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Ion Exchange and Liquid Column Chromatography

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This review spans the time from the 1976 review to the journals available to the author on December 31, 1977. I depended heavily on the new "CA Selects" in high-speed liquid chromatography, along with a "Dialog" computer-based reference search in ion-exchange separations. Together, these lists gave well over 2000 references. I wanted to keep the bibliography to 500 items. The selection was arbitrary as usual, but preference was given to new materials and methods. Many applications are cited, but they are illustrative, not exhaustive. The reader who wants a list of all papers on amino acids, for example, should order his own computer search. Preference was given to publications accessible to readers in English-speaking countries.

Paper and thin-layer chromatography are not included in this review, nor is size-exclusion chromatography, save for two references to "hydrodynamic chromatography". "Affinity

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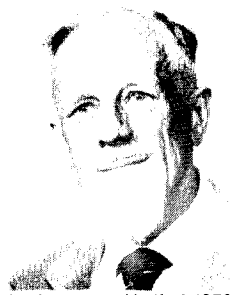

Harold F. Walton joined the staff of the University of Colorado in 1947. His research interests in ion exchange date from 1938 when he went to work for the Permutit Co. as a research chemist; from there he went to Northwestern University in 1940. He obtained the B.A. and D.Phil. degrees at Oxford University. He is the author of three textbooks on inorganic and analytical chemistry and coauthor with William Rieman III of "Ion Exchange in Analytical Chemistry" published in 1970. He has contributed chapters on the physical and analytical chemistry of ion exchange to several cooperative works. In 1961 he was chairman of the Gordon Research Conference on Ion Exchange. Dr. Walton spent the 1966–67 academic year and half of 1970 as a Fulbright visiting professor at the University of Trujillo, Peru. He was given the Colorado Section Award of the ACS in 1976. Dr. Walton has also served on ANALYTICAL CHEMISTRY's Advisory Board.

chromatography" is excluded.

The past two years have seen special activity in new bonded phases for liquid chromatography. The hydrophilic "Glycophases", capable of accommodating the large molecules of peptides and proteins, are of special interest here. Nonionic porous polymer gels and adsorbents based on carbon are other novel stationary phases. The interpretation of solute–sorbent–solvent interactions advances steadily, with special reference to nonpolar stationary phases, "reverse-phase" chromatography and hydrophobic interactions. More and more chromatographers are using 5-μm particles and heeding the guidelines of Guiochon and others (1975) on column optimization. Techniques are being refined, leading to high precision and high resolution. Much attention is paid to detectors, especially electrochemical detectors and diode arrays that allow one to scan the absorption spectrum of the effluent in the moment that it emerges from the column. On the other hand, the much-desired LC–mass spectrometer interface seems to have made little progress.

Ion-exchange methods for concentrating and separating inorganic ions have apparently reached a plateau, with few radically new developments; however, high-speed, high-resolution "ion chromatography" is coming into general use, and new absorbents based on crown ethers have promise. The method of paired-ion chromatography has found wide acceptance.

Applications of ion exchange and liquid chromatography to the analysis of organic compounds are multiplying fast. To choose one small example, the separation of optical isomers has advanced notably.

In September 1977, an international conference on liquid chromatography was held in Austria. A report by Karger in the December *Journal of Chromatographic Science* gives a fine overview of the current state of the art.

BOOKS AND REVIEWS

The number of books that have appeared testifies to the vitality of the field; see references *A1–A13*. Most of them are practical guides to liquid chromatography techniques. One is a collection of papers on environmental analysis (*A6*) that includes important work on liquid chromatography. Another (*A13*) traces the historical development of ion-exchange chromatography through a selection of reprinted papers, from the first publication of Samuelson in 1939 to the present day.

References *B1–B21* are to bibliographies and reviews whose scope will be evident from the titles. Many of the papers cited in the main reference list include reviews of previous work as well as original contributions, so it was difficult to decide which papers should be included in the "B" section.

The new *Journal of Liquid Chromatography*, published by Marcel Dekker, New York, N.Y., will begin publication this year. Those of us with limited library budgets will groan, but we must grant that the editorial board of the new journal is very distinguished indeed.

ION EXCHANGE

New Exchangers. (*a*) *Inorganic.* (See Table I). Combinations of hydrous oxides of highly-charged ions, with one oxide more acidic than the other, all have ion-exchanging properties. Some products are more chemically inert, others less so. The amorphous precipitates have greater surface area than the crystalline compounds, and therefore have higher exchange capacities. On the other hand, amorphous precipitates are more soluble than crystalline compounds, and it is desirable to produce a certain degree of crystallization by heating or refluxing the precipitates.

By now, almost all conceivable combinations of acidic and basic oxides have been prepared. Those described recently are: Al–Sb(V) (*380*); Fe(III)–As(V) (*379*); Fe(III)–Sb(V) (*378*); Cr(III)–Sb(V) (*302*); Zr–Sb(V) (*301*); Nb–As(V) (*370*); Sn(IV)–Sb(V) (*303*); Sn(IV)–V(V)–P(V) (*367*); Sn(IV)–W(VI)–As(V) (*371*); Ta–As(V) (*377, 381*). Titanium arsenate has been used to absorb alkaloids (*369*), and anion-exchange distributions in zirconium oxide have been studied (*424*). The use of inorganic sorbents in chemical analysis has been reviewed (*311*). A study was made of the exchange equilibria of K and Rb ions with hydrogen and other ions on hydrous antimony pentoxide (*41*).

The time is ripe for a general review and evaluation of the dozens of hydrous-oxide ion exchangers that have now been described.

(*b*) *Resinous Exchangers.* Resins selective for iron, copper, vanadium, and uranium, based on *N*-substituted hydroxylamine, were made and studied (*482, 483*); work on condensation-type polymers of 8-hydroxyquinoline continues (*484*). Acrylamide, copolymerized with DVB, gives an anion-exchange resin very similar to the strong-base polystyrene resins (*23*). Poly(vinyl alcohol) has been cross-linked and provided with various functional groups, sulfonic, carboxylic, phosphonic, secondary and quaternary amines, to give a new family of ion-exchange resins (*182, 305*). On macroporous styrene–divinylbenzene polymers, thioglycolate and propylenediamine tetraacetic acid groups have been grafted to give resins selective for gold, mercury, bismuth, and copper, and capable of recovering these and others metals from extremely dilute solutions (*329*). From chlormethylated styrene–divinylbenzene, carboxylic cation exchangers with mono- and difunctionality have been made which have a strong affinity for silver ions (*292*). A review of the properties of carboxylic exchangers which stresses their selectivities for di- and trivalent ions comes from the India atomic energy commission (*481*). Carboxyl and DEAE functionalities can be added to the "Enzacryl" or poly(acrylomorpholine) gel-permeation resins (*119*). A new strong-base resin based on weakly-cross-linked styrene–DVB polymer is described (*10*). Hydrazide (*112*) and diphenylcarbazide (*102*) groups have been incorporated into styrene–DVB polymers, giving special selectivity for mercury and chromium, respectively. A dithiocarbamate resin made from polyethylene imine is selective for lead and zinc (*228*), and a resin selective for fluoride ions was made by attaching $Sb(C_6H_5)_2$ groups to polystyrene (*68*).

In a special class are the resins carrying cryptate (*36*) or crown-ether (*37*) groups. These are not ion exchangers, strictly speaking, but their cyclic "anchor groups" can coordinate cations of the alkali- and alkaline-earth metals, and other cations besides, which then bind anions electrostatically. They are thus "salt exchangers" that can be used for the chromatography of anions as well as cations. Cation selectivities are related to ionic radii. Alkali-metal selectivities peak at K^+ for the crown-6 compounds, and *tert*-butylammonium cations are held more strongly than *n*-butylammonium. Alcohol or water eluents may be used. Columns loaded with an *l*-amino acid serve to separate the optical isomers of this acid (*34, 37*). Resins have been prepared with noncyclic, uncharged ion-carrying groups (*161*).

New light has been shed on the importance of the polymer structure in ion-exchange resins by Davankov and co-workers (*88*). They have shown that replacement of the usual divinylbenzene cross-link by longer cross-links, like diphenyl or tetraphenyl units, gives polymers with entirely different swelling properties that swell considerably in nonaqueous solvents. They call them "macronet" polymers, and use such polymers in their separations of optical isomers (*89, 415*).

Pyrolysis–mass spectrometry is used to characterize ion-exchange resins. It reveals matrix structure, cross-linking, functional groups, and counterions (*35*).

Cross-linking styrene with pure *meta*-divinylbenzene gives a more uniformly cross-linked resin with better chromatographic properties than the common resins (*373*). Exchangers

Table I. Inorganic Ions[a]

Elements	Separated from	Exchanger	Eluent	Elution order	Notes	Ref.
Alkali metals	Each other	I	NH_4NO_3	Li-Na-K-Rb	P-Sb oxide	(*1*)
Alkali metals	Each other	Special	H_2O, MeOH	Li-Na-K	Crown-ether resin	(*36, 37*)
Alkali metals	Each other	C	...	...	Electromigration	(*192*)
Alkali metals	Alk. earths	Special	H_2O, EtOH	Na-Ba-Ca	Uncharged ion carrier	(*161*)
Alkali earths	Each other	Special	H_2O, MeOH	Mg-Ca-Sr-Ba	Crown-ether resin	(*37*)
Rb	K	I	HNO_3	...	On Sb_2O_5	(*41*)
Cu	Other elts.	Chel.	HCl or HI	...	Environmental	(*152, 162*)
Cu, Zn	Waters	Chel.	HNO_3	...	Pb, Cd, Mn also	(*43, 400*)
Cu	Uranium	A	MeOH-HBr	Cu retained	Elute Cu by HCl	(*256*)
Cu, Fe	Hard water	Special	...	Cu, Fe retained	For hardness titrations	(*138*)
Ag	Others	C	...	...	Carboxylic xgr	(*292*)
Au	Ores	Nonionic	Acetone-HCl	Au retained	...	(*456*)
Au	Zn, Cd, In	C	Acetone-HCl	Au passes	For traces	(*453*)
Be	Other elts.	C	THF, MeOH	Be first	Environmental	(*253, 254*)
Be	K, Mg., etc.	C	HNO_3-MeOH	K-Be-Mg	In rocks	(*450, 454*)
Mg	Ca	C	Ethylene diammonium	Mg-Ca	Automated	(*15*)
Mg	Ca	I	4 M HNO_3	Mg-Ca	Nb antimonate	(*366*)
Ca	Cu, Ni, Pb	Chel.	KNO_3	Ca-Cu-Ni	Plus complexing eluent	(*43*)
Ca	Mg, Sr	A	Complexing	Mg-Sr-Ca	Uses EGTA	(*296*)
Sr, Ba	Ca, etc.	C	Complexing	Ca-Sr-Ba	In waters	(*355*)
Ba	Ca, etc.	C	HCl	Ba last	Trace in rocks	(*132*)
Zn, Cd, Al	Plating baths	A	HCl	Al-Zn-Cd	Elute Cd by H_2O	(*431*)
Zn, Cd	Many elts.	C	Acetone-HCl	Cd-Zn	For traces	(*224*)
Zn	Cd	I	NH_4I-acetone	Cd-Zn-Pb	Zr-P, Zr-W oxides	(*397, 398*)
Ca, Zn, Hg	Others	Chel.	...	...	Hydrazine resin	(*112*)
Hg	Pb	I	HNO_3	Hg-Pb	...	(*371*)
B	Mo	C	Dil. acid	B passes	...	(*339*)
Al	Urine	C	1 M HNO_3	Al abs.	For traces	(*38*)
Al, Fe	Others	A	Malonate	Al, Fe abs.	Fe el. by HCl	(*430*)
Sc, Y	Others	A	Malonate	Sc last	Ti, Zr also	(*64*)
Y	La, Ce	C	Citrate	Y first	...	(*394*)
Pm	Nd	A	NO_3-dioxane	...	...	(*4*)
Ce	Ba, Hg, U	C	NH_4OAc	Ce first	Many elts.	(*123*)
Lanthanides	Each other	C	Buffers	...	By HPLC	(*106*)
Lanthanides	Each other	C	EDTA-Fe	...	Displacement	(*304*)
Ga	Al, In, Tl	A	Malonate, HNO_3	Tl, Al, Ga	...	(*64*)
Ga	In	A	Phosphate	Ga, In	Complex equil.	(*108*)
Tl	Others	C	HBr	...	Partially sulfonated resin	(*354*)
Tl	Mo, V, Mn	A	HCl	Tl retained	Manganese nodules	(*255*)
Ti	Fe, Mg, Zn	C	Tartrate	Ti, Fe, Mg	At pH 5	(*422*)
Zr	Many elts.	A	HCl	...	Absd. from malonate	(*429*)
Zr	Th, Pa	A	HCl	Zr last	Radiochem.	(*316*)
Zr	U, Th	Chel.	HCl, H_2SO_4	U, Th, Zr	From seawater	(*32a*)
Ge	As	A	HOAc, H_2SO_4	Ge, As	...	(*327*)
Pb	U_3O_8	A	HBr	Pb retained	Pb el. by HCl	(*247*)
Pb	Others	Chel.	...	Pb retained	Special resin	(*228*)
Pb	Others	I	...	Ba, Pb retained	Ce-Mo oxide	(*91*)
NH_4	Na, amines	C	HCl	...	Ion chromatogr.	(*42*)
PO_4	SO_4, SO_3, Cl	A	NaOH	...	Ion chromatogr.	(*448*)

Table I. (*Continued*)

Elements	Separated from	Exchanger	Eluent	Elution order	Notes	Ref.
P_2O_7	PO_4, etc.	A	HCl	...	Use EDTA	(*141*)
P	Condensed phosphates	A	Acetate	...	Several species	(*285, 328*)
V	Others	C	HNO_3-H_2O_2	V first	In rocks	(*452*)
V, Mo	Waters	A	HCl-$HClO_4$	V, Mo	Absd. as citrate	(*252*)
V	Lanthanides	A	5 M HNO_3	La, V	H_2O elutes La	(*124*)
V	Others	A	MeOH-HCl	V, Cu, U	Nuclear raw mat.	(*256*)
Bi, Hg	Others	I	...	...	Th-W oxide	(*90*)
SO_4, Cl	Organic cpds.	A	$NaHCO_3$	Cl, SO_4	Ion chromatogr. after combustion	(*435*)
SCN	Cl, Br, I, etc.	A	Na_2SO_4	Cl, Br, I, SCN	Sephadex G15; metal ions also sep.	(*266*)
Polythionates	Each other	A	Citrate	Lower *n* first	HPLC; linear log *K*: *n*	(*503*)
Cr	Fe, Al, Ca	C	Acid	Cr, Fe, Al	In chromite	(*208*)
Cr	Waste water	A	Acid	Cr retained	Elute Cr by NH_2OH	(*420*)
Cr	Other elts.	Chel.	...	Cr retained	Special resin	(*102*)
Cr	Isomeric complexes	A	...	...	...	(*327*)
Se(IV)	Pb, As, Sb, Cd	C	$HClO_4$	Se first	Det. by anodic stripping	(*14*)
Te(IV)	I	C	HCl-NH_2OH	I, Te	Radiochemical	(*3*)
Organic Se	Amino acids	C	Citrate	...	Selenodiamines	(*295*)
Halides, PO_4, SO_4	Organic cpds.	A	$NaHCO_3$-Na_2CO_3	Cl, Br, PO_4, SO_4	Ion chrom.; Schöniger combustion	(*75*)
Halides	Each other	Liq.	...	...	On porous glass	(*350*)
Cl	Water	A	...	...	Trace concn.	(*462*)
Mn	Ni	C	Glycine	Ni, Mn	Rising pH	(*513*)
Mn	Nodules	A	PrOH-HCl	Fe, Mn, Zn	Several elts. separated	(*250, 255*)
Mn	U_3O_8, etc.	C	Hexone-HNO_3	U, Mn	Elute Mn with HNO_3	(*248*)
Fe(II)	Fe(III)	A, C	Bipyridyl-HCl	...	Opp. charged complexes	(*362*)
Fe(III)	Seawater, rocks, slag	Chel.	...	...	Also Cu, U; hydroxamic resin	(*482*)
Fe	Ni, Cu	Cell.	...	...	Salicylate cellulose	(*56*)
Rh, Ir, Ru	Complexes	Nonionic	...	...	Various complexes sep.	(*117, 118*)
Os	Cl complexes					(*22*)
Th	Fe, U	A	HCl-acetone	Th, Fe, U	From waters	(*251*)
Th	U	A	H_2SO_4	Th, U	Radiochem.	(*109*)
Th	U	ZrO	Iodate, HNO_3	U, Th	Radiochem.	(*31*)
Th, U	Lanthanides	Chel.	H_2SO_4, HCl	La, Th, U	...	(*203*)
Th, Pa	U	A	HOAc + HCl, HF	Th, Pa, U	...	(*110*)
U	Waters	A	SCN-HCl	U retained	By irradiation	(*53*)
U, Th	Waters	A	Citrate-HCl	Th, Fe, U	Elute by HCl	(*251*)
U, Th	Other elts.	A	Oxalic-HNO_3	...	Sediments	(*257*)
U	Other elts.	A	THF-HCl or MeOH-HCl	U retained; U, Th sep.	Elute U with aqueous HCl	(*249, 256*)
U	Other elts.	I(SiO_2)	Complexing	U retained	Elute U w. HCl	(*457*)
U	Th, Zr, Ti	A	Malonate	U, Th last	Elute U, Th w. HCl, H_2SO_4	(*64*)
U	Fe, Zr, etc.	A	HCl	Fe, Zr, U	In rocks	(*451*)
Am	Fe	C	Complexing	Fe, Am	...	(*30*)
Cf	Cm	...	...	...	...	(*11*)
Transuranium	Each other, lanthanides	Liq.	...	...	On porous silica	(*191*)

[a] See Table II for metal separations by chromatography of organometallic and chelate compounds. Abbreviations: A = anion exchanger, C = cation exchanger, Chel. = chelating exchanger, I = inorganic exchanger.

based on cellulose have a new interest for chromatographers now that bead-type cellulose is available; a variety of functional groups have been attached (*353*), and columns of microparticulate ion-exchanging cellulose mixed with diatomaceous earth have promise in medical and pharmaceutical applications (*480*). Cellulose carrying hydroxamic groups (*259*) and salicylate-bonded cellulose (*56*) will recover traces of iron from sea water.

(*c*) *Bonded Exchangers.* Research continues on ion exchangers chemically bonded to microporous silica. Several reactions serve to introduce different ionic functional groups (*16*, *82*). An anion-exchanging product made by reacting aminopropyltriethoxysilane with silica is a good stationary phase for carbohydrates (*406*), and also for removing traces of copper and iron from water before hardness titrations (*138*).

Most bonded ion exchangers have a rather low capacity, about 0.1–0.6 mequiv/g. A new class of bonded exchangers have been prepared and evaluated by Rosset and collaborators (*63*, *146*, *275*); their capacities range from 0.7 to 2.2 mequiv/g, and on a volume basis the capacity equals or exceeds that of the familiar polystyrene-type resins. They are made by reacting vinylmethoxysilanes with silica. Weakly-basic exchangers are made by attaching epoxy resins to silica. Selectivity orders and ion-exchange equilibrium constants are like those of conventional resins.

Several ion exchangers have been made by bonding appropriate organic groupings like 8-hydroxyquinoline to controlled-pore glass; some of these are made commercially (*162*). Weakly basic amino groups (*278*) and chelating diamine and dithiocarbamate groups (*279*) have been bonded. The products are used to collect trace amounts of oxy-anions and heavy metals from natural waters. After loading, the glass particles are mixed with cellulose and pelletized for x-ray fluorescence measurements.

New Techniques. (*a*) *Ion Chromatography.* This is the name given to the fast, sensitive technique introduced by Small, Stevens, and Baumann in 1975. The chromatographic column is packed with low-capacity surface-active polystyrene beads; this column is followed by a "suppressor" column of high-capacity exchanger that removes the excess of the acid or base eluent and leaves the ions of interest to be measured by electrical conductivity. The technique has been applied to the Schöniger combustion for determining halogens, sulfur, and phosphorus in organic compounds (*75*, *435*), to ammonia–methylamine mixtures (*42*), and to the anions in boiler water (*448*).

(*b*) *Ligand Exchange.* There have been new advances and insights into what is now an old technique. As "ligand exchange" we classify all methods that use association with metal ions to absorb and separate organic compounds.

Two novel approaches were taken by Karger and colleagues (*80*) in an effort to overcome the slow kinetics of conventional ligand-exchange chromatography, where the ligand must form an inner-sphere complex with a metal ion inside an ion-exchange resin. One of these approaches uses a chelating diamine bonded to silica and coordinated with cadmium ions, preferred to copper because their complexes are weaker and form and dissociate rapidly. The other uses a hydrophobic triamine in solution with zinc ions in an acetonitrile–water solvent. The stationary phase is a C_8 or C_{18} reverse-phase bonded packing. Organic anions are drawn into the stationary phase, apparently by outer-sphere complexing or ion pairing with the 4-coordinated zinc–triamine–CH_3CN cations. Sulfa drugs and peptides were beautifully separated with high plate numbers.

Outer-sphere complexing with optically active $Co(en)_3^{3+}$ cations in a carboxylic ion-exchange resin was used to separate optical isomers of amino acids (*145*). The separations were excellent. The well-known work of Davankov on optical-isomer separations has been continued (*89*, *415*), and the mechanism has been further elucidated. Special "macronet" resins loaded with copper ions are used on the preparative scale. The disadvantage of this method is the slowness of ligand exchange.

Chelating exchangers prepared from polysaccharides (Sepharose) were loaded with copper–ammonia complex ions and used to separate peptides and proteins (*158*), and amine-bonded phases loaded with copper ions separated aromatic amines in nonaqueous solvents (*70*). Silica gel, loaded with cadmium by a hydrothermal treatment, was also used to separate aromatic amines (*486*). Silver-loaded silica gels (*7*, *177*, *268*, *290*) and ion-exchange resins (*313*, *314*, *471*) separated cis-trans isomers of various compounds as well as electron-donor aromatic compounds in general: see Table II. A variant on this procedure is to use thallium(I), which binds ligands less strongly than silver(I) but reacts much more rapidly, giving better column efficiencies (*427*).

The association of metal ions with ligands within a resin has been studied by ESR (*493*). Two groups have looked closely at the equilibria involved in metal-ligand associations in an ion-exchange resin in contact with an aqueous solution containing ammonia and metal ions (*104*, *289*). The first of these studies (*104*) explores experimentally the partition of amino acids between a solution and a copper-loaded phosphonic resin, interprets the effects of metal loading and ammonia concentration in terms of a minimum number of ion-exchange and complex-forming reactions, and uses the results to predict optimum conditions for separating amino acids.

Trace Concentration of Inorganic Ions. Chelating ion-exchange resins continue to find new uses in this area. Usually the familiar iminodiacetate resin, Chelex-100, is used (*125*, *129*, *223*, *273*, *479*); other chelating resins serve also (*9*, *400*, *482*). After absorption, the metals are identified and measured by x-ray fluorescence, neutron activation, or atomic absorption spectrometry. Common anion-exchange resins can collect trace metals from water if the proper complexing agent, like hydrobromic acid (*162*), thiocyanate (*53*), or citrate (*251*, *252*) is added. Chromate ions are absorbed so strongly that they can be collected from industrial waste waters by simple anion exchange (*420*). A macroporous resin impregnated with a liquid ion exchanger was used to take up copper (*348*), and an anion-exchange paper absorbed traces of inorganic mercury from water (*74*).

Multielement Separations. Schemes for the separation of metallic elements in silicate analysis by ion exchange continue to be developed (*218*, *306*, *475*). Separations on macroporous resins (*224*) and in the presence of tartaric (*422*) and triethylenetetramine hexaacetic acid (*510*) are reported. The prolific group of Korkisch (*248*) has separated many minor elements from uranium oxide and "yellow cake" by cation exchange in nonaqueous solvent mixtures. Distribution ratios of 73 elements were measured in solutions of hydroxylamine and hydrazine (*470*). DEAE-cellulose in mixed hydrochloric–acetic acid solvents gives high selectivities for multielement separations (*507*). Columns of mixed resins were used to separate transition-metal ions (*321*).

Isotope Separations. Isotopes of boron (*71*, *216*), beryllium, calcium, and cobalt (*272*), and uranium (*86*, *361*) are separated on ion-exchange columns. Electromigration in an ion-exchange membrane is used by Kakihana (*181*) to separate isotopes as well as cations of different elements. Calcium isotopes and transuranium elements were separated on porous silica microspheres impregnated with di-(2-ethylhexyl) phosphate (*190*). Isotopic molecules of benzene and toluene, with ^1H and ^2H, were effectively separated by liquid chromatography on a C_{18} bonded phase (*61*).

Nonchromatographic Uses. A batch method was used to separate zinc from copper alloys after dissolution (*458*). The color of resin beads, produced by in situ reactions of metal ions with color-producing reagents, served to detect subnanogram amounts of chromium (*200*) and to measure quantitatively small quantities of chromium, iron, cobalt, and copper (*508*).

Equilibrium and Kinetic Studies. The distribution of the components of mixed solvents, one of which is always water, inside and outside a cation-exchange resin was intensively studied by Poitrenaud and colleagues (*389*), who also measured the distribution of chelating agents, like acetylacetone and 8-hydroxyquinoline, between solution and resin (*360*), as well as silver–alkali metal ion distributions in solutions of weak and strong acids, where they showed that the measured distributions could be used to evaluate the ionization constant of nitric acid in acetic acid solvent (*390*). The thermodynamics of cation exchange in mixed solvents were studied over a wide range of conditions (*439*), and the dynamics of simple exchanges studied by the displacement method (*237*). Ion-exchange kinetics in surface layers (*114*) and elution curves with nonlinear isotherms (*217*) were treated. The exchange equilibria Al^{3+}–H^+ and Fe^{3+}–H^+ in nitric acid

solutions were measured (*208*). Measurement of interstitial volumes of ion-exchange columns by two methods is described and critically discussed (*271*).

LIQUID CHROMATOGRAPHY

Bonded Phases: Preparation and Properties. Techniques of attaching various organic groups to silica surfaces are now well established and have been reviewed by several authors (*B12, 83, 219, 235, 385*). The commonest method is to treat dry silica with $RSiCl_3$ or $RSi(OCH_3)_3$, and then to add water to form oxygen cross-links between the newly-bound silicon atoms. Kingston and Gerhart describe the preparation of octadecyl-bonded silica by this means (*229a*). The structure of these cross-linked materials is called "molecular fur" by Horvath and Melander (*188*). More hydrophobic surfaces are produced by the reaction of R_3SiCl (*230*). When R is a large group like the familiar $C_{18}H_{37}$, the silica surface is never completely covered (*83*). Unreacted Si–OH groups remain and affect the adsorptive properties of the product. Treatment with trimethylchlorosilane, $(CH_3)_3SiCl$, esterfies most of the free hydroxyl groups and gives a more hydrophobic product (*219, 229a, 230, 235*). Condensation of two Si–OH to give siloxane bridges, Si–O–Si, may occur (*385*). The silica surface is modified by heating (*24*).

"In situ" treatment of packed silica columns does not seem to be widely used, but it does have the advantage that the packed column can be tested for its chromatographic efficiency before the silanizing process is performed (*151*).

The production of silica-bonded ion exchangers was described above. The use of vinylsilanes like $CH_2{=}CHSi(OCH_3)_3$ deserves special mention, as these relatively small molecules seem to give efficient surface coverage (*63, 83, 146*). Once bonded to silica, the vinyl groups serve as points of attachment for other molecules like those of styrene. Another way to make vinyl monomers combine with silica surfaces is by radiation-induced graft polymerization (*140*).

To attach polar groups to silica surfaces the usual way is to use cyanopropyl- or aminopropyltrimethoxysilane, but 3-phthalimidopropyltrichlorosilane gives a bonded phase that is particularly effective for polycyclic aromatic hydrocarbons (*199*). Amide and sulfonamide groups may be bonded to silica by first making the amide or sulfonamide from aminopropyltrimethoxysilane and then causing the product to react with silica (*116*). Polyamide coatings have been deposited on porous glass (*79*).

Polar bonded sorbents of a radically new kind, the so-called "Glycophases", are made by first treating controlled-porosity glass or porous silica with an epoxide, $CH_2(O)CHCH_2O(CH_2)_3Si(OCH_3)_3$. The product retains its terminal epoxy group, which is then opened by a nucleophilic reagent to give $P_s{\cdot}CH_2CHOH$-, where P_s stands for a wide variety of functional groups, ionic or nonionic. The products combine high permeability with mechanical rigidity and are used for high-efficiency chromatography of proteins (*66, 67*).

The properties of nonpolar hydrocarbon-like bonded phases have been studied in detail. Scott and Kucera (*410*) compared five commercial reverse-phase bonded packings that differed in their hydrocarbon chain length and degree of surface coverage. They found that a popular C_{18} packing has a considerable proportion of free Si–OH groups, which adsorb polar solutes and molecules of solvents (including water and acetonitrile) and at the same time make the surface more easily wet by water. The wettability of reverse-phase packings is often taken for granted, but these authors showed that some packings are not wetted if there is much water in the solvent. When the solvent does not come into proper contact with the adsorbent, mass transfer is slow and incomplete.

The longer is the bonded carbon chain, the stronger is the sorption of nonpolar solutes (*179, 219, 235*). The quantitative correlation is not clear. Mixed retention mechanisms seem to operate, particularly with shorter hydrocarbon chains and less surface coverage, where the solute has more access to the silica surface (*235*). Silica treated with $RSiCl_3$ retains nonpolar solutes more strongly than that treated with R_3SiCl (*410*), given the same proportion of carbon. Adsorbent surface heterogeneity can be evaluated from chromatographic data (*490*).

Porous Polymers: Carbon Adsorbents. Nonionic macroporous polymers, the XAD resins of Rohm and Haas Co., are excellent general-purpose absorbents, but they are produced in large grain sizes and must be ground and sized before they can be used in high-performance chromatography (*205, 356, 357*). Recently a series of macroporous polymers has been produced in Japan, expressly for chromatographic use. They have particles in the 5–10 μm range with different pore sizes and chemical types. The styrene–divinylbenzene polymers are hydrophobic and behave somewhat like octadecyl silica in chromatography, with the important difference that they are stable over the entire pH range (*333, 476*). Their aromatic character causes them to bind aromatic compounds in preference to aliphatic. Various applications have been reported (*143, 167, 172, 334, 363*). They are also used in size-exclusion chromatography. Though their selectivities are good, their mass-transfer rates are rather slow.

Cross-linked poly(vinylpyrrolidone) is used in chromatography (*12, 135, 155, 341*). An interesting series of macroporous hydrophilic absorbents, the Spherons, are made by copolymerizing ethylene glycol bis-methacrylate with hydroxyethyl methacrylate and then introducing functional groups (*318, 433*).

Carbon adsorbents have been used in gas chromatography for several years, but it was hard to make them in small, uniform particles that would withstand a pressure gradient. This difficulty has now been overcome, and carbon is now a practical adsorbent for liquid chromatography. Two products have been made, one a graphitized carbon black (*76, 232*), the other a porous silica coated with carbon that has been deposited by pyrolysis of benzene vapor (*28, 77, 78*). Pyrocarbon–silica can be made in smaller particle sizes than graphitized carbon black, and it gives better chromatographic selectivity, at least for the polychlorinated biphenyls (*168*).

Solvent Systems. There is much interest in the role of the solvent in "reverse-phase" chromatography. When hydrophobic bonded phases first came into general use, Locke (*283*) recognized that their selectivities were determined by solute–solvent interactions, and he correlated the retention of aromatic hydrocarbons on octadecyl silica in water–alcohol mixtures with the solubilities of the hydrocarbons in water. The more soluble they were, the less they were retained. This idea was re-examined by Karger (*220*), who noted that retention did not always go inversely as solubility, and that the shape of the solvent molecule was important. Cyclo-alcohols were retained more strongly than straight-chain alcohols even though they were more soluble. He related retention to the effective molecular surface through a function called the "connectivity".

A theory of solvophobic interaction in reverse-phase liquid chromatography was developed by Horvath, Melander, and Molnar (*187–189*). They calculated the energy needed to remove a solute molecule from the solvent and attach it to the long carbon chain of the stationary phase, using bulk properties of the solvent and solute. Solute retention is related to the "hydrocarbonaceous surface area" of its molecules. Among other things, the theory interprets the linear relation between the free energy of partition (or the logarithm of the capacity factor) and the volume fraction of water in a mixed solvent like water–methanol or water–acetonitrile. This relation has been observed by many authors in reverse-phase systems (*168, 219, 220, 356, 499*).

Tanaka and Thornton (*462a*) measured the retentions of many compounds in several homologous series on a C_{18} column and showed that the energies of partition could be expressed by additive constitutive terms. They also studied the effect of solvent composition.

In mixtures of polar with nonpolar solvents, the effects of solvent composition are more complicated. They were studied for silica adsorbents by Saunders (*403*) and others (*156, 428*). Thin-layer chromatography served as a guide to the conditions for column chromatography. The role of water in normal-phase adsorption chromatography was the subject of several studies (*39, 115, 464, 465*), and the use of small amounts of acetonitrile to deactivate silica, instead of water, was proposed (*402*).

The solubility parameter concept has been refined to show contributions of different kinds of solvent–solute interactions, including dipole–dipole, hydrogen-bonding, proton donor and acceptor effects, and dispersion forces (*19, 221, 469, 478*). Scott (*409*) formulated these interactions in a general way for both polar and nonpolar solvents and sorbents, differentiating the polar from the nonpolar forces, and relating dispersion forces

Table II. Organic Compounds[a]

Compounds	Stationary phase	Moving phase	Notes	Ref.
Acids, aliphatic:				
Mono- and dicarboxylic	C	HCl	Maleic, fumaric, acrylic	(*387*)
Dicarboxylic	C	Butanol-H_2O	In fruit juices	(*376*)
From plant tissues	SiO_2-H_2SO_4	$CHCl_3$-cyclohexane		(*326*)
Maleic, fumaric	A	Borate, pH 9	Also acrylic, methacrylic	(*63, 274*)
Polycarboxylic	A	NaOH	Also amino acids	(*105, 111*)
Polycarboxylic, hydroxy	A	$Mg(OAc)_2$	Also on R	(*120, 501*)
Chloracetic acids	AB	CH_3CN-H_2O		(*63, 193*)
Fatty acids	A	HCl	Derivatized	(*222*)
Fatty acids	R	...	*p*-methoxyanilides	(*184*)
Fatty acids	R	MeOH-H_2O	*p*-bromophenacyl esters	(*351*)
Fatty acids	N-Ag	...	Esters on Ag silica gel	(*268*)
Acids, aromatic				
Carboxylic	P	...	On polyamide	(*79*)
Carboxylic, sulfonic	R	TBA	Paired ion	(*488*)
Carboxylic, phenolic	C	HCl, HOAc	Sulfonation of resin varied	(*204*)
Naphthalene-acetic	R	Pr_4N^+	Paired ions studied	(*499*)
Phenolic, flavonoids	R	H_2O-HOAc	Incl. caffeic, salicylic	(*505*)
Nicotinic, benzoic	L	TBA	Paired ion; several acids	(*489*)
Various	AB	Aq. EtOH	pH effect shown	(*16*)
Various, incl. phenols	A	Aq. acids	Benzene, naphthalene solutes	(*205*)
Phenylacetic, urinary	R	H_2O-MeOH	Polarity effect shown	(*323, 492*)
Pyridine carboxylic	CB	KNO_3-H_3PO_4	Isomers	(*63*)
Incl. phenols, aldehydes	A	Aq. EtOH	With sugars	(*347*)
Aflatoxins	N, R	Various	In wine	(*460, 461*)
Aflatoxins, PNA	R	CH_3CN-H_2O	Also cannabis	(*212*)
Alcohols, polyhydric	R	...	Structure correlation	(*58*)
Aldehydes, aromatic	PP	EtOH-H_2O	Incl. cinnamic	(*12*)
Formaldehyde	N	$CHCl_3$	Hydrazone used	(*294*)
Alkaloids	CB	MeOH-H_2O	Also amphetamine, etc.	(*474*)
Ergot	R	CH_3CN-H_2O	Includes LSD	(*96*)
Ergot	R	CH_3CN-H_2O	Trace enrichment	(*405*)
Various	N	Propyl ether	Dansyl derivs.	(*137*)
Various	R	C_7 sulfonate	Paired ion; also phenethylamines	(*286*)
Opium	R	CH_3CN-H_2O	Buffers	(*504*)
Opium	R	C_7 sulfonate	Paired ion	(*340*)
Nicotine	N	EtOAC etc.	In smokers' urine	(*495*)
Nicotine	Ti-As	...	Titanium arsenate absorbs	(*369*)
Various	N	...	Ag-loaded silica	(*7*)
Various	N	$CHCl_3$	Picrate-loaded silica	(*399*)
Amines	N	...	Cd-modified silica	(*486*)
Hydrazines	R	CH_3CN-H_2O	Buffered eluent	(*2*)
Polyamines	C	Citrate	Automated	(*6, 148*)
Polyamines	CB	Pyridinium	Peptides also	(*372*)
Hexosamines	C	HCl	Amino-acid analyzer	(*165, 511*)
Polyamines	P	Various	Fluorescent derivs.	(*338, 345, 396*)
Aliphatic	N	$CHCl_3$-alc.	Paired ion	(*85*)
Aromatic	C	Acid	Incl. catecholamines	(*202*)
Aromatic	PP	EtOH-H_2O	Incl. phenols, acids	(*356*)
Aromatic	N	Cyclohexane	Plus $CHCl_3$ eluent	(*509*)
Aromatic	N	Heptane	Tl-loaded silica	(*427*)
Aromatic	N	Cyclohexane	Cu-loaded SiO_2; isomers	(*70*)
Biogenic	C, CB	Various	Several methods given	(*16, 408, 412*)
Biogenic	N-H_2O	Hexane	Paired-ion	(*352*)
Heterocyclic	N	...	Ag-loaded silica	(*7*)
Aminophenols	R	MeOH-H_2O	Solvent contains Ni	(*447*)
Aminophenols	CB	Phosphate	Isomers sep.	(*395*)
Amino acids	C	Buffers	Li gradient	(*194*)
General	C	Buffers	Improved methods	(*144, 331, 332, 392*)
General	C	Buffers	Meta-DVB resin	(*373*)
Basic	C	Borate	...	(*121*)
General	N	C_6H_6-pyridine	Dansyl derivs.	(*27*)
General	R	CH_3CN-H_2O	Phenylthiohydantoins	(*512*)
General	R	Sulfonates	Paired-ion	(*260*)
General	R	...	Incl. peptides	(*323*)
Anthocyanins	R	MeOH-H_2O	Plus HOAc	(*5, 183*)
Barbiturates	R	MeOH-H_2O	In blood, saliva	(*467*)
Barbiturates	P	Octane-HOAc	On polyamide	(*79*)
Cannabinoids	R	MeOH-H_2O	Plus acid	(*212, 436*)
THC	N	Heptane	...	(*147*)

Table II. (*Continued*)

Compounds	Stationary phase	Moving phase	Notes	Ref.
Carbohydrates	C	Aq. EtOH	Glucose, mannose	(*466*)
General	A	Borate	Automated	(*25, 226, 485*)
General	AB	CH_3CN-H_2O	...	(*406*)
Oligomers	A	Acetate	...	(*271*)
Methyl esters	A	Borate	Also unsubst. sugars	(*426*)
Sugars	A	Water	Aluminate resin	(*386*)
Sugars	R, P	CH_3CN-H_2O	Incl. amino sugars	(*210, 280*)
Catecholamines	C	Borate	...	(*413*)
General	C, R	Formate	Dopa, adrenaline, etc.	(*407, 408*)
General	CB, R	Acetate	Dopamine, adrenaline, etc.	(*16, 312, 388*)
Epinephrene	C, CB	$HClO_4$	Electrochem. det.	(*33, 139*)
Metabolites	R	Phosphate	pH effect shown	(*322*)
General	R	C_{12} sulfonate	Paired-ion	(*153, 233, 242*)
Diethylstilbesterol	N	$CHCl_3$-MeOH	GC also	(*229*)
Drugs, analgesic	N	MeOH-EtOAc	Incl. xanthines	(*62*)
Analgesics	A, C	Buffers	Micro scale	(*196, 449*)
Analgesics	AB	Borate	...	(*82*)
Acetaminophen	A, R	MeOH-H_2O	In urine, serum	(*32, 189a, 243*)
Phenacetin	N, R	Various	...	(*107*)
Aspirin	N, P	Hexane–dioxane	and acetaminophen	(*69, 207*)
Drugs, amphetamine	R	CH_3CN-H_2O	4-Nitrobenzamides	(*72*)
Drugs, sulfa	P	...	Amino-bonded phase	(*418*)
Drugs, sulfa	R, P	CH_3CN-H_2O	With metal ions; paired-ion	(*80*)
Drugs, sulfa	L	Heptane-BuOH	TBA stat. phase; paired-ion	(*455*)
Drugs, phenothiazine	N	Hexane	Short columns	(*164*)
Dyes, incl. food	A	Aq. HCl	Xylenol orange, etc.	(*134, 401*)
EDTA, chelating	A	$(NH_4)_2SO_4$	As Cu complexes	(*211*)
Esters, phthalate	PP, R	MeOH-H_2O	Environmental	(*325, 344*)
Explosives	C, PP	Acetone	...	(*135*)
Explosives	N	$C_2H_4Cl_2$	Mass spec. ident.	(*487*)
Hydrocarbons, aromatic	C	CH_3CN-H_2O	Fe-loaded resin	(*343*)
aromatic	R	CH_3CN, heptane	Carbon sorbent	(*28, 77, 78, 349*)
polycyclic aromatic	R	Alcohol-H_2O	...	(*113, 199, 263, 421*)
polycyclic aromatic	R	MeOH-H_2O	From coal	(*87, 404*)
polycyclic aromatic	N	Pentane	Retention index system	(*131a, 159 364*)
polycyclic aromatic	P, R	Hexane-CH_2Cl_2	Other sorbents compared	(*502*)
benzo[*a*]pyrene	N, R	Various	Also on cellulose	(*163, 239, 414*)
heterocyclic	R, C	CH_3CN-H_2O	From tobacco smoke	(*101, 240*)
Hydrocarbon derivatives:				
nitroxylenes	N	Hexane-acetone	Isomers sep.	(*438*)
chloronaphthalenes	N	Hexane	...	(*48*)
brominated biphenyls	N	Hexane	GC also	(*246*)
PCBs	N	Hexane	Exhaustive study	(*46, 47, 49*)
PCBs	N	...	Combined with GC	(*265*)
PCBs	C, R	CH_3CN-H_2O	Carbon-SiO_2 abs.	(*168*)
Lipids	N	Hexane, MeOH	Incl. phospholipids	(*8, 17, 213*)
Phospholipids	N	Hexane-PrOH	...	(*149*)
Nucleotides	A	Buffers	Automated	(*258, 425*)
Nucleotides	A, R	...	Use band width	(*227, 319*)
Nucleotides	R	MeOH-H_2O	Ion pairing with TBA	(*185*)
Nucleosides, bases	C, A	...	...	(*44, 82, 130*)
Nucleosides, bases	R	...	Evaluation of C_{18}	(*171*)
Organometallic compounds,				
Alkyl and aryl As, Sn, Hg	R	MeOH	Meast. by atomic absorption	(*45*)
Diethyldithiocarbamates	N	CH_3CN-hexane	Light absorption	(*477*)
Dithizonates	N	Benzene	Several metals	(*284*)
Ferrocene	N	Several	And derivatives	(*175*)
Peptides, proteins	P, R	CH_3CN-H_2O	And aqueous buffers	(*169, 264, 324, 337*)
Nonapeptides	R	...	Fluorescence detn.	(*136*)
Dipeptides, amino acids	P	Citrate-H_2O	Tripeptide bonded phase	(*131*)
Proteins, peptides	P	NH_3-NH_4^+	Cu-loaded chelating exchanger	(*158*)
Proteins, nucleic acids	P	...	On "Glycophase"	(*384*)
Glycoproteins	A	Acetate	Sialic acid detn.	(*330*)
Pesticides	N	...	Ag-silica	(*268*)
Pesticides	N	Hexane-PrOH	In milk	(*97, 98*)
carbamate	N, R	...	UV abs. given	(*446*)
carbofuran	N, R	...	Int. standards disc.	(*236*)

Table II. (*Continued*)

Compounds	Stationary phase	Moving phase	Notes	Ref.
Phenols, phenolic acids	PP	Various	Incl. chlorophenols	(*60*, *341*, *357*)
Polyethylene additives	N	CH_2Cl_2-toluene	...	(*92*)
Prostaglandins	R	CH_3CN-H_2O	Used oximes	(*127*, *128*)
Prostaglandins	R	$AgClO_4$ soln.	Cis-trans sep.	(*497*)
Prostaglandins	C-Ag	...	Phenacyl derivs.	(*313*, *314*)
Saccharin	P	Phosphate	In pharmaceuticals	(*13*)
Shale oil sulfonates	A	...	...	(*180*)
Steroids	N, R	MeOH-H_2O	Stat. phases compared	(*40*, *99*, *170*, *267*, *382*)
Steroids	N	CH_2Cl_2-MeOH	Preparative	(*154*)
Steroids	A, C	...	Ion-xch cellulose	(*480*)
Sulfur compounds	R, C	MeOH-H_2O	Amperometric detn.	(*84*)
Surfactants, nonionic sulfonates	N, R	Varied	Glycerides on SiO_2, polyglycols on C-18	(*335*) (*473*)
	PP, A	EtOH-aq. NH_3	$HClO_4$ for anion res.	(*172*, *334*)
Tetracyclines	R	...	Urine, plasma	(*419*)
Urethanes	PP	MeOH-H_2O	...	(*143*)
Urine, UV-absorbing	A	Acetate	High-resolution	(*282*)
Vitamins				
water-soluble	R, P	Also paired-ion	Systems compared	(*500*)
nicotinamide	C	Phosphate	In urine	(*417*)
D group	R	aq. MeOH + Ag^+	...	(*471*)
ascorbic acid	A, R	Acetate, MeOH	Paired-ion on R	(*346*, *442*)
Xanthines				
General	A, N	NH_4OAc on resin	In biol. samples	(*57*)
General	R	Citrate-phosphate	In tea	(*183*)
Caffeine	CB	Dil. HNO_3	In coffee	(*287*)
Theophylline	C	...	In serum	(*215*)
Theophylline	N	$CHCl_3$-MeOH	In serum	(*122*, *291*)
Theophylline	R	CH_3CN-aq. NaOAc	In body fluids	(*81*, *133*, *342*, *444*)

[a] Abbreviations: A = anion exchanger, B = cation exchanger, AB and CB = bonded anion and cation exchanger, N = normal phase (silica), R = reverse phase (including carbon), P = polar bonded phase, PP = porous, nonionic polymer.

to the density of the mobile phase. Mixtures of polar and nonpolar solvents in adsorption chromatography were discussed.

Unger (*478*) addressed the general subject of phase systems and solvent effects in liquid chromatography, including adsorption, partition, and ion exchange.

The partition of weak acids and bases between water and a nonpolar stationary phase, either macroporous polystyrene (*356*, *357*) or C_{18}-silica (*189*), over a range of pH has been studied. The results are consistent with the idea that the uncharged solute species is distributed between two homogeneous phases with a constant partition ratio, while ionic species, the anions of weak acids or the cations of weak bases, are distributed with another and smaller partition ratio. Usually the ionic species are not absorbed at all by the nonpolar stationary phase, but some absorption was found in certain cases. An ionic species cannot be absorbed by itself, and the observed retention must be due either to an electrical double layer (in which case the partition ratio would not be constant) or to ion pairs. In the second case the nature of the counterion would be significant. This point is not discussed in the papers cited.

Paired-Ion Chromatography. Where one or both of the ions is large and hydrophobic, ion pairing can and does occur, and it is more marked in the phase of lower dielectric constant. There are two ways to do paired-ion chromatography; in one, the stationary phase is an aqueous solution containing the hydrophobic pairing ion in concentrated solution and held on an inert porous support, while the mobile phase is a nonpolar liquid like hexane (*85*, *242*, *352*, *463*); in the other, which is now more usual, the stationary phase is octadecyl silica or another nonpolar sorbent, or else a nonpolar liquid supported on silanized silica (*489*), while the mobile phase is a polar solvent like water–methanol, and it contains the hydrophobic pairing ion. This ion could be cetyl trimethylammonium ion or a long-chain sulfonate detergent. Hence the name "soap chromatography", which was given to this technique by Knox (*242*, *244*). Ionic species to be separated by chromatography are drawn into the nonpolar phase (the stationary phase in the second method) more strongly, the more stable are their ion pairs. The concentration of the ion-pairing reagent affected the retention as one would expect from the mass-action law, except that at high concentrations micelles were formed that solubilized the ion pairs and decreased the retention. There was thus an optimum concentration of the pairing ions that gave the strongest retention (*244*).

Kissinger (*233*, *234*) suggested that long-chain alkyl sulfonate ions are absorbed into a bonded octadecyl stationary phase to form what is in effect a cation-exchanging stationary phase which binds cations as an ion exchanger would, and not by forming ion pairs in solution. This idea has some defenders (*260*), but other workers feel that ion pairing in solution is the ruling mechanism (*186*, *410*, *455*, *499*). Many applications of paired-ion chromatography are reported (*463*, *488*).

Chromatographic Columns: Packing, Operating Techniques. The advantages of very fine particles are now recognized. Five-μm particles are now in common use, and some chromatographers are using 3-μm particles (*496*). Attention is therefore being paid to the technique of packing fine-particle columns. The balanced-slurry method is cumbersome, and other methods are being tested (*21*, *225*, *281*, *297*). Upward slurry packing has some advantages (*51*).

Wide columns, 5-mm diameter and more, are better than narrow columns because of wall effects (the Knox–Parcher ratio) (*231*, *245*, *496*). Band broadening and skewing can be caused by wall effects (*245*) as well as by guard columns, sample injectors, and detectors (*231*). The theory of column optimization and the use of reduced plate height–reduced velocity graphs in practical applications was presented by Knox (*241*), Snyder (*441*), Huber (*197*), and Rohrschneider (*391*), while Guillemin et al. (*164*) described an arrangement of short columns packed with 5–7 μm silica that could be operated at moderate pressures with minimum cost (*164*). Factors to be considered in column design, and the proper way to treat columns, were explained by Bristow and Knox (*52*), who defined a "performance index". Effects of sample size (*100*) and solvent compressibility (*298*) were noted. Temperature programming (*236*) and a new high-pressure injection system (*445*) were described. Column-switching, whereby strongly-retained solutes need only travel along a short length

of column, is sometimes useful (*59, 97, 98*). Recycling, with its band-broadening and the limiting resolution that can be achieved, was the subject of a theoretical and experimental study (*299*). It is possible to reach very large plate numbers by coupling several 25-cm columns (*261*).

A gradient device (*403*) and a method of computing the results of gradient elution (*359*) were described. Knowing how retention depends on solvent composition allows one to predict the course of gradient elution, but one must allow for the "demixing" that occurs when the stationary phase absorbs one solvent component preferentially. Rapid changes in solvent composition may produce highly misleading false peaks in an ultraviolet detector (*29, 206*). Mixing two solvents of very different viscosities may cause concentration instabilities and oscillations; this even happens with methanol and water (*178*).

Micro-scale liquid chromatography is performed in columns of PTFE tubing whose volume is less than 0.01 cm^3 (*201*). At the other end of the scale, attention is being paid to preparative chromatography (*393, 498*) and its automatic control (*50*).

Finally, it is reassuring to see the high precision that can be attained in conventional high-performance liquid chromatography if conditions are properly controlled (*18*). It is possible to reproduce retention times to within 0.1% and to analyze mixtures with closely overlapping peaks by simply observing the position of the peak maximum (*411*).

Detectors. (*a*) *Electrochemical.* Electrochemical detectors are gaining in popularity, if the number of publications is any indication. Several applications are noted in Table II. Amperometric detectors are the most common, usually of the wall-jet type (*300*); nonconducting effluents can be rendered conducting by mixing them with a conducting solution after they leave the column and before they enter the detector (*276*). The detector may be operated in the differential-pulse mode (*459*) and cyclic voltammetry can be performed (*55*). Though carbon working electrodes are favored, mercury or mercury-plated platinum may be used (*55, 374, 494*). A coulometric detector with a glassy carbon electrode is described (*270, 468*).

Changes in dielectric constant may be used for LC detection (*238, 315, 365*), and the effect on the potential of a mercury drop serves to detect surface-active compounds (*269*). For a general review of electrochemical detectors, see (*B7*).

(*b*) *Optical.* The different kinds of optical detectors and their sensitivities were compared (*26*). Undoubtedly the most sensitive method of optical detection is laser-induced fluorescence; 7×10^{-13} g of an aflatoxin was so found (*95*). Laser two-photon excited fluorescence is highly sensitive (*416*), as is fluorescence detection with short excitation wavelengths (*262*). Stopped-flow fluorescence spectroscopy facilitates identification (*432*). A fluorescence detector is described which uses a tungsten halogen lamp and has a very small flow cell (*212*).

In light absorption, multiwavelength measurements help resolve overlapping peaks (*307, 437*). The ultimate in multiwavelength detection is the use of photodiode arrays to scan the spectrum of an effluent continuously as it leaves the column. Twenty spectra can be recorded every second with 1-nm resolution and stored in a computer (*93, 94, 320, 320a*).

Halasz (*166*) discusses the limitations of concentration-dependent detectors, which include light-absorption detectors, and concludes that the quantity of an eluted constituent can be measured to 0.5%. Scott (*411*) obtained better precision than this.

Infrared spectroscopy is being used for LC detection (*173, 277*).

Post-column derivatization, followed by optical detection, is a well-known technique. Its applications have been reviewed (*288*), a reaction selective for nitrosamides and nitrites is described (*423*), and the use of air segmentation to limit band broadening is noted (*136, 440*). A novel derivatization reaction is the formation of strongly-absorbing charge-transfer complexes between aromatic amines and iodine (*73*). A novel technique is to spot drops of eluate on a moving thin-layer plate, then evaporate the solvent and expose the spots to ammonium bicarbonate vapor; sterols produce fluorescent derivatives (*40*).

(*c*) *Various.* Only one paper describes a mass-spectrometer interface, a steel conveyor band (*308*), though chemical-ionization mass spectrometry was used in another case (*487*) to examine fractions after they had been collected.

In our 1976 review, we noted the use of the electron-capture detector and stated erroneously that Dolphin had used a moving wire to transfer LC effluents to the detector. Actually he fed the effluent into a heated stream of nitrogen which evaporated the solvent (hexane) and carried the entire effluent into the detector, a very efficient procedure (*97, 98*). The polar solvents commonly used in reverse-phase chromatography swamp the amplifier system, but this problem can be solved, and an electron-capture detector can be used in reverse-phase systems, by mixing hexane with the polar influent (*65*).

The heat-of-adsorption detector has reappeared (*309*), and continuous-flow radioactivity detectors with scintillation are described (*103, 383*). A detector specific for nitrogen compounds uses the chemiluminescent nitric oxide–ozone reaction (*126*), and a sulfur detector burns the sample to SO_2, absorbs the SO_2, and measures electrical conductivity (*293*).

Special Applications. (*a*) *Inorganic Complexes.* Transition-metal cluster complexes are analyzed by reverse-phase chromatography (*118*), rhodium and iridium triphenylphosphines by LC on silica (*117*). Stereoisomeric osmium(IV) complexes were separated by ion-exchange chromatography (*22*).

(*b*) *Trace Organic Compounds from Water.* Absorbents used in columns to collect trace organic compounds from large volumes of water include octadecyl silica (*198*), polyurethane prepared in situ (*336*), carbon (*160*), methacrylate and styrene polymers (*54, 214*). Once collected, they are often analyzed by liquid chromatography (*198, 209*); see the chapter by Pitt et al. in Ref. (*A6*). This important area of trace analysis is now in rapid development.

(*c*) *Separation of Geometrical Isomers.* Liquid chromatography is an important tool for this purpose. Often a simple stationary phase like silica suffices, because cis and trans forms differ greatly in polarity (*229*). For more difficult separations, such as those of vitamins (*472*) and insect sex attractants (*195, 491*), silica or another stationary phase loaded with silver ions is effective (*176, 290*). Cis-trans isomers of abscissic acid, a plant hormone, were separated on a silver-loaded cation exchanger (*375*).

(*d*) *Optical Isomers.* Chiral bonded phases serve to separate optical isomers by forming charge-transfer complexes with the solute molecules. This powerful method was used to resolve *d*- and *l*-sulfoxides (*358*), helicenes (*317*), and diols of benzo[*a*]pyrene (*506*). Amines were resolved into *d*- and *l*-forms by chromatography on a polar stationary phase after conversion to amides with *d*-camphorsulfonyl chloride (*443*), or to esters (*142*). Diastereoisomers were separated on silica gel, using recycle (*20*).

Resolution of *dl*-mixtures through their metal complexes on an ion exchanger was mentioned above under the heading "Ligand Exchange". Optically-active ion-exchange resins were made by condensing chlormethylated cross-linked polystyrene with optically-active amines. They separated *d*- and *l*-mandelic acid (*310*).

(*e*) *Hydrodynamic Chromatography.* Because we have cited a recent comprehensive review of this topic (*B16*), it seemed appropriate to include Small's definitive paper on this ingenious technique for size fractionation (*434*).

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Paper and Thin-Layer Chromatography

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This review covers the literature on paper and thin-layer chromatography (PC and TLC) cited in *Chemical Abstracts* for the period December 8, 1975 to December 12, 1977. Also searched were the bibliography sections of the *Journal of Chromatography*, *Analytical Abstracts*, and about one dozen other journals devoted to analytical chemistry and chromatography.

During the past two years it became apparent that there was a significant reduction in the literature devoted to PC compared to that covering TLC. Also, a number of papers include results both from PC and TLC. For these reasons, we have decided that from now on the sections formerly covering specifically PC and TLC will be combined.

Because of space limitations imposed by the Editors of ANALYTICAL CHEMISTRY, we restricted our review to select papers representing, in our opinion, the most significant advances in PC and TLC. For this reason, many papers appearing in the following journals had to be omitted from this review: *Analytical Biochemistry*, *Planta Medica*, *Helvetica Chimica Acta*, *Zeitschrift fuer Physiologische Chemie*, *European Journal of Biochemistry*, *Journal of Steroid Biochemistry*, *Journal of Biological Chemistry*, *Biochimica et Biophysica Acta*, *Clinica Chimica Acta*, *Journal of Agricultural and Food Chemistry*, *Journal of Antibiotics*, *Steroids*, *Biochemical Medicine*, and *Journal of Clinical Chemistry and Clinical Biochemistry*. We are hoping, however, that most of these significant papers on applications of PC and TLC to specific classes of compounds will be cited in the *Analytical Chemistry* Applied Reviews, published in alternate years.

During the past two years, increasing use has been made of in situ densitometry for the quantitative analysis of a variety of organic compounds; these methods will be covered in both the General and Applications Sections.

High Performance TLC (HPTLC) has been recently reviewed (*192A*) and is expected to find wider acceptance, once commercial equipment becomes available. HPTLC is usually performed on commercial glass plates coated with very fine silica gel particles of uniform size (5 to 10 μm). Resultant chromatograms are very tight, well separated bands and spots, and the detectability is in the nanogram to picogram range. Samples must be applied in volumes less than one microliter for full realization of the increased efficiency of this technique. Solvent development is carried out radially with commercial equipment or in a small glass tank (N-Tank). If larger volumes are required to be applied, as in the case of residues and biological extracts, HPTLC can best be accomplished by programmed multiple development (Apparatus available from Regis Chemical). Alternatively, a two-zoned commercial plate is available from Merck and contains a microfine silica gel for HPTLC and a nonadsorptive synthetic silica gel, effecting the desired band narrowing at the interphase. Chemically bonded C_2, C_8, and C_{18}-HPTLC plates are also commercially available for reversed-phase HPTLC. With the advent of HPTLC, separation efficiency by TLC is now comparable to that achieved by gas chromatography or High Performance Liquid Chromatography (HPLC).

The following symposia on paper- and thin-layer chromatography have been published: the FACSS Second National Meeting (*J. Chromatogr. Sci.* **1975**, 13 (12) and **1976**, 14 (1)); International Camag Symposium on TLC (*J. Chromatogr.* **1976**, 123 (1)), and the 1977 Meeting on Advances in Chromatography (*J. Chromatogr.* **1977**, 142 (11)).

Short-courses on TLC continue to be offered at various times and locations by the American Chemical Society, Center for Professional Advancement, and Kontes, Inc. The ACS course is available in audioform (*132A*) on tape cassettes with a 75-page manual; this course has been reviewed in ANAL. CHEM. **1976**, 48(9), 778A.

Kontes, Inc. publishes a quarterly newsletter on quantitative TLC, which is available from the company at no cost. *A Dictionary of Chromatography*, authored by R. C. Denney, contains entries related to PC and TLC (J. Wiley, Publ.: New York, N.Y., **1976**); a review of this dictionary may be found in *J. Assoc. Offic. Anal. Chemists* **1976**, 59 (6) 1426. The 20th Harvey W. Wiley Award was given to Gunter Zweig "for his outstanding achievement in the development of chromatographic techniques for pesticide analytical methodology" (Oct. 1977).

For nomenclature used in PC and TLC, the reader is referred to the following article: *Chromatographia* **1968**, 1, 338; for HPTLC, ANAL. CHEM. **1977**, 49(12), 1867.

GENERAL CONSIDERATIONS

Books and Reviews. The second edition of Randerath's *Thin-Layer Chromatography* was published in German (*127A*); the bibliography of paper- and thin-layer chromatography covering the period 1970 to 1973 was prepared by Macek, Mais, and co-workers (*95A*); and Heftmann completed his *Chromatography of Steroids* in 1976 (*58A*).

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