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(*S*)-2-Hydroxypropyl-β-cyclodextrin, a new chiral stationary phase for reversed-phase liquid chromatography

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ABSTRACT

(*S*)-2- and (*R,S*)-2-hydroxypropyl-β-cyclodextrin have been bonded to silica gel and evaluated as stationary phases for reversed-phase liquid chromatography. Stationary phases also were prepared on two silicas having different pore sizes and surface areas. Dissimilarities were observed in enantiomeric selectivities between these columns and also between these and the native β-cyclodextrin columns. With the exception of compounds **5** and **10**, all other racemates reported here which have been successfully resolved on the new phases are enantiomers which have not been previously reported as separated on the β-cyclodextrin stationary phase. In some cases, there were also differences in enantioselectivities observed between the (*S*)- and the (*R,S*)-hydroxypropyl-β-cyclodextrin phases on the same silica. The results are discussed in terms of the retention mechanism and compared to results reported earlier for β-cyclodextrin columns.

INTRODUCTION

Cyclodextrins (CDs), cyclic oligomers of glucose bonded through α-1,4 linkages, have been used successively and extensively both as mobile phase additives (CMA)[1–4] and as chiral bonded stationary phases (CBP)[5–7] in liquid chromatography for the separation of enantiomers. Although the native CDs have proven useful as stationary phases for liquid chromatographic applications, their use as mobile phase additives has been restricted because of the low solubility of the native CDs in aqueous solutions. Derivatizing the CD increases the solubility of the CD; however, derivatization has been found to cause changes in the chromatographic behavior[8]. In reversed-phase applications, the separation mechanism is thought to be the result of the formation of inclusion complexes in which the solute is included into the cavity of the CD. For enantiomers, separation is possible if these inclusion complexes have different binding

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constants. The mouth of the CD hydrophobic cavity is surrounded by secondary hydroxyls which are locked into position and are considered to be important in chiral recognition. In the derivatized CD, some of these hydroxyls are substituted with hydroxypropyl groups. When using hydroxypropyl-β-CD as a CMA, it has been found that as the degree of substitution on the derivatized CD increases, it can sometimes affect the binding process as well as the enantioselectivity. It is probable that the hydroxypropyl groups partially occlude the mouth of the CD cavity and sterically influence the formation of inclusion complexes. Computer modeling[9,10] as well as considerable chromatographic data have revealed that enantioselectivity using CDs is enhanced when there is close proximity of substituents on the "chiral center" of the enantiomer capable of forming hydrogen bonds with the secondary hydroxyls of the CD. Unfortunately, many chiral compounds do not meet this requirement. In an effort to extend the range of chiral separations obtainable using CD-based stationary phases, an additional chiral center was incorporated as a side-chain on β-CD and the derivatized CD was used to synthesize a bonded stationary phase. Both the racemic and the optically pure forms of the hydroxypropyl-β-CD were used. The (*R*) and (*S*) designations are used to specify only the configuration of the hydroxypropyl group and not the configuration of the CD. The resultant hydroxypropyl-β-CD stationary phases were evaluated chromatographically. Numerous separations were obtained on the new hydroxypropyl-β-CD bonded phases that were not possible on the β-CD stationary phase. Differences in selectivity also were observed between the racemic and enantiomerically pure stationary phases. In addition, the effect of bonded-phase loading on selectivity was examined.

EXPERIMENTAL

Materials

The 5-μm silica (Nucleosil, 300 Å, and Spherisorb, 120 Å) was obtained from Alltech. (*R,S*)-2- and (*S*)-2-hydroxypropyl-β-CD were synthesized as previously described[11]. Briefly, β-CD was dissolved in aqueous sodium hydroxide (5%, w/w) and the solution was cooled in an ice bath. Propylene oxide of the desired chirality was slowly added with stirring. After about 6 h in an ice bath, the reaction was allowed to proceed for a day at room temperature, neutralized and dialyzed briefly in order to remove the contaminating salts. The retained solution was filtered and the product obtained by freeze drying. The (*S*)-2-hydroxypropyl-β-CD used for the bonded phase was a mixture of homologues with an average molar substitution of 7.9, calculated per cyclodextrin molecule.

The structures of the solutes used are presented in the tables. Idazoxan and methylidazoxan are drugs under investigation at Reckitt & Colman and were supplied by N. A. Hyde from Danson Lane, Kingston-upon-Hull, U.K. Compounds **8–14** were supplied by Dr. David Kimball at the Squibb Institute for Medical Research, Princeton, NJ, U.S.A., and are listed in the tables using the Squibb designation. The thyroid drugs, 3,3',5-triiodo-D,L-thyronine and 3,5-diiodo-D,L-thyronine, were obtained from Dr. H. J. Cahnmann of the National Institutes of Health, Bethesda, MD, U.S.A. The other solutes were obtained from various sources and used without further purification. Methanol, acetonitrile, triethylamine and glacial acetic acid were obtained from Fisher Scientific (St. Louis, MO, U.S.A.). Water was distilled,

TABLE I

LIST OF BONDED SORBENTS

Column, length × I.D. (cm)	*Bonded ligand*	*Pore size (Å)*	*Surface area (m^2/g)*	*Spacer ($\mu mol/m^2$)*	*CD ($\mu mol/m^2$)*	*%C*
A, 25 × 0.46	*S*-Hydroxypropyl	300	100	1.16	0.19	3.1
B, 15 × 0.46	*rac*-Hydroxypropyl	300	100	0.97	0.21	4.3
C, 25 × 0.46	*S*-Hydroxypropyl	120	170	3.77	0.12	5.6

subsequently deionized using a Barnstead Cartridge, filtered and used without further purification.

Columns

The bonded phases were prepared under anhydrous conditions as reported previously[12]. The bonded sorbents were submitted for carbon analysis. The pertinent sorbent parameters are listed in Table I. The surface concentration was calculated according to the equation[13]

$$\text{surface concentration } (\mu\text{mol/m}^2) = \frac{\%\text{C} \cdot 10^6}{1200N_c - \%\text{C}(M-1)} \cdot \frac{1}{S}$$

where %C is the percent carbon (w/w) from elemental analysis, N_c is the total number of carbons in the bonded ligand, S is the surface area of the bare silica and M is the molecular weight of the bonded ligand. The molecular weight used for the derivatized CD determination of the surface concentration is based on an average molar substitution of 7.9.

RESULTS AND DISCUSSION

Bonding results

As can be seen from Table I, better CD coverages were obtained on the silica with the larger pore size (300 Å). This is indicative of some exclusion of the CD from the smaller pores of the silica used in the preparation of column C. Although column C has a lower CD density, the absolute amount of bonded CD is greater because of the greater surface area of the substrate.

Chromatographic results

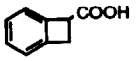
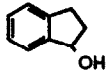
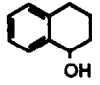
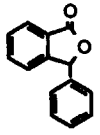
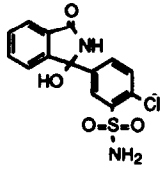
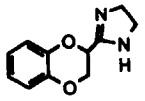
The chromatographic data for the solutes used to evaluate the columns are tabulated in Tables II–IV. If racemates were unresolved on a particular column (A, B or C) no data were reported for these columns in Tables II, III or IV. Unfortunately, due to the different bonding characteristics of each sorbent, it was not possible to obtain a given separation under identical mobile phase conditions on all three columns. Mobile phase conditions were therefore optimized for each column.

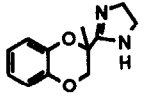
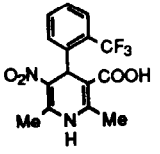
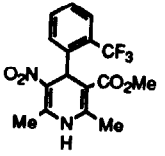
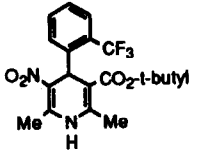
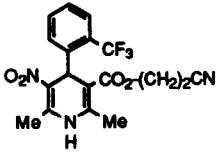
The solutes that were used to evaluate the columns can be loosely classified into

TABLE II

ENANTIOMERIC SEPARATION OF RACEMATES IN WHICH THE CHIRAL CENTER IS A PART OF A RING

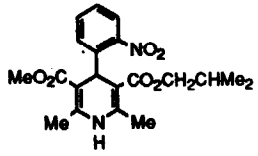
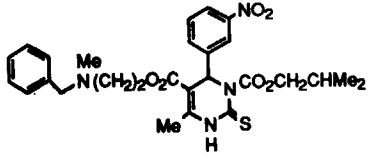
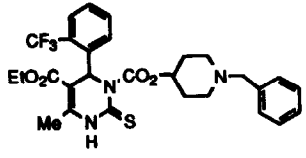
k' = Capacity factor of the first eluted enantiomer; α = separation factor; R_s = resolution; Et = ethyl; Me = methyl; t = *tert*. The buffer used for all of these separations was 1% triethylamine acetate, pH 4.1.

Compound	*Structure*	*Column*[a]	k'	α	R_s	*Mobile phase*
(1) (±)-1-Benzocyclobutene carboxylic acid		A	2.98	1.14	1.0	100% Buffer
		B	1.60	1.10	0.6	95% Buffer in acetonitrile
		C	3.30	1.09	0.6	90% Buffer in acetonitrile
(2) 1-Indanol		A	1.27	1.07	0.6	100% Buffer
		B	1.00	1.11	0.6	100% Buffer
		C	3.00	1.04	0.5	95% Buffer in acetonitrile
(3) (±)-1,2,3,4-Tetrahydro-1-naphthol		A	2.36	1.08	0.7	100% Buffer
		B	1.92	1.08	0.5	100% Buffer
		C	1.71	1.03	0.6	80% Buffer in acetonitrile
(4) 3-Phenylphthalide		B	5.92	1.04	0.5	90% Buffer in acetonitrile
(5) Chlorthalidone		A	1.33	1.38	2.2	95% Buffer in acetonitrile
		B	1.50	1.31	2.8	95% Buffer in acetonitrile
		C	2.80	1.21	1.6	90% Buffer in acetonitrile
(6) Idazoxan		A	0.89	1.07	0.55	100% Buffer
		B	0.63	1.06	0.50	100% Buffer

(7) Methylidazoxan		A	0.75	1.19	1.09	100% Buffer
		B	0.62	1.11	0.60	100% Buffer
		C	1.17	1.08	0.55	95% Buffer in acetonitrile
(8) BAY COOH		A	1.16	1.28	1.56	98% Buffer in acetonitrile
		B	0.65	1.12	0.8	95% Buffer in acetonitrile
		C	3.74	1.11	0.7	95% Buffer in acetonitrile
(9) SQ 28 873		A	2.66	1.06	0.6	95% Buffer in acetonitrile
(10) SQ 30 840		A	4.32	1.22	2.00	85% Buffer in acetonitrile
		B	1.33	1.38	1.50	80% Buffer in acetonitrile
		C	5.46	1.14	1.07	80% Buffer in acetonitrile
(11) BAY CNET		A	3.27	1.10	0.7	98% Buffer in acetonitrile
		B	1.54	1.12	0.5	95% Buffer in acetonitrile

(Continued on p. 186)

TABLE II *(continued)*

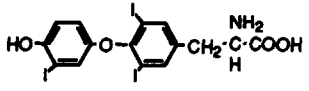
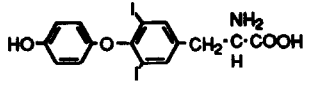
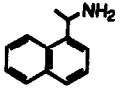
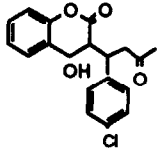
Compound	*Structure*	*Column*[a]	*k'*	*α*	R_s	*Mobile phase*
(12) Nisoldipine		A	4.81	1.13	1.04	90% Buffer in acetonitrile
		C	3.17	1.07	0.6	80% Buffer in acetonitrile
(13) SQ 31 236		A	2.00	1.21	2.00	85% Buffer in acetonitrile
		B	10.86	1.28	1.25	80% Buffer in acetonitrile
		C	2.54	1.13	0.83	95% Buffer in acetonitrile
(14) SQ 31 579		A	5.08	1.14	0.55	95% Buffer in acetonitrile

[a] See Table I for column designation.

TABLE III

ENANTIOMERIC SEPARATION OF RACEMATES IN WHICH THE CHIRAL CENTER IS EXTERNAL TO A RING

k' = Capacity factor of the first eluted enantiomer; buffer A = 1% triethylamine acetate, pH 4.1; buffer B = 1% triethylamine acetate, pH 7.1.

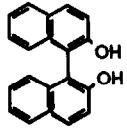
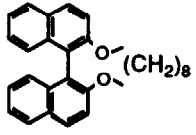
Compound	*Structure*	*Column*[a]	k'	α	R_s	*Mobile phase*
(15) 3,3′,5-Triiodo-D,L-thyronine		A	7.18	1.05	0.65	85% Buffer A in acetonitrile
(16) 3,5-Diiodo-D,L-thyronine		A	8.42	1.07	1.12	90% Buffer A in acetonitrile
		C	4.16	1.05	0.5	80% Buffer A in acetonitrile
(17) α-(1-Naphthyl)ethylamine		A	2.10	1.05	0.3	100% Buffer B
		B	2.58	1.06	0.6	90% Buffer B in methanol
		C	5.40	1.06	0.4	80% Buffer B in methanol
(18) DL-3-(α-Acetonyl-4-chlorobenzyl)-4-hydroxycoumarin		A	4.41	1.06	0.6	95% Buffer B in acetonitrile
		B	5.38	1.04	0.6	95% Buffer B in acetonitrile

[a] See Table I for column designation.

TABLE IV

ENANTIOMERIC SEPARATION OF ATROPIC ANALYTES

k' = Capacity factor of the first eluted enantiomer. Buffer = 1% triethylamine acetate, pH 4.1.

Compound	*Structure*	*Column*[a]	k'	α	R_s	*Mobile phase*
(19) (±)-1,1′-Bi-2-naphthol		A	4.40	1.08	0.6	20% Buffer in methanol
(20)		A	0.64	1.08	0.5	30% Acetonitrile in water
		C	4.16	1.15	1.1	30% Acetonitrile in water

[a] See Table I for column designation.

three groups. The first group consists of those compounds in which the chiral center is incorporated into a ring system. The data for these solutes are tabulated in Table II. Table III contains the data for solutes for which the chiral center is external to an aromatic ring. The third group of solutes consists of atropic compounds, or compounds which contain an axis of dyssymmetry. In almost all cases, the best selectivities were achieved on the larger-pore silica. This is undoubtedly due to the higher derivatized CD density on the silica surface (see Table I).

The spacer chain concentration on the smaller-pore silica is approximately twice that found on the wider-pore silica. This suggests the possibility of an alternative retention mechanism in which the solute interacts with the spacer chain rather than with the CD. The spacer chain, lacking a chiral center, does not confer any enantioselectivity but can contribute to retention. This may account for the lower enantioselectivity of column C despite the generally larger capacity factors relative to the other 25-cm column. Alternatively, there is more likelihood of the CD being linked by more than one spacer chain to the smaller-pore silica. This could limit the mobility of the CD and also sterically restrict access of the CD to the chiral analytes.

As stated in the introduction, the mechanism thought to be responsible for enantiomeric separations using underivatized CDs is related to the formation of a diastereomeric inclusion complex between the CD and the chiral compound. Earlier chromatographic studies using CDs have revealed several solute structural factors affecting chiral recognition[14]. Hydrogen bonding groups adjacent to the "chiral center" capable of interacting with the hydroxyls which line the mouth of the CD cavity enhance enantioselectivity. The hydrophobic interior of β-CD can accommodate a solute of about the same size as a naphthyl or biphenyl group. There must be a relatively tight fit between the solute and the CD cavity for chiral recognition. Better resolution is obtained when the solute has between two and four rings with at least one being aromatic. It also has been found that enantioselectivity is enhanced when the chiral center is "sandwiched" between π systems. In general, the more rigid the substituents on the "chiral center", the better the resolution between enantiomers.

In the case of derivatized CDs, the additional hydroxyl groups external to the CD may play more than one role in chiral recognition. In some cases, the external hydroxyl groups may provide additional sites for hydrogen bonding and may assist in "immobilizing" the solute in the diastereomeric inclusion complex. In contrast to the secondary hydroxyls which are locked into position on the native CD, the OH moiety of the hydroxypropyl groups is free to rotate. This flexibility may allow for a closer approach between the OH and any hydrogen bonding moiety present in the solute leading to stronger or more stereospecific interactions than are possible with the native CD. This effect would be more important for the smaller solutes such as indanol (**2**) and 1,2,3,4-tetrahydro-1-naphthol (**3**). In these cases, the optical purities of the additional chiral centers on the derivatized CD would not play a role in the chiral recognition process. This type of role is also demonstrated by the fact that some enantiomeric pairs (*i.e.*, **5** and **10**) are well resolved on the racemic stationary phase despite the fact that the racemic column was shorter than the optically pure columns (Table II). The fact that the additional hydroxyls do make a contribution to chiral recognition is established because most of these solutes were unresolved on a β-CD column. Compounds **5** and **10** were recently reported as separated on a β-CD column[15]. The β-CD column exhibited better selectivity for these compounds.

In the case of compounds **8–14**, the aromatic ring presumably is the portion of the molecule included into the CD cavity (Fig. 1). The presence of the polar trifluoromethyl (or nitro, compound **12**) substituent on the aromatic ring probably precludes full penetration of the aromatic ring into the CD cavity. Others have found that for geometrical isomers of dipolar substituted aromatics, *ortho*-substituted isomers elute before the *para* isomers[8]. Although the exact substitution patterns of the hydroxypropyl groups on the CD are not known, the presence of these groups no doubt alters the types of interactions possible at the mouth of the CD cavity. These alternate types of interactions possible with the hydroxypropyl-β-CD include not only the hydrogen bonding interactions with the hydroxypropyl OH mentioned above but also include steric interactions with these groups as well. These additional steric

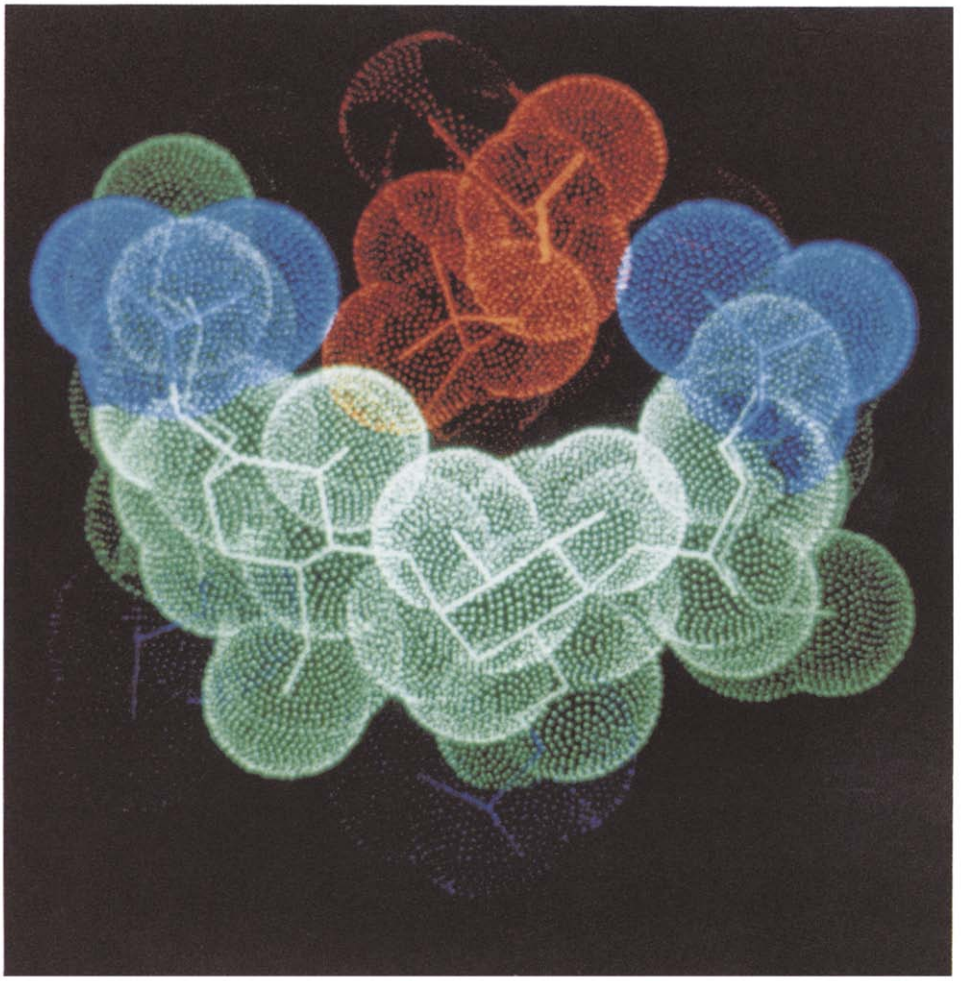


Fig. 1. Computer graphic image of the inclusion complex of BAY COOH (compound **8**), a calcium-channel blocker, and hydroxypropyl-β-CD.

interactions may account for some of the enantioselectivity observed for enantiomers which have bulky substituents on the heterocyclic ring β to the chiral center (*i.e.*, **13** in Fig. 2).

As in the case of β-CD-based separations, selectivity is more likely if the substituents on the chiral center are capable of hydrogen bonding or if the chiral center is adjacent to an aromatic ring. However, the restriction that the hydrogen bonding or aromatic substituents be α to the chiral center seems to be much less stringent for the hydroxypropyl phases. The effect of substitution on selectivity can be seen by comparing the selectivities obtained for compounds with different substituents (Table II). The relative selectivity obtained for the enantiomers of Idazoxan (**6**) is less than that obtained when the chiral center has an additional methyl substituent (**7**). Compounds **9**–**11** are esterified derivatives of compound **8**. The acid is fairly well resolved on all three columns (Fig. 3). This is probably due to hydrogen-bonding interactions through the acid. Some of this resolution is lost, however, when the ester is formed (compounds **9** and **11**) unless the ester has a bulky substituent which can hinder the possible orientations of the molecule in the diastereomeric association complex (compound **10**). In this case, the hydrogen bonding interactions are replaced by steric interactions. Also, for compounds **9** and **12**, the external chiral center of the derivatized CD seems to play a role in the chiral recognition process because the racemic column was unable to resolve the enantiomers. However, the racemic column exhibited better selectivity than the other two columns for compounds **10** and **11**, probably because of the higher bonded CD density.

For some compounds (*i.e.*, **9**, **12**, **15**, **16** and the atropic compounds **19** and **20**), the additional chiral centers undoubtedly play a role in the chiral recognition process because of the significantly greater selectivities achieved on the A and C columns as opposed to the selectivity obtained on the B column (Tables II–IV). Bulky substituents on the chiral centers of the optically active molecules sterically restrict access to the CD cavity and preclude full penetration of the solute into the CD cavity. For example, the presence of an additional iodine on the phenolic ring of 3,3′,5-triiodo-D,L-thyronine (**15**) interferes with the formation of an inclusion complex. Only the optically pure column with the highest bonded ligand density is able to resolve the enantiomers

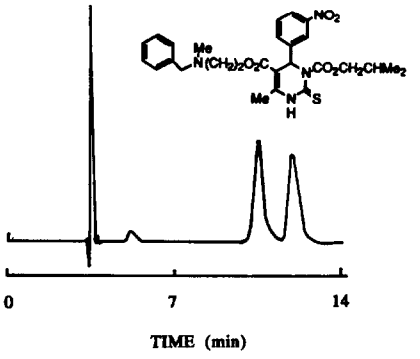


Fig. 2. Chromatogram of a calcium-channel blocker (compound **13**) on the wide-pore (*S*)-hydroxypropyl-β-CD column A. Mobile phase as specified in Table II.

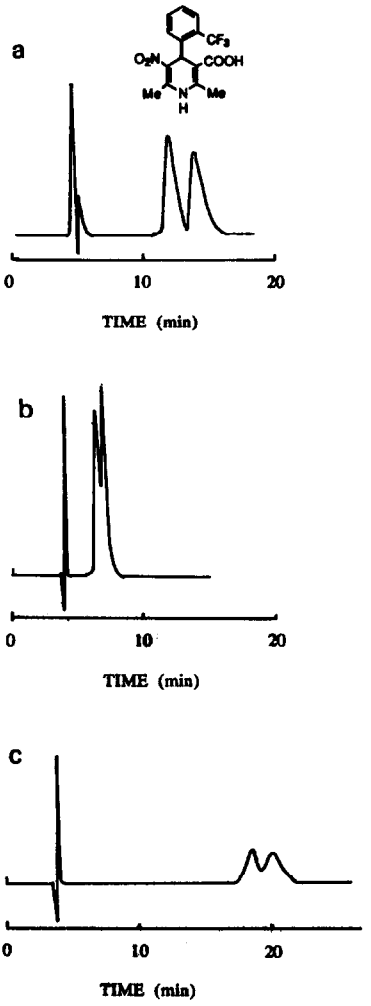


Fig. 3. Chromatograms of BAY COOH (compound **8**) on (a) column A (wide pore, (*S*)-hydroxypropyl); (b) column B (wide pore, racemic hydroxypropyl and (c) column C (narrow pore, (*S*)-hydroxypropyl). Mobile phase as specified in Table II.

(Fig. 4). 3,3′,5,5′-Tetraiodo-D,L-thyronine was unresolvable on any of the columns. The atropic molecules also are sterically restricted from fully entering the CD cavity; therefore, the enantioselectivity must arise through interaction with the chiral center external to the CD. As pointed out by Han and Armstrong[16], it is not necessary for the entire molecule to be included in the CD cavity. In the case of hydroxypropyl-β-CD, it is possible for the hydroxypropyl groups to assume a configuration which would allow stereoselective hydrogen bonding with the chiral portion of the molecule even though the chiral center sits outside the CD cavity as long as a portion of the molecule is tightly complexed within the CD cavity. In order for enantioselectivity to occur, however, the hydroxypropyl groups would have to be optically pure. This would explain the lack of stereoselectivity on the racemic column (B) for these particular solutes.

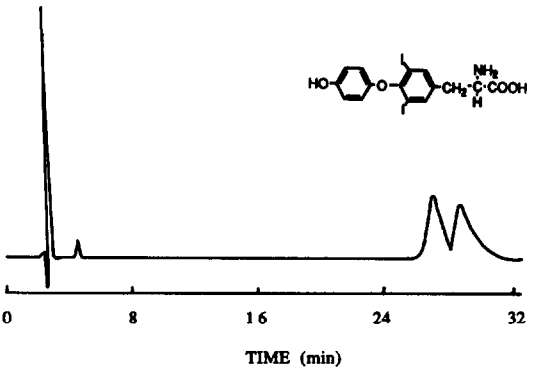


Fig. 4. Chromatogram of 3,5-diiodo-D,L-thyronine (compound **17**) on the wide-pore (*S*)-hydroxypropyl-β-CD column A. Mobile phase as specified in Table III.

In summary, hydroxypropyl-β-CD has been shown to be a useful liquid chromatography packing for the separation of enantiomers which are difficult or impossible to separate using β-CD columns. In most cases, the separation mechanism appears to be, as in the case of the native CD, due to the formation of diastereomeric inclusion complexes. However, the hydroxypropyl-CD phase seems to have more than one mechanism for distinguishing between enantiomers. For smaller solutes, the flexibility of the hydroxypropyl group allows for a closer approach between the hydroxyls and any hydrogen bonding moiety present in the analyte leading to stronger but not necessarily stereospecific interactions than is possible with the native CD. The hydroxypropyl groups can provide not only additional sites for hydrogen bonding but steric interactions as well. In addition, the additional centers of chirality on the hydroxypropyl group may also contribute to enantioselectivity through stereospecific hydrogen-bonding interactions. This is particularly important in cases where the analyte is too big to fully enter the CD cavity.

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