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Chromatography

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THIS REVIEW covers the literature of liquid column, paper, and thin layer chromatography abstracted in *Chemical Abstracts* in the period between mid-December 1969 and December 20, 1971. Chromatography is today the most widely used analytical technique, and this fact is reflected by the many thousands of references during this period on these topics. The authors have selected those contributions which, in their judgment, are most important and will be of most use to analytical chemists. Because ion exchange is the subject of a separate article in this Fundamental Reviews issue, most work involving cellulose-, dextran-, and polystyrene-based ion exchangers and liquid ion exchangers are not covered. In setting this limitation, it is understood that there is great difficulty in accurately drawing the line between the mechanisms of separation on the different liquid chromatographic media. Gas chromatography and electrochromatography (electrophoresis) are also treated elsewhere and are therefore not reviewed here.

The two-year period under consideration was marked by a continued trend toward treatment of chromatography theory in a unified manner. Modern high pressure liquid chromatography emerged as a widely used analytical technique which promises to become one of the most popular and powerful of all chromatographic procedures. High speed, selective separations of compounds which may not lend themselves to analysis by gas chromatography are achieved by adsorption, partition, gel permeation, and ion-exchange procedures. Great strides have been made in column technology with the application of superficially porous supports, porous-layer beads, pellicular ion exchangers, and bonded-phase packings. Commercial equipment for high speed liquid chromatography is available from numerous companies. One drawback is that a research-grade, optimum performance instrument with recorder is priced at $10,000 to 20,000, while a minimum performance instrument without recorder requires $2000 to $8000. The detector remains the weak link in the modern liquid chromatography system. Ultraviolet and refractive index detectors have been by far the most widely used. Of the other types which have been developed, the transport detector has the greatest promise for the future. Many applications were reported of traditional, low-pressure liquid chromatography using shorter, wider columns for adsorption, partition, and ion-exchange chromatography, as well as for gel filtration chromatography, in which the use of soft gel packings precludes the use of high pressures. Although these traditional procedures have the disadvantage of lower column efficiency and slower speed, they still are, and will continue to be, of great importance to most analysts. A method termed affinity chromatography was developed and applied for the purification of macro-

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molecules during the past two years. Confusion may arise in the use of this term; because "affinity chromatography" is also used to collectively designate partition, adsorption, and ion-exchange chromatography as opposed to exclusion (size) chromatography.

Paper and thin-layer chromatography have continued to be applied to analytical problems involving all types of compounds. Although paper chromatography is most often performed by ascending or descending development on Whatman No. 1 or an equivalent paper in simple, vapor-saturated tanks, increasing use is being made of impregnated and loaded papers. Thin-layer chromatography is usually carried out by ascending development in saturated tanks on silica gel layers. When silica gel chromatography is not successful, polyamide, aluminum oxide, or magnesium oxide layers, or layers impregnated with other materials, have been applied. Commercial preformed layers on plastic or aluminum are becoming increasingly popular as replacements for homemade layers on glass plates. Use of unsaturated tanks and vapor or adsorbent programming improves the resolution of some mixtures, and drum thin-layer chromatography provides the most efficient separations yet achieved in thin-layer chromatography. Many new reagents and methods were reported for the detection of separated compounds on paper and thin layers with increased selectivity and/or sensitivity. Paper and thin-layer chromatography remain only semiquantitative methods when instrumental procedures, with or without elution, or traditional visual comparison procedures are used to estimate the amounts of materials in the spots.

The American Chemical Society held short courses in thin-layer chromatography and modern liquid chromatography during the past two years, in addition to several courses in gas chromatography. A 16-mm color, sound film introducing the theory and principles of chromatography was released in 1970 by Educational Services, John Wiley and Sons, New York, N.Y., for use in analytical and separation courses.

The following symposia were published: Symposium on the Identification of Substances by Paper and Thin-layer Chromatography, held in Frascati, Italy, September 1969, published in Volume 48 of the *Journal of Chromatography;* Second Russian-Italian Symposium in Memory of M. Tswett, held in Milan, Italy, October 1969, published in Volume 49 of the *Journal of Chromatography;* Sixth International Symposium on Advances in Chromatography, held in Miami Beach, Fla., June 1970, published in Volume 8 (June, July) of the *Journal of Chromatographic Science;* Ninth International Gel Permeation Seminar, held in Miami, Fla., October 1970, published in Volume 55 of the *Journal of Chromatography;* ACS Symposium on Gel Permeation Chromatography, held in Houston, Texas, at the 159th National Meeting, February 1970, published in Volumes 5 and 6 of *Separation Science;* and the Seventh International Symposium on Advances in Chromatography, held in Las Vegas, Nev., November–December 1971, published in Volume 43, No. 14, of ANALYTICAL CHEMISTRY. The first Camag International Symposium on Thin-Layer Chromatography and Electrophoresis was held in Cherry Hill, N. J., in April 1971.

During the September 1972 meeting of the American Chemical Society in New York, the Analytical Chemistry Division will hold a symposium commemorating the centennial of the birth of Tswett, the 30-year anniversary of the invention of partition chromatography by Martin and Synge, and the 20-year anniversary of the first paper on gas–liquid chromatography by James and Martin. This year also marks the 30-year anniversary of the publication of "Chromatographic Adsorption Analysis," by Harold H. Strain, a former author of this Chromatography Review and the first recipient of the ACS Chromatography Award. This classic book, the first in English on chromatography, undoubtedly attracted many workers to the field. A symposium honoring Tswett will also be held during the same meeting by the Division of the History of Chemistry. Also during 1972, The Chemical Rubber Co. "Handbook of Chromatography," edited by the authors of this review, is scheduled for publication.

The ACS Chromatography Award, sponsored originally by Lab-Line Instruments, was discontinued in 1971. The award was reinstated in 1972 sponsored by Supelco. The initial winner of the new award was J. J. Kirkland of E. I. Dupont de Nemours and Co., Wilmington, Del., for his contributions in gas chromatography and high speed liquid chromatography.

GENERAL CONSIDERATIONS AND THEORY

New books have been published on the principles and techniques of chromatography in English (*355*) and French (*223, 1503*). Helfferich (*565*) authored a book on nonlinear multicomponent chromatography which presents the subject in a unified manner with a common nomenclature. This book provides the analyst with a clear understanding of displacement and frontal development methods. Volume 9 of *Advances in Chromatography* (*464*) and a four-language technical dictionary of chromatography (*40*) were published.

Reviews were published on the development of and the distinctions between the various types of chromatography (*929*), the classification of chromatographic methods (*712*), the principles of chromatography (*1344*), and some diverse applications of chromatography to the non-chemist (*1270*). Historical reports were written on developments in the nineteenth century (*1307*), developments from biblical times to the present (*1302*), and the influence of M. Tswett (*556, 1201*).

General chromatographic theory was developed, and fundamental studies were carried out as follows. Kaiser discussed random and systematic errors in chromatography (*664, 665*), and methods for properly locating base lines and separation lines (*661*). Guiochon wrote a series of papers on the transformation of finite signals in a chromatographic column in which the propagation of a chromatographic peak is described by a system of five differential equations (*506*), the basic mechanism of all chromatographic processes is accounted for mathematically by a single type of equation (*507*), and a computer program is derived from a mathematical model for preparative scale chromatography with which the yield and output per unit time for the separation of a mixture of two compounds are obtained (*508*). Rony applied the extent of separation to time normalization and minimum time analysis in elution chromatography (*1175, 1176*), and studied retention time and the first time moment in elution chromatography in a series of three papers (*429, 1177, 1178*). Grushka (*500*) found that the peak capacity of a chromatographic system depends upon the number of plates and the ratio of the retention time of the last component to that of the inert compound; peak capacity is increased by judicious choice of column length, mobile phase velocity, column temperature, and amount of stationary phase. The same author (*501*) discussed length-temperature time normalization chromatography, a method of increasing the resolution of two components while keeping the retention time of the last solute constant. The increase in resolution is achieved by changing both the column length and its temperature. Smuts and Pretorius derived an equation for calculating the plate height for a packed chromatographic column under various operating conditions (*1305*) and derived basic equations (*1306*) which relate chromatographic separation time to experimentally measurable operational parameters.

Kinetics of first-order reactions and first-order equilibria occurring on chromatography columns are studied by their effect on the shape of chromatographic peaks (*1484*). A computer can

be used to calculate the optimum selectivity of a fixed phase for a multicomponent mixture (*15*). An apparatus was constructed for integration of incompletely separated peaks with an accuracy of 1–1.5% (*22*), and a computer study was made of the efficiency and accuracy of some simple methods for determining peak areas (*24*). The contribution of eddy diffusion and the macroscopic mobile phase velocity profile to plate height has been investigated (*308*). A digital logic system has been developed for the evaluation of instrumental contributions to chromatographic band broadening (*466*). A computer program is available for the resolution of fused chromatographic peaks (*537*). Interpretation of chromatographic peaks is made by Fourier analysis (*440*). Normalization techniques in computer evaluations are used to correct for shifts in elution peaks due to variations in experimental parameters (*650*). Five programming (algorithms) methods are available for input of information from chromatographic pickup units into digital computers (*744*). The calculation of dynamic characteristics of porous adsorbents (*697*) and the use of digital computers in the analysis of chromatographic data have been discussed (*889*). Static bed and moving bed chromatography are compared (*934*). The distortion of chromatographic peak shapes has been examined (*1014*). Stochastic models are developed by treating column chromatography as a diffusional process, interrupted at random for exponential delay times (*1045*). Practical experience with least squares approximation of chromatograms has been described (*1161*). A small on-line computer can be used for distinguishing and measuring the positions and areas of overlapping peaks in a complex chromatogram (*1239*). Random input and cross correlation methods are used to improve the signal-to-noise ratio in chromatographic trace analysis in order to detect a peak buried in background noise (*1297*). A theoretical study has been made of the coupled transfer of two independent components in a fixed bed with instantaneous equilibrium (*1443*). Peak heights can be estimated from the volume of the sample and the molecular weight of a compound in a homologous series (*1366*). The dependence of the partition coefficient and the retention volume on the molar refraction of the stationary phase has been established when stationary phases are substances of one homologous series (*1274*). The separation speed of chromatographic columns has been studied (*1171*). High separation speeds are achieved on short columns with high permeability and with high inlet pressures.

A general method of automated multidimensional fractionation termed cascade chromatography (*354*) can provide completely automatic separation of pure components from complex mixtures. Membrane partition chromatography (*118*) makes use of selective, protein-retentive membranes for the fractionation of protein mixtures. Spherical foam chromatography (booster bubble fractionation) (*859*) is a foam separation technique in which a current of air or nitrogen saturated with a volatile organic or inorganic liquid as the supporting phase is circulated through aqueous solutions for the rapid separation of surfactant substances from dilute solutions.

COLUMN ADSORPTION AND PARTITION CHROMATOGRAPHY

Books and Reviews. Kirkland has edited a book on the modern practice of high speed liquid chromatography (*721*) which includes chapters by him on liquid–liquid partition chromatography (*722*), and by L. Snyder on liquid–solid adsorption chromatography (*1309*). Halasz gives an overview of the field in the same book (*530*). Reviews of column liquid chromatography have appeared in English (*356, 725, 1065, 1066, 1160, 1381*), German (*353*), and Dutch (*298*). Dry column chromatography (*845*) and preparative liquid chromatography (*1560, 1561*) have been reviewed.

Affinity chromatography is a new procedure for the purification of macromolecules (*e.g.*, enzymes). A solution containing the macromolecules is passed through a column containing an insoluble polymer or gel to which a specific competitive inhibitor or other ligand has been covalently attached. Proteins not exhibiting appreciable affinity for the ligand pass unretarded through the column, whereas those interacting with the inhibitor under the experimental conditions will be more or less retarded. Specifically bound protein can be eluted by altering the composition of the solvent so that dissociation occurs. The practice and applications of affinity chromatography have been reviewed (*274, 275, 419*).

Theory and Fundamental Studies. Karger (*678*) has reviewed the effect of physical parameters on the efficiency of high speed liquid chromatography, discussing resolution, retention, theoretical plate characteristics, packing, porous layer effects, capacity, and optimization of columns and comparisons between liquid and gas chromatography. L. Snyder (*1310*) compared various techniques for solving the general elution problem in liquid chromatography and finds solvent programming (best) > coupled – columns > temperature programming ≈ flow programming > normal elution. Bombaugh compared separation mechanisms for liquid chromatography applications (*136*) and discussed resolution in liquid chromatography (*133*). The influence of the polar and steric effects of various substituents on the adsorption energy of thiazoles on alumina (*1505*) and silica (*1506*) was discussed in terms of Snyder's theory on linear adsorption chromatography.

Relatively large diameter liquid partition columns packed with controlled surface porosity supports exhibit superior efficiencies compared to narrow-bore columns (*315*). Flow rates must be accurately determined and controlled for accurate quantitative analysis by high pressure liquid chromatography using linear gradient elution (*166*). The relation between the Hildebrand solubility parameter and the liquid–solid solvent strength parameter has been studied (*704*). In a liquid–solid adsorption chromatography column, the optimum adsorbent fraction (ϕ_{opt}) corresponding to maximum resolution is given by $\phi_{opt} = 1/[1 + K_2(n/2)]$, where K_2 is the distribution coefficient and n, a velocity parameter (*598*). Criteria for the choice of liquid–liquid systems for column chromatography are based on the production of partition coefficients and characterization of the systems (*599*). The dispersion of inert components in liquid flow through columns packed with glass beads has been studied (*297*).

Extra-column peak broadening of inert and retarded solutes has been experimentally studied and theoretically explained (*302*). A relation between the stability constant of a complex and basic chromatographic parameters was established for chromatography on modified sorbents (*1089*). The kinetics of gradient elution chromatography has been analyzed mathematically (*1088*). The use of continuous and incremental methods of mixing to provide solvent gradients have been compared and the theory of each developed (*1243*). Incremental mixing is the simplest and most versatile method, and a simple computer program will provide curves relating mobile phase composition with time. Macroporous silica gels with 80–300 m^2/g sp. surface area and >80 Å average pore radius are preferred for analytical liquid–solid chromatographic separations based on a study of adsorbent structures, surface chemistry, and band broadening (*804*).

An equation has been derived to predict the effect of temperature on the retention volume of a solute in liquid–solid chromatography (*866*). The thermodynamics of liquid–liquid partition chromatography has been reviewed with emphasis on the theory of solute retention and selectivity and the use of this theory for physicochemical measurements by liquid–liquid chromatography (*844*). The thermodynamic basis for selectivity in liquid–liquid

chromatography is further considered in terms of a new approach to solution free energies (*893*). Factors limiting peak capacity in liquid chromatography include low detector sensitivities, poor solute solubility, and/or low sorptive capacity of the stationary phase (*1244*).

Experimental conditions for column chromatography can be chosen by referring to the results of thin-layer chromatography in a similar system (*364, 1403*). The mechanism of reaction between HDEHP stationary phase and rare earths, actinides, and other elements during extraction chromatography with aqueous acid eluents has been studied in terms of the ionic parameters of the species in solution (*1094*). A computer-aided frontal analysis chromatography apparatus has been designed for the determination of adsorption isotherms and surface areas for a wide range of adsorbates and adsorbents (*178*). An expression has been derived for the production rate in preparative elution chromatography (*296*).

Chromatographic Systems. The following column materials have been used for affinity chromatography: 5′-(4-aminophenylphosphoryl) uridine-2′-(3′)-phosphate coupled to Sepharose for the purification of ribonuclease A (*1580*), chicken ovomucoid bound to Sepharose for the separation of bovine α- and β-trypsin (*1547*), Sepharose–4-phenylbutylamine for isolation of chymotrypsinlike enzymes (*1346*), *N*-(ϵ-aminocaproyl)-*p*-aminophenyl trimethylammonium bromide hydrobromide coupled to Sepharose 2B for purification of acetylcholinesterase (*667*), methotrexate coupled to Sepharose via a C_6 chain for purification of dihydrofolic reductase from chicken liver (*691*), chymotrypsin–Sepharose for isolation of chicken ovoinhibitor (*388*), various agarose bead derivatives for protein purifications (*273*), and Gly-Gly-Tyr(Bzl)-Agr linked to an agarose resin for purification of papain (*122*). Tyrosine aminotransferase-synthesizing ribosomes from hepatoma tissue culture cells have been partially purified by affinity chromatography (930).

Kirkland has reviewed different column packings for modern liquid chromatography (*724*). Packings with chemically bonded silicone polymers (Permaphase) are efficient and stable phases for high speed reversed-phase liquid partition chromatography (*723*). Esterified siliceous materials (Durapak; brushes) are another type of surface-reacted (bonded) stationary phases for high speed liquid partition chromatography. The nature and uses of chemically bonded phases have been reviewed (*675*). Pellicular ion exchange beads composed of a thin coat of resin on a glass sphere permit faster, more efficient separations because of improved mass transfer but with a low capacity (*720*). Applications of these supports for ion-exchange chromatography and of Zipax and Corasil porous layer supports for liquid partition chromatography have been described (*1227*). A comparison of Zipax and Corasil as support materials for liquid–liquid partition chromatography has been reported (*870*). Surface-etched glass beads can be used as the support for liquid–liquid partition chromatography (*681*), and advances in the technology of lightly loaded glass columns have been reviewed (*579*). For high speed adsorption chromatography, Porasil porous silica and Corasil pellicular silica packings are recommended as high performance packings (*838*). Porous silica gel can also be used as a support material for high speed liquid-liquid partition chromatography (*1139*).

A large assortment of chromatographic systems were employed for traditional column separations. Silica gel packings were characterized by the elution volumes of nitrophenols (*743*). Silica gel is treated with PVP to prevent irreversible adsorption of proteins (*728*). The surfaces of glass powders and beads were studied using a scanning electron microscope (*313*), and the use of glasses for chromatography was discussed (*527*). Florisil provides separation of polychlorinated biphenyls from chlorinated pesticides (*103*) and remains the most widely used adsorbent for the cleanup of chlorinated pesticide residues prior to gas or thin-layer chromatographic analysis. Charcoal is the preferred adsorbent for the cleanup of phosphate pesticide residues (*1357*). The preparation of porous carbon useful for the chromatographic separation of strongly polar compounds was described (*663*). Magnesium oxide and zinc carbonate columns are used to separate carotenoid pigments (*1349*), and polyethylene for the separation of chlorophylls c_1 and c_2 (*1358*). Magnesium silicate separates furazolidone and nifuroxime (*1589*). Celite columns are used for the assay of multicomponent drug formulations (*1595*) and progesterone in blood (*1356*). Hydroxylapatite columns separate polar lipids (*1296*) and polysaccharides and nucleic acids (*1513*). Amberlite XAD-2 adsorption resin developed with an ethanol–water gradient separates flavonoid compounds (*590*).

Teflon is not a totally inert support for partition chromatography (*258*). A partition column of Sephadex LH20 supporting aqueous methanol and a mobile phase of isooctane gives a separation of oil-soluble vitamins from interfering food constituents (*87*). The following systems have been used to separate metal ions: columns of Amberlyst XAD-2 supporting MIBK and developed with aqueous HBr solutions for the separation of Ga(III), In(III), and Tl(III) (*421*); stationary tributyl phosphate or di-(2-ethylhexyl)-orthophosphoric acid developed with aqueous HNO_3–hydrazine solutions to separate Pu(III), U(IV), and U(VI) (*376, 1223*); stationary tributyl phosphate developed with 6*N* HCl to separate Po from Bi (*1135*); and Porasil C supporting tributyl phosphate and developed with mineral acids for the separation of noble metals (*1091*). Nineteen organic ammonium compounds were separated as inorganic anion pairs by reversed-phase column chromatography (*1532*). Dithizone is an effective stationary phase for the separation of various metals (*1248, 1249, 1327*). Radionuclides from biological materials are separated on Microthene 710 supporting TOPO (*1417*).

Peptides and amino acids in urine are separated on a DEAE-cellulose column eluted with 0.2*M* acetic acid (*1010*), human casein is fractionated on a DEAE-cellulose column eluted with 3.3*M* urea (*988*), ribonucleic acids are resolved on benzoylated DEAE-cellulose developed with NaCl-urea gradients (*1343*) or ethanol-NaCl mixtures (*1464*), sugars and nucleic acid components are specifically complexed on columns of *N*-(*m*-dihydroxyborylphenyl)carbamoylmethyl cellulose (*1572*), amino acid mixtures are separated, and organic acids purified on columns containing water-insoluble derivatives of cellulose crystallite aggregates (*77*), and amines are separated on metal-loaded cellulose columns developed with organic solvents due to complexing between the metal ion and the amine and interaction of the amine with the cellulose (*978*).

Optically active supports are used for the resolution of optical isomers (*53, 183, 1170*). PVP columns developed with HCl–ethanol solutions separate anthocyanins which differ in the number of phenolic groups and glycosides (*1598*). The separation of olefins by column or thin-layer chromatography on Porapak Q with water–propanol solvents is improved by adding $AgNO_3$ to the mobile phase so that the olefins and silver ions form π-complexes (632). Chitosan columns are used to purify thallium nitrate (*979*). 1- and 2-Methylnaphthalenes are separated on a clathrate complex column (*147*). An open pore polyurethane support can be formed *in situ* for the preparation of columns of any size and configuration (*1186*); liquid phases are incorporated by adding them to the precursor reagents. Columns consisting of a bundle of parallel fibers of porous glass and exhibiting uniform flow resistance have been prepared (*345*).

Ribonucleic acid has been fractionated on columns of polylysine

kieselguhr (*47*) and aminoacyl-tRNA on a methylalbumin–kieselguhr column with linear NaCl gradients (*444*). *Escherichia coli* ribosomal ribonucleic acids were chromatographed on a column consisting of methyltrialkyl (C_8–C_{10}) ammonium chloride supported on silanized diatomaceous earth and developed by NaCl gradient elution (*358*). Nucleic acids were fractionated on a magnesium pyrophosphate column (*1069*). Leucine tRNA of *E. coli* was chromatographed on acid-washed, silanized Chromosorb W equilibrated with Freon 214 buffer containing 0.35*M* NaCl and developed with an NaCl gradient (*1562*).

Techniques and Apparatus. Apparatus for high speed liquid chromatography has been reviewed (*567*) and design considerations have been discussed (*138*).

Automatic sample application systems have been described (*337, 456, 596, 1036, 1329*) along with an apparatus and procedure using conventional syringes for collecting liquid or gas samples from moderately pressurized containers for injection into a chromatograph (*1021*).

A method for preparing narrow-size fractions of Hyflo Super-Cel for high speed liquid chromatography is a modification of the method of Scott for ion-exchange beads (*299*). An improved method for treating aluminum oxide for catechol amine chromatography involves washing 12 times with 2 volumes of distilled water, 3 times with 2 volumes of double distilled water, and heating to 400 °C (*312*). A mechanical mixing apparatus was described for the preparation of large batches of reversed-phase chromatographic packing (*536*).

Precision bore and seamless stainless steel, aluminum, and copper provide columns of equivalent efficiency for high speed liquid chromatography. Teflon-coated aluminum columns are not reproducible in regard to HETP (*679*). Inert, all-plastic columns are useful with HF solvents (*1300*). Commercially available acrylic columns consist of different length sections with threaded joints for easy combination to obtain any desired length. Nylon screens can be screwed into the joints to permit the use of different packings within one column (*635*). Chromatographic columns packed with vitreous carbon or bismuth telluride can be heated by passing an electrical current through the packing. Transferrable, alternate hot and cold zones increase the separation rate within the column (*161*). Baffled columns provide convective flow at low flow velocities (*1113*). A chromatographic column having a core of square cross section with the edges contacting the wall provides separated channels in the longitudinal direction. The roughened core surface is covered with the adsorbent (*94*). Modular metal, glass, and plastic components can be combined to give micro columns of various lengths and widths for high-pressure chromatography (*1336*). A special quartz column permits continuous loading and photometric recording of the eluate; the dead volume between the column and the detector is minimized (*729*). Columns with deflection elements give increased efficiency (*933*), and columns with a small number of mixing devices overcome the effects of flow maldistribution (*932*). Columns in which the mobile phase passes through the stationary phase in such a manner that a net effect of increasing cross-sectional area transverse to the flow path is attained, permit decreases in plate heights and increases in band migration rates relative to the mobile phase (*1112*). Kirkland described a column consisting of an open tube lined alternately with several identical monolayers of microparticulates (silica) and colloidal organic particles (gums) of opposite charges. Improved separation of two dimethylureas was obtained (*719*).

The known methods for flow control in high pressure liquid chromatography have been compared, and a new unit serving as a pressure controller (1–500 atm), pulse smoother, safety valve, pressure transducer, and flow programmer was designed (*303*). A simple gas pressure regulator controls liquid chromatography elution rates at pressures below 2 lb/in.^{2} (*176*). A small pressure-elution pump operating efficiently on compressed nitrogen gas was designed for spacecraft missions (*141*). A pump consisting of a compressible Teflon container has been designed (*1242*). A chromatograph providing inlet pressures >60,000 psi allowed use of very fine particles and increased resolution and separation speed (*107*). A chromatograph with a very small column and a refractometer cell housed in a single heat exchanger provided temperature uniformity and low dead volume and sharp, narrow peaks (*1559*). Design of a chromatograph with a thermostated U-shaped column and microadsorption detector sensitive to 10^{-5} mole and reproducible within 4% was reported (*131*).

Solvent programming (gradient elution) is the primary method for altering k' values and increasing resolution in liquid chromatography. This approach has been discussed (*134*), and an apparatus described for the simultaneous and automatic programming of solvent flow and composition with time (*58*). A gradient mixer using a black and white paper curve for programming and magnetic valves for producing the gradient has been developed (*699*). A system for achieving gradient elution with axial equilibrium consists of heptane moderated with isopropanol as the mobile phase and a temperature programmed silica gel column initially equilibrated with isopropanol (*1245*). A general equation is proposed relating the eluting solvent composition gradient and elution time for the partition chromatography of organic acids on Celite (*194*).

Solvents are purified prior to use if they contain impurities which interfere with sensitive detectors; for example, KH_2PO_4 was passed over Dowex 1-X8 ion-exchange resin to remove an ultraviolet absorbing impurity (*1280*). When dry-packing silicic acid, the adsorbent should be added in increments to obtain more efficient columns (*656*). Heavily-loaded columns (up to 50 wt %) have been studied and applied to steroid separations using stepwise pressure programming (*531, 630*). Column separations are precisely designed from TLC separations if the elution volume of the first peak and the points of solvent change are known (*1006*). Recycle techniques in preparative liquid chromatography are discussed (*137*), and a discontinuous recycle method has been described (*950*).

Centrifugal column chromatography has been reviewed (*674*), and separations of sugars (*36*) and steroid hormones (*1156*) achieved in columns of microparticulate silica.

Automatic collection of radioisotopes is obtained using a siphon containing a small floating ^{60}Co source (*1382*). An automatic fraction collector in which the driving and blocking mechanism of the collector consists of couples of magnets has been designed (*90*). A device is described which marks on a recorder chart the points at which a fraction collector changes tubes (*1004*).

A computer program for the acquisition and on-line analysis of data from a high-resolution liquid chromatograph has been described (*239*). An apparatus for converting the signals from a detector into a hologram has been designed (*1405*). Simultaneous multicolumn liquid–liquid and liquid–solid methods with a computerized readout system were used to analyze steroids (*1508*). The method of buffer storage increases the productivity of quantitative liquid chromatographic systems by allowing off-line quantification at a different time than the separation (*685*).

Countercurrent chromatography is a liquid–liquid partition method without a solid support (*619*). Supercritical fluid chromatography uses a supercritical fluid as the moving phase with pressure programming to enable separation of compounds with a wide molecular weight range (*638*). Molecular weights and distributions of oligo-

meric materials are determined by this method (*28*). A simultaneous 25-column, capillary column chromatograph using compressed nitrogen to drive the eluent is superior to a system using pumps (*1509*). Sputtering chromatography is a method for the separation of inorganic cations in which a solution of the cations is placed in a flask attached to an impregnated cellulose column. When the flask is heated at about 100 °C under reduced pressure, the solution boils with bumping and is transferred onto the column to produce a chromatogram (*1606*). A horizontal nylon column packed with cellulose powder, drawing solvent from a constant level reservoir through a wick of cellulose powder, gives improved separations of sugars. The solvent flows out of the column via a second wick (*1024*). Low temperature precipitation chromatography was used to fractionate poly(methyl methacrylate) in a column with temperature cycles (*286*). Electrolytic chromatography involves the use of a column electrode to obtain a separation and localized deposition of metals followed by stepwise dissolution by using controlled-potential electrolysis (*427*). Oxygen-sensitive substances can be successfully chromatographed by anaerobic techniques (*1153*).

The use of high-pressure liquid chromatography on anion-exchange resin (Aminex-27) has been described for UV-absorbing substances from urine (*181*).

Detection and Quantitative Analysis. Developments in the theory, design, and availability of liquid chromatography detectors have been reviewed (*184, 1494–1496*). A miniature photometer for monitoring liquid chromatographic effluents continuously at 2 ultraviolet or visible wavelengths has an effective cell volume of <10 μl (*1421*). A miniaturized ultraviolet flow photometer for continuous monitoring at 254 and 280 nm has also been described (*1422*). Small volume UV photometers exhibit high sensitivity, for instance 0.01 ppm diphenyl (*1104*). A special purpose UV absorption detector was found to be 100 times more sensitive than heat-of-adsorption or refractive-index detectors (*967*). The stability and sensitivity of a UV flow-cell detector was improved by a modification permitting temperature control of the detector and effluent stream passing through the detector cell. The modified detector determined <15 picomoles cyclic AMP in biological materials (*163*). A photometer with a single interference filter which can be tilted to several positions by a cam-operated mechanism may be synchronized with the program of an Auto-Analyzer (*595*).

Derivative formation prior to chromatography increased the sensitivity of the UV detector to 1 ng for steroid 2,4-dinitrophenylhydrazones (*570*). Conversion into detector-sensitive fluoro derivatives after leaving the column was carried out in conjunction with a transport electron capture detector (*865*).

A heat exchanger which causes only slight peak broadening has been described for controlling temperature in a differential refractometer detector (*301*). A commercial differential refractometer was capable of detecting ≧9 ng of sugar in aqueous solution at room temperature (*1353*). A photoelectric line locator was used as a flow-type refractometer in column chromatography (*1022*).

The conventional moving wire liquid chromatography detector functions by wetting the surface of a wire with a thin film of the column effluent. The wire passes into an evaporator to remove the mobile phase and then to a pyrolyzer where the nonvolatile solutes are pyrolyzed. The pyrolysis products are swept by a gas stream into an argon or hydrogen flame ionization detector. A modification (*1246*) which increases the sensitivity of detection to about 1 μg/ml of solute in the mobile phase involves burning the solute in oxygen to form CO_2 and H_2O; the CO_2 and excess oxygen are mixed with hydrogen and passed over a nickel catalyst to convert CO_2 to CH_4 which is then detected by the flame ionization detector. Sugars (*407*) and steroids (*204, 205*) have been analyzed by the conventional transport flmae ionization detector after column chromatography.

Polarographic detectors have been designed for monitoring liquid chromatographic columns (*657, 1393*) and applied to the quantitative analysis of pesticides (*740, 741*). Methylnaphthalenes were analyzed by AC polarography after separation (*147*).

Microadsorption detectors for liquid chromatography (*965, 966*) and for supercritical fluid chromatography (*200*) have been designed. Emission (*1040*), coulometric (*977*), capacitance (*521–523*), and gas density balance (*1125*) detectors have also been applied to liquid chromatography.

A simple radioactivity recording system monitors column chromatographic fractions of ^{32}P-labeled products at lower cost than with a flow-through counting system (*652*). An anthracene packed flow-cell detector mounted in a liquid scintillation counter was used to continuously monitor mercury-203 labeled methylmercury (*277*).

Components separated on a column packed with silica gel containing a fluorescent indicator may be determined by measuring the lengths of the bands visible by ultraviolet radiation (*636*). An apparatus for separating solutes from solvents after chromatography has been described (*1281*). General guidelines for precise quantitative analysis with a stable, high speed liquid–liquid chromatography column have been enumerated (*820*). In this study, a single column was used for more than 12 months.

COLUMN GEL CHROMATOGRAPHY

Books and Reviews. An introductory book on gel chromatography (*392*), general reviews (*39, 558*), reviews of theory (*931*) and peak broadening (*706*), and a review of applications in organic analysis (*877*) have appeared. A general review of gel filtration chromatography (*188*) and chromatography on Sephadex H-20 (*305*) have been published. General aspects of gel permeation chromatography (*123, 135, 206, 207, 789, 1059, 1466, 1585*), theory (*1516*), terms and relations (*124*), calibration of GPC columns (*254*), and polymer characterization by GPC (*76*) have been reviewed.

Theory and Fundamental Studies. Mechanisms controlling salt separations (*93*), effect of sample concentration on the behavior of alkaline earth metal ions on Sephadex columns (*1621*), separation of purines and pyrimidines by adsorption chromatography on Sephadex G-10 columns (*1378, 1557*), band spreading of carbohydrates on Bio-Gel and Sephadex gels (*1105*), interaction of metal ions with Sephadex G-15 columns (*1467*), separation mechanisms in different solvents on Sephadex LH-20 columns (*1364*), effect of anions on the chromatographic behavior of magnesium ions on Sephadex G-15 (*1029*), the relation between column chromatography and electrophoresis of proteins on polyacrylamide gels (*953*), the effect of loading on separation efficiency on Sephadex G-25 columns (*873*), models of gel filtration columns (*426*), a method of boundary analysis for elution profiles in frontal analysis on Sephadex G-200 (*227*), the selectivity of polyacrylamide gels for sugars (*168*), and the influence of solvent strength and temperature in dextran gel chromatography (*167*) have been investigated. A good relation was found between separation of xylodextrans in dextran gel columns and on kieselguhr thin layers, indicating that partitioning effects are an important factor (*169*). Partition effects in addition to exclusion are important in the separation of maltodextrins on polyacrylamide gel (*310*). Particles greater than 400 nm were irreversibly adsorbed on Sephadex, agarose, and cellulose gels (*459*). Boundary spreading during frontal gel chromatography of purified proteins through Sephadex G-100 columns was independent of flow rates and linearly related to the mean elution volume (*574*). Blue Dextran dye used to determine the void volumes of gel columns can be retained at the bottom or top of the gel

bed and can subsequently cause artifacts (*888*).

Studies of the following in gel permeation have been reported: diffusional phenomena in dilute polymer solutions flowing in capillaries (*1044*), the importance of chemical structure on the separation of small molecules (*1027*), instrumental spreading calibration and correction (*1119, 1181, 1182, 1456*), prediction of elution behavior of branched alkanes (*1235*), multiple peak resolution (*1233*), a graphic procedure for obtaining average molecular weights from a chromatogram (*1232*), calculation of long-chain branching distribution from GPC experiments (*1455*), use of fast finite Fourier transforms to solve Tung's equation (*1523*), a rapid iteration method to solve Tung's equation (*1298*), separation mechanisms and theories (*1615*), effect of degree of nitration of cellulose on molecular weight distribution data (*1250*), determination of functionality distributions of carboxy and hydroxy terminated polybutadiene (*800*), axial dispersion of solute zones (*534*), relation between preparation procedure and properties of gels (*562*), correction of zone broadening using a nonpermeating solute (*623*), models of GPC assuming flow through and around each bead (*512*), formation of acceptor–donor complexes in the gel network (*410*), a mathematical method for calculating weight and number functions of molecular weight distributions (*377*), effects of long- and short-chain branching in polymers on GPC results (*342*), thermodynamics of GPC (*199*), estimation of polymer solution intrinsic viscosity from GPC data (*208*), determination of the unperturbed end-to-end distance of polymers (*220*), theory to explain the behavior of micellar solutions (*255*), adsorption isotherms of polystyrene in CCl_4 on macroporous silica gels (*128*), effect of solubility on elution order (*97*), absolute error in determining molecular weight distributions (*96*), and determination of chain transfer constants and some Arrhenius parameters for the first few steps in polymerizations (*74*). Solvents containing 1% of their predeuterated analogs can be used to determine GPC column flow characteristics with differential refractometer detectors (*193*). The elution volume of maltodextrins on polyacrylamide gel exhibited a negative temperature dependence (*311*). Reduction of analysis time in GPC under fixed experimental conditions results in reduced resolution; shorter analysis times at the same resolution are obtained by reducing both column length and gel particle size (*563*). Dispersion arising from permeation is a major contribution to peak broadening for a porous glass (Porasil) packing; for Styragel packing, dispersion due to mobile-phase effects is predominant (*707*). Reliable molar volumes can sometimes, but not always, be calculated from GPC elution behavior of small molecules (*790*). Viscous fingering is a leading cause of peak skewing and broadening (*948*).

In fast GPC, elution volumes are independent of flow rate at ≤ 35 ml/min, and peak width volumes at this flow rate are less than predicted by Van Deemter's equation. The effect of viscous fingering decreases at high flow rates and peak symmetry improves. Increased temperature decreases peak width and improves resolution (*840*). By using very large gel particles, a decrease in the elution volume with flow is observed for polymer molecules in the permeation range of the gel (*839*).

Precipitation chromatography theory was studied by choosing a mathematical model to approximate conditions during the precipitation chromatographic fractionation of a polymer (Baker–Williams fractionation) (*1303*). Organic acids are separated on gelatin columns by this method (*904*).

Column Packings. Swellable polymethacrylates (*561*), agarose derivatives (*1217*), dried agarose gels capable of rehydration (*1151*), benzoylated DEAE-Sephadex (*924*), and hydrophobic long-chain alkyl ethers of Sephadex (*367*) have been synthesized. DEAE-Sephadex A-25 was used for the separation of steroids (*581*) and nucleic acid derivatives (*1145*), Sephadex G-10 (CM) for aflatoxins (*875*), Sephadex G-25 eluted with phenol–acetic acid–water (1:1:1) for acidic plant constituents (*787*), hydroxyalkoxypropyl Sephadex for fat-soluble vitamins (*547*), mercurated dextran for mononucleotides (*499*), and a double column of anion-exchange resin and Sephadex LH-20 for measurement of serum triiodothyronine (*344*).

Porasil and Styragel packings gives approximately equivalent results (*1046*), and Merkogel SI has good properties (*1041*) for high speed gel permeation chromatography. Other suitable packings include porous glasses (*260, 1625*), polystyrene gels prepared by anionic polymerization (*1571*), porous alumina (*49, 50, 1209*), and carbon molecular sieves (*662*). The synthesis of styrene–divinylbenzene (*871, 995*), ethyl(vinyl)-benzene–divinylbenzene, and vinyl acetate–divinyl adipate (*559, 560*) polymeric porous gels has been described.

Calibration Procedures. Tightly cross-linked gel filtration media can be calibrated by a method based on the elution characteristics of Blue Dextran 2000, stachyose, raffinose, maltose, propylene glycol, glycerol, ethylene glycol, and methanol (*475*). GPC columns can be calibrated with polymers with broad molecular weight distributions (*27*), by using an internal standard polystyrene sample (*1584*), with a universal calibration curve obtained by plotting molecular weight times intrinsic viscosity *vs.* elution volume (*954*), using a simple computer approach (*1577*), with unfractionated polymers (*1570*), or by use of log molecular weight *vs.* elution volume graphs (*1627*). The effect of column size, theta conditions, and flow rate on calibration was studied (*1379*).

Apparatus and Techniques. Steric programming was applied by Giddings (*463*) to the chromatography of proteins. Retention was controlled and extended by adding a high molecular weight polymer (*e.g.*, dextran) to the incoming solvent.

Design of high-speed, continuous GPC chromatographs (*71, 504*) and characterization and evaluation of a Pye liquid chromatograph for GPC (*143*) has been made. A system with an upper pressure limit of 2200 psi was designed (*841*). A chromatograph was modified for automatic sample injection and on-line data acquisition (*486*). The use of both UV and refractive index detectors gives qualitative information superior to that obtained with either detector alone (*1196, 1584*). Modifications of a chromatograph can be made to achieve improved precision in determinations of molecular weight averages and polydispersity of polymers (*601*). A recycle device designed to improve resolution in polymer fractionation is based on an alternate pumping principle (*109–111, 346*). Equipment for translating GPC chromatograms to the digital form (*171*) and for simultaneous direct acquisition of data from two chromatograms (*949*) is available. Treatment of glass beads with hexamethyldisilazane at 130 °C reduces adsorption and low recovery of polystyrene from GPC (*622*). IR spectra of GPC fractions were used to identify polymer additives (*594*). A preparative chromatograph incorporating an in-line solvent stripper was used for structural evaluation of copolymers (*72*).

An automated procedure for elution of Sephadex columns with four different solvents has been described (*92*). Modified piston columns are suitable for dextran gel chromatography (*808*). Centrifugal force can be used to accelerate samples through gel columns (*1070*). A small peristaltic pump can be used for ascending gel filtration (*179*). Compressed beds of gel provide increased resolution (*357, 398*). Elution of CM-Sephadex C-50 columns with 0.005*M* aqueous $MgCl_2$ (salting out chromatography) separates the condensates of β-naphthalenesulfonic acid and formaldehyde (*425*), and $AgNO_3$-impregnated Sephadex G-25 eluted with benzene–ethyl acetate–acetic acid–H_2O (11:5:6:10) separates gibberellins (*1518*). Proteins or conjugated proteins are separated by gradient elution of isoelectric precipitates from a gel filtration column (*116*). Stacked and

sectioned columns for preparative gel filtration are described, and problems and applications of large-scale GFC are discussed (*631*). The reproducibility of data obtained by Sephadex G-200 gel filtration of human serum albumin monomer has been reviewed (*577*).

PAPER CHROMATOGRAPHY

Books and Reviews. A new book updating the classic work on paper chromatography by Block, Durrum, and Zweig has been written (*1273*), and a book by Ivor Smith (Interscience Publishers, 1969) concentrates especially on clinical applications of paper and thin-layer chromatography. The *Journal of Chromatography* published an R_F index for Volumes 11–40 (1963–69) in Volume 62(2), November 11, 1971. A general review of paper chromatography (*891*), and a review of temperature gradient paper chromatography (*835*) have been written. Student experiments concerning the paper chromatography of phenols (*1355*) and inorganic ions (*1137*) have been devised.

Sample Preparation Methods. The desalting of urine samples prior to the determination of sugars by paper chromatography is best carried out with the White and Hess system composed of Amberlite IR-120(H^+) and IRA-410(acetate) resins or their monobed mixture Amberlite MB-3 (*1521*). Amino acids (*1198*), phenolic and indolic acids (*1199*), and mucopolysaccharides (*861*) are detected in urine without any pretreatment. Samples are applied to the paper in the form of spots (*861*) or streaks (*1522*). Inorganic ions have been chromatographed as *m*-nitrobenzoic acid complexes (*1259*), acids as substituted benzyl and phenacyl esters, alcohols as *p*-[*p*-(dimethylamino)phenylazo]benzoates, and amines as *p*-[*p*-(dimethylamino)phenylazo]benzamides (*243*) in order to facilitate detection.

Fundamental Studies. The parameters affecting spot broadening were evaluated for Whatman No. 2 paper and Camag cellulose powder, and the dependence of plate height on the breadth of the paper or layer was determined (*309*). Evaporation was found to stabilize solvent velocity in paper and thin-layer chromatography, to have minimal influence on capacity, and only a modest effect on resolution (*1348*). Studies of the mechanism of reversed-phase paper chromatography indicate that lipophilic solutes are partitioned between the organic stationary phase and the mobile phase while hydrophilic compounds behaved as if the separation process involved only the cellulose–water complex and the mobile phase (*446*).

Relations between chromatographic mobility and molecular structure were determined for pyridyl alkyl ketones in systems composed of organic solvent–aqueous HCl (*108*), phenols in liquid–liquid partitioning systems (*1321*), nitrophenols on alumina papers and thin layers (*70*), alkyl phenols on formamide-impregnated paper (*1390*), alkylated phenolic alkaloids in partition systems (*1565*), and phenols in six solvent systems (*1147*). The latter study includes R_F values for 240 compounds in the solvents and color reactions obtained with 15 standard reagents. The relation between mobility and the anabolic activity of steroids was determined (*602*).

The mechanism of temperature gradient paper chromatography was discussed and confirmed (*467*). Simple relations between paper chromatographic R_M values and the composition of liquid chromatography solvent systems were derived (*1315*) and used (*1316*) in selecting an optimum solvent system for column partition separations. The variation of partition coefficient was studied as a function of ligand concentration for several metals in binary systems of the type *n*-amyl alcohol–aqueous NH_4CNS (*1319*). The appearance of multiple spots of chromium was attributed to formation of complex species (*895*). Deviations in R_F values on impregnated papers are caused by nonstandard impregnation, and drying of and impurities in the impregnating agent (*242*). The factors determining the accuracy with which a purity figure can be derived from a paper or thin-layer chromatogram of a radioactive substance were enumerated (*908*).

Chromatographic Systems. Different unmodified papers can give different resolution and speed of development and each should be tested for its quality in a particular application (*626*, *819*). Silica gel and aluminum hydroxide loaded papers provide separation of chloroplast pigments similar to those on thin layers (*1269*). The separation of aromatic amines on carboxymethylcellulose papers was compared to cellulose papers and CM-cellulose thin layers (*1271*). Cellulose phosphate paper chromatography separates the common bases in nucleic acid hydrolyzates (*1193*). The valency states of rhenium were separated on DE-20 ion-exchange paper by development with 4*N* HCl at 0 °C (*1057*). Various metal-ion mixtures were separated on SA-2 and SB-2 ion-exchange paper with mixed aqueous-organic solvents containing mineral acid and a selective extractant (*1272*).

Papers have been impregnated with the following inorganic materials: NH_4SCN acidified with HNO_3 for the separation of metals by development with alcohol solvents (*1320*); 0.02*M* $Na_2B_4O_7$ for the determination of pangamic acid by development with butanol–0.02*M* $Na_2B_4O_7$ (87:13) (*1293*); KCl for the separation of rare earth elements with a developer containing oxine and chloroform (*983*); ammonium tungstosilicate for the separation of uranium by development with dilute $HClO_4$ (*885*); Ag_2CrO_4 for the determination of thiocyanates (*715*); aqueous HNO_3 or $LiNO_3$ for the separation of metals by development with tri-*n*-hexylamine nitrate in various diluents (*401*); $AgNO_3$ for the separation of halogens and thiocyanate (*25*); phosphate buffer for the separation of strychnine and brucine by development with isobutanol–toluene (1:1) (*1586*); 6–8% LiCl for the separation of precious metals by development with 10% HCl in methyl ethyl ketone (*1487*); 2% $(NH_4)_2SO_4$ or NH_4Cl for the separation of alkaloids by development with isobutanol–acetic acid–water (4:4:1 or 20:2:5) (*1418*, *1419*); 4% KCl for the separation of rare earths by development with an acetate buffer containing dioxane and oxine (*982*); HF, HBr, or HI for the separation of metal ions by development with benzene solutions of liquid anion exchangers (*1121*, *1122*), and urea for the separation of fatty acids by development with methanol (*1092*). Addition of traces of NaCl to the developing solvent reduces tailing of antibiotics separated on paper or silica gel layers (*1363*).

Papers have been impregnated with alkaline formamide for the separation of basic drugs (*1493*), formamide or dimethylformamide for the chromatography of glycidyl ethers (*1469*) and oximes (*1449*), formamide or dimethylsulfoxide for chromatography of aromatic *p*,*p*′-diamines (*447*), a mixture of thenoyltrifluoroacetone and tri-*n*-octyl phosphate for the separation of U and Th (*280*), thenolytrifluoroacetone for metal ion mixtures (*291*), bis(2-ethylhexyl)phosphate for metal ions in alloys (*399*), tetrabutyl hypophosphate for actinide elements (*753*), cetyl alcohol for *s*-triazine derivatives (*1063*), and chlorinated paraffin for the separation of trialkyl and triaryl phosphates (*1275*).

Silica gel impregnated glass fiber paper is suitable for the purification of gibberellins (*651*). Chromatography on membrane filters fractionates transfer RNA (*362*) and polyethylene glycols of different molecular weight (*1115*).

Apparatus and Techniques. The techniques and apparatus used for paper chromatography are often applicable as well to TLC. Automatic devices for applying spots or bands of sample (*100*, *334*, *461*), for the starting and stopping of development (*119*, *201*, *716*), and for application of stationary liquid phases (*1491*) have been described.

Development can be carried out at an elevated temperature (*1359*, *1587*) or under the influence of a temperature gradient (*837*) for increased efficiency. Descending development can give better

resolution of amino acids than ascending development (*626*). Circular paper chromatography has been used to separate fungicides (*112*) and alkaloids (*1524*). Phosphates were separated on paper twisted into a spiral (*981*). Diagonal chromatographic techniques (flat bed two-dimensional chromatography or electrophoresis involving a physical or chemical treatment between the first and second dimensions) is useful for detection of artifacts, improved resolution of mixtures, or identification of separated compounds (*528*). The theory and applications of unidimensional multiple development chromatography for the resolution of substances with very close R_F values has been discussed (*1202*), and a graphical method has been presented for determining the number of developments required for the maximum separation of two species by this technique (*469*). In comparison to conventional developing tanks, chromatography on paper strips held in glass test tubes has advantages of simplicity, low cost, and increased speed with some solvents (*1058, 1465*).

A chromatographic ruler in the shape of a right triangle was devised for reading R_F values directly from chromatograms (*1015*), and another device gave readings from circular chromatograms (*45*). A device was described for the direct quantitative transfer of microgram amounts of substances from one paper or thin-layer substrate to another without change in spot shape or size or R_F value (*1531*). Preparative paper chromatography was used to purify compounds labeled with carbon-14 (*378, 379*).

Detection and Identification of Solutes. New detection reagents included chloroplatinic acid for detecting sulfur compounds on starch-impregnated paper or silicic acid thin layers (*1593*); dipping in 3% vanillin + 0.5% H_2SO_4 in ethanol, heating at 90 °C, and dipping in 2.5% $AgNO_3$–0.5% bromophenol blue in acetone (1:1) for adenine compounds (*1596*); mercurated fluorescein for cations (*1599*); 1% 2,4,6-trinitrophenol in 95% ethanol followed by drying and treatment with 5% KOH in 80% ethanol for catechols (*1440*); benzoquinone for amino acids (*852*); 2-nitro-1,3-indandiones for proline, hydroxyproline, and ornithine (*361*); oxidized-hematoxylin reagent for metal ions (*330*); and dimethylaminobenzaldehyde reagent for sulfonamide groups (*154*). A comparative study of oxidizing agents for detection of thiamine was made (*612*).

In addition to characteristic colors formed with chemical reagents, identification of spots can be made by use of summarized chromatograms (*1324*), spot elution followed by spectrophotometry (*483*), outline/unit areas (*1106*), mobility–structure correlations (*1148*), mass spectrometry (*1365*), mass spectrometry plus IR spectrometry (*1386*), and mass spectrometry plus nuclear magnetic resonance spectroscopy (*405*). Detection can also be made by X-ray fluorescence (*600*) and by autoradiography or α- or β-counting for radioisotopes (*851*).

Quantitative Analysis. Visual comparison of spot area after spraying with Aluminon reagent was used to analyze for copper and aluminum (*1367*), and direct densitometry of acids was carried out after spraying with bromocresol blue (*1013*). Nonlinearity effects in optical scanning due to a finite spatial or spectral width of the illuminating window was treated mathematically (*1096*).

Colored derivatives formed on the paper by chromogenic reagents may be eluted and determined photometrically (*1476*). Drugs could be eluted from paper chromatograms stored for several years (*142*). Polyphenols were estimated by location with $FeCl_3$–$K_3[Fe(CN)_6]$ spray reagent, decolorization with 1% Na_2CO_3, treatment of cut-out areas with Folin–Denis reagent and saturated Na_2CO_3, and subsequent colorimetry (*1262*).

Electrometric determination of alkaline earth metals was made during ascending development (*238*). Kinetic methods were used to analyze ruthenium (*1486*) and iridium (*1614*). Cobalt in steel was determined by equilibration with cobalt-60, separation by paper chromatography, autoradiography, and densitometry (*955*). A computerized automatic scanner for bidimensional radiochromatograms was described (*217*). Radionuclides on paper may be simultaneously eluted and electrodeposited on a planchet for α- or β-counting (*1051*).

THIN-LAYER CHROMATOGRAPHY

Books and Reviews. Volumes 1 and 2 of a series of books recording progress in TLC, edited by Niederwieser and Pataki, were published (*1011, 1012*), and also a book on techniques and applications in organic chemistry (*1504*). A second, revised and enlarged edition of Stahl's laboratory handbook was published in 1969 (Springer-Verlag). General reviews (*321, 801, 1345, 1483*) and reviews of applications for the organic chemist (*1124*), theory (*1384*), thin-layer densitometry (*814*), instrumentation (*853*), vapor-programmed TLC (*322*), and combination of planar chromatography with column chromatography (*629*) have been written. A laboratory experiment for student use concerning the TLC separation and spectrophotometric analysis of *o*- and *p*-nitroaniline was described (*606*).

Thin-layer chromatography abstracts, a bimonthly publication from Science and Technology Agency, London, was first issued in January 1971.

Theory and Fundamental Studies. Snyder's theoretical study (*1312*) indicates that the order of decreasing performance in the separation of complex samples is gradient layer TLC > gradient elution TLC > polyzonal TLC. The mechanisms of separations with activity gradients were explained (*453*). The solvent vapor was found to have a large influence on TLC separations (*1400*). The mechanisms of separations with unsaturated chambers and gradient elution were discussed (*325, 452*).

Studies of mobility–structure relations included the following compounds: polycyclic hydrocarbons and heterocyclics chromatographed with different kinds of solvents on silica, alumina, and kieselguhr layers (*1536, 1539, 1540*), cephalosporins chromatographed by reversed-phase TLC (*106*), nitrophenols (*69*) and methylated phenols (*68*) on cellulose impregnated with polyamide, alkyl and halogen substituted aromatic ring compounds (*1073*), Strychnos alkaloids (*1079*), phenols on amide-impregnated cellulose (*481*), and aliphatic acids on cellulose layers (*505*).

Numerous other fundamental TLC studies have been made: The separation of ion pairs in reversed-phase systems (*497*); empirical formulation of multicomponent solvents (*847*); temperature effects during development (*1052*); the effects of various factors on precision (*793*); derivation of an equation relating the R_M value of a proton-donor solute with the composition of a binary solvent system of the type electron donor solvent plus neutral diluting solvent (*1317*); determination of maximum sensitivity in microthin-TLC (*84*); characterization of thin-layer systems using plate numbers (*266*); use of aluminum oxide of varying activity for the determination of chromatographic spectra of quinolines and phenols (*1535*); application of silica gels with different microporous structures for naphthalene separations (*1533*); influence of adsorbent and mobile phase on spot areas (*1534*); comparison of trace metal separations on silica gel to paper chromatographic separations (*925*); estimation of partition coefficients of phenothiazine derivatives from reversed-phase R_M values (*914*); and methods for studying chemical kinetics by use of programmed temperature pyrolysis combined with TLC (*1168*). R_F values on silica gel layers are related to polymer molecular weights (*83*). The geometric index (Ig), which equals the spot height-to-width ratio, is useful in combination with R_F values for predicting degrees of separation (*488*). R_F values depended linearly on the amount of stationary phase in both conventional and reversed-phase thin-layer partition separations of aldehyde 2,4-dinitrophenylhydrazones

on kieselguhr (*326*). The R_F correction method devised by Galanos and Kapoulas for paper chromatography, which makes use of two reference R_F values, is valid for TLC (*329*).

Sample Preparation Methods. Recoveries of individual amino acids differed with the desalting procedure used prior to TLC. Desalting improved resolution, but remaining peptides may interfere with the analysis of the basic amino acids. Desalting with Bio-Rad AG 11A8 resin was most efficient and removed peptides to give a reproducible and quantitative recovery of amino acids (*548*). Desalting of untreated urine can be carried out on a plate that supports both a strongly-acidic cation exchanger and a cellulose layer. Urine is applied to the ion-exchange layer and strong acids and neutral substances are removed with water. The amino acids are then chromatographed into the cellulose layer and separated there two-dimensionally (*764*). Preparation of two-layer plates for amino acid cleanup has been described (*915*). Multiband plates are also used for cleanup of vegetable extracts for pesticide analysis by TLC (*788*). Desalting of urine can be achieved on SA-2 ion-exchange paper prior to the TLC detection of drugs (*75*).

Frozen tissue sections may be applied directly to silica gel plates for lipid analysis (*228*). Amino acids in blood have been chromatographed after direct application of untreated serum or blood-impregnated paper disks onto the layer (*278, 1023*). Amino acids in urine are screened on cellulose foils without sample preparation (*373*).

The following derivatives have been formed to facilitate TLC: 4′-hydroxyanilide derivatives of aromatic sulfonic acids (*1612*); 4-acetyl-2-nitrophenyl derivatives of phenols (*1582*); metal thiocyanates (*1501*); the 2,4-dinitrophenylhydrazone of tetrahydrobenzaldehyde for air pollution analysis (*1451*); dithizonates of mercury and alkylmercury compounds (*1397*); trifluoroacetylacetone rare earth chelates (*1411*); azo dyes formed from cresols, phenols, and naphthols (*1427*); 2,4-dinitrobenzyl and *p*-(dimethylamino)benzeneazophenacyl esters of fatty acids (*246*); diethyldithiocarbamates of metal ions (*1133, 1134*); 2,4-dinitrophenylthio derivatives of indoles (*1136*); azobenzene-4-sulfonic acid esters of estrogens (*1062*); trinitrophenyl derivatives of amino acids in plasma and urine (*1082*); *p*-bromoanilides and *p*-toluidides of lower fatty acids (*803*); acetyletiocholanolone dansylhydrazone (*479*); chlorophenyl fluorosulfonyl benzenesulfonates (*395*); and dansyl estrogens (*348*). Hydrazone derivatives of steroids (*834*) and 2,4-dinitrophenylhydrazones of phenolic aldehydes (*424*) may be formed along the starting line directly on the plate.

Chromatographic Systems. Many different types of layers have been employed in addition to the usual homemade 250-μ silica gel layers: charcoal with polyethylene binder (*1118*); magnesium oxide for lipids (*693*), wax esters (*1007*), sterols (*688*), and plant constituents (*687*); commercial precoated cellulose and silica gel sheets for aflatoxins (*1149*) and trace metals (*926*); silicic acid mixed with glass fibers and cationic starch (*872*); porous glass for organic sulfur compounds (*1590*) and for steroids, drugs, and alkaloids (*887*); 5-μ silica gel layers on cellophane sheets (*886*); microcrystalline cellulose for metal–EDTA complexes (*896*); silica gel with added kaolin and montmorillonite for clay minerals (*690*); magnesium hydroxide for azaaromatic compounds (700); pH-gradient layers for phenothiazines (*769*); ZnS for metal complexes (*776*); Synachrom porous organic polymer beads developed with solvents of varying pH for quinolines and phenols (*633*); prefabricated roll preparations of silica gel and cellulose for steroids, amino acids, and dyes (*532*), polyamide for flavonoids (*1462*), estrogens (*597*), and amino acid dervatives (*1154*); silica gel fiber glass sheets for serum barbiturates (*618*) and steroids (*1247*); superfine Sephadex G-25 to G-200 for dansylated proteins (*441*); calcium carbonate with gypsum binder for morphine and morphinan derivatives (*350*); cellulose layers on aluminum foil for amino acids (*373*); poly(vinyl acetate) for 2,4-dinitrophenylhydrazones (*328*); silica gel impregnated with basic lead acetate for sugars, anthocyanins, and anthocyanidins (*1081*); cellulose mixed with cortical cells from wool for tannin extracts (*1188*); silica gel containing 5% starch and 5% Na_2CO_3 for inorganic anions (*696*); silanized silica gel for *p*-hydroxybenzoic acid and its esters (*1140*); 4:1 microcrystalline nylon Aviamide – 6 - polytetrafluoroethylene Fluoroglide 200 mixed layer on Mylar for hydrocarbons (*1128*); silica gel containing 2% disodium EDTA for xanthones (*43*); silica gel impregnated with 15% thallium nitrate for monoterpene hydrocarbons (*56*); silica gel containing silver nitrate for Cannabis constituents (*494*), pyridine homologs (*1389*), triglycerides (*151, 180*), and amines and carboxylic acids (*1388*); keratin layers for inorganic ions (*155*); esterified keratin for DNP-amino acids and heterocyclic bases (*156*); kieselguhr G + 10% Dowex 50W-X8(K^+) + 4% potassium acetate for anions (*157*); silica gel G + 10% Dowex 50-X8(K^+) + 2% potassium acetate for organic base polythionates (*1304*); silica gel–aluminum oxide (1:1) for heptachlor and heptachlor epoxide (*175*); silica gel buffered to pH 8 for phospholipids (*177*); silica gel impregnated with paraffin oil for nonionic surfactants (*272*); polyamide–silica gel layers for indicator dyes (*237*), water-soluble vitamins (*235*), and fat-soluble dyes (*234*); polyamide–kieselguhr G (2:1) for antioxidants (*236*) and antipyretics (*233*); silica gel impregnated with 0.5% triethanolamine for dansyl hydrazine derivatives of ketosteroids (*226*); cellulose–silica gel (2:3) for nucleosides, purines, pyrimidines, and vitamins (*1444*), for nonvolatile organic acids (*222*), and for L- and D-amino acids following reaction with specific oxidases (*898*); starch for cations (*191*) and organic acids (*190*); acetylated cellulose for polynuclear hydrocarbons (*1215*); silica gel impregnated with oxalic acid for mycotoxins (*1350*); calcium carbonate impregnated with sodium acetate for carbohydrates (*1338*); silica gel–potassium carbonate for phenols, cresols, and naphthols as diazo coupling products (*1428, 1437*); talc-fluorescein for glycosides (*1406*); cellulose or kieselguhr impregnated with 5% tetralin for carbonyl 2,4-dinitrophenylhydrazones (*1401*); calcium sulfate for benzimidazole derivatives (*1472*); acetylated polyamide for quinones (*1548*); silica gel impregnated with urea for hydrocarbon waxes (*331*); silica gel bound with agar-agar for inorganics (*1588*); silica gel–zinc dust for tetryl and related compounds (*1613*); silica gel impregnated with cadmium sulfide for aromatic amines (*1611*); a strip of silica gel or silica gel–cellulose placed between acetylated cellulose strips for two-dimensional separation of phthalodinitriles (*901*); a cellulose layer and an anion-exchange layer for detection of iodide (*445*); silica gel–alumina–kieselguhr (1:1:1) for alkaloids (*465*); kieselguhr impregnated with McIlvain buffer containing 5% glycerol to pH 3.7 for tetracycline antibiotics (*1026*); silica gel impregnated with DMF for Cannabis constituents (*918*); wedge-shaped Chrom AR sheets impregnated with copper for hexosamines (*894*); silica gel impregnated with oleylamine for separating K^+ and Na^+ (*909*); silica gel impregnated with Alamine 336-S oxide for metal ions (*813*); cellulose impregnated with polyethylenimine for catechol amines (*763*); cellulose impregnated with Amberlite LA-2 and developed with HCl-NH_4CNS solutions for metal ions (*777*); silica gel impregnated with bis(2-ethylhexyl)phosphate for rare earth elements (*575*); silica gel sheets treated with diethylamine for cannabinoids (*492*); cellulose impregnated with counter ions for chromatography of ion pairs (*496*); silica gel buffered to pH 11 for coumarins (*282*); and cornstarch layers coated with liquid paraffin for mercury dithizonates (*1397*). Homemade layers were compared with commercial flexible precoated layers for a variety of applications (*95*). The uses

of poly(tetrafluoroethylene) layers for TLC have been reviewed (*1127*). Adsorbent contamination has been blamed for the appearance of two lecithin spots on silica gel plates (*620*).

Solvents giving stepwise pH changes on cellulose plates were used to separate semi-xylenol orange from xylenol orange (*1605*). Bush solvent systems devised for paper chromatography are applicable to steroid separations on silica gel plates (*1563*). Development of silica gel plates with EDTA separates noble metals (*1528*). Development of poly(tetrafluoroethylene) layers on Mylar with 0.5*M* bis(2-ethylhexyl)hydrogen phosphate separates metal ions (*1129*). Azeotropic mixtures were used as solvents for the separation of local anesthetics (*1165*). Investigation of these solvents by GLC before and after they were used several times showed no change in their composition if >15% polar component was present (*1164*). Diagrams relating R_F values to the composition of three-component mobile phases can be used to optimize separation conditions for sample mixtures (*1537*).

Patents have been issued for the following: a glue-free cardboard support fixed on an Al or Sn sheet (*1286*); a plastic sheet support bonded to an aluminum sheet (*1287*); a process for binding layers onto supports using a binder material which can be converted by thermal decomposition to a partitioning adsorbent after application, in the layer, to the support (*1220*); a polyethylene binder for preparing layers on polyterephthalate supports (*1251*); alkali metal silicate binders for TLC plates (*1260*); coating a polymer support with TiO_2 on ZrO_2 before covering with a silica gel layer (*533, 1167*); silicic acid-loaded fiber matrix sheets (*1087*); potassium salts of various acids as binders for silica gel (*555*); polyacrylate resin dispersions as binders (*862*); sintered chromatographic plates (*1035*); dual-thickness plates (*306*); coarse, uniform metal plates (*268*); and plates with alternate transparent and nontransparent segments (*1231*).

Apparatus and Techniques. Automatic and semi-automatic devices for applying spots and streaks for analytical and preparative TLC have been desdribed (*29, 406, 411, 730, 881, 903, 968, 975, 1545, 1546*). Stahl designed a cartridge for application of volatile samples onto thin-layer sheets (*1332*). A device for the direct coupling of a column to a TLC plate was developed (*1482*). Apparatuses for preparative and quantitative (*216*) and descending preparative (*641*) TLC have been described, as well as an adsorbent extractor (*539*) and continuous rotating disk apparatus (*1458*) for preparative TLC. Improved chambers (*825, 991, 1396*), sandwich microapparatus (*316, 317*), and an automated tank (*1229*) have been designed. Portable units including solvent stored in a breakable capsule (*57*) or in a semisolid form (*1441*) have been described. A semiautomatic scraper for precoated plates allows fast, accurate zone separation (*689*).

Drum TLC (*1210*) is a procedure allowing unlimited migration of a pair of sample bands along a TLC bed at constant, high, solvent flow rates. Total resolution is increased by an order of magnitude compared to other TLC methods, or equivalent separations can be carried out in a much shorter time. Vapor-programmed TLC is a method suitable for the separation of closely related compounds which are inadequately separated by classical TLC (*320*). This approach has been discussed (*1009*) and applied to the separation of sterols (*1579*) and aldehydes (*1375*). Gradient elution TLC has been surveyed (*1008*) and applied to the chromatography of lipids (*1414*) and polymers (*669*). TLC has been carried out on layers with pH gradients (*1334*) and thickness gradients (*81*). Improved separations result from using single-component solvents in an unsaturated chamber (*323*). Chromatography on extra-thin films of silica gel provides increased sensitivity in the detection of drugs (*162*). Thin evaporated films of In_2O_3 and etched, anodized aluminum surfaces permitted detection of separated compounds at the 10^{-10}–10^{-16} gram level (*267*). Narcotics in urine were detected at the 500 pg level using mini-thin layer plates developed for 1–3 min with 1–2 ml of solvent (580).

Sugars have been separated by descending development on cleaned aluminum foil (*287*). Unidimensional multiple development with different solvents (*13, 1205*) or with the same solvent (*1578*) can provide increased resolution. DDT formed multiple spots with multiple development and intermediate exposure to UV light (*115*). Equipment for continuous TLC has been described (*541, 1097*). Adsorption of solvent vapor prior to continuous development proved advantageous (*542*). Nucleosides (*849*) and polar lipids (*1190*) were separated by development at right angles with two different solvents. Planavin herbicide was separated by developing twice in the first dimension and once in the second (*182*). Monocarbonyl dinitrophenylhydrazones were developed on 1:1 MgO–Microcell T-38 plates once with the first solvent and twice in the same direction with a second solvent, followed by impregnation with Carbowax 400 and development in the second dimension with a third solvent (*265*). Organophosphorus pesticides are detected with more specificity when they are oxidized with bromine vapor after one-dimensional development on silica gel before development in the second direction (*443*). Circular TLC has been evaluated for microanalysis (*544*) and used to separate aflatoxins (*1150*). Horizontal TLC was used in the determination of testosterone in human urine (*856*). Three-dimensional TLC, in which the directions of the second and third developments are at 90° and 180° angles to the first direction, separates complex mixtures (*640*). Very long development paths are achieved by using Z-shaped layers on 20 × 20 cm cm plates (*639*). Conical TLC is carried out on frustums coated with silica gel (*1264*). Spotting samples on a plate between parallel vertical pencil lines causes rectangular instead of elliptical spots to be formed during development; it is claimed that R_F values are thereby easier to calculate (*1002*). Steroid R_F values increase linearly with development temperature (*1289*). Chromatography under the influence of a temperature gradient separates some mixtures having nearly equal R_F values by isothermal TLC (*836*).

Light-sensitive compounds such as porphyrin methyl esters are developed in the dark (*366*), while Vitamin A is developed under nitrogen to avoid destruction (*62*).

Tropinone was reduced with a $NaBH_4$ spray reagent at 50 °C after being spotted on a silica gel layer; chromatographic development separated the reaction products (*1093*). Tetracyclines are determined by spraying spotted plates with buffered disodium EDTA prior to development (*842*). Steroid biotransformations are investigated by using glucose-treated silica gel plates spotted with fungal spores and steroid substrates (*526*).

Preparative TLC of purines was carried out on ready-made cellulose plates (*339*). Plastubol spray can be used to preserve chromatograms for documentation files (*1385*). Aflatoxins are separated on silica gel coated glass cylinders more quickly than by conventional TLC (*868*). A simple method for the analyses of clinical samples consisting of amino acids, phenolic acids, and sugars utilizes thin-layer chromatography combined with electrophoresis in two-dimensional analysis (*375*).

Miscellaneous chromatographic techniques including thin-layer chromatography and gas chromatography as well as spectrographic methods aid in the investigation of small samples (less than 1 mg) of art objects for preservation and restoration (*956*).

Detection and Identification of Solutes. The following chemical methods have been described for solute detection: Saturated aqueous $KMnO_4$ for detecting dimethylurea herbicides (*850*); an improved starch–iodide method for imides (*717*); different ninhydrin sprays for combined use in detecting amino acids (*770*); HCl gas

plus UV light for steroids (*637*); gaseous formaldehyde for tryptophanyl–dipeptides (*529*); fuchsine dyes for hydrophobic organic compounds (*490, 491, 1295*); $AgNO_3$-treated silica gel sheets for Cannabis constituents (*493*); addition of $AgNO_3$ to the solvent to facilitate detection of DDT (*389*); pyrrole as a universal reagent (*360*); azoresorcinols and naphthols for metal ions (*925*); disodium chromotropic acid for the fluorescent visualization of trioses (*993*); perchloric acid for sugar derivatives (*985*); diphenylamine HCl as a general pesticide reagent (*1071*); methyl yellow for phosphate pesticides (*1130*); Co(II) for amine derivatives (*18*); 1,2-dichloro-4,5-dicyanobenzoquinone as a fluorogenic reagent for S-containing compounds (*88*); HNO_3 for methylated phenolic compounds (*105*); iodide–iodate–amylose reagent for carboxylic acids (*215*); iodine vapor followed by *o*-tolidine for organochlorine pesticides (*1374*); primuline for steroids (*1597*); and 4-acetamidobenzaldehyde thiosemicarbazone–H_2SO_4 for steroids (*1632*). A copying procedure allows the nondestructive detection of monoterpenes: the developed layer is covered with a second identical layer and heated so that part of each spot is transferred to the second layer for treatment with a chromogenic reagent (*892*).

Detection of lipids was achieved by charring on silica gel layers impregnated with ammonium sulfate prior to chromatography so that H_2SO_4 was evenly generated upon heating (*1543*). Noble metals are detected by heating the chromatogram directly with the oxidizing flame of an aerated Bunsen burner (*1607*). Cholinesterase inhibiting phosphate and carbamate pesticides are detected in the subnanogram range by enzymic methods. Bovine liver esterase (*451*) or bee-enzyme solution (*372*) can be used in conjunction with various fluorogenic spray reagents (*1238*). Esterase specificity and selectivity has been evaluated (*912*). Plasma cobalamins (*829*) and streptomycins (*553*) are detected bioautographically. Hydrocarbons (*1128*) and oligonucleotides (*1169*) were detected by 77 °K luminescence. Estrogens in urine were detected using an automatic conductivity detector (*1001*).

Autoradiography combined with thin-film chromatography detected 10^{-11} gram of ^{14}C-labeled amino acids (*269*). An autoradiographic cassette was described for obtaining radiochromatograms on black-and-white or color Polaroid film without conventional photographic processing (*1439*). Addition of scintillators to adsorbents allows luminescence detection of β-emitting radionuclides (*795, 1120, 1492*). Methods and equipment for automatically scanning radiochromatograms have been described (*448, 1575*).

The following methods have been used for characterization and identification of solutes: TLC plus gas chromatographic retention volumes for alkyl iodides (*202*); a numerical analysis method for identifying a trace amount of a substance overlapped by a large amount of another substance (*1457*); derivative formation for confirmation of dieldrin residues (*225*); use of R_M values on different layers for sugars (*59*); chromatographic spectra for aromatic compounds (*1538*); mass spectrometry for air pollutants (*869*), amines (*1624*), and aflatoxins (*520*), and mass spectrometry plus gas chromatography for steroids (*164*). Autotransfer chromatography combined with mass spectrometry was used to characterize pyrroles and indoles (*614*). A rapid transfer system for infrared spectroscopy involves removal of zones from the chromatogram, elution through a KBr microcolumn, and pressing of the KBr zone into a disk (*1335*). Procedures for properly handling TLC spots for subsequent IR examination have been enumerated (*35*). Infrared and ultraviolet spectrometry have been used to identify furan nitration products (*1500*) and Baytex insecticide (*1369*).

A simple device for collecting spots and eluting compounds for examination by spectroscopy has been described (*251*). Drugs must be eluted for positive identification by other means (*262*). TLC on silica gel allows assignment of relative configurations of some aliphatic diastereomeric compounds (*1048*).

Quantitative Analysis. Quantitative procedures in TLC have been reviewed (*1257*). The equation stating that spot area is a linear function of the logarithm of the material present was examined for use in visual *in situ* analyses (*139, 1072*). An automatic planimetric measuring technique for fluorescent bands was described (*170*). Flying-spot scanning densitometry for quantitative analysis was achieved with a commercially available densitometer by oscillating the thin-layer plate while the optical assembly is moved with a constant velocity in a perpendicular direction (*749*). Elution-spectrophotometric methods were compared with densitometry for determining glyceryl nitrates (*1187*). The elution method was used to determine trimethoxybenzoic acid (*608*), antioxidants (*1322*), sugars (*1241*), bile acids (*173*), and DDT (*389*). Direct spectrophotometric methods have been widely used. The instrumentation and techniques for scanning chromatograms has been reviewed (*854, 962, 1447*), and various instruments have been designed or evaluated (*11, 468, 709, 937, 1055, 1226, 1237*). Double-beam difference photodensitometric scanning was studied theoretically (*1095*). Reproducibility of direct spectrophotometry and fluorimetry (*1635*) and effects of adsorbent properties and sample application methods on remission measurements (*653*) were investigated. Plates can be photographed with Polaroid projection film which is then scanned by a normal recording densitometer–integrator (*703*). A computer program (*783*) makes possible the direct calculation of percentage values on the basis of measured reflectance values. *In situ* densitometric and fluorescence methods were applied to lipids (*14, 1163, 1519*), dinitrophenylamino acids (*146*), estriol (*1445*), aflatoxins (*1098*), LSD (*1017*), alkaloids (*899*), carbohydrates (*818*), TAR (*413*), acridine (*416*), porphyrins (*340*), and catechol amine (*349*).

A disposable, suction device collects samples from plates prior to analysis by gas chromatography (*826*). Nitro compounds (*584*), organophosphate pesticides (*1517*), estrogens (*1448*), polynuclear hydrocarbons (*160*), and metal chelates (*60*) were all analyzed by gas chromatography following TLC and elution. Lipids separated by TLC were methylated without elution from silica gel and the resultant methyl esters analyzed by GLC (*607, 1454*). The total-spot injection technique allows GC analysis without prior extraction of the solid phase. The spots are located by UV irradiation and scraped from the plates into melting point tubes. The sealed tubes are introduced into an ampoule crusher injection port of a gas chromatograph (*304*).

TLC has been combined with ring colorimetry for the microanalysis of platinum metals (*649*) and other metals (*645, 646, 648*). Emission spectroscopy after elution was used to determine alkaline earth metals (*415*). Polarographic determination of pesticides following TLC was carried out (*175, 1253*). Isotopic dilution analysis combined with preparative TLC was used in pharmacokinetic studies (*1224*). A flame ionization detector was adapted to scan TLC strips for the quantitation of organic compounds (*1383*).

The liquid scintillation radioassay of thin-layer chromatograms (*1308*) and the radio-TLC of weak β-emitters (*390*) have been reviewed.

A new method for quantitative detection in TLC makes use of a support consisting of silicic acid plus an oxidant, *e.g.*, CuO, coated on the inner wall of a cylindrical glass tube. Chromatograms are developed and solvent is evaporated. The tube is placed in a closed gas circuit, where a thermal conductivity detector monitors the outlet *vs.* inlet carrier gas (He). A zonal furnace scans the TL tube, whereby organic solute zones are converted *in situ* to CO_2 and H_2O. The H_2O is trapped in a tube of P_2O_5, and the CO_2 is monitored (*519*).

APPLICATIONS

Acids and Related Substances. Quantitative paper chromatography

of fatty acids, dicarboxylic, hydroxy, and keto acids has been reviewed with hundreds of references (*692*). Preparatory silica-gel chromatography has been applied to the isolation of pure lower fatty acids arising from Schmidt degradations and permanganate oxidation of olefins (*994*). Reversed-phase paper chromatography was successfully applied to the separation and identification of carbon-14-labeled long-chain fatty acids (*540*). Various esters of fatty acids have been separated by chromatography: phenyl esters of fatty acids by thin-layers of silica gel G (*616*); *p* - (dimethylamino)phenylazophenacyl esters by paper chromatography (*247*); sucrose esters by gel chromatography on Sephadex LH-20 (*747*). 12-Aminostearic acid from the reductive amination of 12-oxostearic acid was monitored by thin-layer and gas-liquid chromatography (*408*). Carboxylic acids from petroleum were isolated as one fraction by gel permeation chromatography (*253*), while several hydroxy acids from soluble tars were identified by paper chromatography (*621*). Organic acids have been identified by thin-layer chromatography, *e.g.*, methylmalonic acid from urine (*511*); citric acid components (*980*); metatartaric acid from wines (*1340*). Liquid–liquid partition chromatography on silicic acid resulted in high-resolution of organic acids from biological samples (*1183*). Orotic acid (*1576*), phorbic acid and piscidic acid have been detected by thin-layer chromatography (*774*). Natural auxins, mainly derivatives of indoleacetic acid have been separated and identified by chromatographic techniques: a new natural auxin, 4-chloro-indolyl-3-acetic acid by paper- and thin-layer chromatography (*883*); tryptamine, β-indolepyruvic acid, β-indoleacetic acid, and indole from plants by thin-layer chromatography on kieselgel G and silica gel (*1155*); the quantitative determination of IAA by paper chromatography and thin-layer chromatography on silica gel and cellulose (*1032*); diffusable IAA from corn and oat coleoptiles by thin-layer chromatography (*1033*).

Pyrrolidonecarboxylic acid has been determined quantitatively from tomato juice by paper chromatography, using densitometry of the spots colored with bromphenol blue (*627*). Semi-quantitative determination of sulfonate esters in grapes, grape juice, and wine has been accomplished by thin-layer chromatography using an ammoniacal silver nitrate spray (*472*). Diastereoisomers have been separated and studied by chromatographic techniques: derivatives of α-hydroxy-β-(3-indolyl)butyric acids by thin-layer chromatography (*927; 1111*); separation of *cis*- and *trans*-aconitic acids by thin-layer chromatography (*1042*). Substituted esters and diamides of malonic acids have been studied by thin-layer chromatography on silica gel F (*1544*). The estimation of 3,4-dihydroxybenzoic acids and methylated metabolites in urine of schizophrenic patients and controls by thin-layer chromatography showed no differences (*1114*); a batch process involving adsorption and elution from alumina has been described for the simultaneous analysis in neural tissue of dopamine, 3-methoxy-4-hydroxyphenylacetic acid and 3,4-dihydroxyphenylacetic acid (*1325*).

Alcohols. Lower carbon-chain aliphatic alcohols have been separated by paper- and thin-layer chromatography as xanthates (*797*); 3,5-dinitrobenzoates to detect fusel oil impurities in ethyl alcohol (*52, 1480*); colored esters by reacting with *p* - [(dimethylamino)phenylazo]benzoyl chloride (*244, 245*). Neutral diol plasmologens have been synthesized and chemically characterized by chromatography (*767*). Polyols have been separated by TLC on alumina layers (*145*).

Alkaloids. The main metabolite from cannabis in urine, 7-hydroxy-Δ′-tetrahydrocannabinol, was extracted, dehydrated to cannabinol with *p*-toluenesulfonic acid, and examined by thin-layer chromatography (*943*). Marihuana was examined for tetrahydrocannabinol, cannabinol, and cannabidiol by thin-layer chromatography (*192*). A method has been described to detect opium contamination in tea and to distinguish it from caffeine, using thin-layer chromatography (*149*); morphine has been separated from opium using column chromatography on neutral alumina and following the elution by thin-layer chromatography (*509*). The thin-layer chromatography of caffeine, theophylline, and theobromine has been reviewed with particular reference to the pH of the layers (*768*). The direct quantitative evaluation of opium alkaloids by thin-layer chromatography has been accomplished using reflectance and fluorescence measurements (*1166*); belladonna alkaloids have been determined quantitatively following thin-layer chromatography by elution of the spots (*1047*) and direct densitometry of the thin-layer plates (*1279*). Caffeine and related methylxanthines have been separated by thin-layer chromatography (*554*); similar techniques have been adapted to the separation of seven tropane alkaloids (*1569*).

The quantitative estimation of quinine from the Cinchona bark has been accomplished by column chromatography on alumina, followed by separation and elution of thin-layer chromatograms (*126*); four principal alkaloids of cinchona have been detected by thin-layer chromatography (*38*). Direct spectrophotometric and fluorescence determinations of thin-layer chromatograms of digitoxin are applicable to other digitalis alkaloids (*338*); saturated and unsaturated steroidal alkaloid glycosides can be separated by thin-layer chromatography of the brominated addition compounds (*383*) and quantitation was achieved by direct photometry of the spots stained with phosphomolybdic acid. Seven ergotoxine alkaloids have been separated by thin-layer chromatography in two solvent systems (*462*). Paper and thin-layer chromatography were utilized to separate different pyrrolizidine alkaloids and their hydrolysis products (*848*).

Amino Acids. Many of the recent advances in the chromatography of amino acids have been in the thin-layer technique using cellulose as adsorbent material; the second edition of Pataki's book on "Techniques of Thin-Layer Chromatography in Amino Acid and Peptide Chemistry" has been published in English (*1054*), and a review by the same author has also appeared which has not been reported in this space previously (*1053*). A study on different solvent systems for the paper chromatographic separation of amino acids showed that isobutyric acid–water and isopropanol–pyridine–water–acetic acid gave best results for accuracy of quantitative determinations (*858*). Two-dimensional thin-layer chromatography using cellulose powder and the solvent systems isopropanol–formic acid–water (40:2:10, v/v); *tert*-butanol–ethyl methyl ketone–91% ammonia–water (50:30:14:6) produced good results although alanine and γ-aminobutyric acid overlapped (*1030*); another two-dimensional thin-layer chromatographic system has been described (*773*). A novel *in situ* desalting technique utilizes cellulose–thin-layer plates having an Amberlite 120 (H^+) band at the starting line (*261*). Direct reflectance densitometry at 490 nm (amino acids) and 620 nm (imino acids) after ninhydrin-Cd and isatin-Cd treatment, respectively, was used for quantitative evaluation of two-dimensional thin-layer chromatograms (*551*). Copper complexes of amino acids have been resolved by paper chromatography with dioxane–water (4:1, v/v) (*677*).

Micro-column liquid–liquid chromatography has been utilized to separate aromatic amino acids as cations partitioning with chloride and perchlorate ions and the liquid organic phases such as 1-pentanol or cyclohexane (*1067*). Liquid–solid open glass capillary column chromatography can be used to separate 1-dimethylamino-5-naphthylsulfonyl (DNS)–amino acids (*1020*). Separation of amino acid racemates has been accomplished by paper adsorption chromatography (*1567*). Amino acid tetrazole analogs have been separated

on paper and silica gel thin-layer plates (*503*).

Newly described detection reagents for amino acids and derivatives after paper or thin-layer chromatography include copper acetate–4-amino-4′-methoxydiphenylamine (*779*); modified Dragendorff reagent (*1461*); ninhydrin–collidine reagent for phenyl thiohydantoins of amino acids (*1180*); sequential staining with toluidine blue for confirmation of sulfated mucopolysaccharides in urine (*279*).

Several screening techniques have been selected for the diagnosis of pathological conditions or hereditary defects: two-dimensional paper and thin-layer chromatography of amino acids in urine (*550*, *1328*), thin-layer chromatography of free amino acids in human sperm plasma (*1342*); thin-layer chromatography screening test for detection of hyperaminoacidemias (*765*); thin-layer chromatography of amino acids in cerebral tumors using frozen tissue (*944*); thin-layer chromatography of amino acids in the blood of premature infants (*101*); TLC to study histidinemia (*605*).

Iodoamino acids (*1413*) including inorganic iodide (*1600*) have been separated by thin-layer chromatography. Mono- and dichloroalanines have been separated by thin-layer chromatography (*1623*). Gamma-aminobutyric acid has been determined quantitatively by one-dimensional paper chromatography (*307*), while urinary β-aminoisobutyric acid has been separated from phenylalanine and tyrosine (*1291*). Urinary tryptophan metabolites have been studied by thin-layer chromatography on silica gel plates (*739*).

Paper chromatography has been applied to amino acid analysis of feeds and other biological materials (*1211*), for the rapid estimation of added monosodium glutamate in food (*55*), and for the qualitative determination of amino acids in mixed plankton (*20*).

Antibiotics. A comprehensive study on the separation of forty-two antibiotics by thin-layer chromatography has been reported (*1228*); feed preservatives and antibiotics in mixed feeds were analyzed by TLC after a three-solvent extraction system (*144*). Different antibiotics like rifamycin, chloramphenicol, novobiocin, and tetracycline were determined quantitatively by reversed-phase thin-layer chromatography (*1141*). For process control of antibiotic manufacture, thin-layer chromatography was employed to yield semiquantitative data (*750*).

Thin-layer chromatography has been widely used for the assay of penicillins and derivatives: mixed penicillins, ampicillin, and cloaxillin in body fluids (*970*); separation of phenoxymethylpenicillin and its acidic inactivation products (*751*); procaine penicillin G (*402*); penicillin derivatives (*513*); phenoxymethyl- and phenoxyethyl penicilloic acids in urine in the presence of parent penicillins (*113*).

The streptomycin group of antibiotics has been resolved by paper chromatography as the bis(2-ethylhexyl)phosphate salts (*552*). A more rapid technique than existing methods for thin-layer chromatography of glycosidic antibiotics like streptomycin, neomycin, kanmycin, and others, employs adsorbents of silica gel G and kieselguhr G (*148*). Neomycins A, B, and C have been separated in eight hours by paper chromatography and only three hours by TLC (*314*). Neomycin, polymyxin B, and bacitracin have been separated by paper and thin-layer chromatography as well as paper electrophoresis (*197*). Polymyxin B sulfate has been identified by hydrolysis and subsequent analysis of amino acids (see above) by thin-layer chromatography and the fatty acid component by gas–liquid chromatography (*525*).

Tetracyclines have been separated by thin-layer chromatography (*1313*) as well as paper chromatography (*5*). Gentamycin sulfate has been studied by thin-layer chromatography and the antibiotic-protein complex by zonal gel filtration on Sephadex (*1471*). Amphoteric and acidic polyene antibiotics have been tested on silica gel thin-layer plates (*1025*). Cephalothin and its metabolite deacetylcephalothin in urine and biological fluids have been studied by paper chromatography (*582*). The production of cyathin has been monitored by thin-layer chromatography on silica gel G (*643*). Tylosin has been determined in biological materials and feeds by thin-layer chromatography (*293*).

Bases and Amines. This is not a well defined class of compounds, and other applications may be found under Amino Acids, Pharmaceuticals, etc. Simple mono- and dialkylamines (methyl- and ethyl-) are separated by TLC on cellulose (*78*). Putrescine has been determined by paper chromatography with the solvent butanol–methyl ethyl ketone–ammonium hydroxide–water (5:3:1:1, v/v) (*270*). Fatty alkanolamides have been separated on silica gel columns using chloroform in mixtures with diethyl ether, diethyl ketone, acetone, methanol in varying proportions, and methanol alone, as elutrients (*564*). The occurrence of cyclohexylamine in urine as a breakdown product of cyclamate has been detected by thin-layer chromatography (*121*). Arylamines have been analyzed by thin-layer chromatography as phthalimidomethyl derivatives (*33*) or directly as arylamines (*203*) on silica gel; aromatic amines have also been analyzed by thin-layer chromatography on cellulose impregnated with bis(2-ethylhexyl) H phosphate and aqueous perchlorate–chloride solvent as mobile phase (*343*). The complexing of diphenyl amine and nitrobiphenyls has been studied by paper chromatography (*1603*).

Catechol amines (dopamine, epinephrine, norepinephrine, and related compounds) have been investigated as their respective dansyl derivatives by paper chromatography (*332*) or directly on precoated cellulose plates by thin-layer chromatography (*400*); quantitative estimation has been accomplished by two-dimensional thin-layer chromatography of ^{14}C-labeled compounds (*400*) or by converting the *O,O,N*-triacetyl derivatives into fluorescent compounds by spraying with ferricyanide–ethylenediamine (*404*, *454*).

In situ fluorometric analysis of bufotenin, serotonin, *N*-methylserotonin, and 5-methoxy-*N*,*N*-dimethyltryptamine has been performed on thin-layer chromatograms using *o*-phthalaldehyde as a spray reagent (*999*). Bipyridines and related compounds have been analyzed by thin-layer chromatography on silica gel G (*487*) and alumina layers (*403*).

The reaction between a monosubstituted guanidine (*e.g.* arginine), 1-naphthol, and hydrobromite in an alkali medium was stabilized with β-thiodiglycol as shown by paper chromatography (*23*). Quantitative determination of urea in urine has been accomplished by thin-layer chromatography followed by treatment with Ehrlich's reagent (*549*). *s*-Triazines, specifically melamine, have been separated and determined qualitatively by thin-layer chromatography on cellulose powder plates (*209*, *942*). Impurities present in melamine have been identified by paper chromatography (*1542*).

Hydrogenation products of quinoline have been identified by liquid–solid column chromatography on alumina containing 4% water as stationary phase and a mobile solvent containing water, dichloromethane, and *n*-pentane (*1311*). The partition of several weak organic bases (substituted quinolines) between HCl and a series of aliphatic alcohols was studied by means of paper chromatography (*1318*).

Ceramides have been studied by thin-layer chromatography (*684*); ceramides from human plasma have been isolated, identified, and quantitated by silicic acid chromatography, thin-layer chromatography, as ceramide acetates, and found to contain mostly sphingosine (*1206*).

Bile Acids (see also under Hormones and Steroids). Individual bile acids have been determined in biological fluids by thin-layer chromatography and fluorimetry (*1049*). Several improved detection reagents for TLC have

been recommended: I_2 vapors followed by benzyl alcohol–concd H_2SO_4–glacial acetic acid (0.15:0.15:20, w/v/v) produced brown-yellow colors for different bile acids (*476*); manganous chloride in sulfuric acid and heating at 100 °C (*477*) produce an immediate color for cholesterol and delayed for bile acids; a spray reagent consisting of 8-hydroxypyrene-1,3,6-trisulfonic acid sodium salt in methanol produces a visible spot under long-wave UV light for as little as 1 μg of bile acids (*708*).

Carbohydrates. Two recent publications are cited for general references, "Chromatography in Wood Chemistry," translated from the Russian into English (*713*) and a review of gel chromatography of carbohydrates (*248*).

High pressure column chromatography of carbohydrates for clinical analysis has been evaluated and promises to yield important data in the investigation of metabolic disorders (*1619*). Many of the papers reviewed here deal with the determination of sugars by thin-layer chromatography: Quantitative analysis of free sugars after TLC on cellulose powder plates with ethyl acetate-pyridine–acetic acid–water (5:5:1:3, v/v) as solvent was performed by eluting the spots and reacting with gallic acid-H_2SO_4, heating and reading the color at 550 nm (*1609*). Similar procedures have been described for mixtures of hexoses and pentoses separated by TLC on cellulose powder (*1126*) and kieselguhr G (*85*). TLC determinations of simple sugars have been reported in fruits (*1138*) and tannin extracts (*973*), paper chromatography of carbohydrates in pineapples (*10*); gas–liquid chromatography of simple sugars from cocoa products as trifluoroacetate derivatives using TLC as confirmatory technique (*857*); carbohydrates in urine by TLC following ion-exchange cleanup (*172*).

Aniline–diphenylamine color reagent has been described as a sensitive test for carbohydrates on thin-layers of silica gel G (1-2 μg detection limit) (*1107*). Uronic acids, neutral sugars, and hexoseamines have been quantitated by paper chromatography with the color reagent 2,3,5-triphenyltetrazolium chloride (*920*).

Fucose has been analyzed from urine by TLC (*86*) and separated from seven other neutral monosaccharides on kieselguhr G in less than one hour (*1408*). Derivatives of carbohydrates have been separated by TLC on cellulose powder on silica gel G layers: tetra-*O*-acetyl-D-glucopyranosides (*46*); glucose methyl ethers (*832*), and glucosamine methyl ethers (*812*); methyl derivatives of D-xyloses (*766*). Reversed-phase paper chromatography has been applied to the separation of sugar osazones (*745*).

Oligosaccharides have been separated by thin-layer chromatography, as for example: the first seven homologs of fructosan series on phosphate-buffered cellulose layers (*683*); homologous fructooligosaccharides from chickory rootstock on kieselguhr G (*257*); the separation of oligosaccharides from from mono- and disaccharides on cellulose thin-layers (*285*); maltooligosaccharides (*882, 1261*). Acetylated formylated, and pyruvylated oligosaccharides have been separated by preparative paper chromatography (*1371*).

Gel permeation chromatography has been applied to the characterization of cellulose fibers cross-linked with formaldehyde (*120*) and the determination of chain length distribution in wood celluloses and derivatives (*26*). Molecular weight distribution of nitrocellulose has been determined by elution chromatography on activated carbon and carbon black (*1601*).

Paper chromatography has been used to identify native and modified corn starches (*1068*) and the partial hydrolysis products of plant polysaccharides (*1192*). The separation of isomeric chondroitin, dermatan, and keratan sulfates has been accomplished by thin-layer chromatography on silica plates using the sequential application of acidic alcoholic solvents containing calcium ions (*831*).

2-Deoxypolyols have been separated by thin-layer chromatography and detected with 3,5-diaminobenzoic acid and periodate solution, applied sequentially (*1568*). Other miscellaneous carbohydrate derivatives have been studied by various chromatographic techniques: urinary cardiotonic glucosides by TLC (*1478*); phenolic glucosides by gel filtration on Sephadex (*1152*); glucosaminoglycans and galactosaminoglycans by column chromatography as cetylpyridinium complexes (*1110*); inositol polyphosphates from soil by gel filtration on Sephadex (*1347*).

Carbonyls. Different carbonyl compounds have been determined as 2,4-dinitrophenylhydrazones by thin-layer chromatography: aliphatic aldehydes and ketones (*1402*); cycloketones (*1074, 1294*); aliphatic and terpene carbonyls (*327*); chlorinated benzoquinones and benzaldehydes (*1581*). Gossypol and derivatives have been resolved by thin-layer chromatography on several types of adsorbents (*66*). Hydrogenated and methoxylated aurones have been separated by paper chromatography (*1203*).

Dyes. The chromatography of dyes has been covered in a book written in Polish (*659*), and thin-layer chromatography as applied in the dye industry has been reviewed (*913*). The identification of dyes from dyed fibers has been accomplished by paper chromatography (*538*), and over 160 solvents have been tested for the paper chromatography of basic dyes (*1391*). Basic dyes have also been separated and detected by polyamide thin-layer chromatography (*1398*). Xanthene derivatives have been detected in nanogram quantities by thin-layer chromatography (*1392*) as well as the greenish fluorescent substance (2,3,4-trichloro-5,7-dibromo-6-hydroxyxanthone-1-carboxylic acid) found in commercial phloxine (Food red 104) (*668*). Acidic anthraquinone dyes have been identified by paper chromatography (*455*). The ionic azo- and azomethine dyes have been separated and purified by column gel filtration chromatography using Sephadex (*1144*). Paper chromatography on acetylated cellulose was able to distinguish between metal-free and metal-containing sulfonic acid dyes and sulfonamides (*1222*). The azo dye, D & C Red No. 12 was determined spectrophotometrically after thin-layer chromatography (*1339*). Chromatography of monoazo disperse dyes on alumina and silica gel columns was used to overcome some problems of deacetylation (*158*).

Edible dyes from coal tar have been studied and estimated quantitatively by paper and thin-layer chromatography (*726*) and polyamide absorption chromatography (*64*). Rhodamines have been investigated by paper chromatography (*1225*) and methylene blue and related thiazine dyes by thin-layer chromatography on silica gel G layers (*843*). Two-dimensional paper chromatography was capable of resolving dyes used in the tanning process (*1463*). Inks and dyes have been studied by thin-layer chromatography (*1481, 1502*), paper chromatography and paper electrophoresis (*610*).

Enzymes, Proteins, and Peptides. Structurally isomeric peptides have been differentiated by gel chromatography on Sephadex G-10 and G-15 (*1629–1631*); specifically isomeric peptides of phenylalanine, tyrosine, and tryptophan, and leucine–glycine were studied. Detailed investigation of over 140 dipeptides by column and paper chromatography has been reported (*187*). Different techniques have been developed for the qualitative and quantitative estimation of peptides as well as molecular weight determinations—all of them involving chromatographic techniques: coupling of fluorescein isocyanate with peptides on paper chromatograms followed by elution for further analysis (*727*); a combination spray of potassium permanganate and ninhydrin for peptide-mapping after thin-layer and paper chromatography of the amino acids (*1530*); molecular weight estimation of polypeptides by thin-layer gel chromatography (*557*); the detection of peptide-active esters

by thin-layer chromatography (*N*-hydroxysuccinimide and pentachlorophenol esters) (*418*).

Recently developed fractionation methods of proteins by various chromatographic methods have been reviewed (*1100*). Analytical gel chromatography of proteins and chromatographic methods for the identification of *N*-terminal amino acids in peptides and proteins have also been reviewed (*12*, *1184*).

The molecular weight of proteins ranging from 10^4 to 10^6 has been determined by gel chromatography on agarose (*815*). The chromatography of proteins on hydroxyapatite has not been generally accepted and factors for this have been discussed (*99*). The heterogeneity of serum proteins in the pig (*1056*) has been demonstrated by gel chromatography on Sephadex G-200; the polydispersity of gelatin has been shown by gel chromatography on high porosity agarose gel (*230*).

Chromatographic techniques continue to be the favored method for isolation of proteins from different sources: basic proteins and polypeptides from salivary gland secretions by adsorption chromatography on polyacrylamide gel (*140*); two proteins from plasma of patients with neuropsychiatric disorders by exclusion chromatography on Sephadex G-25 (*153*); isolation of nucleoproteins form *Escherichia coli* by chromatography on methylated albumin (*762*); purification of bovine kininogen I by batch-wise adsorption and elution with DEAE- and CM-Sephadex (*1610*); purification of serum lipoproteins by agarose gel filtration (*585*) and Sephadex LH-20 (*1194*); molecular sieve chromatography of myosin (*1189*). The isolation of human retinol-binding protein has been accomplished both from urine and serum, involving chromatographic separation on DEAE-Sephadex, gel and affinity chromatography (*1477*). Protein-bound iodine-131 was determined by gel chromatography, but a faster method uses ion-exchange resin and is more suitable for routine work (*333*).

Various enzymes have been isolated and characterized by chromatography: cytochrome c from plants by successive chromatographic purifications on Amberlite CG-50, Sephadex CM-50, Biogel P-30, and CM-52 cellulose (*1157*); transaminases from pig heart by affinity chromatography on a Sepharose column substituted with *N'*-alkyl derivatives of pyridoxamine-5'-phosphate (*256*); isopentyl pyrophosphate isomerases from pumpkin by chromatography on hydroxylapatite columns (*1031*); human plasma naphthylamidases on Sephadex G-200 and DEAE-cellulose (*989*); isolation of lysozymes by column chromatography on deaminated chitin (*229*).

Thin-layer chromatographic techniques have been developed for the direct determination of peptidase and cholinesterase activity (*471*, *1634*); a simple column chromatographic method for peroxidase detection has been modified (*1216*).

Human brain specific antibodies and influenza virus antibodies have been highly purified by affinity- and immunochromatography (*923*, *1450*). Immunochemically pure IgG from human serum has been obtained by chromatography on QAE-Sephadex A-50 which can be regenerated with acetate buffer treatment (*655*). The subject of plant virus purification on cellulose columns with solvent containing polyethylene glycol has been reviewed (*1499*).

Fats and Lipids. Serum lipid analysis by chromatography and electrophoresis has been reviewed and shown to be a useful tool for clinical analysis (*125*). Chromatographic analysis of plasmalogens has been reviewed several years ago and is reported in this space now (*1520*). Different chromatographic techniques for lipid analyses have been described during the past two years: free and esterified cholesterol by alumina column chromatography (*34*) and TLC (*772*); lipids and phospholipids by two-dimensional TLC on silica gel (*34*); lipid screening by TLC (*1101*); direct densitometric determination of serum lipids on TLC plates (*335*); determination of serum and blood lipids by TLC (*8*, *609*, *671*); blood lipid analysis by Sephadex column chromatography and TLC coupled with infrared spectrophotometry and gas–liquid chromatography (*1005*).

Quantitative estimation of phospholipids by one- and two-dimensional thin-layer chromatography has become a prominent technique: elution of ^{32}P phospholipid spots for radio-counting (*938*); elution of spots after two-dimensional TLC and development with ammonium molybdate (*1142*); estimation with Rhodamine B–Tinopal reagent after two-dimensional TLC (*731*); wet *in situ* combustion and phosphorus determination by Fiske-Subbarow after TLC on silica gel (*984*); direct densitometry after TLC as molybdenum blue (*809*, *1354*); two-dimensional TLC of yeast phospholipids (*460*); two-dimensional TLC alone or in combination with triethylaminoethyl-cellulose column chromatography (*1459*). Vapor-programmed thin-layer chromatography has been used to effect the separation of phospholipids from cell membranes (*324*). The elution behavior of acidic phospholipids has been studied on columns of silicic acid, cellulose, and Sephadex LH-20 (*1278*); high-pressure chromatography through small-diameter silicic acid columns has been used to separate the main types of phospholipids from human bile (*947*).

Several other reviews are brought to the attention of the reader: chromatographic methods for separation and quantitative analysis of triglycerides (*1265*); recent analytical methods for oils and fats covers the analysis of glycerides (Japanese) (*591*); chromatographic detection of adulteration of oils and fats (*880*).

A mixture of glycerides has been separated by TLC and estimated quantitatively by densitometry (*1208*); triglycerides in milk have been separated by column (silicic acid) and gas–liquid chromatography (*1266*); neutral hydroxy lipids have been resolved on Sephadex LH-20 (*186*). Red cell glycosphingolipids have been separated by thin-layer chromatography from other lipids (*351*); cerebrosides and sulfatides have been determined by TLC on silica gel G (*921*, *922*). Cardiolipin from beef heart has been purified by a single pass through a silica gel column (*263*). Tobacco lipids have been determined quantitatively by reflectance densitometry on TLC plates (*1592*). Ganglioside fractions from rat brain have been purified on a Sephadex G-100 column (*380*).

Flavonoid Compounds. Fully and partially methylated biflavone ethers have been examined by thin-layer chromatography (*231*). Qualitative and quantitative determination of flavonols in grapes and wines has been accomplished by TLC (*132*). Paper chromatographic identification of flavonoids has been applied to the identification of *Cucumis* species but did not confirm classifications based on morphology and sexual criteria (*165*). Flavanoid polyphenols from beer have been examined by thin-layer chromatography (*1351*) and soybean isoflavones have been separated from their 5-hydroxy derivatives also by TLC (*1549*). Sephadex LH-20 has been used to separate theaflavines (*802*) and Sephadex G-25 or G-50 for the analysis of flavonoids (*63*).

Food Additives (see also Dyes). Chromatographic techniques for the analysis of food additives and constituents have been reviewed by a number of authors (*212*, *213*, *784*, *1394*). Artificial sweeteners including cyclamate, saccharin, and dulcin, have been separated and detected by thin-layer chromatography (*288*, *546*, *986*, *1551*). Synthetic food colors and dyes have been analyzed by thin-layer, paper, and column chromatography: British food colors by TLC (*482*); food dyes licensed in Czechoslovakia by TLC (*1387*); dyes approved for use in pharmaceuticals by column chromatography on microcrystalline cellulose (*295*). Synthetic water-soluble food colors have been identified by TLC on

silica gel G and microcrystalline cellulose plates (*588*, *1064*, *1131*). Reversed-phase TLC has been used to separate and identify oil-soluble food colors (*589*). Yellow food dyes have been identified by TLC on polyamide layers (*232*) and paper chromatography, especially to detect adulteration (*543*). Colors used in carbonated drinks have been identified by paper chromatography (*44*).

p-Hydroxybenzyl alcohol from vanilla has been detected by TLC using cellulose plates (*1109*). Food preservatives of the type mimosine (in soybean sauce), benzoic acid, salicylic acid, dehydroacetate) have been determined by paper chromatography (*1552*), thin-layer chromatography (*811*, *821*), polyamide layers, columns and powder (batch process) (*987*, *816*). Antioxidants have been separated on TLC plates using polyamide–silica gel layers (*810*) or by polyamide batch process (*817*). Emulsifiers, such as monoglycerides, sucrose esters have been analyzed by TLC and infrared spectrophotometry (*1330*). Thin-layer chromatography has been used to study toxic chemicals extracted from plastics in the food industry (*1460*).

Steroids and Hormones (see also Pharmaceuticals). The chromatography of steroids was reviewed several years ago but is cited for the first time here (*906*); a review of thin-layer chromatography of steroids is recommended reading (*833*). High-resolution liquid chromatography has been applied to the analysis of steroid hormones (*336*, *1282*). Column chromatography on Sephadex LH-20 is especially useful for the separation and recovery of plasma steroids (*196*, *974*). A variety of steroids has been resolved by TLC on Adsorbosil 4 and detected with phosphomolybdic acid spray (*992*).

Urinary aldosterone has been determined by thin-layer chromatography (*37*, *1525*) and separated from 18-hydroxycorticosterone and corticosterone (*430*). Urinary corticosteroids have been separated by TLC and quantitated after elution of the spots (*613*) and by direct photometry (*6*). Corticosteroids from human liver have been analyzed by paper chromatography (*384*) and urinary corticosteroids by multi-column capillary liquid/liquid chromatography (*1510*). Corticosterone and acetate derivatives have been separated by TLC on kieselgel; TLC-separation of closely related 6-fluoro-16α-hydroxy corticosteroids has been reported (*114*). 17-Ketosteroids and adrenocortical steroids have been separated by gradient elution chromatography on silicic acid columns (*792*).

Identification of androgens by various chromatographic techniques has been discussed in a review (*1162*). Members of the androstane series have been separated by TLC on silica gel plates (*385*). Δ^4-3-Ketosteroids and Δ^5-3β-hydroxy steroids have been separated on digitonin–silica gel thin-layer chromatograms (*1415*) and the keto-steroids quantitated by direct thin-layer densitometry based on fluorescence quenching (*734*). Testosterone and progesterone have been separated by TLC using silica gel G (*369*). Thin-layer chromatography has been applied to the separation of C_{18}–C_{21} steroid triol sulfates (*630*); C_{18}, C_{19}, and C_{21} steroids (*1204*); C_{19}-steroid 2,4-dinitrophenylhydrazones on polyamide gel layers (*1061*).

Estrogenic steroids have been studied by various chromatographic techniques: free estrogens by liquid-partition chromatography (*775*); separation of synthetic and natural estrogens by TLC (*1416*); estrogens by Sephadex G-25 column chromatography (*592*); separation and quantitative analysis by TLC of synthetic progestatives (*1438*); separation of estrone, 17β-estradiol, and estriol on Sephadex G-10 (*593*); pregnancy urinary estriol by TLC of the dansyl derivative (*347*); qualitative and quantitative determination of urinary pregnanediol by TLC (*7*, *386*); epimeric estriol separation by TLC (*1446*); fluorometric determination of progesterone in plasma after TLC (*480*); urinary pregnane-17,21-diol-20-ones by paper chromatography (*1019*); steroid assay in human pregnancy blood by paper chromatography (*1185*).

The deleterious effect of iodine vapors for the detection of estrogens after thin-layer chromatography has been noted (*935*, *936*).

Urinary testosterone and epitestosterone have been separated and estimated as Girard complexes on TLC (*439*); an elaborate method is described for the determination of urinary testosterone involving column and gas–liquid chromatography (*1564*). Steroid impurities in prednisolone have been detected by thin-layer chromatography (*1018*).

Steroid glucuronides have been separated by paper and thin-layer chromatography (*1230*, *1252*). Thin-layer chromatography has been used to study sterol esters isolated from the nuclei of nucleated chicken erythrocytes (*1323*). Photochemical isomers of ergosterol have been separated by thin-layer chromatography on silica gel G or alumina (*919*).

The group separation of prostaglandins has been accomplished by liquid–gel chromatography on Sephadex LH-20 columns (41). Monkey prolactin has been isolated by affinity chromatography using Sepharose-coupled human placental lactogen (*515*). ACTH has been separated from other pharmaceutical preparations by paper chromatography (*240*). Insulin has been determined by paper chromatography (*1512*). Radio-labeled triiodothyronine and thyroxine have been separated by gel filtration (*517*).

Hydrocarbons. Atmospheric polycyclic aromatic hydrocarbons have been determined by various chromatographic techniques: thin-layer chromatography, gas–liquid chromatography, UV-spectrophotometry, and spectrofluorometry for qualitative studies (*224*); chromatographic methods have been compared and reviewed (*1214*); thin-layer chromatography on silica gel (*1077*) and alumina (*1213*); high-temperature paper chromatography (*874*); hydrocarbons from cigarette smoke by high-resolution liquid chromatography (*807*); methyl-substituted anthracenes as fluorescent indicators in the presence of olefins (*1292*).

Benzo-(1,2)pyrone (coumarin) in essential oils has been identified by thin-layer chromatography after isolation on alumina columns (*79*). The paper chromatography of anthraquinones and glycosides has been reviewed (in Russian) (*1172*). The rapid determination of paraffins in woolen knit goods utilizes thin-layer chromatography (*798*).

Crude oil fractions have been identified by gel permeation chromatography (*1028*), chromatographed on columns of ion exchange resins, silica gel, and alumina (*576*); lubricating oils and additives have been analyzed by thin-layer chromatography (*252*). Alkylbenzenes have been separated by chromatography on alumina columns (*1099*).

Monomers and Polymers. Acrylate, methacrylate, and tiglate ions have been separated by paper chromatography (*860*). Poly(methyl methacrylate) has been fractionated by precipitation chromatography (*290*). Molecular weight analysis of block copolymers (*e.g.*, styrene–butadiene) has been performed by gel-permeation chromatography (*221*). Structural studies and changes in molecular weight distribution of polyethylene have been carried out by gel-permeation chromatography (*586*, *1555*). Diamines, dicarboxylic acids, and ω-amino acids recovered from copolyamides have been determined by thin-layer chromatography and densitometry (*951*); polyethylenepolyamines have been determined by paper chromatography (*733*); the cyclic monomers and oligomers of nylons 6 and 66 have been determined by gel permeation chromatography using Sephadex and Bio-Gel (*952*).

Polyglycerols were partially acetylated and identified by thin-layer chromatography (*283*); the molecular weight distribution of polyethylene glycols has been determined by thin-layer chromatography coupled with densitometry (*381*, *382*); a purity test for certain polyethylene glycols has been based on a thin-layer chromatographic

technique (*458*); column and thin-layer chromatography have been compared for the molecular weight distribution of polyethylene glycol derivatives (*195*).

Novolak resins have been studied by gel-permeation chromatography (*946*); epoxide polymers have been analyzed by thin-layer chromatography (*1352*). Plasticizers have been analyzed by thin-layer chromatography, in PVC compounds (*189*); epoxy plasticizers (*1174*); phthalate plasticizers (*1380*).

Natural Products. Pure gibberellins from *Gibberella fujikuroi* have been isolated by partition chromatography on Sephadex columns (*1086*). The polydispersity of lignins has been standardized by gel-permeation chromatography (*423*). Thin-layer chromatography has been applied for the fingerprinting of commercially available saponins (*284*). Juglone from walnuts has been separated by TLC and visualized with a spray of 4-aminoantipyrene (*1431*).

Thin-layer chromatography has been applied to the analysis of miscellaneous natural products for pharmaceutical use (see also there): tinctures and fluid extracts (*371*); glycyrrhizin in liquorice root (*778*); plant drugs from *Arctostaphylos uva-ursi, Euphorbia hirta, Hedera helix* (*1191*); digitalis glycosides (*185*).

Lichen products have been analyzed by thin-layer chromatography (*276*) using a standardized procedure; the differentiation and identification of vegetable oils have been done by thin-layer chromatography (*686*). The limitation of the paper chromatographic technique for auxin analysis has been reviewed (*701*). Astaxanthin from marine products in its free and esterified forms has been analyzed by silica-gel chromatography (*791*).

Pesticides. A review has been published on the application of thin-layer chromatography to the quantitative analysis of food additives and pesticides in foods (*1395*). A screening method for the detection of organochlorine and organophosphorus insecticides in vegetables utilizes TLC on $AgNO_3$-impregnated alumina and silica gel G plates (*1207*); another modified TLC method is applicable for the identification of the three major classes of insecticides (*1453*). Chromatography of 20 pesticides on alumina columns has been studied (*1373*). Other thin-layer chromatographic methods for pesticides included: chlorinated and organophosphorus insecticides in fruits and vegetable (*498*); in blood and viscera (*264*); reversed-phase TLC for the three major classes (*1550*). Channel layer chromatography with alumina G was applied to a rapid cleanup procedure for pesticide residues (*573*). Various chromogenic spray reagents for TLC of pesticides have been reviewed (*198*).

Seventeen of the most commonly used chlorinated insecticides have been identified by TLC on four types of adsorbents (*352*); chlorinated pesticide residues in fruits and vegetables have been determined by TLC on plates washed with water and treated with $AgNO_3$ (*281*). DDT, lindane, and related chlorinated insecticides have been determined in milk, meat, and eggs by similar thin-layer chromatographic methods (*1, 516, 1037, 1474, 1626*).

Direct densitometric analysis of chlorinated insecticides separated by TLC has been accomplished, using *o*-tolidine spray and UV irradiation; the sensitivity and linearity of reflectance measurements was in the range of 3–40 μg for lindane (*1075*). The effect of chlorinated insecticide on bovine liver esterase enzymic activity was studied by thin-layer chromatography (*449*). DDT and analogs have been separated from interfering polychlorinated biphenyls (PCBs) by thin-layer chromatography (*387*). PCBs have been analyzed by thin-layer chromatography after solvent-partitioning and Florisil cleanup (*318, 964*). Polychloropinene (toxaphene or Strobane) has been separated from DDT in water and soil by TLC on silica gel (plus 5% gypsum) with the solvent n-hexane–methanol–NH_4OH (10:4:0.3)–polychlorinated pinene gave a single spot (*732*).

Thin-layer chromatography has been applied to 23 organophosphate insecticides on layers of alumina G; the method was used for the analysis of river water (*822*). The cholinesterase-inhibition method was modified for the detection of organophosphorus insecticides on thin-layer chromatograms using bee brain enzymes (*957*) and animal liver esterases (*910*). Parathion and suspected metabolites have been analyzed by thin-layer chromatography (*2, 628, 1420, 1507*). Thimet, parathion, lindane, and DDT from green tobacco leaves have been separated and estimated by TLC (*294*). Dipterex in plant samples has been determined by reversed-phase paper chromatography (*756*) and in milk by thin-layer chromatography (*1085*). Water samples were analyzed for Dylox (Dipterex), DDVP, and malathion by thin-layer chromatography (*1636*). Purified plant extracts were analyzed for diazinon by paper chromatography (*755*). High-speed liquid chromatography was applied for the quantitative analysis of Abate in water (*569*); this was the only reported use of high-speed liquid chromatography for pesticide analyses but more applications are anticipated during the next two years to be covered by the 1974 review.

Malathion, its metabolites, and the isomers of malathion monocarboxylic acid have been separated and analyzed by thin-layer chromatography (*660, 1574*). Fenthion residues in apples and plums have been determined by thin-layer chromatography after charcoal-cleanup (*1622*). Formulations of *O,O*-diethyl-S-(2,5-dichlorophenylthiomethyl) dithiophosphate were analyzed by TLC on layers of silica gel HF (*757*). TLC was also applied for the formulation analysis of S-benzyl diisopropyl phosphorothiolate (IBP) (*941*). Two Russian papers reported the determination of phthalophos (Imidan?) and Butiphos (butonate?) by thin-layer chromatography (*1407, 1633*). (Readers are invited to supply the authors with the correct identification of these two insecticides.)

The chromatography of carbamates, including pesticidal thiolcarbamates, has been reviewed several years ago but is reported only new in this review (*397*). Twelve carbamate insecticides have been detected on thin-layer plates by pig liver esterase and 5-bromoindoxyl sulfate as substrate (*911*). Sevin (carbaryl) has been detected on thin-layer chromatograms with bee brain enzymes at a sensitivity of 0.01 ng (*960*).

Sevin residues have been determined by thin-layer chromatography in water and plant tissues (*945*), apples (*682*), and food products (*67*). Impurities in formulations of formetanate and chlorphenamidine have been determined by TLC and reflectance measurements *in situ* (*781*). *o-sec*-Butylphenyl *N*-methylcarbamate has been separated from phenolic impurities by TLC on silica gel plates and after hydrolysis brominated quantitatively (*971*). Methyl carbamate insecticides found in rice grain have been determined by TLC as the corresponding 4-nitrobenzeneazo derivatives (*617*).

Phenylcarbamate and phenylurea herbicides and their metabolites have been separated by two-dimensional thin-layer chromatography using the same solvent in both directions (*1326*). *N*-Chlorophenyl-*N'*-methoxyureas have been separated by TLC (*1314*); fourteen different solvents have been tested for thin-layer chromatography of substituted ureas (*535*). Formulation analysis of the biscarbamate herbicide Betanal was accomplished by TLC on silica gel F plates and direct UV reflectance measurements (*782*); Betanal and the corresponding ethoxy compound could be separated by double development on TLC and determined by direct reflectance readings of the coupled diazo-2-naphthol compound (*782*). Analysis of dithiocarbamates has been reported; Vapam by paper chromatography (*1173*), ziram and metabolites by TLC on alumina plates (*1498*). The thiocarbamate herbicide vernolate and suspected metabolites have been separated by TLC on silica gel plates (*571*).

A procedure has been developed for the simultaneous analysis of 19 herbicides in soils and water commonly used in Saskatchewan, based on thin-layer chromatography (*1299*). Phenoxyalkyl herbicides have been determined by thin-layer chromatography, as for example: 2,4-D in milk, meat, and animal tissue (*3*); 2,4-D and 2,4,5-T from poisoned bees (*959*); the separation of 2,4-dichlorophenoxypropionic acid and the corresponding 2- and 4-methyl analogs (*130*); 2,4-D and MCPA from water (*129*).

The arsenicals cacodylic acid, MSMA, sodium arsenate, and arsenite have been separated by paper chromatography (*1197*). Two different solvent systems are capable of separating the active ingredients of any herbicide formulation commonly used, by thin-layer chromatography (*4*).

The literature of chromatography of triazines has been recently reviewed (*394*). A modified thin-layer technique has been reported for the separation and detection of triazine herbicide residues and corresponding hydroxy derivatives (*759*). A semi-quantitative method for the determination of diquat and paraquat is based on thin-layer chromatography and Dragendorff's detecting reagent (*961*).

The chromatographic analysis of methylenedioxyphenyl derivatives used as pesticides has been reviewed several years ago but is reported in this space only now (*396*). *o*-Phenylphenol residues from fruits and vegetables have been determined by TLC (*289*). Dinitrocresol (DNOC) has been analyzed by paper chromatography (*928*) and thin-layer chromatography (*958*). Similar methods are recommended for DNBP and dinoseb.

Two organo-tin pesticides, Brestan and duTer, have been isolated from soil and analyzed by thin-layer chromatography (*210*). *n*-Dodecylguanidines from sewage lagoon effluents have been determined by thin-layer chromatography (*241*). Warfarin and other rodenticidal coumarins have been isolated from plasma and stool and residues determined by thin-layer chromatography (*827, 1195, 1573*).

Pharmaceuticals. Several reviews on the application of chromatography to the analysis of pharmaceuticals have appeared (*1333, 1409*). Thin-layer chromatography has been used to test the decoloration and deterioration of drug solutions (*998*). Two universal solvents and several support materials have been investigated for thin-layer chromatography of a variety of drugs (*359, 670*). Phenolic-type drugs have been determined by TLC on alumina (*736*). Drugs containing unrelated components (*e.g.*, codeine phosphate, phenobarbital, caffeine, acetylsalicylic acid, and phenacetin) have been analyzed for each component by TLC using a chromatogram spectral photometer (*363*). Pharmaceutical emulsifiers and solubilizers have been analyzed qualitatively by TLC (*1003*).

The active ingredients of analgesic tablets have been rapidly analyzed by high-speed liquid chromatography (*568*). Local anesthetics have been determined by direct densitometry in the UV range and at 440 nm after I_2-exposure following thin-layer chromatography (*900*).

Sulfonamides have been analyzed by thin-layer chromatography (*249, 250, 735, 737*) and paper chromatography (*442*). Other sulfur-containing drugs, hypoglycemics, and biguanidine derivatives have been separated by thin-layer chromatography (*1218, 1219*).

For routine identification of drugs of abuse in urine, thin-layer chromatography on silica gel plates is being widely used (*963*); preliminary screening could be accomplished by fluorometry, and gas–liquid chromatography was used for final confirmation of results from TLC. For the identification of components from cannabis and hashish, thin-layer and gas–liquid chromatography have been employed (*518, 1360*).

The separation and identification of 28 widely used narcotics and some of their metabolites have been accomplished by thin-layer chromatography (*127, 150, 370, 1146*). One- and two-dimensional thin-layer chromatography has been applied to the determination of narcotics (*319, 391, 746, 1159*). Common sedatives and stimulants have been identified by rapid thin-layer chromatography (*1361, 1362*). Phenothiazine derivatives have been studied by thin-layer chromatography (*259, 502, 752, 939*) including chlorpromazine metabolites (*694*). Thin-layer chromatography has been widely applied to the determination of a large variety of psychotropic drugs (*702, 799, 1039, 1078*). Benzodiazepines have been analyzed by liquid-solid chromatography (*1240*) and thin-layer chromatography (*799, 1212, 1442, 1511*).

Thalidomide and its hydrolysis products have been separated by thin-layer chromatography (*1084*). Thin-layer chromatography has been applied to the rapid determination of diuretics (*864*) and chlorothiazides in particular (*1553*). Anthelmintics (piperazine, pyrvinium, etc.) have been separated and determined by paper chromatography (*1554*). The colorimetric analysis of chlorpheniramine with cobaltothiocyanate could form the basis of a detection agent for paper and thin-layer chromatography (*1038*).

Eleven synthetic laxatives have been studied by thin-layer chromatography (*104*). Pyrimethamine and the aryl,-alkyl derivatives of 2,4-diaminopyrimidine in biological fluids and tissues have been determined by Sephadex G-25 column chromatography (*470*). Pyridinium oxides and other quaternary ammonium drugs have been separated by column chromatography on Sephadex G-10 (*271*) and TLC (*990*). Thin-layer chromatography has also been applied to the identification of germicides in personal care products (*478*).

Lysergic acid amide and its isomer have been separated and isolated from morning glory seeds by column chromatography (*1016*). Another natural product, this one by fermentation, cymarin, was purified by column chromatography on alumina (*572*).

A large variety of other drugs has been analyzed by thin-layer chromatography in commercial preparations or isolated from body fluids or tissues: chlorzoxazone (*1470*); coumarin (*890*); 5,5-diphenylhydantoin in blood (*1285*); acridine derivatives (*1604*); hesperidin methyl chalcone (*1283*); usnic acid (*393*). The purity of radiopharmaceuticals has been checked by thin-layer chromatography (*30*).

Phenols. Thin-layer and paper chromatography have been applied to the study of phenols, cresols, dimethylphenols, and naphthols by separating them as the coupled products with diazotized 4-benzoylamino-2,5-diethoxyaniline (Fast Blue Salt BB) or anthraquinone diazonium chloride (*1424, 1430, 1433*). TLC separation of xylenol isomers is not possible unless they are condensed with 1-phenyl-2,3-dimethyl-4-amino-5-pyrazolone or Fast Blue Salt BB (*1432*). Phenols in air samples were trapped and coupled with *p*-nitrodiazobenzene for TLC separation (*830*). The estimation of hydroxyl groups in phenolic inhibitors was accomplished by thin-layer chromatography of the parent and acetylated compounds and a comparison of R_F values (*473*).

Other substituted phenols have been resolved and analyzed by TLC: Fast Blue Salt BB complexes of catechol, hydroquinone, resorcinol, pyrogallol, and pholoroglucinol (*1429*); eugenol and isoeugenol (*514*); 4,4′-isopropylidenediphenol, its isomers and impurities (*1050*); nitrophenols as coupled with anthraquinone-1-diazonium chloride (*1434*).

Urinary phenolic acids have been separated by thin-layer chromatography on cellulose or silica gel layers (*374, 603, 604*). Plant phenolic acids like chlorogenic, caffeic, etc. have been separated by TLC on a mixed adsorbent cellulose-silica gel G (*495*); mycophenolic acid and related compounds have been studied by TLC (*438*). Urinary phenolic amines have been studied by a modified paper chromatographic separation (*1200*); two-dimensional paper chromatography was able to re-

solve *N*-acetyl-*p*-aminophenol isolated from human urine (*611*).

Gallic acid and its alkyl esters have been separated by TLC on polyamide thin-layers (*1399*). Tannins have been separated from chlorophyll on thin-layer plates coated with polyamide (*1475*) or silica gel (*972*). Tannic acid components have been separated by gel permeation chromatography on columns of Sephadex G-25; the products were identified as high-molecular weight polyphenols (*718*). Phenolic oxidants have been determined by TLC on silica gel G (*218*). Phenol-formaldehyde reaction products have been studied by reversed-phase chromatography on a Teflon 6 column (*1423*).

Pigments. The separation of free dicarboxylic acid porphyrins was effected by thin-layer and paper chromatography (*82*); the porphyrin methyl esters were separated by preparative thin-layer chromatography (*341*); protoporphyrin IX monomethyl ester has been separated in milligram quantities by silica-gel TLC (*368*). ^{14}C-labeled chlorophylls a and b have been separated for specific-activity determinations by TLC on confectioner's sugar as adsorbent; algal pigments have been separated on preformed cellulose thin-layers (*1268*); chloroplast pigments have also been separated on Sephadex LH-20 columns (*1277*).

Carotenoids have been separated by two-dimensional, two-adsorbent thin-layer chromatograms (*738*); pigments extracted from tomatoes have also been separated and identified by TLC (*65*). Anthocyanins have been chromatographed on poly(vinylpyrollidone) columns (*1485*).

Purines, Pyrimidines, RNA, and DNA. Purine bases of pharmacological importance (theobromine, theophylline, etc.) have been separated by TLC (*855*) and quantitated by direct fluorescence and reflectance measurements (*1236*); other purines and related compounds have been studied on Bio-Gel P-2 columns (*1284*). Adenine and its metabolites, adenosine and AMP and other nucleic acid bases, nucleosides, and nucleotides have been studied by one- and two-dimensional TLC (*417, 748, 976*). Formic acid hydrolysis of DNA has been followed by paper chromatography (*1263*).

A recent review discusses the analysis of nucleotides, nucleosides, and purine-pyrimidine bases by high-pressure liquid chromatography (*457*); the separation and quantitative elution of nucleosides and nucleotides has been reported, by TLC on polyethyleneimine cellulose-Avicel SF plates (*485*) and bi-dimensional TLC on cellulose powder-thin layers (*1288*). Nucleosides have been separated on polyacrylamide columns (*1083*) and Sephadex G-10 (*1558*); diribonucleoside monophosphates on Bio-Gel P-4 (*796*). Cyclic 3′,5′-adenosine monophosphate has been chromatographed on a matrix of silicic acid–glass microfiber; samples were isolated from animal tissue (*1594*).

Nucleic acids have been isolated by column chromatography on hydroxyapatite (*98*), polynucleotide-coated kieselguhr (*828*), poly-L-lysine–kieselguhr (*48*), methylated albumin on kieselguhr (MAK) (*510*). Similar techniques have been used to purify RNA bacteriophage MS2 by Agarose-column chromatography (*1618*); separation of 5S RNA from other nucleic acids by chromatography on polyamino acid–kieselguhr columns (*846*); RNA from pathological human thyroid glands by MAK column chromatography (*102*).

Present chromatographic methods for fractionating DNA have been reviewed (*758*). DNA has been studied by gradient-partition chromatography on columns of Sephadex LH-20 with increasing Li^+ concentration in the eluting solvent (*714*). Newly synthesized DNA has been characterized by column chromatography on MAK (*1116, 1117*); DNA has been fractionated on columns of poly-L-lysine–kieselguhr (*566*) and wool cortical cell proteins (*409*); separation of T-even bacteriophage DNA from host DNA has been accomplished by chromatography on hydroxyapatite columns (*1034*). Partition chromatography has been applied to fractionate and characterize nonpolar tRNA from rat liver, yeast and *E. coli* (*1566*) and serine tRNA from rat liver (*1331*). Sephadex chromatography has been applied to the removal of aggregations during purification of yeast serine tRNA (*524*). MAK column chromatography has been used to fractionate aminoacyl-tRNA from goldfish brain (*673*). A commercial sample of poly (dA-dT), copolymer of 2′-deoxyribosyladenosine and 2′-deoxyribosylthymidine, has been fractionated by column chromatography on Agarose gel (*634*).

Toxins. Aflatoxins have been detected and quantitated by thin-layer chromatography on polyamide thin-layers (*420*), by two-dimensional TLC and fluorodensitometric evaluation (*1234*) and by TLC and liquid chromatography (*876*). The interference of ethoxyquin was removed by TLC on plates of silica gel G and Silufol-254 (*1337*). Resolution was obtained for aflatoxins B_1, B_2, G_1, and G_2 by TLC on silica gel (*1143*). Flavatoxins in foods have been detected by a rapid thin-layer chromatographic technique (*152*).

Paper chromatography was used to detect the presence of the toxin from *Taxus baccata* (*174*). Diphtheria toxins and toxoids have been studied by gel filtration on Sephadex G-75 and G-200 (*42*).

Vitamins. Vitamins A, D_3, and E have been separated by thin-layer chromatography on silica gel G (*54*). Vitamin B_1 has been isolated by thin-layer chromatography by similar techniques with a different solvent system (*625*). Riboflavine content in urine has been estimated by *in situ* fluorometric densitometry after thin-layer chromatography on silica gel G plates (*545*); purification was first effected by selective adsorption on a talc powder column. Vitamin B_6 has been separated by TLC (*1301*) and thin-layer electrophoresis (*19*). Nicotinic acid and nicotinamide have been separated by TLC and estimated quantitatively by *in situ* fluorometry (*412*) and spot size (*1556*). Thiamine, *N*′-methylnicotinamide, and related compounds have been separated by TLC (*1628*); *N*-(hydroxymethyl)nicotinamide and derivative have been separated by paper chromatography (*583*).

L-Ascorbic acid and erythorbic acid in foods have been determined by paper chromatography (*666*), but the thin-layer chromatographic technique appears to be more rapid than the former (*760*). Vitamin D has been analyzed by thin-layer chromatography (*428, 1158*). Dimeric tocopherol products have been separated on a dry column of silica gel (*292*) and alumina column chromatography (*786*). Pantothenic acid, coenzyme A, and 4′-phosphopantetheine have been isolated on a column of DEAE-cellulose and identified by means of paper chromatography (*996*). Anomalous behavior of radioactive folic acid on thin-layer chromatogram in acidic solvents has been explained by dimerization (*117*).

Miscellaneous Organic Compounds. Chlorinated *p*-benzoquinones have been separated by TLC on absorbents of silica gel DF 5 and β-phenoxyethanol-impregnated Kieselguhr G plates (*1377*) and paper chromatography (*1376*). Thin-layer chromatography has also been used to determine dianilino-*p*-benzoquinones (*940*), 4,4′-dichlorobenzophenone and decomposition products (*450*), and 1-aminoanthraquinone (*823*). As a spray reagent for thin-layer chromatograms of 1,4-benzoquinone and anthraquinone, 1-phenyl-2,3-dimethyl-4-amino-5-pyrazolone has been recommended (*1435*).

Organic sulfur compounds have been analyzed by chromatographic methods: alkyl sulfides, thiols, sulfoxides, and others by TLC (*676*); thiourea and ammonium thiocyanate by alumina column chromatography (*884*); separation of isomeric thioureas, thiazoles, and thiazolines by TLC (*867*); thiosemicarbazones by TLC (*1473*); separation of sodium salts of carbazole sulfonic acids by TLC (*1541*); analysis of β,β′-bis(chlorocyclohexyl) sulfides by TLC (*863*); separation of 1-dimethylaminonaphthalene-5-sulfonylamides by gel permeation chromatography on Sepha-

dex LH-20 (*1258*); detection and determination of mixture of naphthalenesulfonic acids by paper and thin-layer chromatography (*1108*); separation of sulfonic acid esters produced by the sulfonation of 1-dodecene, by TLC (*1123*); separation and identification of fluorescent whitening agents (derivatives of flavonic acid) by TLC on cellulose acetate (*1221*). Color reactions for thiols and sulfoxides on thin-layer chromatograms have been described (*484, 1591*).

Organophosphorus compounds (see also under Pesticides) have been studied by chromatographic techniques: paper and thin-layer chromatography of organo P(III) and P(V) acids has been reviewed (*1102*) and studied (*1103*); di- and monoesters of phosphoric acid have been resolved by TLC (*1341*); neutral organophosphorus compounds have been separated by TLC (*794*); phosphines and derivatives by TLC (*474*); zinc dialkyl dithiophosphates have been analyzed by TLC (*578*); nitrogenated derivatives of phosphorus have been separated by TLC (*907*); exo-trimethylenenorbornyl-2-exo-phosphates have been separated on columns of Sephadex LH-20 (*1410*). Aminoalkylphosphonates separated by paper chromatography have been detected with a ninhydrin-molybdate spray reagent system (*1179*).

Amidines have been characterized as picrates by TLC (*1497*); 4,4′-dinitrodiphenyl and benzidine and their metabolites have been separated by paper chromatography (*785*). 2,4-Tolylenediisocyanates and the 2,6- isomer have been separated by TLC and made visible with Ehrlich's reagent (*824*). Preparative isolation of the diastereoisomers of 2,4-dicyanopentane has been accomplished by column chromatography on silica gel (*1479*). Nonionic detergents have been separated by thin-layer chromatography on silica gel GF, impregnated with oxalic acid (*742*). *N,N*-Dialkylnitrosamines have been resolved by TLC on silica gel GF or PF (*365*). Good separation was obtained for a mixture of *N*-(*m*-nitrophenyl)-D-glucosylamine and the corresponding *p*-nitro isomer by TLC on silica gel-$CaSO_4$ layers (*1372*).

Benzofuran derivatives have been identified by thin-layer chromatography on silica gel and silica-gel–cellulose layers (*997*). The chromatography of cosmetic products has been reviewed with emphasis on final identification by spectrophotometric or derivatization techniques (*1076*). Thin-layer chromatography has also been applied to the analysis of accelerators, anti-oxidants, stabilizers, and rubber-compounding ingredients (*672, 698, 771*).

Organo-metallic compounds have been analyzed mainly by thin-layer chromatography, although a few techniques involve paper chromatography: ferrocenes and biferrocenes have been separated by TLC (*1412*); metal dialkyldithiocarbamates (see also Pesticides) by paper partition chromatography (*1276*) and thin-layer chromatography (*61*); organo-mercury compounds by thin-layer chromatography (*1043*), in formulations (*642*), and industrial waste water (*969*). Organo-titanium compounds have been separated and determined by paper chromatography (*761*).

Inorganics. A number of reviews have appeared dealing with the chromatography of inorganic ions and compounds: inorganic thin-layer chromatography (*805, 917, 1526*); reversed-phase extraction chromatography (*214*); chromatographic separation of metal complexes in nonaqueous solutions (*711*); paper partition chromatography for the analysis of precious metals (*1488*). A laboratory manual has also been published on the chromatographic analysis of inorganic substances (in Russian) (*89*).

Systematic cation analysis on alumina columns has been described recommending potassium thiocarbonate as color reagent for resolved bands on the column (*1290*). Cations have been resolved on silica gel H thin-layer plates with the solvent acetone–37% HCl (*902*), but TLC on cellulose layers by the ascending technique (*916*) or circular development seemed to give superior results (*159*). Paper chromatography has been applied to the determination of trace elements in geological materials (*16*) and TLC for the authentication of paintings by Rembrandt (*422*); in the latter study, the yellow pigment ($2PbO, SnO_2$) was identified.

Rubidium was separated and estimated in the presence of potassium by paper chromatography (*80*); Li^+ in the presence of other alkaline metals was separated by TLC on cellulose thin-layer plates and was resolved from Na^+ and K^+ (*897*). Group II cations have been separated by TLC and visualized with potassium thiocarbonate (*644*). Ni, Co, Cu, and Fe from sulfide ore and rocks were separated and determined quantitatively by paper chromatography (*1514*); paper chromatography was also utilized to separate Cu, Cd, Hg, Zn, Cr, and Ni from industrial electroplating waste liquor (*1436*). Fe, Cr, Mo, and V carbides from alloyed steels have been separated by paper chromatography (*1368*).

A chromatographic separation of Fe(III)–Co(II) and Co(II)–Cu(II) was achieved (*1267*). The chromatographic separation of hydroxy ions of Nb, Mo, and W on Al_2O_3 columns was studied (*710*). By the use of selective detection reagents, a mixture of metal ions was identified by ascending paper chromatography—the mixture contained ions of Be, Ti, V, Mn, Co, Fe(III), Cu, Ga, Se(IV and VI), Mo, Pd, In, Te(IV), Ba, Re, Os, Hg(II), Tl(III), and Bi (*1000*). The various oxidation states of vanadium could be separated by column chromatography on Fluoroplast-4 (*1452*) and thin-layer chromatography (*1255*). Different metal ions are resolved by TLC with solvents containing alkyl esters (*1132*).

Different metal complexes result in improved resolution of the metal ions: inert Co(III) complexes by TLC (*51*); dithizone complexes of metals (*91, 489, 1404, 1608*). The chromatographic behavior of metal–EDTA complexes has been studied on a Sephadex G-15 column (*300*). The facial and meridianal isomers of tris[S-(+)-α-alaninato]-Co(III) have been separated by TLC (*658*); the chromatographic and electrophoretic behavior of hexammine-type Co(III) complexes was studied (*1616*).

Displacement extraction chromatography was used to separate Pb and Bi (*1370*) and Cd and Cu (*1602*). Trace amounts of rare earth elements have been separated from uranium by the use of cellulose column chromatography (*654*). Thin-layer chromatography on cellulose plates was used to study the migration of 27 transition metals (*431*).

Numerous papers have been published on the chromatographic separation of precious elements and related metal ions: the chromatography of Re(VII) has been reviewed (*435*); Rd(VII) has been determined by TLC (*434, 436*); Os was separated as $(OsCl_6)^{2-}$ by paper chromatography from other platinum metals (*905*); TLC separation of Pt metals on cellulose adsorbent (*432*); the separation of Re, Mo, V, and W on alumina thin-layers (*433*); separation and determination of indium by paper chromatography (*695*); separation of Pt, Pd, Re, and Ir by paper chromatography (*1090*); the separation of precious metal chloride complexes by TLC (*1527*); the paper chromatographic behavior of Ir (*1489*); the quantitative determination of individual precious metals after paper partition chromatography (*1490*); Pt, Pd, Rh, Ir, and Au have been separated by paper chromatography (*1515*) and reversed-phase partition chromatography on a column with tributyl phosphate adsorbed on Daiflon (polytrifluorochloroethylene) (*21*); the microdetermination of Au, Ag, and Ru by Weisz ring technology after TLC (*647*); TLC of Au, Se, and Te in an alkaline medium (*437*).

The semiquantitative determination of thallium has been accomplished by paper chromatography by *in situ* colorimetric methods (*17*). The chromatographic behavior of technitium has been studied on paper (*1583*). Tri- and pentavalent antimony has been sepa-

rated by paper chromatography (*879*); arsenic(III) and (V) ions have been separated by paper chromatography (*878*).

Radioisotopes have been studied by a number of chromatographic techniques: complex catalytic processes involving radioisotopes and chromatography have been reviewed (*615*); ^{210}Po from irradiated bismuth has been separated by counter-current partition chromatography on a silica column (*624*); nuclides of the decay chain $^{125}Sn \rightarrow {}^{125}Sb \rightarrow {}^{125}Te_n$ have been separated by TLC (*1254*); carrier-free scandium has been prepared by extraction chromatography on a hydrophobic celite column (*32*); molybdenum in fission products has been separated by solvent extraction and extraction chromatography (*1617*); $^{133}In^m$ has been separated from ^{113}Sn by thin-layer chromatography (*1256*); the chromatographic separation of short-lived protactinium-234m and ^{234}Pa by elution chromatography on an alumina column (*9*); the separation of radioactive iodide from iodine-labeled compounds by means of thin-layer chromatography (*31*).

Gel chromatography of condensed phosphates, molybdic-, molybdophosphoric-, and silicic acids, and Fe(III) hydroxide complexes has been reviewed (*1620*). Cyanide and cyanate ions have been resolved by TLC (*1425, 1426*), in addition to ions of SCN^-, and CO_3^{2-} (*1426*); perrhenate, molybdate, and selenite ions have also been separated by silica-gel TLC (*1080*). The quantitative analysis of As(III), Sb(III), and Bi has been reported following separation by TLC (*1529*); arsenate ions in two valence states have been resolved by TLC on cellulose and silica-gel layers (*414*). Boric acid and its complexes have been studied by TLC (*211*).

Condensed phosphates have been studied by paper chromatography and gel filtration methods (*1060*); selenites, sulfates, and tungstates in the presence of condensed phosphates have been separated by paper chromatography (754); the gel chromatographic behavior of linear phosphates has been studied (*1468*). Perbromate ions have been separated from ClO_4^- and IO_4^- by thin-layer and paper chromatography, as well as paper electrophoresis (*806*). Sulfate, thiosulfate, thiocyanate, trithionate, and tetrathionate ions have been completely resolved by thin-layer chromatography (*705*).

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Electroanalysis and Coulometric Analysis

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THIS REVIEW COVERS the literature appearing during 1970 and through part of December 1971. A few papers published before 1970 have been included which have not previously appeared in this series. A few others are mentioned because of the importance as are a few outside the confines of the title because of their basic interest to workers in the fields of controlled potential electrolysis or coulometry.

BOOKS AND REVIEW ARTICLES

The series "Electroanalytical Chemistry" edited by A. J. Bard continues to contribute to the field in general. Of particular interest are chapters on thin layer coulometry (*73*), the application of controlled potential electrolysis to the study of electrode reactions (*14*), and a fundamental treatment of metal deposition (*71*). A complete catalog of the electrochemistry of inorganic and organic substances in non-aqueous solvent (*119*) should be of great use. The series "Modern Aspects of Electrochemistry" also continues to appear. A recent chapter on the mechanism of charge transfer between metal electrode and ions (*122*) may be of benefit to various workers. Reviews covering electrogravimetry (*30*), electroanalysis (*75*), constant current coulometry (*76*), coulometry (*113, 140*), coulometric analysis of oil products (*132*), and the electroanalysis of the platinum metals and gold (*57*) have appeared.

RECENT TRENDS

The trend in electroanalysis is clearly away from electrogravimetry and to coulometric methods. Interest remains high in the use of controlled potential electrolysis in organic chemistry for fundamental studies and for synthesis. Much work has appeared recently in the area of continuous coulometric titrations. This includes work on gas chromatography detectors and elemental analyzers which involves the combustion of the sample and then sweeping the products into the electrochemical cell. Often but not always, the generation current is variable and a peak shaped current–time curve results. The area under this curve is equivalent to the coulombs used. The combustion method can also be used in a one-shot batch mode with either constant current or variable current. At times the distinction between these methods is difficult.

Considerable work on all types of instrumentation has been developed in the last two years. Noteworthy is the use of computers and digital electronics, microanalysis utilizing combustion, air pollution monitors, and solid state coulometers. Potential scanning coulometry has been examined (*185*) and a number of other variations of potential and current control reported. All of these will be considered under their applications.

Of special interest is the announcement of gas phase coulometry (*116*) which essentially uses electron capture detectors to titrate various compounds with electrons.

ELECTROSEPARATIONS AND ELECTROGRAVIMETRY

Although less work in this area than in the past has been reported, a number of studies and a few innovations are worthy of note. The determination of Ag in the presence of Bi, Cu, and Fe at controlled potential has been studied both gravimetrically and coulometrically (*188*)—the standard deviation of the latter being significantly better. Application of very careful potential control to the determination of Cu, Ag, and Cd has been reported (*107*) as has radiographic studies on the deposition of Ag (*200*). Cd has been separated from Ni in ammonium tartrate solutions (*123*) and Co deposited from tripolyphosphate solution (*130*). Anodic depositions in-