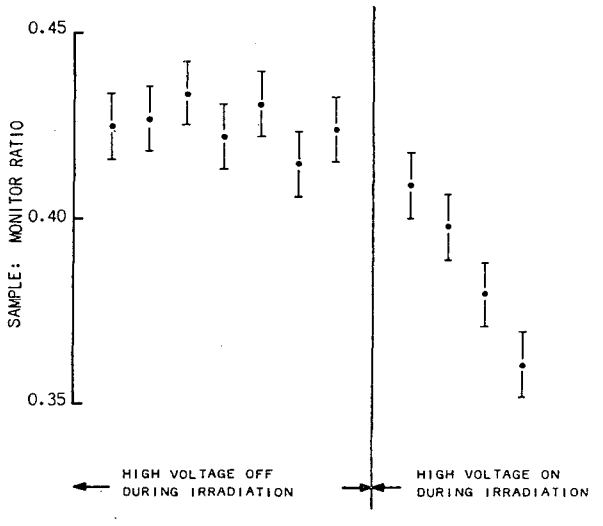

Figure 3. Successive irradiations showing phototube gain shift

Table I. Effect of Variables on Sample to Monitor Ratio

Variable	t (sec.)	Sample to monitor ratio	$\sigma_{exp.}$	$\sigma_{obs.}$
Irradiation period	5	0.417	0.0062	
	10	0.431	0.0053	
	15	0.442	0.0050	
	20	0.427	0.0046	0.0069
	25	0.418	0.0045	
	30	0.433	0.0045	
Transfer time	5	0.421	0.0062	
	7	0.431	0.0070	
	10	0.421	0.0077	0.0095
	12	0.437	0.0086	
	15	0.427	0.0102	
Counting period	5	0.420	0.0068	
	10	0.421	0.0054	
	15	0.426	0.0049	0.0040
	20	0.427	0.0046	
	25	0.429	0.0045	
Neutron flux		0.441	0.0075	
		0.417	0.0073	
		0.435	0.0074	0.011
		0.446	0.0073	
		0.420	0.0071	
		0.431	0.0073	

Table II. Sample to Monitor Ratios for Typical Samples

Oxygen, 200 p.p.m.			Oxygen, 0.418%		
Sample to monitor ratio	$\sigma_{exp.}$	$\sigma_{obs.}$	Sample to monitor ratio	$\sigma_{exp.}$	$\sigma_{obs.}$
0.0522	0.0026		0.355	0.0061	
0.0488	0.0024		0.349	0.0060	
0.0538	0.0026		0.351	0.0060	
0.0472	0.0023	0.0025	0.339	0.0058	0.0052
0.0498	0.0024		0.353	0.0061	
0.0511	0.0025		0.346	0.0059	
0.0548	0.0027		0.347	0.0060	
0.0508	0.0025		0.344	0.0059	

the monitor preamplifier with the amplifier and scaler 30 feet away in the counting area. Optimum operating conditions for the monitor amplifier were determined from curves (Figure 2) of N^{16} counts *vs.* discriminator bias settings for various phototube high voltages. The operating point chosen was a high voltage of 1100 volts and a discriminator bias of 100 mv. These conditions give a negligible background and a relatively low rate of change of count rate with discriminator level. Variation in count rate due to high voltage and discriminator drift is less than 3% over a 6-hour period.

During irradiation, high voltage to the phototube is turned off. If the high voltage is left on, a recoil proton count rate in the scintillator of about 2×10^6 c.p.s. causes substantial phototube gain shift. An example of the shift in sample to monitor ratio resulting from such a gain shift is shown in Figure 3.

The predicted independence of the sample to monitor ratio *vs.* variations in neutron flux and duration of irradiation, transfer, and counting times was confirmed by varying each of these factors and measuring several values of the ratio (Table I). Expected standard deviations ($\sigma_{exp.}$) given in Table I were determined from counting statistics only. Observed standard deviations ($\sigma_{obs.}$) for variation of irradiation and counting periods and transfer time correspond approximately to these expected standard deviations. For the set of observations where neutron flux is varied, $\sigma_{obs.}$ is about 50% greater than $\sigma_{exp.}$ Flux variation was produced by deliberately oscillating the beam current between 40 μa. and 100 μa. several times during the run; attendant shifting of the beam spot on target changed irradiation geometry and contributed to the additional uncertainty.

In Table II, sample to monitor ratios are given for samples containing 200 p.p.m. and 0.418% oxygen. The position of the monitor was not the same for the two series of observations so that concentration and ratio are not in the same proportion. Here, $\sigma_{obs.}$ corresponds closely to $\sigma_{exp.}$, indicating that the precision of analysis is limited primarily by counting statistics. In the typical irradiation where 10^4 monitor counts are accumulated, expected relative standard deviation of the monitor value would be 1%.

IRRADIATION AND COUNTING PROGRAMMER

While the irradiation, transfer, and counting times may be completely arbitrary through use of the monitor, control of these functions by a sequence timer has proved convenient for routine analysis. A small synchronous motor driving six cams operates microswitches for the following operations:

Time, seconds	
0	Turns beam on
13	Resets scalers
15	Turns beam off, turns monitor high voltage on, turns transfer air on
19	Turns transfer air off
20	Turns sample and monitor counters on
40	Turns sample and monitor counters off
43	Turns monitor high voltage off

To reduce statistical uncertainty, most samples are irradiated and counted five or six times. Sample weighing and calculation brings the total analysis time to about 10 minutes. This technique has been used to analyze for oxygen contents from 20 p.p.m. to 35%. At the lowest concentrations, a 50- to 70-gram sample is irradiated and counted; but when the oxygen content is 1% or more, samples as small as 10 mg. can be analyzed.

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A Spectrophotometric Study of the Complexes Formed between Uranyl and Thiosulfate Ions in Water and Water–Ethanol Solvents

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▷ Using the method of continuous variations at 25° C., the complex $UO_2S_2O_3$ was proved to exist in dilute aqueous solutions and in dilute solutions in 30-, 60-, and 90-volume per cent ethanol. The complex $(UO_2)_3(S_2O_3)_2^{+2}$ was found by the same method in more concentrated aqueous solutions. The equilibrium constants for the dissociation of $UO_2S_2O_3$ in the various solvents were determined at 25° C. and the free energy of the process was calculated. In water and 60 volume per cent ethanol the equilibrium constant for the dissociation of this complex has been determined at three or more temperatures and the thermodynamic functions $\Delta F°$, $\Delta H°$, and $\Delta S°$ have been calculated. The limit of identification and the limit of dilution for the detection of uranyl ion by a thiosulfate spot test have been investigated, and some of the ions that interfere with the test listed.

In studying the activation energy for the reaction between iodide and persulfate ions in the presence of different salts, Indelli and Amis (*5*) noted an accelerating effect of uranyl ion upon the reaction. These observers also noted that uranyl ion markedly catalyzed the reaction between persulfate and that the spectrum of UO_2^{+2} ion was strongly modified by the presence of thiosulfate. They attributed these effects to the formation of a complex between uranyl and thiosulfate ions.

Hefley and Amis (*4*) observed that UO_2^{+2} ions formed UO_2Cl^+, UO_2Cl_2, and $UO_2Cl_3^-$ complexes with chloride ions, depending on the concentrations of the ions and on the solvent used. It was felt that the complexes between UO_2^{+2} and $S_2O_3^{-2}$ ions and the equilibrium constants for their dissociations should be investigated at different concentrations of the ions and in different solvents.

The equilibrium constants for the complexes were determined using the preferable method of Foley and Anderson (*2*). In this method the calculations of the equilibrium constants are based on comparisons made in solutions of equal or closely similar absorbances.

EXPERIMENTAL

The instruments and procedures used for determining the empirical formulas and equilibrium constants were those described by Hefley and Amis (*4*), and the uranyl perchlorate solutions were prepared and analyzed in the same manner as described by those authors. The $NaClO_4$ was Mallinckrodt Analytical Reagent which was dried and used without further purification. The Baker Analytical Reagent grade $Na_2S_2O_3$ was used without further purification but was standardized using KIO_3.

The compounds used in determining the cation and anion interferences with the color given by the complex were either Baker or Fisher Analytical Reagents and were used without further purification.

Solutions of the above compounds were prepared at the different temperatures used in the experiments with distilled water which had been deionized by passing through an ion exchange column containing a mixed bed of both anion and cation exchangers, previously generated in separate tubes (*3*). The water prepared in this way had a specific conductance of 3 to 5 $\times 10^{-7}$ mhos. The absolute ethanol used was further purified by the method of Smith (*9*).

The accuracy of the absorption values was found to be ±0.2% by using the same volumetric delivery and dilution techniques as in the experimental runs on a solution of potassium chromate as described by the National Bureau of Standards (*10*). The precision was found to be ±0.2% by repeating the preparation and also by repeated measurements on the same sample.

The data for the equilibrium constants were neither as precise nor as accurate as those for the empirical formulas as is illustrated in Tables I and II. These phenomena are discussed by Hefley and Amis (*4*).

Table I. Equilibrium Constants and Thermodynamic Functions of $UO_2S_2O_3$

Vol. % ethanol	Temp., ° C.	Wavelength range, mμ	Av. value of K	$\Delta F°$ cal./mole	$\Delta H°$ cal./mole	$\Delta S°$ cal./ mole/deg.
0	15	325–345	$3.36 \pm 0.08 \times 10^{-3}$	3290	18,500	53.0
0	20	325–345	$5.14 \pm 0.36 \times 10^{-3}$	3070	18,500	52.9
0	25	325–345	$9.06 \pm 1.52 \times 10^{-3}$	2750	18,500	53.0
30	25	320–450	$5.17 \pm 0.29 \times 10^{-4}$	4480	...	...
60	20	320–350	$1.07 \pm 0.05 \times 10^{-5}$	6680	12,000	18.1
60	25	320–350	$1.45 \pm 0.07 \times 10^{-5}$	6600	12,000	18.0
60	30	320–350	$2.00 \pm 0.00 \times 10^{-5}$	6520	12,000	18.0
60	40	320–350	$3.99 \pm 0.13 \times 10^{-5}$	6300	12,000	18.1
60	20	420–430	$1.19 \pm 0.05 \times 10^{-5}$	6610	18,800	41.4
60	25	420–430	$1.83 \pm 0.04 \times 10^{-5}$	6460	18,800	41.2
60	30	420–430	$3.10 \pm 0.10 \times 10^{-5}$	6250	18,800	41.2
60	40	420–430	$9.04 \pm 0.00 \times 10^{-5}$	5800	18,800	41.4
90	25	350–370	$3.91 \pm 0.51 \times 10^{-6}$	7380	...	...
90	25	430–465	$5.54 \pm 1.40 \times 10^{-7}$	8540	...	...

Table II. Equilibrium Constants for the Dissociation of $UO_2S_2O_3$ in 60 Volume Per Cent Ethanol at 25° C. as Function of Wavelength

λ in mμ	$K \times 10^5$
320	1.34
330	1.51
340	1.51
350	1.42
Av.	1.45 ± 0.07^a
420	1.80
424	1.80
430	1.90
Av.	1.83 ± 0.04^a

[a] Precision numbers are average deviation from average.

EMPIRICAL FORMULAS OF THE COMPLEXES

Since more than one complex was to be sought and their empirical formulas were to be determined, the method of continuous variations described by Vosburgh and Cooper (*11*) was used. It was necessary, however, to assume complexes with different general formulas than the general formulas assumed by Vosburgh and Cooper. Let

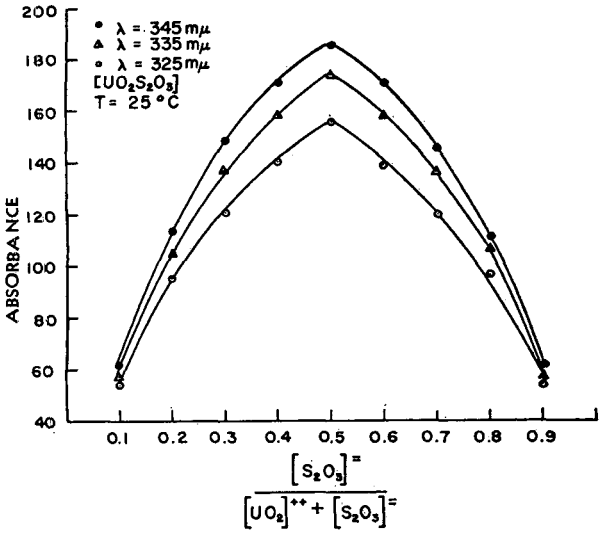

Figure 1. Ionic species in water in dilute solutions

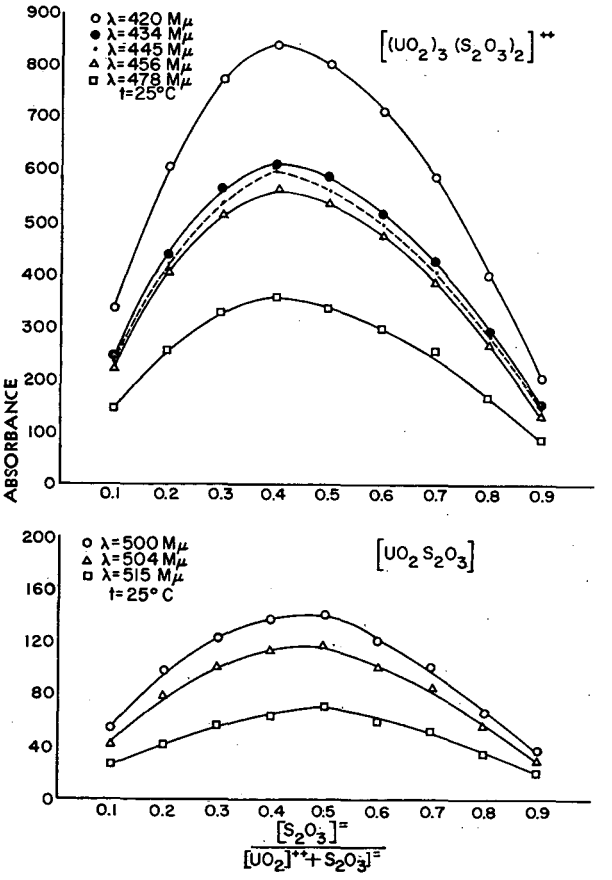

Figure 2. Ionic species in water in more concentrated solutions

the following be the reaction for the formation of a single complex:

$$nA + mB \rightleftarrows A_nB_m \qquad (1)$$

where A represents the UO_2^{+2} ion and B the $S_2O_3^{-2}$ ion. To determine n and m, solutions of the uranyl and thiosulfate ions of the same molar concentrations are mixed in varying proportions and the absorbances of the resulting solutions are measured. Let the concentration of each of the two solutions be M moles per liter, the mixtures be made by the addition of $(1-X)$ ml. of the solution of thiosulfate ion to X ml. of the solution of uranyl ion, and the total volume of the solution be kept constant at 1 ml. If C_1, C_2, and C_3 represent the equilibrium concentrations of $S_2O_3^{-2}$, UO_2^{+2}, and $[(UO_2)_n(S_2O_3)_m]^{(2n-2m)+}$, respectively, the following equations apply for any mole ratio of A to B

$$C_1 = M(1 - X) - mC_3 \qquad (2)$$

$$C_2 = MX - nC_3 \qquad (3)$$

$$C_1^m C_2^n = KC_3 \qquad (4)$$

Following the Vosburgh and Cooper procedure it can be shown that

$$n = \frac{mX}{1 - X} \qquad (5)$$

Vosburgh and Cooper assume the following reaction for the formation of a single complex

$$A + nB \rightleftarrows AB_n \qquad (6)$$

which resulted in the relationship

$$n = \frac{X}{1 - X} \qquad (7)$$

It can be seen that if m is one in Equation 1 then m is one in Equation 5 and the results presented here become identical with the results of Vosburgh and Cooper. Equation 5 is a rearranged form of an equation obtained by Woldbye (*13*). This confirms the correctness of our method and lends support to the development of Equation 13 by extension of our procedure.

If a second complex is formed according to the equation

$$A_nB_m + qA \rightleftarrows A_{(n+q)}B_m \qquad (8)$$

and the concentration of the second complex is C_4, then terms in C_4 must be added to Equations 2 and 3 and an additional equation included, so that the new set of equations become

$$C_1 = M(1 - X) - mC_3 - mC_4 \qquad (9)$$

$$C_2 = MX - nC_3 - (n + q)C_4 \qquad (10)$$

$$C_1^m C_2^n = KC_3 \qquad (11)$$

$$C_3 C_2^q = K'C_4 \qquad (12)$$

and by a proper manipulation of the equations, and by making similar assumptions to those made by Vosburgh and Cooper, it can be shown that

$$\frac{n}{m} = \frac{X}{1 - X} + \frac{qC_4(n + q + m - 1)}{M(1 - X)} \qquad (13)$$

For the formation of the second complex, Vosburgh and Cooper assume the following reaction,

$$AB_n + qB \rightleftarrows AB_{(n+q)} \qquad (14)$$

which yields

$$n = \frac{X}{1 - X} + \frac{qC_4(n + q)}{M(1 - X)} \qquad (15)$$

Again it is seen that if m is one as assumed by Vosburgh and Cooper, then Equation 13 reduces to Equation 15. Our formulas are more general. Vosburgh and Cooper's formulas are for the particular case when $m = 1$.

Katzen and Gebert (*6*) likewise extended the Vosburgh and Cooper formula. In their case the extension was to the formation of a third complex, but always limiting the molecular composition of any complex to one molecule of substance A.

If the stability of the complex ion is such that when C_3 is a maximum C_4 is small, then n/m can be found experimentally. Similarly, $(n + q)/m$ can be found from the composition corresponding to a maximum in C_4, provided that at the maximum C_3 is small.

If three complex ions can be formed from the same two components, and if they are stable enough, the treatment given here can be extended so as to apply.

DATA AND DISCUSSION

The results of the spectropho ometric investigations of the uranyl ion–thiosulfate ion complexes are presented in graphical form in which the absorbance is plotted against the mole fraction of thiosulfate ion in the complexing components.

In dilute aqueous solutions of 2×10^{-4} to $18.00 \times 10^{-4}M$, maxima occurred at a 1:1 ratio of complexing components at 325, 335, and 345 mμ. These data correspond to a formula $UO_2S_2O_3$ for the complex. The data are reproduced in Figure 1.

In more concentrated solutions, 0.0020 to 0.0180M, maxima were observed at 420, 434, 445, 456, and 478 mμ at a 2:3 ratio of thiosulfate ion to uranyl ion, corresponding to the empirical formula $(UO_2)_3(S_2O_3)_2^{+2}$. Maxima were also found at 500, 504, and 515 mμ corresponding to a 1:1 ratio of complexing components and representing an empirical formula $UO_2S_2O_3$. These data are shown in Figure 2.

In 30% by volume ethanol, maxima were observed at 320, 330, 340, 350, 420, 430, 440, and 450 mμ. The solutions were dilute, 2×10^{-4} to $18 \times 10^{-4}M$ and the maxima corresponded to an empirical formula $UO_2S_2O_3$.

In 60% by volume ethanol, maxima in dilute solutions corresponding to the empirical formula $UO_2S_2O_3$ occurred at 320, 330, 340, 350, 420, 424, and 430 mμ.

Using dilute solutions in 90% by volume ethanol, maxima corresponding to an empirical formula $UO_2S_2O_3$ were observed at 350, 360, 420, 440, 450, and 465 mμ.

With reference to the $UO_2S_2O_3$ complex, the difference in the number of absorption bands and the difference in wavelength at which the bands occur as the proportion of ethanol in the solvent increases probably arise from the influence of changing solvation on the electronic oscillations and the atomic vibration frequencies present in the complex. Presumably the nature and the extent of solvation of the solute changes as the proportion of ethanol in the solvent varies. Solvation arises from bonding of some nature and as the solvent varies, the nature of this bonding no doubt alters to some extent, and the spectrum will correspondingly be modified.

In all these measurements the ionic strength of the samples was held constant, and the pH was maintained between 4.08 and 4.88. The ionic strength in dilute aqueous solutions and in all water–ethanol solutions at all temperatures was held constant at 6.33×10^{-3}. In the more concentrated aqueous solutions the ionic strength was maintained at 0.133.

At all wavelengths in the range $\lambda =$ 320 mμ to $\lambda =$ 600 mμ, the difference in the absorbance between the sample and its blank at pH 2.15 was the same as the difference between the sample and its blank at pH 4.58. Moreover, the spectra of the two samples at the two pH values were practically identical, as were the spectra of the two uranyl perchlorate blanks. The difference in the acidity was obtained by adding perchloric acid in the correct amount to the stock $UO_2(ClO_4)_2$ solutions to obtain the desired pH. The ionic strength of all samples used in absorbance measurements was the same. From the data of Mathews, Hefley, and Amis (*7*) on the hydrolysis of UO_2Cl_2, the uranyl ion UO_2^{+2} is 15% hydrolyzed in the solution at pH 4.58 and 2% hydrolyzed in the solution at pH 2.15. The fact that the difference in the absorbance between the sample and its blank is the same at the two pH values, and the fact that the respective spectra were practically identical at the two values of pH were interpreted to mean that the same complex and the same amount of complex were formed at these two widely separated pH values in spite of the difference in the extent of hydrolysis of the UO_2^{+2} ion in the two cases. The hydrolysis effects in the actual runs should therefore be insignificant since the pH range was less than one pH unit in these cases.

Rush, Johnson, and Kraus (*8*) treat hydrolysis schemes involving species higher than dimers. But since the degree of hydrolysis did not seem to affect our results, the monomer⇌dimer and other hydrolysis equilibria were justifiably neglected.

It was found that in the pH range from 4.00 to 5.00, the samples were stable for such a time as to permit temperature equilibration and spectrophotometric measurements. Hydrogen ion concentrations in aqueous solutions were determined from pH measurements using a Beckman Model G pH meter. It was assumed that the hydrogen ion concentrations were the same as the molar concentrations of acid in all water–ethanol solutions. In order to keep the pH of the samples within the range indicated, the stock solution of uranyl perchlorate was titrated with NaOH until the pH of the stock solution was such that when the solutions to be studied were made up, their pH values fell within the range 4.08 to 4.88 as determined in aqueous solution by the pH meter. These solutions were also stable when ethanol was made a part of the solvent.

Equilibrium constants for the dissociation of the $UO_2S_2O_3$ complex at various solvent compositions, temperatures, and wavelengths are presented in Table I. Each of the recorded values is the average of the values of K measured at from 3 to 9 wavelengths of light. In 60 volume per cent ethanol the values of K at 320, 330, 340, and 350 mμ are averaged and those at 420, 424, and 430 mμ are averaged. This was done because there was an increasing divergence of the average values of K in the two wavelength regions as the temperature was raised, and because the precision in each wavelength region was much greater than between the two regions. In addition, there is an asymmetry of the absorbance curves in the 420- to 430-mμ region, due perhaps to a slight absorption in this region by a second complex which probably occurs in limited concentration and hence is not detected. An example is shown in Table II which contains typical data.

The other precision numbers at the compositions of solvent indicated are given to show that the deviations in K in the two wavelength regions are experimentally real.

The data in 90 volume per cent ethanol likewise show two values of K as determined experimentally in the two wavelength regions.

The dependence of K on volume per cent ethanol at 25° C. is shown in Figure 3. The experimentally permissible variation of K in 90 volume per cent ethanol is included in the figure to show that the experimental variation of K is real. Hefley and Amis (*4*) found that the value of K for the UO_2Cl^+ complex depended strongly on solvent composition and exhibited a maximum between 60 and 90 volume per cent ethanol. In the present case at 25° C. there is a linear decrease of K with increasing volume per cent ethanol down to 60 volume per cent ethanol. The curve then splits depending on the wavelength region chosen. One branch of the curve continues on in a linear fashion, and the other branch curves upward. In each of the solvents, 60 and 90 volume per cent ethanol, one of the two values of K is probably not real. For example, at 25° C. and 90 volume per cent ethanol the real value of K is perhaps the value that is linear with the values of K at the other solvent compositions. Hence the value in the 350- to 360-mμ region is obtained no doubt owing to the influence in this wavelength region of the second complex on the absorbance measurements. This complex or some other influence does affect the absorbance measurement for the $UO_2S_2O_3$ complex as is shown in Figure 4, where the left hand sides of the absorbance curves at 350 to 360 mμ are seen to be concave upward whereas the absorbance curves at 420 to 465 mμ are seen to be more symmetric. Certain limitations prevented our studying the higher complex in greater percentages of alcohol, but it is reasonable to suppose that, even in dilute solutions, the higher complex would be more pronounced than in lesser percentages of alcohol. In the greater percentages of alcohol, the dielectric constant would be lower and the forces between complexing particles greater, thus favoring higher complex formation.

The free energies $\Delta F°$ of dissociation of the $UO_2S_2O_3$ complex were calculated from the equilibrium constants in water

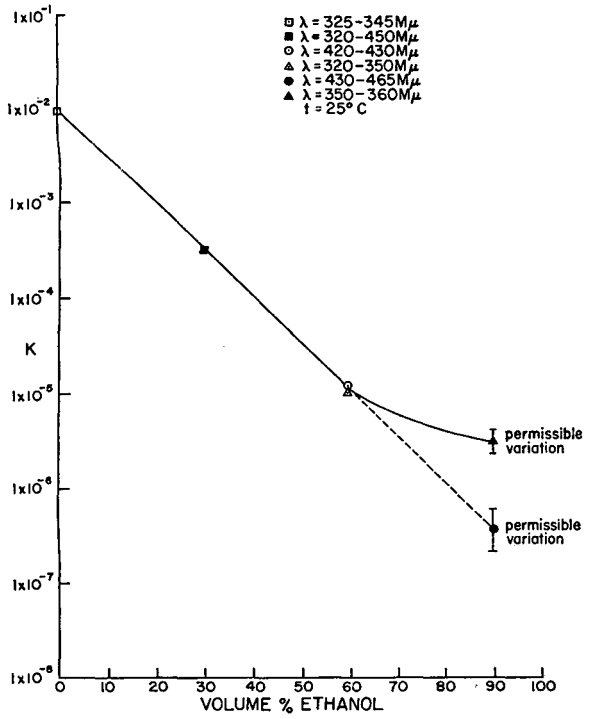

Figure 3. Dependence of equilibrium constant for $[UO_2S_2O_3]$ on nature of solvent

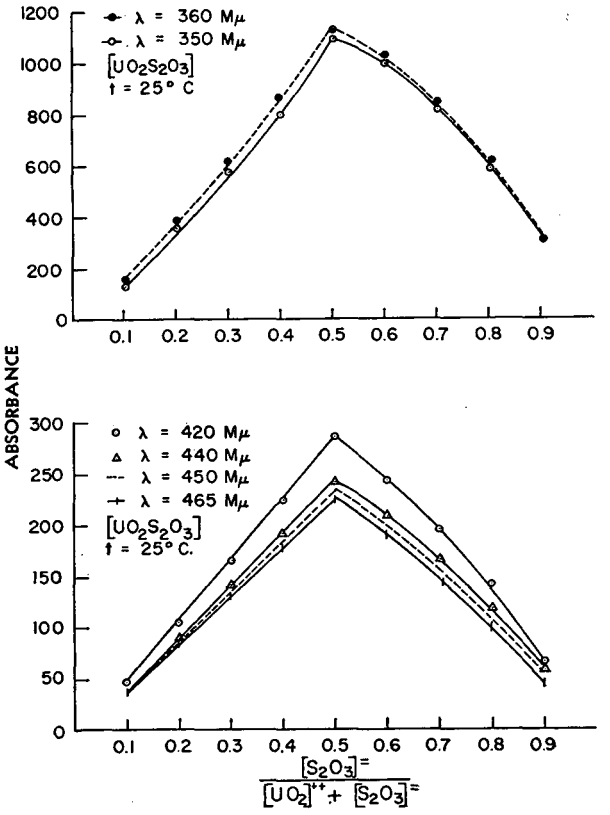

Figure 4. Ionic species in 90% by vol. ethanol

and 60 volume per cent ethanol solvents using the equation

$$\Delta F^\circ = -RT \ln K \qquad (16)$$

and the enthalpies ΔH° of dissociation were calculated by multiplying the slope of the log K *vs.* $1/T$ plots shown in Figure 5 by $-2.303\ R$ using the equation

$$\log K = -\frac{\Delta H}{2.303RT} + \text{constant} \qquad (17)$$

The entropies ΔS° of dissociation were calculated using the equation

$$\Delta F^\circ = \Delta H^\circ - T\Delta S^\circ$$

The values of these thermodynamic quantities for water and 60 volume per cent ethanol are listed in Table I. Since there were two values for K at each temperature in the two wavelength regions in the case of 60 volume per cent ethanol, there are two sets of values for ΔF°, ΔH°, and ΔS°. It is probable that the values of these quantities in the 420- to 430-mμ region is real since the K values in this region were linear with the K values in other solvents. The values of ΔF°, ΔS°, and ΔH° for 60 volume per cent ethanol in the 320- to 350-mμ region are perhaps influenced by the effect of the higher complex on the experimental determination of K as discussed previously. Since 30- and 90-volume per cent solvents were investigated at 25° C. only, the thermodynamic functions ΔH° and ΔS° were not calculated in the case of these solvents.

The dark yellow color resulting from

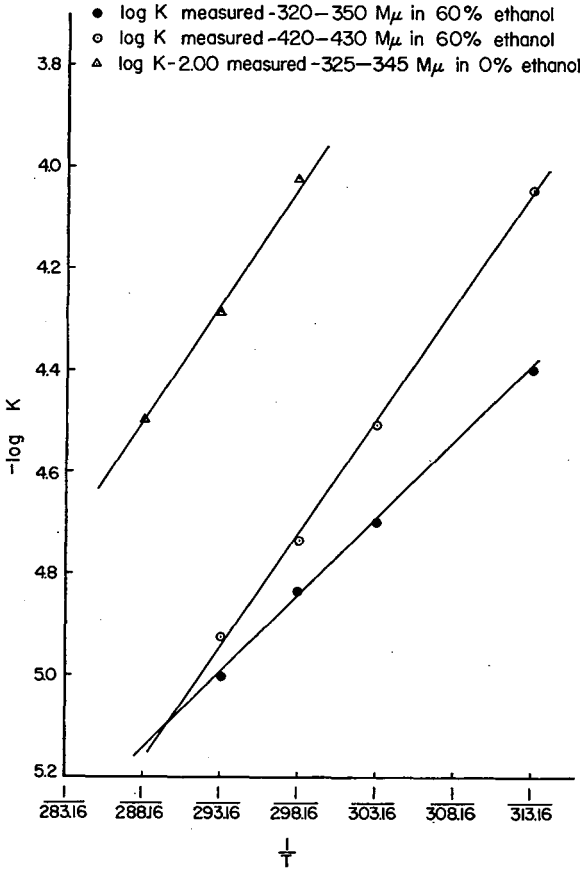

Figure 5. Dependence of equilibrium constant for $[UO_2S_2O_3]$ on temperature

the formation of the uranyl–thiosulfate complex was studied as a possible spot test for uranyl ion. The limit of identification and the limit of dilution in aqueous solution were determined using a spot plate technique (*12*). The limit of identification was found to be 10 μg., and the limit of dilution 1:50,000. Perchlorate, nitrate, sulfate, and chloride anions did not interfere with the test; but oxalate, sulfate, nitrite, cyanide, and sulfide anions mask the color of the complex. Sodium, potassium, lithium, and manganous cations did not interfere with the test; but chromic, nickelic, vanadyl, ferric, ferrous, cupric, and cuprous cations mask the color of the complex. In the case of ferric and cupric ions a black color formed when they were introduced into the solution of complex. The black color faded to a light brown color after a short period of time. This brown color was exactly the same color resulting from the introduction of ferrous or cuprous ions into the solutions of the complex. The ferric and cupric ions were reduced by the thiosulfate ions to the ferrous and cuprous state which still interfered with the test. Feigl (*1*) describes a spot test for uranium which has a limit of identification of 0.92 μg. and a limit of dilution of 1:54,000. Ferrous and cuprous ions do not interfere with this test.

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Precipitation of Zinc and Cadmium 8-Quinolinates from Homogeneous Solution

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▶ **Zinc and cadmium 8-quinolinates have been precipitated by means of homogeneous release of cations from an ammonia complex. Precipitates of excellent physical characteristics were produced by the ammonia volatilization technique. Evidence for the formation of a tris-8-quinolinol complex of zinc was found. An approximate formation constant for this complex has been calculated.**

A VARIETY of methods have been used to produce insoluble 8-quinolinates from homogeneous solution. The hydrolysis of urea (*4, 7, 11*) has been used to lower the hydrogen ion concentration and gradually cause precipitation of the insoluble 8-quinolinates. The hydrolysis of 8-acetoxyquinoline (*2, 6*) has been used to precipitate several metallic cations. A more recent variation is the volatilization of mixed solvent (*5*) to create a precipitation medium. These methods produce precipitates with better physical characteristics than the heterogeneous methods.

This paper describes a new procedure whereby the zinc and cadmium ions are generated in the presence of the precipitant. A metal-ammonia complex is dissociated using volatilization of ammonia, thus releasing the cation. Large crystals of a definite geometric shape are produced. Cobalt has also been precipitated by this technique (*3*).

The purpose of this work was to see if a separation and analysis of zinc and cadmium were possible using the ammonia volatilization technique.

This precipitation method is relatively simple. Zinc or cadmium will not precipitate in a strongly ammoniacal solution because of the very low concentration of free cation. On heating, ammonia leaves the solution, dissociating the ammonia complex. The pH and free cation concentration are influenced by its removal. As the cation is released from its complex in the solution, it is precipitated by the anionic 8-quinolinol. The precipitate is composed of large geometric crystals, which are much easier to filter and wash than crystals formed by conventional methods.

EXPERIMENTAL

Apparatus and Reagents. Unless otherwise specified, all reagents were reagent grade.

Methanol, CH_3OH, 95% technical.

8-Quinolinol, C_9H_7ON, Eastman.

Approximately 0.1*M* zinc and cadmium solutions were prepared by dissolving 0.2 mole of $Zn(C_2H_3O_2)_2$ and $CdCl_2 \cdot 2^1/_2H_2O$ in 2 liters of deionized water.

The apparatus consisted of a Therm-O-Shake shaker hot plate manufactured by the Precision Scientific Co., Beckman Zeromatic pH meter with glass electrodes, and Beckman Model DU spectrophotometer.

Procedure. Pipet a known quantity (approximately 0.5 mmole) of zinc or cadmium solution into a 250-ml. beaker. Add 6 mmoles of ammonium ion (2 ml. of 3*M* ammonium chloride solution) and about 2 moles of ammonia (125 ml. of concentrated ammonium hydroxide). Place the beaker on the shaker hot plate set for approximately 80° C.

Prepare the precipitant by mixing in a separate container at 10 to 50% excess of 8-quinolinol (0.1*M* in methanol) and about 0.75 mole of ammonia (50 ml. of concentrated ammonium hydroxide). Add the precipitant solution, by rapid pouring, to the original solution.

Allow the resulting solution to agitate and heat (about 90 minutes) until a pH of less than 10.5 is attained, filter (either hot or cold) through a medium-porosity sintered-glass crucible, and wash the precipitate with water. Dry the precipitate at 125° C. for at least 1 hour and weigh as the anhydrous 8-quinolinate.

RESULTS AND DISCUSSION

Preliminary investigations were conducted to determine the appropriate concentration of reagents, temperature, and pH that would result in a precipitate with the best physical characteristics.

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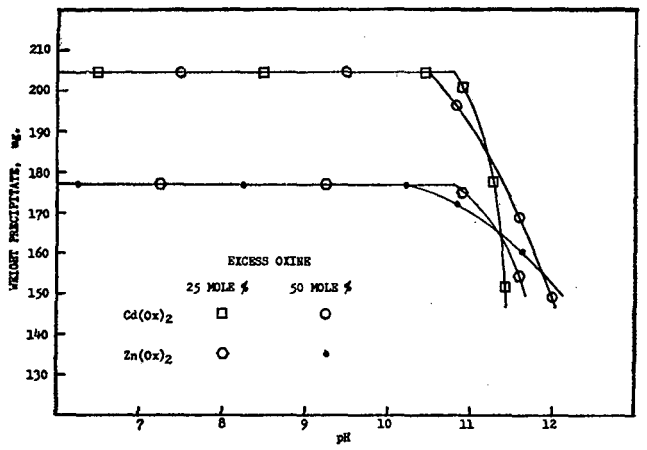

Figure 1. Effect of 8-quinolinol concentration on pH of quantitative precipitation

A large ammonia to metal ratio is necessary to attain an initially homogeneous solution. The added ammonium chloride concentration (0.03*M*) was large when compared to the ammonium ion from the dissociation of ammonium hydroxide.

The temperature of the precipitation is near 80° C. Constant agitation, at this temperature, aids in the volatilization of ammonia.

The precipitates of zinc and cadmium 8-quinolinates obtained by the ammonia volatilization method are stoichiometrically the same as those obtained through a standard precipitation procedure (*12*)—i.e., the bis form. This was determined by a comparison of precipitate weights between the two procedures. The ratio of averages of four determinations each between the different methods is 1.005 for zinc and 1.009 for cadmium. The standard heterogeneous method values were slightly higher, probably because of increased coprecipitation.

Spectrophotometric analyses (*10*) of the filtrates show that for an average sample (25 to 50 mole % excess of 8-quinolinol and 0.5 mole of metal initially taken) about 0.2 mg. of zinc and 0.06 mg. of cadmium are present in the filtrates.

The pH of quantitative precipitation for both zinc and cadmium 8-quinolinates is 10.5 to 10.9 and is influenced by the 8-quinolinol concentration (Figure 1).

The effect of 8-quinolinol concentration on quantitative precipitation was investigated by performing determinations with varying amounts of the reagent (Table I). The first experiment was a direct gravimetric analysis by weighing the precipitates. These precipitates were dissolved in hydrochloric acid and reprecipitated with only a slight excess (10 mole %) of 8-quinolinol. The quantity of zinc in the original filtrates at the various moles per cent of 8-quinolinol was also determined by spectrophotometric analysis.

The quantitative nature of the zinc precipitation is definitely dependent on the 8-quinolinol concentration, while for cadmium it is questionable (Table I). The original precipitates for zinc show that as the excess of 8-quinolinol concentration is increased, the quantitative nature of the precipitation decreases, then at about 300 mole % excess of 8-quinolinol increases again. Post-precipitated reagent was clearly seen deposited on the surface of the precipitates, when a microscopic examination was made. Photomicrographs confirmed this visual observation. Repeated washings showed a marked decrease in this reagent deposit.

Table I shows that the original and reprecipitated gravimetric values are reproduced through 300 mole % excess of 8-quinolinol, while after 300 mole % excess the values are irregular.

The quantity of zinc in the filtrates at the various levels of excess 8-quinolinol, as determined spectrophotometrically, corresponds to that originally obtained gravimetrically through 0 to 300 mole % excess of 8-quinolinol (Table I).

Inspection of Table I reveals two important facts: As the excess of 8-quinolinol is increased, the total amount of zinc in solution increases, and the data obtained from the three types of experiments appear to be consistent from 0 to 300 mole % excess but less consistent from 300 to 700 mole % excess.

The increased zinc concentration was rather troublesome to explain. Several possible series of soluble complexes of zinc—ammine, hydroxo, and 8-quinolinato—might account for it.

At any pH the concentration of hydroxide ion, ammonium ion, and ammonia will be constant in the sample solutions maintained at a constant volume. With this knowledge and equilibrium and formation constants taken from the literature, it is possible to estimate the concentrations of the various species in solution.

The simplest mathematical methods were used in calculating the concentrations of the various forms of zinc in solution. Even though activity coefficients could be approximated for these concentrated solutions, it is questionable if their use would be justified because of the uncertainty of the physical constants taken from the literature. In fact, the use of activity coefficients may imply an accuracy that is not valid.

The concentration of zinc ion in solution may be calculated from a modified *Ksp* relationship:

Table I. Effect of Excess 8-Quinolinol on Precipitation

Mmoles oxine	pH	Weight of $Zn(Ox)_2$, mg. Original[a] ppts.	Reprecipitated ppts.	Spectrophotometric analyses
1		177.8	177.6	0.36
2		177.1	177.2	1.82
3		174.9	174.6	3.98
4		171.7	168.6	7.55
5		176.6	168.9	5.86
6		182.9	170.5	7.05
8		194.7	167.7	...
		Weight of $Cd(Ox)_2$, mg.		
1	10.2	204.0	...	...
2	10.3	204.0	...	...
3	10.2	204.0	...	...
4	10.4	203.8	203.9	0.16
5	10.3	204.1	...	...
6	10.4	204.0	203.2	0.17
7	10.3	201.4	200.8	0.49
8	10.3	206.0	203.1	0.40

[a] Filtered at pH = 10.4.

$$[Zn^{+2}] = \frac{Ksp}{(\alpha\, C_{HOx})^2} \quad (1)$$

where

$$Ksp = [Zn^{+2}]\,[Ox^-]^2 = 5.6 \times 10^{-28} \quad (2)$$

and

$$[Ox^-] = \alpha\, C_{HOx} \quad (3)$$

with α the fraction of total 8-quinolinol (C_{HOx}) in solution in anionic form and defined as

$$\alpha = \frac{Ka}{[H^+] + Ka} \quad (4)$$

$$Ka = \frac{[H^+]\,[Ox^-]}{[HOx]} = 2 \times 10^{-10} \quad (5)$$

The concentrations of the various forms of the ammine and hydroxo complexes may be calculated from an equation (*8*) of the type

$$\frac{C_M}{[Zn^{+2}]} = \sum_{i=0}^{i=n} Ki\,[X]^i \quad (6)$$

where by definition $K_0 = 1$, K is an over-all formation constant, and C_M is the total amount of metal in solution uncomplexed plus that complexed by complexing agent X.

The concentrations of the species $Zn(C_9H_6ON)^+$ and $Zn(C_9H_6ON)_2$ may be calculated from their formation constants (*9*) ($k_1 = 8.1 \times 10^{10}$ and $k_2 = 8.0 \times 10^9$).

The concentrations of the various known species in solution at the 300 mole % excess of 8-quinolinol level and pH 10.4 are listed in Table II. The elementary method of calculating the hydrogen, hydroxide, ammonium ions, and ammonia concentrations is omitted.

The total zinc concentration, calculated from data given in Table I, is about $10^{-4}M$. The highest concentration of zinc that can be accounted for is about $10^{-7}M$. This indicates that some other species may be present in solution.

A higher 8-quinolinato complex of zinc could account for this relatively large amount of zinc in solution, if it is assumed that a salting-in effect or an activity coefficient effect would not bring about the increased solubility.

Most zinc complexes are $4s4p^3$ type. Yet complexes of zinc with apparent $4s4p^34d^2$ bonding have been reported (*1*). There are no higher complexes of zinc reported, so a 8-quinolinato complex beyond the tris-8-quinolinato is unlikely.

The third formation constant in the zinc-8-quinolinato series can be calculated, assuming the presence of the tris(8-quinolinato)zincate(II) ion in solution accounts for the unusually high concentration of zinc.

Table II. Concentration of Various Forms of Zinc

(Moles per liter)

Zn^{+2}	2.7×10^{-24}	$Zn(NH_3)^{+2}$	2.0×10^{-22}	$Zn(OH)^+$	1.3×10^{-23}
$Zn(Ox)^+$	3.0×10^{-15}	$Zn(NH_3)_2^{+2}$	1.9×10^{-20}	$Zn(OH)_2$	1.6×10^{-20}
$Zn(Ox)_2$	3.2×10^{-7}	$Zn(NH_3)_3^{+2}$	1.9×10^{-18}	$Zn(OH)_3^-$	1.0×10^{-20}
		$Zn(NH_3)_4^{+2}$	9.4×10^{-16}	$Zn(OH)_4^{-2}$	8.1×10^{-23}

The formation of the tris(8-quinolinato)zincate(II) ion may be depicted in equation form as

$$Zn^{+2} + 3Ox^- = Zn(Ox)_3^- \quad (7)$$

with

$$k_1k_2k_3 = \frac{[Zn(Ox)_3^-]}{[Zn^{+2}]\,[Ox^-]^3} \quad (8)$$

The Ksp relationship may be introduced into the above equation to yield

$$k_1k_2k_3Ksp = \frac{[Zn(Ox)_3^-]}{[Ox^-]} \quad (9)$$

The $\frac{Zn(Ox)_3^-}{Ox^-}$ ratio is determined from experimental data (Table III) assuming that essentially all the zinc in solution is present as the tris(8-quinolinato)zincate(II) ion. Ksp, k_1, and k_2 are obtained from the literature (*9*), allowing the calculation of k_3. Calculations of k_3 are listed in Table III.

Table III. Calculated Formation Constant (k_3) of the Tris(8-quinolinato)-zincate(II) Ion

Mmoles 8-quinolinol	Calculated $k_3 \times 10^{-4}$	Concentration, moles per liter: Ox^- $\times 10^3$	Concentration, moles per liter: $Zn(Ox)_3^-$ $\times 10^5$
2.0	2.1	4.6	3.05
3.0	2.2	9.2	6.5
4.0	2.7	13.7	12.0
5.0	5.2	18.2	9.6
6.0	1.6	22.9	11.5
	Av. 2.8		

Additional methods for determining the postulated tris-8-quinolinato complex were considered. Polarographic and absorption spectra studies would meet with severe interferences because of the high concentration of 8-quinolinol and the presence of colored decomposition products.

No conclusive evidence for the formation of a tris-8-quinolinato complex of cadmium was obtained; therefore, no attempt was made to calculate a third formation constant.

The only diverse ions investigated were nickel(II), cobalt(II), and copper (II), which form stable ammonia complexes. All of these ions interfere with an analysis of zinc or cadmium. Silver was not studied because of its ease of reduction. From Figure 1 it is seen that zinc and cadmium cannot be separated by the ammonia volatilization method.

These restrictions greatly reduce the effectiveness of the ammonia volatilization method as an analytical tool. Determination of zinc and cadmium can be made in the absence of multivalent cations. This method is an excellent preparation of highly crystalline 8-quinolinates with metals that form stable ammonia complexes.

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Determination of Four Closely Related Triaryl-s-Triazines by Infrared Spectrometry

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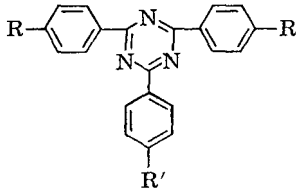
▷ **A method was sought for the determination of four closely related s-triazines(I to IV), which may appear together in difficultly separable mixtures. Two of these (II and III), which are reported here for the first time, were separated from other members by column chromatography on silica gel. Well resolved out-of-plane carbon-hydrogen bending vibrations located between 750 and 825 cm.$^{-1}$ are sufficiently intense to permit quantitative determination of each component in mixtures of all four triazines.**

RECENTLY, a reaction under investigation in this laboratory was found to give one or more members of a family of four closely related triaryl-*s*-triazines: 2,4,6-triphenyl-*s*-triazine(I), 2-(*p*-chlorophenyl)-4,6-diphenyl-*s*-triazine(II), 2-phenyl-4,6-(*p*-chlorophenyl)-*s*-triazine



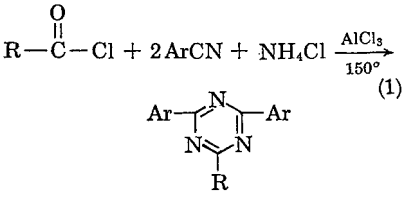
I. R = R′ = H (triphenyltriazine)
II. R = H; R′ = Cl (monochloro)
III. R = Cl; R′ = H (dichloro)
IV. R = R′ = Cl (trichloro)

(III), and 2,4,6-tri(*p*-chlorophenyl)-*s*-triazine(IV). For the sake of brevity, these will be referred to in this paper as triphenyltriazine, "monochloro," "dichloro," and "trichloro," respectively.

To understand the mechanism of the reaction in which these compounds were formed, it was necessary to know with certainty which ones were present and, if possible, to determine how much of each had been produced. Physical separation of each triazine from the other three was extremely difficult and tedious and did not provide an accurate estimate of the relative amounts of each member present in the mixture.

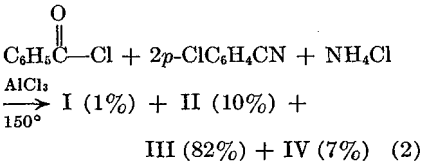
Infrared spectra of crude products indicated that a spectroscopic method might be developed which would permit individual triazines to be recognized, or even determined quantitatively, in the presence of the others. Pure samples of all four compounds were required to test the feasibility of developing an analytical procedure. Although triphenyltriazine and trichloro were readily synthesized simply by trimerizing benzonitrile and *p*-chlorobenzonitrile, respectively, the two unsymmetrical *s*-triazines were more difficult to obtain pure.

The only convenient method of preparing unsymmetrical *s*-triazines of the desired type was reported by Eitner and Krafft (*2*) in 1892 and has been used by other workers since that time (*5*):



(1)

However, attempts in this laboratory to prepare monochloro and dichloro by this reaction gave not only the desired product, but resulted in one or more of the other *s*-triazines, as well. The reaction expected to give pure dichloro produced a mixture of all four triazines, even triphenyltriazine:



$$\xrightarrow[150°]{AlCl_3} \text{I (1\%)} + \text{II (10\\%)} + \text{III (82\%)} + \text{IV (7\%)} \quad (2)$$

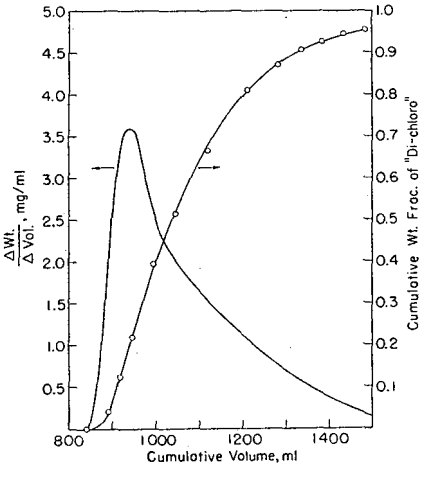


Figure 1. Column chromatography of "di-chloro"

The monochloro preparation, however, resulted in a cleaner *s*-triazine product, with only 1 or 2% each of triphenyltriazine and dichloro being present as contaminants.

The impure samples of dichloro were particularly difficult to purify. Fractional crystallization was without avail, possibly because the unsymmetrical *s*-triazines are much more soluble than the adjacent symmetrical compounds, which are usually the major contaminants.

The method which finally gave the pure mono- and dichlorotriazines was column chromatography on silicic acid. The elution behavior of crude dichloro with the composition given in Equation 2 is illustrated in Figure 1. Cumulative wt. per cent of dichloro in the eluate is plotted against cumulative volume of eluting solvent. The second curve is the first derivative, obtained by estimating slopes along the first curve. The derived curve is expressed as concentration of dichloro in the eluate in mg. per ml. It has the steep front and long tail that are characteristic of compounds which display a convex adsorption isotherm (*3*).

After all four *s*-triazines were obtained in the pure state, it was possible to determine whether an infrared analytical procedure was feasible. A comparison of the infrared spectra of carbon disulfide solutions of the triazines in Figure 2 shows clearly that there are strong bands between 12 and 14 microns which are characteristic of each compound and therefore potentially useful for analytical purposes. Those that were actually used are indicated on the curves. Assignments in every case are to an out-of-plane bending mode either of monosubstituted or disubstituted benzene (*6*). The bands owe their importance in analysis not only to their considerable intensity, but to the fact that their locations shift so much from one triazine to the next that there is little or no overlap.

Despite the reasonably good resolution of the analytical bands, the "hill and valley" method (*4*) of drawing the base line was not very successful when mixtures of the four triazines were being analyzed. However, satisfactory results were obtained by using single beam spectroscopic techniques, as described in the experimental section.

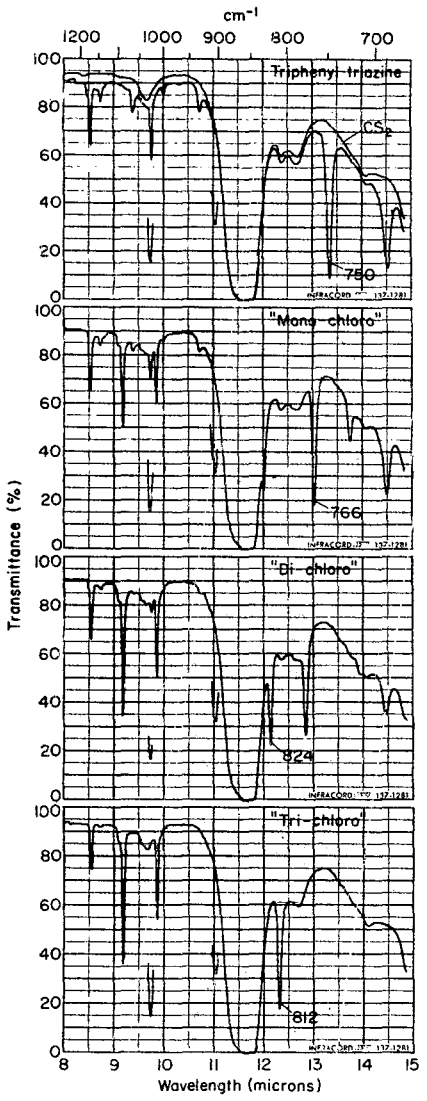


Figure 2. IR spectra of triazines in CS_2 solution

Polystyrene reference peaks at 9.724 and 11.035 microns

EXPERIMENTAL

Triazine Synthesis. Triphenyltriazine and trichloro have been previously reported (*5*). Pure samples were readily prepared by the chlorosulfonic acid-catalyzed trimerization of benzonitrile and *p*-chlorobenzonitrile, respectively (*1*).

TRIPHENYLTRIAZINE. Obtained as needlelike crystals from toluene, m.p. 235.0° to 235.4° C. (corr.); $\lambda_{max}^{CCl_4}$ 268 mμ (ε 6.3 × 10⁴); $\lambda_{max}^{H_2SO_4(95\%)}$ 335 mμ, 400 mμ (ε 5.6 × 10⁴, 5.2 × 10⁴), shoulder between 270 and 305 mμ. Triphenyltriazine sublimes readily at 200° C./0.01 mm.

TRICHLORO. Crystallized from chlorobenzene as very long, thin needles that tended to mat together in a feltlike mass, m.p. 348° to 349° C. (corr.), with prior sintering at 346.5° C.; $\lambda_{max}^{CCl_4}$ 282 mμ (ε 8.0 × 10⁴); $\lambda_{max}^{H_2SO_4(95\%)}$ 368 mμ, 431 mμ (ε 7.3 × 10⁴, 7.6 × 10⁴), shoulder between 290 and 325 mμ.

MONOCHLORO. A mixture of 15.0 grams (0.086 mole) *p*-chlorobenzoyl chloride, 21.9 grams (0.212 mole) benzonitrile, 18.9 grams (0.35 mole) ammonium chloride, and 16.5 grams (0.124 mole) aluminum chloride was heated overnight at 150° C., and then treated with ice and concentrated hydrochloric acid. The filtered mixture was washed well with methanol to give the crude triazine fraction, 12.6 grams (43%, based on *p*-chlorobenzoyl chloride). This was analyzed by the procedure described below, after first passing a solution in carbon tetrachloride through a short column of activated alumina to remove polar contaminants. Infrared analysis: 93% monochloro (or 37% overall yield based on *p*-chlorobenzoyl chloride), 1.2% triphenyltriazine, 1.9% dichloro, and no trichloro. Pure monochloro was obtained as the main fraction of eluate when crude triazine was chromatographed from carbon tetrachloride on a column containing one hundred times the sample weight of silicic acid (Mallinckrodt 100 mesh, analytical grade). Infrared spectra of peripheral fractions confirmed the presence of both triphenyltriazine and dichloro. This confirmation is important since both triazines are present in the mixture before chromatographing at a level too low to be detected with certainty by the infrared spectroscopic analysis. Recrystallization of the pure monochloro fraction from toluene gave loose aggregates of very small needles, m.p. 197.2° to 197.8° C. (corr.); $\lambda_{max}^{CCl_4}$ 276 mμ (ε 6.4 × 10⁴); $\lambda_{max}^{H_2SO_4(95\%)}$ 345 mμ, 413 mμ (ε 5.0 × 10⁴, 5.4 × 10⁴), shoulder between 275 and 310 mμ. Calcd. for $C_{21}H_{14}N_3Cl$: C, 73.36; H, 4.10; Cl, 10.31. Found: C, 73.48, 73.70; H, 4.24, 4.52; Cl, 10.27, 10.47.

DICHLORO. A mixture of 16 grams (0.11 mole) benzoyl chloride, 30 grams (0.22 mole) *p*-chlorobenzonitrile, 18 grams (0.34 mole) ammonium chloride, and 17 grams (0.13 mole) aluminum chloride was reacted and worked up in the same manner as that described for monochloro. The methanol-insoluble fraction weighed 9.7 grams (24% yield based on *p*-chlorobenzonitrile). A chlorobenzene solution was passed through a short alumina column to reduce polar impurities. Infrared analysis of the resulting crude triazine: 82% dichloro (or 11% overall yield based on *p*-chlorobenzonitrile), 10% monochloro, 7% trichloro, and 1% triphenyltriazine. Chromatography on silicic acid, as described above, gave a pure dichloro fraction, with the elution behavior illustrated in Figure 1. The trichloro fraction was eluted just prior to the dichloro, with very little overlap of the zones. The portion of the dichloro elution curve shown in Figure 1 represents the interval from 10- to 18-column volumes of eluting solvent.

The presence of triphenyltriazine, indicated in analysis of the crude triazine at an inconclusive 1% level, was fully confirmed by analysis of the triazines left on the column after elution was discontinued. This remnant was removed by exhaustive Soxhlet extraction with methanol–carbon tetrachloride azeotrope, and was found to consist of dichloro, monochloro, and triphenyltriazine.

Recent work done in this laboratory has demonstrated that dichloro may be obtained in higher yield (40% crude triazine) and with much less triazine as by-product, if *p*-chlorobenzonitrile is used in excess (30%) rather than the approximately equivalent amount employed in the procedure described above. Infrared analysis indicates that the only other triazine produced under these conditions is monochloro, and this is present in the mixture to the extent of only 2%. The presence of monochloro in the product has been fully confirmed by thin layer chromatography (*6*).

Recrystallization of dichloro from chlorobenzene gave long thin needles, m.p. 250.8° to 251.2° C. (corr.); $\lambda_{max}^{CCl_4}$ 281 mμ (ε 7.0 × 10⁴); $\lambda_{max}^{H_2SO_4(95\%)}$ 359 mμ, 422 mμ (ε 4.9 × 10⁴, 5.4 × 10⁴), shoulder between 275 and 325 mμ. Dichloro, like triphenyl triazine, was found to sublime readily at 200° C./0.01 mm. Calcd. for $C_{21}H_{13}N_3Cl_2$: C, 66.68; H, 3.46; Cl, 18.75. Found: C, 66.68, 66.50; H, 3.68, 3.51; Cl, 18.40, 18.60.

Solubility Data. Solubility was determined in several solvents at 30.8° ± 0.5° C. and is listed as grams per liter in the order triphenyltriazine, monochloro, dichloro, and trichloro; carbon disulfide, 7.14, 29.1, 2.99, 0.80; carbon tetrachloride, 3.58, 13.8, 2.24, 0.32; methanol, 0.13, 0.18, 0.03, <0.02; toluene, 4.95, ..., 2.00, 0.46; chlorobenzene, ..., ..., 2.80, 0.61; 95% ethanol, 0.16, ..., ..., ...; pyridine, 4.84, ..., ..., ...

Infrared Analysis Procedure. The analytical method was developed on a Perkin-Elmer Model 137B Infracord linear in per cent transmission and wavelength. All spectra were recorded from 8 to 15 microns at the minimum slit width of 0.025 mm. Carbon disulfide (Baker Technical) was the solvent chosen because not only is it quite transparent at the analytical frequencies but, fortunately, it also appears to be the best solvent available for these sparingly soluble compounds. Even so, because of the particularly low solubility of trichloro and dichloro, it was necessary to use a thick cell, 2.57 mm., for all determinations.

As mentioned above, the "hill and valley" method of determining absorbances (*4*), often used in quantitative infrared analysis, could not be used in this case. It was not possible to obtain a stable base line which would hold for the full range of mixtures of all four triazines. Because of the thick cell used, attempts to improve the base lines by balancing sample solutions against pure solvent with a variable cell in the reference beam were not successful. Quantitative results obtained in preliminary applications of this approach were unsatisfactory.

The best results were obtained when a single cell was used both for pure solvent and for sample solution. The base line was established by comparison of the spectrum of the analytical solution

Table I. Analysis of Synthetic Mixtures

Synthetic mixtures[a]		CS_2 soln., mg./10 ml. Actual[a]	Found	Triazine in mixture, % Actual	Found	Deviation
1	TPT	1.72	1.75	21.3	21.7	+0.4
	Mono-Cl	6.38	6.27	78.7	77.5	−1.2
2	Di-Cl	5.94	6.21	82.0	85.7	+3.7
	Tri-Cl	1.30	1.47	18.0	20.3	+2.3
3[b]	Di-Cl	5.58	6.11	79.7	87.5	+7.8
	Tri-Cl	1.42	1.51	20.3	21.6	+1.3
4	TPT	4.90	5.28	42.2	45.6	+3.4
	Mono-Cl	4.60	4.87	39.7	42.1	+2.4
	Di-Cl	2.09	1.90	18.1	16.4	−1.7
5	Di-Cl	5.92	5.92	79.4	79.4	0
	Tri-Cl	1.54	1.62	20.6	21.7	+1.1
6[c]	TPT	3.69	3.41	36.1	33.4	−2.7
	Mono-Cl	2.68	2.61	26.2	25.5	−0.7
	Di-Cl	3.01	3.09	29.4	30.2	+0.8
	Tri-Cl	0.84	1.27	8.3	12.5	+4.2
7[c]	TPT	0.22	0.20	4.1	3.7	−0.4
	Mono-Cl	5.14	5.15	95.9	96.1	+0.2
8[c]	Mono-Cl	0.16	0.17	2.0	2.1	+0.1
	Di-Cl	7.80	7.74	95.9	95.2	−0.7
	Tri-Cl	0.17	0.10	2.1	1.2	−0.9
9[c]	Di-Cl	8.06	8.23	98.0	100.1	+2.1
	Tri-Cl	0.16	0.17	2.0	2.1	+0.1

[a] Carefully weighed samples of pure *s*-triazines dissolved in carbon disulfide and diluted to the indicated concentrations.
[b] Average of duplicate spectra on the same solution, not run consecutively.
[c] Average of duplicate spectra on the same solution, run consecutively.

with the spectrum of pure solvent, and absorbances were calculated as the differences between curves of sample solutions and the pure carbon disulfide solvent. Instrument variations were guarded against by running a solvent reference curve just prior to each sample.

Dichloro and trichloro were the most difficult triazines to determine with accuracy because the base line (pure solvent curve) is rather steep at their analytical frequencies (topmost curve in Figure 2). The dichloro absorbance *vs.* concentration plot also has a much lower slope than those of the other triazines (Figure 3).

Absorbances were calculated in the usual way (*4*), but it was more convenient and accurate to measure distances between the zero transmittance line and the solvent or sample curves by means of calipers and use these values in lieu of transmittances read off the charts.

The validity of Beer's law for this system was tested by running a large number of samples of each pure *s*-triazine. Determinations were made in the following manner. Samples were weighed to an estimated fifth decimal place on a Mettler B-6 semimicro balance, dissolved in carbon disulfide, and brought to the desired concentration in volumetric flasks. For the sake of greater accuracy, flasks 25 ml. or larger were generally used, although 5 ml. of solution was more than sufficient to fill the cell; this volume could have been used if only a small amount of sample were available. Concentrations were conveniently expressed as mg. per 10 ml. Partially diluted solutions were allowed to come to the temperature of the instrument room before diluting to volume. The results were plotted against concentration in mg. per 10 ml. Although they pass through the origin, the plots are not linear (Figure 3).

This deviation from Beer's law, coupled with a very slight interference among some of the triazines, makes it convenient to use a method of successive approximations in the determination of mixtures. The lower composite curve on each graph represents the very small and approximately equal absorbance of the other three triazines at each analytical frequency; however, trichloro absorbance is negligible at triphenyltriazine and monochloro frequencies. As a first step in calculating results, the components are read directly off the curves to give their approximate concentrations. The second and final approximation is made by subtracting, at the same wavelength, the small absorbances due to the other triazines, then reading the concentrations of the corrected absorbances.

RESULTS AND DISCUSSION

The results of analysis of nine synthetic mixtures of various combinations of the four *s*-triazines are given in Table I, together with deviations in the per cent of each triazine present expressed in absolute units. Except for one analysis, the maximum error was only about 4%, and the average deviation from the true values (shown in Table II) was less than 2%. One of the two spectra responsible for the results given for Synthetic mixture 3

Table II. Precision and Accuracy in Analysis of Mixtures

	Average precision[a] %	No. of detns.	Reproducibility[b] %	Pairs of duplicates
Triphenyltriazine	1.7	4	0.5	9
Monochloro	0.9	5	1.2	10
Dichloro	2.4	7	1.5	10
Trichloro	1.7	6	0.6	10
Av. overall triazines	1.7	22	0.9	39

[a] Summary of data from Table I. Deviations of the determinations in per cent absolute were averaged.
[b] Reproducibility is expressed as the difference in per cent absolute between the determination of a triazine solution and its duplicate, averaged over the number of pairs of duplicates run.

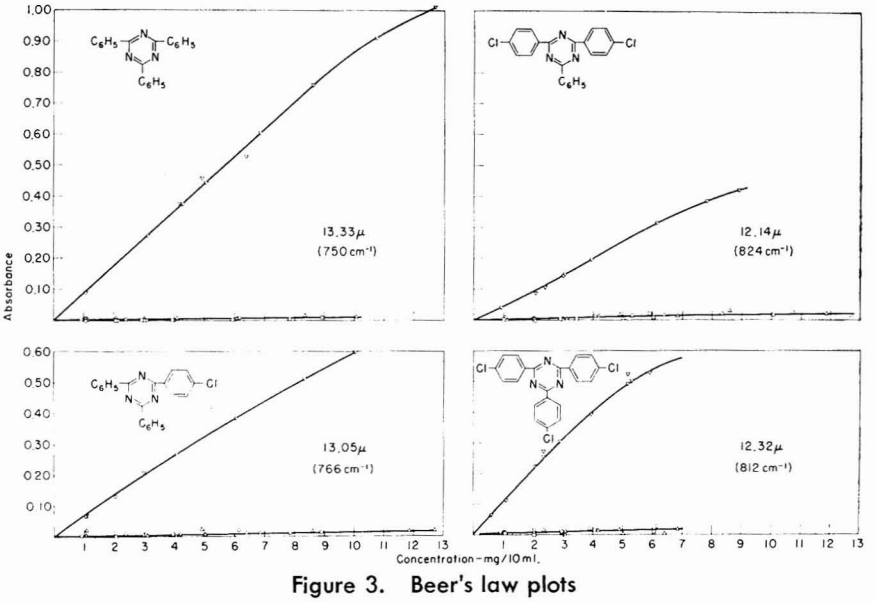


Figure 3. Beer's law plots

The bottom, composite curve in each panel represents the small absorbance due to any of the other triazines, except that trichloro absorbance may be disregarded altogether at the triphenyltriazine and monochloro frequencies

was seriously out of line, hence the very high value for dichloro in this example. No reason for the discrepancy could be found, but we believe it was caused by occasional erratic behavior of the instrument, due possibly to extreme line voltage fluctuation.

Table II shows that analyses of triazine solutions run in duplicate do not on the average show much less variability than the difference between determinations of known samples and the true values. Thus, most of the uncertainty in the method appears to be bound up in the instrumental reproduction of the absorption curves and the translation of these data into absorbance values. Consequently the absolute error in the determination of a triazine by this method is about the same no matter how much of it may be present in the sample.

The analytical bands fall in a region where there are relatively few strong interfering bands, which minimizes the possibility of spectral interference due to impurities when crude triazine mixtures are analyzed. This possibility may be reduced still further by washing the mixtures with methanol, in which all four triazines are very sparingly soluble but in which the likely contaminants such as nitriles and amides readily dissolve. By-products in the crude triazine mixtures may also be removed by passing carbon tetrachloride solutions through a short column of activated neutral alumina, which permits the triazines to pass through directly, but which adsorbs the more polar impurities to a significant degree.

The amount of an *s*-triazine mixture which gives optimum analytical results by this method is a mere 2 to 3 mg. per 5 ml. of carbon disulfide solution. The smallest amount of any one triazine component in such a mixture which can be detected spectroscopically is about 40 to 60 μg.

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Temperature Programmed Capillary Columns in Gas Chromatography

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▶ An F & M Model 609 gas chromatograph, with modified analyzer gas controls and column oven heating system, has been used to temperature program capillary columns. This technique has been investigated for the analysis of dilute hydrocarbon gas mixtures (99.8% N_2). For qualitative and quantitative analyses, the precision of the retention volume and peak area measurements have been determined.

FOR THE ANALYSIS of complex mixtures, the capabilities of capillary columns and temperature programming have been amply demonstrated when used separately, but only recently have the two been combined. Teranishi, Nimmo, and Corse (*6*) and Boys (*3*) have described laboratory-constructed apparatus for temperature programming capillary columns, but gave few details of their results.

In the development of an improved method for analyzing automobile exhaust gas, this technique was investigated using dilute hydrocarbon gas mixtures. Rather than build a laboratory setup, a commercial gas chromatograph featuring flame ionization detection and column temperature programming was modified to use capillary columns. Recently, a suitable commercial instrument has become available (*4*).

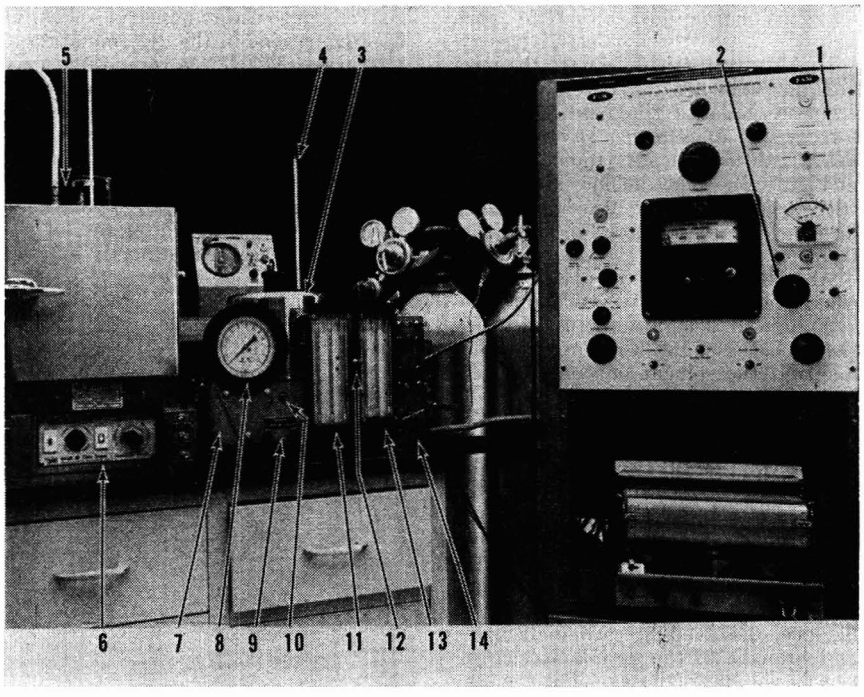

Figure 1. Gas chromatographic apparatus

EXPERIMENTAL

Gas Chromatograph Modifications. Figure 1 shows the modified gas chromatograph (F & M Scientific Corp., Model 609) that was used in this work. This instrument consists basically of the control cabinet, **1**, and the analyzer section, **7**. The principal changes that were made to the apparatus include: modifying the gas controls of the analyzer, adding a gas sampling valve with a thermostating oven, rewinding the capillary columns, and modifying the column oven heating system. The first three changes were

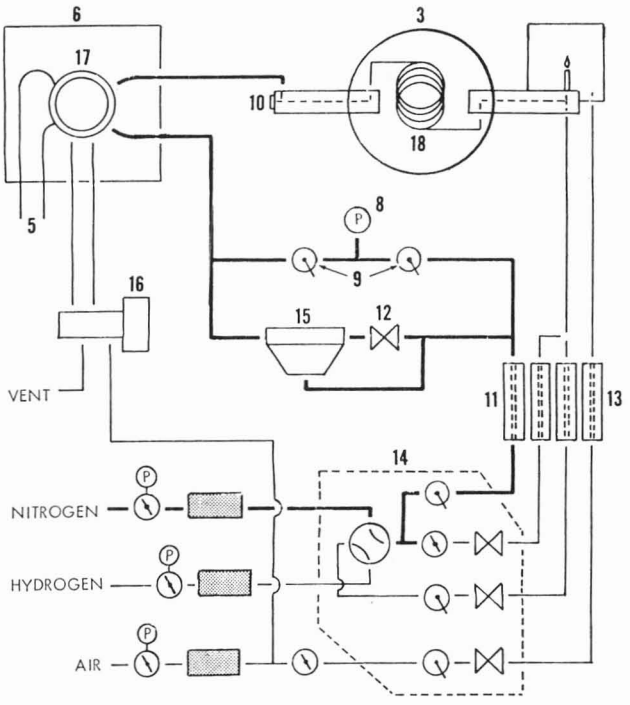

Figure 2. Schematic flow diagram

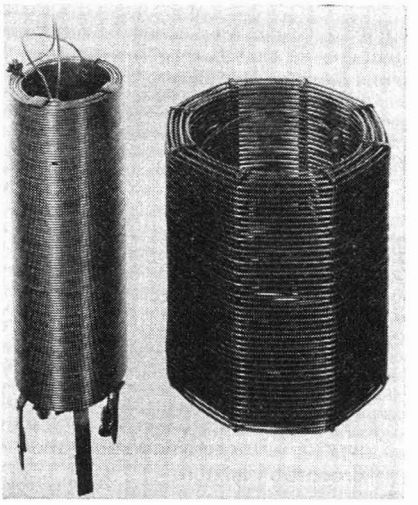

Figure 3. 300-Foot capillary column as received (left) and rewound (right)

made to adapt the instrument to gas analysis using capillary columns, whereas the fourth was made to improve performance.

Gas Controls. A schematic flow diagram for the modified analyzer is shown in Figure 2. The heavier lines represent the carrier gas; the lighter lines, the hydrogen, nitrogen diluent, and air. The added panel, **14** (refer also to Figure 1), has a four-way valve for using either nitrogen or hydrogen as the carrier gas, and toggle and needle valves for setting the various gas flows that are measured with the rotameters on panels **11** and **13**.

The rotameter for the carrier gas was originally between the flow controller, **15**, and the injection block, **10**. It was moved upstream of the flow controller, as shown, to be independent of varying column inlet pressure. Regardless of what column is used or the variation in the column inlet pressure during temperature programming, the carrier gas pressure upstream of the flow controller is set the same for all experiments and only one flow calibration curve is required.

The pressure gauge, **8**, (Marsh Equipment Co., type 220) was added between two toggle valves, **9**, for measuring either the rotameter or column inlet pressure. The flow controller can be bypassed by opening both toggle valves, if column operation at constant inlet pressure is desired.

For easier adjustment of the carrier gas flow, the original valve that was attached to the flow controller at the rear of the analyzer was replaced with a vernier needle valve, **12**, (Ideal Aerosmith Inc., Model V-52-2-14) and attached to the rotameter panel, **11**.

Gas Sampling Valve. The gas sampling valve, **17**, (Greenbrier Instruments Inc., type D) has been described (*5*). It is pneumatically operated by the solenoid valve, **16** (Ross Operating Valve Co., PAL type), and thermostated in the oven, **6** (Blue M Electric Co., Model OV-8A). The connections to the sample gas inlet and outlet ports of the valve are made at the top of the oven at **5**.

Capillary Columns. The capillary column, **18**, was obtained from the Perkin-Elmer Corp. in the form shown on the left in Figure 3. This form, however, was unsuitable for temperature programming because of slow heat transfer to the inner layers of tubing. To avoid this limitation, the tubing was rewound with spaces between the layers of tubing for rapid air circulation as shown on the right.

To coil the tubing in this form, metal spacers were placed about 1.5 inches apart around the periphery of a mandrel and held with rubber bands. The spacers were made by cutting a perforated steel sheet ($^1/_{16}$-inch holes at $^7/_{64}$-inch staggered centers) along the centers of adjacent rows of holes. While slowly rotating the mandrel in a lathe chuck, the tubing was guided into the *U*-notches of the spacers. After completing the coiling operation, the tubing was held in place with wire ties at the ends of the spacers. The coil was slid off the mandrel and the inner row of

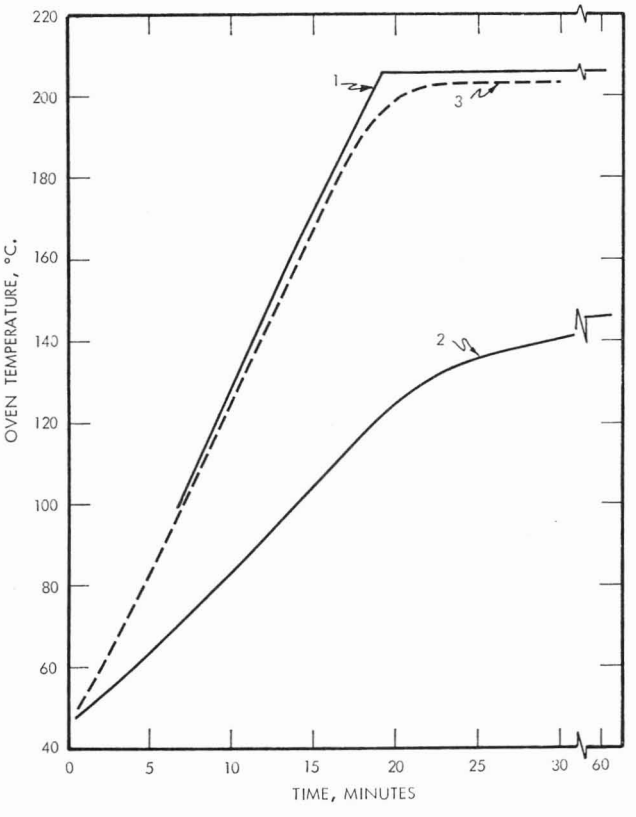

Figure 4. Column oven temperature lag

spacers removed. For the 150-foot columns, each was coiled a different diameter so that two or three columns could be connected in series.

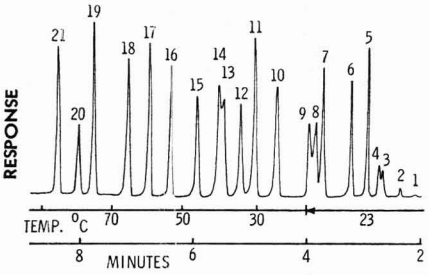

Figure 5. Programmed separation of hydrocarbon mixture

See Table II for the peak identification

Table I. Instrument Conditions for Chromatographic Experiments

Figure	5	6	7	8
Sample	Liquid hydrocarbons	C_1 to C_4 hydrocarbons	C_1 to C_5 hydrocarbons	C_2 to C_8 olefins
Sample volume, ml.	12×10^{-6}	0.17	0.086 to 0.80	0.18
Electrometer, range	10	10	64 to 400	1
attenuation	128	as noted		128
Column[a]	1	1	2	2
Column temperature, ° C.	25 to 90	−50	23	0 to 50
Inlet pressure, p.s.i.g.	37	35	48	58
Air, ml./minute	620	620	260[b]	620
H_2, ml./minute	35	35	20	35
Carrier, ml./minute	4.6 N_2	4.4 N_2	7.1 H_2	4.1 N_2
Auxiliary N_2, ml./minute	30	30	27	30

[a] Column 1 is a 150-foot capillary tube coated with silicone oil; column 2 is a 300-foot capillary tube coated with Ucon LB-550-X.
[b] A Perkin-Elmer flame ionization detector was used for this experiment.

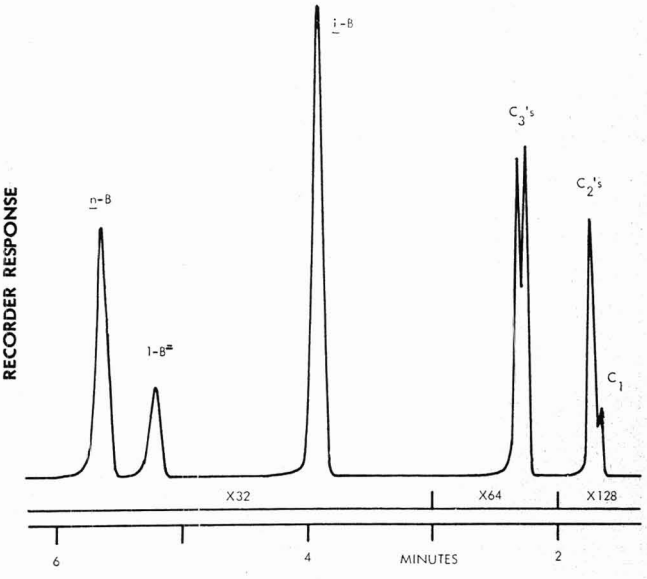

Figure 6. Low-temperature separation of light hydrocarbons

gram after the following changes were made to the oven heating system. The portion of the perforated shield above the fan was wrapped with aluminum foil to force the air more directly over the heater wires. The original fan motor was replaced with one (Bodine Electric Co., Model B2190) run at 7400 r.p.m. The speed of the motor can be adjusted between 3000 and 9000 r.p.m. with the variable transformer, **2** (Figure 1). The controller thermo-

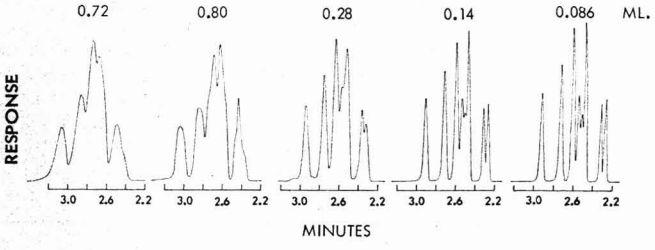

Figure 7. Effect of gas sample size on component separation

Column Oven Heating System. Initial chromatographic experiments using temperature programming indicated large temperature differences between the column and the controller thermocouple. To compare the temperatures of the thermocouple and the air around the column, a thermometer, **4** (Figure 1), was inserted through a hollow bolt in the oven cover, **3**, so that the mercury bulb was close to the column. The controller was set to start at 45° C., increase at a nominal heating rate of 9° C. per minute, and hold the temperature at 206° C. Curve 1 of Figure 4 shows the indicated thermocouple temperature for this heating program. Curve 2 represents the thermometer temperature and illustrates the large temperature difference between the thermocouple and the air around the column. Even after holding the controller at a thermocouple temperature of 206° C. for 41 minutes, the oven temperature only reached 146° C.

Curve 3 represents the thermometer temperature for the same heating pro-

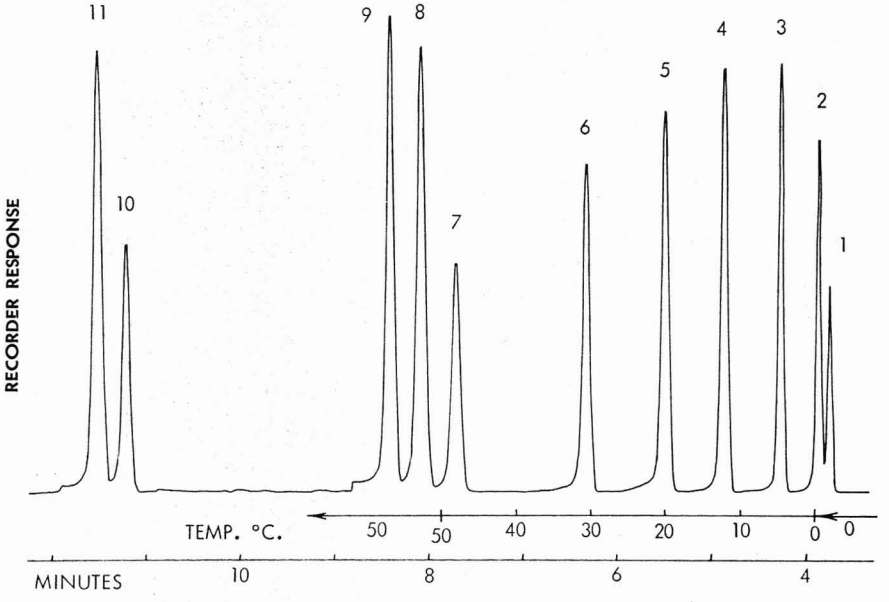

Figure 8. Separation of dilute hydrocarbon gas mixture

See Table III for the peak identification

couple, originally wedged in a hole at the top of the heater support cylinder, was extended through the hole to the center of the cylinder. These changes reduced the temperature lag of the column to within 2% of the prescribed temperature program.

Procedure. Table I lists the instrument conditions that were used for the chromatographic experiments. Gas samples were used for all experiments except the one for measuring the retention volumes. The smallest gas sample that could be measured with the sampling valve was 0.086 ml., and all gas samples were injected directly into the capillary column.

The flame ionization detector, was operated with 1:1 hydrogen:total nitrogen as the fuel mixture for maximum detector response.

For those experiments started at subambient temperature, the column was cooled with chipped dry ice. The dry ice was added through a hole made in the cover handle and held on a wire screen at the top of the oven heating assembly.

RESULTS

Figure 5 shows the separation of a 21-hydrocarbon mixture, and is an example of the sharp peaks and short analysis time obtained with a temperature programmed capillary column. The column was operated isothermally at 23° C. for the first 4 minutes to separate the lower boiling hydrocarbons, then programmed at 16° per minute. This temperature program was chosen from the several investigated, because it afforded a good separation of the hydrocarbons in this mixture. This mixture and five others, containing a total of 65 C_5 to C_8 hydrocarbons, were used to catalog peak retention volumes for component identification purposes.

Retention Volume Precision. To determine the precision of the retention volumes (which is an indication of the reproducibility of the temperature program), the six hydrocarbon mixtures were repeatedly chromatographed during a 3-day period using the conditions of Figure 5. Table II lists the values of the average retention volume, $\overline{V}_N$, and the range of the retention volumes, $V_{N\max} - V_{N\min}$, for the indicated number of runs for each hydrocarbon. V_N was corrected for the volume of the apparatus by subtracting the retention volume of methane. For the 41 hydrocarbons having three or more V_N measurements, the relative standard deviation was calculated from the range (*2*) and averaged ±1.8%. This precision is satisfactory for using retention volumes as a means for component identification.

Subambient Temperatures. A capillary column was also cooled to subambient temperatures in an attempt to separate the light hydrocarbon gases. Figure 6 shows the partial separation of a dilute mixture of C_1 to C_4 hydrocarbons in 99.8% nitrogen (*5*). With the column at −50° C., the C_1 to C_2 hydrocarbons were not separated, but the separations improved with the C_3's and C_4's. This separation is poorer than that reported by Averill and Ettre (*1*) because of the effect of the diluent nitrogen in the sample.

Gas Sample Size. The diluent nitrogen limits the sample size because it spreads out the hydrocarbon components in the column and causes peak broadening. Figure 7 shows that, by injecting a dilute C_1 to

Table II. Retention Volume, V_N, for a Number of Hydrocarbons

Peak number (Fig. 5)	Compound	B. P., ° C.	$\overline{V}_N$, ml.	$V_{N\max} - V_{N\min}$, ml.	No. of runs	Source[a]
	Isopentane	28.0	0.89	0.10	3	P
	1-Pentene	30.0	1.01	0.11	7	P
	2-Methyl-1-butene	31.2	1.05	...	1	P
1	*n*-Pentane	36.0	1.15	0.06	4	P
	Isoprene	34.1	1.21	0.11	7	P
	2-Pentene	36.7	1.29	0.08	3	P
	2-Methyl-2-butene	38.6	1.38	...	1	P
2	2,2-Dimethylbutane	49.7	1.68	0.05	3	P
	Cyclopentene	44.2	1.89	0.05	2	P
	4-Methyl-1-pentene	53.9	1.99	0.07	3	P
3	Cyclopentane	49.3	2.18	0.09	6	P
	2,3,-Dimethyl-1-butene	55.6	2.23	...	1	GM
4	2,3-Dimethyl butane	58.0	2.29	0.01	2	P
	4-Methyl-*cis*-2-pentene	56.4	2.35	0.22	9	P
	4-Methyl-*trans*-2-pentene	58.6	2.41			P
	2-Methylpentane	60.3	2.37	0.06	2	P
5	3-Methylpentane	63.3	2.73	0.20	9	GM
	2-Methyl-1-pentene	62.1	2.80	0.04	2	P
	1-Hexene	63.5	2.78	0.10	2	P
	2-Ethyl-1-butene	64.7	3.16	...	1	GM
6	*n*-Hexane	68.7	3.26	0.22	9	P
	3-Hexene	66.8	3.24	0.13	3	P
	2-Hexene	68.4	3.37	0.12	2	P
	2-Methyl-2-pentene	67.3	3.51	0.24	7	P
	3-Methyl-*cis*-2-pentene	67.7	3.54	0	2	GM
	3-Methyl-*trans*-2-pentene	70.4	3.89			
	Methylcyclopentane	71.8	4.18	0.28	10	GM
7	2,2-Dimethylpentane	79.2	4.16	0	2	P
	2,3-Dimethyl-2-butene	73.2	4.34	...	1	GM
8	2,4-Dimethylpentane	80.5	4.43	0.12	4	P
	2,3,3-Trimethyl-1-butene	77.9	4.48	0.22	4	GM
9	2,2,3-Trimethylbutane	80.9	4.65	0.07	4	GM
	3,4-Dimethyl-1-pentene	80.8	4.93	0.29	8	GM
	Benzene	80.1	5.08	0.18	3	P
	3,3-Dimethylpentane	86.1	5.56	0.17	6	GM
10	Cyclohexane	80.7	5.83	0.18	2	GM
	Cyclohexene	83.0	6.40	0.35	5	GM
	2,3-Dimethylpentane	89.8	6.32	0.09	4	GM
11	2-Methylhexane	90.1	6.33	0.06	5	GM
12	3-Methylhexane	91.9	6.80	0.38	9	P
	5-Methyl-*trans*-2-hexene	88.1	6.94	0.40	9	GM
	5-Methyl-*cis*-2-hexene	89.5				
13	3-Ethylpentane	93.5	7.30	0.12	4	GM
	1-Heptene	93.6	7.46	0.12	2	HW
14	2,2,4-Trimethylpentane	99.2	7.57	0.24	5	P
15	*n*-Heptane	98.5	8.16	0.13	3	P
	trans-3-Heptene	95.7	8.07	0.40	7	P
	cis-3-Heptene	95.8	8.18			
	trans-2-Heptene	98.0	8.33	0.13	3	P
	cis-2-Heptene	98.4	8.77			
	2,4,4-Trimethyl-1-pentene	101.4	8.50	0.18	3	P
16	Methylcyclohexane	100.9	9.06	0.17	5	GM
	2,4,4-Trimethyl-2-pentene	104.9	9.44	0.24	4	P
	4-Methylcyclohexene	102.9	9.55	0.07	2	P
17	2,2,3-Trimethylpentane	109.8	9.8	0.50	9	GM
	Toluene	110.6	10.5	0.2	4	P
18	2,3,4-Trimethylpentane	113.5	10.6	0.3	4	P
19	3-Methylheptane	118.9	11.6	0.4	4	GM
	2-Ethyl-1-hexene	120.0	12.0	0.5	8	HW
	1-Octene	121.3	12.1	0.1	2	P
20	2,2,5-Trimethylhexane	124.1	12.2	0.5	3	P
	2,3-Dimethyl-2-hexene	121.8	12.6	0.2	2	GM
21	*n*-Octane	125.7	12.7	0.5	4	E
	trans-2-Octene	125.0	12.9	0.5	7	P
	cis-2-Octene	125.6	13.2			

[a] P, Phillips Petroleum Co.; E, Eastman Kodak Co.; HW, Humphrey-Wilkinson, Inc.; GM, prepared at the General Motors Research Laboratories or of an unknown source.

C_5 hydrocarbon mixture (200 p.p.m. each), the peaks were unduly broad for sample volumes of 0.28 ml. and larger. The chromatograms for the 0.72-ml. and 0.80-ml. volumes are included to show the effect of similar volumes injected from sample tubes of widely different internal diameters. The tube bores are 2.35 and 0.51 mm., respectively. The latter volume gave a slightly better separation, even though it is larger, because of less mixing of the carrier gas with the sample in the narrow-bore tube.

Peak Area Precision. There are two factors which can affect the precision of the peak area measurements made with temperature programmed capillary columns: the relatively small area of the sharp peaks, and the baseline drift caused by increased column liquid bleeding. Base-line drift becomes more pronounced at the high detector sensitivities required for analyzing dilute hydrocarbon gas mixtures. This is shown in Figure 8, for the separation of a mixture containing 10 olefins and *n*-hexane in 99.9% nitrogen. Although the column was programmed from only 0° to 50° C., some base-line drift occurred, which the operator periodically corrected with the detector balance control.

To compare the peak area precision obtained for different chromatographic conditions, the range of the peak area measurements for each of the 11 hydrocarbons was calculated and is given in Table III. These data were obtained with capillary or packed columns that were temperature programmed or operated isothermally using nitrogen or hydrogen as the carrier gas. The range values vary to a similar extent for the different conditions except for those of Figure 8. These values are lower, even though some of the peak areas had to be corrected for base-line drift. The results of an independent laboratory are included in Table III to make a further comparison between packed and capillary columns. On the basis of these values, it is concluded that similar quantitative results for dilute hydrocarbon gas mixtures are obtained with capillary or packed columns.

Table III. Peak Area Precision for a Dilute Hydrocarbon Mixture

Peak no., Fig. 8	Hydrocarbon	Concn., p.p.m.	Range of peak areas (% of average peak area) A[a,b]	B[c]	C[d]	D[e]	E[f,g]	F[g,h]
1	Ethylene	83	1.2	16.1	1.7	5.3	8.0	2.2
2	Propylene	100	2.1	7.0		6.2	2.5	9.0
3	1-Butene	102	1.0	5.0	1.3	3.5	6.6	2.0
4	3-Methyl-1-butene	97	1.3	5.3	2.7	12.4	8.1	4.3
5	1-Pentene	98	2.2	3.8	3.2	11.3	11.7	2.1
6	2-Methyl-2-butene	93	1.9	3.5	6.4	15.4	5.3	1.4
7	Cyclopentene	75	3.5	9.6	2.9	8.1	5.4	12.4
8	*n*-Hexane	115	4.2	6.5	2.3	6.7	5.6	9.1
9	1-Hexene	104	1.1	6.5	4.5	7.3	6.1	6.8
10	1-Heptene	51	6.2	8.0	11.8	5.7	1.7	17.0
11	2,4,4-Trimethyl-1-pentene	81	6.9	2.7	15.5	2.1	2.2	3.0

[a] Chromatogram for these conditions shown in Figure 8.
[b] Runs, 3; carrier gas, N_2; capillary col.; temp. prog.
[c] Runs, 4; carrier gas, H_2; capillary col.; temp. prog.
[d] Runs, 4; carrier gas, H_2; capillary col.; isothermal.
[e] Runs, 4; carrier gas, N_2; packed col.; isothermal.
[f] Runs, 3; carrier gas, N_2; packed col.; isothermal.
[g] Results of an independent lab.
[h] Runs, 3; carrier gas, N_2; packed col.; isothermal.

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A Gas-Density Detector for Gas Chromatography

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▶ **To better understand how a gas-density detector senses minute changes in composition of the effluent gas, a theory based on the conservation of energy was developed. Equations were developed to show the interrelationship of change in density, flow, and electrical response. On the basis of these equations a simplified gas-density detector was developed. Analyses of complex mixtures—aromatics, saturates, chlorinated and oxygenated compounds—gave accurate results without calibration.**

IN A gas chromatographic system, the detector must respond to minute changes in the composition of the gas exiting from the column. Usually the relationship between composition and detector response is complicated and unpredictable and the detector must be calibrated for the individual components (*2*). With a gas-density detector, calibration is eliminated because response depends on a predictable relationship, the difference in the molecular weights of component and carrier gas (*11*). Because only the carrier gas and not the sample component touches the sensing elements, problems caused by the corrosion of the elements are avoided. Numerous different carrier gases may be used, but the less expensive nitrogen is quite satisfactory for most determinations. A workable gas-density detector was first described by Martin (*3*, *4*). However, these detectors have remained so complex in design and construction, despite efforts to simplify them (*7*, *8*), that they have not come into wide use (*10*). A simpler gas-density detector has been needed.

To explain the response of the detector to changes in density, a concept based on the conservation of energy in the gas was developed. To show this dynamic interrelationship quantitatively, simplified equations were derived. Validity of the equations was confirmed experimentally, and they furnished the basis in attaining an optimum design for a simple gas-density detector (*9*).

THEORY

The basic flow pattern in a gas-density detector is diagrammed in Figure 1. With the conduit network mounted vertically, the reference gas stream enters at A and divides, part flowing up to B and part down to D. The other stream, the effluent from a chromatographic column, enters at C and also divides, part flowing up to B and part down to D. The divided effluent streams combine with the divided reference streams at B and D, flow through BE and DE, and exit together at E. Conduit BD is always filled with effluent gas. When components heavier than the carrier gas are present, the density of the gas in conduit BD is greater and the pressure at D is increased. With a component lighter than the carrier gas, the pressure at D is decreased.

The change in pressure can be measured in two ways. In one way, used for the Martin gas-density detector, a flowmeter in a conduit parallel to BD senses change in flow caused by changes in pressure at B and D. The other way is to measure all of the change in flow in the conduits AB and AD and thus eliminate the parallel conduit.

The flow in conduit loop $ABCDA$ is related to the pressure drop around this loop:

$$\Delta P = 0 = (P_A - P_B) + (P_B - P_C) + (P_C - P_D) + (P_D - P_A) \quad (1)$$

where

P = pressure (at A, B, C, and D). A simple form of Bernoulli's theorem can be derived by assuming that pressure drop develops as potential energy and dissipates as friction, and by neglecting other forms of energy:

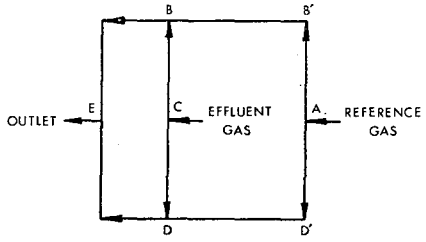


Figure 1. Flow in gas-density detector

$$\Delta P = \rho(X_B - X_D) + \rho(X_D - X_B) - [F(U)]_{AB} - [F(-U)]_{BC} - [F(U)]_{CD} - [F(-U)]_{DA} \quad (2)$$

where

ρ = density
$(X_B - X_D)$ = vertical height of the gas in conduit BD
F = constant for friction, and
U = flow rate in conduits AB, BC, CD, and DA.

The first term, involving density and height, represents potential energy. The remaining terms represent the dissipation of energy. Because the terms for the development and dissipation of energy balance out, pressure drop around loop $ABCDA$ is zero.

Changes in ΔP around loop $ABCDA$ are related to changes in ρ and U.

$$\Delta P = \rho(X_B - X_D) + \rho(X_D - X_B) - [F(U)]_{AB} - [F(-U)]_{BC} - [F(U)]_{CD} - [F(-U)]_{DA} = 0 \quad (2)$$

$$\Delta P' = \rho'(X_B - X_D) + \rho(X_D - X_B) - [F(U + \Delta U)]_{AB} - \{F[-(U - \Delta U)]\}_{BC} - [F(U + \Delta U)]_{CD} - [F(-U - \Delta U)]_{DA} = 0 \quad (3)$$

$$\Delta(\Delta P) = \Delta\rho(X_B - X_D) - [F(\Delta U)]_{AB} - [F(\Delta U)]_{BC} - [F(\Delta U)]_{CD} - [F(\Delta U)]_{DA} = 0 \quad (4)$$

or

$$\Delta(\Delta P) = \Delta\rho(X_B - X_D) - \Sigma F_{ABCD}(\Delta U) = 0 \quad (5)$$

Equation 2 applies when a component is not present in the effluent gas; Equation 3 applies when a component is present in the effluent gas in conduit BD. The difference between these equations is Equation 4 and may be simplified to Equation 5 by assuming ΔU to be the same in all conduits. The first term in Equation 5 represents the development of potential energy because of $\Delta\rho$ in the gas in BD. The second term represents the dissipation of energy because of ΔU caused by $\Delta\rho$.

By assuming viscous flow, factors from Poiseuille's equation may be substituted in the second term:

$$\Delta(\Delta P) = \Delta\rho(X_D - X_B) - \frac{128\eta L\Delta U}{\pi g D^4} = 0 \quad (6)$$

$$\Delta\rho = \frac{128\eta L\Delta U}{\pi g D^4 (X_D - X_B)} \quad (7)$$

where

η = viscosity
L = length of conduit
U = flow rate
g = gravitational constant
D = diameter of conduit

In the loop $ABCDA$ the presence of a component changes the viscosity only in BD. To simplify the experimental evaluation of the terms in Equation 7, the effect of any change in viscosity is considered negligible.

EXPERIMENTAL VERIFICATION OF THEORY

To evaluate each term in Equation 7, an apparatus with gas flow as shown in Figure 1 was constructed. Filament

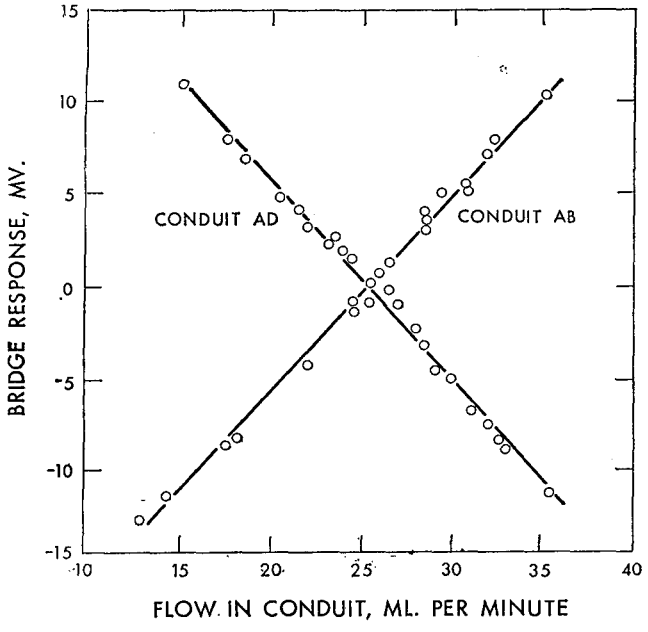


Figure 2. Effect of change in flow on response

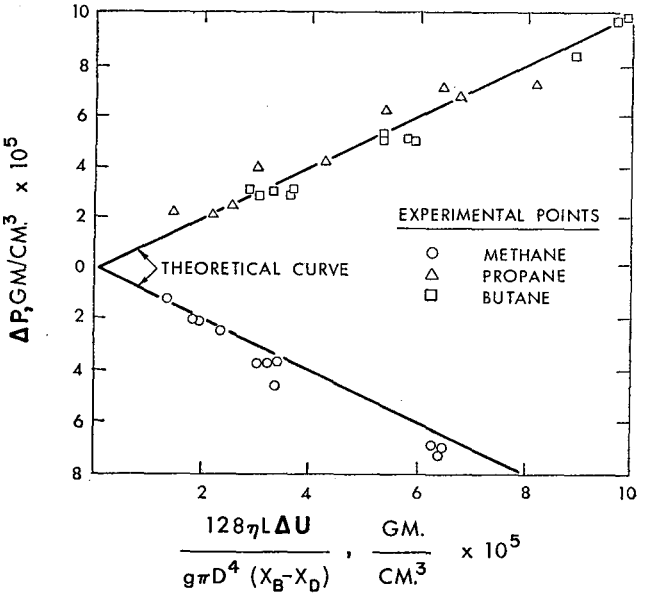


Figure 3. Agreement of experiment and theory

flowmeters located in conduits BB' and DD' were connected via an electrical bridge to a recording potentiometer.

To establish the relationship between ΔU and electrical response, the conduits $BCDE$ were removed, and rotameters with needle valves were connected to the flowmeter conduits. With nitrogen flow to A maintained constant at 50 ml. per minute, flow was diverted from AD to AB and vice versa by adjusting the needle valves. For each conduit, response varied linearly with flow. Therefore, the linear relationship between ΔU and response was established as shown in Figure 2.

The linearity, unlike the nonlinear relationship of conventional flowmeters, may be explained as a direct response to ΔU as this increment of flow is diverted from one flowmeter to the other. With the diversion of flow shown by the sign, the established nonlinear relationship between flow and response is shown by each flowmeter:

$$E_1 = K(U + \Delta U)^2$$

and

$$E_2 = K(U - \Delta U)^2$$

Because the electrical bridge responds to the difference, $E_1 - E_2$, which equals $4KU\Delta U$, response is linear for this apparatus.

To determine ΔU produced by $\Delta\rho$, conduits $BCDE$ were attached, and with reference and effluent streams flowing at 50 ml. per minute, known volumes of methane, propane, and butane were injected at C. The response to the introduction of "sample" was a Gaussian peak. The height of the peak represented ΔU.

By assuming that the gases behaved ideally, $\Delta\rho$ was calculated from the peak width at half peak height by the following equation:

$$\Delta\rho = \rho_n \left[\frac{\left(\frac{V}{V + (B)(C)(R)}\right) M_y + 1.00 - \left(\frac{V}{V + (B)(C)(R)}\right) M_n}{M_n} \right] - \rho_n \qquad (8)$$

where

ρ_n = density of nitrogen, grams/cm.^{3}
M_y = molecular weight of component
M_n = molecular weight of nitrogen
V = volume of component charged, cm.^{3}
B = band width at half peak height, cm.
C = 1/chart speed, minute/cm.
R = flow rate of carrier gas, cm.^{3}/minute.

Of the remaining terms in Equation 7, the viscosity was assumed to be that of nitrogen; the diameter and length of each conduit were measured directly; and various values of effective height, $X_B - X_D$, were obtained by tilting BD from its usual vertical position to angles of 60, 45, 30, and 0 with the horizontal.

In Figure 3, $\Delta\rho$ for methane, propane, and butane is plotted against $128\eta L\Delta U/\pi g D^4 (X_B - X_D)$. Data for components heavier than nitrogen fit the theoretical curve with a positive slope.

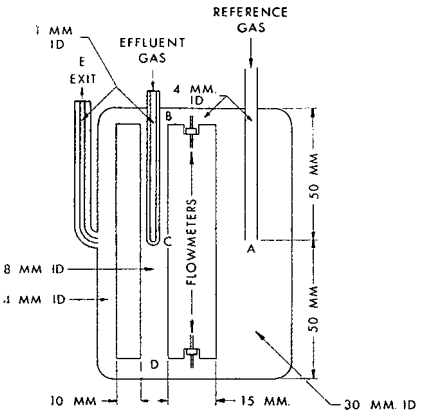


Figure 4. Gas-density detector

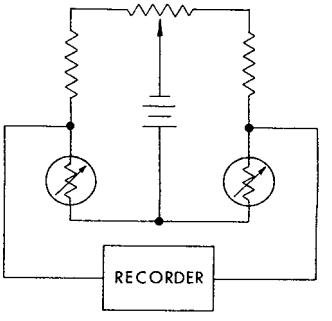
Data for the component lighter than nitrogen fit the theoretical curve with a negative slope. The positive slope shows that increased density diverts flow through AB, while negative slope shows that decreased density diverts flow through AD. Although $(X_B - X_D)$, and hence the potential energy, varied by a factor of 10, no significant deviations from experimental error were observed. Despite the scatter of the data, the correlations provide fundamental evidence that Equation 7 describes the operation of the detector and justifies the simplifying assumptions.

THE APPLICATION OF THEORY IN DEVELOPING A DETECTOR

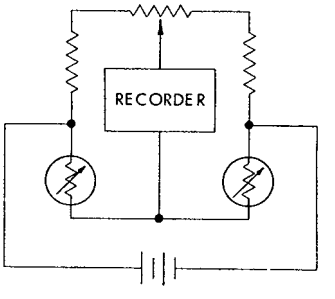
The practical problems involved in designing and operating a detector can be assessed quite satisfactorily from Equation 7. The flow rates of reference and effluent gas must be high enough to maintain ΔU and thus dissipate the energy developed by $\Delta\rho$. Increasing the flow rates of reference and effluent gases increases the linear dynamic range; increasing the flow rate of effluent gas decreases response time because the volume of conduit BD is filled and emptied more quickly. Linear dynamic range and response time—two important performance characteristics—may be enhanced by increasing flow rate. On the other hand, both may also be enhanced by decreasing detector dimensions, but at the expense of sensitivity. Enhancement of one characteristic at the expense of another necessitates compromise in design.

To minimize the compromise in design, the basic conduit flowmeter system was modified as shown in Figure 4. Restricting the diameter around the flowmeters to increase flow velocity increases both sensitivity and dynamic range. Restricting the diameter and length of the effluent gas conduit BD while increasing the diameter of reference gas conduits AB and AD can reduce response time without loss of sensitivity. With these modifications, the flow developed by the potential energy may be used more effectively.

To utilize each kind of flowmeter most effectively, two different electrical bridges, shown in Figure 5, were used. A parallel bridge was used with thermistors and a series bridge was used with filaments. In the parallel bridge, the current through the element with the lower resistance increases and that through the element with higher resistance decreases. Because of the negative temperature coefficient of the thermistors, the change in current in the parallel bridge enhances the decrease of resistance in the heated element and the increase of resistance in the cooled element. A cumulative effect—increasing current coupled with decreasing resistance in one element paralleling a simultaneous decreasing current coupled with increasing resistance in the other—results in a larger net change in resistance than that obtainable using a series bridge. In a series bridge, the same amount of current goes through each of the sensing elements. Because of the positive temperature coefficient of the filaments, a net change in resistance is larger than that obtainable with a parallel bridge. No cumulative resistance change occurs with filaments in either bridge.



PARALLEL BRIDGE, FOR THERMISTORS



SERIES BRIDGE, FOR FILAMENTS

Figure 5. Electrical bridges for flowmeters

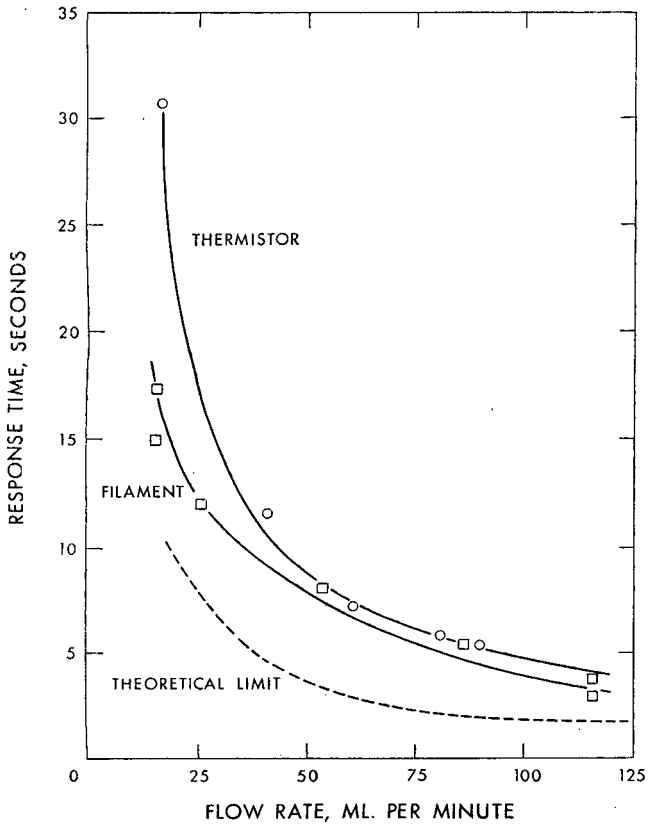


Figure 6. Effect of flow rate on response time

The bridges were connected directly to a recording potentiometer. Because response was adequate, the preamplifier required with some detectors was not needed. In special cases only, such as operation at high temperature, a preamplifier may be helpful.

Testing Detector Performance. Based on these refinements in conduit–flowmeter design, two glass detectors were made (*9*). One contained a matched pair of 15Ω filaments, the other a matched pair of 8K thermistors. Each pair was provided with appropriate electrical bridges.

Data for response time were obtained from the response of the detector to an instantaneous change in gas density (*9*). All other determinations were made using conventional gas chromatographic procedures. A reference and a chromatographic column were attached to the detector in an insulated case at room temperature. Nitrogen was used in both columns at the same flow rate of 60 ml. per minute. Samples were injected to the inlet of the chromatographic column with a hypodermic syringe.

Response time is directly proportional to the time of filling or emptying conduit *BD*, but cannot be less than the theoretical limit defined by (*1*):

$$\tau = 0.623 \frac{Vd}{F} \qquad (9)$$

where

τ = response time, seconds
Vd = volume of *BD*, 4.9 ml.
F = flow rate, ml./second

Response time decreases with flow for both detectors as shown in Figure 6 and approaches the theoretical limit. Because the gas-density detector is a flow-through rather than a diffusion device, only peak shape but not peak area is affected by the response time (*12*).

Sensitivity was calculated from a peak area produced by 0.24 mg. of butane. The limit of detection was defined as twice the noise level divided by sensitivity (*15*). Limit of detection varied with current; therefore, an optimum current was determined for each type of flowmeter as shown in Table I. To determine current giving lowest limit of detection, the values were determined for a range of currents. Because the limit of detection is six times better for the thermistor detector, it is preferred to the filament detector at room temperature. The greater sensitivity of thermistors probably results from the advantages of a higher temperature coefficient and resistance outweighing the disadvantages of small current and element area when used in the parallel bridge.

Table I. Comparison of Detector Performance with Filament and Thermistor Flowmeter Elements

Flowmeter element	Optimum current, ma.	Sensitivity, mv. ml./mg.	Noise, mv.	Detection limit, mg./ml.
Filament	110	250	0.006	48×10^{-6}
Thermistor	5	3200	0.013	8×10^{-6}

Response in the form of chromatographic peaks produced by 5 μl. of two known blends was used to obtain precision and accuracy data shown in Table II. To convert the peak area to quantity of component, a factor was derived from Equation 8:

$$\Delta\rho = \frac{\rho_n Y M_y + \rho_n M_n - Y\rho_n M_n - \rho_n M_n}{M_n} \qquad (10)$$

where Y = mole fraction.

$$\Delta\rho M_n = Y\rho_n(M_y - M_n) \qquad (11)$$

$$Y = \frac{1}{M_y - M_n} \frac{\Delta\rho M_n}{\rho_n} \qquad (12)$$

By multiplying through Equation 12 with the molecular weight of the component, mole fraction (Y) was converted to a measure of the weight of the component (YM_y).

$$M_y Y = \frac{M_y}{(M_y - M_n)} \frac{\Delta\rho_n M_n}{\rho_n} \qquad (13)$$

The appropriate factor, $M_y/(M_y - M_n)$, was used to convert peak area to a measure of the weight of component. Errors ranged from 1 to 3% in both blends. Although one blend contained only hydrocarbons and the other contained hydrocarbons and non-

Table II. Typical Precision and Accuracy Obtained with the Gas-Density Detectors

Component	Thermistor flowmeter Wt. %	Thermistor flowmeter Mean error	Filament flowmeter Wt. %	Filament flowmeter Error
Blend I				
n-Pentane	11.6 ± 0.3	−0.2	11.6 ± 0.3	−0.2
2,2-Dimethylbutane	18.9 ± 0.3	+0.1	18.9 ± 0.3	+0.1
2-Methylpentane	31.2 ± 0.4	0.0	31.1 ± 0.4	−0.1
n-Hexane	38.3 ± 0.4	+0.1	38.4 ± 0.5	+0.2
Blend II				
Ether	14.2 ± 0.3	+0.2	...	...
2-Methylpentane	13.2 ± 0.3	+0.3	...	...
Chloroform	29.7 ± 0.4	−0.3	...	...
Benzene	42.9 ± 0.4	−0.1	...	...

hydrocarbons, accurate results within the confidence interval were obtained without calibrating. These results substantiate the assumption that effects other than change in density are not significant under these conditions. Furthermore, the validity of eliminating calibration by using the gas-density detector was independently substantiated by the analysis of the synthetic blends (*6*).

DISCUSSION

Modifications of the approach are required for two components, hydrogen and ethane. Hydrogen causes a large $\Delta\rho$ and ΔU (about 20 times larger than that of other components for a given weight). Flow rates must be increased not only to dissipate the energy developed by a large $\Delta\rho$, but to prevent highly diffusive hydrogen from reaching the sensing elements. Even with these precautions, results, although linear, are 5% low. Apparently other effects, such as viscosity effects, become significant with the 20-fold increase in flow rates.

Ethane causes an extremely small $\Delta\rho$ and ΔU because its molecular weight is nearly that of nitrogen. The response is low enough to cause poor precision. The obvious solution is to use a carrier gas of a different molecular weight.

For over five years, gas-density detectors have been used to analyze many different samples including olefins in naphtha, products of paraffin isomerization and light naphthas in crude (*5, 6, 14*). Calibrations of thermal-conductivity cells would have been difficult and time consuming because of the large number of components, the presence of both liquids and gases in the samples, and the extreme nonlinear thermal-conductivity behavior of hydrogen in helium (*13*). With the gas-density detector, no calibration was needed other than the 5% correction for hydrogen.

Commercial models of the gas-density detector have been developed by the Gow-Mac Instrument Co. These more sophisticated detectors are made of metal, have interchangeable flowmeters, and may be operated at 300° C.

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Preparation of Methyl Esters for Gas Liquid Chromatography of Acids by Pyrolysis of Tetramethylammonium Salts

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▷ **The tetramethylammonium salts of carboxylic acids are converted to the corresponding methyl esters in high yield when they are injected into a commercial gas chromatographic unit at a vaporizer temperature of 330° to 365° C. The tetramethylammonium salts are prepared either by titrating the acids with tetramethylammonium hydroxide or by ion exchange on an anion exchange resin. This reaction has several advantages compared with existing methylation procedures in the gas chromatographic analysis of acids. However, the yield of methyl ester is lower if the sample size is less than 50 μg.; and oxalic, malonic, malic, and citric acids do not yield any methyl ester by this procedure.**

THE GAS LIQUID chromatography of carboxylic acids is difficult because their polarity and tendency to dimerize cause trailing, poorly shaped peaks, and nonreproducible retention times unless recourse is had to chromatographic phases especially designed for acids. In addition, their polarity results in excessively long retention times for the higher molecular weight acids.

For these reasons mixtures of acids are commonly converted to their methyl esters before they are chromatographed, and a considerable number of papers have dealt with methods for methylating acids. Vorbeck *et al.* (*9*), and Kirkland (*6*) have discussed the relative merits of commonly used methylation procedures.

Recently two techniques have been described in which acids are converted to esters by a pyrolysis reaction, the esters formed being swept directly onto a chromatographic column. In one procedure a mixture of the dry potassium salts of the acids and ethyl potassium sulfate is heated in a pyrolysis device (*5, 8*). In the other, the acids, mixed with boron trifluoride etherate and alcohol, are injected into a heated chamber (*2*).

In an attempt to devise a procedure in which the methyl esters would be formed during the chromatographic step, but which would not require any special apparatus, the authors considered the pyrolysis of tetramethylammonium salts of acids. This reaction was reported by Prelog and Picentanida (*7*), who found that when the tetramethylammonium salts of acids were heated, trimethylamine was driven off and a residue of nearly pure methyl ester remained. The reaction was later applied to the methylation of some sterically hindered benzoic acids by Fuson, Corse, and Horning (*3*). They reported that pyrolysis at 200° to 250° C. gave 60 to 90% yields of methyl esters.

We have found that this reaction proceeds rapidly at the temperatures obtainable in the injection ports of commercial gas chromatography units, so that when solutions of tetramethyl-

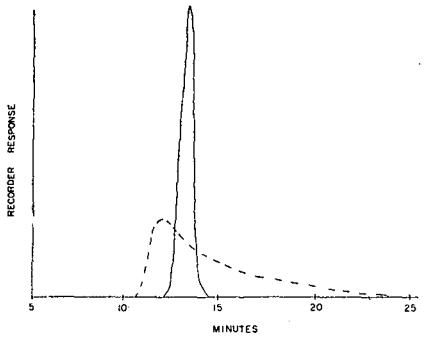

Figure 1. The methyl benzoate peak in the gas chromatography of tetramethylammonium benzoate

—. On F & M Model 500 programmed temperature gas chromatography unit
– – –. On Perkin-Elmer PR 154 Vapor Fractometer. Both at column temperature of 150° C.

ammonium salts of acids are injected into the instrument, the methylation reaction occurs in a nearly quantitative yield in the vaporizer and the methyl esters formed are swept onto the column and chromatographed.

EXPERIMENTAL

Chromatographic Equipment and Conditions. An F & M Scientific Corp. Model 500 programmed temperature chromatographic unit was used with a 4-foot by 1/4-inch o.d. Carbowax 20 M column (20% on 40/60 mesh Chromosorb W). The helium flow rate was 120 ml. per minute, and the detector temperature was 267° C. The column temperature was maintained at 75° C. for 2.5 minutes after injection of the sample, then was programmed at 11° C. per minute to a maximum temperature of 220° C. Except where specified as being otherwise, the injection port temperature was 365° C., and sample sizes were 1 to 5 μl. of a solution containing 20 to 25 mg. per ml. of tetramethylammonium salt.

Procedure: Preparation of Tetramethylammanium Salts. By Titration. The acid was weighed out, dissolved in methanol, and titrated to a phenolphthalein end point with tetramethylammonium hydroxide in methanol, prepared from tetramethylammonium chloride and silver oxide by the procedure of Cundiff and Markunas (*1*). The titrated solution was diluted with methanol to a volume such that the concentration of the acid was between 20 and 25 mg. per ml.

By Ion Exchange. Amberlite IRA-400 ion exchange resin, 2.0 grams, was converted to the hydroxide form by the method of Hornstein *et al.* (*4*). The resin was washed with four 50-ml. portions of anhydrous methanol by stirring and decantation. Then a solution of 0.203 gram of benzoic acid in 10 ml. of methanol was added to the resin. After 10 minutes, the resin was transferred to a column (10 × 250 mm.) and washed with 200 ml. of methanol. A solution of 0.50 gram of tetramethylammonium chloride (Eastman Organic Chemicals, practical grade) in 15 ml. of methanol was added to the column, and the eluate (volume 15 ml., elution rate 0.6 ml. per minute) was collected and a 5-μl. aliquot was chromatographed.

Separation of a Mixture of Acids. A solution of 0.375 gram each of butyric, valeric, caproic, caprylic, capric, lauric, myristic, and palmitic acids in 25 ml. of methanol was prepared. Five milliliters of this solution was titrated with tetramethylammonium hydroxide as described above and diluted to 25.0 ml. with methanol. A 10-μl. aliquot was chromatographed. A second 5.0-ml. portion of the solution of the acids was added to 6.25 grams of Amberlite IRA 400 which had been converted to the hydroxide form and washed with methanol as described above. The resin was then filtered, washed on the filter with 200 ml. of methanol, and added to a solution of 5.0 grams of tetramethylammonium chloride in methanol. The mixture was stirred for 10 minutes and filtered, and the filtrate was diluted to 25.0 ml. with methanol. A 10-μl. aliquot was chromatographed.

Determination of Ester Yields. Purified samples of the methyl esters were prepared from commercial samples by preparative scale gas chromatography. Standard solutions of the esters in methanol were chromatographed and linear plots of peak area *vs.* concentration were obtained. Peak areas were measured by cutting out the peaks and weighing the paper. The amount of methyl ester formed when the tetramethylammonium salts were chromatographed was then estimated by comparing the area of the ester peak with the standard plot. All yield figures are the average of two or more determinations. The difference between duplicate determinations was about 3%.

RESULTS AND DISCUSSION

In a preliminary experiment, a solution of tetramethylammonium benzoate was chromatographed on two commercial gas chromatography units, the Perkin-Elmer Model 154 and the F & M Model 500, using the same column and chromatographic conditions. In both cases, peaks due to trimethylamine, methanol solvent, and methyl benzoate were observed. There was no benzoic acid peak. In both cases, the yield of methyl benzoate, calculated from the peak area, was 91%. However, there was a great difference in the shape of the two peaks, as can be seen in Figure 1. The authors believe this difference is due not only to the difference in temperature in the injection ports of the two instruments, but also to differences in the geometry of the vaporizer heating elements. If some parts of the vaporizer chamber are cooler than others, as is the case in the Perkin-Elmer unit, the tetramethylammonium salt left on the cooler parts when the solvent evaporates will be converted to methyl ester more slowly, causing trailing of the methyl ester peak. In any case, all subsequent work was done on the F & M instrument, and sharp, symmetrical ester peaks were always obtained.

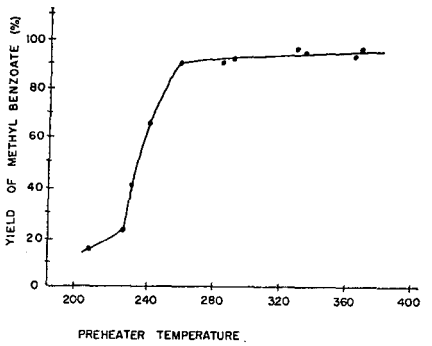

Figure 2. Yield of methyl benzoate as a function of injection port temperature

The optimum injection port temperature was then determined by chromatographing tetramethylammonium benzoate in methanol on the F & M Model 500 unit at a series of different injection port temperatures. The results are shown in Figure 2. Only small amounts of ester are formed below 220° C.; the yield increases sharply as the temperature is increased to 260° C., and a plateau is found between 330° and 370° C. In this range the average yield of methyl benzoate was 99%. All subsequent work was done at an indicated injection port temperature of 365° C. It is possible that the optimum decomposition temperature of other tetramethylammonium salts may be quite different from that of tetramethylammonium benzoate; however, the high yields of methyl esters (Table I) indicate that this is not the case for the acids studied.

Table I. Yields of Methyl Esters from Various Acids

Acid	Yield of methyl ester, %
Acetic	94
Butyric	87
Valeric	92
Caprylic	87
Lauric	93
Myristic	86
Palmitic	88
Benzoic	99
Cinnamic	87
Oxalic	0
Malonic	0
Succinic	94
Levulinic	89
Glycolic	93
Lactic	97
Malic	0
Citric	0

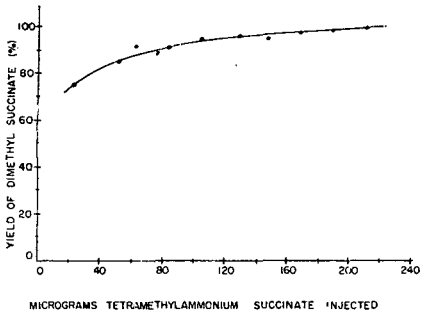

Figure 3. Yield of dimethyl succinate as a function of sample size

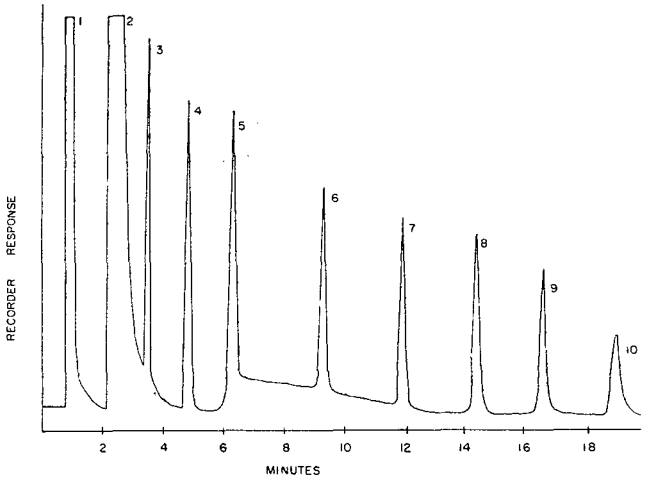

Figure 4. Chromatogram of a mixture of tetramethylammonium salts. Sample preparation and chromatographic conditions are described in Experimental Section

1. Trimethylamine
2. Methanol
3. Methyl butyrate
5. Methyl valerate
5. Methyl caproate
6. Methyl caprylate
7. Methyl caprate
8. Methyl laurate
9. Methyl myristate
10. Methyl palmitate

To ascertain the generality of this reaction we next investigated a number of different acids. The yields of methyl esters are listed in Table I. The fatty acids gave yields in the range 85 to 95%, with no apparent relationship between yield and chain length. Substantially complete conversions to methyl esters were found for aromatic acids, hydroxy acids, and dicarboxylic acids, except for oxalic, malonic, citric, and malic acids. In the case of these four acids no ester peak was observed. Evidently the salts of these acids decompose entirely to lower molecular weight products at 365° C.

During this study we observed that the yields of ester depended somewhat on the sample size. This effect was investigated systematically by chromatographing various volumes of a solution of tetramethylammonium succinate, and also by chromatographing a constant volume of each of a series of tetramethylammonium succinate solutions of varying concentration. In both cases the yield of ester depended on the amount of tetramethylammonium succinate being chromatographed, the yield being less with very small amounts of the salt. The combined data from these two experiments are plotted in Figure 3.

A low yield of methyl ester for small amounts of acids would constitute a serious disadvantage of this procedure, especially if it were used to study mixtures of acids in which some of the acids occur in very small quantities. However, in a mixture of acids, the yield of a given methyl ester does not depend on the amount of the corresponding tetramethylammonium salt but on the total amount of salts in the mixture. This was shown by the following experiment.

A mixture of butyric, succinic, valeric, and cinnamic acids was titrated with tetramethylammonium hydroxide and diluted so that the concentration of each acid in the solution was 5 μg. per microliter and the total concentration of acids was 20 μg. per microliter. A 5-μl. sample of this solution was chromatographed. From Figure 3, we can predict that if the amount of each acid determines the yield of its ester, each ester should be formed in about 75% yield. On the other hand, if the total amount of acids present governs the yield of each ester, the yields of esters should be greater than 90%. The observed yields were: methyl butyrate 99%, methyl valerate 102%, methyl succinate 102%, and methyl cinnamate 91%. This result implies that, in the analysis of a mixture of acids, a reasonably complete conversion of each acid to its methyl ester can be expected as long as the total amount of acids being chromatographed is greater than approximately 50 μg.

The tetramethylammonium salts of carboxylic acids can be conveniently prepared by ion exchange chromatography. Acids are quickly and completely absorbed from methanol solution by a strongly basic anion exchange resin such as Amberlite IRA 400 in its hydroxide form (*4*). Addition of methanolic tetramethylammonium chloride to the resin causes the acids to appear in the eluate as their tetramethylammonium salts. The eluate can be injected into the gas chromatographic unit as it comes off the resin, or it can be concentrated by evaporation of the solvent before gas chromatography. In an experiment using benzoic acid, a yield of 102% of methyl benzoate was observed in the gas chromatogram, based on the amount of benzoic acid added to the resin.

In another experiment, an aliquot of a solution containing eight aliphatic acids was titrated with tetramethylammonium hydroxide, and a second aliquot was added to the ion exchange resin, and the acids were removed from the resin with tetramethylammonium chloride. The two solutions of tetramethylammonium salts thus obtained were chromatographed. In the two chromatograms the peak heights for corresponding esters were the same within the experimental error. This demonstrates that losses of acids in the ion exchange procedure are negligible. The chromatogram from the titrated solution is shown in Figure 4. The trailing peak underlying the methyl caproate and methyl caprylate peaks is due to water formed in the neutralization reaction.

Methanol was used as the solvent for the tetramethylammonium salts throughout this study. Under the chromatographic conditions used, methanol covers the peak from methyl propionate. However, any other solvent capable of dissolving these salts could be used in place of methanol. We have found, for example, that ethylene glycol monomethyl ether is a good solvent for tetramethylammonium salts. In a particular application of this method, a solvent could undoubtedly be found which would not interfere with any of the ester peaks being studied. The trimethylamine formed in the pyrolysis reaction did not interfere with any of the ester peaks, since its retention time was less than that of methyl formate on the Carbowax column.

This method of methylation appears to offer several advantages for the gas

chromatographic analysis of acids. Most notable are its convenience and rapidity. A separate methylation step is not required. Furthermore it can be used with a commercially available gas chromatographic instrument without any modification. The method is particularly convenient when combined with the use of ion exchange resins for the analysis of mixtures, since acids can be separated from neutral and basic compounds, converted to their methyl esters, and examined by gas chromatography in a short, single step procedure. A further advantage here is that the acids can be concentrated after their conversion to nonvolatile tetramethylammonium salts, eliminating the possibility of losing volatile acids or methyl esters during solvent removal.

The variable yields of esters (Table I) make this procedure inapplicable for assay-type analyses. The yields are high enough that the procedure can be used for qualitative analyses. It should also be adequate for trace analyses where high precision is not required, particularly if the standard plots of ester peak area *vs.* concentration are prepared by putting known amounts of acids through the entire procedure.

The most serious defect of the method is that the salts of some acids—e.g., oxalic, malonic, malic, and citric among the acids we have studied— are decomposed instead of being converted to their methyl esters, and so cannot be detected or analyzed by this procedure.

ACKNOWLEDGMENT

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Gas Chromatographic Determination of Free Toluene Diisocyanate in Adducts with Trimethylolpropane

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▶ **The volatile components of toluene diisocyanate (TDI)–trimethylolpropane (TMP) adducts are vaporized and the amount of free TDI is determined by gas liquid chromatography. Conditions are chosen to minimize the thermal dissociation of the adduct. Peak heights of the free TDI and an internal marker added to the unknown are compared to the corresponding peaks of a sample of known free TDI concentration. The method has been used in laboratory studies of TDI–TMP reactions. When compared with a wet chemical method, the gas chromatographic method exhibits greater precision and accuracy in the range of 0.5 to 10% free TDI.**

URETHANE COATINGS are now receiving increasing attention from the paint industry. They are solutions of resins prepared by reaction of the —NCO group of diisocyanates with the —OH group of polyols. A typical component of two-package urethane coatings is the adduct formed by reacting toluene diisocyanate (TDI) and trimethylolpropane (TMP). Reaction of TDI and TMP at sufficiently low temperature takes advantage of the relatively greater reactivity of the —NCO group para to the methyl group and produces a triisocyanate adduct of high molecular weight containing little unreacted TDI (*10*). Because they are free of residual TDI, polyisocyanates of this type offer the advantage of decreased toxicity in addition to greater ease in formulation. Although preparation of polyisocyanate adducts of this type is reasonably successful with Nacconate 100 (2,4-TDI), preparation from the more economical Nacconate 80 (80–20 mixture of 2,4-TDI–2,6-TDI) is complicated by the presence of a higher proportion of the less reactive —NCO groups in the ortho position.

Various statements in the literature indicate that residual TDI content of adducts prepared from the 80–20 mixture ranges from 0.1 to 15% depending upon conditions of the reaction (*2, 3, 5, 10*). Measurement of the free TDI content of these adducts is complicated by the fact that classical methods for the determination of isocyanates are end-group analyses—reaction with amines and infrared spectroscopy—which do not differentiate between mono-, di-, and triisocyanate (*1, 12, 13*). Methods to separate unreacted TDI from the adduct have been suggested. In certain cases, the low molecular weight diamine produced by the acid hydrolysis of TDI can be separated from the high molecular weight triamine produced by the hydrolysis of the adduct. Estimation of the diamine can then be made colorimetrically (*8*). Free TDI has been separated from adducts by vacuum distillation in specially designed apparatus (*11*) and by solvent extraction (*9*). These methods, however, do not lend themselves to routine analysis of a large number of samples.

Gas liquid chromatography (GLC) has been discussed briefly as a method for determination of mono-, di-, and triisocyanates (*4*). The present study concerns the gas chromatographic determination of free TDI in TDI–TMP adducts.

EXPERIMENTAL

The TDI–TMP adducts were prepared in a solvent mixture consisting of 2 parts Cellosolve acetate (Union Carbide Chemical Corp.) and 1 part xylene by weight. The final product consisted of 40% solvent by weight. The preparation of the TDI–TMP adducts was carried out by conventional laboratory procedure. The reaction was considered complete when the measured NCO content was constant and about equal to the calculated value.

Apparatus. A Perkin-Elmer Model 188 vapor fractometer was used with a hot-wire detector. Modification was made to permit control and measurement of the vaporizer temperature.

Table I. Comparison of Methods for Free TDI

Calcd. free TDI, %	Found, % GLC	Found, % Chemical
0.45[a]	0.45	0.6; 0.6
1.5	1.2	4.0; 3.1
2.5	2.5	5.1
4.5	4.6	9
8.5	9.0; 9.4	over 11

[a] Free TDI content of starting material found by GLC.

Table II. Effect of Reaction Temperature on Free TDI Content

80–20 Mixture of 2,4- and 2,6-TDI

NCO–OH group ratio 1.8

Reaction temperature, ° C.	Free TDI, %
65	5.1
50	4.8
40	3.7

Table III. Effect of Isomer Ratio and NCO–OH on Free TDI Content

Reaction temperature 40° C.

NCO–OH group ratio	Free TDI, %
80–20 Mixture of 2,4- and 2,6-TDI	
2.0	5.7
1.9	5.2
1.8	3.7
2.4-TDI	
2.0	3.3
1.9	2.0
1.8	1.4

The column was 2 meters in length, of $^1/_4$-inch stainless steel tubing packed with 10% by weight of Dow Corning High Vacuum Silicone grease on unsized Fluoropak 80. A weighed amount of the support material was added to a chloroform solution of the silicone grease. The solvent was evaporated on a steam bath with agitation to assure uniform coating. Refrigeration of the coated Fluoropak at 0° to 10° C. aided in packing the column by producing a free flowing material. The column was conditioned at 190° C. for several hours in a slow stream of helium.

The separation was carried out at 125° C. with a helium flow of 80 ml. per minute measured at the column exit. Detector current was held at 250 ma. Samples were introduced by means of a Hamilton microliter syringe.

Procedure. The peak height of TDI in the sample was compared to the peak height of TDI in a standard of known free TDI content. Correction for difference in amount of sample and that of standard injected was made by using 1,2,4-trichlorobenzene (TCB) (Eastman P-1641) as an internal marker. Trichlorobenzene was chosen because it is readily available, it has a convenient retention time, and it does not react with material normally found in adducts to be analyzed.

The sample was prepared by adding 1 gram of TCB, accurately weighed, to 8 to 10 grams of the adduct, accurately weighed. Viscosity of the mixture was reduced by addition of 5 ml. of xylene dried over 5A molecular sieve pellets.

The standard used for comparison is prepared in the same manner as the sample, with the addition of a known weight of TDI, approximately 2 to 5% by weight of the adduct. A chromatogram is obtained from 5 μl. of sample and 5 μl. of standard.

Calculations. The ratio of amounts of sample and standard introduced into the chromatograph is determined by the ratio of TCB peak heights after correction for its weight fraction in sample and standard. The TDI content of the original adduct is calculated from the increase in peak height due to TDI added to the standard, after correction is made for any difference in sample size. Once the TDI content of one adduct has been determined, it can be used as a standard to determine the TDI content of other adducts by direct comparison of ratios of TDI to TCB peak heights.

DISCUSSION

The linearity of peak height of TDI with concentration was established from a series of synthetic mixtures of a commercially available adduct with weighed amounts of free TDI in the range of 2 to 10%. After peak heights of TDI and TCB were corrected for weight fraction of internal marker added, the ratio of TDI peak height to TCB peak height was plotted *vs.* per cent TDI added. A straight line resulted having a standard error of the estimate of 0.1%. During this experimentation, it was shown that the peak height ratio depends greatly upon carrier gas flow, column and vaporizer temperatures, but not upon sample size injected. It is for this reason that the sample and standard must be analyzed under identical instrumental conditions rather than relying on reference to a previously prepared calibration curve (*7*).

With the column maintained at 125° C., the vaporizer temperature was varied between 140° and 190° C. The ratio of TDI to TCB peak heights for a single synthetic mixture was strongly influenced by the vaporizer temperature within these limits. The ratio increased with an increase in temperature, but a plateau appeared in the range 150° to 155° C. This is interpreted as the temperature above which thermal decomposition of the adduct occurs, with the release of additional free TDI. [This conclusion is in agreement with recently published results concerning the thermal behavior of urethanes (*6*).] Below this temperature, vaporization is incomplete resulting in low values of TDI found. Operation of the vaporizer at 175° ± 2° C. results in more reproducible results. Any error introduced from thermal decomposition is largely compensated for by vaporization of the standard under the same conditions.

Nonvolatile residue remaining in the vaporizer has an adverse effect on reproducibility. After approximately 10 samples have been injected, accumulated residue causes tailing of peaks. At this point, heat transfer through the residue is not sufficient to cause instant vaporization. As the residue builds up, spurious peaks appear as bubbles burst, giving, in effect, multiple sampling. Shortly after tailing of the peaks begins, the ratio of peak heights will decrease and at this point the vaporizer must be cleaned.

The gas liquid chromatographic method was compared with a wet chemical determination of toluene diamine after hydrolysis of the synthetic mixtures in dilute sulfuric acid. The results of the comparison are shown in Table I.

The method was used to determine the free TDI content of a number of laboratory prepared samples of TDI–TMP adducts. The TDI–TMP reaction temperature was correlated with the free TDI content of the resulting product. Data are given in Table II.

In Table III, the TDI content of the adducts is correlated with the isomer ratio of the TDI. As expected, the presence of the 2,6-TDI tends to increase the free TDI content of the resulting product. The effect of varying the ratio of NCO equivalents to OH groups (NCO–OH) on the free TDI content of the resulting product is also shown in Table III. The theoretical NCO–OH for a pure TDI–TMP adduct is 2.0. Reducing this ratio should result in a decrease of free TDI in the product. This conclusion is supported by the data.

CONCLUSION

Gas liquid chromatography has advantages over other methods of determination of free TDI in adducts with TMP. It permits rapid and quantitative separation of unreacted TDI from the adduct in the systems studied and shows promise of application to other polyisocyanates.

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Chromatographic Liquid Leak Detection

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▶ A method for the detection of volatile silicone fluid leaking from sealed containers has been established. The separating properties of the gas chromatograph were employed satisfactorily in the determination of leak rates of sealed containers. The results show that this technique can be used in many cases in preference to the more common helium and Radiflo leak detectors.

THERE ARE several leak-detection schemes which permit the determination of gas leak rates; the most notable being the helium and the Radiflo tests. However, in cases of liquid leakage, the helium leak test is not applicable because: If a sealed container is liquid filled, there is no space for helium; even if space is purposely left for helium, the liquid may mask the helium from the leak and thus prevent its detection; there is not necessarily a correlation between helium leak rate and liquid leak rate; and the liquid leak rate is of concern, not the helium leak rate. The Radiflo technique, which requires the use of a radioactive gas, also is unsatisfactory for determining liquid leak rates for the same reasons, plus one additional reason: The Kr^{85} diffuses into any exterior organic encapsulating compound, thereby yielding erroneous results.

The containers of interest to us were filled with 0.65-centistoke silicone oil, DC 200. Although the use of a gas chromatograph as a liquid-leak-detection device is unique, gas chromatograph techniques have been used previously to detect and determine silicone oil, and have utilized silicone polymer columns (*1-3, 5*).

EXPERIMENTAL

Apparatus. A Perkin-Elmer Vapor Fractometer, Model 154D, with recorder, integrator, and gas-sampling valve was employed for these experiments. Columns packed with Apiezon L grease and a high-molecular weight polymer of silicone oil, DC 200, were found by experiment to be very suitable for this work. The test apparatus schematic is shown in Figure 1.

Procedure. After cleaning the container with trichloroethylene to remove any surface silicone oil, the container was dried by placing it in a stream of nitrogen. The cleaned container was then placed in the chamber and the vacuum lines were attached as shown in the schematic (Figure 1). The container to be tested was placed in a vacuum to speed the vaporization of any liquid that may leak from the container, to reduce the amount of air in the chamber and thereby improve sensitivity and permit the use of the ideal gas law, and to increase the leak rate. The sample chamber was then flushed thoroughly by opening valves *B*, *C*, and *D*, and pulling air through with the vacuum pump. The sample chamber was then closed by closing valve *B*. The pressure was reduced in the sample chamber to approximately 20 mm. Hg. The time at which a pressure of 20 mm. of Hg was reached was recorded. The sample chamber was then isolated by closing valve *C* and the manometer valve *A*.

The evacuation of the sampling tube was done by opening valve *E*. The evacuation was continued until just before the sample was extracted from the sampling chamber. The sampling-tube pressure was reduced to a pressure of 1 mm. of Hg or less.

After a predetermined time, valve *D* was closed, isolating the sampling tube from the vacuum pump. Then valve *C*, between the sample tube and the sample chamber, was opened for a short interval (approximately 10 seconds) and closed immediately. The time when this operation was completed was recorded.

The gas now contained in the sampling tube was flushed through the chromatograph, using helium gas as a carrier, by opening valves *F* and *G*. The plot obtained on the recorder represented the fractions into which the gas sample was separated. From this

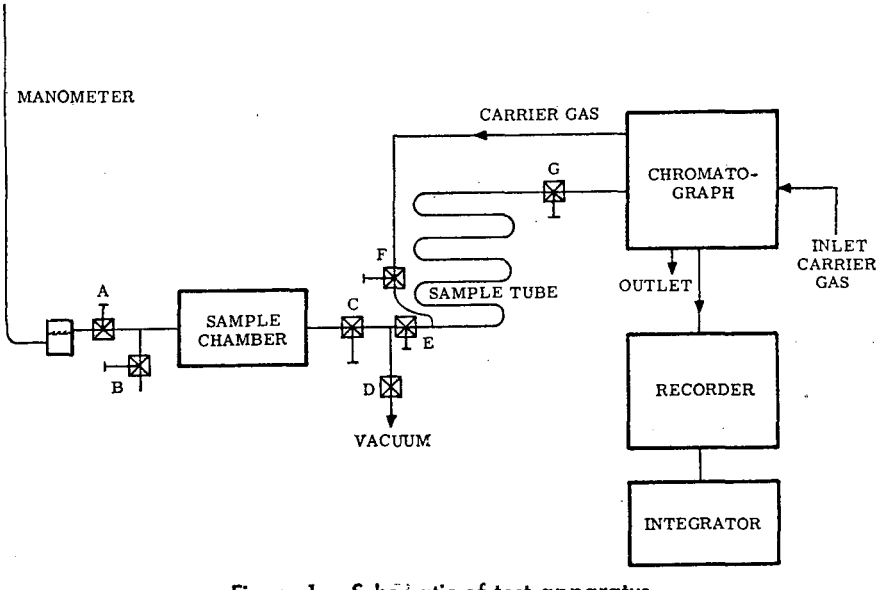

Figure 1. Schematic of test apparatus

Table I. Data from Leak-Test Experiments

Sample	Chamber temp. (° K.)	Time, sec.	Area of air peak	Area of silicone peak	Chamber vol., cc.	Absolute pressure, mm. Hg
a	300	38,800	102,400	...	35	22.9
b	300	47,500	113,000	146	35	45.7
c	300	1,200	63,740	503	29	22.9
d	300	1,200	87,540	32	29	25.4

plot, the mole per cent of DC 200 in the sample was calculated. From the mole per cent of DC 200, the leak rate for the leaking container, in turn, was determined.

Known amounts of silicone oil were injected into the sample chamber to simulate a leak in a container. The recovery was complete enough to ensure that all the oil injected was detected. The accuracy of detection improved as the quantity of oil was increased, while the accuracy was decreased as lesser amounts of the oil were used. However, in no instance was the indicated amount of oil less than the actual amount injected. The sample chamber was baked out under vacuum and then checked with the chromatograph to ensure that all residual silicone oil was removed before placing the next container into the chamber.

To leak-test containers containing silicone oil, DC 200, with a viscosity greater than 0.65 centistoke, a small amount of 0.65-centistoke oil was mixed with the more viscous, less volatile oil before sealing the container. The only change in the test procedure was that the container was held in a vacuum in the sample chamber for periods of about 12 hours, rather than periods of about 20 minutes.

RESULTS AND DISCUSSION

The fractions collected for typical containers are illustrated in Figure 2. The trichloroethylene peak indicates that not all the trichloroethylene was removed from the outside of the container. However, this did not lead to erroneous results because the calculations involved mole fractions. In fact, the trichloroethylene peak, along with the air peak, brackets the silicone-oil peak, thereby making the silicone-oil peak easily identifiable. Figure 2*a* shows the results obtained from a container which did not leak; Figure 2*b* shows the results of a leaky container which contained 3.0-centistoke silicone oil and a small added amount of 0.65-centistoke silicone oil; Figures 2*c* and 2*d* illustrate the results obtained from containers which were filled with 0.65-centistoke oil, which leaked at different rates. The data for these peaks, which will be used in later calculations, are given in Table I. The free volume of the sample chamber with the container inside was established by vacuum-filling the chamber with alcohol and measuring the weight change which was then converted to cubic centimeters.

Table II. Calculated Leak Rates

Sample	Cc. per second
a	0
b	5.0×10^{-10}
c	5.0×10^{-8}
d	2.6×10^{-9}

Since pressures involved in this work are relatively low, the number of moles of silicone oil (n) which leaks into the chamber and becomes vaporized can be calculated from the ideal gas law (*4*).

$$n = \frac{PV}{RT} \qquad (1)$$

where P, V, R, and T are the pressure, volume, gas constant, and temperature, respectively. To find the leak rate, θ, in cc. per second, one multiplies the value for n by NM/td, where N is the mole fraction of silicone oil in the sample chamber, t is the time the container was in the chamber under vacuum, d is the density of the oil at 77° F. and 1

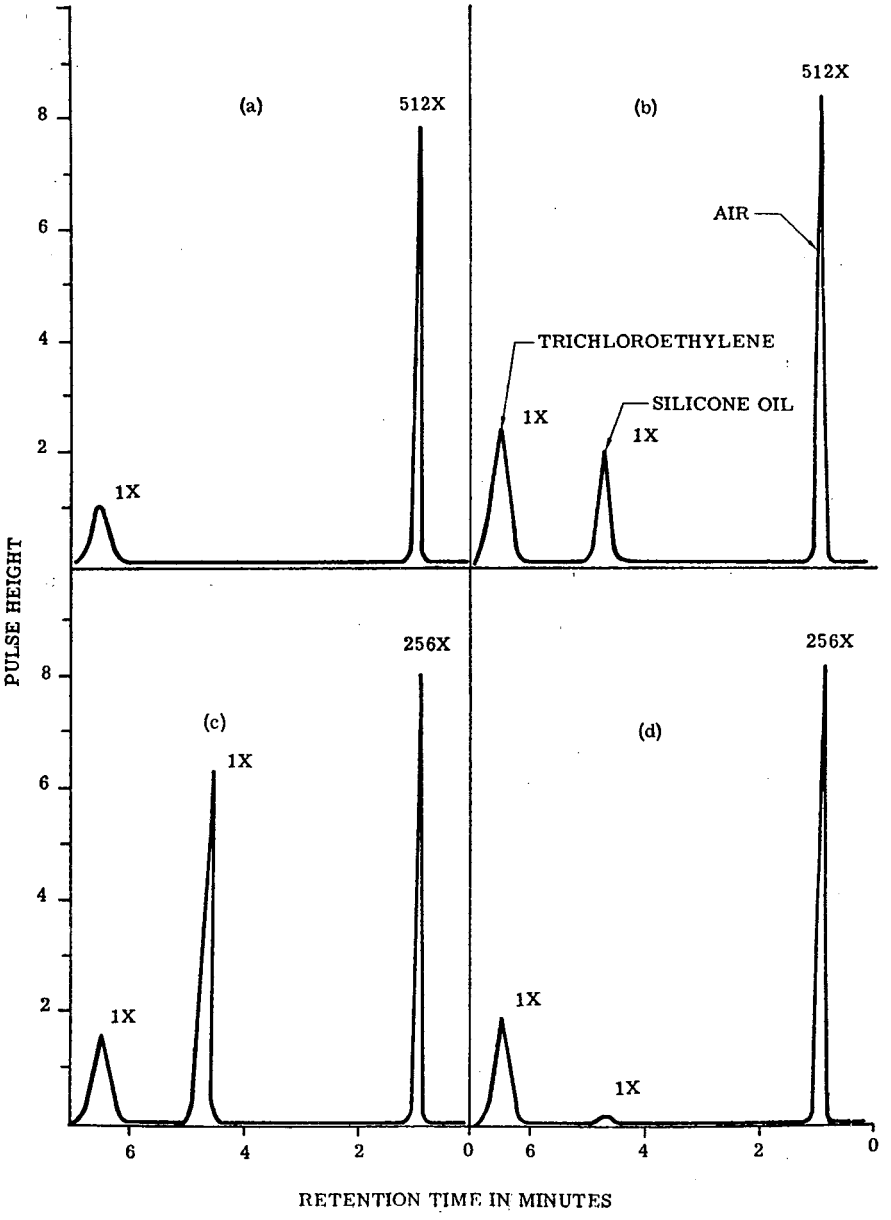

Figure 2. Reproduction of chromatograms of samples from typical containers tested

atmosphere, and M is the molecular weight of the oil. Thus,

$$\theta \text{ cc. per second} = MPVN/RTtd \quad (2)$$

N may be found by dividing the peak area of silicone oil by the sum of the peak areas of the silicone oil and air (and trichloroethylene if present). Neglecting the area of the trichloroethylene peak introduces only about 1% error in the final result. Constant factors in Equation 2 were collected as shown in Equation 3.

$$\theta = 1.15 \times 10^{-5} \frac{(PNV)}{t} \quad (3)$$

Using Equation 3 and the data in Table I, the results shown in Table II were calculated.

The main limiting factor of sensitivity in this technique is the length of time the container remains inside the evacuated sample chamber. Using representative values of 45 mm. of Hg pressure for 20 minutes yields a leak-rate accuracy of 10^{-9} cc. per second. Using 200 minutes will increase the limit another order of magnitude, which is sufficiently sensitive to detect leaks as small as 1 cc. per 30 years. Obviously the surface of the container in question and the sample chamber must be free of residual DC 200.

Any containers which are silicone oil or liquid filled may be leak tested by this technique, provided the liquid can be partially vaporized at temperatures below the working temperature of the column used, 175° C. For higher temperatures, different columns must be used. The results gathered in this work show that this technique is valid and yields more meaningful data for a liquid leak test than does the helium or Radiflo tests.

This leak-detection method is applicable to all components, both electrical and mechanical, that must be stored for extended periods of time, and which, after storage, must perform properly. These components may be filled with any volatile liquid or any gas. Thus, the chromatographic leak-detection method is applicable for both gas and liquid leak-rate determinations. There are several acceleration-sensing and integration devices used in the aerospace and missile field that are leak checked using this method.

ACKNOWLEDGMENT

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Gas Chromatographic Analysis of Chlorophenol Mixtures

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▷ **The use of a phosphoric acid additive and a thermally stable substrate capable of forming strong hydrogen bonds at elevated temperatures makes possible the determination of free chlorophenols on one GLC column without sample pretreatment. The ortho effect is successfully applied to obtain the necessary retention time reversals for resolution of phenol and eight chlorophenols. Double peak formation is observed for the first time in a nonaqueous system.**

THE numerous approaches to the analysis of mixtures of chlorophenols include infrared spectrometry (*6*), nonaqueous spectrophotometric titrations (*7*), ion exchange chromatography (*13*), and gas liquid chromatography (*1, 2, 5, 14*). Of these techniques the titrimetric method (*7*) is the least promising, being limited to relatively simple mixtures of known components in roughly equal amounts. Ion exchange (*13*), while giving good resolution of most of the chlorophenols, requires 3 to 4 hours for a qualitative determination of *o*-chlorophenol (OCP), 2,4-dichlorophenol (2,4-DCP), 2,6-dichlorophenol (2,6-DCP), and 2,4,6-trichlorophenol (2,4,6-TCP), with quantitative work requiring still more time. Phenol and *p*-chlorophenol (PCP) are not determined. Infrared spectrometry (*6*) is versatile and will give a complete analysis of complex mixtures of chlorophenols, but sensitivity and precision are limited, a large number of standard curves are required, and the calculations are laborious (graphical solution of simultaneous equations by successive approximation).

Gas chromatography offers a simple, rapid, and sensitive method of analysis but to date has suffered major drawbacks—e.g., a lack of suitable substrates with high temperature stability, poor resolution, and tailing of the chlorinated phenols on many polar substrates. Extensive gas chromatographic work on alkyl-substituted phenols has been done (*2, 3, 8, 9, 11, 12*), but extension of the techniques to chlorophenols has been relatively unsuccessful. Fitzgerald (*2*) recommended sample pretreatment (methylation) and GLC analysis of the chlorinated anisoles, rather than direct determination of the chlorophenols, particularly when the 2,6- and 2,4-DCP isomers were to be separated. Harvey and Norman (*5*) also used sample pretreatment (titration with diazomethane) and stated that mixtures of chlorophenols could not be analyzed directly. Barry, Vasishth, and Shelton (*1*) did analyze chlorophenols directly, but for mixtures containing PCP, 2,4-DCP, and 2,6-DCP, two different GLC columns (polar and nonpolar) and two separate analyses were required. In addition, 2,6-DCP was not completely resolved from 2,4-DCP but rode on the 2,4-DCP tail.

In the method described below, 2,6-DCP is clearly resolved from and is eluted before 2,4-DCP, despite its higher boiling point. Because of this position reversal, it is possible for the first time to determine small amounts of 2,6-DCP in 2,4-DCP quickly and accurately (Figures 1, 2, and 3). Complete resolution of eight chlorophenols and phenol can be obtained directly on one column (Figures 1 and 2), and no sample pretreatment or additional analyses are required. A phosphoric

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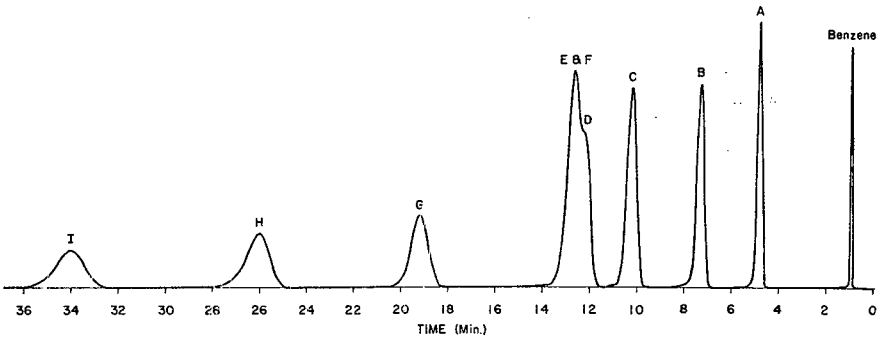

Figure 1. Chromatogram of approximately equal amounts by weight

A. OCP
B. Phenol
C. 2,6-DCP
D. 2,4-DCP
E. 2,3-DCP
F. 2,5-DCP
G. 2,4,6-TCP
H. PCP
I. 2,4,5-TCP

1/4 inch o.d. by 4 meters of 20% Carbowax 20 M plus 1.7% H_3PO_4, 200° C., ca. 120 ml. He/min., Perkin-Elmer 154-D gas chromatograph, 25 μl. of ca. 50% solution injected

acid additive to the substrate is used to eliminate heavy tailing in the same way as in the analysis of fatty acids (*10*).

EXPERIMENTAL

Apparatus. A Perkin-Elmer 154-D Vapor Fractometer (thermistor detector) and a Leeds and Northrup Speedomax G recorder (1-mv., 1-second response) equipped with a disk chart integrator (K3-1, 90-r.p.m. motor) were used in establishing the method. An Aerograph A-90 with four-filament hot wire detector and the above recorder were used for routine analyses.

A Beckman IR-5 (NaCl optics) and ultramicro sampling accessories including beam condenser (Beckman 46075), ultramicro liquid cell (NaCl, 0.1-mm. spacer, Beckman 23532), and wedge cell (Beckman 23532) were used to verify GLC peak identities.

Chromatographic Column. Three (Figures 2 and 3) or four (Figure 1) meters of 1/4-inch o.d. and 0.035-inch wall, stainless steel tubing are packed with 20% (by weight) Carbowax 20 M plus 2% H_3PO_4 (85% aqueous solution) on Gas Chrom P, 60- to 80-mesh, using an electric vibrator. (Carbowax 20 M and Gas Chrom P are available from Applied Science Laboratories, State College, Pa.) The packing is prepared by evaporation of a methanol–Carbowax 20 M–H_3PO_4 solution slurried with the solid support in a rotary vacuum evaporator over a steam bath. The packed column is purged with helium at room temperature and then given a static (no gas flow) 12- to 24-hour conditioning at 225° C., after which the temperature is lowered to 200° C. and the column again purged with helium until a steady base line is obtained.

GLC Operating Conditions and Sample Analysis. Perkin-Elmer detector voltage = 8 volts, power setting = 100; oven temperature = 200° C., flow rate = ca. 100 ml. of He per minute (20 p.s.i. at inlet). Aerograph: filament current = 235 ma., injection block temperature = 250° C., detector block temperature = 280° C.; oven temperature = 200° C., flow rate = ca. 100 ml. of He per minute (20 p.s.i. at inlet).

Twenty - five microliters of 25% (w./v.) solution of chlorophenols in benzene or ca. 5 μl. of molten or liquid chlorophenols may be injected into the chromatograph using a microliter syringe. Molten samples may be easily handled by preheating the syringe from 80° to 100° C.

The Perkin-Elmer injection system was particularly sensitive to overloading. A fast (<0.5-second) injection of 25 μl. of sample and benzene caused a broadening of the eluted peaks and an increase in tailing, apparently because some of the sample was flashing back into the cold inlet of the injection block, causing the peak distortion. Slowing the injection down somewhat (25 μl. in 1.0 to 1.2 seconds) eliminated this effect. This peak distortion was not noticed with the Aerograph A-90.

GLC-IR Analyses. Sample fractions were collected from the Perkin-Elmer using a 20 gauge × 1 inch stainless steel syringe needle slipped on the heated outlet. The shaft of the needle was cooled with dry ice. Sample collections from the Aerograph were made with capillary glass tubing of approximately the same inside diameter as the needle, but 2 to 3 inches long and bent in a U, the bottom of which was immersed in a dry ice–methanol bath.

From 50 to 100 μl. of molten chlorophenols can be easily handled on the 1/4-inch column. The collected fraction is dissolved in 10 to 20 μl. of solvent (CS_2 or CCl_4) and the infrared spectrum of the resultant solution is run in the usual manner, using the wedge cell to cancel out solvent bands.

Standards and Area Correction Factors. Area correction factors (ACF) were determined relative to ACS reagent grade phenol by the internal standard technique, the detector response factor for phenol arbitrarily being set at unity.

The 2,4-DCP (Eastman Organic Chemicals), 2,6-DCP (Aldrich Chemicals), and 2,4,5-TCP (Hooker Chemical

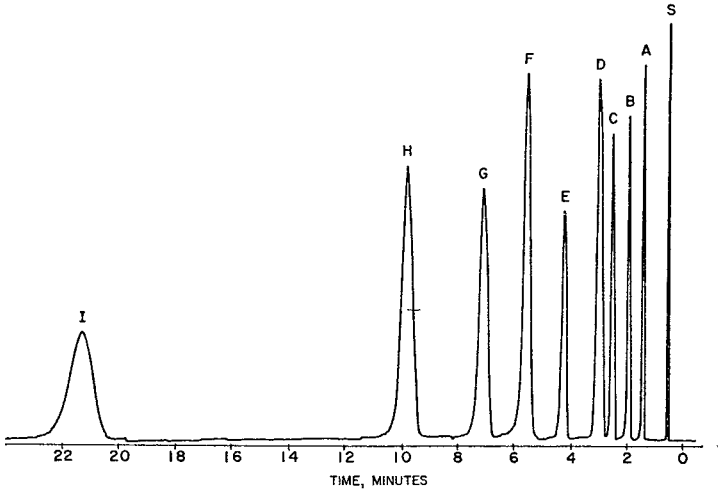

Figure 2. Chromatogram of approximately equal amounts by weight

A. OCP
B. Phenol
C. 2,6-DCP
D. 2,3-, 2,4-, 2,5-DCP
E. 2,4,6-TCP
F. PCP
G. 2,4,5-TCP
H. 2,3,4,6-tetrachlorophenol
I. Pentachlorophenol
in benzene, S

1/4 inch o.d. by 3 meters of 20% Carbowax 20 M + 1.7% H_3PO_4. (This column was used for better than 6 months before this run. Loss of substrate is substantial, as indicated by reduced retention times.) 220° C., 120 ml. He/min., Aerograph A-90 gas chromatograph

Co.) standards were zone refined (*2*) to 99.7, 100.0, and 100.0% purity, respectively, by infrared and GLC analyses.

PCP and 2,4,6-TCP (Eastman Organic Chemicals) assayed 100% by infrared and GLC. OCP (Fisher Scientific Co.) was relatively impure and its area correction factor was obtained by a series of successive approximations utilizing direct internal normalization as well as the above internal standard technique.

DISCUSSION

Of the substrates listed in the literature, tritolyl phosphate (*5*), diisodecyl phthalate (*1*), butanediol succinate (*4*), 4,4'-diaminodiphenyl sulfone (*2*), silicone grease (*1*), silicone fluid (*2*), and Apiezon M and L (*2*) have been recommended for the determination of chloroanisoles (*5*), chlorophenylphenols (*4*), and chlorophenols (*1, 2*). Apiezon L (*2, 9*), silicone fluids (*2, 9*), *o*-phthalate of 3,3,5-trimethylcyclohexanol (*12*), erythritol (*9*), ethylenemercaptal of D-glucose (*9*), sodium dodecylbenzenesulfonate (*2*), Gardilene 40-SD (*2*), and diglycerol (*2*) have been recommended and used with varying success for alkyl substituted phenols.

The "ortho" effect, described in the analysis of alkyl-substituted phenols on polar substrates (*2, 3, 8, 9, 11*), requires a greater substrate polarity (used here to represent the tendency for substrate and solute to form hydrogen bonds) in the case of chlorophenols than for alkyl phenols to get significant deviation from the normal boiling point elution (Table I). This is because ortho-substituted alkyl phenols are sterically hindered from forming hydrogen bonds to a greater degree than are chlorophenols and because their boiling points enhance rather than hinder selective retardation through hydrogen bonding. To date, all reported attempts at achieving adequate resolution of chlorophenol mixtures on polar columns had been unsuccessful because of insufficient hydrogen bonding with the substrate (*1, 2, 5*), low column efficiency due to the Teflon support (*1*), strong absorption on substrate and solid support, and a general lack of high temperature, polar substrates (*2, 5, 8, 9, 12, 14*).

Of the polar substrates tested, polyesters such as polypropylene glycol sebacate (Paraplex G-25, Table I), neopentylglycol adipate, diethylene glycol succinate, and diethylene glycol succinate plus 2% H_3PO_4 gave unsatisfactory resolution, the 2,6-DCP peak either coinciding with or eluting only slightly in front of 2,4-DCP. A fluorinated silicone, D. C. FS-1265, exhibited no hydrogen bonding with phenols whatsoever and resolution was extremely poor. Nonpolar substrates were particularly ineffective (see silicone rubber SE 30, Table I).

Table I. Retention Times Relative to Phenol on Typical Substrates

	Boiling point, ° C.	Carbowax 20 M + H_3PO_4 (at 200° C.)	Carbowax 1540 (at 215° C.)	Paraplex G-25 polyester (at 200° C.)	G. E. silicone rubber SE 30 (at 172° C.)
Benzene	80.1	0.12	...	...	0.433
OCP	175.6	0.61	0.65	0.80	1.22
Phenol	182	1.00	1.00	1.00	1.00
2,6-DCP	220	1.40	1.52	1.89	2.64
2,4-DCP	210	1.68	1.59	1.90	2.36
2,5-DCP	211	1.74	...	...	2.36
2,3-DCP	...	1.74	...	...	2.36
2,4,6-TCP	244.5	2.64	2.74	3.98	4.75
PCP	217	3.57	3.47	3.39	2.36
2,4,5-TCP	246	4.67	4.46	5.29	4.75
Specific ret. vol. for phenol, V_g		63.7	...	...	...

Carbowax 20 M gave the desired resolution and column life (better than 2 months at 225° C. and better than 6 months at 200° C.), but tailing was serious and sensitivity was poor, because of the strong absorption. The addition of a small amount (1.7%) of phosphoric acid eliminated this tailing (Figures 1 and 2), sharpened the peaks significantly, and improved sample recovery and sensitivity. The absolute retention times were reduced considerably but retention times remained essentially unchanged from untreated Carbowax columns (Table I). Too much H_3PO_4 (3.4%) reduces retention volumes drastically and resolution is lost.

The effects of ortho substitutions and of hydrogen bonding between the substrate and the chlorophenols is amply illustrated in Table I, where retention times on Carbowax columns are compared to those on a typical boiling point silicone column. The most significant retention reversals involve: 2,4-DCP eluting behind 2,6-DCP despite its 10° C. lower boiling point; PCP eluting behind 2,4,6-TCP despite its 27° C. lower boiling point; and 2,4,5-TCP eluting well behind 2,4,6-TCP despite an approximately equal boiling point. The less common 2,3- and 2,5-DCP isomers are not resolved sufficiently from 2,4-DCP for quantitative identification. Fortunately, these isomers have distinctly different infrared spectra and may be quickly determined spectrophotometrically after trapping the combined fraction from the gas chromatograph.

The Paraplex G-25 substrate (Table I) is typical of most polyesters, in that weaker hydrogen bonds are formed and the 2,6- and 2,4-DCP isomers are not adequately resolved. The addition of phosphoric acid to the polyglycol substrates reduces the degree of hydrogen bonding (presumably through the formation of phosphate esters), gives resolution equal to untreated polyglycol columns without the unfavorable tailing, and exhibits retention volumes similar to the polyester substrates tested.

On the Carbowax 4000–H_3PO_4 column, the column efficiency decreases with temperature for 2,4-DCP but increases with a decrease in temperature for 2,6-DCP (Table II, C). This phenomenon may be due to the much stronger hydrogen bonding of the 2,4-DCP (as compared to 2,6-DCP) with the substrate. This bond increases in strength as the column temperature decreases and causes a band widening over and above that normally observed. This hypothesis is borne out by the increase in column efficiency for 2,4-DCP on the Carbowax 20 M column, which is somewhat less polar than Carbowax 4000, whereas the HETP for 2,6-DCP remained constant.

Table II. Column Efficiency

Height equivalent to a theoretical plate (*HETP*)

Conditions as described under Experimental, except as noted below

A. *HETP vs.* Sample Size

Sample size, 2,4-DCP mg.	*HETP*, cm.
0.0625	0.132
0.625	0.147
6.25	0.234
12.5	0.390

B. *HETP vs.* Flow Rate (at 210° C.)

Flow rate, ml./min.	*HETP*, cm.	
	2,4-DCP	2,6-DCP
170	0.326	0.101
90	0.326	0.098
55	0.351	0.152

C. *HETP vs.* Temperature (at 170 to 190 Ml./Min.)

	Carbowax 4000, 1.7% H_3PO_4		Carbowax 20 M 1.7% H_3PO_4	
Temp., ° C.	2,4-DCP	2,6-DCP	2,4-DCP	2,6-DCP
225	0.373	0.107	0.316	0.100
200	0.395	0.088	0.326	0.101
175	0.410	0.060	...	...

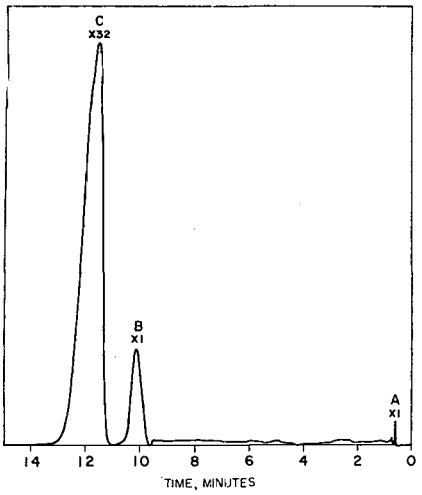

Figure 3. Chromatogram of zone-refined 2,4-DCP

A. Air
B. 0.4% 2,6-DCP
C. 99.6% 2,4-DCP
1/4 inch o.d. by 3 meters of 20% Carbowax 20 M plus 1.7% H_3PO_4, 201° C., ca. 100 ml. He/min. Aerograph A-90 gas chromatograph, 5-μl. molten sample injected

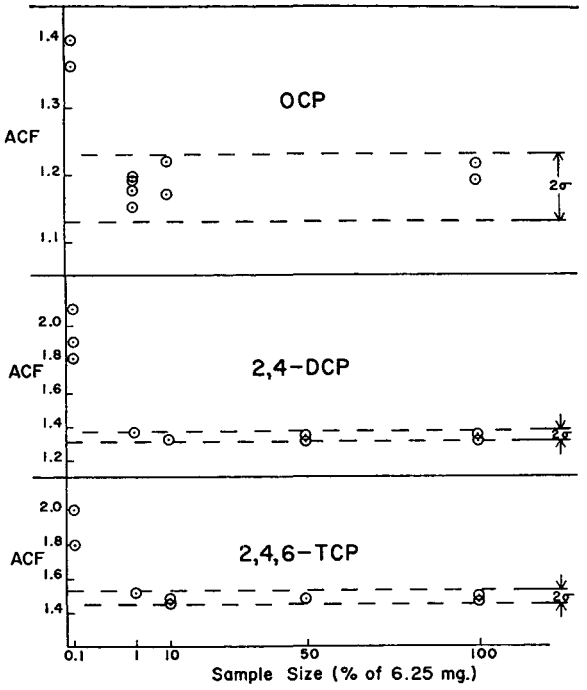

Figure 4. Sample size vs. area correction factors

Figure 3 illustrates the sensitivity obtainable while still retaining good resolution and symmetrical peaks. The ACF's in Figure 4 were determined against a constant amount of phenol in each case, and the volume of solvent injected was kept constant in all cases. It is apparent that, for very small samples (<6 μg.) or for components of less than 0.1% of a 6.25-mg. sample, not only does precision suffer but the magnitude of the ACF's takes a statistically significant jump.

An attempt was made to correlate the molar ACF's with the log of the molecular weight; a linear relationship was obtained for phenol, OCP, PCP, 2,4-DCP, 2,6-DCP, and 2,4,6-TCP. 2,4,5-TCP did not fit the pattern, but the reason for this discrepancy remains unknown at the present time. Pure samples of other chlorophenols were not available at the time to make possible the extension of this correlation.

Double Peak Formation. A heretofore unreported phenomenon of double peak formation (peak splitting) in nonaqueous systems was observed at 175° C. on untreated Carbowax 4000 columns. An unknown peak appeared between the 2,6- and 2,4-DCP peaks. When trapped and examined by infrared, it proved to be 2,6-DCP. This peak was not found using Carbowax columns treated with H_3PO_4. No rigorous explanation can be given for peak splitting, but one possibility can be suggested: Some 2,6-DCP is irreversibly adsorbed and separated from the main peak and then is displaced by the more strongly adsorbed 2,4-DCP peak following closely behind.

Table III. Method Sensitivity, Precision, and Accuracy

A. Sensitivity (Aerograph, 4-Filament Thermal Conductivity Detector, 235-Ma. Filament Current, 200° C., ca. 100 Ml. He/Min.)

1 chart div. = 2.1 mm., full scale = 210 mm.
Maximum sensitivity = 0.7 μg. OCP/chart div. (or ca. 0.01% OCP in 6.25-mg. sample)
Maximum sensitivity = 3.0 μg. 2,4,5-TCP/chart div. (or ca. 0.04% 2,4,5-TCP)
Minimum detectable amount of 2,6-DCP (in 6.25-mg. sample) = ca. 0.02%

B. Precision, Internal Ratio Normalization Analysis of Chlorophenol Mixtures

Species	Level, %	Repeatability, $\bar{s}$, % absolute
OCP	0.5 to 1	0.02
2,6-DCP	5 to 10	0.03
2,4-DCP	90	0.10
2,4,6-TCP	1 to 2	0.07

C. Area Correction Factors (ACF) and Accuracy of Determination (by Internal Standard Technique)

	Av. ACF	n (No. of detns.)	Std. dev., $\bar{s}$	95% confidence limits for av. of n ACF detns., %
OCP	1.188	8	0.024	±1.6
2,6-DCP	1.349	8	0.022	±1.15
2,4-DCP	1.337	8	0.015	±0.81
2,4,6-TCP	1.488	6	0.021	±0.95
PCP	1.189	6	0.007	±0.50

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Gas Chromatographic Determination of Nitrogen in Refractory Materials

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▶ A new and convenient method has been developed for the determination of nitrogen in refractory materials. The method utilizes a conventional induction furnace for combustion of the material in an oxygen atmosphere at approximately 1500° C. The liberated nitrogen gas is collected in a modified Hempel buret and determined with a gas chromatograph. The new method has been routinely applied to several refractory nitride formulations. These include AlN, BN, CrN, Si_3N_4, TiN, and ZrN, as well as AlN·SiC and B_6Si samples.

REFRACTORY NITRIDE RESEARCH at this laboratory required chemical analysis of the new materials to augment crystallographic and spectrographic data. Existing methods, with a few modifications, were satisfactory for all of the elements present except nitrogen. The conventional sulfuric acid method failed to dissolve the new refractories, despite extended digestion periods. Fusion with alkali hydroxides to produce ammonia was considerably better, but incomplete decomposition frequently occurred. Sodium peroxide fusions were always complete, but partial conversion of the nitrides to nitrogen gas could be expected, and was observed.

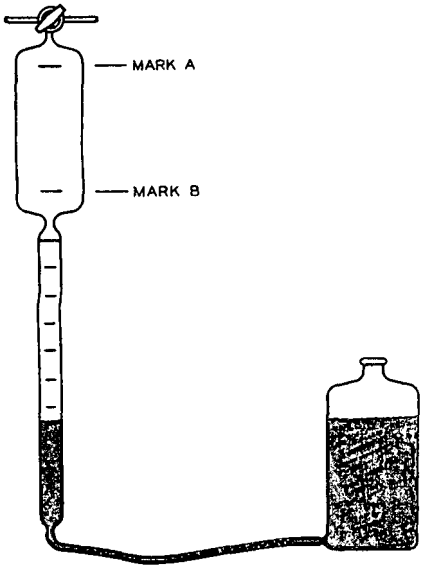

Figure 1. Combustion gas collector

EXPERIMENTAL

The conversion of nitrides to nitrogen and the corresponding metal oxide by high temperature oxidation was reported by Mellor (*2*), but detailed information was not included. To confirm this reaction, equipment designed for routine carbon and sulfur determinations was used. A sample of silicon nitride of known composition was burned in oxygen and the combustion gases were collected in the carbon determinator apparatus. The concentration of nitrogen in these gases was determined (by difference) with a Burrell gas analyzer. The results indicated that nitrogen was quantitatively evolved from the nitride.

This preliminary experiment led to the development of a more convenient apparatus for the collection and volumetric determination of the nitrogen. The apparatus, which removed interfering gases by chemical absorption, and the satisfactory results obtained with it, have been described previously (*1*). Subsequently, a gas chromatograph was found more sensitive and convenient to use for the measurement of the evolved nitrogen. Since the gas chromatograph made possible a direct measurement of nitrogen, it was unnecessary to remove other gases. Consequently, a new and considerably simplified device was constructed for the collection of combustion gases. This collector consists of a conventional 100-ml. graduated Hempel gas analysis buret joined to a cylindrical glass container which has a three-way stopcock at the top (Figure 1).

During the initial development work, combustions were occasionally incomplete. The combustion assembly was eventually modified by shortening the oxygen jet to provide a more diffuse flow of oxygen to the sample. Also, a small piece of rubber tubing was used to prevent tilting of the jet (Figure 2).

Apparatus. CHROMATOGRAPH. Beckman, GC-2 or equivalent, with a 6-foot stainless steel column packed with Linde 5A Molecular Sieves. A 5-ml. sample loop was used for this work, but a 10-ml. loop may also be used.

COMBUSTION GAS COLLECTOR. A 100-ml. Hempel gas analysis buret is joined permanently to one end of an 8-inch cylindrical glass tube, the ends of which have been tapered to match the buret taper (Figure 1). A 3-way stopcock (2-mm. bore) is also joined to the upper end of the tube. (Two of these collectors, one of 600-ml. and one of 900-ml. total capacity, are needed for the <5% N and >5% N ranges, respectively. The diameter of the smaller collector is 2 inches, that of the larger, 3 inches.) The volume of the upper, ungraduated portion from the stopcock to the buret junction is determined by water displacement and the volume is recorded permanently on the glass surface. Two combustion "on and off" marks (*A* and *B*, Figure 1) are also inscribed about 100-ml. from each end of the upper portion of the collector. The collector is attached with a large 3-pronged, rubber-jawed clamp to a 24-inch support rod on a 6- × 8-inch rectangular base.

COMBUSTION TUBE AND JET ASSEMBLY. A 35-mm. quartz or ceramic

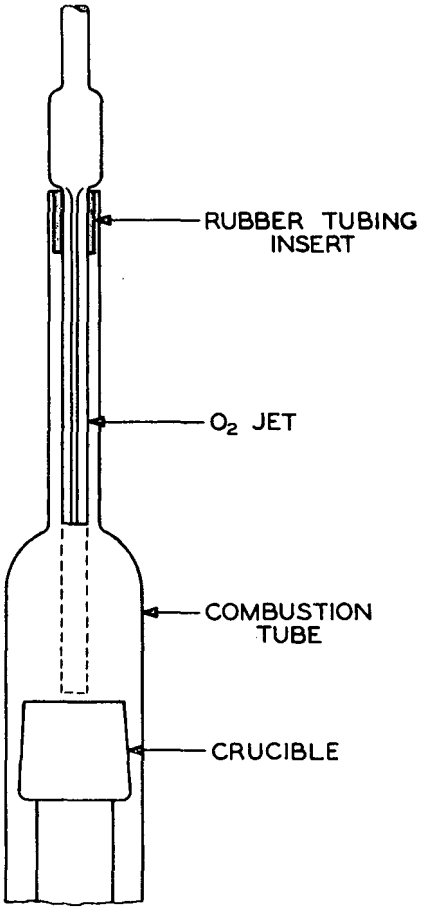

Figure 2. Modified jet assembly

Table I. Limit of Detection

Std. dev. of blank[a]	Sensitivity factor[b]	Limit, % N
±0.13 (5 D.F.)	1.80	0.22
	1.67	0.23
	1.10	0.36

[a] Expressed in *AVP* units, × 10^{-4}.
[b] Expressed in *AVP* units, per mg. N, × 10^{-4}.

Table II. Relationship of Nitrogen Sensitivity Factor Variance and Sample Size

Set No.	Sensitivity factor[a] 75 mg.	100 mg.
1	1.110	1.106
2	1.109	1.119
3	1.184	1.192
4	1.121	1.136
5	1.125	1.125
6	1.159	1.182
7	1.125	1.151
8	1.163	1.174
9	1.179	1.207
10	1.185	1.213
11	1.207	1.216
12	1.160	1.127
Mean	1.15[2]	1.16[3]
Variance	0.00110	0.00155
Variance ratio, calcd.		1.41
Variance ratio, theor. (5%)		2.82
Student "t," calcd.		0.74
Student "t," theor. (5%)		2.20

[a] Expressed in *AVP* units per mg. N, × 10^{-4}.

combustion tube, and a quartz jet are assembled as shown in Figure 2. The jet is shortened and a short length of rubber tubing is inserted as indicated.

Reservoir for Gas Collector Solution. A 1000-ml. aspirator bottle is connected to the lower tip of the gas collector with 3 feet of amber rubber tubing. The reservoir is filled with 1000 ml. of a solution containing 200 grams of Na_2SO_4 and 50 ml. of H_2SO_4. A few drops of methyl orange are added to impart color to the solution to aid in reading the buret.

Procedure. Part A. Combustion of Sample and Collection of Gases. Transfer 100 mg. of 100-mesh sample to a conventional ceramic induction crucible. Cover with 2 grams of iron chip accelerator and 1 gram of tin accelerator (a scoop may be used here) and place on the combustion pedestal of the induction furnace. Raise the pedestal assembly until the unit is securely closed.

Attach the collector apparatus (600-ml. size for <5% N, 900-ml. size for >5% N) to the combustion train. (The combustion train need only contain a dust filter.) Flush the combustion tube and train with 1.5 liters per minute of oxygen for 5 minutes. Turn the collector stopcock from the by-pass to the collect position and allow the collector solution to recede to mark *A*. When mark *A* is reached, start the combustion process. (Note: The solution must be allowed to recede to mark *A* before combustion begins because a partial vacuum will be produced, particularly when there is no nitrogen in the sample. Be prepared to temporarily turn the collector stopcock to an intermediate, closed position until positive pressure is restored. This action will avoid contamination of the combustion train with collector solution.)

When the collector solution has receded to mark *B*, turn off the induction current, turn the stopcock to the by-pass position and allow residual internal pressure to force the collector solution downward into the buret. If more pressure is needed, turn the stopcock to admit more oxygen. Then close the stopcock and disconnect the collector apparatus from the combustion train. Raise the solution reservoir until the solution levels in the buret and reservoir are coincident. Record the buret reading and add to it the volume of the upper, ungraduated portion.

Part B. Chromatographic Analysis. Attach the collector stopcock to the sample port of the gas chromatograph with rubber tubing. Flush the port with the sample gases, then introduce a 5-ml. or 10-ml. sample into a 6-foot Linde 5A Molecular Sieve column at 40° C. under 20-p.s.i. helium pressure. A filament current of 250 ma. and attenuations of 1X, 2X, and 5X will provide adequate peak heights for most nitrogen concentrations. Measure and record the height and half-height width of the nitrogen peak in millimeters. (If an integrator is used, substitute counts for square millimeters.)

Repeat this process for the rest of the samples, standards, and blanks. Duplicate determinations are recommended for each category.

Part C. Calculation of Results. Calculate the area-volume product for each sample, standard, and blank determination as follows:

$$AVP = \frac{(A)(V)(\text{Attn.})}{S}$$

where

A = area under curve, sq. mm.
V = volume of collected gases, ml.
Attn. = instrument attenuation
S = gases injected, ml.

Subtract the blank *AVP* value from each sample and standard *AVP* value. Divide the net standard *AVP* value by the milligrams of nitrogen present in the standard to obtain the nitrogen sensitivity factor, then calculate the nitrogen content of the sample as follows:

$$\% \text{ N} = \frac{AVP_s}{WF} \times 100$$

where

AVP_s = net sample area-volume product
W = sample weight, mg.
F = nitrogen sensitivity factor.

DISCUSSION

A review of the data accumulated over a 7-month period has been made to establish limits of application and precision. Wilson (*3*) recommends that the standard deviation of the blank be reported when a new method is proposed to facilitate its evaluation without detailed experimentation. The standard deviations of the blanks in the two ranges of application (<5% N and >5% N) were calculated by the method for duplicate values (*4*). (This method was used because the range of individual blank values exceeded the mean difference of duplicate values by a factor of nearly 10.) The standard deviations of the blanks in the low and high ranges are, respectively, ±0.13 (5 D.F.) and ±0.085 (11 D.F.) *AVP* units.

To assure a 98% probability that the nitrogen detected is actually present, a 3-fold multiple of the blank standard deviation for the low nitrogen range must be used (*3*). Multiplying, (±0.13 × 3 = 0.39), and converting to % N (dividing by sensitivity factors obtained in the low nitrogen range), produces three limits of detection as shown in Table I. These limits clearly reflect their inverse proportionality to the sensitivity factors. Since the sensitivity factors vary as a function of chromatograph response and of oxygen purity, a single limit of detection cannot be given. The limits in Table I indicate the capabilities of the proposed method, however.

The possibility of differences in nitrogen evolution in the high range as a function of sample size was also investigated. Two samples sizes, 75 mg. and 100 mg., were tested. The data obtained from 24 replicate analyses of a silicon nitride (36.15% N) are shown in Table II. The calculations summarized in Table II clearly show that either sample size may be used, since the calculated variance ratio and Student "t" values do not exceed theoretical values at the 5% probability level.

The data in Table II also clearly reflected other operative effects. A com-

Table III. Between and Within Set Variance of Nitrogen Sensitivity Factors

Series	Mean diff.[a]	Variance	*F* ratio Calcd.	*F* ratio (5%) Theor.
75 mg., Between set	0.031	0.000584 (10 D.F.)	5.36	2.86
100 mg., Between set	0.035	0.000842 (10 D.F.)	7.73	2.86
75 to 100 mg., Within set	0.016	0.000109 (11 D.F.)		

[a] Expressed in *AVP* units per mg. N, × 10^{-4}.

parison of sensitivity factor variations as a function of sets, summarized in Table III, shows a significantly larger difference occurring between sets of analyses than between sample sizes within a set (mean differences between sets are greater by a factor of two and calculated variance ratios exceed the theoretical at the 5% probability level). This is a reflection of the effects arising from daily fluctuations of ambient temperature and pressure and of instrument controls. These effects are controlled and minimized by including standards and blanks with each analysis of a group of unknowns.

The evolution of a nitrogen-bearing gas other than nitrogen was observed only in the case of boron nitride or refractories which contained boron nitride. This was anticipated from a comment in (*2*) pointing out that some nitric oxide is produced upon oxidation of boron nitride. The formation of this secondary by-product was readily observed by the appearance of the reddish-brown vapor of N_2O_3 in the collector apparatus. A colorimetric estimate after absorption of the N_2O_3 vapor in potassium hydroxide indicated that only 0.17% of the nitride nitrogen was converted to nitric oxide during combustion. This bias is automatically eliminated, however, by calibration with a boron nitride standard.

Table IV. Analysis of Various Nitrides and Nitride-Containing Refractories

Sample	Nitrogen, %	
Amorphous boron	1.65,	1.65
$B_{13}P_2$ 879-32	3.81,	3.92
B_6Si 974-25-1	0.84,	0.81
B_6Si 974-18-1	0.43,	0.44
AlN·SiC (impure)	7.11,	7.02
AlN (impure)	10.8,	11.3
CrN[a] (impure)	9.6,	9.7
ZrN[b] (impure)	11.4,	11.4
AlN·SiC	29.6,	29.7
AlN·SiC	27.4,	27.3
AlN·SiC	29.0,	28.8
AlN·SiC	30.9,	30.9
AlN·SiC	29.7,	30.6
Si_3N_4 (36.2% N)	36.4,	36.6

[a] With Kjeldahl method: 9.4% N.
[b] With Kjeldahl method: 11.5% N.

The proposed method has been applied to a variety of refractory materials. These include carbides, nitrides, oxides, phosphides, and silicides of aluminum, boron, chromium, silicon, titanium, zinc, and zirconium. Individual results typical of some of these materials are shown in Table IV. Using the method recommended for duplicate pairs (*4*), the relative standard deviation of all of the pairs in the <5% N range (mean = 2.70% N) is 1.37%. Similarly, for the >5% N range (mean = 27.2% N), the relative standard deviation is 1.43%.

The proposed method offers a relatively simple solution to the problem of rapidly evaluating refractory nitride materials. After the blanks and standards have been run to establish the nitrogen sensitivity factor (30 to 40 minutes), the elapsed time of an analysis in duplicate is 15 minutes. The equipment needed is generally found in analytical laboratories, requiring only minor modifications.

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Gas Chromatographic Technique for Determination of Hydridic and Active Hydrogen in Organic Materials

Microanalysis of Borane Compounds and Alcohols

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▷ The technique described here is based on the chemical liberation of active or hydridic hydrogen in a microreaction cell incorporated in a gas chromatographic flow system and measurement of the hydrogen gas band in a nitrogen gas carrier by thermoconductivity detection. The differential in thermal conductivity between hydrogen and nitrogen was detected with a Teflon-coated hot wire detector. This technique was used for the determination of hydridic hydrogen in borane compounds and for the determination of hydroxyl hydrogen in alcohols. The hydrogen gas was formed from the borane compounds by acid hydrolysis and from the alcohols by reaction with lithium aluminum hydride. The sample sizes were in the milligram range, and analysis was completed in less than 5 minutes.

DETERMINATION of active hydrogen plays an important role in the analysis of organic compounds. By this determination, it is possible to obtain information on the presence and amounts of hydroxyl, amino, or carboxyl groups. A number of methods for the determination of active hydrogen involve the use of methyl magnesium iodide or lithium aluminum hydride and manometric measurement of the liberated hydrogen (*1*, *8*, *10*, *11*). In other methods, an excess of a standard solution of lithium aluminum hydride is back-titrated with a standard solution of alcohol (*4*). The microcombustion method requires only a few milligrams of the sample, but the technique is laborious and time consuming (*9*). A more recent method involves exchange of the active hydrogen with deuterium followed by an assay of the isotopic hydrogen content of the sample (*3*). Diborane has also been used as the hydridic agent for the liberation of hydrogen (*7*). A specially designed gas chromatograph with an elaborate valve system and a system of membrane switches has been designed for the analysis of active hydrogen after liberation by means of lithium aluminum hydride reagent (*2*). The main advantage of the method described is that it can be applied for the analysis of a variety of organic materials and borane compounds using conventional commercially available equipment—i.e., gas chromatograph of any make with a gas sampling valve of any design.

The method described deals with determination of two kinds of chemical hydrogen: the hydridic type, such as that found in the compounds containing a boron-hydrogen bond, and the active hydrogen type, such as that in hydroxyl, amino, and carboxyl groups. Both

types were determined by means of chemical liberation and gas chromatographic detection of hydrogen gas; the hydrogen gas was evolved from the borane compounds by reaction with a protonic agent and from the compounds containing active hydrogen by reaction with lithium aluminum hydride. The generated hydrogen gas was determined in nitrogen carrier gas stream by thermoconductivity detection. The technique used provides an excellent means for the quantitative measurement of minute quantities of hydrogen gas. The high sensitivity of the readout system, combined with extreme simplicity of procedure, permits fast micro- and submicroanalyses of a variety of organic functional groups.

EXPERIMENTAL

Apparatus. The gas chromatograph used in this study has been described previously (*6*). The Beckman valve used to introduce the sample was modified by removing one loop and replacing it with two 1.5-inch-long stubs of pressure tubing. This arrangement allowed placement of the microcell in or out of the carrier gas stream. The remaining loop of the valve was used for calibration.

This system was chosen only because of the availability of the particular instrument for this study. Any other commercially available or custom built gas chromatograph with a gas sampling valve could be used. Since no gas chromatographic separation is involved, the column serving only to slow down elution, any type of column can be used.

Procedure. Determination of Hydridic Hydrogen. Approximately 1 mg. of the sample was introduced into a three-necked reaction microcell (Figure 1). Two necks of the reaction cell were connected to the Beckman gas sampling valve by means of pressure tubing; the third neck was closed by a septum. The sample valve was rotated to place the reaction cell into a stream of carrier gas. After a 1- to 2-minute sweeping period, the valve was rotated 45°, closing off the reaction cell from the stream of carrier gas. By means of a hypodermic needle, 0.2 to 0.3 ml. of hydridic agent (water, diluted acid) was introduced through the rubber septum into the reaction cell. To accelerate hydrogen gas generation, the cell was heated by a small flame until the solution began to boil. After the boiling point was reached, the sample valve was rotated back 45° to place the reaction cell into the stream of carrier gas.

The magnitude of the gas chromatographic response to the generated hydrogen gas was recorded in terms of integral counts obtained from a Perkin-Elmer integrator. The instrument was operated under the following conditions: gas, nitrogen; inlet pressure, 5 p.s.i.g.; flow, 25 cc. per minute; column, 12 feet long by 0.25 inch in diameter; 50% Halocarbon oil, 13–21 on 50% Kel-F molding powder; temp., 26° C.; recorder, 5 mv.; and speed, 0.5 inch per minute. The instrument showed excellent base line stability and absence of noise with nitrogen as the carrier gas. Moreover, the recorder maintained a stable and noiseless base line even when nitrogen flow was stopped. This greatly simplified the analytical procedure.

Determination of Active Hydrogen. A standard solution of lithium aluminum hydride in ether (0.3 to 0.7*M*) was used for active hydrogen liberation. A 0.5-ml. amount of the solution was introduced into the reaction cell; the cell was then connected to the Beckman valve and swept with nitrogen carrier gas. The valve was rotated 45°, and the aliquot of the sample (in dry benzene, or tetrahydrofuran) was introduced by means of a Hamilton microsyringe through the rubber septum into the reaction cell. After completion of the reaction (30 to 60 seconds for alcohol), the hydrogen gas was determined in the same manner described in the procedure for determination of hydridic hydrogen.

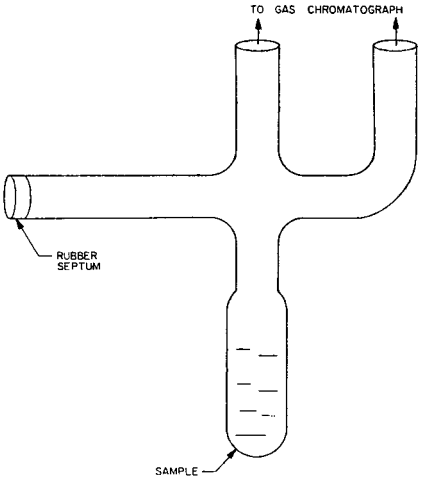

Figure 1. Reaction microcell (to scale)

Calibration and Calculations. To determine the volume of the calibration loop, the following procedure was used. A gas ampoule of known volume was filled with carbon dioxide to a known pressure. The gas from the ampoule was distilled into the loop which was cooled to −196° C. with liquid nitrogen. The loop was then rotated into the helium gas stream and the cooling bath was removed. The carbon dioxide was passed through the gas chromatograph and the output of the recorder was integrated with a Perkin-Elmer integrator. The number of counts per millimole of carbon dioxide was then calculated. The loop was then filled with carbon dioxide to a known pressure, and the gas in the loop was passed through the column. From the ratio of the two carbon dioxide peaks, the pressures, and the calibration bulb volume, the volume of the loop was calculated. This constant volume loop was used later for periodic calibration of the instrument, using hydrogen gas as a standard. The instrument response in terms of integral counts was established for 1 cc. of hydrogen gas. The calibration was accomplished by introducing a standard volume of hydrogen gas from the calibrated loop of the Beckman valve into the flow of nitrogen carrier gas and analyzing it according to the method described above. The instrument's response to the hydrogen gas evolved from the sample was established and the amount of hydrogen present was calculated from a comparison of the two values.

$$\text{Per cent recovery} = \frac{C_s \times MW \times 100}{C_H \times N \times 22.4 \times \text{wt. (mg.)}}$$

where:

- C_s = number of integral counts for the sample
- C_H = number of integral counts for 1 cc. of hydrogen at standard pressure and temperature
- N = number of moles of H_2 generated per mole of analyzed compound
- MW = mol. wt. of analyzed compound

RESULTS AND DISCUSSION

The primary concern of this investigation was the study of the boron-hydrogen bond. A modification of the Hoffman-Lysyj method for the determination of amino nitrogen, in which evolved nitrogen is determined gas chromatographically using helium as the carrier gas (*5*), was used for the determination of hydridic hydrogen in borane compounds.

Such compounds were reacted with protonic agents in a microreaction cell (Figure 1) connected to the gas chromatographic inlet system, and the evolved hydrogen was determined gas chromatographically in the helium stream. However, the small difference in thermal conductivity between the carrier gas (helium) and hydrogen (λ He·10^5 at 0° C. = 33.60, λ H_2·10^5 at 0° C. = 39.60) produced insufficient sensitivity. To overcome this impediment, nitrogen (λ N_2·10^5 at 0° C. = 5.68) was used as the carrier gas, thereby greatly increasing the sensitivity of detection.

It was found that the highest base line stability, especially when nitrogen flow was stopped, was obtained with a Teflon-clad hot wire senser. To compensate for reduced response of such a detection system, a higher impedance (8000 ohms maximum) of the output was used. Such a system seems to provide the best results as far as stability and sensitivity of response are concerned. The thermistor detection system was also tried, but it was found to be too sensitive for detection of hydrogen liberated from compounds containing it in per cent range. However, it might be very satisfactory for the analysis of materials containing active hydrogen in trace amounts.

Since the technique was to be used for determination of boron-hydrogen bonds, it was decided to check the reliability of the method with known compounds. The two compounds selected were trimethylamino monoborane, $(CH_3)_3N{\cdot}BH_3$ and borazole $B_3N_3H_6$. They were prepared and purified by the Synthetic Chemistry Unit of the Rocketdyne Chemistry Section. Methanol was originally tried as the protonic agent and later water was used, but neither gave a sufficiently fast reaction rate. During the long time required to attain equilibrium, some hydrogen diffused out of the tubing at the joints connecting the reaction cell to the chromatograph.

In the final procedure, 10% aqueous hydrochloric acid was used to liberate hydrogen from borane compounds. Application of heat to the reaction cell accelerated the reaction rate and provided rapid and stoichiometric release of hydridic hydrogen from the compounds investigated. Determinations were performed for the hydridic hydrogen in trimethylaminomonoborane and borazole (Table I).

Since the technique investigated depends only on the detection of hydrogen, any reaction that produces hydrogen gas stoichiometrically can be utilized as a basis for an analytical method. An experiment was conducted to study the feasibility of such an analytical approach to the determination of organic functional groups on a micro- and a submicroscale. A common reagent used in the determination of active hydrogen in organic compounds is lithium aluminum hydride. The usual procedure is to react the lithium aluminum hydride with the sample and measure the hydrogen liberated manometrically. To study this reaction in the microcell, benzyl alcohol was used. Benzyl alcohol has a convenient equivalent weight, can be obtained pure, and can be dissolved in dry benzene. The results of this analysis are given in Table II.

On the basis of this result and available literature on the use of lithium aluminum hydride reagent (*4*), the method should find wide application for the quantitative micro and submicroanalysis of functional groups in the following types of organic materials: alcohols, amines, nitriles, glycols, phenols, carboxylic acids, and aromatic nitro compounds.

Only 4 to 5 minutes are required to complete the analysis compared with the 20 minutes and more required for the same task using manometric or combustion methods, depending on the size of the sample. The extreme simplicity of the procedure is based on the use of one reagent (which does not have to be standardized) and simple glassware—one microreaction cell combined with a gas chromatograph. The possibility of micro and submicroquantitative determination of functional groups will provide an extremely useful tool for the analysis of materials available only in minute quantities, such as those of biological research.

ACKNOWLEDGMENT

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Table I. Determination of Hydridic Hydrogen in Organoboron Compounds

	Sample size, mg.	Recovery, %
Trimethylamino-borane, $(CH_3)_3N{\cdot}BH_3$	1.623	95.43
	1.2984	88.65
	0.9738	90.63
	0.6492	99.73
	0.3246	94.43
	0.1623	108.71
Av. and std. dev.		96.3 ± 5.3
Borazole, $B_3N_3H_6$	2.1312	90.43
	1.5984	87.48
	1.0656	96.88
	0.5328	97.07
	0.2664	95.87
Av. and std. dev.		93.6 ± 3.5

Table II. Determination of Active Hydrogen in Benzyl Alcohol

Sample, mg.	Recovery, %
11.670	99.81
	105.37
	99.67
9.336	98.68
	100.32
	100.54
7.002	101.30
	106.29
	103.85
4.668	98.61
	95.63
	96.60
2.334	106.05
	97.76
	105.72
Av. and std. dev.	101 ± 2.9

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The Brown Front in Paper Chromatography

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▷ **The brown front in filter paper chromatography and elution is apparently caused by some modification of the filter paper by the wet–dry interface as it travels along the paper. This effect cannot be eliminated by as many as 20 elutions with water. The brown residue is made up of water-soluble organic material and salts, which can modify the chromatographic behavior of sugar acids.**

THE FLUORESCENCE so readily detected at the solvent front in paper chromatography was investigated as early as 1934 by Bone (*3*). Several papers on this subject have appeared, the latest by Schaffer, Appel, and Forziati (*7*), and it has been shown that the continuous evaporation of water at a wet–dry interface on the fibrous material (cotton strips were used) resulted in chemical modification of the cellulose (evidence for oxidized polymer was found) and the formation of water-soluble, organic degradation products. The reaction appears to require oxygen. Ambler and Finney (*1*) investigated the brown front on filter paper and believe that the mechanism is the formation of a degradation product of cellulose by oxidation at the wet–dry boundary, along with the capillary concentration of the same or a similar product already present in the paper. This research is an extension of that work into the nature of the brown front and its effect on filter paper chromatography and the elution process.

EXPERIMENTAL

Materials and Equipment. The chemicals used in this research were D-glucono-1,4-lactone (Fisher, purified) and D-glucurono-6,3-lactone (Eastman, highest purity). All chromatograms were 19 cm. wide and 40 cm. long and developed by the descending method using 1-butanol–acetic acid–water (4:1:5 v./v.) as the organic phase. The spray reagent used to locate the spots was periodate–permanganate (*6*). The water used for elution was triply distilled (once from permanganate). Unless otherwise specified, the filter paper was Whatman No. 1. Glass-fiber filter paper (Hurlbut Paper Co., No. X-934-AH) was used in one study. The pieces of filter paper from which the eluate was collected were 2 cm. × 10 cm. and tapered at one end.

Stability of the Sugar Acids in Solution. The sugar acids can exist in solution in various forms: as free acids, as salts, or as γ- and δ-lactones. A number of authors (*2, 4, 8*) have discussed the relative stability of these forms. From the chromatographic analysis in this research, it was observed that D-glucono-1,4-lactone was converted into D-gluconic acid when it dissolved in water. D-Glucurono-6,3-lactone dissolved in water as the lactone, but was gradually converted into the free acid at room temperature. After a period of about 3 weeks, there was an equilibrium mixture of the two forms, with the acid form in excess.

Effect of Elution on R_f. When the sugar acids were first chromatographed (as received from the manufacturers without further purification), they showed R_f values in the range of 0.15 to 0.20. However, when these chromatographed spots were eluted and rechromatographed, the R_f range was decreased by about one half. Table I gives representative R_f values for 0.2-mg. samples of the sugar acids. Since the R_f values after elution were about the same as R_f values for the salts of the sugar acids, conversion of the sugar acids to their salts during the elution process seemed probable. The following experiments were devised to determine the cause of this.

Two samples of D-gluconic acid, about 0.2 mg. each, were spotted on a chromatogram. One of these samples was overspotted with the eluate from a filter paper strip. The overspotted sample showed the same R_f value as sodium D-gluconate, whereas the other sample was not retarded. From this, it was concluded that something from the filter paper was causing the salt formation.

In another experiment, a filter paper strip was eluted continuously with water, and the successive drops were collected. Each of these drops was overspotted on a separate sample of D-gluconic acid. The salt formation diminished with each successive drop so that, by the eighth and ninth drops, there was little or no conversion of the sugar acid into its salt. The brown color of the eluate had also disappeared. However, when the strip was dried and re-eluted, the salt formation caused by the first drop of new eluate was about the same as that caused by the first drop in the initial elution. The color of the eluate was now yellow instead of brown. These results seem to indicate that the brown front formed at the wet–dry boundary was in some way responsible for the formation of salt from the sugar acid. This material was easily washed out of the paper, the elution occurring at the solvent front, but when the paper was dried and re-eluted it was formed once more.

Experiments with D-glucuronic acid gave the same results.

Examination of the Residue from the Eluate. When the eluate from the filter paper was evaporated to dryness, a yellow-brown residue remained. When this residue was heated to combustion in a porcelain spoon the organic material was burned away, but a white salt remained which exhibited a crystalline structure under microscopic examination. There was not enough of the salt for ordinary chemical analysis. Activation analysis of the ash from Whatman No. 1 filter paper revealed the presence of sodium, but no other element was positively identified by this method. No attempt was made to identify the organic material which was assumed to be degradation products of cellulose. However, to examine the effect of the organic material on the sugar acids, a drop of the eluate was first treated with Dowex 50-H^+ resin to remove cationic material and then overspotted on a sample of D-gluconic acid. The results can be seen in Figure 1, which is a tracing of a chromatogram. Sample 3 shows only a little tailing, whereas sample 4, which was untreated by the resin, is definitely retarded (formation of salt of the sugar acid) with a small amount of bearding due perhaps to the hydrolysis of the salt to the sugar acid during chromatographic development. The brown color of the eluate was virtually unchanged by this resin treatment. It may be assumed that the organic material was left in the eluate. Dowex 1, an anion-exchange resin, removed the brown color from the eluate, but the

Table I. Representative R_f Values of the Sugar Acids

Acid	Before elution	After elution
D-Gluconic	0.17	0.09
D-Glucuronic	0.15	0.07

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salt-forming property (shown by overspotting technique) remained. Sample 1 of D-gluconic acid was overspotted with water. This was done to show that the salt formation was not caused by something in the water.

In another experiment, 1 drop of 10% hydrochloric acid was added to 1 drop of eluate and overspotted on a sample of D-gluconic acid. Very little salt formation was noted, but the strong acid introduced irregularities in the solvent *front.*

Efforts to Eliminate the Brown Front. Whatman No. 1 strips were eluted 20 times, with drying between each elution. Each elution produced residue containing organic material and salt. The amount of residue was small, but the average of about 200 samples was approximately 0.1 mg. of residue per strip per elution. Less than half of this was salt.

Other papers were tried, for example, Whatman Nos. 2 and 42. Both of these gave the same type of residue. The Whatman No. 42 (acid washed) paper was eluted 13 times, with drying between each elution. The eluate from each elution was overspotted on separate samples of D-gluconic acid. The effect of converting the D-gluconic acid into its salt was about the same for each elution.

Whatman No. 1 paper was washed with 10% hydrochloric acid solution for 24 hours and then washed with water for the same length of time. When this paper was dried and re-eluted with water, residue containing the salt was produced.

Glass-Fiber Paper. Glass-fiber filter paper should not be affected by the wet–dry interface. Some strips of this paper were eluted, and a yellow substance appeared to move down the paper, along with the wet–dry interface which was rather irregular. When this eluate was evaporated to dryness a residue remained which was made up of salt and organic material. When overspotted on D-gluconic acid, this residue altered the R_f value of the acid in the same way as the residue from cellulose fiber paper. Five or six successive elutions, with drying between each, produced residue, but the yellow front was not noticeable after the second elution. After the sixth elution, the glass fiber became fluffy and would no longer transport water by elution. This behavior might indicate that there is an organic binder in the glass-fiber paper. The organic material might also have been introduced during the manufacturing process. For example, sometimes the glass fibers are beaten in a citric acid solution to give a good dispersion.

DISCUSSION

As others have shown, the conditions that prevail at the wet–dry interface which travels along the filter paper in the elution of a chromatogram cause some degradation of the paper to take place. This is evidenced in part by the production of a small amount of water-soluble material which comes from the paper, concentrates in the solvent front, and, on elution and evaporation, gives a brown residue. It is now found that, in the presence of this material, the chromatographic behavior of eluted sugar acids is altered. The changes in R_f values can be attributed to neutralization of the acids or to a salting-out effect (*5*). The change in R_f values appears to be due to inorganic cations, a component of the eluate that has not been recognized previously. This cationic material may be hydrolyzed during chromatography, with formation of salts of the sugar acids.

On combustion, the brown residue is converted to a white, crystalline, salt-like material which also has the ability to change the R_f values of the sugar acids. As would be expected if they contained cationic materials, the brown and white materials lose their ability to affect the acids upon treatment with Dowex 50-H^+.

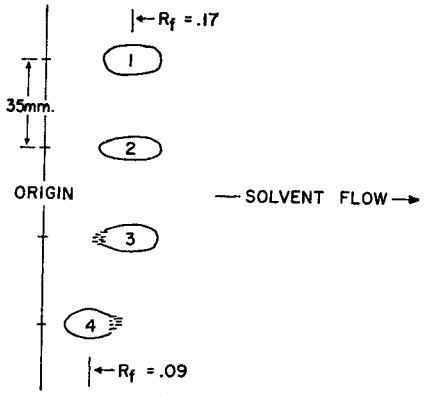

Figure 1. D-Gluconic acid chromatogram

1. Overspotted with water
2. No overspotting
3. Overspotted with eluate treated with Dowex 50
4. Overspotted with untreated eluate

Some inorganic, cationic material is present in filter paper, probably in association with the carboxyl groups that cellulose is known to possess. Passage of molecules of an aldonic acid in the vicinity of a cellulose–carboxylate salt residue would be expected to result in a transfer of the cations by mass-action effects with the resulting formation of aldonate salt. However attractive, this explanation is inadequate for three reasons. First, this process should occur as well during chromatographic development of the sugar acid on paper before elution, but it does not to any significant extent. Second, extensive washing of the paper with dilute hydrochloric acid should be more effective in replacing the cations held in this simple fashion. Third, water alone is capable of effecting the release of the cationic material. The key condition appears to be the presence of a wet–dry interface on the paper. Observations can support only an explanation that relates the oxidative degradation at the interface to an enhanced liberation of cationic impurities. On the one hand, the cations behave as though they are located in inaccessible (crystalline) regions of the cellulose where protons from mineral acid are unable to replace them. On the other hand, the degradative process at the interface is able to effect replacement of the cations by protons from the relatively weak acids resulting from the degradation. In the latter situation, only a portion of the cations associated with the paper is thus removed, and drying the paper and re-eluting liberate more.

The eluted material is not basic; however, it is able to effect this neutralization. More surprisingly, overspotting with comparable amounts of sodium chloride was found to produce similar lowering of the R_f values of the sugar acids. This is clearly related to the experiments of Westall, who has shown that, during chromatography in a 1-butanol–acetic acid–water developer, sodium chloride hydrolyzes with separation of the sodium and chloride ions (*9*).

Apparently, long exposure to air makes the paper more susceptible to modification by the wet–dry interface. It was noted in this work that the first elution of a paper generally produced the brownest residue, but not necessarily the most salt. During successive elutions, the brown color changed to yellow and finally to a light cream color. If a paper was exposed to air for a period of 2 weeks or more between elutions, the color of the residue from the elution following this period was a bit darker than the residue from the previous elution.

In regard to chromatography, it does not seem practical to wash the filter paper repeatedly in an effort to get rid of the residue from the brown front, since 20 elutions were not successful. To minimize the amount of residue, it seems best to elute the paper once with water shortly before use in chromatography.

If one is attempting to purify a substance by chromatography, it seems best to purify as large a sample as possible on one chromatogram, within limits, to minimize the relative amount of residue from the brown front; or to remove salts, the eluate, containing a sugar acid or other organic acid, could be treated with a cation-exchange resin in the acid form.

Further work in this area is currently in progress.

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Determination of Nicotinic Acid in Coffee by Paper Chromatography

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▷ A new method has been developed for the determination of nicotinic acid in coffee. A mild acid treatment of the coffee solution or suspension was followed by neutralization and decolorization with basic lead carbonate. After filtration, the supernatant was resolved by paper chromatography, and the nicotinic acid spots were revealed by a cyanogen bromide–benzidine reagent. The density of the spots was determined with a photoelectric densitometer, and the concentrations were calculated through the use of a standard curve. A study was made of the nicotinic acid content as related to the degree of roast.

MOST METHODS presently used for the quantitative determination of nicotinic acid are based upon biological procedures which are lengthy and require specialized equipment. Chemical methods which have been applied are complex and unsuitable for the determination in coffee. The most common microbiological methods for the quantitative determination of nicotinic acid are those used by Teply, Krehl, and Elvehjem (*14*), Bressani and Navarrete (*2*), and Cravioto, Guzman, and Suarez (*3*). A bioassay method has been based on the growth rates of rats (*13*), and a colorimetric method based on the König color reaction has been used by Gassman and Ehrt (*5*) and by Adamo (*1*). This color reaction was investigated thoroughly by Gassman (*4*) and applied to the analysis of foods. However, the method is complex, requiring three corrections for blanks, and the relative standard deviation is approximately ±10%. Kuznetsova and Chagovets (*10*) used a paper chromatographic separation of nicotinic acid, followed by a colorimetric determination in the eluate from the paper using a color reaction with methyl-*p*-aminophenol.

A reliable and rapid method for nicotinic acid analysis would be of distinct advantage, especially in the food industry. With modifications of a method for the determination of caffeine and trigonelline in coffee (*9*) and with use of a solvent system suitable for the separation of nicotinic acid (*7*), a method of quantitative paper chromatography similar to that described by McFarren, Brand, and Rutkowski (*11*) has been developed. This method is rapid and reliable and appears to be adaptable to the analysis of various food products.

EXPERIMENTAL

Procedure. The roasted coffee beans were ground to a fine powder. When soluble coffee powders were used, no special preparation was required. A 10.00-gram sample of coffee was mixed with 110 ml. of 1.1*N* hydrochloric acid. The sample was refluxed for one hour and allowed to cool to room temperature. The acid was neutralized with 40 grams of basic lead carbonate powder

$$[Pb(CO_3)_2 \cdot Pb(OH)_2$$

Matheson, Coleman & Bell Chemical Co.]. The mixture was transferred to a 200-ml. volumetric flask and diluted to volume. An aliquot was filtered, and the filtrate was used for analysis.

The filtrate was spotted with a micrometer syringe (Burroughs Wellcome Co., London, England) on Whatman No. 3MM chromatography paper in amounts of 0.02 ml., 0.04 ml., and 0.06 ml. for instant powder samples, and 0.05 ml., 0.10 ml., and 0.15 ml. for roast coffee samples. Five spots of a nicotinic acid solution, prepared from sublimed and dried nicotinic acid, were applied to the same chromatogram in amounts ranging from 0.3 to 1.5 μg. per spot.

The papers were allowed to develop by descending chromatography for 16 hours using 1-butanol–ammonium hydroxide–water (100:2:16) as a solvent system. The chromatograms were dried for 30 minutes in a stream of warm air. The dried papers were placed for 2 hours in a closed tank containing cyanogen bromide vapors (fume cupboard). These vapors were produced by placing cyanogen bromide crystals in the bottom of the chamber. After the cyanogen bromide treatment, each paper was sprayed with a solution of 0.25% benzidine in 50% ethanol and allowed to dry in air for 30 minutes. The preparation of fresh spray reagent each day was necessary. The nicotinic acid (R_f = 0.13) appeared as an intense pink spot on a white background. The maximum density of the spot was determined with a Photovolt electronic densitometer, Model 525 (Photovolt Corp., 95 Madison Avenue, New York 16, N. Y.) using a green filter and a slit width of 6 mm. The instrument was first standardized on a blank portion of the chromatogram, and then the maximum density of each nicotinic acid spot was determined by moving each spot slowly over the slit of incident light until maximum deflection was obtained on the galvanometer. The densities of spots of known concentration were plotted on semilogarithmic paper with concentration as ordinate on the

Table I. Nicotinic Acid Recovery

Type of coffee	Original nicotinic acid content found, mg./100 g.	Nicotinic acid added, mg.	Calculated total, mg./100 g.	Amount found, mg./100 g.	Recovery, %
Instant	32.2	15.0	47.2	46.7	98.8
Roast	13.4	15.0	28.4	27.5	97.0

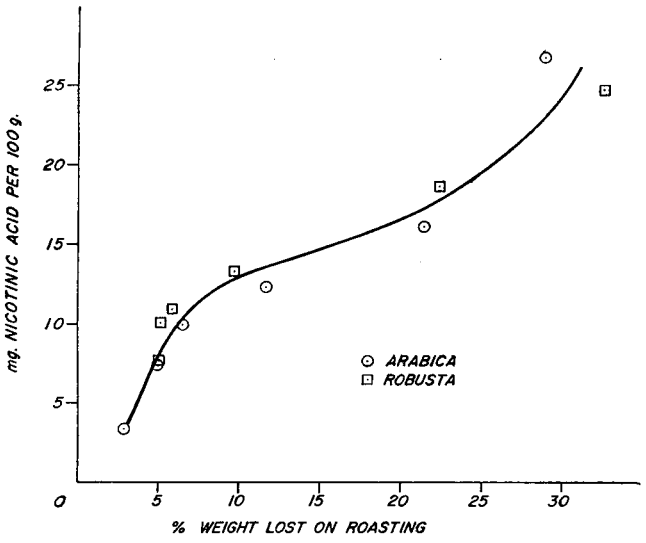

Figure 1. Relation between nicotinic acid content of coffee and degree of roast

log scale and density as abscissa. From these standard curves and the recorded densities, the concentrations of the experimental samples were calculated.

Recovery Experiments. Measured amounts of resublimed nicotinic acid were added to roast coffee and instant coffee samples. These samples were analyzed by the described procedure, and the recovery was calculated. The theoretical total was determined by adding the quantity which had previously been found in the coffee samples to the amount which was added. The recovery data are given in Table I.

The Change in the Nicotinic Acid Content with Degree of Roast. A series of samples of *Arabica* and *Robusta* coffees was roasted to varying degrees. Each sample consisted of 300 grams of unroasted coffee beans. After roasting, the sample was weighed to determine the weight lost on roasting. The nicotinic acid content of these coffee samples of different roasts was determined as outlined previously. The data are plotted in Figure 1.

RESULTS AND DISCUSSION

The preparation of the sample was important in obtaining a reliable nicotinic acid analysis. When roast coffee samples were used, the reaction medium was heterogeneous since the roast coffee remained largely insoluble; therefore, assurance of the complete extraction of the desired component was necessary. Preliminary experiments showed that grinding the roast beans to a fine powder ensured complete extraction. Hydrolysis with dilute hydrochloric acid was introduced to free the nicotinic acid from any chemical complexes. The neutralization of the hydrochloric acid with lead carbonate resulted in a precipitate of lead chloride which removed most of the dark coffee color. The light brown filtrate was suitable for paper chromatographic resolution. Exactly 40 grams of lead carbonate was found by experimentation to result in best recovery of nicotinic acid. Therefore, this amount was used for neutralization of each sample to ensure that there were no variations in analytical results due to different amounts of insoluble material in the volumetric flasks. The recovery experiments showed that the lead chloride precipitate did not remove any of the nicotinic acid. They also indicated an adequate degree of reproducibility.

The precision of the method was determined by analyzing the nicotinic acid content of an instant coffee powder. The average of six determinations was 25.6 mg. per 100 grams of coffee. The standard deviation was ± 1.05 mg.

Green coffee has been reported to contain very little nicotinic acid. Commercial green beans have been processed by drying to stop enzyme action. By a microbiological assay, Bressani and Navarrete (*2*) found 0.6 to 1.3 mg. of nicotinic acid per 100 grams of green coffee. The cyanogen bromide–benzidine test did not indicate any nicotinic acid in green coffee. The more sensitive *p*-aminobenzoic acid spray reagent (*8*) showed a faint trace of nicotinic acid. However, the amount present was too small to be quantitatively determined.

Several researchers (*1–3*) report that the nicotinic acid content of the coffee increases with darker roasts. Trigonelline is converted to nicotinic acid by removal of the methyl group from the nitrogen of the pyridine ring. Since the trigonelline content of coffee has been shown to decrease during roasting (*12*), this conversion is assumed to be the source of the increased nicotinic acid content. This assumption is promoted by the work of Hughes and Smith (*6*) who heated trigonelline in a sealed tube and produced nicotinic acid.

Therefore, using the analytical technique described, a study was undertaken to determine the nicotinic acid content in coffee samples with increasing degrees of roast. Two varieties of coffee beans were used, *Robusta* and *Arabica*. For comparative purposes the data from both these varieties were plotted on the same graph (Figure 1). The data from the two types of coffee are very similar, and a single curve is sufficient to represent both varieties. The rate of nicotinic acid formation varies with the degree of roast.

Several instant coffee powders were analyzed, and the nicotinic acid content was in the range of 18 to 40 mg. per 100 grams of instant coffee. This is in accordance with the data reported by Teply (*13*) and by Hughes and Smith (*6*).

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Attenuated Total Reflectance *vs.* Transmission Infrared Spectrometry in the Quantitative Evaluation of Paint Vehicles

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▷ Certain problems are encountered in using infrared transmission spectrometry for the quantitative evaluation of paint vehicles; therefore, an investigation of attenuated total reflectance spectrometry was conducted. In thin-film analysis, it is very difficult, if not impossible, to know the sample thickness; this knowledge is essential in quantitative infrared transmission spectrometry. The attenuated total reflectance technique eliminates this problem. Therefore, it was concluded that this method is superior to transmission measurements for quantitative evaluation of paint vehicles.

A SERIOUS PROBLEM encounted in infrared transmission studies of paint vehicles is that of controlling sample size and thickness in order to produce infrared spectra of quantitative value. The intensity of the measured spectrum is related to the thickness of the sample and the concentration of the functional groups. Quantitative analysis of the spectrum is dependent on the intensity of the variations in absorption peaks caused by the concentration of the functional groups.

A very new technique, attenuated total reflectance (ATR), developed by Fahrenfort (*2*), offers a solution to this problem. It differs from infrared transmission spectrometry in that the infrared beam is reflected from the sample into the slit of the spectrophotometer instead of passing straight through the sample as in the case of infrared transmission. Consequently, the sample thickness will not affect the spectrum if the sample is at least 5 microns thick, because the reflection is from the first 5 microns.

EXPERIMENTAL

Infrared Transmission and ATR Studies. In the field study to determine the quantitative value of infrared transmission, samples of a paint vehicle (alkyd resin) were prepared and spectrograms were made at NCEL and two other laboratories also using IR-5's. The data were evaluated at NCEL.

To compare ATR with infrared transmission, the field study was repeated by the three laboratories.

Sample Preparation. The samples evaluated by infrared transmission were prepared as follows: the pigment and vehicle were separated, using a Federal test method (*3*) with extraction mixture *A*, and diluted to a volume of 25 ml. with ether. Aliquots of 0.2 ml. were delivered on a salt window (NaCl) and dried for 15 minutes at 50° C.

For the evaluation of the ATR technique the samples were prepared as follows: the pigment and vehicle were separated, using the Federal test method (*3*) with extraction mixture *A* and diluted to a volume of 25 ml. with ether. Aliquots of 0.4 ml. were placed on the prism of the ATR unit (KRS-5) and dried for 15 minutes at 80° C.

RESULTS

The two techniques are compared in the tabulations shown in Table I; four random spectrograms were used to obtain the average peak values for each laboratory. Ranges were calculated as follows:

X_i = average peak value arranged in ascending order (i = 1, 2, 3,).

DISCUSSION

Because of the sensitivity of infrared transmission to sample size and thickness, its application to quantitative evaluation in thin-film studies is limited (*4*). In quantitative evaluation, sample size must remain constant, because the intensity of the functional groups is directly proportional to the concentration and sample size. If the thickness is known, it can be compensated for. In the case of thin films, such as paint vehicles, it becomes a problem to determine the exact thickness. In some cases, variations in thickness can be compensated for mathematically by the absorbance-ratio method (*1*). A practicable technique for the infrared evaluation of paint vehicles should be free of problems related to sample thickness.

These results show that infrared transmission has a high variability (3 to

Table I. Summary of IR-5 Infrared Transmission Results

Peak location, microns	Average peak values			Overall average	Range
	Bremerton	Cal-Colonial	NCEL		
3.4	3.25	3.23	1.91	2.80	1.34
3.5	1.90	2.18	1.46	1.85	0.72
5.8	67.80	6.71	3.13	25.88	64.67
7.9	23.00	5.26	2.76	10.34	20.24
8.9	4.66	2.92	1.81	3.13	2.86
9.4	3.66	2.63	1.71	2.67	1.95
13.5	1.91	1.73	1.23	1.69	0.69

Table II. Summary of IR-5 ATR Results

Peak location, microns	Average peak values			Overall average	Range
	Bremerton	Cal-Colonial	NCEL		
3.4	1.28	1.26	1.45	1.33	0.19
3.5	1.25	1.20	1.25	1.23	0.05
5.8	2.12	2.34	2.25	2.24	0.21
7.9	2.03	1.09	1.87	1.96	0.16
8.9	1.82	1.94	1.78	1.85	0.17
9.4	1.59	1.69	1.60	1.62	0.10
13.5	1.43	1.38	1.42	1.41	0.05

62%) and range (0.7 to 64.6), as shown in Table I.

The ATR technique shows a much lower variability (4 to 16%) and range (0.05 to 0.21), as shown in Table II. Thus, ATR offers a solution to the problem of sample size and makes quantitative evaluation of paint vehicles practicable.

ACKNOWLEDGMENT

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Attenuated Total Reflectance Infrared Analysis of Aqueous Solutions

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▷ **The attenuated total reflectance (ATR) technique is shown to be a practical infrared method for the qualitative and quantitative analysis of aqueous solutions. Characteristic spectra are obtained throughout the rock salt region except in the immediate vicinity of the very intense 3300 cm.$^{-1}$ water band. Water soluble components that are difficult or impossible to determine by conventional infrared transmittance techniques are easily identified and measured using ATR. Spectral data for carboxylic acids and salts, sulfonic acids, amino acids, phenols, amides, carbohydrates, and inorganic components are presented. Data are included to show that two-phase systems containing suspended matter in water, such as dispersed solids or emulsions, can be analyzed directly by this method. Advantages and limitations of the method are discussed.**

THE LOW SOLUBILITY of many water-soluble inorganic, polar organic, and biological materials in the common organic infrared solvents limits the infrared measurement of these materials. Spectral data for materials of this type must be obtained by the analysis of water solutions or of the compound in the solid state. Solid state spectra are often unsatisfactory because additional absorption bands due to the crystalline state can be confused with fundamental vibrations. The use of water as an infrared solvent for transmittance studies was demonstrated as early as 1905 by Coblentz (*6*). Gore, Barnes, and Petersen (*12*) and Blout and Lenormant (*4*) have shown that deuterium oxide can be used, in conjunction with water, to obtain infrared spectra in the rock salt region.

Plyler and Acquista (*25*) have shown that the transmittance spectrum of water contains very intense absorption bands at 3300 cm.$^{-1}$ for the OH stretching vibration and at 1640 cm.$^{-1}$ for the HOH deformation vibration. They attribute a third, very intense band at 660 cm.$^{-1}$ not observed in the water vapor spectrum to an intermolecular vibration arising in groups of water molecules that exist in the liquid state. The latter vibration interacts with the fundamental water vibrations to produce combination bands at 3920 and 2110 cm.$^{-1}$ They showed also that a window is present from ≈1540 cm.$^{-1}$ to ≈1000 cm.$^{-1}$ where there is still enough infrared transmittance in reasonable path lengths of water to permit its use as a solvent for quantitative purposes. Potts and Wright (*26*) proposed the use of a transmittance screen in the reference beam of a double-beam infrared spectrophotometer to obtain a more useful I_0 in the 1540 to 1000 cm.$^{-1}$ region. Kaye (*16*) and Sternglantz (*27*) have investigated optical window materials suitable for use with water.

Reviews by Blout (*3*) and Goulden (*13*), covering applications of infrared transmittance spectra of aqueous solutions of inorganic, organic, and biological materials, and recent papers dealing with studies of lactates (*14*), amino acids (*23*), vitamins (*22*), biogenetic amines (*17*), metal chelate compounds of α-amino acids (*19*), and iminoacetic acids (*20*) attest to the growing interest in the use of water as a solvent for infrared spectrometry. Spectral data are usually observed only in the 915 to 1540 cm.$^{-1}$ region using water and in the 1500 to 1850 cm.$^{-1}$ region with deuterium oxide as the solvent. Cells with barium fluoride or silver chloride windows with path lengths of 10 to 50 microns are the usual experimental conditions. The use of deuterium oxide introduces the complications of hydrogen–deuterium exchange reactions which may result in complex spectra.

The attenuated total reflectance (ATR) technique developed by Fahrenfort (*11*) offers an attractive alternative to conventional transmittance methods to overcome or minimize the intense water absorption bands which limit the range in which useful infrared data can be observed. A simple explanation of the principles of the ATR effect has been given by Wilks (*28*). The absorption-like infrared spectra obtained result from an extremely shallow (less than 5 microns) penetration of energy into the sample. ATR has been applied mostly to solid samples (*7–10, 15, 24*). The work reported in this paper shows that ATR can be applied equally as well to aqueous solutions. Except in the region of the 3300 cm.$^{-1}$ water band, infrared data can be obtained throughout the entire rock salt region. Since ATR is independent of sample thickness, cell designs which accommodate large volumes can be used to simplify filling, emptying, and cleaning of the cells.

The results of the application of the ATR technique to the qualitative and quantitative infrared study of organic and inorganic compounds in water are presented in this paper.

EXPERIMENTAL

The ATR spectra were obtained with a Connecticut Instrument Co. Model ATR-1 attachment and an Irtran-2 (Eastman Kodak Corp.) prism at a 40° angle of incidence. The spectra were recorded with a Perkin-Elmer Model 221 double beam infrared spectrophotometer with rock salt optics. Energy losses were compensated with a Model BA-1 (Connecticut Instrument Co.) variable beam attenuator in the reference beam.

Materials for this study were reagent grade or C.P. quality and were used without further purification.

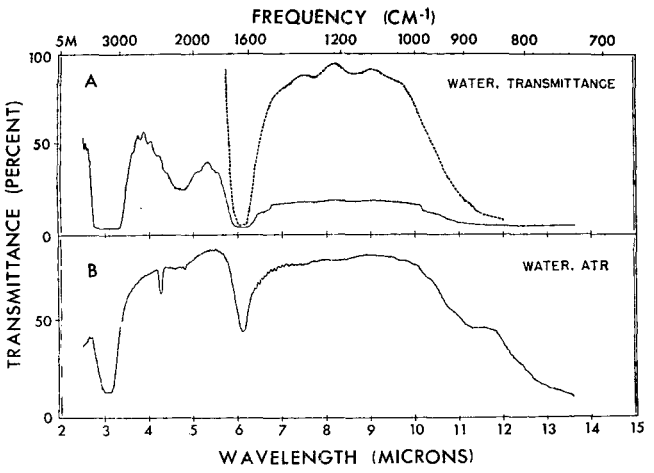

Figure 1. Water spectrum

A. Transmittance, 28 microns, Irtran-2 windows; (——) air reference, (- - - -) transmittance screen reference
B. ATR, Irtran-2 prism, $\theta = 40°$

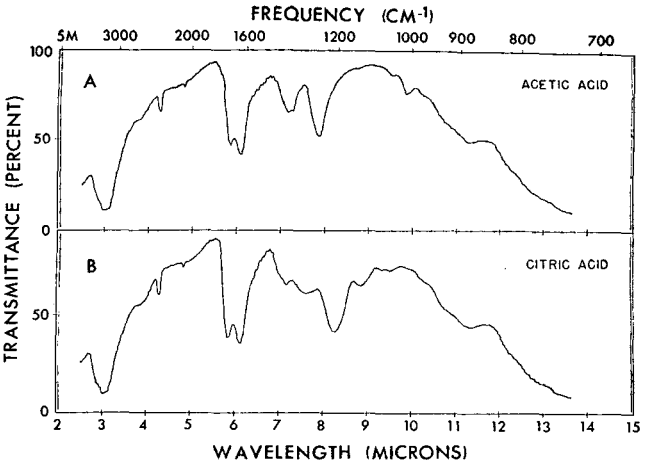

Figure 2. ATR spectra of carboxylic acids in water

Irtran-2 prism, $\theta = 40°$
A. Acetic acid, 4M
B. Citric acid, 2M

RESULTS AND DISCUSSION

The infrared transmittance spectrum of water in a 28-micron path length cell with Irtran-2 windows is shown in Figure 1*A*. Superimposed on this spectrum is shown the effect of placing a transmittance screen in the reference beam of the spectrophotometer to produce a more useful I_0 in the 950 to 1540 cm.$^{-1}$ region. The decrease in transmittance at lower frequencies is due principally to the absorption of the 660 cm.$^{-1}$ water band. Figure 1*B* shows the ATR spectrum of water. The intensity of the 1640 cm.$^{-1}$ water fundamental is greatly decreased, the combination band at 2100 cm.$^{-1}$ produces a very weak absorption, and only the region of the OH stretching band at 3300 cm.$^{-1}$ is opaque. The 2335, 2070, and 890 cm.$^{-1}$ bands in the ATR water spectrum are due to the Irtran-2 prism. The fine structure on either side of the 1640 cm.$^{-1}$ water band is due to atmospheric water vapor resulting from an approximately 4-inch increase in the optical path of the sample beam introduced by the optics of the ATR attachment. The Irtran-2 prism absorbs strongly in the 5000 cm.$^{-1}$ region and becomes opaque to infrared radiation at about 710 cm.$^{-1}$ Spectra were obtained, therefore, only in the 4000 to 740 cm.$^{-1}$ region.

Carboxylic Acids and Salts. Figure 2 shows the ATR spectra of acetic acid and citric acid in water. The position of the C—O stretching band at 1270 cm.$^{-1}$ and the minor absorption bands in the acetic acid spectrum agree with the aqueous transmittance spectra reported by Goulden (*13*) and Sternglantz (*27*) for the 950 to 1510 cm.$^{-1}$ region. The acid carbonyl band at 1705 cm.$^{-1}$ is well defined above the water background. This region is opaque in transmittance spectra of water solutions. The citric acid spectrum shows the carbonyl band at 1705 cm.$^{-1}$ and the positions of the C—O stretching band at 1210 cm.$^{-1}$ and the minor bands in the 950 to 1510 cm.$^{-1}$ window region agree with Parker's (*21*) transmittance data.

The ATR spectra of sodium acetate and sodium citrate in water are shown in Figure 3. The positions of the symmetrical carboxylate band at 1400 cm.$^{-1}$

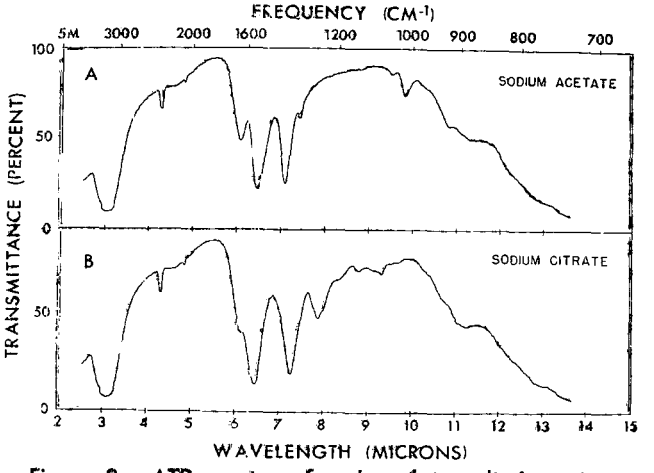

Figure 3. ATR spectra of carboxylate salts in water

Irtran-2 prism, $\theta = 40°$
A. Sodium acetate, 4M
B. Sodium citrate, 2M

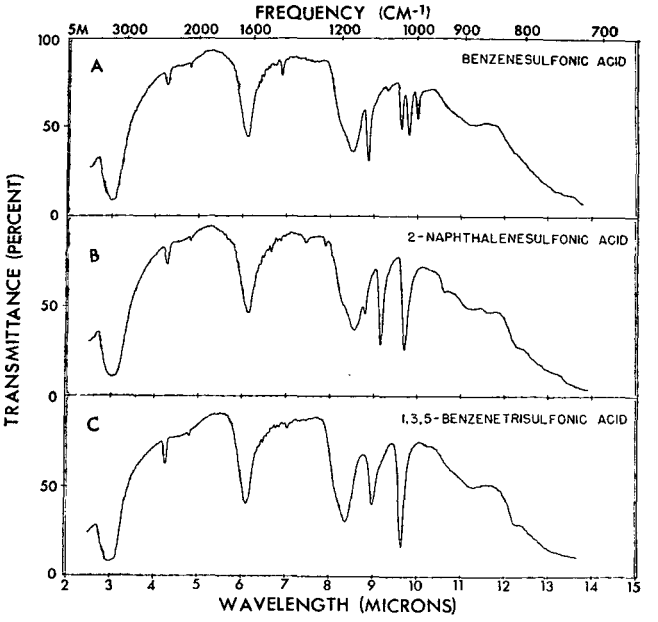

Figure 4. ATR spectra of sulfonic acids in water

Irtran-2 prism, $\theta = 40°$
A. Benzenesulfonic acid, 2M
B. 2-Naphthalenesulfonic acid, 2M
C. 1,3,5-Benzenetrisulfonic acid, 1.5M

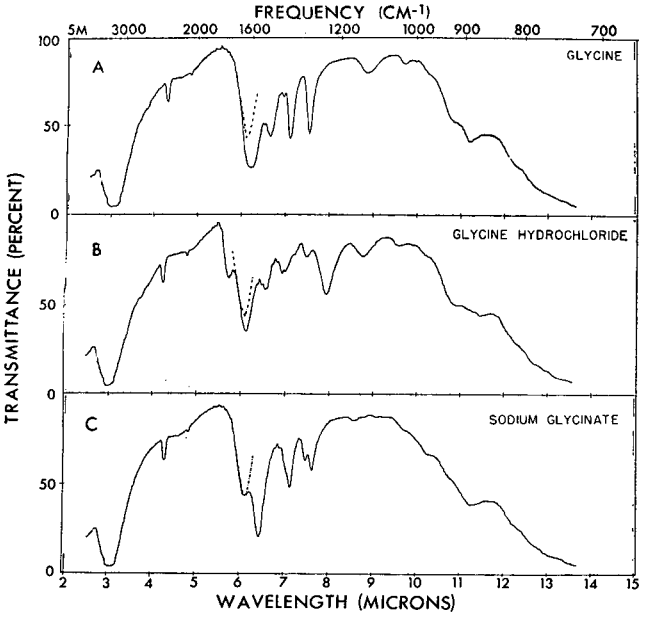

Figure 5. ATR spectra of glycine and salts in water

Irtran-2 prism, $\theta = 40°$
(— — — ATR water blank)
A. Glycine, 3M
B. Glycine hydrochloride, 3M
C. Sodium glycinate, 3M

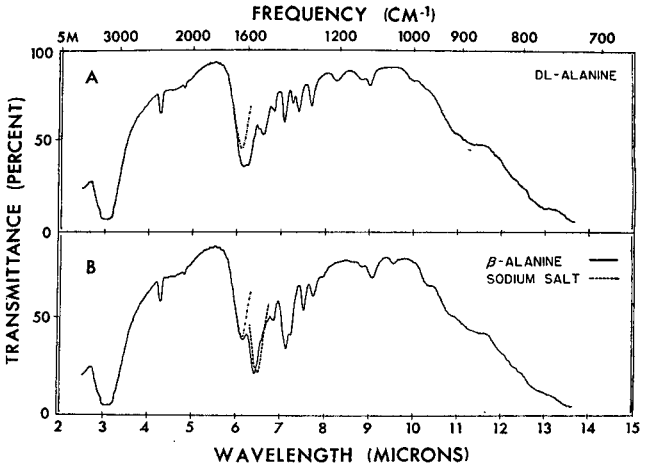

Figure 6. ATR spectra of amino acids in water

Irtran-2 prism, $\theta = 40°$
(— — — ATR water blank)
A. DL-Alanine, 2M
B. β-Alanine, 3M (- - - - sodium salt 3M)

and the minor bands in the spectrum of sodium acetate complement the aqueous transmittance data reported by Parker (*21*). The position of the asymmetrical carboxylate band at 1540 cm.$^{-1}$ agrees with the data obtained by Gore, Barnes, and Petersen (*12*) for heavy water transmittance spectra. The ATR spectrum of sodium citrate shows the symmetrical and asymmetrical carboxylate bands at 1380 and 1560 cm.$^{-1}$ and agrees with the aqueous transmittance data reported by Sternglantz (*27*).

Sulfonic Acids. The ATR spectra of benzenesulfonic acid, 2-naphthalenesulfonic acid, and 1,3,5-benzenetrisulfonic acid in water shown in Figure 4 exhibit some large differences over the solid state spectra of these materials. A doublet band system in the 1200 cm.$^{-1}$ region degenerates into a single broad band and a band in the 1020 to 1040 cm.$^{-1}$ is enhanced. By analogy with carboxylic acid ions, ionic sulfonic acids should produce a pair of bands in the 1000 and 1200 cm.$^{-1}$ regions and Bellamy (*1*) has assigned the ranges of 1260 to 1150 cm.$^{-1}$ and 1080 to 1010 cm.$^{-1}$ to the ionized sulfonic acid group. In water, benzenesulfonic acid absorbs at 1175 and 1020 cm.$^{-1}$, 2-naphthalenesulfonic acid at 1170 and 1032 cm.$^{-1}$, and 1,3,5-benzenetrisulfonic acid at 1190 and 1035 cm.$^{-1}$

Amino Acids. The ATR spectra of glycine, glycine hydrochloride, and sodium glycinate in water illustrate the advantages of this technique for obtaining infrared data even in the immediate area of the 1640 cm.$^{-1}$ water band, which is opaque in transmittance spectra. Figure 5*A* shows the ATR spectrum of glycine in water. The absorption pattern matches exactly the aqueous transmittance spectrum obtained by Parker and Kirschenbaum (*23*) for the window region of 950 to 1510 cm.$^{-1}$ The dipolar ion (zwitterion) appears as a broad band at 1615 cm.$^{-1}$ and is clearly distinguishable over the background of the 1640 cm.$^{-1}$ water band. Figure 5*B* shows the spectrum obtained when an equivalent of hydrochloric acid was added to an aqueous glycine solution. The free acid appears at 1740 cm.$^{-1}$ and additional absorption is found superimposed on the 1640 cm.$^{-1}$ water band that is probably associated with the NH_3^+ ion. The addition of an equivalent of sodium hydroxide to an aqueous glycine solution produced the spectrum shown in Figure 5*C*. The carboxylate ion absorption band shifts to 1500 cm.$^{-1}$ Gore, Barnes, and Petersen (*12*), resorted to the use of D_2O, DCl, and NaOD to obtain the spectra of the three ionic species of glycine.

The ATR spectra of DL-alanine and β-alanine in water are shown in Figure 6. The dipolar ion absorption for DL-alanine appears as an unresolved doublet with bands at 1600 and 1625 cm.$^{-1}$ The band for β-alanine, however, is shifted to 1560 cm.$^{-1}$, the region where simple carboxylate ions usually absorb. The fact that the 1560 cm.$^{-1}$ band is due to the dipolar ion is demonstrated in Figure 6*B* where the carboxylate ion band for the sodium salt of β-alanine in water is superimposed on the spectrum of the neutral solution. The two absorption bands are very close, but the sodium salt band at 1540 cm.$^{-1}$ is displaced by 20 cm.$^{-1}$ from that of the dipolar ion.

The ATR spectrum of L-lysine monohydrochloride in water shown in Figure 7*A* is interesting in that only a single broad band is observed at 1615 cm.$^{-1}$ and no absorption is found in the 1700 cm.$^{-1}$ region for the free acid. The presence of only one half an equivalent of hydrochloric acid apparently results in salt formation of only the ε-NH_2 group and has little effect on the α-NH_2. Therefore, only the normal dipolar ion absorption is observed. Figure 7*B* shows the ATR spectrum of L-cysteine hydrochloride and, like its homolog glycine hydrochloride, shows the free acid band at 1740 cm.$^{-1}$ and some additional absorption at 1629 cm.$^{-1}$ which is probably associated with the NH_3^+ ion.

Phenolics. Figure 8 shows the ATR spectra of resorcinol, pyrocatechol, and pyrogallol in water. These spectra match exactly the solid state transmission spectra of these materials. In each case the C=C skeletal vibration in the 1600 cm.$^{-1}$ region can be readily detected over the 1640 cm.$^{-1}$ water band.

Amides. Figure 9 shows the ATR spectra of acetamide, *N*-methylacetamide, and *N*,*N*-dimethylacetamide in water and presents an interesting series for studying the amide I and amide II bands. The over-all spectral patterns match those of the solid state and capillary liquid film transmittance spectra of these materials. The amide I and amide II bands for acetamide are found at 1655 and 1600 cm.$^{-1}$ in water. For *N*-

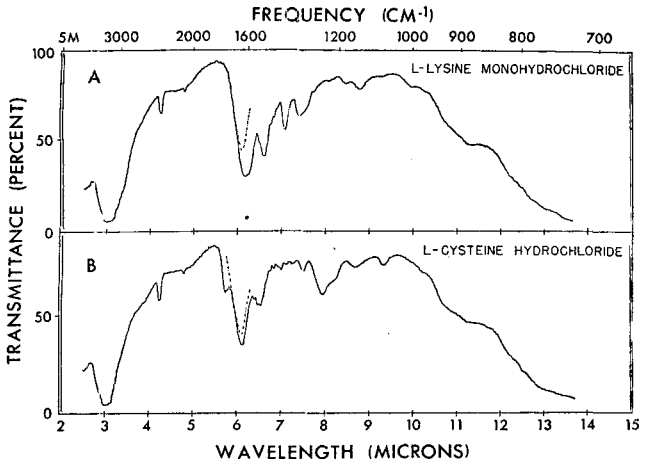

Figure 7. ATR spectra of amino acids in water

Irtran-2 prism, $\theta = 40°$ (– – – – – – ATR water blank)
A. L-Lysine monohydrochloride, 2M
B. L-Cysteine hydrochloride, 2M

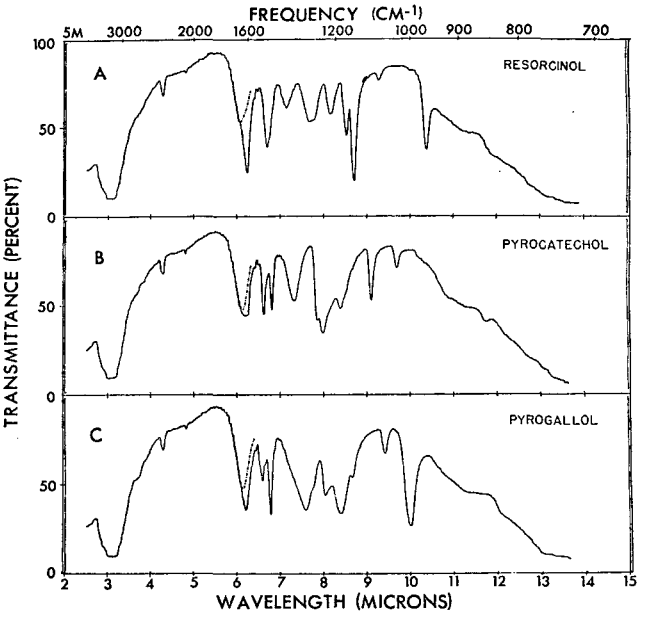

Figure 8. ATR spectra of phenols in water

Irtran-2 prism, $\theta = 40°$ (– – – – – – ATR water blank)
A. Resorcinol, 4M
B. Pyrocatechol, 4M
C. Pyrogallol, 4M

methylacetamide, the amide I and amide II bands are found at 1620 and 1570 cm.$^{-1}$ The highly polar solvent has little effect on the primary and secondary amides. In the case of the tertiary amide, *N,N*-dimethylacetamide, the amide I band shows a large low frequency displacement, from 1647 cm.$^{-1}$ in the liquid film spectrum to 1600 cm.$^{-1}$ in water. This shift probably arises from an interaction or association of the amide carbonyl with water resulting in a bond with some enol character. Bellamy (*2*) reports that methanol produces the same type of shift. In all three cases the amide bands can be readily observed in the region of the 1640 cm.$^{-1}$ water band with minimum interference from the solvent absorption.

Carbohydrates. Goulden (*13*) has made an extensive study of the transmittance spectra of mono- and disaccharides, both in water and the solid state. Figure 10 shows the ATR spectra of glucose, fructose, and sucrose in water. These agree very well with the transmittance spectra and with Goulden's (*13*) observation that much of the fine structure shown in the crystalline state is absent in the solution spectra. It is apparent, however, that sufficient differences exist in the spectra that can be used for either qualitative or semiquantitative analytical purposes.

Inorganic Anions. Figure 11*A* shows the ATR spectra of potassium bicarbonate and potassium carbonate in water. The bicarbonate ion absorbs

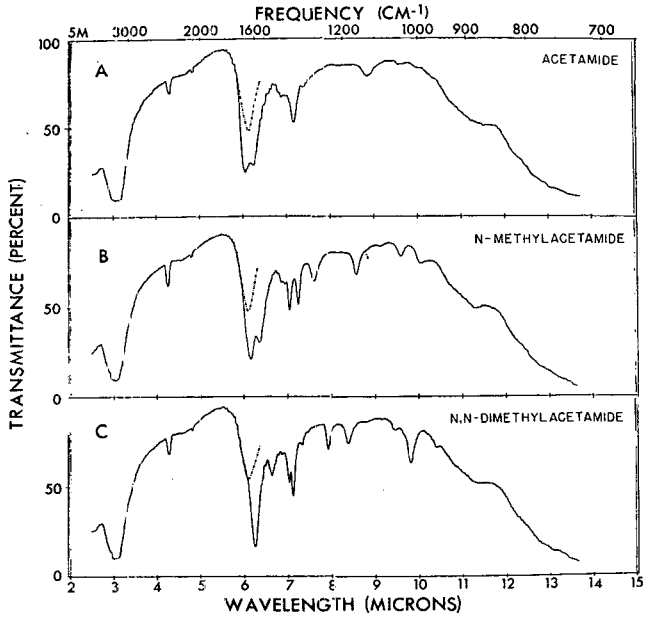

Figure 9. ATR spectra of amides in water

Irtran-2 prism, $\theta = 40°$ (– · – · – · – ATR water blank)
A. Acetamide, 5M
B. *N*-Methylacetamide, 5M
C. *N,N*-Dimethylacetamide, 4M

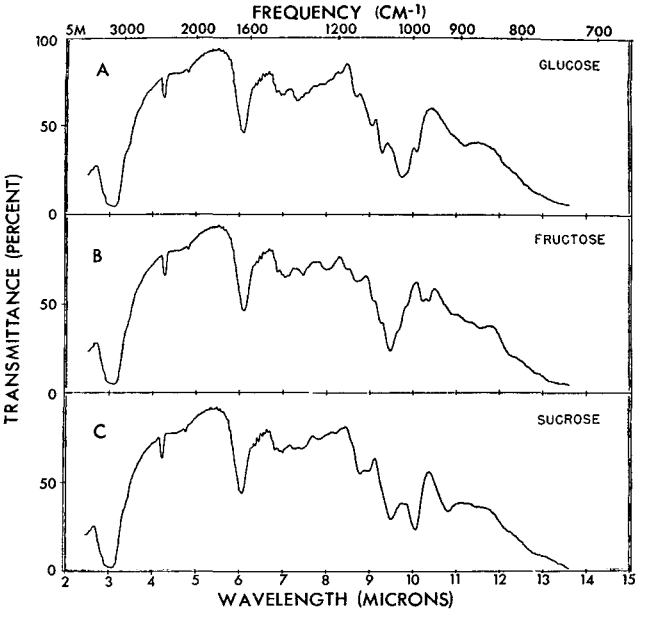

Figure 10. ATR spectra of carbohydrates in water

Irtran-2 prism, $\theta = 40°$
A. Glucose, 3M
B. Fructose, 3M
C. Sucrose, 1.5M

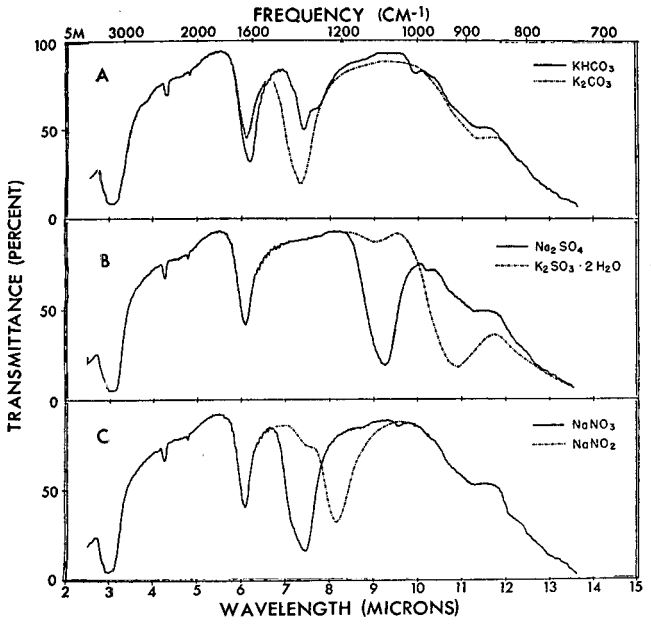

Figure 11. ATR spectra of inorganic ions in water

Irtran-2 prism, $\theta = 40°$
A. Potassium bicarbonate, 2M (———), potassium carbonate, 2M (— — —) ATR water blank (- - - -)
B. Sodium sulfate, 2M (———), potassium sulfite, 2M (— — —)
C. Sodium nitrate, 4M (———), sodium nitrite, 4M (— — —)

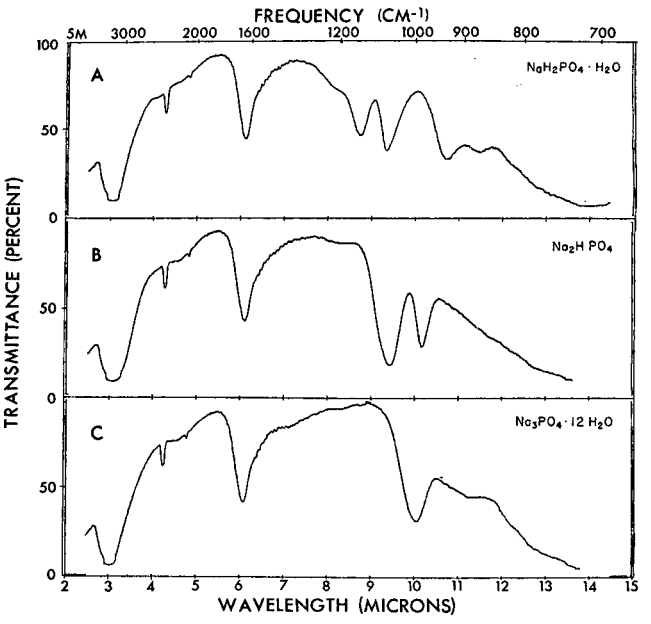

Figure 12. ATR spectra of sodium phosphates in water

Irtran-2 prism, $\theta = 40°$
A. Sodium phosphate, monobasic, 2.5M
B. Sodium phosphate, dibasic, 2.5M
C. Sodium phosphate, tribasic, 0.7M

at 1626 and 1355 cm.$^{-1}$ with a shoulder at about 1310 cm.$^{-1}$ The 1626 cm.$^{-1}$ band appears in the same position as in the solid state spectrum (*18*) and can be readily seen over the 1640 cm.$^{-1}$ water band. The other two bicarbonate ion bands exhibit a low frequency shift of about 60 cm.$^{-1}$ from solid state positions. The carbonate ion absorbs at 1370 cm.$^{-1}$ in water, a considerable displacement from the 1450 cm.$^{-1}$ position in the solid state (*18*).

The ATR spectra of sodium sulfate and potassium sulfite are shown in Figure 11*B*. The sulfate ion absorption at 1078 cm.$^{-1}$ and the sulfite ion absorption at 915 cm.$^{-1}$ in water are both about 30 cm.$^{-1}$ lower than the solid state positions (*18*).

Figure 11 *C* shows the ATR spectra of sodium nitrate and sodium nitrite in water. The nitrate ion absorption at 1335 cm.$^{-1}$ and the nitrite ion at 1225 cm.$^{-1}$ are both displaced about 25 cm.$^{-1}$ lower than the positions in the solid state spectra (*18*).

The ATR spectra of monosodium, disodium, and trisodium phosphate are shown in Figure 12 and are in good agreement with the aqueous transmittance spectra reported by Goulden (*13*) and Sternglantz (*27*). The disodium and trisodium phosphate water spectra also show good agreement with the solid state spectra (*18*), with band positions being displaced by about 10 cm.$^{-1}$ toward lower frequencies. The ATR solution spectrum of monosodium phosphate is much simpler than the corresponding solid state spectrum. A pair of bands at 982 and 1050 cm.$^{-1}$ in the crystalline state are replaced by a single band at 1070 cm.$^{-1}$ in solution.

Two-Phase Systems. The ATR technique is especially applicable to the infrared analysis of aqueous solutions containing suspended matter such as dispersed solids or emulsions. Light scattering by dispersed solids in any solvent system interferes with obtaining transmittance spectra and the high viscosity of most emulsions introduces problems of filling and emptying the very short path length transmittance cells required with water systems. Figure 13*A* shows the ATR spectrum of a 30% solution of sodium 1,3,5-benzenetrisulfonate in water and Figure 13*B* shows the same system containing a 2% carbon dispersion. The combined effects of absorption and scattering of radiation by carbon are believed to cause the increase in the background absorption observed. However, the absorption spectrum for the 1,3,5-benzenetrisulfonate remains distinct and characteristic.

Figure 14 shows the ATR spectra of a polyvinyl acetate and an acrylic resin water emulsion. These spectra match exactly the transmittance spectra obtained for films of these materials (*5*) cast on silver chloride plates. The ATR technique for examining the emulsions per se eliminates the problems associ-

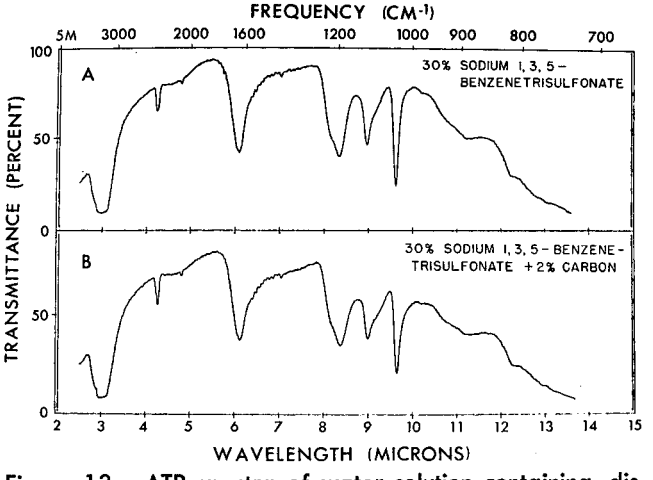

Figure 13. ATR spectra of water solution containing dispersed carbon

Irtran-2 prism, $\theta = 40°$
A. 30% Sodium, 1,3,5-benzenetrisulfonate
B. 30% Sodium, 1,3,5-benzenetrisulfonate containing 2% carbon

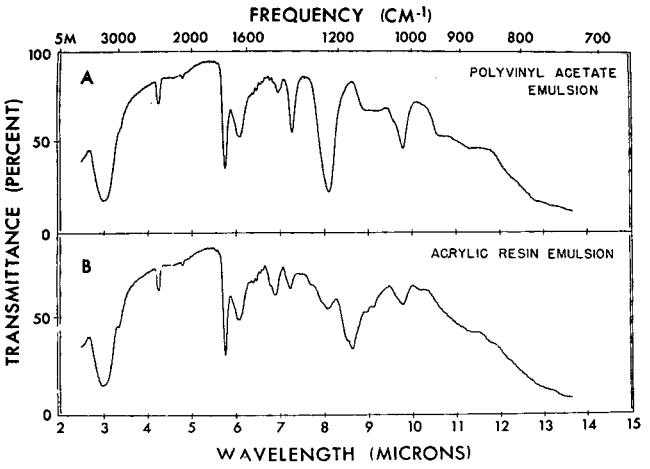

Figure 14. ATR spectra of water emulsions

Irtran-2 prism, $\theta = 40°$
A. Polyvinyl acetate
B. Acrylic resin

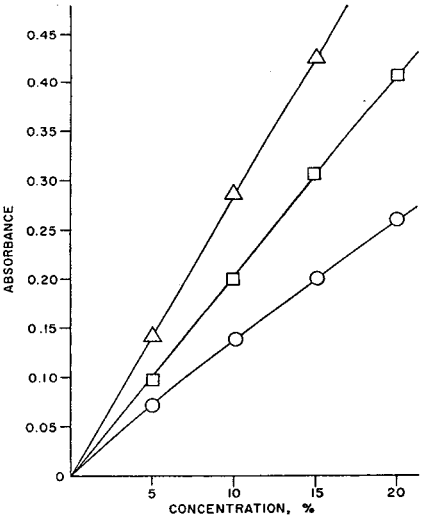

Figure 15. ATR calibration curves for water solutions

Irtran-2 prism, $\theta = 40°$
△, Sodium sulfate
□, Sodium nitrate
○, 2-Methyl-2-nitro-1-propanol

ated with casting the very thin films required for transmittance spectra.

Quantitative Applications. Figure 15 demonstrates the feasibility of ATR for quantitative applications. A plot of the absorbance of sodium sulfate at 1078 cm.$^{-1}$ and sodium nitrate at 1335 cm.$^{-1}$ in water relative to the solvent blank at these frequencies as a function of concentration gives an ideal Beer's law plot. A plot of the absorbance data for the nitro group of 2-methyl-2-nitro-1-propanol at 1534 cm.$^{-1}$ (base line points 1563 and 1493 cm.$^{-1}$) shows a slight deviation from linearity at the higher concentrations. However, this demonstrates the ability to obtain quantitative infrared data in a region where water absorption interferes with transmittance measurements.

Sensitivity. This study has demonstrated the feasibility of ATR to obtain infrared data for aqueous solutions. The very short effective path length used with this method, however, requires high concentrations of materials (in the order of 20% or greater) to obtain suitably intense spectra.

Maximum spectral band intensities can be obtained by chosing the proper prism material and angle of incidence. The dependence of band absorption intensity on the refractive index of the ATR prism and on the angle of incidence has been reported for solids (*8, 10, 15*). Generally, more intense spectra are obtained when the refractive index of the prism is higher than but closely matches that of the sample and the angle of incidence is decreased. Studies in this laboratory have shown that these conditions apply to water solutions as well.

An Irtran-2 prism was selected for the study reported in this paper because of its chemical inertness. More intense spectra for many of the materials examined could be obtained at the same concentration level if a silver chloride prism were used. KRS-5, a more commonly available prism in most laboratories, will in most cases provide solution spectra comparable with Irtran-2. However, since the index of refraction of KRS-5 is greater than that of Irtran-2, less intense spectra are obtained. In addition, the potential toxicity hazards with the double thallium salt might be increased when using solutions. For these reasons, Irtran-2 was used for the work in this report.

The 40° angle of incidence used in this work was chosen because it gave spectra with maximum band intensities and minimum band distortion compared to those obtained at higher angles examined at 5° increments. The equipment used did not permit studies using angles of incidence less than 40°.

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Determination of Plutonium in Biological Material by Solvent Extraction with Primary Amines

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▶ **An accurate and simple procedure for the determination of plutonium in biological materials has been developed. Concentrated urine, or a solution of bone ash, is made at least 1M in H_2SO_4, and plutonium is extracted with a mixture of C_{18} to C_{23} highly branched primary amines in xylene. Plutonium is then extracted from the organic phase with 8M HCl, and measured by alpha counting.**

COLEMAN *et al.* (*1*) have reviewed the use of amine extractants. Petrow, Sohn, and Allen (*6*) have reported an extraction method for the radiochemical determination of thorium in uranium process streams. Thorium is extracted with a mixture of amines. The great advantage of their procedure is the low degree of interference from cationic and anionic impurities, especially phosphate. This suggests its application to the determination of thorium in biological materials such as bone tissue and excreta. Furthermore, it should be applicable to elements which demonstrate similar extraction behavior, such as plutonium.

Most methods for the determination of plutonium, especially in urine samples, either lack reliability or involve lengthy combinations of sensitive coprecipitation methods and solvent extraction procedures. Although much progress has been made to increase accuracy and reproducibility (*4*, *7*, *9*), there still is a need for uncomplicated but reproducible methods of high sensitivity. The method here described has been successfully applied to the study of low level plutonium metabolism but no attempt has been made, as yet, to determine extremely low levels as normally encountered in routine bioassay.

The extractability of plutonium was first determined using synthetic samples. The plutonium was obtained from the Argonne National Laboratory and its isotopic composition was approximately 95% Pu^{239} and 5% Pu^{240}. The amounts of plutonium used were 2.3 $\mu\mu$c. and 2.3 mμc. in approximately 100 ml. of the aqueous phase. The extractant used was the same as that used by Petrow, Sohn, and Allen in their thorium procedures—Primene JM-T, a mixture of *tert*-alkyl primary amines. However, instead of benzene, xylene was chosen as the solvent because of its higher boiling point and lower toxicity. As shown in Table I, the chemical yields for both concentrations, which differed by 1000, were high and reproducible.

Table I. Plutonium Recoveries from Synthetic Samples

Plutonium added, $\mu\mu$c.	Recovery, %
2.3×10^3	100.4 ± 3.1
2.3	96.2 ± 3.4

EXPERIMENTAL

Apparatus and Reagents. The alpha-detection instrument is a 2π proportional counter of conventional design capable of accepting 2-inch stainless steel planchettes; counter performance is checked with a U_3O_8 standard supplied by the National Bureau of Standards.

Primene JM-T, a mixture of *tert*-alkyl primary amines, 5% by volume in xylene, was purchased from Rohm & Haas, Philadelphia, Pa. The Primene solution is washed with half its volume of 1*M* H_2SO_4 prior to use.

Method of Development. The effect of the various oxidation states of plutonium and the possible interference of chloride and nitrate ions on the extraction behavior were evaluated in preliminary experiments.

There exists spectrophotometric evidence (*8*) that excess of SO_2 reduces an equilibrium mixture of plutonium to Pu(III) and that nitrite ion oxidizes Pu(III) spontaneously to Pu(IV), especially at elevated temperatures, but prevents oxidation to Pu(VI). Accordingly, these two chemicals were used to obtain the respective valences. An excess of $KMnO_4$ was used to oxidize plutonium to Pu(VI).

Procedure. URINE ANALYSIS. Urine is collected in polyethylene bottles over 10 ml. of concentrated formic acid to avoid excessive hydrolysis of urea, which would render the specimen basic and could result in loss of plutonium by adsorption on the wall of the container. An aliquot of urine is transferred to a Kjeldahl flask containing enough 5*M* H_2SO_4 to attain an acid concentration of 2*M* in the final sample. The solution is boiled without charring for about 1 hour. Thus far, volumes up to 500 ml. have been extracted. For larger volumes amounts of reagents are increased proportionally. The following amounts of reagents are used for 100-ml. aliquots. The sample is filtered through borosilicate glass wool into a separatory funnel and is allowed to cool to room temperature. Twenty milliliters of a 5% solution of Primene JM-T in xylene is added to the sample and the mixture is shaken for 15 minutes. After separation of the two phases, the aqueous phase is put aside for a second extraction. The organic phase is washed twice with 25 ml. of 1*M* H_2SO_4. Plutonium is removed from the organic phase with two 20-ml. portions of 8*M* HCl. The aqueous phase is again extracted with 20 ml. of 5% Primene, and plutonium is back-extracted from the Primene with 20 ml. of 8*M* HCl. The combined HCl fractions are dried under a heat lamp and then the organic contaminants are destroyed by heating over an open flame or in a furnace at 500° C. The plutonium residue is dissolved in concentrated HNO_3 and transferred to a 2-inch-diameter stainless steel planchette for alpha counting.

Evaporation of the HCl and elimination of the transferred organic material are the points where losses most frequently occur. Acid evaporation is safely done under a heat lamp; careful use of hydrogen peroxide facilitates the combustion of the organic material when an open flame is used.

BONE ANALYSIS. Bone is ashed for 4 hours at 600° C. and, after cooling, is dissolved in as little HNO_3 as possible and diluted with H_2O to a known volume, and a suitable aliquot of this solution is taken for analysis. The amount of bone ash should not exceed 250 mg. for every 75-ml. volume of the aqueous phase. The aliquots are evaporated to dryness under a heat lamp to minimize the amount of HNO_3 and then put in solution with 4 ml. of concentrated HCOOH, and a 60-ml. portion of 2.5*M* H_2SO_4 is added. This solution is heated in a water bath until it is clear, and then transferred to a separatory funnel. Transfer is completed with a 10-ml. rinse of distilled water. Any $CaSO_4$ that forms does not interfere. Plutonium is extracted as described above.

PREPARATION OF SYNTHETIC SAMPLES. Urine samples containing a known

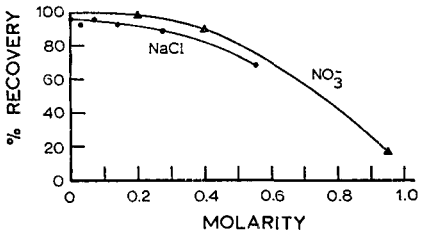

Figure 1. Effect of chloride and nitrate ions on plutonium extraction

amount of plutonium were prepared in the following way. Urine from a beagle was collected over 10 ml. of concentrated formic acid in a bottle to which a known amount of plutonium had been added. A day's output was made up to 500 ml. and aliquots were taken for analysis.

Bone samples containing a known amount of plutonium were prepared by dissolving bone ash containing no plutonium in as little nitric acid as possible and then adding a known amount of plutonium. This was diluted to a known volume and aliquots were taken for analysis.

RESULTS AND DISCUSSION

The extraction behavior of the various oxidation states of plutonium was similar. The extractability decreases in the order:

$$Pu(IV) > Pu(III) > Pu(VI)$$

Since other constituents of the urine may influence the ease of extraction, it is necessary to state the extractability under definite conditions in terms of the amount of plutonium extracted over the total plutonium, instead of in terms of the more general extraction coefficient. The following yields were obtained in a single extraction of 75 ml. of spiked urine with 20 ml. of Primene JM-T 5%: Pu(III) 90.6%, Pu(IV) 98.9%, and Pu(VI) 85%. Plutonium could be extracted from aqueous phases having acid concentrations as high as 8*M* for HAC, 7*M* for HCOOH, and 6*M* for H_2SO_4, but extraction was not feasible with HCl, HNO_3, $HClO_4$, or CCl_3COOH.

Since Cl^- and NO_3^- are the ions most frequently encountered in practical work, an upper limit for their concentration was determined as shown in Figure 1. Phosphate interfered in formic and acetic acid solutions and to a lesser, but significant, degree for concentrations of sulfuric acid less than 1*M*. In 0.5*M* H_2SO_4 acid, Pu(III) still behaves as a cation, but above 1*M* H_2SO_4 an anion complex is formed which is probably stronger than the positively charged phosphate complex. This suggests that it is probably a negatively charged sulfate complex which is extracted with Primene when phosphate is present. Pu(IV) and Pu(VI) form negatively charged complexes readily at lower acid concentrations (*3*, *5*).

Table II. Comparison of Pu^{239} in Bone Determined Directly and by Extraction with Primene

Sample No.	Mean count rates, c.p.m. 250 μl. aliquots[a,b]	Extracted aliquots[b]	% Difference in amounts of Pu^{239} found[c]
2-1	391 ± 7	385 ± 4	1.5 ± 2.3
2-2	1276 ± 20.6	1272 ± 8	0.3 ± 1.7
2-3	809 ± 13.4	804 ± 6	0.6 ± 1.8
6-1	836 ± 11.6	797 ± 9	4.7 ± 1.7
6-2	2713 ± 42.4	2525 ± 16	6.9 ± 1.6
6-3	1229 ± 19.8	1133 ± 34	7.8 ± 3.1
7-1	1662 ± 13.4	1649 ± 13	0.8 ± 1.1
7-2	2190 ± 100	2142 ± 14	2.2 ± 4.5
7-3	1317 ± 20.4	1317 ± 11	± 1.8
9-1	539 ± 8.4	550 ± 7	−2.0 ± 2.0
9-2	1403 ± 20.2	1395 ± 12	0.6 ± 1.7
9-3	609 ± 8	615 ± 8	−1 ± 1.8
10-2	2080 ± 28.2	2216 ± 15	−6.5 ± 1.4
10-3	743 ± 10.6	691 ± 8	7 ± 1.8
11-1	692 ± 11.2	671 ± 8	3.0 ± 2
11-2	3150 ± 56.6	3303 ± 18	−4.8 ± 1.8
11-3	1831 ± 29.6	1751 ± 13	4.4 ± 1.7
12-2	2240 ± 106.4	2316 ± 15	−3.4 ± 4.6
12-3	695 ± 11.2	710 ± 8	−2.2 ± 2
31-3	2550 ± 42.4	2553 ± 16	−0.1 ± 1.8
35-1	666 ± 11.2	720 ± 8	−8.1 ± 1.9
35-2	3010 ± 42.4	3148 ± 18	−4.6 ± 1.4
38-1	480 ± 7.2	485 ± 7	−1.0 ± 2.1
38-2	2340 ± 36	2211 ± 15	−5.5 ± 1.6
38-3	1155 ± 18.6	1168 ± 11	−1.1 ± 4.17
Arithmetic mean			0.42 ± 4.17

[a] Count rates of 250-μl. aliquots adjusted to same volume as extracted aliquots.
[b] Standard deviation of mean is given.
[c] $\left(1 - \frac{\text{Pu by extraction}}{\text{Pu directly}}\right) \times 100$.

Although an H_2SO_4 solution seemed undesirable as the aqueous phase for the extraction of plutonium from biological material because of the presence of varied quantities of calcium, it proved to be the only acid tested that could be successfully applied. As long as H_2SO_4 was added to an already dissolved sample, precipitating calcium sulfate did not affect the recovery of plutonium. It can either be filtered off with insignificant loss of activity, or left in the solution.

In addition to the work with the synthetic samples, the chemical yield of Pu^{239} by the Primene-extraction method was determined for bone samples from dogs injected with Pu^{239} 6 weeks to 5 years before death (*2*). The samples were analyzed by this method and by direct plating. The bones contained sufficient Pu^{239} that 250-μl. aliquots of the solutions could be plated directly for alpha counting to determine Pu^{239}. These solutions were sufficiently diluted to make mass absorption negligible. Then 5 or 10 ml. of each of these solutions was extracted with Primene. This comparison has the disadvantage that the precision of the extraction method is greater than that of the direct plating, since the counting rates, of course, are much greater for the larger aliquots.

The results are presented in Table II. The count rates of the 250-μl. aliquots have been adjusted to the same volumes as those used in the extraction procedure. Both the direct platings and the extractions were done in duplicate. First is given the mean count rate and standard deviation obtained by the direct plating method, followed by the mean count rates and standard deviations obtained by the extraction method. The last column shows the percentage difference in amounts found by the two methods. The standard deviation of this quantity is calculated from the measured standard deviations of the mean count rates of the samples done by the two methods. The difference in amounts of Pu^{239} found by the two methods is small. The mean of the differences for the 25 samples studied is only 0.42% with a standard deviation of 4.17%.

The method described above is proving to be valuable in the accurate analysis of biological materials for small amounts of Pu^{239}. The absence of a coprecipitation step results in a higher and more reproducible chemical yield and a more effective separation from contaminants. The risk of mechanical loss through transfers is also decreased.

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Amperometric Titration of Plutonium(VI) with Iron(II)

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▶ **A method has been developed for the precise titration of plutonium(VI) with iron(II) in which the end point is detected amperometrically with a rotating platinum electrode. The plutonium is oxidized to the sexivalent state in a sulfuric acid medium with argentic oxide, the excess oxidant destroyed by heating, and the plutonium titrated by the addition of standard iron(II) sulfate from special weight burets. For 15-mg. samples, precisions of ±0.06% relative standard deviation are obtainable with no significant bias. For 0.2-mg. samples, the relative standard deviation is 0.4% with a 0.6% negative bias.**

As RECENTLY as 1957 a review by Metz (*8*) of the analytical chemistry of plutonium revealed that only one precise method was available for the determination of plutonium. This method, which was first reported by Boaz, *et al.* (*1*) was the potentiometric titration of plutonium(III) to (IV) with cerium(IV) sulfate. By the use of weight burets for both aliquoting and the addition of titrant a precision of ±0.05% is obtainable (*8*). Iron, the most common contaminant of plutonium metal and its compounds, interferes and a correction for the iron by means of a spectrophotometric determination is necessary. Several variations in the titration of the (III–IV) couple, such as photometric end point detection (*2*), and use of potassium dichromate as the titrant (*10*), have since been introduced.

More recently several new high-precision analytical methods for plutonium have been reported (*11, 13, 14*) in which the interference of iron has been either eliminated completely or significantly reduced. Scott and Peekema (*11*) and Shults (*13*) used controlled potential coulometry to titrate plutonium(III) to (IV). At the 20-mg. level, a precision of ±0.06% is obtainable. At iron to plutonium molar ratios of one or greater (an unusual situation) iron does interfere, but otherwise the method was remarkably free of interferences.

In a method developed by Waterbury and Metz (*14*) plutonium(VI) (obtained by perchloric acid oxidation) is reacted with excess standard iron(II) sulfate to form plutonium(IV) and iron(III). The excess iron(II) is then back titrated potentiometrically with standard cerium(IV). With 500-mg. samples, a precision of ±0.02% can be obtained. Shults (*12*) demonstrated that the excess iron(II) could be determined equally well by controlled potential coulometry. Chromium, manganese, and vanadium interfere. Sulfate cannot be tolerated as it inhibits the preliminary oxidation of plutonium to the sexivalent state.

Plutonium(VI) can be titrated directly with iron(II) by incorporating an amperometric end point detection of the type used by Kolthoff and May (*4*) in the chromium(VI)–iron(II) titration. In both the plutonium and chromium titrations essentially no current is passed by the rotating platinum microelectrode until the end point is reached and thereafter the current is proportional to the amount of excess iron(II) titrant. Argentic oxide, the reagent introduced by Lingane and Davis (*7*) for the oxidation of chromium, manganese, and cerium, has also been found to be particularly satisfactory for oxidizing plutonium to the sexivalent state. The precision and accuracy of the method are comparable to those obtained in other titrations.

During the preparation of this paper for publication, it came to the authors' attention that Helbig (*3*) had also titrated plutonium(VI) amperometrically with iron(II). In principle the titration which Helbig reports is nearly identical to the one reported herein, but the titrations are quite different in practice. Helbig carried out his titrations at the 0.1- and 1-μg. levels with relative standard deviations of ±6 and ±3%, respectively; titrations carried out in our laboratory were at the 0.2- and 15-mg. level with standard deviations of ±0.4 and 0.06%, respectively.

A thorough and comprehensive review of this phase as well as the other phases of plutonium analytical chemistry can be found in the recently published treatise by Metz and Waterbury (*9*).

EXPERIMENTAL

Apparatus. The titration assembly used in this work consisted of an E. H. Sargent and Co. "Ampot" with the microammeter replaced by the more sensitive Ealing Corp. "Scalamp" (No. 29-222), a 600-r.p.m. syncronous rotator for the platinum microelectrode, and a low resistance mercury–mercurous sulfate reference electrode. Saturated potassium sulfate solution was the electrolyte used in the reference electrode and salt bridge. Diffusion of the electrolyte from the bridge into the solution being titrated was prevented by a closure of unfused vycor (Corning No. 7930). Operation of the assembly is proper if a plot of current *vs.* weight of 0.01 meq. per gram of iron(II) (corrected for volume changes) is linear in the range 0 to 50 μa.

When not in use the platinum microelectrode is stored in 1*N* sulfuric acid. Before use it is operated for 2 minutes at +1.2 volts *vs.* the reference electrode. Without this treatment, the residual current in the first titration may be high.

The weight buret used in this analysis is shown in Figure 1. Its particular uses and advantages are covered in the "Discussion" section.

Reagents. The plutonium, iron(II), and chromium(VI) solutions used in this analysis are prepared and aliquoted by weight rather than volume. Concentrations are expressed in milliequivalents per gram of solution.

Plutonium chloride, 0.05 meq. per gram, is prepared by dissolving National Bureau of Standards (NBS) high purity plutonium metal in 3*N* hydrochloric acid.

Ferrous ammonium sulfate, 0.05 meq. per gram, in 1*N* sulfuric acid is standardized daily by amperometrically titrating against standard 0.05 meq. per gram potassium dichromate (NBS). The spread between duplicates should be 0.05% or less. Lower concentrations are prepared by dilution with 1*N* sulfuric acid.

Silver(II) oxide is obtained from Handy and Harmon, 82 Fulton Street, New York 38, N. Y.

Procedure. Dissolve or dilute the samples to a concentration of 5 to 10 mg. of plutonium per gram of solution. Transfer 20 to 25 ml. of this solution to one of the special weight burets. Weighing the buret before and after to the nearest 0.1 mg., transfer an aliquot containing from 10 to 20 mg. of plutonium to a 50-ml. beaker. Add approximately 0.5 ml. of 18*N* sulfuric acid and fume without spattering to dryness. Cool the sample to room temperature and dissolve the plutonium sulfate in about 10 ml. of 0.5*N* sulfuric acid. While swirling the solution, slowly add 25-mg. portions of silver(II) oxide until an excess is present. The presence of excess oxidant is determined by a characteristic blackish-brown color and the presence of a black precipitate of unknown composition (possibly Ag_2O_3). After 10 to 15 minutes, dilute the solution to about 25 ml. with 1*N* sulfuric acid and warm on a hot plate to 85° C. to destroy the excess oxidant. (Both the dark color and the black precipitate disappear during this step.)

After the solution has cooled to 40° C. or less, insert the platinum microelectrode and salt bridge arm of the reference electrode in the solution, start the rotator, and add 10 ml. of 18*N* sulfuric acid. With the microammeter shorted (to protect it from the charging current) apply a potential of +0.6 volt *vs.* the reference electrode. After 1 to 2 minutes reconnect the microammeter and record the current as soon as it is steady. This may take as long as 5 minutes but the time is usually much shorter. The value should be 0.2 μa. or less. (Persistently higher "residual currents" indicate the presence of interfering ion species which are being oxidized at the platinum electrode. Hydrogen peroxide formed by alpha particle radiolysis has been shown to give this effect.) Weigh the weight buret containing the standardized iron(II) solution and slowly add increments to the sample solution until a steady current of about 2 μa. is obtained. Because of the slowness of the reaction, c.f. "Discussion," the current after the addition of each increment increases rapidly to 5 to 10 μa. and then decreases in 1 or 2 minutes to less than 2 μa. (This is particularly true at the start of the titration.) Reweigh the buret and record the weight and current reading. Add more iron(II) solution until the current reaches approximately 20 μa. Again weigh the buret and record the weight and current reading. The end point in the titration is the intersection of the horizontal residual current line and the iron(II) diffusion current line. The residual current line which has a slope of zero is fixed by the initial current measurement, c.f. "Discussion"; the iron(II) diffusion current is fixed by the second and third current-weight measurements. The end point can be determined either by calculation or graphically. In the latter case, the plotted data has the form of a reversed letter "L."

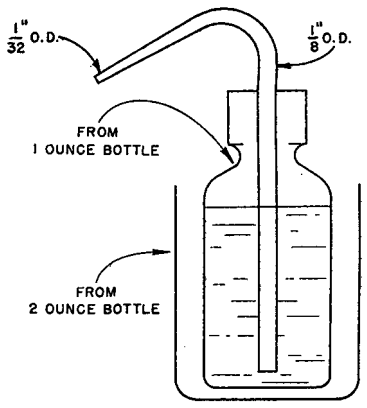

Figure 1. Weight buret

Material—Polyethylene

DISCUSSION

Plutonium(VI)–Iron(II) Reaction. The rate of plutonium(VI)–iron(II) reaction is markedly slower than the chromium(VI) – iron(II) reaction. This is demonstrated by comparing the observed current–time relationships prior to end points in the respective titrations.

In the chromium(VI)–iron(II) amperometric titration (*4*) the current may rise momentarily after the addition of a 100-mg. increment of the iron(II) titrant but returns to the initial value within the response time of the microammeter. Thus several points may be taken prior to the end point to help fix the residual current line. However, only the determination of the initial residual current is necessary to fix the residual current line since its slope is zero.

As has been noted above in the "Procedure," the current in the plutonium(VI)–iron(II) amperometric titration rises to about 10 μa. after the addition of the first 100 mg. of titrant and then drops off exponentially. The current reaches 2 μa. in 1 or 2 minutes and the initial residual current value of 0.1 to 0.2 μa. in 30 minutes or more. As the titration progresses, the current rise becomes smaller and the subsequent current fall becomes more rapid, but the fall is never rapid enough to warrant other measurements of the residual current. For this reason, only the initial current reading is used to establish the residual current line. The assumption that the slope of this line is also zero, as in the chromium(VI) titration, appears to be warranted by the precision and accuracy of the present results.

The increase in current response as the titration progresses suggests that the reaction products have a catalytic effect on the reaction rate. This is borne out by the pronounced effect that iron(III) ions have on the rate of the plutonium(VI)–iron(II) reaction. The addition of 0.2 meq. of iron(III), the amount formed in titrating 24 mg. of plutonium, increases the current response at the start of a titration by about a factor of 5. (This is true of both the magnitude of the current rise on the addition of titrant and the rate of current decrease thereafter.) This effect is used to advantage in the titration of smaller amounts of plutonium where the reactant concentrations may be lower by a factor of 100. By adding a milliequivalent of iron(III) prior to the start of the titration, the titration can be carried out without undue delay.

Oxidation. The oxidation of plutonium to the sexivalent state prior to its titration with bivalent iron requires an oxidant which can be readily destroyed once the oxidation has been completed. Waterbury and Metz (*14*) and Shults (*12*) found fuming perchloric acid to be a satisfactory oxidant when the reaction was carried out under closely controlled conditions. The authors of the present work, however, were unable to obtain reproducible results with this reagent. This difficulty is probably due to a cooling effect created by the unusually strong draft in the fuming facility. When the oxidation was carried out in uncovered beakers, results were invariably low; when the oxidation was carried out in covered beakers, the results were often as much as 1% higher than the theoretical value. Since high results were always associated with small initial cathodic currents, it is presumed that the high results were caused by the decomposition of the perchloric acid and the subsequent titration of these decomposition products.

Argentic oxide which was demonstrated by Lingane and Davis (*7*) to be particularly useful for the oxidation of chromium, cerium, and manganese has been found to be an equally satisfactory oxidant for plutonium. At room temperature and in 0.5*N* sulfuric acid, the plutonium is oxidized rapidly to the sexivalent state. Heating the solution to about 85° C. is sufficient treatment to destroy the excess oxidant.

Incomplete oxidation of the plutonium can occur at higher sulfuric acid concentrations even if a large excess of argentic oxide is added. In 3*N* sulfuric acid, for example, the oxidant is rapidly destroyed by reaction with water before the plutonium is oxidized. To ensure a satisfactorily low acidity the samples are fumed to dryness with sulfuric acid

and then dissolved in 0.5*N* acid. Allowing the dry samples to stand for a number of days before dissolving and titrating them did not affect the results. However, prolonged baking of the sample after the last of the sulfuric acid had been removed made it difficult to redissolve the plutonium.

Buret. Syringe-type microburets were used in the initial stages of this investigation to deliver the small volumes (2 to 4 ml.) of titrant. However, as the method was being refined, it became apparent that the precision of the analysis was being limited by the reproducibility of the buret deliveries and readings.

A weight buret of the type shown in Figure 1 has proved to be particularly convenient in this and other amperometric titrations where the increments of titrant are finite—i.e., no drop splitting is required. The buret is essentially a 30-ml. polyethylene wash bottle with the delivery tip drawn down to a diameter of about 0.03 inch. Because of the nonwetting characteristics of polyethylene and the small diameter of the delivery tube, the unused titrant will return in its entirety to the bottle if finger pressure is carefully applied and relieved. Drop size is about 15 μl. The buret is handled within a polyethylene secondary container and transferred from the secondary to the balance pan and back with forceps. This avoids weight changes and contamination which might occur as the result of direct contact between the buret and the analyst's gloved hand. Aliquoting of the plutonium solutions to be analyzed is done with the same equipment and technique. Weight loss of the buret, presumably the result of diffusion of water vapor through the sides of the polyethylene bottle, amounts to 0.5 mg. per 24 hours.

Interferences. Such elements as vanadium, manganese, chromium, and cerium, which, like plutonium, are oxidized by argentic oxide and reduced by ferrous sulfate, will interfere in this analysis. There are, however, a number of proved separation methods which will with comparative ease selectively remove plutonium from all these interfering elements. In strong hydrochloric acid plutonium(IV) can either be extracted with 30% tributyl phosphate-inert diluent (*6*) or adsorbed on Dowex-1 anion exchange resin (*9*). Back extraction or elution is effected with dilute hydrochloric acid plus a reducing agent. Plutonium(IV) is also adsorbed onto Dowex-1 from strong nitric acid (*5*). Elution in this case is effected with 0.3*N* hydrochloric acid–0.01*M* hydrofluoric acid. Results of our studies confirm the findings of Waterbury and Metz (*14*) that the precision and accuracy of results obtained with plutonium samples that are subjected to separation procedures are seldom as good as those obtained with pure materials. However, Kressin and Waterbury (*5*) have recently reported excellent results when using the nitric acid–Dowex-1 system on larger samples (200 mg).

Difficulty is, of course, encountered in the sulfuric acid fuming step when the amount of salts other than plutonium becomes large. Spattering, precipitation, and/or incomplete chloride and nitrate removal may be encountered. Since the precision and accuracy requirements become correspondingly less severe as the concentration of plutonium in alloys or processing samples decreases, the extraneous salt problem can be circumvented to some extent by reducing the amount of plutonium to be titrated from 10 to 1 mg. or less and titrating with a correspondingly more dilute iron(II) sulfate solution. Particularly careful consideration should be given to the interference problem in samples where the plutonium concentration is low, since seemingly insignificant impurity concentrations may affect the accuracy of the results. For example, in a 1% plutonium–uranium alloy, 10 p.p.m. of manganese (equivalent weight equals 11.0) will introduce a +1% error in the plutonium (equivalent weight equals 119.5) result.

Table I. Results of Amperometric Titration of 99.98% Plutonium Metal

Plutonium taken, mg.	No. of detn.	Rel. std. dev., %[a]	Plutonium purity, %[b]	Error, %
10–20	10	0.06	99.97	−0.01
0.10–0.30	13	0.4	99.4	−0.6

[a] For a single determination.
[b] Average of all determinations.

RESULTS

The precision and accuracy of the method were checked by analyzing a plutonium solution which had been prepared from high purity plutonium metal. The results of two typical tests are summarized in Table I. The purity of the metal, 99.98%, was determined by difference after spectrochemical, carbon, oxygen, and nitrogen analyses. The combined concentration of impurities such as chromium, vanadium, and manganese which would lead to high results was negligible. The titration of the more concentrated plutonium solution was done with 0.05 meq. per gram of iron(II) sulfate; the less concentrated solution with 0.001 meq. per gram of iron(II) sulfate. The small negative error, 0.01%, at the 15-mg. level is not statistically significant. No explanation can be offered for the small negative bias at the 0.2-mg. level.

The method was also tested on a limited number of samples which required a preliminary separation of the plutonium from interferences. Coprecipitation of plutonium with lanthanum fluoride (*6*) from a hydrochloric acid solution of a uranium–20% plutonium–5%-fission element alloy resulted in plutonium recoveries of 98.6 ± 0.5%. The low recovery and lack of precision are very probably attributable to incomplete precipitation rather than difficulties in the titration. Separation from a 200-fold excess of magnesium was effected by an extraction of plutonium(IV) from hydrochloric acid into 30% tributyl phosphate–carbon tetrachloride solution (*6*). Recovery was 99.9% with a relative standard deviation of 0.2%.

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Direct Mass Spectrometric Analysis of Petroleum Samples Boiling in the Kerosine Range

Heavy Reformates and Hydrodealkylated Products

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▷ A mass spectrometer method for the analysis of petroleum streams and products boiling between 350° and 550° F. is described. Nine compound types, including eight hydrocarbons and alkyl benzothiophenes, are reported. Additionally, a breakdown of alkyl naphthalenes by carbon number is presented. Olefins, thiophenes, and/or sulfides may be independently determined and the data from these analyses integrated with those obtained by mass spectrometric analysis. The method was developed primarily for the analysis of feed and product streams associated with the production of naphthalene by hydrodealkylation. Maximum accuracy (±1% absolute) is obtained with such samples. Slightly lower accuracy is found for other sample types boiling in the kerosine range. The method requires no separation of the sample prior to analysis and is carried out in less than 2 man-hours per sample.

Mass spectrometric procedures for the analysis of a wide variety of petroleum fractions have been reported in the literature. They include methods for gasolines (*6*), heavier distillates (*1*, *3*, *4*), certain nonhydrocarbons isolated from petroleum (*5*, *7*, *9*), saturates (*10*), and paraffin waxes (*2*). Apart from the rather specialized case of waxes, all procedures except those for gasolines require preliminary separation of the sample into two or more fractions. Each fraction must be analyzed individually and the results combined to give values for the total sample.

Analytical procedures which require prior separation of the sample have several shortcomings relative to direct mass methods: increased cost and elapsed time, reduced precision and, in some cases, reduced accuracy. In any separation process, certain material losses are unavoidable. The repeatability of an analysis based on sample separation will depend in large measure on the repeatability of the separation process. The accuracy of the analysis will similarly suffer, unless the errors from the separation step are offset by increased accuracy in the mass analysis of each fraction. At the same time the cost of preseparation and the need for mass analysis of several fractions, rather than a single sample as in direct mass methods, increase the cost of an analysis to the point where full advantage of mass spectrometer techniques may not be taken. A review of the literature emphasizes both the lack of, and the need for, direct mass spectral methods which can analyze samples boiling above the gasoline range with requisite detail and accuracy.

Interest in 350° to 550° F. petroleum fractions has been accentuated by the recent advent of commercial hydrodealkylation processes for the production of naphthalene, such as the Unidak process of the Union Oil Co. Research process studies and routine control of commercial units require the ability to analyze streams associated with this process for various molecular types, particularly the alkyl naphthalenes by carbon number. Heavy reformates from the production of gasoline are currently one of the more popular feedstocks for hydrodealkylation.

A direct mass procedure has been developed in this laboratory for the compositional analysis of heavy reformates and related hydrodealkylated streams with a high level of accuracy (±1% absolute). Other sample types in the same boiling range are also analyzed with somewhat lower, but generally satisfactory accuracy. The molecular classes reported include eight hydrocarbon and one sulfur type; naphthalenes are also determined by carbon number. At the same time provision is made in the case of cracked samples and high sulfur samples for integration of data from the independent analysis of three other compound types —olefins (*12*), thiophenes (*11*), and alkyl sulfides (*11*)—with data obtained from the mass spectrometric method.

EXPERIMENTAL

Apparatus. A consolidated Engineering Corp. Model 21-103C mass spectrometer with a Houston Glass Fabricating heated inlet system was used. The magnet current was 0.465 ampere, and the inlet temperature 150° C. The isatrom temperature was about 270° C., giving the following *n*-hexadecane pattern:

m/e^+	Ratio
43	1.20
57	1.00
71	0.55
85	0.35
226	0.30

Method Development. Fragment Analysis. The nine compound types listed in Table I are determined by a conventional fragment analysis similar to that followed by previous workers (*4*). The fragment peak summations of Table I are similar to the normal parent-minus-one sequence, with the exception of the indane-Tetralin and alkyl naphthalene classes. The compounds Tetralin and naphthalene may be present in high concentrations in

Table I. Fragment Mass Groupings[a]

Alkanes	$\Sigma\ 43 = 43 + 57 + 71 + 85 + 99$
Noncondensed cycloalkanes	$\Sigma\ 69 = 69 + 83 + 97 + 98 + 111 + 112$
Condensed cycloalkanes	$\Sigma\ 67 = 67 + 81 + 95 + 96 + 109 + 121$
Alkyl benzenes	$\Sigma\ 91 = 91 + 105 + 119 + 133$
Indanes-Tetralins	$\Sigma\ 104 = 104 + 117 + 131 + 145 + 159$
Alkyl naphthalenes	$\Sigma\ 128 = {}^1/_2\,(128) + 141 + 155 + 169 + 183$
$-14z$	$\Sigma\ 153 = 153 + 167 + 181 - 0.1\,(\underline{156} + \underline{170} + \underline{184})$
$-16z$	$\Sigma\ 165 = 165 + 179 + 193 + 207$
Benzothiophenes	$\Sigma\ 147 = 147 + 161 + 175$

[a] All peaks used in these summations are polyisotopic, with the exception of the three alkyl naphthalene parent peaks (156, 170, 184) used in the acenaphthene summation correction. These are monoisotopic.

Table II. Matrix Inverse

	43	69	67	91	104	128	153	165	147
Alkanes	+0.089517	−0.023595	−0.017067	+0.000000	−0.006500	−0.009000	+0.007223	−0.000809	−0.003471
Noncondensed cycloalkanes	−0.023152	+0.223519	−0.057318	+0.000000	+0.000000	−0.006000	−0.008154	−0.014542	−0.003524
Condensed cycloalkanes	+0.008031	−0.050556	+0.178171	−0.007000	+0.008847	+0.000000	+0.000579	−0.001539	−0.023871
Alkyl benzenes	−0.000268	−0.001356	−0.002377	+0.127272	−0.021128	+0.001901	−0.010184	−0.007289	+0.000551
Indanes-Tetralins	−0.000528	+0.000319	+0.000146	−0.003422	+0.174703	−0.002050	−0.001669	−0.000042	+0.000025
Alkyl naphthalenes	−0.003960	−0.001402	−0.000482	−0.006056	+0.005198	+0.266707	−0.016209	+0.002668	+0.003017
−14z	+0.003960	−0.024000	+0.006579	−0.002640	+0.000852	−0.000007	+0.495535	+0.003405	+0.002193
−16z	+0.008052	−0.003832	−0.033782	+0.000000	+0.000384	−0.001525	−0.061310	+0.415882	−0.027234
Benzothiophenes	+0.002390	−0.002000	−0.003780	−0.000510	−0.007280	−0.001860	−0.001070	−0.002400	+0.168500

350° to 550° F. samples, yet are not adequately represented in a parent-minus-one type summation. Hence, the indane-Tetralin fragment summation is modified to include Tetralin by adding the 104 mass, while the alkyl naphthalene summation is similarly modified for naphthalene by adding 0.50 times the 128 mass. Additionally, the contribution of alkyl naphthalenes to the -14z (from empirical formula C_nH_{2n+z}) fragment summation is markedly dependent on the average molecular weight of the naphthalenes in the sample (naphthalene and the methylnaphthalenes cannot contribute to Σ153). This effect is handled by correcting Σ153 for alkyl naphthalene interference prior to application of the matrix inverse to the various fragment summations. The correction uses the monoisotopic naphthalene parent peaks —e.g., 156, 170, 184—as shown.

The sensitivity data for the compound types of Table I were derived from the mass spectra of pure compounds and chromatographic concentrates, and, where necessary, extrapolation of these data. The final matrix inverse corresponding to the types and summations of Table I is shown in Table II.

Carbon Number Distribution of Alkyl Naphthalenes. The molecular weight distribution of the alkyl naphthalenes is usually of interest only in samples associated with hydrodealkylation. Such samples are normally highly aromatic and enriched with naphthalenes. In these cases, the slight contribution of paraffins to the naphthalene parent peaks can be ignored and the naphthalene concentrations calculated from the monoisotopic parent peaks. Relative weight concentrations of the C_{10} through C_{15} alkyl naphthalenes are calculated by Equations 1 to 6.

$$C_{10}H_8 = 0.38\,[(128) - 0.13\,(132) - 0.30\,(146) - 0.13\,(156) - 0.40\,(160) - 0.40\,(174)] \quad (1)$$

$$C_{11}H_{10} = 0.61\,(\underline{142}) \quad (2)$$

$$C_{12}H_{12} = 0.84\,(156) \quad (3)$$

$$C_{13}H_{14} = 1.00\,(\underline{170}) \quad (4)$$

$$C_{14}H_{16} = 1.00\,(\underline{184}) \quad (5)$$

$$C_{15}H_{18} = 1.00\,(\underline{198}) \quad (6)$$

The underlined quantities refer to monoisotopic peak heights at the indicated mass numbers; the nonunderlined values refer to polyisotopic peak heights. These relative alkyl naphthalene concentrations by carbon number are then normalized to the total alkyl naphthalenes value from the inverse fragment analysis. The interference values and sensitivities in Equations 2 to 6 were derived primarily from the mass spectra of pure compounds and extrapolation to higher carbon numbers.

Olefinic and/or High-Sulfur Samples. The analysis of cracked samples or of high-sulfur stocks requires supplementary determination of olefins (*12*) and/or alkyl thiophenes plus alkyl sulfides (*11*). The relative concentrations of the sample components determined by the inverse of Table II are then corrected for the presence of these supplementary components by means of Equations 7 to 11, following which the matrix-determined components are renormalized to 100% minus the total concentration of supplementary components (olefins plus thiophenes plus sulfides).

Corrected % alkanes = inverse value −0.23 (% olefins) − 0.07 (% thiophenes) − 0.06 (% sulfides) (7)

Corrected % noncondensed cycloalkanes = inverse value −0.35 (% olefins) − 0.18 (% thiophenes) − 0.27 (% sulfides) (8)

Corrected % condensed cycloalkanes = inverse value −0.35 (% olefins) − 0.07 (% thiophenes) − 0.67 (% sulfides) (9)

Corrected % alkyl benzenes = inverse value − 0.04 (% olefin) (10)

Corrected % indane—Tetralins = inverse value − 0.01 (% olefin) (11)

The above correction factors were derived from the mass spectra of olefin-, sulfide-, and thiophene-containing fractions isolated from several different sample types by liquid adsorption chromatography. The sulfur-type corrections are generally small for cracked and low-sulfur samples, since thiophenes and sulfides are generally trace constituents of cracked samples (*11*). Where no correction is made for sulfur types present, renormalization of the corrected inverse concentrations is not required.

DISCUSSION

All of the hydrocarbon and sulfur types reported in the present method have been previously observed in straight-run gas-oil fractions (*4*). Their presence in the various sample types currently analyzed was verified by detailed characterization studies in this laboratory. The only compound types not included in the present method which do occur in appreciable concentrations in some sample types are the indenes and dihydronaphthalenes. These latter compounds are restricted to cracked samples, and may be independently determined (if desired) as in the case of the aliphatic olefins, thiophenes, and sulfides. Low ionizing voltage mass analysis of the total sample or of an aromatic concentrate (*8*) is a convenient technique.

The present method was designed specifically for the analysis of heavy reformates and hydrodealkylated streams, and maximum accuracy for the method is realized in the analysis of these sample types. Extension of the present method to other sample types in the 350° to 550° F. range shows some reduction in accuracy, although in this respect the present method compares favorably with other routine compositional analysis procedures for samples boiling above 400° F.

Validity of Method. Heavy Reformates and Hydrodealkylated Streams. The accuracy of the present analysis as applied to heavy reformate and hydrodealkylated samples was evaluated in several ways. Original samples were subjected to detailed characterization by conventional combinations of distillation, liquid-adsorption chromatography, and low and high ionizing voltage mass spectrometry; the "true" analyses of these samples derived from this nonroutine characterization were then compared with the routine application of the present direct mass procedure. Synthetic blends were also made up from chromatographically isolated fractions and/or pure compounds. In each case the purity of the chromatographic fraction was ensured by independent ultraviolet absorption and low voltage mass analysis. The absence of saturates in most samples of the type under discussion simplified the simulation of actual samples by blends.

Table III. Blends of Heavy Reformate Monoaromatic Fractions and Pure Alkyl Naphthalenes

(Weight per cent)

Blend	Saturates		Monoaromatics		Naphthalene		Methyl naphthalene		C_{12} Naphthalenes		Sulfur compounds	
	Known	Method	Known	Method	Known	Method	Known	Method	Known	Method	Known	Method
1[a]	0.0	0.4	41.3	39.8	17.6	18.8	41.1	40.1	0.0	0.1	0.0	0.8
2[a]	0.0	0.8	54.2	53.3	0.4	0.7	45.4	44.2	0.0	0.1	0.0	1.4
3[a]	0.0	0.0	38.7	36.7	33.4	33.4	26.3	24.9	3.6	3.6	0.0	0.7
4[b]	0.0	0.0	46.8	40.2	5.4	7.1	1.9	0.2	45.3	49.5	0.0	0.8
5[b]	0.0	0.0	56.7	53.1	6.1	7.0	24.6	25.0	12.6	13.3	0.0	1.8

[a] C_{12} naphthalenes are mixtures of dimethylnaphthalenes.
[b] C_{12} naphthalenes are ethylnaphthalenes.

Table IV. Synthetic Blends of Heavy Reformate Monoaromatics and C_{12}^+ Naphthalenes, Hydrodealkylation Monoaromatics, and Pure C_{10} and C_{11} Alkyl Naphthalenes

(Weight per cent)

Blend	Saturates		Monoaromatics		Naphthalene		Methyl Naphthalenes		C_{12} Naphthalenes		Sulfur compounds	
	Known	Method	Known	Method	Known	Method	Known	Method	Known	Method	Known	Method
1[a]	0.0	0.0	44.7	43.3	12.7	13.6	30.4	30.3	12.3	12.4	0.0	0.2
2[a]	0.0	0.0	28.3	27.9	6.3	6.6	47.6	47.2	17.8	17.9	0.0	0.0
3[a]	0.0	0.0	33.6	32.7	1.3	1.5	48.6	48.4	16.6	17.0	0.0	0.0
4[b]	0.0	0.2	25.4	24.3	39.1	38.2	32.0	32.1	3.6	5.0	0.0	0.0
5[b]	0.0	0.1	8.1	8.0	49.1	49.2	33.0	32.9	9.6	9.6	0.0	0.0
6[a,b]	0.0	0.3	37.2	35.6	21.8	23.0	31.2	31.3	9.7	9.7	0.0	0.0
7[b]	0.0	0.2	23.4	22.1	39.8	41.1	31.2	30.6	5.5	5.8	0.0	0.0
8[b]	0.0	0.2	9.7	9.6	50.3	50.2	30.2	29.9	9.8	10.0	0.0	0.0

[a] Monoaromatics isolated from heavy reformate.
[b] Monoaromatics isolated from hydrodealkylated reformate.

Table V. Synthetic Blends of Chromatographic Fractions from a Stove Oil, Kerosine Extract, and Heavy Reformate

(Weight per cent)

	Saturates		Monoaromatics		Diaromatic		Sulfur compounds	
	Known	Method	Known	Method	Known	Method	Known	Method
Stove oil								
1	28.0	28.8	39.0	35.9	25.0	27.7	8.0	7.7
2	66.0	63.6	22.0	21.7	9.0	11.7	2.9	3.1
3	47.0	45.7	23.0	23.1	23.0	24.5	7.2	6.6
Heavy reformate								
1	0.0	0.6	52.0	50.3	47.0	48.2	0.0	0.6
2	0.0	0.5	32.0	31.2	68.0	68.2	0.0	0.0
Kerosine								
1	98.5	98.4	1.5	1.4	0.0	0.1	0.0	0.0
2	9.7	11.6	81.2	73.4	7.5	13.0	1.5	2.1
3	47.5	47.1	28.0	26.7	20.6	22.6	3.9	3.9

Table VI. Comparative Analysis by Nonroutine Characterization and the Present Mass Spectrometer Method

(Weight per cent)

	Saturates		Monoaromatic		Diaromatic		Sulfur compounds	
	Chromo	MS	Chromo	MS	Chromo	MS	Chromo	MS
Jet fuel								
1	94.3	96.4	5.6	3.7	0.1	0.1	0.0	0.2
2	94.8	92.7	5.1	5.1	0.1	1.9	0.0	0.5
Kerosine extract	23.6	21.3	57.5	56.5	15.8	18.9	3.0	3.6
Stove oil	77.5	76.5	12.8	14.1	7.1	7.6	2.3	2.1
Heavy reformate								
1	0.2	0.6	25.1	26.5	74.5	72.8	0.0	0.0
2	0.3	0.7	43.2	42.8	56.2	56.6	0.0	0.2
3	0.0	0 9	89.9	92.4	11.1	6.8	0.0	0.0

Table III summarizes the results from the analysis of blends of monoaromatics (separated from reformate fractions) plus pure naphthalenes in the C_{10} to C_{12} range. For blends 1 to 3, where the C_{12} naphthalenes are simulated by various pure dimethylnaphthalenes, the accuracy for each compound type reported is about ±1% absolute. Blends 4 and 5, where ethylnaphthalene simulates the C_{12} naphthalene, show a somewhat greater average error. It is currently believed that ethylnaphthalenes are a minor constituent in reformate samples, so that this discrepancy in blends 4 and 5 is of little importance.

In Table IV, a comparison similar to that of Table III is offered. Monoaromatic fractions were isolated from both heavy reformate and hydrodealkylated samples, a C_{12}^+ naphthalenes fraction was isolated from a heavy reformate sample, and synthetic blends were made up from these fractions and the pure C_{10} and C_{11} alkyl naphthalenes. Again an accuracy of about ±1% is indicated for each component reported. In Table V the analysis of two reformate samples synthesized by blending chromatographically isolated monoaromatics and diaromatics is shown, while in Table VI three reformate samples whose composition had been determined by nonroutine means are shown. For brevity and clarity in presentation, no comparisons are shown in these tables for a breakdown of the various monoaromatics subtypes, but comparisons with low voltage mass analysis indicate comparable accuracy in these compound types as well. Similarly, in the following discussion of the analysis of other sample types no mention is made of the breakdown of saturate subtypes, although the accuracy of the determination of these compound types was also independently established.

Other Nonolefinic Sample Types. Further tests were also made of the accuracy of the present method in the case of such sample types as stove oils, jet fuels, kerosines, and kerosine ex-

Table VII. Analyses of Olefinic Samples

(Weight per cent)

Sample	Saturates Routine	Saturates Known	Olefins	Monoaromatics Routine	Monoaromatics Known	Diaromatics Routine	Diaromatics Known	Sulfur compounds Routine	Sulfur compounds Known	Nitrogen compounds[a]
360–460° F. FCC gasoline–gas oil fraction	22.5	26.7	14.7	42.9	34.9	15.8	17.5	3.3	5.0	1.2
Blend of above	18.5	16.4	9.1	39.1	35.5	26.1	28.8	5.4	8.2	2.0
Blend of above	39.2	40.9	22.4	26.0	25.3	8.5	8.3	2.3	2.4	0.0
460–510° F. FCC gas-oil fraction	25.8	25.4	10.7	29.1	33.5	25.9	21.5	5.8	7.0	1.9

[a] Determined independently.

tracts. These samples consist predominantly of straight-run and hydrotreated streams, and have low olefin contents. Several samples of these types were independently analyzed by nonroutine techniques, and blends of some of the chromatographic fractions incidental to these analyses were made up to simulate new samples. Comparative data for the analysis of these samples and blends by the present method are summarized in Tables V and VI. With one exception, kerosine blend 2, in Table V, all of these samples are analyzed with an accuracy exceeding ±3% absolute. In the case of this sample it is possible that a blending error was made; insufficient amounts of the chromatographic fraction blending samples remained to check this possibility. Such an event appears likely from the good agreement in the analysis of kerosine blends 1 and 3 in Table V, made up from the same blending fractions.

Cracked Samples in 350° to 550°F. Boiling Range. The final application of the present method was to cracked materials, which contain appreciable amounts of olefins. Table VII summarizes comparisons of the routine analysis on blends and samples of predetermined composition. With the exception of the first sample, an accuracy of about ±4% absolute is indicated. The first sample was light, and this resulted in a poorer than average material recovery from the chromatographic separation used to characterize the sample independently of the routine method. Cracked samples of this type show a very pronounced variation of composition with boiling point (*12*), and it is believed that this resulted in an erroneous nonroutine analysis. In verification of this assumption, blends of the fractions recovered from this sample give much higher accuracy (Table VII). This example also emphasizes our previous remarks on the greater lack of reproducibility of nondirect compositional analysis methods.

All fragment-type mass spectrometric methods are limited in accuracy by the variation of mass spectral cracking patterns with changing molecular weight, and the present method is no exception. Because the boiling range of the samples nominally within the scope of the present method is relatively narrow (200° F.), this problem is less serious than in other reported methods where an effort has been made to analyze samples covering a much wider boiling range. Optimum accuracy in the present method requires that the sample fall completely within the specified boiling point limits (350° to 550° F.).

Table VIII. Analyses of Several Representative Sample Types by Present Methods

Component	Weight per cent: Gasoline bottoms fraction	FCC 460° to 510° F. fraction	Finished kerosine	Kerosine extract	Stove oil
Total saturates	2.3	36.1	92.7	21.3	76.5
Alkanes	2.2	12.9	36.8	5.4	24.3
Noncondensed naphthenes	0.0	12.5	31.6	7.8	28.8
Condensed naphthenes	0.1	10.7	24.3	8.1	23.4
Total aliphatic olefins	—[a]	10.7	—[a]	—[a]	—[a]
Total monoaromatics	44.2	29.7	5.1	56.5	14.1
Alkyl benzenes	31.8	7.6	2.1	22.8	6.2
Indanes + Tetralins	12.4	22.1	3.0	33.7	7.9
Total diaromatics	53.5	25.9	1.9	18.9	7.6
Alkyl naphthalenes	48.9	24.7	0.6	16.9	6.0
Acenaphthenes + biphenyls	2.5	1.0	0.8	2.0	1.6
Fluorenes	2.1	0.2	0.5	0.0	0.0
Total sulfur compounds	0.0	7.6	0.5	3.6	1.8
Thiophenes	[a]	2.7	[a]	2.1	0.3
Benzothiophenes	0.0	4.9	0.5	1.5	1.3
Alkyl sulfides	[a]	0.0	[a]	0.0	0.2
Total nitrogen compounds	—	—	—	—	—
Alkyl naphthalenes by C No.					
$C_{10}H_8$	7.0	1.9	—	1.7	—
$C_{11}H_{10}$	24.9	9.8	—	6.0	—
$C_{12}H_{12}$	11.9	12.3	—	6.1	—
$C_{13}H_{14}$	3.9	0.2	—	2.2	—
$C_{14}H_{16}$	0.8	0.1	—	0.6	—
$C_{15}H_{18}$	0.2	0.2	—	0.2	—

[a] Not determined.

Table VIII illustrates the application of the present routine method to several samples falling within its scope, both to illustrate the detail provided by the method and to contrast the differences in composition found in stocks falling in the present boiling range. Total analysis time for the present method is less than 2 man-hours.

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Ion Exchange Separation of Brominated Salicylanilides and Hydroxybenzoic Acids

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▶ The application of nonaqueous ion exchange chromatography to hydroxybenzene derivatives has been extended to cover two new groups of compounds. Mixtures of brominated salicylanilides or of benzoic acid and hydroxybenzoic acid isomers are quantitatively adsorbed on Dowex 2-X8, ion exchange resin, acetate form. Gradient elution with acetic acid–methanol mixtures selectively desorbs the compounds. Concentrations are determined by ultraviolet spectrophotometry. Salicylanilide, 4′-bromosalicylanilide, 5-bromosalicylanilide, and 4′,5-dibromosalicylanilide are separated by this method. The 3,5-dibromo- and 3,4′,5-tribromosalicylanilide are not resolved. Benzoic acid and the three isomers of hydroxybenzoic acid are also separated by the above procedure.

In recent years, brominated salicylanilides have become increasingly important because of their germicidal activity. When added to soaps, they reduce greatly the growth and metabolism of skin flora which give rise to the so-called body odor.

Brominated salicylanilides are usually prepared by the bromination of salicylanilide. The choice of conditions depends on the desired product. In most instances, the product is a mixture and marketed as such. It therefore was necessary to develop methods of analysis for such mixtures.

Infrared measurements are generally satisfactory for the determination of the major components in brominated salicylanilide mixtures. However, this technique lacks the necessary sensitivity for the determination of minor components. It is in this area that the ion exchange method has been found extremely useful.

Nonaqueous ion exchange chromatography has found increasing use in recent years (*9, 12, 13*). Thus compounds having even very slight water solubility may be easily separated. The use of continuous gradient elution (*1, 2*) is often helpful in the separation of compounds that are not resolved by stepwise elution. Techniques for the separation of chlorophenols (*14*) have been extended to the brominated salicylanilides and hydroxybenzoic acids. Chromatograms were monitored with an ultraviolet scanner having a 290-mμ filter.

The separation of the ortho, meta, and para isomers of hydroxybenzoic acid has been of interest for many years. Numerous papers (*3, 4, 6, 7, 11*) have been published on this separation by paper chromatography. Liquid partition chromatography (*5, 10*) with silicic acid as the adsorbent has also been used. The *o*- and *m*-hydroxybenzoic acids may be determined by their fluorescence spectra (*15*). The proposed ion exchange method separates benzoic acid and the three isomers of hydroxybenzoic acid.

EXPERIMENTAL

Apparatus and Reagents. The gradient elution equipment, resin preparation, ultraviolet monitoring apparatus, and techniques have been described (*14*).

Brominated Salicylanilides. These were prepared by condensing aniline or *p*-bromoaniline with the proper bromosalicylic acid so as to give an unambiguous product. Melting points were comparable with those reported (*8*). A 13- × 400-mm. chromatographic tube was used for some of the separations.

3,4′,5-Tribromosalicylanilide. For mixtures containing predominantly 3,-4′,5-tribromosalicylanilide, dissolve a 0.1-gram sample by warming in 50 ml. of methanol. Adsorb the mixture on a 13- × 150-mm. bed of Dowex 2-X8, ion exchange resin, acetate form, 200 to 400 mesh. Connect the column to the gradient elution equipment and proceed with a 0.2% acetic acid–methanol gradient elution. After salicylanilide and 4′-bromosalicylanilide are removed as shown in Figure 1, continue the gradient elution with 2% acetic acid–methanol solution. Do not change the solution in the mixing flask for this second elution. When the 5-bromosalicylanilide and the 4′,5-dibromosalicylanilide are completely eluted, terminate the gradient elution. Attach a reservoir to the column and remove the 3,5-dibromo- and/or 3,4′,5-tribromosalicylanilide by elution with 300 ml. of glacial acetic acid. Determine concentrations of the components in the eluates by ultraviolet spectrophotometry.

4′,5-Dibromosalicylanilide. When 4′,-5-dibromosalicylanilide is the major component, adsorb a 10-mg. sample on a 13- × 330-mm. resin bed. The minor components will be 5-bromosalicylanilide and 3,4′,5-tribromosalicylanilide with possible traces of salicylanilide and 3,5-dibromosalicylanilide. Proceed with a 4% gradient elution. Salicylanilide will emerge between 170 and 260 ml. of eluate; 5-bromosalicylanilide, between 500 and 750 ml.; and 4′,5-dibromosalicylanilide, between 750 and 1250 ml. Remove the remaining components as described above.

Hydroxybenzoic Acids. Adsorb a 0.05- to 0.1-gram sample of *m*-hydroxybenzoic acid on a 13- × 330-mm. bed of the ion exchange resin from a small amount of methanol. Proceed with a gradient elution of 15% acetic acid–methanol solution. After the benzoic, *p*-hydroxybenzoic, and *m*-hydroxybenzoic acids are removed as shown in Figure 2, terminate the gradient elution. Remove any salicylic acid remaining on the resin by elution with glacial acetic acid. The salicylic acid will emerge between 100 and 300 ml. of the glacial acetic acid eluate.

DISCUSSION

The brominated salicylanilides are separated on the basis of their acidity. The weakest acid is eluted from the ion exchange column first. Acidity of the brominated salicylanilides depends on the number and position of the substituted bromine atoms. Substitution on the salicylo ring contributes much more to the ionization of the hydrogen ion of the hydroxyl group than substitution on the prime ring. Therefore, although 4′,5-dibromosalicylanilide has two bromine atoms as compared with one for 5-bromosalicylanilide, the dibrominated compound is only slightly more acidic than its monobromo counterpart. Analogously, 4′-bromosalicylanilide is slightly more acidic than salicylanilide and 3,4′,5-tribromosalicylanilide than 3,5-dibromosalicylanilide. However, salicylanilide, 5-bromosalicylanilide, and 3,5-dibromosalicylanilide show a considerable difference in acidity as a group, as do the structures 4′-bromosalicylanilide, 4′,5-dibromosalicylanilide, and 3,4′,5-tribromosalicylanilide.

In the separation of any unknown mixture, the ion exchange separation

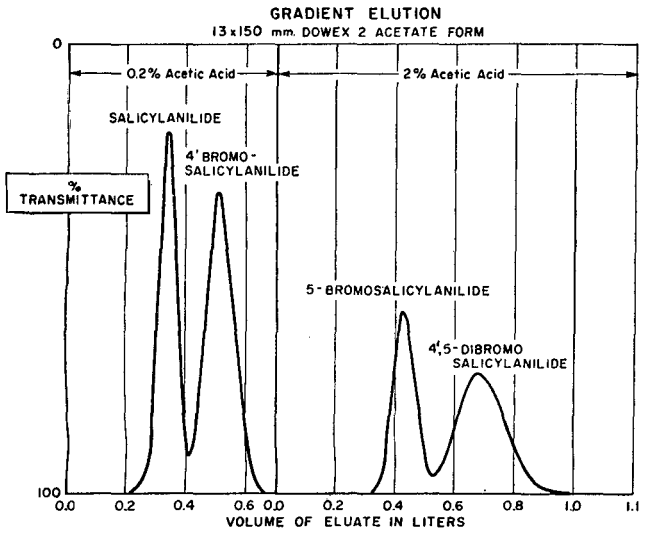

Figure 1. Separation of brominated salicylanilides on Dowex 2-X8 ion exchange resin, acetate form, using gradient elution

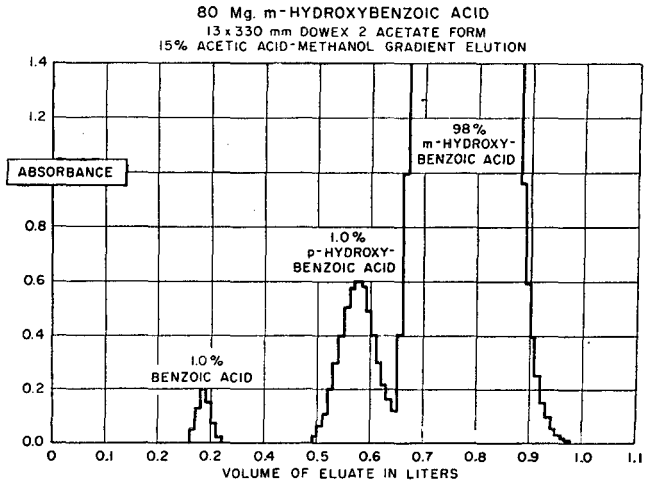

Figure 2. Separation of benzoic, *p*-hydroxybenzoic, and *m*-hydroxybenzoic acids on Dowex 2-X8 ion exchange resin, acetate form, using gradient elution

should be run, using conditions that give the best possible resolution. This will include the use of the continuous gradient elution procedure. However, once the composition of the mixture has been ascertained, the elution procedure can be modified to fit a certain sample composition as a control analysis.

Salicylanilide and 5-bromosalicylanilide can be separated from 3,5-dibromosalicylanilide and/or 3,4',5-tribromosalicylanilide by stepwise elution with fixed concentrations of acetic acid in methanol.

After adsorption of the mixture on a 13- × 150-mm. bed of the resin, the column is eluted with 100 ml. of 0.3% acetic acid–methanol solution. This is followed by 100 ml. of 3% acetic acid solution to remove salicylanilide and 200 ml. of 20% solution to remove 5-bromosalicylanilide. Highly brominated salicylanilides are removed with 300 ml. of glacial acetic acid. 4'-Bromosalicylanilide and 4',5-dibromosalicylanilide may be separated by the same procedure by substituting 0.5 and 5% acetic acid solutions for the 0.3 and 3% solutions, respectively. The salicylanilide–4'-bromosalicylanilide and 5-bromosalicylanilide–4',5-dibromosalicylanilide pairs, however, must be separated by the continuous gradient elution technique.

The adsorption and desorption of the brominated salicylanilides are quantitative, as illustrated in Table I. Mixtures 1 and 2 were separated by continuous gradient elution, whereas mixtures 3, 4, and 5 were separated by stepwise elution.

If there is an appreciable concentration of 5-bromosalicylanilide in samples that are predominantly 4',5-dibromosalicylanilide, the sample size should be reduced to prevent overlap of the chromatograms. However, some overlap can be tolerated, because the ultraviolet sensitivities (A per mg.) of the two compounds are almost identical.

The ultraviolet spectra for the brominated salicylanilides are characterized by two absorption maxima (Table II). The pairs of compounds having identical absorption maxima above 300 mμ can be easily confused even after separation into pure fractions. Besides their position of elution from the column, a further identification method consists of determining the ratio of the absorbances of the two maxima.

As shown in Figure 2, the isomers of hydroxybenzoic acid can be separated from each other and benzoic acid. Salicylic acid, although not shown on the chromatogram, is removed with glacial acetic acid after removal of the other acids. With the hydroxyl group in the ortho position, salicylic acid is a considerably stronger acid than its isomers and benzoic acid. The meta and para isomers are similar in their acidity, as shown in Table III. Thus

Table I. Analysis of Known Mixtures of Brominated Salicylanilides

Mixture	Salicylanilide, mg.	4'-Bromo-, mg.	5-Bromo-, mg.	4',5-Dibromo-, mg.	3,5-Dibromo-, mg.	3,4',5-Tribromo-, mg.
1						
Added	1.02	1.02	0.95	0.99		
Found	1.02	0.92	0.92	1.00		
2						
Added	1.02	1.02	0.95	0.99		
Found	0.98	0.91	0.91	0.87		
3						
Added		2.03		1.98		
Found		2.00		2.02		
4						
Added		2.03		2.49		96.9
Found		2.05		2.62		96.1
5						
Added	2.04		1.90		1.96	
Found	2.06		1.99		1.91	

Table II. Ultraviolet Absorption Data of Brominated Salicylanilide in 5% Acetic Acid–Methanol Solution at 25° C.

Compound	Absorption max., mμ	Molar absorptivity	Absorption max., mμ	Molar absorptivity
Salicylanilide	303	8,380	268	11,100
4'-Bromosalicylanilide	302	11,500	274	15,700
5-Bromosalicylanilide	316	7,200	272	10,000
4',5-Dibromosalicylanilide	316	9,030	276	14,000
3,5-Dibromosalicylanilide	328	8,080	277	8,830
3,4',5-Tribromosalicylanilide	328	9,350	280	12,200

Table III. pK_a for Benzoic and Hydroxybenzoic Acids

Acid	pK_a
Benzoic	4.20
p-Hydroxybenzoic	4.48
m-Hydroxybenzoic	4.08
o-Hydroxybenzoic	2.97

their separation is much more difficult. The separation of 0.8 mg. of *p*-hydroxybenzoic acid from 78.4 mg. *m*-hydroxybenzoic acid is a severe test for this separation. If more nearly equal quantities were ion exchanged, the separation would be much greater.

Although benzoic acid has a pK_a value between that of *p*-hydroxybenzoic and *m*-hydroxybenzoic, it emerges from the ion exchange column before either of these compounds. Other factors such as molecular configuration, adsorption, solubility, etc., besides pH probably have some effect on the separation. Other acids having pK_a values greater than 3 should be separated by this technique.

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Quantitative Analysis of Aspirin, Phenacetin, and Caffeine Mixtures by Nuclear Magnetic Resonance Spectrometry

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▷ A procedure is described utilizing NMR spectrometry in the rapid quantitative analysis of mixtures of aspirin, phenacetin, and caffeine. Several known mixtures have been analyzed as well as some commercial preparations. The average deviations from the correct results were: aspirin, 1.1%; phenacetin, 2.2%; and caffeine, 3.2%. The time required for the analysis is about 20 minutes.

CONSIDERABLE EFFORT has been devoted to the problem of developing suitable methods for the routine quantitative analysis of commercial analgesic preparations containing aspirin, phenacetin, and caffeine (APC). Procedures have been published utilizing separation by extraction, partition chromatography, and ultraviolet, visible, and infrared spectrophotometry. Conners cites a number of references on this subject (*1*). The method of the National Formulary (*2*) is of the extraction type but is not suitable for routine quality control work because of the time required to complete the analysis. Of the other procedures the infrared method of Parke *et al.* (*3*) seems to be most advantageous in terms of accuracy and speed.

Nuclear magnetic resonance spectrometry (NMR) can also be used very conveniently to analyze APC mixtures. The speed and accuracy of the NMR method to be described is about the same as that of other spectrometric methods, but it has the advantages of being more direct in the sense that a separate analytical peak of known origin is present for each component and that no calibration curves are required since the absorptivities for the protons giving rise to the various analytical peaks are constant and unaffected by solvent or solute interactions. In common with other spectrometric methods, no separation of the components is required for NMR analysis.

The basic principle which permits the use of magnetic resonance absorption as a quantitative measure of a particular substance is that the signal strength is proportional to the number of magnetic nuclei. The dependence of the signal intensity on the relaxation times T_1 and T_2, the intensity of the driving radio frequency field, and the field sweep rate have been discussed in the literature (*5*, *6*). It should be remembered that the signal strength is also inversely proportional to the absolute temperature. In the present work the fact that the instrumental conditions chosen for the analyses are such that the integrated intensities of the peaks employed are proportional to the number of protons present was demonstrated by the accuracy with which the composition of known standard mixtures could be determined.

EXPERIMENTAL

Apparatus. Spectra were obtained at 60 mc. per second using the Varian A-60 analytical NMR spectrometer, and in one case at 100 mc. per second using the Varian HR-100 NMR spectrometer.

Procedure. Weigh accurately into an A-60 NMR sample tube about 60 mg. of the carefully powdered sample. Using a micropipet, introduce exactly 0.500 ml. of $CDCl_3$ into the tube. It is important to avoid exceeding the solubility of aspirin which is least soluble of the three components. Cap the sample tube and shake, and gently warm the sample to effect complete solution of the APC. Binder materials such as starch and lactose will not dissolve but they do not interfere with the analysis and can be left in the sample tube. The sample is placed in the A-60 spectrometer and the NMR spectrum is obtained, the instrument gain, *RF* field, and sweep rate being adjusted to give a convenient integral presentation. The integral of each peak of interest should be run several times and the average

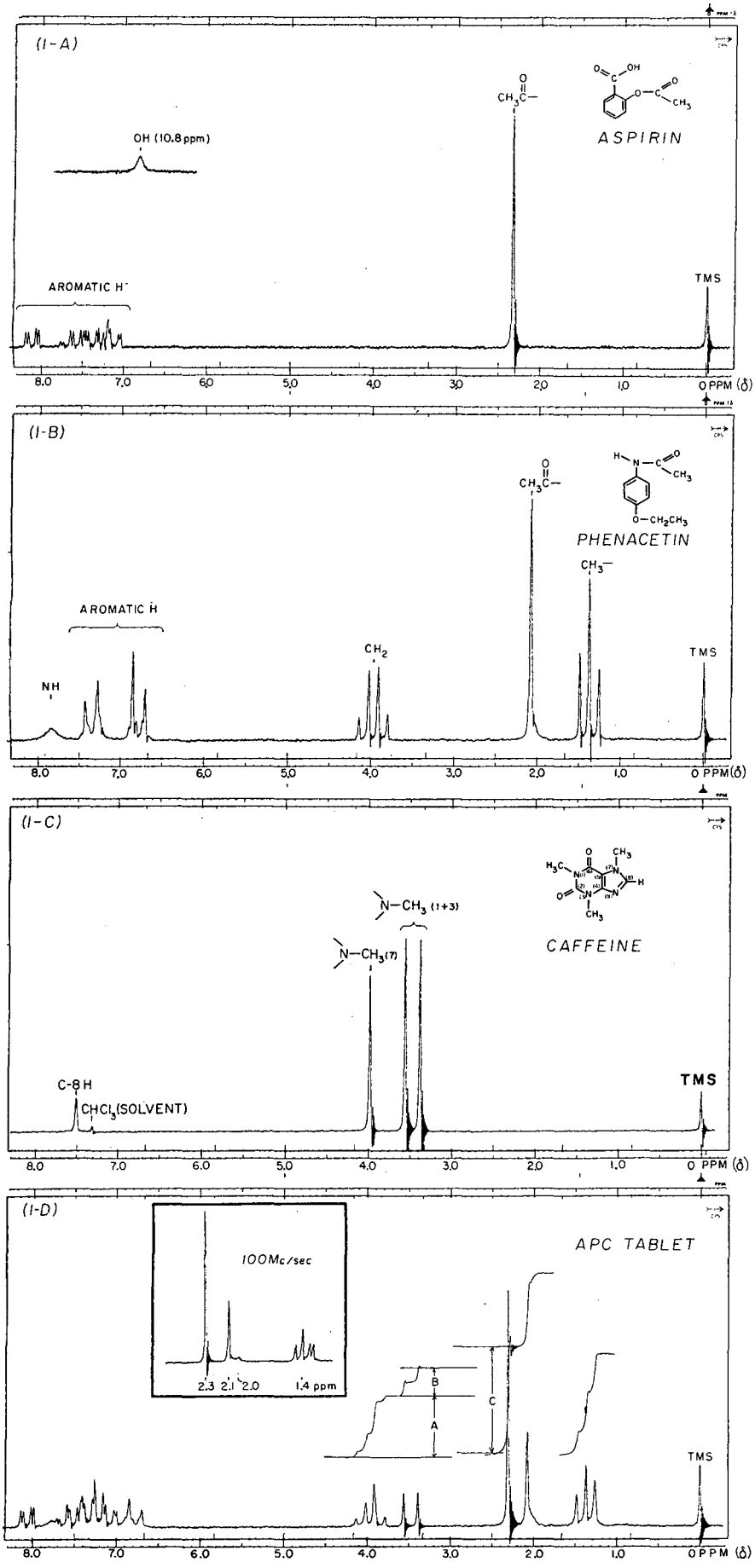

Figure 1. NMR spectra of aspirin, phenacetin, caffeine, and mixture

A. Aspirin
B. Phenacetin
C. Caffeine
D. APC mixture

value taken. To obtain an absolute integral calibration for a particular set of instrument settings, a standard sample containing a known concentration of one of the components, or for that matter a known concentration of any proton-containing compound, is substituted for the sample to be analyzed and its integral is taken. In this work a sample containing 50 mg. of caffeine per ml. of solution served as a standard.

RESULTS AND DISCUSSION

The proton NMR spectra of aspirin, phenacetin, and caffeine in deuterochloroform solution are shown in Figures 1*A*, 1*B*, and 1*C*, respectively. The solvent contains about 1% of tetramethylsilane which produces the reference signal at zero on the parts per million or "δ" scale shown on the charts. Chemical shifts in δ units can be read directly from this scale. The interpretations of these spectra are simple and are indicated on the charts. Figure 1*D* shows the spectrum of 60 mc. of a commercial APC tablet dissolved in 0.500 ml. of $CDCl_3$. The latter spectrum also illustrates the form in which the integral data are obtained. The height of the integral step above each peak is proportional to the area under that peak.

For aspirin the sharp peak at about 2.3 p.p.m. which represents the ester methyl group was chosen as the analytical peak. For phenacetin a choice of characteristic peaks is available. The quartet at about 4.0 p.p.m. and the triplet at about 1.3 p.p.m. in Figure 1*B* represent the CH_2 and CH_3 groups, respectively, of the O-ethyl group while the intense peak at about 2.1 p.p.m. is due to the amide methyl group. The latter peak is actually made up of two peaks as can best be seen in Figure 1*D* where a broad shoulder appears on the up-field side of the peak in question. This peak is seen even more clearly in the 100 mc. per second spectrum shown as an insert in Figure 1*D*. The origin of this peak is not known by the author but it is probably due to the existence of rotational isomers about the —C—N— bond since the position of this peak is temperature dependent and its intensity needs to be included if the $—CH_3$ integral in pure phenacetin is to be consistent as representing three protons relative to the remaining peaks. The triplet at 1.3 p.p.m. was rejected as an analytical peak because in several commercial APC preparations an unknown impurity gives a peak overlapping the highest field component of the triplet as can be seen by comparing Figure 1*B* with 1*D*. This peak is also shown clearly in the 100-mc. spectrum. The quartet at about 4.0 p.p.m. is suitable as an analytical peak for phenacetin although one of the methyl peaks of caffeine overlaps the

Table I. NMR Analysis of APC Mixtures

Sample description	Mg. added/ml. or mg./tablet (LABEL)			Found by NMR			Absolute error, %		
	Aspirin	Phenacetin	Caffeine	Aspirin	Phenacetin	Caffeine	Aspirin	Phenacetin	Caffeine
Aspirin ($CHCl_3$)	50.0	...	...	50.0	...	...	0	...	...
Caffeine ($CHCl_3$)	...	...	8.0	...	...	8.2	0	0	2.5
Phenacetin ($CHCl_3$)	...	36.0	...	...	35.7	...	...	0.8	...
APC mixture ($CHCl_3$)	50.0	36.0	8.0	50.0	35.4	8.2	0	1.7	2.5
APC mixture ($CHCl_3$)	40.6	32.6	7.3	40.5	33.8	7.5	0.25	3.7	2.7
APC mixture ($CDCl_3$)	29.8	22.0	8.6	29.2	21.4	8.4	2.0	2.7	2.3
APC mixture ($CDCl_3$)	41.6	37.6	9.0	41.1	36.6	8.7	1.2	2.7	3.3
APC mixture ($CDCl_3$)	40.2	41.6	6.0	39.0	40.0	6.3	3.0	3.8	4.8
APC mixture ($CDCl_3$)	29.7	30.6	5.4	29.8	30.6	5.1	0.41	0	5.5
APC mixture ($CDCl_3$)	26.2	28.8	19.0	25.8	28.4	18.6	1.5	1.3	2.1
APC tablet A ($CHCl_3$)	226	162	32.4	217	165	30.6	...	...	...
APC tablet B ($CHCl_3$)	226	162	32.4	219	174	31.6	...	...	...
APC tablet A ($CDCl_3$)	226	162	32.4	221	164	31.1	...	...	...
APC tablet B ($CDCl_3$)	226	162	32.4	220	171	31.4	...	...	...

quartet at 3.9 p.p.m. Correction for the latter peak can be made easily by subtracting the intensity of one of the CH_3 peaks at 3.4 and 3.6 p.p.m. which are due to the remaining two methyl groups of caffeine. For caffeine these two methyl resonances are suitable analytical peaks.

The number of milligrams of each component per milligram of sample can be calculated as follows:

$$\text{Aspirin: } \frac{\text{mg. aspirin}}{\text{mg. sample}} = \left[\frac{C}{I\text{caf}}\right] \times \left[\frac{MW\text{asp}}{MW\text{caf}}\right] \times \left[\frac{\text{mg. caf}}{\text{mg. sol.}}\right]_{STD} \times \left[\frac{0.500 \text{ ml.}}{\text{mg. sample}}\right] \quad (1)$$

$$\text{Phenacetin: } \left[\frac{\text{mg. phenacetin}}{\text{mg. sample}}\right] = \frac{A - 1/2(B - .0055C)}{I\text{caf}} \times \frac{3}{2} \times \frac{MW\text{phen}}{MW\text{caf}} \times \left[\frac{\text{mg. caf}}{\text{ml. sol.}}\right]_{STD} \times \left[\frac{0.500 \text{ ml.}}{\text{mg. sample}}\right] \quad (2)$$

$$\text{Caffeine: } \frac{\text{mg. caffeine}}{\text{mg. sample}} = \frac{1/2\,(B - .0055C)}{I\text{caf}} \times \left[\frac{\text{mg. caf}}{\text{ml. sol.}}\right]_{STD} \times \left[\frac{0.500 \text{ ml.}}{\text{mg. sample}}\right] \quad (3)$$

where A, B and C are the integral values as shown in Figure 1*D* and may be measured in arbitrary units using a ruler, *I*caf is the integral of one CH_3 group of the caffeine standard sample, *MW*asp is the molecular weight of aspirin, etc. The term 0.0055C represents a correction for the Carbon-13 sideband from the aspirin peak which falls beneath the caffeine CH_3 peak at 3.4 p.p.m. Carbon-13 has a natural abundance of 1.11% and a nuclear spin of $^1/_2$. The 1.11% of the protons which are bonded to C^{13} will therefore have their NMR signals split into a doublet by spin-spin coupling. The magnitude of this coupling is a function of the type of hybridization in which the carbon orbitals are involved (*4*). For saturated carbon atoms there will be satellite signals at $\pm\sim 60$ c.p.s. from the main C^{12}-H peak, each amounting to 0.55% of the main band. Therefore in the NMR spectrum of the APC mixture (Figure 1*D*) the low field C-13 satellite of the aspirin peak is included in the area of the caffeine peak at 3.4 p.p.m. and necessitates the correction mentioned above. The high field satellite from the caffeine peak, of course, is included in the aspirin peak but no correction for this is necessary in this case because the amount of caffeine present is small compared to the amount of aspirin.

Ordinary chloroform may be substituted for deuterochloroform in this analysis and good results can still be obtained. This solvent is less convenient however because it contains a small amount of ethyl alcohol as a stabilizer. If $CHCl_3$ is used, a solvent blank must be run under the conditions to be used for the sample and an appropriate correction made for the contribution of the $-CH_2-$ resonance of the alcohol to the integral in the 4.0 p.p.m. region, which overlaps with the phenacetin CH_2 resonance and two of the caffeine methyl peaks. It may be possible to remove ethanol from $CHCl_3$ by some simple procedure making it a more attractive solvent for this analysis, but this was not attempted in the present work.

Table I presents typical results obtained on several solutions of various known concentrations of APC both alone and in synthetic mixtures. Also included are results obtained on two commercial APC tableted preparations chosen at random from a drug store shelf, along with the quantities of each component contained as given on the labels. The solvent used is indicated in the column headed "Sample Description." Each result given was obtained from the analysis of a single sample, but the integral data used is the average of at least seven successive runs. The integrals were measured between points 5 mm. to the right and to the left of the point of maximum slope of the integral curve, and from a base line parallel to the chart edge.

The accuracy of the results depends on the quantity of each component contained in the sample. The mixtures of Table I cover the range of compositions usually found in commercial preparations. The average deviations from the correct result for all of the known mixtures shown are: aspirin, 1.1%; phenacetin, 2.2%; and caffeine, 3.2%. These results show that NMR can be used to analyze commercial APC preparations with an accuracy sufficient for quality control purposes and comparable to that obtained by other instrumental methods.

The time required for a single analysis is about 15 to 20 minutes including weighing and calculations and this could probably be shortened further if a number of samples were to be analyzed simultaneously.

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Spectrophotometric Determination of Vanadium in High Purity Aluminum

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▶ A method for determining trace quantities of vanadium in aluminum powder is presented. Vanadium as the vanadium-phosphotungstate is concentrated by extracting into *n*-hexanol and determined spectrophotometrically at 436 mμ. The vanadium is subsequently calculated from a prepared calibration curve. Ions, such as iron, molybdenum(VI), titanium, nickel(II), chromium(III), cobalt(II), and including aluminum salts in concentrations normally found in aluminum powder, do not interfere with the analysis. The procedure, although relatively fast requiring only 25 minutes to complete after the complex is formed, is dependent on the rate with which aluminum powder can be dissolved and the excess acid removed.

Of the numerous spectrophotometric methods available for the determination of vanadium, only a few are applicable for the low concentration of vanadium present in 99.9% pure aluminum powder. Of the applicable methods, none is suitable without first concentrating the vanadium by extraction from the aqueous solution.

Pearse describes a procedure utilizing 2,6-pyridine dicarboxylic acid which is selective for vanadium under specified conditions (*7*). It is not applicable for our work because the colored species has a low molar absorptivity, and is not readily extractable from the aqueous system into an organic solvent. Tributylphosphate is used as an extractant by Majumdar and De for the removal of vanadium(V) from solution (*4*). No data are given by them and the quantitative nature of the procedure is not discussed. Additionally, it is unsuitable because the vanadium must be reextracted into water before it is analyzed, and aluminum salts in high concentrations interfere with the tributylphosphate extractant. Benzoylphenylhydroxylamine is used by Ryan to complex vanadium (*8*). The vanadium–benzoylphenylhydroxylamine complex is extracted with chloroform. The reaction is quite selective and sensitive, but the complex stability is affected by sulfuric acid concentrations and large concentrations of aluminum salts.

Sodium tungstate and phosphoric acid are used by several workers to complex and determine small quantities of vanadium. Among these, Cooper and Winter (*1*) describe the separation and determination of vanadium in steels. Hopps and Berk (*3*) and Milner *et al.* (*5*) describe the determination of vanadium in fuel oil ash and petroleum oils, while an extensive study of the phosphotungstate method is discussed by Wright and Mellon (*9*).

We chose the phosphotungstate method for the work described here because it is sensitive at the low level of vanadium under examination, and only a minimum of interference is observed with the constituents, including aluminum salts, normally found in 99.9%. pure aluminum powder. Morrison and Freiser (*6*) discuss the extraction of hetero poly acids from aqueous solutions with oxygenated organic solvents. They describe the extraction of the phosphomolybdate but not the vanadium phosphotungstate complex. The oxygenated solvents they describe for molybdovanadophosphoric acid are not satisfactory for the extraction of the vanadium phosphotungstate complex system. A study of oxygenated organic extractants resulted in a rapid quantitative extraction of the desired complex with alcohols ranging from *n*-hexanol to dodecanol. In the work described here, the vanadium-phosphotungstate colored complex is extracted with *n*-hexanol and the vanadium determined at 436 mμ is calculated from a prepared calibration curve. The procedure, although rapid requiring only 25 minutes to complete after the complex is formed, is dependent on the rate with which aluminum powder can be dissolved and the excess acid removed.

EXPERIMENTAL

Reagents and Apparatus. *n*-Hexanol, purified by distillation. All other chemicals used were reagent grade.

Sodium Tungstate (0.5*M*). Dissolve 16.5 grams of sodium tungstate dihydrate in distilled water and dilute to 100 ml.

Standard Vanadium Solution. Vanadium pentoxide (0.0535 gram) is dissolved in 50 ml. of 5% sodium hydroxide. The solution is acidified with 10 ml. of 1:1 sulfuric acid then diluted to exactly 1 liter with distilled water. The vanadium concentration is determined by the sulfur dioxide and standard permanganate procedure (*2*).

Mixed Acid. Concentrated sulfuric, nitric, and hydrochloric acids, and distilled water are mixed in the volume ratio of 1:2:2:3, respectively.

All spectrophotometric measurements were made at 25° C. with either a Beckman DU or Beckman DB spectrophotometer using 1-cm. matched silica cells.

Recommended Procedure. Weigh an appropriate size sample of aluminum powder (2 to 5 grams, but not to exceed 0.4 mg. of V) into 600-ml. beakers. Dissolve by careful repeated addition of 0.5- to 1.0-ml. portions of mixed acid. (Violent reaction and frothing occur with larger repeated acid additions.) After the reaction subsides (85% dissolved), add more of the acid mixture to make a total volume of ca. 175 ml. Evaporate this mixture to strong fumes of sulfuric acid (ca. 50 ml.). Cool, add 15 ml. of 85% phosphoric acid, and dilute to 200 ml. with distilled water. Heat to boiling and add 1.5 ml. of 0.5*M* sodium tungstate solution. Add 4% sodium permanganate solution to the boiling mixture until it remains pink after boiling for 5 minutes. Then carefully discharge pink color with 1 to 2 drops of 1:4 HCl. Destroy excess HCl (if any) with 1 to 2 drops of the 4% sodium permanganate to just the first pink color. Cool the mixture, transfer it to a separatory funnel, and dilute it to 350 ml. with distilled water. Add 18 ml. of *n*-hexanol to the solution in the separatory funnel. Shake for 30 to 40 seconds, let stand 3 to 5 minutes, and remove the aqueous phase. Add 30 ml. of 2.5*M* H_2SO_4 to the *n*-hexanol extract, shake for 30 to 40 seconds, and let the phases separate. Remove the aqueous phase and transfer the *n*-hexanol extract to a 25-ml. volumetric flask. Rinse the separatory funnel twice with 2-ml. portions of *n*-hexanol, transferring each to the 25-ml. volumetric flask. Add 2 ml. of acetone to the 25-ml. volumetric flask and make to volume with more *n*-hexanol. Mix and let stand for 10 minutes. Read absorbance at 436 mμ *vs.* a reference prepared the same way, except with no aluminum powder.

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Table I. Results of Vanadium Found in 5-Gram Samples of Aluminum Powder

Sample	No. of replicate determinations	Vanadium, mg. Added	Vanadium, mg. Recovered	Vanadium, mg. Range	Recovery of added vanadium, %	Rel. std. dev., %
101-A	5	None	0.348	0.346–0.350		0.348 ± 0.59
120	6	None	0.056	0.055–0.057		0.056 ± 1.8
	2	0.061	0.115	0.114–0.116	96.7	
	2	0.305	0.355	0.353–0.358	98.0	
828	4	None	0.021	0.021–0.022		
	3	0.183	0.203	0.201–0.204	99.5	
815	4	None	0.090	0.089–0.092		
	2	0.183	0.272	0.272–0.272	99.5	
101-2D	4	None	0.077	0.075–0.080		
	2	0.061	0.139	0.139–0.139	101.6	
	3	0.305	0.383	0.381–0.384	100.3	
101-L	4	None	0.740	0.736–0.742		
113	4	None	0.440	0.437–0.444		
$(Al)_2(SO_4)_3 \cdot 18H_2O$	3	None	0.016	0.019–0.014		
$(Al)_2(SO_4)_3 \cdot 18H_2O$	2	0.092	0.111	0.110–0.111	103.3	
$(Al)_2(SO_4)_3 \cdot 18H_2O$	2	0.244	0.265	0.262–0.267	102.1	
Spectro Grade Al	3	None	0.004	0.004–0.004		
Spectro Grade Al	2	0.092	0.097	0.097–0.097	101.1	

Preparation of Calibration Curve. Pipet appropriate aliquots of standard vanadium solution into 600-ml. beakers. Add 175 ml. of mixed acid and evaporate to strong fumes of sulfuric acid. Then proceed as outlined in the recommended procedure above. A reference solution is similarly prepared without the standard vanadium solution.

DISCUSSION

Mixed acid is added in small increments to guard against frothing and violent reaction with the aluminum powder. The reaction from each acid increment is allowed to subside before more is added. After the sample is 20 to 30% dissolved, larger and more frequent increments of acid are added.

Evaporation of the solution to strong sulfuric acid fumes ensures removal of interfering chloride and nitrate anions present in the acid mixture. A minimum volume of 50 ml. is highly recommended to keep aluminum salts in solution. Also, the recommended volume assures the removal of all or nearly all of the interfering anions.

The colored vanadium–phosphotungstate complex has only a moderate molar absorptivity. Aluminum salts require a relatively large volume to remain in solution as well as present a certain amount of interference for which allowance must be made unless the colored complex is extracted. Since the vanadium concentration covered in this work is very low, it is desirable to extract the colored complex into an organic solvent.

Probably the most critical step in the entire analysis is the oxidation of all the vanadium to the quinquevalent state. Permanganate is used to oxidize the vanadium as well as any other ions which could subsequently reduce vanadium(V). At the same time, the permanganate concentration is critical because intermediate oxides of manganese form during the oxidation step, especially if interfering chloride anion removal is inadequate. The intermediate manganese oxides result in an abnormally colored solution and interfere by extracting with the colored vanadium–phosphotungstate complex. Excess permanganate and intermediate manganese oxides are destroyed by very careful addition of 1:4 hydrochloric acid. If excess 1:4 hydrochloric acid is added, the oxidation step is repeated.

Extraction of the colored vanadium–phosphotungstate complex into *n*-hexanol proceeds rapidly and smoothly. Only one extraction is required at the low concentration of vanadium studied. However, it is reasonable to assume that higher concentrations of vanadium would require multiple *n*-hexanol extractions. The extraction is not affected by a wide range of sulfuric acid concentrations, and the system obeys the Lambert-Bougert-Beer law in the concentration range of 0.02 to 0.6 mg. of vanadium in 25 ml. of solution. Minor quantities of the aqueous system are trapped by the *n*-hexanol during extraction. This results in a two-phase system in the final extract. Acetone is soluble in both water and *n*-hexanol and is added to the mixture to form a clear single phase. The vanadium is then determined by measuring the absorbance of the colored complex at 436 mμ and the vanadium calculated from a prepared calibration curve. Acetone does not interfere at the prescribed wavelength. Additionally, the colored complex in *n*-hexanol is stable for 72 hours.

RESULTS

Table I summarizes typical results as well as the precision obtained with various high purity aluminum powders. It also includes results on recovery of known quantities of vanadium added to these aluminum powders.

The recoveries of added vanadium are quite good in all cases, ranging from 96.7 to 103.3%. The relative standard deviation calculated on replicate determinations of different aluminum powder samples at the 0.061-mg. level of added vanadium is less than ±3.5% while at the high level of 0.305 mg. of added vanadium, the relative standard deviation is less than ±1.4%.

Table II summarizes the results obtained for the extraction of the vana-

Table II. Extraction Study of Vanadium–Phosphotungstate Complex

Extraction solvents	Comments
Amyl alcohol	Inconclusive results. A stable emulsion formed at the phase interface
Diethyl ether	No extraction of the colored complex
Amyl acetate	Partial extraction of the vanadium complex
Ethyl acetate	Partial extraction and mutual solubility of organic and aqueous phases. An emulsion formed at interface
Cyclohexanone	Partial extraction. The colored complex was unstable in extractant. An emulsion formed at the interface
Acetyl acetone	Extraction only 85–90% complete
Ethyl acetoacetate	Complete extraction. Mutual solubility of phases occurred causing unstable colored complex
n-Butanol	Complete extraction of the complex. Mutul solubility betweea phases occurred and the colored complex was very unstable
n-Hexanol	Complete extraction of complex. No discernible mutual solubility of the phases. The colored complex was stable for 72 hours in *n*-hexanol. The phases separated cleanly with a sharply defined interface in 3 to 5 minutes
n-Octanol	Complete extraction of complex. Similar to *n*-hexanol above except a very slight interface emulsion
n-Decanol	Complete extraction of complex. Similar to *n*-hexanol above except the interface emulsion was more pronounced than with *n*-octanol
n-Dodecanol	Complete extraction of complex. Several minutes required to allow interface emulsion to separate

dium-phosphotungstate complex into several oxygenated organic solvents. Extraction of the vanadium complex is quantitative at room temperature with the normal alcohols through dodecanol. With tetradecanol and heavier alcohols, extraction becomes difficult because the extraction mixture must be heated, emulsions difficult to break form at the interface, and the absorbance of the vanadium complex must be determined at elevated temperatures.

Concentrations of chromium(III), cobalt(II), iron(III), nickel(II), and molybdenum(VI) normally found in the high purity aluminum powder used for this work do not interfere in the analysis. Table III describes the results obtained by adding the various ions to a known concentration of vanadium prior to formation of the colored vanadium phosphotungstate complex. In concentrations 25 times that of vanadium, nickel(II) does not interfere while iron(III) interferes only slightly. Chromium(III) and cobalt(II) interfere markedly in 25-fold excess and in 1000-fold excess cobalt interference is quite large. Both chromium(III) and cobalt(II) interference are eliminated by cathodic reduction. Molybdenum (VI) interferes in 25-fold and greater excess. Cathodic reduction of molybdenum does not eliminate the interference and has little, if any, effect on it. Molybdenum(VI) interference is eliminated by extraction of the chloride with diethyl ether. Ordinarily a minimum of five extractions with diethyl ether is required to remove the molybdenum(VI) interference.

Table III. Interference in the Vanadium Determination by Diverse Ions Added to 0.18 Mg. of Vanadium

Ion	Mg. added	Absorbance: Before cathodic reduction	Absorbance: After cathodic reduction	Mg. vanadium after reduction
Ni(II)	5.0	0.199	0.196	0.185
Cr(III)	5.0	0.260	0.206	0.194
Fe(III)	5.0	0.182	0.196	0.185
	5.0	0.183	0.192	0.180
Co(II)	5.0	0.244	0.199	0.189
	200.0	0.750	0.209	0.201
Mo(VI)[a]	5.0	0.312	0.322[b]	0.312
	500.0	0.520	0.570[b]	0.553

[a] Cathodic reduction not applicable to removal of interference from molybdenum(VI).
[b] Solutions hazy and absorbance not representative.

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Fluorometric Determination of Selenium in Plants and Animals with 3,3′-Diaminobenzidine

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▶ **Two methods of oxidizing samples (Parr bomb and Schöniger flask) for the fluorometric determination of selenium in plants and animals have been developed and/or improved. Intensive and detailed studies have been made on the variables affecting the results. These methods are superior to previous methods in that only a small sample is required; extraneous fluorescence is more efficiently removed; the time of analysis is materially decreased. The combustion techniques, measured by 13 determinations with each technique on the same ground sample of alfalfa, gave for the Parr bomb an average of 1.14 p.p.m. of Se with a standard deviation of ±0.019; for the Schöniger flask, an average of 1.17 p.p.m. of Se with a standard deviation of ±0.012. Results obtained by the fluorometric 3,3′-diaminobenzidine (DAB) method agree reasonably well with neutron activation determinations. The fluorometric DAB method has been tested on alfalfa, grass, linseed meal, wheat, corn, oats, lettuce, and pear, apple, and radish leaves; and on blood, muscle, liver, brain, and milk.**

FOR many years the interest of research workers has been directed toward the investigation of selenium (Se) toxicity in farm animals. Forage containing Se in excess of 5 p.p.m. is toxic to cattle.

Recently investigators (*3–5, 8, 12*) have shown that a Se deficiency may exist in cattle and sheep; muscular dystrophy (white muscle disease) may occur if the feed ingested is low in Se. Furthermore, sheep are unthrifty on low Se diets even if white muscle disease does not occur (*8*).

Conventionally the analyst may use the AOAC (*1*) method of determination in plant material exceeding 2 p.p.m. of Se. The ground plant sample is mixed with H_2SO_4 and HNO_3 in a beaker and after digestion the digest is transferred to a distillation flask. The digest is distilled with HBr and Br_2 and the distillate collected in a cooled Erlenmeyer flask. SO_2 is passed into the flask to remove excess Br_2, and hydroxylamine added to reduce selenious acid to elemental Se. The precipitated Se is measured either gravimetrically or colorimetrically.

There are serious objections to this method, particularly when plant or animal samples are analyzed for Se. The method requires 5 grams of plant sample; it has a relatively low sensitivity, since it is applicable only to

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Table I. Abridged Comparison of Cousins' (3) and Watkinson's (17) Methods for Se in Biological Material

Step	I (Cousins)	II (Watkinson)
1	Drying sample Plant tissue, yes Animal tissues, usually not	Dry at 30°–40° C. (plant tissue)
2	Grinding (for plants)	Plant tissue, finely ground
3	Weighing, 1 to 10 grams of animal tissue	Amount of material to give not less than 0.02 μg. Se
4	Oxidation, HNO_3 + $HClO_4$ digestion	Same
5	Isolation of Se a. Reduction to Se(IV) b. Coprecipitation of Se with As c. Filtration d. Digestion (HNO_3 + $HClO_4$)	Isolation of Se Reduction to Se(IV) (HCl) Complexation of Se (zinc dithiol) Extraction ($C_2H_2Cl_2$ + CCl_4) Digestion (HNO_3 + $HClO_4$)
6	Complexation (HCOOH + EDTA)	Same
7	Reaction with 3,3′-diaminobenzidine (DAB)	Same
8	Extraction (toluene)	Same
9	Measure fluorescence	Same

Table II. Fluorometric Determination of Se in Plant Material by Method III

Step	Parr Bomb	Schöniger Flask
1	Grind air-dried plant sample (80-mesh screen)	Same
2	Pellet and weight accurately 0.10- to 0.20-gram sample into fused silica ignition capsule	Weigh accurately <0.12 gram of sample into Schöniger ignition paper
3	Attach platinum fuse wire and nylon yarn (16). Add 10 to 15 ml. H_2O as absorbing liquid and assemble bomb. Use 30 atm. of oxygen and ignite as per instructions (16). Cool bomb in tap water for 10 minutes. Release excess oxygen over a period of 2 minutes	Add 25 ml. H_2O to 2-liter Schöniger flask and ignite sample in usual manner. Let flask sit for 10 minutes
4	Wash contents of bomb into 150-ml. beaker, using small portions of H_2O from wash bottle	Wash contents from flask into 150-ml. beaker using small portions of H_2O from wash bottle
5	Filter solution through S & S white ribbon paper into 250-ml. beaker	Same
6	Make filtrate (about 30 to 35 ml.) approximately 4*N* by adding 10 ml. of concd. HCl	Same
7	Boil for 10 minutes to reduce all Se to Se(IV) state	Same
8	Add 1 ml. of concd. HCOOH	Same
9	Add 5 ml. of 0.1*M* EDTA (disodium salt)	Same
10	Add 1 ml. of 0.5% 3,3′-diaminobenzidine hydrochloride solution (freshly made)	Same
11	Adjust pH 2.0-2.4 (NH_4OH)	Same
12	Set in subdued light 30 minutes	Same
13	Adjust pH with NH_4OH to 6.8–7.2. Make solution volume to 50 ml. with H_2O	Same
14	Extract (VirTis) with 5.00 ml. of toluene (or mesitylene for low concentrations of Se)	Same
15	Read fluorescence of Turner fluorometer using filters 2A + 47B for primary filter and 2-A-12 for secondary filter. Note sensitivity and range as well as dial reading. For extremely low concentrations of Se, use Turner high sensitivity kit	Same

materials containing > 2 p.p.m. of Se; it is very laborious and time consuming.

Hoste (9) and Hoste and Gillis (10) introduced 3,3′-diaminobenzidine (DAB) as a selective colorimetric reagent for selenium. The piazselenol or selenium–diaminobenzidine complex (Se-DAB) formed when Se(IV) reacts with 3,3′-diaminobenzidine or its hydrochloride has an intense yellow color. Se-DAB can be readily extracted from a slightly acid or neutral solution and measured colorimetrically. Cheng (2) and Parker and Harvey (15) have studied the chemistry of this reaction; Cheng reported the limit of sensitivity to be 0.05 p.p.m. of Se using a 1-cm. absorption cell. Handley and Johnson (7) have used the same reaction with plant material and report that 0.25 μg. of Se may probably be determined with confidence. Magin *et al.* (14) use this method for measuring Se in water.

Cousins has been credited with discovering that the piazselenol fluoresces. Cousins (3, 4) and Watkinson (17) have developed fluorometric methods (Methods I and II) for measuring low concentrations of Se in biological material. An abridged comparison of these methods is shown in Table I. The Se-DAB complex formed by the reaction of Se(IV) with 3,3′-diaminobenzidine or its hydrochloride, when activated with light at wavelengths of 420 to 430 mμ, fluoresces at wavelengths of 560 to 585 mμ; although the blank has a rather high fluorescence, the fluorometric method is more sensitive than the colorimetric method.

Methods I and II are still laborious and time-consuming and subject to extraneous fluorescence which some workers ascribe to lipides or lipoidal material in the sample. It also seems likely that in a plant tissue sample much of the extraneous fluorescence is due to unoxidized plant sample, or, rather, unoxidized chlorophyll and/or xanthophyll; both of these plant pigments are known to fluoresce strongly. This extraneous fluorescence following HNO_3 or HNO_3+$HClO_4$ treatment apparently cannot be removed by organic extraction; neither can it be removed by adsorption using the common adsorbents such as charcoal, Darco G-60, or diatomaceous earth. Cousins (3) avoids this difficulty by isolating Se by coprecipitation with As; Watkinson (17) separates Se from a plant digest by complexing the Se with zinc dithiol and extracting. The improvement of proposed Methods IIIa (Parr bomb) and IIIb (Schöniger flask) over the methods of Cousins (3, 4) and Watkinson (17) is due to two factors:

1. A small sample (0.10 to 0.20 gram) of ground plant tissue is oxidized in a 2-liter Schöniger flask or a Parr bomb. This is much more convenient

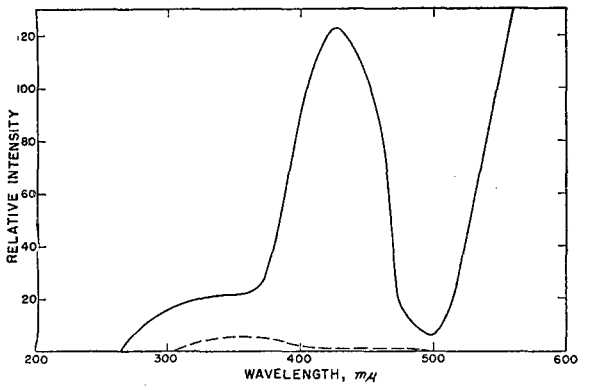

Figure 1. Activation spectra on Aminco-Bowman spectrophotofluorometer

——— Se(IV)-DAB fluorescence setting, 565 mμ
– – – Reagent blank fluorescence setting, 565 mμ

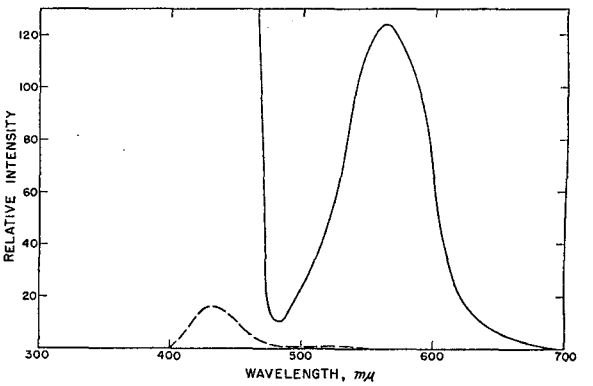

Figure 2. Fluorescence spectra on Aminco-Bowman spectrophotofluorometer

——— Se(IV)-DAB activation setting, 425 mμ
– – – Reagent blank activation setting, 425 mμ

and rapid than oxidizing or digesting a larger plant sample in acid. For very low concentrations of Se in plants (less than 0.10 p.p.m.), three methods are readily available to increase the magnitude of the fluorometer readings. If only an occasional low Se measurement is required, a second sample may be oxidized in the Parr bomb or Schöniger flask after cooling the previous sample; it is not necessary to wash out the bomb or flask contents between ignitions. If a series of low Se measurements is required, the Turner high sensitivity kit attachment can be used to increase the sensitivity of the fluorometer by a factor ranging from 8 to 10. Gutenmann and Lisk (*6*) have also used a 5-liter Schöniger flask (Thomas-Lisk style) with a larger sample for the determination of Se in plants. Figure 10 shows that the use of mesitylene instead of toluene as the extractant will also increase the sensitivity of the method.

2. The weight of unburned plant material particles of Methods IIIa and b ranges from 0.1 to 0.2 mg. per determination, which is an insignificant amount of the sample; these unburned particles are filtered off immediately and contribute no extraneous fluorescence from their chlorophyll and/or other plant pigment content. This simple method removes the extraneous fluorescence easily and effectively.

EXPERIMENTAL

Apparatus. Parr bomb. Fused silica capsules (Parr List No. 43A3) with platinum wire (26-gauge) and nylon yarn (*16*). Schöniger flask, Thomas-Lisk style, 2-liter capacity. Spectrophotofluorometer, Aminco-Bowman. Fluorometer, Turner Model 110, with and without high sensitivity kit. Fifteen-millimeter cuvettes. VirTis Extractomatic with 100-ml. tubes and Teflon stopcocks. pH meter, Beckman Zeromatic. Scintillation counting assembly, Nuclear-Chicago scaler (Model 181A) and pulse-height analyzer (Model 1810) or a Technical Associates Scaler (Model DS-58) and pulse-height analyzer (Model SA-20) utilizing a thallium-activated sodium iodide crystal. Homogenizer, VirTis or equal. Lyophilization apparatus: a desiccator connected to a Pirani gauge and a vacuum pump through a cold trap is adequate.

Reagents. All reagents were of analytical grade and included NH_4OH, HCl, HNO_3, toluene, mesitylene, formic acid (88 to 90%), EDTA (disodium salt), and 3,3'-diaminobenzidine hydrochloride (J. T. Baker). Water redistilled from glass was used in making up all solutions, since it contained less fluorescent impurities than distilled, deionized (mixed bed) water.

All glassware was washed with a nonfluorescing detergent solution and rinsed with distilled water, followed by rinsing in redistilled water and oven drying. Care was taken to keep fingerprints off cuvettes.

Standardization. A selenium(IV) standard stock solution was prepared by dissolving 0.500 gram of elemental Se (99.9%) in a few drops of HNO_3 and diluting with 4*N* HCl to 1 liter. This 500 p.p.m. Se(IV) standard stock solution was further diluted with 4*N* HCl to prepare standard solutions of 50, 5, 0.5, 0.05, and 0.005 p.p.m. of Se. From these dilute standard solutions, aliquots were taken to make standard curve *A* shown in Figures 3 and 4. Air (oxygen) will slowly oxidize Se(IV) to Se(VI) unless the solutions are boiled with 4*N* HCl or prepared with 4*N* HCl. Se(VI) reacts with DAB to produce a compound with greatly decreased fluorescence when activated at 420 to 430 mμ. The detailed steps for making the standard curves (Figures 3 and 4) are shown by steps 7 to 15 of Table II.

Figure 1 shows the activation curve obtained with the Aminco-Bowman spectrophotofluorometer for the DAB blank and Se(IV)-DAB complex. The activation peak of Se-DAB is at 425 mμ; Figure 2 shows the fluorescent curve taken with the same instrument. The fluorescent peak of the Se(IV)-DAB complex is at 565 mμ.

The standard curves shown in Figures 3 and 4 were made using the Turner fluorometer. The primary filters used were a sharp-cutoff 2A filter plus a narrow-pass 47B filter; the secondary filter was 2A-12 sharp-cutoff filter (designations are Wratten numbers). Figures 3 and 4 show standard working curve *A* to be linear up to at least 0.20 μg. of Se per 5 ml. of toluene. The standard working curve is adequate for sample weights of alfalfa ranging from 0.10 to 0.20 gram.

PROCEDURE

Plant Samples. Representative air-dried samples were first ground in a standard model Wiley mill (40-mesh screen) and a small subsample was taken and reground in an intermediate Wiley mill (80-mesh screen). Detailed steps using the Parr bomb (Method IIIa) and the Schöniger flask (Method IIIb) are shown in Table II. Safety precautions for both ignition procedures included: use of small samples in the bomb or flask; a balloon attached to Schöniger flask; all flask ignitions made behind a plastic safety shield. The fluorescence measurements for plant sample solutions and standard solutions were made in exactly the same manner.

Animal Samples. Blood. Parr Bomb. Adsorb 1 ml. of serum on a 11-cm. ashless filter paper. Lyophilize (or dry) the sample to dryness (approximately 4 hours). Cut paper into small squares (approximately 1/8 inch) and place in silica ignition capsule. Alternately the paper may be pelleted after cutting. Follow the procedure of IIIa beginning with step 3.

Schöniger Flask. Adsorb 1-ml. serum sample as for Parr bomb. Fold the filter paper into a square (approximately 1/2 inch). Insert Schöniger wick (made from Schöniger ignition paper) into folded sample. Follow procedure of Method IIIb beginning with step 3.

Milk. Same as serum.

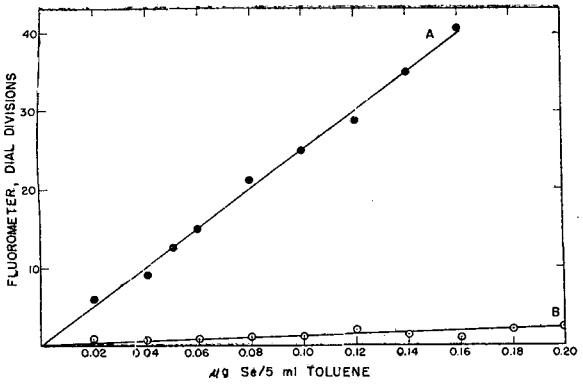

Figure 3. Effect of valence of Se on standard curves

A. Se(IV)
B. Se(VI)
Range 30×; sensitivity 3

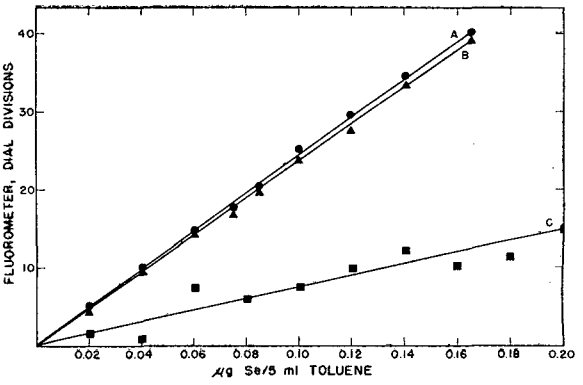

Figure 4. Effect of reduction methods on fluorescence of Se-DAB complex

A. Se(IV)
B. Se(VI) reduced with HCl
C. Se(VI) reduced with NH_2OH
Range 30×; sensitivity 3

Tissue. Homogenize at least 2 grams of sample. Spread sample in a thin layer on a planchet and lyophilize (12 to 20 hours). Powder the sample, using a small mortar and pestle. Weigh out 0.1 to 0.2 gram of pulverized sample into Schöniger ignition paper. Follow the procedure of Method IIIb, beginning with step 3.

STUDY OF VARIABLES AFFECTING FLUORESCENCE OF Se-DAB

An extended and detailed study was made of all variables which might reasonably affect the fluorescence of Se-DAB solutions. In general, standard solutions of Se were used; the reagents and their concentrations were the same as previous workers had found to be satisfactory. Figures 3 to 10 show the effects of these variables, and since the Turner fluorometer was used for this study the ordinate of each curve is expressed as the dial reading at a designated range for this instrument. The dial reading is proportional to the fluorescence of the Se(IV)-DAB toluene extract.

Valence. Selenium(IV)-DAB has a maximum activation peak at approximately 425 mμ (Figure 1), and a maximum fluorescence peak at approximately 565 mμ (Figure 2), which are suitable for fluorescence measurements. Se(VI)-DAB does not activate or fluoresce at these wavelengths. It is necessary to ensure that all the selenium is in the +4 valence state; otherwise the results of an analysis will be too low, as shown by standard curve *B* in Figure 3. If sufficient time is permitted to elapse, standard solutions of Se(IV) will slowly change to Se(VI) because of atmospheric oxygen. This is not surprising, since it can be shown thermodynamically using the data of Latimer (*13*) that:

$$O_2 + 2H_2SeO_3 = 2SeO_4^{-2} + 4H^+ \qquad E_o = +0.08 \text{ volt} \quad (1)$$

This reaction, although slow, proceeds as written in Equation 1. It should be possible to study the rate of this reaction by using a spectrophotofluorometer, or a fluorometer with the proper filters.

Reduction Methods. The selenium solution obtained in steps 6 and 7 should be at least 4*N* in HCl and boiled approximately 10 minutes. Figure 4 (curves *A* and *B*) indicated good agreement for the fluorescence results when two standard solutions, Se(IV) and Se(VI), are so treated. Hydroxylamine is too strong a reducing agent for this reaction; some of the Se(VI) is converted to elemental Se.

Varying pH before Development. Figure 5 shows the fluorescence results when the pH is varied immediately following the addition of DAB. A pH of 2.0 to 2.4 (step 11) is satisfactory, as was also noted by Cheng (*2*), who was studying the colorimetric measurement of Se-DAB.

Varying pH before Extraction. The fluorescence curve obtained in Figure 6 also corroborates fluorometrically the colorimetric results of Cheng (*2*). A pH of 7 is therefore used in step 13.

Varying Reaction Time before Extraction. The effect on fluorescence of varying reaction time of Se(IV) with DAB is shown in Figure 7. The solution was kept in subdued light at room temperature (23° to 24° C.). The curve shows that a time of 30 minutes is adequate for step 12.

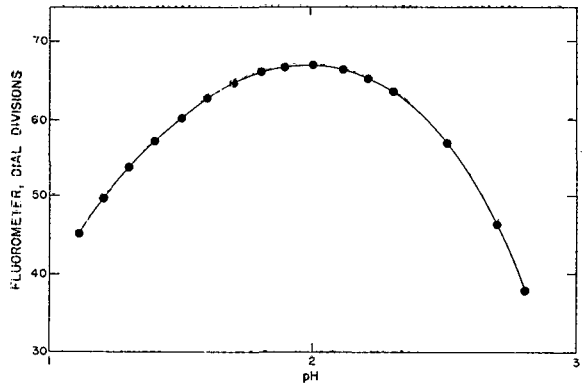

Figure 5. Effect of varying pH immediately following addition of DAB on fluorescence of Se-DAB complex

Range 30×; sensitivity 3

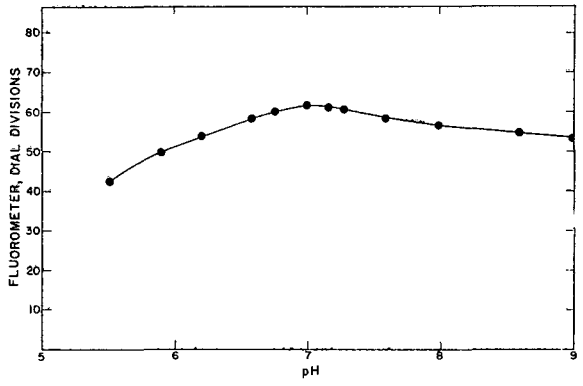

Figure 6. Effect of varying pH of aqueous phase before extraction, on fluorescence of Se-DAB complex

Range 30×; sensitivity 3

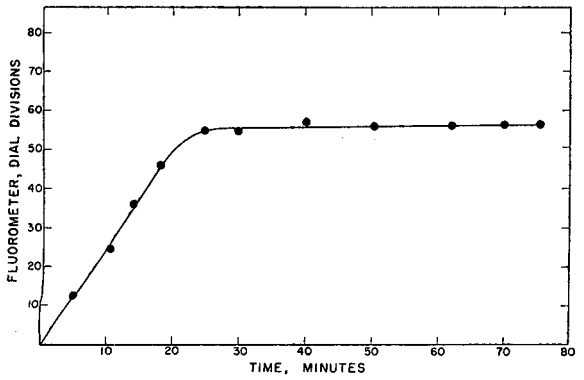

Figure 7. Effect of varying reaction time of Se(IV) with DAB (in subdued light), on fluorescence of Se-DAB complex

Range 30×; sensitivity 3

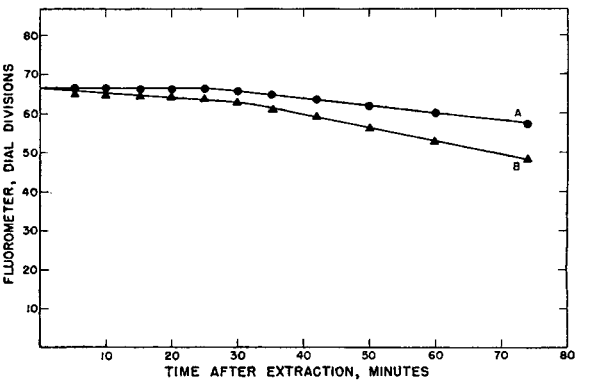

Figure 8. Effect of standing time on fluorescence, after extraction of Se-DAB complex

A. Subdued light
B. Sunlight
Range 30×; sensitivity 3

Standing Time in Subdued Light of Extracted Se-DAB. A toluene solution containing Se-DAB was divided into two parts: Solution A kept in subdued light (semidarkened room) remained more fluorescent than Solution B in sunlight, as indicated in Figure 8. These results indicate that: all steps following step 11 should be made in subdued light, or when possible, in darkness; fluorescence of Se-DAB in toluene begins to decrease after standing 30 to 35 minutes even in subdued light.

Temperature during Standing Time. Figure 9 illustrates the effect on fluorescence of varying the temperature of Se-DAB toluene extracts kept at constant temperatures in darkness. The fluorescence decreases with temperature; in this experiment, a rise of 1° C. reduced the measurement of Se by 0.0084 p.p.m. When measuring small amounts of Se, it is advisable to let the toluene extracts stand in an oven or a constant temperature room at the specified temperature.

Varying Extractants. Figure 10 shows the fluorescence results obtained with standard solutions using five different extractants. Mesitylene appears to be the most favorable; however, toluene is cheaper and more easily obtained; for most purposes a good grade toluene is satisfactory.

Inorganic Ions. The effect of diverse inorganic ions on fluorescence is shown in Table III. The samples of Te and its salts probably had Se as an impurity, judging from the erratic results.

If a large amount of iron is present in a sample, more EDTA should be used.

Table III. Effect of Diverse Inorganic Ions on Fluorescence of Se-Dab Complex

In organic ion	Amount added,[a] (μg.)	Increase of fluorometer dial readings[b]
Al(III)	1,000	0
As(V)	2,000	0
Ca(II)	30,000	0
Cl^-	1,000	0
Cu(II)	1,000	0
Ce(III)	1,000	0
Co(III)	100	0
Cr(II)	1,000	0
Fe(II)	1,000	0
Fe(III)	1,000	0
Fe(II)	10,000	78
K(I)	1,000	0
Mn(II)	4,000	0
Mo(IV)	1,600	0
Mo(IV)	1,000	0
Na(I)	1,000	0
Ni(II)	1,000	0
Pb(II)	1,000	0
Si(IV)	1,000	0
Sn(IV)	1,000	0
Te(IV)	2,000 (Te metal)	98
Te(IV)	2,000 ($TeCl_4$)	42
Te(IV)	2,000 (TeO_2, Baker)	26
Te(IV)	1,000 (TeO_2, Baker)	14
Te(IV)	100 (TeO_2, Baker)	0
V(II)	1,000	0
Y(III)	1,000	0
W(II)	2,000	0
Zn(II)	1,000	0
NO_3^-	1,000	0
PO_4^{-3}	1,000	0
SO_4^{-2}	1,000	0

[a] To 50-ml. aliquot of standard solution containing 0.05 μg. of selenium. This solution has a fluorometer dial reading of 12.5 divisions.

[b] Range 30×; sensitivity 3 on Turner fluorometer.

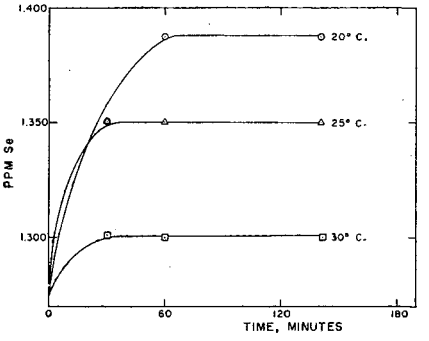

Figure 9. Effects of temperature and standing time (in darkness) on toluene extracts of Se-DAB

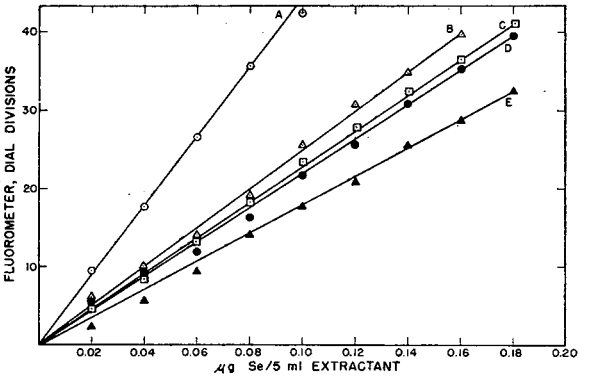

Figure 10. Effect of various extractants on standard Se-DAB fluorescence

A. Mesitylene
B. Toluene
C. o-Xylene
D. p-Xylene
E. Benzene

RECOVERY EXPERIMENTS

Radiotracer methods were used in checking the recoveries of a number of different steps in Method III (Tables IV, V, and VI).

Alfalfa was grown in the greenhouse using a modified Hoagland macronutrient solution with micronutrient concentrations as recommended by Johnson (*11*). Each 19-liter nutrient solution contained 100 μc. of Se^{75} (3000 μc. of Se^{75} per gram of Se). The alfalfa was harvested at $^1/_{10}$ to $^1/_4$ bloom stage. Grinding and other analytical steps were according to steps in Method III. Radiotracer measurements were made, preceding and following each step or experiment. Total recovery calculations are based on the assumption that total Se is recovered to the same per cent as Se^{75}.

Use of Various Absorption Media. Radiotracer recoveries were made on 0.10 to 0.12 gram of alfalfa containing Se^{75} after combustion in a 2-liter Schöniger flask using various liquid media. Table IV shows that the recovery from the flask is complete; water, 0.1*N* HCl, and 0.1*N* NaOH absorb equally well; and 25 ml. of liquid is sufficient. Water was chosen for the absorbent, because the absorbing solution after combustion is acid and the pH has to be raised.

The Parr bomb gave similar results, and required only 10 to 15 ml. of H_2O.

Extraction Results. A stock solution of Se(IV) containing some Se^{75} was made up and an aliquot taken for fluorometric analysis (steps 6 to 15, Method III). Radiotracer measurements were made before and after extraction with toluene. The results for 50 ml. of aqueous phase and 5 ml. of toluene indicated that a recovery of 74 $\pm$2% would be obtained with one extraction; there is no advantage (*14*) in attempting to increase the per cent extracted by increasing the number of extractions, because the volume of the organic phase will also be increased.

Table V shows the radiotracer recovery results for various organic extractants. It appears that many organic extractants are superior to toluene for extracting Se from aqueous Se-DAB solutions; unfortunately, Se-DAB does not fluoresce in many of these organic extractants. An exception is mesitylene (Figure 10).

Plant Samples. Radiotracer recoveries were run on alfalfa samples using Methods IIIa and IIIb (Table VI). Slightly better precision is obtained with the Schöniger flask; there is little to choose between the Parr bomb and the Schöniger flask with respect to the average value. The bomb will oxidize larger samples than a 2-liter flask, but the cost of the bomb is greater. More samples can be oxidized per day with two flasks than with one bomb.

Table IV. Radiochemical Recoveries of Se-75 Using Various Absorption Media

Oxidation	Absorbing media	Recovery, %
Schöniger	H_2O	100 ± 1[a]
	0.1*N* NaOH	100 ± 1[a]
	0.1*N* HCl	100 ± 1[a]
Parr bomb	H_2O	100 ± 1[a]
	0.1*N* NaOH	100 ± 1[a]
	0.1*N* HCl	100 ± 1[a]

[a] Relative standard deviation.

Table V. Recovery of Se-75 Using Various Organic Extractants[a]

Solvent	Selenium-75 recovery, %
Benzene	77.8 ± 3[b]
Toluene	73.8 ± 3
o-Xylene	69.9 ± 3
p-Xylene	70.1 ± 3
Mesitylene	80.0 ± 3
Cumene	56.1 ± 3
Bromobenzene	56.2 ± 3
Chlorobenzene	69.2 ± 3
Nitrobenzene	79.0 ± 3
o-Nitrotoluene	92.0 ± 3
m-Nitrotoluene	76.2 ± 3
Chloronaphthalene	77.5 ± 3
Nitrobiphenyl	74.4 ± 3
Cyclohexane	25.2 ± 3
Cyclochlorohexane	83.0 ± 3
Methylcyclohexene	20.0 ± 3
Hexane	0.5 ± 3
Chlorohexane	34.1 ± 3
Carbon tetrachloride	45.8 ± 3
Chloroform	84.5 ± 3
Bromoform	41.3 ± 3
Trichloroethane	71.0 ± 3
Ethylene chloride	86.7 ± 3
Chloromethylbutane	22.1 ± 3
Isoamyl alcohol	80.0 ± 3
Caprylic alcohol	80.0 ± 3
Octanol	82.5 ± 3
Decyl alcohol	61.3 ± 3
Diethyl carbinol	69.0 ± 3
Butyl acetate	73.6 ± 3
Amyl acetate	75.4 ± 3
3-Heptanone	80.4 ± 3

[a] 50 ml. of aqueous solution of selenium-75 and 5 ml. of organic extractant.
[b] Relative standard deviation.

ANALYTICAL RESULTS

Comparison of Analytical Methods. Methods IIIa and IIIb were compared by analyzing 13 replicates of a ground alfalfa sample by each method. The

Table VI. Fluorometric Determination of Se in Plant Material[a]

Recovery Experiment Method III Step	Se^{75} recovery through individual steps, %
1. Air-dry sample	...
2. Grind (60-mesh screen)	...
3. Weighing	100 ± 2[b]
a. Parr bomb (0.1–0.2 gram)	
b. Schöniger flask (<0.12 gram)	
4. Oxidation (Parr bomb)	95 ± 3
5. Filtration	...
6. Reduction (4*N* HCl)	100 ± 3
7. Complexation of Fe	...
8. Reaction with DAB	...
9. Extraction (toluene)	
First extraction	74 ± 2
Second extraction	14 ± 1
Third extraction	...
Over-all recovery (3 extractions)	96 ± 2
Over-all recovery (steps 3–9 inclusive)	90.7 ± 2.4

[a] Alfalfa containing Se^{75}.
[b] Relative standard deviation.

Table VII. Comparison of Fluorometric and Neutron Activation Methods

Material analyzed	Fluorometric Parr combustion, p.p.m.	Activation analysis, p.p.m.
Trefoil grass hay	0.094	0.053
	0.094	
	0.101	0.062
	0.100	
Linseed oil meal	1.12	1.10
	1.12	
Wheat bran	0.076	0.064
	0.076	
Oats	0.024	0.026
	0.026	
Cull red kidney beans	0.010	0.012
	0.012	

Table VIII. Fluorometric Determinations of Se[a] in Various Plants

Plant tissue	Selenium duplicates,[b] p.p.m.		Plant tissue	Selenium duplicates,[b] p.p.m.	
Pear leaves	0.44	0.46	Sample 2, Alfalfa[c]	0.17	0.20
Apple leaves	0.55	0.52	Sample 3, Alfalfa[c]	0.09	0.07
Radish leaves	0.28	0.27	Sample 4, Alfalfa[c]	0.13	0.09
Lettuce leaves	0.64	0.57	Sample 5, Alfalfa[d]	0.07	0.08
Corn	0.30	0.34	Sample 6, Alfalfa[d]	0.44	0.44
Wheat	0.28	0.30	Sample 7, Alfalfa[d]	0.46	0.41
Sample 1, Alfalfa[c]	0.15	0.19	Sample 8, Alfalfa[d]	0.62	0.58

[a] Measured on dry basis.
[b] Parr bomb combustion.
[c] White muscle disease ranch.
[d] Nonwhite muscle disease ranch.

results using the Parr bomb indicated an average of 1.14 p.p.m. of Se, with a standard deviation of ±0.019; for the Schöniger flask, an average of 1.17 p.p.m. of Se, with a standard deviation of ±0.012.

Table VII compares neutron activation with fluorometric DAB method of analysis for several types of plant samples. The results obtained agree well on four of the six samples. Duplicate results were not available on the neutron activation analyses.

Plant Samples. Table VIII shows the analytical results for Se in a number of diverse plant materials.

In this group of analyses alfalfa samples, from ranches where white muscle disease is prevalent, fall below 0.20 p.p.m. of Se; results from all nonwhite muscle ranches except one were greater than this value. These samples were taken some years ago before the use of bore-type hay samplers became prevalent; it is not certain how representative the samples are.

Animal Samples. Table IX shows the preliminary results obtained on a variety of animal samples. This type of analysis is being continued in our current white muscle research.

ACKNOWLEDGMENT

The authors are indebted to D. E. Hogue of Cornell University for plant samples and the neutron activation results shown in Table VII.

Table IX. Fluorometric Determination of Selenium in Animal Tissues and Secretions

	Cattle[a] duplicates, p.p.m.		Sheep[b] duplicates, p.p.m.		Rat[b] duplicates, p.p.m.	
Liver[c]	2.24	2.25	1.98	1.98	2.03	2.13
Muscle[c]	3.25	3.30	2.49	2.31	1.29	1.64
Brain[c]	2.64	2.49	1.42	1.75	1.92	2.00
Heart[c]	2.35	2.30	1.28	1.28	2.66	2.84
Kidney[c]	0.63	0.61	3.19	3.10	2.65	2.63
Serum[d]	0.017	0.019				
Milk[d]	0.027	0.029				

[a] Parr bomb combustion.
[b] Schöniger flask combustion.
[c] Measured on dry basis.
[d] Measured on "as received basis."

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Determination of Water and Alkali Metal Oxide in Alkali Metal Salts of Selenium and Tellurium by Karl Fischer Titration

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▶ **The moisture content or water of crystallization in alkali metal salts of selenium and tellurium can be determined accurately with the Karl Fischer reagent. Sodium selenite, potassium tellurite, and sodium tellurate behave like a mixture of the respective oxides, and the alkali metal oxide in these salts reacts quantitatively with the Karl Fischer reagent. The results for the alkali metal oxide content offer an indirect method for the determination of combined selenium or tellurium. Anhydrous sodium selenate is not affected by the reagent. The data obtained compare favorably with analyses of weight loss by thermal drying and of selenium or tellurium by potentiometric titration.**

A GROWING interest is evidenced in the application of the Karl Fischer reagent in the inorganic field. Bryant *et al.* (*3*) studied the behavior of 25 hydrated inorganic salts and concluded that the Karl Fischer method is more rapid and precise than thermal drying procedures. In addition, base metal oxides, either free or as hydroxides or salts of weak acids, are the most frequent type of interfering materials, since water is formed by the action of these oxides upon hydroiodic acid. In general, since the interference is stoichiometric, it is possible to calculate the water content where the reaction is known.

This paper demonstrates the application of the Karl Fischer reagent to the alkali metal salts of selenium and tellurium. Both the water content and the alkali metal oxide content can be determined, with an indirect estimation of combined selenium or tellurium.

EXPERIMENTAL

Preparation of Samples. Sodium selenite was prepared by neutralizing a selenious acid solution with a slight excess of sodium hydroxide. A snow-white precipitate was formed on evaporating the resulting solution and anhydrous sodium selenite was obtained by drying the product to constant weight in a vacuum desiccator over potassium hydroxide. Sodium selenite pentahydrate crystals were obtained by allowing a saturated sodium selenite solution to stand at room tem-

perature for about one month. Sodium selenate decahydrate crystals, snow-white, were obtained from technical grade sodium selenate by recrystallization. This operation was carried out at temperatures below 40° C. to avoid the formation of lower hydrates.

Potassium tellurite was prepared from tellurium dioxide and potassium hydroxide by following the same procedure as for the preparation of sodium selenite. Anhydrous potassium tellurite was obtained by oven-drying the product at 105° C. to constant weight.

Potassium tellurite dihydrate, colorless crystals, was obtained from the alcohol layer upon adding ethyl alcohol to a saturated solution of potassium tellurite and allowing to stand for about one month. Disodium tetrahydrogen tellurate dihydrate, $Na_2H_4TeO_6 \cdot 2H_2O$, was prepared by Fouasson's method (*4*). The product was dried in air. Anhydrous disodium tetrahydrogen tellurate was obtained by drying the dihydrate at 50° C. to constant weight.

Preparation of Karl Fischer Reagent. The reagent was prepared following reported procedures (*1*, *6*).

Standardization of Reagent. The standardization was performed one week after the reagent had been prepared. In the first half of the present work, the reagent was standardized against a standard solution of water in methanol. In the second half, the reagent was standardized against sodium acetate trihydrate as recommended by Roy and Mabon (*5*).

Analytical Procedure. A sample (0.1 to 0.4 gram) equivalent to 2 to 4 mmoles of water was suspended in 25 ml. of anhydrous methanol in a 100-ml., glass-stoppered volumetric flask. Then the sample was titrated with standard reagent until the pale yellow of the liquid was permanently tinged reddish brown with free iodine. The water content of the suspending methanol was determined by a blank titration and the titer of the sample was corrected by this amount. (The selenium and tellurium salts are insoluble in methanol, and the reaction rate and the sharpness of the end point are dependent on the particle size of the sample. The time required to complete one titration is usually less than 30 minutes. In all samples, except sodium selenate, a dark-gray metallic selenium or tellurium precipitate was formed during the titration. Since the precipitate settled out readily, there was very little interference in the detection of the end point.)

Table I. Anhydrous Salts

Compound	% Na_2O (or K_2O) Found	Calcd. from Se or Te analyses	Theoretical
Na_2SeO_3	35.91 36.05 35.89	35.90	35.86
K_2TeO_3	37.05 37.03	...	37.11
$Na_2H_4TeO_6$	22.70 22.55	22.55	22.66

Table II. Hydrated Salts

(Powder form)

Compound	% Na_2O (or K_2O) Found[a]	Calcd. from Se or Te analyses	% H_2O Found[a]	Found[b]	From wt. loss on drying	H_2O/anhyd. salt, mole ratio, x
$Na_2SeO_3 \cdot xH_2O$	30.92 31.01	...	13.77	...	13.90 (dried in vacuum desiccator over KOH overnight)	1.5
$K_2TeO_3 \cdot xH_2O$	30.86 30.99 31.10	30.74	16.86 16.50 16.20	16.86 16.52 16.20	16.25 (15 days at 105° C.)	2.8

[a] Calculation based on Method A.
[b] Calculation based on Method B.

RESULTS AND DISCUSSION

Typical data on the determinations of alkali metal oxide and/or water in sodium selenite, sodium selenate, potassium tellurite, and disodium tetrahydrogen tellurate are given in Tables I, II, and III. The calculation of the results was based on the following stoichiometric reactions. The salt-forming function of pyridine has been ignored in order to simplify the equations.

$$H_2O + I_2 + SO_2 + CH_3OH \rightarrow 2HI + HSO_4CH_3 \quad (1)$$

$$Na_2SeO_3 + I_2 + 3SO_2 + 3CH_3OH \rightarrow 2NaI + Se + 3HSO_4CH_3 \quad (2)$$

$$Na_2SeO_4 + I_2 + SO_2 + CH_3OH \rightarrow \text{no reaction within 24 hours} \quad (3)$$

$$K_2TeO_3 + I_2 + 3SO_2 + 3CH_3OH \rightarrow 2KI + Te + 3HSO_4CH_3 \quad (4)$$

$$Na_2H_4TeO_6 + 3I_2 + 6SO_2 + 6CH_3OH \rightarrow 2NaI + 4HI + Te + 6HSO_4CH_3 \quad (5)$$

In many cases, parallel potentiometric determinations of selenium or tellurium content (*2*), as well as deter-

Table III. Hydrated Salts

(Crystal form)

Compound	% Na_2O (or K_2O) Found[a]	Calcd. from Se or Te analyses	Theor.	% H_2O Found[a]	Found[b]	From wt. loss on drying	Theor.
$Na_2SeO_3 \cdot 5H_2O$	23.59 23.74 23.50	23.50	23.59	34.20 33.75 34.65	34.13 33.74 34.54	34.45 (2 days at 105° C.)	34.22
$Na_2SeO_4 \cdot 10H_2O$	...	16.73	16.80	49.50 49.50 49.45 49.60	...	49.18 (2 days at 105° C.)	48.78
$K_2TeO_3 \cdot 2H_2O$	32.29 32.59	...	32.48	12.96 12.11	...	12.54 (2 days at 105° C.)	12.41
$Na_2H_4TeO_6 \cdot 2H_2O$	20.00 20.00	19.89	20.02	11.75 11.75	11.83 11.82	12.11 (3 days at 50° C.)[c]	11.63

[a] Calculation based on Method A.
[b] Calculation based on Method B.
[c] Temperature recommended by Fouasson (*4*).

minations of the water content by thermal drying, were made. The results are given for comparison.

The results given in Table I indicate that the anhydrous salts of sodium selenite, potassium tellurite, and disodium tetrahydrogen tellurate react quantitatively with Karl Fischer reagent by following Reactions 2, 4, and 5, respectively. Hence, it is possible to calculate the water content of the hydrated salts. It is clear that the titer for the hydrated salts is a measure of the combined alkali metal oxide and water content. The latter, in this case, was calculated by two methods: Method A, assuming the sample is composed of pure anhydrous salt and water only; and Method B, estimating the alkali metal oxide content from separate selenium or tellurium analyses, then obtaining the water content from the titer by difference.

The calculated results are compared with the water content found from weight loss on thermal drying. The two sets of data agree very well, as shown in Tables II and III, although the thermal drying procedure is more time-consuming.

Sodium selenate is not affected by the Karl Fischer reagent and the titer is derived exclusively from water content. This is in line with Bryant *et al.* (*3*), who found that the normal and acid alkali salts of sulfuric acid are not affected by the Karl Fischer reagent.

The sulfur dioxide, present in the Karl Fischer reagent, reduces Se(IV), Te(IV), and Te(VI) in sodium selenite, potassium tellurite, and disodium tetrahydrogen tellurate, respectively, to metallic selenium and tellurium during the titration. No redox reactions take place between iodine and the selenium or tellurium species.

The nonintegers of the H_2O/Na_2SeO_3, and H_2O/K_2TeO_3 mole ratios in the case of the powder-form salts either represent the average number of moles of water of crystallization, or indicate that some of the water present is adsorbed on the surface of the solids. The preparation of potassium tellurite dihydrate crystal, $K_2TeO_3 \cdot 2H_2O$, has not been described in the literature previously.

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Determination of Phosphorus in Iron and Steel with Quinoline Molybdate

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▶ An improved method for volumetric or gravimetric determination of phosphorus in all classes of iron and steel is based on precipitation of phosphorus as quinoline phosphomolybdate from a perchloric–hydrochloric acid solution after oxidation to orthophosphate by refluxing with perchloric acid. Modifications overcome interference by iron, arsenic, zirconium, niobium, tantalum, and tungsten. Inhibition of precipitation by iron is overcome by adding more reagent or digesting the mixed solutions; of quinoline arsenomolybdate, by volatilization of arsenic with hydrobromic acid. Niobium, tantalum, and tungsten occlude phosphorus with their oxides, which are precipitated as the perchloric acid solution is taken to reflux. Zirconium phosphate is simultaneously precipitated. Phosphorus is recovered from these precipitates. Precipitation of zirconium phosphate later is avoided by stopping evaporation of the hydrobromic–perchloric acid solution as bromine ceases to be evolved. Precipitation of quinoline niobio- and tantalomolybdates from small amounts remaining in solution after perchloric acid reflux is prevented by addition of tartaric and nitric acids. Further precipitation of tungsten oxide with occlusion of phosphorus is also prevented by tartaric acid. Tests on NBS samples and current alloy types of iron and steel have established reproducibilities of ±0.001, ±0.002, ±0.01, and ±0.03 for phosphorus levels of 0.01, 0.1, 0.5, and 1.5%.

AMERICAN, Australian, and British standard methods for phosphorus in iron and steel (*2*, *4*, *5*) have deficiencies which can be grouped into those affecting the time-length of the procedure and accuracy. The first group includes the necessity for supplementary oxidation of phosphorus, recovery of phosphorus from precipitated oxides, and conversion of ammonium phosphomolybdate (AmPMo) to magnesium ammonium phosphate (*2*) or lead molybdate (*4*, *5*) when a gravimetric measurement is desired.

The second group includes the gravimetric determination of small contents of phosphorus by the magnesium ammonium phosphate method (*2*). This may not give the accuracy desired because of limitations imposed by the low factor weight for phosphorus in magnesium pyrophosphate.

The presence of sulfuric acid, derived from sulfurous acid added to dissolve precipitated manganese oxides formed from the use of potassium permanganate for supplementary oxidation (*2*), may affect the yield. Small amounts of sulfuric acid increase the yield and large amounts decrease it when phosphorus content is measured volumetrically. The effect is negligible for small phosphorus contents, when the technique of dropwise addition of reagents is used to avoid excess. It becomes noticeable, however, when high phosphorus contents are determined and 10-ml. volumes of potassium permanganate and correspondingly large amounts of sulfurous acid are used—for example, Sections 31 and 39, ASTM methods for phosphorus in ferrous materials (*2*).

The standard methods yield low results for phosphorus contents of the order of 0.01%, because of the inhibiting effect of iron, and is not overcome by increasing the reagent addition to twice the usual amount and/or by standing for a long time. Yields of 0.004, 0.003, and 0.007% phosphorus by the standard methods for NBS samples 55a,

Table I. Determination of Phosphorus in Standard Analyzed Iron and Steel Samples by Quinoline Phosphomolybdate Method

Natl. Bur. Standards samples	Recommended phosphorus content, %	Phosphorus found, %	
		Volumetric	Gravimetric
No. 50b 18W-4Cr-1 V steel	0.029	0.030 0.031 (3)	0.030 (4)
No. 115 15 Ni-6Cu-2Cr cast iron	0.113	0.107 (6) 0.108 (5)	0.107 0.108 (3) 0.109
No. 121 18Cr-8Ni-0.4Ti steel	0.016	0.017 (5)	0.018 (4)
No. 153 8Co-8Mo-4Cr-1.5W-2V steel	0.025	0.025 (3)	0.025 0.026 (2)
No. 7e high phosphorus cast iron	0.878	0.858 0.855 0.848 0.846 (2)	0.855 [range 0.850 to 0.860 (7)]
British Chemical Standard sample 206	1.51	1.51 1.52 (5)	1.51 1.52 (2)
British Chemical Standard sample 206/1	1.38	1.38 (7) 1.39 (2) 1.40 (2)	—

Numbers in parentheses show individual determinations giving results stated.

55b, and 123, were raised to 0.010, 0.007, and 0.010 by the method described.

Inaccuracies arising from the use of potassium permanganate and from weighing as magnesium pyrophosphate led the author to undertake the development of an improved method based upon the precipitation of phosphorus as the quinoline-phosphomolybdate complex (*15*). As a result, a method was formulated along the lines of the present Australian and British methods (*4*, *5*) and tested on a number of NBS samples of iron and steel. Table I shows good agreement between volumetric and gravimetric measurements and with NBS and BCS recommended values, except for NBS samples 115 and 7e.

The method, the investigations leading to its development, and the results were submitted to the Ferrous Metals Analysis Committee of the Standards Association of Australia and examined by a panel of seven members. The members of the panel, each representing a different laboratory and organization, then carried out determinations by the method on a number of samples of current alloy types of iron and steel. Volumetric measurement was used for most of the determinations (laboratories 1 to 5), since gravimetric measurement was introduced late in the program.

The results by each laboratory showed a high degree of repeatability (Table II). Close agreement between results for the different laboratories showed that systematic errors were absent, and that reproducibility limits could be set. These were determined, after consideration of all the factors involved, as ±0.001, ±0.002, ±0.01, and ±0.03 for the 0.01, 0.1, 0.5, and 1.5% phosphorus levels.

The committee has recommended to the Standards Association of Australia that the method be issued as an Australian Standard for phosphorus in all classes of iron and steel, replacing the present standard A.S.K.1, Part 3.

EXPERIMENTAL

Reagents. Quinoline Molybdate Reagent Solution. Add 150 grams of molybdenum trioxide (100%) to 600 ml. of water containing 50 grams of sodium hydroxide. Heat to dissolve. [Alternatively, dissolve 250 grams of sodium molybdate (free from ammonium salts) in 600 ml. of water.] Cool.

Slowly add 500 ml. of hydrochloric acid (sp. gr. 1.16). Heat to dissolve. Cool. Add 100 volume % hydrogen peroxide dropwise until the green color is removed. Dissolve 28 ml. of distilled quinoline in 600 ml. of hydrochloric acid (50%). Mix the two solutions, heat to boiling, allow to cool, and stand for 24 hours. Dilute to 2 liters, and filter into a plastic bottle.

Arsenate Reagent. Dissolve 0.25 gram of arsenious oxide (As_2O_3) in 3 ml. of 10% sodium hydroxide. Add 5 ml. of hydrochloric acid (sp. gr. 1.16), 50 ml. of water and 1 gram of sodium chlorate. Heat to boiling and boil for 2 to 3 minutes. Allow to cool and dilute to 100 ml.

Magnesia Mixture. Dissolve 15 grams of magnesium chloride crystals ($MgCl_2 \cdot 6H_2O$) and 15 grams of ammonium chloride in water and dilute to 100 ml.

Solvent Mixture. Mix together 100 ml. of water, 100 ml. of acetone, and 5 ml. of ammonium hydroxide solution (sp. gr. 0.880).

Standard Solutions. Solution 1. Sodium Hydroxide, 0.084*N* (approximately). Prepare a solution of sodium hydroxide by dissolving 3.4 grams in water and diluting to 1 liter.

Solution 2. Nitric Acid, 0.084*N* (approximately). Prepare a solution of approximate concentration by diluting 5.5 ml. of nitric acid (sp. gr. 1.42) to 1 liter.

Standardization. Transfer 25 ml. of sodium hydroxide solution to a 100-ml. conical flask, add 5 drops of phenolphthalein solution, and titrate with the nitric acid solution.

Weigh out 0.4283 gram of pure dry acid potassium phthalate ($HKC_8H_4O_4$) into a 300-ml. conical flask and add 50 ml. of water. Heat to dissolve, boil 2 to 3 minutes, cover with a glass, and allow to cool in a cold water bath. Add 35 ml. of sodium hydroxide solution, and 5 drops of phenolphthalein solution, and titrate with nitric acid solution until the pink color is discharged. Calculate the normality of the sodium hydroxide solution.

0.4283 of pure dry acid potassium phthalate is equivalent to 25 ml. of 0.0839*N* sodium hydroxide solution.

1 ml. of 0.0839*N* hydroxide solution is equivalent to 0.1 mg. of phosphorus.

Procedure. General. Transfer a suitable weight of sample to a 400-ml. conical beaker. The following weights and aliquots are suggested:

Gravimetric Determination

% P	Sample, g.	Aliquot
0–0.35	2	
0.35–0.7	1	
0.7–1.4	1	1/2
1.4–2.0	1	1/4

Volumetric Determination

% P	Sample, g.	Aliquot
0.0.1	2	
0.1–0.2	1	
0.2–0.5	2	1/5
0.5–1.0	1	1/5
1.0–2.0	1	1/10

Where necessary, take aliquots from the solution after the hydrobromic acid treatment.

Add cautiously a mixture of 15 ml. of nitric acid (sp. gr. 1.42) and 25 ml. of hydrochloric acid (sp. gr. 1.16) and digest until dissolved. Hydrofluoric acid or sodium fluoride may be used to assist the decomposition of high silicon irons. Glassware used in the decomposition of the sample and fuming of the solution must then be free from phosphorus.

Add 25 ml. of perchloric acid (sp. gr. 1.67) and cover the beaker with a cover glass. Evaporate to fumes of perchloric acid and continue the fuming for at least 5 minutes. Fume the solution at a temperature to maintain a steady reflux of acid, in the form of a continuous film of liquid, at least two thirds the way up the sides of the beaker.

Cool, add 50 ml. of water, mix to dissolve soluble salts, and allow the residue to settle. Filter through a medium-texture paper into a 300-ml. conical beaker and wash with small quantities of hydrochloric acid (2%

v./v.). Reserve the filtrate if treatment of the residue is necessary and proceed with the appropriate residue treatment.

Residue Treatment. Transfer the residue and filter paper to a platinum crucible and ignite at a dull red heat. Cool, moisten the residue with 2 ml. of hydrofluoric acid (sp. gr. 1.13) and 5 drops of nitric acid (sp. gr. 1.42), and evaporate to dryness.

Fuse the residue with 5 grams of potassium bisulfate until a clear melt is obtained, extract in 25 ml. of tartaric acid solution (20%, w./v.), and transfer to a 200-ml. conical flask. Add 8 ml. of arsenate reagent and 10 ml. of magnesia mixture and adjust the volume of the solution to 50 ml. Make the solution just alkaline to bromocresol purple (0.2%) with ammonium hydroxide solution (sp. gr. 0.880), and then add 5 ml. in excess. Cool to 15° C., shake the solution vigorously, and let stand for 1 hour.

Collect the precipitate on a tightly packed paper-pulp pad, washing five times with small amounts of cold ammonium hydroxide solution (3%, v./v.). Dissolve the precipitate from the pad into a 150-ml. beaker with two washes of approximately 10 ml. of hydrochloric acid (50%, v./v.) each and complete the washing of the pad with six small washes of hot water. Transfer the solution to the filtrate from the insoluble residue.

When zirconium occurs separately —i.e., not in association with niobium, tantalum, or tungsten—treat the residue for the removal of silica as above. Fuse with 0.5 gram of sodium carbonate, extract with boiling water, filter, and wash with hot water. Acidify with hydrochloric acid (50%, v./v.) and return the solution to the filtrate from the insoluble residue.

To the filtrate, or to the combined filtrate and acid extract from the insoluble residue treatment, add 15 ml. of hydrobromic acid (sp. gr. 1.46) and evaporate just to removal of bromine. Add water to a total volume of 150 ml.

When vanadium is present in the quinquevalent state and in appreciable quantity, both the color and texture of the quinoline phosphomolybdate precipitate are affected. Color is orange instead of yellow, and texture fine instead of granular. These modifications of the normal precipitate do not measurably affect the result obtained, but may be eliminated by reduction of the vanadium to the quadrivalent state with sulfurous acid before addition of quinoline molybdate reagent. Remove excess sulfur dioxide from the reduction of vanadium by boiling before addition of quinoline molybdate reagent.

For steels containing niobium, tantalum, or tungsten, interference in the quinoline phosphomolybdate step should be avoided: dilute the solution to 150 ml. and add 1 ml. of nitric acid (sp. gr. 1.42) and 5 grams of tartaric acid. Heat to dissolve, and boil gently for 10 to 15 minutes.

Make aliquot volumes to 150 ml. with amounts of water and perchloric acid (sp. gr. 1.67) sufficient to give a total acidity between 1*N* and 1.5*N*. Bring to a boil and add quinoline molybdate reagent dropwise to the continuously boiling solution. Cool slightly. Add 20 ml. of quinoline molybdate reagent except when tartaric and nitric acids are present, when the amount should be increased to 25 ml. To ensure quantitative separation of low amounts of phosphorus, give the mixture a digestion time, at a temperature near boiling point, increasing from zero at 0.025% phosphorus, to 10 minutes at 0.015%, and 2 hours at 0.005% phosphorus. Alternatively, for the rapid separation of amounts of phosphorus less than about 0.015%, add 50 ml. of reagent followed by 10- to 15-minute digestion.

GRAVIMETRIC DETERMINATION. Filter under suction through a sintered Gooch crucible (of No. 4 porosity), and wash the precipitate twice with hydrochloric acid solution (2% v./v.), and then with water until the washings are free from acid. Transfer Gooch and contents to an oven maintained at 250° C., and heat to constant weight. Cool and weigh. The color of the precipitate is normally yellow, but a green tinge may be present. This does not measurably affect the result.

Dissolve the precipitate from the Gooch with solvent mixture. Heat the Gooch to constant weight at 250° C., cool, and weigh. The difference between the two weights is the weight of quinoline phosphomolybdate $(C_9H_7N)_3H_3(PO_4 \cdot 12MoO_3)$. Carry a blank determination through all steps of the procedure.

VOLUMETRIC DETERMINATION. Filter under suction through an asbestos pad supported by a removable perforated disk held in a filter funnel. Wash the precipitate twice with hydrochloric acid solution (2%, v./v.) and then with water until the washings are free from acid.

Transfer the pad and precipitate to

Table II. Reproducibility of Determinations of Phosphorus in Iron and Steel by the Recommended Method by Different Laboratories

	Phosphorus found, %								
	1	2	3	4	5	6		7	
Laboratory						Volumetric	Gravimetric	Volumetric	Gravimetric
Stalloy, 4 Si-0.3 Al	0.012 (3) 0.013	0.009 (3)	0.010 0.011	0.010 0.011 0.012	0.011 0.011	0.009 (3)	—	0.009 (5) 0.010 (2) 0.011	—
Ti-stabilized 18/8 steel	0.020 (3) 0.019	0.018	0.018 0.019	—	0.019 0.020 0.020	0.020 0.021	0.019 0.020	0.018 (3) 0.019 (3)	—
Nb stabilized stainless steel, 1 Nb	0.021 0.021 0.018	0.020 0.019 0.020	0.018 0.020	—	0.019 0.020 0.021	0.020	0.021 (2) 0.022 (2)	0.020 (4) 0.021 (5)	0.018 (2) 0.019 (6) 0.020
Molybdenum-bearing hot die steel, 5 Mo, 6 W, 1.9 V	0.036 0.036	0.038 (3)	0.038 0.040	—	0.041 0.041	0.040	0.039 0.040	0.037 (4) 0.038 (3) 0.039 (2)	0.037 0.038 (4)
Plain carbon steel	0.087 0.087 0.089	0.084 0.084 0.085	0.088 0.089	0.087 (3)	0.088 0.088 0.086	0.086 0.086	0.087 0.087	0.087 (4) 0.089 (6)	—
Austenitic iron, 14 Ni, 2.5 Cr, 6 Cu, 2 Si	0.165 0.167 0.168	0.163 0.164 0.165	0.162 0.165 0.165	0.163 0.169	0.163 0.165	—	—	0.167 (3) 0.168 0.169	
Stove iron, 0.6 P	0.576 0.576 0.572	0.574 0.579 0.576	0.562 0.563	0.558 0.562 0.565	0.560 0.570	—	—	0.566 (2) 0.568 (2) 0.564	0.571 0.572 0.574 (2)
High-phosphorus cast iron, BCS 206	1.50 (2) 1.51 (2)	1.54 1.54 1.55	1.52 1.53 1.55	—	—	1.51 1.51	1.50 (3) 1.51	1.51 1.52 (5)	1.51 1.52 (2)

Volumetric determination only used by laboratories 1–5.
Numbers in parentheses show individual determinations giving results stated.

a 300-ml. conical beaker and add 50 ml. of water. Boil for several minutes. Cool. Add 5 drops of phenolphthalein solution (0.5%, w./v.) and a volume of standard sodium hydroxide solution sufficient to give an excess of at least 5 ml. Shake to dissolve the precipitate.

Titrate the excess sodium hydroxide with the standard nitric acid until the pink color is discharged. Read the volumes of acid and alkali used. (Carry a blank determination through all steps of the procedure.)

CALCULATIONS. Percentage of phosphorus $= \frac{(B-C)}{A} \times 1.4$, gravimetric finish,

or

$\frac{F(D-EG)}{A} \times \frac{1}{100}$, volumetric finish

where A = weight of sample taken, grams
B = weight of Gooch and precipitate, grams
C = weight of Gooch, grams
D = ml. of standard sodium hydroxide solution used (0.084N, approx.)
E = ml. of standard sodium hydroxide solution (0.084N, approx.) equivalent to 1 ml. of nitric acid solution (0.084N, approx.)
F = factor of standard sodium hydroxide (0.084N, approx.) in terms of 0.084N
G = ml. of standard nitric acid (0.084N, approx.) used.

DISCUSSION

Basis for Procedure. The British method (*5*) was adopted by reason of its familiarity to metallurgical chemists in Australia (as A.S.K.1, Part 3), and and the applicability of the introductory steps to all classes of iron and steel. These included sample decomposition, supplementary oxidation of phosphorus, filtration of insoluble material after taking the solution to reflux with perchloric acid, and arsenic elimination by hydrobromic acid. These steps were adopted with slight modification. Also it appeared likely by reason of similarities in the properties of the two complexes, that modifications to overcome interference by other elements incorporated in the AmPMo method, could be adopted for the QPMo method.

Acidity for Precipitation. Wilson reported (*15*) that solution acidity should be such as to prevent the precipitation of quinoline molybdate and yet enable QPMo to be completely precipitated. Alfelt (*1*) showed that hydrochloric, nitric, and perchloric acids were suitable for the precipitation medium, but imposed upper limits on the acidity which could be tolerated even when large excesses of reagent were added. Fernlund and Zechner (*8*) preferred to work within the limits of 0.9 to 1.6N and this was adopted. By

Table III. Determinations of Phosphorus in National Bureau of Standards Samples 55a, 55b, 11e, and 13d with Quinoline and Ammonium Molybdate Reagents

(Effect of variations in procedure)

Expt. no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
NBS sample no.	55a						55b					11e		13d	
Pptn. reagent and vol. added, ml.		100		100		80		60		40			40		40
Quinoline molybdate															
Ammonium molybdate	100		100									100		100	
Standing time, min. at 95° C., after reagent addition	21[a] hours	10	21[a] hours	10	60	10	30	5	120	30	180	2½[a] hours	10	2[a] hours	10
Volumetric detn.	0.0049	0.0098	0.0024	0.0066	0.0065	0.0058	0.0063	0.0037	0.0066	0.0036	0.0062	0.0155	0.0167	0.0166	0.0182
	0.0051	0.0097	0.0024	0.0065	0.0068	0.0055	0.0068	0.0035	0.0065	0.0038	0.0066	0.0154	0.0163	0.0167	0.0184
	0.0048	0.0098	0.0024									0.0154	0.0163	0.0168	0.0183
													0.0167	0.0169	0.0181
Phosphorus found, % Gravimetric detn.		0.0099					0.0070								0.0177
		0.0101					0.0066								0.0178
		0.0101					0.0070								0.0177
		0.0102					0.0074								0.0180
							0.0075								

[a] Time at room temperature.

Results obtained by two analysts working independently.
Sample weight of 4 grams taken for each determination, with double amounts of reagents and reaction volumes. Volume for ammonium phosphomolybdate precipitation 150 ml., including 25 ml. nitric acid (sp. gr. 1.42) and 10 ml. ammonia (sp. gr. 0.880). Ammonium molybdate reagent made up according to directions for ASTM preparation 101.

taking 25 ml. of acid of specific gravity 1.67 for the perchloric acid reflux step, all danger of salts separating and becoming baked on the bottom during reflux was eliminated, and about 20 ml. of perchloric acid remained after refluxing, equivalent to about 1.6*N* for a 150-ml. precipitation volume.

Quinoline Molybdate Reagent (QMoR). Wilson (*15*) and Perrin (*12*) had added the constituents separately. However, the prepared reagent was used because of its convenience. Any phosphate present as an impurity in the constituents is eliminated during the preparation of the reagent. The proportion of constituents employed was varied only slightly from that used by Fernlund, Zechner, and Andersson (*9*).

Technique for Precipitation. A number of techniques have been reported for the precipitation of QPMo (*1, 9, 12, 15*), and it seemed that they would yield similar results. Investigation showed, however, that precipitation technique was important, and that the reagent should be added slowly, dropwise, to the boiling phosphate solution. With this technique, of 2.500 mg. of phosphorus taken (as phosphate) 2.498 mg. was found, with a standard deviation of ±0.0008. Repeatabilities by other techniques were good, but results were lower—for example, 2.480 mg. from adding QMoR in one amount to the boiling solution, and 2.460 mg. from adding it to the cold solution and then heating to coagulation.

Volumetric and Gravimetric Measurement. The equivalence of the two methods of measurement had been indicated by previous investigators. Wilson (*15*) found by volumetric measurement that QPMo precipitated with stoichiometric composition $(C_9H_7N)_3H_3(PO_4 \cdot 12MoO_3)$ and later investigators (*5–7, 12, 14*) confirmed this by gravimetric measurement. Nevertheless, the aspect of equivalence was re-examined for other factors which might affect accuracy. None was found for volumetric measurement, employing the ASTM alkalimetric titration procedure (*2*) with changes only in amounts and concentrations of reagent solutions. For gravimetric measurement, heating the wet Gooch and precipitate for 30 minutes at 250° C. gave results satisfactory for practical purposes. On continued heating, 2% in weight (for 0.2 gram of precipitate) was slowly lost over 16 hours. This loss would not have been detected in the experiments of Wendlandt and Hoffman (*14*), but confirmed the findings of Perrin (*12*).

Effect of Other Elements. IRON. Wilson (*15*) reported that 1 gram of iron had no effect on the precipitation of QPMo, but he was precipitating appreciable amounts of phosphorus. Alfelt (*1*), working with 0.6 mg. of phosphorus, found 2.2 grams of iron to have a marked inhibition effect on the precipitation of QPMo from solutions of hydrochloric and nitric acids. This was overcome by adding more reagent. To determine the intensity and range of the effect, a series of experiments (Table III) was therefore designed using NBS samples of plain iron and carbon steel. Four-gram samples were used to reduce the effect of variations in the blank upon the result. Iron exerted a marked inhibiting effect at the 0.005% phosphorus level, but at 0.015% the effect was almost negligible. It was overcome by digesting the mixed solutions near the boiling point. Digestion times required were almost zero for a sample of 0.025% phosphorus, about 10 minutes for 0.015% and 2 hours for 0.005%. The addition of larger amounts of QMoR gave rapid quantitative precipitation of QPMo, a digestion time of about 15 minutes being required for this procedure at the 0.005% phosphorus level.

Determinations were also made volumetrically by the ASTM method (*2*). Results after the mixed solutions had been shaken and let stand for 21 hours were considerably lower at the 0.005% phosphorus level than by the QPMo method. At the 0.015% level, the difference was slight.

ARSENIC. Interference by arsenic in the precipitation of AmPMo is slight under appropriate precipitation conditions, even when appreciable amounts are present. However, quinoline arsenomolybdate coprecipitated with QPMo under all conditions of precipitation, confirming the findings of Meyer and Koch (*11*). Consequently arsenic must be removed before precipitating QPMo. Volatilization as bromide as in the British method (*5*) was adopted.

VANADIUM. Fuchs and Veiser (*10*) reported that up to 0.2% vanadium had no effect on the precipitation of QPMo, but that larger amounts should be reduced to the quadrivalent state, suggesting that quinquevalent vanadium had interfered. The effect of vanadium at the highest level which might be encountered in steels (approximately 2%) was therefore determined. Quinquevalent vanadium inhibited the precipitation of QPMo from pure phosphate solutions (at the 0.1-mg. phosphorus level), but not from solutions containing iron. Qualitative effects observed in the presence of iron were the somewhat later stage in the addition of QMoR when precipitation of QPMo began and the deep orange color of the precipitate in contrast with the normal yellow. No modification of the basic method was therefore required, but it was deemed advisable to include a provision for its reduction to the quadrivalent state, should this be desired.

TITANIUM. Tests on solutions of NBS samples 11e and 13d to which the equivalent of 1% titanium as perchlorate had been added showed that there was no quantitative effect upon the precipitation of QPMo. Precipitation occurred, however, at a somewhat later stage during the addition of QMoR.

ZIRCONIUM. For the recovery of phosphorus precipitated as zirconium phosphate, as the perchloric acid solution of a steel containing zirconium is taken to fumes, the British method (*5*) specifies a sodium carbonate fusion followed by an aqueous extraction. This was adopted for the QPMo method. Return of the recovered phosphate to the filtrate from the perchloric acid refluxing step, followed by the hydrobromic acid treatment for the elimination of arsenic and final evaporation to fumes of perchloric acid, was unsatisfactory. In all experiments zirconium phosphate was reprecipitated on evaporation of the solution to fumes of perchloric acid. It could not be dissolved by any treatment except fusion. The difficulty was resolved by ceasing to evaporate the solution when the last traces of bromine (from the decomposition of hydrobromic acid) had been volatilized. With this modification zirconium phosphate equivalent to 2.5 mg. of phosphorus was soluble in 150 ml. of solution containing 2 grams of iron, 20 mg. of zirconium, and 20 ml. of perchloric acid (sp. gr. 1.67). Consequently QPMo could be separated from the boiling solution without interference.

NIOBIUM, TANTALUM, AND TUNGSTEN. Elimination of interference by these elements presented a major difficulty. Phosphorus, occluded by the oxides precipitated as the perchloric acid solution of the sample was concentrated and taken to reflux, was conveniently recovered using modification 2 of the British method (*5*). Precipitation of the oxides at this stage was, however, incomplete, although inferred complete in respect to niobium by Silverman (*13*). Additional amounts separated at the conclusion of the hydrobromic acid treatment step and as the solution was evaporated further to fumes of perchloric acid, or, alternatively, allowed to stand for some time. Phosphorus was again occluded and could be recovered as described above.

When this procedure was used for niobium- (and tantalum) bearing steels, the QPMo precipitate was contaminated much more with steels containing a small phosphorus content (NBS 123, P=0.007%) than with those containing more phosphorus (NBS 123b, P=0.024%). Precipitates were fine and filtered slowly, in contrast to the coarse, easily filterable precipitates normally obtained. They became dark

green, from an initial yellow, after heating for 30 minutes at 250° C. When heated at 160° C. for some hours, the color remained unchanged. A slow, continuous loss in weight occurred on heating at 160° C., and when after heating at this temperature for 3 hours the precipitates were heated at 250° C., a rapid loss in weight amounting to about 5 mg. maximum took place, followed by the slow rate of loss found for pure precipitates.

The volumetric finish gave titers much larger than expected from the NBS values for phosphorus and revealed the presence of considerable amounts of titratable impurities in the precipitates. Extraction of precipitates heated at 250° C., using a solvent mixture consisting of water, acetone, and ammonia (sp. gr. 0.88) in the proportion 20:20:1, showed the contaminant to be largely soluble. The insoluble residues, after heating at 900° C., consisted of approximately 25% niobium oxide, a large proportion of chromium oxide, and a small proportion of iron oxide, with traces of other elements. It was obvious that niobium had not been completely removed by filtration following the hydrobromic acid treatment. The evidence suggested the formation of an insoluble compound between niobium oxide and quinoline molybdate which precipitated more or less simultaneously with QPMo.

In experiments to test this hypothesis, it was found that addition of QMoR to clear, stable solutions of niobium in 1.5*N* perchloric acid caused the separation of precipitates with properties precisely similar to those described. The amount of contamination of QPMo precipitates was dependent on the volume of QMoR added, but still marked when small volumes of QMoR were added and digestion times were extended to effect complete precipitation of phosphorus.

Elimination of Niobium Interference. Possible solutions appeared to be complete removal of niobium before the addition of reagent, reprecipitation of QPMo to remove niobium, and prevention of formation of the quinoline niobiomolybdate complex (QNbMo).

Attempts to remove niobium from solution were based on the use of hydrolytic precipitation procedure of the ASTM method for niobium in steels (*3*). Several hydrolytic separations with an intermediate step of taking to fumes of perchloric acid were required to remove niobium almost wholly from solutions of NBS steels 123 and 123b. The procedure was lengthy and was not considered further. When the method was modified so that solutions were allowed to stand for some days before the hydrolyzed niobium was filtered off, niobium was almost completely removed from solutions of Standards Association of Australia Steel (SAA) No. 108 with four results of 0.019% phosphorus being obtained (0.020% phosphorus by the AmPMo method). Hydrolytic precipitation of niobium from solutions containing large amounts of nitric acid also resulted in incomplete separation with one precipitation. The possibility of utilizing phenyl arsonic acid was also explored. Niobium could be almost entirely removed from solution with this reagent, but difficulty was experienced in completely volatilizing arsenic from the excess reagent added. As to reprecipitation of crude QPMo, it was found that a number of such steps would be required to remove niobium completely. The procedure would be lengthy and consequently was not considered further.

Prevention of the separation of QNbMo by (A) retaining niobium in solution as an oxy salt, or (B) forming a stable, soluble niobium complex was then explored.

(A). It was thought that if evaporation of the steel solution were stopped as soon as all bromine had been vola-

Table IV. Determination of Phosphorus in Samples of NBS Ingot Irons 55a and 55b under Various Experimental Conditions

Sample	Experimental conditions	Phosphorus, % (gravimetric)
55a	Basic method	0.0099, 0.0101, 0.0102
	2 ml. nitric acid (sp. gr. 1.42) and 10 g. tartaric acid added	0.0095, 0.0103
55b	Basic method	0.0070, 0.0074, 0.0073 0.0069, 0.0068
	2 ml. nitric acid (sp. gr. 1.42) and 10 g. tartaric acid added	0.0074, 0.0070, 0.0073
	4 mg. niobium added after hydrobromic acid treatment, 2 ml. nitric acid (sp. gr. 1.42) and 10 g. tartaric acid added	0.0073, 0.0070
	As for above, but 2/3 hydrochloric acid in quinoline molybdate reagent replaced by nitric acid	0.0098, 0.0085, 0.0084

Sample weights of 4 g. taken. Amounts of reagents and volumes double those for 2 g. sample.

Table V. Determination of Phosphorus in Niobium-Bearing Steels

Sample	Quinoline molybdate reagent added, ml.	Digestion time, min.	Tartaric acid added, g.	Nitric acid added, ml.	Phosphorus found, % Gravimetric	Volumetric
NBS 123[a]	40	15	5	1	0.0094, 0.0094	
					0.0097	
			10		0.0098	
			5	4	0.0095	
		120	5	1	0.0098	
	25	120	5	1	0.0096	
		60	5	1	0.0092	
SAA 108[b]	25	15	5	1	0.021, 0.021	0.019, 0.019
					0.021, 0.020	0.020
NBS 123b[c]	25	15	5	1	0.024, 0.024, 0.024	0.024, 0.024

NBS National Bureau of Standards.
SAA Standards Association of Australia.
2 g. samples taken.

[a] NBS value 0.007.
[b] SAA value 0.020.
[c] NBS value 0.024.

tilized, niobium might be retained in solution as oxyperchlorate and so not interfere in the separation of QPMo. The results of tests with solutions of NBS steel 13d, to which various amounts of niobium were added, indicated this was possible. However, when these conditions were applied to solutions of NBS steel 123 and SAA 108, gross contamination was found with the former, but only slight contamination with the latter.

(B). The use of tartaric acid as a complexing agent was explored. The tartaro complexes of niobium are hydrolyzed when boiled with mineral acid, but it was considered that if a mineral acid containing niobium in solution, or hydrolyzed but in colloidal form, were boiled with a sufficient amount of tartaric acid, the reverse reaction should take place with formation of a stable complex. When this treatment was applied to solutions of 4-gram samples of NBS steel 123, fine-textured, green precipitates separated on adding QMoR. The green color was thought to be due to reduction by tartaric acid. A small amount of nitric acid was therefore added to the solutions before adding QMoR. This resulted in yellow, coarse precipitates which could be filtered easily. When heated at 250° C. a tinge of green was given to the original yellow color, varying in depth from precipitate to precipitate.

To evaluate the modification, a number of tests were conducted under different experimental conditions with solutions of NBS ingot irons 55a and 55b and then on niobium-bearing steels, NBS samples 123 and 123b, and SAA 108 (Tables IV and V). The use of tartaric and nitric acids was without effect on the precipitation of QPMo from solutions of the ingot irons, and eliminated any significant effect by niobium. Large amounts of tartaric acid could be tolerated. Smaller amounts of nitric acid were also without effect, but large amounts led to some hydrolysis of the quinoline molybdate reagent with coprecipitation of molybdic acid with the QPMo. With the niobium-bearing steels, amounts of QMoR added and digestion times could be varied in a related way, so that there was no effect. A figure of 0.010% phosphorus was taken to be the correct result for NBS steel sample 123. This is a little higher than the NBS value of 0.007% and is considered to reflect the lower solubility of QPMo as compared with AmPMo. Results for NBS 123b show that the solubility effect becomes negligible with higher phosphorus contents. Heating the precipitates for 30 minutes at 250°C. resulted in colors ranging from slightly greenish yellow to pale green. No quantitative effect was detected.

Elimination of Tantalum Interference. Tantalum is similar to niobium in many of its chemical properties, and could be expected to behave similarly in the QPMo procedure. Experiment showed this to be the case. Tantalum was more readily separated at the close of the hydrobromic acid evaporation step than niobium and in a granular form, contrasting with the colloidal appearance of the niobium precipitate. A compound precipitated with QMoR in a manner similar to niobium, although there appeared to be a smaller tendency for the compound to form. Precipitate color, after heating at 250°C., in the presence of tantalum contamination was somewhat orange instead of the yellow of the pure precipitate. Table VI shows the contamination was eliminated by the use of tartaric and nitric acids. Interference by tantalum in phosphorus determinations in solutions of NBS steels 123 (Ta = 0.03%) and 123b (Ta = 0.20%) would therefore be eliminated by the use of this modification.

Elimination of Tungsten Interference. Precipitation of tungstic oxide was not complete when the solution of a tungsten-bearing steel was taken to reflux with perchloric acid. Additional amounts precipitated at the close of the hydrobromic acid evaporation step and occluded phosphorus. The addition of QMoR appeared to complete the precipitation. Interference was eliminated by the use of tartaric and nitric acids as for niobium and tantalum (Table VI).

Although the addition of tartaric and nitric acids, followed by boiling the solution for 10 to 15 minutes, eliminated interference, it did not entirely dissolve oxides of niobium, tantalum, and tungsten which might have precipitated at the close of the hydrobromic acid evaporation step. Presumably, the trace of precipitate remaining was "aged" material of small surface area which dissolved extremely slowly. Niobium and tantalum oxides would have no effect on the gravimetric difference method because of their insolubility in the QPMo extraction solvent. Table VI shows that tungsten was likewise insoluble in the dilute ammoniacal solvent.

Table VI. Effect of Variations in Experimental Conditions on Gravimetric Determination of Phosphorus with Quinoline Molybdate in NBS Steels 13d and 55b

(Tantalum and tungsten added)

Element	Amount added, mg.	NBS sample[a]	Added: Tartaric, g.	Added: Nitric, ml. (sp. gr. 1.42)	Phosphorus found, %
Tantalum	Nil	13d	Nil	Nil	0.0154, 0.0157
	2	13d	Nil	Nil	0.0165, 0.0165
					0.0168, 0.0168
	2	13d	5	1	0.0154, 0.0157
Tungsten	3	13d	5	1	0.0157, 0.0157
	3	13d	Nil	Nil	0.0143, 0.0143
	5	55b	5	1	0.0065, 0.0065
					0.0068
	Nil	55b	Nil	Nil	0.0070, 0.0068

[a] 4 g. (NBS 55b) and 2 g. (NBS 13d) taken.

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Application of the Pararosaniline–Formaldehyde Spectrophotometric Method to the Determination of Sulfur in Blister and Refined Copper

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▷ **The sensitive pararosaniline–formaldehyde chromogenic system has been applied to the determination of sulfur as sulfur dioxide in blister and refined copper. Several factors affecting the combustion, furnace temperature, use of fluxes, and combustion accelerators and retarders have been investigated. Conditions established are quite different from those recommended by other researchers in the analysis of high melting metals and alloys. The effect of temperature on color development of the sulfonic acid derivative of p-rosaniline and its stability has been established for the first time. Interferences from common impurities present in copper such as selenium, tellurium, arsenic, and antimony in concentrations up to 10 times that of sulfur have been investigated. A procedure has been devised allowing accurate determination of sulfur in copper in the range 2 to 500 p.p.m. The standard deviation at the 10-p.p.m. level is less than ± 1, and at the 100-p.p.m. level less than ± 4.**

SULFUR IN electrolytic copper has been determined in this laboratory during the past 10 years by the direct combustion–iodate titration method analogous to the ASTM method E30-56T for sulfur in steel and cast iron (*1*). The concentration range covered by the ASTM method is from 0.005 to 0.40% sulfur. Since the sulfur content of the electrolytic copper is well below the lowest concentration level given for steels, and since the size of the sample is limited by the size of the combustion boats to about 2 grams, considerably weaker potassium iodate solutions had to be used in the analysis of copper than in the analysis of steels. In this laboratory, a solution 0.0007*N* KIO_3 was used for titrating sulfur dioxide evolved from a 2-gram sample. It takes only 1 ml. of this solution to titrate the equivalent of 10 p.p.m. sulfur. Since the end point of such a dilute solution is quite elusive, variations in sulfur concentration of the order of several parts per million, although physically significant, can hardly be detected with any acceptable degree of accuracy. Thus the lack of precision of the combustion–iodate titration method is attributable mainly to the subjective evaluation of a highly deceptive end point. Because of this shortcoming, inherent in the titration of any dilute solution, efforts have been made by a number of researchers to devise methods allowing spectrophotometric evaluation of sulfur. A method that has had considerable success is that developed by West and Gaeke (*7*). It involves the absorption of sulfur dioxide in a solution of sodium tetrachloromercurate followed by addition of *p*-rosaniline hydrochloride and formaldehyde to form a purple-colored sulfonic acid derivative of pararosaniline (*4*). Larsen, Ross, and Ingber (*3*) applied the method to the analysis of sulfur in uranium trioxide, sodium zirconium fluoride, and hydrofluoric acid while Burke and Davis (*2*) applied the same technique to the analysis of sulfur in nickel and steel and their alloys. Unfortunately, Burke and Davis, in analyzing the only copper-base sample (Cast Bronze, NBS #52A) obtained a sulfur assay twice as high as indicated by the National Bureau of Standards. Invariably, and unmistakably, high results were also obtained in this laboratory when the same procedure was tried on a number of copper samples for which accurate assays obtained by the long gravimetric method were available.

Since the melting temperature of copper and its alloys is approximately 400° C. below that of steel and nickel alloys, and since the common impurities present in copper are quite different from those found in nickel and steel, we have undertaken to establish the exact furnace conditions, oxygen flow rate, use of fluxes, and effect of impurities as applicable to the determination of sulfur in electrolytic, blister, and anode copper by the *p*-rosaniline method.

At the time of reviewing this paper our attention was drawn to a recent work by Stange and Lehmann (*6*) dealing with the sulfur determination in copper alloys at the very high concentration level of 0.15%. Formaldehyde and fuchsine were used as the chromogenic reagents and the standardization was accomplished by means of steels of known sulfur content.

EXPERIMENTAL

Apparatus. FURNACE ASSEMBLY. Combustions were carried out in a Dietert High Temperature Varitemp Furnace Model No. 3420 (H. W. Dietert Co., Detroit, Mich.). The four Globar heating elements connected in series when new, can be reconnected in parallel or series-parallel as they age and the desired temperature can no longer be reached.

The combustion tube, "Sulcum-Burrell," has an overall length of 27 inches, with a restricted end 8 inches long × $^3/_{16}$-inch i.d. × $^1/_2$-inch o.d. and the larger section 19 inches long × $^7/_8$-inch i.d. × $1^1/_8$-inch o.d.

SAMPLE CONTAINERS. A white variety of Lecotherm combustion boats, long style, $3^3/_4$ inches long × $^9/_{16}$ inch wide × $^7/_{16}$ inch high (Canlab# 24-414) is sulfur-free and requires no blanking. A grey variety of Lecotherm boats should be ignited in a stream of oxygen for 10 minutes at 1150° C. and stored in a desiccator.

SAMPLE FORM. Samples in the form of drillings, rods, or grindings can all be run for sulfur. However, because of the possibility of surface contamination, samples of the smallest area, such as rods, are preferred. They are the easiest to clean, too, by abrasion with a file or by acid treatment.

SPECTROPHOTOMETER. Beckman B Model, with matched 1-cm. cells.

Reagents. SODIUM TETRACHLOROMERCURATE, 0.2*M*. Dissolve 23.4 grams of sodium chloride in about 200 ml. of water and transfer the solution into a beaker containing 54.4 grams of mercuric chloride. Mix to dissolve and dilute to 1 liter with water.

PARAROSANILINE HYDROCHLORIC ACID SOLUTION, 0.04%. Weigh 1 gram of pararosaniline hydrochloride into a 100-ml. beaker. Add 2 ml. of water and triturate with a glass rod to a smooth paste. Add gradually 85 ml. of water while stirring, then allow to stand for 24 hours. Stir again and filter through a 30-ml. medium porosity fritted glass crucible into a 100-ml. volumetric

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flask. Make up to the mark with water.

Pipet 4 ml. of the above solution into another 100-ml. volumetric flask and add 6 ml. of hydrochloric acid. Mix well, and dilute to the mark with water.

FORMALDEHYDE SOLUTION, 0.2%. Pipet 5 ml. of the 40% v./v. (37.2% w./v.) formaldehyde solution into a 1000-ml. flask and make up to the mark with water.

STANDARD SODIUM SULFITE SOLUTION, 100 P.P.M. OF SULFUR. Dissolve 0.396 gram of anhydrous sodium sulfite (approximate assay 99.4%) in water and make up to 1 liter.

CALIBRATION

Prepare, in accordance with the conditions set below, two calibration curves, one to be used for the lower sulfur contents found in electrolytic copper, the other for the higher sulfur contents found in blister copper.

(a) To the first series of eight 100-ml. volumetric flasks containing 12 ml. of sodium tetrachloromercurate solution add the equivalent of 0, 5, 10, 15, 20, 30, 40, and 50 μg. of sulfur. Now add from a buret 5 ml. of pararosaniline hydrochloric acid solution and 5 ml. of formaldehyde solution. Dilute to the mark with water and mix well.

(b) To the second series of eight 250-ml. volumetric flasks containing 30 ml. of sodium tetrachloromercurate solution add the equivalent of 0, 20, 40, 60, 80, 100, 120, and 150 μg. of sulfur. Add from a buret 12.5 ml. of pararosaniline hydrochloric acid solution and 12.5 ml. of formaldehyde solution. Dilute to the mark with water and mix well.

Place the flasks in a water bath at 25° ± 0.5° C. for 30 minutes and then read the absorbance at 560 mμ *vs.* water in the reference cell.

Deduct the absorbance of the reagent blank from that of the synthetic solutions. Plot two graphs showing absorbance due to the sulfur–pararosaniline compound in abscissa *vs.* micrograms of sulfur in the ordinate.

PROCEDURE

Pickle in 1:1 nitric acid for 3 to 5 seconds a portion of electrolytic or anode copper containing up to 40 μg. of sulfur and a portion of blister copper containing approximately 100 μg. of sulfur. Rinse the sample first in water, then in acetone, and finally air-dry.

Where air may be contaminated by sulfur dioxide, or other sulfur compounds such as mercaptans, it should be purified before being used for drying purposes. In such a case, the sample is placed in a 50-ml. beaker tightly closed with a two-hole rubber stopper provided with inlet and outlet tubes. Suction is applied at one end of the beaker and air drawn at the other end after passing, first, through an "acid tower" filled to one-third its capacity with 1:1 glacial acetic acid to remove sulfur dioxide, and second, through a "dry tower" filled with magnesium perchlorate to remove moisture.

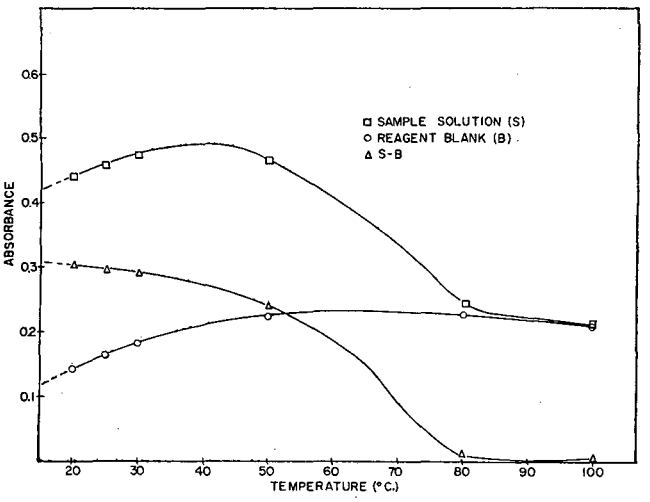

Figure 1. Absorbance of sulfur–pararosaniline compound as function of temperature

50 μg. of sulfur added to all sample solutions

Weigh the dry sample and place it in a combustion boat. Introduce the boat into the hot zone of the combustion tube with the furnace temperature set at 1150° ± 10° C. and oxygen flow at 1 liter per minute. Sulfur dioxide gas is scrubbed into a 50-ml. cylinder containing 12 ml. of sodium tetrachloromercurate. The total combustion time is 5 minutes signaled by an alarm clock. Treat similarly an empty combustion boat for the reagent blank.

Now remove the bubbler from the scrubber solution after rinsing it with water. In the case of electrolytic and anode copper, transfer the scrubber solution into a 100-ml. volumetric flask and add from a buret 5 ml. of pararosaniline solution and 5 ml. of formaldehyde solution. In the case of blister copper, transfer the scrubber solution into a 250-ml. flask and add 12.5 ml. of both pararosaniline and formaldehyde solution. Make up to the mark with water and mix.

Place the flask in a water bath at 25° ± 0.5° C. for 30 minutes and read the absorbances of the reagent blank and sample solutions at 560 mμ.

DISCUSSION

Color Development. Because of the existence on the market of a variety of pararosaniline substances of varying dye potency, the conditions of the color development established by other researchers could not be applied without verification. Pate, Lodge, and Wartburg (*5*) listed 18 pararosaniline compounds from four suppliers with identical or vaguely differing designations, each having a different label assay. The rosaniline hydrochloride used in this laboratory (Eastman P 1378) was not among the 18 listed. The sulfonic acid derivative of the *p*-rosaniline hydrochloride solution showed the maximum absorbance at 560 mμ. Beer's law was strictly obeyed for the concentration range from 5 to 120 μg. of sulfur. The maximum absorbance was reached when the color was allowed to develop for 30 minutes. In the course of this investigation it was also established that temperature has considerably greater effect on color development than realized by any previous researchers. West and Gaeke (*7*), who did pioneering studies related to the formation of the sulfur–pararosaniline complex, had measured absorbances of standard solutions containing varying amounts of sulfur against the reagent blank after the color was developed in a thermostat at six selected temperatures in the range 11° to 30° C. No appreciable variation in the absorbances of the colored compounds was observed from one temperature to another. This experience, however, although basically confirmed in the course of our investigation, was somewhat misleading. This is due to the fact that for the limited temperature range considered, the absorbances of the blank and the sample solution had increased with temperature by approximately the same amount, the difference between the two remaining about constant by mere coincidence. As a result, the absorbance attributable to the colored sulfur compound was assumed unaffected by the temperature variations. However, if the absorbances of the reagent blank and the sample solutions were measured separately for each temperature, and moreover, the temperature range extended to higher values, then the unequivocal relationship between the intensity of the color and the temperature at which the color was developed would have been established. The relevant data obtained in this laboratory are plotted in Figure 1. The graph shows clearly that the absorbance of the reagent blank increases appreciably with temperatures up to 80° C. when it levels off. The

absorbance of the sample solution, on the other hand, first increases with temperature reaching a maximum at about 40° C., then rapidly decreases as apparently the sulfur – pararosaniline compound decomposes at higher temperatures, until finally at 100° C. the absorbance of the sample solution approximates that of the reagent blank. All solutions were heated for 30 minutes in a water bath set at the temperatures shown on the graph, followed (where required) by rapid cooling to 25° C. in ice water. Absorbances were measured immediately thereafter. The decomposition of the colored sulfur compound was complete after heating the solution in the steam bath for only 10 minutes. The solution, however, remained perfectly clear.

In the temperature range 20° to 30° C., and for the sulfur concentration considered, the absorbance of the reagent blank increases with temperature more, proportionally, than the absorbance of the sample solution. The difference between the two absorbances under the experimental conditions remained constant by mere coincidence. In view of the great variety of pararosaniline compounds of differing properties available on the market, and in view also of the desirability of extending the application of the procedure to as wide a sulfur concentration range as possible, it is felt that the knowledge of the mechanism of the color development as a function of temperature is essential.

Factors Affecting Combustion. Setting free all sulfur present in the sample as sulfur dioxide is a prerequisite of an accurate sulfur analysis. The efficiency of combustion and the resulting recovery of sulfur depend on the exact control of the following factors: oxygen flow rate, furnace temperature, time of combustion, use of fluxes, and combustion accelerators or retarders. We have found that the same oxygen flow rate of 1 liter per minute used in accordance with our combustion–iodate titration method is also the most satisfactory in the combustion – spectrophotometric method. The minimum safe furnace temperature assuring complete fusion of copper and release of sulfur dioxide in a 5-minute period is 1150° C., or about 70° higher than the melting temperature of copper. At higher temperatures, molten copper tends to leak through the boat causing breakages of the combustion tube. Fluxes, such as iron powder, added when combustion is carried out in induction furnaces are not needed when the radiant-heating Dietert Varitemp Furnace is used. Addition of accelerators, such as tinned copper strips, customary in the analysis of high melting nickel and steel alloys, is not required in the analysis of copper which melts promptly. Thus, in eliminating fluxes and accelerators causing high sulfur blanks, better accuracy can be achieved. The use of combustion retarders such as alundum powder was also considered in this laboratory with a view to slowing down the rate of sulfur dioxide evolution. For example, the addition of retarders was important in estimating sulfur dioxide by titration. However, in this instance, the sodium tetrachloromercurate solution was an excellent absorbent of sulfur dioxide under the conditions given.

Accuracy of the Procedure. As mentioned in the introduction, Burke and Davis (*2*) in applying their procedure devised for the determination of sulfur in nickel and steel to the only copper-base metal obtained a result twice as high as given by the NBS. The presumable reason for obtaining a high result, suggested from our own experience, is that the combustion was carried out at a temperature approximately 400° C. above the melting temperature for copper. At such a high temperature molten copper penetrates completely the combustion boat, releasing sulfur contained in the boat itself that otherwise is not liberated in the process of blanking the empty boat at the same temperature.

In analyzing the NBS Cast Bronze No. 52C for sulfur, the result obtained in this laboratory agreed closely with the NBS certified value. The data obtained from quadruplicate runs performed on two consecutive days are listed in Table I. The results obtained in this laboratory may appear to be slightly on the high side. However, it has to be pointed out that the fact that NBS does not report the fourth decimal figure is indicative of the inadequacy of the combustion–iodate titration procedure used by the laboratories cooperating in the NBS program.

The great accuracy of the pararosaniline procedure for higher sulfur contents was substantiated by analyzing three anode and blister copper samples containing, on the basis of the most careful gravimetric determination of sulfur as barium sulfate, 40, 115, and 370 p.p.m. sulfur. The results obtained by the spectrophotometric method agreed within ±2 p.p.m. for the first sample, and within ±4 and ±10 p.p.m. for the second and third samples, respectively.

Table I. Sulfur in NBS Cast Bronze No. 52C

(Certified Value, 0.002%)

	S, %		S, %
First day	0.0026	Second day	0.0024
	0.0028		0.0024
	0.0026		0.0026
	0.0025		0.0026
Av.	0.0026	Av.	0.0025

Table II. Precision of Sulfur Determination

	S, %	
	Wire bar	Anode copper
First day	0.0010	0.0116
	0.0008	0.0120
	0.0009	0.0116
	0.0010	0.0124
Av.	0.0009	0.0119
Second day	0.0009	0.0118
	.0009	.0121
	0.0009	0.0120
	0.0010	0.0112
Av.	0.0009	0.0118
Std. dev.	0.0001	0.00037

Table III. Effect of Some Common Impurities Present in Copper on Sulfur Analysis

All solutions contained 25 μg. of sulfur

	Impurity added, g.	Sulfur found, g.
Selenium (Se^{+4})	0	25
	25	26
	125	24
	250	25
	Av.	25
Tellurium (Te^{+4})	0	25
	25	26
	125	26
	250	24
	Av.	25
Arsenic (As^{+3})	0	24
	25	23
	125	25
	250	24
	Av.	24
Antimony (Sb^{+3})	0	24
	25	25
	125	25
	250	26
	Av.	25

Table IV. Typical Analyses of Various Copper Materials

Material	Lot	S, %
Wire bar	10024-1	0.0009
		0.0010
	10026-2	0.0011
		0.0012
	11009-1	0.0008
		0.0007
	11012-1	0.0009
		0.0009
High purity research copper		0.0002
		0.0002
		0.0002
		0.0001
Blister copper	10662	0.0503
		0.0510
	15068	0.0385
		0.0375
Anode copper	14475	0.0065
		0.0060
	15842	0.0046
		0.0048

Since the results above 100 p.p.m. are customarily reported as percentage to the third decimal figure, the agreement between the two procedures can be stated as being within ±0.001% sulfur.

Precision. A sample of wire bar copper and a sample of anode copper were each run in quadruplicate on two consecutive days. The results are shown in Table II.

While the standard deviation for both wire bar and anode copper calculated on eight runs is extremely low, it is still more gratifying to note that the averages of quadruplicate runs obtained on two different days are practically identical.

Interferences. Selenium, tellurium, arsenic, and antimony would all interfere in the sulfur analysis of copper by the combustion–iodate titration method if introduced into the reaction vessel. This is why no attempt was ever made to determine sulfur in anode or blister copper by the combustion–iodate method. These materials were always analyzed for sulfur by the long gravimetric method as barium sulfate. It is, therefore, obvious that considerable saving in time would be realized if the pararosaniline method were applicable to the analysis of sulfur in anode and blister copper. To establish the possible interferences, increasing amounts of selenium, tellurium, arsenic, and antimony were added to synthetic solutions containing 25 μg. of sulfur and the color was developed in accordance with the standard procedure. The results obtained are shown in Table III. The data indicate clearly that none of the four impurities considered, present in concentrations up to 10 times that of sulfur, has any effect whatsoever on the precision and accuracy of sulfur analysis.

RESULTS

The versatility of the procedure as to the various copper products and the wide range of applicability as to the actual sulfur content are self-evident from Table IV.

The duplicate assays agree closely for the whole wide range of sulfur concentrations.

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Flame Photometric Determination of Manganese in Copper and Steel Alloys

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▶ Manganese is extracted from copper and steel alloys with 2-thenoyltrifluoroacetone (TTA) for a rapid flame spectrophotometric determination of manganese. Complexing agents are employed to prevent the extraction of other ions by TTA. Triplicate samples of alloys containing from 0.02 to 1.00% manganese (0.05 to 0.50 mg. of manganese) were analyzed within 45 minutes with a standard deviation of ±0.01. The effect of foreign ions and reaction conditions for the determination is reported.

ANALYTICAL APPLICATIONS of 2-thenoyltrifluoroacetone (TTA) have been reviewed by De and Khopkar (*3*). Although this reagent has been employed primarily for the separation of metals, it has also been employed as a reagent for the flame photometric determination of aluminum (*7*) and yttrium (*1*). Cheng (*2*) reported in a study on masking that in the presence of tartrate at pH 6 to 10, only manganese and cobalt formed a precipitate with TTA.

Flame photometric methods for manganese have been reported (*5, 6, 9*). Dippel and Bricker (*6*) reported serious inhibition of the manganese emission from constituents normally present in manganese bronze, nickel–copper alloys, aluminum alloys, and stainless steels, and employed the method of standard addition to overcome this inhibition. Dean and Cain (*4*) employed a selective extraction procedure for manganese with diethyldithiocarbamate which required several extractions to remove manganese. Very little work has been performed to investigate the properties of solids formed in TTA reactions. This investigation reports a study of the reaction of manganese with TTA and the application of this reaction for a rapid determination of manganese by means of flame spectrophotometry.

EXPERIMENTAL

Reagents. Standard manganese chloride solutions (0.0670*M* and 0.0500 mg. of manganese per ml.) were prepared by dissolving 13.00 grams of $MnCl_2 \cdot 4H_2O$ and 0.1805 gram of $MnCl_2 \cdot 4H_2O$, respectively, in water and diluting to 1 liter. These solutions were standardized by titration at pH 10 against standardized EDTA using Calmagite (G. Frederick Smith Chemical Co., Columbus, Ohio) in ethanol as the indicator. Other solutions were made by appropriate dilution.

2-Thenoyltrifluoroacetone solution 0.055*M* for gravimetric investigations, was prepared by dissolving 6.2 grams of the technical reagent in 125 ml. of acetone and diluting to 500 ml. with water.

2-Thenoyltrifluoroacetone solution, 0.055*M*, for the extraction of manganese was prepared by dissolving 6.2 grams of the reagent in 166 ml. of 4-methyl-2-pentanone and diluting to 500 ml. with 2-octanone.

Phosphate buffer, pH 8, was prepared by mixing 468 ml. of 0.1*M* NaOH, 500 ml. of 0.1*M* KH_2PO_4, and 32 ml. of water.

Ammonia buffer, pH 8, was prepared by dissolving 70 grams of NH_4Cl in 500 ml. of 15*M* NH_4OH, adding sufficient hydrochloric acid to adjust to pH 8 as indicated on a pH meter, and diluting to 1 liter with water.

Mixed buffer, pH 8, was prepared by mixing 200 ml. of the ammonia buffer and 100 ml. of phosphate buffer.

Apparatus. A Beckman Model DU flame spectrophotometer with SERA unit, Beckman Model A. C. power supply, and RCA 1P28 photomultiplier tube was used. Data were recorded on a 5-mv. Bristol recorder with a 1-second pen response. A Millivac-Poly-Functionist (Millivac Instrument Corp., Schenectady, N. Y.) was employed to adjust the zero position of the pen on the recorder.

Table I. Qualitative Tests

Ion	Phosphate buffer + TTA	Ammonia buffer + TTA	Masking mixture + mixed buffer + TTA[b]
Al(III)	N.C.[a]	N.C.	turbid
Au(III)	sl. turbid	sl. turbid	sl. turbid
Ba(II)	N.C.	N.C.	N.C.
Bi(III)	N.C.	N.C.	N.C.
Ca(II)	N.C.	N.C.	N.C.
Cd(II)	white ppt.	white ppt.	white ppt.
Ce(IV)	N.C.	yellow ppt.	N.C.
Co(II)	yellow ppt.	yellow ppt.	yellow ppt.
Cr(III)	sl. turbid	sl. turbid	white ppt.
Cu(II)	yellow ppt.	yellow ppt.	yellow ppt.
Fe(II)	purple ppt.	blue ppt.	blue soln.
Fe(III)	sl. turbid	brown ppt.	turbid
Hg(II)	N.C.	turbid	sl. turbid
La(III)	N.C.	white ppt.	N.C.
Mg(II)	N.C.	white ppt.	white ppt.
Mn(II)	yellow ppt.	yellow ppt.	yellow ppt.
Ni(II)	yellow ppt.	yellow ppt.	white ppt.
Os(VIII)	N.C.	yellow soln.	N.C.
Pb(II)	turbid	white ppt.	white ppt.
Pd(II)	N.C.	loss of color	N.C.
Pt(IV)	N.C.	yellow ppt.	turbid
Rh(III)	N.C.	N.C.	N.C.
Sb(III)	N.C.	N.C.	N.C.
Sn(II)	N.C.	N.C.	N.C.
Sn(IV)	N.C.	N.C.	N.C.
Sr(II)	N.C.	N.C.	N.C.
Th(IV)	sl. turbid	sl. turbid	sl. turbid
Ti(IV)	N.C.	white ppt.	N.C.
U(IV)	N.C.	yellow ppt.	N.C.
Zn(II)	N.C.	N.C.	white ppt.
Zr(IV)	N.C.	N.C.	N.C.

[a] No change.
[b] Masking mixture consisting of equal parts by volume of 2*M* tartaric acid and 2*M* sodium fluoride.

Table II. Weights of Manganese–TTA Precipitates, Grams

All samples contained 50 ml. of 0.006700*M* manganese solution.

Phosphate-buffered	Phosphate-buffered, NaOH	Ammonia-buffered	Ammonia + phosphate buffered
0.3165	0.2461	0.2451	0.2377
...	0.2475	0.2309	0.2396
...	0.2332	0.2631	0.2375
...	0.2119	0.2611	0.2382
...	...	0.2835	0.2339
...	...	...	0.1106[a]
...	...	...	0.1102[a]

[a] 25 ml. of 0.006700*M* manganese solution.

These instrument settings were employed unless otherwise specified:

Sensitivity control, SERA unit	5 turns counter-clockwise
Phototube resistor, megohms	22
Slit, mm.	0.040
Sensitivity switch position of power supply	4
Millivac, voltage range ($E_{d.c.}$)	100%
Oxygen pressure	6 p.s.i., gauge
Acetylene pressure	2 p.s.i., gauge

A Kofler micro hot stage (Arthur H. Thomas, Philadelphia, Pa.) was used.

Procedure. The Precipitation of Manganese with TTA. In aqueous solutions at pH 8, manganese reacts with TTA to form a yellow precipitate. Qualitative tests, as indicated by the formation of a precipitate, were performed to ascertain if other ions reacted with TTA. These tests included experiments to indicate the effect of buffer addition, buffer composition, masking agents, and the sequence of addition of the reagents. The procedure employed in the qualitative tests consisted of mixing 2 ml. of 0.01*M* solution of the cation to be tested with 2 ml. of the buffer solution, 2 ml. of masking mixture, and 2 ml. of TTA in acetone solution according to the sequence indicated in Table I. The masking mixture was prepared by mixing equal volumes of the tartrate and fluoride solutions. The results of the qualitative tests indicated that the least interference for a gravimetric procedure of manganese would be the addition of the masking agent mixture to the sample, followed by the addition of the phosphate buffer and finally TTA.

To ascertain if the reaction were quantitative, 15 ml. of buffer and 75 ml. of TTA solution were added to samples containing 50 ml. of 0.0067*M* manganese solution. The samples were digested on a steam bath for 2 to 6 hours, primarily to evaporate acetone. The precipitate was filtered through a fine-porosity sintered-glass funnel, washed with water, and dried to constant weight at 110° C.

Manganese–TTA precipitates were prepared using phosphate buffer, phosphate buffer and sodium hydroxide, ammonia buffer, and varying ratios of phosphate and ammonia buffer. The phosphate buffer and sodium hydroxide mixture were used because the phosphate buffer did not maintain a basic pH during the precipitation. For the samples listed in Table II, the manganese was completely precipitated, since the titration of the filtrate with EDTA at pH 10 required only 1 to 2 drops of 0.0245*M* EDTA to change the color of the indicator. As indicated in Table II, the mixed buffer gave reproducible weights of precipitate of 0.2374 gram, ±3.5 mg. for 50 ml. of manganese solution. However, when half of the amount of manganese was taken, the weight of the precipitate was not half of the previous weight, but 0.1104 gram, ±0.02 mg.

The precipitates of manganese with TTA under the reaction conditions were yellow in appearance; the precipitate formed in the phosphate-buffered solution was darker yellow and coarser than the precipitate formed with the ammonia buffered or mixed buffered solution. Using a Kofler hot stage, the precipitates formed in the ammonia-buffered or mixed-buffered solution were observed to decompose at 225° to 226° C. The phosphate-buffered precipitate darkened but did not decompose at 346° C. The precipitates were soluble in acetone and other ketones like 2-octanone, 4-methyl-2-pentanone; they were insoluble in toluene, carbon tetrachloride, and benzene. The infrared spectra (*8*) of the ammonia- and mixed-buffered precipitates showed an

Table III. Microanalysis of Manganese–TTA Precipitates

Sample	Mn, %	C, %	H, %	N, %	P, %	S, %
Phosphate buffered solution + NaOH	14.84	37.74	2.03	...	...	12.25
	14.52	38.13	1.94	...	...	12.12
Ammonia buffered solution	6.37	40.34	4.23	1.47	...	13.45
	5.53	40.70	4.33	1.36	...	13.15
Mixed buffered solution	6.61	38.71	3.13	3.78	...	13.15
	6.19	38.92	3.23	3.48	...	13.38
Theoretical percentages						
$MnTTA\ (MnC_8H_4O_2SF_3)$	19.92	34.78	1.45	...	...	11.59
$Mn(TTA)_2$–$(MnC_{16}H_8O_4S_2F_6)$	11.06	38.63	1.61	...	...	12.87
$Mn_2(TTA)_3$–$(Mn_2C_{24}H_{12}O_6S_3F_9)$	14.23	37.25	1.55	...	...	12.41
$Mn(TTA)_4$–$(MnC_{32}H_{16}O_8S_4F_{12})$	5.85	40.89	1.70	...	...	13.63

additional absorption peak at 3.15 microns and an alteration of the absorption peaks at 6.8 and 6.9 microns compared to the phosphate-buffered material. These spectra peaks are indicative of N–H bonding and are in agreement with the presence of nitrogen reported in the microanalysis of the precipitate. The spectra were recorded on a Perkin-Elmer Infracord, using the KBr pellet technique. Microanalyses of the precipitates are reported in Table III.

Extraction of Manganese from Aqueous Solutions with TTA. Because of the solubility of the manganese–TTA complex in ketone solvents, it seemed possible that manganese might be separated by an extraction procedure in which TTA would be dissolved in an organic ketone immiscible with water. Although the manganese–TTA complex is soluble in 2-octanone, when only 2-octanone was employed as the extractant solvent, excess TTA tended to precipitate out of the solution on extraction. Incomplete extraction of manganese was observed when 4-methyl-2-pentanone was employed as the extracting solvent. Different ratios of these two ketones were tested for extraction and a mixture (by volume) of 2 parts of 2-octanone and 1 part 4-methyl-2-pentanone proved most effective. The completeness of the extraction and the effect of pH were determined by adding to 2 ml. of 0.0067*M* manganese solution, 4 ml. of mixed buffer and sufficient hydrochloric acid or ammonium hydroxide to vary the pH between 1.2 and 10.1, and diluting the aqueous solution to a volume of 50 ml. with water. The manganese in the aqueous solution was then extracted by shaking for $^1/_2$ minute with 20 ml. of 0.055*M* TTA dissolved in the ketone mixture. Following extraction, the aqueous solution was titrated with standardized EDTA at pH 10, using Calmagite as indicator to determine the amount of manganese which remained unextracted. The results, reported in Figure 1, show that more than 97% of the manganese was separated by one extraction at pH 8.4.

Calibration Curve for Flame Photometry. To a series of 250-ml. separatory funnels containing 20 ml. of water was added, respectively, 0.00, 1.00, 2.00, 4.00, 6.00, 8.00, and 10.00 ml. of standard manganese solution containing 0.0500 mg. of manganese per ml., and sufficient mixed buffer (4 to 10 ml.) to adjust the solution to pH 8. The manganese was extracted with 10.00 ml. of 0.055*M* TTA mixed-ketone solution by shaking the solution in a separatory funnel for $^1/_2$ minute. The aqueous layer was discarded and the organic layer was transferred to the sample cup of the instrument. The flame photometer was set at a fixed wavelength of 405 mμ (maximum sensitivity for manganese in this system) and the emission of manganese from the organic solvent was recorded. The recorder was set at zero on the chart paper by means of the Millivac unit, with the flame of the atomizing burner as the reference standard. The sample of highest manganese concentration was employed as the standard for setting the full scale range of the recorder, using the SERA unit for recorder span adjustment. The sample was burned for 1 to 2 minutes and the average distance from the base line to the emission intensity, as indicated on the recorder, was measured. A linear calibration curve was obtained by plotting the emission intensity *vs.* the concentration of manganese.

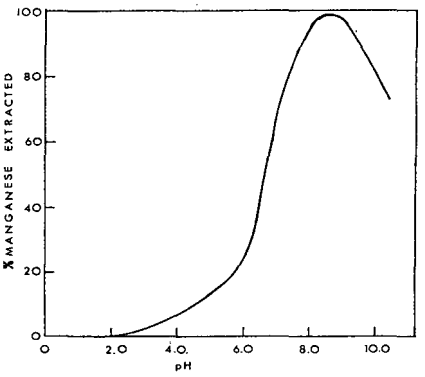

Figure 1. Effect of pH on manganese extraction

RESULTS

Effects of Foreign Ions and Masking Agents. To test the effect of foreign ions and masking agents on the emission of manganese, a series of samples were prepared as indicated in Table IV. The measurements for manganese were made at wavelength of 405 mμ, and also by scanning over this wavelength with the SERA unit. In both cases the results for the percentage of manganese detected were the same within experimental error and are reported in Table IV.

Analysis of Alloys. National Bureau of Standards zinc base alloy and manganese bronze samples, weigh-

Table IV. Manganese Recovered (%) Flame Photometrically in the Presence of Foreign Ions

Foreign ion[a]	Absence of masking agent	NaF[b]	Tartaric acid[c]	Masking mixture[d]
Mn(II) alone	100.0	100.0	100.0	100.0
Al(III)	89.4	101.0	99.3	97.3
Au(III)	101.1	100.0	100.0	100.0
Ba(II)	91.9	97.6	99.3	98.3
Bi(III)	93.5	98.5	95.1	96.2
Ca(II)	92.3	97.0	103.0	103.0
Cd(II)	100.0	100.0	100.0	100.0
Ce(IV)	84.0	88.7	96.9	88.7
Co(II)	86.2	81.1	80.4	78.8
Cr(III)	82.7	71.8	52.7	59.0
Cu(II)	98.7	100.0	100.0	100.0
Fe(II)	81.9	91.0	94.8	90.4
Fe(III)	81.4	84.2	94.4	93.4
Ga(III)	100.0	100.0	100.0	100.0
Hg(II)	102.3	100.0	100.0	100.0
K(I)	98.0	97.8	98.0	97.9
La(III)	58.7	87.8	90.7	96.6
Mg(II)	79.4	100.2	99.6	99.6
Na(I)	100.0	100.0	100.0	100.0
Ni(II)	88.3	97.6	94.8	95.9
Os(VIII)	100.0	100.0	100.0	100.0
Pb(II)	101.1	96.0	100.2	99.4
Pd(II)	102.0	100.0	100.0	100.0
Pt(IV)	98.8	100.0	100.0	100.0
Rh(III)	100.0	100.0	100.0	100.0
Sb(III)	98.8	100.0	100.0	100.0
Sn(II)	100.0	100.0	100.0	100.0
Sn(IV)	100.0	100.0	100.0	100.0
Sr(II)	100.0	100.0	100.0	100.0
Th(IV)	70.8	92.9	93.6	92.4
Ti(IV)	97.1	100.0	100.0	100.0
U(IV)	96.4	100.0	100.0	100.0
Zn(II)	98.8	100.0	100.0	100.0
Zr(IV)	94.6	94.5	95.3	100.5
EDTA	27.3	27.6	27.3	27.5
NTA	42.7	42.9	42.7	42.7
Cyanide[e]	60.2	60.2	60.2	60.2
Trien sulfate[f]	100.0	100.0	100.0	100.0

All samples contained 2 ml. of 0.0067*M* manganese solution.

[a] 2 ml. of 0.01*M* foreign ion solution.
[b] Ion in presence of 5 ml. of 2*M* NaF.
[c] Ion in presence of 5 ml. of 2*M* tartaric acid.
[d] Ion in presence of 5 ml. of an equal mixture of 2*M* NaF and 2*M* tartaric acid.
[e] 1 ml. of 2*M* sodium cyanide.
[f] Ion in presence of 230 mg. of triethenetetraamine disulfate.

Table V. Analysis of Manganese in Alloys

			Manganese, % Present	Determined
NBS	94b	Zinc Base Alloy	0.014	0.015 0.014 0.014
NBS	62D	Manganese Bronze	0.66	0.66 0.67
NBS	86C	Aluminum Alloy	0.041	0.043 0.040 0.039
NBS	159	Steel	0.802	0.78 0.80 0.80
NBS	10d	Steel Bessemer	0.914	0.92 0.91

ing from 0.500 to 0.200 gram (so as to contain from 0.05 to 0.50 mg. of manganese), were dissolved in 2.5 ml. of 1:1 nitric acid by heating on a hot plate. The samples were evaporated to one quarter of their original volume; 5 ml. of water were added, and the solution was digested for 10 minutes. After cooling to room temperature, the solution was neutralized with ammonium hydroxide. Ten milliliters of 2*M* tartaric acid and 10 ml. of 2*M* sodium fluoride were added. The solution was reneutralized with ammonium hydroxide, 4 ml. of mixed buffer were added, and manganese was extracted with 10 ml. of 0.055*M* TTA. The extract was transferred to the flame photometer, the emission compared with the calibration curve, and the percentage of manganese calculated. The results are reported in Table V.

The aluminum alloy was dissolved in a mixture of 2.5 ml. of 1:1 nitric acid and 2.5 ml. of 1:1 hydrochloric acid. The above procedure was then followed for the analysis of the alloy (Table V).

Steel samples were dissolved in 10 ml. of 1:30 nitric acid. The above procedure was followed except that the complexing agent was added prior to pH adjustment and the amount of tartaric acid was doubled (Table V).

DISCUSSION

Extraction of manganese with TTA in the presence of masking agents prevents inhibition in flame photometry of manganese emission by constituents normally present in samples. Consequently, the percentage of manganese present in alloys could be determined directly from the calibration curve, eliminating the necessity of a standard addition method. Samples containing between 0.05 to 0.50 mg. of manganese in 50 ml. of aqueous solution were extracted with 10 ml. of the TTA–ketone solution. Aspirating the organic extract containing the manganese directly into the atomizing burner enhanced the emission of manganese over 10-fold, compared with an aqueous solution containing an equivalent amount of manganese, and permitted the construction of a calibration curve that covered the range of 0.05 to 0.50 mg. of manganese per 10 ml. of organic extract. Triplicate samples of alloys which contained from 0.02 to 1.00% manganese (0.05 to 0.50 mg. of manganese) were dissolved and analyzed within 45 minutes. From the calibration curve run in triplicate (18 points) a standard deviation of ±0.01 was calculated for this method.

Although the masking mixture must be added before manganese is extracted, the masking mixture can be added prior to, or subsequent to the adjustment of the samples to pH 8. It was observed with the copper samples that a small amount of precipitate formed when the masking mixture was added prior to pH adjustment. However, by rapid addition of ammonium hydroxide for preliminary pH adjustment, the precipitation of copper hydroxide could be prevented. No precipitate then formed in these samples when the masking mixture was added subsequent to final pH adjustment. In steel samples it is necessary to add the masking mixture prior to pH adjustment to prevent the precipitation of iron hydroxide. The tests conducted for possible interferences (Table IV) are indicative of the interferences which might be expected. The presence of other ions in the sample affect the procedure; for example, NBS 159 steel sample, which was satisfactorily analyzed, contains a higher chromium content than manganese. In interference tests, chromium of this concentration in the presence of manganese inhibited manganese emission.

Considerable work has been done to investigate the reaction and type of complex formed in solution by various ions with TTA. However, no work appears to have been done to characterize and isolate these organic chelates of manganese as precipitates. Microanalyses of the precipitates indicates that nonstoichiometric Mn–TTA precipitates are formed. It is interesting to point out from the gravimetric results that the mixture of Mn–TTA complexes which is formed depends on the precipitating conditions. In ammoniacal solution, an ammonium–Mn–TTA salt appears to be formed. The infrared and thermal decomposition studies confirm that the formation of precipitates is dependent upon the buffer composition. Microanalysis shows that even in the case of the phosphate buffered precipitate, no phosphate was present in the precipitate. Under these reaction conditions, therefore, no manganese phosphate was formed. It is noteworthy that a highly selective manganese determination can be performed under such conditions where nonstoichiometric precipitation takes place, by extracting all mixtures of the manganese precipitate with an organic solvent.

This flame photometric method of manganese analysis does not appear to be limited only to the samples reported; it could probably also be applied to the determination of manganese in tissue, plants, glass, food, etc. Although other masking agents may be used, the choice is somewhat limited since (ethylenedinitrilo)tetraacetic acid, nitrilotriacetic acid, and cyanide formed sufficiently strong complexes with manganese to prevent its complete extraction with TTA. Fluoride, tartrate, or their mixture may be used as masking agents. In the steel samples, tartrate alone did not prevent the precipitation of iron. Fluoride also complexes aluminum and thorium. The analysis of magnesium alloys by this method was unsuccessful. With the buffer composition used, the masking mixture was unable to prevent the precipitation of magnesium (probably as magnesium ammonium phosphate), when magnesium was present in large excess. Triethenetetraamine disulfate may also be used as a masking agent, and at present studies are being made to determine its applicability to samples for this type of flame photometric analysis.

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A Continuous Electrolytic Analyzer for Acidic or Basic Components of Process Gas Streams

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▶ An apparatus for continuously determining trace concentrations of acidic or basic components of a gas stream is described. The analysis is carried out by scrubbing the gas stream with an aqueous salt solution and titrating the dissolved species with electrolytically generated hydronium or hydroxide ions. A constant pH is maintained within the titration vessel, the current required for this being a measure of the acid or base content of the gas stream. The apparatus has been used successfully in monitoring levels of HCl content from 0.1 to over 300 p.p.m. by volume.

IT IS FREQUENTLY necessary, particularly in the case of petroleum refinery processes, to measure the concentration of acidic or basic constituents present in trace amounts in gas streams. Ordinarily this is accomplished by batch techniques, whereby the gas sample is scrubbed of the trace component which is subsequently determined by one of a variety of neutralization, redox, or gravimetric methods. The advantages of a continuous method for carrying out this type of analysis are obvious; and the electrolytic method herein described is simple, sensitive, and rapidly responsive to changes.

Instruments and methods similar to the one below have been described by Coulson and Cavanagh (*2*) and Briglio, Brockman, and Shaffer (*1*). The apparatus employed by them were not designed for long-range, continuous use, however. A discussion of helpful experimental techniques in coulometric titrations in general has been given by Fuchs and Quadt (*3*).

The method employed with the present apparatus consists of continuously titrating the acidic or basic constituent absorbed in aqueous solution from the gas stream with electrolytically generated hydronium or hydroxide ions. A constant pH is maintained within the titrating vessel, the current required for this being a measure of the acidic or basic content of the gas stream. The concentration of these components in the gas is readily obtained with a knowledge of the total gas flow through the titrating vessel.

To exemplify the reactions taking place within the electrolytic cell, assume the determination of an acidic component such as HCl wherein the titration is carried out in the pH region 5 to 8. The reactions occurring at the smooth platinum electrodes within the complete electrolytic cell are the following:

$$2H_2O + 2\bar{e} = 2OH^- + H_2 \quad \text{(Cathode)}$$

$$3H_2O = 2H_3O^+ + {}^1/_2O_2 + 2\bar{e} \quad \text{(Anode)}$$

Mixing of the anode and cathode compartments is prevented, and absorption with subsequent neutralization of HCl is carried out in the cathode chamber. If the neutralization reaction is sufficiently rapid and absorption of HCl is quantitative, the rate of addition of titrating agent—i.e., the magnitude of the titrating current—is proportional to the concentration of HCl present in the gas stream. As proved by comparison with batch methods, these conditions are met with the present apparatus.

The titrating current generated by the apparatus is related to the quantity of HCl titrated per unit time by Faraday's law:

$$e = 3.727 \times 10^{-2} I$$

where e is equivalents of HCl titrated (or OH^- generated) per hour. I is the generating current in amperes.

Like considerations also apply to the titration of basic components where the titrating vessel is now the anode compartment of the electrolytic cell. Mixtures of acids or of bases, whether all strong or some strong and some weak, may be determined by proper choice of control pH in the titrating vessel. Titration of a strong acid component only in the presence of a weak one is possible in this manner. This is analogous to the titration of mixed acids by conventional batch methods. When dealing with mixtures of acids and bases, the net acidity in aqueous solution would be titrated. The present apparatus features an automatic reversal of polarity for conversion from acid-type to base-type titrations. This finds use in situations where the gas stream may contain both acidic and basic components which may alternate in being dominant.

EXPERIMENTAL

Apparatus. The titration procedure is carried out by means of the arrangement shown schematically in Figure 1. The gas stream is scrubbed of HCl (in our sample case) by passing through the cathode chamber of the electrolytic cell. A Beckman No. 39183 combination electrode assembly within the cathode compartment allows monitoring of the pH. The pH signal is fed to a Beckman Model J pH Analyzer which serves as amplifier for the coulometer controlling the hydroxide generating current. These basic ions are produced at the smooth platinum electrode within the cathode chamber. A recorder may be used for following the level of HCl being determined.

Titration Vessel. Figure 2 is a detailed drawing of one model of the glass electrolytic titration vessel. To provide mixing of the anode compartment (in the case of acidic titration), and thereby prevent polarization of

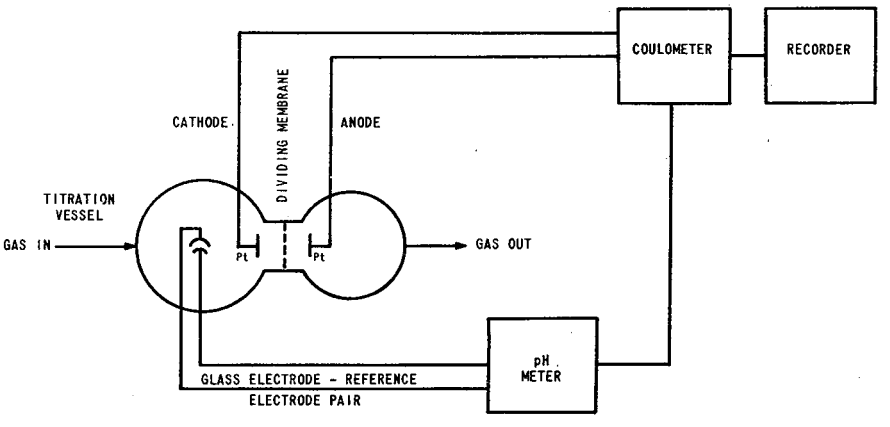

Figure 1. Schematic diagram of continuous acid-base analyzer

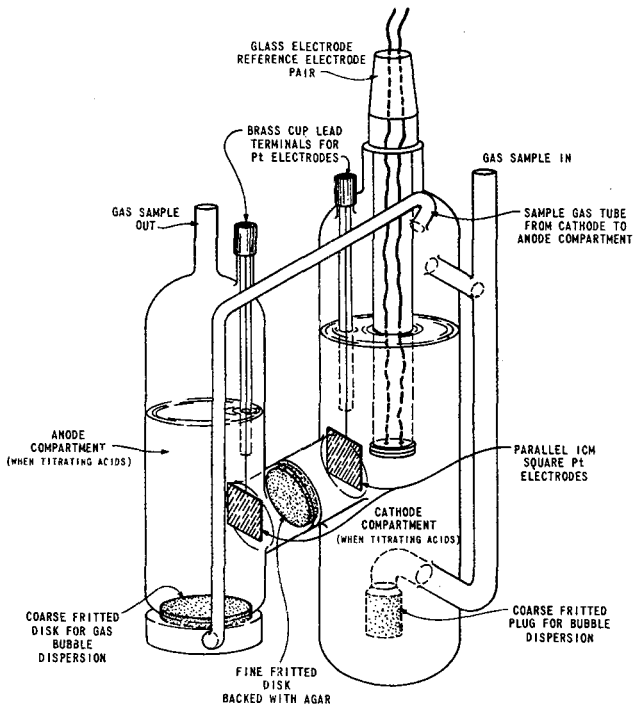

Figure 2. Titration vessel

the electrode by adhering oxygen bubbles, the gas stream scrubbed of acid in the cathode compartment is then passed through the anode chamber before venting. Fritted glass is employed for dispersal of the gas in each chamber. Anode and cathode compartments are separated by a fritted glass disk backed on the upstream side with agar.

The electrolyte used in the titration vessel is approximately 0.5 to 1.0*M* KNO_3. Salts of other strong, nonhalogen acids may be used. Since, in the titration of acids, the electrode reaction in the anode chamber produces hydronium ion, the possibility of diffusion of those ions across the agar boundary exists if the acid concentration is allowed to build up. This may be prevented by adding a few crystals of slightly soluble $BaCO_3$ to the anode chamber. The acid produced will then react with carbonate to form CO_2 and water.

Since the gas streams analyzed will generally be quite dry, there will be a continual drop in water level in the electrode compartment on the inlet side of the cell. It is necessary to replenish the level by addition of water at intervals of 2 to 5 days, depending on the rate of throughput of gas. The slow change in pH due to concentration of the solution in the titrating compartment exerts a negligible effect upon the titrating current.

Controller. A simplified diagram of the controller is shown in Figure 3. The output of the pH Analyzer is fed to one of the input windings of a magnetic amplifier (Airpax Preac No. 5549), the other input winding receiving a bias current from the potentiometer marked "control point." If the two input currents are equal, the output of the mag-amp is zero; and no current flows through the current electrodes. If the pH rises above the set point, because of constant rate addition of base, the output of the mag-amp will be amplified by T_1 and provide a current to the electrodes as indicated by the solid arrows. This in turn tends to decrease the pH, the final result being a constant current proportional to the rate of addition of base. If acid is being titrated, the pH drops below the set point; and the current polarities now are as indicated by the dotted arrows, amplification being provided by T_2. Gain control through negative feedback is provided by the potentiometer marked "feedback." The full-scale current is adjusted by the "range" rheostat which actually consists of a number of fixed resistors selected with a multipoint switch to provide 9 fixed ranges between 25 μa. and 12.5 ma. full scale. The detailed diagram is shown in Figure 4.

The original reason for using a magnetic amplifier was to provide isolation between the pH electrodes and current electrodes which was important with some earlier pH analyzers used in this fashion. With the Beckman Model J analyzer this isolation, however, is not required; and other d.c. amplifiers may be used. The circuit is connected to ground at Electrode No. 1. If Electrode No. 2 were grounded, capacitively-coupled a.c. ground currents from the mag-amp would be partially rectified by the cell resulting in a d.c. titrating current which would not be indicated by the meter, giving erroneous results. This has been demonstrated in actual use. Therefore, some care has to be taken also when connecting a recorder to the circuit.

RESULTS AND DISCUSSION

Performance. Acid and base levels producing neutralization currents on the continuous analyzer from 5 μa. to approximately 20 ma. have been monitored successfully. These have represented concentrations from 0.1 to 300 p.p.m. by volume at the rates of gas throughput employed. It is not the concentration, of course, but the rate of throughput of acidic or basic components which determines the operating limits of the equipment. The maximum gas rate desirable with the cell pictured in Figure 2 was approximately 60 liters per hour.

At high levels of titrating current—e.g., above 500 μa.—the sensitivity of the system is so great as to make damping necessary. This can be accomplished either electronically or by moving farther from the equivalence point pH (where pH changes are the greatest) in conducting the titration. For example, at low titrating currents, HCl may be determined at a pH near 7; but at higher currents it is desirable to carry out the titration at a pH of 5.5 to 6.0.

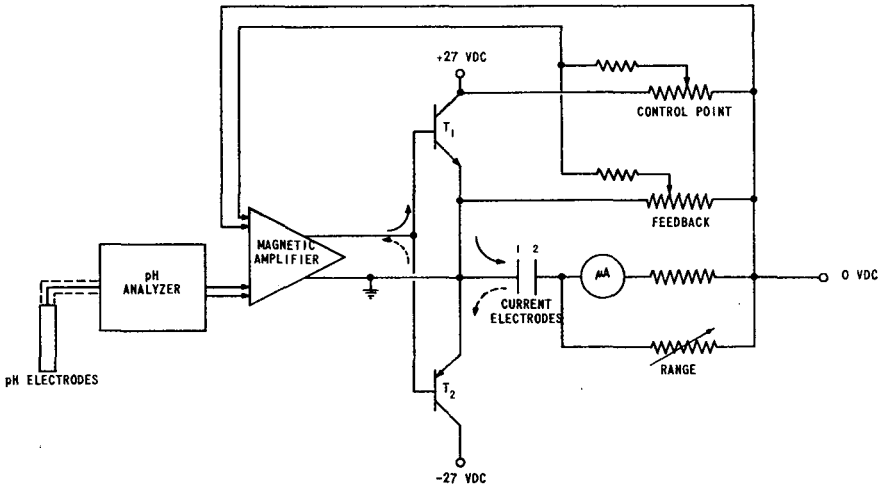

Figure 3. Simplified circuit diagram of acid-base analyzer

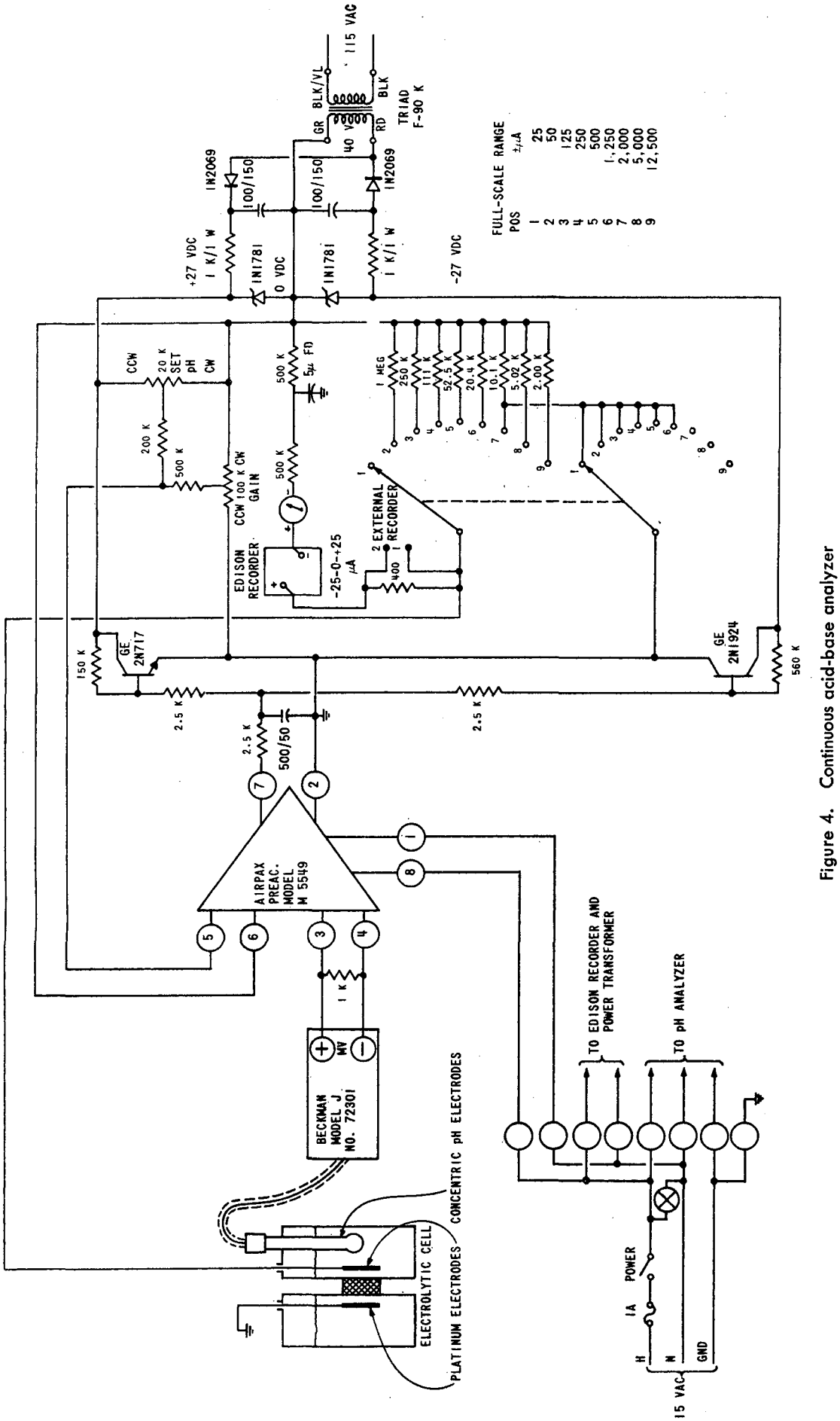

Figure 4. Continuous acid-base analyzer

Table I. Comparison of Continuous and Batch Methods on Four HCl-Containing Gas Streams

Continuous analyzer 1000 cc./min. gas flow rate at 15° C.			Batch samples: 283 liters of gas scrubbed by Na_2SO_3		%
I, μa.	HCl, μeq./hr.	p.p.m.	HCl, μeq.	p.p.m.	Deviation
270	10.1	4.0	46.1	3.85	+4.1
585	21.8	8.6	107	8.9	−3.5
1,060	39.6	15.6	184	15.4	+1.3
20,900	778	306	3870	323	−5.3

The pH chosen for the reference point of the titration of a strong acid or base does not affect the level indicated by the generating current other than to influence the degree of cycling.

As with all potentiometric titration methods, best results are obtained when temperature fluctuations are not great. Changes in temperature will cause shifts in pH and in the reference electrode potential. These changes, if they occur rapidly and in one direction, can be troublesome. Cycling temperatures with a frequency of several minutes will cause minor fluctuations; but the average titration current read will be a true indication of the amount of acid or base being determined. It was shown experimentally that application of heat to the titrating vessel at a uniform rate of 5° F. per hour would result in a reduction in observed titrating current of 10 μa. (when determining an acid). Lining out at an increased temperature, however, would result in the titration current's returning to its original level. These figures enable one to estimate the degree of temperature constancy required for any given level of acidity being determined. Normal ambient temperature variations have not caused difficulties with our apparatus within the over-all ±5% limits of accuracy which we have experienced with the equipment. This level of accuracy was determined by comparison with batch methods on the same gas stream during periods of constant HCl level. The batch analyses were conducted by passing 10 cubic feet of sample gas through a sodium carbonate scrubber and subsequently analyzing the solution for chloride by potentiometric titration with silver nitrate. Table I shows the results of four such comparisons.

We have had one of these analyzers in continuous service on a refinery gas stream for eight months. During that time, only replenishment of evaporated water from the titration chamber of the cell has been required for continued troublefree operation.

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A Rapid Radiochemical Procedure for Tin

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▷ A very rapid radiochemical procedure has been developed for the isolation of radioisotopes of tin from their fission-product isobars. An irradiated uranium solution containing tin and antimony carriers is added to a solution of sodium borohydride. The volatile stannane (SnH_4) formed is decomposed in a hot quartz tube to the metal, which is collected on a cold surface. Stibine (SbH_3), which is also formed under these conditions, is removed by absorbtion on an Ascarite column. The tin chemical yield ranges between 15% for an antimony decontamination factor of 2×10^4 to 60% for an antimony decontamination factor of 10^3. The time required for separation of the tin metal from the other fission product elements is about 10 seconds. Decontamination factors of other antimony descendents are: I = 7×10^4, and Te > 2×10^4. Arsenic is also volatilized as the hydride; however, it is not necessary to eliminate it in this work for the determination of the Sn fission yield.

A VERY RAPID radiochemical procedure for the separation of tin from mixed fission products (MFP) was required to determine the fission yields and radiation characteristics of short-lived tin isotopes. A simple one-step procedure was sought which would have the required speed and would give an exact time for the tin separation. The decontamination factors for antimony, tellurium, and iodine had to be large since the fission yield of the tin nuclide is finally determined from the amount of a longer-lived radioactive iodine descendent arising from the decay of this separated tin.

The Nuclear Science Series Monograph, "The Radiochemistry of Tin" (*11*), lists a number of radiochemical methods for separating tin from MFP. These procedures include separations by multiple sulfide precipitations, distillation of the halides, solvent extraction, and combinations of these methods. The time for separation varies from 20 minutes to 4 hours, and chemical yields vary from 50 to 95%. Decontamination factors for descendent and parent elements are not given. Since these methods are unsatisfactory for studies of short-lived tin isotopes, other methods were investigated.

Production of tin hydride with an alkali borohydride has been reported (*1, 2, 6, 12*). This paper describes an application of these methods to give a rapid, clean radiochemical separation of tin from MFP. Slight modifications were made to this general tin procedure so that irradiated samples could be handled rapidly for determination of tin fission yields.

EXPERIMENTAL

Reagents. The concentration of carriers and reagents used are: antimony(III), 10 mg. of Sb per ml. in concentrated HCl; tin(IV), 400 mg. of Sn per ml. in 3*N* HCl; and sodium borohydride solution, 120 mg. of $NaBH_4$ per ml. in 0.2*N* NaOH.

Apparatus. The apparatus for the determination of tin is shown in Figure 1. A simpler apparatus may be used if a rapid separation is not necessary. Unit *A* holds the solution of carriers, acid, and mixed fission products. It is fitted with a three-way stopcock to provide vacuum for introducing the solution of MFP into the vessel and nitrogen for forcing the solution rapidly into unit *B*. Unit *A* also has a side arm with a needle valve in series to introduce nitrogen for flushing the air out of the system before starting the reaction. This unit and the method of introducing the sample to it are the same as unit *A* described previously in the procedure for the rapid separa-

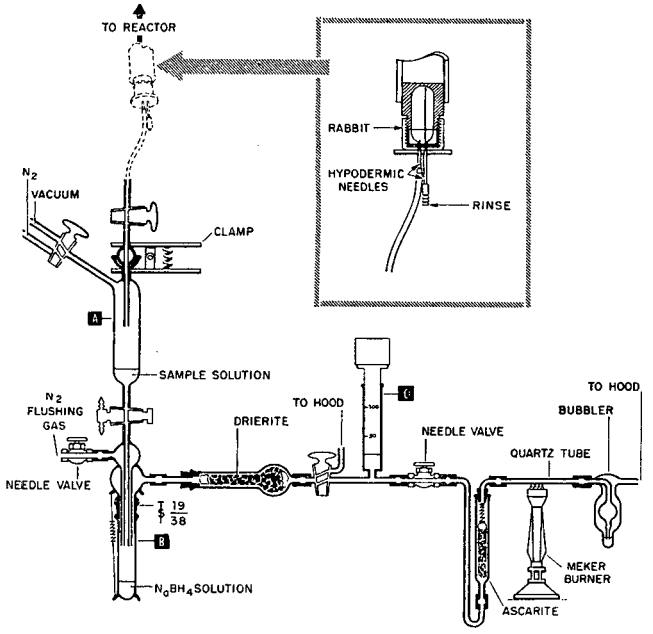

Figure 1. Apparatus for tin separation

tion of antimony and arsenic hydrides from MFP (*4*).

Stannane is formed in unit *B*, which is a borosilicate glass tube of 18-ml. volume with a ground female joint that fits onto section *A*. The gases from unit *B* pass to a calcium chloride drying tube, of 12-mm. i.d. and 100 mm. long, which contains Drierite (8-mesh anhydrous $CaSO_4$). This tube is fitted with a three-way stopcock, one leg of which is vented to a hood. The other leg is in series with a 100-ml. syringe, unit *C*, fitted with a weighted piston to give a pressure of about 1 p.s.i.g. The syringe acts as a surge chamber to hold the rapidly evolved gas from unit *B*. A needle valve in line directly after unit *C* is used for controlling the flow rate of the gas through the rest of the system. Next, the gases pass through a borosilicate glass tube of 11-mm. i.d. and 150 mm. long, of which 110 mm. is filled with Ascarite (20- to 30-mesh sodium hydroxide-impregnated asbestos absorbent); a quartz tube heated by a Meker-type burner; and a bubbler to indicate the flow rate of the flushing gas. Rubber tubing carries the waste gas from the bubbler to the hood. Since stibine and stannane are extremely toxic, it is imperative that they be vented to a hood and thus diluted with air to a nontoxic concentration.

Procedure. In Figure 1, unit *B* contains 1 ml. of sodium borohydride ($NaBH_4$) solution. This unit and the train of apparatus following it is flushed for a few minutes with nitrogen before starting the analysis. The needle valve between unit *C* and the Ascarite column is adjusted to a flow rate of 10 ml. per second. This adjustment is made in the following manner. With nitrogen flowing through the system, the rubber tubing leading out of the bubbler is pinched so that no gas escapes past the bubbler. The valve which lets the flushing gas into *A* is turned off when the piston of the syringe reaches the 100-ml. graduation on the barrel. If the plunger remains stationary, the system is gas-tight. A stopwatch is started at the same time the gas is allowed to flow out of the bubbler. It should take the syringe 10 seconds to empty. In a few trials the correct adjustment of the needle valve can be made. This adjustment should have to be made only once after the apparatus is assembled.

Unit *A* contains 1 ml. of 0.6*N* HCl and 1 mg. of antimony(III) carrier. The quartz tube is heated until it just starts to glow. (It is best to heat the central portion of the quartz tube just prior to use so the ends will remain cool. This prevents damage to the rubber tubing and provides a cool portion for the deposition of the tin mirror. While the temperature of the tube does not seem to be critical, overheating will cause apparent migration of the tin into the quartz.) The vacuum is applied (unit *A*) and the MFP solution containing 10 mg. of tin(IV) carrier is drawn into unit *A* followed by 1 ml. of 0.6*N* HCl rinse solution. It is convenient but not necessary to have both tin tracer and tin carrier present during the irradiation. The vacuum line is turned off, and the inert gas (under 2 lb. pressure) is applied by a half-turn of the three-way stopcock. The reagents are then added to the $NaBH_4$ solution in unit *B*. The tin metal deposits on the quartz tube within about 2 seconds from the start of the reaction. After 10 seconds the three-way stopcock between the Drierite and unit *C* is given a half-turn. This vents any residual gas to the hood so that the quartz tube with the tin deposit can be safely and quickly removed and that the time from the end of irradiation to the tin separation can be better known. The collected sample is dissolved from the tube in a closed system with cold 50% H_2SO_4 containing a carrier of the appropriate descendent element (iodine in the present studies). Other reagents may be used for this solution step or the radiations from the separated tin may be measured directly from the quartz tube depending upon the purpose of the analysis.

Tin-113, which is not formed in fission, may be used for the chemical yield determination if it is added with the tin carrier. An aliquot of the dissolved tin mirror is counted or it is assayed through gamma pulse-height analysis after the fission product tin and its descendents have decayed or have been otherwise separated. The tin-113 counting rate, after correcting for aliquot size and any decay of the tin-113, is a measure of the chemical yield. Luke's (*8, 9*) photometric method for the determination of tin in microgram amounts is an alternative method of finding the chemical yield.

Except for the small amount of gas vented to the hood, all fission product liquids and gases are retained within the apparatus. Since the hydrides of tin and antimony are extremely toxic, a fully enclosed system such as has been described, affords a maximum of safety.

RESULTS

Effect of Tin Oxidation State. Our experiments showed that the oxidation state of the tin carrier solution had no detectable effect on the production of stannane. This is not unexpected since $NaBH_4$ is a very strong reducing agent. In the application of this procedure to independent fission yields, tin(IV) was used because most of the fission-product tin is expected to be initially in its highest oxidation state, and stannous chloride is readily oxidized by the air (*7*) dissolved in irradiated solutions.

Separation of Volatile Materials from Stibine and Stannane. A clean separation of tin from iodine was essential to determine the independent fission yields of tin. The desiccant Drierite, anhydrous calcium sulfate, which works well in removing iodine activities from stibine, (*4*) was used. It holds back other volatile fission product activities as well. A glass wool plug just before the desiccant stops most of the spray and enables one to make several runs before changing the desiccant. A small spring between the one-hole rubber stopper and the Drierite maintains constant pressure on the column so that the gases cannot channel.

Separation of Stibine from Stannane. While the reaction of a tin salt with sodium borohydride gives a good yield of stannane, stibine, which has similar physical properties, is also formed in significant amounts. The major effort in developing this procedure was directed toward a separation of the gases stannane and

stibine. As mentioned previously, the quantity of iodine descendent is a measure of the cumulative tin yield. Therefore, a high decontamination from all elements in the mass chain was required. In previous work (*4*) it was found that arsine (AsH_3) and stibine (SbH_3) can be separated from each other by passing the mixture of gases through a quartz tube at 600° C. where only the stibine is decomposed to the metal. The arsine passes through this tube and is decomposed to the metal in a second quartz tube heated to a higher temperature. The contamination of antimony in the arsenic mirror was only 0.05%. It was not possible to make a satisfactory separation of tin and antimony in this manner.

From Mellor (*10*) a list of possible materials which would preferentially absorb stibine from a stibine–stannane mixture was compiled and studied. At the high flow rates needed to give a rapid separation of the tin from MFP, liquid traps were ineffective. The gas was not scrubbed well enough to reduce the stibine contamination to an acceptable level. Solid traps found to retain varying amounts of stibine were: mercurous salts, freshly-reduced copper powder, silver nitrate, potassium hydroxide, and sodium hydroxide. These solids were mixed about 1:1 with asbestos to maintain a fast flow of gas through the absorbant. Of these reagents, potassium and sodium hydroxide gave the best separation of the hydrides under the experimental conditions established. Since these hydroxides are hygroscopic and difficult to handle, a commercially-available mixture of NaOH and asbestos, developed by J. B. Stetser (*13*) and called Ascarite, was used. A trap 11 mm. in diameter and 80 mm. long passed only 0.039% of the original amount of stibine entering. The amount of antimony holdback carrier was 1 mg. Figure 2 illustrates the absorption of stannane and stibine individually on an Ascarite trap. Initially, 1 mg. of tin carrier was used in this separation since Schaeffer and Emilius (*12*) have shown that the tin yield has an inverse dependence upon the concentration of tin in solution. Figure 2 shows that by increasing the tin concentration a better relative separation of stannane from stibine can be obtained. These curves show the absorption of the independent gases. With a mixture of stannane and stibine, the absorption behavior is somewhat different. When 33 mg. of tin(IV) carrier was used with 1 mg. of antimony (III) carrier, more stibine came through the Ascarite than when 11 mg. of tin (IV) was used with 1 mg. of antimony (III). [One milligram of Sb(III) carrier was used because with only 1 μg. of Sb (III) carrier, 0.25% of the antimony activity deposited with the tin, and with larger amounts of Sb(III) carrier the Ascarite column might be overloaded.] In the procedure adopted, 10 mg. of tin(IV) carrier is used with 1 mg. of antimony(III) holdback carrier.

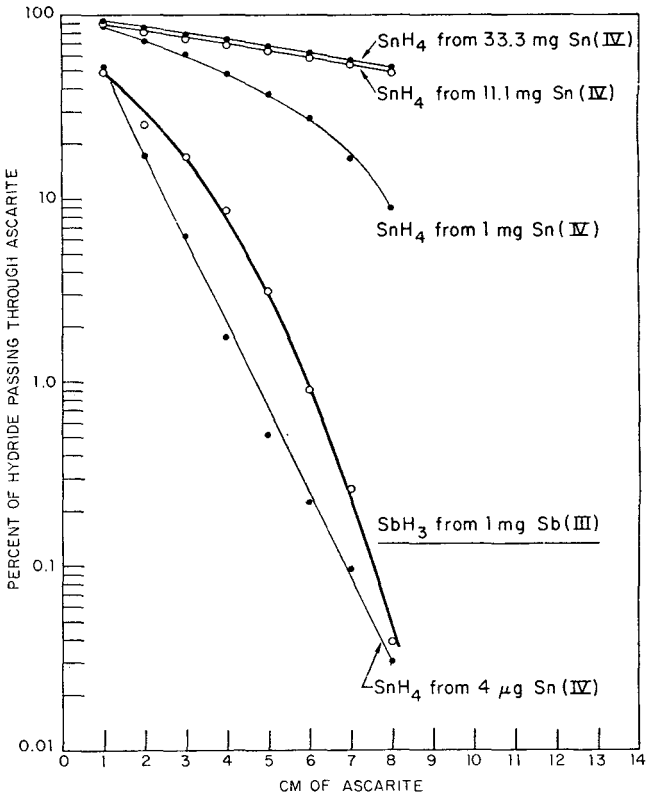

Figure 2. Absorption of stannane and stibine on Ascarite trap

To get a still better decontamination from antimony, the column length was increased to 11 cm. with a resultant sacrifice in tin yield. The Ascarite column was filled in the following manner: a plug of glass wool was placed in the constricted end of the 11-mm. i.d. glass tube; the Ascarite was poured into the column up to a mark 11 cm. from the glass wool plug with gentle tapping to settle the Ascarite; a short glass wool plug was placed over the Ascarite filling; and, a small steel compression spring was placed between the glass wool plug and the one-hole rubber stopper to prevent channeling.

DISCUSSION

A specific application of the rapid separation of tin from its fission product descendents has been made in the determination of the fission yields of tin-131, 132, and 133 in the thermal neutron irradiation of uranium-235. Use was made of the same pneumatic system mentioned in a previous paper (*5*). After leaving the reactor, the pneumatic sample carrier containing the MFP solution, 10 mg. of tin(IV) carrier, and tin-113 tracer (for chemical yield determination) impales itself upon two hypodermic needles. The solution is drawn through a hypodermic needle by vacuum into *A* (Figure 1), which contains 1 ml. of 0.6*N* HCl and 1 mg. of antimony(III) carrier. The rabbit and needle assembly are then quickly rinsed with 1 ml. of 0.6*N* HCl, which is added through the second hypodermic needle. Emptying and rinsing the rabbit takes about 2 seconds. The tin fission products are then separated as described in the tin procedure. The time of the tin separation is determined at five seconds after the addition of the fission-product solution to the borohydride solution. Five seconds is chosen because the SnH_4 is passing through the Ascarite column at a constant rate over the 10-second period.

In the determination of the independent fission yield of a given tin isotope, the separated tin was allowed to decay to its known iodine descendent. The tin was then dissolved in cold 50% H_2SO_4 containing a measured amount of iodine carrier, and a radiochemical determination of iodine was carried out similar to Glendenin's (*3*). The amount of iodine found is a direct measure of the amount of tin originally present. The chemical yield of tin (which varied between 10 and 14%) was determined by counting the added tin-113 tracer after allowing the fission product tin and its daughters to decay to an insignificant level of activity. The interference of the tin-113 produced from the tin-112 in the tin carrier

amounted to only 10^{-3} disintegrations per second.

It has been possible by the above technique to determine independent fission yields of tin fission products in thermal neutron fission of uranium-235, tin half-lives, generic relationships, and also prominent gamma photopeak energies from pulse-height distributions taken of the rapidly separated tin fractions. The results in Table I have been obtained from many different tin separations made at times after fission varying from 10 seconds to 30 minutes. Independent experimental values of these tin half lives were obtained through continuous evolution of SnH_4 from a basic $NaBH_4$ solution of uranium-235 during neutron irradiation and collection of the tin descendents on a charged wire. Tentative values by this method are 44 seconds for tin-131, 50 seconds for tin-132, 43 seconds for tin-133, and 20 seconds for tin-134.

Table I. Half Lives and Fission Yields of Tin Isotopes

Nuclide	Half life, seconds	Fission yield, %
Sn^{131a}	65 ± 10	0.7
Sn^{132}	50 ± 10	0.5
Sn^{133}	39 ± 15	0.2

[a] The 1.6-hour isomer is not observed. Its fission yield would be less than 0.03%.

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Automatic, Amperometric, Cupferron Titration of Zirconium in Highly Radioactive Solutions

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▶ **The amperometric titration of zirconium with cupferron reported by Olson and Elving has been modified and adapted for hot cell analysis. A titration has been devised in which the current is monitored continuously while the titrant is fed in at a constant rate. A stirred mercury pool is the indicating electrode which senses the point where cupferron comes into excess, and a platinum wire is used for the reference. Precision of 1% relative standard deviation is obtainable for benchtop analysis and 1.5% for titrations in a hot cell. This titration was specifically devised for the analysis of zirconium in molten salt reactor fuel, and the titration can be performed directly following dissolution without the need for separations.**

THE INITIATION of the Molten Salt Reactor Experiment (commonly referred to as MSRE) program brought out the need for a method to determine zirconium in highly radioactive solutions. The activity of the sample material makes it mandatory to perform all the operations in a hot cell, thereby imposing certain limitations on the procedure to be used. Zirconium analysis is routinely carried out by x-ray fluorescence. The relative ease of this analysis coupled with the good results obtainable have given little incentive to develop chemical methods for zirconium. Since the installation of an x-ray fluorescence unit into a hot cell involves serious problems related to cost, operation, and maintenance, attention was directed to the development of a chemical method of analysis.

Titrimetric procedures are preferred to gravimetric or spectrophotometric procedures in hot cell work from the standpoints of the ease of installation, manipulation, and replacement. Furthermore, procedures utilizing electrometric end point devices are best because the components within the hot cell require a minimum of space and moving parts, and the bulk of the instrumentation can be located outside. Therefore, it was natural that the search for a suitable method was directed toward a volumetric method utilizing an electrometric end point device.

Most of the available volumetric procedures for zirconium depend upon the complexation reaction with EDTA. The direct titration with EDTA at a pH of 1.5 has been described by Fritz and Fulda (*3*). At a pH of 1.5, the hydrolysis of zirconium becomes a serious problem and a back-titration of excess EDTA (*4*, *5*) is generally more reliable. Olson and Elving (*8*) reported the amperometric titration of zirconium using cupferron for titrant and a dropping mercury electrode for the indicator electrode.

The amperometric titration has many attractive features which can be utilized to good advantage for this analysis. The titration is performed in a 2*M* sulfuric acid medium in which only a few metals beside zirconium can react with cupferron. The high acid medium also minimizes errors due to the partial hydrolysis of zirconium. Best of all, this titration is feasible in the presence of moderate amounts of fluoride, and even large excesses of fluoride can be tolerated by the simple expedient of adding some aluminum ion. This is important to this particular analysis because the starting material is a mixture of fluoride salts, and the repeated fuming necessary to remove the last traces of fluoride can be avoided.

On the other hand, the entire process is tedious and lengthy and is not suited for adaption to hot cell use. A small increment of titrant has to be added, the solution deaerated, and the current determination made. This series has to be repeated several times for a single titration. Finally, the current *vs.* titrant volume graph has to be constructed for the location of the end point.

It was thought that if the principle of this titration could be adapted to one in which the titrant is fed in at a constant rate and the current monitored

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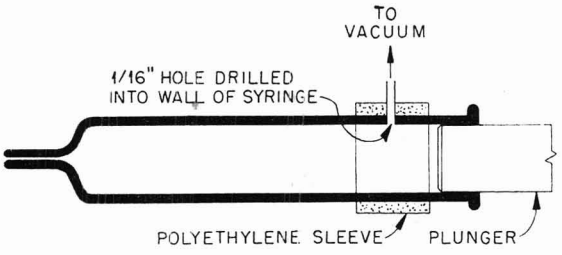

Figure 1. Vacuum connection to syringe

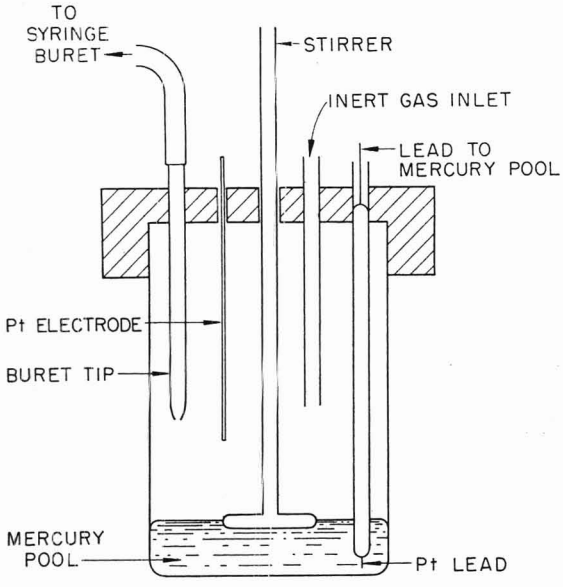

Figure 2. Titration cell

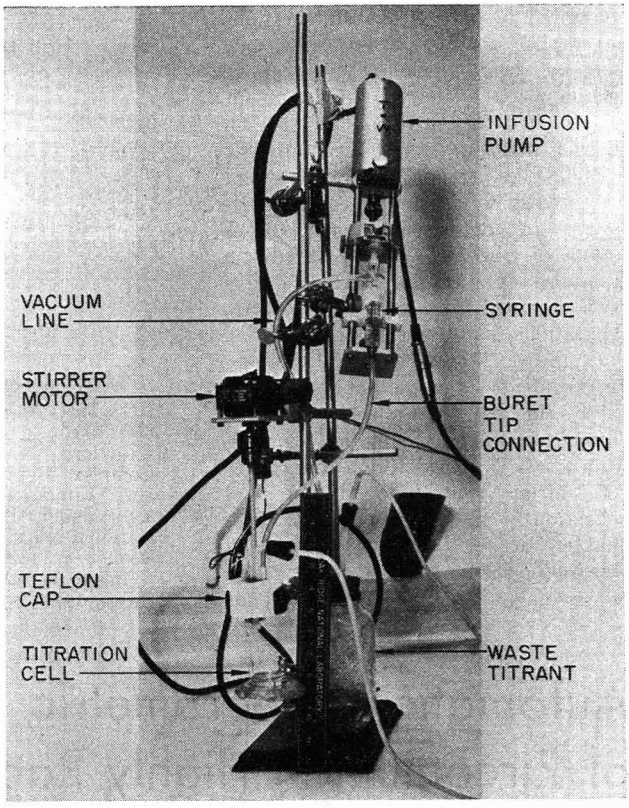

Figure 3. Titration assembly

continuously, a procedure well suited for hot cell use would result. The results of this investigation showed that by the proper choice of electrodes and working conditions such a titration was possible and could be easily performed in a hot cell with good precision. The electrode system can be utilized for many other titrations in which the titrant contains a substance which is reducible at the dropping mercury electrode.

EXPERIMENTAL

Reagents. Cupferron solution, 0.05*M*. Dissolve 775 mg. of cupferron (Eastman White Label) in water that has been deoxygenated with a stream of inert gas for 10 minutes. Dilute to 100 ml.

Zirconium standard solution, 5 mg. Zr per ml. Accurately weigh about half a gram of high purity zirconium metal (99.9%) into a platinum dish. Cover the metal with 10 ml. of nitric acid and place the dish on a hot plate. Add a few drops of hydrofluoric acid to the warm solution to start dissolution. Add hydrofluoric acid dropwise whenever the dissolution reaction slows down. When dissolution is complete, carefully add 10 ml. of sulfuric acid and heat till brown fumes are completely gone. Transfer the sulfuric acid solution to a volumetric flask. Use 1*N* sulfuric acid washing during the transfer. Dilute to 100 ml.

Equipment. Constant delivery buret assembly. A portable infusion-withdrawal pump (Catalog No. 1100, Harvard Apparatus Co., Inc., Dover, Mass.) equipped with a 2-r.p.m. motor and 10-ml. syringe is used to deliver the titrant at a rate of 0.34 ml. per minute. The delivery tube from the syringe is a polyethylene tube of $^1/_8$-inch i.d. and $^1/_4$-inch o.d. drawn to a tip at the terminal to the solution. A $^1/_8$-inch hole is drilled into the side of the syringe barrel near the far withdrawal end (Figure 1). This portion of the barrel is covered with a machined plastic piece that fits snugly over the barrel. A hole is drilled into this plastic in line with the hole in the barrel to receive the outlet tubing for a vacuum connection. For filling the buret the plunger is backed to the rearmost position, the delivery tip is immersed in the titrant solution, and the vacuum connection is made until the barrel of the syringe is filled up to the vacuum line level. The vacuum is turned off and the plunger moved forward till the vacuum line is sealed off.

Titration cell. The titration cell assembly is shown in Figure 2. The cell itself is a 50-ml. lipless beaker. A plastic cap (Teflon) is machined to fit over the beaker and is provided with openings for the buret tip, stirrer, reference electrode, lead to the mercury pool, and inert gas inlet. Since there will always be a positive inert gas pressure within the cell, the passages through this cap do not have to be sealed. The stirrer blade is positioned to stir both the solution and the mercury pool at the same time. The complete titration assembly in place in the hot cell is shown in Figure 3.

Polarograph. A recording polarograph with a 10- or 20-μa. full scale sensitivity range is used.

Procedure. Place 10 ml. of mercury and 10 ml. of 1*N* sulfuric acid in the titration cell. Transfer 1 ml. of standard Zr solution (5 mg. per ml.) by means of a micropipet followed by one drop of 0.1% gelatin solution. Position the titration cell under the cap and deoxygenate the solution for 10 minutes. Start the stirrer. Apply −0.5 volt to the mercury pool *vs.* the platinum electrode and start the current recorder and the infusion pump. Stop the titration when an abrupt and nearly vertical change in current is indicated. Locate the end point by extrapolation and, subsequently, determine the titer by comparing the volume of titrant delivered *vs.* the amount of zirconium taken.

In a similar manner, titrate an unknown sample. Use the titer obtained from the standardization to calculate the zirconium content of sample.

RESULTS

A typical titration curve is shown in Figure 4B.

Precision studies conducted over a period of 10 working days showed that a

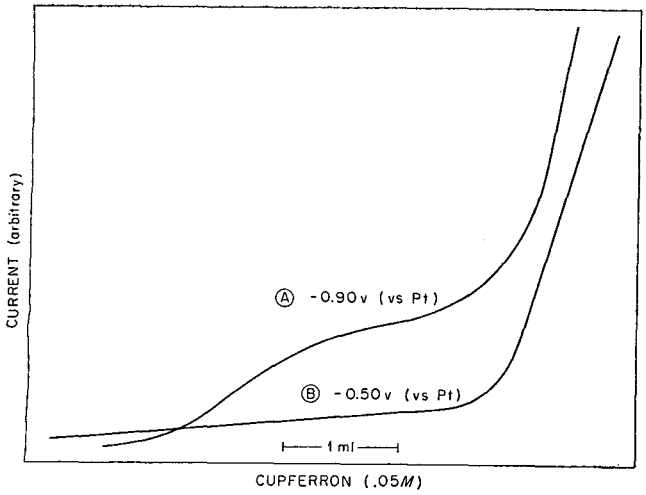

Figure 4. Effect of potential on shape of titration curve

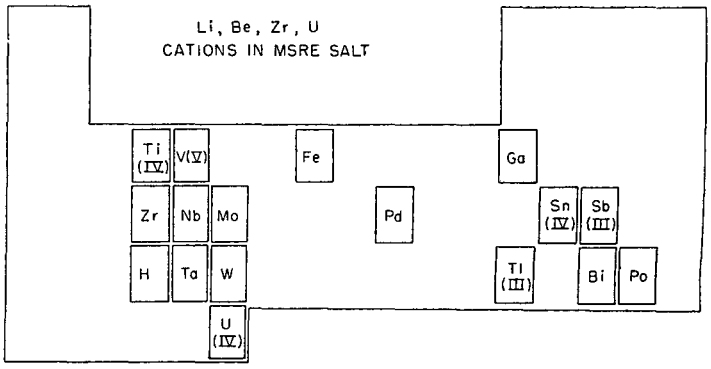

Figure 5. Elements precipitated by cupferron in 1–4N acid

relative standard deviation of 1% or better is attainable for benchtop analysis. With the assembly in place in the hot cell, the precision is 1.5%. The precision is the same for titrating zirconium either as the pure solution or as a component in a simulated molten salt reactor material.

DISCUSSION

The titration assembly was devised with the problems of the ease of installation, manipulation, and replacement as foremost considerations. The setup that is described is comprised of equipment that is easily available or material simple to fabricate and occupies a very limited area within a hot cell. The manual operations that have to be performed with the master slave manipulators have been reduced to transferring the requisite reagents to the titration cell, positioning the titration cell under the Teflon cap, and moving the buret delivery tip to the proper site for either the filling operation or the titration. All other controls are performed from outside the hot cell. The equipment is of a simple nature such that in the event of a major malfunctioning, a new setup can be installed rapidly and economically.

The critical problem in this adaptation was the search for a suitable electrode system. The dropping mercury electrode is not practical for this work because the solution has to be stirred throughout the titration. The rotating platinum electrode and a coated mercury electrode were also found to be unsatisfactory. The stirred mercury pool that is used in many coulometric procedures was found to be admirably suited for this purpose. A calomel electrode making contact with the solution via a sulfate bridge is an excellent reference electrode. In view of some difficulties experienced at this laboratory on the erratic behavior of calomel electrodes under the effects of radiation, other electrodes were tried, and a simple platinum wire electrode was found to be suitable. It is thought that this electrode pair (with either the calomel or platinum) can be used to detect the end point of many titrations in which the titrant itself is reducible at a dropping mercury electrode, provided the titration reaction is feasible at the operating potential.

The end point mechanism is dependent upon the electroactive nature of cupferron. The polarography of cupferron was studied in detail by Kolthoff and Liberti (*6*) and Elving and Olson (*2*). The reduction is thought to be a stepwise affair involving six electrons giving a final reduction product of phenylhydrazine. The compound was slightly decomposed by acid solutions below pH 3; however, the degree of this decomposition is relatively small such that cupferron can be used for amperometric titrations in acid media where changes in current flow rather than the absolute magnitude of the current are important. The half-wave potential for this reduction in an acid medium varies according to the following relation:

$$E_{1/2} = -0.58 \text{ volt (vs. S.C.E.)} - 0.128 \text{ pH.}$$

Olson and Elving operated at −0.84 volt, a potential well on the plateau of the diffusion wave in 2*M* sulfuric acid, assuming that the above relation can be extended to such a strong acid medium. A 1*N* sulfuric acid medium was the medium used in this work, and it was best to perform the titration at −0.55 volt (*vs.* S.C.E.) or −0.50 volt (*vs.* platinum).

Cupferron decomposes slowly even in the dry state, and the rate of deterioration is even faster in aqueous solution. The cupferron solution was prepared fresh daily by dissolving a weighed portion in deoxygenated water and used for that day only. The change in titer during the course of a day is not distinguishable; however, a slight discoloration is observed after 24 hours. It was not necessary to continue purging the cupferron solution once it was inside the syringe.

Table I. Composition of MSRE Salt

Compound	Mol %	Element	Weight %
LiF	66.8	Li	12.2
BeF_2	29.0	Be	6.8
ZrF_4	4.0	Zr	9.6
UF_4	0.15	U	0.93
		F	70.5

Maxima effects are observed in the polarography of cupferron which can be removed very readily by the addition of gelatin. The addition of gelatin to this titration medium is necessary to ensure good titration curves.

The two titration curves shown in Figure 4 show the effect of operating potential upon the shape of the titration curve. At a potential of −0.90 volt, a potential on the diffusion wave plateau, considerable current begins to flow from a little after the start of the titration (Curve *A*), and it is difficult to construct the proper line corresponding to the pre-end-point stage for the extrapolation of the end point. At −0.50 volt, a potential near the foot of the diffusion wave, the pre-end-point portion is nearly a straight line as shown by curve *B*. In either case, the current rise after the end point is very sharp.

The elements precipitated by cupferron in 1 to 4*N* acid are shown in Figure 5 (*7*). Oxalate, citrate, tartrate, phosphate (*1*), and EDTA are some of the commonly used complexers which can be present and not interfere. Moderate amounts of fluoride (up to 10 times zirconium) also will not interfere. Larger amounts of fluoride can be complexed with aluminum which is unreactive toward cupferron. In this acid medium nitrate is a very bad interference because it reacts with cupferron, while chloride reacts with mercury.

The composition of MSRE salt is

shown in Table I. The salt is dissolved in a nitric–sulfuric acid medium with the addition of a little boric acid and the nitric acid is later fumed away. Since the only possible cationic interference of U(IV) has been converted to U(VI) during the dissolution and no anionic interferences are present, the titration for zirconium can be made directly after the removal of the nitrate.

The titration of 5 mg. of zirconium takes about 5 minutes using the equipment and reagents that have been described. The entire procedure including the makeup of the solution, deoxygenating, and the titration takes about 20 minutes. The 1.5% precision for determining zirconium at this level is within the limitations imposed upon this analysis. If necessary, the precision can be improved by taking larger samples or, to a limited extent, using more dilute titrant. The reproducibility in taking samples seems to be the chief cause for the lower precision of the analysis in the hot cell.

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Hydrogen Cell Assembly for Standard Electromotive Force Measurements

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▷ **A simple hydrogen cell assembly for the calibration of buffer solutions, glass electrodes, and reference electrodes is described. The design of the cell permits such calibrations to be made in 20 to 30 minutes with a maximum standard deviation of ±0.6 mv. (±0.01 pH).**

MODERN ELECTRONIC pH instrumentation now permits refinement of the readout to 0.001 pH. Such a high degree of precision, for example, is desired in the measurement of the pH of blood and other body fluids where small changes of pH may have significance. The importance of high precision pH measurements has further been recognized by the assignment of revised standard pH values, given to three decimal places, for the seven National Bureau of Standards (NBS) reference solutions which define the pH scale (*5*).

For very accurate pH measurements, frequent calibrations of a pH cell containing a glass electrode are necessary. This requires a reliable reference buffer, the pH of which has been determined previously with great accuracy. Second, the pH response of the glass electrode must be checked, preferably in the pH range in which it is to be used. It is known that even the best modern electrodes show small variations in response to pH with time. Third, the stability of the reference electrode with time must be verified. This paper describes a simple hydrogen cell assembly in which standardization of buffers, glass electrodes, and reference electrodes can be made accurately and with relative ease. The hydrogen cell assembly is similar in principle to that described by Bates, Pinching, and Smith (*6*), modified to obtain more rapid thermal and chemical equilibrium and designed to fit the Beckman Model 28505 Thermomatic constant temperature block.

The standardization of a buffer solution against a NBS primary standard is accomplished by measuring the e.m.f of cells with liquid junctions of the following type:

Pt; H_2 (gas), Primary Standard/Saturated KCl/Buffer X, H_2 (gas); Pt

The design of the cell assembly permits the formation of physically well defined liquid junctions. The residual liquid junction potential—i.e., the difference of the two liquid junction potentials formed between the saturated potassium chloride solution and the primary standard buffer on one side and the test solution on the other—manifests itself as an indeterminate error in the derived pH. In the ideal case, where the liquid junction potentials are numerically identical and opposite in sign, the residual liquid junction potential equals zero. It is, therefore, of great importance to be able to produce and reproduce closely identical liquid junctions at both sides of the salt bridge.

A minimum of two buffer standards is needed for the calibration of a glass electrode. Its pH response can be determined by measuring the e.m.f. of cells without liquid junctions of the type

Pt, H_2 (gas), Buffer Soln./Glass Electrode

Similarly, the stability of a reference electrode can be verified by measuring the e.m.f. of the cells:

Pt, H_2 (gas),
Buffer Soln./Reference Electrode

EXPERIMENTAL

Apparatus. A sketch of the hydrogen cell assembly is given in Figure 1. It consists of two electrode compartments, *a* and *a'*, connected by a saturated KCl bridge, *b*. Each electrode compartment holds approximately 3 ml. of solution. The liquid junctions are formed at *c* and *c'* and protected from turbulence caused by the bubbling hydrogen gas, by stainless steel balls, *d* and *d'*, that imperfectly separate the standard and test solutions from the KCl solution. The bottom of each electrode compartment is ground at *c* and *c'* to match the contour of the stainless steel balls. Compartment *e* holds a stopper, *f*, that prevents displacement and mixing of the saturated KCl solution in the capillary bridge with the solutions of the two electrode compartments. Stopper *f* is ground in place at *g*. The presence of saturated KCl in this area maintains electrical continuity. Purified hydrogen gas enters the system through *h* and *h'*; *i* and *i'* are platinum electrodes, and *j* and *j'* are outlets for hydrogen gas. Copper wire leads covered with phone tips, *k* and *k'*, are soldered to the platinum electrodes for electrical connections.

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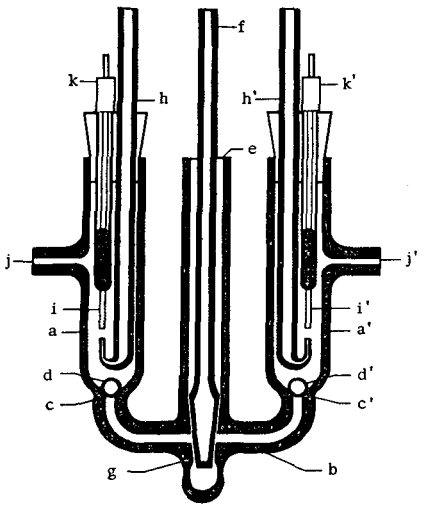

Figure 1. Hydrogen cell assembly

a, a'	electrode compartments
b	capillary bridge
c c'	liquid junction area
d, d'	stainless steel balls
e	compartment for saturated KCl
f	stopper, ground in place at *g*
h, h'	inlets for hydrogen gas
i, i'	platinized platinum electrodes
j, j'	outlets for hydrogen gas
k, k'	phone tips

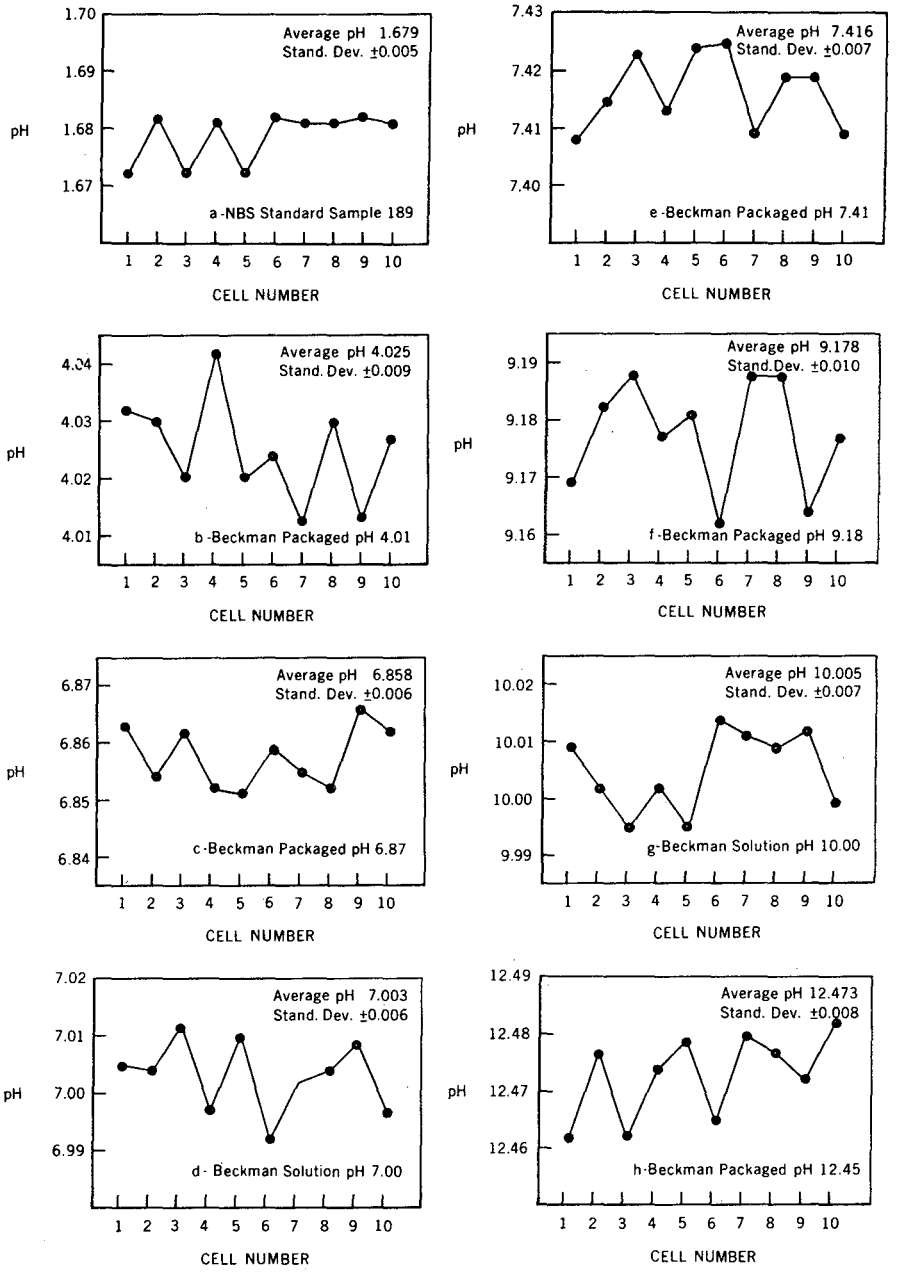

Figure 2. Performance of hydrogen electrode cell assembly in standardization of buffers at 25° C.

The preparation of platinizing and palladizing solutions and the electroplating of the platinum electrodes were carried out in the manner described by Bates (*2*). The plated electrodes were stored in distilled water when not in use.

Before entering the cell assembly, the hydrogen gas was passed through a Deoxo purifier (Engelhard Industries, Inc.), a safety flask, a wash bottle containing distilled water, and another safety flask, respectively.

Electromotive force measurements were made with the Beckman Model 1019 research pH meter. All measurements were carried out at 25° C. ± 0.01° C.

Reagents. Reagent grade potassium chloride and NBS standard samples were used for the salt bridge and primary standard buffer solutions, respectively. All solutions were prepared with doubly distilled water.

Procedure. A. Standardization of Buffer. Thoroughly clean and dry the cell vessel. Carefully introduce saturated potassium chloride solution into compartment *e* until the capillary bridge is filled to *c* and *c'*. Insert stopper *f* into tube *e* and seat it in ground joint *g*. Seat the stainless steel balls *d* and *d'* in place. Make sure no air bubbles are trapped in the salt bridge. Remove any excess saturated potassium chloride solution in the electrode compartments *a* and *a'* with absorbent tissue. Fill the electrode compartments *a* and *a'* with NBS primary standard buffer and the buffer solution to be standardized, respectively, to a level slightly below outlets *j* and *j'*. Place the cell vessel in a Thermomatic constant temperature block or any other constant temperature bath. Rinse the hydrogen electrodes *h, i, h'*, and *i'* with distilled water and carefully dry the glass parts with a clean soft tissue. Insert the electrodes firmly in place. Make gas and electrical connections. Bubble purified hydrogen into the assembly at a rate of about three bubbles per second. Allow the system 20 to 30 minutes to reach thermal and chemical equilibrium. Measure the e.m.f. of the cell. Compute the pH difference of the two buffers by dividing the measured e.m.f. by the appropriate Nernst coefficient (*4*).

B. Standardization of Glass Electrode. Clean and dry the cell vessel. Fill compartments *a, a'*, and *e* and capillary bridge *b* half way with a NBS primary standard buffer or previously calibrated buffer. Insert stopper *f* in compartment *e* to seat in ground joint *g*. Fill compartment *a* with buffer to a level slightly below outlet *j*. Place the cell assembly in a constant temperature bath. Rinse the hydrogen electrode *h, i* with distilled water and carefully dry the glass parts. Insert the electrode in compartment *a*. Make gas and electrical connections. Place the glass electrode to be standardized into compartment *a'* in such a position that it does not rest on the bottom of *a'*. Allow 20 to 30 minutes for the system to reach equilibrium, then measure the e.m.f. Calculate the standard potential of the glass electrode using the equation

$$E^{\circ}_{GE\ vs.\ NHE} = E_{GE\ vs.\ HE} + Corr.$$

where $E^{\circ}_{GE\ vs.\ NHE}$ = standard po-

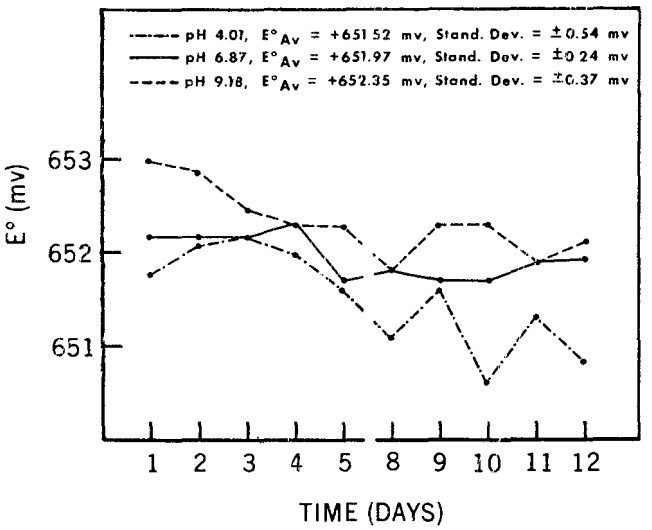

Figure 3. E° of No. 41263 glass electrode measured in pH 4.01, pH 6.87, and pH 9.18 buffers

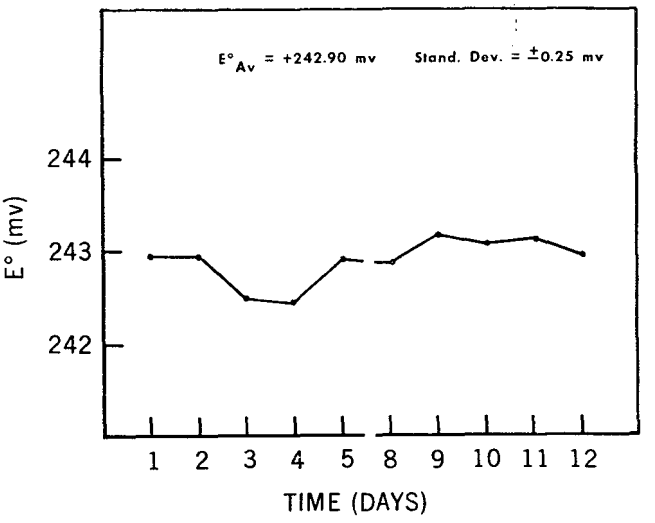

Figure 4. E° of No. 39071 saturated calomel reference electrode measured with standard hydrogen cell assembly

tential of the glass electrode, $E_{GE\ vs.\ HE}$ = measured cell potential, and Corr. = hydrogen pressure correction factor (*1*).

C. Standardization of Reference Electrode. The same sequence of operation as in B is followed here. Calculate the standard potential of the reference electrode using the equation

$$E°_{RE\ vs.\ NHE} = E_{RE\ vs.\ HE} + \text{Corr.} - \frac{2.3026\ RT}{F}\ pH$$

in which $E°_{RE\ vs.\ NHE}$ = standard potential of the reference electrode, $E_{RE\ vs.\ HE}$ = measured potential of the reference electrode, Corr. = hydrogen pressure correction factor (*1*), 2.3026 RT/F = Nernst coefficient (*4*), and pH = pH of the buffer solution used.

RESULTS AND DISCUSSION

The results of the standardizations of commercially available Beckman buffers against an NBS equimolar phosphate buffer (pH_s = 6.865 at 25° C.) are presented in Figure 2. Also included in Figure 2 are results obtained in standardizing a sample of NBS tetroxalate buffer. Ten successive measurements of each buffer were made on 10 different cells. Maximum standard deviations of 0.006 pH units were observed in the intermediate pH range. In the high acidity and high alkalinity regions, higher maximum standard deviations may be anticipated. The reproducibility of the e.m.f. measurements in these regions is not as good as in the intermediate pH range. This probably results from greater variations in the residual liquid junction potential due to the abnormal mobilities of hydrogen and hydroxide ions. The exact nature of liquid junction potentials is not known, although this has been the subject of many studies through the years. An extensive discussion of the characteristics of several types of liquid junction is given by Guggenheim (*7*). Bates (*3*) also gives an excellent summary on this subject.

An additional problem of a different nature enters into the picture in the standardization of a pH 4.01 phthalate buffer solution. Here, the platinum black coating of the hydrogen electrode acts as an active catalyst in the hydrogenation of potassium hydrogen phthalate and stable potentials cannot be obtained. As recommended in the literature, palladium black, a less active catalyst, was used for these measurements. However, the difficulty is believed to be not completely remedied.

The Beckman No. 41263 glass elec-

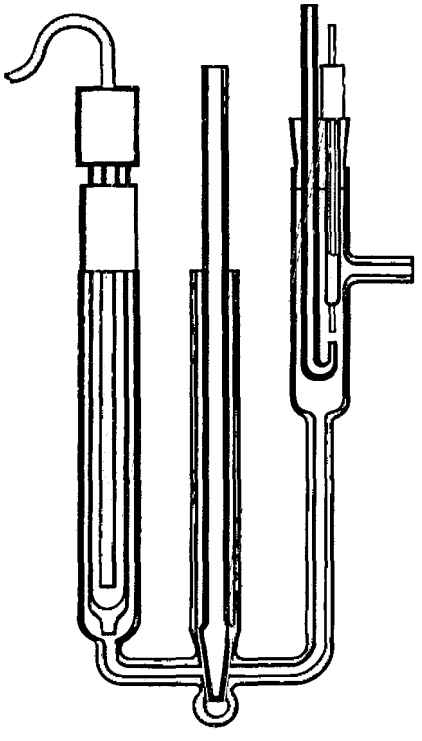

Figure 5. Modified hydrogen cell assembly

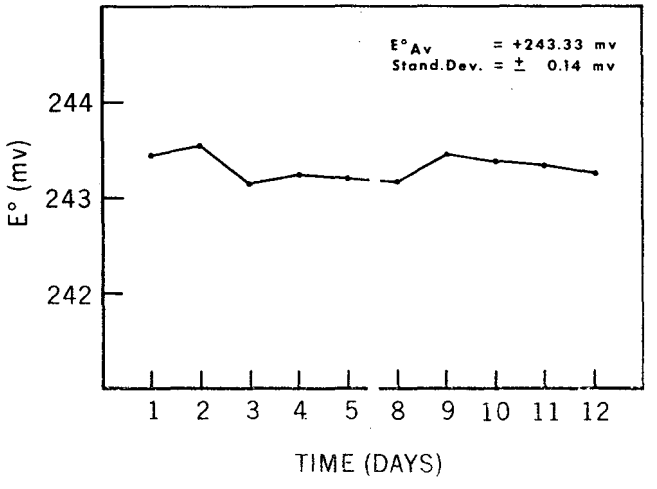

Figure 6. E° of No. 39071 saturated calomel reference electrode measured with modified hydrogen cell assembly

trode is recommended for use with the pH meter. The variation with time and buffer solution of the standard potential (E°) of such an electrode is shown in Figure 3. Ten successive measurements in pH 4.01, pH 6.87, and pH 9.18 buffer solutions were made over a period of 2 weeks. Again, the measurements in pH 4.01 buffer show the largest standard deviation although palladized electrodes were used. Moreover, a slightly *lower* value for the E° was observed.

Similarly, 10 successive measurements were made on the No. 39071 saturated calomel reference electrode. Figure 4 shows the stability of such an electrode in pH 6.87 buffer over a time interval of 2 weeks. The standard deviation obtained verifies the good reproducibility and stability of the carborundum frit junction of the reference electrode.

The standard potential of the reference electrode could not be determined accurately in the hydrogen electrode cell assembly since the electrode compartment was too short to accommodate the entire electrode. This caused temperature gradients to exist in the reference electrode internal and its electrolyte. To overcome this difficulty, a modified cell assembly as shown in Figure 5 was used. This cell assembly was immersed in a deeper constant temperature bath to keep the entire electrode at the desired temperature. The results obtained with this modified cell assembly are presented in Figure 6. The average E° value is 0.43 mv. higher than the corresponding potential of Figure 4.

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Sensitive Method for the Determination of Submicrogram Quantities of Manganese and Its Application to Human Serum

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▶ Oxidation of leucomalachite green with periodate in the presence of manganese is a catalytic type reaction which has been used for the quantitation of submicrogram amounts of manganese. There is not, however, linear proportionality between the absorbance at any given time during this reaction and the content of manganese in the system. Experimental data indicated that this reaction is complicated by a subsequent reaction which destroys the dye. Oxidation of malachite green with periodate in the presence of manganese follows a first order reaction catalyzed by manganese. This subsequent reaction explains the behavior of the leucomalachite green oxidation curves. A method for determination of manganese in the millimicrogram range based on the oxidation of malachite green is proposed and applied to human serum. The normal level of serum manganese was found to be 0.36 to 0.90 mμg. per ml.

THE OCCURRENCE of minute amounts of manganese in certain biological materials has prompted the development of a variety of procedures (*4-10, 12*). The most sensitive of these employs neutron activation analysis, a procedure not practical for most analytical laboratories (*2, 8, 10*). An excellent review by Cotzias (*3*) provides a comprehensive background for this subject.

The more sensitive of the chemical methods involve oxidation of colorless leucomalachite green (LMG) to the dye, malachite green (MG). Manganous ions are converted by periodate to a higher state of oxidation and then oxidize the leuco base to the dye. Many times the manganese present would necessarily be consumed if the reaction proceeded through stoichiometric reactions involving Mn^{+2} or its oxidation products. This then would imply a catalytic effect of manganese upon the reactions which may be written

$$Mn^{+2} \xrightarrow{NaIO_4} Mn^{Ox}$$

$$Mn^{Ox} \xrightarrow{LMG} Mn^{+2}$$

and the observed effect would be appearance of MG. If periodate and LMG are in large excess, the reaction should be zero order and, if Beer's law is obeyed, absorbance plotted *vs.* time should yield a straight line. This, however, is not the case (*12*). The reaction, therefore, seems to be somewhat complicated and provided the impetus for this investigation leading to the development of a new method for determination of trace amounts of manganese of the order of 1 to 15 mμg. A simple test with this sensitivity would be desirable for the determination of manganese in human blood serum.

EXPERIMENTAL

Reagents. All reagents were reagent grade or the purest commercially available. Water used in preparing all reagents was redistilled from all-glass apparatus. The solutions were stored in acid-washed polyethylene bottles.

PHOSPHATE ACETATE BUFFER. $NaH_2PO_4 \cdot H_2O$ (27.6 grams) in water and 12.0 ml. of glacial acetic acid were diluted to 100 ml. with water. Then 21.0 ml. of 1.0*N* NaOH was added. The pH of a 1 to 4 dilution of this solution was 3.6 to 3.7.

LEUCOMALACHITE GREEN (*p,p'*-tetramethyldiaminotriphenylmethane), obtained from Hopkins and Williams, Ltd. Fifty milligrams was dissolved in 100 ml. of 0.5% (v./v.) HCl. The reagent was stored in a refrigerator and discarded after one week.

MALACHITE GREEN, obtained from Matheson, Coleman and Bell. Ten milligrams was dissolved in 100 ml. of water, and discarded after one week.

MANGANESE STANDARD SOLUTION, 5 μg. per 100 ml. $MnSO_4 \cdot H_2O$ (30.8 mg.) was dissolved in 1 liter of 0.1*N* H_2SO_4; 0.5 ml. of this solution plus 0.5 ml. of 0.1*N* H_2SO_4 were freshly diluted to 100 ml. with water.

Methods. All glassware used was treated with 6*N* HCl at 60° C. for about 30 minutes and then washed with redistilled water. Experiments designed to study the kinetics of the reaction were performed as follows. Test tubes were set up to contain 0, 0.02, 0.05, and 0.10 μg. of manganese. The following then were added in order: 1 ml. of 1*N* HCl, 7.5 ml. of phosphate-acetate buffer, a volume of 1*N* NaOH determined to give a final pH of 3.6, and 1.0 ml. of leuco-base or dye. Water was added to 14.5 ml. and then 0.5 ml. of 1% sodium periodate solution was added to start the reaction. The tubes

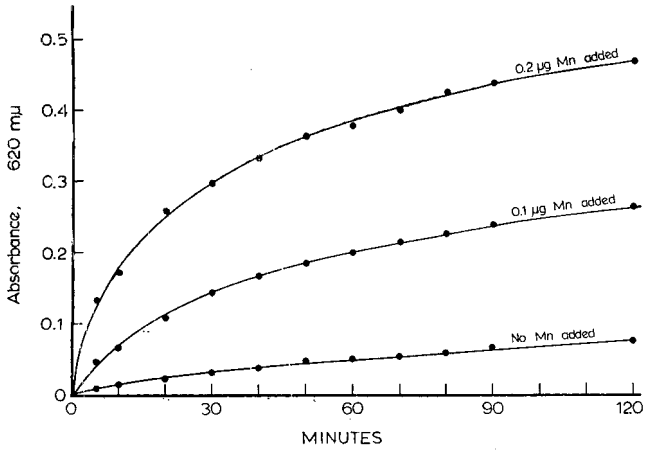

Figure 1. Catalytic oxidation of leucomalachite green by periodate in the presence of manganous ions

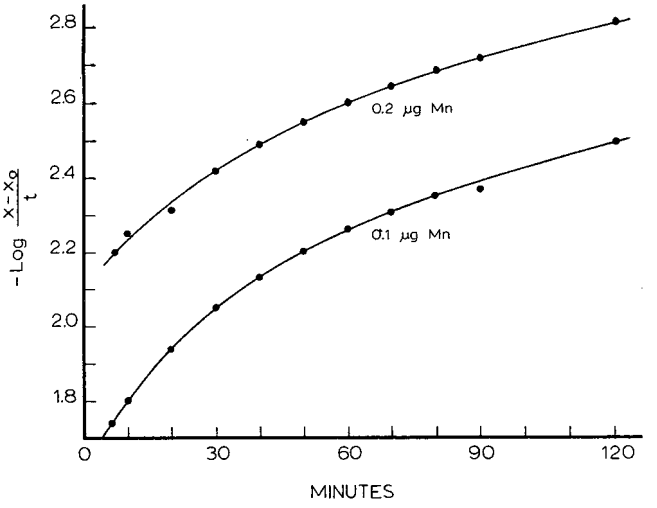

Figure 2. Test for a first order loss of manganese

were mixed after the addition of reagents, stoppered with plastic covered corks, and placed in a water bath at 26° C. Readings were taken hourly at 620 mμ with water as a reference blank in a Beckman Model B spectrophotometer equipped with a test tube adapter.

Treatment of Serum. Blood obtained by venipuncture was allowed to clot and centrifuged. For analysis, 3.0 ml. of serum was placed in 25- × 100-mm. Vycor test tubes (Corning Glass No. 7915) which contain less than 1 p.p.m. of manganese. (Borosilicate glass contains too much manganese to be used in this part of the procedure.) For recovery experiments, additional tubes were set up containing serum, and 0.2 ml. of manganese solution (equivalent to 10 mμg. of manganese) was added.

The tubes were placed in an aluminum heating block and the samples were dried at 116° C. in about 2 hours. Ashing of dried residues was performed in the same tubes in a muffle furnace at 540° C. After 1 hour the cooled residues were moistened with 1 drop of water, redried, and reashed. After an additional wetting, drying, and reashing treatment, the ashes were completely free of visible carbon. They were dissolved in 0.3 ml. of 1*N* HCl; the tubes were stoppered with Saran-wrapped corks and heated in a 70° C. water bath for 5 minutes.

After the tubes cooled to room temperature, 2.30 ml. of buffer, 0.85 ml. of water, sodium hydroxide (0.45 ml. was used to achieve a pH of 3.6), 0.3 ml. of MG, and 0.3 ml. of 0.5% sodium periodate were added as described in the general method above. A blank, devoid of sample ash, was prepared in the same way (including the HCl), and standards containing 5, 10, and 15 mμg. of manganese were set up by substituting 0.1, 0.2, and 0.3 ml. of manganese standard for part of the water.

A sufficient volume of each solution was transferred to the cuvette tubes u ed for spectrophotometric reading. These tubes were stoppered with Saran-wrapped corks and placed in a 26° C. water bath. The hourly spectrophotometric readings were commenced 3 hours after the addition of sodium periodate and were taken for 5 hours. The absorbances were plotted on semilogarithmic paper *vs.* time. The slopes of these lines were used in calculating the manganese concentrations.

The total volume of solution for the catalytic reaction using serum was reduced to 0.3 of that employed in the preliminary investigations. This was necessary to achieve the sensitivity required in assaying levels of manganese found in convenient volumes of serum. Concentrations of all reagents in the final solutions were not altered.

RESULTS AND DISCUSSION

Kinetics of Reactions. If the concentrations of LMG and $NaIO_4$ are in large excess relative to products formed, the reaction

$$\text{LMG} + IO_3^- \xrightarrow{Mn^{+2}} \text{MG} + \text{other products}$$

should be zero order, and the plot of absorbance (which in the case of MG is proportional to concentration) against time must be linear. The plot of experimental data of absorbance *vs.* time, however, was not linear (Figure 1). There was an apparent loss of velocity in the formation of the MG color with time. This deceleration in the reaction might be logically attributed to an irreversible loss of manganese in its catalytically active form. Because of the low concentration of manganese, that supposed loss should be a first order reaction, and in that case it may be shown that

$$\log \frac{x - x_o}{t} = \log \alpha\,[\text{Mn}] - \frac{k_1 t}{2.3} \quad (1)$$

where x is the absorbance in a test solution (to which Mn^{+2} has been added) at the time t, x_o is the absorbance in the blank at the same time, and α and k_1 are constants. Then, plotting $\log (x - x_o)/t$ *vs.* t should give a straight line for each concentration of Mn. This was not observed (Figure 2) and the hypothesis of an irreversible first order loss of Mn was abandoned.

A second possible explanation for the behavior of the system as described by the curves in Figure 1 may be made with the assumption of a secondary reaction culminating in destruction of dye. In that case, the low concentration of dye justifies the assumption of a first order reaction. Then, using MG instead of LMG, there ought to be decolorization of the dye. If the reaction is catalyzed by manganese, then

$$\frac{dx}{dt} = -k_1 x \quad (2)$$

where x is the absorbance of the solution and k_1 a constant proportional to manganese concentration. Actually, k_1 should be proportional to the active form of manganese, Mn^{Ox}—i.e., the oxidized form.

At the outset, only Mn^{+2} is present and it is therefore necessary that an initial lag period pass until a constant value for the relationship $[Mn^{Ox}]/[Mn^{+2}]$ be attained. Equation 2 is valid only after the lag period and when integrated between t_1 and t_2

$$\int_{x_1}^{x_2} d \ln x = -k_1 \int_{t_1}^{t_2} dt \quad (3)$$

therefore

$$\frac{\log x_1 - \log x_2}{t_2 - t_1} = k_1 = b + \beta[\text{Mn}] \quad (4)$$

Equation 4 describes the slope, k_1, of the straight line obtained by plotting the readings taken periodically *vs.* the corresponding times. These slopes should be proportional to the Mn content of the system and also, therefore, to $b + \beta$ [Mn] where b is the slope when no manganese has been added to the system. This

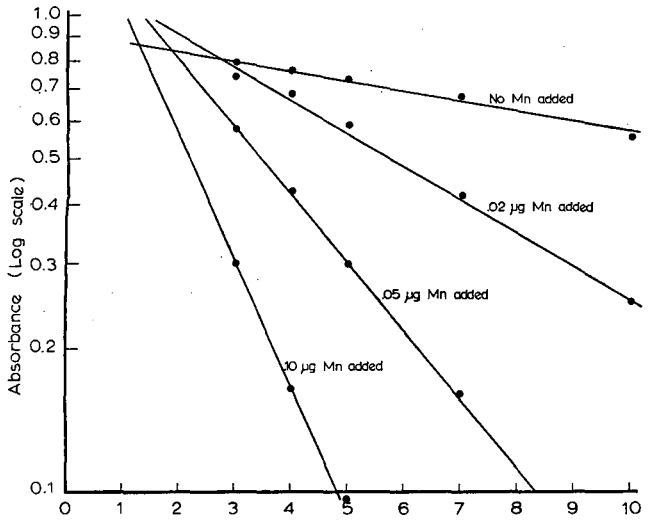

Figure 3. Catalytic oxidation of malachite green by periodate in the presence of manganous ions

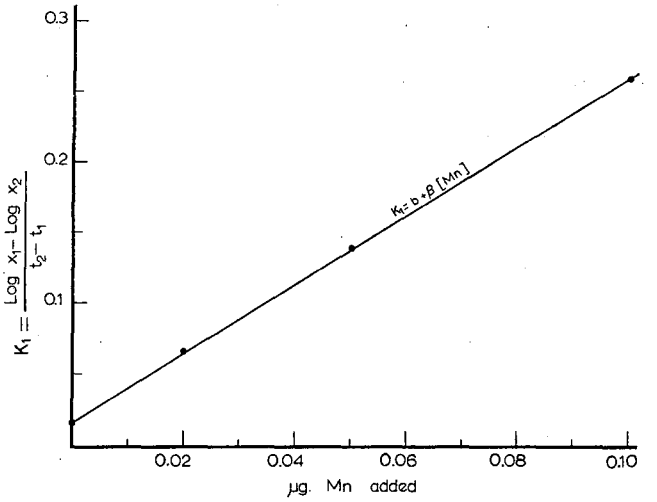

Figure 4. Relationship between the slopes k_1 and manganese concentration

conclusion was confirmed experimentally as shown in Figure 3 and by the linear relationship, shown in Figure 4. The deceleration in catalytic oxidation of LMG can now be explained by this subsequent reaction. Furthermore, after a certain period a decrease in absorbance may be expected, this period being shorter for higher concentrations of manganese. This was experimentally demonstrated (Figure 5). A similar subsequent reaction can also explain the observations of Single (*11*) using tetrabase in place of LMG.

The method now proposed depends on the first order reaction

$$\text{MG} \xrightarrow{\text{Mn}^{0x}} \text{colorless products}$$

and is suited to analytical determinations in the range of 0.2 to 3.0 mµg. of manganese per ml. of final solution read. An operating temperature of 26° C. and pH of 3.6 were chosen because higher temperatures and pH's increased the rate, thus limiting the range over which the reaction could be followed. Reaction velocities were too low at lower temperatures and pH's.

APPLICATION TO SERUM

To calculate the level of manganese in a sample of serum, Equation 4 was used in the following form

$$\text{mµg. Mn} = \frac{K_x - b}{K_s - b} \times S \qquad (5)$$

where K_x is the slope for the sample, K_s is the slope for the standard containing S mµg. of manganese, and b is the slope of the blank.

Recovery. Manganese equivalent to serum concentrations of 3 mµg. per ml. was added to each of 7 serums. The mean recovery was 76% (range 74 to 80%). The above normal values were not corrected for recovery.

Table I. Normal Values for Manganese in Human Blood

Author	Method	Mµg. Mn/ml.[a]
Bowen (*2*)	Activation analysis	14–34 (B) 11–25 (S)
Papavasiliou and Cotzias (*10*)	Activation analysis	9–15 (B) 2–3 (S)
Bentley, Snell, and Phillips (*1*)	Microbiological	50–80 (B)
Kehoe, Cholak, and Storey (*7*)	Spectrographic	0–240 (B) 0–100 (P)
Miller and Yoe (*9*)	Spectrophotometric	35–120 (P)
Fernandez, Sobel, and Jacobs	(This work)	0.36–0.90 (S)

[a] B = whole blood; S = serum; P = plasma.

Normal Levels in Human Serum. Normal values were determined on 12 laboratory workers. The 95% normal range obtained by calculation of the mean and standard deviation was 0.36 to 0.90 mµg. per ml. Data for males and females showed no significant difference by the t test (5% level); therefore, the results were pooled. A comparison of these normals with those obtained by other methods is shown in Table I. The divergence of these data from those published by others may be due to a variety of causes, including specificity of methods and contamination of sample preceding and during analysis.

Interference by Other Ions. The following ions were tested in the method for interference. The figure after each ion indicates the concentration of the element in micrograms per milliliter equivalent to one milliliter of serum. These ions are not likely to be found in serum in concentrations greater than those given. Therapeutic levels of certain elements are included. Fe^{+2}, 10; Cu^{+2}, 5;

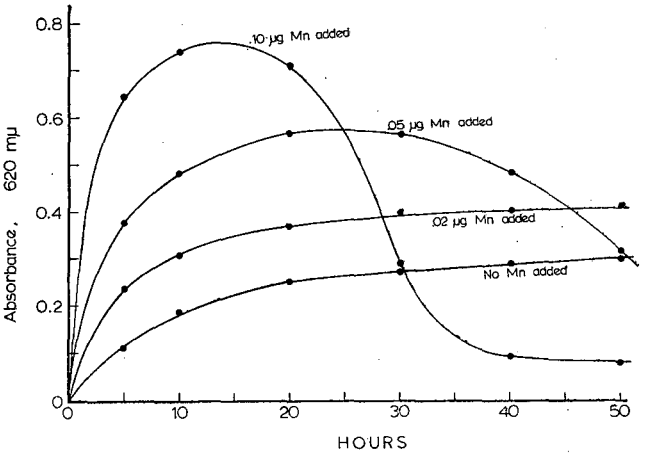

Figure 5. Reaction of leucomalachite green with periodate in the presence of manganous ions over an extended period

Sn^{+2}, 0.6; AsO_2^-, 3; Ba^{+2}, 3; Ag^{+2}, 3; Sb^{+3}, 15; Cr^{+3}, 0.6; F^-, 15; I^-, 30; $Mo_7O_{24}^{-6}$, 0.6; Ca^{+2}, 300; VO_3^-, 0.6; Br^-, 300; Ni^{+2}, 1.5; Sr^{+2}, 0.6; Co^{+2}, 1.5; Li^+, 0.6; Zn^{+2}, 15; Pb^{+2}, 15; Al^{+3}, 15; Bi^{+3}, 108.

No interference was observed except with iodine, where rapid partial decolorization was observed during the lag period after which the interference ceased—i.e., curves in Figure 3 are displaced downward without changing their slopes. Levels of 0.3 μg. of I^- per ml. of serum (normal total serum iodine is less than 0.1 μg. per ml.) do not produce any effect.

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Fast Qualitative and Quantitative Microanalysis of Plasticizers in Plastics by Gas Liquid Chromatography

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▷ Heavy esters used as plasticizers, such as dioctyl phthalate or dioctyl adipate and related compounds, may be analyzed on gas chromatographic columns at 200° to 240° C. using SE30 or poly(neopentylglycol adipate) on glass beads. Use of retention indices greatly helps in identifying the compounds eluted from the column. A special technique of mild pyrolysis of the sample in the injection port of the apparatus allows analysis of the plasticizers without any kind of extraction step. Good qualitative and semiquantitative results may be obtained.

PLASTICIZERS are very commonly used to increase flexibility, workability, or distensibility of plastics or elastomers. The more important compounds used are esters of aliphatic dicarboxylic acids (oxalic, succinic, adipic, suberic, and sebacic acids), phthalic and phosphoric acids, and of aliphatic alcohols from C_1 to C_{10}, mainly *n*-butyl and 2-ethylhexyl alcohols. The analysis of these compounds must be made on elaborated materials which may contain from a few per cent to as much as 50% of plasticizers.

Conventional chemical methods of analysis which include solvent extraction, saponification, and identification of acids and alcohols are time consuming and quite difficult. Little information is available for the analysis of plasticizers by mass spectrometry. Infrared (IR) spectrometry is quite useful for identifying the major component of a mixture, but like all these methods does not seem efficient enough to distinguish between mixtures of closely related compounds such as *n*-butyl and *n*-hexyl phthalate, or *n*-butyl suberate and *n*-hexyl adipate (*4, 10, 11*). However, it is sometimes possible to use this method without the lengthy extraction step. Polarography (*9*) and fluorescence (*2*) were limited until recently to phthalic esters, and were also time consuming. Paper chromatography does not seem to have given good results (*3, 5, 7, 8*); it is not specific enough to allow direct identification, and does not give enough sample to get an IR spectrum. Column chromatography separates sufficiently large samples but is slow and needs a large amount of material. Gas chromatography theoretically allows the separation and identification of all these esters, but many difficulties arise and no study of the whole mass of cited esters was made until recently. Bassemir (*1*) gave some analyses of phthalates as example of an application of gas chromatography to the separation of high molecular weight compounds; Lewis and Patton (*12*) gave retention data for a number of phosphates, phthalates, and related compounds on Apiezon K at 283° C., using short columns with high load (30% on Celite 545). A large volume of solute must be used (10 μl.), and the low vapor pressure of these components results in important leading of the peaks because of over-loading of the column. Cook *et al.* (*6*) used a very short column (9 inches) of 25% silicon grease to obtain the separation of dibutyl-, butylbenzyl-, and dibenzyl phthalates.

Zulaica and Guiochon discussed (*15, 16, 18*) the use of low-loaded columns to analyze the cited esters. Using 0.5% of silicon gum SE30, or of poly(neopentylglycol adipate), on glass beads etched by hydrofluoric acid, they showed that the elution of heavy esters boiling up to 400° C., under atmospheric pressure, was possible at 200° to 240° C.; no loading is noticed, provided a sufficiently small sample is introduced (0.05 to 0.20 μl.). The behavior of these columns, and especially the reproducibility of retention data, was studied. Retention volumes and retention indices

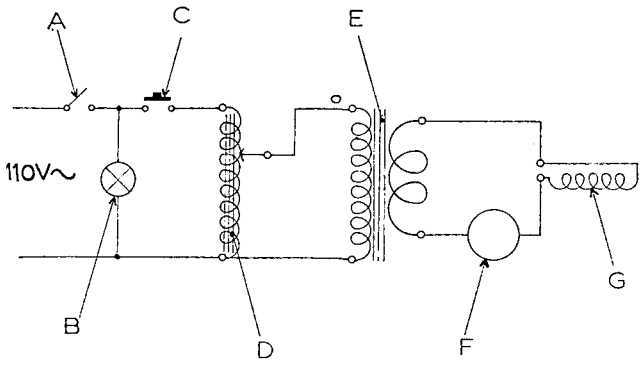

Figure 1. Diagram of electrical circuit for pyrolysis unit

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were given for esters of methyl to nonyl alcohols, and phthalic, phosphoric, and dicarboxylic (oxalic to sebacic) acids. From all these data, some simple rules could be drawn up since the variations of indices with carbon number in all the homologous series studied here are linear. This allows the qualitative and quantitative analysis of plasticizers in bulk.

In this paper we wish to discuss the application of their work to the analysis of plasticizers in plastics. The cited esters are external plasticizers—i.e., they are dissolved between the macromolecules of plastics without being bonded to them in any way. It is not difficult to extract them, either by solvent or by heating. Only the first of these methods has been used until now. It appears to be tedious and time consuming. The method described here bypasses the extraction step by fast heating of a plastic sample of a few milligrams in the injection port of a chromatograph at a sufficiently high temperature to cause the plasticizers to evaporate in the carrier gas. The temperature must not be too high, to minimize the pyrolysis of the plasticizers, and/or other components of the plastic material.

EXPERIMENTAL

Apparatus. The chromatograph used is a Perkin-Elmer Model 116E. It is a German-made version of the 154D vapor fractometer. The detector is a thermistor katharometer.

Columns. Two columns were used, both copper tubing, 4-mm. i.d., 6-mm. o.d. One is 2 meters long with 0.5% silicon gum SE30 on glass beads, previously etched for a few minutes by concentrated hydrofluoric acid and washed, the sieved beads (125- to 160-micron fraction) is used. The other column is 3 meters long with 0.5% poly(neopentyl glycol adipate) on the etched glass beads. Hydrogen is used as carrier gas with a flow rate of about 60 cc. per minute. Full details on treatment of glass beads and efficiency of columns are given elsewhere (*18*).

Pyrolysis Unit. The main part is a platinum coil welded to two copper wires, very similar to those used in the pyrolysis of polymers—for example, (*13*). The device is fitted to the chromatograph in place of the conventional silicon rubber cap. The platinum wire is 0.4 mm. in diameter and 35 mm. long. The coil is 2-mm. o.d.; its resistance is 0.02 ohm. The electrical circuit is given in Figure 1. By adjusting the variable transformer to a preset value, it is possible to get a given current in the platinum wire on closing the switch *C*. The pyrolysis time is given by a chronometer. Alternatively a time switch may be used. This is quite advisable when working on a routine basis.

The variable transformer is calibrated by measuring with the ammeter, *F*, the current obtained with no sample in the coil, on closing the switch *C*. This current decreases markedly in the first few seconds because of the increase resistance of platinum with heating.

Materials. The esters were either prepared from pure *n*-alcohols and acids or purchased from Eastman-Kodak (Rochester).

Samples of plastics were made using a mixer (Brabender, Duisburg, Germany) heated between 160° and 200° C., as was convenient. The plastic used was mainly poly(vinyl chloride) (PVC) Solvic 229 (Solvic, Paris, France); plasticizers and the ratios used are given in Tables I and II. Other samples, as indicated in these tables, came from industrial sources and were analyzed by conventional methods (extraction and IR spectrometry).

Procedure. A tube with a sharpened end is used to take samples from the product to be tested. The inner diameter of the tube is chosen so that the samples fit well in the platinum coil. Samples are then weighed. Their mean weight is about 5 mg. (Tables III and IV). Less heavy samples might be used in connection with a more sensitive detector and a thinner pyrolysis coil. One sample is then inserted in the pyrolysis coil which is screwed on the chromatograph and the hydrogen pressure set at the desired value. When base line drift is no longer noticeable, the electric circuit (Figure 1) is energized (*A*), the variable transformer (*D*) is set at the chosen value, and the current is allowed to flow in the platinum coil (*G*) for the preselected time by closing switch *C*. Current value is read on the ammeter, *F*.

The most important draw-back of this system comes from the first step: the flow of carrier gas must be stopped before removing the platinum wire, cleaning it, inserting a new sample, and

Table I. Materials Used in Qualitative Analysis

PVC + di-*n*-butyl phthalate + tri-*n*-butyl phosphate
PVC + di-*n*-butyl phthalate + di-*n*-butyl sebacate
PVC + di-*n*-butyl phthalate + di-*n*-butyl sebacate + tri-*n*-butyl phosphate
PVC + di-*n*-butyl phthalate + bis(2-ethylhexyl)adipate
PVC crystal tubing + di-*n*-butyl phthalate
PVC flask + dimethyl-, diethyl phthalate
PVC transparent film + bis(2-ethylhexyl)-phthalate
Granulated copolymer PVC–poly(vinyl acetate) + di-*n*-butyl phthalate

Table II. Materials Used in Quantitative Analysis

Ref. no.	Plastic	Plasticizer	Di-*n*-butyl phthalate, %
1	PVC	None	0
2	PVC	Di-*n*-butyl phthalate	5
3	PVC	Di-*n*-butyl phthalate	10
4	PVC	Di-*n*-butyl phthalate	20
5	PVC	Di-*n*-butyl phthalate	30
6	PVC	Di-*n*-butyl phthalate	40
7	PVC	Di-*n*-butyl phthalate	50
8	PVC	Di-*n*-butyl phthalate + 10% di-*n*-butyl sebacate + 10% tri-*n*-butyl phosphate	10
9	PVC	Di-*n*-butyl phthalate + bis(2-ethylhexyl)adipate	15
10	PVC	Di-*n*-butyl phthalate + di-*n*-butyl sebacate	7.5
11	PVC	Di-*n*-butyl phthalate + tri-*n*-butyl phosphate	25
12	PVC	Commercial di-*n*-butyl phthalate	40[a]
13	PVC + PVA	Commercial di-*n*-butyl phthalate	20[a]

[a] Determined by infrared spectrophotometry after solvent extraction.

Table III. Quantitative Calibration for *n*-Butyl Phthalate

Ref. no. (see Table II)	Per cent *n*-butyl phthalate, sample	Peak height[a] (mm.)	Sample weight[a] (mg.)	Height per mg. of sample (mm.)
1	0.0	...	...	...
2	5.0	71.0	6.33	11.2
3	10.0	93.0	4.26	21.9
4	20.0	340	7.84	43.4
5	30.0	455	5.89	77.2
6	40.0	475	4.75	100.0
7	50.0	590	4.85	121.6
8	10.0	192	6.71	28.6
9	15.0	242	6.35	38.1
10	7.5	131	6.78	19.3
11	25.0	458	6.14	74.6
12	40.0	360	3.72	96.7
13	20.0	169	3.29	51.6

[a] Mean value for a series of 10 samples.

Table IV. Reproducibility of Results

Peak height (mm.)	Sample weight (mg.)	Height per mg. of sample (mm.)
31	3.95	78
12	5.52	90
48	5.65	85
54	6.15	87
56	6.22	90
54	6.45	83
57	6.45	88
71	7.40	95
69	7.62	90
...	...	...
	Mean	87
	Std. dev.	±4.9

putting the device in place again. Several minutes are required for the base line to become stable again. The highest sensitivity of the apparatus is needed because the glass bead columns accept only minute samples—less than 1.0 μl. The whole step may require 10 minutes, but may be greatly reduced by using two pyrolysis devices and a bypass system, provided it is sufficiently heated to avoid any condensation of the heavy esters. This system may also avoid the introduction of a large amount of air into the column and the further oxidation of the stationary phase which progressively happens. This, and the deposit of tar from the pyrolysis of plastic itself, may alter the behavior of the columns, but after several months of use no modification of retention data or efficiency were observed. No deterioration of the sensing elements of the katharometer was noticed.

RESULTS

Calibration of the Pyrolysis Unit. The temperature of pyrolysis is poorly defined. Temperature increases in the sample from center to surface, in the platinum coil from both ends to the middle, and in the platinum wire from surface to center. These variations in temperature may be very important. Moreover, there is not enough time to attain a steady state, and all the pyrolysis occurs during the dynamic step of increasing temperature. The pyrolysis time is scarcely longer than 10 seconds. A study of the variations of temperature with time during the pyrolysis will be published elsewhere (*17*). All that is necessary in this study is reproducibility under well-defined conditions. An idea of the temperature reached by the sample is given by Figure 2, where temperature is plotted *vs.* current in the wire. The first curve is obtained in the following way: a crystal of a pure compound, the melting point of which is well-known, is inserted in the coil. The same procedure as for pyrolysis of plastics sample is followed and a known current allowed to flow in the wire for 10 seconds. Then the coil is removed. If the sample has not melted, the procedure is followed again with a current 0.1 amp. higher; if melted, the procedure is followed, after cleaning the coil, with a current 0.1 amp lower and a new crystal. For the reasons discussed above, this gives only an approximate temperature. The measurements of electrical resistance of the wire made under the same conditions—without sample, after 10 seconds of heating—is in good agreement with this curve (see Figure 2, second curve). The systematic discrepancy of about 30° C. is attributed to the systematic variation of temperature across the wire. We were unable to make optical measurements under 800° C. Above this temperature the three methods are in good agreement. However, in this paper we prefer to give the pyrolysis current.

The minimum temperature required for rapid enough evaporation, in the carrier gas, of all the plasticizers dissolved in the plastic sample is well above the temperature above which thermal decomposition of plasticizers seems to become noticeable. However, fairly reproducible results have been obtained by this technique.

Figure 3 shows a series of pyrolyses made with different samples of the same product. Each sample is pyrolyzed twice to determine the plasticizer remaining after the initial pyrolysis. With a current of 5 amps. there is only incomplete volatilization, since the peak of the ester is as high in the second pyrolysis as in the first. With a current of 7 amps. (about 560° C.), the volatil-

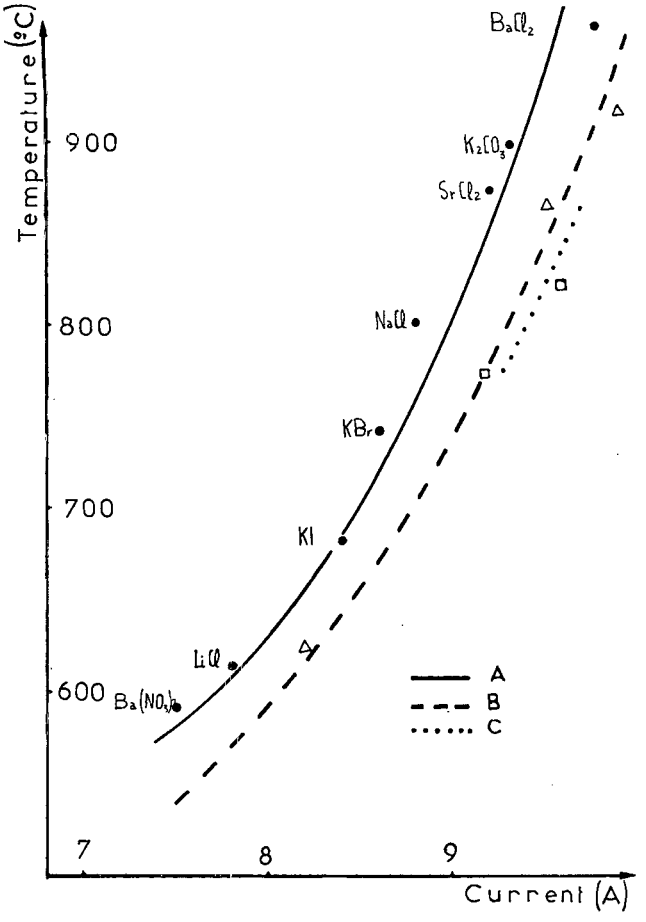

Figure 2. Temperature of pyrolysis *vs.* current

A: Melting of salts
B: Resistance of the platinum wire
C: Optical pyrometer

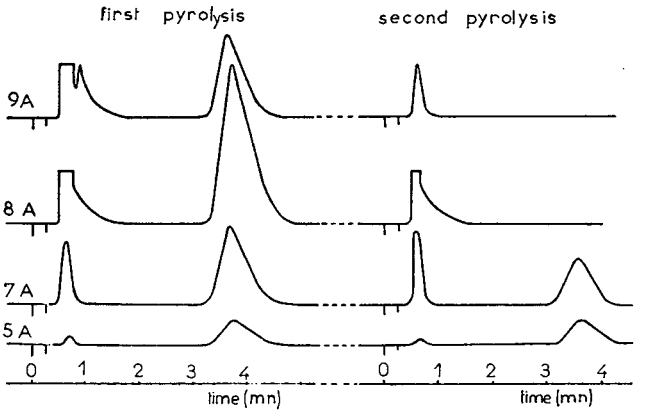

Figure 3. Influence of pyrolysis current on plasticizer recovery

Di-*n*-butyl phthalate in PVC
Apolar column

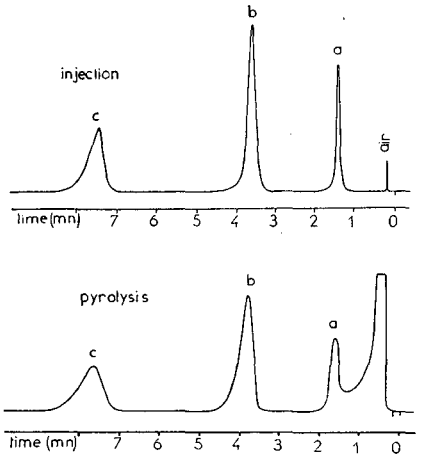

Figure 4. Apolar column

Bottom curve: Analysis by pyrolysis of a sample of PVC plasticized with (a), tri-n-butyl phosphate; (b), di-n-butyl phthalate; and (c) di-n-butyl sebacate
Upper curve: Analysis of a mixture of the same plasticizers using conventional injection with a syringe

ization is not complete. There is already a noticeable degradation of the plastic itself. With a current of 8 amps., (about 625° C.), the thermal degradation of the plastic is very marked, and there remains no detectable trace of plasticizer after the first pyrolysis. Above 8 amps., the higher the current, the lower is the plasticizer peak, showing that the thermal degradation of platicizers is increasing. Of course this phenomenon varies widely according to the plasticizer. Lewis and Patton (*12*) reported that bis(2-ethylhexyl)-*o*-phthalate is quite stable when the preheater of their chromatograph was at 580° C., but that bis(2-ethylhexyl)-*m*-phthalate is partially decomposed at 440° C. Thus, although the estimated maximum temperature of the surface of the wire is about 650° C., some kinds of plasticizers may be almost completely decomposed. However, in all the plasticizers studied in our work (see Table I) this never happened.

The same experiments were made at different current levels around 8 amps. while varying the pyrolysis time. The best results were obtained with a current of 8 amps. and a time of 10 seconds or more. Lengthening this time gave no further modification of the chromatogram obtained.

Figure 4 shows the chromatogram obtained with a sample of a PVC containing tri-*n*-butyl phosphate, di-*n*-butyl phthalate, and di-*n*-butyl sebacate and the corresponding chromatogram obtained by injection of a liquid mixture of these three esters. For approximately the same quantity of solute, peaks are narrower and higher when liquid is injected than when the plasticizer is obtained on pyrolyzing the polymer. The pyrolysis peaks tail somewhat, while with liquid injection they are Gaussian and give leading when the column is overloaded. This tailing seems to come from the injection which is not instantaneous, but takes 5 to 10 seconds. During that time the rate of vaporization of plasticizer into the carrier gas stream is not constant. The smallness of the discrepancies between retention data obtained with the two systems of injection (see Table V) seems to be related to the good stability of experimental parameters: temperature and flow-rate. The latter is obtained in spite of the interruptions of gas flow rate to introduce the samples because a toggle valve is used for this purpose and not the regulator valve.

On all the chromatograms shown in this paper it may be noticed that the pyrolysis of the plastic itself gives mainly low boiling compounds with very short time of elution. On the columns, the tailing observed is due to the polarity of some of these compounds (mainly hydrogen chloride) and to the injection function. No high boiling compounds can be detected so that there can be no interference with the plasticizers.

Qualitative Analysis. The main difficulties come from the total absence of an air peak and the impossibility of adding known standards, such as *n*-paraffins, to the mixture. The retention indices must thus be computed from the logarithms of retention volumes using the expression of Wehrli and Kovats (*14*):

$$I = 200 \frac{\log V_N(nP_{z+2}) - \log V_N(X)}{\log V_N(nP_{z+2}) - \log V_N(nP_z)} + 100\,z$$

where I is the retention index, $V_N(X)$ the absolute retention volume of substance X, and nP_z, the *n*-alkane with z carbon atoms (z is an even number). The computation of the absolute retention volume is made from the retention time of the peaks of the plasticizers as measured on the chromatogram, the origin of time being taken at the middle of the pyrolysis time. Correction for column gaseous volume is taken from a calibration curve relating air elution

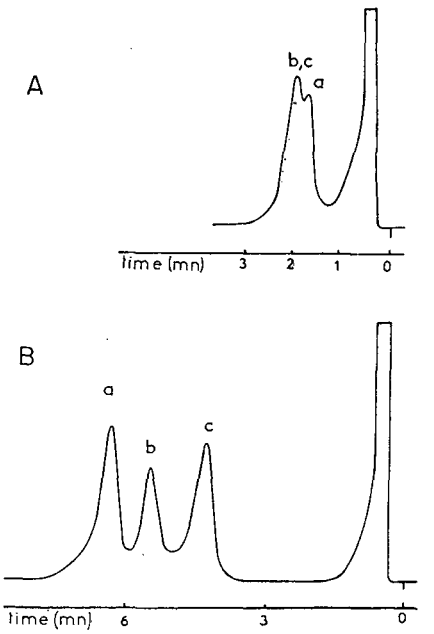

Figure 5. Analysis of a sample of plasticized PVC

a: Diethyl phthalate
b: Dimethyl sebacate
c: Tri-*n*-butyl phosphate

A: Apolar column
B: Polar column

time to the carrier gas flow rate. Table V shows the retention indices of some plasticizers: in the first row the values are computed from the rules previously given (*15*), in the second row they are measured from pure compounds injected as liquids, and in the third row they are measured from the pyrolyses of plastic products containing known plasticizers at different concentrations. The difference between the last two values are less than ±1% of the indices. This is equal to the relative standard deviation of a series of measurements of the indices obtained by this experimental technique. When *n*-alkanes may be added to the plasticizers, in bulk or in solution, a relative standard deviation of ±0.3% may be obtained (*15*).

Figure 5 shows the chromatograms obtained on the two columns for samples of the same material, PVC plasticized

Table V. Computed and Measured Retention Indices

Compounds	I_R Computed	I_R Injection	I_R Pyrolysis	$I_{R(pyr.)} - I_{R(inj.)}$
Dimethyl phthalate	1457	1450	1469	19
Diethyl phthalate	1587	1583	1594	11
Di-*n*-butyl phthalate	1926	1932	1920	−16
Bis(2-ethylhexyl)phthalate	2482	2470	2496	26
Tri-*n*-butyl phosphate	1663	1648	1633	−15
Di-*n*-butyl sebacate	2169	2150	2156	−6
Bis(2-ethylhexyl) adipate	2412	2391	2400	9

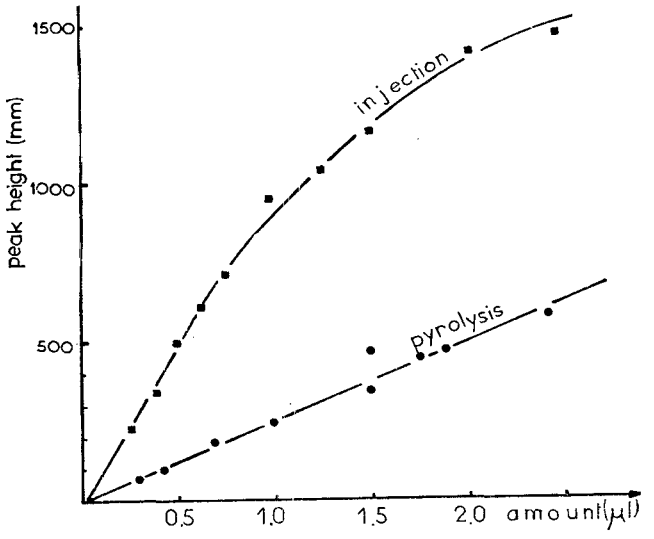

Figure 6. Quantitative analysis of di-*n*-butyl phthalate

Peak height *vs.* plasticizer amount, either injected with a syringe or present in the pyrolyzed plastic sample. Apolar column

with diesters which may be identified only by the comparison of their retention data on the two columns.

Quantitative Analysis. Quantitative analysis may be made by gas chromatography if separation of the significant peaks from others in the chromatogram can be obtained. With our columns the most commonly used plasticizers may be separated. Most plastics contain only one to three plasticizers. As an example, we shall discuss in detail only analysis of di-*n*-butyl phthalate which is a very important plasticizer.

Figure 6 shows the plot of peak height *vs.* quantity of liquid injected. A straight line is obtained from 0 to 0.8 μl., then some curvature appears, because of overloading of the column. The minimum detectable quantity is about 0.002 μl. Figure 6 also shows the plot of peak height *vs.* quantity of plasticizer when pyrolysis is used. These points are obtained from samples of plastic prepared in this laboratory and containing known percentages of di-*n*-butyl phthalate (with or without other plasticizers). These mixtures are listed in Table II with their compositions. Table III gives the mean height of the peaks obtained when a series of 10 samples of each material is pyrolyzed, the mean weight of the samples, and the height per milligram of sample—which is approximately proportional to the percentage of plasticizer. The relative error of the analyses is about 5%.

Table III shows that there is no action of other plasticizers on the eluted quantity of di-*n*-butyl phthalate. The results obtained are not modified by the nature of the plastic itself: numerous samples of PVC and some copolymers of various origins were tested with the same results.

Peak height was preferred to peak area for quantitative measurements because for liquid samples the correlation does not seem better with the area in the linear part of the curve, and because in the curved part the peak does not remain Gaussian but shows some leading. It is difficult to get an accurate value of this area without an integrator. In pyrolysis the same difficulties arise from the tailing that peaks generally show. The use of an integrator should greatly improve the precision of the analysis.

In Figure 6 a great difference between the peak height obtained for the same quantity of di-*n*-butyl phthalate injected or introduced by pyrolysis may be noticed. This difference arises partly from the previously-discussed difference in peak shape, as shown in Figure 4. Injection gives a peak of di-*n*-butyl phthalate which is, for the same quantity of plasticizer, 2.10 times that obtained by pyrolysis. The ratio of the peak areas measured with a planimeter, however, is only 1.21. This difference cannot come from a loss of plasticizer during the fabrication: a loss of more than 10% would have been quite improbable, and the actual concentrations of plasticizer were analyzed by IR spectrometry after solvent extraction. This loss is likely to come from pyrolysis of the plasticizer. If this is true it means that the experimental conditions—such as platinum wire dimensions, injection port geometry, etc. —can have a very marked influence on the quantity of plasticizers actually injected into the column.

As may be seen from Table IV, the relative standard deviation of results for a series of 10 measurements made on the same plastic is ±5.5%. The same deviation is obtained for samples holding more than 7.5% of di-*n*-butyl phthalate.

With the apparatus described here, one analysis may be made in half an hour or less; with some technical refinements it seems possible to complete the analysis in less time.

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Aerodynamic Forces in Thermogravimetry

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▶ In thermogravimetry, small samples permit better accuracy and resolution for the same scanning rates. With balances of adequate sensitivity, aerodynamic "noise" is of interest. Aerodynamic noise is found to be strongly affected by hangdown tube diameter and pressure, proportional to the horizontal area of the sample pan, only slightly affected by changes in sample temperature, and independent of sample weight. Precision to the 1-μg. level can be obtained at atmospheric pressure with very small diameter tubes, or with large tubes at pressures below 150 torr.

IN THERMOGRAVIMETRY, small samples offer substantial advantages. Sample temperature is more homogeneous, and exposure to atmospheres present is more uniform. Transitions are sharper, and both transition temperature and mass are better defined. In addition, the transition temperature value obtained will generally be closer to the true value for the molecule. An example is shown in Figure 1.

To measure thermogravimetric curves for samples of a few milligrams or tens of milligrams with good accuracy, precision of the order of 1 μg. is desirable. The first requirement for such work is a controlled-atmosphere balance of adequate sensitivity. Several such instruments have been described by the authors (*1*, *2*). With such an instrument, the question arises as to the level of precision to which the phenomena themselves permit measurement. Much important thermogravimetric work requires significant partial pressures about the sample. Heating in such an atmosphere will cause aerodynamic forces, which introduce uncertainty into the balance readings. The magnitude and nature of these forces, the effect of operating parameters upon them, and the conditions for optimum precision were investigated.

The aerodynamic forces applied to the balance can be divided into two components, a constant offset and a varying component. The varying component always looks like the "noise" seen on spectrophotometer traces (Figure 2). Like that noise, it can be characterized in magnitude by the peak-to-peak variation for the degree of precision significant here. The uncertainty in this measure of the uncertainty is about ±20%. The rule of thumb that an average value can be defined to within one fifth of the peak-to-peak (*pp*) value by visual estimation from a noisy trace was applied. The constant offset was never larger than the *pp* noise in these experiments. It could be determined by a blank run and corrected for, if desired, so it is not considered further here.

The resulting situation can be compared to that in infrared spectroscopy, where resolution, signal to noise ratio, and scanning time are all important and interrelated. Both resolution and scanning time can be improved by using smaller samples, but at the possible expense of signal to noise ratio. To achieve both high resolution and high signal to noise ratio may require longer scanning times.

Two well known boundary conditions may be noted. The first is weighings in air at ambient temperature and pressure. These are the conditions under which balances are commonly rated, and much data is available on them. While

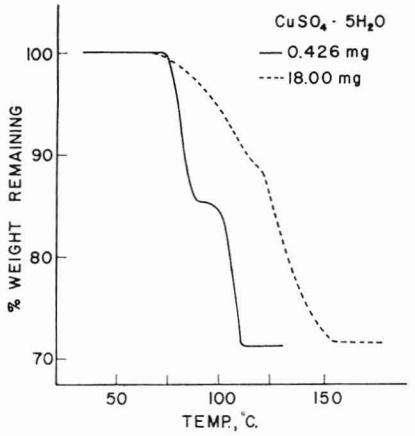
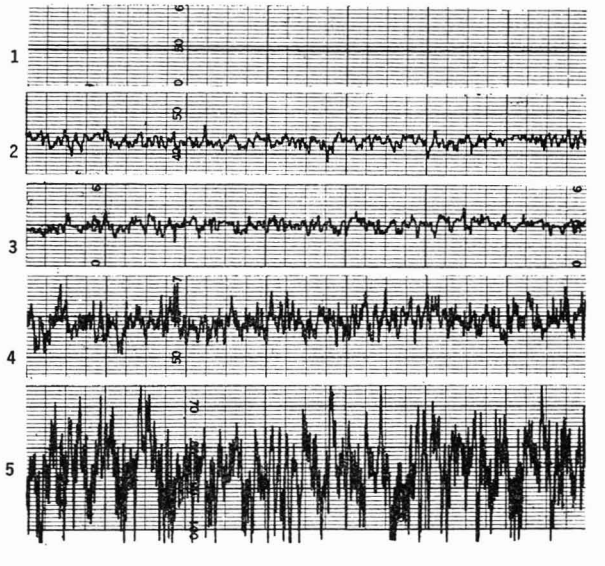


Figure 1. Thermogravimetric curves for copper sulfate pentahydrate in air at atmospheric pressure with samples of 0.426 mg. and 18.00 mg. under identical conditions

Heating rate was 13° C./minute. Two moles of water are lost at about 75° C. and two more at about 100° C., in separate reactions. Resolution and reaction temperature values are much better with the smaller sample. Temperature error with the larger sample was more than 40° C.; mass error at the break about 20% of the true change



AREA

Figure 2. Typical noise curves at 100° C. and atmospheric pressure in a 41-mm. diameter tube; sensitivity 0.01 mg./div.

(1) no hangdown wire
(2) hangdown wire only
(3) 100 mg. vertical foil added
(4) hangdown wire, stirrup, and 12-mm. pan
(5) 25-mm. pan

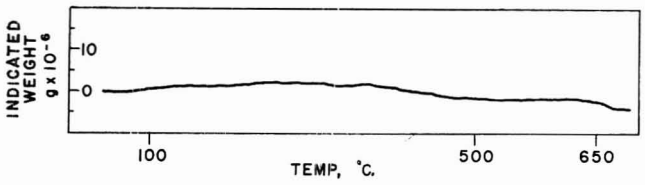


Figure 3. Blank run on platinum pan using RG Electrobalance and No. 2006 TGA kit

Hangdown tube is 8 mm. i.d., scanning rate about 20° C./minute. Blank correction must be made for work finer than ±2 μg.

air currents are believed to be a limiting factor, they do not prevent stability at the 0.1-μg. level in a properly designed balance. The second is weighings in high vacuum, as is often done in surface chemistry. Values to 0.1 μg. have been reported for such weighings (*6*).

In the pressure range from about 10^{-3} to 10 torr there is another phenomenon, thermomolecular flow, which also exerts real forces on heated samples and hangdown wires. It is discussed elsewhere (*4, 5*). Measurements in this work were confined to the pressure ranges where thermomolecular flow is not a factor.

EXPERIMENTAL

The curves in Figure 1 and Figure 3 were taken using an RG Electrobalance®[1] and No. 2006 TGA accessory.

All other measurements were made using an RG Electrobalance mounted in a glass vacuum bottle with hangdown tubes (Figure 4). This instrument is sensitive to 0.1 μg., and can be set to full scale recorder ranges from 0.02 mg. to 1 gram. The sample was suspended near the bottom of the hangdown tube, which is 500 mm. long and 41-mm. i.d., for all runs except one noted below. The suspension wire was 0.1-mm. diameter nichrome. The sample space was heated by a heating tape. Temperature values were measured outside, underneath the heating tape, and corrected by the results of a run made with a thermocouple inside at the sample position. The difference between inside and outside temperature values did not exceed 10° C. There was no thermal insulation between the glass bottle and the heating tape. Time constant was 0.5 second for all runs except Figure 3. More filtering would reduce noise further.

The first run was made without anything suspended from the balance beam to evaluate the effects of air currents flowing up the tube and impinging directly on the beam, of heat conduction up the tube, and of radiation directly from the heating tape to the balance mechanism. Sample space temperature was 100° C., and pressure was atmospheric. Noise was less than 1 μg. *pp*, and thus negligible for this study. All subsequent forces measured are believed to be real forces acting on the sample and its suspension, and not balance errors.

Area. All of the following runs were made at 100° C. and atmospheric pressure.

The second run was made with hangdown wire only. The noise increased to about 40 μg. *pp*. Then a vertical foil weighing 100 mg. and about 2 sq. cm. in area was added. There was no perceptible increase. With a small stirrup and 12-mm. diameter aluminum pan in a horizontal plane, the noise increased to about 100 μg. *pp*. With a large stirrup and 25-mm. pan, it increased to about 350 μg. *pp*. The conclusion drawn was that vertical area has no effect on the noise, but horizontal area suspended from the balance increases it almost proportionally.

Geometry. Two different geometries were investigated in an attempt to reduce the noise at atmospheric pressure. In the first approach, the stirrup with 12-mm. pan was put inside a metal enclosure suspended from the bottle (Figure 5). It was completely enclosed except for a small hole about 1-mm. diameter in the top to pass the wire. At 100° C. with 100-mg. sample, the noise was reduced from 100 μg. *pp* to 40 μg. *pp* by the

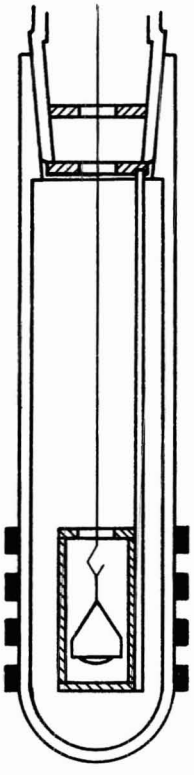


Figure 5. Enclosure for sample pan and stirrup is suspended from bottle so hangdown tube can be removed easily

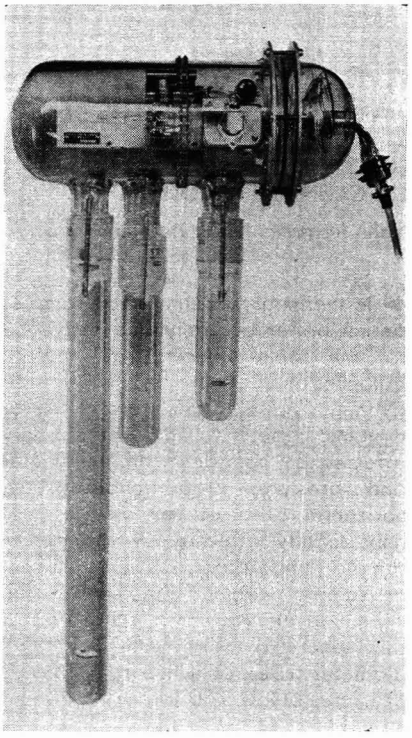


Figure 4. RG Electrobalance weighing mechanism in glass vacuum bottle as used in measurements

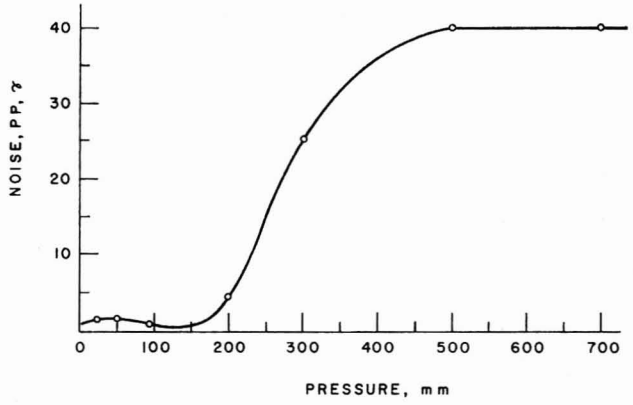


Figure 6. Effect of air pressure on aerodynamic noise, μg. *pp* vs. absolute pressure in torr

enclosure. This is the amount observed on the hangdown wire alone. It appears that the enclosure eliminated the forces on the pan, leaving only those on the hangdown wire, which set a limit to further improvement in this direction.

In the second approach, a much smaller diameter hangdown tube was used (7-mm. i.d. and 150 mm. long) (*3*). A special quartz pan, 5-mm. i.d. and weighing about 50 mg., was suspended by the same wire, with no additional load. Temperature was 100° C., pressure was atmospheric. Noise was only 1.2 μg. *pp*, permitting measurements to about 0.24 μg. This geometry appears ideal for samples below about 20 mg., eliminating all concern with aerodynamic noise.

Temperature. The effect of temperature was determined with the 12-mm. pan, 100-mg. load, at atmospheric pressure in the enclosure (Figure 5). At ambient temperature, 25° C., the noise was only 0.4 μg. *pp*, which appears consistent with a large body of performance data considering the effect of the unusually long hangdown wire. At 100° C., the noise had increased to about 40 μg. *pp*. In other work the authors have often observed major increases in noise immediately after beginning to heat at sample temperatures as low as 40° C. At 300° C., the noise had only increased to 50–60 μg. *pp*. Similar results were obtained without the enclosure and in other hangdown geometries at temperatures up to 1250° C. It was concluded that relatively slight heating causes major amounts of noise, and that further increases in temperature cause only minor increases in noise. This appears to make other measurements at 100° C. applicable to much higher temperatures.

Mass. The effect of mass in the pan was evaluated with the 12-mm. pan at 100° C. and atmospheric pressure, in the enclosure, with samples of 10, 100, and 1000 mg. An effect was expected since the wire and pan had moved in other experiments. However, no difference was observed between the three runs. The noise remained about 40 μg. *pp* for all loads. For maximum precision as a fraction of total load, the sample should then be as heavy as possible. If to one fifth of the *pp* noise can be read, a precision of 10^{-5} of total load can be obtained in the large hangdown tube at atmospheric pressure, using an enclosure.

Pressure. The effect of pressure was determined at 100° C. in air with a 100-mg. sample, in the enclosure (Figure 6). Up to about 150 torr, the noise was not more than 1 μg. *pp*. There seems to be a slight minimum just above 100 torr. Above 200 torr, the noise increases rapidly, so that 500 torr is just as bad as 760. Identical results were obtained without the enclosure up to 200 torr; beyond that, the noise increased in a similar way to 100 μg. at 760, as expected. A pressure of 150 torr is enough atmosphere for many experiments. Where it is, aerodynamic noise can be eliminated easily, even in large tubes.

Blank. A blank run on a 40-mg., empty platinum pan with 8-mm. i.d. hangdown tube, in air at atmospheric pressure is shown in Figure 3. The indicated weight varies ±2 μg. between 50° and 650° C. Such blank curves are reproducible, and blank corrections should be made for the finest work. An electrical filter period of 2.5 seconds in this run eliminated all noise. At this sensitivity, the recorder chart can be read to about 0.4 μg. With quartz pans, the effect of reduction in air buoyancy produces a blank curve ranging from 0 to 40 μg. which agrees with theoretical values within ±2 μg.

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Spectrophotometric Determination of Bromide Ion in Water

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▶ **Bromide ion is determined spectrophotometrically in water as a function of its inhibition in the chlorination of ammonia to form trichloramine. The determination is rapid and applicable to concentrations of bromide ion in the range of 0.02 to 1.20 p.p.m. Reactions proceed in solutions buffered to pH 5.6 between 23° and 26° C., and the concentration of bromide ion is measured as a function of the ammonia lost because of oxidation by hypobromite to nitrogen. A simplified preparation for a modified cadmium iodide–linear starch reagent, which produces the blue starch–triiodide complex used for spectrophotometric determination, is described.**

IN A RECENT PAPER (*11*) the authors established the feasibility of determining ammonia spectrophotometrically in the parts per billion range as trichloramine, through the starch–iodide reaction. As part of that study, it was found that bromide ion interfered because of its oxidation by hypochlorite to hypobromite which then oxidizes ammonia to nitrogen; thus the yield of trichloramine is decreased leading to low values for ammonia. Interference due to bromide ion was eliminated by the use of salicyclic acid, but the fact that its interference was significant and specific at concentrations of less than 1 p.p.m. suggested the applicability of this reaction to its determination in water. With some slight modification in the original procedure for ammonia, the method has been adapted to the determination of the bromide ion in the range of 0.02 to 1.20 p.p.m. Under these circumstances, ammonia becomes the standard reagent, and bromide ion is estimated as a function of the amount of ammonia not oxidized to nitrogen.

A determination is made by adding an accurately measured amount of ammonia to the bromide ion sample buffered to pH 5.6 between 23° and 26° C. Upon addition of hypochlorite, competitive reactions between ammonia and hypobromite, which is formed almost immediately, result in the simultaneous formation of trichloramine and nitrogen. The amount of each product formed is dependent upon the quantity of bromide ion present initially if the ammonia and hypochlorite concentrations remain constant for a series of determinations. Unreacted hypochlorite and hypobromite are then selectively destroyed with nitrite ion and the trichloramine–nitrite oxidation product is measured spectrophotometrically as

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the blue starch–triiodide complex. Absorbance decreases linearly with increased bromide ion concentration.

Samples containing ammonia would give high absorbance values, thus leading to a low estimate of bromide ion. To circumvent this interference, a method for prior ammonia removal utilizing ion exchange techniques has been incorporated into the procedure. The only other significant interference is iron, which can be removed in the same manner. Chlorine would be expected to interfere, but its incompatibility with bromide ion prevents the presence of the latter in chlorinated samples, and a determination under these circumstances is unnecessary.

EXPERIMENTAL

Special Reagents and Equipment. All solutions were made with ammonia-free water prepared by ion exchange (*1*).

Standard bromide solution, 1000 p.p.m., 1.489 grams of potassium bromide per liter of solution.

Stock ammonia solution, 1000 p.p.m. NH_3, 3.142 grams of ammonium chloride per liter of solution.

Working ammonia solution, 20.00 ml. of the stock solution diluted to one liter.

Buffer solution, pH 5.6, 136 grams of sodium acetate trihydrate and 8.2 ml. of glacial acetic acid per liter of solution. The pH of this solution should be carefully adjusted after preparation with the aid of a pH meter.

Hypochlorite solution (*11*), 25.00 ml. of Zonite per liter of solution.

Nitrite solution, 0.5% sodium nitrite.

Modified cadmium iodide–linear starch reagent. Dissolve 2.50 grams of Superlose HAA-11-HV (Stein, Hall and Co., 285 Madison Ave., New York, N. Y.) in 500 ml. of water and stir well until an almost clear solution results (about 15 minutes). In another 400 ml. portion of water, dissolve 11.0 grams of cadmium iodide and boil gently for 15 minutes to expel any iodine. Combine the two solutions and heat the mixture for twenty minutes at a temperature just below its boiling point. After cooling to room temperature dilute the solution to 1 liter and set it aside over night. Filter with suction through a fine filter paper, such as Whatman's No. 42, on a Büchner funnel. Superlose HAA-11-HV is a water-soluble hydroxyethyl derivative of amylose potato starch fraction. The absorption curves of the triiodide ion complex in the visible region are similar to those of previously reported linear starch reagents (*2–10*), although it is not so pure a blue color to the eye. Adherence to Beer's law at 615 mμ was confirmed using iodate ion in 2.5*N* hydrochloric acid as the oxidizing agent. The molar absorptivity is 16,900 per equivalent of oxidizing agent.

Cation exchange column. A standard borosilicate glass chromatographic tube, 12 inches in length, $^1/_2$-inch i.d., is fitted with a sealed-in, coarse porosity, fritted disk and filled with enough 16- to 50-mesh nuclear sulfonic cation exchange resin, in the hydrogen form, to produce a 6-inch column. This column should contain no other packing material which may affect the rate of exchange due to surface adsorption phenomena. Disturbance of the upper resin layers, when the column is in operation, can be prevented through careful introduction of the sample.

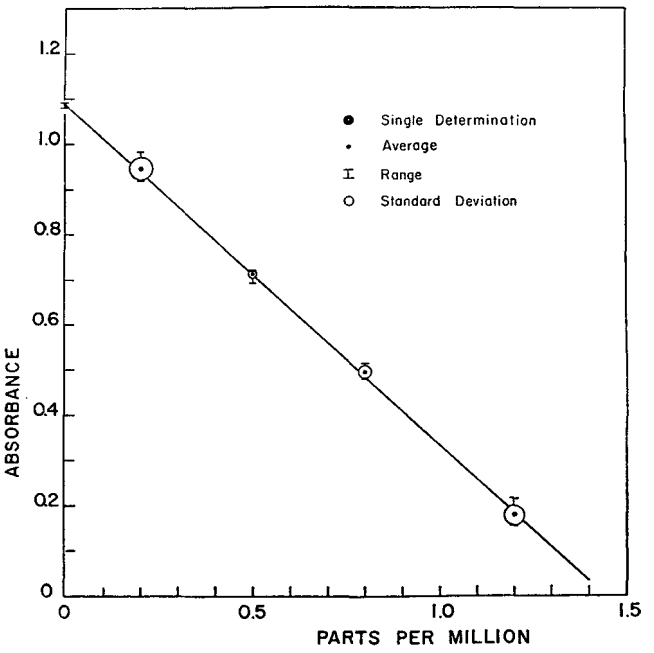

Figure 1. Calibration curve

Procedure. Adjust the temperature of bromide ion samples in the range of 0 to 1.2 p.p.m. to between 23° and 26° C. To 50.0 ml. of the sample in 125-ml. Erlenmeyer flasks add 1.00 ml. each of the buffer and working ammonia solutions. With stirring add 1.00 ml. of the hypochlorite solution and continue stirring for several seconds to ensure intimate mixing. After $3^1/_2$ minutes, add 1.00 ml. of the nitrite reagent and stir for at least 40 seconds, making certain to include the solution adhering to the walls of the flask. Add 2.00 ml. of the cadmium iodide–starch reagent stir well, and measure the absorbance at 615 mμ. after 10 minutes.

If the capacity of the buffer is insufficient to bring the pH of the samples to 5.6, this should be done roughly with either 0.1*N* nitric acid or sodium hydroxide prior to its addition. Excess buffer should not be added to effect the same result, because the reactions involved in the chlorination step are dependent upon the acetate to hypochlorite concentration ratio.

If ammonia is present in excess of 0.01 p.p.m., pass a 100-ml. sample through the cation exchange column at a flow rate of about 10 ml. per minute. Discard the first 40 ml. of solution and run a determination for bromide ion on the next 50-ml. portion as described above. In waters containing large amounts of

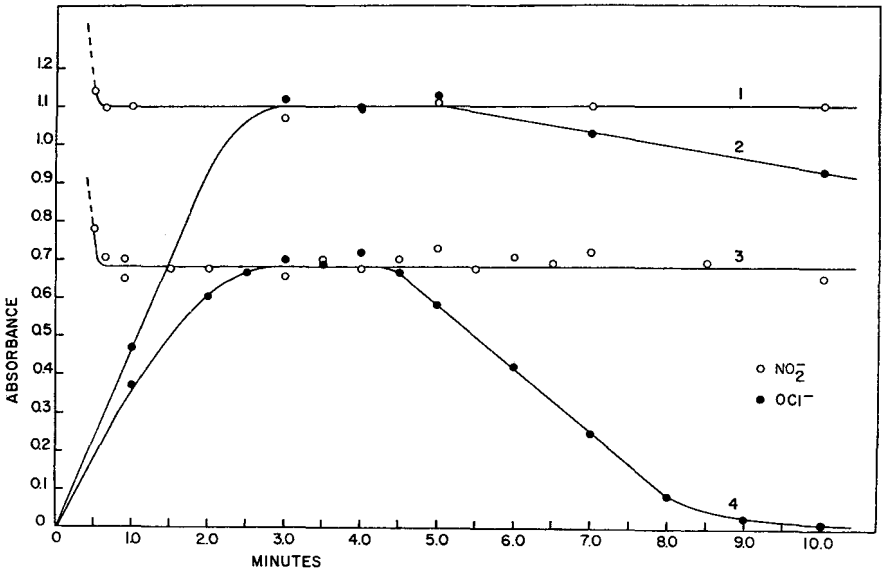

Figure 2. Time dependence of chlorination and nitrite reactions

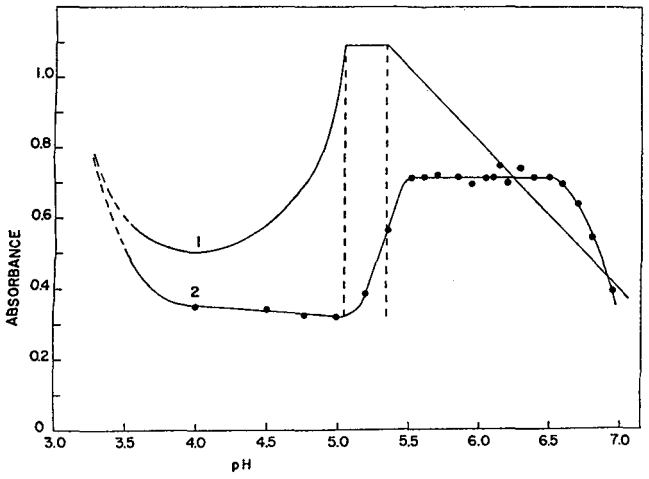

Figure 3. Effect of pH on trichloramine recovery

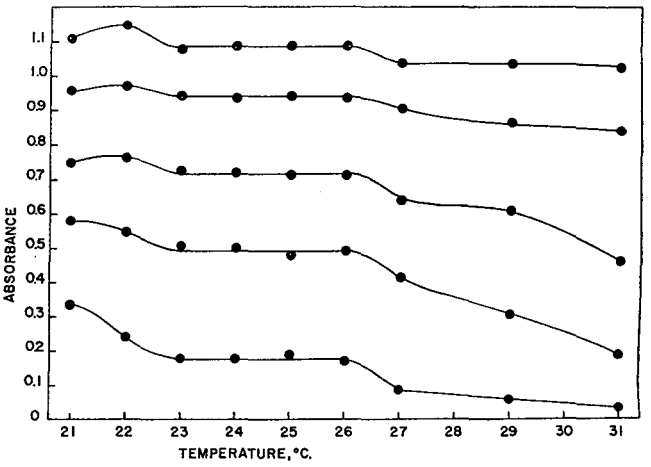

Figure 4. Effect of temperature on trichloramine recovery

ionic species the pH of the sample may be lowered, because of hydrogen ion liberation from the resin, to a point where the buffer cannot overcome the high acidity, thus necessitating a pH adjustment as mentioned previously. This procedure was used successfully on samples containing up to 20 p.p.m. of ammonia.

DISCUSSION

Figure 1 shows the relationship among the average, the range, and the standard deviation for a series of five consecutive bromide determinations at each concentration shown. The standard deviations at each concentration were ±0.005 at 0 p.p.m., ±0.030 at 0.020 p.p.m., ±0.016 at 0.50 p.p.m., ±0.020 at 0.80 p.p.m. and ±0.029 at 1.20 p.p.m. The average standard deviation for 25 readings was ±0.020. The value 0.020 was therefore taken as the lower limit of the applicable range. A coefficient analogous to the molar absorptivity calculated from Figure 1 was −60,600.

A series of time studies involving the chlorination and nitrite reactions for 0- and 0.5-p.p.m. bromide ion samples are presented in Figure 2. Curves 1 and 2 show the effect on absorbance (produced at 615 mμ by the starch–I_3^- complex in a subsequent reaction) of the 0-p.p.m. bromide samples as the reaction time is varied. Curves 3 and 4 represent the same for the 0.5-p.p.m. bromide samples. The reaction times in curves 1 and 3 are those of the nitrite solution between its addition to the ammonia and ammonia–0.5-p.p.m. bromide solutions, which previously have reacted for $3^1/_2$ minutes with hypochlorite according to the procedure given for the method. The reaction times in curves 2 and 4 are those of the hypochlorite prior to the addition of the nitrite solution. It is apparent from curves 2 and 4 that maximum formation of trichloramine occurs in somewhat less than three minutes as ammonia reacts with hypochlorite. In curve 2 a gradual decrease in absorbance indicates a slow decomposition of trichloramine after about 5 minutes. The sharper decrease in absorbance shown in curve 4 indicates that bromide ion hastens the trichloramine decomposition. Curves 1 and 3 show that no decrease in absorbance occurs even ten minutes after the nitrite addition regardless of whether or not bromide ion is present. Since it has been established from curves 2 and 4 that decomposition of trichloramine results after four to five minutes, it must be assumed that a reaction between trichloramine and nitrite occurs to produce a relatively stable product which subsequently oxidizes the iodide ion in the starch–iodide reagent. If this is the case, one would expect the oxidizing agent formed to be a nitrogen oxychloride, possibly NOCl. Based on this reasoning, the following equations may be depicted as taking place,

$$OCl^- + Br^- \rightarrow OBr^- + Cl^- \quad (1)$$

$$3H^+ + NH_3 + 3OCl^- \rightarrow NCl_3 + 3H_2O \quad (2)$$

$$3OBr^- + 2NH_3 \rightarrow N_2 + 3H_2O + 3Br^- \quad (3)$$

$$2NCl_3 + 6Br^- \rightarrow N_2 + 6Cl^- + 3Br_2 \quad (4)$$

$$OCl^- + OBr^- + 2NO_2^- \rightarrow Cl^- + Br^- + 2NO_3^- \quad (5)$$

$$NCl_3 + NO_2^- \rightarrow X \quad (6)$$

$$X + 3I^- \rightarrow I_3^- + Y \quad (7)$$

$$I_3^- + \text{starch} \rightarrow \text{starch–}I_3^- \quad (8)$$

where X and Y represent the product of the trichloramine–nitrite reaction and its decomposition products.

Only Equations 2, 6, 7, and 8 lead eventually to the formation of a blue complex. The other reactions all yield products which subsequently lose their ability to oxidize iodide ion through nitrite reduction, or are already in the reduced state. Reaction 4 probably does not occur to any great extent in this procedure because the time of chlorination is stopped at $3^1/_2$ minutes and, as shown in curve 4, it would not proceed significantly until about $4^1/_2$ minutes. The high absorbance below 30 seconds in curves 1 and 3 is attributed to incomplete hypochlorite or hypobromite destruction by nitrite ion.

The effect of pH on absorbance is shown in Figure 3. Curve 1 is similar to the one published earlier (*11*) for an ammonia sample containing no bromide, and curve 2 represents the same system containing 0.5 p.p.m. of bromide ion. Obviously the greatest sensitivity would be realized if the determination were run at a pH about 5.2, which is on the maximum plateau of curve 1. However, when this was done, the relationship between absorbance and bromide ion concentration was not linear. Above pH 5.5 there is a linear relationship and this region was therefore chosen for the present method. Because the stability of the blue starch–triiodide complex decreases above pH 5.5, due to incipient precipitation of cadmium hydroxide, the buffer system of 5.6, (which is as acidic as practicable without being on the shoulder of the plateau in curve 2) was selected.

In Figure 4 the effect of temperature on the reproducibility of the determination is presented. It is apparent from the curves, which cover the concentration range of the calibration curve, that solutions should be brought to between 23° and 26° C.

The relationship between color formation of the blue starch–triiodide complex and time is shown in Figure 5. Maximum development is reached in somewhat less than ten minutes and then remains constant.

Presented in Table I are the results of an interference study. The substances studied were added to samples containing 0.5 p.p.m. of bromide ion and run according to the regular procedure. Substances which caused a decrease in color intensity are listed as positive interferences. Aliphatic amines, such as methylamine, react similarly to ammonia and lead to a low estimation of

Table I. Effects of Interferences

Substance	Concentration, p.p.m.	Type of interference
K^+	100	None
Zn^{+2}	100	None
Na^+	150	None
Cu^{+2}	10	Positive
	5	None
Mg^{+2}	100	None
Ca^{+2}	100	None
Mn^{+2}	100	None
Co^{+2}	10	None
Fe^{+2}	1	Positive
	5	None[a]
Fe^{+3}	1	Positive
	5	None[a]
CO_3^{-2}	100	None
$C_2H_3O_2^-$	100	None
PO_4^{-3}	100	None
SO_4^{-2}	400	None
NO_3^-	300	None
F^-	100	None
Cl^-	125	None
I^-	1	Negative
NH_3	0.01	Negative
	20	None[a]
Methylamine	1	Negative
Aniline	1	Positive
Benzidine	1	Positive
1Pheno	1	None

[a] Samples passed through cation exchange column before determination.

Table II. Analyses of Water Samples by Standard Addition

Source of sample	Bromide,[d] p.p.m. Added	Bromide,[d] p.p.m. Recovered	Std. dev.	Rel. error, %
A[a]	0.10	0.12	±0.02	+20
	0.50	0.51	±0.03	+2.0
	1.00	1.04	±0.03	+4.0
B[b,c]	0.20	0.22	±0.03	+10
	0.70	0.71	±0.04	+1.4
	1.20	1.13	±0.03	−5.8

[a] Treated sample, Manhattan, Kan., city water supply.
[b] Blue River water collected at the Tuttle Creek reservoir near Manhattan, Kan.
[c] Suspended solids removed by filtration.
[d] Each value represents average of five consecutive determinations.

bromide ion when present at concentrations of 1 p.p.m. and below. Aromatic amines, such as aniline, develop a yellow hue immediately upon the addition of hypochlorite which masks the blue starch complex. Copper can be tolerated up to about 5 p.p.m. while iron interferes at the 1-p.p.m. level. However, samples containing up to 5 p.p.m. of iron(II) and iron(III) gave no interference after being passed through the cation exchange column under the same conditions employed to remove ammonia. Anions such as carbonate and phosphate will interfere above 50 p.p.m. unless the pH is adjusted with nitric acid, as described above, before addition of the buffer. The values shown in Table I for both these substances are based on their determination following this type of pH adjustment.

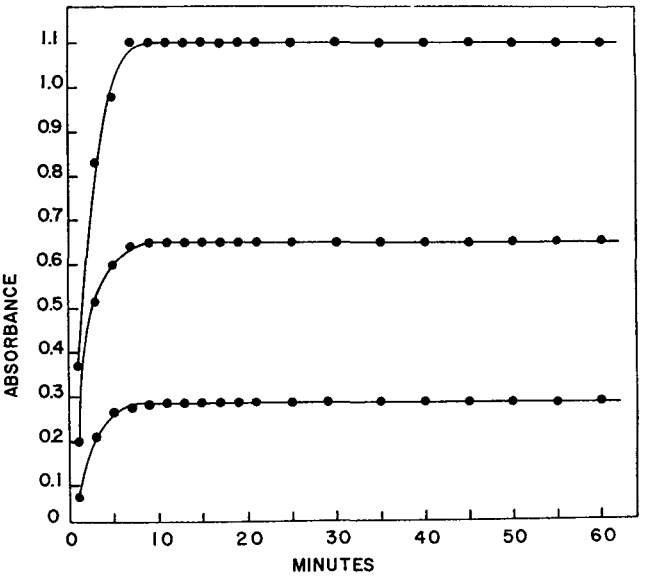

Figure 5. Color development of starch–triiodide ion complex

The applicability of the determination was tested by running a series of standard additions of bromide ion to natural and treated water samples. The results of this study are shown in Table II. The treated samples were collected on a day when the water gave a positive test for residual chlorine and negative tests for ammonia and bromide. Samples were heated gently to remove chlorine before the bromide additions were made. The natural samples contained about 0.02 p.p.m. of ammonia and less than 0.05 p.p.m. of bromide prior to addition, and in these determinations the cation exchange procedure was used.

The hydroxyethyl amylose derivative employed here has not been recommended previously for the preparation of a cadmium iodide–starch reagent. It was comparable in stability and color formation to reagents reported earlier (*2–11*), while having the advantage of simpler preparation. Solutions stored for more than five months gave the same color reaction as those freshly prepared, and exhibited no signs of discoloration or cloudiness.

Some variation in the position of the calibration curve may result when the hypochlorite solutions are changed. It is recommended, therefore, that several points on the curve be reestablished as fresh solutions are prepared. In general the absorbance will not vary by more than 0.05 units between different batches. The hypochlorite reagent described here can be used without further calibration for periods of several weeks if stored in a dark, cool place.

ACKNOWLEDGMENT

The authors are grateful to Stein, Hall and Co. for providing samples of Superlose HAA-11-HV.

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Determination of Lithium in Aluminum by Flame Photometry

Utilization of Organic Solvents for Submilligram Amounts

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▶ **An improved flame photometric method for the determination of submilligram amounts of lithium in commercial purity aluminum is described. The addition of isopropyl alcohol and acetone to acid solutions containing lithium and aluminum permits the determination of microgram levels of lithium without separations. The effects of various organic solvents and of some diverse ions on lithium emission are given. Background correction techniques, gas pressures, and solvent addition techniques are also described.**

FLAME PHOTOMETRIC METHODS are often considered when the determination of alkali metals is required. In spite of the inherent sensitivity of these methods for alkalis, problems exist when aluminum is the base metal. It is desirable to have available an analytical procedure which eliminates the need for separations and incorporates the specificity and simplicity of flame methods. The improved flame photometric method to be described accomplishes both these objectives—analysis without separation.

The effects of aluminum on the flame emission intensity for lithium have been investigated in detail (*6, 10*). Practically all observers noted a decrease in lithium emission when aluminum was present. This effect, when only microgram quantities of lithium are present, makes quantitative measurement a formidable task. In addition to lower emission intensity, the presence of gross amounts of aluminum results in viscous solutions which are difficult to atomize with conventional burners unless there is considerable dilution. The minimum practical volume for a 1-gram aluminum sample is 100 ml. With these restrictions, the lowest lithium concentration measurable with confidence has been on the order of 10 μg. per gram, or 0.001%. It is apparent then that a more sensitive system is required if measurements are to be made at 0.0001%.

The use of organic solvent systems provides a convenient means for increasing the net emission in the flame for a number of elements. Methanol was used by Brewster and Claussen (*1*), and Hourigan and Robinson (*8, 9*), in their procedures for sodium in aluminum, which have been used extensively in the aluminum industry for several years. Other investigators (*2, 3, 7*) have utilized various solvents to enhance the emission of elements such as aluminum, boron, arsenic, antimony, bismuth, and lead, all notoriously difficult to excite. Solvents such as acetone, butanone, propanol, and ethanol are particularly effective for enhancement when employed singly, decreasing in effectiveness in the order named (*3*). Binary combinations of these solvents are even more effective.

Organic solvents offer a twofold advantage in the analysis of aluminum. The enhancement effect is pronounced for lithium. In addition, the viscosity of the solution is considerably lowered, providing the proper solvents are chosen, resulting in a more regular and more rapid sample feed rate, with very little atomizer clogging.

The utilization of certain organic solvent systems added to acid solutions of aluminum affords a means of increasing the sensitivity of flame photometric methods for lithium in aluminum down to a concentration of 0.0001% with reliability. It is felt that the method is equal in precision and accuracy to methods for other elements at this level, and that the desired results may be obtained in a reasonable length of time.

EXPERIMENTAL

Reagents. Standard lithium solutions were prepared by dissolving Johnson-Matthey "Spec-Pure" lithium carbonate, dried at 110° C., in a minimum amount of HCl, and diluting to volume with distilled water. The aluminum used in the preparation of standards was 99.9% Al, in which no lithium could be detected by emission spectroscopy; 99.5% aluminum may be used satisfactorily, provided it is lithium free. All other reagents were reagent grade, and were used without further purification.

Instrumentation. A Warren Spectracord, Model 3000, with Beckman flame attachment and a 1P28 multiplier phototube was used.

Procedure. For a sample containing from 0.0001 to 0.01% of lithium, a 1-gram sample is dissolved in 20 ml. of 1:1 hydrochloric acid. After solution is nearly complete, 1 ml. of 1:1 nitric acid is added to oxidize the iron and aid in the solution of small amounts of copper. If the reaction proceeds slowly, 3 or 4 drops of a saturated solution of mercuric chloride may be added. After solution is completed, the volume is adjusted to 20 ml. and cooled. The solution is then transferred to a 100-ml. volumetric flask. Twenty milliliters of isopropyl alcohol, added incrementally, are used as a transfer solution. The volume is adjusted to 100 ml. with acetone and mixed. The sample is excited with an oxygen–hydrogen flame; the emission intensity is recorded and compared with calibration curves prepared from synthetic standards containing lithium-free aluminum.

RESULTS

Precision and Accuracy. While the procedure described is simple, it is capable of producing analytical data which are precise and accurate at a level of less than 0.001%. Analytical working curves in the 0.0001 to 0.001% range contain no points which vary from the curve by as much as 0.00005% Li. Any variation of these points from the curve is within the limits of instrumental reproducibility. It should be emphasized that the standards used in the preparation of these curves are absolute. Aluminum in which no lithium is detectable is present in amounts equivalent to that present in the samples. Known quantities of lithium are added.

Repetitive analysis of numerous samples resulted in excellent precision. Comparative analytical data are given in Table I. All values shown were obtained at different times with different portions of the gross metallic samples being used.

Working Curves. Analytical working curves prepared from samples containing 1 gram of aluminum, the organic solvents, and lithium ranging

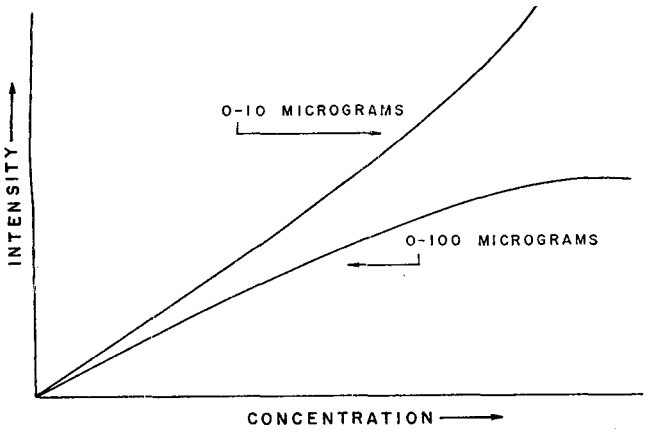

Figure 1. Examples of calibration curves showing effect of Li concentration on slope

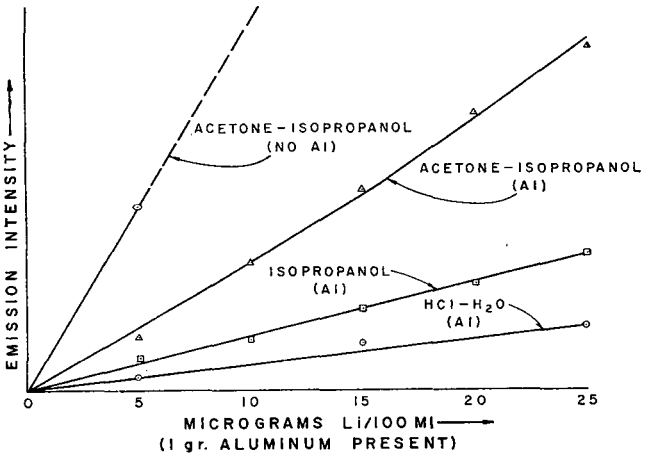

Figure 2. Effect of various solvents

from 10 to 100 μg. per 100 ml. exhibit the traditional shape of alkali metal curves, curving toward the concentration axis as self-absorption begins. However, on expanded curves covering the range of 1 to 10 μg. per 100 ml. and full instrumental sensitivity, a slight curvature toward the intensity axis occurs. Dean (*5*) mentions the possibility of this effect, and attributes it to a nonproportional increase in the number of neutral atoms entering the flame, in comparison to the number of ionized atoms. This type of curve has been obtained in every instance over the 1-to-10 μg. range.

It is desirable to construct new four- or five-point calibration curves for every set of samples when determinations of less than 0.001% lithium are made since the instrument is operated at maximum sensitivity. In the preparation of these curves, synthetic standards are made by dissolving lithium-free aluminum, adding the desired amount of lithium, and diluting with isopropyl alcohol and acetone. The exact reproduction of previously obtained curves is difficult at this concentration. Examples of calibration curves are shown in Figure 1.

DISCUSSION

Solvent System. In choosing a solvent system for use in flame photometric measurements, the solubility of the salts in the organic material is of considerable importance. Attempts to dissolve dehydrated salts in absolute solvents are generally unsuccessful, but 80% concentrations of many alcohols provide solutions usable with aluminum. Isopropyl alcohol solutions resulted in approximately threefold enhancement for lithium over aqueous solutions. Preliminary work had shown that in the absence of aluminum, acetone increased the intensity for lithium more than twice as much as isopropyl alcohol, indicating that acetone should provide an excellent medium for the determination. However, 1 gram of aluminum in a 80-ml. acetone–20-ml. acid solution resulted in precipitation of salts, so a mixture of 20 ml. of isopropyl alcohol and 60 ml. of acetone per 100 ml. was investigated. If the aluminum sample in acid solution is transferred to the volumetric flask with the isopropyl alcohol, acetone may be added, and a clear solution will result. The order of solvent addition must be maintained. This mixture gave a twofold increase in lithium intensity over isopropyl alcohol alone, and was used for all succeeding work. The relative intensities of lithium in the various solvent solutions investigated are illustrated in Figure 2.

Excitation Conditions. The excitation conditions for the determination are quite critical, especially for values of less than 0.001%. Solutions containing organic solvents produce flame configurations that are considerably different from those produced by aqueous samples, and the portion of the flame producing maximum emission intensity varies with the individual element to some extent (*2*). Flame heights were adjusted carefully, and the focusing mirror of the burner housing was profiled across the flame until maximum emission was obtained. Fuel and oxygen pressures were adjusted to give maximum sensitivity above background on samples containing 5 μg. of lithium per gram of aluminum in a 100-ml. volume. On the instrumentation employed, oxygen pressures of 12 to 13 p.s.i. and hydrogen pressures of 4 to 5 p.s.i. were satisfactory. Small variations in hydrogen pressure caused erratic results, so frequent monitoring of this setting was necessary. With these gas pressures, and a medium bore Beckman burner, the sample consumption rate was about 3 ml. per minute, allowing sufficient time for the spectrum to be scanned over the wavelength range of interest.

Detectors. All samples were run by recording the spectrum from 700 to 650 mμ, with the lithium line at 671 mμ being used for measurement. A 1P28 multiplier phototube was used as the detector. While this tube is not especially sensitive for measurements in this region of the spectrum, it has been reported (*6*) that the lithium response of this tube is approximately 20 times that of an ordinary red-sensitive phototube. Some 1P28 detectors were less responsive than others at this wavelength. It therefore might be necessary to select a detector for this region.

Background Correction. Considerable emission occurs in the 600- to 700-mμ. region when lithium-free aluminum solutions are examined. Pungor and Zapp (*10*) have reported flame emission from aluminum oxide formed from aluminum chloride, being especially noticable at wide slit widths. While the slit widths used in our

Table I. Interlaboratory Comparisons

% Li			
Laboratory A; 3 determinations		Laboratory B; 5 determinations	
Mean	Range	Mean	Range
0.0003	0.0003–0.0004	0.0003	0.0003–0.0004
0.0006	0.0006–0.0006	0.0006	0.0004–0.0006
0.0008	0.0007–0.0009	0.0009	0.0008–0.0011
0.0012	0.0011–0.0013	0.0012	0.0010–0.0014
0.0020	0.0019–0.0022	0.0021	0.0021–0.0021
0.0038	0.0037–0.0040	0.0041[a]	0.0040–0.0042

[a] Four determinations.

experiments were smaller than those employed by Pungor and Zapp, the contribution of aluminum to total emission at 671 mμ had to be taken into account.

The use of lithium-free metal as a blank caused erratic results when determinations at low lithium levels, which required considerable signal amplification, were attempted. However, the use of the base-line method of background correction, as described by Dean (4), gave much more reproducible measurements. In this method, transmittance values are read first at the emission peak, then at wavelengths on either side of the peak, and the interpolated value is used as background. On recording instruments, a line is drawn through the minimum readings on either side of the peak. The background intensity is determined by the point of intersect of this baseline with a vertical line drawn through the transmittance peak (Figure 3).

Interfering Elements. Cu, Mn, Cr, Ni, Zn, and Ti in concentrations up to 0.2% in aluminum do not interfere from the standpoint of emission enhancement or suppression. However, if silicon is present in larger amounts, some atomizer clogging may occur. Filtration on a rapid paper is recommended in this case. Magnesium suppresses lithium when present in amounts greater than 5%, but in pure metal, the effect is insignificant. Iron may be tolerated up to 0.5%. Sodium has no measurable effect up to 5 mg. per 100 ml. of sample.

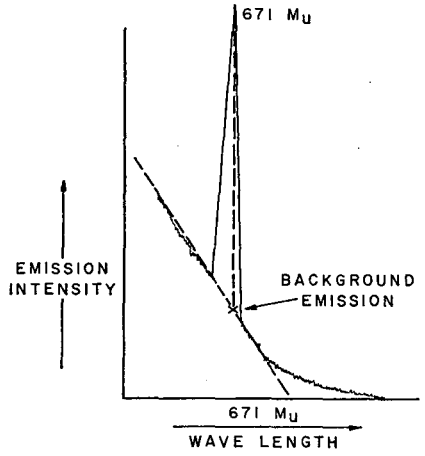

Figure 3. Method of background correction

In the wavelength region under consideration, spectral interferences are at a minimum. Interference from the Sr lines at 666 mμ and 680 mμ was not investigated, since this element and others having significant emission in this region are not usually found in aluminum. At the recommended slit width of 0.1 mm., resolution of the 671-mμ lithium line by the DU monochromator of the Spectracord is satisfactory.

ACKNOWLEDGMENT

The authors are indebted to C. A. Broyles, Reynolds Metals Co., Malvern, Ark., for his assistance in evaluating the method.

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Improved Spectrophotometric Method for the Determination of Small Amounts of Chloroform

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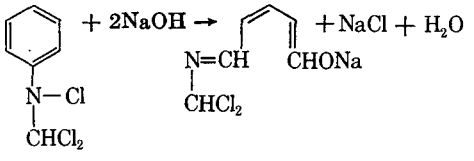
▶ The reddish color obtained on addition of pyridine to a chloroform solution in the presence of sodium hydroxide (Fujiwara reaction) was used for the qualitative and quantitative determination of chloroform. By measuring the absorbance at 366 mμ instead of 525 mμ, and working at optimum conditions established in this report, the sensitivity of the test was considerably increased. The method developed has a relative standard deviation of ±5% and permits the detection of 0.2 p.p.m. of chloroform.

ON ADDITION of pyridine to an aqueous chloroform solution and heating for some minutes in presence of sodium hydroxide, a reddish color is obtained which depends on the concentration of chloroform. The mechanism of the reaction is not known, but the following has been proposed (3).



Fujiwara (4) used this reaction for detecting chloroform in body fluids and tissues. Vole (1) employed it for quantitative determinations. He compared the color produced by known concentrations of chloroform against standards obtained from acidified alcoholic fuchsin solutions. Though he claimed a limit of detection of 1 p.p.m., he reported that the accuracy of the method was practical only for higher chloroform concentrations.

Later authors who determined chloroform in different media did not obtain a sensitivity better than 10 p.p.m.

By establishing optimal working conditions, less than 1 p.p.m. of chloroform in water was determined in the present work. A new absorbance peak, more intense than the maximum at 525 mμ used by previous groups (1–3), was discovered at 366 mμ.

The 525-mμ peak and the red color faded on standing, while the 366-mμ peak became more intense.

Chloroform may be determined accurately by this method only in the absence of other polyhalogen compounds such as chloral and trichloroacetic acid, which react similarly.

Under certain conditions, the interfering effect of these compounds can be allowed for.

EXPERIMENTAL

All the chemicals used were of analytical reagent quality.

A Beckman DU spectrophotometer was used.

Procedure. Pipet 5 ml. of an aqueous solution containing from 2

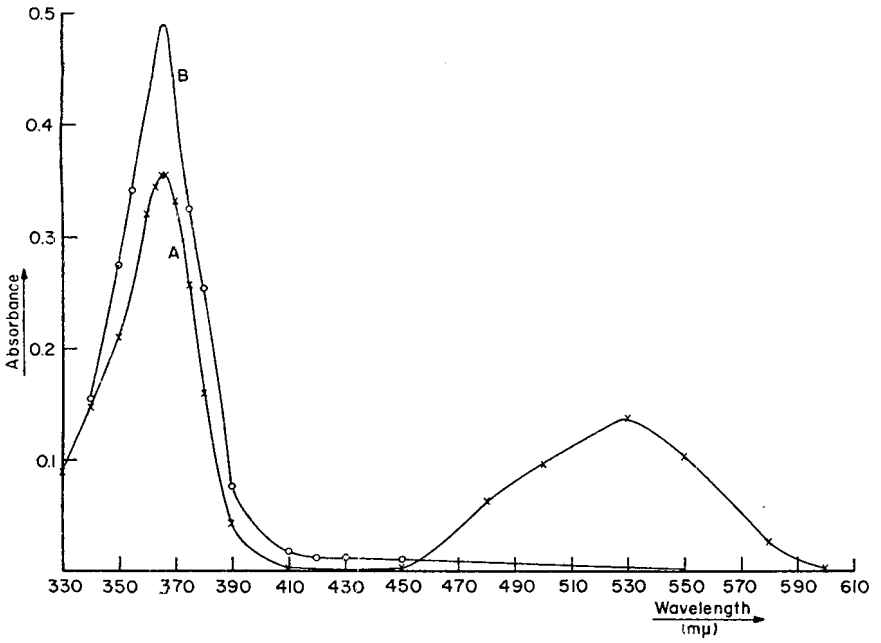


Figure 1. Absorbance spectrum of pyridine layer

2 μg. chloroform per ml.
1-cm. light path cells
A. 25 minutes after separation
B. 24 hours later

to 40 μg. of chloroform into a 25-ml. glass-stoppered bottle. Add 5 ml. of pyridine and 10 ml. of 40% (w./w.) sodium hydroxide, close the bottle, and shake. Heat on a water bath at 70° C. for 15 minutes, shaking from time to time, cool to room temperature under the tap, transfer to a 40-ml. separatory funnel, shake, allow to stand for a few minutes until the two layers separate completely, and discard the aqueous layer. Transfer the colored pyridine layer into a 10-ml. volumetric flask, and make up to volume with distilled water. Read the absorbance at λ = 366 mμ, using 2-cm. light path cells in a Beckman DU spectrophotometer, 1 hour after removal from the water bath, and determine the chloroform content of the sample from the standard curve. Rigorously maintain the time and warming conditions the same as those for the preparation of the standard calibration curve.

Calibration Curve. Prepare a standard chloroform stock solution containing 1 mg. of $CHCl_3$ per ml. by weighing 1 gram of chloroform in a very small weighing bottle, introducing it into a 1-liter volumetric flask, and diluting with distilled water.

Pipet 1 ml. of the stock solution into a 100-ml. volumetric flask and make up to volume with water. Introduce aliquots of this solution ranging from 0.2 to 5 ml. into 25-ml. glass-stoppered bottles, make up to 5 ml. with water, and treat as in "Procedure."

A series of standards ranging from 0.2 to 5 p.p.m. of chloroform was obtained. A straight-line calibration curve was obtained by plotting the absorbance of these solutions *vs.* chloroform concentration.

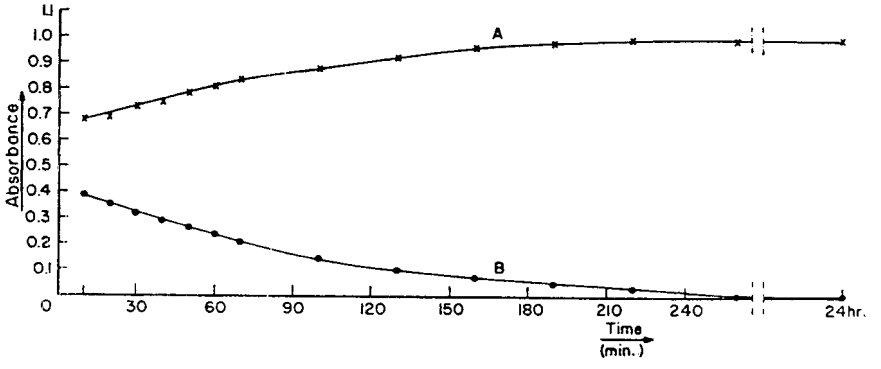


Figure 2. Effect of time factor on absorbance peak

2-cm. light path cells
A. λ = 366 mμ
B. λ = 530 mμ

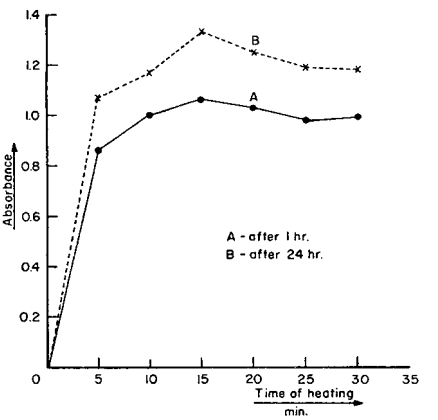


Figure 3. Effect of length of heating time

λ = 366 mμ
2 μg. chloroform per ml.

DISCUSSION AND RESULTS

Absorbance Spectra. The absorbance spectrum was examined in the range 330 to 600 mμ within 30 minutes after separation of the pyridine layer. In addition to the known maximum at 530 mμ, a more intensive one was found at 366 mμ (Figure 1).

The spectrum was redetermined after 24 hours. The maximum at 366 mμ increased, whereas that at 530 mμ disappeared (Figure 1).

Determination of Optimum Conditions. The optimum conditions for the determination of chloroform were found by investigating the following parameters.

Effect of Time Factor on Absorbance Peak. The red color of the pyridine solutions faded on standing. Absorbance at the two maxima of a sample containing 2 p.p.m. of chloroform was measured at periods ranging from 10 minutes to 24 hours after preparation of the pyridine extract. As shown in Figure 2, the intensity at λ = 530 mμ decreased, while that at λ = 366 mμ increased. Both processes were complete after about 260 minutes. The respective minimum and maximum values attained remained constant. Figure 3 shows that the 366-mμ absorbance obtained after 1 hour (curve *A*) was about 80% of the maximum.

The other parameters were investigated using only the 366-mμ peak and samples containing 50 μg. of $CHCl_3$.

Length of Heating Time of Alkaline Solutions. Heating the reaction mixture for 15 minutes in a 70° C. water bath gave optimal absorbance values. This optimum heating time was independent of the time delay required for development of the 366-mμ absorbance peak (Figure 3). Prolonging the heating made the determination somewhat less sensitive.

Sodium Hydroxide Concentration. Ten milliliters of NaOH at concentra-

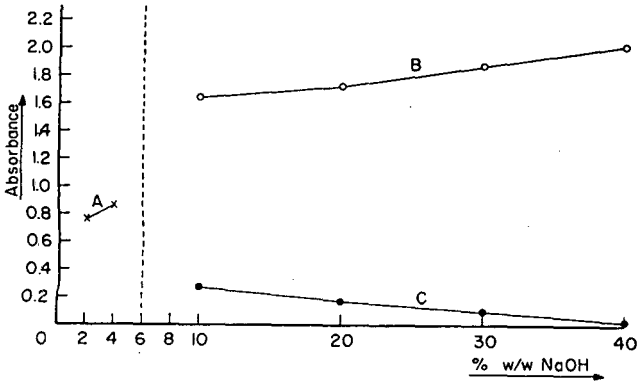


Figure 4. Effect of NaOH concentration

A. One phase
B. Pyridine layer
C. Aqueous layer
λ = 366 mμ
2-cm. light path cells

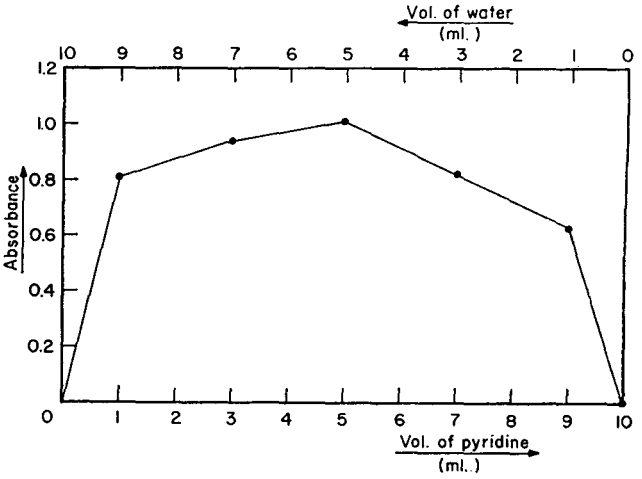


Figure 5. Effect of water-pyridine ratio

λ = 366 mμ
2.5 μg. chloroform per ml.

tions from 2 to 40% (w./w.) were used to prepare the samples. The results are presented in Figure 4.

At concentrations below 6% there was no phase separation, no red color was observed, and there was no absorbance at 530 mμ. Nevertheless, the solutions absorbed at 366 mμ.

With concentrations higher than 6% (w./w.), two phases, a colorless aqueous layer and a red pyridine layer, separated. The absorbance of both layers was measured at 366 mμ. The absorbance of the pyridine layer increased with increasing NaOH concentration, while that of the aqueous layer decreased. The highest absorbance of the pyridine layer was obtained with 40% NaOH with almost no absorbance by the aqueous layer.

Calibration curves obtained using 10, 20, 30, and 40% (w./w.) of sodium hydroxide indicated that sensitivity increased with more concentrated sodium hydroxide.

Water-Pyridine Ratio. Samples containing 50 μg. of chloroform were prepared in volumes of water varying from 1 to 9 ml. Pyridine was added, to give a total volume of 10 ml.

The results obtained (Figure 5) show that for the conditions described under "Procedure," a 1 to 1 ratio of water to pyridine gives maximum absorbance.

Interference of Other Polyhalogens. Several polyhalogenated compounds also give the Fujiwara reaction (4). Samples containing 40 and 400 μg., respectively, of each were prepared as described under "Procedure" for chloroform. The results are presented in Table I.

Precision. The precision of the method was found by repeated analysis of three standard samples containing 1, 2, and 4 μg. per ml. of chloroform, respectively. The standard deviation, calculated for each series, is given in Table II.

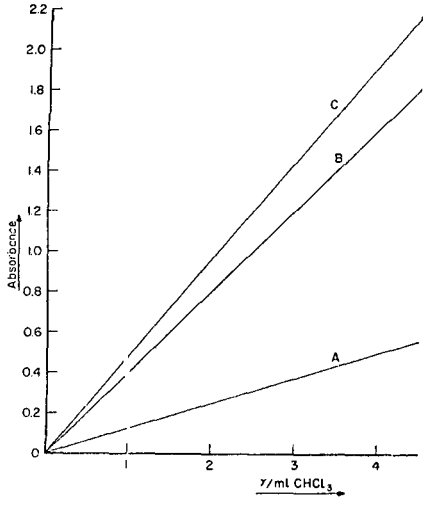


Figure 6. Calibration curves

A. λ = 530 mμ after 1 hour
B. λ = 366 mμ after 1 hour
C. λ = 366 mμ after 24 hours
2-cm. light path cells

Table I. Absorbance of Various Polyhalogens at 366 Mμ

Reagent	μg. per ml. in final solution	Absorbance at 366 mμ	Remarks
Chloroform	4	1.58	
Carbon tetrachloride	4	0.025	
	40	0.19	
Iodoform	4	0.095	No red color obtained
	40	0.88	
Trichloroacetic acid	4	1.28	
Chloral	4	1.40	

Table II. Precision of Method

$CHCl_3$, μg. per ml. Taken	Found	Mean	Std. dev.	Precision, rel. std. dev., %	Rel. error, %
1.0	0.9	0.97	±0.0465	±4.79	3.3
	0.94				
	0.96				
	1.0				
	0.99				
	1.03				
2.0	1.98	2.006	±0.061	±3.06	0.3
	2.04				
	2.02				
	1.90				
	2.02				
	2.08				
4.0	3.8	3.86	±0.208	±5.4	1.0
	4.08				
	4.06				
	3.74				
	3.60				

Table III. Comparison of Methods for Determination of Chloroform Based on Fujiwara Reaction

Author and medium	Pyridine added, ml.	NaOH Ml.	NaOH Concn., %	Temp. of reaction, ° C.	Heating time, min.	λ = mμ	Sensitivity, p.p.m.
Feigl (3)	2 drops	1 drop	20	100	Few	Visual	20
Fujiwara (4), body fluids	2	3	10	100	Just to boiling	Visual	1
Cole (1), tissue extracts	1	2	20	100	1	Visual	1
Gettler (5), tissue extracts	5	10	20	100	1	Visual	10
Daroga (2), soil and air	20	10	20	100	5	525	30
Hildebrecht (6), CCl_4	15	5 drops	10	100	3	525	10
Milton (9), urine and blood	5	2.5	20	100	5	525	50
Hunold (7), air	10	2	0.08	100	5	Filter S 49 Elko II	10
Our method (8)	5	10	40	70	15	366	0.2

CONCLUSION

It is obvious that the absorbance at 366 mμ is not due to the product that absorbs at 530 mμ, and that the compound that absorbs at 366 mμ is formed from the red compound, since the time necessary for attaining maximum absorbance in the ultraviolet is identical with that necessary for complete fading of the red color (Figure 2).

Using the conditions described under "Procedure," a considerable increase in sensitivity over previous techniques for chloroform determination was obtained (Table III). By measuring the absorbance after 4 hours, the sensitivity may be increased by a further 25%. Even by reading the absorbance at 530 mμ, as in previously reported procedures (3–5), the sensitivity is increased. Figure 6 shows a comparison of these three possibilities for determination of absorbance.

Table III compares the methods used for the determination of chloroform in different media by different authors using the Fujiwara reaction.

By changing the working conditions it would probably be possible to develop sensitive methods for other polyhalogen compounds.

ACKNOWLEDGMENT

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Spectrophotometric Determination of Acetylenic Compounds as Mercuric Acetate Complexes

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▷ **A method is presented for determining monosubstituted and disubstituted acetylenic compounds via the triple bond on the molecule. The method is based on the formation of the mercuric acetate addition products of the acetylenic compounds and measurement of the ultraviolet absorption of these addition compounds. The method is selective for acetylenic compounds and is sensitive to low ranges of concentration.**

THE EXISTING methods for determining acetylenic compounds are of three types: determination of unsaturation via the addition of bromine, hydrogen, or iodine halide (iodine number) (12); determination of the acetylenic hydrogen in the case of monosubstituted acetylenes (HC≡CR) (1–5, 7–9, 13); and solvation of the acetylenic compound to the corresponding ketal or ketone and determination of the ketone (11, 12).

The methods based on addition reactions lack specificity. Ethylenic compounds exhibit the same addition reactions and cause high results. Many organic compounds substitute bromine for hydrogen and oxidizable compounds consume bromine. The same interferences occur to some extent with iodine halide methods. When the composition of the sample permits, bromination is a rapid and easy means for determining acetylenic compounds. Other reducible compounds interfere with hydrogenation methods by consuming hydrogen, and the methods are usually less precise than other methods for determining acetylenic compounds.

The methods for the determination of monosubstituted acetylenes by replacing the acetylenic hydrogen atom with a metal ion have a higher degree of selectivity than the methods involving addition reactions, but interferences still exist. These methods are either acidimetric or argentometric in nature. Thus, acids and bases in samples limit the use of the acidimetric methods, though in some cases the analysis can be accomplished by first neutralizing the sample. Compounds such as halides, mercaptans, cyanides, and others react with silver, limiting the use of the argentometric methods. These methods, of course, do not apply to disubstituted acetylenes (RC≡CR′) since

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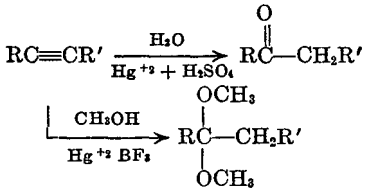
no replaceable hydrogen exists in these cases.

Among the most specific methods for determining acetylenic compounds are those which involve reaction of the acetylenic triple bond with water (*10, 11*) or an alcohol (*14*) to produce a ketone or ketal, respectively



Both the latter compounds are determined volumetrically with hydroxylamine hydrochloride or, if acidimetry is impossible because of interferences, the carbonyl products can be measured gravimetrically with 2,4-dinitrophenylhydrazine. Free carbonyl compounds in the sample constitute the only interference and these can be determined in a separate reaction and proper corrections can be applied.

A method similar to the solvation approach exists (*6*) which involves reaction of the acetylenic group with mercuric acetate, but in this case the acetic acid liberated is titrated. The reaction between mercury compounds and the triple bond is the intermediate step in the solvation of the acetylenic compound to a ketone. This method lacks precision and general applicability to acetylenic compounds, most probably because the mercury addition product does not remain as such but solvates to some degree to the corresponding ketal or ketone.

Analytical methods utilizing solvation to the ketone or ketal lack sensitivity in the low ranges of acetylene content with one exception (*10*). This procedure is sensitive to very small amounts of certain acetylenes; however, some acetylenes containing other functional groups cannot be determined by this method. The 2,4-dinitrophenylhydrazones obtained are water soluble and do not extract into the cyclohexane layer.

In the work with which the authors have been involved, the need became apparent for a method both selective for acetylenic compounds and sensitive in the low ranges of concentration. In the ensuing investigations it developed that the mercury addition product generally absorbed in the ultraviolet region of the spectrum. Early attempts to utilize this approach were unsuccessful since water or alcohol were used as solvents and the solvation reaction which consumed the mercury addition intermediate proceeded at too rapid a rate. It was then decided that acetic acid should be tried as the solvent, in which case the solvation should not be possible. The mercury addition product was found to be quite stable in this medium and the analysis was possible.

The main advantage of the method described here is the ability to determine accurately and specifically small amounts of acetylenic compounds. However it should be added that for some acetylenic materials the absorption of the mercury addition product is such that it will permit precise assay (±1%) of high purity acetylenic compounds. The necessity of preparing standards is the one disadvantage of the method, but once a standard curve is obtained for a particular acetylenic compound, samples can be rapidly and easily analyzed.

Table I. Results Obtained for Pure Acetylenic Compounds

Compound	Mg. Calcd.	Mg. Found	Wavelength, mμ	Molar absorptivity × 10^{-2}
2-Propyn-1-ol	4.8	4.7	287	4.2
	4.8	4.7		
	4.8	4.7		
3-Butyn-1-ol	1.7	1.7	285	4.1
	1.7	1.7		
	1.7	1.7		
1-Hexyne	1.9	1.9	295	5.5
	1.9	1.8		
	1.9	1.8		
1-Octyne	3.5	3.5	295	4.3
	3.5	3.5		
	3.5	3.3		
1-Dodecyne	4.0	4.0	295	4.7
	4.0	4.0		
	4.0	4.0		
2-Methyl-3-butyn-2-ol	3.6	3.6	300	3.6
	3.6	3.7		
	3.6	3.6		
3-Octyn-1-ol	1.1	1.2	320	31.8
	1.1	1.1		
	1.1	1.2		
Propargyl acetate	5.0	5.0	280	3.5
	5.0	5.0		
	5.0	5.1		
Dipropargyl ether	3.4	3.4	285	10.0
	3.4	3.5		
	3.4	3.4		
2-Butyne-1,4-diol	3.0	3.0	285	3.6
	3.0	3.0		
	3.0	3.0		

EXPERIMENTAL

Reagents. Mercuric acetate solution. Twenty grams of mercuric acetate were dissolved in 1 liter of acetic acid.

Procedure for Standards. A solution of the acetylenic compound in acetic acid is prepared which contains 1 mg. per ml. Aliquots of this solution are pipetted into a series of 50-ml. volumetric flasks and 25 ml. of mercuric acetate solution are added with a pipet to each flask. The solutions are diluted to 50 ml. with acetic acid and are allowed to stand at room temperature for 30 minutes. A blank solution is prepared in the same manner except that the acetylenic compound is omitted. After 30 minutes the absorbances of the standards are determined at the proper wavelength against the blank with a Beckman Quartz Spectrophotometer or any other suitable instrument. One-centimeter cells are used for all measurements. A plot of absorbance *vs.* milligrams of compound is prepared.

Procedure for Samples. A sample which contains between 1 and 10 mg. of acetylenic compound is weighed into a 50-ml. volumetric flask, or, for samples which contain high concentrations of the acetylene, an aliquot of an acetic acid solution of the sample may be used as in the procedure for the standards. Mercuric acetate reagent (25 ml.) is pipetted into the flask, and the solution is diluted to the mark with acetic acid. A reagent containing 20 grams of mercuric acetate in 1 liter of acetic acid was used except in the determination of low concentrations of certain acetylenic compounds in olefins. In these determinations, listed in Table I, a reagent solution containing 60 grams of mercuric acetate per liter of acetic acid was used.

After 30 minutes, the absorbance of the solution is determined at the proper wavelength against the same blank as used for the standards. Milligrams of compound are read from the standard curve and per cent is calculated.

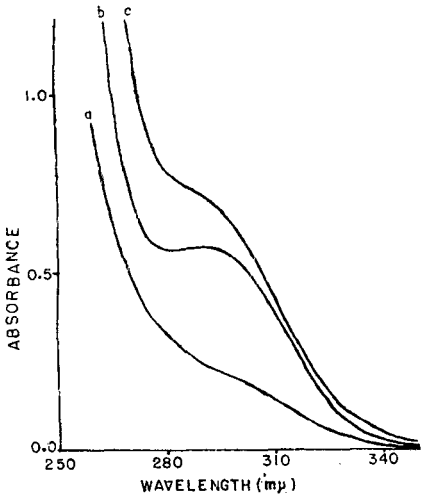


Figure 1. Spectra of acetylenic compounds in an acetic acid solution of mercuric acetate

(a) 1-Dodecyne
(b) 3-Butyn-1-ol
(c) Dipropargyl ether

DISCUSSION AND RESULTS

The method was tested on both mono- and disubstituted acetylenic compounds. The spectra obtained for those compounds are presented in Figures 1 to 4. An examination of these curves will show that the complexes formed by mercuric acetate with certain acetylenic compounds have absorption spectra which exhibit maxima, while those formed with other acetylenic compounds do not have definite maxima. When possible, the absorbance was measured at the maximum, but when no definite maximum was present in the spectrum of a complex, the absorbance was measured at the point of least slope.

In general, the spectra obtained for the complexes of acetylenic hydrocarbons did not have definite maxima. Where the acetylenic compound contained a second functional group besides the triple bond, a maximum is usually exhibited by the spectrum of its mercuric acetate complex.

All monosubstituted acetylenes tested could be determined, but the method could not be used to determine certain disubstituted acetylenic compounds. Disubstituted acetylenic hydrocarbons (RC≡CR′) compounds where R and R′ are alkyl groups) formed complexes which showed too small an increase in absorbance over that of the reagent to be determined. The complexes of 3-hexyne and 3-octyne did not absorb

Table II. Determination of Acetylenes in the Presences of Ethylenes

Compound		Alkyne, %		Reagent concentration, grams mercuric acetate per liter acetic acid
Acetylenic	Ethylenic	Added	Found	
1-Octyne	1-Decene	0.12	0.14	20
		0.73	0.81	20
		5.75	5.94	20
1-Dodecyne	1-Decene	0.19	0.19	20
		1.13	1.24	20
		8.68	8.86	20
1-Hexyne	1-Pentene	0.12	0.11	20
		0.72	0.79	20
		5.80	5.80	20
2-Propyn-1-ol	Allyl alcohol	0.20	0.16	60
		0.60	0.60	60
		2.91	2.91	20
		2.91	2.91	20
		4.76	4.67	20
1,4-Butynediol	1,4-Butenediol	0.29	0.26	60
		0.40	0.34	60
		0.40	0.37	60
		0.72	0.68	60
		1.18	0.99	20
		1.18	1.09	20
		5.30	4.74	20
		5.30	4.74	20
Propargyl acetate	Allyl acetate	0.10	0.06	20
		0.60	0.40	
		4.76	4.48	

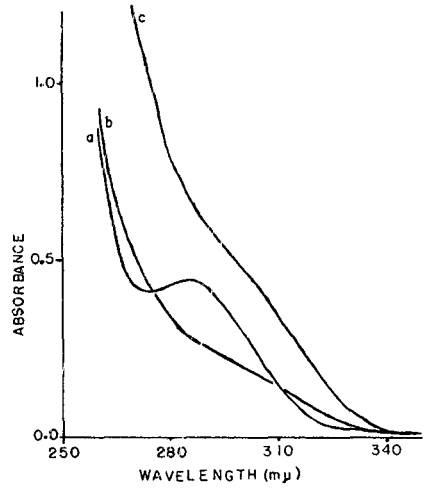


Figure 2. Spectra of acetylenic compounds in an acetic acid solution of mercuric acetate

(a) 2-Butyne-1,4-diol
(b) 1-Hexyne
(c) 1-Octyne

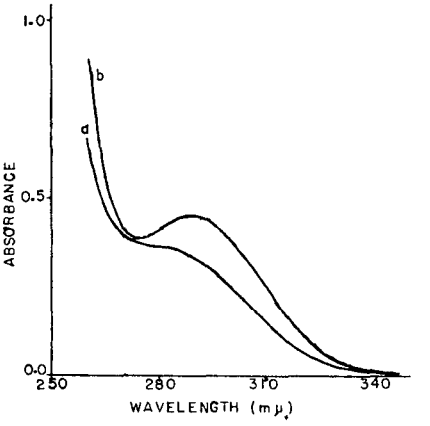


Figure 3. Spectra of acetylenic compounds in an acetic acid solution of mercuric acetate

(a) Propargyl acetate
(b) 2-Propyn-1-ol

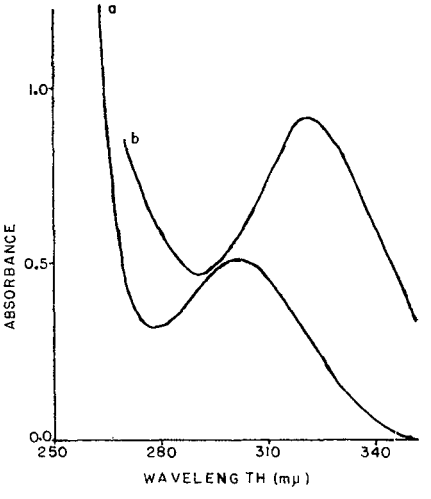


Figure 4. Spectra of acetylenic compounds in an acetic acid solution of mercuric acetate

(a) 2-Methyl-3-butyn-2-ol
(b) 3-Octyn-1-ol

sufficiently to be used for determining these compounds. In some cases no change in the absorbance of the reagent was noted on the addition of hydrocarbons of this type. No increase in absorbance of the mercuric acetate solution was caused by 3,6-dimethyl-4-octyn-3,6-diol. In this particular instance, the absence of complex formation is probably responsible for the failure of the method since it was previously observed (*11*) that compounds of this type react very slowly with mercuric salts even at elevated temperatures. With the exception of the above compound, all the disubstituted acetylenes tested which contained a second functional group other than the triple bond could be determined by the method.

Acetylenic compounds could be determined in the presence of ethylenic compounds in all cases tested where the ratio of double bond to triple bond was not more than one hundred to one without change in the method. Where the ratio of double bond to triple bond was greater than one hundred to one, it was necessary to increase the concentration of the reagent from 20 grams of mercuric acetate per liter of acetic acid to 60 grams per liter in the determination of some compounds to obtain complete reaction of the triple bond. The concentration of reagent used for determination of acetylenic compounds in the presence of ethylenic compounds is given in Table II. Ethylenic compounds in general appear to form complexes with mercuric acetate in acetic acid, but most of the complexes do not cause an increase in absorbance. 2,4,4-Trimethylpentene was the only olefin which was found to give an increase in absorbance. A decrease in absorbance was noted in some cases when mercuric acetate complexed with ethylenic compounds, but except in the case of allyl acetate this decrease was not great enough in the region of measurement to cause serious errors. Low results were obtained for propargyl acetate in allyl acetate.

The results obtained for acetylenic compounds alone are tabulated in Table I and those obtained for mixtures of ethylenes and acetylenes are given in Table II. From Table I it can be seen that the number of milligrams of acetylenic compounds found by analysis was within one tenth of a milligram of that taken in all determinations. Therefore, the precision and accuracy of a determination should be within 1% if a 10-mg. sample is taken. The molar absorptivities of the complexes are included in Table I.

The absorbances for all compounds tested except 3-octyn-1-ol remained the same from 30 to 60 minutes. The absorbance of the 3-octyn-1-ol complex decreased continuously over the period of measurement, but the rate of decrease was slow enough that good results could be obtained.

Compounds which absorb in the region of measurement, or form mercury complexes which absorb in this region, will interfere with the method. With the exception of the olefin mentioned previously no interference has been encountered from mercury complexes. In most cases in which samples contain compounds which absorb in the ultraviolet region it is possible to eliminate the interference by using an acetic acid solution of the sample as a blank. The mercuric acetate causes only a small increase in absorbance for which a correction can be applied. The quantity of mercuric acetate used in the above procedure begins absorbing at approximately 320 mμ and the absorbance increases slowly to 280 mμ. At 285 mμ the absorbance is 0.05. Below 280 mμ the absorbance increases rapidly. As much as 5 grams of water may be present without interference. The effect of higher concentrations was not investigated, but the production of ketone by hydration may cause a significant decrease in absorbance if the water concentration is too high.

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Determination of Oxygen, Hydrogen, Nitrogen, Argon, and Carbon in Thin Films by Flash Discharge Lamp

SIR: There is a current interest and need for a method suitable for analysis of thin films. Such an analysis presents a challenge to the chemist for several reasons: the amount of material for analysis is extremely small, the film is normally deposited on a glass or ceramic substrate, and the metals used generally form very stable compounds—i.e., the refractory metals.

The analytical methods normally used for such measurements become borderline when applied to thin film analysis. Also, the recovery of nitrogen from metals like tantalum, zirconium, etc., has never been feasible by vacuum fusion, yet nitrogen is known to be present in considerable amounts.

The principle of this new method is based on the application of a xenon flash discharge lamp capable of inducing a film temperature on the order of 5000° to 8000° C. When the metal film is flashed in vacuum, this extreme temperature is sufficient to evaporate the film directly from a glass substrate and to dissociate such stable compounds as tantalum oxide and nitride. The gases evolved are collected by a high speed mercury diffusion pump and later transferred with a Toepler pump to a measuring pipet. The gases are compressed to a known volume, and the pressure is measured by means of a cathetometer.

By means of low pressure techniques, a sample is transferred to a calibrated sample loop. The gas mixture within the loop is swept out by means of the helium carrier gas and into a gas chromatograph. The gases are separated with a 2-meter 5A molecular sieve refrigerated with dry ice in series with a similar sieve at room temperature and detected with a Gow-Mac thermistor. The chromatograms are obtained in about 30 minutes.

A standard curve is readily obtained by admitting micro quantities of oxygen, nitrogen, argon, and carbon monoxide to the chromatograph to determine the area under the peak per microgram of gas.

The hydrogen value is obtained best by difference since the thermal conductivities of the hydrogen and the helium are so near alike that the sensitivity is not adequate. The presence of oxygen in fair amounts has made it possible to measure the carbon as carbon monoxide, part of this peak is oxygen and the remainder the carbon.

The detection level with the current apparatus, which is not designed for optimum sensitivity, is of the order of 0.1 atom %. Since the films may contain as much as a hundred times this sensitivity level, the method is very suitable for this application.

This method offers a unique and rapid approach to the simultaneous analysis of thin films for oxygen, hydrogen, nitrogen, argon, and carbon, and is being applied to film studies to be described in detail later.

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Use of Molten Salts as a Solvent in Thin Layer Chromatography

SIR: Only a few applications of molten salts as solvents for chromatography have been reported although the high ionic character of these liquids should be useful in the separation of inorganic and polar organic substances. The higher temperatures available with these liquids could make changes in equilibrium in reactions an advantage. Juvet and Wachi (*5*) and Hanneman, Spencer, and Johnson (*4*) have reported the use of a molten salt substrate in gas chromatography to separate volatile inorganic halides and organic substances, respectively. Gruen *et al.* (*3*) investigated the use of $LiNO_3$-KNO_3 eutectic with added amounts of chloride to separate several transition metal ions on an alumina column. More recently, Alberti, Grassini, and Trucco (*1*, *2*) have used the $LiNO_3$-KNO_3 eutectic as a solvent on glass fiber paper and in electrophoretic separation.

This communication reports preliminary experiments on the applicability of molten salts to thin layer chromatography.

EXPERIMENTAL

Glass plates, 1 inch × 12 inches, were coated with silica gel from which cation impurities had been removed by washing with 1 to 1 HCl to water and distilled water. The plates were activated by drying at 110° C. for a minimum of one hour. The chromatographic chamber consisted of a long borosilicate glass tube containing 1 to 2 inches of the $LiNO_3$-KNO_3 eutectic (43 mole % $LiNO_3$). To maintain isothermal conditions, this tube was immersed in a large molten salt bath of $LiNO_3$-KNO_3 eutectic at 270° ± 2° C. Applied to the plate were 0.002 ml. of 0.1*M* aqueous solutions of the ions as nitrates and the chromatograms were run in the usual manner. Because of the low volatility of the molten salts, and the large bath used, no cover or thermal shields were necessary. Approximately one hour was required for the solvent to travel 10 cm. The plates were then removed and cooled to room temperature. The excess silica gel above the solvent front could be rubbed off leaving the solvent front well defined. Location of the ions used in these preliminary experiments was accomplished by spraying the plates with dilute aqueous $(NH_4)_2S$.

RESULTS

In the trial experiments, the various ions of the first group in the qualitative analysis scheme were tried using the simple $LiNO_3$-KNO_3 eutectic. The values of R_f are listed in Table I. In a mixture of ions it has been possible to separate silver, lead, and mercury(I) using this method. Other than the

Table I. R_f of Inorganic Ions Using Molten Salt Solvent

Ion	R_f	Color of spot with $(NH_4)_2S$
Ag	0.98	black
Tl(I)	0.94	brown
Pb	0.30	brown
Hg(I)	0.00	yellow-brown
Hg(II)	0.00	black

necessity for a large molten salt bath or a well regulated furnace, this method offers no complications.

The solidified solvent on a developed chromatogram gives a resulting plate less prone to abrasive damage. This may cause difficulties if one wants to remove a separated fraction.

Further experiments are under way to extend the use of molten salts in thin layer chromatography and to investigate the usefulness of various complexing anions on inorganic separations.

ACKNOWLEDGMENT

The author thanks James Schlegel for the use of his molten salt bath.

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Preparation of Thin-Film Gas Chromatographic Columns with Polyvinylpyrrolidone-Inactivated Supports

SIR: Gas chromatographic columns used for analytical purposes with steroids and other polyfunctional compounds of comparable molecular size and structural complexity are best prepared with inactivated supports. Various silanizing procedures for the deactivation of diatomaceous earth supports have been published [see (*3*, *4*) and references cited therein], but the separation of hydroxyl-substituted compounds without partial adsorption and tailing is often difficult even when a silanizing procedure is used. The extent to which tailing may occur depends upon both the structure of the solute and the nature of the support and the phase.

In a continuing search for ways of preparing superior thin-film columns with polar phases, the possibility of deactivating supports by a coating process using an organic polymer was considered. In pursuit of this possibility, it was found that polyvinylpyrrolidone (PVP) did not itself function as a satisfactory liquid phase at 200° to 220° C., but that supports coated with 1 to 2% PVP could in turn be coated with a liquid phase to yield an excellent column packing. The column used to obtain chromatogram *B* of Figure 1 was prepared with a thin-film packing containing 1% neopentyl glycol succinate (NGS) on an acid-washed (*3*) diatomaceous earth support (Gas Chrom P) coated with 1% PVP before the polyester film was applied. The slurry coating technique of Horning, Moscatelli, and Sweeley (*2*) was used both for the PVP coat (methanol solution) and the NGS (acetone solution) coat. For comparison, the result obtained with a packing containing 1% NGS on an acid-washed and silanized support (*3*) is shown in Figure 1*A*.

In separate experiments it was found that PVP-treated supports were not suitable for the preparation of thin-film packings with nonpolar silicone polymers. This may be due to poor wetting of the polar PVP surface by these

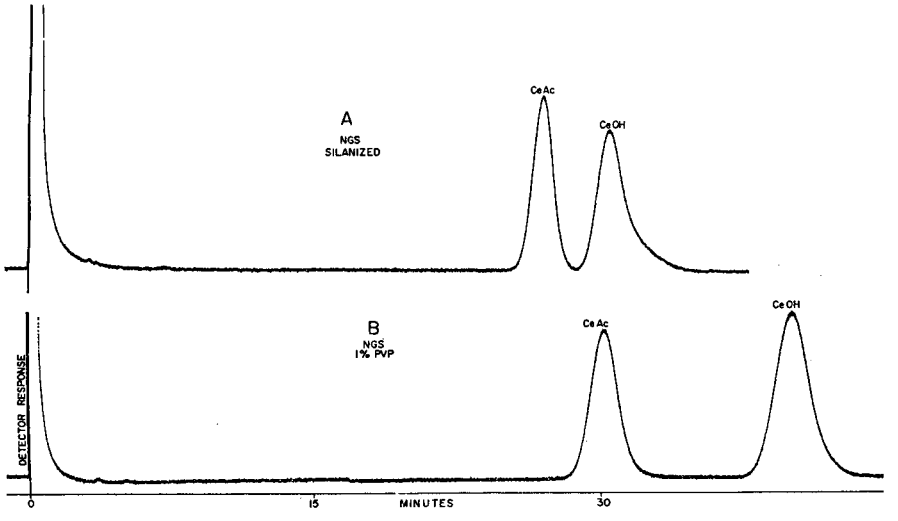

Figure 1. Gas chromatographic separation of mixture of cholesteryl acetate (CeAc) and cholesterol (CeOH) with 1% NGS on differently inactivated supports (*A*, silanized; *B*, PVP-treated)

Column conditions given in Table I

Table I. Comparison of Effects Found With a Silanized Support and a PVP-Inactivated Support

Compound	Relative retention time, 1% NGS[a] Silanized	1% PVP
Cholestane	1.00[b]	1.00[c]
Cholestanyl methyl ether	2.75	2.78
i-Cholesteryl methyl ether	1.27	1.30
Cholestanol	6.59	7.87
Cholesterol	7.11	8.42
Cholestane-3-one	7.16	7.22
Cholesteryl acetate	6.37	6.32
Androstane-3β,17β-diol	5.24	9.51
Pregnane-3α,20α-diol	7.19	11.3
Androstane-3,17-dione	5.43	6.08
Pregnane-3,20-dione	6.70	7.27
5α-Pregnane-3,20-dione	7.37	8.04

[a] Both columns (6-ft. × 4-mm. i.d. glass U-tubes) were operated in same column oven with same detector (argon ionization) and conditions (216° C., 100 ml./min.). The support (100–120-mesh Gas Chrom P) was acid-washed (*3*). One portion was then silanized and coated according to (*3*); the other portion was coated with 1% PVP before application of the polyester.
[b] Cholestane time, 4.4 min.
[c] Cholestane time, 4.6 min.

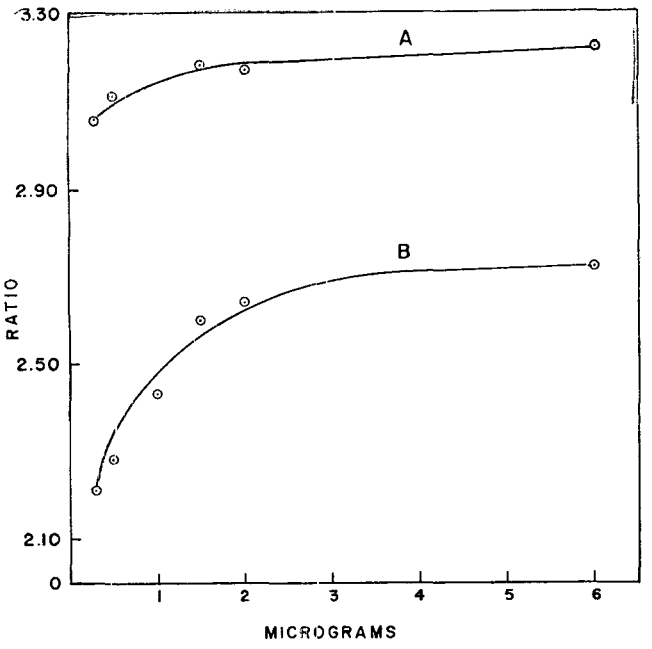

Figure 2. Variation of response ratio (ratio of peak areas) of cholestanol–tetratriacontane mixture with sample size (micrograms of cholestanol) with two differently prepared column packings (*A*, PVP-treated; *B*, silanized)

Column conditions given in Table I

materials. However, polar phases could be coated on PVP-inactivated supports without difficulty. Columns made with these packings showed several interesting properties. The almost complete absence of tailing seen with a sterol is illustrated in chromatogram *B* of Figure 1; the shape of the peak suggests that adsorption of the sterol is less than that found when a silanized support is used. This was confirmed by a study using the approach of Horning *et al.* (*1*). Figure 2 shows the variation of response ratio (ratio of peak areas) of a cholestanol–tetratriacontane mixture with sample size for two types of columns. The superiority of the PVP-coated packing with respect to lack of adsorption is evident.

Table I contains relative retention time data comparing effects found with a silanized (with dichlorodimethylsilane) support and a PVP-inactivated support. Since the retention times observed for a number of steroids are about the same under the two conditions, it appears that PVP is not itself acting as a liquid phase. Nevertheless, PVP coating modified the retention behavior found for steroids with hydroxyl groups. Alcohols were retained much longer than the corresponding ketones. It is not certain if the changed selective retention behavior of the solute is due to interaction with the PVP surface beneath the film of NGS or results from a partial solution of PVP in the polyester phase.

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Vanadium(II)–1,10-Phenanthroline as an Oxidation-Reduction Indicator

SIR: Vanadium in its +2, +3, and +4 oxidation states forms complexes with 1,10-phenanthroline (*1*), but no complete description of any of these systems has been published. The review article quoted (*1*) describes the vanadium(II) complex as being intensely blue-violet. During a systematic study of all of the vanadium-1,10-phenanthroline complexes, the possibility of using this intensely colored compound as an oxidation-reduction indicator was explored. In dilute acid solution the color is bleached by oxidizing agents at about 0.0 volt *vs.* S.C.E.; the color is restored by chromous ion at about −0.1 volt *vs.* S.C.E. It is as an indicator for titration with strong reducing agents such as Cr(II) that this complex may be useful. Chromous chloride and chromous sulfate are well established as reducing agents (*2*, *4*) but are generally used with potentiometric end point detection; the green color of the chromic ions produced when Cr(II) is oxidized tends to obscure a visual end point. Some organic indicators which have transition potentials near 0.0 volt *vs.* S.C.E. have been described, but many of them are insoluble, unstable, or otherwise unsuitable (*3*). This communication describes the use of the vanadium(II) complex with 1,10-phenanthroline as an indicator in titrations with chromous ion; the details of the formulas and stability of the species involved are being investigated. The exact nature of the colored species is not particularly relevant to this type of application because equilibrium between V(II) and 1,10-phenanthroline is very slowly attained; a sufficient color intensity is developed in 2 to 3 seconds, however, to serve as a titration indicator.

A solution of V(II) and 1,10-phenanthroline (1:4) in 0.01*F* HCl shows a broad absorption band with a maximum at 645 mμ. The molar absorptivity is 5400 liters per mole-cm. (calculated for the vanadium) when the solution is fresh and it increases to over 8000 in 36 hours. The colored complex formed by the reduction of vanadyl ion with Cr(II) in the presence of 1,10-phenanthroline has the same spectrum as the V(II) preparation.

The indicator is prepared from $VOCl_2$ and 1,10-phenanthroline. Take 0.228 gram (1.25 mmoles) of V_2O_5 and heat it briefly in 10 ml. of 3*F* HCl. Cool to room temperature, add 0.25 gram of Na_2SO_3, and heat gently until all the V_2O_5 has been converted to $VOCl_2$ and the excess SO_2 driven off. Dilute to 50 ml., add 1.49 grams (7.50 mmoles) of 1,10-phenanthroline monohydrate, and

stir until it dissolves. Add 6*F* NaOH dropwise until the brown precipitate which forms just redissolves; if the solution is too acid the complex will not fully form. This gives a green solution 0.050*F* in the indicator; 2 to 3 drops in 50 to 75 ml. are sufficient for a titration.

The use of this indicator is illustrated by the data given for the titration of ferric iron with $CrCl_2$. A solution of ferric chloride in HCl was prepared and standardized against $K_2Cr_2O_7$. A chromous chloride solution was prepared by electrolytic reduction of $CrCl_3$ and was standardized by coulometric titration with Br_2. One-milliliter aliquots of the iron solution were titrated as follows: 50 ml. of 1*F* H_2SO_4 and 3 drops of indicator were placed in a cell and oxygen was removed with N_2. Chromous chloride was added until the first perceptible blue color, the iron solution was added, and the solution was titrated again to the blue color. The color change is rapid, even at room temperature, and is easily seen, even though the solution is colored green by the chromic ions. The color change is obscured if more than 3 ml. of 0.1*F* $FeCl_3$ are titrated in 50 ml. Figure 1 shows that the indicator end point effectively coincides with the equivalence point in this titration. The

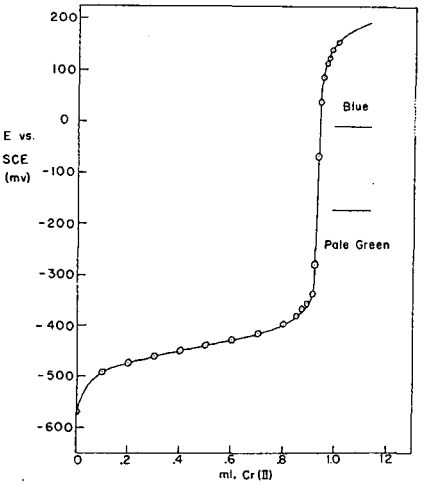

Figure 1. Titration of 0.896 ml. of 0.08906*F* $FeCl_3$ in 50 ml. of 1*F* H_2SO_4 with 0.08533*F* $CrCl_2$

results of some visual titrations are shown in Table I.

The use of this complex, as well as substituted phenanthroline complexes of vanadium, as indicators for other oxidation-reduction titrations is being investigated and the results will be reported later.

Table I. Results of Titrations

Fe taken, mg.	Cr(II) solution, ml.	Fe found, mg.
	1.040	4.96
4.93	1.032	4.92
	1.036	4.94
		av. 4.94

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Separation of the Methyl Esters of Resin Acids by Gas Liquid Chromatography

SIR: At the Forest Products Laboratory, work has been under way with the objective of developing a gas liquid chromatography method for the separation of the fatty and resin acids found in extractives from wood and wood products. At this stage in the study several results may be stated. First, the polar liquid phase, diethylene glycol succinate (DEGS), gives better separation of the methyl esters of the principal resin acids (*7*) than previously used polar solvents (*5*) and appears also to be capable of separation of some pairs of these esters to an extent not indicated by a recent publication (*1*). Second, methyl levopimarate, though quite labile both chemically—e.g., acid medium—and thermally, has been found to traverse the GC column unchanged both chemically and reproducibly in the chromatographic sense. Finally, when stored in capped vials as a solution in a low boiling solvent (Skellysolve B, *n*-hexane), methyl levopimarate is unstable and slowly converts to methyl dehydroabietate. The change is accompanied by four additional peaks having retention times shorter than that of the parent material; one of the four peaks has a retention value corresponding to that of methyl pimarate.

EXPERIMENTAL

Apparatus. A Research Specialties Company Model 600 gas chromatograph equipped with thermal conductivity detector is being used. Operation has been in the isothermal mode with 275°, 200°, and 215° C. as the respective temperatures of injection port vaporizer, column, and detector. The detector is operated with a bridge current of 200 ma. Sample size (0.25 to 1.0 μl. of solution) and solute concentration are adjusted so that the detector may be operated at full sensitivity (XI scale) into a recorder (Leeds and Northrup, Speedomax Type G, AZAR) with 1-mv. full scale sensitivity and 1/4-inch-per-minute chart speed. The helium carrier gas is adjusted to a nominal flow (soap-film flowmeter) of 150 ml. per minute, where $p_i/p_o = 2.18$ (p_o = atmospheric pressure).

Microliter size hypodermic syringes (Hamilton Co.) were used for sample injections.

The apparatus described by Lohr and Kaier (*11*) was adapted to the instrument for the purposes of peak trapping from the effluent steam. A J-tube-shaped trap was used to collect solvent bleeding from the packing.

Column. The column used was 6-foot stainless steel tubing, nominal 1/4-inch o.d. The effective dimensions of the packing were 0.54 cm. (i.d. of tubing) by 182.7 cm. long; 14.6 grams of DEGS packing were loaded into this column volume.

The packing was prepared from materials available commercially: DEGS, "pretested polyester," (Applied Science Laboratories, Catalog No. M-306); NSG, nitrile silicone gum, General Electric Company, XE60 (Analytical Engineering Laboratories, Inc., Catalog No. GS91A); solid support, Anakrom ABS, 70- to 80-mesh (Analytical Engineering Laboratories, Inc.). The support was coated in each case by slurrying it and the appropriate liquid phase in acetone and removing the acetone in a rotary evaporator with heating.

While the solvent loading was initially a nominal 20% by weight for each packing, subsequent bleeding reduced this figure. By trapping the column effluent almost continuously during operation, it is estimated that the loading was reduced to approximately 16% (maximum) for the DEGS data of Table I and 225° C. data of Table II; the loading for the remainder

Table I. Relative Retention Data for Methyl Esters of Resin Acids[a]

Ester	DEGS r_{18}	DEGS r_{LP}	DEGS α_{12}	NSG r_{LP}	NSG α_{12}
18:0[b]	1.00	0.183	...	...	...
Pimarate	4.08	0.75	...	...	...
Levopimarate	5.47	1.00	1.34	1.00	...
Palustrate	5.50	1.01	1.01	...	...
Isopimarate	5.98	1.09	1.09	...	...
Abietate	8.64	1.58	1.45	1.40	...
Dehydroabietate	9.52	1.74	1.10	1.24	1.13
Neoabietate	10.2	1.86	1.07	...	

[a] Column temperature, 200° C. [b] Stearic acid, t_R' = 4.3 minutes, r_{16}(16:0 = 1) = 1.72 for methyl stearate.

Table II. Temperature Function of Relative Retention for Test Pairs of Fatty Acid and Resin Acid Esters on DEGS

	Temperature, ° C. 189	200	216	225[a]
Resin acid esters: methyl dehydroabietate–methyl abietate				
α_{12}	1.11	1.09	1.07[b]	1.07
r_{18}[c]	9.94	9.42	8.80	8.57
n[c] (plates)	2060	1880	1800	2080
R	1.13	0.94	0.66	0.72
Fatty acid esters: methyl oleate–methyl stearate				
t_R'[d] (min.)	5.02	3.34	1.96	2.19
$\alpha_{12} = r_{18}$	1.17	1.18	1.17	1.19
n[d] (plates)	1690	1530	1560	1480
R	1.50	1.42	1.31	1.4

[a] From a previous set of measurements (*12*). [b] Calculated from initial retention times, t_{R1}' (*6*). [c] Methyl dehydroabietate. [d] Methyl stearate.

of the data in Table II and for that in Table III is something less than the above figure. For the NSG data (Table I) the loading was not larger than 10%, excessive bleeding having occurred.

Each packing was conditioned and operated at a number of temperatures. With the DEGS column, the maximum temperature of exposure was 235° C.; for the NSG, this was 265° C. Normal use of each was at 200° C.

Methyl Ester Standards. The fatty acid methyl esters were of 99+% purity (Applied Science Laboratories, State College, Pa.) Levopimaric (*9*), neoabietic (*10*), and isopimaric (*2*) acids were isolated from oleoresin; dehydroabietic acid was prepared from dehydroabietonitrile (*4*), and abietic acid was prepared by isomerization of levopimaric acid (*8*). Palustric and pimaric acids were obtained from R. V. Lawrence, Naval Stores Laboratory, U. S. Department of Agriculture, Olustee, Fla.

Methylation of the resin acids was accomplished using diazomethane in a method similar to that of Schlenk and Gellerman (*13*). Freshly distilled diazomethane in ethyl ether was added dropwise to a solution containing 10 mg. of the resin acid per ml. (9:1) ethyl ether–methanol at 0° C. until the solution had a yellow tinge against a white background. The slight excess of diazomethane and solvent were removed with a stream of high-purity nitrogen. Methyl levopimarate, isopimarate, and dehydroabietate were each recrystallized before use.

RESULTS AND DISCUSSION

Table I lists the relative retention values obtained for the seven principal resin acid methyl esters. With the exception of methyl pimarate, all data have been computed from chromatograms in which one or both of the standards, methyl stearate (r_{18}) and/or methyl levopimarate (r_{LP}), were also present. The use of the two reference compounds has been found convenient and is felt to be necessary also because of the great differences between the properties of the two types of acids, chemically and structurally. Since reported data (*1*, *5*) have been given with respect to methyl palmitate, conversion of the data in the tables to this basis may be made from $r_{16} = 1.72 \times r_{18}$. [Relative retention values for practically all members of the fatty acid esters in the series 12:0 through 24:0, plus 18:1 and 18:2, have been measured; these data are omitted here since they agree essentially with that summarized elsewhere (*3*)].

The degree of separation realized between adjacent pairs of resin acid esters is more effectively seen from the respective separation factors, α_{12} (column 4, Table I). Each value of α is computed relative to the previous compound as the standard—i.e., as component 2.

In the published data (*1*, *5*), GC separation of the ester pair abietate-dehydroabietate has not been achieved. Recent data (*12*) obtained in the course of this work reported α_{12} = 1.07 for this pair at 225° C. Extension of the data to several lower column temperatures has been made with the results shown in Table II. For comparison, similar data are included for the fatty acid pair methyl oleate–methyl stearate which is used as a test mixture in following the history of the packing. The improvement in both separation factor and resolution is very evident as column temperature is decreased; an

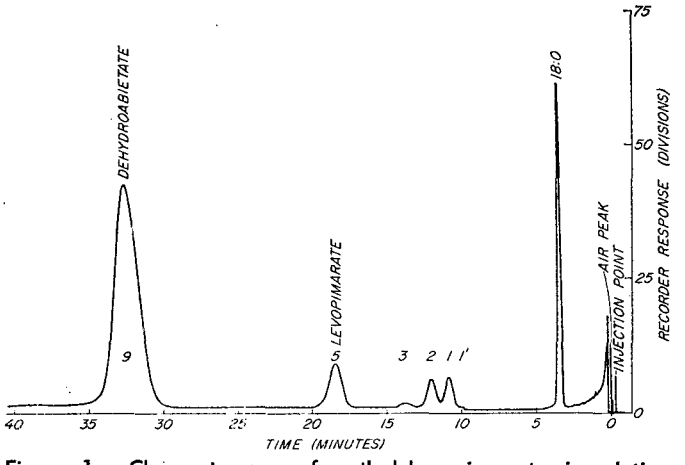

Figure 1. Chromatogram of methyl levopimarate, in solution 97 days

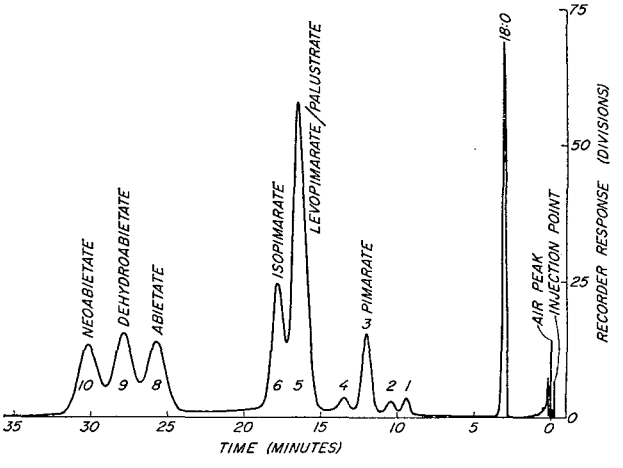

Figure 2. Chromatogram of methyl oleoresin, filtered through alumina

effective separation of this pair is thus possible. Presumably, further improvement in separation would result by decreasing column temperature further, but this at the expense of an increase in retention time which could be offset only by decreasing the liquid phase loading. Additional work is planned in this respect.

The retention values for methyl levopimarate as reported (*5*) appear to be attributed to a peak derived from isomerization or disproportionation. In the course of our work (*12*), injection of a sample of pure, crystalline methyl levopimarate dissolved in low boiling solvent (C_6 range) resulted in a single peak on the chromatogram. The solute band corresponding to the chromatogram peak identified as methyl levopimarate was collected and its identity verified by means of UV spectrometry. Various samples of methyl levopimarate, both pure and impure, were used in this phase of the study, all with identical results. We believe, therefore, that this resin acid ester is stable to the gas chromatography conditions applied herein and traverses the column unchanged chemically and with a reproducible GC retention value. Special attention has been given to minimizing the possibility of acid-catalyzed isomerization of the resin acid esters by use of catalyst-free prepurified DEGS on siliconized diatomaceous earth support (Anakrom ABS).

In an unknown mixture the accurate identification of methyl levopimarate from GC retention data alone is limited, however, by the fact that other resin acid esters have (methyl palustrate, Table I), or may have, relative retention values essentially equal to that of levopimarate. Verification of the peak due to the latter would be subject then to independent analysis or to gas chromatography with a liquid phase other than DEGS that affords the needed separation. The latter aspect is also under investigation.

Sufficient evidence has been accumulated to show that solutions of a crystalline, pure methyl levopimarate in low boiling solvents such as purified Skellysolve B and *n*-hexane, though stored under nitrogen in capped glass vials, are not stable. The resin acid ester is converted to methyl dehydroabietate (predominantly) plus four other compounds with retention times shorter than that of methyl levopimarate (Figure 1). One of these four (No. 3) has a relative retention value corresponding to that of methyl pimarate. Peak 1′ is observed distinctly in a "younger" solution where peak 1 is smaller than here. The identity of both methyl levopimarate and methyl dehydroabietate peaks was verified from the characteristic ultraviolet spectrum of each for samples trapped from the chromatograph effluent. Experiments aimed at observing the effect of the sample container on the stability of the ester showed that conversion could not be prevented even if glass was rinsed with aqueous ammonia solution, and that the rate of conversion was affected by the nature of the sample container and was slower for samples stored in "test tubes" of Teflon and polyethylene. Work is in progress to determine the source of this problem and how to avoid it so that the integrity of wood extracts will not be impaired.

Table III. Relative Retention Data for Methylated Oleoresin and Rosin

Peak no.	r_{18}	r_{LP}	Compound
18:0[a]	1.00	0.180	Stearate
1	3.19	0.57	...
2	3.54	0.64	...
3	4.08	0.73	Pimarate
4	4.57	0.82	...
5	5.55	1.00	Levopimarate/ palustrate
6	6.03	1.09	Isopimarate
7	6.95	1.25	...
8	8.75	1.58	Abietate
9	9.46	1.71	Dehydro-abietate
10	10.2	1.85	Neoabietate

[a] Methyl stearate, t_R' = 2.94 minutes.

As a demonstration of the practical value of the technique and data described above, samples of gum rosin and of the resin acids obtained from an oleoresin sample by amino-cellulose ion exchange (*14*) were methylated and chromatographed. A portion of each ester mixture was filtered through an alumina column to remove oxygenated compounds. Four samples were run; each was "spiked" with a small amount of methyl stearate. The resulting data are summarized in Table III and illustrated by the chromatogram for the filtered methyl oleoresin sample (Figure 2). With one exception (the unfiltered methyl rosin sample), the four chromatograms were alike qualitatively, each exhibiting the same nine peaks listed in the table; peak 7 disappeared when the methyl rosin sample was filtered. Since the adjusted retention times for the four samples were within ±1% of each other, they were averaged and the relative retention values computed.

The slightly higher value of r_{18} for the levopimarate-palustrate peak apparently reflects the fact that the rosin sample contained no levopimarate, and in the oleoresin, this peak contained both esters in the ratio of approximately 3 to 1. These supplementary data were obtained by a thin layer chromatography technique wherein the levopimarate-palustrate pair was separated (*15*).

Quantitative application of the data is not possible at this stage in the study. However, as an indication of the quantity of material applied to the column, solutions of known concentration of methyl stearate and methyl levopimarate were chromatographed and interpreted in terms of peak height and peak area. In the sample size range of 10 to 400 μg. represented by these data, the response was closely linear with respect to both peak height and peak area. For methyl levopimarate, the slope was 0.44 μg. per sq. mm. and 0.57 μg. per division for methyl stearate. Thus, the respective ester peaks in Figure 1 represent 50 μg. of methyl levopimarate and 34 μg. of methyl stearate.

Preliminary work (*12*) with the nitrile silicone gum XE60 (NSG) at 200° C. provided the data in Table I for three resin acid esters. While the relative retention with respect to methyl levopimarate is less than that for DEGS, the separation factor for methyl abietate–methyl dehydroabietate is not, but the order of elution has been reversed.

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Anomalous Capillary Melting Points for Sugars Obtained with Borosilicate Glass Capillaries

SIR: Capillary melting points for organic compounds have been commonly obtained with the use of soft-glass capillaries, usually hand-drawn. However, commercially available capillaries are now in extensive use, being inexpensive, conveniently packaged, and highly uniform in shape. These capillaries are made of borosilicate glasses, which at first thought should not occasion any difference in the melting temperatures of compounds. We have found, however, that this glass does influence the melting points of some of the sugars.

When the melting points of three samples of pure glucose, two of the alpha and one of the beta forms obtained commercially from three different sources, were being checked using the commercial capillaries (one end sealed), they were found to be 5° to 10° C. higher than the literature values. But the anomalous capillary melting points continued to be obtained when other capillaries from another package were used. Infrared spectra of the samples verified the anomer structures and failed to show the presence of any impurities. The melting point of a test standard with a certified melting point of 150° C. (micro) placed in one of the commercial capillaries showed that the thermometer was not at fault. Portions of the sugars placed on a Parr-Dennis melting point bar, a Fisher-Johns melting point apparatus, and a Kofler micro hot stage melting point apparatus all melted at temperatures that were in agreement with the published values. The glass of the commercial melting point capillary then became suspect because in the above equipment the latter two apparatus make use of microscope cover glasses and slides which are not made of borosilicate glass and the melting point bar is, of course, all metal.

With a return to established practice being desirable to confirm the suspicion, capillaries were hand-drawn from short, open-end sections of soft-glass tubing. These sections, 12-mm. o.d. with a 1-mm. wall thickness, were first boiled in two changes of fresh distilled water and dried. Melting points obtained with the capillaries thus prepared checked the published values for the sugars. Borosilicate capillaries were also washed and dried (done with open-end tubes before sealing and use); the melting point still agreed with those from the already-sealed tubes. When sample-containing capillaries, one of soft glass and the other of borosilicate, were attached to the same thermometer in the oil bath, the difference in behavior of the sugars was very striking—the sample in the soft-glass capillary was completely melted while the other was still solid. Similar effects were observed when either pure D-fructose or pure cellobiose was substituted for glucose. In the latter case, melting was followed immediately by decomposition.

This difference in observed melting points cannot be explained by tube dimensions. These were determined by using a micrometer eyepiece microscope. The outside diameter of both sets of capillaries averaged 1.5 to 2.0 mm.; wall thickness for the soft-glass tubes was 0.05 to 0.09 mm., while that for the commercial ones was about 0.2 mm. The thicker wall of the commercial capillary did not influence the melting points of the glucoses because holding the bath temperature at 148° and 150° C. did not produce sample liquefaction with these capillaries. Furthermore,

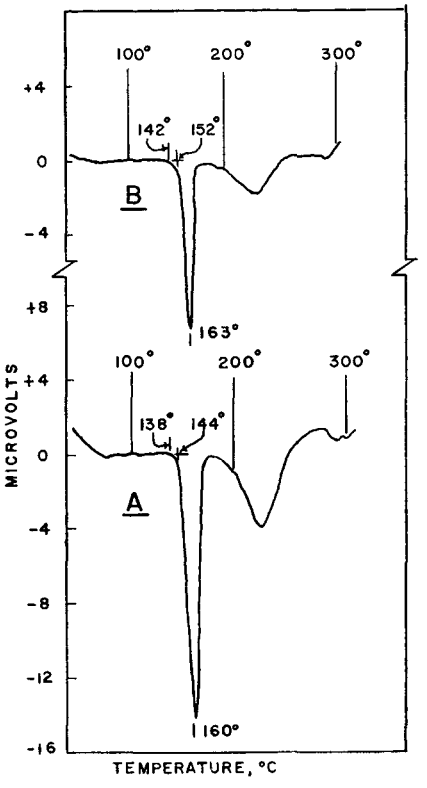

Figure 1. Differential thermal analysis curves for (A) alpha-D-glucose and (B) alpha-D-glucose and powdered borosilicate glass

elevated melting points were obtained with the soft-glass capillaries when powdered borosilicate glass (obtained by grinding a capillary in a mortar and pestle) was added to the sugar before loading the capillary. These visual observations were confirmed in a differential thermal analysis apparatus. In Figure 1 are shown the curves for an alpha-D-glucose sample—the shift of temperatures caused by the glass is readily apparent here.

There is mention in the literature that the glass of the capillary can have an effect on the melting point obtained. Morton (*4*) has referred to the reported effect of unwashed soft glass on lowering the melting points of glucose, fructose, mannitol, phenacetin, and other compounds, an effect which is apparently overlooked in other texts on laboratory methods and technique. Here the melting point is lowered because of alkali and products of devitrification remaining on the surface of the unwashed soft glass. However, correct melting points are obtained when the same glass is clean. Dieckmann (*1*), however, reported three different melting points for keto-acetyldibenzoylmethane when capillaries of three different glasses, Jena, ordinary, and Thuringer, were used. With Jena glass the melting point was some 20° C. higher than with ordinary glass. Jones (*2*) found different melting points for pure rotenone when capillaries of Pyrex No. 774, ordinary soft glass, and Corning electrode glass No. 015 were used. The melting points were 163–4°, 159.5–60.5°, and 155–6° C., respectively. This finding was further extended by Jones and Wood (*3*) with alpha-toxicarol, beta-toxicarol, and deguelin. Higher melting points were obtained in borosilicate capillaries. Jones and Wood also used six commercially available glasses, plus transparent silica, with alpha-toxicarol. The melting points fell into two groups, 230–1° C. for borosilicate glasses and transparent silica, and 200–7.5° C. for alkaline soft glasses. In all of the above, alkali again was the factor involved, but this time coming from the glass itself in sufficient quantity to apparently cause enolization of the compound.

In the present instance, both forms of D-glucose, the D-fructose, and the cellobiose are affected similarly by borosilicate glass. This could be explained by assuming that the melting points as

obtained with the soft-glass capillaries might be low because of the effect of alkali—except that the melting points of the glucoses as obtained with the soft-glass capillaries agree with those melting points obtained on the Dennis melting point bar where there are no glass surfaces. Furthermore, the mixture of powdered borosilicate glass and the sugar still gave higher melting points in the soft-glass capillary and the differential thermal analysis apparatus. Perhaps boron from the borosilicate has interacted with the sugars (boron does complex with the hydroxyl group) or has catalyzed some molecular rearrangement. The cause of the phenomena should be further investigated.

Finally, we believe that as glass capillaries are used extensively there is some need for further testing borosilicate melting point capillaries for suitability, particularly in connection with those compounds that are capable of mutarotation, bear hydroxyl groups, or have aldehydic or ketonic groups capable of undergoing keto-enol transformations.

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Determination of Total Cholesterol in Blood Serum with Perchloric Acid–Phosphoric Acid–Ferric Chloride Reagent

SIR: The current methods for the determination of total cholesterol in blood serum are based on either the Liebermann-Burchard reaction (*4*) or the reaction with ferric chloride–sulfuric acid reagent (*6*). However, the former reaction requires many strict conditions of performance to obtain a reproducible color development; while in the latter reaction, small amounts of impurities in sulfuric acid frequently give higher cholesterol values (*1*, *2*, *5*). The method presented here is very simple to use with a large number of samples, and gives cholesterol values analogous to those determined by Schoenheimer and Sperry's method, which is generally accepted as a standard method for the determination.

EXPERIMENTAL

Reagents. Color reagent. Dissolve 8.0 grams of $FeCl_3 \cdot 6H_2O$ (reagent grade) in a mixture of 200 ml. of perchloric acid (70%, reagent grade) and 600 ml. of phosphoric acid (85%, reagent grade). This reagent is very stable and can be stored at room temperature for a long time.

Ferric chloride, 0.1%, glacial acetic acid solution.

Ferric chloride, 0.2%, aqueous acetic acid solution. Dissolve 1.18 grams of $FeCl_3 \cdot 6H_2O$ in a mixture of 590 ml. of glacial acetic acid and 20.0 ml. of water.

Standard cholesterol stock solution, 0.2 mg. per ml. Dissolve 50 mg. of pure cholesterol (*3*) in 250 ml. of glacial acetic acid.

Working standard cholesterol solutions. Dilute 10.0, 20.0, 30.0, 40.0, 50.0, and 60.0 ml. of the standard stock solution with glacial acetic acid to give a total volume of 61.0 ml. in each case. The working standard solutions thus obtained have equivalent concentrations of total serum cholesterol of 100, 200, 300, 400, 500, and 600 mg. per 100 ml., respectively.

Procedure. To 6.00 ml. of 0.1% $FeCl_3$ solution in a centrifuge tube is added 0.100 ml. of serum. The mixture is stirred thoroughly with a stirring rod to complete coagulation of proteins. The rod is removed and the tube is centrifuged at about 3000 r.p.m. for 10 minutes. To 4.00 ml. of the supernatant solution in a test tube is added 2.00 ml. of color reagent. A blank tube with a mixture of 2.00 ml. of glacial acetic acid and 2.00 ml. of 0.2% $FeCl_3$ aqueous acetic acid solution is also prepared. In the case of calculating unknown cholesterol values from the absorbance of a single working standard solution, another tube with a mixture of 2.00 ml. of a single working standard solution, 2.00 ml. of 0.2% $FeCl_3$ aqueous acetic acid solution, and 2.00 ml. of color reagent is also prepared. The contents of all tubes are mixed well by shaking. Then the tubes are heated in a boiling water bath for 20 minutes in such a way that the surfaces of the contents are kept under the surface of boiling water. The tubes are cooled in running water. When the contents of the tubes are thoroughly chilled, the absorbance of the sample (and of a single working standard solution) is read at 450 mμ on a spectrophotometer with a glass cell of 10 mm. sample path length against the reagent blank.

Calculation. The cholesterol values of the samples are calculated from the calibration curve which is described below or conveniently from the ratio of absorbance of unknown to that of a single working standard solution employed.

Calibration Curve. Three aliquots of 2.00 ml. of each working standard cholesterol solution are pipetted into test tubes, and 2.00 ml. of 0.2% $FeCl_3$ aqueous acetic acid solution and 2.00

Table I. Influence of Composition of Color Developing Agents on Coloration

Concentration of $FeCl_3$ (%, w./v.)	Ratio of $HClO_4$ to H_3PO_4 (v./v.)	Color of blank tube	λ_{max} (mμ)	$E_{450\ m\mu}$
0	1:3	colorless	455	0.350
0.5	2:1	yellow		0.600
	1:1	faint yellow		0.560
	1:2	colorless	450	0.500
	1:3	colorless	454	0.450
	1:4	colorless	453	0.400
1.0	2:1	yellow	379	0.800
	1:1	yellow		0.520
	1:2	faint yellow	452	0.500
	1:3	faint yellow	452	0.440
	1:4	colorless	453	0.440
2.0	1:2	yellow	376	0.450
	1:3	faint yellow		0.420
3.0	1:3	faint yellow	383	0.390
	1:4	faint yellow	363	0.340

Table II. Influence of Concentration of Color Developing Agent on Coloration[a]

Ratio of $HClO_4$ to H_3PO_4	Volume of color developing agent (ml.)	Volume of glacial acetic acid (ml.)	Volume of final mixture (ml.)	$E_{450\ m\mu}$[b]
1:2	1.00	1.00	6.00	0.130
	1.50	0.50	6.00	0.184
	2.00	0	6.00	0.214
1:3[c]	1.00	1.00	6.00	0.185
	2.00	0	6.00	0.423
	3.00	0	7.00	0.386

[a] In each case 2.00 ml. of cholesterol solution and 2.00 ml. of 0.2% $FeCl_3$ aqueous acetic acid solution were used.
[b] Av. of three tubes.
[c] Cholesterol solution used was different from that used in 1:2 series.

Table III. Influence of Ratio of Perchloric Acid to Phosphoric Acid in Color Developing Agent on Cholesterol Value in Serum

	Cholesterol value (mg./100 ml.)			
		Ratio of $HClO_4$ to H_3PO_4		
Serum	Schoenheimer and Sperry's method	1:2	1:3	1:4
1	223	230	223	217
2	187	194	184	178
3	176	198	186	175
4	99	99	91	85
5	182	185	184	171
6	192	208	204	193
7	173	187	182	176
8	196	210	202	188
9	244	246	242	223
Mean	186	195	189	178

Table IV. Comparison of Present Method to Schoenheimer and Sperry's and to Zak's Methods

	Cholesterol value (mg./100 ml.)		
Serum	By Schoenheimer and Sperry's method	By present method	By Zak's method
10	358	350	358
11	178	186	197
12	312	318	319
13	237	235	250
14	190	199	212
15	229	224	228
16	165	161	176
17	204	195	205
18	168	174	184
19	183	172	188
20	190	187	198
21	178	181	181
22	158	160	172
23	152	159	163
24	190	178	193
25	127	130	140
26	176	181	179
27	162	163	166
28	129	128	136
Mean	194	194	202
34[a]		189	

[a] Hyland Normal Clinical Chemistry Control Serum which contained 185 mg./100 ml. of cholesterol. Hyland Laboratories, Los Angeles, Calif., U. S. A.

ml. of color reagent are successively added. A blank tube with 2.00 ml. of glacial acetic acid and 2.00 ml. each of both reagents is also prepared. The subsequent treatment is identical with the one described under procedure. The absorbances are read at 450 mμ against the reagent blank. The calibration curve thus drawn up is a straight line.

RESULTS AND DISCUSSION

The deproteinization of serum was successfully carried out with 0.1% $FeCl_3$ in glacial acetic acid (*1*). Since a larger amount of $FeCl_3$ was given to the deproteinized solution by adding the color reagent, the diminution of $FeCl_3$ by deproteinization had no influence upon the coloration of the final liquid.

From our preliminary experiments it became clear that cholesterol developed a color by heating with perchloric acid (70%) in acetic acid and the addition of $FeCl_3$ intensified the color. A yellow color of ferric chloride in the mixture was lightened by the addition of phosphoric acid (85%). Therefore, a mixture of perchloric acid, $FeCl_3$, and phosphoric acid was used as a color developing agent.

To determine the composition of the color developing agent, 20 kinds of mixtures of three chemicals were prepared. To 2.00 ml. of the same cholesterol solution in glacial acetic acid were added 2.00 ml. of each mixture and 2.00 ml. of 0.2% $FeCl_3$ aqueous acetic acid solution. The resulting 20 mixtures were heated in a boiling water bath for 20 minutes. Their visible-light absorption spectra were measured by a Beckman DK-2 ratio recording spectrophotometer with their reagent blanks and summarized in Table I, except five mixtures which gave yellow blank tests. This table clearly shows the following points: yellow blank tests were given when the concentration of $FeCl_3$ exceeded 1.0% or when the ratios of perchloric acid to phosphoric acid were 2:1 and 1:1. A less intense color was developed when a mixture was lacking in $FeCl_3$. The preferable concentration of $FeCl_3$ was thought to be 1.0% to avoid the influence on the coloration which might be caused by the diminution of $FeCl_3$ in the deproteinization.

Next, the influence of concentration of color developing agent in the final mixture on the coloration was examined. Here the final mixture means a mixture which contains color developing agent, 0.2% $FeCl_3$ aqueous acetic acid solution, and cholesterol solution. The final mixtures prepared were heated in a boiling water bath for 20 minutes and their absorbances were read at 450 mμ against the respective blanks. The result shown in Table II reveals that the most intense color is given in the presence of 2.00 ml. of color developing agent in 6.00 ml. of the final mixture.

It is important to note that the ratio of perchloric acid to phosphoric acid in the color developing agent affects the cholesterol value in serum even when the concentration of $FeCl_3$ is fixed as 1.0%. To select the best condition, three reagents were prepared which contained perchloric acid in the ratios of 1:2, 1:3, and 1:4 to phosphoric acid, respectively. These reagents were applied to the same serum by the method described under procedure, and the cholesterol values were read from each calibration curve. The resulting data are shown in Table III with those of parallel tests by Schoenheimer and Sperry's method. The cholesterol values increase with increasing concentration of perchloric acid, and almost agree with those of Schoenheimer and Sperry's method when the ratio of perchloric acid to phosphoric acid is 1:3.

The necessary time of heating to reach a maximum absorbance was between 15 and 25 minutes, and 20 minutes' heating was thought to be preferable in routine work.

Both visible-light absorption curves of developed color with a standard cholesterol solution and serum had practically the same shape. They had a tendency of gradual increasing intensity toward shorter wavelength region with a shoulder at 452 mμ.

Table V. Recovery Tests of Method

	Cholesterol value (mg./100 ml.)		
Serum	Initial	After addition of cholesterol (equivalent to 100 mg./100 ml. in serum)	Recovery, %
29	228	327	99
30	216	318	102
31	191	291	100
32	165	266	101
33	165	263	98
34[a]	189	291	102

[a] See footnote of Table IV.

The color was stable and its absorbance was constant for about 4 hours not only in the dark but also in the light. It followed Beer's law when a concentration of cholesterol in a standard solution lay between zero and 0.2 mg. per ml. Therefore, when serum has a higher cholesterol value than 600 mg. per 100 ml. it should be diluted with water before determination.

Table IV presents a result of comparison of the present method to Zak's and to Schoenheimer and Sperry's methods.

Recovery tests of this method gave 100 ± 2% recovery as shown in Table V.

Cholesteryl acetate and oleate also developed colors, both of which had an identical visible-light absorption spectrum with that of cholesterol. And 100 ± 1% and 97 ± 1% recoveries of equivalent cholesterol values were given by cholesteryl acetate and oleate, respectively (Table VI).

The relative standard deviation of this method at a level of 225 mg. per 100 ml. cholesterol in serum was 0.76%.

Table VI. Recovery Tests of Cholesteryl Esters

Acetate			Oleate		
Equivalent cholesterol values (mg./100 ml.)		Recovery, %	Equivalent cholesterol values (mg./100 ml.)		Recovery, %
Added	Recovered		Added	Recovered	
	181	101		118	98
180	180	100	121	116	96
	181	101		117	97
	270	100		355	98
271	271	100	362	356	98
	269	99		350	97

ACKNOWLEDGMENT

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Methyl Orange Spectrophotometric Method for Schiff Bases

SIR: In a recent issue of ANALYTICAL CHEMISTRY, Silverstein (*3*) described the development of a methyl orange method for measurement of high molecular weight amines in aqueous solution. It was based on reaction of the amines with methyl orange at pH 3 to 4 to form a yellow complex soluble in an organic liquid. The color intensity of the organic phase was used as a direct measure of total amine concentration. This procedure was made specific for the detection and measurement of primary, secondary, and tertiary amines.

The basic methyl orange colorimetric method for amines was introduced by Brodie and Udenfriend (*1*) in 1945 for the determination of cinchona alkaloids and other organic bases in plasma and urine. Later, the writer and Ellenbogen (*2*) applied this technique to the estimation of *d*-amphetamine and *dl*-amphetamine in blood and urine. In the interim period this method has been used by the writer and/or colleagues for many analyses involving detection and measurement of amines. Several variations have been used compared to the method reported by Silverstein (*3*).

The methyl orange technique is made more reliable, especially at the low concentration levels, by coating the glass equipment with a silicone film such as Desicote, which works very well. This prevents the loss of amines by deposition on the glass surfaces and makes it possible to obtain reproducible results under a variety of experimental conditions. The sensitivity of the methyl orange procedure is increased for measurement of total amines by extracting the yellow methyl orange complex from the organic phase into aqueous acid solution. The absorbance of the resulting red solution at 508 mμ is significantly higher than for the organic phase at 430 mμ. Other method variations exist including the use of benzene as a solvent instead of ethylene dichloride and the use of a different buffer system.

This report describes a further application of the methyl orange procedure as used in this laboratory for measurement of 100 p.p.m. of Schiff bases in No. 2 fuel oils. In general, Schiff bases represent the class of compounds derived by chemical reaction (condensation) of aldehydes or ketones with primary amines. The general formula for the weakly basic Schiff base is RR′—C=N—R″. The Schiff base for this work, I, is represented by the formula *tert*-$C_{12-15}H_{25-31}$—N=CH_2, which is a mixture of highly branched primary amines having a tertiary alkyl structure (nitrogen attached to the tertiary carbon atom).

The oil sample containing Schiff base I is dissolved in benzene and given an alkaline wash. The benzene is shaken with methyl orange reagent at pH 5 and the excess methyl orange is removed. The methyl orange which dissolves in the benzene through complex formation with Schiff base I is returned to an acid solution and measured spectrophotometrically.

EXPERIMENTAL

Apparatus. Coat the inside of glass stoppered bottles, 100-ml. capacity, with a silicone product such as Desicote, produced by Beckman Instrument Co.

A Cary Model 11 spectrophotometer with 2 cm. fused quartz cells was used for measurement in the visible range.

Reagents. Benzene Containing 1.5% Isoamyl Alcohol. A technical grade of benzene is purified by successive washings with 1*N* sodium hydroxide, 1*N* hydrochloric acid, and distilled water. A reagent grade of isoamyl alcohol is washed with 0.2 volume of 1*N* hydrochloric acid followed by several washes of distilled water. The absorbance of the reagent blank should not exceed 0.010.

Methyl Orange Reagent. Dissolve 500 mg. of methyl orange crystals in 100 ml. of warm water. Immediately before use, mix equal volumes of the methyl orange solution and saturated boric acid solution.

Standard Solution of Schiff Base I. To prepare a stock solution, weigh

approximately 0.040 gram of Schiff base I accurately to ±0.0001 gram into a 100-ml. volumetric flask, dissolve in benzene containing 1.5% isoamyl alcohol, and dilute to volume.

To prepare a working solution, pipet 3 ml. of stock solution into a 100-ml. volumetric flask and dilute to volume with benzene containing 1.5% isoamyl alcohol.

Procedure. Oil Clean-Up. Weigh 4 to 5 grams of fuel oil containing Schiff base I at the 0.01% concentration level accurately to ±0.01 gram into a 50-ml. volumetric flask. Dissolve the oil in benzene containing 1.5% isoamyl alcohol and dilute to volume. Transfer approximately 50 ml. of the benzene solution to a 125-ml. separatory funnel. Wash the benzene with 10 ml. of 10% sodium hydroxide solution followed by 10 ml. of distilled water with 2-minute shakings and discard the wash solutions. Centrifuge approximately 25 ml. of the washed benzene phase for 2 to 3 minutes.

Color Development. Transfer approximately 25 ml. of the centrifuged benzene to a 100-ml. silicone-treated glass stoppered bottle. Pipet 1 ml. of methyl orange reagent into the bottle, stopper, and shake mechanically for 5 minutes. (The mechanical shaker should at least simulate a very vigorous hand shaking.) Decant approximately 15 ml. of the supernatant benzene into a centrifuge tube, being careful to exclude any methyl orange reagent, and centrifuge for 3 minutes at 1500 to 2000 r.p.m. Pipet 20 ml. of 1*N* hydrochloric acid solution into a 60-ml. separatory funnel. Pipet 10 ml. of the centrifuged benzene into the separatory funnel, using special care not to pipet any methyl orange reagent from the bottom of the centrifuge tube, and shake by hand for 1 minute. Allow the phases to separate, isolate the acid phase, and centrifuge to clarify if necessary. Read the absorbance of the acid phase at 508 mμ (absorption peak) and 700 mμ using a 2-cm. cell and water as a reference solvent. Determine the micrograms of Schiff base I from the reference curve and calculate the p.p.m. found.

Standard Reference Curve. Prepare a standard reference curve covering the range of 0 to 6 μg. of Schiff base per milliliter of final acid solution by pipetting 5-, 10-, 15-, 20-, and 25-ml. aliquots of Schiff base I working solution into 100-ml. glass stoppered bottles, diluting to exactly 25 ml. by pipetting in benzene containing 1.5% isoamyl alcohol, and analyzing according to the color development procedure. Plot (absorbance at 508 mμ minus absorbance at 700 mμ) as the ordinate and (micrograms of Schiff base I per milliliter of 1*N* hydrochloric acid solution) as the abscissa. Once the reference curve has been established, one or two points suffice for checking. A slight deviation from Beer's law at the lower concentration levels necessitates the use of a reference curve.

DISCUSSION

Recoveries of Schiff base I at the 100-p.p.m. level from fuel oil are 98 ± 2%. The overall relative error is within 4% of the amount present when applied to 11 out of 14 commercial base stock oils. These oils are representative of products from 14 companies and geographical locations in the United States and Canada. Three of the oils have interfering constituents accounting for errors equivalent to 12 to 46% of the amount of Schiff base I at the 100-p.p.m. level. The oil colors ranged from light yellow to black with no correlation between color and amount of interfering constituents.

The method has a degree of specificity in that common low molecular weight organic bases such as quinoline in fuel oils offer no interference. However, components with basic strength, structure, and molecular weight comparable to Schiff base I are likely to interfere.

The molar absorptivity (ε) of Schiff base I using an average molecular weight of 218 is 36,200. Under the conditions of the method, 3.01 μg. of Schiff base I per milliliter of 1*N* hydrochloric acid will give an absorbance of 1.0 using a 2-cm. cell. The sensitivity limit based on an absorbance of 0.1 is approximately 0.3 μg. per ml.

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Dual-Chamber Micro Cross-Section Detector for Permanent Gas Analysis

Sir: Recently Lovelock, Shoemake, and Zlatkis (*3*) reported on the design and response characteristics of a highly sensitive cross-section ionization detector. They were able to increase the sensitivity of this detector by simply decreasing its internal volume.

For more than six months we have been using a dual-chamber micro cross-section detector based on Lovelock's micro parallel plate design for the routine analysis of H_2, O_2, N_2, and CO_2 in microbiological studies and for the nonroutine analysis of H_2, CH_4, CO, CO_2, C_2H_6, C_2H_4, C_2H_2, and C_3H_8 produced during the pyrolytic decomposition of organometallics. The cross-section detector was chosen for this application after we had verified that thermal conductivity detectors have insufficient sensitivity for the analysis of H_2 when using helium as a carrier and that anomalous H_2 peak reversal effects occur. The anomalous H_2 responses can be eliminated by using a mixed carrier gas containing 60% helium and 40% hydrogen (*1*); however, we preferred to use pure helium because of its availability.

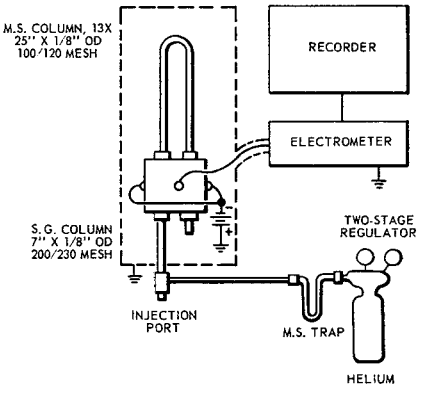

Figure 1. Series column arrangment with dual-chamber detector

EXPERIMENTAL

A series arrangement of a silica gel column and a molecular sieve column as employed by Roxburgh (*5*), who used a thermal conductivity detector, was chosen. Preliminary experiments demonstrated that the parallel column, single detector method employed by Brenner and Cieplinski (*2*) for the analysis of mixtures of O_2, N_2, and CO_2 was not feasible when H_2 was present; the relatively great mass difference between H_2 and the remaining gases resulted in nonlinear stream splitting. The series arrangement utilized for our analysis is shown in Figure 1 with the dual-chamber cross-section detector shown in Figure 2. This detector

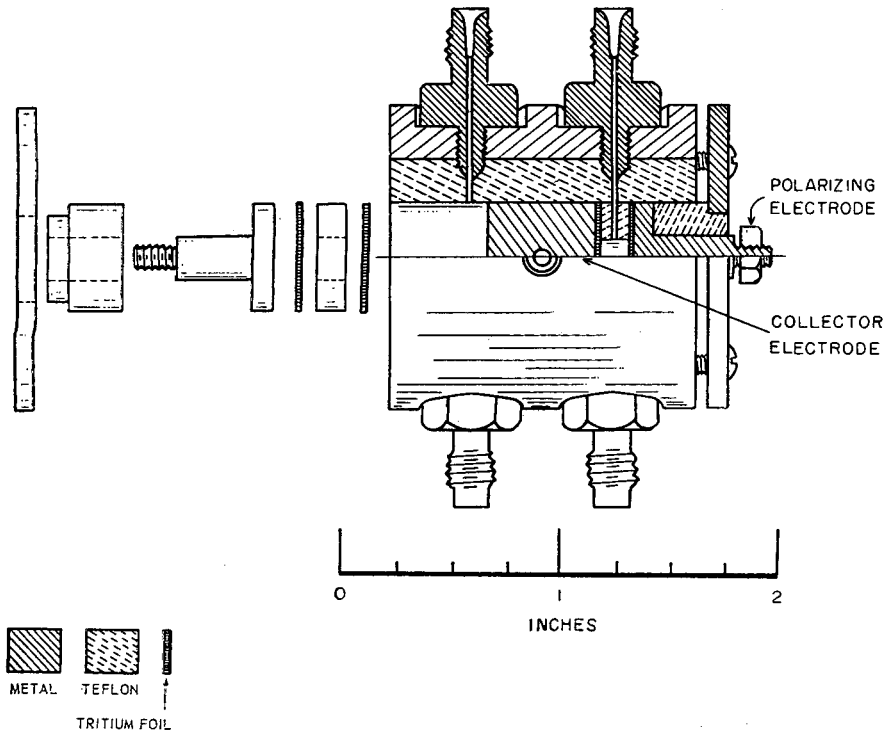

Figure 2. Dual-chamber micro cross-section detector, quarter-section view

utilizes four tritium titanate-coated foils, two polarizing electrodes (+45 volts with respect to ground), and a single collector electrode separating the two chambers. With this arrangement, the response of gases eluting from both columns is positive (as opposed to the reversal of polarity obtained by Roxburgh with conventional thermal conductivity detectors).

DISCUSSION

Although it has been stated that the response of a cross-section detector is linear to 100% gas or vapor concentration (*3*), we have not found this to be true with all the gases we have analyzed when utilizing helium as a carrier gas. The most apparent deviation from this is in the case of H_2, where peak reversal occurs similar to that found when mixtures of H_2 and helium are analyzed by thermal conductivity detectors. This is indicated in Figure 3 which is a calibration curve used in the analysis of H_2, O_2, N_2, and CO_2. Although not shown in Figure 3, carbon monoxide and methane also show nonlinearity of response but do not exhibit peak reversal. The cause of peak reversal of H_2 in helium in the cross-section detector is not clear; however, Otvos and Stevenson (*4*) reported that the value obtained for the apparent ionization cross-section of H_2 in helium was different from that obtained when only H_2 was present and speculated that this might be due to interaction of helium in its metastable state. This peak reversal was not serious for our analysis because under the conditions of analysis, the response was sufficiently linear and reproducible up to 15 μl. of H_2 to allow quantitative analysis with a satisfactory accuracy.

For our work a syringe transfer and

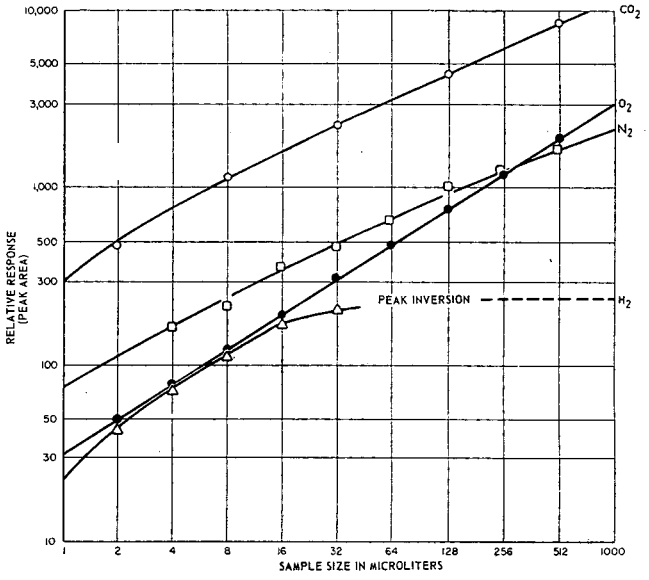

Figure 3. Response curves for H_2, O_2, N_2, and CO_2

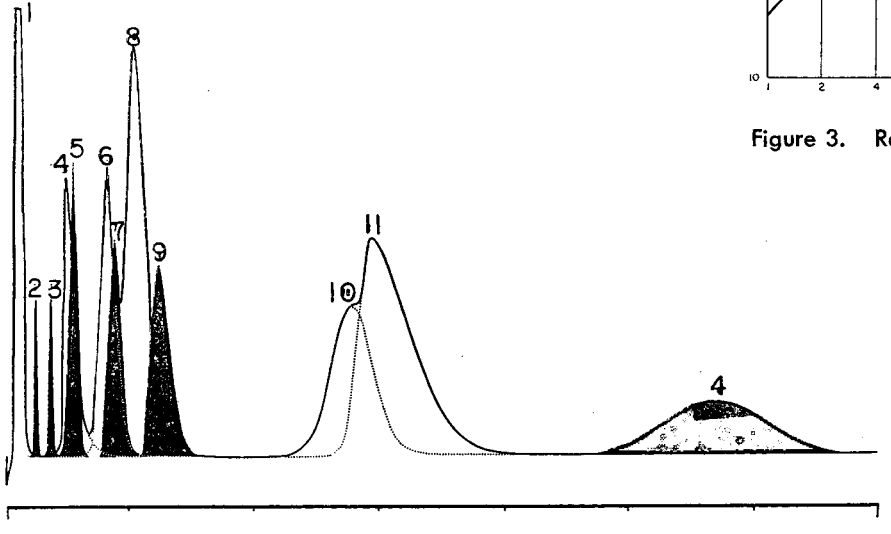

Figure 4. Composite chromatogram

Gray peaks are components eluted from the molecular sieve column: (1) unresolved components, (2) H_2, (3) O_2, (4) C_2H_6, (5) N_2, (6) CO_2, (7) CH_4, (8) C_2H_4, (9) CO, (10) C_2H_2, (11) C_3H_8

Table I. Relative Retention Times

Sample	Retention time (min.) Silica gel	Molecular sieve
H_2	0.35	1.0
O_2	0.5	1.75
N_2	0.5	2.7
CH_4	0.6	4.4
CO	0.75	6.2
C_2H_6	2.6	28.5
CO_2	4.2	...
C_2H_4	5.1	...
C_2H_2	14.0	...
C_3H_8	14.8	...

Flow rate = 4 ml./min., temperature = 25° C.

injection was required. With syringe injections for a 100-unit response (10-μl. injection of O_2) the standard deviation over an 8-hour analysis period, sampling every 10 minutes, is 2.18 which corresponds to a relative error of $\pm$ 2.2%. Better accuracy and reproducibility are obtained by using a gas sampling valve. The base line drift is less than 10% per 8-hour day when utilizing a commercial gas chromatographic electrometer-amplifier (Jarrell-Ash Model 26-770) at a sensitivity setting to provide a 2% noise level on a 1-mv. recorder (about 10^{-10} amp.). The sensitivity of response at this amplification level is sufficient to provide a signal twice the noise level for 1×10^{-6} gram of CO_2 (about 0.05 μl.).

In Figure 4, a composite chromatogram is shown of all the gases we have analyzed in this system to date, while the relative retention times for each component are given in Table I.

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Effect of Pectinol 100 D on the Spectrophotometric Determination of Pectic Substances

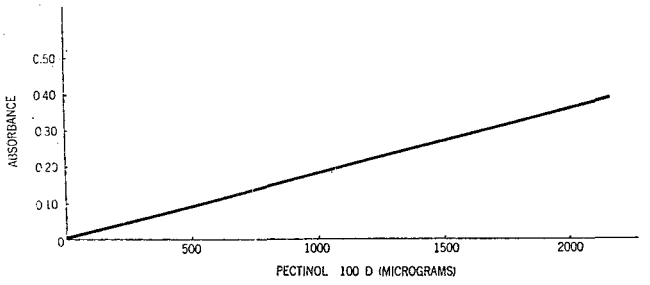

Figure 1. Color produced by reaction of Pectinol 100 D with carbazole reagent

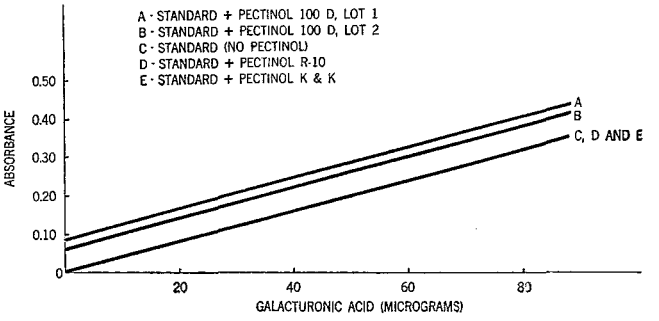

Figure 2. Effect of 400 μg. of various pectinols on color reaction of galacturonic acid and carbazole reagent

SIR: Work on the determination of pectins by the procedure of Postlmayr, Luh, and Leonard (*6*) indicates that use of the pectic enzyme preparation Pectinol 100 D (Rohm and Haas Co., Philadelphia, Pa.) in the amounts specified may lead to erroneously high values for protopectin and correspondingly low values for water-soluble pectin.

The Postlmayr procedure is an adaptation of the method of McCready and McComb (*5*) which makes use of Pectinol 100 D, and makes possible the determination of protopectin as well as total pectin. Since water-soluble pectin is assumed to be the difference between total pectin and protopectin, the Postlmayr procedure has found wide use as a means of determination of the various pectin components.

The determination is based on Dische's sulfuric acid-carbazole reaction with hexuronic acids (*1*, *2*) which produces a pink color. Potter and McComb (*7*) have found that Pectinol 100 D also reacts with sulfuric acid and carbazole to produce a pink color. They state, however, that for the analysis of fruits by the method of McCready and McComb (*5*) the color produced by pectinol is insignificant.

In our laboratory we have found the color contribution due to Pectinol 100 D to be insignificant only at very low concentrations, Figure 1. The interfering color has an absorption spectrum identical to that produced by the reaction of galacturonic acid with the carbazole reagent and the intensity of the color is directly proportional to the concentration of pectinol. The amounts used by McCready and McComb (*5*) for total pectin determinations resulted in a pectinol concentration of 16 μg. per 2-ml. aliquot, which produced no significant interfering color.

Postlmayr *et al.*, in their protopectin determinations, used an amount which gives a final concentration of 400 μg. of pectinol per 2-ml. aliquot. In this laboratory, use of the larger quantity of pectinol in the determination of protopectin in fresh peaches gave values which averaged 20% higher than the actual values. Figure 2 illustrates the effect of 400 μg. of pectinol in increasing the intensity of color produced by reaction of galacturonic acid and carbazole reagent. It can be seen that 400 μg. of Pectinol 100 D gives a significant amount of color, and that the amount varies among samples from different lots. Therefore, if Pectinol 100 D is used, each lot should be checked to determine whether a correction must be made for the color produced by the pectinol.

Figure 2 also indicates that Pectinol R-10 (Rohm and Haas Co.) which has been used by Esau, Joslyn, and Claypool (*3*) and by Joslyn and Deuel (*4*), and Pectinol (K & K Laboratories, Jamaica, N. Y.) do not interfere with the color reaction.

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A Simplified Spectrophotometric Method for Determination of Total and Esterified Cholesterol with Tomatine

SIR: The classical method of cholesterol and cholesterol ester determination is by precipitation of free cholesterol by digitonin in acid medium and of total cholesterol after saponification. The amount of precipitated digitonide is then determined by the Liebermann-Burchard color reaction after mixing with the acetic anhydride-sulfuric acid reagent (*1*, *3*). Two problems are involved. One, digitonin is not a specific cholesterol precipitant and digitonin itself gives a color reaction; therefore, excess digitonin must be removed by many washings which is time consuming. Second, the color reagent is not stable; one has to prepare the reagent for each determination.

A number of reports of rapid total cholesterol assay indicated that if certain proteins and bilirubin can be excluded from the blood sample, extraction of fat and precipitation is not necessary (*4*, *6*, *8*). These methods failed to separate free from esterified cholesterol. Kabara *et al.* (*5*) found a glycoside tomatine which is more specific than digitonin in cholesterol precipitation and also is negligible in giving a Liebermann-Burchard color reaction. However, repeated centrifugations and washings were still required. We formulated a color reagent, which is stable for 2 weeks at room temperature and 4 weeks in the refrigerator and a solvent which is able to precipitate protein and bilirubin but able to dissolve the cholesterol and cholesterol ester. Combining these methods, we present a total and esterified cholesterol assay procedure which eliminates the ordinary repeated washings and centrifugations and shortens the entire procedure from a day to an hour.

EXPERIMENTAL

Reagents. COLOR REAGENT. A mixture containing 30% glacial acetic acid, 60% acetic anhydride, and 10% sulfuric acid by volume is prepared in an ice water bath. Anhydrous sodium sulfate is added to the mixture to a concentration of 2%. It is stored in the refrigerator and used without warming.

FAT SOLVENT. 95% ethyl alcohol–acetone–ether (6:3:1) by volume.

CHOLESTEROL STANDARD. Cholesterol acetate (144 mg., assayed equivalent to 130 mg. cholesterol) and 70 mg. of cholesterol are dissolved in a 100-ml. mixture of 95% ethyl alcohol and ether (98:2).

TOMATINE REAGENT. Two grams of tomatine (manufactured by Chemical Concentrates, Fort Washington, Pa., and also by Nutritional Biochemical, Inc., Cleveland, Ohio) is dissolved in a 100-ml. mixture of 95% ethyl alcohol–water–glacial acetic acid (20:79.5:0.5).

Procedure. Add 15 ml. of fat solvent at room temperature to a test tube containing 0.5 to 1.0 ml. of unknown serum, other body fluid, or homogenized tissue. Rubber stoppers are used for all the tubes to prevent evaporation. Invert the mixture slowly and completely 30 times and centrifuge it for 5 minutes at 1500 r.p.m. Transfer 2 ml. of the supernatant to a 50-ml. beaker for total cholesterol, and another 10 ml. of it to a 15-ml. centrifuge tube. Add 1 ml. of tomatine reagent to the centrifuge tube, mix, and let stand for 10 minutes. Centrifuge the tube and transfer 2 ml. of its supernatant to another 50-ml. beaker for the cholesterol ester. Dry the beakers on a steam bath. After drying, add 0.5 ml. of glacial acetic acid to each beaker, followed by 5.0 ml. of color reagent. Let the solutions stand for 20 minutes and read against the stable color reagent (as the blank) at 610 mμ with a spectrophotometer. A standard is also run with the unknown throughout the procedure. Since the results previously reported showed that the present reagents for Liebermann-Burchard follow Beer's law, the unknown cholesterol values are obtained by Beer's law using the cholesterol standard (*4*).

RESULTS AND DISCUSSION

Extraction. The common fat extraction solvents used by Bloor (*1*), Schoenheimer and Sperry (*9*), and Kabara, McLaughlin, and Riegel (*5*) are combinations of ethyl alcohol, acetone, and ether. Our results from experiments with various combinations of alcohol, acetone, and ether show that the greater the quantity of ether, the better the extraction (Table I). However, the difference is only hundredths of the actual value obtained. Since we attempted to save time by avoiding the adjustment of volume due to evaporation, we used the lower percentage of ether. The second reason for choosing the 6:3:1 is due to some precipitating effects observed in solvents 1 and 3. When C.P. cholesterol acetate was added separately to these solvents along with tomatine, immediate precipitates were observed in solvents 1 and 3. No precipitation was observed in these two solvents containing no cholesterol ester or solvents 2 and 4 containing cholesterol acetate (Table I). The 6:3:1 solvent was also tested to show its incapability of dissolving C.P. bilirubin, which is one of the most important interference materials in blood for the Liebermann-Burchard reaction.

Table I. Cholesterol Extraction Ability of Various Solvent Combinations

	Solvent composition			Cholesterol extracted, % (unknown serum)	Precipitation of cholesterol acetate[a]
	Alcohol	Acetone	Ether		
(1)	1	1	1	100	+
(2)	2	1	1	99	−
(3)	4	4	1	98	+
(4)	6	3	1	98	−

[a] Precipitate observed when using tomatine manufactured by Chemical Concentrates.

Table II. Cholesterol Esters of Human Serum Determined at Different Tomatine Precipitation Times

Precipitation time, min.	Unknown serum, mg. %: Normal (approx. 0.2 mg. free cholesterol)			Low (approx. 0.1 mg. free cholesterol)		
	1	2	3	4	5	6[a]
0[b]	205	198	220	98	90	105
5	143	125	155			
10	143	119	155	78	63	91
20	137	119	154			
30	143	114	155			
60	141	110	154	76	67	93
12 hrs.	139	114	150	70	63	67

[a] This was done using tomatine manufactured by Nutritional Biochemical, Inc.
[b] Represents total cholesterol; other values represent cholesterol esters after precipitation.

Precipitation of Free Cholesterol by Tomatine. Precipitation of free cholesterol by digitonin and tomatine requires hours for completion. The present tomatine solution, which is nearly saturated, will precipitate free cholesterol immediately. We observed no difference among precipitation times varying from 5 to 60 minutes (Table II). Kabara, McLaughlin, and Riegel (*5*) observed that a low level of cholesterol requires a longer period of precipitation. This was seldom noticed in the present trial. In case no immediate precipitation was observed, as in sample 6 in Table II, this solution was left to stand overnight. The failure of immediate precipitation was mainly due to improper preparation of the tomatine reagent and incomplete mixing of tomatine reagent before using.

Variation of Replicates. To study the reproducibility of the present method, 100 unknown serum samples were determined in duplicate. We used Bennett and Franklin's method (*2*) to determine the standard deviation (S. D. = $\sqrt{\Sigma d^2/2k}$ where d represents difference between duplicates and k, the number of such differences obtained). Values obtained were 229.2 ± 3.9 and 158.5 ± 4.4 for the total and esterified cholesterol, respectively.

Comparison with Other Methods. The present method was compared with various methods. One human serum was used as the testing material. The results are shown in Table III. The present method gave significantly smaller values for both total and esterified cholesterol than Bloor's method. The data also show that the present method gave a slightly higher value than the Schoenheimer and Sperry method. Therefore, the present cholesterol values are between those of Bloor and Schoenheimer and Sperry.

Practical Findings. It has been shown that in humans, the female has consistently lower serum cholesterol than the corresponding male until the sixth decade (*7*). This difference among infants, however, is not definite, because the extremely low infant serum cholesterol value is rather hard to detect. We used the present method to compare the cholesterol levels among a number of day-old white infants. The infant serum cholesterol is less than 100; therefore, it was necessary to use 0.5 ml. (or 100 mg.) of cholesterol standard to ensure that the absorbance produced remained within the best range on the spectrophotometer. The results in Table IV show a significant difference of cholesterol values between male and female infants according to a t-test (*10*) for group comparison.

Under normal conditions, human serum cholesterol rarely rises higher than 400 mg.%. The latter can be determined easily by the present method providing that a higher cholesterol standard of (2 ml. or 400 mg.%) is used to ensure that the absorbance produced remains within the best range on the spectrophotometer. However, in experimental work, expecially in animals or organ assay, the cholesterol level quite often rises to more than 1000 mg.%. The present method was also tested for its applicability. Four sets of rabbit serum with abnormally high cholesterol values were tested. One milliliter of serum was extracted and total and esterified cholesterol were assayed as described. It was found that their absorbance values were too high for the spectrophotometer. Since we still had enough supernatant left from total and cholesterol esters, we redid the assays by using 0.5 ml. of filtrate instead of 2 ml. We obtained the values of 1058, comparable to 1119 with the previous method (Table IV), (*4*). This shows that even when one obtains unreadable values, the present method can be used to obtain true values from the material available.

Tissue Cholesterol. Four samples of high cholesterol rabbit liver were analyzed individually. After homogenization in a Potter-Elvehjem homogenizer (Kontes Glass Co., Vineland, N. J.), 0.2 ml. of homogenate was used. The present method was compared to the boiling method of Schoenheimer and Sperry. The results show that the present method gave slightly higher values than the boiling method (Table IV).

Table III. Cholesterol Determinations of Unknown Serum in Mg. % by Different Methods

No. samples		Method			
Total cholesterol	Cholesterol ester	Bloor[a]	Present	Schoenheimer	Huang (*4*)
10		170 ± 7	156 ± 4	153 ± 9	
	10	144 ± 6	113 ± 2	103 ± 7	
20			78 ± 16		78 ± 17

[a] Significantly higher than those of present method according to t-test for group comparison (*10*).

Table IV. Total and Esterified Cholesterol in Various Samples

Samples	No. assayed	Method	Total cholesterol, mg. %	Cholesterol ester, mg. %
Serum from day old:				
boy	12	Present	85 ± 12[a]	60 ± 6
girl	12	Present	66 ± 6	55 ± 9
Serum from rabbit	4	Present	1058	334
	4	Huang (*4*)	1119	
Rabbit liver homogenate	4	Present	1625	1374
	4	Boiling	1420	1210

[a] Significant difference from female according to t-test for group comparison (*10*).

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Separation of Diethylbenzenes by Gas Chromatography Using Packed Columns

SIR: Recent publications (*1*, *2*) provided data on successful gas liquid chromatographic separations of xylene isomers utilizing a column of tetraalkylammonium bentonite (Bentone-34) modified with Silicone Oil or diisodecylphthalate and supported on Chromosorb, Celite, or firebrick.

A separation of diethylbenzene (DEB) isomers was also obtained on a similar column. Figure 1 shows a resolution of the constituents in a commercial DEB. Studies of the separation became desirable when it was found that the stability of DEB–TBP (tri-*n*-butylphosphate) solutions against nitration by nitric acid varied widely with the source of the DEB. Nitration of the DEB was shown when unwanted fission products extracted along with uranium when the TBP–DEB solution was used to solvent extract a solution which simulated a nitric acid dissolution of a spent reactor fuel element.

Controlled nitration of solutions of TBP in pure *ortho*-, *meta*-, and *para*-diethylbenzenes proved the *meta* isomer to be most stable toward nitric acid, and the *para* isomer to be least stable. It was, therefore, desired to correlate the compositions of several commercial DEB's with their extraction behavior after nitration.

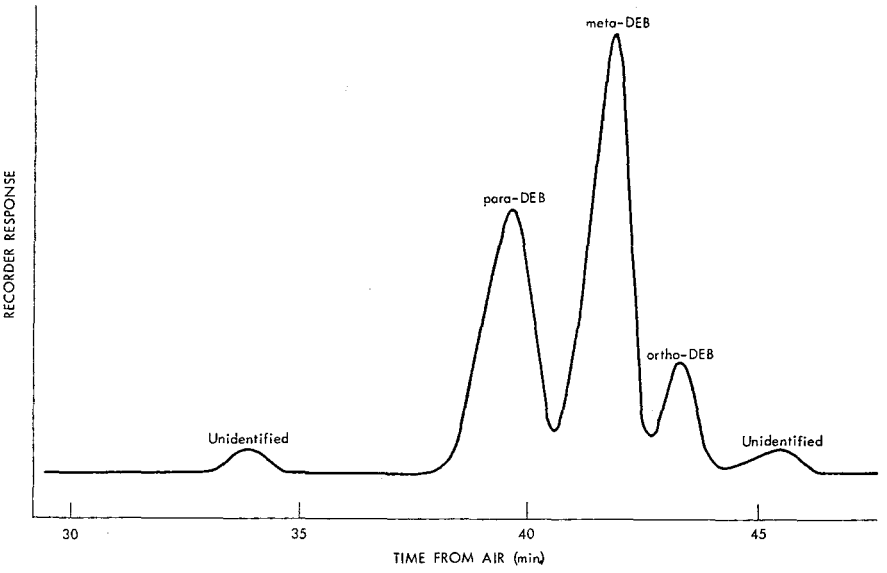

Figure 1. Chromatogram of commercial diethylbenzene

EXPERIMENTAL

Apparatus. An Aerograph Autoprep Model A-700 (heated metal-filament detector) gas chromatograph equipped with an L&N Speedomax H recorder was used in this work. The column consisted of a copper tube (20 feet long and $^1/_4$-inch o.d.) packed with 65- to 80-mesh Celite, onto which had been absorbed Bentone-34 and Dow-Corning No. 550 Silicone Oil.

Operating Conditions

Column temperature, initial, ° C.	70
final, ° C.	150
rise, degrees per minute	~3
Injection-port temperature, ° C.	225
Detector temperature, ° C.	225
Helium flow, cc. per minute	35
Column pressure, p.s.i.	50
Filament current, ma.	175
Attenuation	16
Sample size, μl.	10

Column Preparation. Two grams of Silicone Oil (Dow-Corning 550) were dissolved in 100 ml. of benzene and then slurried with 4.24 grams of Bentone-34 (National Lead Baroid Division, Houston, Texas). About 90 ml. of the benzene was evaporated from the slurry, with stirring, at room temperature. The Bentone–Silicone Oil mixture was then slurried in 200 ml. of benzene and then mixed with 40 grams of Celite. The solvent was evaporated to dryness slowly, with constant stirring, under a stream of air at room temperature. A straight length of copper tubing (20 feet long and $^1/_4$-inch o.d.) was filled with the dry material, plugged at each end with glass wool, and then coiled to fit the column housing. Finally, the column was conditioned at 150° C. for about 30 minutes.

DISCUSSION

The order of elution is: *para*-, *meta*-, and *ortho*-DEB. The proportions of Bentone-34 and Dow-Corning 550 Silicone Oil were important to the gas liquid chromatographic identification of the isomers. If a greater proportion of Silicone Oil is used [for example, equal parts of Bentone-34 and Silicone Oil, the proportions recommended for optimal resolution of the xylene isomers (*1*)], the *meta–para* resolution decreases. Also, if a higher proportion of Bentone-34 is used, the *ortho–meta* resolution decreases. No substrates other than Dow-Corning 550 were tested in combination with Bentone-34.

Mixtures of the pure isomers of DEB were tested and proved the feasibility of using the chromatographs for quantitative analysis of each isomer to within 0.5%. The relative areas under the curves were determined by weighing. Subsequent results of analyses of several

Table I. Discussion of Isomers in Commercial Diethylbenzenes

Source of DEB	Composition, % *ortho*	*meta*	*para*	Unidentified
Koppers Plastic Division	10	50	36	4
Dow Chemical Co.—Dowetch brand	9	56	30	5
Dow Chemical Co., lot 05252	5	61	28	6
Eastman Organic Chemicals (technical, *meta* and *para* mixture) T-1931	17	60	14	9

commercial DEB's are shown in Table I.

No attempt was made to identify the materials that emerged just before and after the DEB peaks.

CONCLUSIONS

Because this column gave good resolution of the DEB isomers it was possible to correlate the stability of commercial DEB's toward attack by nitric acid with their compositions. The stability of TBP–DEB mixtures, as measured by fission product extraction, increased with increased *meta* and decreased *para* concentrations and verified the results of tests with the pure isomers.

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Spectrophotometric Determination of Fatty Amines in Aqueous Solution

SIR: Spectrometric analysis of aqueous solutions of fatty amines has recently been reported (*2*). This method was extended by this laboratory to field use for samples containing 0 to 1.0 p.p.m. amine. We found that by combining the buffer and dye reagents, a combination-single reagent could be used. The procedure requires less sample and shows increased sensitivity over field methods previously reported which use bromophenol blue as the dye and chloroform as the extraction solvent (*1*).

EXPERIMENTAL

Combination Reagent. Dissolve 0.1 gram of methyl orange in deionized water. Dissolve 29.6 grams of sodium acetate trihydrate and 50 grams of potassium chloride in deionized water. Combine the two solutions, add 100 ml. of glacial acetic acid, and dilute to 500 ml. with deionized water.

Table I. Data for Standard Curve

ODA, p.p.m.	Methyl orange procedure	Previous procedure
Blank (O)	96	100
0.10	86	94
0.20	77	86
0.33	70	77
0.50	53	68
0.54	50	65
0.76	40	55
1.00	34	47
1.09	30	43

Transmittance at 420 mμ, %.

Procedure. To a 250-ml. separatory funnel add 100 ml. of the sample to be tested. Add 5 ml. of the combined reagent and set aside for 10 minutes. Add 20 ml. of ethylene dichloride and shake for 5 minutes. Allow the layers to separate for approximately 5 minutes. Drain off the lower layer and measure transmittance immediately at 420 mμ.

RESULTS AND DISCUSSION

Data for a typical standard curve are shown in Table I. The curve is linear over the range of 0 to 1 p.p.m. Table I also shows the sensitivity of previously used procedures and of this method, the latter being approximately 50% more sensitive.

A single reagent system presents an advantage for field use. The buffer and dye reagents were combined in proportion so that 5 ml. of the single reagent was essentially equivalent to 4 ml. of buffer and 2 ml. of dye solution. This reagent is stable for at least 1 month without refrigeration.

Silverstein (*2*) reports the reaction of various amines to this procedure. Since this modification is similar in its reaction with octadecylamine (ODA), interferences are the same. Sample handling and sampling techniques should be the same. Silverstein showed that morpholine and cyclohexylamine gave slightly positive results. Table II shows this effect to be negligible except where a relative error of better than ±10% is required. There is only a slight variation between choloroform and ethylene dichloride, the latter being more sensitive. Ethylene dichloride is preferred also because of its higher boiling point.

Table II. Effect of Neutralizing Amines on ODA Determination

ODA, p.p.m.	Amount: Morpholine, p.p.m.	Amount: Cyclohexylamine, p.p.m.	Amount ODA recovered, p.p.m.
0.0	10		0.0
0.0	500		0.02
0.54	10		0.54
0.54	50		0.58
0.54	500		0.62
0.0		10	0.00
0.0		500	0.06
0.54		10	0.59
0.54		500	0.61
0.54	Ethylene dichloride extraction		0.58
0.54	Ethylene dichloride extraction		0.57
0.54	Chloroform extraction		0.50
1.09	Chloroform extraction		0.95

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Automatic Recording of Spectrophotometric Titration Curves with the Beckman Model DU Spectrophotometer

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THREE BASIC APPROACHES to the performance of photometric titrations are the manual plotting of a series of individual measurements, automatic titrant shutoff at the end point utilizing the amplified (and sometimes differentiated) signal from the photodetector, and automatic recording of the titration curve. The last technique possesses the advantages of speed and convenience over the first, and it is better suited than the second to less favorable titration reactions bordering upon nonfeasibility where photometric titrations are best able to demonstrate their inherent superiority to other end point methods.

Automatically recorded photometric titration curves have been reported, based upon a Cary recording spectrophotometer (*2*, *3*), a Beckman Model B spectrophotometer (*4*), and instrumentation incorporating both the Beckman Model B and the Precision-Dow Recordomatic titrator (*1*, *5*). An automatic recording photometric titrator has now been assembled based upon the Beckman Model DU spectrophotometer, a log recorder, and a constant rate buret. All of the components can be purchased commercially, and no knowledge of electronics or instrument design is required. Rapid and convenient titrations can be performed over a wider wavelength range and with higher precision than was possible with any but the most expensive setups previously described. Examples of acid-base, redox, and chelometric titrations, both with and without indicators, are presented which demonstrate the capability of the instrument.

APPARATUS

A Sargent constant rate, motor driven buret (No. S-11120-1) was used for titrant delivery; this buret has a 10-ml. capacity and delivers at the rate of 1 ml. per minute. Titrant was introduced into the titration vessel through Tygon tubing terminating in a glass capillary tip. The tubing was wrapped with black tape as a precaution against stray light. The Model DU cell compartment was replaced by a larger one described previously (*6*) which accommodated a 150-ml. beaker of ultraviolet-transmitting Corning Vycor 7910 glass; the path length through the beaker was 5.5 cm. Magnetic stirring was employed as before (*6*). This cell compartment is mounted by means of the four screws that are ordinarily on hand to attach the phototube housing and large cell compartments to the plate on the Model DU monochromator case. Thus it takes only a minute or two to return the instrument to its normal condition, and it remains readily available for other measurements.

The Beckman Energy Recording Adapter (ERA, No. 5800) was wired into the DU according to the instructions provided. This is an easy operation requiring less than half an hour. The ERA has a simple circuit, and an inexpensive substitute could be built very easily. A switch on the ERA restores the DU circuit to its normal operation. Two different log recorders were used in the present work: good results were obtained with both the Sargent Model SRL and the Photovolt Linear/Log Varicord 43. If a log recorder were not available, a simple, easily constructed, and inexpensive logarithmic attenuator circuit such as that described by Marple and Hume (*4*) could presumably be used with the other instrumentation described here.

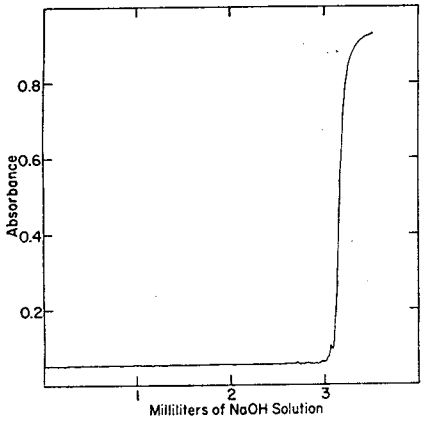

Figure 1. Titration of potassium acid phthalate with sodium hydroxide

83 mg. KHP titrated with 0.1N NaOH at 550 mμ with phenolphthalein indicator

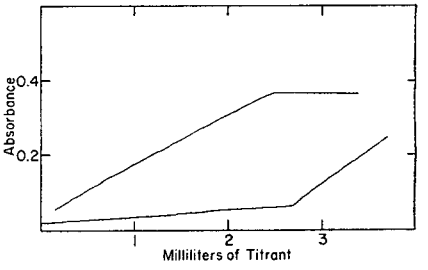

Figure 2. Titration curves

Lower curve. 104 mg. Fe^{+2} titrated with 0.1N $K_2Cr_2O_7$ at 440 mμ; no indicator
Upper curve. 15 mg. Cu^{+2} titrated with 0.1M EDTA at 745 mμ; no indicator

RESULTS

Figures 1 and 2 show typical titration curves obtained with the instrument described above, and Table I summarizes the results of a large number of acid-base, redox, and chelometric titrations. The precision is seen to be

Table I. Results of Titrations

Substance titrated	Quantity, mg.	Titrant	Indicator	Wavelength, mμ	No. of titrations	Av. dev., p.p.t.
KHP	30–250	NaOH	Phenolphthalein	550	9	3.4
NaOH	4–35	HCl	Phenolphthalein	550	12	4.9
Na_2CO_3	33–93	HCl	Phenolphthalein	550	10	6.0
Na_2CO_3	33–93	HCl	Methyl red	540	10	6.4
As^{+3}	50–150 as As_2O_3	I_2	None	320	8	3.4
Fe^{+2}	50–350	$Cr_2O_7^{-2}$	None	440	12	5.8
Fe^{+2}	50–350	$Cr_2O_7^{-2}$	Diphenylamine sulfonate	585	12	5.1
Cu^{+2}	15–55	EDTA	None	745	8	7.6
Cu^{+2}	15–55	EDTA	Pyrocatechol violet	440	8	4.8
Ni^{+2}	60–220	EDTA	None	1000	14	6.6
Ni^{+2}	60–220	EDTA	Pyrocatechol violet	440	14	4.7
$Ca^{+2} + Mg^{+2a}$	0.6–4.0 as $CaCO_3$	EDTA	Eriochrome black *T*	640	6	7.0

[a] Water-hardness titration using local tap water.

somewhat less than that of the best visual titrations, but it is considered good for an automatic titrator. The principal limitation is that the titration reaction must be rapid, a condition which must be satisfied in any automatically recorded titration.

Because the instrument is assembled largely from commercial components, the worker who is unsophisticated in instrumentation or is not even primarily a chemist but who needs a titrator to serve his own ends may readily make use of it. If a Model DU spectrophotometer is at hand, the cost is modest by present standards; if both a DU and a recorder are available, the cost is small.

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Sample Injector for Ion Exchange Chromatography and Flow Cell for Continuous Photometry at 210 Millimicrons

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IT IS often desirable to determine the concentration of substances in the effluent from a fractionation column by measurement of absorbance in the ultraviolet. While automatic sampling photometers are available for use with fraction collectors, it is simpler to pass the effluent through a flow cell in a recording photometer. However, optimal separations are obtained only if the flowing stream is of sufficiently narrow cross-sectional area. The construction of a micro flow cell for use with a Zeiss PMQ II spectrophotometer and columns of about 1 cm. or larger in diameter is described here.

This equipment has been used to advantage in studies on ribonuclease reported elsewhere (*2*, *3*). In that work, the sensitivity in detection of proteins was increased by use of 210 mμ (*4*) rather than 280 mμ as usually employed. To obtain a steady baseline, it was necessary to keep the flow rate constant at all times, even during addition of the sample, since the ion exchange resin itself released materials which absorb at 210 mμ. The device developed for injection of the sample onto the column provides other advantages to make it of more general interest. Therefore, this sample injector is described here too.

EXPERIMENTAL

Chromatographic Unit. In the present work, a solution of NaCl is employed as eluent. Only the stationary phase of the column (a carboxylic resin) acts as the buffer. The procedure for the preparation of such a column is described elsewhere (*3*); other solutions may be used provided the absorbance at 210 mμ is sufficiently low. Columns with an internal diameter of about 0.9 cm. were equipped with a supporting disk of porous Teflon (Grade 50-55, Fluoro-Plastics, Inc., Filbert and Cuthbert Sts., West of 36th, Philadelphia 1, Pa.). Disks of porous Teflon are added to 50% aqueous acetic acid, deaerated with a water aspirator for 30 minutes, rinsed several times with a solution of NaCl, and then deaerated again, this time without acetic acid. The bottom of the column is a 12/1 semiball joint. A PVC connector of the type used on the Spinco amino acid analyzer (Beckman/Spinco swivel fitting 120-558, PVC 120-561 connector, 12-mm. socket to 1/16 inch o.d. Teflon tubing) provides convenient attachment of the 22-gauge Teflon tubing (Pennsylvania Fluorocarbon Co. Inc., 1115 N. 38th St., Philadelphia 4, Pa.) leading to the flow cell.

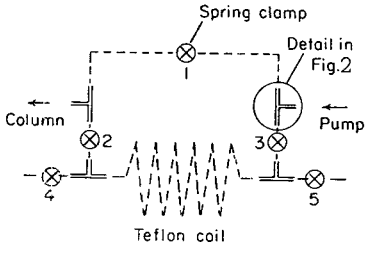

Figure 1. Sample injector

The detection system is sensitive to air bubbles which appear in the effluent stream when air pressure is used to drive the sample into the column at the beginning of a chromatogram. Some other means of adding the sample is needed, therefore. The photometer also detects any alteration in the small, steady concentration of ultraviolet absorbing material leeched from the resin and the other components. The concentration of the extraneous ultraviolet absorbing material in the effluent is dependent upon the rate of flow through the system. During operation, the flow rate, therefore, must be held constant by a suitable pump. A Milton-Roy minipump fitted with Teflon or polyethylene tubing, and a pressure gauge (200 p.s.i., U. S. Gauge) is operated at 30 ml. per hour (*6*). A section of silicone tubing of 1/16 inch i.d. and length of 6 inches is inserted in the delivery line to provide an elastic component to reduce the magnitude of the pulse of pressure during the stroke of the piston. A section of Tygon tubing is slipped over this silicone tube to prevent it from bursting. Sediment and cations emerging from the pump are removed by a 6-cm. column of coarser IRC-50, equilibrated in the same manner used for the chromatographic column. The effluent from this column of coarser resin is directed to the chromatographic column. The sample is inserted into the pumped stream which feeds the chromatographic column by the use of the arrangement shown in Figure 1. The sample is taken up in a coil of about 10 feet of 22-gauge Teflon tubing. The coil has a capacity of about 1 ml. It is connected to provide an optional parallel path between the pump and the column. The T-connections are constructed of Teflon and Nylon fittings as shown in Figure 2. Flow through the tubing is interrupted by spring clamps adjusted in tension for the pressure employed. The sections of tubing to which the clamps are applied are replaced from time to time as they become damaged.

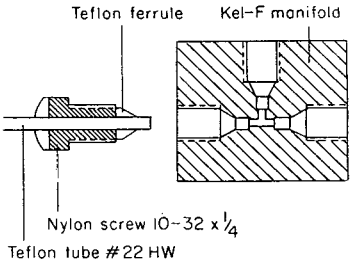

Figure 2. Detail of T-connections shown in Figure 1

At the beginning of a chromatogram, clamps 2, 3, 4, and 5 are closed, clamp 1 is opened, and the pump turned on. Eluent passes through the column and the sample coil is bypassed. After the base line registered by the recorder has become constant (about 1.5 hours), the coil is filled with the sample to be chromatographed. For this purpose clamps 4 and 5 are opened

and suction is applied at one end. The other dips into the sample which contains 10 to 1000 μg. of protein per milliliter of the same solution employed as eluent. When the coil is full, clamps 4 and 5 are closed. To inject the sample into the influent stream, clamps 2 and 3 are opened and clamp 1 is closed. Pumping is continued until the end of the chromatogram.

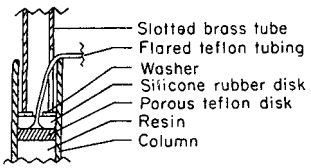

Figure 3. Cross sectional view of top of an ion exchange column for use with sample injector

This method of injecting the sample into the pumped stream feeding the column requires that there be no dead space above the top surface of the resin column. This can be accomplished if the top of the column is arranged as shown in Figure 3. A disk of porous Teflon 50-55, cut with a cork borer and deaerated as described earlier, is forced onto the top of the resin surface. The column is filled with eluent during these manipulations to ensure that air is not driven into the column. Teflon tubing of 22 gauge is passed through a section of a disk cut from $^5/_{32}$-inch silicone rubber sheet and the end of the tubing is flared by a hot tool so that it will not pull out. The rubber disk is inserted into the column and is forced down until it is resting directly on the Teflon disk. An aluminum washer is added on top of the rubber disk, and a suitable length of brass tubing with a slot in the end is then placed on top of the stopper and clamped in a ring stand to prevent movement of the disk. The bottom of the column can be closed off by the same type of device shown in Figure 3 for closing off the top. In this case the chromatograph tube is simply a piece of glass tubing.

Recording Photometric Unit. The construction of a micro flow cell with a light path of 14 mm. and an internal diameter of 1.5 mm. is shown in Figure 4. It is employed with a Zeiss PMQ II spectrophotometer equipped with a microcell compartment and a 3.5-mm. mask and beam condenser. The instrument was selected because the optical system yields light of high spectral purity down to at least 200 mμ. About 35% of the emergent light passes through the narrow bore of the flow cell. Absorbance was proportional to the concentration of protein (ribonuclease) up to an absorbance of 1.1. Stability of this single beam instrument is generally within an optical density of 0.002 for periods of 2 to 8 hours, often longer when the temperature of the laboratory is constant. Light transmission at the shorter wavelengths gradually decreases owing to fogging of the mirror in the lamp housing. Therefore, new mirrors are installed with each new hydrogen lamp. A new lamp is installed when a slit width greater than 1.0 mm. becomes necessary to work at 210 mμ. The half intensity band width is always less than 2 mμ.

The output from the photomultiplier circuit of the spectrophotometer is recorded by a Speedomax Recorder (Leeds & Northrup, Model G, 10 mv., equipped with a variable chart speed, $^1/_2$ to 30 inches per hour) to yield the familiar type of effluent curve.

The flow cell is filled initially with a 5% solution of Brij 35 (Atlas Powder Co., Wilmington, Del.). Suction from a water aspirator is used for filling and to dislodge bubbles. Once the optical channel is free of bubbles, liquid is pumped through the cell until the ultraviolet absorbing detergent is removed. Air bubbles usually travel through the cell, but occasionally one may stick to one of the optical faces from which it may be dislodged by flicking the inlet or outlet tubes sharply with a finger.

DISCUSSION

The equipment and techniques described in this communication were devised to increase the speed and sensitivity of the chromatographic procedures (*2*, *3*) for the detection and estimation of ribonuclease and its derivatives. The method depends upon the maintainance of a constant and rapid rate of flow through the column. Columns of finely divided IRC-50 have already been shown to yield high resolution at flow rates of 45 ml. per hour per sq. cm. (*2*). The sample insertion device as well as the head of the column has been found to operate at pressures as high as 100 p.s.i. By measurement at 210 mμ, with the aid of the present techniques and a 0.9 cm. × 6 cm. column of IRC-50, it is possible to measure in 1 or 2 hours' time the quantity of enzyme —only a few micrograms of protein— actually present in 1 ml of the mixture used for determination of enzymic activity (*1*). The course of the alkylation reaction leading to the formation of the inactive 1-carboxymethylhistidine-**119** and 3-carboxymethylhistidine-**12** derivatives of ribonuclease has also been determined conveniently in this manner (*3*). Since no buffers are needed in the flowing solution, it may be possible to gain an additional increase in sensitivity by use of wavelengths shorter than 210 mμ.

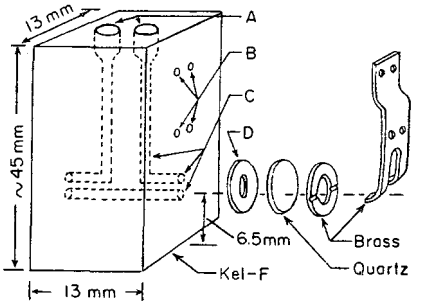

Figure 4. Assembly of one of two optical faces of micro flow cell

A, holes for $^1/_{16}$-inch Teflon tubing connectors (Beckman/Spinco swivel fitting 120-558). *B*, tapped holes for securing brass clamp. *C*, holes, 1.5-mm. i.d., are carefully drilled to avoid shreds. *D*, silicone washer, 0.5 mm. thick. Central, oblong hole is formed from two 1.5-mm. diameter holes connected by straight cuts made with a razor blade

Spectrophotometer cells with narrow channels have been described for use with small volumes by Kirk, Rosenfels, and Hanahan (*5*) and for continuous flow of very narrow streams by Ullrich and Hampel (*7*). The former cell is not adaptable for flow. The smaller cross-sectional area of the latter cell was not compatible with many light sources. The cell presented here offers a combination of features which include small volume, long light path, narrow stream, ease of construction and ease of disassembly for cleaning. A cell of similar dimensions, but constructed of fused quartz, is now available from Pyrocell Manufacturing Co., New York, N. Y.

ACKNOWLEDGMENT

Apparatus was constructed by Werner K. Krug and Nils A. Jernberg of this institute.

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Needle Cup Sampling Device for Gas Chromatography

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The increase in sensitivity of the ionization type detectors—e.g., argon ionization detector—over the hot wire (thermal conductivity) type detectors in gas chromatography has brought about an increased demand for sampling devices suitable for the transfer of microgram quantities of sample. The writer has constructed such a device which is not only simple, but is satisfactory for use with the Model 20 Barber-Colman argon ionization detector gas chromatograph. The proper sample size for this instrument using methyl esters derived from typical fats and oils appears, under the conditions employed, to range from 8 to 35 μg. As the device to be described is essentially of fixed volume, three of these devices were constructed having approximate capacities of 0.008, 0.034, and 0.058 μl. Some 80 chromatograms were run on mixed methyl esters using this device for the transfer or introduction of the sample into the gas chromatograph system. In no case where the proper sample size was chosen, did an overload of the detector and/or double peaking (peak top inversion) occur. Samples which were solid (wax-like) at room temperature but volatilized in the flash heater of the injection port system were easily introduced with this sampling device. No experiments were carried out with powdered crystalline materials. Further, providing no cold spots exist in the injection system (seal included), little or no modification of the conventional silicone-rubber-sealed injection port is required.

This sampling device, as shown in Figure 1, is referred to as a needle cup sampling device and essentially consists of a needle cup supported on the end of a rod or needle stock. One means of fabricating such a device is as follows:

A conventional syringe needle—e.g., a 3-inch 26-gauge syringe needle—is modified by removing a small segment of the pointed end (approximately a 4-mm. length). A needle cleaning wire is inserted in the separated end such that the needle point now constitutes a micro-needle cup. A small space is allowed between the separated ends (about 0.5 mm.) so that a welded seal can be made between the cut ends of the needle. The needle point is ground to a more oblique (blunt) angle to improve the reproducibility in the sampling. The capacity of the needle cup is essentially fixed, being governed by the depth and gauge of the needle stock—e.g., a needle cup made from 26-gauge needle stock having a depth of 1 mm. has a volume of approximately 0.05 μl. Figure 1 is a sketch of such a device constructed from a conventional syringe needle.

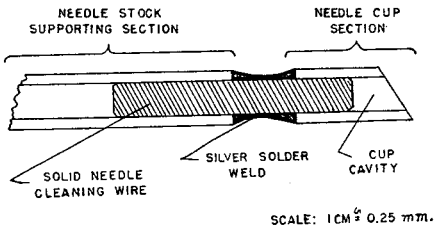

Figure 1. Needle cup sampling device

While this sampling device is of a fixed volume type, it is evident that very small samples (less than microgram amounts) can be introduced into the gas chromatography system either by displacing the bulk of the sample from the loaded cup or by employing a solvent diluted sample. Replicate chromatograms were obtained on the equipment previously mentioned using a mixed methyl ester sample. Table I shows the total areas obtained for a series of injections made with the 0.034-μl. (approximately 28-μg.) sampling device. The chromatogram areas were determined by the conventional triangulation method.

The per cent deviation data in Table I include in part the inherent errors associated with a given gas chromatograph system—e.g., response variations due to electronic and gas velocity fluctuations, etc. Nevertheless, the data show that under the conditions employed sample injections with the 0.034-μl. sampling device were reproducible to approximately 2.5%. Similarly, injections with the 0.008-μl. sampling device were reproducible to approximately 10%. One difficulty experienced with this sampling device was displacement of a portion of the sample by fragments of the seal as the needle cup punctures the injection port seal, and it was found that a well worn (prepunctured) seal was desirable.

Sampling or loading of the needle cup was somewhat more involved than sampling with a Hamilton microsyringe and the positive loading of the needle cup was facilitated by a needle-cleaning wire probe. Loading of the needle cup by an evacuation procedure may be feasible, but sampling by means of capillary action with this device is not recommended. The manipulation procedure for the needle cup sampling device is briefly detailed below:

The needle cup is probed with a short—e.g., 3-mm.—length of needle-cleaning wire to inspect for trapped particles. A rotating and/or plunging action on the wire (the tip of the wire is beveled like the end of a screwdriver) will remove the bulk of the foreign particles.

A short length—e.g., 5 to 6 mm.—of sufficiently transparent plastic tubing is filled with the liquid or solid sample. The plastic section is attached to the needle cup, and a needle-cleaning wire (now referred to as the loading wire) is inserted through the sample (in the case of a liquid sample) and made to enter the needle cup cavity. The open end of the plastic section is tilted upward, and the wire is moved in a manner to promote positive displacement of the air from the needle cup cavity. When the air bubbles have been moved a reasonable distance from the needle cup opening, the wire is slowly removed from the cavity and the entering of the sample into the cup is observed with a lens to ensure that air is not drawn back into the cup.

The previous part of the manipulation procedure may be repeated with fresh sample or solvent in event the needle cup shows signs of contamination. The plastic section is conveniently filled with a microsyringe which is left attached to the plastic section until the plastic section has in turn been attached to the needle cup.

When the needle cup has been filled with sample, the outside of the sampling device below the plastic section is rinsed with hexane and the plastic section carefully separated from the needle cup. Wiping of the needle cup with tissue when it is loaded is not recommended.

The sample contained in the needle cup is then quickly introduced into the flash heater section of the gas chromatograph. The needle cup can be left in the carrier gas train for an extended period—e.g., 1 to 2 minutes.

Table I. Gas Liquid Phase Chromatographic Areas for Approximately 0.034 μl. of Mixed Methyl Ester Sample

Total observed area[a] in sq. cm.				Av. total area, sq. cm.	Deviation in total area, %
Run 1	Run 2	Run 3	Run 4		
5198	5058	5105	4841	5051	2.4

[a] Attenuated areas were related back to 1-mv. sensitivity.

DISCUSSION

It will be noted in the manipulation procedure that this sampling device is not immediately removed from the flash heater section of the gas chromatograph. This can be advocated since uncontrolled needle "boil out" and "blow by" as is sometimes experienced with the conventional microsyringe is limited in the case of "boil out" and eliminated in the case of "blow by" (escape of sample between plunger and barrel). Further, some fractionation of the sample has occasionally been observed with sample introduction by means of the conventional microsyringe. Such fractionation could result in the loading of the gas chromatograph column with a sample of erroneous composition. While fractionation may occur during sample introduction with the needle cup sampling device, the extended injection period apparently minimizes this difficulty. Under the conditions employed, volatilization of the sample from the cavity of the needle cup was essentially complete and no difficulties were encountered with peak tailing or column efficiencies.

In conclusion, introduction of ester samples derived from edible fats and oils with the needle cup sampling device into gas chromatography systems using highly sensitive detectors (such as β-ray ionization detectors) was found very satisfactory. If the present day trend for even more sensitive detectors continues, it is apparent that some form of this sampling device and procedure could be of considerable use. The needle cup sampling device is easily constructed, simple to manipulate, and in many cases requires little or no modification of the conventional silicone-rubber-sealed injection ports.

Column Rejuvenation in Gas Chromatography by Means of Reservoir Sections

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GAS CHROMATOGRAPHIC columns deteriorate with extended use. Among probable factors for such deterioration is a leaching action of the carrier gas and injected samples on the stationary phase at the column head. Samples injected into an old column, therefore, diffuse over a longer distance than in a new column before reaching an effective portion of the stationary phase. The result is a spreading of the peak with accompanying loss in efficiency. When the former becomes excessive, the column is usually discarded.

Since column preparation is both an art and a science, extension or rejuvenation of the life of a good column is highly desirable. A simple method for doing this, utilizing what we call reservoir sections, has been devised in our laboratory.

A chromatographic column [16 feet long × 1/8 inch in diameter filled with Johns-Manville brick dust (60- to 80-mesh) coated with 1% of LAC 1-R-296], which initially gave excellent results for the separation of a mixture of carbonyl compounds, gradually declined after a period of use (isothermally and with programming) to the point where its separating efficiency had dropped drastically. A short column (1/4 inch × 1 foot) containing 5% of the same stationary phase used in the longer column on brick dust (reservoir section) was then mounted between the carrier gas inlet and the main column so that the carrier gas passed through the reservoir section before reaching the main column. Injected samples bypassed the reservoir section. With this modification, the efficiency of the original column was restored.

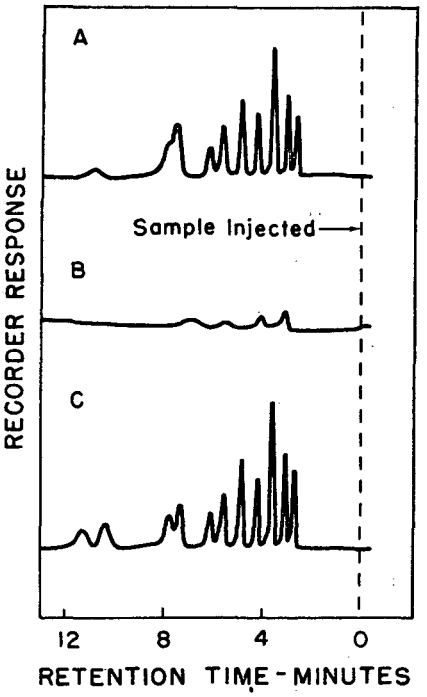

Figure 1. Chromatograms of a mixture of carbonyl compounds made on

A. A freshly prepared column
B. The same column after extended use
C. The used column with the reservoir section in place

Figure 1*A* illustrates the separation of a mixture of aldehydes and ketones made with a freshly prepared LAC 1-R-296 column. Figure 1*B* illustrates a separation of an identical injection made on the same column after it had been in use for several months. Normally it would be discarded as no longer capable of making practical separations. Figure 1*C* illustrates a chromatogram of the mixture on the same column after a reservoir section had been in place in the system for 30 minutes. It is obvious from the illustration that the column had been restored to its original efficiency.

The example cited is particularly striking since it appears to be a case in which the uncoated solid support at the head of the deteriorated column (Figure 1*B*) displays considerable activity. (Most of the sample is actually lost.) Rejuvenation, in this case, serves to re-coat the active sites and to permit only the desired partition process to occur. It is also extremely likely that the stationary phase vapor in the carrier stream serves effectively to displace adsorbed sample vapors from the walls of the injection chamber and inlet tubing, thus enhancing the performance of even brand new columns. As a result new columns with the added column section had double the number of theoretical plates calculated without the reservoir in place.

Reservoir sections may be used at room temperature, or they may be placed in the oven of the gas chromatography apparatus where they receive the same heat treatment as the main column.

Rejuvenation of a column has also been achieved by reversing a spent column. This temporarily resulted in an efficiency equal to that obtained when the column was new. However, with time the efficiency once again declined.

REFERENCE to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

Solvents for the Paper Chromatography of Amino Acids and Sugars without Spraying

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PAPER CHROMATOGRAMS of amino acids and sugars were run with the spraying agents already dissolved in the solvent mixture. This eliminated the need for their drying and subsequent spraying and in the case of amino acids enabled the movement of the spots to be followed up during the running of chromatograms and avoided their elution from the paper when the solvent was left to drip.

Amino acid mixtures containing 1% glycine, alanine, valine, and threonine were successfully separated by this procedure using the solvent systems (*1*) shown in Table I after addition of about 0.1% ninhydrin. Soon after the solvent front reached the amino acids, the spots acquired a pink coloration but their contours were not sharp due to tailing. This, however, disappeared when the chromatograms were removed from the solvent and left to dry at room temperature for 3 to 6 hours. The spots were then sharper than those obtained in the usual manner by spraying the chromatograms, so that a smaller amount of amino acids could be detected.

Amino acids are known to react with ninhydrin at low pH (*2*). We have found however that the R_f values of amino acids are not altered by the addition of ninhydrin to the solvent system suggesting that such a reaction does not take place during the running of the chromatograms. It is probable that this method can be applied to other solvent systems provided they do not contain a compound that gives a color with ninhydrin such as ammonia.

Table I. Solvent Mixtures for Amino Acids

Solvent mixtures	Volumes, ml.	Wt. of ninhydrin, mg.
Ethanol–water	75:25	100
n-Butanol–2*N* acetic acid	20:20	40
n-Butanol–acetic acid–water	40:10:10	60
Methanol–pyridine–water	80:4:20	100
Isopropanol–phenol–water	7:70:25	100
Upper layer of:		
n-Butanol–acetic acid–water	40:10:50	50 (in upper layer)
n-Butanol–acetic acid–water	68:5:27	85 (in upper layer)
n-Butanol–acetic acid–water	40:10:60	50 (in upper layer)
n-Butanol–acetic acid–water	25:6:25	25 (in upper layer)
n-Butanol–phenol–acetic acid–water	20:20:8:40	50 (in upper layer)

Table II. Solvent Mixtures for Sugars

Solvent mixtures	Volumes, ml.	Reagent
Upper layer of:		
n-Butanol–acetic acid–water	40:10:50	Benzidine (100 mg.)
n-Butanol–acetic acid–water	40:10:50	*p*-Anisidine (60 mg.)

Paper chromatograms of sugars were run with the solvent systems shown in Table II which contained benzidine or *p*-anisidine. In this case, the spots appeared only after drying the chromatograms at 100° C. for 10 minutes. Mixtures of glucose, galactose, arabinose, and rhamnose were separated satisfactorily by this method.

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Simplified Method for Determining Sensitivities in Activation Analysis

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SEVERAL RECENT PAPERS have given excellent summaries of sensitivities for activation analysis (*3–5, 10*). However, in the computations, specific flux levels and/or specific irradiation periods were assumed. Meinke (*6–8*) has extended this work by showing how the sensitivity for activation analysis can be varied and discrimination obtained using different times of irradiation. Although these data and compilations are important and serve a valuable purpose, the assumption of specific fluxes and irradiation periods limits the immediate and general applicability of the data.

The method for determining sensitivities in activation analysis described in this paper does not have these limitations. There are two minor drawbacks, however, which will be discussed later. Although this method is not intended to be used exclusively in lieu of other techniques, the user will find that it is a rapid method and that he can obtain either accurate results or valid estimates with it.

Other useful nomograms pertaining to radioactive buildup and decay are available (*2, 11*). However, these do not lend themselves readily to sensitivity calculations. Mesler (*9*) has described an excellent method whereby a rapid assessment of thermal neutron activation, in terms of the activity produced, can be obtained. For some exposure times, however, a correction factor must be applied.

Equation 1 is the basis for sensitivity calculations in activation analysis

$$W_z = \frac{AM_z}{(6.02 \times 10^{23})\sigma f S \theta} \qquad (1)$$

where W_z is the weight of the sample nuclide, Z, in grams; A is the disintegrations per second of the product nuclide; M_z is the atomic weight of Z; σ is the cross section in square centimeters; f is the neutron flux in neutrons per sq. cm. per second; S is the saturation factor; and θ is the fraction of the target isotope in the element.

S is defined by $(1 - e^{-0.693T/t})$, where T is the irradiation time and t is the half life of the radiation product in the same units of time as T.

If one defines a variable, X, as $X = T/t$, the variation of S *vs.* X is as shown in Figure 1. Note that at $X \geq 6$, S approaches the value of 1 very closely. Thus, although saturation is said to occur usually at $X = 10$ ($T = 10t$), one may assume, for approximate calculations, that saturation occurs at $X \geq 6$.

In Equation 1, the true unknowns, which thus far have prevented a formulation of data of essentially general application, are σ, f, and S (or X). While it would be difficult to correlate these on a conventional X–Y or three dimensional plot, it is a relatively easy matter

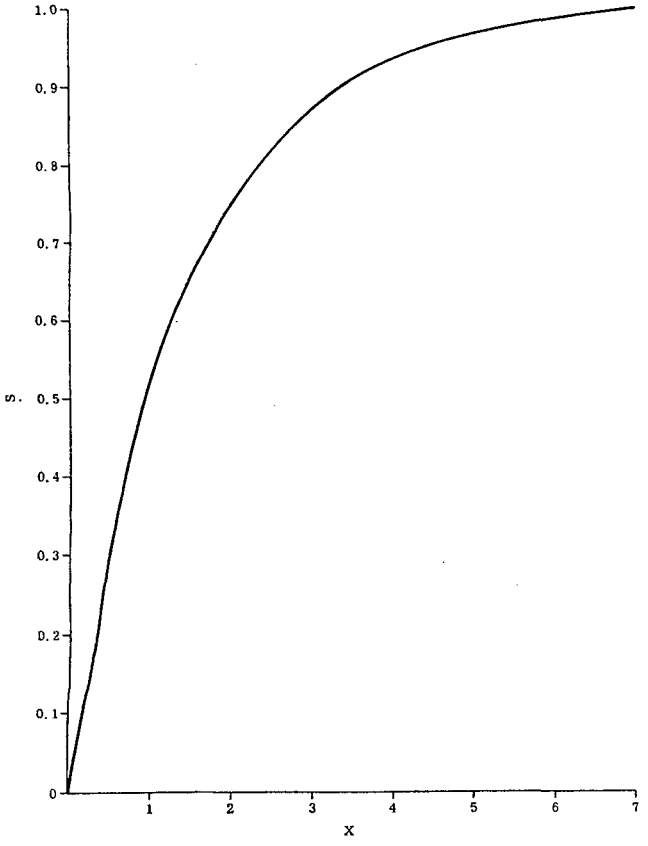

Figure 1. Variation of S with X

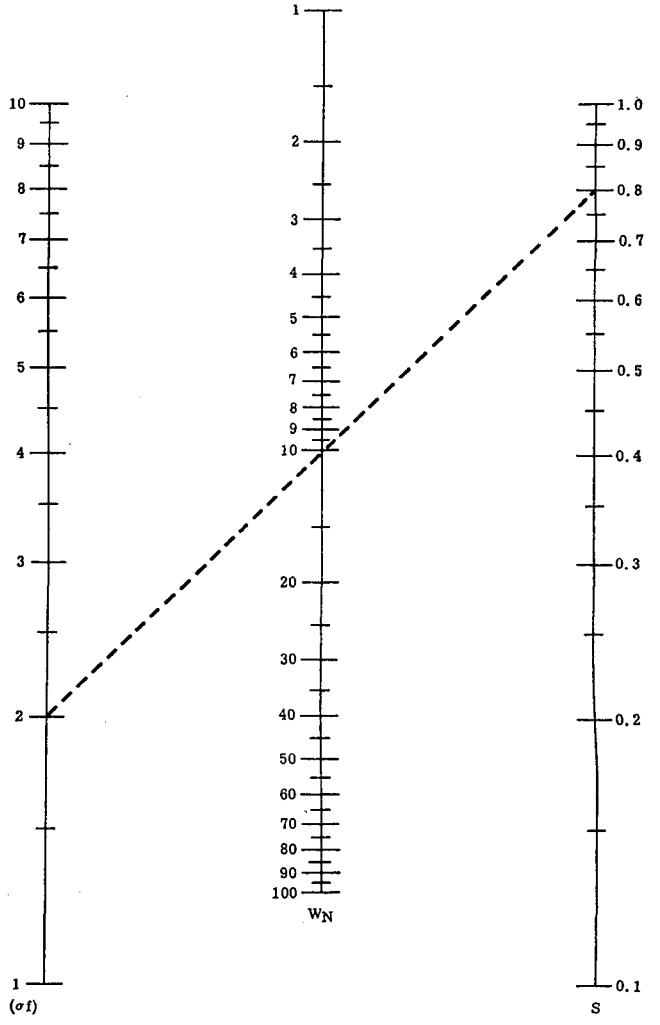

Figure 2. Nomograph of S, (σf), and W_N for activation analysis

to prepare a nomograph correlating these three variables.

To do this, Equation 1 is simplified by assigning the value of one disintegration per second to A. Then

$$W_x = \frac{(16.6 \times 10^{-25})M_x}{\sigma f S \theta} \qquad (2)$$

For the nomograph, W_N, S, and (σf) are used as the three axes and are plotted on logarithmic scales as shown in Figure 2. The S and (σf) axes are one-cycle logarithmic scales, whereas the W_N axis, which is equidistant from the other axes, is a two-cycle logarithmic scale. When a value of W_N for a nuclide is found on the nomograph, the sensitivity for this particular nuclide is given by Equation 3.

$$W_x = \left(\frac{W_N M_x}{\theta}\right) \times 10^{n-25} \qquad (3)$$

The value of n is the exponent of the product, (σf). For example, to determine the sensitivity for $_{56}Ba^{136}$ (θ = 0.0781). With a cross section of about 0.4 barn, assume that the available neutron source has a thermal flux of 5×10^{10} neutrons per sq. cm. per second and that the irradiation time is $S = 0.8$ (~2.3 half lives). Then $(\sigma f) = 2 \times 10^{-14}$. If a line is drawn from $S = 0.8$ to $(\sigma f) = 2$, the value of W_N may be read as 10.03 on a nomograph (Figure 2). Therefore,

$$W_{Ba^{136}} = \left[\frac{(10.03)(136)}{(0.0781)}\right] \times 10^{14-25} = 0.17\ \mu g. \qquad (4)$$

A standard calculation with Equation 1 yields $W_{Ba^{136}} = 0.18\ \mu g$. Thus, the nomograph method is in good agreement with the standard method.

A is not limited to 1 disintegration per second; rather it may be any value which the investigator feels necessary. Increasing A to 100 would alter Equation 3 to 10^{n-23} rather than 10^{n-25}. Apparently assuming $A = 100$, Studier *et al.* (*12*) estimated that in a flux of 3×10^{13} neutrons per sq. cm. per second the maximum sensitivity for I^{127} is of the order of 0.1 nanogram (abbreviated ng.). From Figure 2, knowing that θ is 1.0 and $(\sigma f) \sim 2.01 \times 10^{-10}$, $W_{I^{127}}$ is calculated to be approximately 0.11 ng.

This procedure also may be used to calculate sensitivities in activation analysis utilizing a 14-m.e.v. neutron flux.

Assuming that $f = 10^8$, $S = 1.0$, and $A = 100$, the sensitivity for F^{19} undergoing an (n, p) reaction, as calculated by Equation 1, is 23.4 mg., whereas the nomograph method yields 24.3 mg.

One further value of the nomograph method is that it amplifies Meinke's work, which illustrates how sensitivities can be varied and discrimination obtained. It is evident that the nomograph method facilitates actual calculations of T and any other variable which can influence the discrimination.

As mentioned previously, there are two drawbacks to this method. Many of the papers which have given summaries on sensitivities have incorporated two important items which are absent in the nomograph procedure as it now stands—detector counting efficiencies and time intervals before counting to allow for chemical separation, to permit unwanted activities to die out, etc. These were deliberately omitted to make the nomograph method as generally applicable as possible. The detector counting efficiency is easily disposed of by introducing ϵ, which represents counting efficiency, into the numerator of Equation 4 and incorporating its value into the value of W_N. Then the values

of W_N would be correspondingly different—i.e., if ϵ were 0.25, the values on the W_N axis would be one fourth of that shown in Figure 2. Thus, each investigator may use his own counting efficiency and prepare a nomograph which is applicable to his work. The time interval is included by introducing into the numerator of Equation 4 the term, $e^{\lambda t_i}$, where t_i is the time elapsed from irradiation to detection (*1*). The incorporation of this term into W_N could cause difficulty in obtaining the normal axis for W_N and may have to remain an entity in Equation 3.

This method is cumbersome in that calculations are necessary to obtain quantitative results. However, this is compensated for by the fact that, after a little experience, one can make excellent qualitative estimations without written calculations. After obtaining W_N, rapid approximate mental calculations with Equation 3 are possible.

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WORK was done under the auspices of the United States Atomic Energy Commission. Contents of this paper do not necessarily reflect the position of the U. S. Army.

Liquid Sample Introduction System for a Mass Spectrometer

Albert D. Pattillo and Howard A. Young, Mobil Oil Co., Beaumont Refinery, Beaumont, Texas

DURING the past several years, members of ASTM Committee D-2, RD IV have cooperated in a program to develop a method to analyze petroleum fractions in the gasoline boiling range for hydrocarbon types. One of the principal drawbacks to such a method has been finding a suitable means of sample introduction into the mass spectrometer vacuum. The device shown in Figure 1 has been used routinely at Mobil's Beaumont Refinery Laboratory for two years and has eliminated the objections presented by other methods.

Two CEC Model 21-103C Mass Spectrometers were used to conduct this work. Both instruments are equipped with heated inlet systems operated at 135° C. (275° F.). A project was initiated to determine the best means of introducing a sample in the gasoline boiling range into the mass spectrometer.

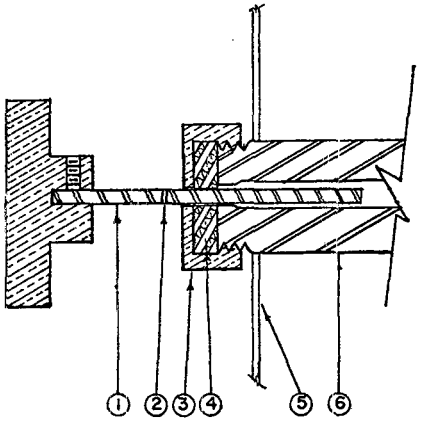

Figure 1. Cross section of liquid introduction system for mass spectrometer

① 1/8-inch drill rod
② Charging volume (approx. 0.0006 cc.). Drill hole in 1/8-inch rod, solder appropriate size needle stock in hole, machine, and polish
③ Compression cap
④ Teflon plug (1/8-inch thick)
⑤ M.S. panel
⑥ Connection to M.S. inlet system

The first method tried consisted of using a microliter syringe to inject a sample through a silicone rubber plug into the vacuum system. Further investigation revealed selective adsorption, predominately with the aromatics. A more commonly used device was then installed consisting of a fritted disk covered with a heated gallium-indium-tin alloy. The sample was introduced through this with a capillary "dipper." Reproduction was poor as a result of the elevated temperature of the alloy, some distillation occurring through the fritted disk.

Since a device similar to the one shown in Figure 1 had been used successfully in this laboratory on gas chromatographs, it was decided to build and install one on the mass spectrometer.

DESCRIPTION AND OPERATION

The sample introduction system is mounted on the front panel of the control cabinet along with the conventional gas introduction system. The liquid introduction system consists of a piece of stainless steel (6, in Figure 1) drilled so that the drill rod, 1, can be moved through it into the mass spectrometer. The drill rod is polished with crocus cloth. The stainless steel, 6, is threaded, and a compression cap, 3, is screwed onto it. The compression cap contains a 1/4 inch thick Teflon plug, 4, drilled so that the drill rod fits into it snugly. The compression cap is pulled up tight enough to maintain the mass spectrometer vacuum. The small hole, 2, in the drill rod has a capacity of approximately 1 μl. To obtain a small hole, install a section of hypodermic needle.

With the drill rod pulled out partially, a sample is introduced into the small hole with a syringe or any other convenient means. The drill rod is then pushed in until the sample reaches the mass spectrometer vacuum. The sample is completely vaporized into the mass spectrometer.

The mass spectrometer has been operated at 204° C. (400° F.) with a satisfactory sample introduction. A fine stream of laboratory air is directed on the drill rod while in the charging position to permit the reproducible introduction of samples boiling at 35° C. (95° F.). Any sample can be introduced that has a boiling point high enough to be volatilized at the temperature of the inlet system maintained at a pressure of less than 5×10^{-6} torr before sample introduction. No difficulty has been observed with samples having a 95% boiling point of 260° C. (500° F.).

Many advantages result from the installation of such a liquid introduction system. Since no distillation occurs, reproducibility of pressure measurements is better. Teflon is essentially inert to hydrocarbons, resulting in no selective absorption. There is little chance to lose high vacuum due to channeling or leakage such as occurs when using fritted disks and silicone rubber plugs. A relatively volatile sample can be introduced at room temperature into the heated inlet system. One such device has been in constant use on a mass spectrometer without replacing any parts for two years, while another one has been in service for a longer period of time on a gas chromatograph.

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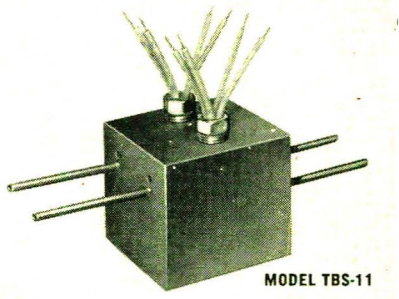

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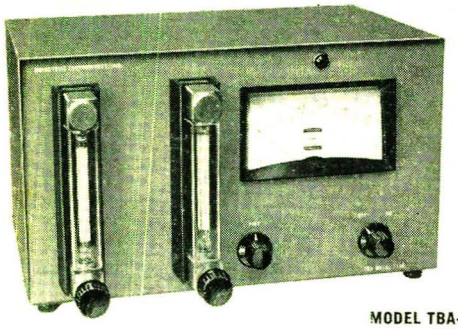

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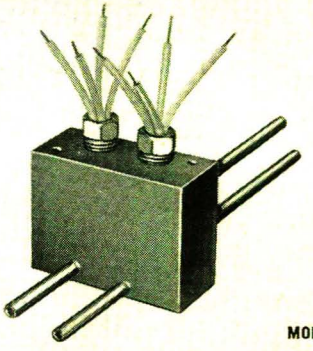

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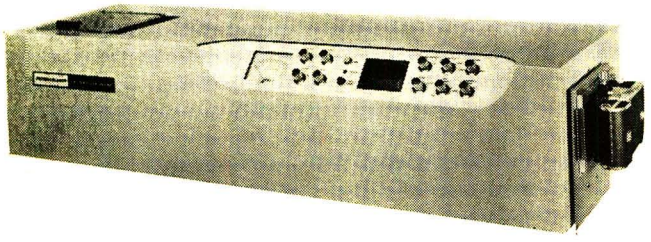
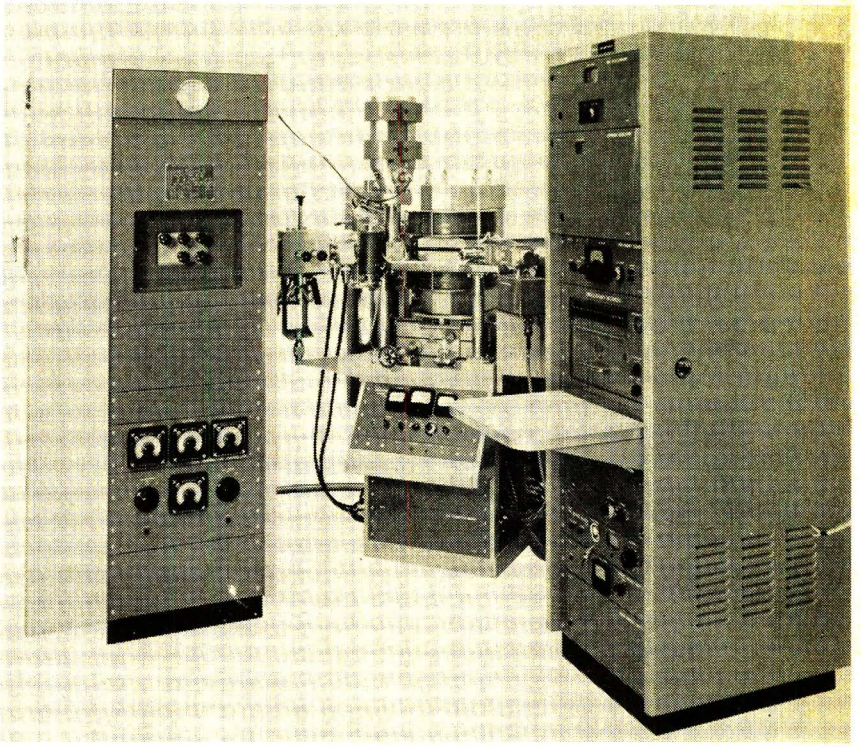
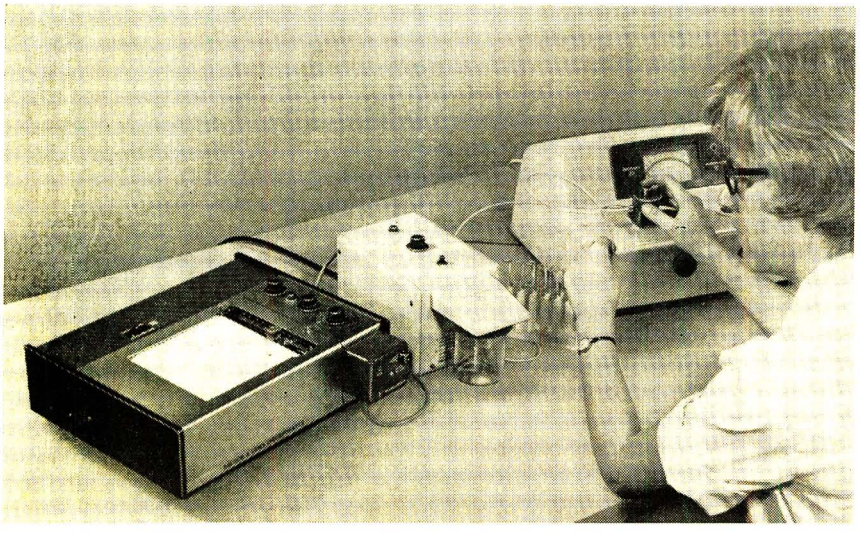


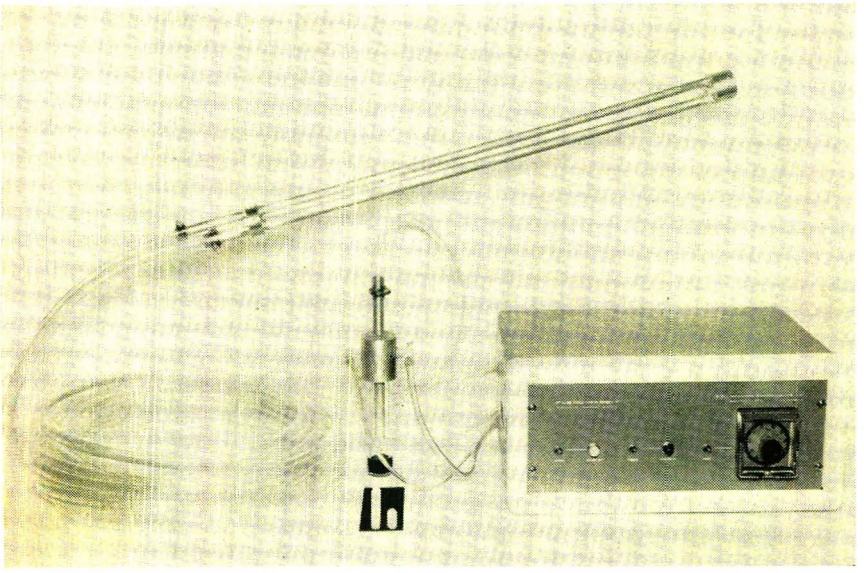
The Model 238 electrophoresis apparatus is a bench-type unit with a viewing port on the front panel. Two camera backs are provided; for standard $3^1/_2$ by $4^1/_4$ film packs and plates, or for a Polaroid Land camera. Two light sources are built into the instrument and the electrophoresis cell is of the type developed by Tiselius. Perkin-Elmer, Norwalk, Conn. **401**



A wide range mass spectrometer, the Model RMU-6A, Hitachi Perkin-Elmer Spectrometer, is the latest of a line of mass spectrometers which has been produced by Hitachi Limited since 1950. Attachments and accessories available include heated metal or heated all glass sampling systems, special ion sources, and a secondary electron multiplier detector for rapid high sensitivity analysis. The primary use of the RMU-6A is expected to be in organic chemistry research where the instrument, with a gas chromatograph, will be used to identify organic and inorganic materials, employing a unique coupling system. The instrument can be used for natural product studies and for a wide range of product quality control applications. It is also applicable to precision analysis of isotopes. Perkin-Elmer Corp., Main Ave., Norwalk, Conn. **402**



The low cost data acquisition system shown provides a rapid, precision method for analyzing and recording colorimetric samples. Component units have been specifically designed to function together in an integrated manner. As a result, ease and speed of operation permit technicians to produce a valid record in a matter of seconds. Components of the system include the Spectronic 20 colorimeter, a Flow-Thru cuvette, the V.O.M.-5 strip chart recorder, and the Spectro Chart-25 (which adapts the recorder for use with the Spectronic 20). Bausch & Lomb, Inc., Rochester 2, N. Y. **403**



The Model 1402 Flexo-Rabbit pneumatic transfer system utilizes flexible plastic tubing to transport samples to and from radiation sources such as neutron generators, reactors, accelerators, and gamma sources. The tubing system has an aluminum irradiation section at one end and a special receiver at the other. Tubing systems have been supplied over 200 feet in length and with inside diameters up to 2 inches. The sample capsule can be driven through 100 feet of tubing in slightly over one second. The entire system is readily portable and may be set up in a matter of minutes. Reactor Experiments, Inc., Belmont, Calif. **404**

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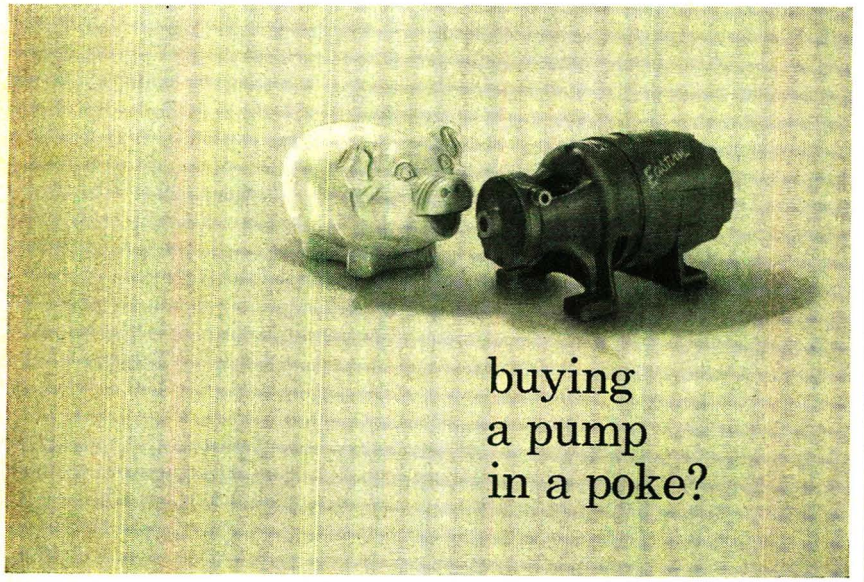

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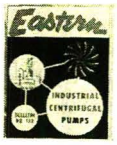

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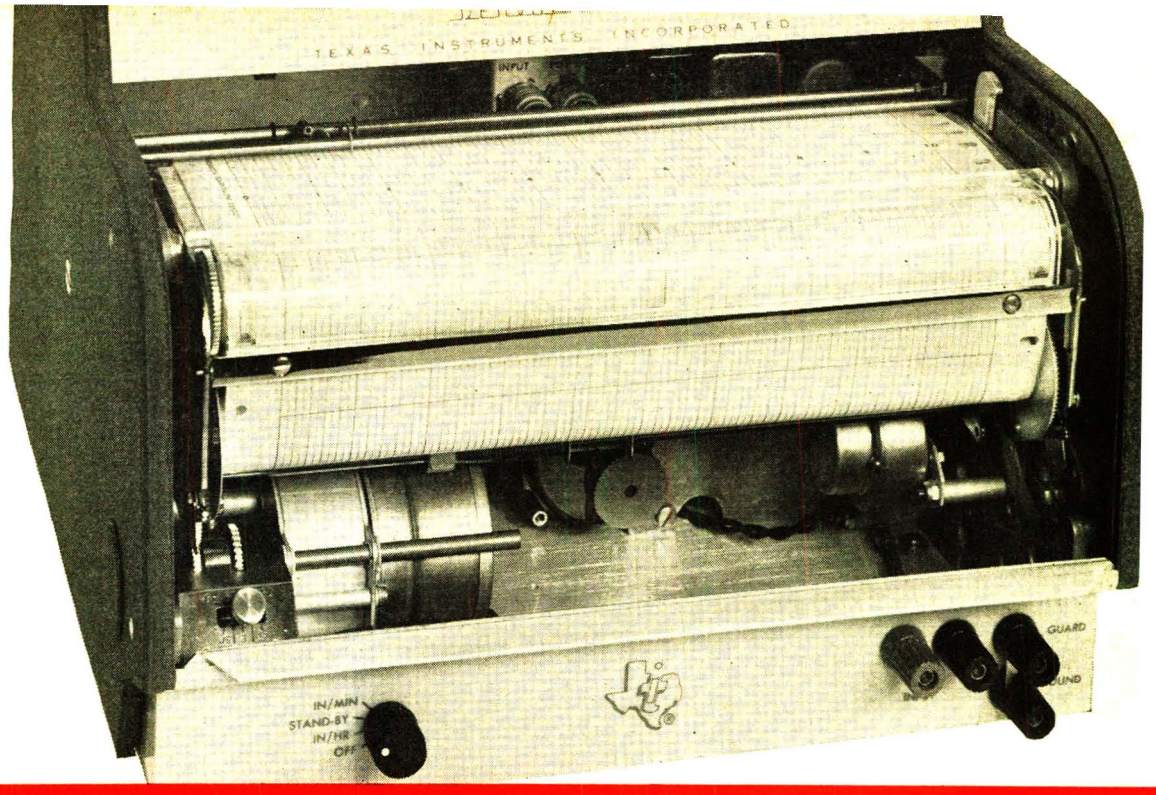

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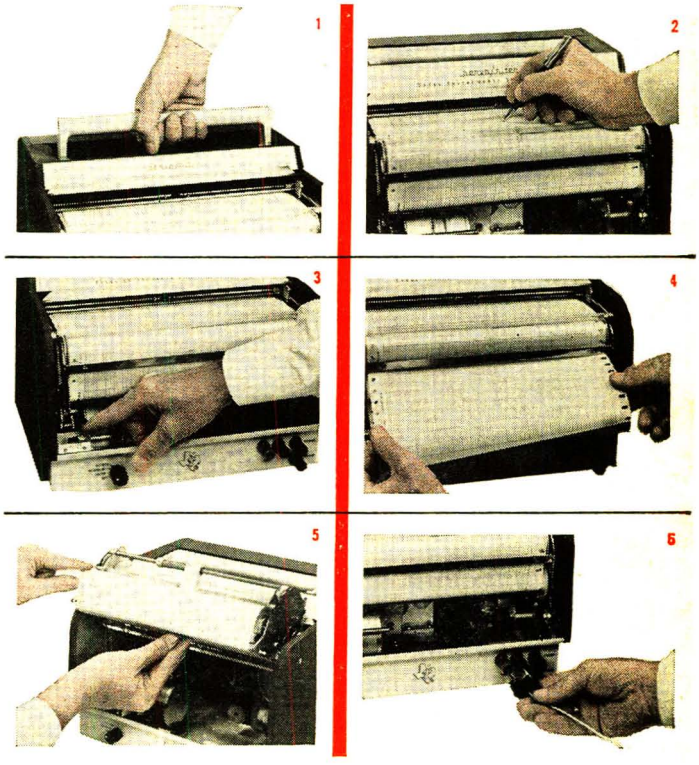

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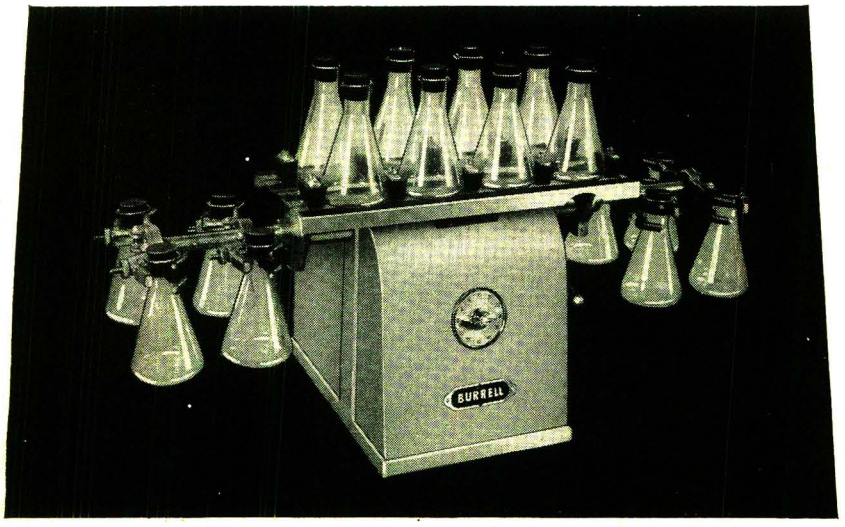

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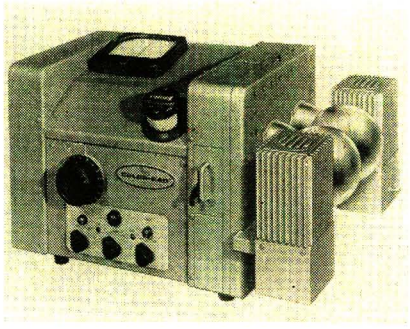

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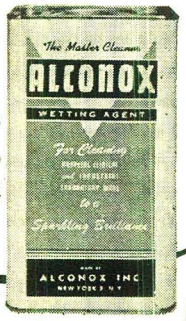

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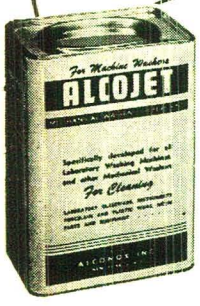

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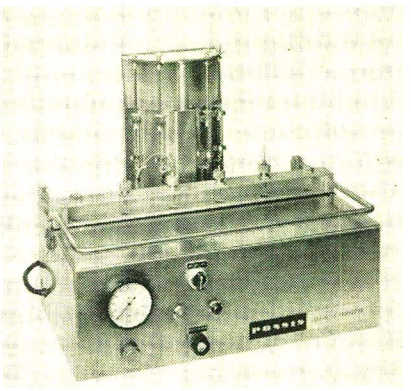
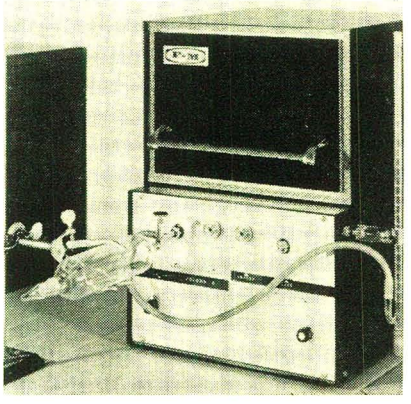
NEW PRODUCTS



The PLE 400, an evaporator for preparation of filter papers in partition chromatography can be used for the completely automatic unattended deposition and evaporation of organic and inorganic solutions in paper partition chromatography. Four filter papers may be prepared simultaneously, and any quantity of fluid or solution up to 1 ml. will be deposited and evaporated, resulting in small discrete spots and negligible loss of yield. Other features include automatic shut off at end of cycle, points of deposition enclosed and protected from airborne contamination, and contact of fluids by only glass and Teflon. Possis Machine Corp., 825 Rhode Island Ave., South, Minneapolis, Minn. **420**



A component collection system for gas chromatography, the Model TCS-3, allows 100% positive collection of both the component effluent and carrier gas and is not dependent upon the condensation of a solute from a moving gas stream. The total collection system consists basically of a 300-ml. glass collection flask, manifold needle valve, and soap film flowmeter. The collection flask is first evacuated and then connected to the exit port of the gas chromatograph by means of a needle valve containing a by-pass manifold. F & M Scientific Corp., Avondale, Pa. **421**

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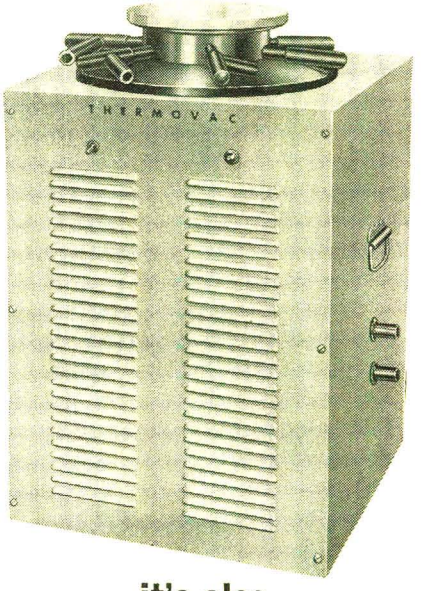

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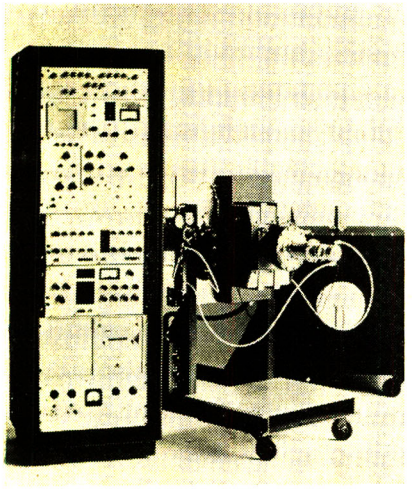
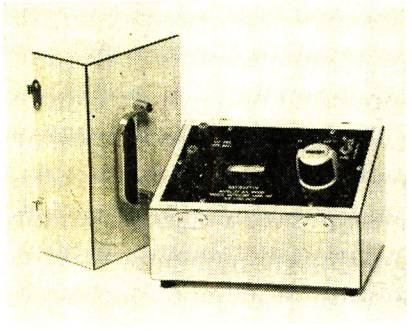
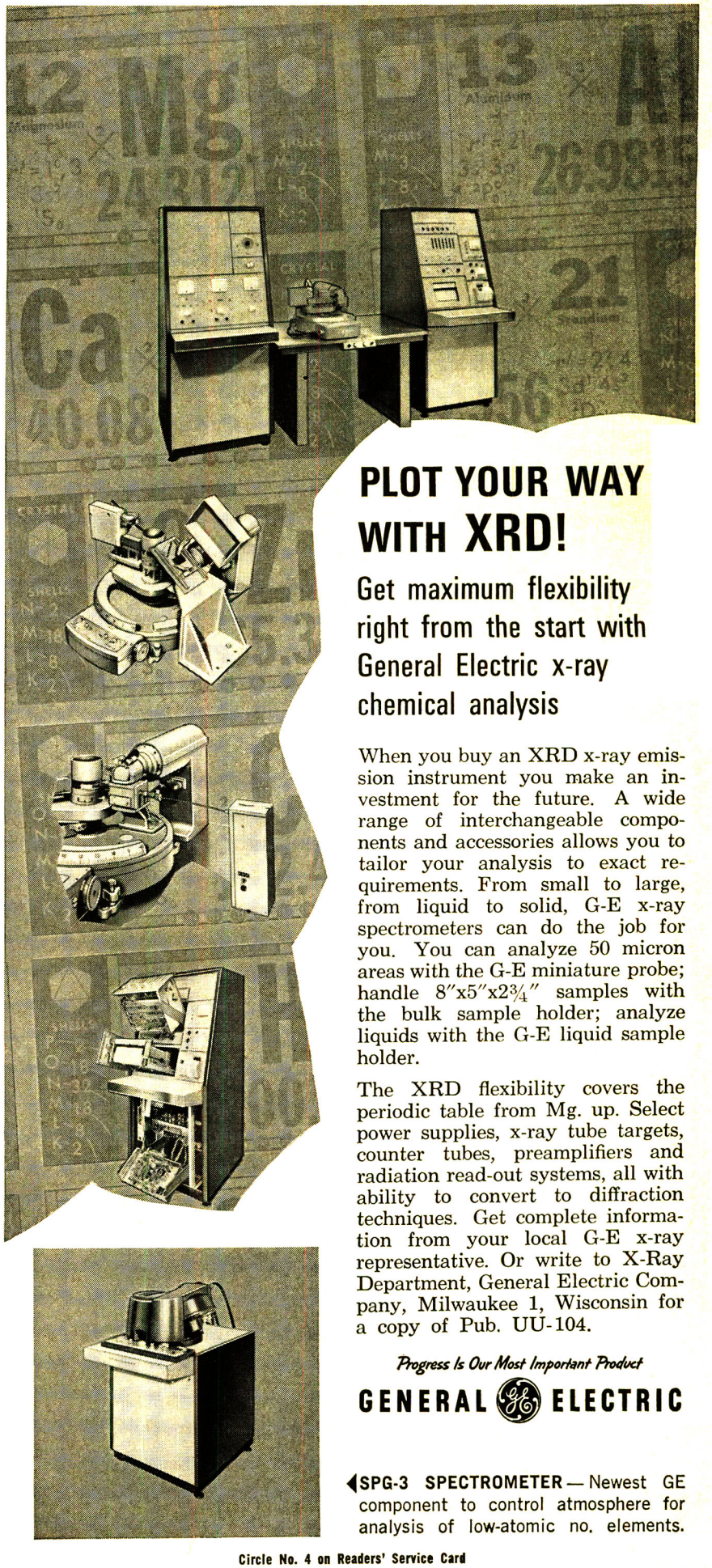


The complete activation analysis laboratory shown, the 9601 system, includes irradiation, sample handling, and counting equipment. The laboratory is built around a newly-developed T(d,n) accelerator, Series 9900, capable of producing a 4 pi yield of 2.5×10^{11} neutrons per second and a fast flux of 5×10^{9} n cm.$^{-2}$ sec.$^{-1}$ The 9601 is a complete data acquisition system for the reduction of complex gamma-ray spectra in activation analysis studies. The counting system may be used for a broad range of analyses. The 9601 system consists of a dual scintillation detector assembly, rapid transfer and system programmer, 400 channel pulse-height analyzer, multiscaler programmer, high voltage power supply, digital printer, and flux monitoring system. Texas Nuclear Corp., P. O. Box 9267, Austin 17, Texas. **422**



The Model 102 portable ratiometer shown indicates voltage-ratio or resistance-ratio of potentiometers, and output of potentiometric transducers, within ±0.03% FS on digital. It operates from a built-in long-life battery and cannot damage or burn out sensitive resistance elements. Pacific Metrology Laboratories, Inc., P. O. Box 3552, San Diego 3, Calif. **423**



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NEWS

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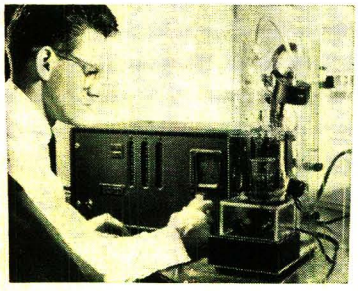

Coulter Counter evaluation by Allis-Chalmers

"The apparatus is both a good research tool and a production line control tool."

Allis-Chalmers research engineers put a good deal of effort into comparing the abilities of their electronic Coulter Counter with other methods of fine particle analysis. Results: The Coulter Counter continues to obtain highly accurate, reproducible size distribution data for silica samples within the 2 to 200 micron range. Routine tests were found to be especially fast. The versatility of Coulter Counter automation for simple size distribution on other materials is also accepted fact.

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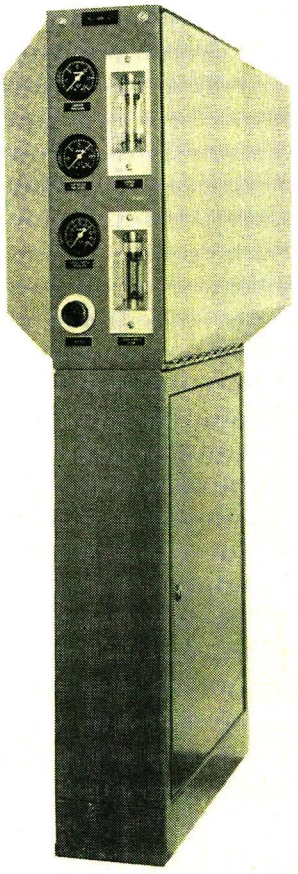
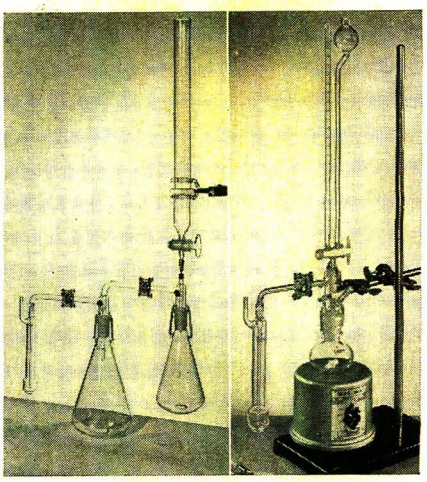
A new gas and liquid purifier, designed to protect delicate instruments, removes oil, water, and solid particles through the use of molecular sieves and other special replacement cartridge filters. The unit is rated for inlet pressures up to 2000 p.s.i.g. (oxygen, 500 p.s.i.g. maximum). The Matheson Co., Inc., P. O. Box 85, East Rutherford, N. J. **424**



The chromatographic analyzer for on-line composition control of gas or liquid streams has several unique features. First, analyzer accuracy has been made comparable to laboratory devices by close control of the temperature of the sensitive detection system. Second, there is access to columns and valves which are contained in a compartment isolated from the explosion-proof housings in which all electrical components are enclosed. Third, instruments used to check analyzer performance are mounted on the front of the analyzer. Finally, both control and readout operate from a continuous trend type of signal representing component concentration. The Foxboro Co., Foxboro, Mass. **425**

New from Delmar...



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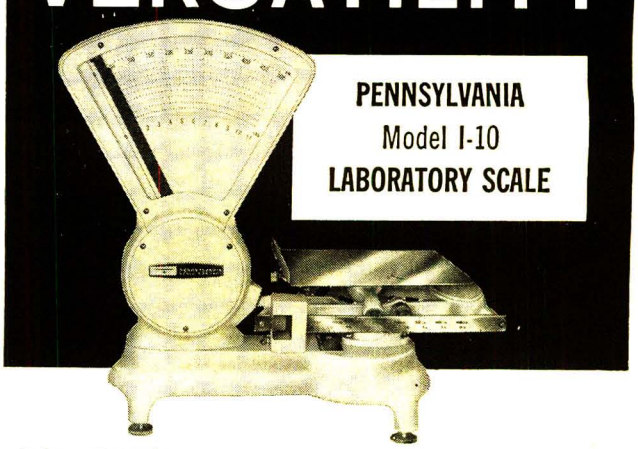

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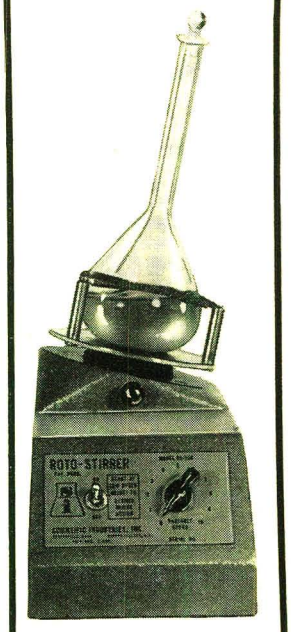

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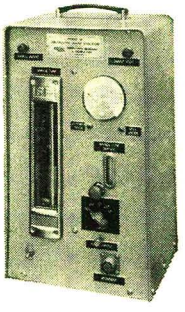

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Lightweight, portable moisture analyzer complete with all necessary components for accurate, precise moisture measurement.

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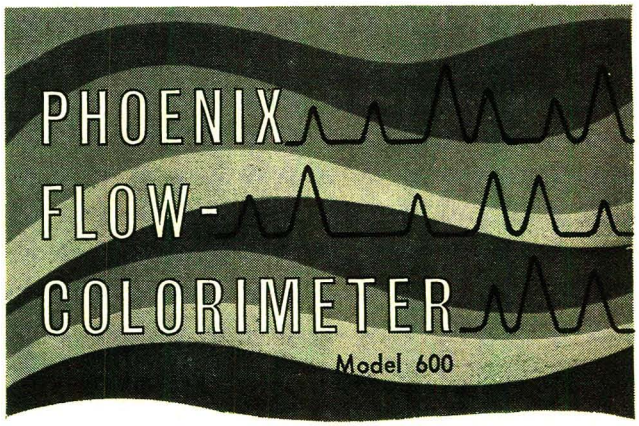

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Radiochemicals

Three new radiochemicals for chromatography standards are available in liquid form. Methyl linoleate-1-C^{14} has a specific activity of 9.3 millicuries per millimole; methyl oleate-1-C^{14} has a specific activity of 1.6 millicuries per millimole, and methyl stearate-1-C^{14} has a specific activity of 1.5 millicuries per millimole. All three have a tested purity of 99%. Tracerlab, 1601 Trapelo Rd., Waltham 54, Mass. **503**

Lead Telluride Single Crystals

Lead telluride single crystals are available for fundamental studies on thermoelectric, electronic, and structural parameters. Determined data for these crystals in random orientation are: resistivity, 1.5×10^{-2} ohm/cm. and Seebeck coefficient, 160 mv. at 450° C. The crystals are available in a standard size (1 cm. diameter) at $250 per cm. of length. Nonstandard diameters can also be supplied. Semi-Elements, Inc., Saxonburg Blvd., Saxonburg, Pa. **504**

Uniformly Tagged C^{14} Chemicals

Fatty acid chemicals in which a uniform proportion of all the different positions are occupied by C^{14} instead of the commonly available material in which only one position is tagged are now available. The radiochemical compounds offered are palmitic acid, palmitoleic acid, oleic acid, linoleic acid, linolenic acid, and the corresponding methyl esters. Applied Science Laboratories, State College, Pa. **505**

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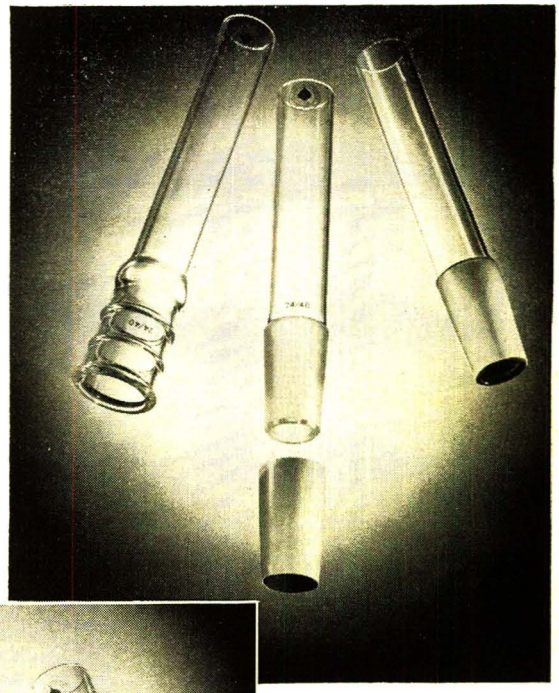

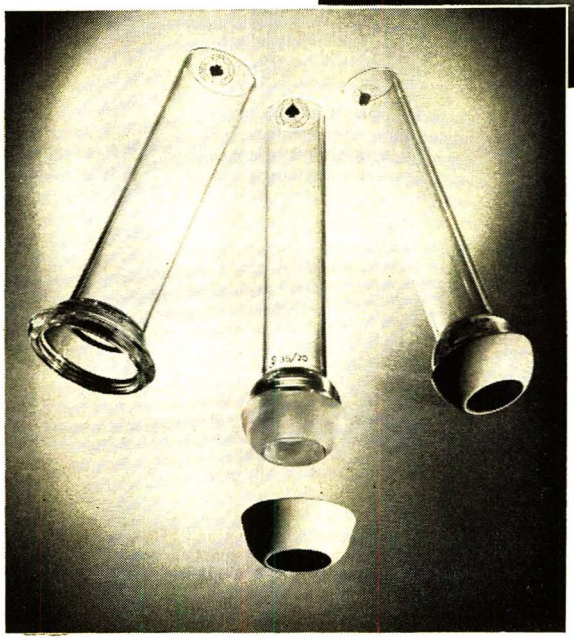

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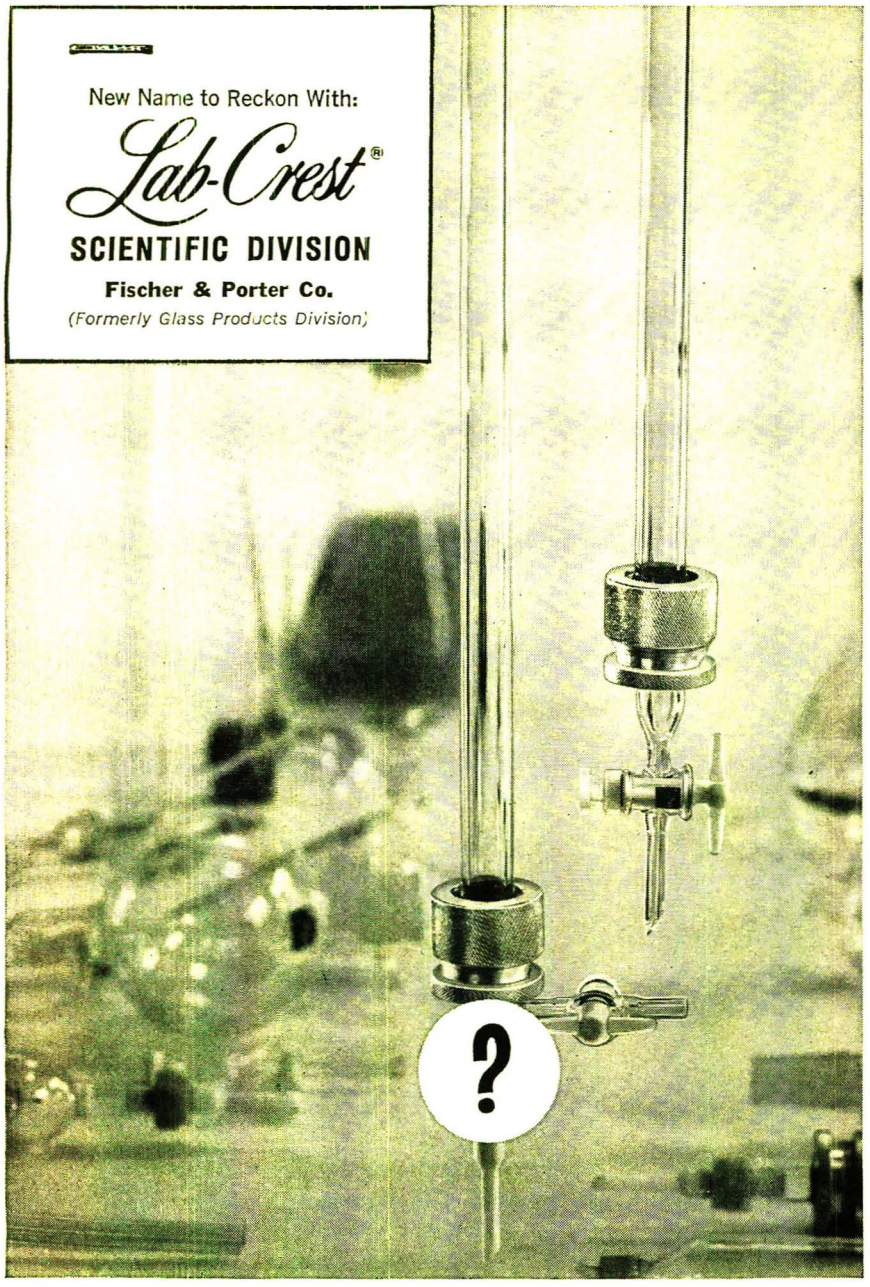

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The valve will never freeze, never need grease. Its body is precision-bore glass tubing; its needle, Teflon.

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All things considered, the built-in's your best bet, but if you just want the valve itself, fine. We'll be glad to oblige. If you'd like information on either the valve, the bottom connections, or both, let us know.

Lab-Crest® SCIENTIFIC DIVISION

FISCHER & PORTER COMPANY, 1603 County Line Road, Warminster, Pa.

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MANUFACTURERS' LITERATURE

Liquid Scintillation Systems. An 8-page bulletin discusses flow monitor and flow detector systems for continuous measurement of radioactivity in liquid and gas chromatography effluents. In addition, an 8-page folder is available which describes the 3000 and 4000 series of spectrometers for analysis of discrete samples containing beta- and alpha-emitting isotopes. Packard Instrument Co., Inc., Box 428, La Grange, Ill. **601**

Gas Chromatography Materials. An 18-page bulletin contains information on gas chromatography stationary phases and stationary media manufactured by May and Baker, Ltd., England. Tables classify stationary phases by name and by application and give references. Gallard-Schlesinger Chemical Mfg. Corp., 1001 Franklin Ave., Garden City, N. Y. **602**

Ultraviolet Analyzer. A 4-page folder describes the compact Model 1056-A UV analyzer. The instrument covers the spectrum from 200 to over 400 $m\mu$ and uses 2 flow cells of optically polished quartz in split-beam operation. Vanguard Instrument Co., P. O. Box 244, La Grange, Ill. **603**

Gas Chromatograph. A 12-page brochure describes the Model 154 D Vapor Fractometer. This is the company's basic instrument and is available with a pyrolysis accessory and electron capture detector. Other choices of detectors and other accessories are also discussed. Perkin-Elmer Corp., Main Ave., Norwalk, Conn. **604**

Dosimetry System. A 6-page folder presents the concept of thermoluminescent dosimetry and the technical characteristics of the company's lithium fluoride thermoluminescent dosimetry system. The dosimeter responds over the range from 0.1 to 100,000 r. with a precision of $\pm 2\%$. Controls for Radiation, 130 Alewife Brook Parkway, Cambridge 40, Mass. **605**

Gas Chromatography Supplies. A 32-page catalog contains information on gas chromatography column materials and accessories. In addition to listing liquid phases and solid supports, information on the composition and preparation of solid supports and application of liquid phases for particular separations are given. Wilkens Instrument & Research, Inc., P. O. Box 313, Walnut Creek, Calif. **606**

Blood Gas Accessory. Bulletin 7019, 4 pages, describes an accessory to the GC-2A gas chromatograph which can be used for the analysis of blood O_2, N_2, CO, and CO_2. Only 0.1 cc. of blood is required and the analysis takes only 4 minutes. Beckman Scientific and Process Instruments Division, 2500 Harbor Blvd., Fullerton, Calif. **607**

Sodium Chloride Crystals. A 4-page bulletin discusses the use of sodium chloride crystals for infrared cell windows. The properties and the techniques for shaping, grinding, and polishing rocksalt crystals are described and illustrated. Optovac, Inc., North Brookfield, Mass. **608**

Stable Isotopes of Oxygen. A 24-page booklet details the availability of the stable isotopes of oxygen in various forms. Both O^{17} and O^{18} labeled compounds are presented. Included in the booklet are references to the use of these materials in different areas of analytical chemistry. YEDA Research and Development Co., Rehovoth, Israel. **609**

Centrifuge Accessories. A 12-page catalog lists tubes, adapters, and accessories for the company's centrifuges. An illustrated index and pictorial charts aid in supplying information on the available products. Ivan Sorvall, Inc., Norwalk, Conn. **610**

Safety Pipetters. A 4-page brochure describes the "Clinac" mechanical pipetter available in micro and standard sizes. These pipetters can deliver fractions of a drop with an accuracy of 0.01 ml. or better. Also pictured and described is the "Pumpett," which accommodates all sizes of pipettes. La-Pine Scientific Co., 6001 South Knox Ave., Chicago 29, Ill. **611**

Chemicals. The 1963 British Drug House, Ltd., catalog lists 4000 packaged chemicals available from The Ealing Corp. Included are inorganics, microanalytical reagents, organic analytical standards, and materials for chromatography. The Ealing Corp., 2225 Massachusetts Ave., Cambridge 40, Mass. **612**

Surface Tension Measurement. "Techniques with the Cahn Electrobalance," No. 5 describes the use of the surface tension accessory to measure and record surface tension automatically. Cahn Instrument Co., 15505 Minnesota Ave., Paramount, Calif. **613**

Fluorine Determination. Method Sheet No. 69 describes a direct spectrophotometric procedure for the determination of fluoride ion. The absorb-

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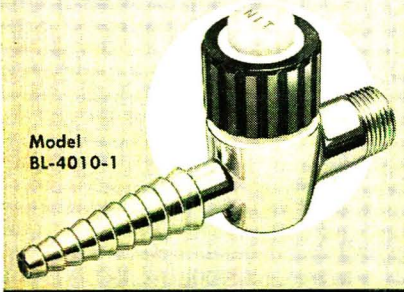

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Refer to 1963 Sweet's Catalog, Code: 35b/Ta

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MANUFACTURERS' LITERATURE

ance of the blue complex formed by fluoride ion with the cerium(III) salt of alizarin complexan is measured at 620 mμ. Unicam Instruments, Ltd., York St., Cambridge, England. **614**

Pour Point Analyzer. A 4-page bulletin describes a pour point analyzer developed jointly by California Research Corp. and Beckman. Designed to monitor and record the melting point of individual process streams, the analyzer can effectively determine pour point from −90° F. to + 50° F. Bulletin PP-4042. Beckman Scientific and Process Instruments Div., 2500 Harbor Blvd., Fullerton, Calif. **615**

Bi-Distillation Apparatus. A 4-page data file contains information on a quartz glass bi-distillation apparatus. A double distillate water product is prepared in a single operation as two distillation stages are incorporated in a single closed system. Engelhard Industries, Inc., 75 Austin St., Newark, N. J. **616**

Ultrasonic Cleaning. A bulletin describes the Metason apparatus for ultrasonic cleaning of small samples. The units, designed especially for metallurgical samples, use wide band frequency generation so that all parts of the transducer are energized by polysonic frequencies. Wm. J. Hacker & Co., Inc., P. O. Box 646, West Caldwell, N. J. **617**

Power Controller. A 4-page bulletin describes the Niatrol. This instrument is a fully proportional a.c. power controller, delivering up to 10 amp. at output voltages from 0 to 115 volts a.c. When used with a thermistor probe, the unit will maintain the temperature of a bath constant to within 0.005° C. for several hours, or within 0.02° C. indefinitely. Niagara Electron Laboratories, Andover, N. Y. **618**

X-Ray Microscopy. A 6-page folder, illustrated with photos, diagrams, and micrographs, discusses terminology, focusing, depth of field, stereo microscopy, differential staining, measurement of absorption index, and diffraction patterns in using an x-ray microscope. Philips Electronic Instruments, 750 South Fulton Ave., Mount Vernon, N. Y. **619**

Research Microscope. A 10-page bulletin describes the Reichert Zetopan research microscope. Included are pictures and descriptions of various accessories for extending the applications. Wm. J. Hacker & Co., Inc., P. O. Box 646, West Caldwell, N. J. **620**

Gel Filtration. A 20-page booklet describes the use of Sephadex, a cross-linked dextran material, for fractiona-

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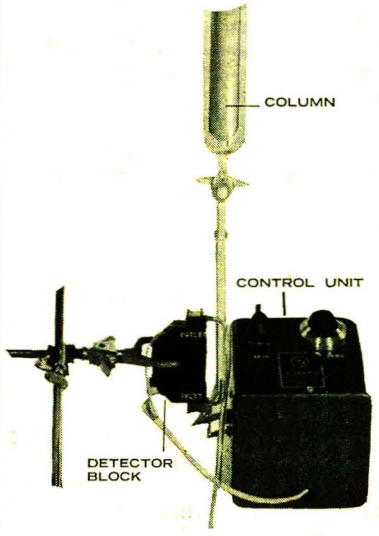

The liquid chromatography technique is a particularly valuable analytical tool to the food, drug, rubber, medical, pharmaceutical and heavy chemical industry because it allows analysis without subjecting the sample to high temperatures which may destroy or break down the true sample. In liquid chromatography the sample is introduced to the column in its true liquid state or as a solid dissolved in a liquid. An eluting solvent then carries the sample through the column. The development of this technique has lagged far behind gas chromatography because of the lack of a universal, sensitive column monitoring device.

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tion according to molecular size. Application of the material to various laboratory procedures is suggested and 6 pages of references are included. Pharmacia Fine Chemicals, Inc., 501 Fifth Ave., New York 17, N. Y. **621**

Hemagglutination. A 4-page brochure describes an automatic, quantitative system for running 30 to 60 hemagglutination determinations each hour. The method is based on colorimetric measurement of residual hemoglobin and results are recorded on a strip chart in terms of absorbance units and expressed in % cells agglutinated. Bulletin H 1. Technicon Instruments Corp., Research Park, Chauncey, N. Y. **622**

Alpha Survey System. A 3-page bulletin describes the Model 1000/63 Pi alpha survey meter. The instrument has 4 scale ranges covering counting rates to 10^6 c.p.m. and can be used as a portable or with a.c. power supply. Physics International Co., 2229 Fourth St., Berkeley 10, Calif. **623**

Polished Optics. An 8-page folder describes the company's optical polishing center. Materials are offered with guaranteed optics from 0.1 to 70 microns. Flats, prism, lenses, and tailor made crystals are available. Harshaw Chemical Co., 1945 East 97th St., Cleveland 6, Ohio. **624**

Gas Chromatographic Techniques. A 21-page paper, "Special Techniques in Gas Chromatography," covers various special applications. Included are trace analysis for several components; polymer pyrolysis; analysis of corrosive constituents; etc. F & M Scientific Corp., Avondale, Pa. **625**

Combustion Tubes. A 4-page bulletin, C363, describes and gives dimensions of high temperature porcelain and "Zirco" combustion tubes. These tubes have a maximum temperature limit of 3000° F. (2900° F. for "Zirco"). McDanel Refractory Porcelain Co., 510 Ninth Ave., Beaver Falls, Pa. **626**

Nuclear Instrumentation. A 6-page brochure presents methods of selecting and grouping nuclear instrumentation according to the purpose of the user. Four basic systems are described including the modules used in the systems and the accessories for input and output functions. Radiation Counter Laboratories, Inc., 5121 W. Grove St., Skokie, Ill. **627**

Digital Instruments. A 16-page short form catalog describes 4- and 5-digit digital multimeters, scanners, buffers,

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MANUFACTURERS' LITERATURE

and differential voltmeters. Auto Data, Inc., 5121 Weeks Ave., San Diego 10, Calif. **628**

UV-Visible Spectrophotometer. An 8-page bulletin describes and gives specifications for the Model 202 spectrophotometer. This instrument scans the ultraviolet in the region of 190 to 390 $m\mu$ and the visible in the region of 350 to 750 $m\mu$. Perkin-Elmer Corp., Norwalk, Conn. **629**

Spectrophotometer Sources. A bulletin describes utlraviolet source lamps and a new dual source unit for the company's spectrophotometers. The dual source unit permits a choice of tungsten or hydrogen lamps at the turn of a knob. Scientific and Process Instruments Div., Beckman Instruments, Inc., 2500 Harbor Blvd., Fullerton, Calif. **630**

Differential Scanning Calorimeter. An 8-page brochure describes the DCS-1. This instrument permits the direct measurement of heats of reaction and transistion in thermal analysis. Samples as small as 0.0001 gram can be used. Perkin-Elmer Corp., Main Ave., Norwalk, Conn. **631**

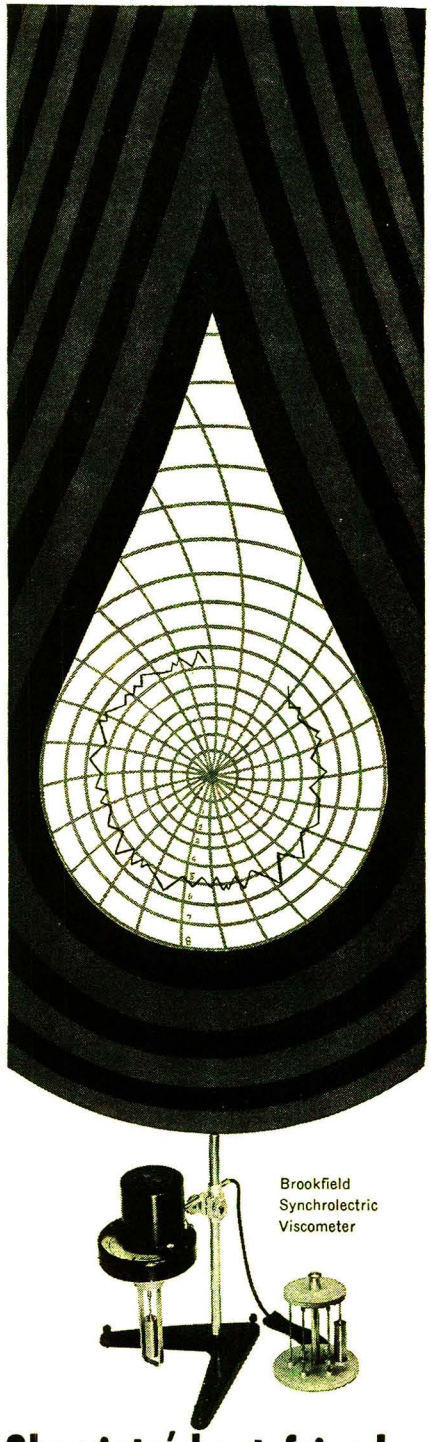
Thermal Gravimetric Analysis. A 4-page bulletin discusses "Component TGA." Illustrated and described are some TGA systems which include the RG Electrobalance. Cahn Instrument Co., 15505 Minnesota Ave., Paramount, Calif. **632**

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.

Chrom Line. August 1963. This 4-page issue describes "molecular sieve flour" for gas chromatography. This material is mixed with an appropriate amount of Chromosorb P and poured into a column. Other materials for gas chromatography are also listed. Chrom-Line Labs., P. O. Box 12131, Kansas City, Mo.

Spex Speaker. Vol. VIII, No. 2. This 8-page issue considers in detail the various factors involved in spectroscopic photography. Included are choosing the photographic emulsion, calibrating the emulsion, and reducing errors. The issue also describes the Shatterbox grinder and blender. Spex Industries, Inc., 3880 Park Ave., Metuchen, N. J.



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MANUFACTURERS' LITERATURE

Arcs and Sparks. Vol. 9, No. 2. This 24-page issue contains news of interest to spectroscopists and features a write-up on NASA's Lewis Research Center, Cleveland, Ohio. Ultra Carbon Corp., P. O. Box 747-A, Bay City, Mich.

Facts & Methods for Scientific Research. Vol. 4, No. 1. This 12-page issue is devoted to new applications and techniques in analysis by gas chromatography. Gas chromatographic analytical techniques as applied to oil of lemon, free sterols, coal tar, and paraffin waxes are discussed and illustrated. F & M Scientific Corp., Route 41 and Starr Rd., Avondale, Pa.

Atomlight. No. 29. This issue describes the use of multiple labeling to study the metabolism of ethanol in man. Another article describes analytical studies using two levels of C^{14} and H^3. New England Nuclear Corp., 575 Albany St., Boston 18, Mass.

Jena Review. No. 2/63. This issue includes a detailed contribution on the "Influence of resolving power on the detection sensitivity in spectral analysis." The publication also describes accessories for the Universal spectrophotometer VSU 1; an instrument for evaluating fluorescent paper chromatograms; a heat microscope; and other related equipment. VEB Carl Zeiss Jena, Jena, Carl-Zeiss, Strasse 1, German Democratic Republic.

Aerograph Previews and Reviews. July 1963. This 4-page issue describes the Hy-Fi A-600-C. This new model gas chromatograph has an improved electrometer. Also described are the Model A-650 hydrogen generator and the Model A-675 steam generator. Wilkens Instrument & Research, Inc., P. O. Box 313, Walnut Creek, Calif.

Spectrographer's ARL News Letter. Vol. XVI, No. 2. This issue describes the company's new spectrographic analyzer, Model 26000-1. This low-cost system complete with source unit, excitation stand, viewer-comparator, film, electrodes, developing materials, etc., fits easily on the top of an average sized desk. Applied Research Laboratories, Inc., P. O. Box 1710, Glendale 5, Calif.

S/P Bulletin. The July issue features the Oxford titrator, a single compact instrument for calcium and chloride determinations. Also described are a microscope projection screen, materials for various blood procedures, reagent dispensers, and a new Warburg apparatus. Scientific Products, 1210 Leon Place, Evanston, Ill.

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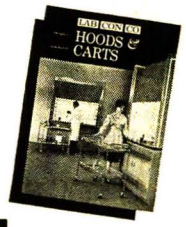

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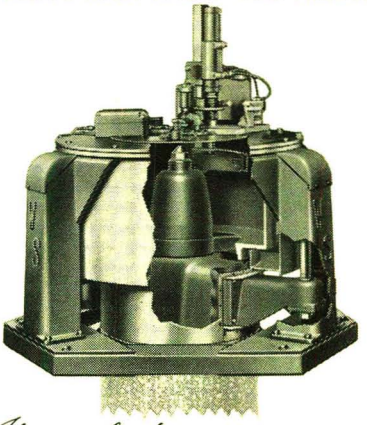

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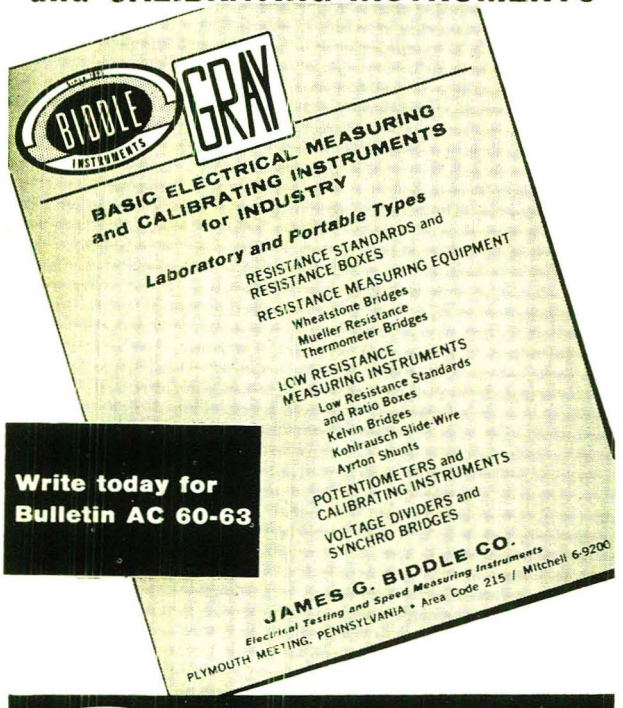

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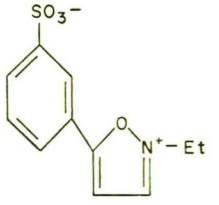

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Chromatographs. New gas unit has temp. controller-programmer. **163**

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(*Continued on page 164 A*)

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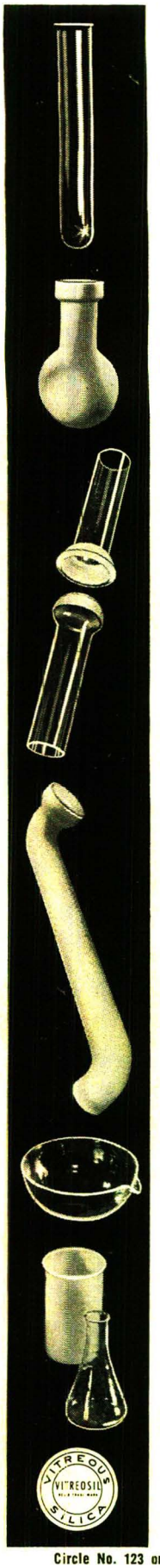

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Generators. Bulletin on neutron generator and 400-channel pulse analyzer. **214**

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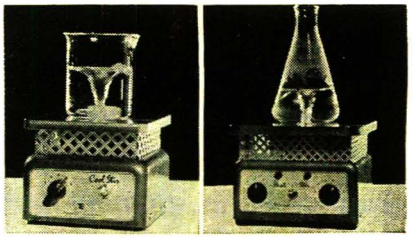
Heaters. Info on electrothermal armoured heaters. **104**



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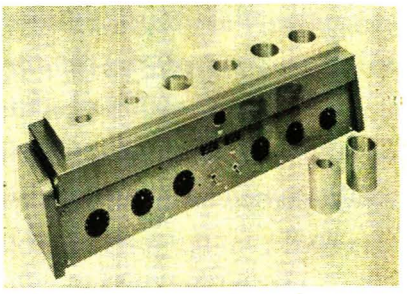

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Nuclear Units. Info on line of nuclear instrument modules. **185**

Oils. Bulletin on oils, waxes, and greases for high vacuum work. **32**

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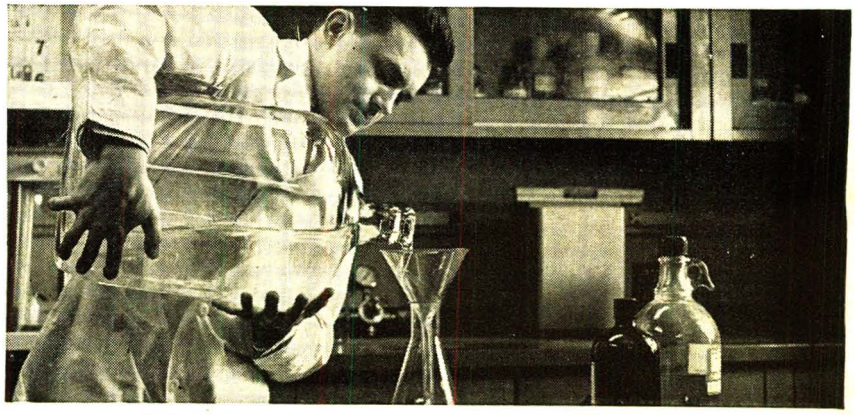

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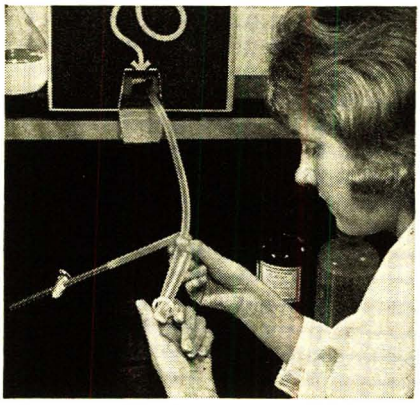
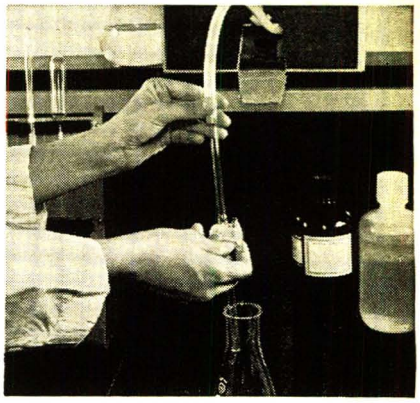


3. Invert carton and remove safety ring from tubing.



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Pumps. Bulletin on plastic pumps for continuous duty. **148**

Pumps. For ion pumping and Ti sublimation pumping. **197**

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Recorders. Bulletin on unit with 250 mm. chart. **134**

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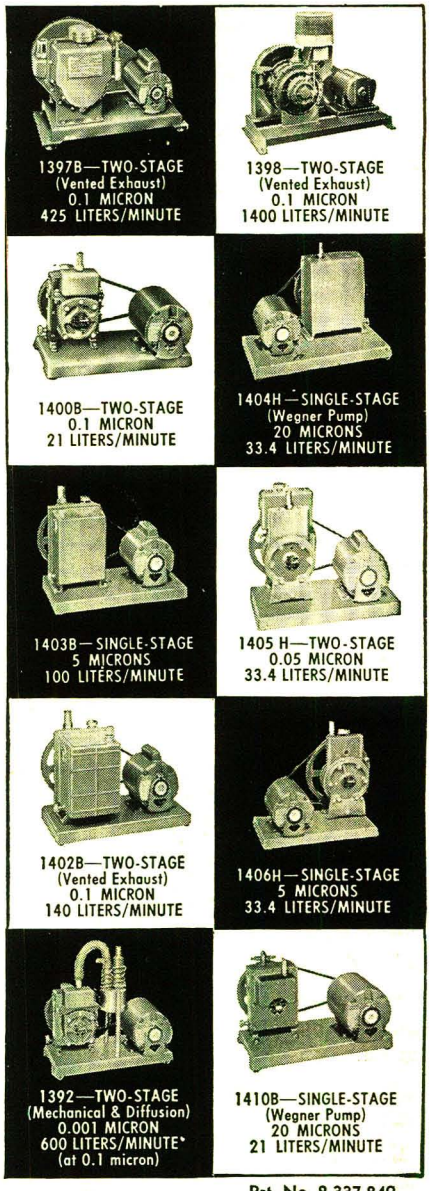

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Spectrometers. Data file on mass unit for solids, liquids, gases. **132**

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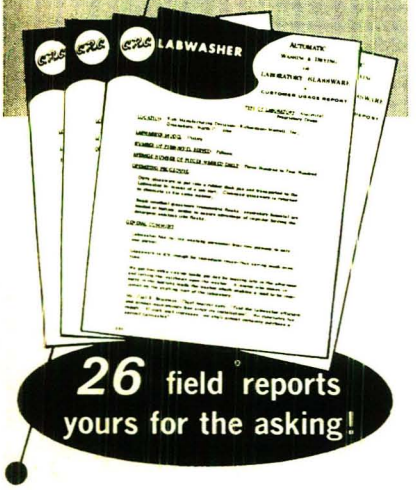

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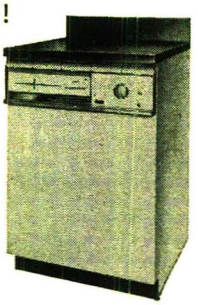

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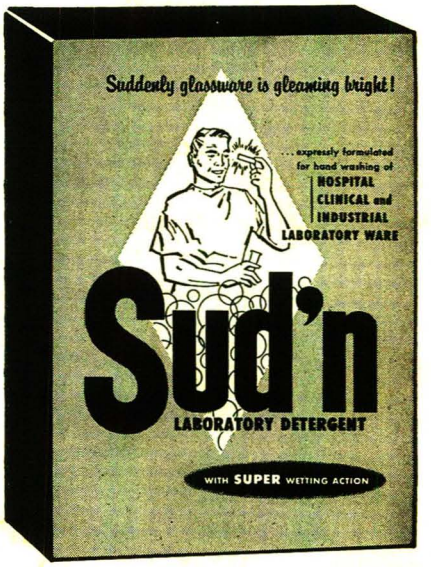

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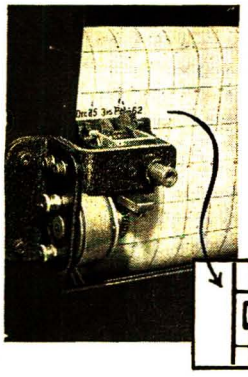

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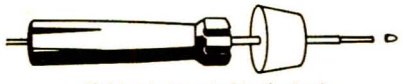

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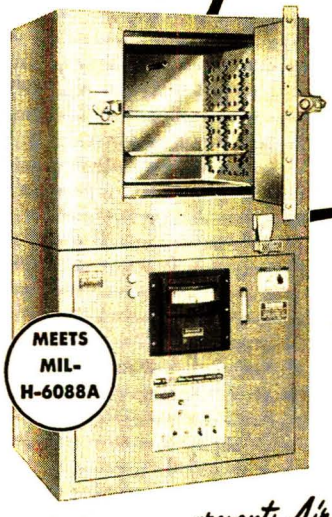

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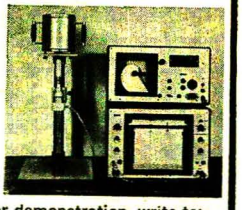

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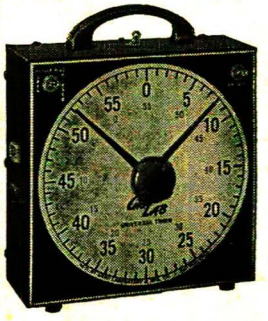

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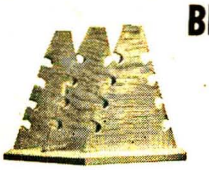

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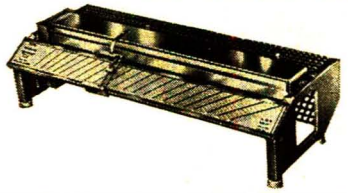

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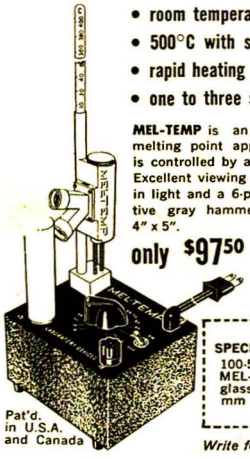

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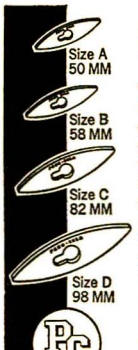

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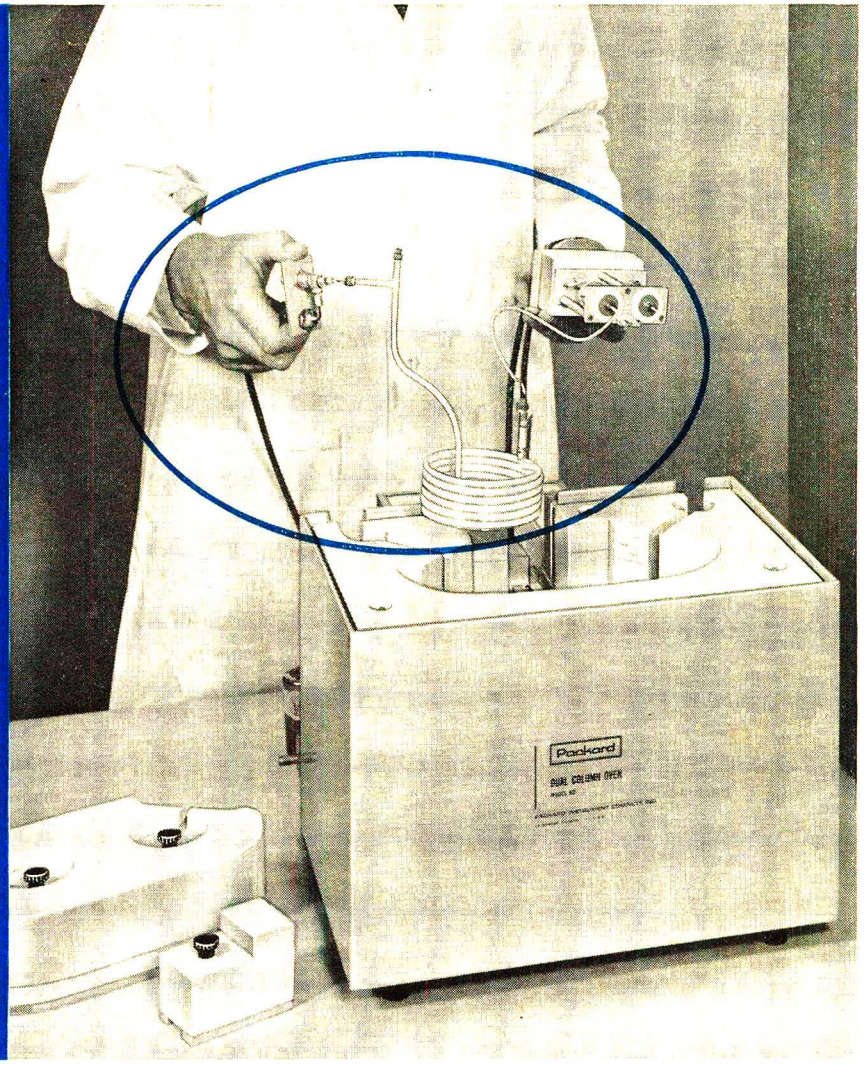

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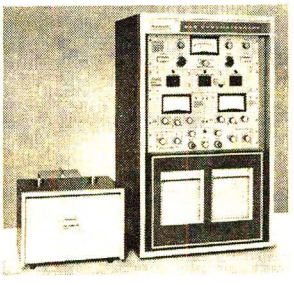

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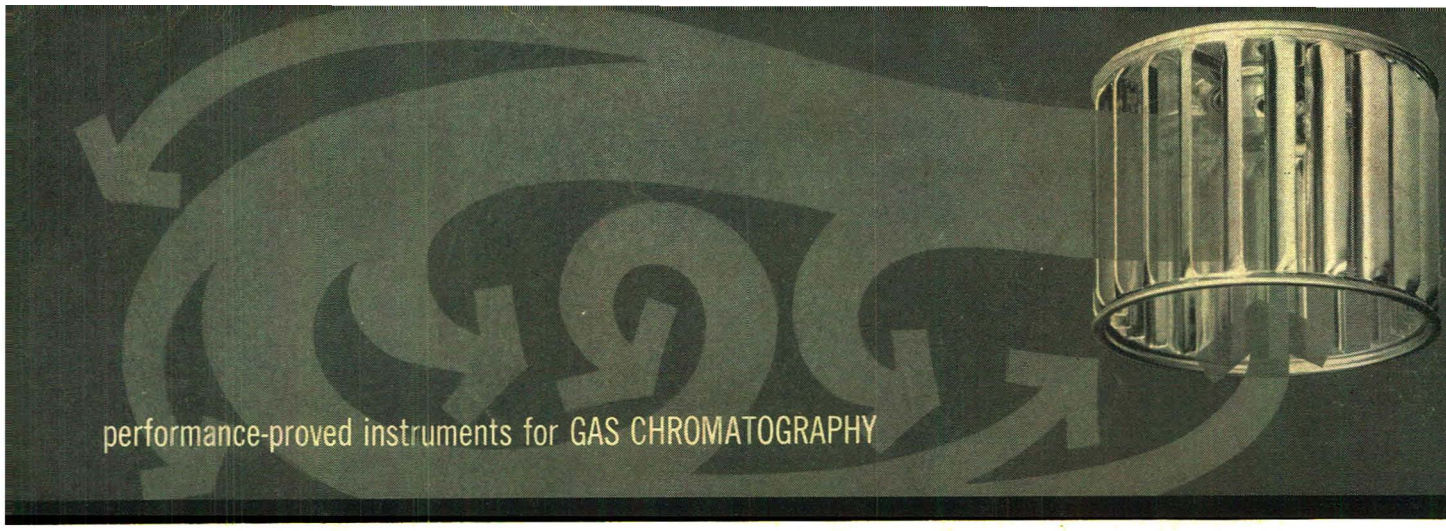

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