Ligand Exchange Chromatography

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FOREWORD

About 20 years ago, a new type of chromatography was developed, namely, ligand exchange chromatography (LEC). It makes use of the formation of labile coordinate bonds between ligands and a metal cation, producing coordination compounds or complexes.

LEC, in its liquid and gas variations, has made it possible to resolve many of the outstanding problems in the separation, purification, and analysis of different substances which other types of chromatography had failed to do. The hallmark of the achievements of this technique is its ability to separate optical isomers. Several interesting modifications of LEC have also been developed.

This book presents a systematic and comprehensive review of the information on chromatographic processes that involve the formation of coordination compounds, aiming not only to demonstrate the achievements that have been made in the theory and praxis of chromatography, but also to point out, as far as possible, the future potential of LEC.

THE AUTHORS

Vadim A. Davankov, D.Sc., Professor of Chemistry, Institute of Organo-Element Compounds, Academy of Sciences of the U.S.S.R., started working at the above institute in 1962. There he received his Ph.D. degree and later, the degree of Doctor of Sciences in Chemistry. Since 1975 Dr. Davankov has been the head of the Laboratory for Stereochemistry of Adsorption Processes.

Dr. Davankov's main research field is separation of optical isomers by means of column liquid chromatography and immobilized enzyme techniques. In 1968, together with Rogozhin, he patented ligand exchange chromatography as a general approach to resolve racemates into enantiomeric pairs. Developing this idea has also included extensive studies on structure and enantioselectivity phenomena in copper(II) complexes with amino acids and diamines, as well as examining new types of polymeric networks, i.e., macronet isoporous polystyrene and hypercross-linked polystyrene "Styrosorb". Dr. Davankov has authored and co-authored more than 250 scientific publications.

Dr. Davankov serves as Vice Chairman of the Scientific Council on Chromatography of the Academy of Sciences of the U.S.S.R. and Chairman of its Liquid Chromatography Section. In 1978 he was awarded the Tswett Medal by the Academy of Sciences of the U.S.S.R.

James D. Navratil, Ph.D. Chemistry, University of Colorado, Boulder, started working at Rocky Flats, operated by Rockwell International for the Department of Energy, in 1961. He has held several positions in the Analytical Laboratories and Research and Development (present position, Manager of Chemical Research), and from 1978 to 1981 was on leave of absence to the International Atomic Energy Agency, Vienna. He is also an Adjunct Professor in the Department of Chemistry and Geochemistry, Colorado School of Mines, Golden.

He was named Rockwell International Scientist/Engineer of the Year in 1977. Dr. Navratil's research interests are mainly chemical separations and actinide chemistry.

He is founder and co-editor of the journal, *Solvent Extraction and Ion Exchange,* and serves on the editorial board of seven other journals. He is active in the American Chemical Society and is founder and past chairman of the ACS subdivision of Separation Science and Technology. Dr. Navratil was the recipient of the Colorado ACS award (1984) and two IR-100 awards (1983 and 1985). He has authored or co-authored more than 100 publications and co-edited nine books and co-authored the book, *Polyurethane Foam Sorbents in Separation Science.*

Harold F. Walton, Ph.D., Oxford University, joined the University of Colorado faculty as a member of the Department of Chemistry in 1947 and remained until his retirement in 1982, when he spent several months in Paris as the guest of Professor R. Rosset in the Ecole Superieure de Physique et de Chimie. He returned to the University of Colorado in an active capacity as Senior Research Associate of the Cooperative Institute for Research in Environmental Sciences and Professor Emeritus of Chemistry.

He was one of the first scientists to pursue a program of research in ligand exchange chromatography, which continued until his retirement. In 1976 he received the Colorado Section Award of the American Chemical Society.

He has taught in several countries of Latin America, especially Peru. He is a Corresponding Member of the Chemical Society of Peru and an Honorary Professor of the University of Trujillo and the University of San Marcos, Lima.

He is the author of seven books, including *Ion Exchange in Analytical Chemistry* (jointly with W. Rieman), several chapters in cooperative works, and some 120 research papers. These deal with ion exchange, chromatography, electrolyte solutions, electrochemistry, geochemistry, and water pollution.

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Chapter 1

INTRODUCTION: HISTORY, PRINCIPLES, TERMINOLOGY

H. F. Walton and V. A. Davankov

No method of chemical separation can equal chromatography in versatility and breadth of applications. Neutral molecules and ions can be separated, as can isotope species, small and large molecules, natural and artificial macromolecules, latex particles of different sizes, and even living cells. Chromatographic separations depend on differences in the distribution of substances between two phases, one stationary and the other moving. The moving phase can be a gas, a liquid, or a supercritical fluid. Because chromatography is a multistage process, even small differences in affinity can be exploited to produce useful separations. The forces that determine distribution ratios can be of many kinds, including dispersion forces, π-bonding, electrostatic forces, charge-transfer interactions, hydrogen bonding, and metal-ligand coordination. It is this last kind of interaction that is the basis of ligand-exchange chromatography.

The term "ligand-exchange chromatography" (LEC) dates from 1961 from a short report by Helfferich[1] entitled "Ligand Exchange: A Novel Separation Technique", which he later amplified in two longer papers dealing with the mechanism of LEC.[2,3] Helfferich had the problem of recovering a 1,3-diamine (1,3-diamino-2-hydroxypropane) from a dilute aqueous solution that also contained ammonia. He solved the problem by packing a glass column with a cation-exchange resin that was loaded with the blue copper(II) ammonia complex ions, mainly $Cu(NH_3)_2^{2+}$ under the conditions he was using, and passing the solution through this column. He saw a deep-blue band forming at the top of the column, which spread downward as the solution continued to flow; it contained the Cu-diamine complex. Each diamine molecule had displaced two molecules of ammonia:

$$Res_2Cu(NH_3)_2 + (\text{diamine}) = Res_2Cu(\text{diamine}) + 2NH_3$$

where Res denotes the functional group of the cation exchange resin, here the carboxyl ion.

After the deep-blue diamine band had spread through the length of the column, Helfferich displaced the absorbed diamine by passing a small volume of concentrated ammonia solution, reversing the reaction written above and restoring the column to its original condition. Displacement was efficient because two ammonia molecules replaced one diamine molecule, a process that is favored at high concentrations. (A similar condition exists in water softening, where one calcium ion displaces two sodium ions from the solid exchanger in dilute solution, and the column is regenerated by a concentrated solution of sodium chloride.) Little or no copper was removed from the resin, only the ligands; ammonia and the diamine changed places, and hence the name "ligand exchange".

The process of ligand exchange can be applied to any amines, and indeed to any compounds, that form labile coordination complexes with copper ions; moreover, the metal ions need not be those of copper, but could be any metal ions that form labile coordination complexes with the compounds to be separated. The process can be applied to analytical elution chromatography. If a mixture of amines, for example, is introduced into a column of metal-loaded ion exchanger and a solution of ammonia is passed, the amines will move along the column at different rates, with those forming more stable complexes remaining behind, while those that form weaker complexes move ahead (see Figure 1). Different selectivity orders can be expected with different metal ions, different ion exchangers, and different eluents. Helfferich wrote: "The method combines two fields of chemistry, namely,

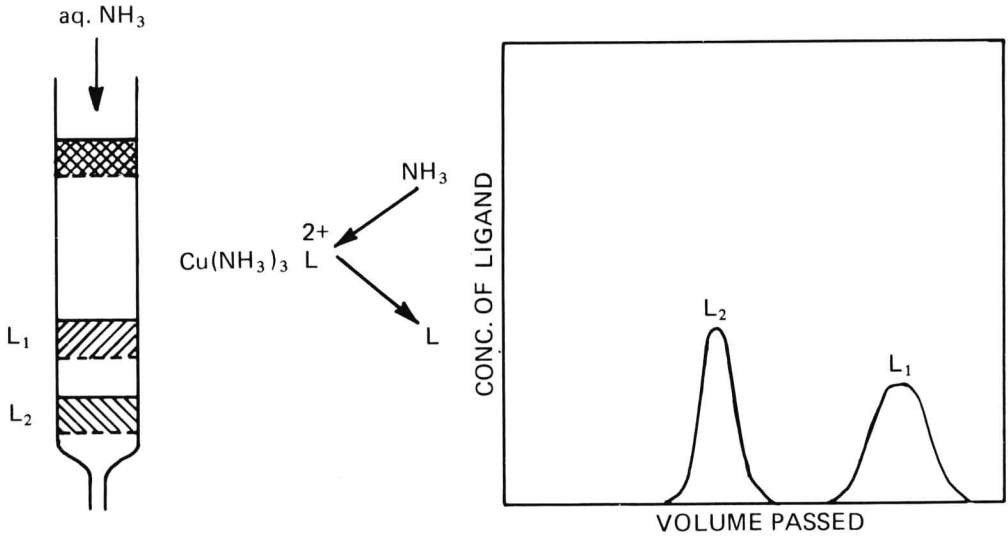


FIGURE 1. Ligand exchange chromatography, schematic.

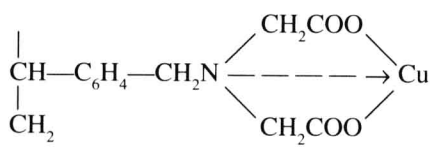
ion exchange and coordination chemistry, in order to accomplish a task that neither could do alone.''

Helfferich noted that the method could be applied to gas-liquid chromatography. One could use a carrier gas containing ammonia, for example, to analyze a mixture of volatile amines. To date, little use has been made of ligand exchange in gas chromatography, though labile metal-ligand complexes are exploited in gas chromatography of olefins on stationary phases carrying silver(I) ions. This topic will be treated later in the book (Chapter 7).

A ''ligand'' is something ''tied on''. In coordination chemistry, it is a neutral molecule or an anion attached by a coordinate link to a metal ion. By incorporating lone electron pairs of the donor atom of the ligand, the central metal cation completes its electron shell, building up to the stable shell structures characteristic of the noble gases. If the metal-ligand bond is labile (that is, easily formed and broken), one ligand can substitute for another. Water is a ligand, and coordination of water molecules is always important in aqueous solutions.

The ligand can be a negative ion. One of the most important uses of LEC is the separation of amino acids, which coordinate as their deprotonated/singly charged anions. The net charge on the metal ion must remain positive, however, or the metal will be stripped from the column.

We have considered that the ligands move in LEC while the metal ions remain stationary. Of course, this statement is an idealization. The metal ions do leave the exchanger and move through the column, because they enter into ion-exchange equilibrium with cations of the mobile phase. Even a dilute ammonia solution contains ammonium ions. Displacement of metal ions is minimized by choosing an exchanger, the functional groups of which themselves form coordinate bonds with the metal ions, like carboxylate or the iminodiacetate ions, which is the functional group of the chelating ion-exchange resin, Chelex®-100:



Coordination to fixed functional groups reduces the capacity of metal ions to bind other ligands, and that may be a disadvantage. The matter will be discussed in Chapter 2.

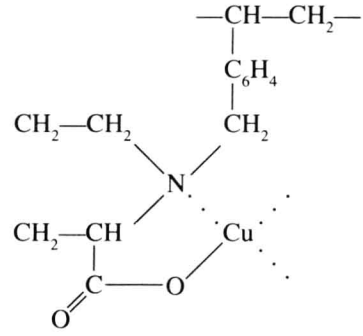
In general, one must accept the fact that some metal ions will be displaced from the exchanger into the mobile phase. To keep a steady concentration of metal ions in the exchanger, it is common practice to add a small amount of metal salt to the mobile phase, at concentrations of, for example, 10^{-3} to 10^{-4} *M*. Coordination and the exchange of ligands now occurs in the mobile phase as well as in the stationary phase, and ligand distribution ratios are affected. The importance of the complex formation equilibria in the two phases to amino acid chromatography will be seen in Chapter 4.

A new development in liquid chromatography uses ligand exchange in the mobile phase. The stationary phase need not be an ion exchanger; it may be nonpolar, like porous silica bonded with octadecyl groups, the familiar reversed-phase packing. The metal ions are added to the mobile phase, and if the ligands are such that they form uncharged complexes, these uncharged metal-ligand complexes are distributed between the stationary and the mobile phase. Amino acids are examples of such ligands, and the technique of ligand exchange in the mobile phase is widely used today to analyze mixtures of enantiomers of amino acids, that is, of D- and L-optical isomers.

A characteristic of LEC is its sensitivity to the molecular structure of the ligands. Small differences in molecular shape or size often have a large effect on strengths of binding to a metal-loaded ion exchanger. In the restricted environment of an ion-exchange resin, substituents on a donor nitrogen atom may greatly hinder the formation of a coordinate link, and groups far removed from the donor atom may interact with the exchanger network. Hence, the great range of selectivity orders, which we have mentioned above and which will be seen in Chapter 3.

Nowhere is the selectivity of ligand exchange better seen than in the distinction between the D and L forms of optically active ligands. The most spectacular triumph of LEC is the separation of optical isomers of amino acids.

The use of a chiral ion exchanger for this purpose was first proposed by Rogozhin and Davankov in 1968.[4] They grafted an optically active amino acid, L-proline, on to a specially cross-linked polystyrene and treated the result with copper sulfate in aqueous ammonia to give a copper-loaded polymer having this structure:



Each copper ion is coordinated with two fixed proline units and forms a bridge between them. The copper-loaded polymer can now absorb dissolved amino acids from an aqueous solution. In doing so, the copper bridges are broken, thus:

$$\overline{\text{RPro}}\text{–Cu–}\overline{\text{ProR}} + \text{HA} = \overline{\text{RPro}}\text{–Cu–A} + \overline{\text{HProR}}$$

where HA stands for a dissolved amino acid in its uncharged or dipolar-ion form, and $\overline{\text{RPro}}$ signifies a negatively charged proline anion bound to the polymer network. (Conventionally,

the bar above formulas in ion-exchange chemistry indicates absorption in the exchanger phase.) This equilibrium, and others that accompany it, are discussed in detail in Chapter 5, Section V.

Once absorbed, the amino acid can be displaced by water if the attachment is weak, otherwise by ammonia:

$$\overline{\text{RPro}}\text{–Cu–A} + \overline{\text{HProR}} + \text{NH}_3 = \overline{\text{RPro}}\text{–Cu–}\overline{\text{ProR}} + \text{NH}_4^+\text{A}^-$$

Hence, the polystyrene-proline-copper resin can be used for the chromatography of amino acids, including proline itself.

In an early experiment, Rogozhin and Davankov packed a glass column, 9 mm wide and 50 cm long, with 12 g of this polymer (particle diameter, 30 to 50 μm), washed it with water, and introduced 0.5 g of racemic DL-proline dissolved in water. Then they passed water, followed by dilute ammonia. The first amino acid fractions to emerge from the column consisted entirely of L-proline; to get D-proline out of the column, they passed 1 *M* ammonia. In this way they recovered 0.25 g of L-proline and D-proline, each in 100% optical purity.[5-7] A small amount of copper was displaced from the column by ammonia along with the amino acid, but it was easily held back by interposing a short column of copper-free, proline-grafted polymer at the exit to the main column. The D- and L-proline were thus isolated in pure, copper-free form.

These results gave the impetus to an extended series of research by many investigators that will be described in detail in Chapter 5. At first, the emphasis was on the synthesis and use of chiral (optically active) polymers. These gave impressive separation factors and were good for preparative purposes, but bands were broad and the separation process was a bit slow. Then, chiral-bonded silicas were prepared and silica-based, reversed-phase supports were dynamically coated with optically active amino acids that carried long hydrocarbon chains to make them stick to the support. In every case, the stationary phases were loaded with copper ions. Another development, which we have noted, was to use an achiral support, sometimes a cation-exchange resin, sometimes a standard reversed-phase bonded silica, and add the chiral resolving agent to the mobile phase. Faster ligand exchange and more narrow chromatographic bands were achieved in this way, but the chiral mobile phase is obviously more suited to analytical than to preparative use. In every case, the chiral resolving agent was a metal complex, usually of copper(II), sometimes of zinc(II), or nickel(II), with a chiral amino acid, L-proline, L-hydroxyproline, or L-phenylalanine.

The function of the metal ion is to bring two amino-acid molecules close together in a fixed orientation. One molecule is of the optically active resolving agent, like L-proline. The other is the D or L form of the acid to be resolved. Each molecule is held in place by two coordinate-covalent bonds. The third interaction point, which is necessary for chiral discrimination, is provided by exchange forces or steric hindrance between the hydrocarbon side-chains of the two molecules. The result is that the free energies of formation of the L–Cu–L and L–Cu–D diastereoisomers may differ by 2 or 3 kJ, which is quite adequate to permit chromatographic resolution.

It is now time to formulate a definition of LEC. At first, it was taken for granted that ligands would be exchanged around metal ions that were immobilized in the stationary phase. We now see that the metal ions can reside in the mobile phase as well; indeed, there is no reason why they might not remain exclusively in the mobile phase. A working definition of LEC might read as follows:

> Ligand-exchange chromatography is a process in which complex-forming compounds are separated through the formation and breaking of labile coordinate bonds to a central metal atom, coupled with partition between a mobile and a

stationary phase. It separates ligands by causing them to change places around metal ions. The exchange can occur in either the stationary or the mobile phase.

In reading this definition we must again bear in mind that water is a ligand that can take the place of other ligands, and that nearly all LEC is performed in solutions that contain water.

An early use of metal-ligand complexing in chemical separations was made by Tsuji in 1960.[8] He absorbed isonicotinic acid hydrazide on a cation-exchange resin that was loaded with various metal ions. The strongest absorption was obtained with copper(II), but other cations, listed in decreasing order of absorption strength, were nickel(II), mercury(II), cobalt(II), cadmium(II), zinc(II), iron(II), lead(II), manganese(II), and aluminum (III). In every case, the compound of interest was displaced from the resin in concentrated form by aqueous ammonia. Both batch and column arrangements were used. Tsuji did not, however, call his process ligand exchange.

A more recent variation of ligand exchange is "metal chelate affinity chromatography," so called by Porath,[9] who has developed it as "a new approach to protein fractionation." To absorb and desorb proteins without denaturation, the stationary phase must be hydrophilic and the binding sites must be easily accessible by large molecules. Porath and co-workers have used agarose to which they attached long hydrophilic side chains terminating in an iminodiacetate group, which holds a metal ion, generally copper(II) or zinc(II). Proteins carrying histidine or cysteine are especially strongly held. The absorbed proteins can be selectively removed by passing aqueous buffers through the column, leaving the metal behind. Though the authors did not call it by that name, the process is clearly one of LEC. It is described in more detail in Chapter 4.

Yet another kind of LEC makes use of what is called "outer-sphere coordination". This is an interaction, a sort of ion-pair formation, between a stable coordination complex and another ion of opposite charge.

It should be reemphasized that very weak intermolecular interactions can serve as a base for a chromatographic process. Only formation of labile coordination compounds can be related to LEC. Numerous examples exist of chromatographic separations of stable, kinetically inert complexes of cobalt(III), chromium(III), platinum(II), platinum(IV), and some other metal ions, but they have little in common with LEC. These complexes can be separated according to an ion-exchange mechanism or reversed-phase technique, but there is no exchange of ligands in the inner coordination sphere of metal ions in these separations.

However, aside from the inner coordination sphere, the above complexes possess a highly organized solvation shell which can be regarded as a second, outer coordination sphere. Its ligands are bonded relatively weakly and can easily be exchanged under chromatographic conditions. And indeed, several interesting separations have been achieved using ligand exchange in the outer coordination sphere of inert complexes. They will be given consideration in Chapter 5, Section VI.

One of the first uses of outer-sphere coordination in LEC was made by Karger and associates in 1978,[10] though the complex ion was labile, not inert. Their mobile phase was a solution of the zinc complex of a hydrophobic triamine, $C_{12}H_{25}N(CH_2CH_2NH_2)_2$, in an acetonitrile-water mixed solvent; a molecule of acetonitrile coordinated with the zinc ion to give a fully coordinated complex ion, but nevertheless, this fully coordinated cation formed ion pairs with anions of sulfa drugs and permitted their chromatographic separation. The stationary phase was a C_8-bonded silica. This research extended to the use of asymmetric, optically active triamines and the separation of optical isomers of dansyl amino acids.[11,12]

Though mobile-phase interaction may have predominated in this system, it is quite likely that some of the hydrophobic complex cations were absorbed into the stationary phase and formed ion pairs there, as well as in the mobile phase. The chapters to follow will show many examples of mobile-phase interaction, ion-pair formation, and outer-sphere coordination.

REFERENCES

1. **Helfferich, F.,** Ligand exchange. A novel separation technique, *Nature (London)*, 189, 1001, 1961.
2. **Helfferich, F.,** Ligand exchange. I. Equilibria, *J. Am. Chem. Soc.*, 84, 3237, 1962.
3. **Helfferich, F.,** Ligand exchange. II. Separation of ligands having different coordinative valencies, *J. Am. Chem. Soc.*, 84, 3242, 1962.
4. **Rogozhin, S. V. and Davankov, V. A.,** A chromatographic method for resolution of racemates of optically active compounds, *German Patent* 1,932,190, Jan. 8, 1970, Appl. U.S.S.R. July 1, 1968; *Chem. Abstr.*, 72, 90875c, 1970.
5. **Rogozhin, S. V. and Davankov, V. A.,** Ligand chromatography on dissymmetric complex-forming sorbents — a new principle of resolution of racemates, *Dokl. Akad. Nauk S.S.S.R.*, 192, 1288, 1970 (Engl. transl., p. 447).
6. **Davankov, V. A. and Rogozhin, S. V.,** Chromatography of ligands as a new method for studying mixed complexes. Stereoselective effects of α-amino acid complexes of copper(II), *Dokl. Akad. Nauk S.S.S.R.*, 193, 94, 1970 (Engl. transl., p. 460).
7. **Rogozhin, S. V. and Davankov, V. A.,** Ligand chromatography on asymmetric complex-forming sorbents as a new method for resolution of racemates, *Chem. Commun.*, p. 490, 1971.
8. **Tsuji, A. and Sekiguchi, K.,** Adsorption of nicotine acid hydrazide on cation exchanger of various metal forms, *Nippon Kagaku Zasshi*, 81, 847, 1960.
9. **Porath, J., Carlsson, J., Olsson, I., and Belfrage, G.,** Metal chelate affinity chromatography, a new approach to protein fractionation, *Nature (London)*, 258, 598, 1975.
10. **Cooke, N. H. C., Viavattene, R. L., Eksteen, R., Wong, W. S., Davies, G., and Karger, B. L.,** Use of metal ions for selective separations in high-performance liquid chromatography, *J. Chromatogr.*, 149, 391, 1978.
11. **Lindner, W., LePage, J. N., Davies, G., Seitz, D. E., and Karger, B. L.,** Reversed-phase separation of optical isomers of Dns-amino acids and peptides using chiral metal chelate additives, *J. Chromatogr.*, 185, 323, 1979.
12. **LePage, J. N., Lindner, W., Davies, G., Seitz, D. E., and Karger, B. L.,** Resolution of the optical isomers of dansyl amino acids by reversed-phase liquid chromatography with optically active metal chelate additives, *Anal. Chem.*, 51, 433, 1979.

Chapter 2

GENERAL CONSIDERATIONS

H. F. Walton

I. EXPERIMENTAL CONDITIONS

A. Metal Ions

The only metal ions that do not form complexes in aqueous solutions are those of the alkali metals, and these ions, of course, are hydrated. The other metallic cations may be conveniently classified into "hard" and "soft" acids, with word "acid" understood to mean an electron acceptor. Hard acid cations are those having a noble-gas electronic structure or a large charge:radius ratio. These associate preferably with hard bases like F^-, OH^-, and ligands containing oxygen. Soft acid cations are those of transition and posttransition metals; they do not have a noble-gas electronic structure. They associate preferably with ligands having nitrogen or sulfur as electron donors.

Ligand exchange chromatography (LEC) is most often performed with metal ions of the soft acid type, and primarily with copper(II). Copper(II) is the ion of choice because it forms very stable complexes. The Irving-Williams series states that as one proceeds along the first transition series, the divalent ions form complexes, the stability of which rises in the order $Mn < Fe < Co < Ni \ll Cu$ with a sharp peak at Cu, then falls going from Cu to Zn. This stability order is the same for all soft-acid ligands. The copper(II) ion has a square planar distribution of coordinate valences, which sets it apart from the others; nickel(II) favors octahedral coordination, and zinc(II) favors tetrahedral. As we know, copper(II) complexes can also be octahedral, but the octahedron is a distorted one with the axial bonds being much weaker than the square planar or equatorial bonds. The axial coordination of copper(II), however, is vitally important in ligand exchange selectivity (see Chapter 5).

In choosing a cation for LEC, we must consider how strongly it is bound to the ion exchanger which is the stationary phase. The sulfonated polystyrene resins that are commonly used do not bind copper(II) very strongly; it is too easily displaced by NH_4^+ and other cations. For this reason, much of the earlier work on LEC was done with nickel ions attached to a sulfonated polystyrene resin. Copper(II) ions were supported on an acrylic or chelating resin, which holds them strongly enough so that they are virtually not displaced by aqueous ammonia. Another circumstance that favors the use of nickel(II) or zinc(II) rather than copper(II) is the exchange of ligands that are bound very strongly, like 1,2-diamines. These compounds are held so strongly by copper(II)-loaded exchangers that the only practical way to get them off the column is to strip the metal ions off with dilute acid.

Yet another circumstance that favors the use of zinc(II) is the speed of the exchange. In the LEC of amino acids on a sulfonated polystyrene exchanger, zinc(II) gives sharper bands than copper(II), indicating faster exchange.[1,2] The extreme case of slow exchange, of course, is in kinetically stable complexes formed by cobalt(III), chromium(III), and platinum(IV). These complexes have their uses in LEC, but only through "outer-sphere" complexing or ion-pair association. This topic will be mentioned later. Meanwhile, note that cobalt(II) is seldom used in LEC, because it is easily oxidized by air to cobalt(III) in the alkaline media commonly used.

After copper(II), zinc(II), and nickel(II), the soft-acid cations that have been most used in LEC are cadmium(II), silver(I), and mercury(II). Cadmium ions have been used in the column chromatography of sulfur ligands, including thiourea,[3] but their main use has been in thin-layer chromatography, where they have been incorporated into silica and used to

analyze mixtures of aromatic amines.[4-7] In effectiveness, there is little to choose between cadmium(II) and zinc(II). Silver ions incorporated in ion-exchanging polymers, as well as in silica, have been used for the chromatography of heterocyclic nitrogen bases[8] as well as various olefinic compounds,[9,10] but since the solvents used were nonaqueous, it is questionable whether the processes should be called LEC. However, separations were due to differences in stability of metal ligand complexes. Mercury(II) has a great affinity for ligands containing sulfur, and a macroporous cation exchanger loaded with mercury(II) may be used to remove sulfur compounds as a class from petroleum;[11] a mercury-loaded resin served for the chromatography of aromatic hydroxy acids.[12] However, mercury(II) salts react irreversibly with polystyrene-based ion-exchange resins with covalent attachment of mercury to the aromatic ring.[13]

Turning now to the hard-acid cations, the one most used is probably calcium(II), which forms complexes with polyhydric alcohols and certain sugars. Calcium-loaded columns of cation-exchange resins are used routinely for the analysis of sugar mixtures, with water as the eluent. The calcium complexes are weak, but their formation constants have been measured in some cases, and the mechanism of chromatographic retention is mainly ligand exchange (see Chapter 6).

Several publications describe the use of iron(III)-loaded ion exchangers for recovery and chromatography of phenols,[14,15] aromatic acids and hydroxy acids,[16] beta-diketones,[17] and even aromatic diamines.[18] Titanium(IV) has been used to separate hydroxy acids.[19] Aluminum(III)-loaded cation-exchange resins were used for fractionation of DNA and RNA, using alkaline glycine buffers as eluents.[20]

A complication in using iron(III) and aluminum(III) is the easy hydrolysis of these ions, which in aqueous solution starts above pH 3 to 4. The lanthanum ion, lanthanum(III), being larger, is much less hydrolyzed — only about 1% at pH 6. A resin loaded with lanthanum(III) selectively retains anions of carboxylic acids and hydroxy acids and can be used for their chromatography[21] using an acetate buffer as eluent. A problem here, which is quite general in LEC, is the poor chromatographic efficiency, i.e., the band broadening which is associated with slow exchange. The rate of ion exchange is limited, as a rule, by the rate at which ions and molecules can diffuse in and out of the exchanger. Conventional gel-type resins loaded with trivalent ions are more compact and have a smaller water content than those loaded with univalent ions, and diffusion in such resins is correspondingly slow.

B. Exchangers

The exchanger used to retain the cations in LEC can be either organic or inorganic, or a combination of both, namely, a bonded silica. Most work has been done with organic exchangers, and principally those derived from cross-linked polystyrene because these are easily available and their particles are sufficiently rigid that they can be used in closed columns under pressure.

The most common organic cation exchanger is sulfonated polystyrene, which can be obtained in various degrees of cross-linking and various particle sizes. For analytical chromatography, small and uniform particles are desired, about 10 μm in diameter. A cross-linking of 8% is sufficient to give enough rigidity, and 6% cross-linked resins — even 4% cross-linked — can be used with divalent counter-ions. (Doubly charged ions act electrostatically to pull the polymer chains together, and cause the resin particles to contract and become more rigid and more tolerant of high-pressure gradients; these are the ions used in LEC.)

The common gel-type resins, which are internally homogeneous, are better for analytical chromatography than are macroporous resins. In our experience, they give narrower and more symmetrical chromatographic peaks. Macroporous resins are aggregates of very small, highly cross-linked microspheres, and it appears that the environment of the ionic functional

groups is not uniform; the peaks show much tailing. Where good chromatographic performance is not needed, as in the recovery of traces of ligands from large volumes of water, macroporous resins can be used to advantage, as they can and must be when treating nonaqueous solutions. Macroporous sulfonated polystyrene, in our experience, retains metal ions more poorly than do the gel-type resins, but this problem is less acute in nonaqueous solvents.

None of these objections apply to the "macronet" and "isoporous" polymers developed by Davankov and co-workers.[22,23] These resins are solvent-modified polymers made by using long rod-like molecules as cross-linking agents. They are distinguished by high internal porosity and uniformity of cross-linking. Their properties are described elsewhere in this book.

When gel-type sulfonated polystyrene resins are used in LEC, it is necessary to add metal salt to the mobile phase, for copper(II) and zinc(II) are not retained with sufficient strength to prevent loss from the column. As we have noted, nickel(II) is held more strongly.

Metal ions are held much more strongly if the resin has other functional groups. Some authors have used phosphonate groups attached to cross-linked polystyrene.[24] More commonly used is the iminodiacetate chelating resin, Chelex®-100, but diffusion in and out of this resin is slow and chromatographic performance is poor; further, the ligand-binding capacity is limited by the coordination of the metal ion to the resin functional group. Nevertheless, a nickel(II)-loaded chelating resin has been used for the column chromatography of organic acids.[25] The most important use of chelating resins, both in ligand exchange and in the exchange of inorganic ions, is the recovery and concentration of trace substances from large volumes of water. A nickel-loaded iminodiacetate chelating resin has been used to recover amino acids from waste water; 11 samples were passed through a bed containing 10 mℓ of nickel-loaded resin, and the absorbed amino acids were later eluted with concentrated aqueous ammonia.[26] Similarly, a copper-loaded chelating resin recovered dissolved amino acids from sea water[17] and from urine.[28,29] Phenols were absorbed from industrial waste water by a chelating resin loaded with iron(III) and stripped from the resin by dilute sodium hydroxide.[30] For applications like these, high affinity is necessary, but high resolution is not.

Functional carboxyl groups retain copper(II) and other metals more strongly than do sulfonate groups, and carboxyl is the functional group of cross-linked polyacrylate-methacrylate resins, sold commercially as Bio-Rex® 70. Acrylic resins whose structure is aliphatic, have the advantage that there is little π-bonding between the resin and solutes of aromatic character. We saw this difference in the LEC of amphetamine drugs;[31] an acrylic resin gave symmetrical and fairly narrow peaks, while a polystyrene-based resin gave broad, asymmetrical peaks with much tailing, indicative of a mixed retention mechanism. An acrylic resin is better for the LEC of alkaloids.[32] However, acrylic resins are less uniform and less reproducible than polystyrene-type resins. We found that an experimental batch of acrylate resin was much better at retaining alkaloids than the regular commercial material. The ligand-binding behavior of the copper in the two resins was correlated with the electron-spin resonance of Cu^{2+}.[33] Subtle differences in polymer structure may make large differences in metal-ligand binding.

A great disadvantage of acrylic resins in high-resolution chromatography is their softness. They are easily deformed under pressure and must be treated with great care in closed columns.

Even softer than the acrylic resins are the exchangers derived from natural polymers like cellulose and dextran. Such exchangers are used in LEC primarily to retain large biological molecules like those of proteins and peptides, but a DEAE cellulose loaded with antimony served to separate aliphatic and aromatic amines, including diamines.[34] Copper-loaded Sephadex®G-25, a dextran carrying carboxyl groups, served to separate amino acids as a group

from peptides.[35] Dextran and Sepharose® (a polysaccharide) have been treated to introduce iminodiacetate chelating groups, and the products, loaded with copper(II) and used in gravity feed columns, served to collect and separate proteins and peptides.[36,37] Loaded with mercury(II) instead of copper(II), these materials were selective sorbents for proteins having sulfhydryl groups, like papain.[38]

In high-performance liquid chromatography (HPLC), it is a great advantage to use packings based on silica. Porous silica is hard and will stand high flow rates and high pressure gradients; it can be obtained in well-defined particle sizes and porosities. A great variety of organic groups can be attached chemically to silica; the techniques for doing this are well known.[39-49] Much high-resolution chromatography is done with silica coated with chemically bonded organic groups, primarily hydrocarbon chains like $C_{18}H_{37}$. Naturally, bonded silicas have been developed for use in LEC.

One of the early attempts to use bonded silica for this purpose was made in 1977 by Chow and Grushka.[39] They took silica carrying amino groups as $-CH_2CH_2CH_2NH_2$, the common amino bonded phase, and impregnated it with copper(II) from a solution of copper sulfate in dry methanol. Aqueous solutions stripped copper from the exchanger. To hold copper and other metal ions more tightly, the next step was to bind diamines, polyamines, and iminodiacetate groups to silica. Masters and Leyden[46] attached diamine groups not to silica, but to controlled pore glass, by refluxing with *N*-β-aminoethyl-γ-aminopropyltrimethoxysilane dissolved in dry toluene. The product carried the groupings $-Si-CH_2-CH(NHC_2H_5)CH_2NH_2$. It was loaded with copper(II) by contact with a copper(II)-ammonia solution in water, taking up about 0.4 μmol copper per gram. Chromatography of amino acids and amino sugars was accomplished with an eluent 0.1 *M* in ammonia and ammonium ions, pH 9.5, which was 10^{-4} *M* in $CuCl_2$. Chromatographic efficiency was poor, probably because the particle size was too great, but it established that the diamine function held copper(II) sufficiently and strongly enough that aqueous eluents could be used. Later, Gimpel and Unger[40] bonded silica with several aminosilanes, introducing the groups $Si-(CH_2)_3NH(CH)_2$, $Si-(CH_2)_3N(CH_2COOH)_2$, $Si-(CH_2)_3N(CH_2COOH)CH_2CH_2N-(CH_2-COOH)_2$. Loaded with copper(II), these materials were used to separate amino acids and mixtures of aliphatic carboxylic acids. Various buffer solutions were used as eluents, generally in the pH range of 3 to 6, each containing 8×10^{-5} *M* copper(II).

Other functional groups can be grafted to silica; there are many possibilities. Starting with γ-aminopropyl-bonded silica, the common commercial "amino bonded phase", Chow and Grushka[47] attached dithiocarbamate ligands by reaction with carbon disulfide, and diketo groups by reaction with ethyl benzoylacetate; the bonded ligands coordinated copper(II). By first attaching a diamine ligand, they produced a bonded silica that carried the cobalt(III)-*tris*-ethylenediamine complex, which was effective for chromatography of nucleotides with buffered phosphate eluents;[48] this separation makes use of "outer-sphere coordination".

Tartaric acid has been attached to silica via the bonded γ-aminopropyl group.[42] Optically active D-tartaric acid was used in the synthesis, giving an asymmetric stationary phase that allowed separation of optical isomers by chromatography. Catecholamines and related compounds, including amino acids like "dopa", were separated on the copper-loaded stationary phase, as was the hydroxy-acid mandelic acid. Eluents were phosphate buffers made 3×10^{-4} *M* in copper(II). Optical isomers were separated with good resolution in running times of 15 min.

Starting again with the amino bonded phase, $Si-(CH_2)_3NH_2$, Shahwan and Jezorek[41] attached 8-quinolinol to silica in this form: