CHIRAL SEPARATIONS

1. Introduction

Chiral separations are concerned with separating molecules that can exist as nonsuperimposable mirror images. Examples of these types of molecules, called *enantiomers* or *optical isomers*, are illustrated in Figure 1. Although chirality is often associated with compounds containing a tetrahedral carbon with four different substituents, other atoms, such as phosphorus or sulfur, may also be chiral. In addition, molecules containing a center of asymmetry, such as hexahelicene, tetrasubstituted adamantanes, and substituted allenes or molecules with hindered rotation, such as some 2,2′ disubstituted binaphthyls, may also be

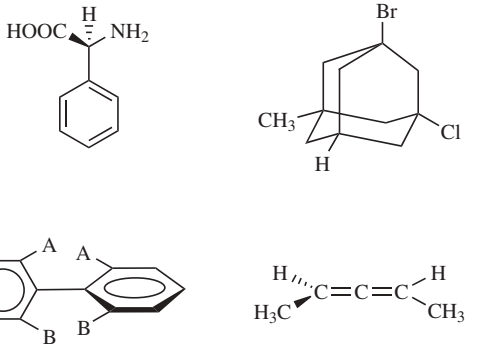
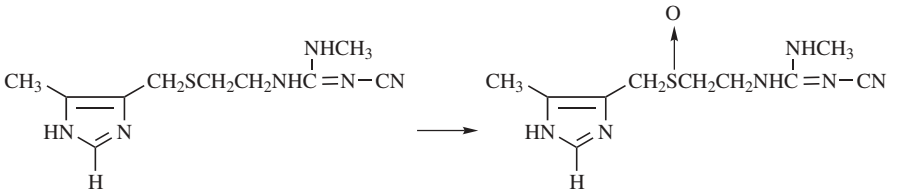


Fig. 1. Examples of chiral molecules.

chiral. Compounds exhibiting a center of asymmetry are called *atropisomers*. An extensive review of stereochemistry may be found under PHARMACEUTICALS, CHIRAL.

Although scientists have known since the time of Louis Pasteur (1) that optical isomers can behave differently in a chiral environment (eg, in the presence of polarized light), it has only been since about 1980 that there has been a growing awareness of the implications arising from the fact that many drugs are chiral and that living systems constitute chiral environments. Hence, the optical isomers of chiral drugs may exhibit different bioactivities and/or biotoxicities.

In the case of enantiomerically pure chiral drugs, the possibility of racemization or inversion either *in vivo* or during storage cannot be ruled out. Ibuprofen is an example of a chiral drug which undergoes rapid inversion *in vivo* (2). In addition, there are several examples of achiral (or *prochiral*) drugs being biotransformed into chiral entities. In some cases, the enantiomeric ratios produced by laboratory animals may differ from that produced in humans. For example, cimetidine (**1**), used to treat peptic ulcers and marketed as Tagamet, is achiral. However, cimetidine sulfoxide (**2**), one of its major metabolites, is chiral by virtue of oxidation of the sulfur atom to a sulfoxide (the lone pair of electrons on the sulfur constitutes the fourth group). In humans, the (+) enantiomer predominates (2.4:1) but in rats, although (+)-cimetidine sulfoxide is produced in excess, the enantiomeric ratio approaches racemic (1.3:1). This raises the question of the suitability of rats as appropriate test models for this particular drug (3).

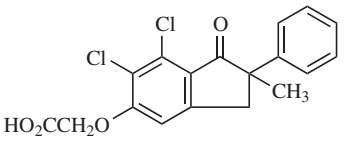


(**1**) (**2**)

For those drugs that are administered as the racemate, each enantiomer needs to be monitored separately yet simultaneously, since metabolism, excretion

or clearance may be radically different for the two enantiomers. Further complicating drug profiles for chiral drugs is that often the pharmacodynamics and pharmacokinetics of the racemic drug is not just the sum of the profiles of the individual enantiomers.

Although it might seem that administration of enantiomerically pure substances would always be preferred, the diuretic indacrinone (3), is an example of a drug for which one enantiomer mediates the harmful effects of the other enantiomer (4). (+)-Indacrinone, the diuretically active enantiomer or *eutomer*, causes uric acid retention. Fortunately, the other enantiomer (*distomer*) causes uric acid elimination. Thus, administration of a mixture of the two enantiomers, although not necessarily racemic, may have therapeutic value.



(**3**)

Although a great deal of the work currently being done in chiral separations is related to pharmaceuticals, the agricultural and the food and beverage industries are affected as well. For instance, several chiral pesticides are used commercially. It is possible that the enantiomers may differ in their persistence in the environment and their effectiveness against specific pests. For example, the neurotoxic action of the pesticide, ethyl-4-nitrophenyl phenylphosphono thionate (EPN), resides almost entirely in the *S* enantiomer while the desired insecticidal activity resides entirely in the *R* enantiomer (5). This raises the question of whether the pesticide may be safer and more effective if applied as an enantiomerically pure formulation. In the food and beverage industry, many of the constituents that confer flavor or aroma in foods and beverages are chiral. For instance, the configuration of the 4-alkyl-substituted γ-lactones responsible for much of the flavor in fruits is almost exclusively *R* (6). Often, the two enantiomers have very different aromas or flavors. The presence of any of the "unnatural" enantiomer may confer an "off-flavor" to the substance and may be indicative of racemization under adverse storage conditions, adulteration, or formulation from nonnatural sources.

The growing awareness of the implications of chirality to the pharmaceutical industry has spurred tremendous effort toward stereoselective synthetic strategies and the development of new chiral catalysts. However, the enantiomeric purity of these substances or their chiral precursors needs to be determined. Also, there are many chiral compounds for which no stereospecific synthetic pathways have been devised. Thus, there is a tremendous need not only for analytical scale ($<5-10$ mg), but bulk-scale chiral separations as well. Whether analyzing drugs or synthetic precursors for enantiomeric purity, monitoring biological or environmental samples for chiral discrimination or trying to enantioresolve kilogram quantities of a racemic drug, there are a variety of reasons for performing chiral separations. The purpose of the separation dictates, to some extent, the method employed.

Traditionally, chiral separations have been considered among the most difficult of all separations. Conventional separation techniques, such as distillation, liquid–liquid extraction, or even some forms of chromatography, are usually based on differences in analyte solubilities or vapor pressures. However, in an achiral environment, enantiomers or optical isomers have identical physical and chemical properties. The general approach, then, is to create a "chiral environment" to achieve the desired chiral separation and requires chiral analyte–chiral selector interactions with more specificity than is obtainable with conventional techniques.

A variety of strategies have been devised to obtain chiral separations. Although the focus of this article is on chromatographically based chiral separations, other methods include crystallization and stereospecific enzymatic-catalyzed synthesis or degradation. In crystallization methods, racemic chiral ions are typically resolved by the addition of an optically pure counterion, thus forming diastereomeric complexes.

Enzymatically based methods depend on the stereospecificity of an enzyme-catalyzed reaction, such as lipase-catalyzed esterification, to degrade enantioselectively the unwanted enantiomer or to produce the desired enantiomer. Because only one enantiomer undergoes the reaction, the subsequent separation is reduced to separating two different species. For example, in the case of enzyme-catalyzed esterification, the originally difficult enantiomeric separation is reduced to the separation of the ester of one optical isomer from the alcohol or acid of the other optical isomer of the original starting material, and may be accomplished using a variety of conventional separation methodologies (7). One disadvantage of enzymatically based methods is that only one enantiomer is obtained and there is usually no analogous method for producing the opposite enantiomer.

An alternative method of creating a chiral environmental is to derivatize a chiral analyte with an optically pure reagent, thus, producing diastereomers. The resultant diastereomers, containing more than one chiral center, have slightly different melting and boiling points and can often be separated using conventional methods. A number of chiral derivatizing agents, as well as the types of compounds for which they are useful, have been developed and are listed in Table 1. Limitations of this approach include lack of suitable functionality in the analyte that can be derivatized with an appropriate enantiomerically pure derivatizing agent, unavailability of a suitable derivatizing agent of sufficiently high or at least known optical purity, difficulty of removing the derivatizing group after the desired separation has been accomplished, enantiodiscrimination during derivatization, potential racemization either during derivatization or removal or the chiral derivatizing group (which is not always possible), and the additional validation required to confirm that the enantiomeric ratio of the final product corresponds to the original enantiomeric ratio.

2. Use of Chiral Additives

Another method for creating a chiral environment is to add an optically pure chiral selector to a bulk liquid phase. Historically, many of the chiral selectors

Table 1. **Analyte Functional Groups and Chiral Derivatizing Reagents**

Analyte functional group	Derivatizing agent	Product	Examples of derivatizing agents
carboxylic acid (acid or base catalyzed)	alcohol	ester	(−)-menthol
	amine	amide	1-phenylethylamine
			1-(1-naphthyl)ethylamine
amine (1°)	aldehyde	isoindole	*o*-phthaldialdehyde−2-mercaptoethanol
amine (1° and 2°)	anhydrides	amide	γ-butyloxycarbonyl-L-leucine anhydride
			O,O-dibenzoyltartaric anhydride
	acyl halides	amide	(*R*)-(−)-methylmandelic acid chloride
			α-methoxy-α-trifluoromethylphenyl-acetyl chloride
	isocyanates	urea	α-methylbenzyl isocyanate
			1-(1-naphthyl)ethyl isocyanate
	isothiocyanate	thiourea	2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate
			α-methylbenzyl isothiocyanate
(1°, 2°; can *N*-dealkylate 3°)	chloroformates	carbamate	(−)-menthyl chloroformate
			(+)-1-(9-fluorenyl)ethylchloroformate
alcohols	acyl halides	ester	(−)-menthoxy acid chloride
			(*S*)-*O*-propionylmandelyl chloride
	anhydrides	ester	(*S,S*)-tartaric anhydride
	chloroformate	carbonate	(−)-menthyl chloroformate
	isocyanate	carbamate	α-methylbenzyl isocyanate

currently available as chiral stationary phases for high performance liquid chromatography originated as chiral mobile-phase additives, particularly in thin-layer chromatography (tlc). Chiral additives have several advantages over chiral stationary phases and continue to be the predominant mode for chiral separations by tlc (8) and capillary electrophoresis (ce) (9). First of all, the chiral selector added to a bulk liquid phase can be readily changed. The use of chiral additives allows chiral separations to be done using less expensive, conventional stationary phases. A wider variety of chiral selectors are available to be used as chiral additives than are available as chiral stationary phases, thus, providing the analyst with considerable flexibility. Finally, the use of chiral additives may provide valuable insight into the chromatographic conditions and/or likelihood of success with a potential chiral stationary-phase chiral selector. This is particularly important for the development of new chiral stationary phases because of the difficulty and cost involved.

Chiral additives, however, do pose some unique problems. Many chiral agents are expensive or are not commercially available, and therefore, must be synthesized. The presence of the chiral additive in the bulk liquid phase may also interfere with detection or recovery of the analytes. Finally, the presence of enantiomeric impurity in the chiral additive may add analytical complications (10).

2.1. Thin-Layer Chromatography. Thin-layer chromatography (tlc) offers several advantages for chiral separations and in the development of new chiral stationary phases. Besides being inexpensive, tlc can be used to screen mobile-phase conditions rapidly (ie, organic modifier content, pH, etc), chiral selectors, and analytes. Several different analytes may be run simultaneously

on the same plate. Usually, no preequilibration of the mobile phase and stationary phase is required. In addition, only small amounts of mobile phase, and therefore, chiral mobile-phase additive, are required. Another significant advantage is that the analyte can always be unambiguously found on the tlc plate.

Two mechanisms for chiral separations using chiral mobile-phase additives, analogous to models developed for ion-pair chromatography, have been proposed to explain the chiral selectivity obtained using chiral mobile-phase additives. In one model, the chiral mobile-phase additive and the analyte enantiomers form "diastereomeric complexes" in solution. As noted previously, diastereomers may have slightly different physical properties such as mobile phase solubilities or slightly different affinities for the stationary phase. Thus, the chiral separation can be achieved with conventional columns.

An alternative model has been proposed in which the chiral mobile-phase additive is thought to modify the conventional, achiral stationary phase *in situ*, thus, dynamically generating a chiral stationary phase. In this case, the enantioseparation is governed by the differences in the association between the enantiomers and the chiral selector in the stationary phase.

Several different types of chiral additives have been used including (1*R*)-(−)-ammonium-10-camphorsulfonic acid (11), cyclodextrins (12,13), proteins, and various amino acid derivatives such as *N*-benzoxycarbonyl-glycyl-L-proline as well as macrocyclic antibiotics (14). Chiral counterions such as (1*R*)-(-)-ammonium-10-camphorsulfonic acid and *N*-benzoxycarbonyl-glycyl-L-proline have been used under normal phase conditions (eg, ca 2.5 m*M* with 1 m*M* triethylamine in methylene chloride on a diol tlc plate), promoting ion-pair associations (15). In contrast, the cyclodextrins (16,17), proteins, and amino acid derivatives have been used exclusively under aqueous mobile phase conditions. In the case of the cyclodextrins, the limited aqueous solubility of β-cyclodextrin (∼0.17 *M* at room temperature), the most commonly used cyclodextrin, can be enhanced by using a saturated urea solution. In addition, it is recommended that 0.6 *M* NaCl can be used to stabilize the binder by which the stationary phase is attached to the glass support (18).

Chiral separation validation in tlc may be accomplished by recovering the individual analyte spots from the plate and subjecting them to some type of chiroptical spectroscopy such as circular dichroism or optical rotary dispersion. Alternatively, the plates may be analyzed using a scanning densitometer. Scanning densitometers irradiate the surface of the plate at a specified wavelength (in the ultraviolet or visible regions) and can measure the intensity of the reflected beam. A trace of the reflected beam vs distance has the general appearance of a chromatogram and an example is shown in Figure 2 (19). The relative peak heights or areas of the two enantiomers obtained at two or more different wavelengths should remain constant because the extinction coefficients of the enantiomers are identical at every wavelength.

2.2. Capillary Electrophoresis. Capillary electrophoresis (ce) or capillary zone electrophoresis (cze), a relatively recent addition to the arsenal of analytical techniques (20,21), has also been demonstrated as a powerful chiral separation method. Its high resolution capability and lower sample loading relative to hplc makes it ideal for the separation of minute amounts of components in complex biological mixtures (22,23).

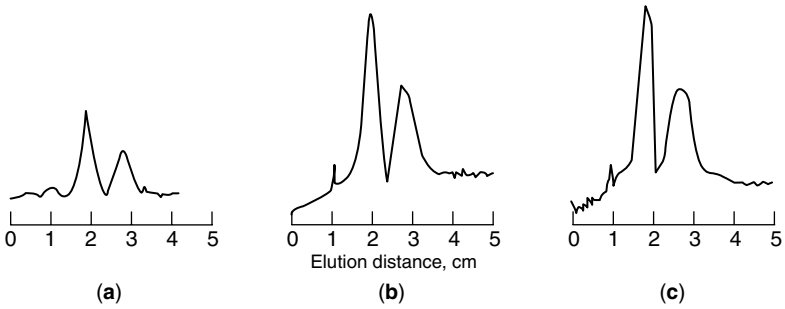


Fig. 2. Tlc densitometer scans showing the resolution of isoproterenol on a hptlc silica-gel plate obtained using a mobile phase consisting of 6.8 m*M* (1*R*)-(−)-ammonium-10-camphorsulfonic acid in 75:25 (v/v) methylene chloride:methanol. (**a**) 254 nm, (**b**) 275 m, (**c**) 300 nm.

In a ce experiment, a thin capillary is filled with a run buffer and a voltage is applied across the capillary. Although a complete treatment of the fundamental principles of ce is beyond the scope of this article, it can be said that the underlying impetus for separations in ce is, in general, derived from the fact that charged species migrate in response to an applied electric field proportionately to their charge and inversely proportionately to their size. Thus, given equivalent charges, lighter analytes have higher electrophoretic mobilities than heavier analytes and, given equivalent sizes, more highly charged species have higher mobilities than lesser charged or neutral species. In fact, neutral species have no intrinsic electrophoretic mobility. Species having opposite charges have electrophoretic mobilities in opposing directions.

Chiral separations by ce have been performed almost exclusively using chiral additives to the run buffer. The advantages of this approach are identical to the advantages mentioned previously with regard to using chiral mobile-phase additives in tlc. Many of the chiral selectors used successfully as mobile-phase additives in tlc and as immobilized ligands in hplc have been used successfully in ce including proteins (24), native (25) and functionalized cyclodextrins (26,27), various carbohydrates (28,29), assorted functionalized amino acids (30), chiral-ion pairing agents (31), and macrocyclic antibiotics (32). Other ce chiral selectors which have not been used as immobilized chiral selectors in hplc include bile salts (33), chiral surfactants (34), and dextran sulfate (35).

Although chiral ce is most commonly performed using aqueous buffers, there has been some work using organic solvents such as methanol, formamide, *N*-methylformamide or *N*,*N*-dimethylformamide with chiral additives such as quinine (36) or cyclodextrins (37,38). Nonaqueous ce requires that the background electrolyte be prepared using organic acids (eg, citric acid or acetic acid) and organic bases (eg, tetraalkylammonium halides or tris(hydroxymethyl)-aminomethane).

Theoretical models (39,40), as expressed in equation 1, where μ represents the mobility

$$\mu_1 - \mu_2 = \frac{\left[\mu_{1,c} - \mu_{1,f}\right]\,[K_1 - K_2][CA]}{[1 + K_1[CA]]\,[1 + K_2[CA]]} \qquad (1)$$

of the analyte in the free and complexed states, K represents the binding constants of enantiomers 1 and 2 and $[CA]$ is the molar concentration of the additive, reveal that, in general, two conditions must be met to achieve chiral separations by ce. First, there must be differences in the binding constants of the two enantiomers with the chiral selector. Second, because the intrinsic electrophoretic mobilities of the enantiomers are identical in the free state, there must be a significant difference in the mobilities of the analyte in the complexed and free state. Chiral selectors have generally been shown to be the most effective when the intrinsic electrophoretic mobility of the additive is in the opposite direction of the intrinsic electrophoretic mobility of the analyte.

Chiral separations by ce is a rapidly growing field and offers the analyst tremendous flexibility with regard to chiral selector choice. In addition, because the additive is typically in the run buffer, there is virtually no column preequilibration. However, ce instruments tend to cost more than most chromatographic systems, sample capacity is much smaller for ce than for an analogous hplc method, sample recovery is not a trivial problem in ce and run-to-run reproducibility for ce tends to be much worse than for most chromatographic methods. Nevertheless, the flexibility, minimal sample and/or chiral selector required and the extremely high resolving power of ce ensure that this technique will continue to play an important role in chiral separations in the future.

3. Chiral Stationary Phases

Most chiral chromatographic separations are accomplished using chromatographic stationary phases that incorporate a chiral selector. The chiral separation mechanisms are generally thought to involve the formation of transient diastereomeric complexes between the enantiomers and the stationary phase chiral ligand. Differences in the stabilities of these complexes account for the differences in the retention observed for the two enantiomers. Often, the use of a chiral stationary phase allows for the direct separation of the enantiomers without the need for derivatization. One advantage offered by the use of chiral stationary phases is that the chiral selector need not be enantiomerically pure, only enriched. In addition, for chiral stationary phases having a well understood chiral recognition mechanism, assignment of configuration (eg, R or S) may be possible even in the absence of optically pure standards. However, chiral stationary phases have some limitations. The specificity required for chiral discrimination limits the broad applicability of most chiral stationary phases; thus there is no "universal" chiral stationary phase. The cost of most chiral columns are typically much higher ($\sim 3\times$) than for conventional columns. In contrast to conventional chromatographic columns, chiral stationary phases are generally not as robust, require more careful handling than conventional columns and usually, once column performance has begun to deteriorate, cannot be returned to their original performance levels. In many cases, chromatographic column choice or mobile-phase optimization for chiral stationary phases is not as straightforward as with conventional stationary phases. In conventional chromatography, there is usually a well-behaved relationship between retention and mobile phase composition or column temperature. However, in many of the chiral stationary

phases, the stationary phase present a multitude of different types of sites with not necessarily equivalent populations for interaction with the analytes. In the case of some liquid chromatographic stationary phases, the different types of sites may result in normal phase type behavior under very nonpolar mobile phase conditions and reversed-phase type behavior under highly polar mobile phase conditions. The multiplicity of types and numbers of sites also confounds thermodynamic considerations (41). Often, there is a narrow window of mobile-phase conditions under which enantioselectivity is observed and these conditions may be unique for a particular chiral analyte. Thus, for many of the chiral stationary phases, adequate chiral recognition models, used to guide selection of the appropriate column for a given separation, have yet to be developed. Column selection, therefore, is often reduced to identifying structurally similar analytes for which chiral resolution methods have been reported in the scientific literature or chromatographic supply catalogues and adapting a reported method for the chiral pair to be resolved.

An additional complication, sometimes arising with the use of chiral stationary phases, may occur when the analytes either exist as *conformers* or can undergo inversion during the chromatographic analysis. Figure 3 illustrates a typical chromatogram obtained for oxazepam, one of the chiral benzodiazepines that can undergo ring opening and inversion at the chiral center (42). As can be seen from Figure 3, the peaks appear to have a plateau between them and are sometimes referred to as "Batman peaks". This effect can sometimes be suppressed by lowering the column temperature. Although the appearance of Batman peaks is not unique to chiral separations, the specificity of chiral analyte–chiral selector interactions may increase the frequency of their occurrence.

3.1. Thin-Layer Chromatography. Chiral stationary phases have been used less extensively in tlc as in high performance liquid chromatography (hplc). This may, in large part, be due to lack of availability. The cost of many chiral

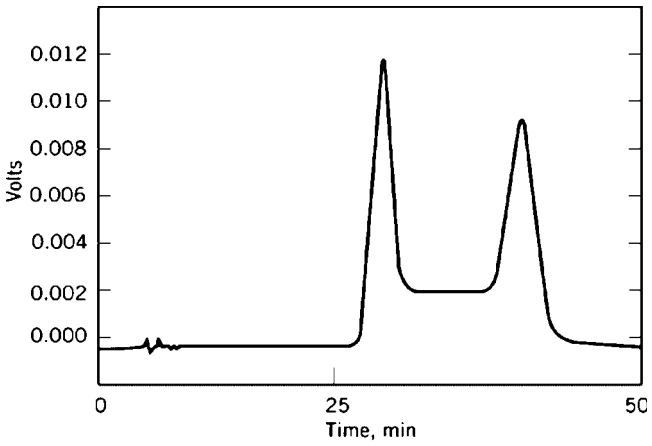


Fig. 3. The chiral separation obtained for oxazepam on a sulfated cyclodextrin hplc column (4.6 mm ID × 25 cm) using a 10% acetonitrile/buffer (25 m*M* ammonium acetate, pH 7).

selectors, as well as the accessibility and success of chiral additives, may have inhibited widespread commercialization. Usually, nondestructive visualization of the sample spots in tlc is accomplished using iodine vapor, uv or fluorescence. However, the presence of the chiral selector in the stationary phase can mask the analyte and interfere with detection (43).

Chiral stationary phases in tlc have been primarily limited to phases based on normal or microcrystalline cellulose (44,45), triacetylcellulose sorbents or silica-based sorbents that have been chemically modified (46) or physically coated to incorporate chiral selectors such as amino acids (47,48) or macrocyclic antibiotics (49) into the stationary phase.

Of the silica-based materials, only the ligand-exchange phases are commercially available (Chiralplate, tlc plates are available through Alltech Associates, Inc.) Supelco, Inc., the Aldrich Chemical Company, and Bodman Industries are all based on ligand exchange. Typically in the case of the ligand-exchange type tlc plates, the ligand-exchange selector is comprised of an amino acid residue to which a long hydrocarbon chain has been attached (eg, (2*S*,4*R*,2′*RS*)4-hydroxy-1-(2-hydroxydodecyl)proline) (50). The hydrocarbon chain of the functionalized amino acid is either chemically bonded to the substrate or intercalates in between the chains of a reversed phase-stationary phase thus immobilizing the chiral selector. The bidentate amino acid chiral selector is thought to reside close to the surface of the stationary phase and participates as a ligand in the formation of a bi-ligand complex with a divalent metal ion (eg, Cu^{2+}) and the chiral bidentate analyte (Fig. 4). Analytes enantioresolvable using ligand exchange are usually restricted to 1,2-diols, α-amino acids, α-amino alcohols, and α-hydroxyacids (51,52). Again, differences in the stabilities of the diastereomeric complexes thus formed give rise to the chiral separation.

3.2. High Performance Liquid Chromatography. Although chiral mobile phase additives have been used in high performance liquid chromatography (hplc), the large amounts of solvent, thus chiral mobile phase additive, required to pre-equilibrate the stationary phase renders this approach much less attractive than for tlc and is not discussed here.

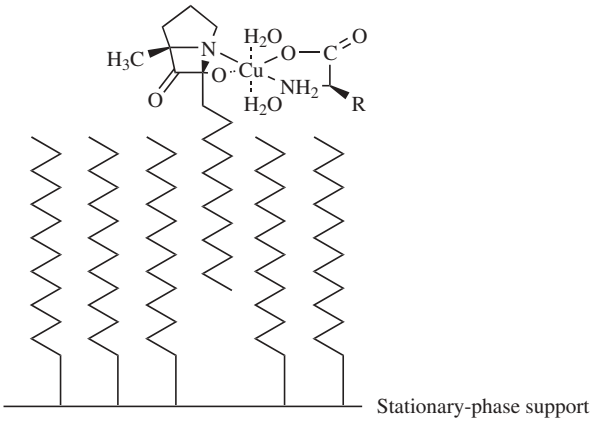


Fig. 4. A ligand-exchange chiral selector complexed with a chiral analyte.

Table 2. **Classes of Hplc Chiral Stationary Phases**

Column chiral selector	Typical mobile phase conditions	Typical analyte features required
pirkle	nonpolar organic; 2-propanol–hexane	π-acid or π-basic moieties for charge transfer complex; hydrogen-bonding or dipole stacking capability near chiral center
protein	phosphate buffers	aromatic near chiral center; organic acids or bases; cationic drugs
cyclodextrin	aqueous buffers; polar organic	good "fit" between chiral cavity or chiral mouth of cyclodextrin and hydro-phobic moiety; hydrogen-bonding capability near chiral center
ligand exchange	aqueous buffers	α-hydroxy or α-amino acids near chiral center; can do nonaromatic
chiral crown ether	0.01 *N* perchloric acid	primary amines near chiral center; can do nonaromatic
macrocyclic antibiotics	aqueous buffers, nonpolar and polar organic	amines, amides, acids, esters; aromatic; hydrophobic moiety
cellulosic and amylosic	nonpolar organic	aromatic

The last decade has seen the commercialization of a large number of different types of chiral stationary phases including the cyclodextrin phases (53), the chirobiotic phases (54), the π–π interaction phases (55,56), the protein phases (57–61), as well as the cellulosic and amylosic phases (62,63) and chiral crown ether phases (64,65). Currently, there are over 50 different chiral columns that are commercially available for hplc. Table 2 briefly summarizes the types of columns available as well as typical applications and mobile-phase conditions. Each of these chiral stationary phases are very successful at separating large numbers of enantiomers, which in many cases, are unresolvable using any of the other chiral stationary phases. Unfortunately, despite the large number and variety of chiral stationary phases currently available, there remains a large number of enantiomeric compounds that are unresolvable by any of the existing chiral stationary phases. In addition, incomplete understanding of the chiral recognition mechanisms of many of these chiral stationary phases limits the realization of the full potential of the existing chiral stationary phases and hampers development of new chiral stationary phases.

4. Ligand-Exchange Phases

Among the earliest reports of chiral separations by liquid chromatography were based on work done by Davankov using ligand exchange (66). These types of columns are available from Phenomenex, J. T. Baker, and Regis Technologies, Inc. As noted previously in the discussion regarding ligand exchange in tlc, chiral separations by ligand exchange in hplc is accomplished using bidentate amino acid ligands, immobilized on a chromatographic substrate, and a divalent

metal cation which participates in the formation of a diastereomeric complex with a bidentate chiral analyte and the ligand. Although almost any amino acid can form the basis for the chiral selector, proline and hydroxyproline exhibit the most widespread utility. Also, although other metals can be used, copper(II) is usually the metal of choice and is added to the aqueous buffer mobile phase.

The dependence of chiral recognition on the formation of the diastereomeric complex imposes constraints on the proximity of the metal binding sites, usually either an hydroxy or an amine α to a carboxylic acid, in the analyte. Principal advantages of this technique include the ability to assign configuration in the absence of standards, enantioresolve nonaromatic analytes, use aqueous mobile phases, acquire a stationary phase with the opposite enantioselectivity, and predict the likelihood of successful chiral resolution for a given analyte based on a well-understood chiral recognition mechanism.

5. Pirkle Phases

The first commercially available chiral column for liquid chromatography was introduced in 1980. This was the first generation of the "Pirkle phases", named after their originator, and was based on *N*-(3,5-dinitrobenzoyl)phenylglycine which was immobilized on a silica support (67). Of all of the commercially available chiral stationary phases for liquid chromatography, the chiral recognition mechanism for the "Pirkle" phases are among the best understood. Chiral recognition on Pirkle phases is thought to depend upon complimentary interactions between the analyte and the selector. These interactions may be $\pi-\pi$, steric, hydrogen-bonding, or dipole–dipole interactions and contribute to the overall stability of the diastereomeric association complexes that form between the individual enantiomers and the chiral selector in the stationary phase. The $\pi-\pi$ interactions arise through the association of aromatic systems with complementary electron withdrawing (eg, nitro) and electron donating (eg, alkyl) substituents. The electron-deficient aromatic system is often referred to as π-*acidic*; the electron-rich system is usually referred to as π-*basic*. Three unique interactions emanating from the chiral centers of the analyte and their chiral ligand in the stationary phase, seem to be required for successful chiral recognition. A model invoking three unique points of interaction is sometimes referred to as the *3-point interaction model* first proposed by Dalgliesh (68). To promote analyte–selector interactions, functional groups are often introduced into the analyte through achiral derivatization. For example, amines may be derivatized with 3,5-dinitrobenzoyl chloride to introduce a π-acid aromatic group to promote diastereomeric complexation with a π-basic (*R*)-*N*-(2-naphthyl)-alanine chiral selector in the stationary phase. Derivatization often has the additional benefit of enhancing solute solubility.

Nonpolar organic mobile phases, such as hexane with ethanol or 2-propanol as typical polar modifiers, are most commonly used with these types of phases. Under these conditions, retention seems to follow normal phase-type behavior (eg, increased mobile phase polarity produces decreased retention). The normal mobile-phase components only weakly interact with the stationary phase and are easily displaced by the chiral analytes thereby promoting enantiospecific

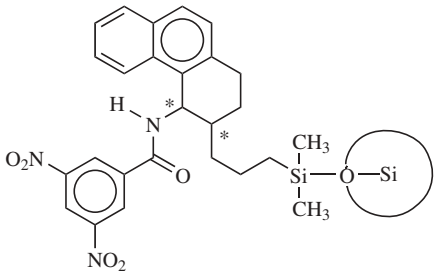


Fig. 5. The structure of the chiral selector in the Whelk-O-1 chiral stationary phase.

interactions. Some of the Pirkle-types of phases have also been used, to a lesser extent, in the reversed phase mode.

Reciprocity, an important concept introduced by Pirkle (69,70), exploited the notion that analytes that were well resolved using a particular chiral selector would likely be good candidates for chiral selectors to enantioresolve analytes similar to the original chiral selector. For instance, the first generation Pirkle phase incorporating *N*-(3,5-dinitrobenzoyl)phenylglycine was very successful at enantioresolving compounds containing naphthyl moieties near the stereogenic center. This insight spawned a second generation of Pirkle phases based on *N*-(2-naphthyl)-α-amino acids (71). These phases were very successful at enantioresolving analytes containing a 3,5-dinitrobenzoyl group, such as 3,5-dinitrophenyl carbamates, and ureas of chiral alcohols and amines (72). These columns are available through a variety of sources including Phenomenex, Regis Technologies, Inc., J. T. Baker, Inc., and Supelco, Inc.

The structure of the Whelk-O-1 phase, the most recent addition to this type of chiral stationary phase, is illustrated in Figure 5. This selector has a wedge-like chiral surface with one edge offering the π-basic tetrahydrophenanthrene ring system; the other edge is comprised of a 3,5-dinitrobenzoyl π-acidic moiety. The amide linkage between the two ring systems presents dipole stacking and hydrogen-bonding interaction sites. The presence of both π-acid and π-base features, as well as the inherent rigidity of the chiral selector, confers greater versatility than any of the previous Pirkle-type phases, imposing fewer constraints on both analyte structural features required for successful enantioresolution and mobile phase conditions. Indeed, this chiral stationary phase has demonstrated considerable chiral selectivity for naproxen, warfarin, and its *p*-chloro analogue under nonaqueous reversed-phase conditions (73) and reversed-phase conditions (74,75). An additional advantageous feature of this phase is its availability with either the (*R,R*) or (*S,S*) configuration, thus, permitting the enantiomeric elution order to be readily changed. The small size of the chiral selector also promotes fairly high bonded ligand densities in the stationary phase, which coupled with the high enantioselectivities often achieved with these phases, facilitates their use for preparative-scale separations (76).

6. Cyclodextrin Phases

Cyclodextrins are macrocyclic compounds comprised of D-glucose bonded through 1,4-α-linkages and produced enzymatically from starch. The greek letter which

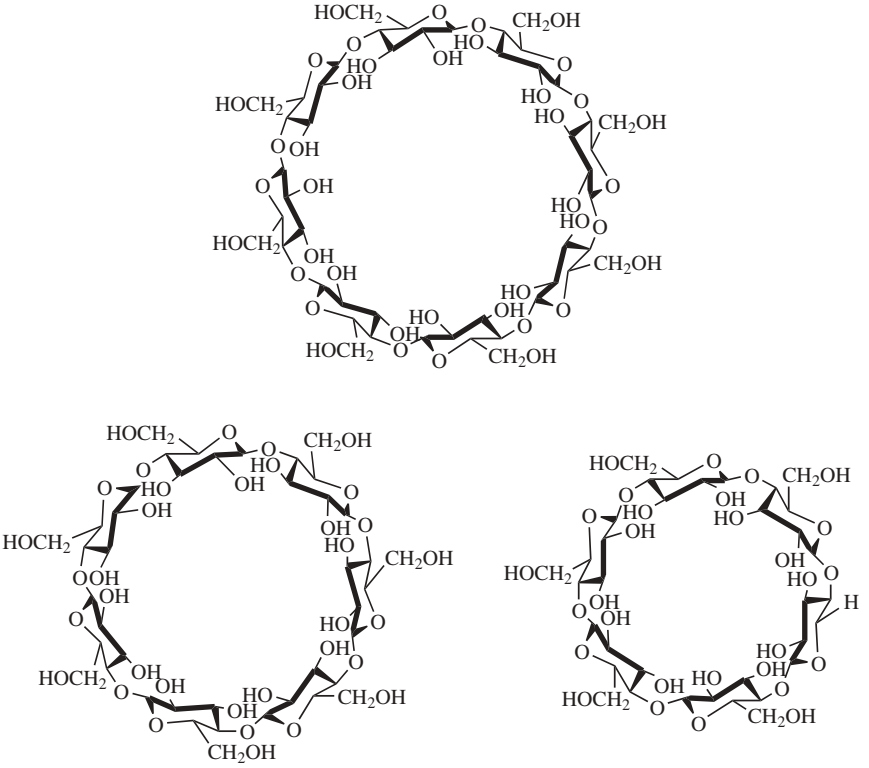


Fig. 6. The structure of the three most common cyclodextrins.

proceeds the name indicates the number of glucose units incorporated in the CD (eg, $\alpha = 6$, $\beta = 7$, $\gamma = 8$, etc). Cyclodextrins are toroidal shaped molecules with a relatively hydrophobic internal cavity (Fig. 6). The exterior is relatively hydrophilic because of the presence of the primary and secondary hydroxyls. The primary C-6 hydroxyls are free to rotate and can partially block the CD cavity from one end. The mouth of the opposite end of the CD cavity is encircled by the C-2 and C-3 secondary hydroxyls. The restricted conformational freedom and orientation of these secondary hydroxyls is thought to be responsible for the chiral recognition inherent in these molecules (77).

Among the most successful of the liquid chromatographic reversed-phase chiral stationary phases have been the cyclodextrin-based phases, introduced by Armstrong (78,79) and commercially available through Advanced Separation Technologies, Inc. or Alltech Associates. The most commonly used cyclodextrin in hplc is the β-cyclodextrin. In the bonded phases, the cyclodextrins are thought to be tethered to the silica substrate through one or two spacer ligands (Fig. 7). The mechanism thought to be responsible for the chiral selectivity observed with these phases is based on the formation of an inclusion complex between the hydrophobic moiety of the chiral analyte and the hydrophobic interior of the cyclodextrin cavity (Fig. 8). Preferential complexation between one optical isomer and the cyclodextrin through stereospecific interactions with the secondary hydroxyls which line the mouth of the cyclodextrin cavity results in the

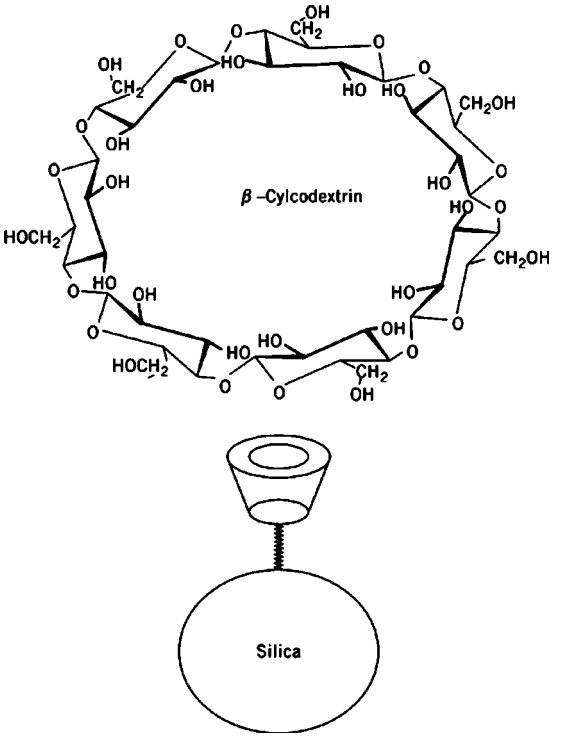


Fig. 7. A tethered cyclodextrin and the structure of β-cyclodextrin, the most common cyclodextrin used as a bonded ligand in liquid chromatography.

enantiomeric separation. Unlike the Pirkle-type phases, enantiospecific interactions between the analyte and the cyclodextrin are not the result of a single, well-defined association, but more of a statistical averaging of all the potential interactions with each interaction weighted by its energy or strength of interaction (80).

Vast amounts of empirical data suggest that chiral recognition on cyclodextrin phases in the reversed phase mode require the presence of an aromatic moiety that can fit into the cyclodextrin cavity, that there be hydrogen bonding

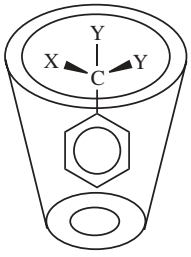


Fig. 8. A hydrophobic inclusion complex between a chiral analyte and a cyclodextrin.

groups in the molecule, and that the hydrophobic and hydrogen-bonding moieties should be in close proximity to the stereogenic center. Chiral recognition seems to be enhanced if the stereogenic center is positioned between two π-systems or incorporated in a ring.

Most of the chiral separations reported to date using the native cyclodextrin-based phases have been accomplished in the reversed-phase mode using aqueous buffers containing small amounts of organic modifiers. However, polar organic mobile phases have gained in popularity recently because of their ease of removal from the sample and reduced tendency to accelerate column degradation relative to the hydroorganic mobile phases (81). In these cases, because the more nonpolar component of the mobile phase is thought to occupy the cyclodextrin cavity, the analyte is thought to sit atop the mouth of the cyclodextrin much like a "lid".

Limitations with the chiral selectivity of the native cyclodextrins fostered the development of various functionalized cyclodextrin-based chiral stationary phases, including acetylated (82,83), sulfated (84), 2-hydroxypropyl (85), 3,5-dimethylphenylcarbamoylated (86) and 1-naphthylethylcaarbamoylated (87) cyclodextrin. Each of the glucose residues contribute three hydroxyl groups to which a substituent may be appended; thus, each cyclodextrin contributes multiple sites for derivatization. Typical degrees of substitution per β-cyclodextrin (with 21 hydroxyls) range from three to ten. Hence, there are many residual hydroxyls on each cyclodextrin.

The substituents of these functionalized cyclodextrins seem to play a variety of roles in enhancing chiral recognition. In some cases, the substituent may only serve to enlarge the chiral cavity or may provide alternative interaction sites. For instance, in the case of the naphthylethylcaarbamoylated cyclodextrin, the naphthyl ring provides a π-basic site and the carbamate linkage provides additional hydrogen bonding and dipole interaction sites not available with the native cyclodextrin. On the sulfated cyclodextrin phase, the sulfate group presents the potential for ion-pair formation unavailable with the native cyclodextrin. The introduction of 2-hydroxypropyl- and 1-naphthylethylcarbamoyl substituents incorporates additional stereogenic centers onto the cyclodextrin. In some cases, the configuration of the substituent dominated the enantiomeric elution order. However, in other cases, the enantiomeric elution order was independent of the configuration of the substituent. In addition, in some cases, the chiral selectivity of the cyclodextrin seemed to synergistically augment the chiral selectivity of one configuration of the substituent while antagonizing the chiral selectivity of the oppositely configured substituent. A particularly attractive feature of these functionalized cyclodextrins is that many of them exhibit enantioselectivity under hydroorganic reversed phase, as well as normal phase and polar organic mobile-phase conditions, and that each set of conditions can provide chiral separations for analytes which are not resolved under any of the other type of mobile-phase conditions. Further, the chromatographic mode (eg, reversed phase to normal phase) can be readily changed with no deleterious impact on chiral recognition as long as routine care is taken to avoid problems with solvent immiscibility. The naphthylethyl-carbamoylated cyclodextrin phase was considered to be one of the first "multimodal" chiral stationary phases (88).

7. Cellulosic and Amylosic Phases

Cellulose and amylose are comprised of the same glucose subunits as the cyclodextrins. In the case of cellulose, the glucose units are attached through 1,4-β-linkages resulting in a linear polymer. In the case of amylose, the 1,4-α-linkages, as are found in the cyclodextrins, are thought to confer helicity to the polymeric chain.

As mentioned previously, cellulosic phases as well as amylosic phases have also been used extensively for enantiomeric separations more recently (89,90). Most of the work in this area has been with various derivatives of the native carbohydrate. The enantioresolving abilities of the derivatized cellulosic and amylosic phases are reported to be very dependent on the types of substituents on the aromatic moieties that are appended onto the native carbohydrate (91). Table 3 lists some of the cellulosic and amylosic derivatives that have been used. These columns are available through Chiral Technologies, Inc. and J. T. Baker, Inc.

With the exception of the microcrystalline cellulose I triacetate (92) and tribenzoate materials, which are sufficiently robust to be used directly as packing material, most of the commercially available cellulosic and amylosic phases are comprised of mixtures of exhaustively derivatized polymers which are coated onto large pore γ-aminopropyl silica. These coated polymeric phases exhibit admirable enantioselectivities, but as is the case with all commercially available chiral stationary phases, they also have some potential disadvantages. The large polymer size requires the use of fragile, large pore silica. The fact that the chiral selector for these phases is coated onto the silica sometimes restricts the types of mobile phases that can be used. In addition, the secondary structure of the polymer, which seems to be important in the chiral recognition mechanism, may be altered irreversibly by storing the columns in polar solvents leading to disastrous consequences for chiral separations. The polymeric nature of the chiral selectors for these phases and the importance of the secondary structure also hamper the development of models for the chiral recognition mechanism for these phases (93). Despite these factors, the cellulosic and amylosic phases have enjoyed tremendous success at enantioresolving structurally diverse compounds (94,95) and have some of the highest capacities of all the chiral commercially available chiral stationary phases, thus, rendering them among the most suitable for preparative chromatography (96).

Table 3. **Carbohydrate Derivatives Used as Hplc Chiral Stationary Phases**

Cellulosic	Amylosic
triacetate	
tribenzoate	
tribenzylether	
tricinnamate	
triphenylcarbamate	triphenylcarbamate
tris-3,5-dichlorophenylcarbamate	
tris-3,5-dimethylphenylcarbamate	tris-3,5-dimethylphenylcarbamate
tris-1-phenylethylcarbamate	tris-1-phenylethylcarbamate

The chiral recognition sites on these polymeric carbohydrate phases are thought to be channels or grooves in the polymer matrix and that analytes are included into these channels. Evidence for inclusion is provided by the enhanced chiral recognition observed for many analytes as the steric bulk of the alcohol mobile phase modifier increases. Chiral recognition seems to require the presence of an aromatic ring, for $\pi-\pi$ interactions, and polar sites of unsaturation or hydrogen bonding functionalities. As in the case of the Pirkle-type phases, these chiral stationary phases are usually used in the normal phase mode and mobile phases typically consist of hexane and 2-propanol although there have been some reports of these phases being used in the reversed phase mode (97).

8. Protein-Based Phases

Proteins, amino acids bonded through peptide linkages to form macromolecular biopolymers, used as chiral stationary phases for hplc include bovine and human serum albumin, α_1-acid glycoprotein, ovomucoid, avidin, and cellobiohydrolase. The bovine serum albumin column is marketed under the name Resolvosil and can be obtained from Phenomenex. The human serum albumin column can be obtained from Alltech Associates, Advanced Separation Technologies, Inc., and J. T. Baker. The α_1-acid glycoprotein and cellobiohydrolase can be obtained from Advanced Separation Technologies, Inc. or J. T. Baker, Inc.

In most cases, the protein is immobilized onto γ-aminopropyl silica and covalently attached using a cross-linking reagent such as *N,N′*-carbonyldiimidazole. The tertiary structure or three dimensional organization of proteins are thought to be important for their activity and chiral recognition. Therefore, mobile phase conditions that cause protein "denaturation" or loss of tertiary structure must be avoided.

Typically, the mobile phases used with the protein-based chiral stationary phases consist of aqueous phosphate buffers (98). Often small amounts of organic modifiers, such as methanol, ethanol, propanol, or acetonitrile, are added to reduce hydrophobic interactions with the analyte and to improve enantioselectivity. In some cases, dramatic changes in chiral recognition occur when small amounts of organic modifiers, such as *N,N*-dimethyloctylamine or octanoic acid are added to the mobile phase. It is thought that these additives may be playing an active role in enhancing chiral recognition through absorption of the organic modifier onto the protein which induces conformational changes in the overall tertiary structure of the protein. In these cases, the *allosteric* modifier-mediated conformational changes in the protein are thought to enhance chiral recognition by a variety of mechanisms including changes in the accessibility of various stereospecific sites on the protein or obstruction of nonstereospecific sites (99).

As in the case of the cyclodextrin and amylosic and cellulosic phases, the chiral recognition mechanism for these protein-based phases is not well understood. In some cases, it is thought that analytes may form inclusion complexes with hydrophobic pockets within the biopolymeric matrix. These hydrophobic interactions may couple with hydrogen bonding, electrostatic interactions, and $\pi-\pi$ or dipole stacking to individual amino acid residues, thus, contributing to stereospecific orientational constraints within the hydrophobic pockets.

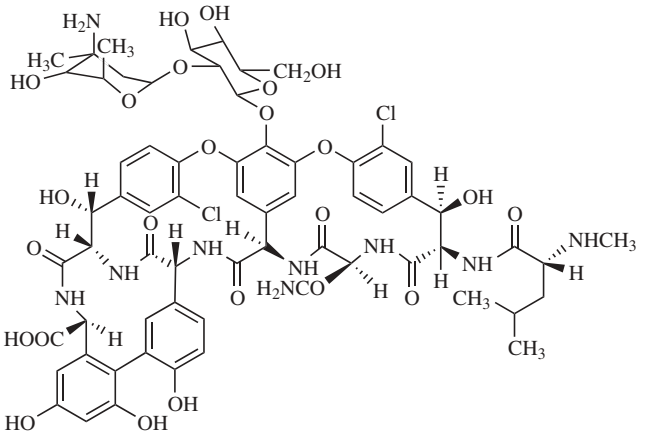
Optimization of chromatographic conditions and selection of analytes that can be successfully resolved on these phases is usually done empirically. In addition, the large molecular weight of these biopolymers dictates that the amount of chiral selector that can be immobilized on the column packing material is very small. Although the protein is large, relative to the analytes, the actual region of the protein that affects the chiral separation may be very small. Thus, the capacity (amount of material resolvable during a single chromatographic run) of these columns is generally fairly small ($<\sim 0.1$ mg) and the columns are easily overloaded.

An interesting application of the protein-based phases is various protein binding and displacement experiments which can be done fairly routinely (100). For instance, the chiral selectivity of chiral stationary phases derived from the serum albumin, one of the most abundant blood proteins which functions as a transport protein, from different animal species including rabbit, rat and human has been compared (101). This work suggests that differences in the enantioselectivity, toward a particular drug, of a column derived from human serum albumin and a column derived from some other animal serum albumin might be indicative that a particular species might not be a good animal model during drug development, thus, obviating the need for animal testing.

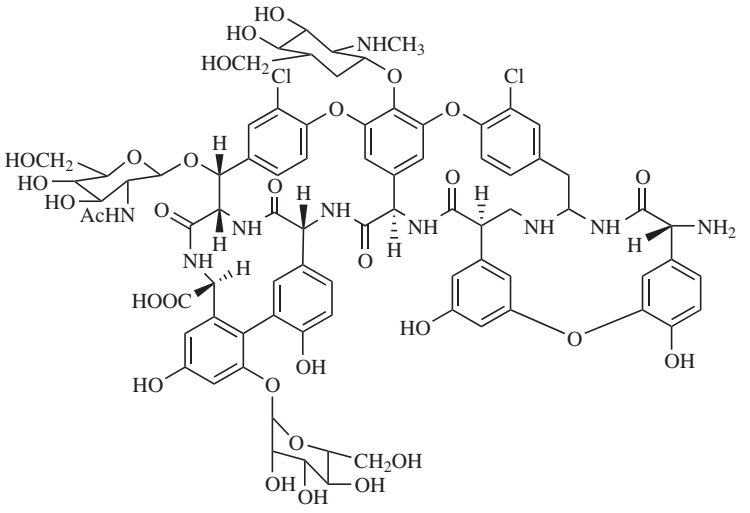
Chiral separations on protein-based phases may also provide useful information on drug interactions. For instance, the effect of the individual enantiomers of warfarin on the enantioselectivity of human serum albumin toward benzodiazepinones has been studied using a human serum albumin column with warfarin as a mobile phase additive (102).

9. Chirobiotic Phases

The chirobiotic chiral stationary phases (103,104) are based on macrocyclic antibiotics such as vancomycin (**4**) and teicoplanin (**5**).



(**4**)



(**5**)

These chiral selectors, originally used as chiral additives in capillary zone electrophoresis, incorporate aromatic and carbohydrate, as well as peptide and ionizable moieties. The presence of aromatic groups, allowing for π–π interactions, and the macrocyclic rings, offering potential inclusion complexation, give these phases some of the advantages of the protein-based phases (eg, peptide and hydrogen bonding sites) and the carbohydrate-based phases but with greater sample capacity and greater mobile phase flexibility. Indeed, these phases seem to be truly "multimodal" in that they have demonstrated chiral selectivity in the normal, polar organic, and reversed-phase modes. In the normal and polar organic phase modes, π–π interactions, and dipole stacking are thought to play a predominant role in chiral selector–analyte interactions. In the reversed-phase mode, hydrogen bonding, inclusion complexation and, for charged analytes, electrostatic interactions are thought to dominate the interactions. In addition, the use of such well-defined chiral selectors facilitate method development and optimization. These columns are commercially available through Advanced Separation Technologies, Inc. and Alltech Associates.

10. Chiral Crown Ether Phases

Chiral crown ethers based on 18-crown-6 (Fig. 9) can form inclusion complexes with ammonium ions and protonated primary amines. Immobilization of these

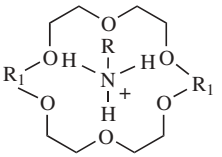


Fig. 9. An inclusion complex formed between a protonated primary amine and a chiral crown ether.

chiral crown ethers on a chromatographic support provides a chiral stationary phase which can resolve most primary amino acids, amines, and amino alcohols. However, the stereogenic center must be in fairly close proximity to the primary amine for successful chiral separation (105,106). Significantly, the chiral crown ether phase is unique in that it is one of the few liquid chromatographic chiral stationary phases that does not require the presence of an aromatic ring to achieve chiral separations. Although chiral recognition seems to be enhanced for analytes containing either bulky substituents or aromatic groups near the stereogenic center, only the presence of the primary amine is mandatory.

Mobile phases used with this stationary phase are typically 0.01 *N* perchloric acid with small amounts of methanol or acetonitrile. One significant advantage of these phases is that both configurations of the chiral stationary phase are commercially available and can be obtained from J. T. Baker Inc. and Chiral Technologies, Inc. (Crownpak CR).

11. Chiral Synthetic Polymer Phases

Chiral synthetic polymer phases can be classified into three types. In one type, a polymer matrix is formed in the presence of an optically pure compound to molecularly *imprint* the polymer matrix (Fig. 10) (107,108). Subsequent to the polymerization, the chiral template is removed, leaving the polymer matrix with chiral cavities. The degree of cross-linking in the polymer matrix and degree of association between the template molecule and the monomer, is governed by the type and concentration of the monomer, the concentration of the template, the solvent and temperature or pressure under which polymerization takes place. All play a role in the chiral selectivities achieved with these phases. The selectivities achieved with these phases are generally excellent, thus, facilitating semipreparative separations. However, the applicability of these chiral stationary phases are generally limited to the analyte upon which the phase is based and a limited number of analogues. In addition, these types of phases generally exhibit poor efficiency in large part because the polymeric matrix contributes

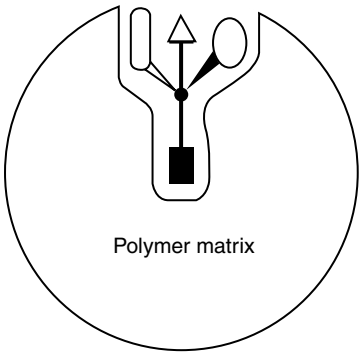


Fig. 10. The relationship between a chiral template molecule and the polymeric matrix formed in the presence of the template molecule.

to nonsterespecific binding. Advantages of this approach include the ability to prepare reciprocal phases and the predictability of the enantiomeric elution order.

Another type of synthetic polymer-based chiral stationary phase is formed when chiral catalyst are used to initiate the polymerization. In the case of poly (methyl methacrylate) polymers, introduced by Okamoto, the chirality of the polymer arises from the helicity of the polymer and not from any inherent chirality of the individual monomeric subunits (109). Columns of this type (eg, Chiralpak OT) are available from Chiral Technologies, Inc., or J. T. Baker Inc.

A third type of synthetic polymer-based chiral stationary phase, developed by Blaschke (110), is produced when a chiral selector is either incorporated within the polymer network (111) or attached as pendant groups onto the polymer matrix. Both are analogous to methods used to produce polymeric chiral stationary phases for gc. The polymers can be either coated onto a silica substrate, comonomers bearing silane functional groups may be added for subsequent reaction with the silica, or the silica may be chemically modified to incorporate monomer-bearing silanes. More recently, L-valine-3,5-dimethylanilide has been bonded to a poly(glycidylmethacrylate-*co*-ethylenedimethacrylate polymer which formed the underlying substrate (112). Chemical bonding of the polymer to the substrate eases the mobile phase restrictions imposed on the coated chiral polymer stationary phases.

In general, the synthetic polymeric phases seem to have polarities analogous to diol-type phases and a wide range of mobile phase conditions have been used including hexane, various alcohols, acetonitrile, tetrahydrofuran, dichloromethane and their mixtures, as well as aqueous buffers.

12. Chiral Separation Validation for Hplc

Chiral separations present special problems for validation. Typically, in the absence of spectroscopic confirmation (eg, mass spectral or infrared data), conventional separations are validated by analyzing "pure" samples under identical chromatographic conditions. Often, two or more chromatographic stationary phases, which are known to interact with the analyte through different retention mechanisms, are used. If the pure sample and the unknown have identical retention times under each set of conditions, the identity of the unknown is assumed to be the same as the pure sample. However, often the chiral separation that is obtained with one type of column may not be achievable with any other type of chiral stationary phase. In addition, "pure" enantiomers are generally not available.

Most commonly, uv or uv–vis spectroscopy is used as the basis for detection in hplc. When using a chiral stationary phase, confirmation of a chiral separation may be obtained by either monitoring the column effluent at more than one wavelength or by running the sample more than once. The same mobile-phase conditions are used, but monitoring is done at different wavelengths. Because enantiomers have identical spectra in an achiral environment, the ratio of the peaks for the two enantiomers should be independent of wavelength.

Although not absolute proof of a chiral separation, this approach does provide strong supporting evidence.

As in tlc, another method to validate a chiral separation is to collect the individual peaks and subject them to some type of optical spectroscopy, such as, circular dichroism or optical rotary dispersion. Enantiomers have mirror image spectra (eg, the negative maxima for one enantiomer corresponds to the positive maxima for the other enantiomer). One problem with this approach is that the analytes are diluted in the mobile phase. Thus, the sample must be injected several times. The individual peaks must be collected and subsequently concentrated to obtain adequate concentrations for spectral analysis.

Alternatively, a chiroptical spectroscopy can be used as the basis for detection on-line using commercially available optical rotary dispersion or circular dichroism-based detectors. Optical rotary dispersion instruments are analogous to refractive index-based detectors for conventional chromatography in that they are universal, do not require the presence of a chromophore in the analyte and have the least sensitivity of the optical detectors. Circular dichroic detection, although more sensitive than optical rotary dispersion-based detection, requires not only the presence of a uv chromophore in the analyte, but that the chromophore be not too distant from the asymmetric center of the analyte. Figure 11**a** illustrates a simulated chromatogram for an enantiomeric separation obtained using a conventional absorption detector. Figure 11**b** illustrates a simulated chromatogram for an enantiomeric separation using circular dichroic detection. Both types of chiroptical detectors produce positive and negative peaks for the two enantiomers. However, neither chiroptical detector can distinguish a fair separation (Fig. 11**d**) from a poor separation (Fig. 11**f**) in which there is considerable overlap of the two peaks. This is because the signals generated by the two enantiomers have opposite signs, and thus, any overlap causes cancellation of signal. Further, peak overlap results in nonlinear detector response vs concentration. Therefore, some other detection method must be used in conjunction with either of these types of detection. Nevertheless, as can be seen from Figure 11**f**, chiroptical detection can be advantageous if there is considerable overlap of the two peaks. In this case, chiroptical detection may reveal that the leading and tailing edges of the peak are enantiomerically enriched which may not be apparent from the chromatogram obtained with nonchiroptical detection (Fig. 11**e**).

Another method for validating chiral separations by lc is to couple the chromatographic system to a mass spectrometer. In mass spectrometry, high energy ions are used to bombard molecules exiting the column. The impact of the high energy ions causes the molecules to "fragment" into various ions which are then sent to a "mass discriminator". The ion fragments are detected and a fragmentation pattern or mass spectrum is reconstructed. Enantiomers have identical fragmentation patterns. Hence, identical fragmentation patterns for two peaks in the chromatogram confirms a chiral separation.

13. Chiral Stationary Phases for Gas Chromatography

Although chiral stationary phases for gas chromatography (gc) were introduced before liquid chromatographic chiral stationary phases, development of gc chiral

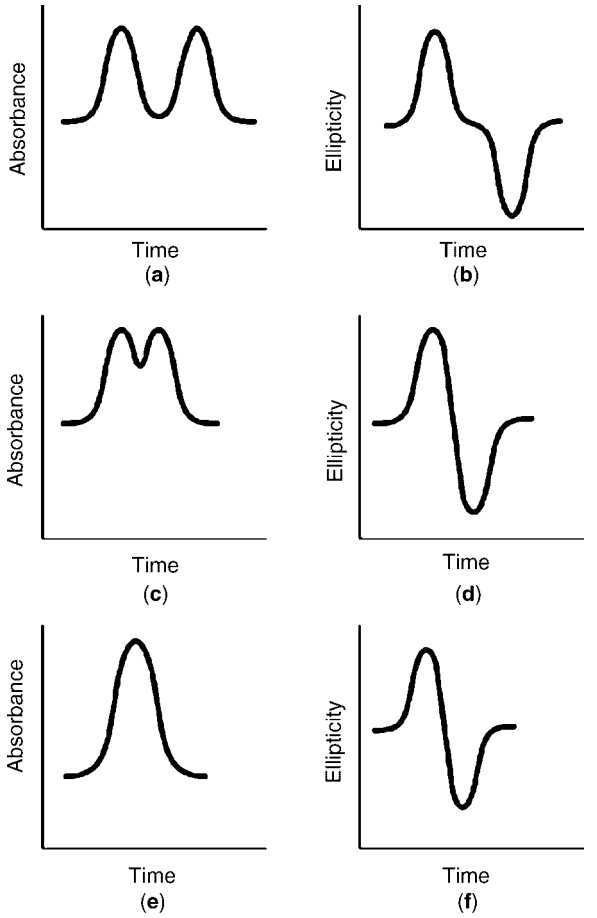


Fig. 11. Simulated chromatograms of chiral separations obtained using nonchiroptical detection (**a, c, e**) and chiroptical detection (**b, d, f**) illustrating the effect of peak overlap on the resultant chromatogram.

stationary phases lagged behind for a variety of reasons. First of all, analysis by gc requires that the analyte be volatile and thermally stable. This condition often requires that the analyte be derivatized with an achiral reagent prior to chromatographic analysis to enhance sample volatility. In some cases, derivatization may actually enhance detector response (eg, trifluoroacetylation amplifies electron capture detection) or chiral interactions. However, it should be noted that the presence of more than one type of functionality (eg, amine and alcohol) in the analyte with differing reactivities toward the derivatizing agent may add additional complications. Typical achiral derivatizing reagents, as well as the appropriate functionality required in the analyte, are listed in Table 4.

The use of gas chromatography for chiral separations was also hampered because the high column temperatures typically used in gc tend to accelerate racemization of the stationary phase, thus, decreasing column longevity. The high column temperatures typically used in gc also tend to accelerate racemization of the analyte. In addition, the differences in the stabilities of the

Table 4. **Analyte Functional Groups and Typical Achiral Derivatizing Reagents**

Type of derivatizing agent	Analyte functional group	Examples of derivatizing agents
alkyl silyl	alcohols thiols carboxylic acid amines	*N*-trimethylsilylimidazole *N,O*-bis(trimethylsilyl)-trifluroracetamide
acyl, haloacyl or anhydride	alcohols amines amides oximes thiols ketones	acetic acid heptafluorobutyryl chloride trifluoroacetyl chloride
alcohol	carboxylic acids	methanol
alkyl halides	carboxylic acids	methyl bromide
diazoalkyl	carboxylic acids sulfonic acids phenols	diazomethane
isocyanate	alcohols amines hydroxy acids	isopropylisocyanate
phosgene	β-amino alcohols β-amino thiols diols *N*-methylamino acids	
alkyl hydroxylamine	ketones	methylhydroxylamine

diastereomeric complexes formed between the enantiomers and the stationary phase tends to be overcome by the high column temperatures. Finally, preparative scale separations are generally harder to implement in gc than in hplc. However, the inability of most liquid chromatographic methods to resolve chirally small nonaromatic compounds that are frequently used as chiral synthetic building blocks, as well as improvements in gc column technology, has led to renewed interest in chiral stationary phases for gc.

Gc chiral stationary phases can be broadly classified into three categories: diamide, cyclodextrin, and metal complex.

13.1. Diamide Chiral Separations. The first chiral stationary phase for gas chromatography was reported by Gil-Av and co-workers in 1966 (113) and was based on *N*-trifluoroacetyl (*N*-TFA) L-isoleucine lauryl ester coated on an inert packing material. It was used to resolve the trifluoroacetylated derivatives of amino acids. Related chiral selectors used by other workers included *n*-dodecanoyl-L-valine-*t*-butylamide and *n*-dodecanoyl-(*S*)-α-(1-naphthyl)-ethylamide. The presence of the long alkyl groups lowered chiral selector volatility thus reducing but not entirely eliminating column bleed and improving column longevity.

The first commercially available chiral column was the Chiralsil-val (Fig. 12), which was introduced in 1976 (114) for the separation of amino acid-type compounds by gas chromatography. It is based on a polysiloxane polymer containing chiral side chains incorporating L-valine-*t*-butylamide. The polysiloxane back-

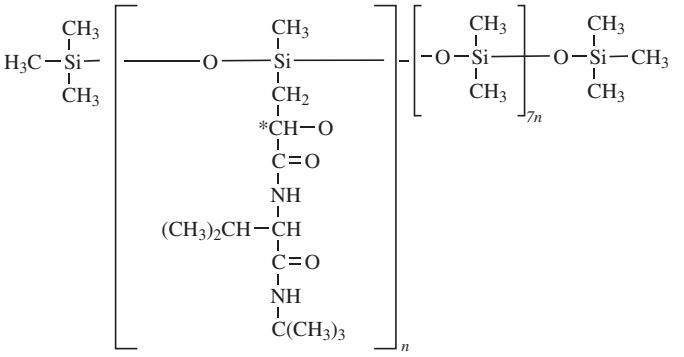


Fig. 12. Structure of Chiralsil-val.

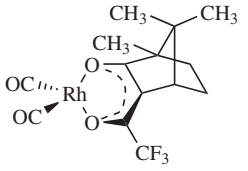
bone improved the thermal stability of these chiral stationary phases relative to the original coated columns and extended the operating temperatures up to 220°C. The column is effective for the separation of perfluoroacylated and esterified amino acids, amino alcohols, and some chiral sulfoxides. Another polysiloxane-based chiral stationary phase incorporating L-valine-(*R*)-α-phenylethylamide appended onto hydrolyzed XE-60 was found to be particularly successful at resolving perfluoroacetylated amino alcohol derivatives (115). Through judicious choice of derivatizing agent, chiral separations were obtained for a wider range of compounds, including amino alcohols, α-hydroxy acids, diols and ketones, than had previously been obtainable using these types of stationary phases (116).

The chiral recognition mechanism for these types of phases was attributed primarily to hydrogen bonding and dipole–dipole interactions between the analyte and the chiral selector in the stationary phase. It was postulated that chiral recognition involved the formation of transient five- and seven-membered association complexes between the analyte and the chiral selector (117).

On each of these amino acid-based chiral stationary phases, the configuration of the most retained enantiomer corresponds to the configuration of the chiral selector in the stationary phase (eg, L-amino acids were retained longer on the *N*-trifluoroacetyl (*N*-TFA) L-isoleucine lauryl ester column). Thus, configuration of the analytes can be assigned even if no optically pure material is available as long as optically pure standard materials are available for structurally related compounds. Another advantage is that stationary phases incorporating the chiral selector with either configuration may be readily prepared or are commercially available. Thus, the elution order may readily be reversed by using a column containing the chiral selector with the opposite configuration, thus, providing another tool for chiral separation validation. Also, quantitation of a very small peak in the presence of a very large peak is generally easier if the smaller peak elutes first.

13.2. Metal Complex. Complexation gas chromatography was first introduced by V. Schurig in 1980 (118) and employs transition metals (eg, nickel, cobalt, manganese or rhodium) complexed with chiral terpenoid ketoenolate

ligands such as 3-trifluoroacetyl-1*R*-camphorate (**6**), 1*R*-3-pentafluoro-benzoyl-camphorate or 3-heptafluorobutanoyl-(1*R*,2*S*)-pinanone-4-ate. In most cases, the chiral selector is dissolved in a polymer matrix coated on the interior walls of a capillary column. This class of chiral columns is particularly adept at enantioresolving some olefins and oxygen-containing compounds such as ketones, ethers, alcohols, spiroacetals, oxiranes, and esters. Many of these compounds lack suitable functionality for derivatization with chiral reagents, and thus, are not amenable to diastereomer formation. Unfortunately, as is the case with many of the chiral stationary phases, the chiral recognition mechanism is not sufficiently refined to allow for prediction of analyte absolute configuration on the basis of retention times except for a very limited number of cases. Nevertheless, these columns allow for the direct chiral separation of compounds that are important synthetic precursors and may be difficult to separate by any other method.



(**6**)

13.3. Cyclodextrins. As indicated previously, the native cyclodextrins, which are thermally stable, have been used extensively in liquid chromatographic chiral separations, but their utility in gc applications was hampered because their highly crystallinity and insolubility in most organic solvents made them difficult to formulate into a gc stationary phase. However, some functionalized cyclodextrins form viscous oils suitable for gc stationary-phase coatings and have been used either neat or diluted in a polysiloxane polymer as chiral stationary phases for gc (119). Some of the derivatized cyclodextrins which have been adapted to gc phases are 3-*O*-acetyl-2,6-di-*O*-pentyl, 3-*O*-butyryl-2,6-di-*O*-pentyl, 2,6-di-*O*-methyl-3-*O*-trifluoroacetyl, 2,6-dipentyl, 2-*O*-methyl-3,6-di-*O*-pentyl, permethyl, permethylhydroxypropyl, perpentyl, and propionyl. Several of these are available commercially. For instance, Advanced Separation Technologies, Inc., Alltech Associates, J. & W. Scientific, and Supelco, Inc., all carry cyclodextrin-based chiral gc columns. Although these derivatized cyclodextrins are often coated, neat, onto the capillary column inner walls, some work has been done to tether the cyclodextrins to a polysiloxane backbone to enhance the thermal stability of the resultant phase (120). Some of the separations obtained with these materials are quite remarkable and include compounds such as halogenated alkanes (Fig. 13) (121), alcohols, alkenes, bicyclic compounds, and simple alkanes.

Although the chiral recognition mechanism of these cyclodextrin-based phases is not entirely understood, thermodynamic and column capacity studies indicate that the analytes may interact with the functionalized cyclodextrins by either associating with the outside or mouth of the cyclodextrin, or by forming a more traditional inclusion complex with the cyclodextrin (122). As in the case of

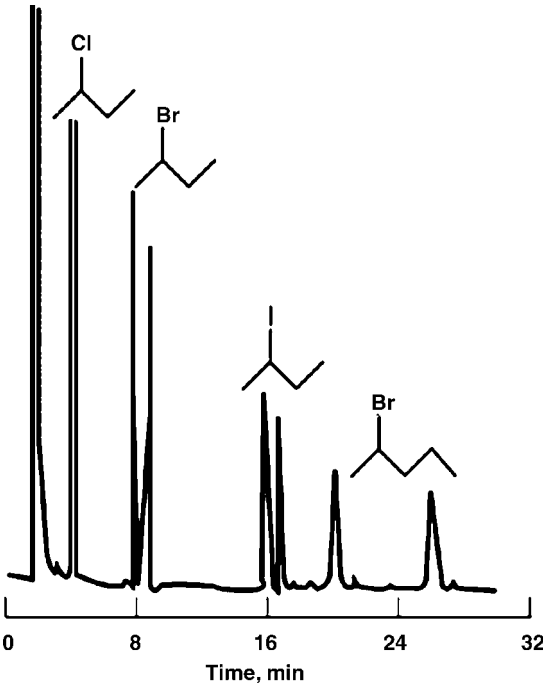


Fig. 13. Enantiomeric separations of monohalohydrocarbons on a 2,6-*O*-dipentyl-3-*O*-trifluoroacetyl-γ-cyclodextrin coated capillary column (10 m, 0.25 mm ID). Column temperature, 30°C; nitrogen carrier gas, 20.7 kPa (3 psi).

the metal-complex chiral stationary phase, configuration assignment is generally not possible in the absence of pure chiral standards.

14. Chiral Separation Validation for Gas Chromatography

The special problems for validation presented by chiral separations can be even more burdensome for gc because most methods of detection (eg, flame ionization detection or electron capture detection) in gc destroy the sample. Even when nondestructive detection (eg, thermal conductivity) is used, individual peak collection is generally more difficult than in lc or tlc. Thus, off-line chiroptical analysis is not usually an option. Fortunately, gc can be readily coupled to a mass spectrometer and is routinely used to validate a chiral separation.

15. Conclusions

The field of chiral separations has grown explosively into a well-developed specialty within separation science in the last two decades. Considerable effort in the field has thus far been directed toward solving analytical separation problems and in the design and development of new chiral separation methods. However,

in the future, chiral separations may have the potential to provide an invaluable tool for probing intermolecular interactions, increasing our understanding of the chemistry behind some biological processes and disease states (123,124). Also the efficacy of drug therapy can be enhanced by eliminating or minimizing untoward side effects (125) produced by distomeric ballast and allowing for better control in the application of chiral or prochiral entities to biological or environmental systems.

BIBLIOGRAPHY

1. L. Pasteur, *Comptes Rendus de l'Academie des Sciences* **26**, 535 (1848).
2. W. J. Wechter, D. G. Loughhead, R. J. Reischer, G. J. Van Giessen, and D. G. Kaiser, *Biochem. Biophys. Res. Comm.* **61**, 833 (1974).
3. R. A. Kuzel, S. K. Bhasin, H. G. Oldham, L. A. Damani, J. Murphy, P. Camilleri, and A. J. Hutt, *Chirality* **6**, 607 (1994).
4. S. A. Tobert, *Clin. Pharmacol. Ther.* **29**, 344 (1981).
5. H. Ohkawa, *Bull. Environ. Contamin. Toxicol.* **18**, 534 (1977).
6. H. G. Schmarr, A. Mosandl, and K. Grob, *Chromatographia* **29**, 125 (1990).
7. T. Aleibi-Kolbah, G. Félix, and I. W. Wainer, *Chromatographia* **35**, 264 (1993).
8. D. W. Armstrong, J. R. Faulkner, Jr., and S. M. Han, *J. Chromatogr.* **452**, 323 (1988).
9. T. J. Ward, *Anal. Chem.* **66**, 632A (1994).
10. C. Pettersson, A. Karlsson, and C. Gioeli, *J. Chromatogr.* **407**, 217 (1987).
11. J. D. Duncan, D. W. Armstrong, and A. M. Stalcup, *J. Liq. Chromatogr.* **13**, 1091 (1990).
12. D. W. Armstrong, F.-Y. He, and S. M. Han, *J. Chromatogr.* **448**, 345 (1988).
13. A. D. Cooper, T. M. Jeffries, and R. M. Gaskell, *Anal. Proc.* **29**, 258 (1992).
14. D. W. Armstrong and Y. Zhou, *J. Liq. Chromatogr.* **17**, 1695 (1994).
15. C. Pettersson and G. Schill, *J. Liq. Chromatogr.* **9**, 269 (1986).
16. M.-B. Huang, H.-K. Li, G.-L. Li, C.-T. Yan, and L.-P. Wang, *J. Chromatogr. A* **742**, 289 (1996).
17. D. W. Armstrong, J. R. Faulkner, Jr., and S. M. Han, *J. Chromatogr.* **452**, 323 (1988).
18. D. W. Armstrong, F.-Y. He, and S. M. Han, *J. Chromatogr.* **448**, 345 (1988).
19. J. D. Duncan, D. W. Armstrong, and A. M. Stalcup, *J. Liq. Chromatogr.* **13**, 1091 (1990).
20. W. G. Kuhr, *Anal. Chem.* **62**, 403R (1990).
21. B. L. Karger, *Amer. Lab.*, 23 (Oct. 1993).
22. J. W. Jorgenson and K. D. Lukacs, *Anal. Chem.* **53**, 1298 (1981).
23. A. S. Cohen, A. Paulus, and B. L. Karger, *Chromatographia* **24**, 15 (1987).
24. P. Sun, N. Wu, G. Barker, and R. A. Hartwick, *J. Chromatogr.* **648**, 475 (1993).
25. S. Terabe, K. Otsuka, and H. Nishi, *J. Chromatogr.* **666**, 295 (1994).
26. Y. Y. Rawjee and G. Vigh, *Anal. Chem.* **66**, 619 (1994).
27. A. M. Stalcup and K. H. Gahm, *Anal. Chem.* **68**, 1360 (1996).
28. A. M. Stalcup and N. M. Agyei, *Anal. Chem.* **66**, 3054 (1994).
29. H. Soini, M. Stefansson, M.-L. Riekkola, and M. V. Novotny, *Anal. Chem.* **66**, 3477 (1994).
30. P. Gozel, E. Gassman, H. Michelson, and R. N. Zare, *Anal. Chem.* **59**, 44 (1987).
31. A. M. Stalcup and K. H. Gahm, *J. Microcolumn Sep.* **8**, 145 (1996).
32. D. W. Armstrong, K. Rundlett, and G. L. Reid, *Anal. Chem.* **66**, 1690 (1994).
33. T. O. Cole, M. J. Sepaniak, and W. L. Hinze, *J. High Resolut. Chromatogr. Chromatogr. Commun.* **13**, 570 (1990).

34. Y. Mechref and Z. El Rassi, *Chirality* **8**, 515 (1996).
35. N. M. Agyei, K. H. Gahm, and A. M. Stalcup, *Anal. Chim. Acta* **307**, 185 (1995).
36. A. M. Stalcup and K. H. Gahm, *J. Microcol. Sep*. **8**, 145 (1996).
37. R. S. Sahota and M. G. Khaledi, *Anal. Chem*. **66**, 1141 (1994).
38. F. Wang and M. G. Khaledi, *Anal. Chem*. **68**, 3460 (1996).
39. S. A. C. Wren and R. C. Rowe, *J. Chromatogr*. **603**, 235 (1992).
40. A. Guttman, A. Paulus, A. S. Cohen, N. Grinberg, and B. L. Karger, *J. Chromatogr*. **448**, 41 (1988).
41. S. Jönsson, A. Schön, R. Isaksson, C. Pettersson, and G. Petterson, *Chirality* **5**, 505 (1992).
42. A. M. Stalcup, S. Gratz, and Y. Jin, unpublished results.
43. L. Witherow, T. D. Spurway, R. J. Ruane, I. D. Wilson, and K. Longdon, *J. Chromatogr*. **553**, 497–501 (1991).
44. H. T. K. Xuan and M. Lederer, *J. Chromatogr*. **635**, 346 (1993).
45. H. T. K. Xuan and M. Lederer, *J. Chromatogr*. **645**, 185 (1993).
46. C. A. Brunner and I. W. Wainer, *J. Chromatogr*. **472**, 277 (1989).
47. R. Bhushan and V. Parshad, *J. Chromatogr*. **721**, 369 (1996).
48. R. Bhushan and I. Ali, *Chromatographia* **35**, 679 (1993).
49. R. Bhushan and V. Parshad, *J. Chromatogr*. **736**, 235 (1996).
50. U. A. Th. Brinkman and D. Kamminga, *J. Chromatogr*. **330**, 375 (1985).
51. V. Mathur, N. Kanoongo, R. Mathur, C. K. Narang, and N. K. Mathur, *J. Chromatogr*. **685**, 360 (1994).
52. M. Remelli, R. Piazza, and F. Pulidori, *Chromatographia* **32**, 278 (1991).
53. D. W. Armstrong and W. DeMond, *J. Chromatogr. Sci*. **22**, 411 (1984).
54. D. W. Armstrong, Y. Tang, S. Chen, Y. Zhou, C. Bagwill, and J. R. Chen, *Anal. Chem*. **66**, 1473 (1994).
55. W. H. Pirkle, J. M. Finn, B. C. Hamper, J. L. Schreiner, and J. R. Pribish, *Am. Chem. Soc. Symp. Ser. No. 185*, Chapt. 18, 1982.
56. W. H. Pirkle and P. G. Murray, *J. Liq. Chromatogr*. **13**, 2123 (1990).
57. J. Hermansson and M. Eriksson, *J. Liq. Chromatogr*. **9**, 621 (1986).
58. G. Schill, I. W. Wainer, and S. A. Barkin, *J. Liq. Chromatogr*. **9**, 641 (1986).
59. S. Allenmark, *J. Liq. Chromatogr*. **9**, 425 (1986).
60. M. Okamoto and H. Nakazawa, *J. Chromatogr*. **508**, 217 (1990).
61. T. Miwa, H. Kuoda, S. Sakashita, N. Asakawa, and Y. Miyake, *J. Chromatogr*. **511**, 89 (1990).
62. R. Isaksson, P. Erlandsson, L. Hansson, A. Holmberg, and S. Berner, *J. Chromatogr*. **498**, 257 (1990).
63. Y. Okamoto, R. Aburatani, K. Hatano, and K. Hatada, *J. Liq. Chromatogr*. **11**, 2147 (1988).
64. T. Shinbo, T. Yamaguchi, K. Nishimura, and M. Sugiura, *J. Chromatogr*. **405**, 145 (1987).
65. M. Hilton and D. W. Armstrong, *J. Liq. Chromatogr*. **14**, 9 (1991).
66. V. A. Davankov, A. A. Kurganov, and A. S. Bochov, *Adv. Chromatogr*. **22**, 71 (1983).
67. W. H. Pirkle, J. M. Finn, J. L. Schreiner, and B. C. Hamper, *J. Am. Chem. Soc*. **103**, 3964 (1981).
68. C. E. Dalgliesh, *J. Chem. Soc.*, 3940 (1952).
69. W. H. Pirkle, D. W. House, and J. M. Finn, *J. Chromatogr*. **192**, 143 (1980).
70. W. H. Pirkle and T. C. Pochapsky, *J. Am. Chem. Soc*. **108**, 352 (1986).
71. W. H. Pirkle, T. C. Pochapsky, G. S. Mahler, D. E. Corey, D. S. Reno, and D. M. Alessi, *J. Org. Chem*. **51**, 4991 (1986).
72. W. H. Pirkle, G. Mahler, and M. H. Hyun, *J. Liq. Chromatogr*. **9**, 443 (1986).
73. W. H. Pirkle and C. J. Welch, *Tetrahedron Assym*. **5**, 777 (1994).

74. C. J. Welch, T. Szczerba, and S. R. Perrin, *J. Chromatogr. A* **758**, 93 (1997).
75. Regis Technologies Application Guide, pp. 30, 31.
76. C. J. Welch and S. R. Perrin, *J. Chromatogr*. **690**, 218 (1995).
77. T. J. Ward and D. W. Armstrong, "Cyclodextrin Stationary Phases" in M. Zief and L. J. Crane, eds., *Chromatographic Chiral Separations*, Marcel Dekker, Inc., New York, 1988, p. 131.
78. S. M. Han and D. W. Armstrong, in A. M. Krstulovic, ed., *Chiral Separations by HPLC*, John Wiley & Sons, Inc., New York, 1989, 208–287.
79. D. W. Armstrong, T. J. Ward, R. D. Armstrong, and T. E. Beesley, *Science* **232**, 1132 (1986).
80. R. E. Boehm, D. E. Martire, and D. W. Armstrong, *Anal. Chem*. **60**, 522 (1988).
81. D. W. Armstrong, S. Chen, C. Chang, and S. Chang, *J. Liq. Chromatogr*. **15**, 545 (1992).
82. A. M. Stalcup, J. R. Faulkner, Y. Tang, D. W. Armstrong, L. W. Levy, E. Regalado, *Biomed. Chromatogr*. **5**, 3 (1991).
83. P. Camilleri, C. A. Reid, and D. T. Manallack, *Chromatographia* **38**, 771 (1994).
84. A. M. Stalcup and K. H. Gahm, *Anal. Chem*. **68**, 1369 (1996).
85. A. M. Stalcup, S. Chang, D. W. Armstrong, and J. Pitha, *J. Chromatogr*. **513**, 181 (1990).
86. D. W. Armstrong, A. M. Stalcup, M. L. Hilton, J. D. Duncan, J. R. Faulkner, and S. C. Chang, *Anal. Chem*. **62**, 1610 (1990).
87. A. M. Stalcup, S. C. Chang, and D. W. Armstrong, *J. Chromatogr*. **540**, 113 (1991).
88. D. W. Armstrong, M. Hilton, and L. Coffin, *LC-GC* **9**, 647 (1992).
89. H. Hopf, W. Grahn, D. G. Barrett, A. Gerdes, J. Hilmer, J. Hucker, Y. Okamoto, and Y. Kaida, *Chem. Ber*. **123**, 841 (1990).
90. Y. Okamoto, Y. Kaida, R. Aburatani, and K. Hatada, *J. Chromatogr*. **477**, 367 (1989).
91. Y. Okamoto, K. Hatano, R. Aburatani, and K. Hatada, *Chem. Lett.*, 715 (1989).
92. J. M. Jansen, S. Copinga, G. Gruppen, R. Isaksson, D. T. Witte, and C. J. Grol, *Chirality* **6**, 596 (1994).
93. T. Shibata, I. Okamoto, and K. Ishii, *J. Liq. Chromatogr*. **9**, 313 (1986).
94. D. T. Witte, F. J. Bruggeman, J. P. Franke, S. Copinga, J. M. Jansen, and R. A. De Zeeuw, *Chirality* **5**, 545 (1993).
95. Y. Okamoto, T. Ohashi, Y. Kaida, and E. Yashima, *Chirality* **5**, 616 (1993).
96. J. Wagner, H.-J. Hamann, W. Döpke, A. Kunath, and E. Höft, *Chirality* **7**, 243 (1995).
97. M. Tanaka, H. Yamazaki, and H. Hakusui, *Chirality* **7**, 612 (1995).
98. J. Iredale, A.-F. Aubry, and I. W. Wainer, *Chromatographia* **31**, 329 (1991).
99. T. A. G. Noctor, I. W. Wainer, and D. S. Hage, *J. Chromatogr*. **577**, 305 (1995).
100. D. S. Hage, T. A. G. Noctor, and I. W. Wainer, *J. Chromatogr. A* **693**, 23 (1995).
101. G. Massolini, A.-F. Aubry, A. McGann, and I. Wainer, *Biochem. Pharm*. **46**, 1285 (1993).
102. E. Domenici, C. Bertucci, P. Salvadori, and I. W. Wainer, *J. Pharm. Sci*. **80**, 164 (1991).
103. D. W. Armstrong, Y. Tang, and S. Chen, *Anal. Chem*. **66**, 473 (1994).
104. D. W. Armstrong, Y. Liu, and K. H. Ekborgott, *Chirality* **7**, 474 (1995).
105. T. Shinbo, T. Yamaguchi, K. Nishimura, and M. Sugiura, *J. Chromatogr*. **405**, 145 (1987).
106. M. Hilton and D. W. Armstrong, *J. Liq. Chromatogr*. **14**, 9 (1991).
107. B. Sellergren, M. Lepistö, and K. Mosbach, *J. Am. Chem. Soc*. **110**, 5853 (1988).
108. L. Fischer, R. Müller, and B. Ekberg, *J. Am. Chem. Soc*. **113**, 9358 (1991).
109. Y. Okamoto, K. Suzuki, K. Ohta, K. Hatada, and H. Yuki, *J. Am. Chem. Soc*. **101**, 4763 (1979).

110. G. Blaschke, W. Bröker, and W. Fraenkel, *Angew. Chem*. **98**, 808 (1986).
111. S. G. Allenmark, S. Andersson, P. Möller, and D. Sanchez, *Chirality* **7**, 248 (1995).
112. Y. Liu, *Anal. Chem*. **69**, 61 (1997).
113. E. Gil-Av, B. Feibush, and R. Charles-Sigler, *Tetrahedron Lett*. **10**, 1009 (1966).
114. H. Frank, G. J. Nicholson, and E. Bayer, *J. Chromatogr. Sci*. **15**, 174 (1974).
115. W. A. König, I. Benecke, and S. Sievers, *J. Chromatogr*. **217**, 71 (1981).
116. W. A. König and E. Steinbach, and K Ernst, *J. Chromatogr*. **301**, 129 (1984).
117. B. Feibush, A. Balan, B. Altman, and Gil-Av, *J. Chem. Soc. Perkin II*, 1230 (1979).
118. V. Schurig, *Chromatographia* **13**, 263 (1980).
119. H. P. Nowotny, D. Schmalzing, D. Wistuba and V. Schurig, *J. High Resolut. Chromatogr. Chromatogr. Commun*. **12**, 383 (1989).
120. D. W. Armstrong, Y. Tang, T. Ward, and M. Nichols, *Anal. Chem*. **65**, 1114 (1993).
121. W.-Y. Li, H. L. Jin, and D. W. Armstrong, *J. Chromatogr*. **509**, 303 (1990).
122. A. Berthod, W. Li, and D. W. Armstrong, *Anal. Chem*. **64**, 873 (1992).
123. D. W. Armstrong, J. Zukowski, N. Ercal, and M. Gasper, *J. Pharm. Biomed. Anal*. **11**, 881 (1993).
124. J. Roboz, E. Nieves, and J. F. Roland, *J. Chromatogr*. **500**, 413 (1990).
125. I. W. Wainer, J. Ducharme, C. P. Granvil, H. Parenteau, and S. Abdullah, *J. Chromatogr. A* **694**, 169 (1995).

GENERAL REFERENCES

C. F. Poole and S. K. Poole, *Chromatography Today*, Elsevier Science Publishers B.V., Amsterdam, The Netherlands, 1991.
I. W. Wainer, *Drug Stereochemistry: Analytical Methods and Pharmacology*, Marcel Dekker, Inc., New York, 1993.
W. A. König, *The Practice of Enantiomer Separation by Capillary Gas Chromatography*, Hüthig Verlag, Heidelberg, Germany, 1987.
P. Schreier, A. Bernreuther, and M. Huffer, *Analysis of Chiral Organic Molecules*, Walter de Gruyter & Co., Berlin, Germany, 1995.
G. Subramanian, *A Practical Approach to Chiral Separations by Liquid Chromatography*, VCH, Weinheim, Germany, 1994.

APRYLL M. STALCUP
University of Cincinnati