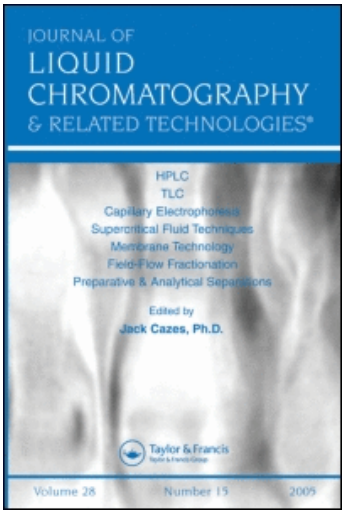

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CHIRAL STATIONARY PHASES FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS: A MINI-REVIEW

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ABSTRACT

There has been a proliferation of papers on the use of chiral stationary phases (CSP's) to separate optical isomers in high performance liquid chromatography. The chemistry, mechanism and stability of these CSP's can vary widely. Furthermore, the applicability, availability and cost of a CSP can mean the difference between its being of passing academic interest as opposed to a technique that could have a significant impact on science and technology. Six different classes of chiral stationary phases are examined and discussed including the new chiral cyclodextrin bonded phases. The separation mechanism, strengths and limitations of the CSP's are also considered whenever such information is available.

INTRODUCTION

In the last decade there has been a tremendous impetus to develop efficient liquid chromatographic techniques for the separation of racemates. There are several reasons for this. For example, an efficient method for determining optical purity would be highly beneficial in many scientific disciplines in-

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cluding: organic and inorganic synthesis, kinetics, pharmacology, geochronology (i.e., using the degree of amino acid racemization to date organic articles of archaeological importance) and so forth. The pharmaceutical industry obviously needs effective analytical and preparative separations for a variety of enantiomeric compounds which are known to have different physiological activities (1-4). The fact that the traditional method of resolving racemic mixtures (i.e., fractional recrystallization of diastereomeric salts) is relatively difficult, inefficient and limited in applicability (5,6) has greatly increased the interest in alternative techniques. The efficiency, speed, wide applicability and reproducibility of the modern liquid chromatograph have made it the instrument of choice for most of the recently reported enantiomeric separations. Be this as it may, it is clear that recent HPLC techniques have evolved and/or benefited from classic column chromatographic methods (6). It also appears that the most interesting research in this area involves the development of new highly selective stationary phases. In this review the chemistry, applicability and limitations of six classes of chiral stationary phases will be examined. This work is not intended to be a comprehensive review of enantiomeric separations. For example, work on the use of chiral mobile phase additives and ligand exchange LC will not be covered. Readers interested in these particular areas are refered to the

the many fine reviews and papers that have recently been published (7-16). The six classes of chiral stationary to be considered in this work are: cyclodextrin bonded phases, π-complex/hydrogen bonding stationary phades, polymeric stationary phases, charge transfer stationary phases, protein bonded phases, and crown ether bonded phases.

I. Chiral Cyclodextrin Bonded Phases

Cyclodextrins are chiral, toroidal shaped molecules formed by the action of Bacillus macerans amylase on starch (see Figure 1)(17-19). These macrocyclic polymers contain from six to twelve glucose units bonded through α-(1,4)linkages. The three smallest homologs, α-cyclodextrin (cyclohexaamylose), β-cyclodextrin (cycloheptaamylose) and γ-cyclodextrin (cyclooctaamylase) are available commercially while larger homologues must be individually produced and isolated. Cyclodextrins have several structural features that make them highly useful in separations (Figure 1). First of all, the interior of the cyclodextrin cavity contains no hydroxyl groups and is relatively hydrophobic. Consequently they are able to complex a variety of water insoluble or sparingly soluble molecules, see Figure 2. This property led to their use as mobile phase modifiers in the TLC separation of a variety of structural isomers (20-22). Traditional column chromatography on polymerized cyclodextrin was investigated as well (23-25).

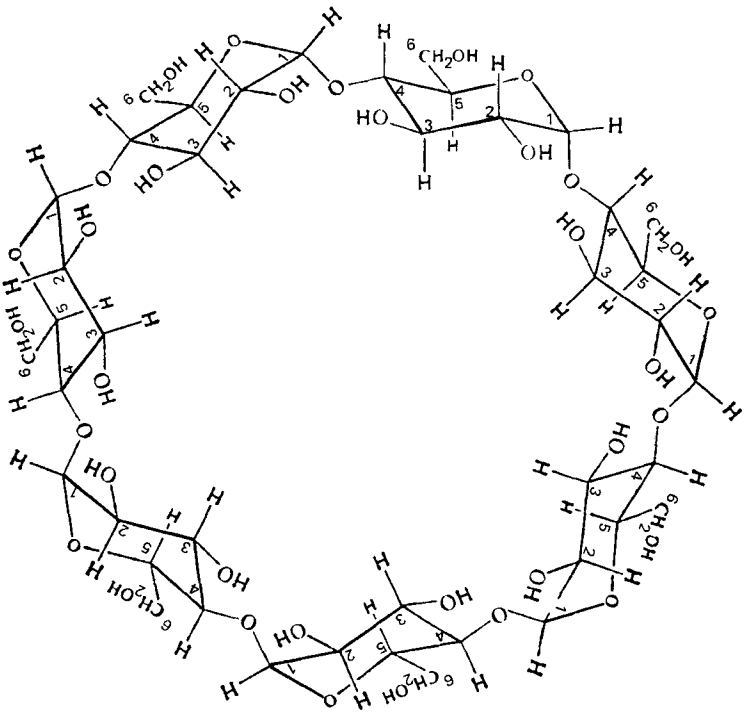


Figure 1. A schematic showing the structure of β-cyclodextrin. The cavity is hydrophobic and is 7 to 8 Å deep. Note that all glucose units are locked in a chair conformation and joined by a stable α-(1,4)glycosidic linkage. All hydroxyl groups are on the outer edges of the molecule with the primary 6-hydroxyls restricting the "bottom side" of the molecule.

Most importantly, each glucose unit contains five chiral atoms and the 2-hydroxyl groups at the entrance of the cyclodextrin cavity project in a clockwise direction (Figure 1). Chiral recognition has been shown to be optimal on a β-cyclodextrin column for compounds the size of biphenyl or a little larger (26,27). If, in addition, the chiral solute also contains a substituent that can hydrogen bond with the 2-hydroxyl

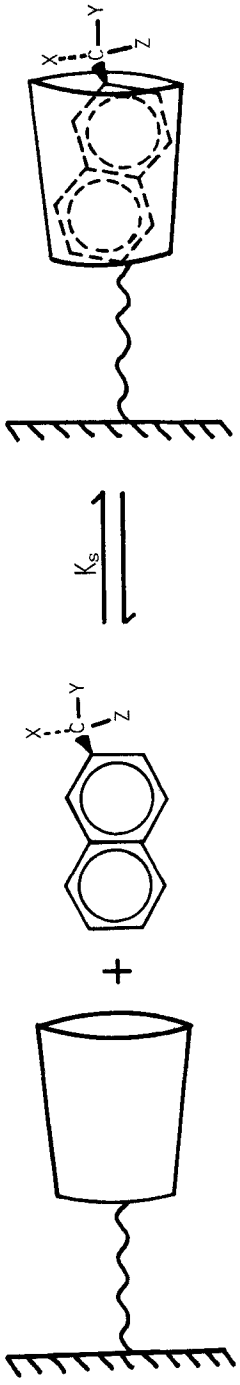


Figure 2. A schematic of cyclodextrin bonded to a silica gel support and reversibly forming an inclusion complex with a chiral molecule. Neither the linkage nor the cyclodextrin contain nitrogen (e.g., amines or amides) in any form.

groups at the mouth of cyclodextrin cavity, then enantiomeric separations are particularly efficient and predictable (see Figure 3). For example, the L-enantiomer of all dansyl or naphthyl amino acids is eluted first. Table I lists typical enantiomeric compounds that have been resolved on chiral cyclodextrin bonded phases.

It is interesting to consider whether or not chiral cyclodextrin inclusion complexes satisfy the three point chiral recognition model (29). Considering the simplified model shown in Figure 2, one could argue that there is a three point attachment via the hydrophobic group in the cavity and at least two of the groups projecting radially from the mouth of the cyclodextrin cavity. This seems to be likely for many of the compounds studied (Table I). One might also argue that there could be more than three points of interaction if the entire molecule is within the cyclodextrin. However small molecules that are completely enveloped by the cyclodextrin tend not to show enantioselectivity (a N-benzoyl amino acid in β-cyclodextrin for example). In these cases one must go to a smaller cyclodextrin (such as α-CD). A "tight fit" with part of an enantiomer extending out of the cyclodextrin cavity seems to produce the desired optical resolution in many cases. It may be possible that a one or two point attachment accompanied by steric restraints (as first suggested by Lochmüller (30, 31)) could in some cases be responsible for the observed

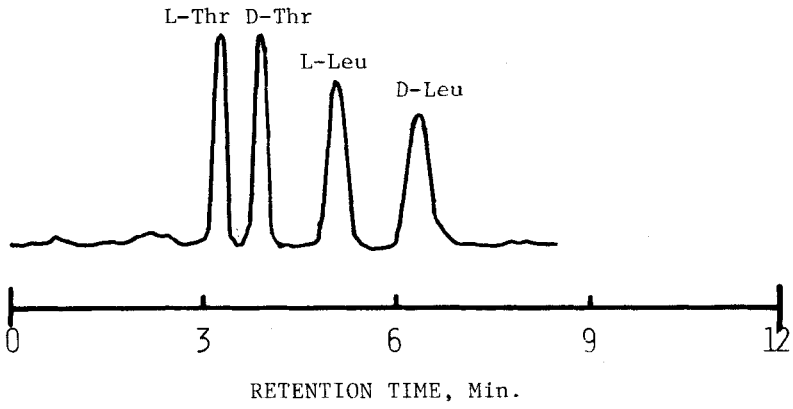


Figure 3. A chromatogram showing the separation of racemic dansyl threonine and leucine. Note that the L-enantiomer is always eluted first. Cromatographic conditions: column = 0.46 x 10 cm B-CD, solvent = 50% methanol + 50% water, flow = 1.0 ml/min, detection γ=254 nm.

enantioselectivity (see section IV). Because of its strict stereochemical requirements and the intimacy of the inclusion complex, cyclodextrin bonded phases may be a more useful means to assign absolute configuration than other CSP's. Certainly a good deal more work must be done in this area.

There are several advantages to chiral cyclodextrin packings. They are, for example, commercially available* and there are several different size cyclodextrins which allows one to separate a variety of different size enantiomers. This packing is water stable and is most often used with aqueous-methanol mobile phases (26-28). This is important since the presence of water is known to ruin most of the other chiral

*Advanced Separation Technologies, Inc. 37 Leslie Ct. P.O. Box 297, Whippany, NJ 07981.

Table I: A List of Typical Enantiomers Resolved on β-Cyclodextrin Columns Using Aqueous-Methanol Mobile Phases (26-28,32).

1.	D,L-alanine β-naphthylamide
2.	D,L-methionine β-naphthylamide
3.	D,L-alanine β-naphyl ester
4.	dansyl D,L-valine
5.	dansyl D,L-threonine
6.	dansyl D,L-norleucine
7.	dansyl D,L-phenylalanine
8.	dansyl D,L-leucine
9.	dansyl D,L-methionine
10.	dansyl D,L-tryptophan
11.	dansyl D,L-serine
12.	dansyl D,L-norvaline
13.	dansyl D,L-α-amino-N-buteric acid
14.	dansyl D,L-arginine
15.	N-benzoyl-D,L-arginine β-naphthylamide
16.	(+,-) hexabarbital
17.	(+,-) mephobarbital
18.	(+,-) usnic acid
19.	(+,-) α-(1-naphthyl)ethylamine
20.	(+,-) cyclohexylphenylacetic acid
21.	D,L-propranolol
22.	(+,-)2,3-O-isopropylidene-2,3-dihydroyx-1,4-bis(diphenylphosphino)butane [a.k.a. DIOP]
23.	(+,-) trans α,α'-(2,2-dimethyl-1,3-dioxalane-4,5-biyl)-bis-(biphenylmethanol)

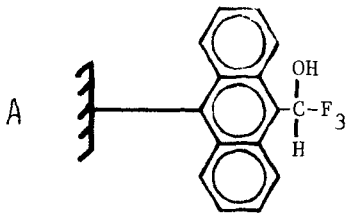
stationary phases. An added benefit of cyclodextrin bonded phases is that they are useful for the resolution of many nonenantiomeric compounds and have been found to be superior to conventional reverse phase packings for many of the more routine separations (27, 32). They can also be used as normal phase packings (e.g., with hexane-alcohol mobiles phases) although the separation is not due to inclusion complex formation but rather to hydrogen bonding and dipolar interactions

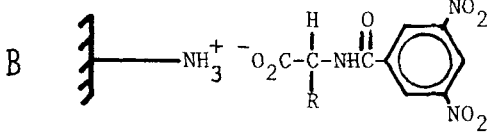
with surface hydroxyl groups (32). Lastly, these columns are currently less expensive than most routine normal and reverse phase columns.

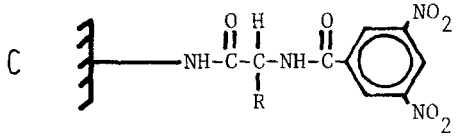
Cyclodextrin packings also have some shorcomings. For example they are generally about 80% as efficient as the best reverse phase columns. This is because the formation of strong inclusion complexes causes somewhat slower mass transfer which results in detectable band broadening. Fortunately, this relatively small decrease in efficiency is usually accompanied by a large increase in selectivity. Currently only β- and γ-cyclodextrin columns are available commercially, however an α-cyclodextrin packing for smaller molecules will undoubtedly be forthcoming. After extensive use (particularly when using mobile phases with a very high percentage of water) one finds that the efficiency and retention begins to decrease. This is because strongly retained impurities are occupying many of the cyclodextrin cavities. The column is easily regenerated by flushing with absolute methanol or ethanol.

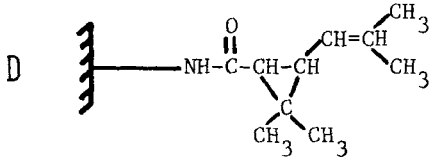
II. "π-Complex-Hydroden Bonding" Stationary Phases

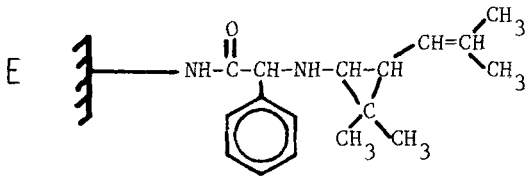
The three-point chiral recognition model has been used as a basis for the design of several chiral stationary phases. Pirkle and co-workers origionally designed CSP's "A" through "C" (see Figure 4) in view of this model and successfully resolved a series of enantiomeric sulfoxides and 3,5-dinitrobenzoyl deriva-

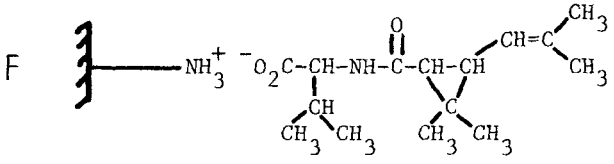












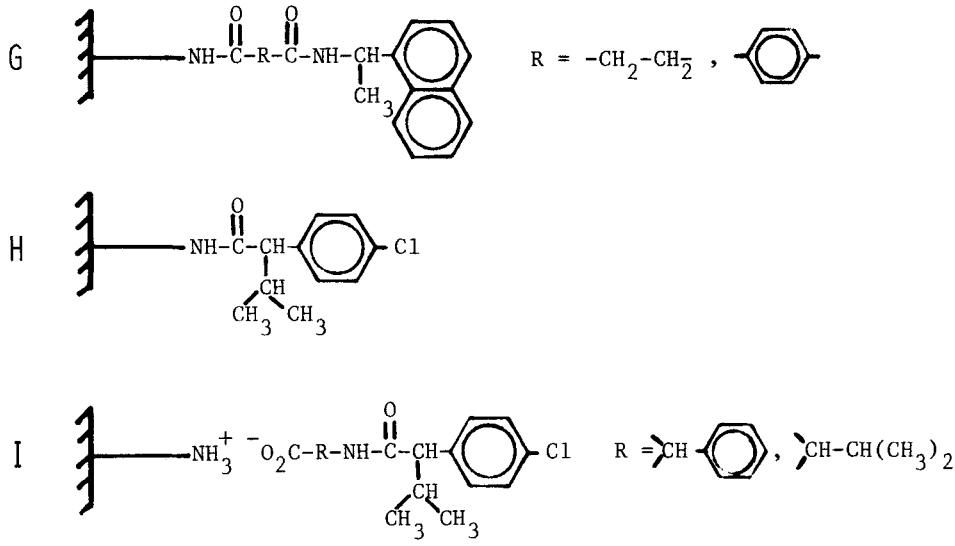


Figure 4. A schematic showing nine different class II chiral stationary phases. All are used under normal phase, conditions (e.g., hexane: isopropanol mobile phases). Packings A through C were developed by Pirkle and co-workers (33-35). Packings D through I were developed by Oi and co-workers (43-47). All packings except for "A" have the chiral molecule attached to the silica support via a γ-aminopropylsilane linkage.

tives of amines, alcohols, thiols, amino acids, amino alcohols and hydroxy acids (33-35). The necessary three point contact between the CSP and enantiomeric solute was thought to be maintained via two hydrogen bonds and a π-π donor-acceptor interaction (33-35). Subsequent work indicated that dipole-dipole stacking and Van der Waals interactions must also be considered in many cases (36). One important feature of these CSP's was that they seemed to be applicable to a broader range of compounds than most of the earlier reported chiral packings.

Recent research in this area involves the extension of applications, the development of a rational for predicting elution order and the developement of another generation of CSP´s of this type. Wainer and co-workers, have largely been involved with the first two pursuits above, particularly in the separation of a variety of chiral compounds of pharmaceutical interest (36-40). Other research groups have evaluated enantiomeric separations of dihydroxy and tetrahydroxy polynuclear aromatic hydrocarbons (41) and a variety of acyclic alkyl carbinols and their derivatives (42). Oi and co-workers (43-46) have focused their attention on the developement of new and modified CSP's (see structures D through I, Figure 4). Their work has shown that there can be significant differences in selectivity for many of the related CSP's. In fact, it has been noted that there are selectivity differences in packings containing the same chiral base molecule but differing in whether they are attached covalently or ionically (structures "B" and "C" in Figure 4) (36). Undoubtedly these types of studies will continue to shed light on the separation mechanism.

There are several advantages to Type II CSP's. Firstly, CSP's "B" and "C" (Figure 4) are available commercially.* Currently, more enantiomeric seperations have been reported on this class of packing than on any other. As a result, the interested

*J. T. Baker, Phillipsburg, NJ

researcher has available a significant quantity of useful data which can be used to formulate future work. Indeed, a recent paper evaluated the role of mobile phase composition on enantiomeric selectivity and resolution (47).

A disadvantage of class II CSP's is that the presence of any water in the mobile phase tends to ruin both the separation and the packing. It is possible, however, to regenerate the ionically bonded packings provided a significant portion of the γ-aminopropylsilanized silica gel has not hydrolyzed. It has been noted that it is more difficult to separate ester derivatives of some enantiomers than the corresponding amides (36). It was also noted that there was a reversal in the elution order of an enantiomeric pair of compounds during the course of a study (36). It is not currently known how common this phenomenon is, but it could restrict the use of these CSP's for assigning absolute configurations.

III. Chiral Polymer Stationary Phases

There is an extensive literature on the use of chiral polymers for traditional column chromatography of enantiomers. Many but not all are naturally occuring polymers. Typical examples include: cellulose, microcrystalline cellulosetriacetate, starch, polymerized amino acids, cross linked polystyrene containing alkaloids, and isotactic (+)-poly(triphenylmethyl methacrylate)(6). The use of analogous stationary phases in

HPLC is much less extensive but will undoubtedly grow as a result of recent successful applications (48-50). There are presently two basic approaches in using chiral polymers as HPLC stationary phases. First, if the crosslinked polymer possess sufficient mechanical strength (to withstand the pressure) and can be obtained in particle sizes of appropriate dimensions (i.e., 5 to 10 μm) and distributions, then it can be used as is. A typical example of this approach is Lindner and Mannschreck's use of microcrystalline triacetylcellulose for the HPLC separation of several racemic compounds (48). Partial resolution of a cyclic allene hydrocarbon was also reported (48). A series of racemic 2,2'-disubstituted 1,1'-binaphthyls and racemic trans-disubstituted cyclic compounds were also resolved by HPLC using a finely ground insoluble (+)-poly(triphenylmethyl methacrylate) (50). A somewhat different approach is to adsorb the chiral polymer onto silica gel. This simple approach was shown to produce a highly effective chiral phase when a low molecular weight (+)-poly(triphenylmethyl methacrylate) was used (49). In addition, the selectivity is often different from that found for the analogous packing made from the "ground" or pulverized high molecular weight polymer. For example, the four stereoisomers of the insecticide phenothrin (i.e., (+) and (-), cis- and trans-3-phenoxybenzyl chrysanthemate) are resolved on the "coated" stationary phase but not on the "ground" type (49). One can expect many

additional enantiomeric separations in the near future as researchers adsorb different chiral polymers on a variety of silica gels. Certainly the use of polysaccharides, polyaminoacids and appropriate derivatives of these polymers will produce interesting results.

Initial experiments seem to indicate that the coated chiral polymer stationary phases may be more widely applicable than packings composed of pure polymer. Certainly one advantage with the "coated" material is that one can easily and inexpensively make a wide variety of different CSP's. A disadvantage is that use of mobile phases containing appreciable amounts of water will likely result in inferior separations and/or damage to the packing. The packing can always be recoated with polymer, however. Some polymer loss has even been noted when using nonaqueous solvents (49).

IV. Chiral Charge Transfer Stationary Phases

In 1955 it was demonstrated that hexahelicene could be resolved from a solution of chiral α-(2,4,5,7-tetranitro-9-fluorenylidenaminooxy) propionic acid (TAPA) by fractional recrystallization (51). By 1960 resolution was obtained by column chromatography with TAPA coated silicic acid (52). Mikes and co-workers extended this technique to HPLC, separating ten racemic helicenes and two double helicenes using a stationary phase of silica coated with TAPA (53). They also examined three

homologues of TAPA and found that the size of the substituent on the chiral carbon affected the selectivity. Alumina impregnated with (S)-(+)-TAPA has been used as well (54). TAPA and 2,2'-diylhydrogen phosphite XL has been bonded to silica gel (see Figure 5) and has shown similar success in the separation of helicenes (55, 56) as has silica gel coated with riboflavin (57). Lochmüller and Ryall utilized a small 2,4-dinitrophenyl group as the change transfer acceptor attached to a chiral atom, see Figure 5 (31). Although partial resolution of 1-aza[6] helicene and heptahelicene was achieved, the real significance of this work was in Lochmüller's conclusion as to the mechanism involved. It was postulated that the small 2,4-dinitrophenyl group could not have achieved the multiple overlaps with the helicenes as proposed for TAPA. Consequently it may be possible to achieve chiral discrimination in chromatography with only one (31) or two (30) strong interactions. It was indicated in previous gas chromatographic work that steric repulsion may be a possible substitute for positive interactions (30).

It is apparent that chiral charge transfer stationary phases are useful for the separation of racemic helicenes. Unfortunately it has not yet been demonstrated that they have any wider applicability. As these stationary phases are either coated on silica gel or bonded via an amide linkage through an aminopropylsilane, they should not be use with aqueous or aqueous-organic mobile phases.

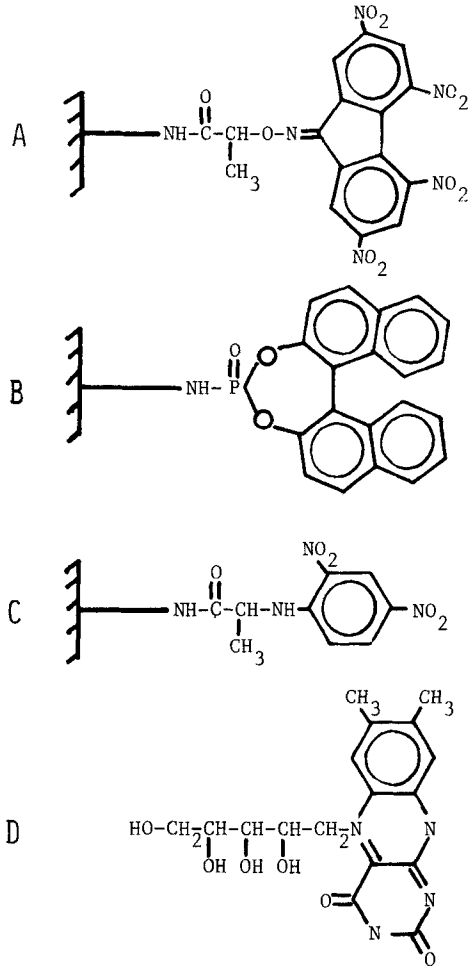


Figure 5. A schematic showing the structure of four different charge transfer adducts that have been bonded to or adsorbed on silica gel to create CSP's capable of separating a variety of helicenes. Again, the chiral molecule in all bonded packings was attached via a γ-aminopropylsilane linkage. Compound A = TAPA, B = 2,2'-diylhydrogen phosphite XL, C = N-2,4-dinitrophenylalanine, and D = riboflavin.

V. Protein Bonded Phases

For protein bonded phases, as for most of the previous chiral stationary phases, there was a legacy of successful applications by classic column chromatography. Generally bovine serum albumin (BSA) was linked to agarose (58), which could then resolve enantiomers of aromatic amino acids, some N-benzoyl-amino acids and aromatic suphinyl and sulphorximine compounds (59). More recently, Allenmark and co-workers successfully attached BSA to 10 μm silica and demonstrated its utility in HPLC (60). A series of ten different N-benzoyl and N-naphthoyl amino acids showed enantioselectivity on this packing.

Two of the advantages of this packing are that it can be used with aqueous or aqueous-organic mobile phases and that it is available commercially.* The only other packing that shares these advantages is the chiral cyclodextrin bonded phase (Part I). Currently it is not known whether or not this packing can separate any enantiomers other than N-aroyl D,L-amino acids. The stability, lifetime and cost of the packing as well as whether or not it can be regenerated is not presently known by this author.

VI. Chiral Crown Ether Bonded Phases

Cram and co-worker's research on the complexation of ions by crown ethers led to the development of cyclic compounds which

*Mackerey Nagel & Co., Duren, G. F. R.

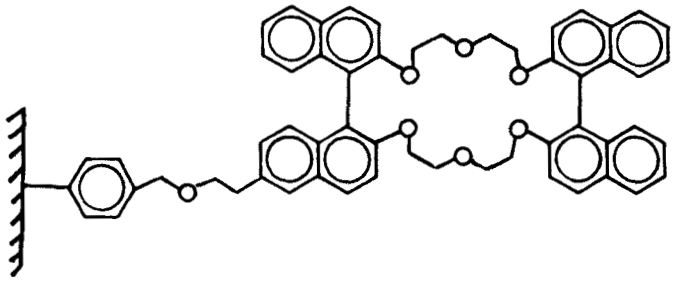


Figure 6. A schematic of a chiral crown ether which can complex protonated primary amines and in some cases show chiral recognition (61-63).

showed enantioselectivity (see Figure 6) (61-63). Crown ethers have the ability to complex a number of cations (e.g., Na^+, K^+, NH_4^+, etc.). The stability of the crown ether-ion complex depends, to a significant extent, on the fit of the ion in the crown ether cavity. For example, the larger crown ether, 18-crown-6, prefers to complex the larger potassium ion over the smaller sodium ion. For the smaller crown ether, 15-crown-5, the selectivity is the opposite. Ammonium ion is about the same size as potassium ion and fits well into the cavity of the crown ether shown in Figure 6. If the ammonium ion is attached to a chiral atom (as in a protonated amino acid) then the possibility exists for the separation of those enantiomers. Indeed, this was shown to be possible for a number of aromatic amino acids and analogous compounds (61-63).

Although these interesting stationary phases effectively separate some racemic compounds that contain primary ammonium

ions, their over all applicability is obviously limited (as are the chiral charge transfer stationary phases, for example). Judging from the current cost of many crown ethers the possibility exists that this packing may be prohibitively expensive for large scale use. It is well known that aqueous solvents and aqueous-organic mixtures tend to decrease the interaction between a crown ether and a guest ion (e.g., as opposed to organic solvents). It is not likely that the enantioselectivity observed in these systems (which utilized organic solvents) would remain in typical reverse phase mobile phases.

ACKNOWLEDGEMENT

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