Considerations of Chiral Recognition Relevant to the Liquid Chromatographic Separation of Enantiomers

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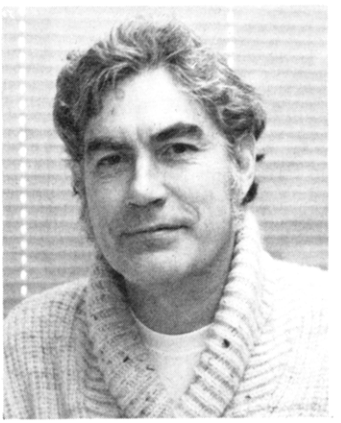
Contents

The dependence of the chemical or biological activity of a substance often depends upon its stereochemistry, a fact recognized for over a century. However, accurate descriptions of the origins of stereo- and enantioselectivity are rare. In this review, rationales purporting to account for liquid chromatographic observations of chiral recognition are considered. Chiral recognition is a subtle aspect of the broader subject of molecular recognition. Both imply the existence of a transient complex formed selectively in a mixture of several species. Chiral recognition, in chromatographic terms, means preferential interaction of one enantiomer of a substance with one enantiomer of a (usually) second substance. In this review, we limit the discussion to situations in which the selector substance has been immobilized on an inert support and the selectand enantiomers are chromatographed upon this chiral stationary phase (CSP).

Stereo- and enantioselective adsorption implies that the CSP "senses" the spatial relationship between structural elements of the analytes, this "sensing" requiring some form of interaction between an adequate number of structural elements of the CSP with those of the analyte. To specify the origin of enantioselective adsorption, one must specify the nature of the various interactions between the species involved.

Many tools are available for studying the structure and dynamics of multimolecular complexes, with NMR (nuclear magnetic resonance) being perhaps the most suited to detailed examination of such complexes in solution. However, it is not yet widely appreciated that liquid chromatography is extremely useful for the study of solution complexes even though, rigorously, liquid–solid chromatography takes place not in solution but rather at an interface. Like NMR, liquid–solid chromatography gives a weighted time-average view of dynamic events. NMR provides direct structural and dynamic information, while chromatography is sensitive



William H. Pirkle was born in Shreveport, LA, on May 2, 1934. He holds a B.S. from the University of California at Berkeley (1959) and a Ph.D. from the University of Rochester (1963). After a year as an NSF Postdoctoral Fellow at Harvard with E. J. Corey, he joined the faculty at the University of Illinois at Urbana–Champaign, where he is now a full Professor of Chemistry. His interests have long focused on chiral recognition as applicable to nuclear magnetic resonance spectroscopy and liquid chromatography. His research has led to the first chiral solvating agents for NMR determination of enantiomeric purity and absolute configuration and to the first commercial chiral stationary phases for HPLC separation of enantiomers. Present research interests include the design of chiral catalysts and enantioselective membranes.

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to changes in the free energies of association which indirectly reflect structure, dynamics, and solvation. Although this review is concerned with the basic aspects of chiral recognition as applied to the development of chiral stationary phases for direct liquid chromatographic separation of enantiomers, many of the considerations discussed here are applicable to achiral systems as well.

CSPs capable of preferentially retaining one of a pair of enantiomers offer a distinct advantage of the study of molecular recognition phenomena in that those transient interactions between the chiral phase and the enantiomeric analytes that do *not* contribute to enantioselectivity will "cancel"; that is, only those sets of interactions that are important for enantioselectivity will cause differential retention of the enantiomeric analytes. To an extent, this deconvolutes the myriad complex interactions between the enantiomers and the CSP, permitting one to focus on a narrower "set" of interactions. By using a homologous or analogous series of analytes and/or CSP's, one can observe the chromatographic consequence of changing a single structural feature of the system.

I. Theory of Enantioselective Complexation

In order to discuss enantioselective complexation, we should first establish what is meant by "interaction" between two molecules. Two molecules might be said to "interact" when they begin to perturb each other's electronic orbital energies (i.e., their orbitals begin to overlap appreciably). All intermolecular forces originate from such perturbations, and it would perhaps be more correct to discuss intermolecular interactions in such terms. However, the usual terms "hydrogen bonding", "dipole stacking", and "steric interactions" all describe specific types of perturbations and are sufficient for our purpose, since it is generally understood what these terms mean. A "set" of interactions then refers to multiple perturbations between two molecules.[1]

A further important distinction to be made when discussing intermolecular interactions is between *single-point* interactions and *multipoint* interactions. If one considers two convex surfaces in contact with one another, this contact is ideally at a single infinitesimal point. Thus, we describe overlap between two "convex" orbitals to be a *single-point* interaction. Hydrogen bonding and other end-to-end dipole–dipole interactions are single point in nature. However, some interactions between linear and/or planar functionality (i.e., dipole stacking and π–π interactions) cannot be described completely by single-point contact and are multipoint in nature. These types of interactions will be discussed in some detail.

Having described these basic ideas, we may now consider how chiral recognition occurs. As the enantiomers pass over the CSP, each forms a transient adsorbate. These adsorbates are diastereomeric and their formation is a necessary but insufficient condition for ultimate separation of the enantiomers. Diastereomeric complexes differ in symmetry and are members of different point groups.[2] If this difference in symmetry was all that was required for chiral recognition, any chiral species would suffice to differentiate between the enantiomers of any other substance as long as they were in proximity. This is obviously not always the case. The diastereomeric adsorbates must differ "adequately" in free energy for enantiomer separation to be observed. The practical problem is to control the degree to which the diastereomeric complexes differ in their free energies of formation.

Recent calculations, which treat the interactions between two chiral species in terms of "overlap-exchange" functions, have shown that energy differences between diastereomeric complexes **1** and **2** do not result solely from individual atom–atom or dipole–dipole interactions (that is, interaction between tetrahedron edges) but are the result of six-center forces occurring simultaneously between triplets of atoms or functionality in the two species.[3] This validates a longstanding precept of chiral recognition, known as the *three-point rule*. We restate this rule now (in a slightly modified form, for clarity): *Chiral recognition requires a minimum of three simultaneous interactions between the CSP and at least one of the enantiomers, with at least one of these interactions being stereochemically dependent.* That is, at least one of the interactions will be absent or significantly altered by replacing one enantiomer with the other *without conformational change in any component*. The requirement for a minimum of three simultaneous interactions can be justified by using simple tetrahedral structures, as shown in Figure 1. As the species approach one another, each enantiomer of the selectand is capable of two of the three potential interations shown. The selector distinguishes between the two selectand enantiomers by the presence or absence of a third interaction *which must not be collinear* with the first two. Thus, the three interaction sites of each omponent must define a plane that, at the instant of chiral recognition, is presented to the approaching partner. The points D and D′ lie outside the aforementioned plane as a consequence of the chirality of the tetrahedron.

Although conceptually simple, the three-point rule is often misinterpreted, being sometimes confused with the "three-point-binding" theory that Ogsten proposed to explain the enantiospecific nature of enzymatic reactions.[4] This variation on the "lock and key" proposal for enzyme–substrate interaction declared that a substrate must be bound to the enzyme at at least three points in order to ensure enantiospecificity in the enzyme-catalyzed reaction. Ogsten put forth this proposal well before the structure of any enzyme had been determined, and in light of present-day knowledge, the proposal is unrealistically simplistic. It does, however, take cognizance of the same geometric necessities as the three-point rule. The three-point rule differs from Ogsten's theory in that it does not require all three interactions to be attractive (i.e., "bonding"). In many cases, repulsive steric interactions are invoked, usually in combination with one or more bonding interactions, to explain chiral recognition.

A second source of confusion regarding interpretation of the three-point rule seems to arise from drawings such as Figure 1 that are used to illustrate the concept. In these drawings, interactions between selector and selectand are typically depicted as being between corners of two tetrahedra centered on the stereogenic centers of the two species. Apparently, it is sometimes assumed that the rule implies that interacting functionality must be similarly arranged with respect to the

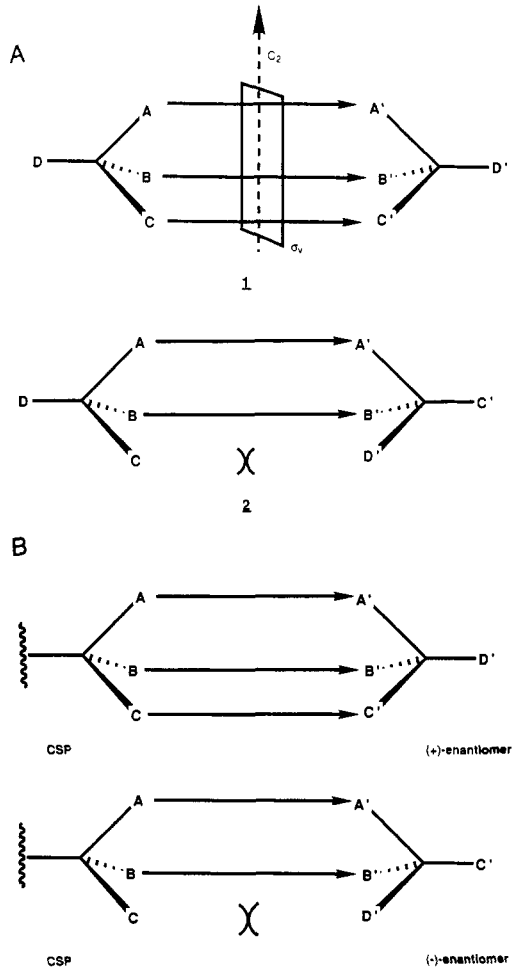


Figure 1. (A) Diastereomeric complexes **1** and **2** differ in symmetry. Complex **1** has plane σ_v and a C_2 axis of pseudosymmetry bisecting the vector between the two stereogenic centers. These elements are absent in complex **2**. If one of the species involved in complex formation is fixed to a stationary support, a CSP is generated as shown in B. The (+) enantiomer of the analyte is shown to be capable of three simultaneous interactions with the CSP (A–A′, B–B′, and C–C′), whereas the (–) enantiomer is capable of only two simultaneous interactions. If all three interactions are free-energy-lowering, the (–) enantiomer will be less retained by the CSP. Alternatively, one interaction might be steric, in which case the enantiomer that affords the free-energy-lowering interactions with the least degree of steric interaction will be most retained.

stereogenic centers of the selector and selectand. Consider the case in which dipoles lie along the vectors AB and A′B′ in Figure 1. "Stacking" of these dipoles is functionally equivalent to two point-to-point interactions and but a single additional interaction, C–C′, suffices to afford chiral recognition. Similar arguments can be adduced for π–π interactions between aromatic rings. The multipoint nature of π–π and dipole-stacking interactions is presumably responsible for the ubiquity of these interactions in chiral recognition rationales. It is not required that the involved species be conformationally locked "tetrahedra" although a degree of conformational preference is typically involved in instances of observed chiral recognition. We reiterate that implicit in the preceding concept is that the conformations of the CSP and the analyte enantiomers are the same in both diastereomeric adsorbates. Though this may not actually be the case, it is on this basis that chiral recognition at a given instant can be rationalized. The reader will be aware that chromatographically observed chiral recognition stems from a weighted time average of the contributions of all possible complexes. This can entail many directions of approach (i.e., relative orientations), different conformers, and different combinations of three (or more) simultaneous interactions, especially for polyfunctional CSPs or analytes. In cases where one diastereomeric adsorbate is significantly more stable than all others, one can rationalize the occurrence and sense of chiral recognition on the basis of preferential formation of this adsorbate.

We now turn to the pratical and theoretical considerations involved in chiral recognition on chiral stationary phases during the chromatographic separation of enantiomers.

II. Chiral Recognition on Chiral Stationary Phases (CSPs)

In order for enantiomers to be chromatographically separated on a CSP, two conditions must be fulfilled. Diastereomeric adsorbates[2] must be formed from the CSP and at least one of the analyte enantiomers and these must differ in their free energy of formation. These requirements will be considered in turn.

Diastereomeric adsorbates are formed as a result of one or more attractive interactions between the CSP and analyte enantiomers, by expulsion of the analyte enantiomers from a reverse mobile phase, or by passive diffusion of the analyte enantiomers into a chiral matrix. Even though no bonding interactions are invoked in the latter two instances, the analyte enantiomers are in a chiral environment. Here, chiral recognition, should it occur, would be entirely steric in origin. In such cases, it would be difficult to state which steric interactions are involved. Such situations are equivalent to a stationary phase containing chiral cavities. In such a situation, enantiomer separation may occur because one analyte enantiomer is better able to enter the cavities than is the other. As in size exclusion chromatography, the entering analyte is most retained. The three-point rule is still in effect during enantioselective "rejection" of the least retained enantiomer. Combinations of binding forces and chiral cavities are also possible and are presumably encountered in some polymeric and protein-derived CSPs.

How does an energetic difference between the implicitly formed diastereomeric adsorbates affect chromatographic behavior? Some chromatographic parameters, retention and selectivity for example, are thermodynamically controlled, whereas band shapes are influenced by the kinetics of mass transfer. Little is yet known of the relation between structure and absolute rates of adsorption–desorption, although the ratio of these rates defines the partitioning of an analyte between the stationary phase and mobile phase. For a given analyte, this partitioning is described by the capacity factor, k_1. Used to describe chromatographic separation of enantiomers, α_{DL} is the ratio of the capacity factors for the D and L enantiomers of the analyte on a given CSP with an achiral mobile phase at constant temperature.

$$\alpha_{DL} = k_D/k_L \qquad (1)$$

Because capacity factors are equilibrium constants, eq 1 may be rewritten

$$\alpha_{DL} = \exp(-\Delta G^{m}_{D}/RT)/\exp(-\Delta G^{m}_{L}/RT) \quad (2)$$

where the ΔG^{m}_{i} are the molar free energies of adsorption, or, expressed in a slightly different form

$$\alpha_{DL} = \exp(-\beta\Delta G_{D})/\exp(-\beta\Delta G_{L}) \quad (3)$$

where β is $(kT)^{-1}$ and ΔG_i are the molecular free energies of adsorption of the ith enantiomer. Rearrangement of eq 2 gives the relationship between α_{DL} and $\Delta(\Delta G)^{m}$, the difference in molar free energy of formation of the diastereomeric adsorbates

$$\Delta(\Delta G)^{m} = -RT \ln \alpha_{DL} \quad (4)$$

Boehm et al. have used statistical thermodynamic considerations to derive an expression analogous to eq 3:

$$\alpha_{DL} = \exp(-\beta\sum_{i}E_{iD})/\exp(-\beta\sum_{j}E_{jL}) \quad (5)$$

where E_i and E_j are the energies of the ith and jth mode of interaction, respectively, of the D and L enantiomers of the analyte with the CSP.[5] Comparing expressions 3 and 5 indicates, not unexpectedly, that all of the enantioselectivity observed is directly the result of CSP–analyte interactions, assuming that the same number of solvent molecules are displaced from the stationary phase upon adsorption of either enantiomer by the CSP. This assumption is experimentally validated, since the temperature dependence of α_{DL} for most liquid chromatographic enantiomer separations near ambient temperature indicates that the entropy contribution to $\Delta(\Delta G)$ is relatively unimportant. In fact, in one reported case where enantiomer separation is entropy-controlled, differing numbers of solvent molecules are involved in forming the diastereomeric adsorbates with the CSP.[6]

Owing to the nature of chromatographic processes, relatively small values of $\Delta(\Delta G)$ suffice to afford observable chromatographic separations. A value of 50 small calories affords a separation of 1.09, easily observable on a high-efficiency HPLC system. There is justifiable skepticism concerning the validity of any mechanism purporting to explain such small energy differences, despite a strong tendency among workers in the field to advance chiral recognition rationales, even when comparatively few data are available upon which to base such a rationale. However, such hypotheses do aid in designing experimental tests of their validity (mechanisms can be disproven but not proven) and frequently have considerable predictive power. In our own experience, these chiral recognition rationales have frequently suggested structural changes in CSPs that have led to enhanced enantioselectivity.

What boundary conditions must be enforced when formulating a chiral recognition model? These depend on the type of CSP–analyte pairing under consideration. Typically, chromatographic separation of enantiomers involves solution interactions between CSP and analyte for which the free energies are small with respect to kT. This implies that the molecules are relatively free to tumble with respect to each other and exert relatively little mutual conformational control. The degree of enantioselectivity will be determined principally by which diastereomeric adsorbate contains the complexed species in (approximately) their lowest energy conformations. This is an extremely important consideration. Chiral recognition models invoked to explain enantioselectivity between nonrigid molecules often involve sets of interactions with the CSP that can obtain for either enantiomer, the energetic difference between the diastereomeric adsorbates arising from the ability of one enantiomer to present the required interaction sites to the CSP from a lower energy conformation than does the other. Those conformations of the species involved that are most populated are expected to contribute most heavily to the overall enantioselectivity of weakly interacting systems. This statement is a generalization and is strictly valid *only* when the free energies of complexation are small with respect to kT and complexation does not provide sufficient energy to populate high-energy conformations of either the CSP or the analyte. It is essential to recognize that it is the *weighted time average* of all possible solution interactions that is important for determining retention and enantioselectivity. In some instances, multiple chiral recognition processes may make significant contributions to the overall chromatographic behavior. This obviously complicates the formulation of a chiral recognition model, and additional parameters may have to be invoked.

A conformationally rigid CSP that interacts strongly with the analytes might be expected to exert considerable conformational control over the latter during interaction. Protein-derived CSPs may, to a significant degree, fall into this category. In this case, those analyte conformers that are most populated in solution may or may not be those populated upon binding. As an aside, we point out that large adsorption energies, ΔG, lead not only to long retention times but also to broad chromatographic peaks. Since enantioselectivity is determined by $\Delta(\Delta G)$, the designer of a CSP must try to maximize $\Delta(\Delta G)$ while minimizing ΔG so as to maintain chromatographic efficiency. Columns of high efficiency are desirable, for they are better able to separate the components of complex mixtures than columns of low efficiency, all other things being equal.

As noted, chiral recognition takes place when the diastereomeric adsorbates formed from the CSP and the analyte differ in free energy of formation. The difference in free energy, $\Delta(\Delta G)$, needed for adequate chromatographic separation is influenced by the efficiency of the system employed. The chromatographic separation factor, α, is related to $\Delta(\Delta G)$ by eq 4. If the chromatographic system is of high efficiency, so that narrow peaks are afforded, relatively small $\Delta(\Delta G)$'s will suffice for the analytical-scale separation of enantiomers. A variety of useful CSPs have been devised more or less empirically and often show rather modest levels of enantioselectivity. Provided they afford adequate levels of chromatographic efficiency, such CSPs can be entirely adequate for analytical purposes. For analytical applications, it is only necessary that peaks be separated; high-level enantioselectivity is not essential and is even undesirable. However, high-level enantioselectivity can always be attenuated, should this be desired, by use of less optimal mobile phases, higher temperatures, or CSPs of reduced enantiomeric purity. High-level enantioselectivity is important for facile preparative separations of enantiomers. For this and other reasons, one often wishes to amplify $\Delta(\Delta G)$, thereby

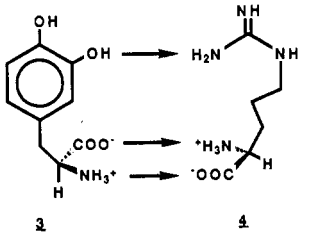


Figure 2. Baczuk's CSP, derived from *l*-arginine, **4**, was used to separate the enantiomers of DOPA (**3**). The CSP was designed based upon consideration of the interactions needed for three-point binding of DOPA, as shown.

enhancing the enantioselectivity of the CSP. This is something that can best be done through an understanding of chiral recognition mechanisms.

III. Historical Background of CSP Research

The potential for separation of enantiomers through the use of a chiral nonracemic adsorbant has long been understood, with a number of attempts at enantioselective adsorption being reported in the 1920s, including the observation of induced optical rotation in racemic dye solutions used to dye wool.[7,8] These early attempts were invariably made by using natural chiral polymeric adsorbants such as wool and cellulose or other polysaccharides. Senoh and co-workers[9] reported the separation of aromatic amino acids by paper chromatography in 1951, work that Dalgleish later extended, justifying the observed results with a variation of the three-point attachment model.[10,11] He noted that derivatization of the amino or carboxyl functionality or replacing the aromatic side chain with an aliphatic group resulted in the loss of separation, and it was concluded that three simultaneous binding interactions are necessary for enantioselective adsorption to occur.

Work by Baczuk provides the first instance in which three-point interaction was applied to the design of a CSP for a specific application.[12] Using space-filling models, Baczuk reasoned that *l*-arginine showed the proper arrangement of ionic sites for three-point interaction with complementary sites on *l*-DOPA (*l*-dihydroxyphenylalanine) (**3**) (see Figure 2). Indeed, when *l*-arginine was covalently bound to Sephadex resin, CSP **4** was generated, which could separate DOPA into its antipodes. Surprisingly, however, it was the *d* enantiomer of DOPA that was found to be most retained on the *l*-CSP, indicating that the mechanism of action was not that originally proposed.[13]

The pioneering efforts noted here reflect two philosophies of CSP preparation. The use of a natural chiral polymer as an enantioselective adsorbant has been and continues to be a widely used approach. Such adsorbants provide the advantage of low cost and wide availability and usually do not require too much investment of time to prepare. However, biopolymeric adsorbants sometimes tend to have poor mechanical and chromatographic properties. An alternate approach entails the use of designed synthetic CSPs prepared for a specific type of separation. This approach typically provides stationary phases with good mechanical and chromatographic properties. Moreover, the mechanisms by which such CSPs separate enantiomers are more readily discerned. Often, such CSPs require some form of analyte derivatization to achieve selectivity for a wide range of client racemates.

IV. Chiral Polymers as CSPs

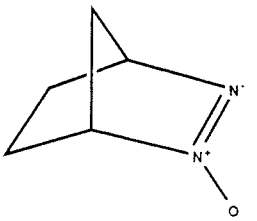
As noted above, early attempts at chromatographic separation of enantiomers were almost exclusively focused on the use of natural chiral adsorbants such as cellulose or Sephadex. Drawbacks to such materials are numerous. These materials have poor mechanical properties and their high polarity and porous structure give rise to unfavorable kinetic behavior. Attempts to improve the chromatographic and enantioselective properties of cellulose have concentrated on derivatization of the cellulose hydroxyl groups to decrease polarity of the material and to provide additional steric bulk for interaction between CSP and analyte. In 1976, Hesse and Hagel introduced cellulose triacetate (CTA-I), which they prepared by acetylation of microcrystalline cellulose under heterogeneous conditions.[14,15] Under these conditions, some of the original structure of the polymer is preserved, this being important for enantioselectivity. Dissolution and reprecipitation of CTA-I lead to poorer enantioselectivity and reversal of elution order in some cases.[16] Electron microscopy of CTA-I indicates that although acylation of microcrystalline cellulose alters the crystal structure, a good deal of order remains in CTA-I.[17] Upon annealing the material, however, there is loss of enantioselective adsorption, indicating that it is the imperfections, the "holes" in the crystal lattice of CTA-I, that provide sites for enantioselective adsorption. In fact, all evidence seems to point to an inclusion mechanism for enantioselectivity on CTA-I by shape-selective adsorption into chiral cavities in the polymer network. It is the size and shape of the molecule, rather than specific functionality, that are important for determining the separability of a given species.

Recently, a promising new material has been developed by Ichida et al.[18] This adsorbant, CTA-II, is also prepared by the peracylation of cellulose, but the peracylated cellulose is then solubilized and reprecipitated onto diphenyl-silanized macroporous silica gel, providing a fairly durable and noncompressible chromatographic medium with improved chromatographic behavior. Wainer and Alembik have studied the separation of a number of analytes on CTA-II and conclude that the mechanism of retention in this case is attractive rather than inclusive, with dipole-stacking interactions proposed to account for observed behavior.[19]

Other recent innovations in cellulose-derived CSPs by Okamoto's group include the preparation of carbamate CSPs by treatment of cellulose and other polysaccharides with aryl isocyanates. As with CTA-II, these materials may be coated onto silica, and there are some indications that they also operate by attractive interactions between analyte and CSP.[20] A novel variation on this theme by Okamoto involves preparation of the *tristrans*-4-(phenylazo)phenyl carbamate of cellulose.[21] This CSP provides base-line separations of the enantiomers of *trans*-1,2-diphenyloxirane and Troger's base. Upon irradiation of the stationary phase with UV light, however, the (phenylazo)phenyl substituent isomerizes to the cis form, and the CSP now shows little enantioselectivity. The isomerization is reversible, as heating will regenerate the trans form. Thus the intriguing possibility of "switchable

selectivity" is presented. Although the practicality of the present example might be limited, such switchable behavior might be very desirable in systems that show a very high degree of selectivity for a given selectand, selectivity which might be desirable to attenuate under certain circumstances.

Although the range of analytes separable on CTA-I and CTA-II is quite broad, it is difficult to determine exactly what structural features are required for separation. If CTA-II does indeed act by attractive interaction as suggested by Wainer, then some polar functionality would be essential. Requirements for separability on CTA-I, however, are not at all clear-cut. Polarity is not required, as the enantiomers of many chiral hydrocarbons have been separated on CTA-I. Chirality may be either axial or atom-centered, and aromatic functionality is desirable, but not essential (witnessed by the separability of the enantiomers of diazoxyalkane **5** on CTA-I). It is reasonable to assume



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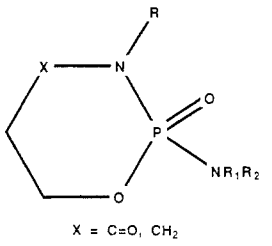
that there is some critical exclusion size beyond which neither enantiomer of the analyte will be adsorbed. Presumably, at least one enantiomer of the analyte should be of the proper shape for entry, thus ensuring that there will be interaction between that enantiomer and the walls of the chiral cavity. If a number of derivatives of a compound are available (i.e., several different esters of the same chiral acid), it is probable that one will show greater chiral recognition than the others. Thus, screening would seem to be necessary to establish which derivative is most suitable for separation of a given pair of enantiomers.

The success of CTA-I and CTA-II has inspired researchers to investigate the enantioselective adsorption properties of a number of synthetic chiral polymers. Blaschke has recently reported the resolution of chiral oxazaphosphorines of type **6** on a CSP prepared by



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polymerizing (*S*)-*N*-(ethoxycarbonyl)-2-phenylethylacrylamide.[22] Separations reminiscent of those observed with CTA-I have been observed on chiral polyamide phases.[23,24]

Japanese workers have recently found that an effective CSP may be generated by coating silica with a copolymer of enantiomerically pure *trans*-1,2-diphenylethylenediamine and one of a number of diacids. The investigators report the separation of the enantiomers of a number of α-hydroxy amides, esters, and ketones as well as Troger's base and β-binaphthol on such polyamide CSPs.[25]

A useful polymeric CSP that is now commercially available is that prepared by the asymmetric polymerization of triphenylmethyl methacrylate (TPMM). With chiral anions as initiators (lithium (*R*)-phenylethylanilide or (−)-sparteine–butyllithium), a TPMM polymer is afforded that exhibits a helical chirality generated by bevel-gear interactions between the triphenylmethyl groups.[26,27] The TPMM CSP is capable of separating the enantiomers of compounds that are themselves helically chiral (i.e., helicenes) or that have an aromatic substituent at the stereogenic center. Typically, the helicene enantiomer most retained is that which has the same helicity as the CSP, indicating that an intercalative mechanism is operative to some degree. A comparison of the relative merits of the TPMM CSP and other commercially available CSPs with respect to the separation of rotenoid enantiomers has been published.[28]

In some industrial processes, it might be desirable to have a CSP that is "customized" for a particular racemate. Work by Wulff et al. on the design of imprinted polymers provides some insight into how this might be done.[29,30] These workers have prepared a copolymer derived from methyl methacrylate, ethylene dimethacrylate, and the 4-styrenyl borate ester of 4-nitrophenyl α-*d*-mannopyranoside as a template. Hydrolysis of the borate ester and subsequent desorption of the mannopyranoside yields a polymer containing chiral cavities. This polymer is selective for the *d* enantiomer of the template and is essentially a CSP specific for one pair of enantiomers. Optimization of such systems has yielded highly specific adsorptive (and catalytic) surfaces in some cases, and it is not unreasonable to expect that very high selectivity (but narrow scope) might be observed with such designed CSPs.

V. Protein-Derived CSPs

Another type of CSP that is of widespread applicability owing to its ease of preparation and commercial availability is that derived from serum transport proteins such as bovine serum albumin (BSA).[31] BSA, isolated from bovine blood, functions as a transport vehicle for compounds not highly soluble in aqueous media but containing ionizable functionality, such as fatty acids. Because BSA must be relatively nonselective in its binding ability, it has a number of different types of binding sites for ionic compounds, and upon being irreversibly bound to a surface such as silica or agarose, it provides a CSP that is capable of separating the enantiomers of a variety of chiral amines and carboxylic acids. In some cases, very high enantioselectivity is observed.[32] However, drawbacks to bonded-protein CSPs are numerous. Low sample capacities are typical, indicating a limited availability of binding sites. In some cases, overloading results in elution order reversal, a result of the presence of more than one type of binding site.[33] Finally, the protein itself is not particularly durable, being subject to degradation over time and under extremes of temperature and pH.[34] Finally, rational prediction of elution order is not simple, since a variety of binding sites may be involved in the retention of a given analyte, and not all may exhibit the same sense of enantioselectivity. Despite these draw-

backs, the availability of chiral precursors and the undeniable effectiveness of these CSPs for a variety of analytes under near-physiological conditions dictate that these phases will be used quite heavily for analytical purposes.

A number of other protein-based CSPs have been prepared and studied, including ones derived from α-acid glycoprotein[35-38] and ovomucoid.[39] The former CSP has been studied more extensively than the BSA-derived CSP and may be of broader scope. A cost and efficiency analysis of protein CSPs with respect to commercially available donor–acceptor CSPs has recently been published.[40]

VI. Ligand-Exchange Chromatography

Ligand-exchange chromatography (LEC) was developed by Davankov and Rogozhin in 1971.[41] As its name implies, LEC involves the reversible formation of complexes between metal ions and chiral complexing agents, typically (but not exclusively) α-amino acids. The mechanism of LEC separations has been thoroughly studied. Many reviews on this topic are available,[3,13] and the authors of the present review will only attempt to highlight the important features of the technique and discuss recent developments.

Models developed to describe enantioselectivity in LEC invoke the presence of multicomponent complexes containing a central metal ion (usually Cu^{2+} or Ni^{2+}) complexed by two chelating chiral bifunctional molecules. One or more solvent molecules complete the first solvation sphere of the metal ion. If the chelators are α-amino acids, the amino and carboxylate groups of the two chelators are arranged equatorially around the metal ion in alternating fashion. If one of the chiral chelators is bound to a support, the CSP can form diastereomeric adsorbates with bidentate analyte enantiomers. The relative stabilities of the adsorbates (the homochiral and heterochiral complexes), if different, lead to chromatographic separation of the analyte enantiomers. If the stationary phase is achiral, but an enantiomerically enriched chelator is present in the mobile phase (chiral mobile phase additive), enantiomer separation may still be obtained by differential partitioning of the diastereomeric complexes between the stationary phase (usually a reverse-phase support) and the mobile phase.[42]

It has been observed that the relative stability of the diastereomeric adsorbates is controlled by several factors, the most critical of which is the choice of bound chiral chelator. Proline and hydroxyproline have been most often used, since their steric rigidity provides a high degree of enantioselectivity. Also of great importance is the choice of underlying support; on polar supports the homochiral complex is generally more retained, while on nonpolar supports, the heterochiral complex is more retained.

Proposed chiral recognition models for LEC are shown in Figure 3. On polar CSPs such as **7**, an axial ligand is provided by the stationary phase for the metal ion. Since the formation of the heterochiral adsorbate interferes with this ligation, this adsorbate is less stable than the homochiral analogue, as shown.[43,44] Polymer-supported CSP **7** was specifically designed to provide axial ligation of the metal ion. Conversely, the relatively high hydrophobicity of the heterochiral adsorbate near to the stationary support provides incentive for its formation when the support is nonpolar (CSP 8). When the analyte is tridentate, as is the case with ornithine, threonine, and aspartic acid, exceptional behavior is noted, for the enantiomer incorporated into the homochiral adsorbate is now the most retained on CSP 8.[45,46] Steric interactions, the presence or absence of axial ligands, and the nature of the second solvation sphere all affect the degree of free energy difference between the homo- and heterochiral adsorbates.[6] Because of the number of species involved in forming these complexes, entropy effects are considerably more important in LEC than in other CSP–analyte interactions. Figure 4 illustrates the entropic control of the separation of *N*-benzylproline enantiomers on CSP 8. This unusual case, noted by Davankov, is reflected by a slight increase in the separability of the enantiomers of *N*-benzylproline upon an increase in temperature.[47] The effect is explained by observing that steric hindrance prevents the complexation of an axial solvent molecule in the heterochiral adsorbate, but not in the homochiral

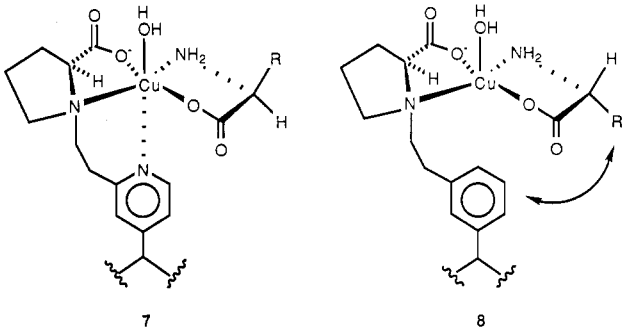


Figure 3. Chiral recognition during LEC. CSP **7**, incorporating a pyridyl ring to provide an axial N ligand for Cu^{2+}, selectively retains the amino acid enantiomer that has the same absolute configuration as the CSP (the *S,S* diastereomeric adsorbate is shown). CSP **8**, prepared with a nonpolar polymer support matrix, selectively retains the analyte enantiomer with the configuration opposite that of the CSP if R is nonpolar. Favorable hydrophobic interactions between the R group of the analyte and the nonpolar polymer support are thought to increase the stability of the heterochiral diastereomeric adsorbate with respect to the homochiral pairing.

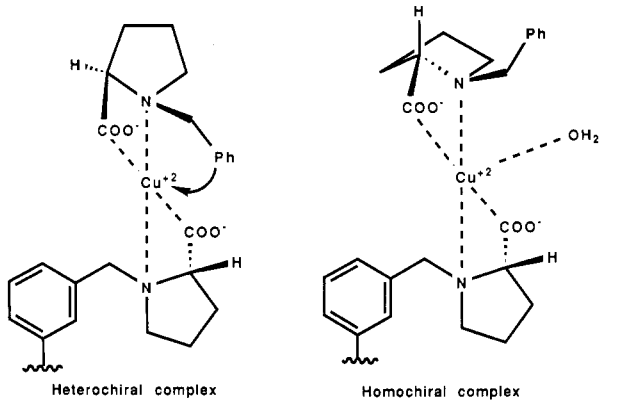


Figure 4. Entropy control of a ligand-exchange enantiomer separation. The heterochiral adsorbate of *N*-benzylproline with CSP 8 contains only three species, with axial ligation of the Cu^{2+} by H_2O prevented by the steric bulk of the analyte benzyl group. The homochiral adsorbate contains four species with the axial ligand present. The homochiral complex is thus enthalpically favored, while the heterochiral complex is entropically favored. Increasing chromatographic separability of the enantiomers with increasing temperature indicates that the $T\Delta S$ term is dominant under the conditions of LEC for this system.

adsorbate. This entropically favors the heterochiral adsorbate, and, in this case, $\Delta(\Delta S)$ is the controlling factor in the separation, causing the homochiral enantiomer to elute first.

When a chiral mobile phase additive is present, the situation is more complex than when the chiral selector is bound (as a CSP). However, recent work by Broge indicates that equilibrium models that accurately describe LEC separation of the enantiomers of valine using Aspartame (*l*-aspartyl-1-phenylalanine methyl ester) as a chiral mobile phase additive can be derived by using identical K_{eq} for the formation of both solution diastereomeric complexes.[48] This seems to indicate that the relative stability of the solution complexes does not generate the observed enantioselectivity. Rather, it is the difference in partition coefficients of the two diastereomeric complexes between the mobile and statioinary phases that is responsible for enantiomer separation. In this sense, the method is equivalent to the chromatographic separation of covalent diastereomers.

Because LEC requires no separate derivatization step and uses an aqueous mobile phase, it is usually the method of choice for analysis of underivatized α-amino acids. Detection of underivatized amino acids may be difficult at low concentrations; hence, these analytes have been prederivatized with a dansyl group or other fluorescent marker to facilitate detection.[49,50] Alternatively, the separated enantiomers may be allowed to react with a postcolumn derivatizing agent such as phthalaldehyde to facilitate detection.[51] Picomolar quantities of amino acid may be detected by such methods. This approach is compatible with biological samples because polar solvents are used and allows one to use nondedicated reverse-phase columns. One simply adds the appropriate chiral mobile phase additive(s) to obtain separations. Since LEC is designed for bidentate analytes, its scope is restricted relative to other types of CSPs and not all bidentate analytes are so resolvable. While the enantiomers of α-amino acids are generally separated by LEC, only poor to modest separations have been obtained for β-amino acids.[52] Weinstein and Grinberg have separated the enantiomers of α-methyl-α-amino acids by LEC using a chiral mobile phase additive,[53] and Feibush and co-workers have separated the enantiomers of catecholamines, amino alcohols, and β-hydroxy-α-phenylethylamines, all as their salicylaldehyde Schiff bases, using a proline CSP.[54] The enantiomers of several aliphatic and aromatic α-hydroxy carboxylic acids have been resolved on a hydroxyproline CSP,[55,56] and catecholamines have also been resolved by LEC on a tartaric acid CSP.[57]

Although LEC is unwieldy for preparative separations, it has been used for this purpose.[58] Difficulties may be encountered in removing and recycling polar mobile phases, in recovering a chiral mobile phase additive (if used), and in removal of trace metal ions. In some cases, metal ion contamination of the eluted enantiomers has been avoided by precharging the CSP with the desired ion prior to separation[59] or, alternatively, by passing the eluent through a scavenger column to remove traces of metal ion that may elute.[60] Other problems with LEC are reflected in the generally poor band shapes and low chromatographic efficiencies. Owing to unfavorable desorption kinetics, poor band shapes and long retention times are often seen in LEC.

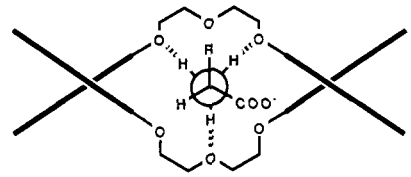


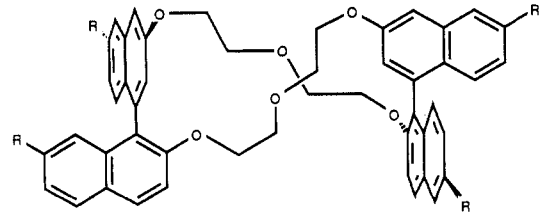
Figure 5. Energetically favored diastereomeric complex between a chiral crown ether and an amino acid zwitterion. Space-filling models of the two diastereomeric complexes predicted this to be the more stable diastereomeric complex based primarily on steric interactions between the R and carboxylate groups of the amino acid zwitterion and the naphthyl rings of the crown ether (see ref 63–70). Amino acids with the largest R groups show the highest degree of chiral recognition with the chiral crown ether.

Attempts to improve the situation have included the addition of monodentate ligands to the mobile phase to increase exchange rates[61] and operation at elevated temperatures so as to increase desorption rates.[62]

VII. Chiral Recognition via Host–Guest Complexation

Much ground-breaking work in the field of molecular recognition in general and chiral recognition in particular has been done with host–guest complexes. The efforts of Cram's research group at UCLA have been particularly fruitful in this regard, with a series of papers published in the seventies bringing into focus many of the problems that must be solved in order to achieve enantioselective complexation.

Initial publications dealt with enantioselective solution complexation of chiral amines by chiral crown ethers of the general type **9**.[63,64] Chromatographic



9a R = H
9b R = $Si(CH_3)_2OCH_3$

separation of enantiomers, first using a chiral crown ether as a mobile phase additive with an achiral support[65] and later as a covalently bound CSP,[66] was reported for a series of chiral amines and amino esters. Several papers published in 1978 provide structural analysis of these complexes, emphasizing the effect of structural changes in both host and guest upon the sense and extent of chiral recognition. Detailed studies were performed on a series of hosts and guests using NMR, chromatographic, and X-ray crystallographic techniques.[67] Based on chromatographic separability, it was concluded that those guests that most completely fill the chiral cavity exhibit the highest degree of chiral recognition for the host. Base-line separations were observed for the enantiomers of the methyl esters of *p*-hydroxyphenylglycine, tyrosine, phenylalanine, and tryptophan on a CSP derived from **9b**. Also significant was the fact that the observed sense of recognition could be rationalized by using the chiral recognition models from which the host was designed (Figure 5). Only the tryptophan separation did not follow the expected course, and this could be rationalized by the absence of π-stacking interactions between the naphthyl groups

of the CSP and the indole ring of the analyte. It was also noted that the mobile phase, analyte counterion (typically PF_6^-), or temperature only affects the extent, but not the sense, of selectivity.[68] Rather, the sense of enantioselectivity was exclusively determined by the structural interactions of host and guest. It was therefore concluded that chiral recognition models may be used to design chiral selectors based on structural considerations and are valid tools for understanding and predicting solution interactions of chiral molecules.

Other work by Cram's group concerns the optimization of structure of the host for best enantioselectivity of amine and amino ester analytes.[69] Changes in the number of ethylene ether bridges between the binaphthyl units as well as substitution of the naphthyl rings were made in order to determine their effect on enantioselectivity.

Finally, ^1H NMR was used to examine the nature of enantioselective host–guest complexation.[70] Solutions of (*S,S*)-**9a** and the enantiomers of α-phenylethylamine (PEA) hydrobromide were used as references, since this salt forms tight ion pairs in chloroform and does not complex with the crown ether. Spectral comparisons of (*S,S*)-**9a**–(*R*)-PEA (PF_6^-) and (*S,S*)-**9a**–(*S*)-PEA (PF_6^-) host–guest complexes in general justified the proposed chiral recognition models. Similarly, analysis of the (*S,S*)-**9a**–*d*-phenylglycine methyl ester and (*S,S*)-**9a**–*l*-phenylglycine methyl ester complexes did not reveal any inconsistencies with the model shown in Figure 5. Analysis of spectral shifts was primarily made in terms of expected shielding of guest substituent protons due to the naphthyl ring currents and deshielding due to hydrogen bonding. Comparison of data obtained by ^1H NMR of the solution complex (*S,S*)-**9a**–*d*-phenylglycine with that obtained from X-ray analysis of the crystalline complex indicated that the crystal and solution structures of the complex are similar.

Another group of chiral cavity containing compounds that have attracted a good deal of attention are the naturally occurring cyclodextrins (CD). Because of the availability of these natural hosts and the relative ease with which functionality may be appended to the hydroxyl groups that rim the larger opening of the cavity, these molecules have inspired a multitude of studies on host–guest phenomena. Since the CD cavity is chiral, it is not unreasonable to expect enantioselectivity to be observed upon complexation of chiral substrates. A number of analytes that can be enantioselectively retained on CD CSPs typically contain aromatic functionality appended to the stereogenic center of the analyte, as well as polar or hydrogen-bonding functionality. The aromatic moiety is thought to be the portion that is included by the hydrophobic CD cavity while the polar group interacts with the hydroxyls that surround the opening. Using this rationale, Armstrong and coworkers have resolved dansyl α-amino acid derivatives[71] and chiral derivatives of ferrocene and other metallocenes.[72]

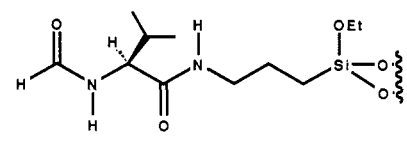
Typically, the kinetics of host–guest complexation are relatively slow on a chromatographic time scale, resulting in poor band shapes. Furthermore, unless simple large-scale synthesis and resolution of chiral crown ethers become possible, general usage of CSPs such as **9b** will be precluded. CD CSPs, on the other hand, are relatively inexpensive to prepare and are seeing extensive usage owing to their commercial availability.

VIII. Donor–Acceptor CSPs

CSPs that act by attractive interactions between nonionic functionality are termed donor–acceptor CSPs (DA CSPs). By optimization of the rearrangement of hydrogen-bonding, π donor–acceptor, dipole-stacking, and steric interactions between CSP and analyte, a number of highly selective CSPs suitable for a broad range of analytes and showing high chromatographic efficiencies have been prepared. Chromatographic efficiencies are reasonably high because these CSPs are generally prepared by covalently linking a monolayer of chiral precursor to a support (typically silica) having good mechanical properties. Free energies of interaction between CSP and analyte are typically small with respect to kT, so mass transfer is efficient and band shapes typical of high-resolution HPLC columns can be obtained. The choice of chiral moiety is limited only by imagination, and so variation and optimization are possible. Finally, the interactions between CSP and analyte are amenable to rationalization using chiral recognition models, making it possible in many cases to predict which analytes will resolve on a given CSP and to relate elution order of the enantiomers to their absolute configurations. Some tradeoffs are necessary, of course. Typically, unfunctionalized molecules will show little or no separability on most DA CSPs, and quite often derivatization is required for separation. Nevertheless, DA CSPs are generally the most practical wide-spectrum CSPs available for general liquid chromatographic use.

Recent years have seen a flurry of activity in DA CSP design, and there is no indication that the advances have slowed. This review will attempt to classify the various types of DA CSPs by mode of action and analyte types resolved and provide some insight into the common denominators that control chiral recognition in this rapidly growing class of CSPs.

Some of the simpler DA CSPs are related to CSPs developed for use in GC enantiomer separations. These have been prepared by covalent attachment of *N*-acyl-amino acids to an γ-aminopropyl-silanized silica gel support via an amide linkage and are capable of separating the enantiomers of *N*-acyl-α-amino amides and esters.[73,74] CSP **10**, derived from *N*-formyl-L-valine, shows enantioselectivity for a number of client analytes. Similar CSPs prepared from ureas of L-valine have also been described.[75]



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Hara proposed a working chiral recognition model for these CSPs based on a two-point hydrogen-bonding model, shown in Figure 6.[76] Pirkle deemed these interactions insufficient for chiral recognition, since either enantiomer should be able to afford both hydrogen bonds simultaneously with the CSP. He instead proposed a π-dipole stacking of the amides of CSP **10** and analyte (Figure 6) which, because it is a face-to-face

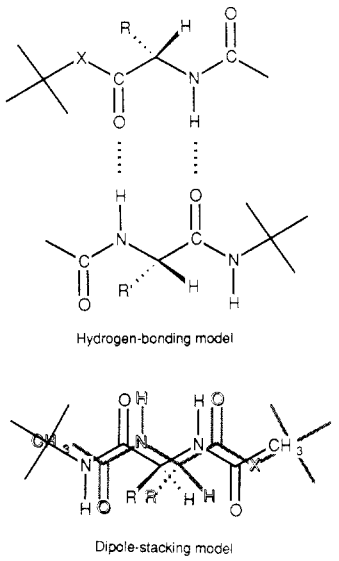
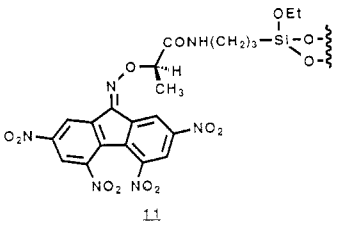


Figure 6. Hydrogen bonding versus dipole stacking for chiral recognition of *N*-acyl-α-amino amides on CSP **10**. Hara's hydrogen-bonding model provides two-point interaction between CSP and analyte. Although this may be the dominant form of interaction in the adsorbate, these interactions alone are incapable of giving rise to chiral recognition. Interactions between the R or H on one stereogenic center with R′ or H on the second seem unlikely owing to the distance involved. A face-to-face approach promoted by stacking of amide (or ester) π-dipoles possibly explains the observed chiral recognition, since it would place the aforementioned substituents in proximity and could lead to the requisite stereochemically dependent interaction.

interaction, is multipoint in nature and allows interaction between the substituents on the stereogenic centers.[77] Recently, Hara has invoked additional interactions between the analyte and silica support (interactions that would be stereochemically dependent) to further support the hydrogen-bonding model.[78]

The controversy over hydrogen bonding versus dipole stacking is central to the understanding of how DA CSPs operate. In almost every case, successful DA CSPs contain some aromatic or extended π-functionality, and this is not accidental. Because of the sterically demanding nature of π–π interactions (face-to-face or face-to-edge), they are often the controlling factor in enantiomer separations on DA CSPs. π–π interactions are inherently multipoint, as indicated by Figure 7, in which a hypothetical diastereomeric complex shows the necessary three-point contact utilizing only a π–π interaction and a single additional interaction.

The first DA CSP, **11**, prepared by Mikes et al., relied almost completely on π-donor–acceptor interactions to separate helicene enantiomers.[79,80] Nearly all DA CSPs



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reported since incorporate π-functionality and rely on π–π interactions for efficacy. However, a number of recent reports do describe DA CSPs that do not rely on π–π interactions for their enantioselective behavior. Sinibaldi et al. discuss CSP **12**, derived from enantiomerically pure *trans*-1,2-diaminocyclohexane, which

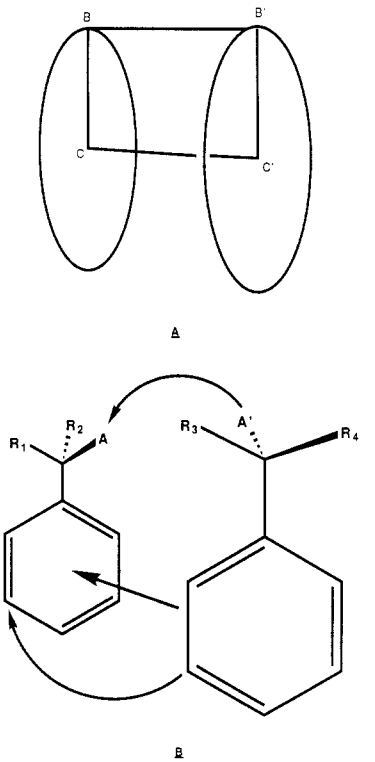

Figure 7. Three-point interactions involving only π–π interactions and one additional interaction. Diagram A shows that the relative orientations of two planar circular objects are completely defined by two vectors, one between the centers and one between points on the circumferences. Thus, π–π interactions can in principle provide two of the three interactions between two species required for chiral recognition. Diagram B illustrates this situation, with only one stereochemically dependent interaction needing to be invoked in addition to the π–π interaction to account for chiral recognition.

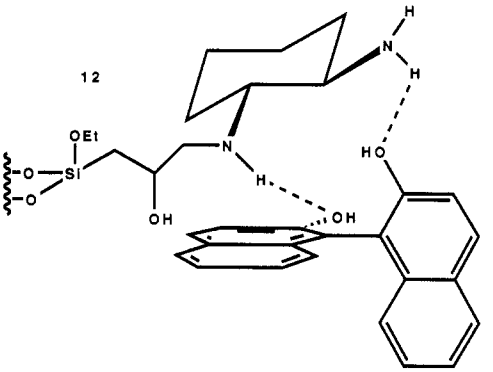

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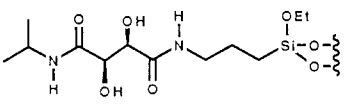
Figure 8. Chiral recognition of β-binaphthol on Sinibaldi's CSP **12**. CSP **12** utilizes two stereochemically constrained hydroxyl groups to provide hydrogen-bonding sites for bidentate analytes.

is capable of separating the enantiomers of 1,2-cyclohexanediol and bi-β-naphthol as shown in Figure 8.[81] Hara describes CSP **13**, derived from tartaric acid,



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which, although it contains amide functionality, seems unlikely to be able to utilize π-stacking very effectively due to the sterically hindering nature of the isopropyl

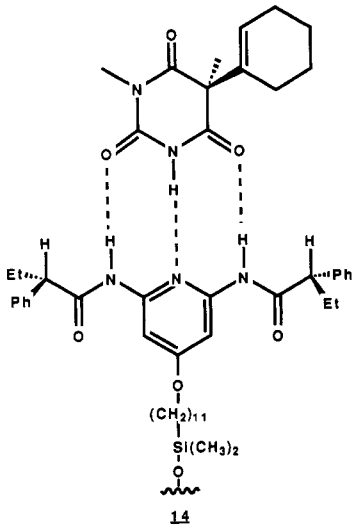

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Figure 9. Three copolanar hydrogen bonds mimicking DNA base pairing provide interaction between an analyte and CSP **14**. Because the bonds are coplanar and may be obtained for either enantiomer of the analyte, they are insufficient to provide chiral recognition in themselves, and the observed enantioselective adsorption provided by CSP **14** must result from incidental steric interactions between the CSP butyramide groups and substituents of the analyte stereogenic center.

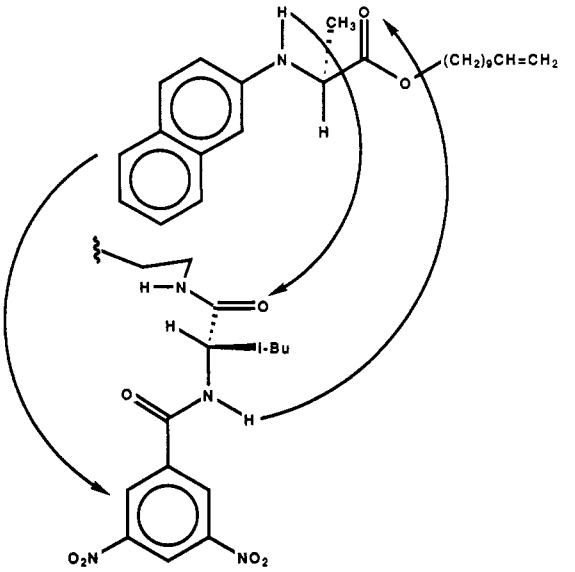
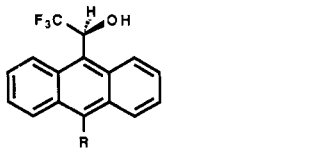


Figure 10. Chiral recognition between CSP **18** and *N*-(2-naphthyl)alanine undecenyl ester **19a**. Multipoint interaction is obtained by π-donor–acceptor overlap between the naphthyl ring of **19a** and the dinitrobenzoyl ring of **18**, a hydrogen bond between the dinitrobenzamide N*H* of **18** and the carbonyl oxygen of **19a**, and a second hydrogen bond between the amino N*H* of **19a** and the C-terminal carboxamide oxygen of **18**.

substituent on the amide nitrogen. This CSP is capable of resolving a variety of analytes that contain dual hydrogen-bonding sites such as barbiturates, succinimides, bi-β-naphthol, diols, α-hydroxy esters, etc.[82] In both of these instances, it might be expected that the two hydroxyl groups are stereochemically restrained with respect to each other in the CSP, a similar situation occurring for the two complementary interaction sites in the analyte. Hence, the dual hydrogen-bonding interactions are able to provide stereochemically dependent multipoint interactions, something a single hydrogen bond cannot do. Recent work by Feibush et al. concerns CSP **14**, which mimics the hydrogen-bonding interactions responsible for DNA base pairing to obtain CSP–analyte interaction (Figure 9).[83] The separation of barbiturates, hydantoins, glutaramides, and succinimides into their enantiomers is described. It should be pointed out that despite the three-point hydrogen-bonding nature of Watson and Crick and Hoogstein base pairing, the three points of interaction are collinear and are insufficient in themselves to account for chiral recognition. Since extensive NMR and X-ray structure data confirm the nature of the diastereomeric complexes as shown in Figure 9, it must be concluded that chiral recognition arises from incidental steric interactions between the substituents of the stereogenic centers of the α-phenylbutyramide groups of the CSP and the substituents on the stereogenic centers of the analytes.

The above examples are exceptions rather than the rule, and most DA CSPs do rely on π–π interactions for efficacy. We now return to a discussion of these CSPs.

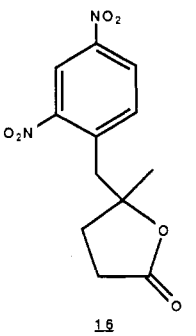
Early experiments by Pirkle and Sikkenga demonstrated that effective π–π overlap between the enantiomerically pure chiral solvating agent **15a** and one enantiomer of chiral lactone **16** increases the stability of the complex with respect to its diastereomer.[84,85]



15a, R = H

15b, R = $CH_2S(CH_2)_3Si(OEt)O_2Si$...

15c, R = $OCH_2CH_2S(CH_2)_3Si(OEt)O_2Si$...



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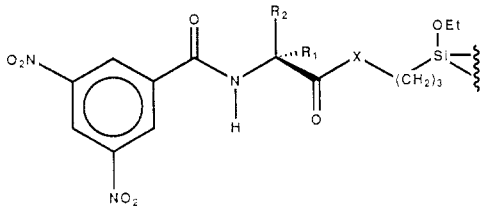
This was shown by competitive binding with achiral lanthanide shift reagents and by column liquid chromatographic separation of the enantiomers of **16** on silica using **15a** as a chiral mobile phase additive. Because of the success of these initial studies, CSP **15b** was prepared and found to separate the enantiomers of a variety of π-acceptor-substituted species.[86] Improvement of enantioselectivity was obtained upon increasing the π-donor character of the anthryl ring, as in CSP **15c**.[87]

A number of analytes resolvable upon CSP **15b** were tested as precursors for reciprocal CSPs themselves. Prominent among these are the *N*-(3,5-dinitrobenzoyl) derivatives of α-amino acids. It became rapidly apparent that CSPs **17** and **18** are useful for the separation of enantiomers of a wide range of π-donor and π-dipole-containing analytes. These CSPs are prepared by bonding the *N*-(3,5-dinitrobenzoyl)-α-amino acid to γ-aminopropyl-silanized silica gel either through an



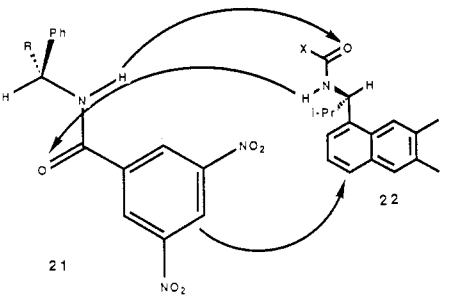
17 R_1 = C_6H_5, R_2 = H a, X = NH b, X = $O^-NH_3^+$

18 R_1 = H, R_2 = *i*-Butyl a, X = NH b, X = $O^-NH_3^+$

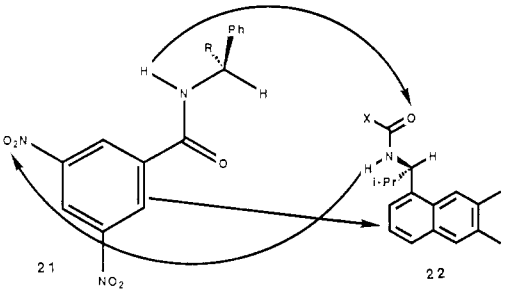
ionic or covalent linkage to the amino nitrogen of the γ-aminopropyl chain. CSPs **17** and **18** are commercially available, and even a partial listing of the types of analytes separable on these CSPs would be long. Some of the more common client analytes include amines (usually N-acylated, often with a 1-naphthoyl group),[88,89] alcohols, sulfoxides, and sulfoxamides,[90,91] epoxides, diols, and oxidation products of polyaromatic hydrocarbons,[92-94] phosphine oxides,[95] a variety of heterocyclic compounds,[96] and binaphthols,[97] among others. Another recent publication describes the use of supercritical CO_2 as a mobile phase to separate phosphine oxides on CSP **17**.[98] Several references highlight the interest in ibuprofen-type analgesics, the anilides of which are separable on these CSPs.[99,100] A number of similar CSPs prepared from other amino acids are possible and of some utility.

The success of CSPs **17** and **18** has spurred a great deal of theoretical interest in the mechanism by which the observed enantioselectivity occurs. Lipkowitz et al. have undertaken a number of molecular mechanics energy minimizations in order to determine the conformations of CSP **17** that are likely to be most populated and hence most involved in chiral recognition.[101-105] These workers have noted that the conformational minima are relatively broad for CSP **17**, and rotational barriers relatively low, so that a number of conformational states might contribute significantly to enantioselectivity on these CSPs, although models typically invoke only the rotamer in which the average position of the methine hydrogen of the CSP is represented as eclipsing the carbonyl oxygen of the dinitrobenzamide group, as shown in Figure 10. Pirkle et al. have undertaken a number of studies in order to clarify the mechanisms by which these CSPs operate.[106] The exact mechanism may vary somewhat for different analytes, but a number of common denominators are evident. Analytes separable on CSP **17** and **18** almost always contain π-donor functionality or dipolar π-groups conformationally influenced by the stereogenic centers. Generally, the analyte contains a basic site that can act as a hydrogen-bond acceptor. The presence of acidic hydrogens in the analyte may sometimes improve separability as well. Figure 10 shows a chiral recognition model for the separation of *N*-(2-naphthyl)alanine undecenyl ester **19a** on CSP **18**. The enantiomer of **19a**, which is homochiral with the CSP (i.e., having the same absolute configuration), is very selectively retained on CSP **18**, with a separation factor of 10.2 having been recorded.[107] This analyte contains all of the elements mentioned above in a suitable arrangement for a high degree of chiral recognition to occur.

Reciprocity has given rise to a large number of "third-generation" CSPs, based on compounds that are

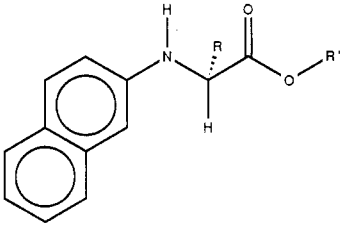


Dipole-stacking model (intercalative)



Hydrogen-bonding model (non-intercalative)

Figure 11. Two competing mechanisms for chiral recognition of *N*-(3,5-dinitrobenzoyl)arylalkylamines **21** on CSP **22** (X = $NHCO(CH_2)_{10}$-SiO_3). The dipole-stacking mechanism involves intercalation of the R substituent of the analyte between adjacent strands of the CSP and is the dominant mechanism for R groups shorter than $(CH_2)_7CH_3$. For such analytes the most retained enantiomer is *R* on the (*R*)-**22** CSP shown. For R groups longer than $(CH_2)_7CH_3$, the nonintercalative mechanism is dominant, and the *S* enantiomer of type **21** analytes are most retained on the (*R*)-**22** CSP.



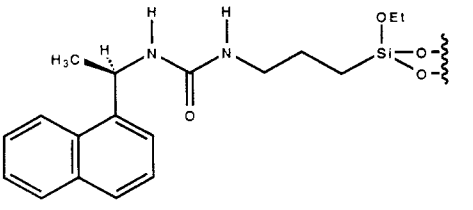
19a R = CH_3, R' = $(CH_2)_9CH{=}CH_2$

19b R = CH_3, R' = $(CH_2)_{11}SiOEt(O)_2Si$...

19c R = *i*-Propyl, R' = $(CH_2)_{11}SiOEt(O)_2Si$...

19d R = CH_3, R' = CH_3

resolved by CSPs **17** and **18**. This "bootstrapping" method of designing reciprocal CSPs from compounds that themselves resolve on existing CSPs is based on the premise that if two molecules show mutual chiral recognition, then it does not matter which of the two is bound to a stationary support for that recognition to occur. This is in practice not strictly correct, for the nature of attachment to the CSP does often affect chiral recognition. Within limits, however, reciprocity is a useful guide to CSP design. Oi and co-workers introduced CSP **20**, which separates the enantiomers of *N*-



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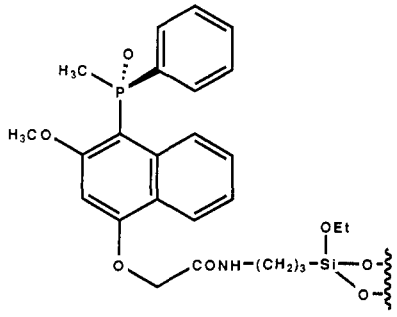
and *O*-dinitrobenzoyl- and dinitroanilido-derivatized amines, alcohols, and related compounds.[108,109] Related CSPs prepared by Pirkle and Hyun were the subject of a series of papers that analyzed in detail the structure–activity relationships of α-arylalkylamine-derived CSPs.[110-113] As expected, increasing the π-donor character of the aryl group enhances the separability of π-acceptor analytes. More importantly, it was demonstrated that the mode of attachment of the chiral moiety to the surface of the silica is important and may determine the sense and extent of chiral recognition, as more than one mode of enantioselectivity is operative on such CSPs. A case in point involves the separation of a homologous series of *N*-(3,5-dinitrobenzoyl)-α-arylalkylamines, **21**, on CSP **22**. Two mechanisms are proposed to account for enantioselectivity, an intercalative ("parallel") mechanism, which places the alkyl group of the analyte in between the strands of the stationary phase, and a nonintercalative ("perpendicular") mechanism, both of which are shown in Figure 11. For analytes having alkyl chains shorter than $n = 8$, the *R* enantiomers are most retained on the *R*-configuration CSP. As the chain length increases, the separability decreases until $n = 8$, for which no separation is observed. The $n = 9$ compound once again resolves, but now the *S* enantiomer of the analyte is more retained. Shortening the connecting arm between the chiral selector and silica support, lessening the average interstrand distance, or packing the space between strands with alkyl groups all lessen retention of the *R* enantiomer and bring about inversion of elution order at chain lengths shorter than $n = 8$. A change of orientation of the chiral selector with respect to the underlying support brought about by changing the mode of attachment changes the balance between the available mechanisms and has the anticipated affect on elution order and selectivity.[111]

Other interesting "third-generation" CSPs have been reported. An alkylarylphosphine oxide CSP **23**, capable



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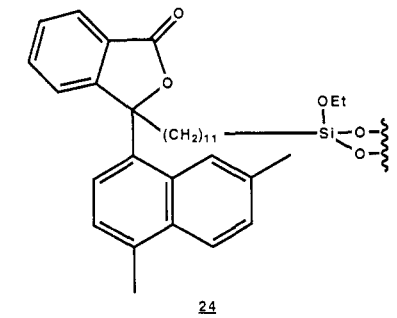
of resolving α-amino acid dinitrobenzamides, has been prepared by Tambute et al. and the mechanism of chiral recognition studied by NMR using *N*-(3,5-dinitrobenzoyl)-α-phenylethylamine as a chiral solvating agent.[114] Pirkle and Sowin have described a phthalide CSP, **24**, which resolves the 3,5-dinitrobenzoyl derivatives of amino acids, amines, and alcohols.[115] Pirkle and Hyun prepared a CSP containing both π-acidic and π-basic sites in order to observe the balance between various modes of chiral recognition,[116] and Yamushita and Numakura reported the preparation of binaphthyl-type CSPs **25** and **26**, which resolve biaryls, *N*-(3,5-dinitrobenzoyl)amino acid esters, and arylalkylcarbinol dichlorophenyl carbamates.[117]



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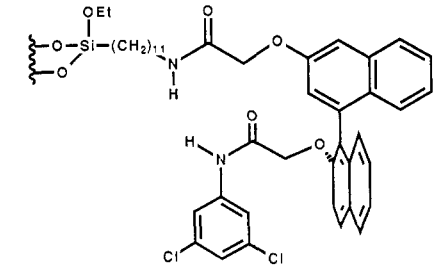

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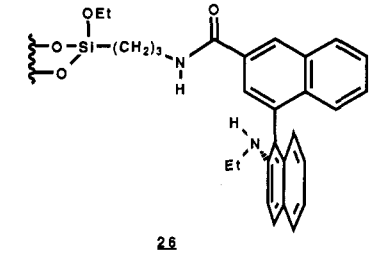

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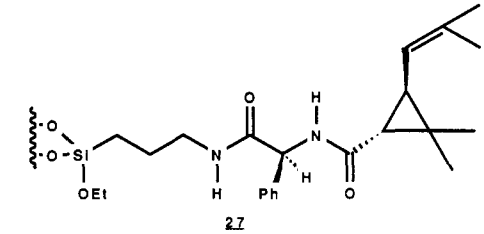
Japanese workers have, in recent years, produced a variety of CSPs that resolve the same types of analytes as other third-generation CSPs but, because they often contain a number of stereogenic centers and a variety of potential interaction sites, they are mechanistically more difficult to study. However, many of these CSPs are commercially available and in general use. Oi and co-workers have prepared CSPs from acylated amino acids and chrysanthemic acid bonded covalently to silica through a variety of linkages (CSPs **27** and **28**).[118,119] The dinitrophenyl carbamates of chiral fatty glycerides have been separated on CSP **29**.[120]



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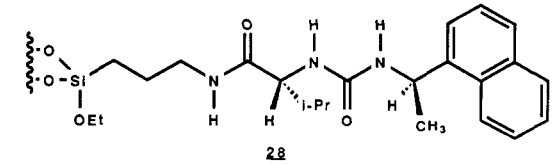

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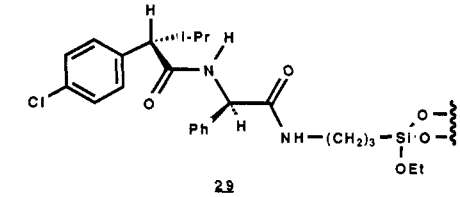

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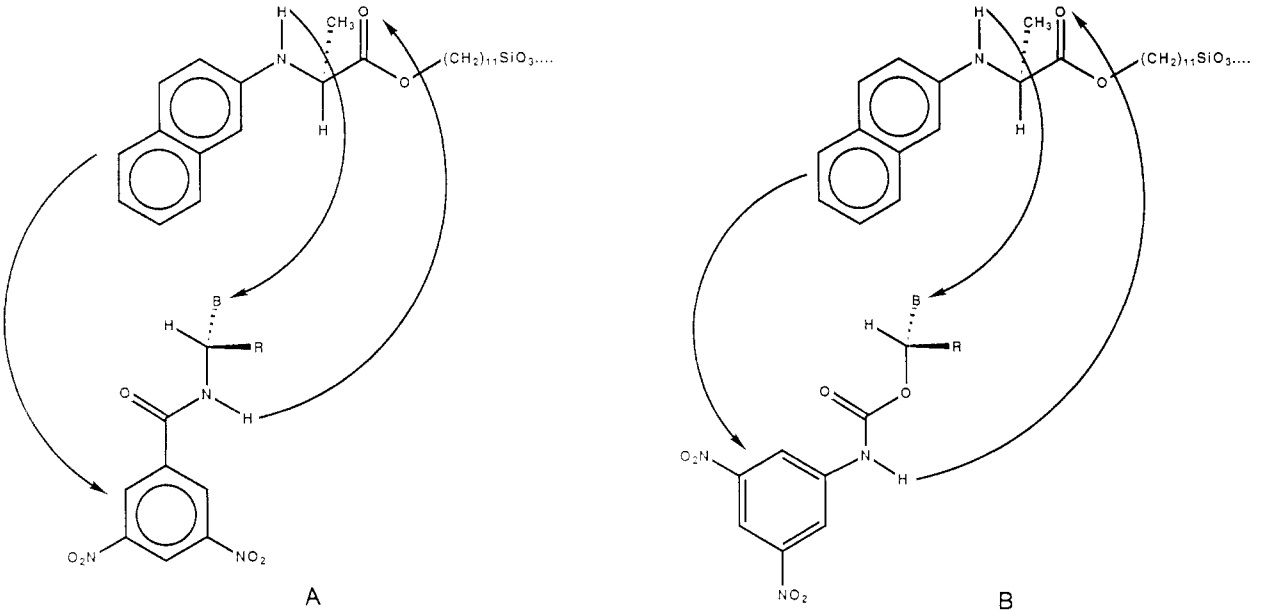
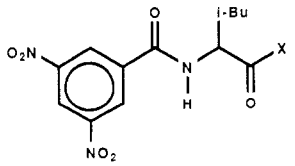


Figure 12. Generalized chiral recognition models of CSP **19b**. Diagram A shows the more retained enantiomer of the *N*-(3,5-dinitrobenzoyl) derivative of a chiral amine, while diagram B shows the same for a chiral alcohol as the *O*-(3,5-dinitrophenyl)carbamate. The interactions are a π-donor–acceptor interaction between the CSP and analyte aromatic groups, a hydrogen bond between an acidic proton of the analyte and the carbonyl oxygen of the CSR, and a hydrogen bond between the amino N*H* of the CSP and some generalized basic site on or near the stereogenic center of the analyte, B. In the absence of this basic site, the enantiomer that places the sterically smaller group at site B while maintaining the other interactions described is retained the longest.

TABLE I. General Classification of Types of CSPs along with Client Analytes, Mobile-Phase Requirements, and Derivatization Requirements

type of CSP	analyte, mobile-phase requirements
chiral polymer	wide scope, generally some sterically bulky group at stereogenic center desirable; derivatization may or may not be required; preparative use feasible, polar and nonpolar mobile phases have been used
protein CSP	ionizable functionality usually present (NR_3, COOH, etc.); no derivitization required; preparative use unlikely, aqueous mobile phases required
LEC	multidentate analytes only (α-amino and α-hydroxy acids, Schiff bases, etc.); aqueous mobile phase required; derivitization optional
chiral crown ether CSPs	chiral amines, amino acids, sterically bulky substituents at the stereogenic center; polar mobile phases; no derivitization required
cyclodextrin CSPs	polar and aromatic substitution at the stereogenic center desirable; preparative separations feasible; polar or nonpolar mobile phases
donor–acceptor CSPs	hydrogen-bond donor or acceptor, π-donor or π-acceptor; functionality may be introduced through derivatization; preparative separations feasible; polar or nonpolar mobile phases used

Recent work in these laboratories has resulted in a series of CSPs related to **19b** and **19c** that show a very high degree of reciprocal chiral recognition with *N*-(3,5-dinitrobenzoyl)-α-amino acid derivatives related to CSPs **17** and **18**.[121,122] Large separation factors are observed for the enantiomers of *N*-(3,5-dinitrobenzoyl)-leucine *n*-butylamide, **30a**, on CSP **19b** derived from



30a, X = NH-n-butyl
30b, X = NH-n-propyl
30c, X = NH-$(CH_2)_{10}$-NHCOCH(i-Bu)NHCO(3,5-dinitro)benzoyl

N-(2-naphthyl)alanine undecenyl ester (α = 15.2). The enantiomers of some *N*-(3,5-dinitrobenzoyl) dipeptide esters show even greater separability, with separation factors of over 20 being reported. For the bisamide of *N*-(3,5-dinitrobenzoyl)leucine, **30b**, a separation factor of 120 was noted on CSP **19b**. This is the expected result of doubling $\Delta(\Delta G)$ with respect to that observed for the monodentate analyte, which leads approximately to squaring of the original separation factor.[123]

The very large separation factors seen for these inverse separations of **30** on CSPs **19a** and **19b** are the result of an optimal arrangement of interaction sites, little interaction between CSP and analyte that does not lead to chiral recognition, and the relative exclusiveness of each interaction. For example, the unshared pair of electrons of the aniline-like N of **19** is somewhat delocalized into the naphthyl ring and hence increases the π-donor nature of that aromatic system. The same delocalization decreases the proton affinity of the nitrogen lone pair of **19** and so reduces the possibility of the nitrogen being a hydrogen-bond acceptor, although the NH proton is still capable of being a hydrogen-bond donor. On the other hand, the carbonyl oxygen of the ester group of **19** serves only as a basic site. Hence, there can be few bonding interactions between selector and selectand other than those shown in Figure 10, thus enhancing the degree of chiral recognition.

By appropriate derivatization, the enantiomers of amines, alcohols, thiols, diols, α- and β-amino acids, and many other analytes can be made separable on CSP **19b**. The mechanism by which analytes interact with the CSP have been studied extensively by chromato-

graphic and spectroscopic methods. Observation of intra- and intermolecular nuclear Overhauser effects has been particularly effective in determining the relative orientations of **19d** and **30b** in the homochiral complex.[124] Lipkowitz has noted that molecular mechanical calculations agree with experimental data regarding the lowest energy conformation populated by CSP **19b**, although he notes that other minima exist that are easily accessible and probably populated to a considerable extent.[126,127] UV–vis and NMR spectroscopic titrations have allowed calculation of K_{eq} for the homochiral complex.[125] From these studies it is clear that to a first approximation, CSP **19b** utilizes essentially one general mechanism for all of its client analytes (Figure 12). Because of this, enantiomer elution order shows a high degree of regularity, and in no case has an elution order been observed that contradicts the models shown in Figure 12. This regularity permits assignment of absolute configuration based on elution order with a confidence previously unknown. The interactions stipulated in Figure 12 have recently been confirmed by X-ray structure analysis of a 1:1 complex of (*S*)-*N*-(2-naphthyl)alanine methyl ester and (*S*)-*N*-(3,5-dinitrobenzoyl)leucine *n*-propylamide. A recent CSP derived from (*S*)-*N*-(1-naphthyl)leucine shows rather larger separation factors than does its predecessor, **19b**.[128] Separation factors exceeding 50 have been observed on this new CSP.

As is typical for all the chiral phases produced in these laboratories, the magnitude of the separation factors for enantiomeric pairs increases as the column temperature of CSP **19b** is reduced. There are occasional instances of inversion of enantiomeric elution order with changing temperature, which would make it difficult in such cases to establish absolute configuration based on elution order at a single temperature.[129] No such case has been observed with any of the DA CSPs prepared in our laboratory. However, one need but determine α at more than one temperature in order to see if α increases or decreases as the temperature is lowered. This establishes whether one is above or below the "inversion" temperature.

IX. Conclusion

Table I gives a brief summary of the types of CSPs that have been prepared, a summary of structural requirements for each CSP's client analytes, and its potential for preparative separations. A fairly complete listing of the types of compounds resolvable on various CSPs by compound type is to be found in a recent review published by the same authors.[130]

X. References

(1) A reviewer of the initial version of this review noted that we make no reference to "through-space effects", which the reviewer considered to be important to chiral recognition. This is intentional for we find the phrase "through-space effects" to be vague, since all intermolecular interactions are through-space. If the phrase refers to electric field effects or steric effects, these are addressed as such.
(2) In the context of this review, *diastereomeric complex* refers to solution complexes between the enantiomers of an analyte and some chiral selector. When referring to such interactions taking place on the surface of a CSP, we will use the phrase *diastereomeric adsorbate*.
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