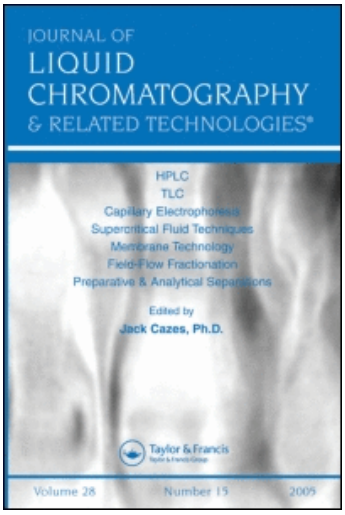

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Improved Cyclodextrin Chiral Phases: A Comparison and Review

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IMPROVED CYCLODEXTRIN CHIRAL PHASES: A COMPARISON AND REVIEW

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ABSTRACT

Cyclodextrin chiral phases have been shown to be widely applicable for the separation of enantiomers, diastereomers, structural isomers and routine compounds. Two innovations have recently occured in this field. First, the efficiency and selectivity of the β-cyclodextrin column has been improved. Second, compatable TLC plates which produce separations analogous to the columns have been developed. These results are discussed subsequent to a brief review of published work. In addition, the mechanism of separation on cyclodextrin bonded media, solvent effects, temperature effects and structural effects on chiral separations are considered.

Introduction

Cyclodextrins (abbreviated CD), are cyclic oligosaccharides traditionally formed by the action of Bacillus macerans amylose on starch (1,2). These natural macrocyclic polymers contain six to twelve glucose units which are bonded through α-(1,4)linkages. The three smallest homologs, α-cyclodextrin (cyclohexamolyose), β-cyclodextrin (cycloheptamylose) and γ-cyclodextrin (cyclo-

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octamylose) are available commercially while the larger homologues must be individually produced and isolated. Cyclodextrin can also be made by isolating the enzyme cyclodextrin transglycosylase (C.T.G.), immobilizing it on an appropriate support and passing starch solutions over the matrix. In this way, one can not only optimize the reaction conditions, but also control the type and amount of cyclodextrin produced by introducing various trace solutes to the reaction mixture (3). The ability of cyclodextrins to form inclusion complexes with a variety of water insoluble, sparingly soluble and soluble compounds make it particularly useful for separations (Figure 1). An additional important feature of all cyclodextrins is that each glucose unit is chiral and the 2-hydroxyl groups at the entrance of the cyclodextrin cavity project in a clockwise direction. If tightly complexed chiral solutes also contain substituents that can interact with the mouth of the cyclodextrin cavity, then there exists the possibility for an enantiomeric separation (4).

One of the earlier effective uses of cyclodextrins in chromatography was as mobile phase modifiers in TLC (2,5,6). In addition, polymerized cyclodextrin gels were used as stationary phases in column chromatography with varying degrees of success (7-9). It was necessary, however, to develop a stable, completely derivatized, HPLC packing before cyclodextrins could reach their full potential as a separations media. Initially, Japanese research groups were able to attach different cyclodextrins to silica gel via ethylene diamine linkages (10,11). Subsequently,

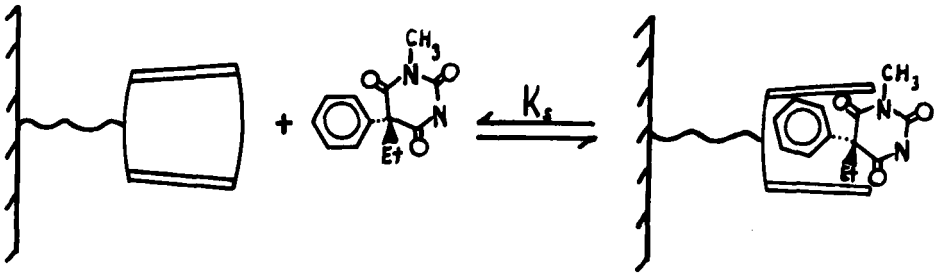

Figure 1. Schematic of a cyclodextrin molecule, represented as a truncated cone, which forms a reversible inclusion complex with mephobarbital.

problems were encountered with these packings: (A) they were hydrolytically unstable, (B) the cyclodextrin loading was often low, (C) the amines present affected selectivity, (D) nitroxide formation occurred during synthesis, (E) the syntheses were often tedious (13). Other packings that contained no nitrogen linkages, which were thought to be more widely useful and commercially feasible, were developed by Armstrong (12). This packing consisted of chiral cyclodextrin molecules linked to silica gel by a six to ten atom spacer. Both the linkage and cyclodextrin were hydrolytically stable under standard L.C. conditions. The use of these LC stationary phases to successfully resolve a variety of difficult-to-separate isomers was demonstrated (13-15).

An improved β-cyclodextrin phase will also be discussed and compared to the original cyclodextrin media. These improved phases have nearly twice the efficiency and loading of the original columns. Also new derivatized β-cyclodextrin phases

which exhibit a completely different selectivity will be discussed.

REVIEW OF PREVIOUS MATERIAL

Previous results have demonstrated that the original B-CD bonded phases were reasonably effective in separating various optical, geometrical and structural isomers (13-15). Table 1 gives examples of separations of a diverse group of stereoisomeric compounds. In addition to the optical isomers mentioned, separations have also been achieved for at least twelve other metallocenes; hexobarbital, β-naphthyl ester derivatives of amino acids, substituted dioxolanes, and selected carboxylic acids (13-15). In some cases, it was possible to detect as little as .20% of one enantiomer in the presence of 99.80% of the other (13). Epimers such as the α and β isomers 20-hydroxy-4-pregnen-3-one, 5-androstan-3-ol-17-one, 17-estradiol, and 11-hydroxy-progesterone have been separated as well as cis/trans isomers of clomiphene, benzo[a]pyrene-7,8-diol, 3-hexen-1-ol and syn/anti azobenzene (13-15). In addition, a wide variety of structural isomers have been separated on the β-CD column including ortho, meta, and para isomers of amino benzoic acid, nitrophenol, nitroaniline, xylene, bromobenzoic acid, and biphenyl. Certain isomeric compounds such as benzopyrene-diols and the four epimeric estriols in TABLE 1 are very difficult to separate by LC or GC (15,16). Taken together, these results clearly demonstrate the potential of cyclodextrin bonded phases in HPLC. Depending on one's particular needs, the CD-column can be viewed as a viable,

TABLE I. A Brief Summary of Stereoisomeric Separations of Different Classes of Compounds. One Specific Example is Given for Each Class.

Enantiomeric Compounds[a]	k'	α	R_s	Mobile Phase[b]
Dansyl Amino Acids				
(L)-norleucine	1.90	1.26	2.30	50:50
(D)-norleucine	2.40			
β-Naphthyl Amino Acid Derivatives				
L-alanine β-naphthylamide	5.1	1.20	2.00	50:50
D-alanine β-naphthylamide	6.1			
Barbiturates				
(-)mephobarbital[c]	14.8	1.14	1.6	20:80
(+)mephobarbital	16.9			
Metallocenes				
(-)s-(1-ferrocenylethyl)-thiophenol	3.1	1.39	2.27	90:10
(+)s-(1-ferrocenylethyl)-thiophenol	4.3			
Carboxylic Acids				
α-methoxy-α-trifluoro-methylphenyl acetic acid[c]	7.5	1.31	0.6	50:50
	9.8			
Misc.				
(-)DIOP[d]	10.56	1.12	1.2	48:52
(+)DIOP	11.84			
Diastereomeric Cpds				
Geometrical Isomers				
cis-stilbene	7.3	1.62	4.7	55:45
trans-stilbene	4.5			
Epimers				
16,17-epiestriol	2.8	1.36	1.49	
estriol	3.8	1.53	1.52	60:40
17-epiestriol	5.8	1.98	5.2	
16-epiestriol	11.5			

a) A 10 cm β-cyclodextrin column was used unless otherwise noted.
b) Numbers represent the volume percent of methanol to water. The flow rate was 1.0 ml/min.
c) 25 cm β-cyclodextrin column.
d) 2,3,0-isopropylidene-2,3-dihydroxy-1,4-bis(diphenylphosphino)butane.

less-expensive alternative to other chiral phases, or as a column that is complimentary to other chiral stationary phases (CSP). Certainly no single CSP can be equally effective for all optical isomers.

The original CD bonded phases have also demonstrated an ability to function as a nonconventional reversed phase. Excellent separations of a series of barbiturates (including barbital, butabarbital, sodium pentabarbital, phenobarbital, secobarbital, and amobarbital) have been achieved. Other routine separations include mycotoxins (i.e., T-2 tetraol, T-2 triol, HT-2 toxin, T-2 toxin, verrucurol and diacetoxyscirpenol) and polycyclic aromatic hydrocarbons on both beta and gamma-CD bonded phases (17). Compounds such as vitamins and quinones have also been reported to be separated on β-CD bonded phases (14,17). These results indicate, it is possible to do routine conventional separations on important classes of compounds using β-CD bonded media. Recently improved β-CD bonded phases are superior to the original β-CD columns and will be discussed in the following section.

One of the advantages of cyclodextrin bonded phases is that compatible TLC plates can be made from analogous media (18). This is advantageous because one can rapidly evaluate a variety of solvent systems and chromatographic conditions via TLC. In addition large numbers of samples can be run simultaneously if need be. Preliminary results indicate that LC and TLC separations are comparable (18). For example, compounds such as the dansyl amino acids have been resolved on CD-bonded TLC plates. Dansyl

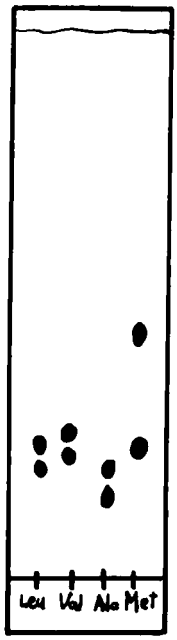

Figure 2. Enantiomeric separation of dansyl-D,L-leucine, valine, alanine and methionine on a 5 x 20 cm β-cyclodextrin TLC plate. Mobile phase: 50% MeOH/50% (1% triethylammonium acetate buffer pH = 4.1). Note that the spots are fully resolved.

D,L-leucine, valine, alanine, and methionine were fully resolved on a 5 cm x 20 cm β-CD bonded TLC plate (Fig. 2). Several geometrical and structural isomers have also been separated with these plates (18).

IMPROVED β-CYCLODEXTRIN MEDIA

Beta-cyclodextrin columns with greater CD-loading and packed via a better technology have now been utilized. Earlier

β-cyclodextrin columns were generally 10-20% less efficient than conventional reversed phase columns. Because of their greater selectivity which allowed one to separate a variety of enantiomers and other isomers, it was thought that this was an acceptable trade-off. Recent columns have comparable efficiency to conventional reversed phases and a considerably higher loading of cyclodextrin which results in separations that were not able to be achieved on the original columns. While the original β-CD columns often had plate numbers between 40,000 - 55,000 plates/meter (5μ support), recent columns typically exhibit from 70,000-100,000 plates/meter. This increased efficiency allows one to use higher methanol or acetonitrile/H_2O ratios, which in turn decreases the analysis time. For example, Fig. 3 shows an optimum separation of dansyl D,L-phenylalanine on an original versus an improved 25 cm β-cyclodextrin column. Note that the second separation is baseline, it utilizes a higher methanol concentration and the analysis time was reduced by two thirds. The improved column also allows the separation of certain compounds that were previously unresolved. Figure (4) shows the baseline resolution of enantiomers of tetrahydroisoquinoline which was difficult to resolve on the original β-CD columns. Other compounds which have been enantiomerically resolved on the improved CD-phases include members of the estrogen β-blocker series and a variety of cancer chemotherapy drugs. There is an obvious need for the pharmaceutical industry to separate enantiomeric compounds which are known to have different physiological activities (19-21). As

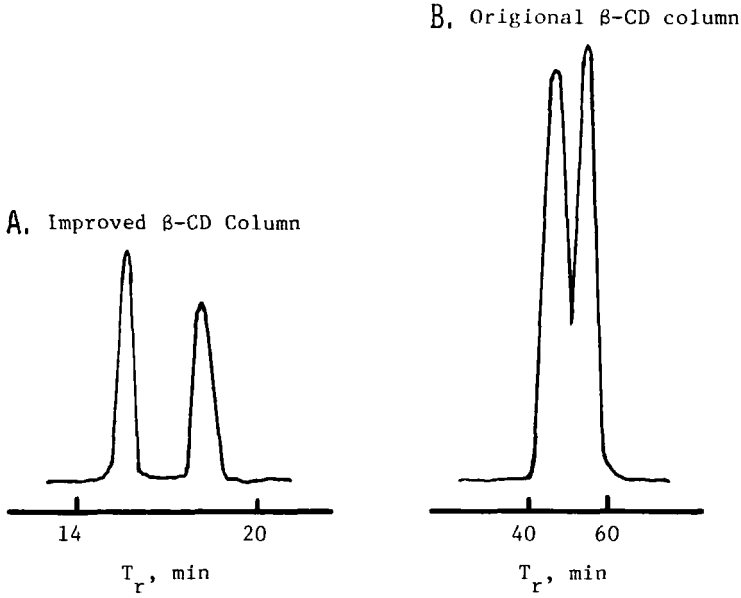

Figure 3. Enantiomeric separation of dansyl-D,L-phenylalanine on an improved and original 25 cm β-cyclodextrin column. Conditions: flow rate 1.0 ml/min, 22°C, mobile phase for chromatogram A was 70% MeOH/30% H_2O; chromatogram B, 50% MeOH/50% H_2O.

improvements continue on the β-CD phases, their versatility and effectiveness will continue to expand.

One of the more interesting developments in cyclodextrin bonded packings is their derivatization. The 2-hydroxy position of cyclodextrins can be functionalized with methyl and acetyl groups. This changes the hydrophobicity as well as the selectivity of the CD phases. Chiral separation requires that the solute enter the cavity, while allowing association between the

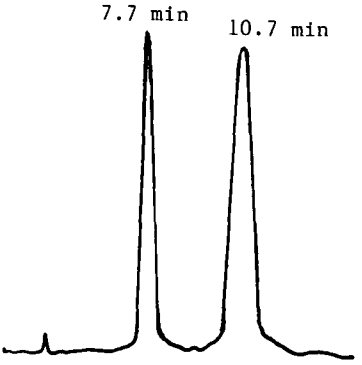

Figure 4. Chromatographic separtion of the enantiomers d,l-1-[5-chloro-2-(methylamino)phenyl]-1,2,3,4-tetrahydroisoquinoline on an improved 25 cm β-CD column. Conditions: flow rate 1.0 ml/min, 25°C, mobile phase 10% acetonitrile/90% TEAA (1%, pH = 4.1).

solutes chiral center (or substituents thereof) and polar groups at the edge of the cavity. An example that illustrates this point is the separation of optical isomers of norgestrel (Fig. 5). By replacing the 2-hydroxyl group with an acetyl group, the cavity becomes more hydrophobic and the entrance is extended to a point where chiral recognition is feasible. The possibility exists to tailor the groups at the entrance of the CD-cavity to meet the spatial requirements of many diverse molecules.

MECHANISM OF SEPARATION

It is widely believed that an inclusion complex should be formed for chiral recognition to be possible (22). This has been verified by performing a normal phase separation (eg. using

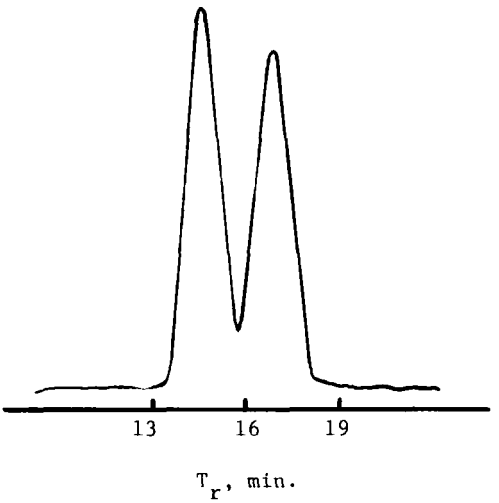

Figure 5. Separation of (±) norgestrel using a 25 cm acetylated-β-CD column. Conditions: flow rate 1.0 ml/min, 25°C, mobile phase 40:60 Methanol-water.

hexanol: 2-propanol mobile phase) on a β-CD column. The hydrophobic solvent occupies the cyclodextrin cavity and the enantiomeric solute is restricted to the outside surface of the cyclodextrin cavity. No enantiomeric resolutions have been achieved in this mode as yet, although excellent routine separations are common. Apparantly, the inclusion complex formed should be a relatively "tight fit" for the hydrophobic species in the cyclodextrin cavity (1,2,22,23). For example, β-cyclodextrin seems to exhibit better enantioselectivity for molecules the size of biphenyl or naphthalene than it does for smaller molecules (4,13,15). Smaller molecules are not tightly held and appear to

move in a manner where they feel the same average environment. It also appears that the chiral center must be near the cavity entrance or have a substituent oriented in a specific position such that it would be able to form at least one strong interaction with the groups present at the cavity entrance (see fig. 1). When an enantiomer is able to fulfill the above conditions, the possibility for chiral recognition is good. Enantioselectivity appears to be due to a combination of cyclodextrin's gross geometry which allows inclusion complex formation and the chirality of the number 2 and 3 glucose carbons at the entrance of the cavity.

It has already been noted that it seems necessary for a solute to interact with the mouth of the cyclodextrin cavity in order to observe enantioselectivity (4,22,24-26). Extensive use of CD-bonded phases made it apparent that small changes in either the structure of the cyclodextrin or the chiral solute, could in some cases cause large differences in enantioselectivity. Norgestrel was an example where the chiral center (the number 17 carbon) and its substituents were, spacially, too far from the mouth of the CD cavity to interact with the 2-hydroxyl groups. By derivatizing the hydroxyl group one effectively changed the enantioselectivity of the stationary phase to enhance chiral recognition. Conversely, if the chiral center of the solute was hidden between large bulky substituents, one could alter the structure of the solute to enhance chiral recognition. This was demonstrated with a series of metallocene compounds (22). (±) α-

Ferrocenylbenzylalcohol was not able to be resolved since the hydroxyl substituent attached to the chiral carbon was apparently hidden between the bulky ferrocene and phenyl groups. By replacing the hydroxyl group with thioethanol, the length of the hydroxy-substituent on the chiral carbon was extended beyond the bulky groups and good resolution was observed. Another example of how small changes in a solute's structure can effect selectivity is the compound binaphthyl crown-5 (27). This crown ether was baseline separated on a 25 cm β-cyclodextrin column. But when one of the crown oxygen atoms was replaced with a nitrogen, (binaphthyl mono-azo- crown-5) resolution was no longer observed. It is apparent that the ability to make small changes in either the cyclodextrin or enantiomer structure provides one with an additional powerful tool to resolve enantiomeric mixtures.

A wide range of solvents can be used with cyclodextrin bonded phases depending upon the particular application at hand. By using mobile phase mixtures such as hexanol: 2-propanol, the cyclodextrin stationary phase is made to function as a normal phase. Separations tend to be analogous to those of a diol column. This is because solutes adsorb to the hydroxyls on the outside of the cyclodextrin while the hydrophobic solvent occupies the cavity. Inclusion complexes usually are formed only in the presence of water and certain organic modifiers such as dimethyl sulfoxide, dimethyl formamide, acetonitrile and alcohols (4,10,11,14). Since the interaction of solutes with cyclodextrin is greatest in water, retention can be increased by increasing the

water concentration in the mobile phase. While broad peaks and tailing are artifacts often associated with long retention times, they can be minimized by the use of buffers. Buffers such as 0.1 to 1% triethylammonium acetate (TEAA), pH = 4.1, sharpens eluting peaks and increases resolution and efficiency. For the derivatives of amino acids and peptides, use of the buffer TEAA (1%, pH=4.1) instead of water, has produced up to four fold increases in efficiency. Other buffers compatable with cyclodextrin bonded phases may also be used such as ammonium acetate and phosphate buffers.

It also should be noted that the elution order of most compounds in the reversed-phase mode on β-cyclodextrin media can be different from that using traditional reversed phase columns. This is indicative of the fact that the retention mechanisms are not the same. For example, the arene tricarbonyl-chromium complex of benzene is retained much longer on β-CD than C_{18} at all solvent compositions measured, while the benzene free ligand is retained much longer on ODS than β-CD at all solvent compositions measured (28).

The effect of mobile phase composition on an enantiomeric separation can be clearly seen in Figure 6. As the methanol concentration is decreased, resolution and retention time are both increased. Therefore, the higher the concentration of the organic modifier, the easier it is for a solute to be displaced from the cyclodextrin cavity. Acetonitrile and ethanol exhibit a greater affinity for the cyclodextrin cavity than methanol, consequently

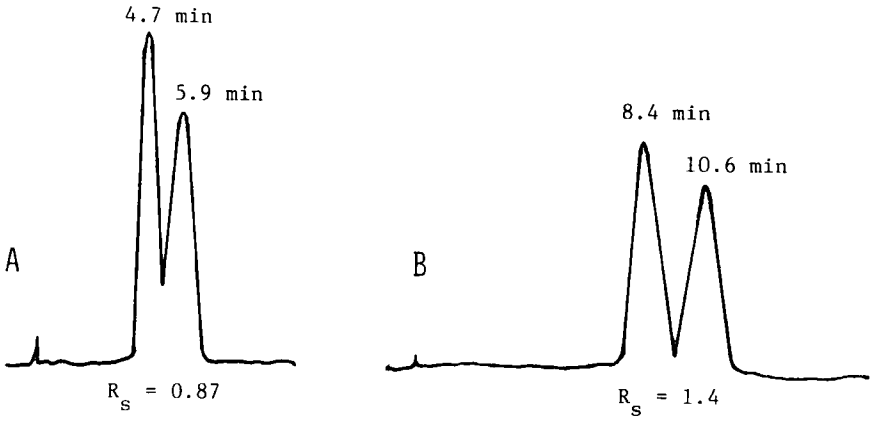

Figure 6. Chromatogram A shows the separation of (±) binaphthyl crown-4 on a 10 cm β-CD column. Flow rate was 1.5 ml/min and the solvent composition 35% methanol, 65% water. Chromatogram B shows the same separation with the organic modifier reduced to 30% methanol.

much lower concentrations of these modifiers are needed to obtain comparable retention times. Furthermore, selectivities of some compounds are very different in MeOH/H_2O and AcN/H_2O. While most compounds studied thus far have exhibited a higher degree of selectivity in methanol/water, a few compounds give better separations in acetonitrile/water mobile phases (29). Further studies are currently in progress to understand fully the mechanisms involved.

Changes in temperature have a greater effect on the retention of solutes on cyclodextrin bonded phases than on comparable reversed phase columns. This is because the binding constant (Ks

in Fig 1) of a solute to the cyclodextrin is significantly effected by temperature. As temperature is increased the binding of the solute to cyclodextrin decreases rapidly. In fact K_s approaches zero between 60° and 80° for most compounds (2,14).

Sometimes during analysis, samples may contain trace impurities which are retained in the cyclodextrin cavity and can eventually decrease their effectiveness. One can usually return the column to its original condition by flushing the column with ethanol or acetonitrile which displaces the impurities in the cyclodextrin cavity.

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